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THE EXPERIMENTS OF BIOSATELLITE II

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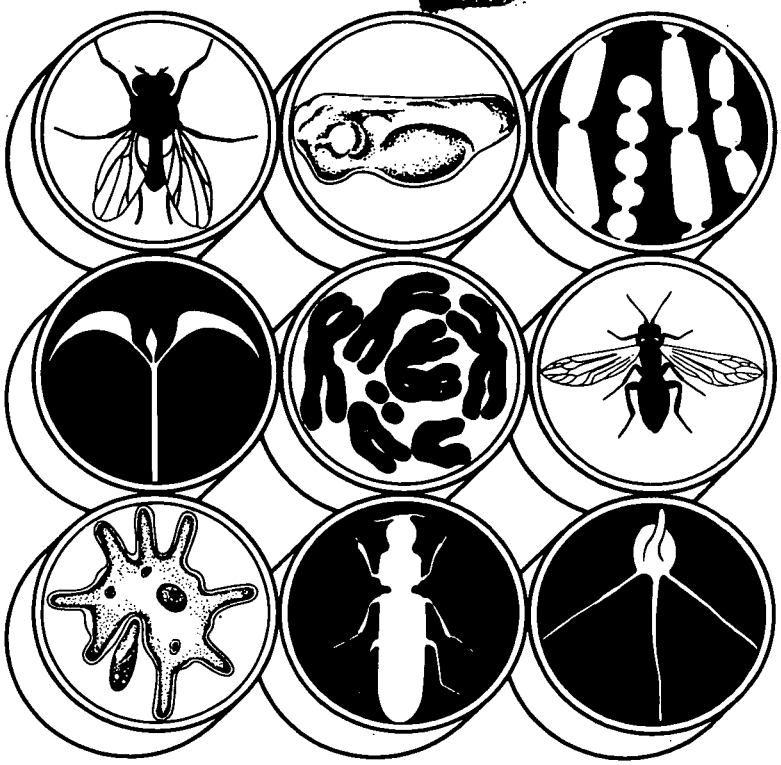

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THE EXPERIMENTS OF BIOSATELLITE II

Edited by
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NASA Office of Space Science and Applications

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Foreword

The Biosatellite Program was the first serious attempt in the United States to design a spaceflight system tailored to the needs of biologic experimentation. In this program, Biosatellite II was the first successfully completed mission. Biosatellite I failed on recovery, and only the engineering tests of the system were completed since the biologic experiments were entirely dependent on recovery for acquisition of data.

For everyone closely connected with Biosatellite II the experience was both painful and rewarding. The novel experience of biologists finding themselves embroiled in a large-scale systems engineering task was probably matched by the frustrations of engineers encountering a new vocabulary and a new set of parameters which, though ill defined, often turned out to be inflexible.

A final frustration for everyone was the requirement, generated by public interest, to present a report on the scientific results long before detailed analyses of experimental material and required postflight ground experiments could be completed.

The document to which this foreword applies finally reports on the matured experimental results. In the interim many of the individual experiments have given rise to papers in the journal literature. At this writing, the 13 experiments have led to well over 100 such contributions which are widely distributed in the scientific literature.

The principal reward, however, for those of us who lived closely with Biosatellite II was a strong sensation of pioneering. For the space program this was the first attempt to carry out classic experimentation, as distinct from observation as in space physics, in the orbital environment. For biologists, there was the new technology of remote and self-contained experimentation with its own potential for generally improving the status of biologic experiments. With this report there will be the reward of completing a long and arduous task and of recording some of the earliest observations of the behavior of Earth life removed from its parent planet.

It is with a great deal of personal pride that I acknowledge the spirit and enthusiasm of the Biosatellite II team. In particular I wish to cite Dr. Joseph F. Saunders, Benny B. Hall, and Thomas P. Dallow, NASA Headquarters personnel, and Dr. Robert T. Seamans, former Deputy Administrator of NASA, who was a motivating force behind the Biosatellite Program.

At the Ames Research Center, Drs. G. Dale Smith, R. H. Schiffman, J. W. Tremor, K. Yokoyama, and J. E. Hewitt played major roles in experiment development, while John Foster, Charles A. Wilson, Bonne C. Look, John W. Dyer, Gary H. Bowman, and the late Robert M. Crane provided managerial skills and engineering talent vital to the project's success. The Biosatellite II pictorial documentary, throughout most of the present volume and in the educational films, is the contribution of Harry R. Zabower.

Eldon A. Volkmer and John C. Tomasello of the NASA Goddard Space Flight Center as well as Donald C. Sheppard and Robert H. Gray of NASA Kennedy Space

Center are cited for their role in tracking and data acquisition, launch vehicle interface, and launch operations. Without the recovery team of the U.S. Air Force and the U.S. Navy, this success story might not have been written.

I gratefully acknowledge the contribution of the General Electric Re-entry Systems Department, especially the work of Vincent C. deLiberato, John T. Glancey, Nicholas J. Dragann, Howard M. Wittner, and Otto Klima and that of Dr. Hilliard Paige and Mark M. Morton.

Throughout the life of the Biosatellite Program we were in constant communication with consultants and advisors for guidance from the scientific community. My appreciation is extended to them all, and specifically to Drs. Nello Pace and W. Ross Adey for the role they played in convincing NASA of the scientific necessity for the Biosatellite Program.

The cover art, designed and illustrated by Paul Bennett of Ames Research Center, pictorially summarizes our experiments.

I would be remiss if I did not extend my deepest appreciation to the experimenters and their scientific associates whose contributions are detailed in the chapters of "The Experiments of Biosatellite II."

ORR E. REYNOLDS,
Director, Bioscience Programs

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Biosatellite II—The Nation's First Biology Laboratory in Space

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With the launch and successful recovery of Biosatellite II, a new dimension has been added to the study of bioscience. Living systems have been removed from the influence of the Earth's gravitational field and periodicities and then recovered in order to determine the effects the experience exerted on fundamental biologic processes. In the early years of spaceflight the status of bioscience experimentation in space was very discouraging in terms of both the prediction of scientific benefit and the dearth of proper instrumentation and reliable spaceflight systems for conducting remotely and recovering well-designed and thoroughly Earth-tested experiments. The concept of the Biosatellite made it apparent that this automated space vehicle could be the unique instrument for studying one of the most fundamental and challenging problems of biology—the relationship of gravity to life processes. A feature of the planned Biosatellite science was that the scientific activities in space would be inseparable from other scientific activities carried out on Earth. A very important facet of the Biosatellite was its interdisciplinary character; a team of scientists, engineers, and managers was assembled to accomplish a national space bioscience objective.

As with every new scientific venture of this magnitude, a formidable problem of organization and management was created. For instance, the complexity of a space mission such as Biosatellite requires large-scale support. Approximately 35 senior bioscientists and 120 technicians from universities, industry, and government played key roles in the development of the experiments. Systems integration, fabrication, communications, launch operations, and other engineering features were the product of approximately 800 contractor personnel. Several hundred U.S. Air Force and U.S. Navy personnel were involved in tracking the satellite and in operations involving recovery of the experiment capsule. Finally, some 200 members of the NASA staff were associated with various phases of the mission.

Years of diligent and personally exhausting scientific and engineering teamwork led to the launch of Biosatellite II[1] on September 7, 1967, at 6:04 p.m. EDT from Cape Kennedy aboard a two-stage thrust-augmented Delta launch vehicle. The 957-pound biology laboratory was placed into a very satisfactory 190-mile near-circular, paraequatorial orbit around the Earth. Unfortunately, the scheduled 72-hour space mission had to be curtailed because of a threatening tropical storm in the Pacific Ocean recovery area and a communication

[1] Biosatellite I, launched on December 14, 1966, with an identical experiment payload, performed successfully for the entire orbital mission until the retrorocket failed to ignite. Thus, reentry was not achieved and no experiment results were obtained.

problem between the spacecraft and its tracking stations (Wilson, 1968). With the highest priority being given to reliable recovery of the Biosatellite and its science, the 280-pound experiment capsule was separated from the spacecraft and deorbited; it reentered the Earth's atmosphere on its parachute and was recovered by the U.S. Air Force over the Pacific Ocean near Hawaii on September 9, 1967, at 3:15 p.m. EDT. Thus, the scientific payload was subjected to only 45 hours of Earth-orbital flight. The successful aerial retrieval of Biosatellite II after almost 2 days in space and its delivery to the scientists for experiment analyses culminated the first phase of the mission. It would be at least 2 years before the second phase was completed—the analyses, data reduction, and scientific conclusions.

BIOSATELLITE II EXPERIMENT SELECTION

The Space Science Board of the National Academy of Sciences (NAS), during its 1962 summer study session, recommended ". . . an exploitation of special features of the 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 (and function) in living systems" (NAS-National Research Council, 1962).

After substantial discussion with prominent representatives of the bioscience community, it was determined that the most desirable procedure with the Biosatellite program would be selection of a fairly large number of well-integrated experiments arranged so as to enable the study of a wide spectrum of biologic phenomena for which there was *a priori* reason for predicting an effect from the conditions of space flight. From this initial "survey," it should be possible to identify fruitful areas of biologic interest for future study.

Although the Biosatellite program was not accepted formally by the Administrator of NASA until December 1962, prior announcements of the program were publicized through scientific media, presentations to research groups, and visits to scientists. The scientific community had enthusiastically submitted more than 185 experiment proposals for consideration for flight in Biosatellites (Saunders et al., 1966). A number of the proposed experiments resulted from projects already sponsored under the NASA Supporting Research and Technology program dealing with basic research related to NASA's missions in space biology, exobiology, and spaceflight.

In December 1962, the Bioscience Subcommittee of NASA's Space Science Steering Committee reviewed the preliminary flight proposals and categorized them according to the following criteria:

Category I. — Well-conceived and scientifically sound investigations pertinent to the goals of the scientific program and the objectives of the particular mission and offered by a competent investigator from an institution capable of supplying the necessary support to insure that satisfactory flight hardware can be delivered on time and that the data can be properly reduced, analyzed, interpreted, and published in a reasonable time after a successful launch. Investigations in category I are recommended for immediate flight and can be displaced only by another category I investigation.

Category II. — Well-conceived and scientifically sound investigations that are recommended for flight, but at a lower scientific priority than those of category I.

Category III. — Scientifically sound investigations that require further development of associated experimental apparatus. Category III investigations should be funded for development and may be reconsidered at a later time for flight acceptance and payload assignment, if possible.

Category IV. — Proposed investigations that are rejected scientifically for the particular mission under consideration.

Forty of the experiments in categories I through III were funded through grants to support their specific definition as flight experiments. It was during this interval of the Biosatellite project that the staff of the NASA Ames Research Center (designated as the experiment and spacecraft management center) became involved with the definition and development of many of the candidate experiments.

In May 1963 panels of specialists were convened to evaluate further these proposals as to their scientific merit, engineering feasibility, and progress achieved. Five panels were formed to deal with proposals in (1) biorhythms in plants and animals, (2) primate and mammalian function, (3) nonmammalian organisms, (4) plant morphogenesis

and photosynthesis, and (5) biologic effects of radiation. The spectrum of scientific areas included genetic and somatic effects of radiation, cosmic radiation, dosimetry, and related physical phenomena. Cardiovascular function, neurophysiologic function, behavior, and metabolic pathways constituted the areas to be studied in primates and mammals. The plant experiments were concerned with morphogenesis, development, growth, and photosynthesis. Body temperature, motor activities, and photosynthetic, metabolic, and growth rhythms were among the phenomena of periodicity. Reproduction, differentiation, cellular phenomena, and longevity completed the proposed panorama of biologic study.

The proposed flight investigations were reviewed and evaluated from the scientific viewpoint by using the following instructions:

(1) The scientific merit of the investigation, including both the desirability of the investigation within the discipline to which it pertains and the probability of acquiring positive scientific results.

(2) The competence and experience of the investigator as an indication of his ability to carry his investigation to a successful conclusion.

(3) The reputation and interest of the investigator's institution, especially from the viewpoint of whether the institution will provide necessary support to ensure that the investigation can be completed satisfactorily.

(4) The scientific adequacy of the proposed apparatus, particularly whether it can supply the data needed.

The recommendations of the panels were reviewed by the NASA Bioscience Subcommittee in December 1963; it expressed unanimous approval of the experiments recommended. In the meantime, three Biosatellite missions were established on the basis of the approved scientific objectives of the various experiments. These are as follows:

(1) *Three-day flight mission.* — To determine the effects of weightlessness and of weightlessness combined with gamma radiation on a variety of living organisms.

(2) *Twenty-one-day flight mission.* — To investigate the effects of the space environment on biologic rhythms as well as the effects of prolonged weightlessness on cellular processes in plants and small animals.

(3) *Thirty-day flight mission.* — To determine the effects of prolonged weightlessness on neurophysiologic function, cardiovascular system, general metabolism, and behavior of primates.

On January 16, 1964, the Bioscience Programs Office recommended to the NASA Space Science Steering Committee a list of 22 experiments for the three missions. Fourteen experiments were recommended for the payload for the 3-day flight, three for the 21-day flight, and five for definition and further development for the 30-day flight. On the basis of its review of January 24, 1964, the Space Science Steering Committee approved the experiments for flight assignment and further development, as recommended.

In February 1964 payload selection was made by the Associate Administrator, NASA Office of Space Science and Applications, for each of the three Biosatellite missions (Saunders et al., 1966). Although 14 experiments originally constituted the scientific payload of Biosatellites I and II, it was determined during experiment development that the experiment dealing with the embryogenesis and development of sea urchin eggs was incompatible because of a seasonal variability and a problem of sperm and egg sensitivity to the plastic housing for the organisms (Willoughby, 1969). The experiment was subsequently withdrawn by the principal investigator; the withdrawal required the approval of the NASA Bioscience Subcommittee, Space Science Steering Committee, and Associate Administrator for Space Science and Applications. The final Biosatellite II experiments are listed in table 1.

The 17 experiments selected by NASA for flight in the three Biosatellites were reviewed again in August 1964 by the Bioscience Subcommittee. Other experiments were recommended in addition to these, provided it were possible to accommodate them in the spacecraft and provided they did not interfere with any of the experiments in the three approved payloads. Eleven experiments were placed in category I and three, in category III. This continuing Subcommittee review of experiments and their progress was maintained up to the time of launch of each Biosatellite.

EXPERIMENT ACCOMMODATION

On March 2, 1963, a request was announced to industry for a proposal for the design and development of a spacecraft for the Biosatellite program.

TABLE 1. – The Biosatellite II Experiments

Experiment No.	Principal Investigators	Institution	Experiment
P–1017	S. P. Johnson[a]	North American Rockwell Corp.	Weightlessness effects on the pepper plant
	T. W. Tibbitts	Univ. of Wisconsin	
P–1020	S. W. Gray	Emory Univ.	Weightlessness effects on wheat seed anatomy
	B. F. Edwards		
P–1138	H. M. Conrad	Resources Planning & Control Corp.	Weightlessness effects on wheat seed biochemistry
	S. P. Johnson	North American Rockwell Corp.	
P–1096	C. J. Lyon	Dartmouth College	Weightlessness effects on wheat seed physiology
P–1035	R. W. Price	Colorado State Univ.	Weightlessness effects on the amoeba
	D. E. Ekberg	General Electric Co.	
P–1047	R. S. Young	NASA Ames Research Center	Weightlessness effects on the frog egg
	J. W. Tremor		
P–1037	F. J. deSerres	Oak Ridge National Laboratory	Radiation-weightlessness effects on bread mold
	B. B. Webber		
P–1039	J. V. Slater	Univ. of California, Berkeley	Radiation-weightlessness effects on flour beetle
	B. Buckhold		
P–1079	R. C. von Borstel	Oak Ridge National Laboratory	Radiation-weightlessness effects on parasitic wasp
	A. R. Whiting		
	R. H. Smith		
	R. L. Amy	Southwestern Univ., Memphis	
	D. S. Grosch	North Carolina State Univ., Raleigh	
P–1123	A. H. Sparrow	Brookhaven National Laboratory	Radiation-weightlessness effects on blue flower (spiderwort)
	L. A. Schairer		
P–1135	R. H. Mattoni	NUS Corp.	Radiation-weightlessness effects on bacteria
	E. C. Keller, Jr.		
	W. T. Ebersold	Univ. of California, Los Angeles	
	W. R. Romig		
	F. Eiserling		
P–1159	E. Altenburg[a]	Rice Univ.	Radiation-weightlessness effects on vinegar gnat adults
	L. Browning		
P–1160	I. I. Oster	Bowling Green State Univ.	Radiation-weightlessness effects on vinegar gnat larvae

[a]Deceased.

The three Biosatellite mission types previously mentioned were described in the announcement so that flexibility and adaptability of the spacecraft would be dictated by these as well as future experiments. Responses came from the Bendix Corp., Aeroneutronics Div. of Ford Motor Co., General Dynamics/Astronautics Corp., General Electric Co., Lockheed Missiles and Space Div. of Lockheed Aircraft Corp., McDonnell Douglas Corp., and Northrop Corp. The proposals were evaluated according to their response to the experiment specifications and engineering restraints provided by the Ames Research Center. After evaluation of all spacecraft design proposals, study contracts were awarded to Northrop Corp., Lockheed Missiles and Space Div. of Lockheed Aircraft Corp., and the General Electric Co. Re-entry Systems Department (GE/RSD). In July 1963, after evaluation of these design studies, GE/RSD was selected as the spacecraft contractor for the design, fabrication, and testing of six Biosatellite spacecraft, a prime and a backup vehicle for each of the three missions.

Early in 1964, North American Rockwell Corp. and GE/RSD were awarded contracts to develop, fabricate, and test hardware items and experiment packages to assist the biologists in carrying out their experiments in the remote space laboratory. As one can imagine, innovations in both experiment procedure and experiment accommodation were mandatory for the successful conduct of the new space bioscience.

Simultaneously with experiment and spacecraft

development, the NASA Goddard Space Flight Center undertook the task of spacecraft tracking and communications. Although experiment data were not telemetered, it was required that capsule temperature and relative humidity, which were vital to the experiments, be transmitted to tracking stations. Goddard personnel determined that the Biosatellite would be compatible with the existing Space Tracking and Data Acquisition Network. A "Multipurpose Satellite Operations Control Center" was tailored to Biosatellite needs; it featured telemetry demodulation stations, digital and analog displays, and other vital functions necessary for directly observing, in real time, spacecraft reactions and responses (Dyer, 1968).

At Cape Kennedy's Unmanned Launch Operations Facility the details were worked out for spacecraft compatibility with the Delta launch vehicle. Biology laboratories were constructed for the final assembly of the experiments and their integration into the spacecraft. Duplicate experiment packages were maintained as Earth controls for those orbiting in the Biosatellite. Extensive training and practice by teams of biologists and engineers were required to meet the rigid demands of schedule and mission constraints.

Special attention was devoted to recovery of the Biosatellite II capsule after its return to Earth. Details were worked out with the U.S. Air Force and the U.S. Navy for rapid recovery procedures by either air retrieval (the primary mode of recovery) or water recovery. Because of the perishable nature of the experiments, it was essential that recovery be as rapid as possible; temporary laboratory facilities were established at Hickman Field, Hawaii, to accommodate the experimenters for quick dismantling of their experiments and for "first look" and preparation of their flight specimens for transport to mainland laboratories.

THE SPACECRAFT LABORATORY

The Biosatellite II spacecraft was 8 feet in length and 4.5 feet in diameter at the area of mating with the Delta launch vehicle (figure 1). The spacecraft systems are shown in figure 2. The adapter assembly remained in orbit after retrofire separated it from the recovery capsule which contained the experiments (Dyer, 1968).

The basic design of the spacecraft was predicated on protection of the experiments from the external environment and provision of a controlled atmosphere within the compartment wherein the experiments were contained. The experiment capsule was made of aluminum varying in thickness from 0.080 inch at the nose to 0.035 inch in the conical aft region (fig. 3 and Look, 1968).

Biosatellite II used bottled air at a presure of 1 atmosphere. Throughout the flight the monitors for total pressure and partial pressure of oxygen indicated that these were maintained at 14.5 psia and 146 mm, respectively. Because of their concern

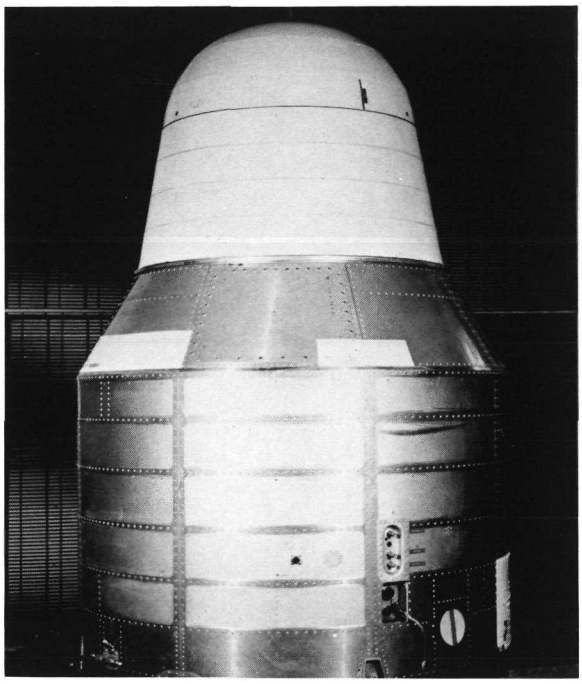

FIGURE 1.— Biosatellite II spacecraft.

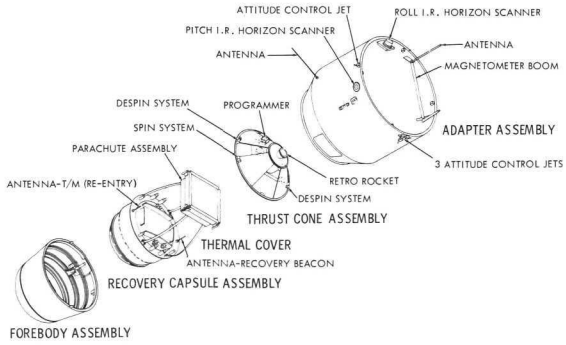

FIGURE 2.— Biosatellite II spacecraft systems.

for atmospheric contaminants which might interfere with experiments, the experimenters established, prior to flight, acceptable levels of major atmospheric contaminants. Samples of the experiment capsule atmosphere were taken prior to the closure of the capsule before launch and prior to its opening following recovery. Table 2 shows the comparison of the flight capsule observations with the preflight specifications. With the exception of formaldehyde, a component of the fixative used in the wheat seedling experiment, all constituents were within the ranges specified.

Preflight and postflight analyses of ethylene in the Biosatellite II capsule atmosphere were not specified. However, in the phase C postflight test (cf. discussion in section entitled "Results") an analysis for ethylene in the atmosphere was made because of the concern that the closed atmospheric system of Biosatellite II could lead to the accumulation of such volatile constituents. A concentration of 0.9 ppm of ethylene was found in the recycled atmosphere of the Biosatellite II capsule, whereas less than 0.05 ppm (limit of instrument sensitivity) was detected in the control capsule.

The specifications were for a temperature of 21.1±2.8 C and a relative-humidity range of 40 to 70 percent. Figure 4 documents the conditions of temperature and relative humidity existing inside the Biosatellite II experiment capsule. Data were obtained from the time of experiment assembly to the time of opening of the capsule following its recovery. Average temperatures are shown for the

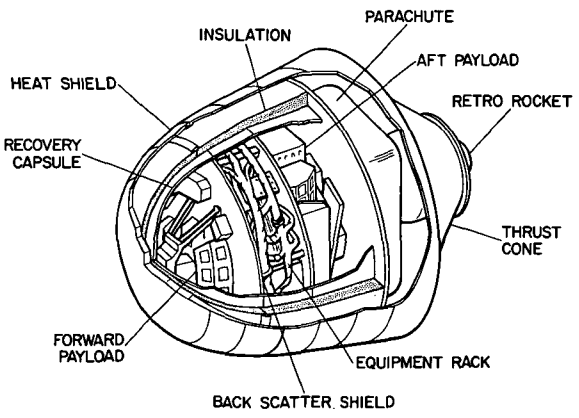

FIGURE 3.– Experiment capsule of Biosatellite II.

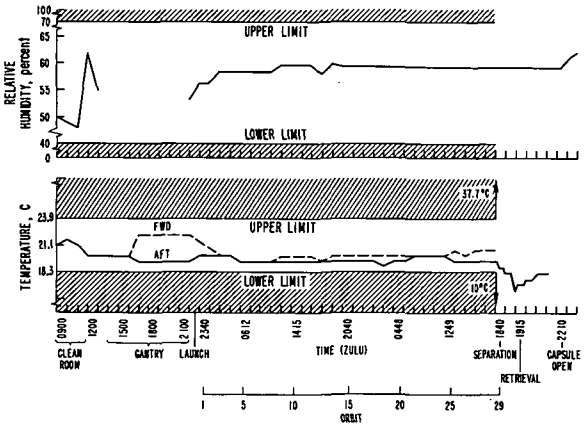

FIGURE 4.– Biosatellite II temperature and relative-humidity profile.

TABLE 2. – Biosatellite II Capsule Atmosphere Analysis

Constituent	Preflight	Postrecovery	Specification
Formaldehyde	<0.8 ppm	0.8 to 2 ppm	1 ppm
Glutaraldehyde	0.4 to 0.8 ppm	0.4 to 0.8 ppm	3 ppm
Oxygen	21.3 mole %	21.5 mole %	17.7 to 21.7 mole %
Nitrogen	80.4 mole %	79.8 mole %	77.3 to 82.3 mole %
Carbon dioxide	0.1 mole %	0.3 mole %	0.026 to 1.0 mole %
Ozone	Not detected[a]	Not detected[a]	0.1 ppm
Hydrogen sulfide	Not detected[b]	Not detected[b]	20 ppm
Carbon monoxide	Not detected[c]	10 ppm	50 ppm
Ammonia	Not detected[b]	Not detected[b]	50 ppm

[a] Sensitivity to 0.1 ppm.
[b] Sensitivity to 1.0 ppm.
[c] Sensitivity to 10.0 ppm.

forward (FWD) and the aft experiments.

A major requirement was that acceleration during orbit should not exceed 10^{-4} *g* and that the spacecraft maintain 10^{-5} *g* for 95 percent of the mission. An exception to these specifications was acceptable during alinement of the spacecraft for its deorbit maneuver. The extent to which the *g* level requirements were satisfied for spacecraft stabilization is shown in figure 5. As illustrated, the 10^{-5} *g* limit was exceeded for short periods of time during the first nine orbits. However, the excessive *g* rates were quickly reduced to within control limits when the rate control mode was activated during the pass of Biosatellite II over tracking stations. It was estimated that the acceleration level was above 10^{-5} *g* for about 11 percent of the total flight period instead of for the designed 5 percent (Look, 1968).

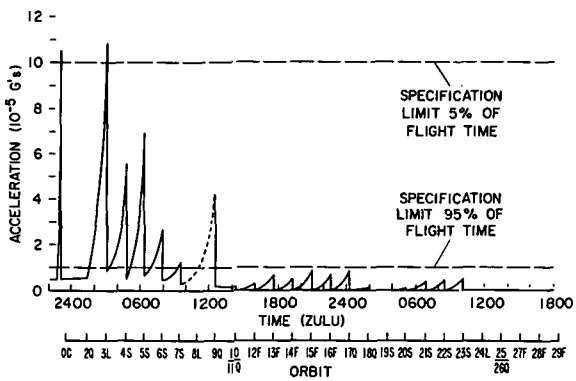

FIGURE 5.— Payload acceleration during the orbital flight of Biosatellite II.

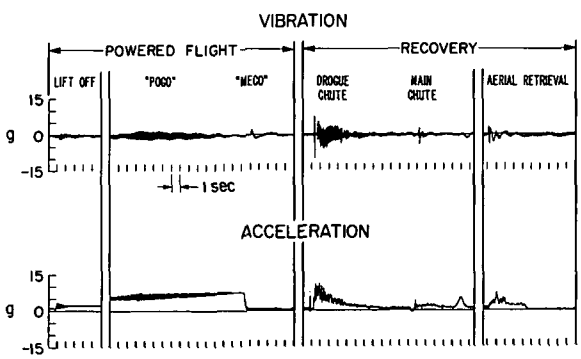

FIGURE 6.— Vibration and acceleration in Biosatellite II along longitudinal (thrust) axis.

The Biosatellite II vibration and acceleration profiles were measured along the longitudinal (thrust) axis during powered flight and recovery. Only the most active periods are shown in figure 6.

In order to measure the vibration, acceleration, and noise levels within the Biosatellite II experiment capsule, a system was devised that was compatible with the spacecraft tape recorder — a seven-channel unit developed for the recording of biomedical data on Gemini (Look, 1968). A schematic diagram of this vibration, acceleration, and noise measurement system is shown in figure 7.

No evidence of noise above the 100-dB lower limit of the capsule sensors was recorded except during the 17-minute period of aerial retrieval. At this stage of flight, sound pressure levels up to 120 dB were recorded in the frequency range of 20 to 10 000 Hz. During launch a peak of 138 dB was reached for only a few seconds.

The power supply, telecommunications, attitude control system, programmers, storage containers, and other equipment required during orbital flight but not recovery were contained in the adapter assembly. Power was supplied by a battery having a capacity of 330 ampere hours at 36 volts.

Data from the spacecraft were received in the form of pulse code words, frequency modulated on a 136-MHz carrier (Dyer, 1968). One frame of data was composed of 265 words of 6-binary bits (plus 1 parity). The transmission rate was 1 frame per second. Data were arranged in 118 channels of information, 62 of which dealt with spacecraft environmental parameters and verifications of the electromechanical actuation of experiments. The

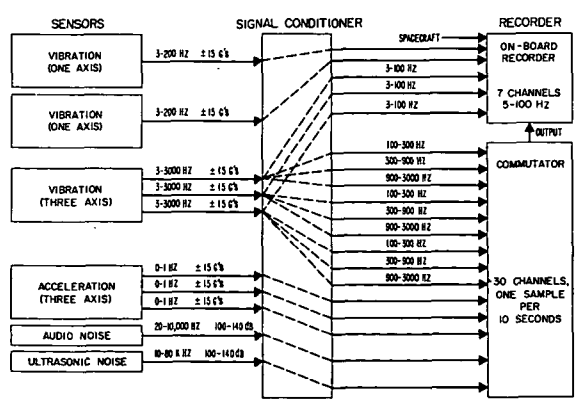

FIGURE 7.— Schematic of the vibration, acceleration, and noise measurement system in Biosatellite II.

remaining channels were utilized for spacecraft performance evaluation and operational control.

When the orbital phase of Biosatellite II was complete, the spacecraft, by command, was alined for deorbit, and the reentry vehicle was separated from the adapter. As the reentry vehicle decelerated and reentered the Earth's atmosphere over the Pacific Ocean, a parachute was deployed, thereby making it possible for aerial retrieval of the Biosatellite II capsule and its experiments.

THE EXPERIMENTS

Thirteen experiments divided into two groups (see table I) were flown in this space biology laboratory in order to study the effects on life processes of (1) weightlessness and (2) weightlessness combined with gamma radiation. The basic goal of the flight was to determine the effects of the space environment on fundamental biochemical processes in cells, the development and growth of plants and animals, the structure of living tissues, and changes, if any, in genetic transmissibility. Specifically, the radiation-weightlessness experiments were expected to show whether weightlessness would be synergistic, antagonistic, or unresponsive in its interaction with radiation. In general, the objective of Biosatellite II was to perform experiments to explore the role of gravity in the maintenance of normal organization and function in living systems. A second objective was to obtain information on molecular, genetic, and cellular changes for a better assessment of the possible hazards to living systems of a new spectrum of physiologic stresses encountered during spaceflight.

Prior to the philosophic approach to the Biosatellite II radiation-weightlessness experiments, various organisms were put into missiles and sent up to high altitudes in balloons and rockets by the United States and the Soviet Union for the study of ambient radiation. Later, as it developed the capability for orbiting spacecraft, the U.S.S.R. initially approached the problem through studies of the effects of weightlessness combined with ambient radiation. Many of these early experiments were performed without precise spacecraft environmental control. The ambient radiation levels encountered in Earth orbital spaceflight were very low, i.e., below the threshold of recognized radiation response, and they were inaccurately measured (Jenkins, 1968).

Thus, the Panel on Radiobiology of the Space Science Board's Environmental Biology Committee undertook a study of those aspects of the space program which involve radiobiologic interests (NAS - National Research Council, 1963). The panel strongly recommended that "a few simple, but discriminating, experiments be performed to ascertain whether additional experiments in space would have any basic radiobiologic interest." The following remarks of the panel constituted the basis for a recommendation for controlled radiobiologic experiments in space:

> There is the remote possibility that the radiobiologic response may be modified by factors as yet unknown and perhaps not susceptible to study terrestrially. Experiments designed to settle this matter must include the exposure of biological materials during space flight and must meet the following criteria of reliability: (1) the use of well known biologic systems, e.g., mutation induction or chromosome breakage; (2) the use of sufficient number of individuals in the experiment to guarantee statistical precision of the results; (3) the exposure of the system to known quantities and qualities of radiation; and (4) the use of adequate Earth controls.
>
> An example of a suitable experiment might be the exposure of a stable cell system to known quantities of radiation from an enclosed source while in orbit. A package no larger than a pencil could be designed for such an experiment. The experiment should provide for a graded series of radiation doses to a set of samples completely sealed, so far as possible, against external influences. Such preliminary experiments could be completed within a year.
>
> If the results of such adequately controlled experiments on diverse systems are negative, then the question of the existence of space factors that modify radiobiologic principles, as we now understand them, would be answered in the negative. The further pursuit of such modifying factors would, at this point, no longer exist as a matter of primary practical importance.
>
> Only after physical studies have provided sufficient information on the temporal and spatial distribution of radiations of different qualities in space should there be investigations with biologic material on the ambient radiations in space to check for effects that are unexpected. For these investigations simple systems are needed that can survive the rigors of space flight without too elaborate environmental control, and that can be measured either automatically in flight or after recovery. Failure of the ambient radiation to produce unexpected results in several systems should lead to de-emphasis of radiobiologic experiments in orbit.

In principle, these criteria were used to design the payload for radiobiologic experiments. The experiments concerned with the effects of combined weightlessness and radiation were exposed to

precise doses of gamma radiation from an 85-strontium nitrate source. These seven experiments were located in the forward section of the capsule (figs. 3 and 8 and table 1). The individual experiments were located at various distances from the radiation source and were mounted on a radiation shield of 0.050-inch tungsten bonded between 0.008-inch-thick aluminum sheets. The ^{85}Sr source was contained in a tungsten-nickel-copper sphere which, on Earth command, was opened by a spring mechanism 1 hour after Biosatellite II was on orbit (cf. paper by Hewitt, p. 333). It was closed, again by command from Earth, 1 hour prior to reentry. Experiment packages identical to those exposed to radiation in the forward compartment were located in the aft compartment to serve as inflight nonirradiated controls.

The biologic species upon which the radiation-weightlessness experiments were performed included bacteria, insects, and plants. In the larvae and adults of *Drosophila melanogaster* (two experiments) well-established genetic effects were studied. These effects included recessive lethal mutations, visible mutations at specific loci, loss of dominant markers from the Y chromosome, translocations, chromosome crossing over in the male, and chromosome nondisjunction. Gross mortality and cytologic studies of chromosome aberrancies in somatic cells, in addition, were quantitated in the *Drosophila* larvae.

In pupae of the flour beetle, *Tribolium confusum,* studies were made of a prominent developmental wing abnormality consisting of median elytral splitting, elytral blistering, and membranous wing protrusion. Pupal periods and dominant lethality were observed in the F_1 offspring (oocytes) of males and females exposed to spaceflight.

The *Habrobracon junglandis,* or parasitic wasp, experiment was designed to survey mature sperm and the various stages of oogenesis for mutations (especially dominant lethality), recessive lethal and visible mutation frequencies, and inherited partial sterility. Of concern also was the effect of spaceflight on survival, lifespan, and biochemical and behavioral differences of the animals.

Tradescantia clone (02), the spiderwort, was studied to determine the effects on frequency of spontaneous and radiation-induced chromosome aberrancies and spontaneous mutations. Specific genetic and somatic endpoints included color changes (from blue to pink or colorless), stunting in stamen hairs, pollen abortion, microspore death, disturbed spindle function, and total chromosome abnormalities.

In the *Neurospora crassa,* or orange bread mold, experiment a two-component heterokaryon was used. The heterokaryon was obtained by fusing two different haploid strains, each with its own genetic markers. The frequency of radiation-induced recessive lethal mutations at two specific gene loci was studied. Point mutations and chro-

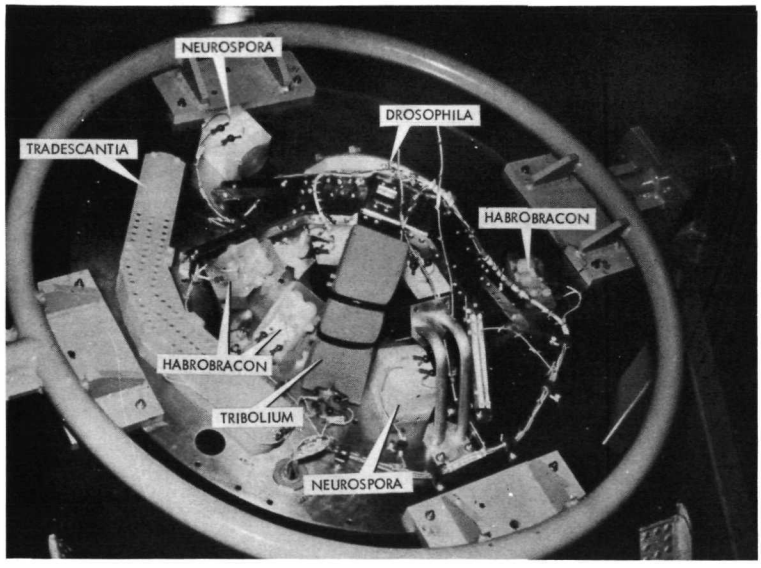

FIGURE 8.— Biosatellite II radiation-weightlessness payload assembly mounted on tungsten shield. Experiments surround the radiation source.

mosome deletions, as well as overall survival, were included in the parameters under study.

Strains of *Salmonella typhimurium* and *Escherichia* coli were used to study the induction of lysogeny, a biologic process extremely sensitive to a variety of environment factors such as vibration and radiation. Free phage production and bacterial population density were included in the study.

The experiments dealing only with the interaction of living systems with greatly reduced gravity included species considered to be sensitive to altered values of *g*. These experiments were placed in the aft compartment (figs. 3 and 9 and table 1) where they were shielded from radiation.

Long ago it was determined that the egg of the frog responds to disorientation with respect to the force of gravity. Thus, it became obvious that the gravity-sensitive stage of embryologic development in the egg of *Rana pipiens* might be adaptable for studying the effects of weightlessness on the ability of the fertilized egg to divide, differentiate, and develop normally.

The amoeba, *Pelomyxa carolinensis,* appears to be independent of the Earth's *g* force, although it does require a gravitational force to attach itself to a substrate for locomotion and feeding. During feeding its protoplasmic movement may be independent of gravity. It was hypothesized, therefore, that weightlessness might alter the manner and rate of reproduction, cell division rate, synchrony of nuclear division, digestive processes, locomotion, and morphogenesis and cause disruption of function in cellular and subcellular processes.

Three different groups worked with the wheat seedling *Triticum vulgare* (Georgia Experiment Station, 1123). Originally each of the three groups submitted independent experiments. Two were concerned with a variety of physiologic, histochemical, and anatomic changes in developing wheat seedlings, whereas the third was devoted to biochemical phenomena in corn. Because of space, weight, and power constraints, the three independent teams of experimenters agreed to merge their experiments and, so that they could still maintain autonomy, to use one experiment package containing a number of wheat seeds that would adequately satisfy the needs of all three teams. The specific experiments were: (1) The physiology of *Triticum vulgare* was determined as a function of time by measuring the lengths of the roots and coleoptiles. Postflight photography enabled measurements of the angles of roots and shoots so as to determine the degree of disorientation caused by the absence of gravity. (2) Morphogenesis, histochemistry, and growth were the principal foci of the anatomic study. (3) A study of key enzymes associated with some of the intermediary metabolic and energetic processes rounded out the wheat seedling experiment. Tissue slices were analyzed for six enzymes, protein content, oxygen consumption, amino acids, and ethylene production.

During the flight of Biosatellite II the geotropic

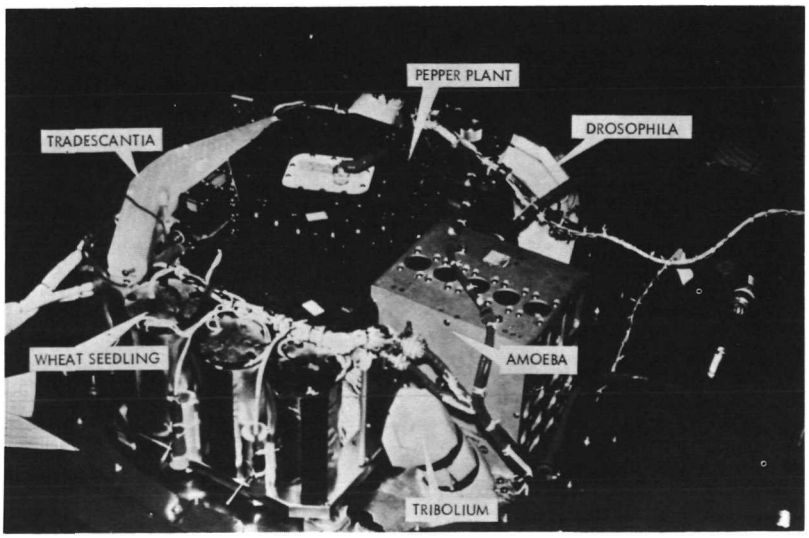

FIGURE 9.— The Biosatellite II nonradiation experiment assembly.

response of the pepper plant, *Capsicum annuum,* was photographed at the rate of 1 frame every 10 minutes. Changes in the liminal angle were determined and mobilization of amino acids and carbohydrate was studied.

All experiments and associated hardware experienced exposure to the expected spaceflight conditions, with the exception of weightlessness, prior to the mission so that background data on possible responses to vibration, linear acceleration, temperature excursion, and other factors could be obtained.

Each experiment prototype package and the experimental biologic systems had been vibrated at 1.5 times the expected flight levels. Most of them had been vibrated with and without a radiation source. Exhaustive tests of vibration, acceleration, and shock as well as the simulated thermal-vacuum of space established the capability and durability of the 13 experiments and the assurance that they would perform as intended. Where feasible, detailed analyses were performed to determine the effects exerted on the living materials by these physical stresses.

Several sets of Earth controls at Cape Kennedy were run simultaneously with the orbital period for comparison with the flight experiments. These were separated into three categories. Control I was the Biosatellite II qualification capsule and was identical to the orbiting vehicle. The environment was held to the nominal constant environment of the flight capsule (temperature, relative humidity, and irradiation). Control II was a growth chamber with temperature and relative humidity identical to those of control I, but without irradiation. Control III consisted of four incubators with temperatures following the average temperature telemetered from Biosatellite II, but at a delay of 4 hours. Radiation exposure was provided by a 1.2 Curie source of ^{85}Sr matched in strength to the flight source and exposed for the same period of time as was the Biosatellite II source. Temperature and relative humidity were controlled by an air-conditioning system.

Because there is, to a limited extent, a similarity between clinostat[2] treatment and the weightless state, this instrument was used prior to flight to study the geotropic response of the plants as well as to study their responses to nullification of the directional component of the gravity force vector. In addition to the foregoing controls, Earth clinostat plant controls were in effect for the entire flight period of Biosatellite II. The wheat seedling Earth clinostat controls were continued beyond recovery for a total experiment period of 65 hours.

Acceleration levels (both vibrating and linear), temperature, and relative humidity in the Biosatellite II spacecraft were maintained under satisfactory control within limits used in preflight testing (figs. 4 and 6). The most serious anomaly in the flight having an effect on the conduct of the experiments was the necessity for terminating the flight about 1 day early, for reasons previously mentioned. Therefore, the experiments which were designed to be completed in 72 hours actually were shortened by one-third. In some experiments this decreased the expression of changes brought on by weightlessness and complicated the analysis.

RESULTS

Five months after the recovery of Biosatellite II the preliminary scientific observations in each experiment were presented at a symposium convened by the National Academy of Sciences and NASA on February 23 and 24, 1968 (Saunders, 1968). Two months later, the experiments dealing with weightlessness combined with radiation were discussed during a Biosatellite II symposium at the Sixteenth Annual Meeting of the Radiation Research Society.

Initially, the experiment results indicated an interaction between radiation and one or more factors encountered during spaceflight (Reynolds and Saunders, 1968). This interaction varied from increasing the effects of radiation severalfold to decreasing the effect slightly, but significantly. Several of the experiments had a background of control studies which assured that vibration alone could not have been the only interacting factor; others did not have sufficient preflight control data. The set of experiments for the study of the biologic effects of weightlessness alone showed a close correspondence to results obtained by exposure to rotation on the clinostat (Thimann, 1968).

For these reasons, postflight laboratory control studies were performed in order to clarify some of

[2] The clinostat provides a time-integrated change of direction of the gravitational vector over 360°; therefore, it avoids the directional influence of gravity. It does not remove gravity or produce a condition of weightlessness.

the ambiguities seen and to interpret more precisely the phenomena observed in the preliminary results. In the process of removing any of these uncertainties, it was hoped that important clues might be uncovered concerning some of the biologic mechanisms involved. It was anticipated, too, that leads for future space bioscience research might be obtained.

The postflight tests were conducted at the NASA Ames Research Center under conditions simulating as closely as possible the environments encountered during the Biosatellite II mission (Dyer, 1969). The tests were divided into three phases, as follows:

(1) *Phase A.* — The objective of the phase A tests was a comparison of the radiation-scattering characteristics of the Biosatellite II capsule and those of the Earth control capsule. Radiation doses were measured within the experiment packages at the positions normally occupied by the biologic specimens. The doses within the packages were compared with those measured at the locations of the lithium fluoride (LiF) tubes mounted on each package. Since this test dealt only with dosimetry, no living systems were used.

(2) *Phase B.* — In the phase B test, living experiment materials were used in both the flight capsule and the Earth control. Simultaneously, the two vehicles and their experiments were subject only to radiation identical with that of the flight. Temperature and relative humidity were of the levels observed during flight. The intent of the test was to detect differences, if any, due only to the capsule environment.

(3) *Phase C.* — The phase C test was performed with biologic experiments in both capsules. Radiation and flight-level vibration and acceleration stresses were simulated, as closely as possible, for the flight capsule, whereas the Earth control capsule served as a static control with radiation. A plot is shown (fig. 10) of the power spectral density levels for the high-frequency lift-off and transonic simulation with the Biosatellite II capsule. Figure 11 shows the acceleration levels recorded by the three transducers during the two-phase, low-frequency, sine-sweep portion of the lift-off vibration simulation. The simulated launch acceleration profile is illustrated in figure 12.

As more information accrued following the postflight tests, it was disseminated as widely as possible by the experimenters. Presentations were made and discussions were held during meetings of the Committee on Space Research (COSPAR) in Tokyo (May 1968), in Prague (1969), and in Leningrad (1970); at a Symposium on the Genetic Effects of Space Environments (Tokyo, 1968); and at the Biosatellite II Symposium sponsored by the Sections on Botany and Zoology of the American Association for the Advancement of Science (Dallas, 1968).

In July 1969 the flight results and all Earth-

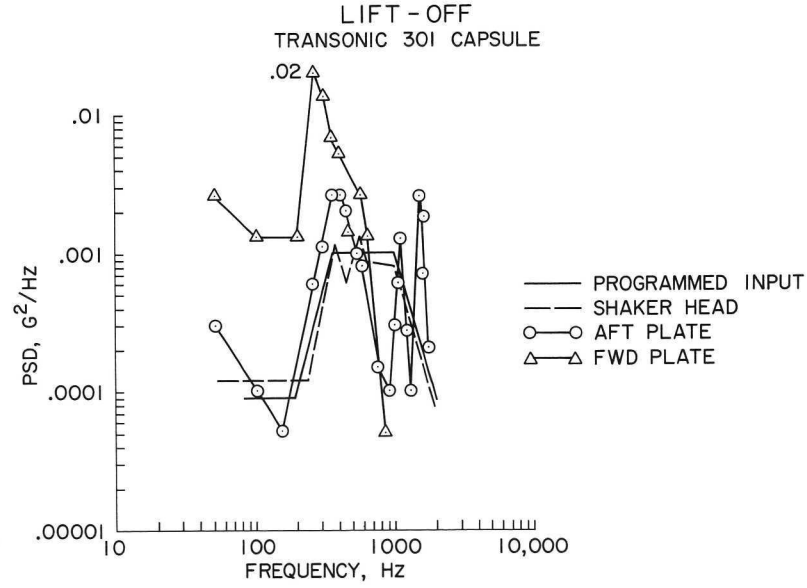

FIGURE 10.— Liftoff transonic vibration profile. Phase C; postflight.

control test findings were reviewed during the National Academy of Sciences Summer Study at Santa Cruz (NAS - National Research Council, 1970). One of the conclusions of the study was: "Radiobiologic experiments carried out on Gemini flights and in Biosatellite II have shown that, for well-defined genetic endpoints, there is no significant synergism between radiation and the spaceflight environment. The question of whether space factors modify radiobiologic principles, as we know them, has been answered in the negative. In this context, the experimental program has been successful . . ."

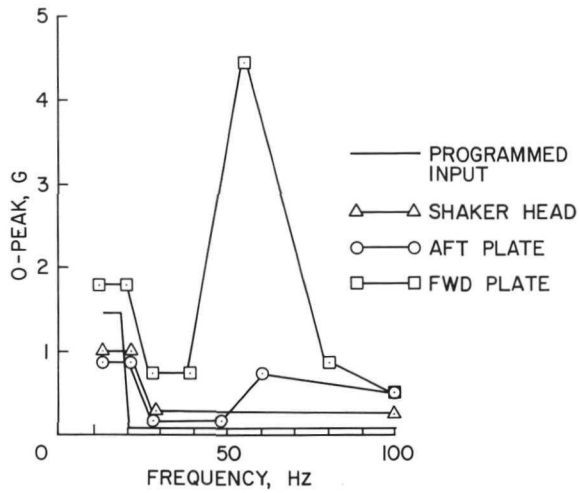

FIGURE 11.— Low-frequency sine-sweep vibration. Phase C; postflight.

During a review of the final results of the Biosatellite II experiments in New Haven in January 1970, a number of findings were considered as significant expressions of weightlessness at the cellular, subcellular, and molecular level. It was surprising that many fundamental biochemical and energetic processes appeared to be unaffected by spaceflight.

The details of each experiment are presented in succeeding papers.

CONCLUDING REMARKS

Still not completely resolved is the question of the effects of interaction of radiation and weightlessness on biologic systems. In the short-term effects observed in the Biosatellite II studies there is certain evidence of strong synergism between radiation and weightlessness. Further, evidence still persists of an interaction between radiation and spaceflight which was not duplicated in postflight acceleration and vibration tests.

The influence on the observed phenomena of possible contaminating gases, e.g., ethylene, formaldehyde, and glutaraldehyde, are uncertain. The latter two gases were used as fixatives in the wheat seedling and in the amoeba and frog eggs, respectively.

Vibration was found to be a prime contributor to many of the aberrancies ascribed originally to spaceflight. Even in those findings considered significant, vibration effects cannot be excluded totally because exact duplication of the combined space-

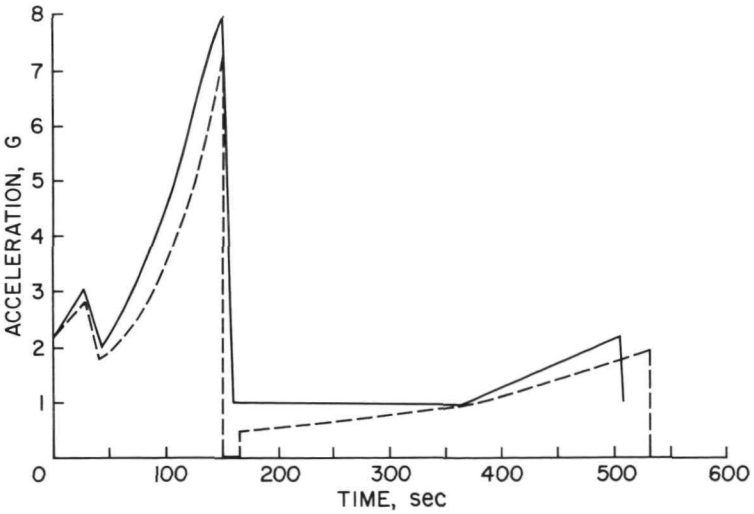

FIGURE 12.— Launch acceleration profile. Phase C; postflight.

flight vibration and acceleration profile is not possible.

In interpreting some of the noted responses to irradiation during spaceflight, caution must be exercised in that cosmic ray primaries (heavy particles) cannot be excluded, at this point, as being contributory.

In general, this variety of Biosatellite II experiments that covered a broad spectrum of biology made it possible to make a preliminary assessment of the effect of the space environment on biologic function in both plant and animal systems. The success of Biosatellite II represents the beginning of a development of understanding of the role of gravity in the evolution of life and its function on Earth. It is obvious, however, that it will take many more well-conceived experiments under carefully controlled conditions before definite conclusions can be expected.

For example, "One hundred years ago the Swiss biochemist, Friedrich Miescher, succeeded in chemically isolating a new and peculiar substance, lying deep within the nuclei of the sperm cells of salmon. It was distinct from protein and because it occurred in nuclei, he called it nuclein. Eighty-four years later, James Watson and Francis Crick published two papers dealing with the molecular structure of that same amorphous white powder" leading to the identity of the substance we now casually refer to as DNA (Haskins, 1970).

Discussions of the experiments of Biosatellite II will go on, but years will pass before their full impact on biology can be realized.

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Experiments With Invertebrates

Mutational and Physiologic Responses of *Habrobracon* in Biosatellite II[1]

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and

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For our investigation of the genetic effects of spaceflight in Biosatellite II we elected to follow six principles: (1) It was essential to use mutational tests that had been used numerous times and ones in which we had considerable faith. The journey into outer space was innovative enough without adding innovations to the biologic test system itself. (2) Our test, in greatest depth, should be restricted to the most homogeneous population of cells, and in this case we chose the sperm for progeny testing. (3) We wished to look at the immediate genetic effects on the cells that had been exposed directly to the space environment and not at later generations of cells. (4) We wanted to test as many different cell types as possible because cells in different stages of gametogenesis not only have vastly different nucleocytoplasmic ratios (from sperm with a ratio $> 10:1$ to mature oocytes with ratio of about 10^{-6}) but also are metabolically different and show different sensitivities to radiation. (5) We were determined to study dose-action kinetics of the organisms placed in a variety of positions away from the radiation source that was placed aboard Biosatellite II. Thus, we would have dose-action curves at our disposal, and we would have a series of doses of radiation for cells of varying sensitivities, with different dose optima for studying effects. Further, with a variety of containers in different positions in the spacecraft, we could look for position effects within the spacecraft itself. (6) Last, we resolved to keep a sharp lookout for anomalies that might arise which had no relation to the sensitive genetic tests we were to use — thus different physiologic criteria also were assayed.

The parasitic wasp, *Habrobracon,* is especially suitable for the types of genetic studies that were performed in Biosatellite II. This small braconid, *Habrobracon juglandis* Ashmead = *Bracon hebetor* (Say), has been the object of genetic investigations for 50 years and has been particularly useful for mutational analyses. It has a short life cycle (approximately 9 days at 30 C), a visible ontogeny (embryonic development is clearly seen through a transparent chorion; the larvae are ectoparasitic; and the pupae dwell in a brittle, easily breakable cocoon), and staging and sensitivity of the gametes have been thoroughly studied and are well understood. One of its more useful features is that unfertilized eggs develop into haploid males, a phenomenon that simplifies dominant lethal analyses and facilitates identification of mutation changes such as recessive lethals and translocations occuring any place in the genome.

A feature of *Habrobracon* that has been exploited during the last two decades is that information on the entire genome at every stage of development can be obtained from every egg laid by a treated female, or by a female mated with a treated male. In order that each egg be followed throughout development, there necessarily is a limit to the

[1] Research jointly sponsored under NASA Grant NsG678 with the Atomic Energy Commission under contract with the Union Carbide Corp.

number of females (about 150 for each day of egg laying) that any one investigator can handle. Since the flight of the Biosatellite II was guaranteed to be a unique experience, we were pleased to have the help of a number of investigators from several different institutions (von Borstel et al., 1968). We worked together for a short time before, during, and after the flight and the postflight tests in order to secure large volumes of data.

EXPERIMENT DESIGN AND METHODS

Experiments

The data reported here were obtained from three experiments with *Habrobracon* in the spacecraft, one from the orbital flight and two from the tests on Earth after the flight. Each of these experiments in the spacecraft was controlled by the *Habrobracon* being exposed to radiation in a setup which was of essentially the same geometrical configuration as the flight vehicle. The postflight tests afforded the opportunity to examine the animals exposed inside the spacecraft when it was not subjected to stress (the phase B test), (cf. paper by Saunders, Reynolds, and Smith, p. 1) and after it had been subjected to vibration, acceleration, and shock that approximated the stresses of the flight itself (the phase C test). Since preparation for a *Habrobracon* experiment requires a 43-day leadtime to build up the genetic stocks necessary for a test and a 6-month posttest period for the numerous genetic analyses of the organisms, we were unable to analyze all of the organisms placed in the flight vehicle and Earth-based control setup during the test phases B and C. Therefore, it was necessary for us to make guesses as to which postflight tests were most crucial—we guessed correctly most of the time.

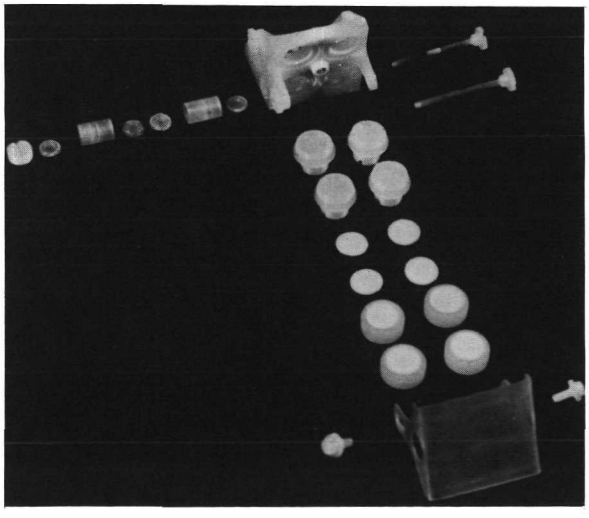

FIGURE 1.— Disassembled flight package used for the *Habrobracon* portion of the Biosatellite II experiment.

Hardware

Five packages, each containing four modules to hold the *Habrobracon,* were used for the experiment. The modules were secured on each package to face the radiation source and named according to their position facing the source; i.e., LR is lower right, UL is upper left, etc. A disassembled pack-

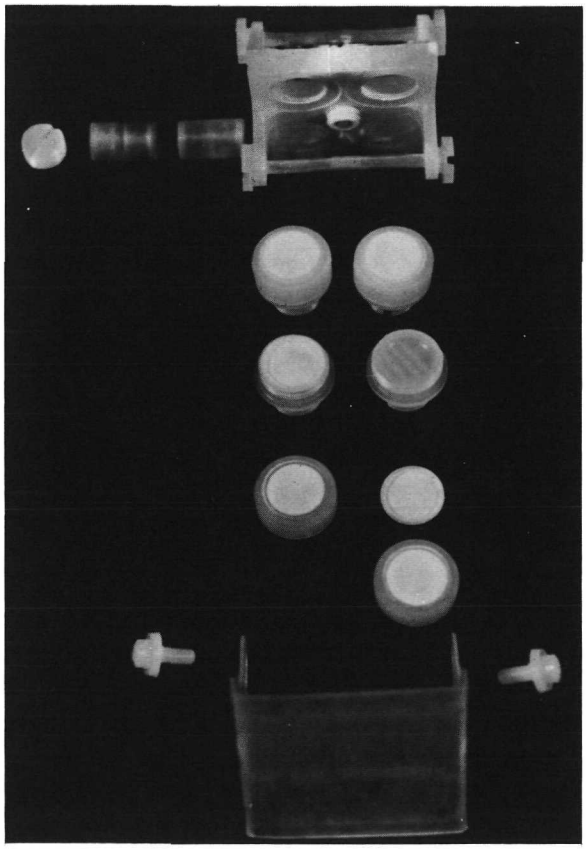

FIGURE 2.— Partially assembled flight package. The tubes for lithium fluoride powder are in place on the bracket; two of the modules are capped; one of the modules has a screen overlying the depression where the *Habrobracon* are held; and the other module is still disassembled. The capped containers for the *Artemia* and *Saccharomyces* are at the upper left of the photograph.

age used for the *Habrobracon* experiment is shown in figure 1. The same package, in different degrees of assembly, is shown in figures 2 and 3. Three of the packages were like the one shown in the figures. The 4000 R nominal exposure package had a concave face for placement of the modules containing the *Habrobracon* to ensure adherence to the isodose requirements. The 0 R nominal exposure package held multilayered modules so that more *Habrobracon* could be contained. Some of the *Habrobracon* in this package were irradiated before the launch.

So that the radiation exposure could be measured, each module carried three Toshiba glass-rod dosimeters. Additional dosimetry was accomplished by placing lithium fluoride (LiF) powder in tubes held in front of and behind the modules. *Artemia salina* (San Francisco strain) and *Saccharomyces cerevisiae* were placed in the containers provided for them at the lower part of the packages. A thermistor to record the local temperature was located centrally between the modules.

Radiation

Most of the *Habrobracon* were exposed to five (including 0 R) different exposures of radiation during the flight. Some of the *Habrobracon* were irradiated with 2000 R at a rate of exposure of approximately 120 R/min just before the launch; these were held in the nonradiation section of the flight vehicle. The *Saccharomyces* was placed in the package primarily to detect scattered radiation, since its response to linear energy transfer is well worked out (Mortimer, Brustad, and Cormack, 1965). *Artemia* blastocysts were used, since they are sensitive to vibration.

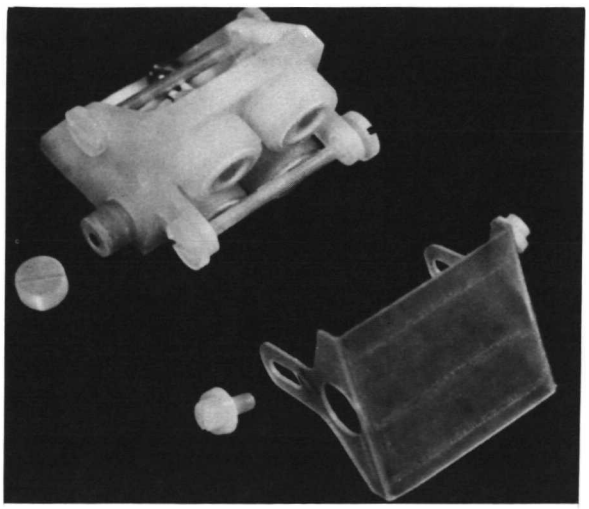

FIGURE 3.— Partially assembled flight package. The modules and tubes for lithium fluoride powder are in place; one of the containers for the *Artemia* and *Saccharomyces* is shown protruding from the side of the bracket. The secondary electron equilibrium shield lies in front of the package.

The five packages in the spacecraft and in the Earth-based control system were placed relative to the 85-strontium source so that the *Habrobracon* would receive nominal γ-radiation exposures of 4000, 2000, 1000, 500, and 0 R. The highest exposure was not achieved in Biosatellite II because of the foreshortened flight. By an unfortunate circumstance the 1000 R nominal exposure package was tilted in the spacecraft. The bracket holding it had been inadvertently turned 180° during spacecraft assembly so that the package was tilted upward at an angle of approximately 30° away from the source. This mistake was not noted and therefore not corrected by any of the inspectors who were responsible for checking the assembly of the spacecraft. It was detected after the flight when the Toshiba glass dosimeters and LiF powder were being measured. The error was verified by examination of photographs taken during disassembly of the spacecraft. This configuration of the 1000 R package was used only in the Biosatellite II flight and in the flight vehicle in the phase C experiment.

For the Biosatellite II flight, the radiation source was activated at 7:04 p.m. EDT on September 7 and closed on the 28th orbit at 12:58 p.m. EDT on September 9, an exposure period of nearly 42 hours. This exposure duration was also used in the phase B and phase C tests. The average exposures calculated for the *Habrobracon* positions inside the modules are shown in table 1.

Habrobracon

The strains of *Habrobracon* used for the experiments were selected for their divers properties (table 2). The Raleigh (*R*) strain, wild type, is vigorous and long lived under conditions of stress. The *lemon* strain has fragile females and reasonably vigorous males, but the *lemon* locus has the useful characteristics of being semidominant. By the time the Biosatellite II vehicle was ready to be launched, we had decided to incorporate a linkage

TABLE 1. – Radiation Conditions (Corrected for Geometry and Scattered Radiation) in the Flight Vehicle and Earth-Based Control

Experiment	Average exposure to *Habrobracon*, R				
Nominal exposure	0	500	1000	2000	4000
Biosatellite II flight	.7	356	589	1272	2425
Biosatellite II Earth control I	1.7	371	729	1245	2431
Phase B flight	1.5	342	689	1219	2376
Phase B Earth control I	1.5	341	723	1261	2442
Phase C flight	1.7	352	575	1232	2471
Phase C Earth control I	1.5	360	726	1241	2420

TABLE 2. – *Habrobracon* Strains Used in Flight and Earth-Based Experiments

[*R*, Raleigh wild-type strain; *le*, lemon strain; *c*, cantaloupe; *ho*, honey]

Date	Experiment	Animals in test		Females used for mating
		Females	Males	
Sept. 7 to 9, 1967	Biosatellite II	$\frac{le++}{+c\ ho}$	*le*	*R*+/+
Mar. 12 to 14, 1968	Phase B	$\frac{le++}{+c\ ho}$	*R*+	$\frac{c\ ho}{c\ ho}$
May 22 to 24, 1968	Phase C	$\frac{c\ ho}{c\ ho}$	*R*+	$\frac{c\ ho}{c\ ho}$

test into our program and had prepared vigorous females, heterozygous for the *lemon (le), cantaloupe (c)*, and *honey (ho)* markers on chromosome I.

Since the phase B and C tests were done shortly after the flight, we were unable to build up the *lemon* strain to a sufficiently large population without jeopardizing the data collecting from the flight animals. We elected to use males from the Raleigh strain for phases B and C and to use our supply of *lemon* males to prepare the heterozygous females. By the time of the phase C test, we were unable even to prepare the heterozygous females without jeopardizing the collection of data from the phase B test. Both post-flight tests followed the flight too closely for optimum control of all parameters of the *Habrobracon* experiment. Nevertheless, by making deliberate choices in one postflight test or the other, we were able to control fully all of the endpoints where spaceflight effects had been found.

Analysis of the males followed the procedures of von Borstel and Rekemeyer (1959) for dominant lethality, recessive lethality, and inherited partial sterility. Analysis of the females followed the procedures of Whiting, Smith, and von Borstel (1968) for total dominant and recessive lethality induced during oogenesis, and of Grosch (1968) for oogonial killing and dominant lethality.

Yeast

The strain X841 yeast is a diploid yeast of the following genetic composition:

$$\frac{a}{a}\ \frac{his5\text{-}2}{his5\text{-}2}\ \frac{trp1\text{-}1}{trp1\text{-}1}\ \frac{+}{pet1}\ \frac{arg4\text{-}1}{ARG4\text{-}1}\ \frac{ARG4\text{-}2}{arg4\text{-}2}\ \frac{thr1}{THR1}\ \frac{met1}{MET1}\ \frac{URA1}{ura1}\ \frac{lys1}{LYS1}\ \frac{LEU1}{leu1}\ \frac{ADE2}{ade2}$$

This multipurpose stock has a variety of uses (Mortimer et al., 1965). Since most of the experiments for which this stock was designed were already being carried out in the Biosatellite II experiment, we were interested primarily in two tests: (1) A determination of the frequency of recombination within the *arg4* gene, as estimated by the frequency

of revertant colonies on plates lacking arginine but containing all other auxotrophic requirements; and (2) a determination of the frequency of recombination between the *ade2* marker and the centromere, as estimated by the frequency of red colonies and colonies with red sectors.

The yeast was grown at 30 C in 600 ml of complete medium in four 2000-ml flasks which were constantly shaken. They were prepared about 2 to 3 weeks prior to flight. After the yeast growth had saturated at approximately 2×10^8 cells/ml, the flasks were sampled to determine which had the lowest frequency of revertants. These flasks were stored at 2 C.

Two days prior to the flight the yeast suspensions were centrifuged and the supernatant fluid was decanted. The wet yeast was then packed in the small cylindrical containers shown in figure 1 and again refrigerated. The yeast was transported to Cape Kennedy under refrigeration. After termination of the experiment, the yeast was brought back to the laboratory under refrigeration. Assays were carried out on complete medium and on medium containing all requirements except arginine.

Artemia

The San Francisco strain of *Artemia salina* was used for the Biosatellite II experiment. It has the

TABLE 3. – Summary of Dominant Lethality in *Habrobracon* Sperm in the Biosatellite II Experiment

Observation	Nonirradiated packages		Radiation packages			
Nominal exposures, R	0	2000 X-ray	500	1000	2000	4000
Biosatellite II flight						
Actual exposures, R	0.7	2000	356	589	1272	2425
Total eggs	3337	704	2010	1691	1536	1736
Preblastula death	46	177	107	160	308	538
Male offspring	925	236	574	432	423	564
Female offspring	1628	200	869	781	570	411
Frequency of preblastula death of diploid eggs	0.015	0.421	0.076	0.133	0.298	0.509
Biosatellite II Earth control						
Actual exposures, R	1.7	2000	371	729	1245	2431
Total eggs	2309	865	1705	1782	1512	1712
Preblastula death	30	252	119	176	249	552
Male offspring	702	270	524	601	536	587
Female offspring	1186	234	765	732	495	359
Frequency of preblastula death of diploid eggs	0.014	0.466	0.105	0.161	0.284	0.550
Biosatellite II postflight phase B flight vehicle						
Actual exposures, R	1.5	2000	34.2	689	1219	2376
Total eggs	722	739	704	618	582	730
Preblastula death	13	248	38	60	106	276
Male offspring	176	166	146	153	132	184
Female offspring	454	241	440	310	257	201
Frequency of preblastula death of diploid eggs	0.014	0.444	0.062	0.125	0.237	0.522

highest frequency of emergence for any wild-type strain, with approximately 77 percent of the cysts developing to the adult stage. The *Artemia* cysts were loaded from the vacuum-sealed containers in which they are kept into the cylindrical containers used in the experiment (fig. 1). Some of the *Artemia* were preirradiated with 16 kR. Six replicate dishes with 50 cysts per dish were assayed at each dose level.

RESULTS

The first observation made after the flight of Biosatellite II was that every *Habrobracon* had survived and all were in excellent condition.

Genetic Analysis of Males

There were no differences among the flight- and Earth-based controls when spontaneous or radiation-induced dominant lethal mutations in sperm were the criteria (table 3). Table 3 contains the computed frequencies of the preblastula deaths, the class that represents deaths caused by the breakage-fusion-bridge cycle (von Borstel, 1960). Other types of dominant lethal events such as those caused by large deletions or losses of chromosomes were present in the same frequency in the flight and control animals. This is shown in figure 4 where dose-action curves for hatchability are presented; hatchability represents that fraction of the population which escapes death both from bridge breakage and from chromosome imbalance.

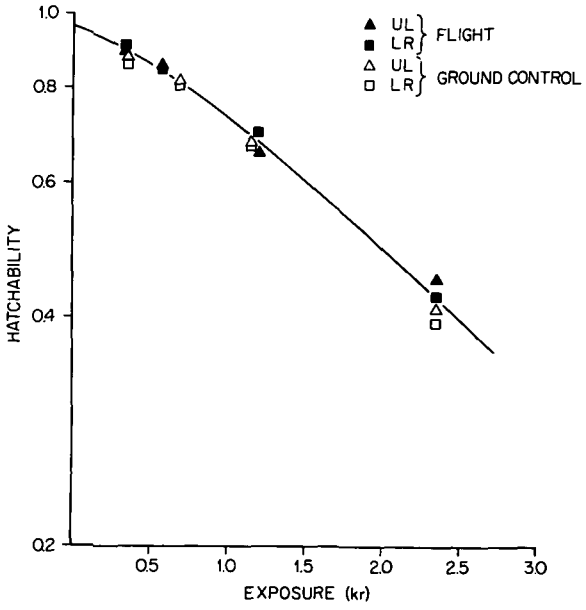

FIGURE 4.— Hatchability of fertilized eggs from females mated to males irradiated in the spacecraft and the Earth-based control. UL, *Habrobracon* from upper left modules; LR, *Habrobracon* from lower right modules (von Borstel et al., 1968).

The spontaneous recessive lethal mutation frequency in sperm in the flight animals was 22/378 = 0.058 (table 4). This was greater by a factor of more than 3 than that of the controls, 12/732 = 0.016.[2] Most of the control data were obtained from the same strains of *Habrobracon* tested before the launch. In the phase C experiment spontaneous lethal mutation frequency in sperm showed an enhancement similar to that found in flight animals when compared with that of animals in the phase B experiment. The phase C animals were from the flight vehicle which had undergone a simulation of the flight profile. Thus, we believe that the enhancement of spontaneous recessive lethal frequency in sperm was induced by some factor of the flight profile other than weightlessness.

There was no effect of spaceflight on radiation-induced recessive lethal mutation frequency or on frequency of inherited partial sterility in sperm (table 4 and fig. 5). The recessive lethal mutation frequencies follow the expected one-hit kinetics, and inherited partial sterility approaches the expected two-hit kinetics.

Genetic Analysis of Females

The females were transferred at daily or subdaily intervals for a 20-day period. The transfers were made at previously established intervals and were designed to permit collection of groups of eggs which were in different stages of oogenesis during the flight.

One of the more puzzling findings in the Biosatellite II experiment was the lethality induced in the *Habrobracon* oocytes which were in the first meiotic metaphase during the spaceflight (table 5). Though fecundity was higher for the metaphase I oocytes from nonirradiated females in the flight vehicle than for those from nonirradiated females in the Earth-based control (379 and 298 eggs, respectively), the viability was distinctly lower (0.491 compared with 0.893). A similar circumstance was obtained for metaphase I oocytes from

[2] Pooled control data from these and other sources.

TABLE 4. – Summary of Recessive Mutations and Translocations in Sperm in Biosatellite II Tests

Observation	Nonirradiated packages				Preirradiated packages		Irradiated packages with nominal dose, R, of —										
					2000		500		1000			2000			4000		
	Flight	Earth control I	Phase B	Phase C	Flight	Earth control I	Flight	Earth control I	Flight	Earth control I	Phase B chronic	Flight	Earth control I	Phase B chronic	Flight	Earth control I	Phase B chronic
Actual dose, R	0.7	1.7	1.5	1.7	2000	.....	356	371	589	729	689	1272	1245	1219	2425	2431	2376
Recessive lethals:																	
Embryonic	1/378	0/73	0/172	2/354	7/86	5/90	4/362	5/137	5/336	4/76	5/202	15/292	5/64	9/179	22/291	20/182	10/146
	(0.003)	(0.0)	(0.0)	(0.006)	(0.081)	(0.056)	(0.011)	(0.036)	(0.015)	(0.053)	(0.025)	(0.051)	(0.078)	(0.050)	(0.076)	(0.110)	(0.068)
Larval	10/378	2/73	3/172	10/354	10/86	12/90	9/362	8/137	15/336	8/76	12/202	25/292	5/64	20/179	51/291	23/182	25/146
	(0.026)	(0.027)	(0.017)	(0.028)	(0.116)	(0.133)	(0.025)	(0.058)	(0.045)	(0.105)	(0.059)	(0.086)	(0.078)	(0.112)	(0.176)	(0.126)	(0.171)
Pupal	11/378	0/73	0/172	5/354	13/86	12/90	15/362	6/137	8/336	5/76	1/202	21/292	3/64	11/179	39/291	17/182	5/146
	(0.029)	(0.0)	(0.0)	(0.014)	(0.151)	(0.133)	(0.041)	(0.044)	(0.024)	(0.066)	(0.005)	(0.072)	(0.047)	(0.061)	(0.134)	(0.093)	(0.034)
Visible mutations	0/378	0/73	0/172	0/354	0/86	0/90	0/362	0/137	1/336	0/76	1/202	2/292	0/64	1/179	8/291	5/182	2/146
	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.003)	(0.0)	(0.005)	(0.007)	(0.0)	(0.006)	(0.027)	(0.027)	(0.014)
Translocations:																	
Total	0/378	0/73	1/172	1/354	14/86	10/90	5/362	3/137	5/336	4/76	7/202	12/292	3/64	16/179	52/291	31/182	35/146
	(0.0)	(0.0)	(0.006)	(0.003)	(0.163)	(0.111)	(0.014)	(0.022)	(0.015)	(0.053)	(0.035)	(0.041)	(0.047)	(0.089)	(0.179)	(0.170)	(0.240)
Females with one or more	0/378	0/73	1/172	1/354	11/86	10/90	5/362	2/137	5/336	4/76	5/202	11/292	3/64	15/179	48/291	28/182	30/146
	(0.0)	(0.0)	(0.006)	(0.003)	(0.128)	(0.111)	(0.014)	(0.015)	(0.015)	(0.053)	(0.025)	(0.038)	(0.047)	(0.084)	(0.165)	(0.154)	(0.205)
With position effects	0/378	0/73	1/172	0/354	7/86	4/90	1/362	0/137	2/336	1/76	5/202	4/292	1/64	9/179	35/291	22/182	20/146
	(0.0)	(0.0)	(0.006)	(0.0)	(0.081)	(0.044)	(0.003)	(0.0)	(0.006)	(0.013)	(0.025)	(0.014)	(0.016)	(0.050)	(0.120)	(0.121)	(0.137)
Total wasps tested	378	73	172	354	86	90	362	137	336	76	202	292	64	179	291	182	146

TABLE 5. – Summary of Survival of Offspring from Oocytes Irradiated in the First Meiotic Metaphase Stage (Transfer 1)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	85	81	68	69	76	147	379
Eggs hatched	81	55	50	67	74	114	327
Hatchability	0.953	0.679	0.735	0.971	0.974	0.776	0.863
Stages of death:							
1	0	1	0	0	1	15	2
3	3	6	9	2	0	9	20
4	0	7	4	0	0	4	11
5	1	12	5	0	1	5	19
Adults eclosed	40	21	9	57	59	83	186
Adult survival	0.494	0.259	0.132	0.826	0.776	0.565	0.491
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	99	53	18	62	66	95	298
Eggs hatched	96	49	17	57	61	91	280
Hatchability	0.970	0.925	0.944	0.919	0.924	0.958	0.940
Stages of death:							
1	0	0	0	0	2	3	2
3	3	2	1	3	1	0	10
4	0	1	0	2	1	0	4
5	0	1	0	0	1	1	2
Adults eclosed	94	48	15	54	55	88	266
Adult survival	0.949	0.906	0.833	0.871	0.833	0.926	0.893

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R	.....	.....	356	.....	.....	589	.....	.....	1272	.....	.....	2425
Total eggs	35	48	83	103	104	207	77	94	171	87	99	186
Eggs hatched	31	44	75	44	65	109	24	8	32	11	10	21
Hatchability	0.886	0.917	0.904	0.427	0.625	0.527	0.312	0.085	0.187	0.126	0.101	0.113
Stages of death:												
1	2	1	3	3	1	4	9	0	9	18	0	18
3	0	1	1	13	13	26	32	28	60	54	38	92
4	1	2	3	13	5	18	2	40	42	0	51	51
5	1	0	1	30	20	50	10	18	28	4	0	4
Adults eclosed	8	17	25	9	12	21	17	0	17	8	5	13
Adult survival	0.229	0.354	0.301	0.087	0.115	0.101	0.221	0.0	0.099	0.092	0.051	0.070
Earth control I												
Actual exposure, R	.....	.....	371	.....	.....	729	.....	.....	1245	.....	.....	2431
Total eggs	51	53	104	82	57	139	72	31	103	43	61	104
Eggs hatched	46	47	93	64	44	108	42	18	60	12	16	28
Hatchability	0.902	0.887	0.894	0.780	0.772	0.777	0.583	0.581	0.583	0.279	0.262	0.269
Stages of death:												
1	1	3	4	12	5	17	17	8	25	19	23	42
3	1	0	1	3	8	11	10	3	13	8	20	28
4	2	0	2	3	0	3	2	1	3	2	2	4
5	1	3	4	0	0	0	1	1	2	2	0	2
Adults eclosed	38	38	76	59	36	95	36	17	53	10	13	23
Adult survival	0.745	0.717	0.731	0.720	0.632	0.683	0.500	0.548	0.515	0.233	0.233	0.213

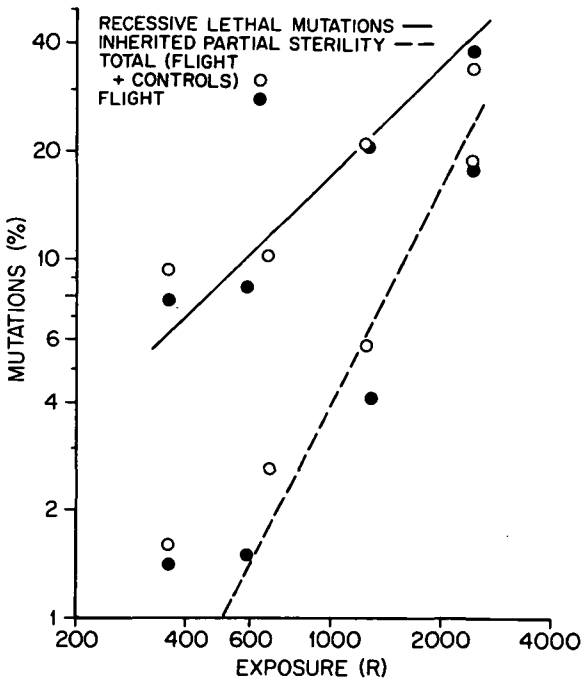

FIGURE 5.— Dose-action curves for total recessive lethal mutations and inherited partial sterility in sperm.

females exposed to 2000 R of X-radiation before the flight; fecundity was 147 and 95 and viability was 0.565 and 0.93, respectively.

The stages at which the developing *Habrobracon* died are shown in figure 6. It can be seen that, with the exception of the earliest stage, the distribution of deaths throughout development is relatively uniform for the metaphase I oocytes from the nonirradiated flight animals. Death at stage 1 connotes dominant lethality, usually from induction of the breakage-fusion-bridge cycle. This was noted in the data for the metaphase I oocytes from the flight animals irradiated before the flight.

An increase in frequency of deaths at stage 3 usually indicates death of the embryo from chromosome imbalance. This can be seen from the distribution of stages of death for unweighted averages of stages of death of offspring from females heterozygous for translocations (fig. 6). The unweighted averages of stages of death of offspring from 50 females heterozygous for recessive lethal mutations shown on the same diagram indicate that a reasonably uniform distribution for times of death is obtained from stage 4 throughout the rest of development.

Thus, a case can be made that the excess of deaths found among the offspring from females that had been in the flight vehicle may be from a mixture of chromosome imbalance phenomena and recessive lethal mutations induced by the spaceflight conditions. This reduction in survival was dependent upon the location of the nonirradiated females both in the package and in the module; those in the innermost and middle layers in the upper right module had the lowest hatchabilities. A similar pattern is shown in table 6 for the slightly younger oocytes that had not been subject to irradiation during the flight. Thus, even though these results were not found in the phase C test, we believe that the responses in the unirradiated *Habrobracon* females, which differed from one position to another within the space vehicle, indicate that the low viability and apparent increase in mutation frequency came from some spaceflight stress other than weightlessness. It can be seen that the metaphase I oocytes of the flight females irradiated either before or during the flight generally had significantly lower hatchabili-

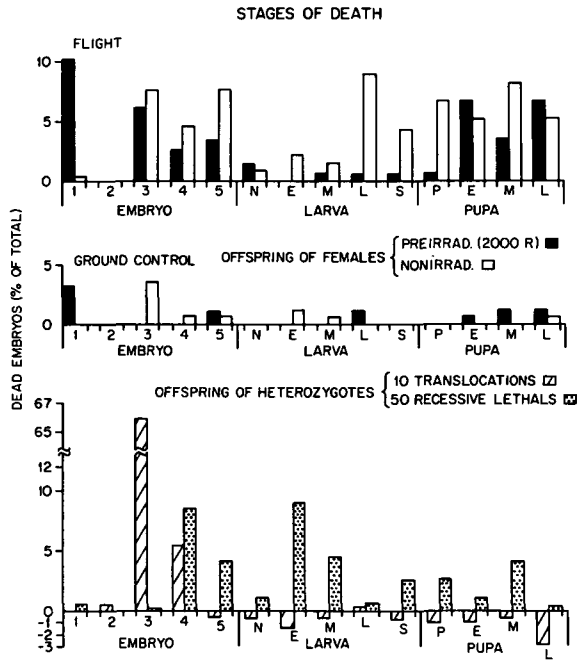

FIGURE 6.— Stages of death of *Habrobracon* from eggs which were in metaphase I in the spacecraft and in the Earth-based control, and stages of death of eggs from a random sample of females heterozygous for recessive lethal mutations and translocations.

ties than those laid by the females from the Earth-based control setup (table 5 and figs. 6 and 7). This is not true for the slightly younger stages (table 6) in which, if anything, the flight oocytes were slightly more resistant to radiation than those in the Earth-based control.

The oocytes in the diplotene stage of the meiotic prophase were quite resistant to any effects of the flight or the concomitant irradiation (tables 7 and 8); thus effects of spaceflight factors on the ooplasm and yolk are excluded.

Data from the phase B and C tests are not listed, since they followed closely those for the females in the Earth-based control.

Hatchability data similar to those compiled in tables 5 to 8 were obtained for all 20 days of the egg-laying period. The data for one exposure only (the highest) are summarized in figures 8 to 11. The day-by-day fecundity data for the Earth-control animals are presented in figure 8. These data clearly show that the fecundity-depressing effects of radiation are obliterated and even reversed in those animals exposed to spaceflight conditions. That is, more eggs than normal were obtained from the oogonia irradiated during the flight. Even the animals which were irradiated before the flight had higher fecundity than the preirradiated females that were kept on Earth; the preirradiated females did not exhibit the higher-than-normal egg production shown by those irradiated during the flight. The animals exposed to the

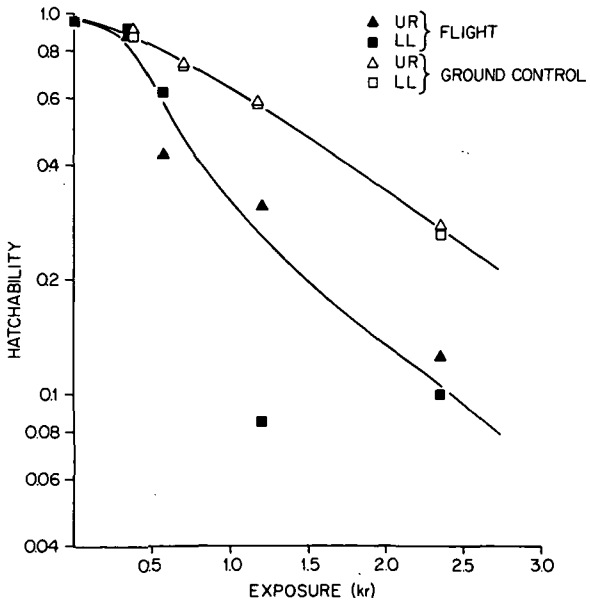

FIGURE 7.— Hatchability of unfertilized eggs from virgin females irradiated in the spacecraft and the Earth-based control. The eggs represent the class that was in the first meiotic metaphase at the time of irradiation. UR, *Habrobracon* from upper right modules; LL, *Habrobracon* from lower left modules (von Borstel et al., 1968).

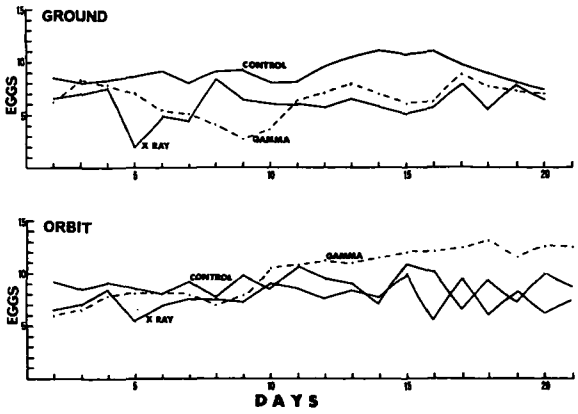

FIGURE 8.— Mean daily egg production for samples of *Habrobracon* virgin females from the spacecraft and the Earth-based control. In each case, the curves have been drawn for unirradiated controls, for females receiving an acute preflight exposure of 2000 R of X-radiation, and for females receiving 2425 R of γ-radiation during the flight itself (from Grosch, 1968).

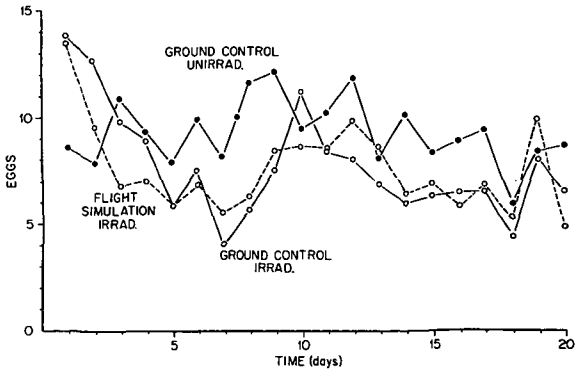

FIGURE 9.— Mean daily egg production for samples of *Habrobracon* virgin females from the spacecraft and the Earth-based control in the phase C test. The curves have been drawn for unirradiated control females, for females in the flight vehicle receiving 2471 R, and for females in the Earth-based control receiving 2420 R (from Grosch, 1970).

TABLE 6. – Summary of Survival of Offspring From a Mixture of Oocytes Irradiated in the First Meiotic Metaphase, Diakinesis, and Late Diplotene Stages (Transfers 2, 3, and 4)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	128	79	82	70	87	85	446
Eggs hatched	125	43	72	65	78	59	383
Hatchability	0.977	0.544	0.878	0.929	0.897	0.694	0.859
Stages of death:							
1	0	0	0	0	1	0	1
3	1	3	1	4	1	5	10
4	1	7	4	0	2	13	14
5	1	26	5	1	5	8	38
Adults eclosed	86	35	52	36	64	39	273
Adult survival	0.688	0.443	0.634	0.514	0.736	0.459	0.612
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	63	61	62	77	71	122	334
Eggs hatched	62	56	61	76	69	117	324
Hatchability	0.984	0.918	0.984	0.987	0.972	0.959	0.970
Stages of death:							
1	0	2	0	1	1	2	4
3	1	2	0	0	0	1	3
4	0	0	1	0	1	1	2
5	0	1	0	0	0	1	1
Adults eclosed	51	47	44	52	44	66	238
Adult survival	0.810	0.770	0.710	0.675	0.620	0.541	0.713

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R	.....	.....	356	.....	.....	589	.....	.....	1272	.....	.....	2425
Total eggs	73	37	110	77	106	183	110	112	222	145	113	258
Eggs hatched	52	28	80	74	100	174	97	89	186	98	74	172
Hatchability	0.712	0.757	0.727	0.961	0.943	0.951	0.882	0.795	0.838	0.676	0.655	0.667
Stages of death:												
1	1	0	1	1	0	1	0	10	10	12	18	30
3	1	0	1	2	3	5	9	4	13	34	17	51
4	4	1	5	0	2	2	2	1	3	0	1	1
5	15	8	23	0	1	1	2	8	10	1	3	4
Adults eclosed	34	16	50	59	81	140	64	68	132	67	48	115
Adult survival	0.466	0.432	0.455	0.766	0.764	0.765	0.582	0.607	0.595	0.462	0.425	0.446
Earth control I												
Actual exposure, R	.....	.....	371	.....	.....	729	.....	.....	1245	.....	.....	2431
Total eggs	52	58	110	54	66	120	103	103	206	118	131	249
Eggs hatched	50	57	107	47	54	101	75	77	152	56	73	129
Hatchability	0.962	0.983	0.973	0.870	0.818	0.842	0.728	0.748	0.738	0.475	0.557	0.518
Stages of death:												
1	0	0	0	3	3	6	15	12	27	39	26	65
3	2	0	2	1	7	8	11	10	21	17	24	41
4	0	1	1	0	2	2	1	2	3	5	8	13
5	0	0	0	3	0	3	1	2	3	1	0	1
Adults eclosed	41	48	89	32	33	65	54	48	102	44	51	95
Adult survival	0.788	0.828	0.809	0.593	0.500	0.542	0.524	0.466	0.495	0.373	0.389	0.382

TABLE 7. – Summary of Survival of Offspring From Oocytes Irradiated in Late Diplotene Stage (Transfer 5)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	96	129	57	88	88	78	458
Eggs hatched	94	125	57	88	82	76	446
Hatchability	0.979	0.969	1.000	1.000	0.932	0.974	0.974
Stages of death:							
1	1	3	0	0	0	0	4
3	0	0	0	0	0	2	0
4	0	0	0	0	0	0	0
5	1	1	0	0	6	0	8
Adults eclosed	86	114	51	80	81	73	412
Adult survival	0.896	0.884	0.895	0.909	0.920	0.936	0.900
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	139	82	122	168	130	122	641
Eggs hatched	137	81	122	164	123	113	627
Hatchability	0.986	0.988	1.000	0.976	0.946	0.934	0.978
Stages of death:							
1	0	0	0	0	1	4	1
3	0	0	0	0	2	3	2
4	1	1	0	3	2	0	7
5	1	0	0	1	2	1	4
Adults eclosed	131	78	120	154	115	101	598
Adult survival	0.942	0.951	0.984	0.917	0.885	0.828	0.933

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R	.....	.....	356	.....	.....	589	.....	.....	1272	.....	.....	2425
Total eggs	49	68	117	100	88	188	90	55	145	85	90	175
Eggs hatched	49	65	114	94	88	182	89	53	142	84	90	174
Hatchability	1.000	0.956	0.974	0.940	1.000	0.968	0.989	0.964	0.979	0.988	1.000	0.994
Stages of death:												
1	0	2	2	0	0	0	0	2	2	0	0	0
3	0	0	0	4	0	4	1	0	1	1	0	1
4	0	1	1	0	0	0	0	0	0	0	0	0
5	0	0	0	2	0	2	0	0	0	0	0	0
Adults eclosed	44	52	96	87	83	170	86	49	135	72	79	151
Adult survival	0.898	0.765	0.821	0.870	0.943	0.904	0.956	0.891	0.931	0.847	0.878	0.863
Earth control I												
Actual exposure, R	.....	.....	371	.....	.....	729	.....	.....	1245	.....	.....	2561
Total eggs	123	132	255	112	121	233	134	93	227	78	118	196
Eggs hatched	122	126	248	109	116	225	129	91	220	76	111	187
Hatchability	0.992	0.955	0.973	0.973	0.959	0.966	0.963	0.978	0.969	0.974	0.941	0.954
Stages of death:												
1	0	1	1	1	2	3	3	0	3	1	1	2
3	0	1	1	0	1	1	1	0	1	1	5	6
4	0	0	0	1	1	2	1	1	2	0	1	1
5	1	4	5	1	1	2	0	1	1	0	0	0
Adults eclosed	115	118	233	96	109	205	120	86	206	72	104	176
Adult survival	0.935	0.894	0.914	0.857	0.901	0.880	0.896	0.925	0.907	0.923	0.881	0.898

TABLE 8. – Summary of Survival of Offspring from Oocytes Irradiated in Diplotene Stage (Transfer 6)

Observation	Nonirradiated package with module-layer preirradiation exposure, R, of—						
	Outer, 0	Middle, 0	Inner, 0	Outer, 0	Middle, 0	Inner, 2000	Pooled, 0
	UR			LL			
Flight							
Actual exposure, R						2000	0.7
Total eggs	Not scored	57	86	Not scored	86	39	229
Eggs hatched	do	57	86	do	80	32	223
Hatchability	do	1.000	1.000	do	0.930	0.821	0.974
Stages of death:							
1	do	0	0	do	2	1	2
3	do	0	0	do	1	2	1
4	do	0	0	do	0	3	0
5	do	0	0	do	3	1	3
Adults eclosed	do	43	76	do	68	27	187
Adult survival	do	0.754	0.884	do	0.791	0.692	0.817
Earth control I							
Actual exposure, R						2000	1.7
Total eggs	130	162	91	58	69	86	509
Eggs hatched	118	160	91	56	68	70	493
Hatchability	0.908	0.988	1.000	0.966	0.986	0.814	0.969
Stages of death:							
1	0	0	0	0	0	8	0
3	0	0	0	1	1	6	2
4	0	2	0	1	0	1	3
5	12	0	0	0	0	1	12
Adults eclosed	110	152	81	36	52	60	431
Adult survival	0.846	0.938	0.890	0.621	0.754	0.698	0.847

Observation	Radiation packages with nominal exposure, R, of —											
	500			1000			2000			4000		
	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled	UR	LL	Pooled
Flight												
Actual exposure, R			356			589			1272			2425
Total eggs	Not scored	Not scored	Not scored	Not scored	Not scored	Not scored	71	71	142	64	63	127
Eggs hatched	do	do	do	do	do	do	71	65	136	58	62	120
Hatchability	do	do	do	do	do	do	1.000	0.915	0.958	0.906	0.984	0.945
Stages of death:												
1	do	do	do	do	do	do	0	0	0	3	0	3
3	do	do	do	do	do	do	0	4	4	3	1	4
4	do	do	do	do	do	do	0	0	0	0	0	0
5	do	do	do	do	do	do	0	2	2	0	0	0
Adults eclosed	do	do	do	do	do	do	65	58	123	42	51	93
Adult survival	do	do	do	do	do	do	0.915	0.817	0.866	0.656	0.810	0.732
Earth control I												
Actual exposure, R			371			729			1245			2561
Total eggs	76	131	207	44	31	75	40	85	125	67	77	144
Eggs hatched	76	128	204	44	30	74	39	78	117	58	69	127
Hatchability	1.000	0.977	0.986	1.000	0.968	0.987	0.975	0.918	0.936	0.866	0.896	0.882
Stages of death:												
1	0	0	0	0	1	1	1	0	1	5	3	8
3	0	1	1	0	0	0	0	4	4	2	4	6
4	0	1	1	0	0	0	0	2	2	1	0	1
5	0	1	1	0	0	0	0	1	1	1	1	2
Adults eclosed	64	110	174	32	26	58	31	67	98	45	54	99
Adult survival	0.842	0.840	0.841	0.727	0.839	0.773	0.775	0.788	0.784	0.672	0.701	0.688

flight simulation profile in the phase C experiment resembled the Earth-based control animals from the flight experiment (fig. 9).

Low hatchability frequencies seen in eggs which were in the early meiotic prophase during the flight (fig. 10) were not found in the Earth-based controls nor in the flight simulation experiment of phase C (fig. 11). It is of interest to note that the high survival of the irradiated oogonial cells in the flight, as seen by fecundity measurements (fig. 8), are also reflected in the hatchability measurements (fig. 10).

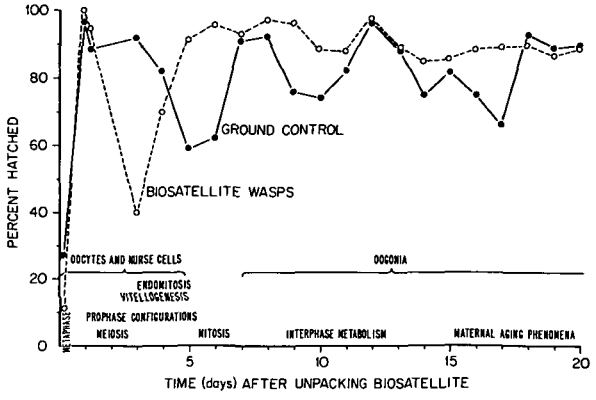

FIGURE 10.— Hatchabilities of eggs from *Habrobracon* virgin females from the Biosatellite II spacecraft (2425 R) and Earth-based control (2431 R). The hatchabilities are from each daily or subdaily transfer (from Grosch, 1970).

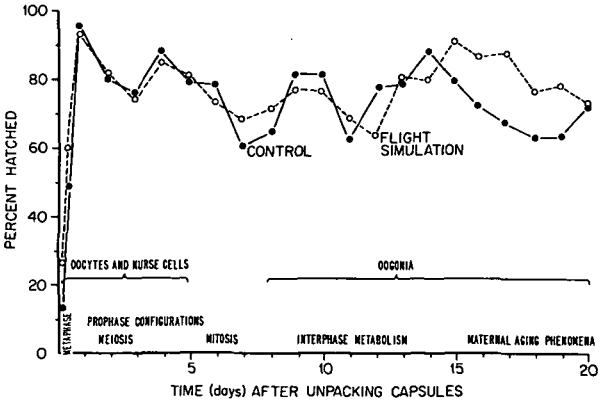

FIGURE 11.— Hatchabilities of eggs from *Habrobracon* virgin females exposed during the phase C test flight simulation (2471 R) and its control (2420 R) (from Grosch, 1970).

Genetic Recombination

Extensive data were accumulated on genetic recombination among markers on chromosome I of *Habrobracon*. These data are not included here because no effects of the spaceflight were found.

Observations on Behavior

The mating behavior of the males that had been in the spacecraft was altered. Observed matings of 243 males from the Earth-based control at Cape Kennedy were achieved in about 3 hours by three investigators. At Hickam Field it took three investigators over 13 hours to achieve with certainty one-third of the 254 planned matings of the flight animals. These males from the spacecraft were disoriented and unable to find or to copulate with the females. Most of the males must have recovered during the trip from Hickam Field to Oak Ridge because genetic studies indicated that 80.3 percent of the females had mated with the males from the spacecraft prior to being set for oviposition. Ninety-three percent of the males from the

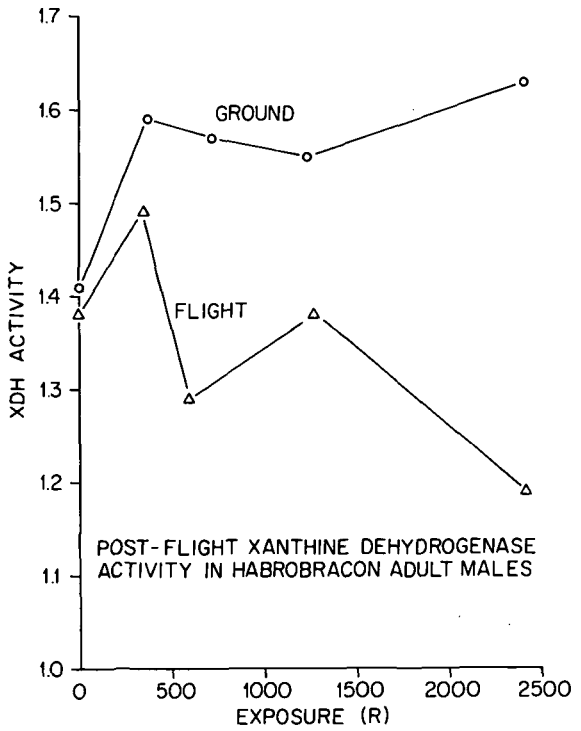

FIGURE 12.— Xanthine dehydrogenase (XDH) activity in the males from the spacecraft and Earth-based controls (Keller, unpublished data).

Earth-based control mated. This abnormal behavior was not observed in the postflight Earth-based control tests.

On the other hand, the *Habrobracon* females did not seem to be disoriented by their flight experience. When placed with host larvae, the females immediately began to paralyze them, feed, and lay eggs.

Physiologic and Late Effects

The fertilizing capacity of the sperm from the males in the spacecraft was greater than that of the sperm from males in the Earth-based control. The increased fertilizing capacity appears to be an enhancing effect of radiation combined with weightlessness, because the males not exposed to radiation and those exposed to the radiation prior to launch did not show this effect.

The *Habrobracon* males, after they had been mated to females, were frozen and shipped to Dr. E. C. Keller, who studied their xanthine dehydrogenase activity. He found that the activity of this enzyme in the flight animals was depressed more than that in the controls at all radiation levels (fig. 12 and unpublished data from Dr. Keller).

One of the most puzzling features of the Biosatellite II experiment was the data obtained for the longevity of females. The *Habrobracon* females that had been subjected to spaceflight conditions had a longevity approximately 10 percent greater than that of the controls (table 9). Although the females lived longer at every exposure to radiation, the data were not significant for any one exposure; taken altogether, however, the data showed a significant difference.

Saccharomyces

The assays used on the *Saccharomyces* were for intragenic and intergenic recombination. The diploid cells in the flight showed no difference for these two endpoints from those in the Earth control.

The survival curve for diploid yeast has a long shoulder, so that cell killing from radiation does not begin until an exposure of 15 kR is reached. The highest exposure for the yeast in the Biosatellite II experiment was 1516 R.

The data for induced intragenic recombination within the *arg4* locus are presented in table 10. It can be seen that there is essentially no difference in frequency at any exposure for yeast in the ground-based control and in the spacecraft.

The data for induced intergenic recombination are shown in table 11. These values show that at only one exposure (other than 0 R) did fewer red variants appear in the yeast that were in the Earth-based control than in those in the flight. The 95-percent confidence limits for the control and flight data do not overlap at the 900 R exposure. All the

TABLE 9. – Mean Lifespans of Females From Biosatellite II and the Associated Postflight Experiments (Grosch, 1968 and 1970)

Biosatellite II Earth-based control		Flight		Postflight (phase C) Earth-based control		Vibrated and centrifuged	
Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days	Actual exposure, R	Lifespan, days
			25.1±0.7				
1.7	23.3±0.9	0.7	[a]32.1±2.5	1.4	23.3±1.8	1.7	18.7±2.0
371	21.6±1.8	356	23.3±1.4	360	.....	352	.....
	17.2±2.7						
729	21.4±2.3	589	24.5±0.5	726	21.8±1.6	575	21.9±1.5
1245	23.8±1.9	1272	24.3±1.4	1241	21.6±1.9	1232	22.7±1.1
2431	24.9±1.5	2425	[a]34.1±4.4	2420	22.7±1.6	2471	21.3±1.8
[b]2000	[b]21.9±1.9	[b]2000	[b]24.2±1.5	.....	.....	.....	.....

[a] Received special care during the last week of life.
[b] Preirradiated with X-rays.

TABLE 10. – Frequency of Revertant Cells From a Requirement for Arginine

Nominal exposure, R	Estimated exposure, R	Number colonies	Number plates, *YEPD*[a]	Number revertants	Number plates, *−ARG*	Dilution difference	Revertants surviving cell
			Flight				
4000	1513	1518	10	3841	7	10^{-5}	0.361×10^{-4}
2000	918	1487	10	3553	10	10^{-5}	.238
1000	547	1604	10	2391	8	10^{-5}	.186
500	298	2092	10	2298	9	10^{-5}	.122
0	0.7	1685	10	1629	10	10^{-5}	.097
			Control I				
4000	1516	2120	10	7019	10	10^{-5}	0.331×10^{-4}
2000	899	1932	10	4154	8	10^{-5}	.269
1000	558	1981	10	3415	10	10^{-5}	.172
500	310	2004	10	2448	9	10^{-5}	.136
0	1.7	1985	10	938	5	10^{-5}	.095

[a] *YEPD*= Complete medium consisting of 1 percent yeast extract, 2 percent peptone, and 2 percent glucose.

remaining data increase with the exposure; thus, we attribute the "dip" in the control data to a chance fluctuation. Such a fluctuation was not seen in data obtained in the phase C experiment.

Artemia

The *Artemia* were placed in that part of each package that had originally been designed for shielded dosimeters. When it became apparent from preflight tests that shielded dosimeters were not needed, this part of the package was found to be suitable for monitoring certain other parameters. *Artemia* cysts were used in these positions since they had been found to have an enhanced adult survival after high-intensity, low-frequency vibrations.[3] The spaceflight did not significantly affect viability of the *Artemia* below the 5-percent level of significance, but there is a strong trend in the emergence data which might reflect the influence of spaceflight factors other than radiation (table 12).

The parameters that were measured, in addition to the emergence of the *Artemia* from the cysts, were the survival to adulthood and the fertility of every surviving individual; also, frequencies of embryologic abnormalities were recorded. In addition, an F_1 test was carried out which consisted of the measurement of frequencies of (1) zygotes encysted, (2) emergence, (3) fecundity, and (4) fertility. These data were obtained from pair matings of individuals that had survived to adulthood from the cysts that had been in the Biosatellite II experiment. By comparison of these data for the flight animals with those for animals that had been in the Earth-based control, we conclude, with the reservation cited above, that the encysted, quiescent form of the *Artemia* was not influenced significantly by the spaceflight or by the gamma radiation received during the flight. Furthermore, an appreciable preflight conditioning dose of gamma radiation (an exposure to 16 kR) did not aid in uncovering deleterious or beneficial influences of orbital spaceflight.

[3] Unpublished data from D. S. Grosch.

SUMMARY AND CONCLUSIONS

There are two major conditions of spaceflight that are difficult, if not impossible, to reproduce in the laboratory — weightlessness, as defined by a freely orbiting condition, and the vibration profile of a spacecraft during its launch and recovery phases. If without further experimentation and data analysis we arrange the differences found be-

TABLE 11. – Frequency of Red Variant Colonies Among Total Colonies Scored

Nominal exposure, R	Estimated exposure, R	Number colonies	Number red colonies	Number red sectors	Red colonies + red sectors	Red colonies + sectors / Total number colonies
			Flight			
4000	1513	19 817	48	32	80	4.0×10^{-3}
2000	918	20 559	43	36	79	3.8
1000	547	18 900	26	20	46	2.4
500	298	22 890	19	11	30	1.3
0	.7	20 087	17	7	24	1.1
			Control I			
4000	1516	25 488	51	43	94	3.7×10^{-3}
2000	899	16 732	16	12	28	1.7
1000	558	19 434	29	19	48	2.5
500	310	18 263	20	12	32	1.8
0	1.7	16 144	10	6	16	1.0

TABLE 12. – Emergence From *Artemia* Cysts in the Biosatellite II Experiment

[Unpublished data from D. S. Grosch]

Package	Radiation in flight, R	Percent emergence	*t* values	*P* values
Spaceflight	0.7	53.7	2.458	0.06
Earth-based control	1.7	45.3		
Spaceflight (pre-X-rayed 16 000 R)	2425	70.7	2.527	.05
Earth-based control (pre-X-rayed 16 000R)	2431	59.3		

tween animals in the spacecraft and animals in the Earth-based control (tables 13 and 14) in these two categories, we could come to the following conclusions.

Vibration

The enhancement of spontaneous recessive lethal frequencies in sperm of *Habrobracon* seen from analysis of the flight animals was reproduced during the phase C test, in which the flight vehicle was vibrated and centrifuged; this effect thus seems clearly to have been induced by something other than weightlessness. The enhancement of embryonic, larval, and pupal deaths in the nonirradiated flight females (deduced by genetic reconstruction to have been caused by chromosomal aberrations and recessive lethal mutations) was restricted to their placement at different positions within a package. Althought the effect was not seen in the postflight vibration and centrifugation test, we believe that the "position effect" nature of the phenomenon excludes an effect from weightlessness. The strong trend for enhanced emergence from *Artemia* cysts might be caused by vibration.

Weightlessness

At this time, the best candidates for an effect of weightlessness appear to be the following: (1)

TABLE 13. – Genetic Effects Obtained in the *Habrobracon* Experiment in Biosatellite II Compared With Those Obtained on Earth

End points	Effect of flight dynamics	
	Flight alone	Flight plus radiation
Sperm:		
Dominant lethal mutations	Null	Null
Recessive lethal mutations	Increased	Null
Translocations	Null	Null
Oocytes:		
Hatchability:		
Metaphase I	Reduced	Reduced
Late prophase I	Null	Null
Early prophase I	Null-reduced	Reduced
Recombination: Late prophase I	Null	Null
Transitional oogonia: Hatchability	Null	Increased
Primitive oogonia: Hatchability	Null	Increased

TABLE 14. – Physiologic Effects Obtained in the *Habrobracon* Experiment in Biosatellite II Compared with Those Obtained on Earth

End points	Effect of flight dynamics	
	Flight alone	Flight plus radiation
Survival:		
Males	Null	Null
Females	Null	Null
Fecundity:		
Transitional oogonia	Null	Increased
Primitive oogonia	Null	Increased
Behavior:		
Mating of males	Disoriented	Disoriented
Oviposition of females	Null	Null
Other effects:		
Fertilizing capacity of sperm	Null	Enhanced
Xanthine dehydrogenase activity in males	Reduced	Reduced
Lifespan of females	Increased	Increased

enhancement of fecundity and hatchability of primitive and transitional oogonia; (2) disorientation of male mating behavior; (3) increased lifespan of females; and (4) decreased xanthine dehydrogenase activity. The two remaining effects of flight, namely, the enhanced fertilizing capacity of sperm and the reduced hatchability of eggs from females irradiated as metaphase I and early prophase I oocytes, could be either from weightlessness or from other factors of spaceflight.

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Genetic Implications of Spaceflight

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Bowling Green State University

Biologic experiments in space offer the scientific community unmatched opportunities replete with theoretical as well as applied implications (Reynolds, 1968; Saunders, 1968a, 1968c, and 1968d). They afford a unique opportunity to investigate the reactions of living material in a novel environmental situation with the ultimate view of enhancing our understanding of its normal functioning capabilities on Earth. The information obtained about the responses of living organisms to the stresses of outer space should also benefit man in his preparations for voyaging forth into this frontier. By way of example, irreparable damage might have been perpetrated on countless generations of humans if the establishment of safe limits for handling and utilizing radiation (ranging from X-rays to isotopes) had to await observations on exposures of man. As a matter of fact, all of our present standards for man concerning genetic damage and its concomitant irreversibility were established solely on the basis of experiments on nonprimate organisms, particularly *Drosophila* (U.N. Committee on the Effects of Atomic Energy, 1958 and 1962; Medical Research Council, 1960). Equally important, of course, were certain physiologic responses peculiar to man which were subsequently established on the basis of observations made directly on him.

It would seem that either of these goals constitutes ample justification for continuing and expanding our efforts in Biosatellite research. Moreover, the recent advances made by our Soviet colleagues (Dubinin and Kanavets, 1962; Glembotskiy et al., 1962; Parfenov, 1962) and the highly interesting results obtained by the United States team of investigators with Biosatellite II (Browning, 1968; Browning and Altenburg, 1968; de Serres and Webber, 1968; Reynolds and Saunders, 1968; Saunders, 1968b; Slater, Buckhold, and Tobias, 1968a and 1968b; Sparrow et al., 1968a and 1968b; Tobias et al., 1967; Von Borstel et al., 1968a; Von Borstel et al., 1968b) point up exciting areas for further investigation while providing us with meaningful knowledge concerning the parameters already examined (Jenkins, 1968).

Shortly after the recovery of Biosatellite II (Dyer, 1968; Wilson, 1968) with our modules containing immature stages of development (i.e., larvae) of the fruit fly, *Drosophila melanogaster,* and following the preliminary analyses, it became apparent that some factor or factors associated with the space environment (perhaps weightlessness, vibration, cosmic radiation, or a combination of some or all of these) acting by itself as well as in combination with ionizing radiation is capable of enhancing the extent of some types of damage to the genetic material in a significant manner (Oster 1968a, 1968b, 1968c, and 1968d; Oster and Good, 1968). Now that these analyses have been largely completed and compared with extensive control tests carried out on Earth, a fairly comprehensive view of the spectrum of effects which has emerged can be presented. Moreover, since several types of organisms were flown in Biosatellite II, we should be able to make interspecies comparisons in order to help distinguish between more generalized reactions and those unique to certain organisms.

EXPERIMENT DESIGN AND METHODS

Highly specialized strains of the fruit fly, *Drosophila melanogaster,* were developed and later modified to meet the needs of the Biosatellite program (Oster 1955a, 1955b, 1956a, 1956b, 1957, 1958a, 1958b, 1958c, 1958d, 1961a, 1961b, 1966,

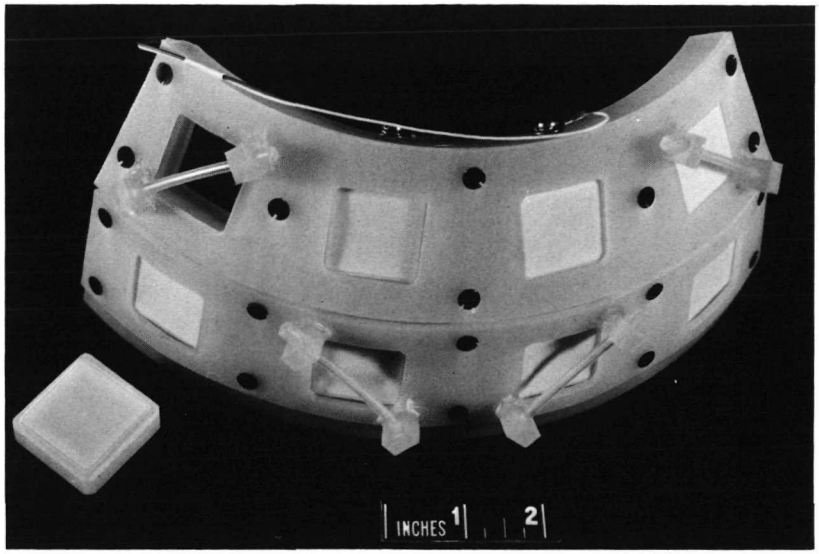

FIGURE 1.—Flight package for *Drosophila* larvae. Left, individual module removed to expose radiation dosimeter.

and 1970; Oster and Balaban, 1962 and 1963; Oster, Schwarz and Binnard, 1963; Muller and Oster, 1963). By flying such suitably marked stocks, which probably represent the most elaborate systems available today for genetic research with multicellular animals, we could detect practically all the known types of damage to which the genetic material is heir. Not only did the markers affect the adult phenotypes but some were used which could be recognized in the preimaginal stages, e.g., the larvae.

A prototype of the strains eventually employed by our group in Biosatellite II was used by Browning (1968) in the experiments essentially involving mature reproductive cells in adults. These strains were constructed largely with the aid of the National Aeronautics and Space Administration and "the building blocks" (or component marker strains) provided by the Mid America *Drosophila* Stock Center maintained under the present author's direction at Bowling Green State University.

Part of the immature material flown on Biosatellite II was exposed to an onboard source of radiation (approximately 800 R from 85-strontium), whereas part of it was shielded during flight. (Apparatus for this Biosatellite experiment is shown in figs. 1 and 2.) Similarly handled individuals (irradiated as well as shielded) served as one of the groups of Earth-based controls. Another group of Earth-based material was subjected to the stresses encountered during flight, among them vibration and shock, exclusive of weightlessness, both with and without radiation. Because of the limitations on available areas for placing material on board the satellite, it was deemed most expedient to use several radiation doses delivered to the material of other experimenters, e.g., von Borstel and de Serres of the Oak Ridge National Laboratory, who had available in their own home laboratories physicists for consultation and the necessary refined dosage calculations.

Following recovery of Biosatellite II, the material as well as all the Earth-based controls were handled with the results shown in figure 3. This scheme allowed us to detect changes in both the somatic and the reproductive cells by a combination of observations involving cytologic and breeding

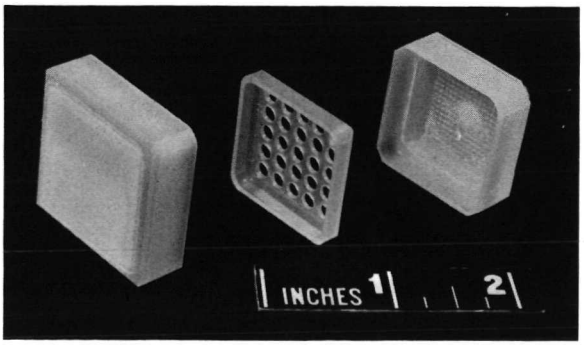

FIGURE 2.— Disassembled module for *Drosophila* larvae.

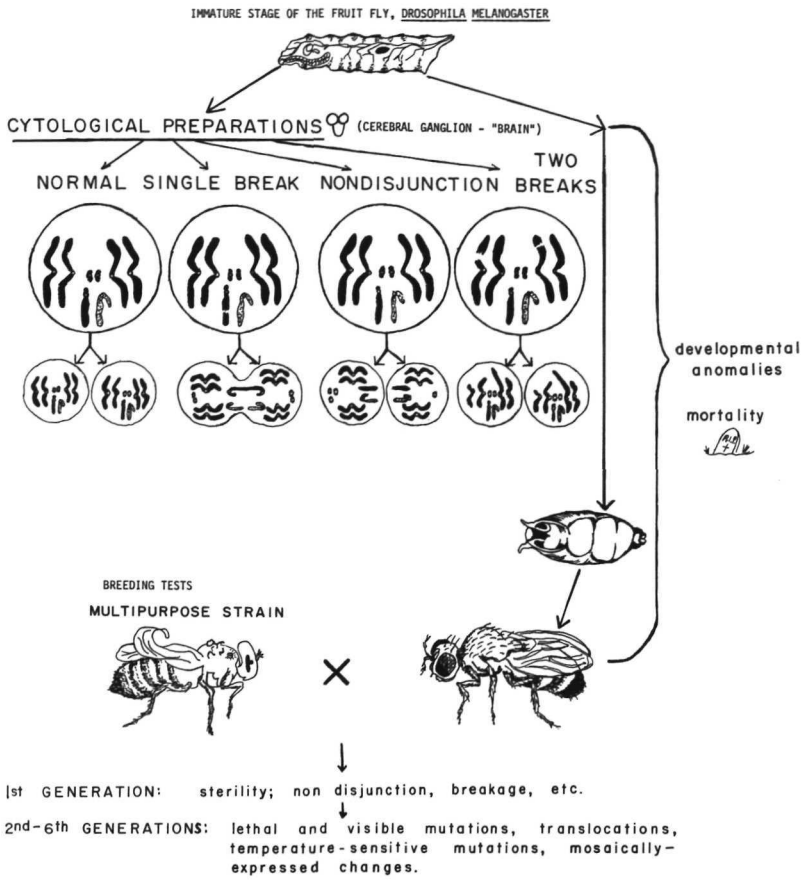

FIGURE 3.—General methodology used for detecting genetic changes following recovery of orbited larvae.

analyses. One important aspect of our technique involved the incorporation of a ring-shaped X chromosome into some of the strains used. The utilization of phenotypic characteristics which could be recognized in the larval stages permitted us to distinguish and subsequently sample individuals bearing different genetic makeups. In eukaryotes, rings have never become established under ordinary circumstances because such chromosomes are highly susceptible to increased loss following breakage. Under experimental conditions, however, ring chromosomes can be incorporated into strains and persist as stable entities until subject to breakage. This served to amplify greatly the sensitivity of our system.

Immature stages of development, i.e., larvae, were used because they contain a preponderance of actively dividing and metabolizing cells and thereby approximate the tissues of most other organisms which may play a role in the exploration of space. Also, since the reproductive tissues of such individuals do not contain any mature cells (some of which are characterized by grossly different qualitative and quantitative responses to radiation) it was possible to insure that homogeneous stages of cell development were being compared. The ability to observe several parameters in the genetic material of the somatic cells (by macroscopic and microscopic analyses of the individuals themselves) as well as the reproductive cells (by breeding and observing their offspring) allowed us to assess the extent of the damage in both the exposed individual and his descendants. Such combinations of observations tend to be complementary since certain changes are more readily detectable by one or the other method. The manner in which a change arises as well as its persistence through several cell generations play an important role here.

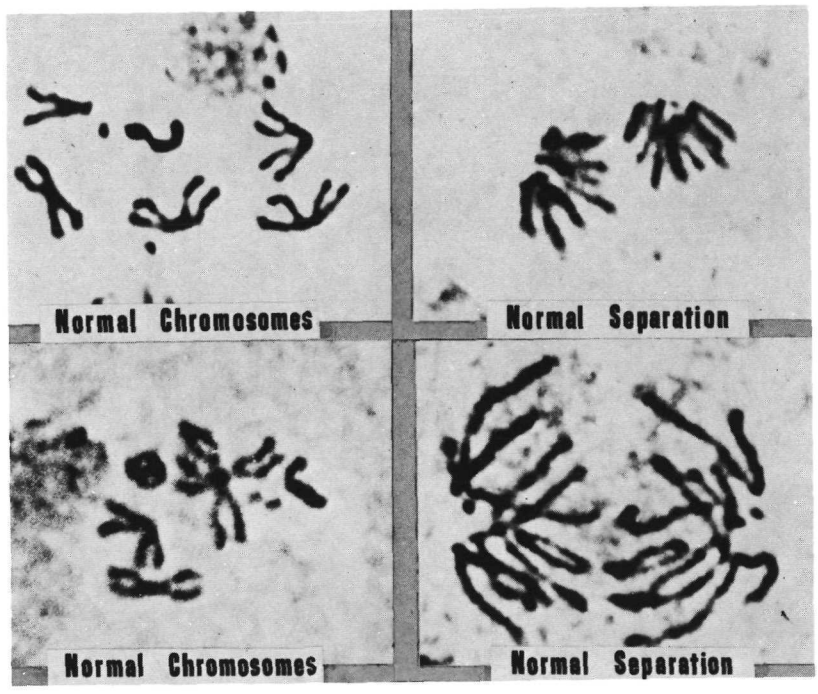

FIGURE 4.—Normal chromosome complements of ring- and rod-bearing strains.

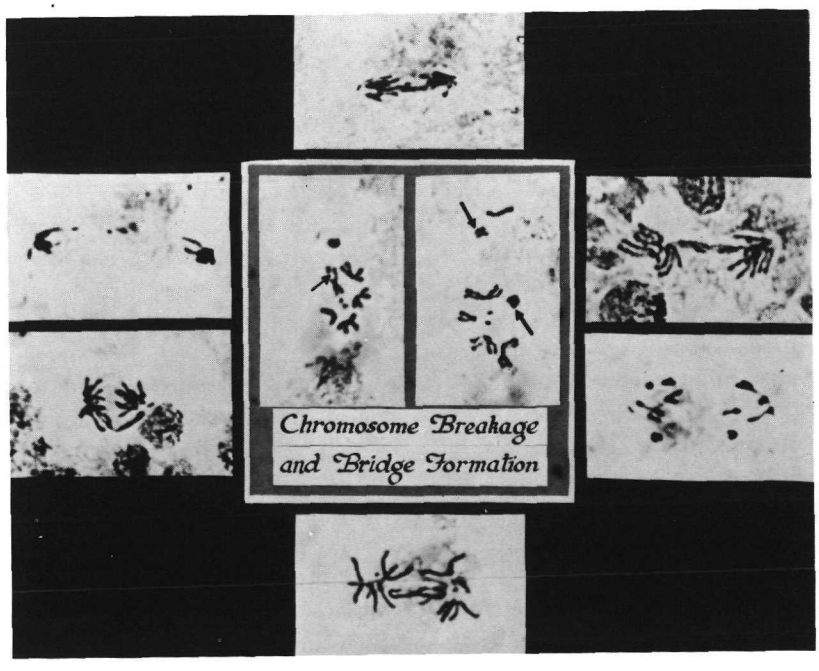

FIGURE 5.—Examples of chromosome breakage leading to loss.

Figure 4 illustrates normal chromosomal configurations in both rod- and ring-bearing stocks of *Drosophila* whereas figures 5 and 6 show instances of breakage and faulty chromosome separation, respectively. Such events can be detected readily. Moreover, *Drosophila* affords an advantage because certain types of disproportionate chromosomal arrangements are compatible with life and therefore can be detected several cell generations after their occurrence. Although a human fetus may survive with some types of chromosomal imbalance, such individuals are usually burdened with severe mental and/or physical defects.

RESULTS

The results of experiments carried out on Earth did not incriminate any condition encountered in space (aside from weightlessness, of course) which could be duplicated on the ground as a possible factor responsible for the events observed following flight. The conditions investigated involved radiation with and without the so-called flight-profile stress (a stress such as vibration and shock) to which the biologic material had been exposed during the flight. Weightlessness was not considered.

Table 1 shows the types of effects which could be detected in the somatic cells by macroscopic observation. As can be seen, although radiation does increase mortality, it does so irrespective of whether the larvae have been subject to the conditions present during flight. Similarly, the flight-profile conditions by themselves were without effect. Table 2 shows the results which could be detected in the reproductive cells, that is, by breeding analysis. Again, although some changes, such as sex-linked lethal mutations and crossing over in the male, are increased by radiation delivered in the doses used for these experiments, the flight-profile conditions did not affect their response, nor did they cause any detectable increases or decreases when compared with results from the completely untreated controls on Earth.

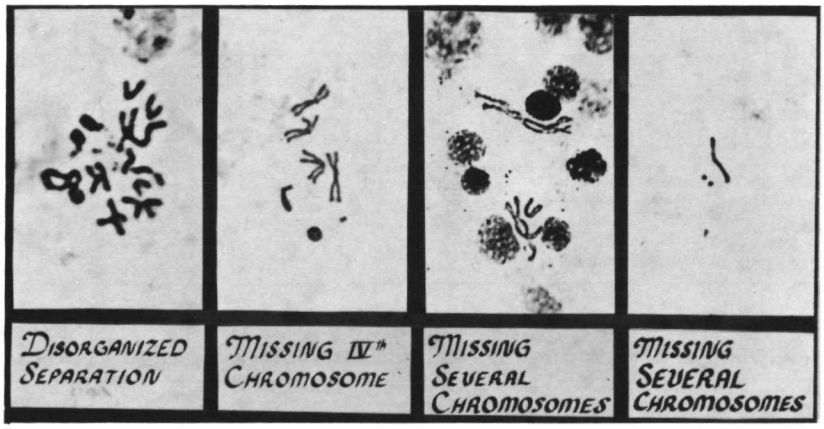

FIGURE 6.—Examples of faulty chromosome separation (nondisjunction).

TABLE 1. — Effects Observed by Macroscopic Observation

Characteristic	Irradiation plus flight-profile stresses	Flight-profile stresses	Irradiation on Earth	Control on Earth
Survival	$\frac{334}{480}=69.58\%$ ± 2.09	$\frac{462}{480}=96.25\%$ ± 0.87	$\frac{331}{480}=68.96\%$ ± 2.11	$\frac{458}{480}=95.42\%$ ± 0.91
Gross developmental anomalies	$\frac{0}{334}=0$	$\frac{2}{462}=0.43\%$ ± 0.30	$\frac{0}{331}=0$	$\frac{1}{458}=0.22\%$ ± 0.22

The effects which could be detected both microscopically, i.e., cytologically, and by breeding tests are shown in table 3. Again, although radiation increases the frequency of three out of the four categories, the flight-profile conditions are completely ineffective both in combination with radiation and by themselves.

The absolute frequencies for the effects to be discussed may seem rather low, but it should be borne in mind that only gonial cells have been

TABLE 2. – Effects Detected in Reproductive Cells

Characteristic	Irradiation plus flight-profile stresses	Flight-profile stresses	Irradiation on Earth	Control on Earth
Fertility	~59 ♂♀/mating	~54 ♂♀/mating	~58 ♂♀/mating	~56 ♂♀/mating
Sex ratio	$\frac{1261}{2480}=50.85\% \pm 1.00$	$\frac{701}{1441}=48.65\% \pm 1.31$	$\frac{884}{1782}=49.61\% \pm 1.19$	$\frac{1794}{3643}=49.25\% \pm 0.83$
Sex-linked lethal mutations	$\frac{5}{1698}=0.29\% \pm 0.13$	$\frac{2}{1822}=0.11\% \pm 0.08$	$\frac{4}{1523}=0.26\% \pm 0.13$	$\frac{4}{3448}=0.12\% \pm 0.06$
Crossing over (X,Y exchanges in ♂♂)	$\frac{6}{2806}=0.21\% \pm 0.09$	$\frac{2}{2455}=0.08\% \pm 0.06$	$\frac{7}{2714}=0.26\% \pm 0.10$	$\frac{4}{4623}=0.09\% \pm 0.04$
Point mutational changes (at the dumpy locus)	$\frac{0}{1945}=0$	$\frac{1}{2161}=0.05\% \pm 0.05$	$\frac{1}{2849}=0.04\% \pm 0.04$	$\frac{2}{3818}=0.05\% \pm 0.04$
Multiple chromosome breakage (leading to translocations)	$\frac{0}{1743}=0$	$\frac{0}{1933}=0$	$\frac{0}{2614}=0$	$\frac{0}{3331}=0$

TABLE 3. – Cytologic Observations of Genetic Effects in Somatic and Reproductive Cells

Characteristic	Irradiation plus flight-profile stresses	Flight-profile stresses	Irradiation on Earth	Control on Earth
Chromosomal nondisjunction	0/16 brain	0/19 brain	1/24 brain	2/39 brain
	$\frac{5}{1243}=0.40\% \pm 0.18$	$\frac{2}{1368}=0.15\% \pm 0.10$	$\frac{8}{1678}=0.48\% \pm 0.17$	$\frac{4}{2836}=0.14\% \pm 0.07$
Single and multiple chromosome breakage (leading to loss)	$\frac{112}{1564}=7.16\% \pm 0.65$	$\frac{8}{1323}=0.60\% \pm 0.22$	$\frac{115}{1687}=6.82\% \pm 0.61$	$\frac{14}{2606}=0.54\% \pm 0.14$
	$\frac{9}{1213}=0.74\% \pm 0.25$	$\frac{2}{1332}=0.15\% \pm 0.11$	$\frac{10}{1209}=0.83\% \pm 0.26$	$\frac{2}{1811}=0.11\% \pm 0.08$

studied in the breeding tests and that this stage is highly radiation resistant. This furnishes all the more reason why certain effects, if they be induced at all, represent highly significant events.

Table 4 shows the results obtained by observing the effects on the individuals themselves following flight. There appears to be a significant decrease in survival following irradiation on orbit. Table 5 indicates of effects noticeable in the reproductive cells that sex-linked recessive lethals and crossing over are enhanced when radiation is delivered under weightlessness. Some of these changes, such as crossing over between the X and Y chromosomes and translocations, may also be detected cytologically, but only under certain circumstances. A striking effect was noted in the case of multiple chromosome breakage leading to the formation of translocations.

TABLE 4. – Postflight Macroscopic Observations of Individuals from Biosatellite II and Their Earth Controls

Characteristic	Irradiation in flight (fore)	Flight (aft)	Irradiation on Earth (fore)	Earth (aft)
Survival	$\frac{271}{480}=56.46\%$ ± 2.25	$\frac{455}{480}=94.79\%$ ± 1.02	$\frac{324}{480}=67.50\%$ ± 2.13	$\frac{451}{480}=93.96\%$ ± 1.05
Gross developmental anomalies	$\frac{1}{271}=0.37\%$ ± 0.37	$\frac{3}{455}=0.66\%$ ± 0.38	$\frac{0}{324}=0$	$\frac{1}{451}=0.22\%$ ± 0.21

TABLE 5. – Observed Genetic Effects in Reproductive Cells from Biosatellite II and Earth Controls

Characteristic	Irradiation in flight (fore)	Flight (aft)	Irradiation on Earth (fore)	Earth (aft)
Fertility	~55 ♂♀/mating	~55 ♂♀/mating	~55 ♂♀/mating	~40 ♂♀/mating
Sex ratio	$\frac{2326}{5005}=46.47\%$ ± 0.71	$\frac{572}{1203}=47.55\%$ ± 1.44	$\frac{1548}{3158}=49.02\%$ ± 0.89	$\frac{1083}{2252}=48.09\%$ ± 1.05
Sex-linked lethal mutations	$\frac{14}{1961}=0.71\%$ ± 0.19	$\frac{2}{1898}=0.11\%$ ± 0.07	$\frac{6}{1724}=0.35\%$ ± 0.14	$\frac{1}{1732}=0.06\%$ ± 0.06
Crossing over (X,Y exchanges in ♂♂)	$\frac{11}{2005}=0.55\%$ ± 0.17	$\frac{3}{2115}=0.14\%$ ± 0.08	$\frac{10}{3412}=0.29\%$ ± 0.09	$\frac{2}{3128}=0.06\%$ ± 0.04
Point mutational changes (at the dumpy locus)	$\frac{1}{1865}=0.05\%$ ± 0.05	$\frac{0}{1873}=0$	$\frac{0}{1688}=0$	$\frac{1}{1703}=0.06\%$ ± 0.05
Multiple chromosome breakage (leading to translocations)	$\frac{4}{501}=0.80\%$ ± 0.40	$\frac{3}{540}=0.56\%$ ± 0.32	$\frac{0}{525}=0$	$\frac{0}{681}=0$

The effects which could readily be observed in both the somatic and reproductive cells are summarized in table 6. Single and multiple breakages were observed to be high for all the chromosomes in the somatic and reproductive cells of the group irradiated during flight. On the other hand, contrary to the reports for mature oocytes, there did not seem to be a discernible effect on the failure of proper chromosome separation or nondisjunction in the reproductive cells. At the same time, both flight and irradiated-during-flight material yielded a marked increase in nondisjunction of chromosomes in the somatic cells.

We conclude on the basis of our macroscopic observations and cytogenetic analyses that (1) radiation appears to interact with weightlessness to produce a higher mortality, more chromosomal breakage followed by loss or exchange of genetic information, and more sex-linked recessive lethal mutations in actively growing and metabolizing individuals than in those irradiated on Earth; and (2) some factor(s) associated with spaceflight, probably extensively or perhaps exclusively involving weightlessness, is capable of causing improper chromosome separation (nondisjunction) and the formation of chromosomal translocations.

DISCUSSION

Our previous indications of an enhancement of genetic damage have now been placed on a firm statistical basis by the accumulation and analysis of a large quantity of additional data. These enhancements of effects are not strictly related to whether a particular type of change is produced by only radiation at the levels presently employed. Moreover, extensive postflight tests involving the environment encountered during the Biosatellite II mission, exclusive of weightlessness, have practically ruled out the possibility that vibration, shock, etc. may have contributed to the effects observed.

Of course, merely observing these phenomena would tell only half of the story. Our eventual aim is to determine the mode of this interaction for both theoretical and practical reasons. At the present time we can only speculate and perhaps guess about the possible mechanisms underlying these observations. It is obvious that further experimentation both on Earth and in space should help with the interpretation of the manner in which weightlessness interacts with radiation and at times seems to increase genetic damage by itself.

Although we can postulate that weightlessness enhances radiation damage at the level of a single gene by interfering with the operation of some ubiquitous cellular repair process, at the moment this is really only a description and not an explanation. Unfortunately, we cannot yet describe the manner in which this interaction takes place, since we do not yet understand the manner in which repair processes are operating and the extent to which such systems are present in multicellular organisms.

As far as space-related effects on chromosome separation are concerned, it may be that some

TABLE 6. – Genetic Changes Observed in Both Somatic and Reproductive Cells

Characteristic	Irradiation in flight (fore)	Flight (aft)	Irradiation on Earth (fore)	Earth (aft)
	31/35 brain	22/32 brain	1/38 brain	1/36 brain
Chromosomal nondisjunction	$\frac{4}{1052}=0.38\% \pm 0.19$	$\frac{3}{2348}=0.13\% \pm 0.07$	$\frac{11}{3007}=0.37\% \pm 0.11$	$\frac{2}{2200}=0.09\% \pm 0.06$
Single and multiple chromosome breakage (leading to loss)	$\frac{236}{2196}=10.75\% \pm 0.66$	$\frac{19}{2946}=0.64\% \pm 0.15$	$\frac{163}{2359}=6.91\% \pm 0.52$	$\frac{17}{3567}=0.48\% \pm 0.12$
	$\frac{21}{1180}=1.78\% \pm 0.39$	$\frac{2}{1254}=0.16\% \pm 0.11$	$\frac{11}{1252}=0.88\% \pm 0.26$	$\frac{1}{991}=0.10\% \pm 0.04$

slight perturbations involving a variety of sites in the orientation of the protein molecule array of the spindle underlies this phenomenon. At least we do know from previous work of our own, as well as that of others, that spindle damage by radiation on Earth follows two-hit kinetics and, as might be expected, exhibits a marked fractionation effect (Oster, 1965).

On the other hand, when we come to breakage phenomena at the chromosome level, our speculations can be couched in somewhat more definite terms, since we understand more about the processes involved here and can at least describe the events mechanically. For one thing, it is perhaps self-evident that once a break has occurred the chance for the broken ends to recombine in their original order, that is, to undergo restitution, *or* to remain free at least momentarily, *or* to unite with other broken ends must depend on the proximity of the sites of breakage. We know that the union of broken ends requires energy and is enhanced by high oxygen tension (Wolff, 1967). Other than this, we are still far away from understanding the structure of the chromosome in higher organisms. Until we do, we can only vaguely speculate about the simple or vastly complicated sequence of events which must intervene between the induction of a chromosome break and its subsequent restitution or reunion. It may be that weightlessness tends to interfere at the enzymatic level with the transfer of energy to sensitive sites and/or to increase the distances between broken ends, thus favoring a lack of restitution and leading to a loss of chromosomes.

As mentioned previously, several chromosomal translocations had been detected in both the orbited and the irradiated-on-orbit groups. From a purely statistical viewpoint, the origination of seven cases of separate origin of this type of aberration in *Drosophila melanogaster* is highly improbable. As a matter of fact, had we found only one case, I would have considered it to be highly suggestive. In addition to its being the result of two separate events in nonpartner chromosomes in very radiation-resistant cells, it is particularly interesting that six of the seven cases detected involved one of the smallest elements of the chromosome set in *Drosophila,* which is again an event entirely unlikely to be due to chance (figs. 7 to 9). Figure 10 portrays still another aspect of the extremely unlikely possibility for such changes to be transmitted

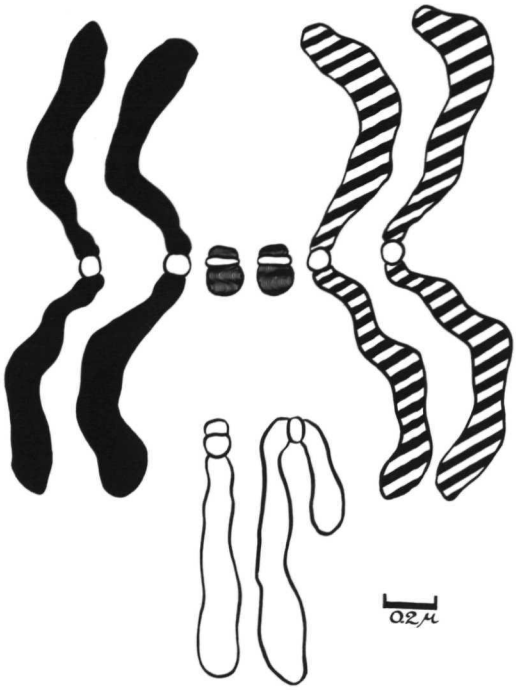

FIGURE 7.— Diagrammatic representation of chromosomes in *Drosophila*.

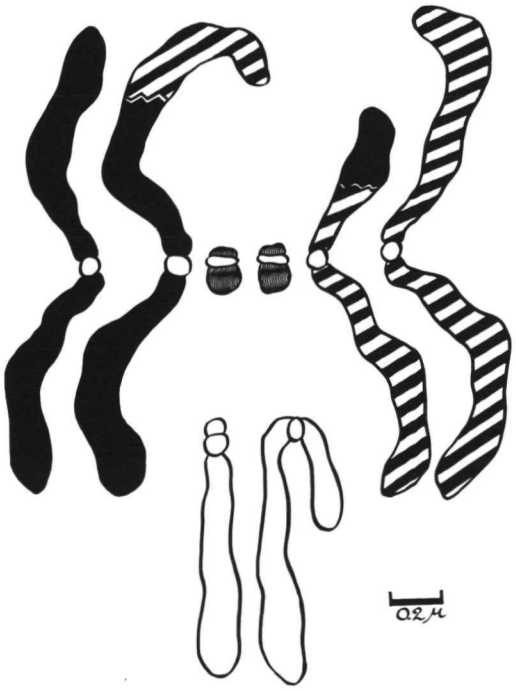

FIGURE. 8.— Diagrammatic representation of chromosomes in *Drosophila* presented to illustrate a translocation involving two of the larger chromosomes.

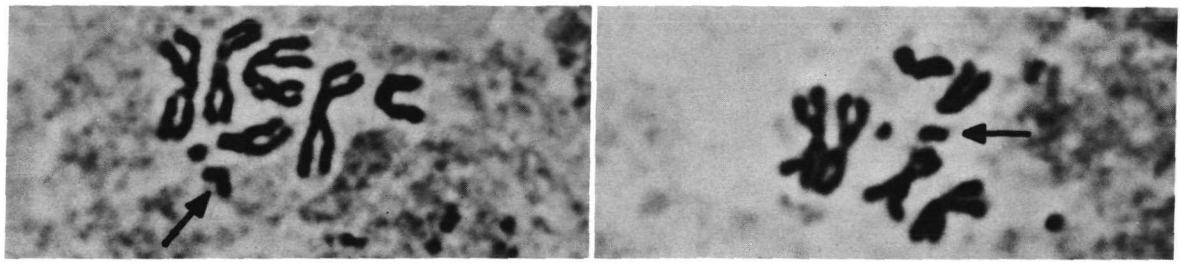

FIGURE 9.— Cytologic appearance of one of the translocations found in the orbited material. Only one portion of the abberration, namely, the segment of the large chromosome which has become attached to the small fourth chromosome, is visible.

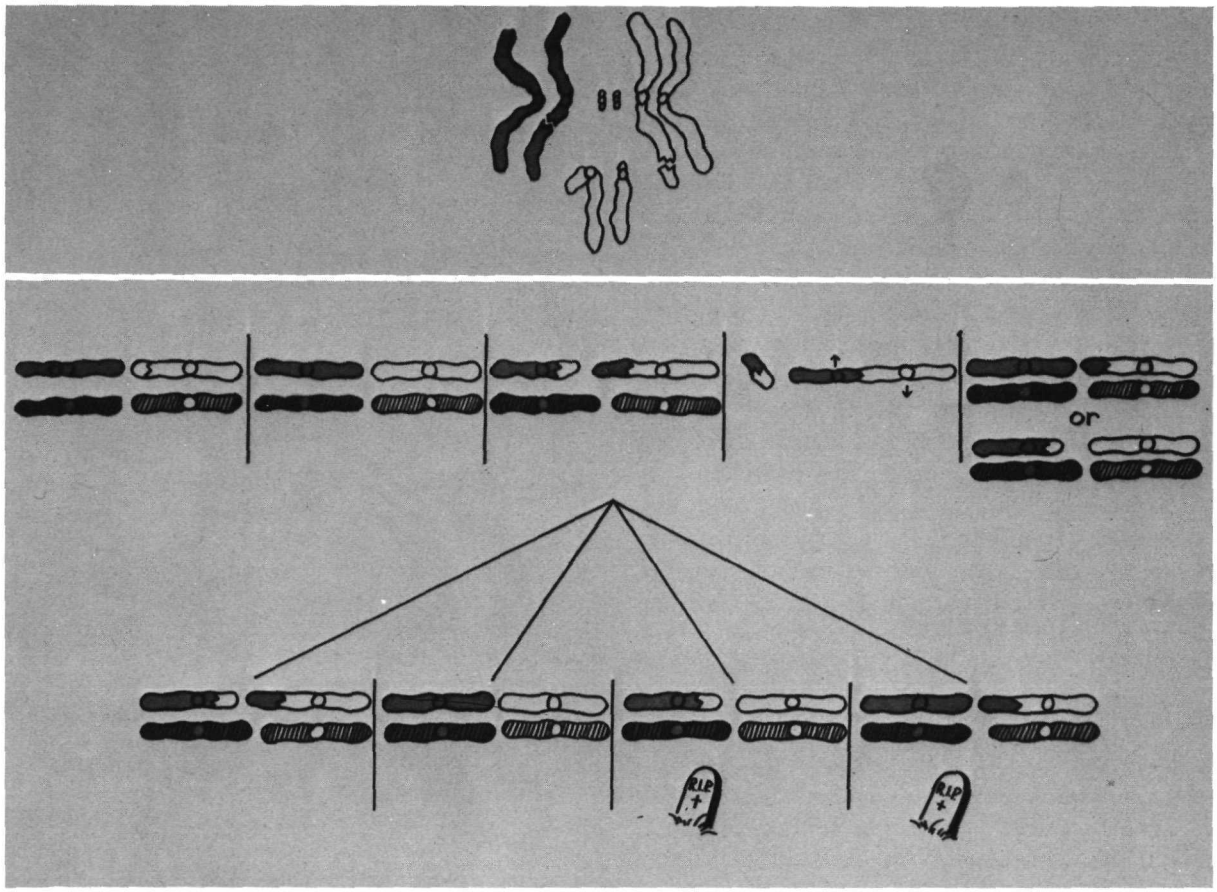

FIGURE 10.—Chances for detecting a translocation which has been induced in a diploid cell. The topmost figure represents the normal diploid chromosome complement in a gonial cell in which two breaks in two nonpartner chromosomes have occurred. The middle row illustrates the possible configurations which may result. The pairs of chromosomes actually involved, from left to right: Normal restitution with no untoward effects; segregation of the originally unbroken chromosomes; exchange of broken ends with the formation of a translocation; exchange of broken ends leading to acentric and dicentric chromosomes which are inviable for mechanical reasons and therefore cannot be transmitted to the offspring; and segregation yielding only one of the chromosomes involved in the translocation and therefore resulting in an unbalanced zygote. The cross-hatched chromosomes represent those contributed by the mother. The bottom row represents the manner in which a translocation, once established, can be detected genetically. The absence of the two classes of individuals which die can be readily detected by employing a suitable combination of markers.

(granted that they do occur in diploid gonial cells such as the ones under present consideration) and, therefore, to be detected by genetic tests. The significance of this observation should be considered from the point of view of space biology in particular and chromosomal behavior in general.

Figure 11 shows the *Drosophila* chromosome complement (in square) and a human chromosome set. The latter has many more small-size elements in relation to medium-size and large-size chromosomes than has the former. Recently Hecht et al. (1968) reported that some of the chromosomes in humans appear to be associated with each other in a nonrandom fashion, thereby favoring the occasional formation of exchanges. If weightlessness does indeed favor such nonrandom association (in this case a small chromosome), it would help to explain our findings. Changes involving at least one small chromosome, when considered from the standpoint of the life of an individual and/or at least a portion of his cells, can have serious consequences. Unfortunately, they would ordinarily not be detectable using the conventional methods of analysis available in organisms other than *Drosophila*. On the basis of available Earth-based data, it is unlikely that the low fluxes of cosmic radiation detected by Hewitt (1968) could have produced the translocations. However, since small chromosomes, primarily because of their size, have not been investigated extensively on Earth, we do not yet know very much about their behavior under the influence of radiation; this is particularly true of those with high linear energy transfers.

Since balanced stocks of the detected translocations, i.e., strains which are heterozygous for the particular change but which carry other markers and complex inversions to insure transmission of the desired combination from generation to generation, are still maintained in our laboratory, further attempts are being made to determine the exact regions where the original breaks have occurred. Genetic tests and examinations of the giant salivary gland chromosomes are being utilized for this. For example, it would be highly interesting to know

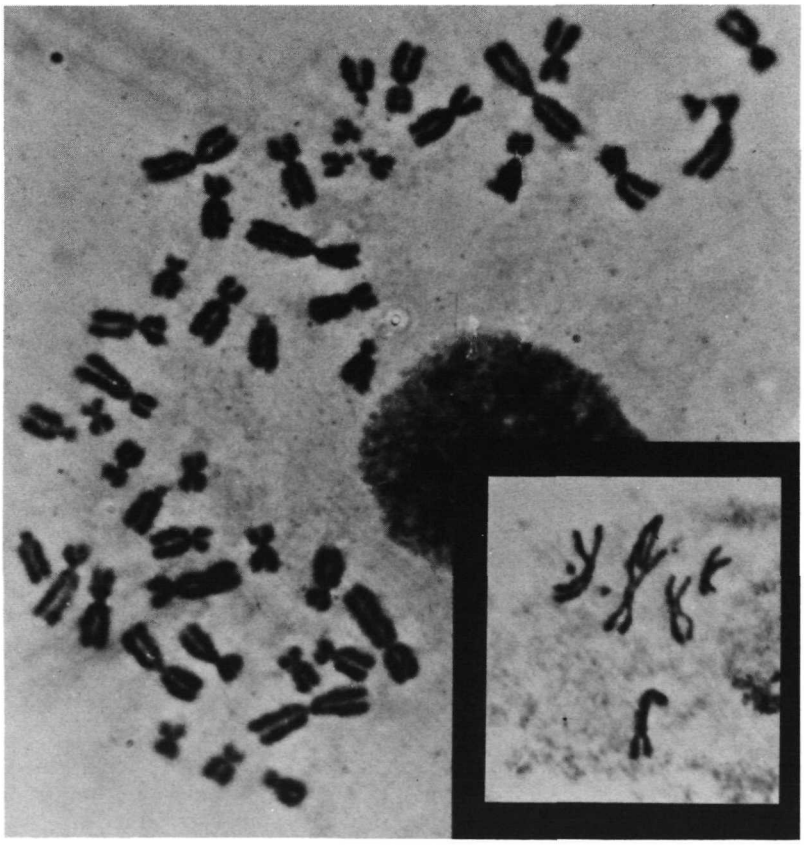

FIGURE 11.—Contrast in numbers of relatively small-sized chromosomes between *Drosophila* (inset) and humans.

whether the breakage distribution resembles that ordinarily encountered for radiation-induced changes on Earth or whether the major portion of those found in the orbited material involve other specific regions (in addition to the seeming selectivity already observed for the involvement of a whole chromosome).

SUMMARY AND CONCLUSIONS

The results reported suggest that radiation appears to interact with weightlessness to produce a higher mortality, more chromosomal breakage followed by loss or exchange of genetic information, and more sex-linked recessive lethal mutations in actively growing and metabolizing individuals than are produced in those irradiated on Earth.

In addition, and more markedly, it was found that some factor(s), perhaps extensively or perhaps exclusively involving weightlessness, associated with spaceflight is capable of causing improper chromosome separation (nondisjunction) and the formation of chromosomal translocations.

It is obvious that the aforementioned results and possible interpretations point up interesting and exciting new avenues of investigation from the standpoint of strictly Earth-oriented as well as space-oriented research (Kondo, 1968a and 1968b; Saunders, 1968d).

ACKNOWLEDGMENTS

We are particularly indebted to several individuals who helped to make this mission a success. We especially wish to mention Drs. J. Hewitt and J. Tremor and Messrs. C. Wilson and G. Bowman of NASA Ames Research Center; Drs. O. Reynolds and J. Saunders of NASA Office of Space Science and Applications; Messrs. D. Good, B. Laban, L. Nicholl, P. Oster, M. Thompson, and E. Wallis at Bowling Green State University; and the staff of the General Electric Co. in Philadelphia, Pa.

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Genetic Effects of the Space Environment on the Reproductive Cells of *Drosophila* Adults and Pupae

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This paper presents an account of some of the experimental findings resulting from the first opportunity to study possible transmissible genetic damage in the fruit fly, *Drosophila melanogaster,* caused by exposure to the conditions of spaceflight; namely, prolonged weightlessness and the inherent vibration, acceleration, and other factors attendant upon launch and recovery of a space capsule. Some of the material was subject to continuous ionizing radiation to determine what interaction, as measured in terms of genetic effects, might occur when spaceflight factors were combined with radiation. The large variety of specimens for both stage of maturity and genotype of the reproductive cells used in these experiments gave the project an exploratory nature; it is to be followed by careful selection, after return of the data, of material most suitable for further testing in space.

Biosatellite II did not orbit the Earth for the planned 72 hours and the dose of radiation received was cut down accordingly; the result was a lowering of mutation rates due to radiation and a shortening of the exposure to weightlessness. Therefore, an intensive effort was made to obtain as much data from the postflight controls as possible in order to determine the effects, if any, that could be assigned to vibration and acceleration, which could be simulated on Earth.

Among the large number of different genetic tests performed only the following showed significant differences between flight material and Earth-based controls. (1) An increase on the borderline of significance was found in the frequency of recessive lethal mutations in mature sperm when the irradiated flight material was compared with the concurrent Earth-based control material. (2) A significant decrease in the translocation frequency in irradiated mature sperm was found in the vibrated and irradiated Earth-based controls when they were compared with either the irradiated flight material of Biosatellite II or the irradiated but nonvibrated Earth-based controls. (3) A probable increase was observed in the production of translocations in the unirradiated flight material and among the vibrated and unirradiated Earth-based controls. (4) A significant increase in frequency of loss of markers from the Y chromosome and in frequency of translocations in male germ cells in all stages of maturation in pupae was found in the material in the irradiated compartment during flight when compared with the irradiated concurrent Earth-based controls. (5) An increase in the translocation and recessive sex-linked lethal frequencies in such cells in males given X-rays of 4000 R before flight was not significant when the flight material was compared with the concurrent Earth-based controls but was highly significant when the material was compared with all the controls. These differences could be attributed to vibration, acceleration, or contamination of the capsule atmosphere with formaldehyde, gluteraldehyde, and ethylene. Therefore, there is no evidence that weightlessness acting either alone or in combination can produce genetic effects, but neither can it be excluded as an interacting agent with these other factors. Additional Earth-based experiments coupled with flights of longer duration will be necessary to separate conclusively a possible

interaction between weightlessness and the other factors associated with spaceflight.

EXPERIMENT DESIGN AND METHODS

Drosophila Flight Packages

The *Drosophila* adult and pupae flight package consisted of eight cubicle polypropylene modules 2.5 cm on a side (fig. 1). Fresh culture medium was added shortly before the etherized flies were loaded. The modules were then mounted on a rack in two horizontal rows of four each (fig. 2). Preflight testing showed that 75 inseminated females could be loaded into a single module with good survival. Food in the same amount as that originally loaded was added after unloading to help support the larvae developing from eggs laid in flight. All packages underwent thorough tests before flight to determine their ability to withstand heat, vibration, and pressure stresses.

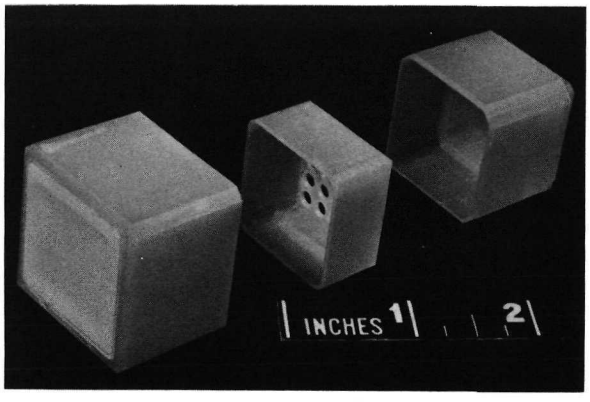

FIGURE 1.— *Drosophila* adult module, open view.

The flight capsule carried its own gas management system, the air within it being constantly recycled and maintained at a pressure of 1 atmosphere. Relative humidity varied from 48 to 62 percent and temperatures ranged from 62 to 73 F in the irradiated and unirradiated portions of the space capsule. One rack of eight modules was loaded into each compartment.

Concurrent Earth-based control flies were loaded like the flight specimens and installed in a capsule similar to the one used for flight. This capsule was not sealed but was open-ended, with air-conditioned ambient air from the laboratory circulated through it at a flow rate the same as that of the air being recycled in the flight capsule. Relative humidity ranged from 49 to 62 percent, and temperatures, from 63 to 72 F. Another group of flies was loaded into modules and housed in an incubator in which the humidity and temperatures were maintained within the same ranges. A third set of controls was kept in an incubator in which the humidity and temperatures were adjusted, with a lag of 4 hours, to those being received by telemetry from the flight capsule.

Radiation

The radiation source was ^{85}Sr-emitting gamma rays of 0.513-MeV energy (cf. paper by Hewitt,

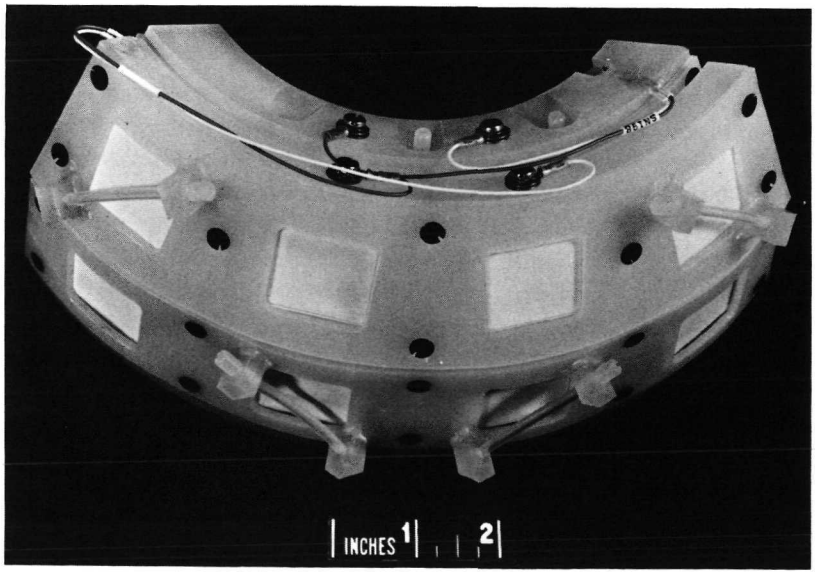

FIGURE 2.— Assembled *Drosophila* package with radiation dosimeter in place.

p. 333). The specimens were irradiated only during orbit. The dose received at the midpoint of the space between the surface of the food and the screen in each module containing the flies was calculated from the readings of three LiF tubes attached to the outside of the package (fig. 2). For the flight specimens the total dose was 1432 R applied at a rate of about 34.2 R per hour for 41 hours and 54 minutes, and for the Earth-based controls it was 1404 R for the same period. Variation in readings between the tubes was less than 4 percent. Total radiation received in the compartment shielded by a tungsten backscatter shield was 0.24 R for the experimental group and 0.31 R for the controls (Hewitt, 1968).

It has been assumed that the adult flies were flying about for at least part of the time during flight. This would allow for a variation in the dose of radiation they received which depended upon their position, at various times, within the module between the surface of the food and the screen lid (a distance of 1 cm). Calculations have shown that this variation would be no greater than 6 percent and that the average dose received by the flies in each module would be expected to be reasonably uniform. It would be a negligible factor except in cases of differences of borderline significance. Any differences in mutation frequencies for which significance is being claimed are of the order of 25 to 200 percent.

A postflight control test simulating the concurrent controls for the flight (irradiated and unirradiated specimens only) was performed on March 12, 1968, and a second postflight control test with hard environments (acceleration, vibration, and acoustic noise) was performed on May 22, 1968. Five sensors had been used to monitor the vibration levels in the capsule of Biosatellite II, three in the irradiated compartment and two in the unirradiated. One of those in the irradiated compartment was a triaxial accelerometer which measured vibration in the longitudinal, tangential, and radial directions. The other accelerometers measured in only the longitudinal direction. The launch maximum acceleration was 8 *g* attained in 150 seconds, and upon reentry it was 9.5 *g* obtained in 40 seconds. Vibration data were telemetered from the interface between the whole Biosatellite spacecraft and the two-stage Delta launch vehicle. The two sets of data were in good agreement in the low frequency range where biologic effectiveness of vibration is known to be maximum.

In the May 22 postflight test, flight vibration was simulated as nearly as possible for lift-off and recovery. All simulated vibration was in the longitudinal direction and was not the random vibration experienced in flight. It was necessary to break down the two phases further because of equipment limitations; e.g., in simulating lift-off the capsule was first subjected to the launch vibration and 1 hour later was placed on the centrifuge and subjected to the launch acceleration profile. Simulation within these limitations was in good agreement with monitored flight experience. The details of these events are given in the paper from NASA Ames Research Center (1968). They have been summarized here inasmuch as the *Drosophila* adult and pupae experiments showed that certain genetic effects might be attributed to these factors.

Genetic Technique

Because there is no question as to homogeneity with regard to germ-cell stage, and therefore with regard to mutagenic sensitivity, of mature sperm cells carried in inseminated females, greater emphasis was placed on these flight specimens and their controls than on any other material, although mutations in germ cells in different stages of development were also detected. The multipurpose genetic technique is described below for each of the four types of flies loaded.

(1) *Type 1:*—Females with yellow bodies *(y)* instead of the normal gray (y^+), dumpy wings *(dp)*, wings shorter than normal and of characteristic shape, and white eyes (a combination of three mutant eye colors: brown *(bw)*, scarlet *(st)*, and pink peach (p^p). The effect of each mutation is to reduce, to a different extent, normal pigment formation, and in combination to reduce it altogether, thereby causing the eyes to look white. The symbols for the mutations are shown in parentheses, with small letters indicating recessive mutations and capital letters indicating dominant mutations. Figure 3 shows a normal female and a normal male *Drosophila,* and figure 4 shows a female with the yellow, dumpy, white characteristics just outlined. The genotype of such females is $y\ sc^{S1}\ In49\ sc^{8};\ dp\ bw;\ st\ p^p$. The symbols $sc^{S1}\ In49\ sc^{8}$ indicate in-

versions or rearrangements of the segments of the X chromosome which prevent crossing over (or breakage and recombination) when in a female possessing one X chromosome having the inversions and the other not. If such precautions are not taken, the "escape" of a lethal mutation from one X chromosome to the other is possible, and detection of the lethal is prevented.

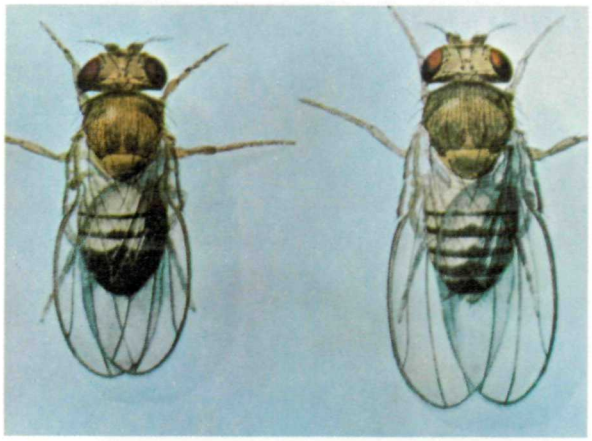

FIGURE 3.— Normal male *Drosophila* on left; normal female, on right.

These females were inseminated by males with forked bristles *(f)*, which are distinctly shorter than normal and are gnarled and forked at the ends. They also had mutant Bar eyes *(B)*, or eyes very much reduced in size and slit-shaped instead of oval (fig. 5). The genotype of these males is $f \,/\, y^{+}\ sc^{8} \cdot Y \cdot B^{S}$. In this inbred strain the Y chromosome (present only in males) possesses a $y^{+}\ sc^{8}$ fragment attached to one end of the Y. Its presence causes the sons of the inseminated females to have gray instead of yellow bodies (the yellow body color comes from the mother's X chromosome and is transmitted to her sons). The B^{S} fragment of the X chromosome had likewise become attached to the end of the Y opposite from that to which the y^{+} fragment had been attached, thus causing the males to have Bar eyes (fig. 6).

Males of this genotype less than 24 hours old were isolated and held for 2 days before being

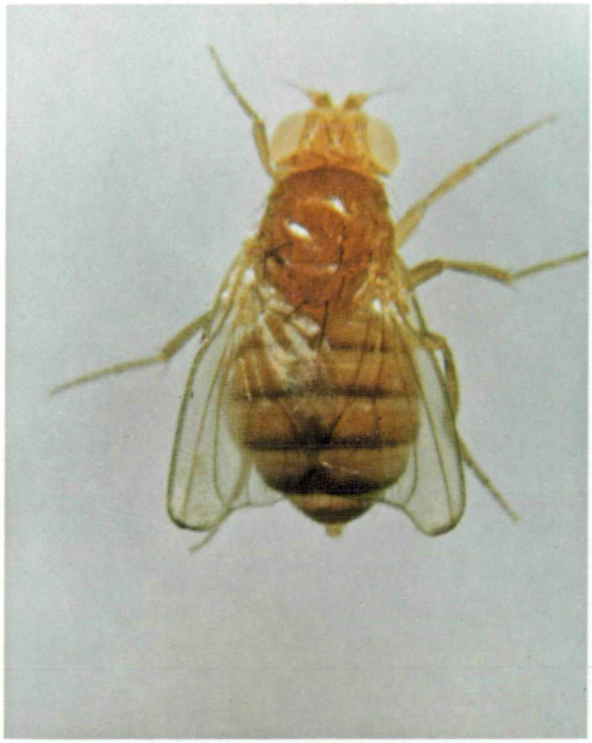

FIGURE 4.— Female *Drosophila* with mutations causing yellow body, dumpy wings, and white eyes.

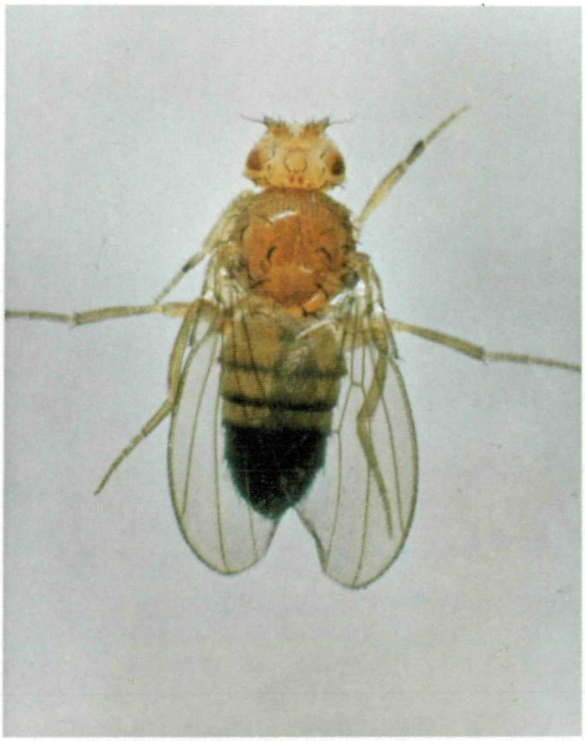

FIGURE 5.— Male *Drosophila* with mutations causing shape of eyes to be Bar instead of round and bristles to be shortened and forked at the ends.

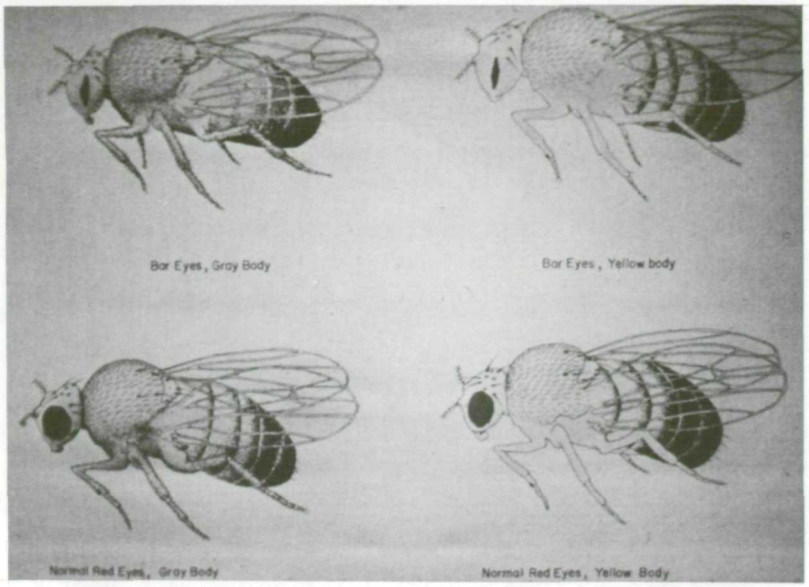

FIGURE 6.—Bar eyes and gray body (instead of yellow) in males caused by B^S and $y+$ fragments of X chromosome attached to Y chromosome (upper left). Other flies show losses of these fragments.

allowed to inseminate young yellow, dumpy, white virgin females that had also been isolated 2 days previously. Twenty-four hours before they were loaded for flight, an excess of males was shaken together with the females without etherization, a procedure which insures almost 100 percent fertilization. About 15 hours before launch, the flies were lightly etherized, and 75 females were separated from the males and loaded into each of five modules to be placed in the irradiated compartment of the capsule and into each of three modules to be placed in the unirradiated compartment. Packages of eight modules were loaded for each of the three types of controls.

Eggs were laid in the modules during flight; those hatching the first 3 days after recovery were considered to have been laid during the period when flight conditions might possibly have affected chromosome rejoining during fertilization. Those hatching afterward were considered as having been fertilized and laid after flight and were included in the offspring being examined for mutations in brood 1, that is, the flies hatching from the vials into each of which three of the inseminated females had been placed immediately after unloading. The three females contained in each vial were transferred every 3 days to a fresh food vial and labeled successively as brood 2, 3, or 4 in a procedure to augment the number of offspring obtained from them.

The normal sons of the inseminated females had gray bodies and Bar eyes. The daughters were entirely normal (fig. 3). Among the first-generation offspring, losses of the dominant markers y^+ and B^S (gray bodies and Bar eyes) could be detected, as well as visible mutations at the *y, dp, bw, st,* and p^p loci. Nondisjunction resulting from the fertilization of an XX egg by a Y-bearing sperm resulted in a y^+ *B* female (gray bodies and Bar eyes instead of yellow bodies and oval eyes), whereas an egg containing no X, when fertilized by an X-bearing sperm, would appear as a y^+B^+f (forked bristles and normal eyes) male. The use of nondisjunctional females as parents was guarded against because the males of the stock from which the females were obtained had a y^+ sc^8 · *Y* chromosome (gray instead of yellow bodies), which made it possible to reject nondisjunctional females because they were phenotypically gray instead of yellow.

Second-generation tests for recessive lethal mutations were made by crossing individual $f/$ y sc^{S1} $In49$ sc^8 daughters (normal appearing) to three males of composition $FM6$, y^{34} sc^8 dm $B/$ $l(X)$ 7. The inversions in the X chromosome of this stock will prevent crossing over between it and either of the two X chromosomes of the mother, thus allowing for the detection of recessive lethal mutations in the paternal X chromosome by the absence of gray, forked sons and in the maternal X chromosome, by the absence of yellow sons.

Translocation tests were made by mating one heterozygous son (of composition $y\ sc^{S1}\ In49\ sc^{8}$; $dp\ bw/+\ +$; $st\ p^{p}/+\ +$) to three $y\ sc^{S1}\ In49\ sc^{8}$; $dp\ bw$; $st\ p^{p}$ virgins. It was possible to detect translocations between the following chromosomes: II and III; Y and II; Y and III; and Y, II, and III. If no translocation had been produced, females with four different eye colors (white, brown, orange, and red) would be found in equal proportions. The females were scanned without etherization; the finding of only two different eye colors in addition to white eyes (which would always be present) would quickly rule out the possibility of a translocation. The presence of a translocation is detectable by the absence of at least one of the four expected classes of eye colors. The males could not be used because they had Bar eyes in which the eye colors are not easily seen.

(2) *Type 2:* — Forty males isolated for 2 days before flight and of genotype $f/\ y^{+}\ sc^{8}\ Y \cdot B^{S}$ loaded into one module along with an equal number of heterozygous males of genotype $y\ sc^{S1}\ In49\ sc^{8}/\ y^{+}\ sc^{8} \cdot Y \cdot B^{S}$; $dp\ bw/+\ +$; $st\ p^{p}/+\ +$. The latter type of males allows for the detection of recombinants between the loci *dp* and *bw* and *st* and p^{p}, sometimes referred to as crossovers in males, which normally do not occur, as well as translocations and lethals in the paternal X.

(3) *Type 3:* — Young $f/\ y^{+}\ sc^{8} \cdot Y \cdot B^{S}$ males irradiated with 4000 R of X-rays as close to loading time as possible. Forty of these males were loaded into each of the two modules in the unirradiated flight compartment, together with an equal number of virgin females of genotype $y\ sc^{S1}\ In49\ sc^{8}$; $dp\ bw$; $st\ p^{p}$, in order to determine whether spaceflight conditions would have any effect on rejoining of the already broken chromosome fragments.

(4) *Type 4:* — Late third instar larvae or prepupae of genotype $y\ sc^{S1}\ In49\ sc^{8}$; $dp\ bw/+\ +$; $st\ p^{p}/+\ +$. The males hatching from these modules were bred individually to three $y\ sc^{S1}\ In\ 49\ sc^{8}$; $dp\ bw$; $st\ p^{p}$ virgins every other day for six broods to detect visible mutations or were crossed to *y ct* females so that the daughters could be tested for recessive lethals in their X chromosome; *ct* was used as a marker to distinguish between the paternal (ct^{+}) X chromosome under test and the maternal X. One-fourth of the males were heterozygous like their fathers and were tested for translocations individually by crossing to three $y\ sc^{S1}\ In49\ sc^{8}$; $dp\ bw$; $st\ p^{p}$ virgins.

RESULTS

Mutations Occurring in Mature Sperm in Inseminated Females

RECESSIVE LETHAL MUTATIONS

As shown in table 1, a recessive lethal frequency of 4.545±0.677 percent (43/946) was recovered from inseminated females carrying mature sperm receiving 1432 R of gamma radiation during flight, as compared with 3.272±0.552 percent (34/1039) recovered from the concurrent irradiated controls housed in the capsule with air-conditioned ambient air. This increase was on the borderline of significance ($P=0.05$). In postflight controls receiving radiation alone in the same capsule, a recessive lethal frequency of 3.223±0.312 percent (103/3196) was found. The almost identical frequencies found in these two controls receiving radiation alone, in the same capsule, demonstrate the reliability of this experimental technique. From postflight controls receiving radiation alone and housed in the flight capsule with recycled air, a frequency of 4.219±0.267 percent (240/5689) was obtained. Also, a frequency of 4.545±0.406 percent (171/2639) was recovered from postflight irradiated controls housed in the flight capsule but receiving, in addition, vibration and acceleration simulating as nearly as possible that received at launch and reentry of the Biosatellite II capsule.

Thus the increase in recessive lethal frequency found in the irradiated flight specimens was almost exactly reproduced both in the vibrated and nonvibrated postflight controls which were housed in the flight capsule in which the gas was recycled.

Table 2 shows that measurable amounts of formaldehyde (Form.), glutaraldehyde (Glu.), and ethylene (Ethyl.) were present in the capsules at the beginning and end of some of the experiments. Glutaraldehyde and formaldehyde were used in some experiments, although with regard to the ambient air the measurements would appear to have little meaning. A marked synergism between formaldehyde and X-rays in the production of recessive lethals in *Drosophila* has been reported by Sobels (1956) (see table 3). *Drosophila* is very sensitive to chemical mutagens in the vapor phase,

TABLE 1. – Sex-Linked Recessive Lethals in Sperm Used To Inseminate Females Before Flight or Control Testing

Program	Date	Phase	Capsule atmosphere	Chromosomes tested	Lethals	Lethal frequency, %±std. dev.
			Irradiated			
Flight	Sept. 1967	Biosatellite	Recycled	946	43	4.545±0.677
Controls:						
Vibrated[a]	May 1968	C	Recycled	2 639	120	4.547±0.406
Not vibrated	Mar. 1968	B	Recycled	5 689	240	4.219±0.267
Not vibrated	Sept. 1967	Biosatellite	Ambient	1 039	34	3.272±0.552
Not vibrated	May 1968	C	Ambient	3 196	103	3.223±0.312
			Unirradiated			
Flight	Sept. 1967	Biosatellite	Recycled	768	2	0.260±0.184
Controls:						
Vibrated[a]	May 1968	C	Recycled	3 366	1	0.030±0.030
						[b]0.230±0.183
Not vibrated	Sept. 1967	Biosatellite	Ambient	495	1	0.202±0.202
Not vibrated	Mar. 1968	B	Recycled	2 881	3	0.104±0.060
Not vibrated	1966–67		Ambient	11 791	9	0.076±0.025
Total not vibrated				15 167	13	0.086±0.024
						[c]0.174±0.185

[a] Includes simulation of vibration, acceleration, and shock to which the flight specimens had been subjected.
[b] Difference, flight and vibrated controls.
[c] Difference, flight and nonvibrated controls.

TABLE 2. – Formaldehyde, Glutaraldehyde, and Ethylene in Atmosphere of Capsules Measured at Time of Loading and About 50 Hours Thereafter

[If no values given, not measured; where measured, sample having maximum reading is shown.]

Date	Flight capsule (recycled) [a]							Earth-based control capsule (ambient) [b]						
	Form., ppm		Glu., ppm		Ethyl., ppm		Recessive lethal frequency, % ± std. dev.	Form., ppm		Glu., ppm		Ethyl., ppm		Recessive lethal frequency, % ± std. dev.
	Start	End	Start	End	Start	End		Start	End	Start	End	Start	End	
Sept. 1967	<0.8	<0.8	0.8	2.1	.....	.....	4.6±0.7	.....	.....	<10.0	<10.0	.....	.....	3.3±0.6
Mar. 1968	.7	1.1	.2	.2	.....	.....	4.2±0.3	1.9	.7	.2	.2	.....	.....	.....
May 1968	< .2	< .2	.7	.6	0.07	0.9	4.6±0.4	< .2	.4	.7	.6	<0.02	<0.05	3.2±0.3

[a] Atmosphere recycled in closed system at 760 mm Hg and 21±3 C.
[b] Ambient air circulated through open system at 760 mm Hg and 21±3 C.

TABLE 3. – Effect of Formaldehyde Pretreatment on the Mutagenic Action of X-rays on *Drosophila*

[Taken from Sobels, 1956]

X-ray, R	Formaldehyde (0.28 mm³ injected)	Chromosomes	Lethals	Lethal frequency, %
.....	0.05M	360	1	0.3
1700	.....	373	39	10.4
1700	0.05M	268	47	17.5
.....	0.033M	500	5	1.0
1700	.....	657	86	13.1
1700	0.033M	317	63	19.8

TABLE 4. – Visible Mutants Occurring in Sperm Carried in Inseminated Females

Program	Date	Phase	Capsule atmosphere	Total F_1 offspring	*dp* (a)	*bw*	*st*	*pp*	*y*	Total visibles	
										No.	Poisson limits of frequency at $P=0.05$, %
Irradiated											
Flight	Sept. 1967	Biosatellite	Recycled	21 537	15	2	4	0	3	24	7.2 to 16.6×10^{-2}
Controls:											
Vibrated[b]	May 1968	C	Recycled	20 934	9	2	2	0	1	14	3.7 to 11.1
Not vibrated	Mar. 1968	B	Recycled	16 537	16	5	3	0	0	24	9.3 to 21.6
Not vibrated	Sept. 1967	Biosatellite	Ambient	26 170	6	5	2	1	2	16	3.6 to 9.7
Not vibrated	May 1968	C	Ambient	9 748	10	2	2	0	1	15	8.6 to 25.4
Total all controls				73 389	41	14	9	1	4	69	7.0 to 11.9
Unirradiated											
Flight	Sept. 1967	Biosatellite	Recycled	14 436	0	0	0	0	0	0	0 to 2.6×10^{-2}
Controls:											
Vibrated[b]	May 1968	C	Recycled	15 728	2	0	0	0	2	4	0.6 to 6.5
Not vibrated	Sept. 1967	Biosatellite	Ambient	31 222	0	0	0	0	0	0	0 to 1.2
Not vibrated	May 1968	C	Ambient	15 066	1	0	1	0	0	2	0.1 to 4.8
Not vibrated	Mar. 1968	B	Recycled	14 267	0	0	0	0	0	0	0 to 2.7
Spontaneous	1966–67	.....	.....	52 000	0	0	0	0	0	0	0 to 0.7
Total not vibrated				112 555	1	0	1	0	0	2	0.0 to 0.6

[a] Only those flies with the oblique wing character were scored in these experiments.
[b] Includes simulation of vibration, acceleration, and shock to which flight specimens had been subjected.

and formaldehyde probably existed as a vapor in the capsule with the recycled gas. No valid comparison can be made between the injected dose in Sobels' experiments and the dose received from recycled contaminated gas in the March 1968 controls. It was decided beforehand to accumulate as much data as possible using the recycled gas capsule in the March 1968 control because it was to be the only opportunity of the experimenters to do such a test. It appears that no matter what mutation frequency had been found in the ambient-air capsule, it would have been of no value if it had been measured, since the concentrations measured at the beginning and end were momentary measurements not indicative of the average concentrations.

VISIBLE MUTATIONS IN MATURE SPERM

No significant differences were observed for visible mutations at the five loci studied when the flight material was compared with Earth-based controls (table 4).

LOSSES OF DOMINANT MARKERS FROM THE Y CHROMOSOME IN MATURE SPERM

No significant differences were observed in losses from the Y chromosome when flight material was compared with Earth-based controls (table 5).

TRANSLOCATION IN MATURE SPERM

A total translocation frequency of 7.117±0.777 percent (78/1096) was found among the male offspring of inseminated females flown in the irradiated compartment of Biosatellite II (table 6).

TABLE 5. – Losses of y^+ and/or B^S Markers From Y Chromosomes in Sperm Carried by Inseminated Females

Program	Normal F1 males ($y+$ B)	$y+$ $B+$	y B	y $B+$	Y Mas	Var B	Total	Percent loss
			Irradiated					
Flight	8 961	24	16	55	6	6	107	1.19
Controls:								
Vibrated[a]	9 282	48	10	45	1	3	109	1.15
Not vibrated:								
Sept. 1967	11 349	25	18	55	1	4	109	.91
Mar. 1968	7 485	35	10	55	5	11	116	1.55
May 1968	3 900	13	4	8	1	6	32	.82
Total not vibrated	22 734	73	32	118	7	21	351	1.54
			Unirradiated					
Flight	5 905	5	0	1	0	0	6	0.10
Controls:								
Vibrated[a]	6 192	4	0	1	0	1	6	.10
Not vibrated:								
Sept. 1967	13 124	3	1	9	0	0	13	.10
Mar. 1968	6 198	2	1	1	0	0	4	.07
May 1968	6 556	5	2	1	0	0	8	.10
Total not vibrated	25 878	10	4	11	0	0	25	.09

[a] Includes simulation of vibration, acceleration, and shock to which the flight specimens had been subjected.

TABLE 6. – Translocations Occurring in Sperm Used To Inseminate Females Before Irradiated Flight or Control Testing

Program	Date	Genomes tested	Translocations							
			2/3, %	Y–2	Y–3	Y–2–3	Total	Frequency, %	Std. dev.	*P*
Flight	Sept. 1967	1 096	45 (4.106)	16	13	4	78	7.117	±0.777	.....
Controls:										
Vibrated[a]	May 1968	2 913	69 (2.369)	25	24	4	122	4.188	±0.371	.....
Not vibrated	Sept. 1967	811	41 (5.055)	11	4	0	56	6.905	±0.890	.....
Not vibrated	May 1968	2 605	84 (3.225)	29	25	2	140	5.374	±0.442	.....
Not vibrated	Mar. 1968	3 902	144 (3.690)	44	50	9	247	6.330	±0.390	.....
Total not vibrated		7 318	269 (3.676)	84	79	11	443	6.054	±0.279	.....
Differences:										
Flight and vibrated controls			+1.737±0.662 ($P<0.01$)					+2.929	±0.861	0.001
Flight and not-vibrated controls			+0.430 (not significant)					+1.0163	±0.825	.20
Vibrated and not-vibrated controls			—1.307 ($P=0.0004$)					—1.865	±0.464	.0001

[a] Includes simulation of vibration, acceleration, and shock to which the flight specimens had been subjected.

This was not significantly different from the irradiated concurrent control frequency of 6.905±0.890 percent (56/811). During the large-scale testing of the postflight irradiated controls a frequency of 6.33±0.390 percent (144/3690) was observed as a result of the March 1968 test and a frequency of 5.374±0.442 percent (140/2605) was observed in the May 1968 test. The total control frequency was thus 6.054±0.279 percent (443/7318) which was not statistically different from that of the flight specimens. Although there was quite a bit of variation between the frequencies in the three tests, the frequency of translocations of each control test was not significantly different from that of each of the other controls, nor was a significant difference indicated when the controls were compared by using the mean of the sum of the controls as the expected value ($\chi^2 = 1.01$).

A frequency of only 4.188±0.371 percent (122/2913) was obtained from the vibrated, accelerated, and irradiated group (table 6). The increase in translocations found in the flight specimens over those found in the vibrated and accelerated group is highly significant, as is the lowering of the number in the vibrated group below that in the other controls. Therefore, it appears that some factor other than vibration and acceleration may be responsible for the high frequency of translocations in the flight specimens, if it be assumed that the vibration received in the postflight control test was similar in biological effectiveness to that encountered in flight. If this were not so, then differences between the flight vibration profile and the postflight simulated vibration profile may account for the difference.

Teratogenic Effects Produced in Cells in Various Stages of Maturation

An abnormality consisting of a deformed thorax and missing wing (fig. 7) was found in 5.3 percent of the flies hatching during the first 4 days from eggs laid by the inseminated females flown in the irradiated compartment of the capsule, as compared with 3.3 percent found among the irradiated controls. Since the abnormality occurred in early embryos that were exposed to varying doses of radiation with respect to their age, the data are presented in table 7 and figure 8 according to the days on which the flies hatched. Muller's weighted harmonic mean method of comparing the flight and control series has been used (Muller, 1941). The increase is seen to be on the borderline of significance when compared with the concurrent controls but highly significant when compared with

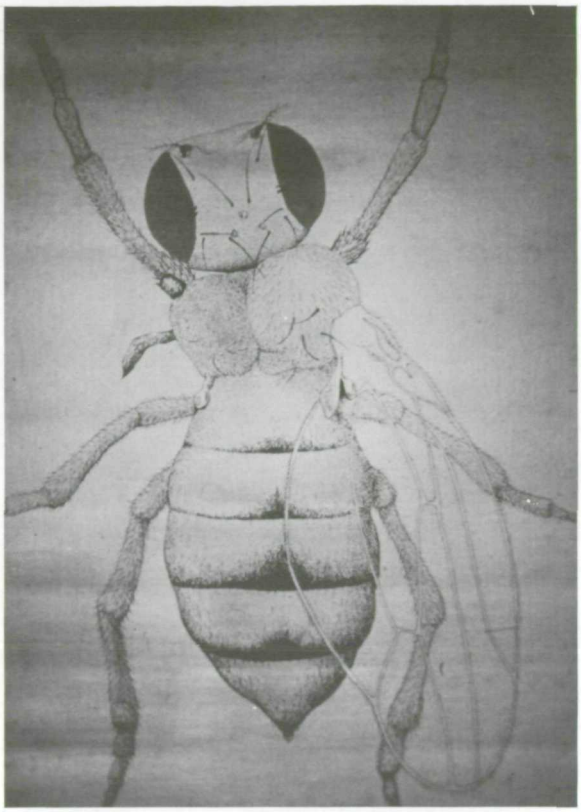

FIGURE 7.—*Drosophila* with deformed thorax and missing wing.

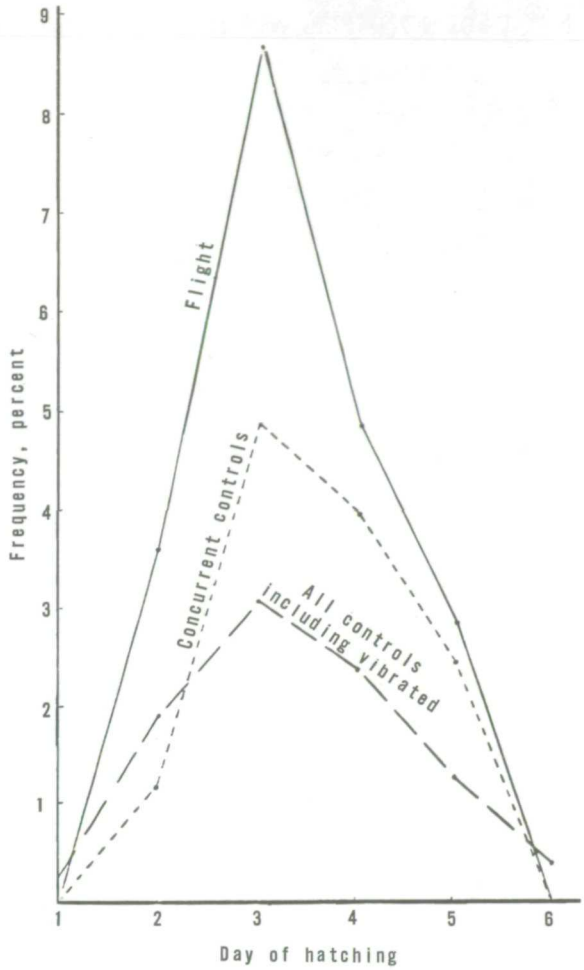

FIGURE 8.—Flies with deformed thorax and missing wing hatching from eggs laid in irradiated compartments during flight of Biosatellite II.

all the controls. When the Poisson limits are calculated, the increase over the concurrent controls is not significant but the increase over the total controls is again significant ($P=0.01$). Figure 8 shows the frequency of the abnormality in graphic form. The vibrated controls did not differ significantly from the other controls.

Genetic Effects Produced in Cells in Various Stages of Maturation

NONDISJUNCTION IN INSEMINATED FEMALES

Since nondisjunction between the X chromosomes was occurring in the female germ line, the cells of which were rapidly proliferating and changing in genetic sensitivity, Muller's weighted harmonic mean method of statistical analysis (Muller, 1941) has been applied. When the inseminated females were unloaded, they were transferred every 3 days to new containers contaning fresh culture medium (broods). As shown in table 8, the dosage of radiation received on the shortened flight of Biosatellite II was too low to give adequate rates for a sensitive comparison of the flight material with the Earth-based controls. No differences were found in the unirradiated groups.

PUPAE

Prepupae and late third instar larvae were collected and mixed in a beaker of water in order to obtain random samples. Approximately 200 were loaded into a single module. Radiation in orbit was begun about 15 hours after loading, at which time all of the specimens had undergone pupation. Following flight the males were mated to fresh virgins at successive 2-day intervals for six broods. Broods represent successive samples of the germ line that

TABLE 7. – Flies With Deformed Thorax and Missing Wing Hatching From Eggs Laid by Inseminated Females in Modules During and After Flight

Day of Hatching	Flight			Concurrent controls					Total controls				
	Total offspring	Deformed thorax	Frequency, %	Total offspring	Deformed thorax	Frequency, %	Weighted harmonic mean difference, %, day 1 to —	*P*	Total offspring	Deformed thorax	Frequency, %	Weighted harmonic mean difference, %, day 1 to —	*P*
Irradiated													
1	45	0	0.0	68	0	0.0	.....	.....	354	1	0.3	.....	.....
2	165	6	3.6	172	2	1.2	1.9	0.14	955	18	1.9	1.3	0.160
3	184	16	8.7	267	13	4.9	2.8	.03	944	29	3.1	3.3	.001
4	249	12	4.8	250	10	4.0	2.0	.05	1 017	24	2.4	3.0	.001
5	315	9	2.9	206	5	2.5	2.0	.05	721	9	1.3	2.6	.001
6	604	0	.0	637	0	.0	2.0	.05	3 634	14	.4	1.4	.001
Total	1 562	44	2.8	1 600	30	1.9	.....	.....	7 625	95	1.3	.....	.....
Unirradiated													
1–21	2 510	0	.....	1 909	0	.....	.....	.....	5 272	0	.....	.....	.....

TABLE 8. – Nondisjunction of X Chromosomes Occurring in Inseminated Females

Program	Date	Total F_1 offspring	Nondisjunctional F_1 offspring			Weighted harmonic mean difference, %	Difference
			Males	Females	Total		
Irradiated							
Flight	Sept. 1967	21 537	22	8	30	0.1	
Concurrent controls	Sept. 1967	26 170	24	23	47	.2	Not sig.
Postflight controls:							
Vibrated	May 1968	20 934	6	14	20	.1	
Not vibrated	May 1968	9 748	7	7	14	.1	
Unirradiated							
Flight	Sept. 1967	14 436	2	9	11	0.1	
Concurrent controls	Sept. 1967	31 222	5	14	19	.0	Not sig.
Postflight controls:							
Vibrated	May 1968	15 728	0	7	7	.0	
Not vibrated	May 1968	15 066	1	4	5	.0	

are in different stages of development at the time of flight and irradiation. Since there are changes in genetic sensitivity during development, Muller's weighted harmonic mean method of statistical analysis (Muller, 1941) has been applied. Because of the rapid change in cell sensitivity, it is inappropriate to make comparisons among experiments conducted at different times. The flight material and concurrent controls can be compared since the pupae were divided at random from the same beaker. Similarly, in the Earth-based controls the vibrated and irradiated specimens can be compared with the concurrent nonvibrated and irradiated. The results follow.

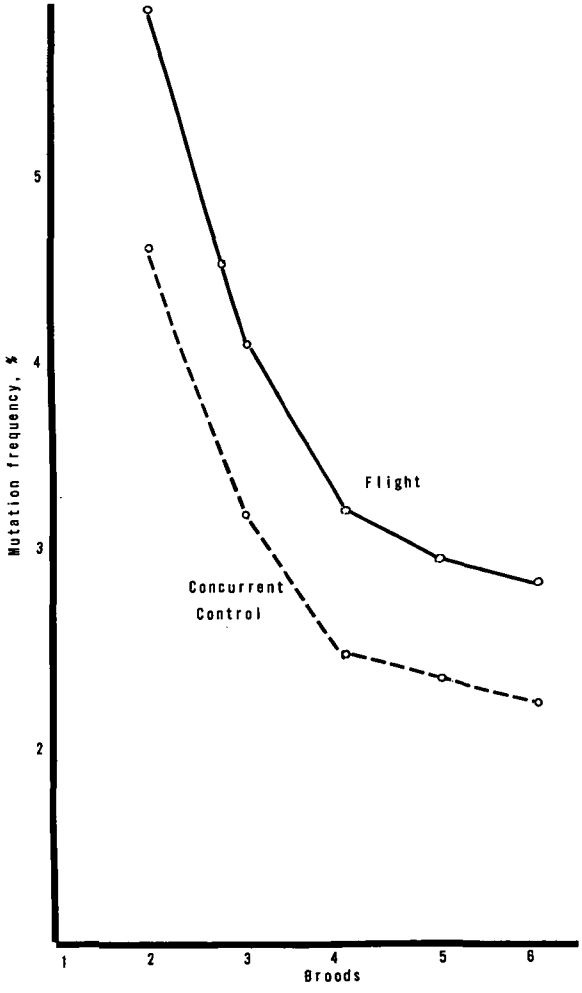

FIGURE 9.— Loss of markers from Y chromosome of irradiated pupae due to flight.

Losses of Markers From the Y Chromosome

The results of the analysis of the losses of markers from the Y chromosome have been reported (Browning, 1968) and are summarized in table 9 and figure 9. Losses of the dominant markers y^+ and *B* from the Y chromosome had been found to be significantly increased by flight in the irradiated compartment over those of the irradiated concurrent controls when the two series were analyzed by the above-mentioned harmonic mean method. In figure 9 the weighted harmonic mean of broods 1 and 2 combined from the irradiated flight material has been plotted as the first point on the upper curve. The second point is the mean of broods 1, 2, and 3 combined, and so on. The lower curve shows the corresponding combined means of the concurrent irradiated controls. The standard errors are not shown, but at each point the difference is significant with values of *P* ranging from 0.03 to 0.001. A significant increase in number in the vibrated and irradiated over those in the nonvibrated but irradiated postflight controls was also found, as shown in table 9 and figure 10. In the absence of radiation, no differences were found.

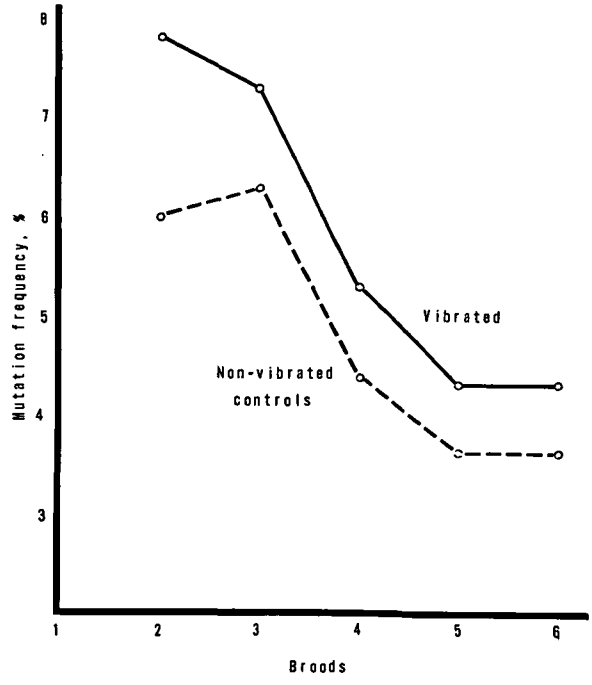

FIGURE 10.— Loss of markers from Y chromosome of irradiated pupae due to vibration.

TABLE 9. — Losses of Markers From Y Chromosome Occurring in Males in Pupal Stage

Program	Total F_1 males	Number of males with—						Weighted harmonic mean difference, %	Difference, %	P
		Loss of *B*	Loss of *y*+	Loss of *B* and *y*+	*y* mos	var *Bar*	Total			
Irradiated										
Flight	14 332	198	75	176	0	3	452	2.8	.....	.....
Concurrent controls	14 503	116	42	119	0	3	280	2.2	0.6±0.2	0.001
Postflight controls:										
Vibrated	6 868	134	49	125	0	8	316	4.3	.....	.....
Not vibrated	6 809	85	45	106	0	2	238	3.6	.7±0.3	.030
Unirradiated										
Flight	17 603	0	0	15	0	0	15	0.1	.....	.....
Concurrent controls	12 118	0	0	14	0	0	14	.1	Not sig.	.....
Postflight controls:										
Vibrated	18 555	7	3	61	1	1	73	.4	.....	.....
Not vibrated	4 096	1	0	12	.....	.....	13	.3	Not sig.	.....

TABLE 10. — Lack of Effect of Spaceflight on Nondisjunction, X•Y Exchanges, and Crossovers Occurring in Males in Pupal Stage

Program	F_2 female offspring					F_2 offspring		
	Total	Nondisjunction	Weighted harmonic mean difference, percent	X•Y exchanges	Weighted harmonic mean difference, percent	Total	Crossovers[a]	Weighted harmonic mean difference, percent
Irradiated								
Flight	20 720	45	0.2	109	0.5	35 052	111	0.3
Concurrent controls	20 271	45	.2	92	.5	34 774	114	.3
Postflight controls:								
Vibrated, May 1968	10 158	22	.2	89	.8	17 044	73	.4
Not vibrated:								
May 1968	9 738	20	.2	65	.7	16 547	74	.5
Mar. 1968	14 659	54	.3	98	.8	25 819	122	.5
Unirradiated								
Flight	24 937	62	0.2	2	0.0	36 720	8	0.0
Concurrent controls	20 287	40	.2	0	.0	24 973	2	.0
Postflight controls:								
Vibrated	27 233	88	.3	7	.0	45 788	3	.0
Not vibrated:								
May 1968	5 744	5	.1	1	.0	9 870	2	.0
Mar. 1968	11 285	21	.2	0	.0	19 422	1	.0

[a] Crossovers or recombinants between *dp*, *bw*, *st*, and p^P markers.

Nondisjunction, X•Y Exchanges, and Crossovers

Table 10 shows that no differences were detected between the flight material and the Earth-based controls with regard to these genetic effects.

Lethals and Translocations

No differences were found with regard to the sex-linked recessive lethal frequencies in the male pupae between those that were flown and those that served as controls (table 11). However, with regard to translocations in the irradiated compartments, there was a significant increase in frequency after flight when compared with that of the concurrent controls (fig. 11). On the other hand, the vibrated postflight controls had a significantly lower translocation frequency than those not vibrated in that test. This result is similar to the significant lowering of the translocation frequency in inseminated females in the postflight simulated vibration and acceleration test. Since the flight material had been also subjected to vibration and acceleration at launch and reentry, but still remained higher than the concurrent nonvibrated

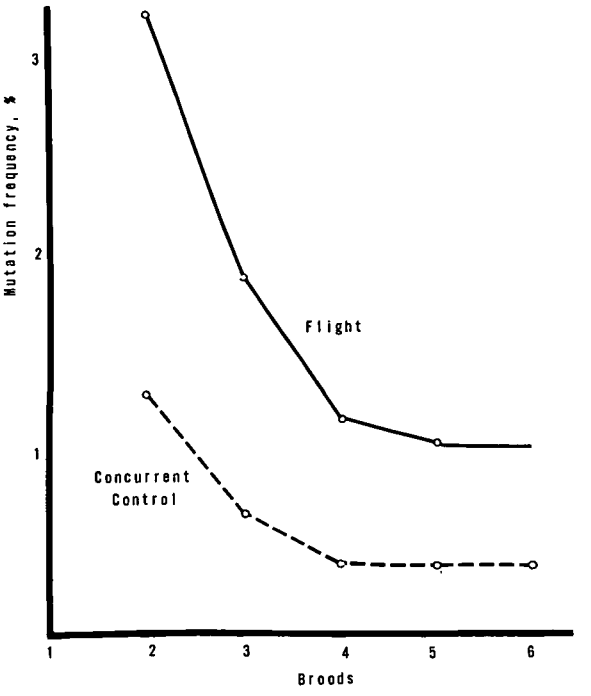

FIGURE 11.—Chromosome translocations in flight material (irradiated pupae).

TABLE 11. – Lethals and Translocations Occurring in Males in Pupal Stage

Program	Date	F_3 lethal tests					F_3 translocation tests				
		Chroms	Lethals	Weighted harmonic mean difference, %	Difference	*P*	Genomes	Trans	Weighted harmonic mean difference, %	Difference	*P*
Irradiated											
Flight	Sept. 1967	2 010	51	2.5	.....	.....	1 303	13	1.1	.....	.....
Concurrent controls	Sept. 1967	2 442	46	1.9	Not sig.	.....	2 924	26	.4	0.7	0.01
Postflight controls:											
Vibrated	May 1968	1 956	73	3.4	.....	.....	849	23	2.2	.....	.....
Not vibrated	May 1968	2 557	78	3.4	Not sig.	.....	1 161	34	4.0	1.8	.03
Unirradiated											
Flight	Sept. 1967	2 045	1	0.1	.....	.....	1 287	0	0	.....	.....
Concurrent controls	Sept. 1967	2 532	0	0	.....	.....	1 000	0	0	.....	.....
Postflight controls:											
Vibrated	May 1968	3 068	4	[a].2	.....	.....	2 001	2	.1	.....	.....
Not vibrated	May 1968	.....	.....	.....	.....	.....	1 025	0	0	.....	.....

[a] Not significantly higher than spontaneous rate.

controls, some flight factor other than vibration and acceleration must be assumed to account for the increase; or, as pointed out for the inseminated females, the simulation of vibration and acceleration in the postflight test may not have been biologically equivalent to that of flight. No differences were noted in the unirradiated groups.

TRANSLOCATIONS IN THE UNIRRADIATED MALE REPRODUCTIVE CELLS IN ALL GERM-CELL STAGES

An unexpected result was the finding that three translocations from three separate experiments had occurred in the unirradiated flight specimens in a total of 2770 tests (table 12). The spontaneous occurrence of translocation is so rare that controls are usually considered unnecessary. Among the vibrated controls two were found in a total of 2001 tests from the specimens in the pupal stage. From all vibrated Earth-based experiments, including specimens with reproductive cells undergoing maturation at the time of vibration, only those two were found, in a total of 8584 tests. For each Biosatellite experiment, some tests were run on unirradiated control specimens, such that a total of 3875 tests was accumulated in which no translocations were detected. More than 4000 translocation tests made by this laboratory as controls for other experiments have shown no translocations.

MALES GIVEN X-RAYS OF 4000 R BEFORE FLIGHT

Even though virgin males of the same age are irradiated, when they are mated individually in successive broods to fresh lots of females some will exhaust their store of the highly sensitive mature sperm sooner than others. Thus the mutation frequencies will vary from brood to brood. However, over many broods the mutations from individual males will tend to become equalized, as germ cells in all stages will have been utilized. It is therefore possible to compare directly the postflight controls with the flight and concurrent controls. As seen particularly in table 13, the mutation frequencies (or weighted harmonic means) vary, the amount depending on the groups compared.

Losses of Markers From Y Chromosomes and Visibles

Table 14 shows that no differences in frequencies of the losses of markers from the Y chromosomes and visibles were detected in the flight material when compared with the Earth-based controls.

Recessive Sex-Linked Lethals

The frequency of lethals in the flight specimens was not significantly higher than that in the concurrent Earth-based controls, but it was significantly higher than that in the postflight controls and the sum of all the controls (table 15 and fig. 12).

Translocations

Table 13 shows that an increase in translocations

TABLE 12. – Translocations Without Irradiation, All Experiments

Male germ-cell stage	Orbited			Vibrated controls			Controls	
	Genomes tested	Translocations		Genomes tested	Translocations		Genomes tested	Translocations
		2.3	Y.3		Y.2	Y.3		
Mature sperm in inseminated females	610	0	1	3 230	0	0	615	0
Spermatogonia in eggs laid during test	211	1	0	732	0	0	254	0
All stages in heterozygous males	486	0	[a]1	895	0	0	1 165	0
All stages in homozygous males	176	0	0	1 726	0	0	197	0
Maturing germ cells in pupae	1 287	0	0	2 001	[a]1	[b]1	1 644	0
Total	2 770	1	2	8 584	1	1	3 875	0

[a] Occurred in mature sperm.
[b] Occurred in spermatogonial cell.

TABLE 13. – Lack of Effect of Spaceflight on Frequencies of Losses of Markers From Y Chromosome and on Recessive Visible Mutations (Broods 1 Through 4)

[Males given 4000 R of X-rays before flight.]

Program	Date	Total males	Y losses	Weighted harmonic mean difference, %	Difference	*P*	Total	Visibles	Weighted harmonic mean difference, %	Difference
Flight	Sept. 1967	5 959	199	3.3			14 676	33	0.2	
Concurrent controls		8 003	274	3.5	Not sig.		18 899	32	.2	Not sig.
Postflight controls:		7 259	254							
Vibrated	May 1968	2 547	84	3.2			8 142	17	.2	
Not vibrated	May 1968	3 440	117	3.5	Not sig.		10 661	18	.2	
	Mar. 1968	7 264	240	3.4	Not sig.		17 282	48	.3	Not sig.

TABLE 14. – Recessive Sex-Linked Lethals Occurring in Males Given 4000 R of X-rays Before Flight (Broods 1 Through 3)

Program	Date	Tested chromosomes	Lethals	Weighted harmonic mean difference, %	Difference	*P*
Flight	Sept. 1967	1 611	151	9.3		
Concurrent controls	Sept. 1967	2 381	197	8.2	Not sig.	
Postflight controls:						
Vibrated	May 1968	1 774	130	7.4		
Not vibrated	May 1968	2 564	180	7.1	Not sig.	
	Mar. 1968	1 131	91	8.0	Not sig.	
Flight versus		1 611	151	9.6		
Vibrated controls		1 774	130	7.0	2.6	0.01
Flight versus		1 611	151	9.3		
Not vibrated controls		6 085	467	7.4	1.9	.01
Flight versus		1 611	151	9.4		
All controls		7 889	597	7.2	2.2	.003

TABLE 15. – Translocations Occurring in Males Given 4000 R of X-rays Before Flight (Broods 1 Through 5)

Program	Date	Genomes tested	Translocations					Weighted harmonic mean difference, %	Difference	*P*
			2•3	Y•2	Y•3	Y•2•3	Total			
Flight	Sept. 1967	1 941	115	29	41	17	202	10.6		
Concurrent controls	Sept. 1967	2 389	126	53	49	18	246	10.4	Not sig.	
Postflight controls:										
Vibrated	May 1968	926	76	23	19	14	132	13.9		
Not vibrated	May 1968	1 373	93	23	26	7	149	11.2	2.7	0.05
	Mar. 1968	1 495	80	30	33	7	150	10.5	3.4	.015
Flight versus								14.5		
Vibrated controls								12.8	Not sig.	
Flight versus								11.4		
Not vibrated controls								9.6	1.7	.04

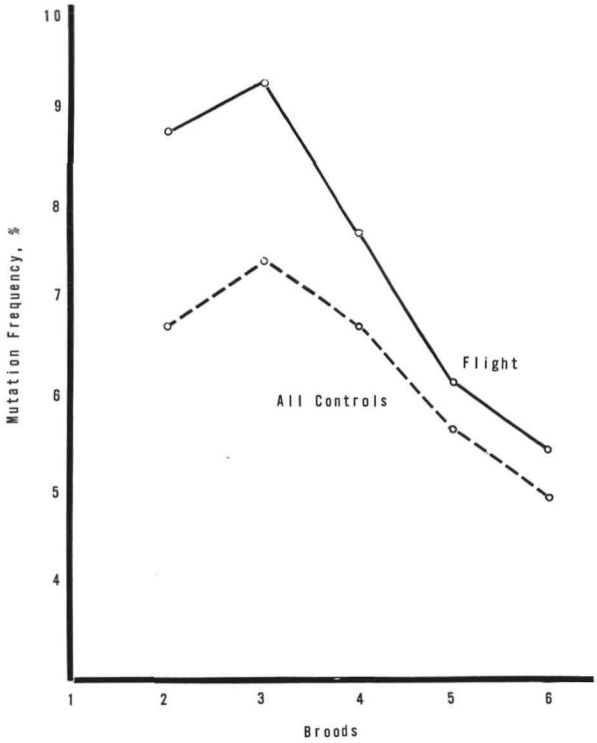

FIGURE 12.— Recessive sex-linked lethals in flight specimens (X-rayed males).

in the flight specimens was not significant when they were compared with the Earth-based concurrent controls or with the vibrated postflight controls. It was highly significant when they were compared with the sum of all the nonvibrated controls. These results are shown in figure 13.

DISCUSSION

An increase in the frequency of recessive lethal mutations occurring in irradiated mature sperm carried in inseminated females flown on Biosatellite II was of borderline significance ($P=0.05$) when compared with that of the concurrent irradiated controls. It appears that this increase can be accounted for by synergism between gamma radiation from ^{85}Sr and vibration, acceleration, or the contamination in the capsule atmosphere referred to in table 2. A similar finding has been made in the insect *Habrobracon,* specimens of which were flown on Biosatellite II.[1] This finding is of general scientific interest and warrants further investigation, pointing as it does to premutational or postmutational damage caused by the flight environment before or after chronic irradiation.

This unexpected result has led to careful reexamination of the data from the *Drosophila* experiment, with particular attention being paid to the variations between modules and between broods within a given series. A few differences have been found, but since the number of mutants found in each such case was well below 50, it is appropriate in these cases to assume a Poisson distribution of the mutants in the population of chromosomes. When this is done, the differences are obviously not significant. Thus, the totals reported in table 1 leave no doubt as to an interaction between chronic irradiation and the flight environment.

Extensive studies have been made by Lefevre (1965) in which he determined that only statistical fluctuations in the frequency of mutations are en-

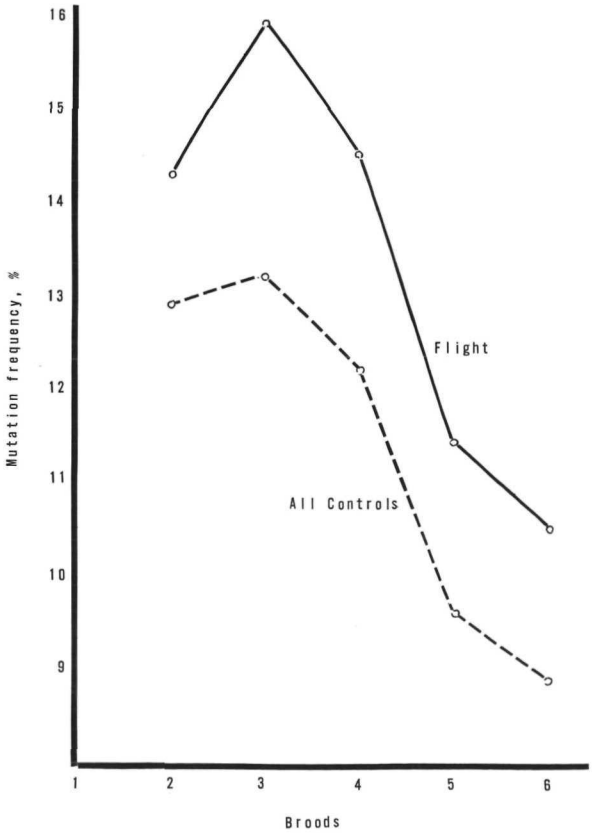

FIGURE 13.— Translocations of chromosomes in X-rayed males.

[1] Personal communication from R. C. von Borstel; cf. pp. 17.

countered. When recessive lethal mutations are detected in the paternal X chromosome of inseminated females of *Drosophila melanogaster* after 5000 R of X-ray irradiation of the females, neither the age of the males used to inseminate the females nor the length of time that the sperm had been mature before being utilized played any part in the recovered frequency.

The combined visible mutation frequency for the *dp, bw, st,* p^p, and *y* loci in the irradiated flight specimens is not significantly different from the controls, but an increase of approximately 25 percent is indicated. Although no increase in visible mutations is indicated in the vibrated group of the irradiated series, the present limited data do not rule it out, and the finding of two *dp* mutations in 16 000 flies in the vibrated group of the unirradiated series is of considerable interest. By analogy with the results of the recessive lethal experiment just described, it is conceivable that the increase in the irradiated flight specimens may be a result of acceleration and vibration. The distinction between lethal and visibles is a matter of convenience, since all gradations of viability exist among visibles. Further study of the effect of vibration and acceleration, when applied before or after irradiation, on the frequency of visible mutations can be done in Earth-based experiments. Genetic techniques are available that would allow for the detection of visible mutations at many more than the five loci studied in the Biosatellite II experiments and would enable meaningful results to be obtained from an experiment of practical proportions.

The frequency of transmissible translocations in the paternal genome in the vibrated and accelerated postflight control group of inseminated females also receiving chronic radiation was significantly lower than that in the corresponding irradiated controls or in the flight group, as shown by large-scale experiments. If one assumes that the vibration and acceleration in Earth-based controls are biologically equivalent to vibration and acceleration in flight, then the correct comparison is between the Earth-based vibrated and irradiated test performed May 1968 and the flight of Biosatellite II in September 1967 (table 6). The observed increase of the flight material, 2.929 ± 0.861 percent, is significant at the 0.0001 level. Since this experiment was conducted in the most homogeneous type of genetic material (mature sperm) the comparison of experiments conducted 8 months apart is reasonable. A more plausible explanation at this time is that the Earth-based vibrations do not match the random vibrations of launch, retrofire, and recovery. From previous experiments with the genetic test of translocation, it should be possible to continue this experiment with great precision and to determine whether the difference between flight material and Earth-based attempts to simulate flight conditions is due to the failure of simulation or weightlessness.

Theoretical studies have been made by Pollard (1965) in which he assumes that an orderly distribution of particles in a cell under the influence of unidirectional gravity on Earth is accomplished by counterbalancing forces between molecules or particles due to thermal agitation, these forces acting as buoyant forces. For bacterial cells he has shown that the difference in the number of DNA molecules per unit volume at a certain level cannot be shown to be statistically different from that of another level in the absence of a gravitational field because of the small size of bacterial cells (1 micron or 10^{-4} cm).

Our result necessitates the assumption that the sperm, most of which are suspended in a fluid medium in the storage organs of the females during flight, are of sufficient volume to allow for the detection of an effect due to absence of gravity, the dimensions being 0.28 to 0.37 micron in diameter and 7.4 to 8.8 microns in length, or about 10 times the size of bacteria. Hence, the possibility of an effect is not ruled out. The state of the DNA molecules in sperm heads is unknown, but broken chromosome fragments do not rejoin until after the sperm head has entered the egg cell after fertilization. The mechanism whereby abnormal chromosome rearrangements take place is not known, but it may be speculated that slight displacements at the molecular level might result in fewer cases of restitution in the normal configuration and also in a higher frequency of abnormal rejoining. The translocations reported here occurred in sperm cells that were used to fertilize eggs after flight.

Very unexpected was the finding, among the progeny of unirradiated but orbited inseminated females, of three translocations in 2770 tests. As is well-known, the spontaneous occurrence of translocations is very rare, and none would be expected

in 50 000 or more tests in the absence of treatment. A total of 3875 translocation tests of control specimens was made in all of the Biosatellite II experiments; no translocations were found. Over the last several years in this laboratory in connection with other experiments, no translocations have been found in over 4000 tests of untreated specimens when using the same strains of flies. These tests are sufficient to conclude that there is nothing unusual about our strains or testing procedures.

In order to show statistical significance of a frequency as low as 3 in 2770, control tests of untreated individuals almost 20 times this number (50 000) would be necessary. If no translocations were found in 50 000 tests, the lower limit of the experimental series would then be 1.08×10^{-4} and the higher limit of the controls, 1.06×10^{-4}, a significant difference being indicated ($P=0.01$). Since it was impractical and indavisable to attempt testing on this scale in the Biosatellite experiments, the results of other workers regarding the spontaneous occurrence of translocations are being utilized.

We conclude that the detection of three translocations offers evidence that spaceflight, without radiation, does produce translocations that can be transmitted to the offspring and that at least some of them may have been caused by vibration and acceleration. The three translocations came from three different inbred strains, thereby ruling out the possibility that more than one could have been of preexisting origin. Similarly, a coincidence of more than one having been caused by heavy particle radiation can be ignored.

The finding of translocations among spermatogonial cells of *Drosophila* flown in the unirradiated compartment of Biosatellite II has been reported.[2] The importance of reciprocal translocations for the human race has been pointed out by Muller (1954). One-half of the offspring of the mating of a person carrying a reciprocal translocation with a normal person will die (or be aborted) because one-half of the germ cells of the parent carrying the translocation will be genetically deficient in some genes and possess duplicates of others. In flies, where many offspring result from one mating, such matings would produce only one-half as many offspring as produced by other matings in each generation, and the translocation would eventually be eliminated from the population. In the case of humans, however, where few children are born to one couple, an unsuccessful pregnancy is usually followed by another fertilization until a viable offspring results that has a 50-percent chance of carrying the mutation. Thus the translocation may continue to spread indefinitely through the population and add to the human load of mutations.

Cytologic preparations of cerebral ganglia and cells of the larval eye disks by the method of Oster (1963) have been made for a sample of surviving fertile males showing losses of the y^+ and *B* markers from the Y chromosomes as well as certain other translocations involving the Y chromosome. The latter are more apt to give cytologic evidence for a translocation than are translocations between the second and third pairs of autosomes. Figure 14 is a photograph of the nuclei of a fertile male that has lost the *B* marker. Since all of the fertility factors which are distributed througout the Y chromosome have been preserved, the deletion of the *B* marker would be expected to be very small and not detectible cytologically. Therefore this preparation appears normal. Figure 15 shows nuclei of the son of a female in which the fragment containing the *B* marker attached to the Y chromosome has been deleted from the Y chromosome and become attached to the X chromosome. The deleted Y chromosome can readily be seen.

In figure 16 are shown nuclei of cells in which a

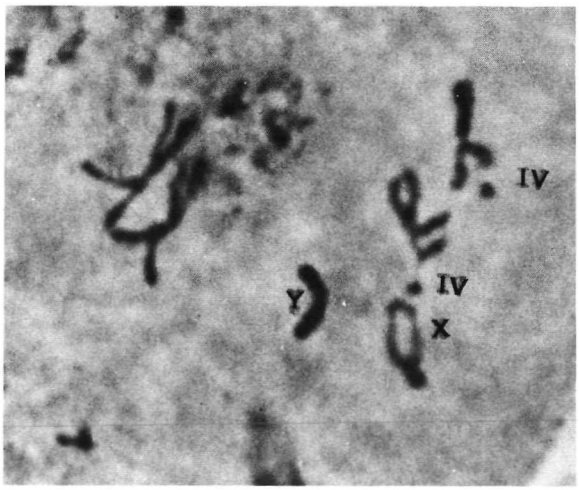

FIGURE 14.— Nuclei of a fertile male showing loss of the *B* marker.

[2] Personal communication from I. I. Oster.

translocation occurred in an irradiated male in the Biosatellite II experiments. The breeding data from the stock of this translocation, which has been maintained for many months, show that the translocation is between the Y chromosome and the second pair of autosomes. In the photograph the shorter Y chromosome (onto which a small part of the second chromosome must have become attached) can be seen as a deleted Y. Also, the attachment of a large segment of the darkly staining Y to the second chromosome can be clearly seen. Figure 17 shows nuclei of a sterile nondisjunctional male containing no Y chromosome. In figure 18 nuclei of the daughter of a nondisjunctional female in which three X chromosomes are present are shown. Figure 19 shows nuclei which contain three of the small fourth chromosomes.

Although an increase in an early developmental abnormality (missing wing and deformed thorax) was reported as occurring in the flies hatching from eggs laid in the irradiated compartment during flight, the numbers in each series (43 and 30) were not very large. The decision to include these data here is based upon the fact that in each of the five

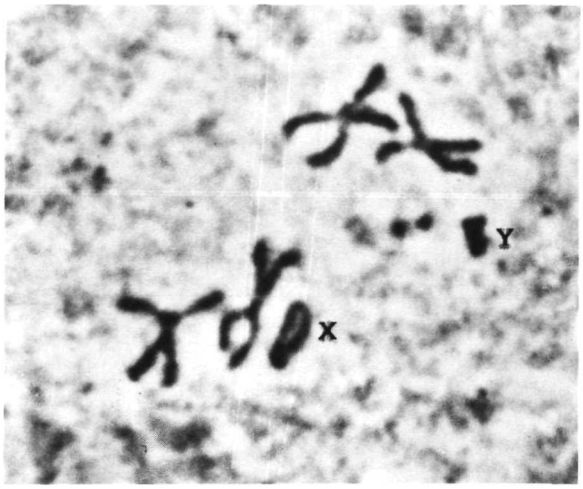

FIGURE 15.— Loss of *B* marker in Y chromosome with subsequent attachment to the X chromosome.

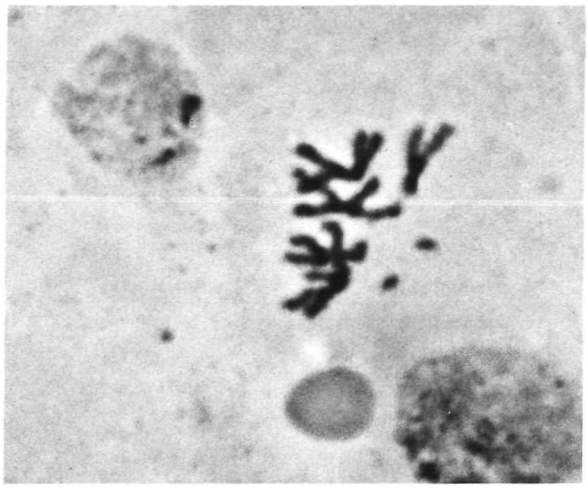

FIGURE 17.— Nondisjunction in male with missing Y chromosome.

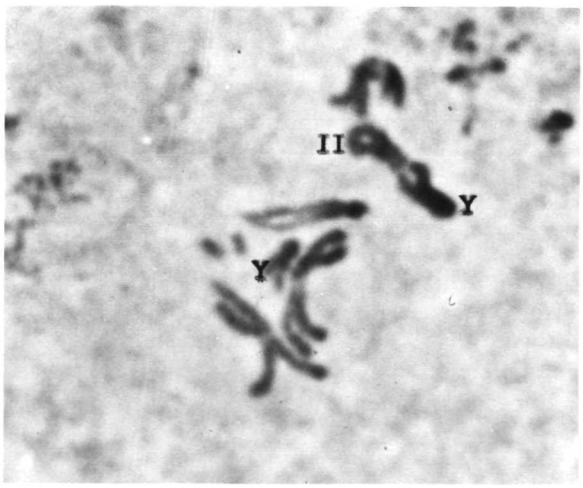

FIGURE 16.— Translocation between Y and IInd chromosomes in Biosatellite irradiated male.

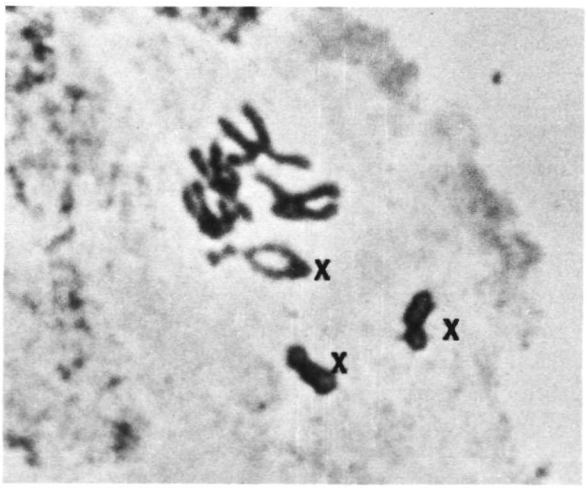

FIGURE 18.— Nondisjunction in female with extra X chromosome.

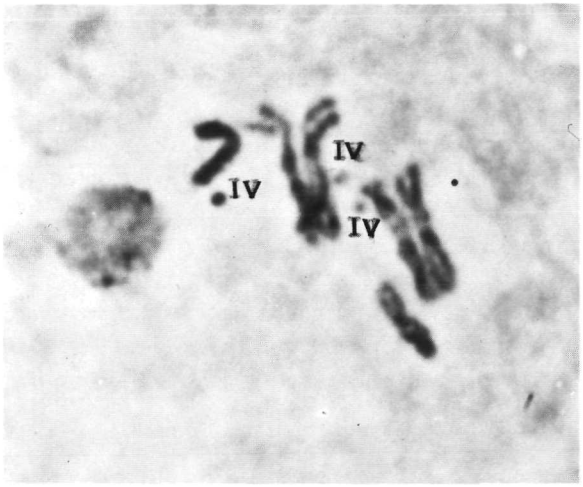

FIGURE 19.— Extra IVth chromosome.

irradiated control series tested, in which all conditions were simulated as nearly as possible as for flight, the frequency has been lower than that in the irradiated and orbited specimens. A correlation between nutritional deficiency, based on the number of flies hatching from each irradiated module, and an increase in abnormal flies sometimes was found in the controls but not always. Offspring hatching later than those shown in table 7 were produced in the Biosatellite series and in the concurrent controls, but no deformed offspring were found. The degree of crowding was greatest in the irradiated controls (1 in 540 flies per module versus 1 in 425). The frequency of developmental abnormality in the vibrated and accelerated series undergoing radiation was lower than that in the irradiated flight specimens. Nevertheless, in an original experiment of such small size, as on the Biosatellite, it might have been that by chance more eggs were laid that were in a more sensitive stage for the development of the abnormality than were laid in the irradiated controls. Therefore, we feel that insufficient evidence exists at present to conclude that an increase in this deformity is related to spaceflight coupled with radiation. Only reproducibility in a future flight would clarify the matter.

Only in instances where mature sperm in inseminated females or spermatogonial cells in very young embryos are treated can complete assurance be had of homogeneity with regard to mutagenic sensitivity of the treated material. Nevertheless, dividing cells, including those of the germ track, are of great importance. *Drosophila* pupae have been shown by Zimmering (1963) to possess reproductive cells that are very sensitive to ionizing radiation because they are rapidly undergoing meiosis. A higher frequency of losses of the y^+ and *B* markers from the Y chromosome was recovered among the offspring of the irradiated male pupae flown in Biosatellite II than was recovered from those of the irradiated concurrent controls. A similar increase was also found in the vibrated and accelerated group receiving radiation when they were compared with the corresponding postflight controls. Thus, as in the case of recessive lethals in inseminated females, the increase can be ascribed to vibration and acceleration when preceding or following chronic irradiation, or possibly to contaminated capsule atmosphere. Zimmering has shown that most of the losses of these markers from the Y chromosome represent deletions, and it is also known that a certain proportion of recessive lethals is the result of small deletions. Reproducibility in dividing cells of pupae is dependent upon careful mixing of the experimental specimens before start of the test, as was clearly seen in this material.

Translocations in irradiated pupae were increased by spaceflight but decreased by vibration when compared with those in their respective controls, thus probably ruling out any effect of atmospheric contaminants on this type of genetic change, since both tests were conducted in the capsule in which the gas was recycled. A lowering of the translocation frequency by vibration was also found in the irradiated inseminated females. Although translocations were also increased in preirradiated males from the flight when compared with those of all the nonvibrated controls, the vibrated controls showed an increase when compared with the other controls. The increase was not significant when compared with the frequency in flight specimens. In the latter experiment the vibration was acting after the fragmentation of the chromosomes. In the first two experiments (inseminated females and pupae), the vibration occurred before and after chronic irradiation. Speculation at this time as to a mechanism to explain the increase in translocations in preirradiated males and the decrease in the other two experiments would not be meaningful. To test the

reproducibility of the results would be highly desirable and of basic scientific interest.

Data from the experiments in which heterozygous and homozygous males were flown are not included in this report except for a mention of the finding of one translocation in the unirradiated flight specimens, since in no instance were any other effects of spaceflight noted. In this material in particular the low radiation dose received in Biosatellite II results in mutation frequencies lower than those in the other experiments, because the male germ cells were undergoing maturation very slowly, when compared with pupae, and contained proportionately fewer sensitive cells when compared with those of the mature sperm of the inseminated females. Nevertheless, especially important are genetic effects of spaceflight on the reproductive cells of the adults of any organism, especially when they are subjected to flights of long duration.

The genetic testing described has been undertaken on offspring from eggs laid after flight, for the most part, because of the greater abundance of such offspring. However, all the flies from the eggs laid during flight by the females that had been inseminated by the preirradiated males were scored for first-generation mutations, and second-generation tests were made for lethals and translocations. In all instances, in the flight specimens there were indications of frequencies higher than those in the nonvibrated controls, although the differences were not significant. Postflight vibration tests likewise gave indications of differences in the same direction. The number that could be tested was less than one-tenth those tested from eggs that were laid after flight. In all cases the frequencies seemed unusually high, which made it appear that this might be a very sensitive test.

The flies hatching during the first 2 days from the eggs laid during flight by the inseminated females were considered to have received most of the gamma radiation during flight. They gave differences between the flight specimens and the controls in the same directions as had been found in the offspring of inseminated females hatching from eggs laid after flight. In addition, a small sample of normal males was tested to the third generation to see whether effects in the early germ track cells of the developing eggs could be detected. As expected, clusters of mutations of common origin were recovered and none of the differences were significant. It appears that further experiments can be designed that would overcome some of the difficulties encountered in the tests of flies hatching from eggs laid during flight.

SUMMARY AND CONCLUSIONS

Genetic effects of spaceflight of 45-hour duration at about 170 miles above the Earth in the presence and absence of continuous gamma radiation of 1432 R have been measured in *Drosophila melanogaster,* with the following differences observed between flight specimens and Earth-based controls.

(1) An increase on the borderline of significance was found in the frequency of recessive lethal mutations in mature sperm when the irradiated flight material was compared with the concurrent Earth-based control material.

(2) A significant decrease in the translocation frequency in irradiated mature sperm was found in the vibrated and irradiated Earth-based controls when they were compared with either the irradiated flight material of Biosatellite II or the irradiated, but nonvibrated, Earth-based controls.

(3) A possible increase in flies with a deformed thorax and abnormal wing which hatched from eggs laid during flight in the irradiated compartment was found over flies with deformities which hatched from eggs laid by any of the flies in the combined irradiated controls.

(4) Three translocations in the unirradiated flight specimens were detected among 2770 tests. That these translocations may have been caused by vibration and acceleration was shown by the detection of two translocations in 8585 tests in the vibrated and accelerated group.

(5) In specimens flown in the pupal stage, losses of the dominant markers y^+ and *B* from the Y chromosome were found to be significantly increased by flight in the irradiated compartment over those of the irradiated concurrent controls. A significant increase of these losses in the vibrated and irradiated over those in the nonvibrated but irradiated postflight controls was also found.

(6) In irradiated specimens flown in the pupal stage, translocations between the II, III, and Y chromosomes were found to be significantly increased by flight when compared with those of the concurrent controls. In irradiated postflight tests, however, a significant decrease in translocations in the vibrated group was found when compared with

those in the concurrent nonvibrated controls. Attention is called to the similarity of this result and that described in (2) above.

(7) In males that were irradiated with 4000 R of X-rays shortly before flight, the frequencies of recessive sex-linked lethals and of translocations were not significantly higher in the flight material than in the concurrent Earth-based controls, but they were significantly higher when compared with those in the concurrent plus the postflight controls.

(8) In postflight testing of preirradiated males, translocations were significantly increased by vibration and acceleration simulating that of flight, when compared with those of the Earth-based controls not receiving vibration. However, the frequency of translocations in the flight specimens remained higher than that in the vibrated controls.

The heterogeneous nature with regard to mutagenic sensitivity of the germ cells in pupae and preirradiated males undergoing maturation makes the results from this material less conclusive than those from mature sperm. In the former, Muller's weighted harmonic means method of comparing the flight specimens with the controls has been used throughout. The great majority of tests showed no significant effect due to the flight environment. The differences found, as described above, could be attributed to vibration, acceleration, or contamination of the capsule atmsophere with formaldehyde, gluteraldehyde, and ethylene. Therefore there is no evidence that weightlessness, acting either alone or in combination, can produce genetic effects, but neither can it be excluded as an interacting agent with other factors. Additional Earth-based experiments coupled with flights of longer duration will be necessary to separate conclusively a possible interaction between weightlessness and the other factors associated with spaceflight.

ACKNOWLEDGMENTS

Acknowledgment is made of the assistance of the late Drs. Edgar Altenburg and H. J. Muller in the planning and design of these experiments, as well as of the helpful suggestions of Dr. W. R. Lee in analyzing the data. Also acknowledged is the assistance of Drs. John Tremor and John Hewitt of NASA during the preparations for launch of Biosatellite II and during the postflight control tests, as well as that of Mr. Charles Wilson, Dr. G. Dale Smith, Mr. Gary Bowman, Mr. L. Polanski, and others of NASA's administrative and engineering staff assigned to the Biosatellite Project.

Much of the credit for the successful carrying out of these experiments belongs to Charles Mylar, Ruth Montgomery, Judith Rowen, John Cargill, Joe Penson, and Arturo Torres, who acted as technical assistants on the project.

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Some Effects of Spaceflight on the Flour Beetle, *Tribolium confusum*

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At the time the Donner Laboratory team of the University of California began experimentation on the biologic effects of the space environment, it seemed important to find a biologic system that was small and light in weight, could live unattended for a period of many hours, could withstand a large unexpected excursion in environmental variables such as temperature, and had an exceedingly simple life-support system. Attention was focused on the flour beetle, *Tribolium confusum,* as the experimental object, not only because it admirably fits some of the above listed specifications, but also because it might be a prototype for the type of organism that could live in symbiosis with primitive plant life on another planet such as Mars.

Tribolium is a well-known organism often used in studies of population genetics. Its genetics has been described by Sokoloff (1966), and certain physiologic studies with this organism have been summarized (Grosch, 1962). Figure 1 shows this organism's typical stages of development—the egg, larval, pupal, and adult stages. The size of the egg is about 0.5 mm and the adult is a few millimeters longer. At 30 C, all stages of development, from fertilized egg to adult, take about 40 days. Our laboratory was initially engaged in a study of developmental abnormalities induced by radiation at various stages of this organism.[1] As with other organisms, the type of abnormality induced depends not only on the dose of radiation but also on the time the radiation was applied during the period of development. Figure 2 shows typical developmental abnormalities obtainable when vari-

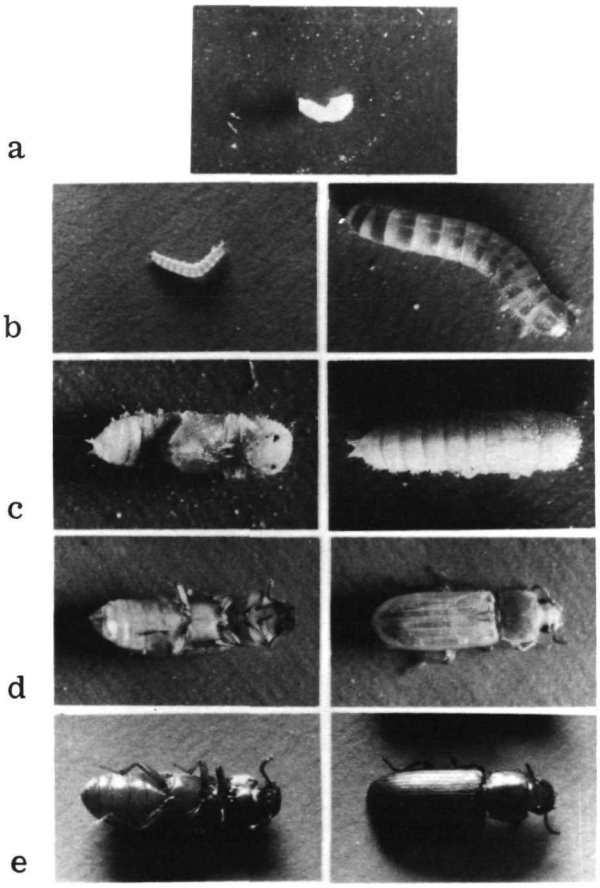

FIGURE 1.—Developmental stages of *Tribolium confusum*. (*a*) Eggs. (*b*) Larva. (*c*) Pupa. (*d*) Immature adult. (*e*) Adult.

[1] We are indebted to Professor Garth Kennington for supplying us with strains of *Tribolium confusum* Duval.

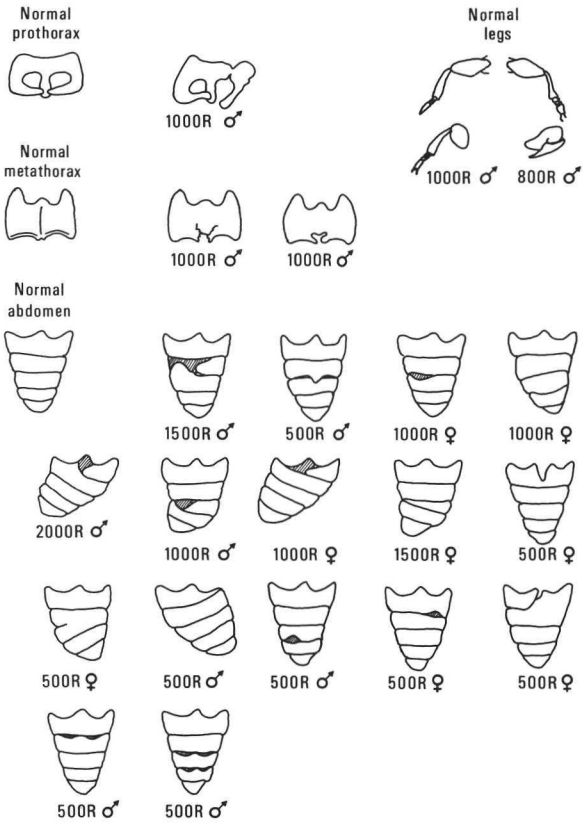

FIGURE 2.— Teratologies in *Tribolium* adults from irradiated eggs.

FIGURE 3.— Typical membraneous wing abnormalities of *Tribolium*.

ous stages of fertilized eggs are irradiated. When the pupal stage was irradiated in our laboratory (Beck 1962; 1963) it was found that the predominant abnormality produced was a morphologic, easily recognizable deformation in the size and structure of the membranous wings. The proliferation of the wings was not checked as it usually is in normal beetle development so the elytra were unable to close. This feature makes the abnormality easily recognizable. Beetles with typical deformities of the membraneous wings are shown in figure 3. Figure 4, which gives the dose-effect relationships, indicates that a low number of abnormalities (1 to 2 percent) occur spontaneously, while at high doses to the pupae, almost 100 percent of the adult animals will develop the abnormality. Scoring of the deformity is easy and reliable.

Microscopically, there is an easily discernible alteration in the structure of the membranous wing which may be seen on the development and arrangements of the spikes on the wing. Usually the spikes, which have the function of keeping the wings at an appropriate distance from the elytra, arrange themselves in a regular, hexagonal formation. Following radiation, and because of an abnormality of phosphorus and calcium metabolism, the hexagonal structure is disturbed. The development of the individual spikes also becomes subnormal, and the cells of the wing proliferate in an irregular and disorganized manner.

By use of localized beams of alpha particles accelerated in the Berkeley Heavy Ion Linear Accelerator (Slater et al., 1964) it was determined that the region most sensitive to radiation-inducing wing abnormalities is well localized in the pupae. Later studies have also shown that the effects of radiation and temperature on wing abnormalities closely parallel the effect of radiation on the eclosion process itself and on the development of the adults from the pupae. The appearance of the wing abnormality is, in a sense, an index of derangement in normal developmental processes from pupal to adult stage.

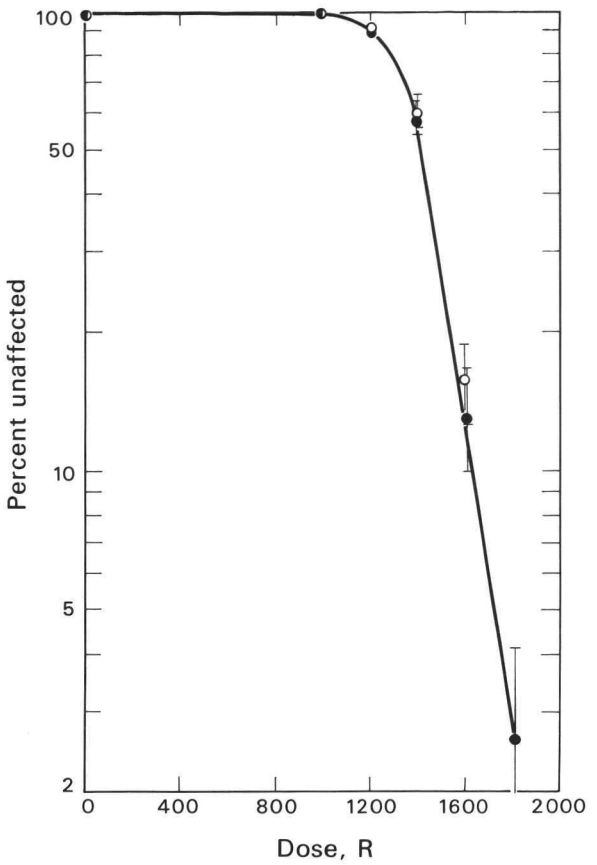

FIGURE 4.— Dose-response curve for incidence of wing abnormalities.

Several mutants are known that cause abnormal wing development. The morphologic appearance of the wing in such mutants is not materially different from those induced in the course of the development. We assumed from the start that the effects of weightlessness might be more marked on early stages of development of the beetles, perhaps immediately following fertilization, than on later stages. Nevertheless, for the Biosatellite II experiment it was determined to use pupae. The main reason for this came from the experimental conditions of the Biosatellite II flight. There was to be a period of several hours prior to launch and attainment of weightlessness. It was feasible to hold the organisms in the pupal stage sufficiently long for the prelaunch waiting period so that most of the development of the wing would take place after reaching weightlessness. It was found that, by choosing an appropriate temperature, the rate of development of the organism could be slowed down or speeded up in such a manner as to accommodate the timing for the Biosatellite II mission.

EXPERIMENT DESIGN AND METHODS

Baseline Experiments

THE ORGANISM

Stock cultures of *Tribolium confusum* Duval pupae were grown at 30 C in whole wheat flour with 2 percent yeast additive; 20 percent of this mixture was "conditioned," i.e., used for a previous culture. Prepupae, or larvae which are ready to pupate, were obtained by clearing a mixed larvae-pupae culture of all pupae and young larvae. This was done by placing a flour culture of larvae and pupae on a 12 mesh sieve and shaking flour through the sieve. The culture was allowed to remain on the sieve for several hours in a 30 C incubator, during which time young active larvae crawled through and dropped onto the flour. Inactive prepupae and pupae remained on top of the sieve. These were then placed in trays and gently sorted out with a camel's hair brush. When the efficiency of collection was tested, 91 percent of the larvae collected in this manner pupated within 50 hours, whereas less than 1 percent of the larvae which passed through the sieve pupated at this time. From these prepupae cultures, pupae were obtained at later times so that their age span could be limited. For pupal period studies and the dose-response measurements for the wing abnormality described below, 8 hours lapsed before the pupal collection, so that there was only an 8-hour age span. The pupal period was therefore considered to be from the middle of this 8-hour interval of pupation to the average eclosion time.

For age sensitivity studies, two types of experiments were done in order to test larval sensitivity as well as pupal sensitivity. For larval (or prepupal) sensitivity, prepupae were X-irradiated and observed every 3 hours subsequently for pupation. Those that had pupated were then put in separate petri dishes until they had eclosed and could thus be scored for wing abnormalities. In this manner, sensitivity as a function of time prior to pupation could be measured. For pupal sensitivity, collections were made every 3 hours for a few days and were then X-irradiated in a divided polystryrene petri dish. Pupae were then placed in dishes until

they could be scored for wing abnormalities after eclosion.

TEMPERATURE

Ambient temperature profoundly alters the rate of the process of development (Slater, et al., 1963; Buckhold and Slater, 1969). Temperature was controlled in floor-model Precision Scientific Incubators. Standard culture temperature was 30.0±0.5 C. In some experiments, cultures were maintained at temperatures ranging from 10 to 35 C for 3 days postirradiation, and then at 30 C until eclosion. Relative humidity was maintained at approximately 15 percent by means of silica gel bags in the incubators.

Pupal periods varied considerably over the temperature range studied (table 1). Spontaneous wing abnormalities also depended on the temperature. These reach a minimum between 30 and 35 C (Slater, et al., 1963). Figure 5 shows some of the results obtained.

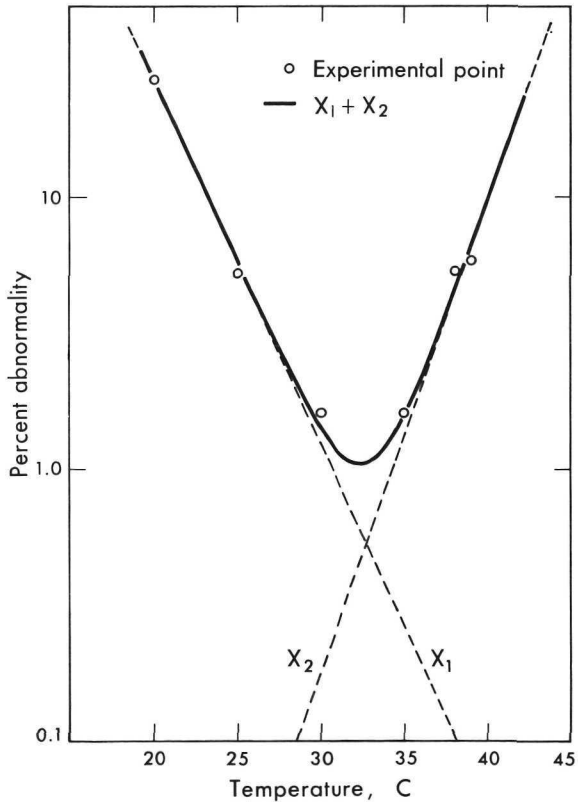

FIGURE 5.— Spontaneous wing abnormalities as a function of temperature (Celsius).

The effect of temperature on X-ray-induced wing abnormalities is shown in the nine experiments presented graphically in figure 6. In each experiment, pupae were kept at two or three separate temperatures including a control at 30 C. Thus, there were 12 separate temperatures which were compared with controls in these nine experiments. The average percent wing abnormalities in the nine controls at 30 C was 32.4±7.6 percent. Pupae were kept at the designated temperature for 3 days after irradiation with 1400 R. Because of the variation between experiments, wing abnormalities at a particular temperature have been compared with those in the 30 C group for that experiment. Minimum damage was incurred at 28 C, which contrasts with the spontaneous wing abnormalities which are at a minimum between 30 to 35 C. Spontaneous wing abnormalities (controls) for 68 percent of the 21 temperature groups (including the nine controls) were less than or

TABLE 1. — Pupal Periods at Different Temperatures

Temperature, C	Pupal period, days
20	24±1
24	[a]10.8±0.3
29	[a]5.8±0.1
34	[a]4.9±0.1

[a]Slater et al. (1963).

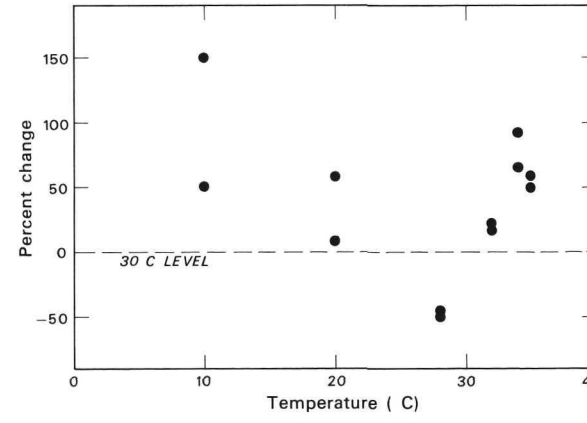

FIGURE 6.— Effect of temperature on X-ray-induced wing abnormalities. Dose, 1400 R; average percent wing abnormality at 30 C is 32.4±7.6.

equal to 2.2 percent, and 91 percent had standard deviations overlapping into this low range.

IRRADIATION

X-irradiation was done on a therapeutic X-ray unit (Phillips) at 15 mA and 180 keV with a 1.0-mm aluminum filter. The distance from the target to the specimen was 30 cm, giving a dose rate of 280 R/min. All baseline experiments, other than dose-response experiments, were done at 1400 R.

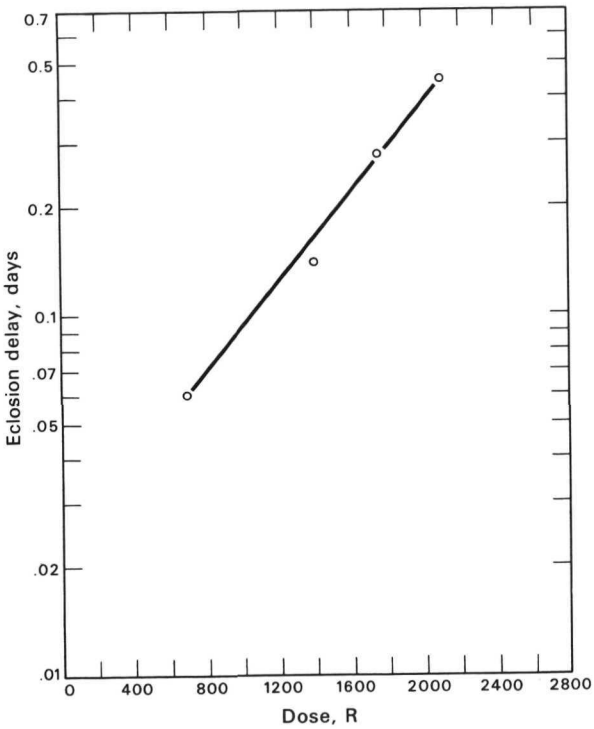

FIGURE 7.—Eclosion delay as function of dose.

Irradiation of pupae not only produced adults with wing abnormalities but also caused a change in the time rate of eclosion. A linear relationship was found between logarithmic eclosion delay and dose over a range of 700 to 2100 R (fig. 7). The large value for the y-intercept implies that a low level of irradiation may result in a perceptible delay. Preliminary results at 50 R support this idea.

Irradiation of larvae, however, did not alter the time distribution of pupation from that of unirradiated larvae; e.g., 50 percent of irradiated larvae and 46 percent of control larvae eclosed after 24 hours, and 92 percent of both sets had eclosed after 51 hours. This indicated that the method of measuring the amount of time for prepupation was valid for irradiated larvae.

The dependence of radiation response on age for wing abnormality production in X-irradiated larvae and pupae is shown in figure 8. The percentage of wing abnormalities is plotted against age prior to, and that following, pupation. Larvae were sensitive at 40 hours prepupation, but sensitivity doubled at 25 hours prepupation. Control larvae averaged 2 percent wing abnormalities. There were two peaks of pupal sensitivity occurring at 10 and 25 hours postpupation. Sensitivity had fallen off to control values after 39 hours.

The demonstrated changes in sensitivity to irradiation correlates well with cytologic studies (Beck, 1962) of wing development in *Coleoptera,* the order of which *Tribolium confusum* is a member. Although wings may start developing early in the larval stage, they develop slowly. During the prepupal stage when larvae stop feeding, the wings increase rapidly in size, largely because of rearrangements of the contents of the hypodermal cells and the entrance of haemolymph into the wing

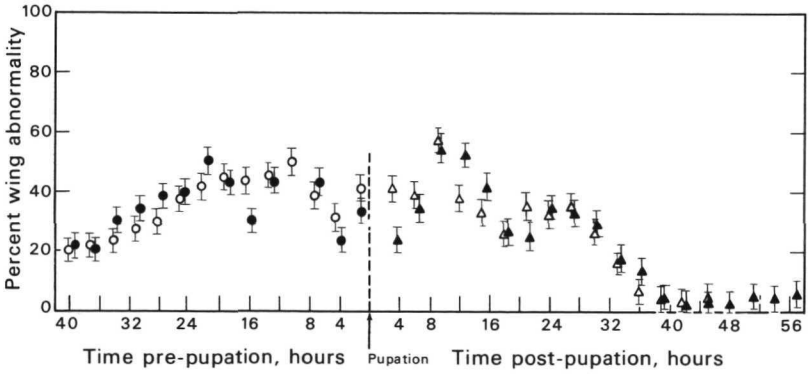

FIGURE 8.—Dependence on age of X-ray-induced wing abnormalities.

cavity. Early in the pupal stage, rods of chitinlike material are formed which offer structural support to the delicate wings and, thus, complete wing development.

The time delay observed in eclosion following pupal irradiation might correspond to the effect of radiation on the kinetics of the juvenile hormone. The results indicate that wing formation at 30 C is essentially completed by 39 hours postpupation. Increased metabolic activity early in the pupal stage might also account for the increased effect of hypothermia, at this time, on the length of the pupal stage.

The prominent shoulder of the dose-response curve (fig. 4) indicates a complex, multihit phenomenon, perhaps representative of the fact that this gross morphologic effect is observable only when many cells are hit. The sharpness of the response above 1200 R makes *Tribolium* pupae excellent biologic material for studying the modification of a radiation effect.

OTHER PARAMETERS

It has been shown (Amer, 1965) that oxygen is essential to the eclosion of *Tribolium* in amounts of 10 percent or more in the ambient gas and that it becomes toxic to the eclosion process when 40 percent or more is present. In separate studies it has also been demonstrated that the eclosion process as well as the amount of radiation-induced wing abnormalities change when a magnetic field of several-thousand-gauss intensity is present during the pupal stage.

The Biosatellite Experiment

The first question asked of this orbital experiment was, "Is there synergism between radiation and weightlessness on pupae that develop to the adult stage?" Another question was, "Are radiation effects on beetle development the same in the space environment as on Earth?" The second question concerned the course of alteration in normal development in the near weightless state. The experiment was carried out in such a manner that exposure on Earth to radiation was possible prior to the flight and an 85-strontium source of gamma rays delivered an additional dose of radiation during the flight.

In view of the limited space available in the flight capsule and the constraints of prelaunch hold time, accuracy of temperature monitoring, etc., an experiment design was chosen that allowed the four different sets of comparisons shown in table 2. Using the same batch of pupae simultaneously, the beetles were divided into two equal groups. Half remained at Earth level (E) and half participated in the flight (F). For each, there were four dose levels: (A) No dose; (B) 950 R gamma rays during 43 hours of flight; (C) 1350 R preirradiation; (D) 1350 R preirradiation and 950 R during flight. The effect of weightlessness alone should show in the difference between FA and EA. The differ-

TABLE 2. – Biosatellite II Flight and Earth Control Parameter

Sample	Pre-launch		Flight		Symbol
	Gravity	Irradiation	Gravity	Irradiation	
Earth level:					
Earth control	1 g	Zero[a]	1 g	Zero[a]	EA
Earth gamma-ray control	1 g	Zero[a]	1 g	950 R in 43 hr	EB
Earth preirrad. control	1 g	1350 R in 13 min.	1 g	Zero	EC
Earth irradiated control	1 g	1350 R in 13 min.	1 g	950 R in 43 hr	ED
Flight:					
Flight no dose	1 g	Zero[a]	Weightless[b]	Zero[a]	FA
Flight gamma-ray only	1 g	Zero[a]	Weightless[b]	950 R in 43 hr	FB
Flight preirrad. only	1 g	1350 R in 13 min.	Weightless[b]	Zero[a]	FC
Rad. and preirrad. in flight	1 g	1350 R in 13 min.	Weightless[b]	950 R in 43 hr	FD

[a]Zero, except for natural cosmic ray background and natural radioactivity.
[b]Near weightlessness, experienced in orbital flights.

ences in postradiation reactions may be observed as the difference between FB and EB. If a small dose of radiation in the weightless state caused an effect, there will be differences between FC and EC (but this is in the flat part of the response curve). If the dose given in flight caused an effect in the sensitive part of the response curve, there may be a difference between ED and FD. If some spaceflight factor affected all flown samples, then all E's should be different from all F's.

It was understood that temperature and humidity were to be monitored in flight and duplicated as closely as possible on Earth. Vibration controls performed preflight were to be retested separately in similar experiments.

The flight was curtailed at about 45 hours, instead of lasting the planned 72 hours. The prelaunch time was longer than that optimum for the organisms. We chose a temperature near optimum for the beetles during flight. It was known that this might minimize synergistic effects, since at an extreme temperature (e.g., 38 C) where the development process is already labile one would observe synergistic effects more readily.

Briefly, all results in flight were, within statistical error, the same as those on Earth, except that sample FD, the flight sample preirradiated and exposed to gamma rays in flight, exhibited more wing abnormalities with some statistical significance than did the ED, the Earth-based irradiated control.

Biosatellite II Flight

The details of the flight experiment were published previously (Slater et al., 1968 and 1969).

DESIGN

In a module in Biosatellite II (fig. 9), 720 pupae of the flour beetle, *Tribolium confusum* Duval, were orbited for 45 hours at an altitude of 196 nautical miles, half in the presence of a ^{85}Sr source and half shielded from it. All pupae were between 19 and 27 hours old at lift-off. Two of the three module compartments, the top and middle, contained groups FC and FD pupae which had been given a preirradiation dose of 1350 R of 180 keV X-rays to bring them into their sensitive dose range so that the additional radiation received during flight would contribute significantly to a major portion of the wing damage. For this X-irradiation, a portable Norelco X-ray unit was used at 4.8 mA with a 1.05-mm aluminum fllter. Pupae were essentially two layers thick in a 2-inch diameter polystyrene petri dish at a distance of 6.63 inches from the head of the unit. Under these conditions, the average dose rate was 105.4 R/min, so that the 1350 R irradiation took about 13 minutes. The profile of the beam was flat within 5 percent across the dish, at a distance of 7.0 inches from the head. Identical Earth controls were maintained at 30 C by means of strip heaters. One identical backup package was kept in a 30 C incubator. All incidents when power was turned off to the heaters, such as during gantry hookups of the capsule, were duplicated in the Earth controls and backup package.

All module components are shown in figure 10. One or two pupae were placed in each hole in the felt inserts which were cushioned between layers of tissue paper. This was necessary to minimize possible vibration effects during launch and recovery. Four precalibrated LiF disk dosimeters (fig. 9) were inserted between the two layers of pupae in each compartment. These were read by Isotopes, Inc., Cambridge. Four LiF tube dosimeters recorded the dose in the front and rear of the module. Temperature varied only slightly throughout the flight, the aft temperature being 84.8±0.4 F. Earth control temperatures were slightly higher, unirradiated ones (the aft) being 85.0±0.0 F and the radiation-exposed ones (forward) being 86.4±0.6 F. Relative humidity was maintained at a desirable low

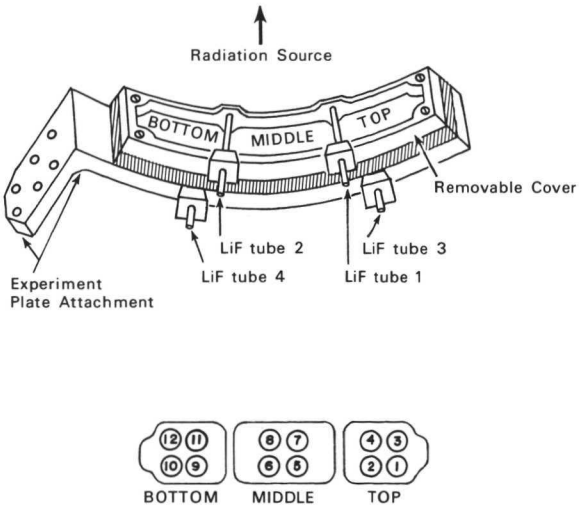

FIGURE 9.— Biosatellite II *Tribolium* module.

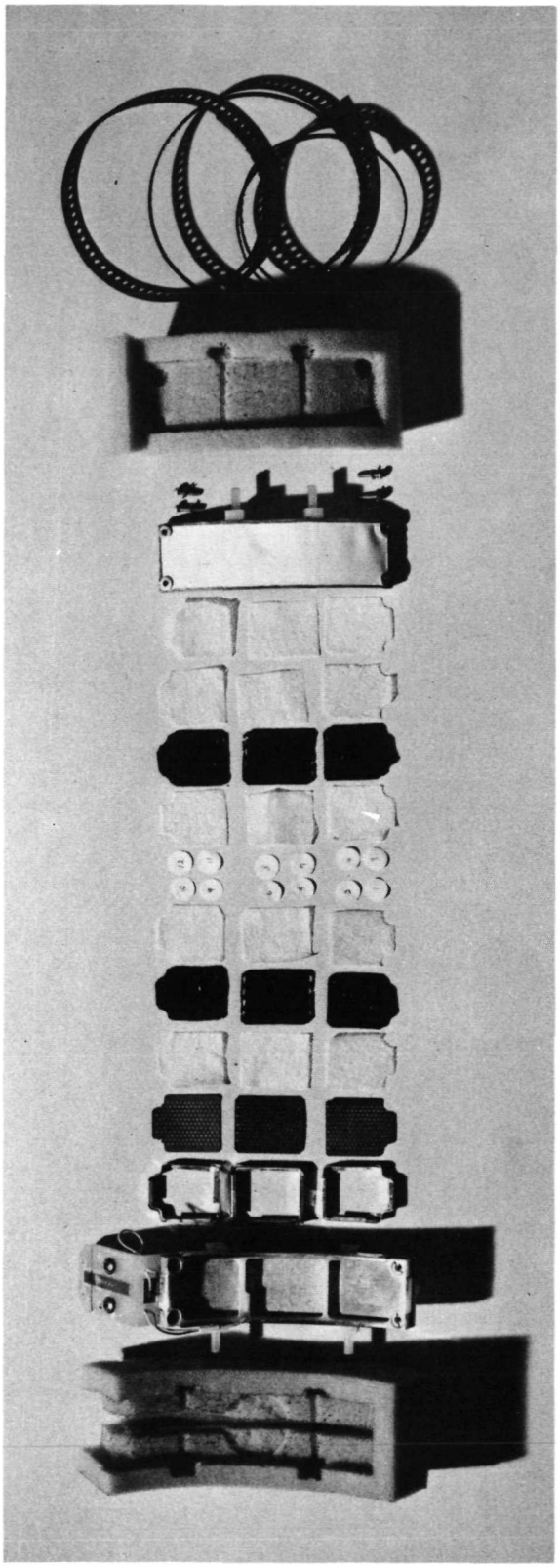

FIGURE 10.—*Tribolium* module components. From the top: (1) nylon straps to attach insulation; (2) foam insulation; (3) screws for module; (4) module top; (5) two tissue papers (6) felt pupae holders; (7) tissue paper; (8) LiF dosimeters; (9) tissue paper; (10) felt pupae holders; (11) tissue paper; (12) cardboard support; (13) aluminum compartment edges; (14) module bottom; and (15) foam insulation.

level of 58 to 60 percent for this experiment throughout the flight. Temperature excursions for flight and Earth control modules prior to launch and during deorbit are shown in figure 11.

DOSIMETRY

The LiF disk dosimetry on the forward packages (table 3) agreed very well with the LiF tube dosimetry observed at the NASA Ames Research Center (table 4), except for the bottom module compartment closest to the experiment plate. In the flight package, this compartment apparently received a dose 23 percent lower than that expected. The analogous Earth control compartment did not show this. These compartments housed only pupae which were not preirradiated (Group FB); therefore, no significant differences were found in these groups, as was expected from the low total irradia-

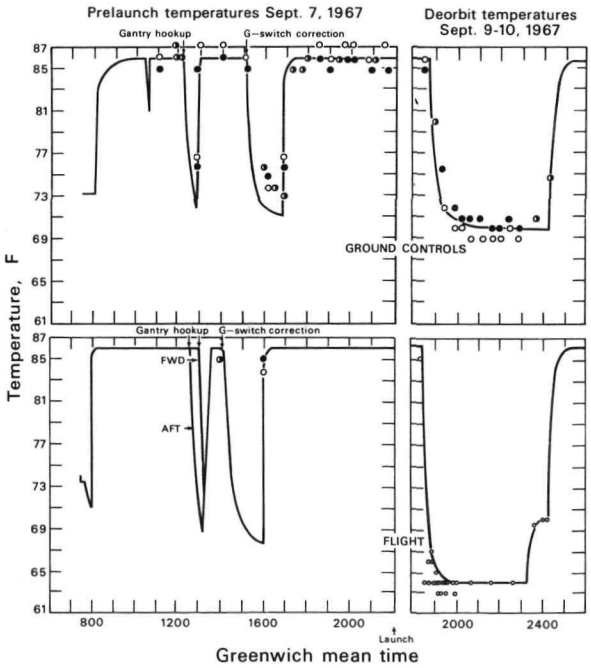

FIGURE 11.—Biosatellite II flight and Earth control temperature excursions.

tion dose during this sensitive pupal stage. Forward compartment pupae received about 950 R during the 43 hours that the capsule radiation source was open. The extensive postflight dosimetry tests at Ames Research Center showed that dosages were matched between capsules to within 0.2 percent for the *Tribolium* module. A gradient existed for both modules with the top compartment receiving a 10 percent greater dose than the bottom compartment. This does not affect the experimental results since, in all cases, comparable groups were in the same compartments of flight and Earth modules.

RESULTS AND DISCUSSION

Flight Results

As an overall picture of the effect of the spaceflight, one can look at the percentage of molting failures, i.e., cases where eclosion has begun but the pupal skin has not been shed completely, and at the percentage of pupal deaths (table 5). There were no meaningful differences between the different sample groups with respect to these two biologic endpoints.

The eclosion data appear to be quite scattered if one examines the pupal period as a function of the total radiation dose received (table 6). However, one can correct all pupal periods to those which would have been obtained were all groups at 86 F by making use of the known relationship between pupal period and temperature (Buckhold and Slater, 1969). When this was done, the differences between flight and Earth control pupal periods were found to be quite similar, the flight being about 1.5 hours longer than the Earth controls. This difference between flight and Earth controls is significant to the 5 percent level when

TABLE 3. – LiF Disk Dosimetry on Biosatellite II

Disk	Dose, R			
	Flight forward	Flight aft	Earth forward	Earth aft
1	1015	<3	930	<3
2	959	<3	947	<3
3	970	<3	924	<3
4	954	<3	924	<3
5	901	<3	973	<3
6	1003	<3	921	<3
7	994	<3	942	<3
8	954	<3	937	<3
9	723	<3	1194	<3
10	721	<3	1118	<3
11	778	<3	887	<3
12	744	<3	929	<3

TABLE 4. – LiF Tube Dosimetry on Biosatellite II Postflight Tests at NASA Ames Research Center

Tube	Dose, R			
	Flight forward	Flight aft	Earth forward	Earth aft
1	1080	0.42	1070	0.48
2	1060	.45	1030	.49
3	775	.33	770	.45
4	750	.36	745	.47

TABLE 5. – Molting Failure and Pupal Deaths on Biosatellite II

Symbol	Sample	Molting failure, %	Pupal deaths, %
FD	Flight forward, preirrad	1.25	0.83
FB	Flight forward, not irrad	1.67	.00
FC	Flight aft, preirrad	.41	.41
FA	Flight aft, not irrad	.85	.00
ED	Earth forward, preirrad	.00	.83
EB	Earth forward, not irrad	.00	.00
EC	Earth aft, preirrad	.84	1.67
EA	Earth aft, not irrad	.00	1.74
	Backup incubated, preirrad	.84	.00
	Backup incubated, not irrad	3.36	.00

TABLE 6. – Pupal Periods Given by Raw Data and Temperature-Corrected Data[a] From Biosatellite II

Sample	Pupal period, days			
	Preirrad. (1350 R)		Not preirrad.	
	Aft	Forward (+~950 R)	Aft	Forward (+~950 R)
Raw data:				
Backup	6.56		6.38	
Earth	6.67	6.60	6.60	6.48
Flight	6.78	6.82	6.69	6.78
Corrected to 86.0 F:				
Earth	6.59	6.63	6.52	6.51
Flight	6.68	6.65	6.59	6.61

[a] Corrected during flight only (45 hours total).

an analysis of variance is done treating all flight samples as one treatment and Earth controls as another. Random block design (Steel and Torrie, 1960) was used to compare, pairwise, the various radiation conditions. A 2-hour delay due to the X-irradiation (Buckhold and Slater, 1969) is apparent in both flight and Earth control samples, but the long-term gamma-irradiation (~950 R) did not cause an eclosion delay, either alone or superimposed on the preirradiation X-ray dose.

Measurements of percent wing abnormality have demonstrated one important difference between flight and Earth control beetles (table 7). Flight adults, obtained from the preirradiated pupae which also received the chronic ^{85}Sr dose, had 44.8 percent wing abnormalities in comparison with Earth control adults which developed only 29.9 percent abnormalities. When an analysis of variance and comparison by means of orthogonal contrasts (Steel and Torrie, 1960) was done, this increase in damage to flight beetles was found to be significant to the 2.5 percent level.

The data on wing abnormalities are also plotted in figure 12 along with probable errors for each point. It is clear that gamma radiation in flight had more effect than did gamma radiation on Earth.

Postflight Tests for Wing Abnormality

Two sets of postflight tests were held several months after completion of the flight. In the first of these, the experimental animals were placed

TABLE 7. – Wing Abnormality Results for *Tribolium Confusum* From Biosatellite II

Symbol	Condition	Temp., F, in flight	Preirrad.	Dose, R / Time, hr	Irrad. during flight	Dose, R / Time, hr	Total dose, R	Flight vibration and acceleration	Sample size, N	Wing abnormalities, %	99% confidence level	P
FD	Flight	84.0±0.2	Yes	1350/0.2	Yes	969/43	2319	Yes	240	44.8±3.2	36 to 53	To 0.025
ED	Earth control	86.4±0.6	Yes	1350/0.2	Yes	942/43	2292	No	240	29.9±3.0	23 to 37	
FB	Flight	84.0±0.2	No	0	Yes	755/43	755	Yes	120	2.6±1.4	Not significant	Not significant
EB	Earth control	86.4±0.6	No	0	Yes	1030/43	1030	No	120	1.7±1.2	Not significant	
FC	Flight	84.8±0.4	Yes	1350/0.2	No	<3	1350	Yes	240	19.0±2.5	13 to 26	Not significant
EC	Earth control	85.0±0.0	Yes	1350/0.2	No	<3	1350	No	240	16.8±2.4	11 to 24	
FA	Flight	84.8±0.4	No	0	No	<3	<3	Yes	120	4.3±1.8	1 to 12	Not significant
EA	Earth control	85.0±0.0	No	0	No	<3	<3	No	120	3.7±1.7	Not significant	

in a spare space capsule to observe whether unexpected differences between the capsules could affect the biologic material. Everything was done as precisely as for the Biosatellite II Earth control. This was the "capsule comparison test" performed in March 1968. The second test was carried out in May 1968. No special capsule was provided but the test samples were subject to the vibration and vibrated acceleration profile of Biosatellite II. This is the "vibration-acceleration test."

Table 8 and figure 13 have wing abnormality data from the vibration-acceleration test. As seen from a comparison of appropriate test and control samples in each class, there is agreement between vibrated and nonvibrated samples. Numerically, the vibrated irradiated groups had fewer wing abnormalities than did the appropriate controls, whereas in Biosatellite II the flight-irradiated sample had more wing abnormalities than did the Earth controls.

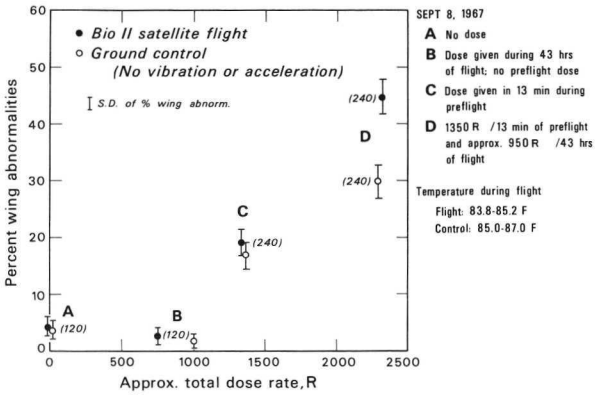

FIGURE 12.— *Tribolium* wing abnormalities in Biosatellite II flight and Earth controls.

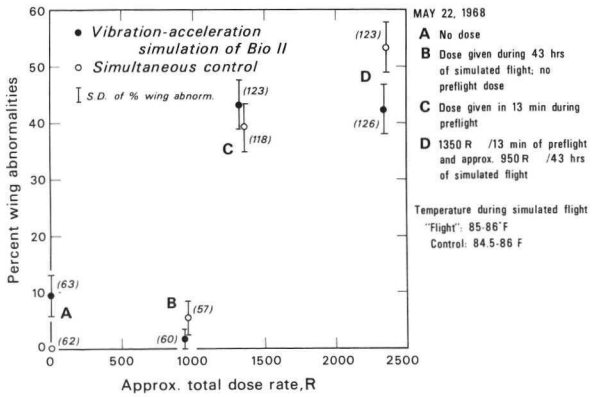

FIGURE 13.—*Tribolium* wing abnormalities in vibration and acceleration postflight test.

Results of the capsule comparison test also indicate that none of the comparable groups differ with statistical significance. Thus, in no instance have vibrated samples at Earth level shown greater percentage wing abnormalities than did the controls. From this result we are inclined to doubt that vibration was the cause of the difference in wing abnormalities between the flight and Earth control samples in Biosatellite II.

The absolute wing abnormality ratios obtained in the flight experiment and, later, in the capsule control and vibration control are not the same for the same dose. It is our contention that such data should be compared only if the experiments were done on the same day and used the same batch of organisms. We know that quite precise dose-effect relationships are obtained on a given day.

Some of the factors that may cause variations in radiosensitivity are: (1) Long term, perhaps annual, rhythms in sensitivity; (2) the time of day when irradiation is performed; (3) population density of the parent sample; and (4) geographic location. The flight data were obtained on populations that became acclimatized to Florida for 2 weeks in September. The capsule test was carried out in March, and the vibration-acceleration test in May, near San Francisco.

We have now analyzed some of the factors involved. There is indeed a variation in percentage wing abnormalities depending on the time of the day when radiation is carried out. In California, radiation exposure carried out at 9 to 10 a.m. shows little scatter, whereas tests at other times of the day show large scatter. By controlling the daily light-dark cycle in the incubator and the time of pupation and irradiation, we have obtained survival curves with good reproducibility at various times during the year.

Genetic Tests

A number of females and males returning from the flight were mated with incubated Earth controls in order to determine the number of induced dominant lethals. It was found that in the same doubly irradiated flight group in which the wing

TABLE 8. – Wing Abnormality Results for *Tribolium confusum* Postflight Vibration-Acceleration Test

Condition	Temp., F in flight	Preirrad.	Dose, R / Time, hr	Irrad. during flight	Dose, R / Time, hr	Total dose, R	Vibration and acceleration	Sample size, N	Wing abnormalities, %	99% confidence level	P
Vibration-acceleration test	85.1	Yes	1350/0.2	Yes	988/43	2338	Yes	126	42.4±4.4	31 to 54	Not significant
Vibration-acceleration control	85.9	Yes	1350/0.2	Yes	1014/43	2364	No	123	53.3±4.5	41 to 65	
Vibration-acceleration test	85.1	No	0	Yes	950/43	950	Yes	60	1.7±1.7	Not significant	Not significant
Vibration-acceleration control	85.9	No	0	Yes	960/43	960(?)	No	57	5.5±3.0	Not significant	
Vibration-acceleration test	85.9	Yes	1350/0.2	No	<3	1350	Yes	123	43.2±4.5	31 to 55	Not significant
Vibration-acceleration control	84.6	Yes	1350/0.2	No	<3	1350	No	118	39.4±4.5	28 to 52	
Vibration-acceleration test	85.9	No	0	No	<3	<3	Yes	63	9.5±3.7	2 to 24	Not significant
Vibration acceleration control	84.6	No	0	No	<3	<3	No	62	0.0	Not significant	

abnormality was increased females laid eggs having a higher percent of dominant lethality than did those in the corresponding Earth control; i.e., there was 78±6 percent dominant lethality for flight females compared with 27±5 percent for Earth control females (table 9). When an analysis of variance (Cochran, 1943) with orthogonal contrasts is done (Gabriel, 1963), this result is statistically significant to the 2.5 percent level. Less data are available on these two groups than on others because of two other effects which may be physiologic effects on the parent female receiving both irradiation doses. First, approximately 60 percent of these females laid no viable eggs compared with an average of 13 percent laying no viable eggs in all other groups. Also, those females which did lay viable eggs in these two groups produced only 50 percent as many eggs as did the control females. The usual difference between preirradiated and nonirradiated males and females was obvious; therefore, groups were compared both within sex classes and also within preirradiation treatment classes. Neither the preirradiated nor the nonpreirradiated males showed any other significant differences due to the flight or ^{85}Sr irradiation. On the other hand, ^{85}Sr irradiation increased dominant lethality for both preirradiated and nonpreirradiated females. No significant differences between flight and Earth controls for females of both preirradiated classes were found other than in the above-mentioned doubly irradiated samples.

In the postflight capsule comparison test, dosimetry again agreed closely for the two capsules with dosages differing by no more than 1 percent of each other. The dominant lethality results were rather ambiguous, since the experimental females from either capsule yielded very few offspring when crossed with incubator control males. The reverse cross of experimental capsule males with incubator control females showed extreme variability in the results from different matings with many instances of 0-percent hatchability. It was quite clear that more crosses should have been made for this portion of the test.

In the vibration-acceleration test, dosages of the vibrated capsule and control capsule differed by less than 1 percent. More matings were done in this test than in the capsule comparison test, and the results for dominant lethality were much more extensive, although all crosses with experimental females yielded fewer eggs than the reciprocal crosses. No significant differences were found for either offspring of matings between experimental males and control females or for offspring of the reciprocal mating (table 10).

SUMMARY AND CONCLUSIONS

Development of *Tribolium* pupae has apparently proceeded in a normal manner in spite of the stress of *g* forces and vibration and of about 45 hours in a nonirradiated weightless condition. The number of wing abnormalities induced in preirradiated beetles by additional gamma irradiation while under weightlessness was, however, greater at a

TABLE 9. – Percentage of Dominant Lethals in F_1 From Biosatellite II

Parent sample[a]	Dominant lethals, %[b]			
	Preirrad. (1350 R)		Not preirrad.	
	Aft	Forward (+~950 R)	Aft	Forward (+~950 R)
Backup females	20.5 (278)	.....	8.0 (196)	.....
Backup males	35.1 (310)	.....	14.0 (136)	.....
Earth females	13.0 (108)	26.7 (75)	0.0 (197)	47.6 (187)
Earth males	27.6 (246)	38.1 (341)	0.0 (177)	8.3 (169)
Flight females	30.1 (252)	78.5 (42)	19.7 (230)	22.8 (181)
Flight males	38.5 (343)	38.2 (317)	4.6 (240)	11.4 (232)

[a] Each sample crossed with incubator controls.
[b] Number of eggs scored for hatchability in parentheses.

borderline significance level (2.5 percent) than was the number of those induced under comparable conditions on Earth. The authors feel that Earth control experiments exclude vibration as a cause for this. Assuming that the finding was not a statistical fluctuation and that it represents a real interaction of radiation effects with weightlessness, we may search for a possible explanation and for methods whereby the effect can be further studied.

We cannot assume that weightlessness interferes with the number of primary molecular radiation lesions. Therefore, we should look for an explanation that relates to repair processes or to the physiologic state of the organism during exposure to radiation. The existence of a repair process in *Tribolium* can be studied by the split-dose technique. The effect of a given dose of irradiation depends on the time of administration of partial dose installments. This process is being studied in some detail in our laboratory. An example is shown in figure 14. The number of wing abnormalities are shown as a function of the time interval between two dose installments of 900 R each, which make at total of 1800 R. Radiation is most efficient if both installments are delivered at the same time.

Since the first dose (preirradiation dose) may disturb and delay the cell divisions that occur and may cause a delay in eclosion, a change of radiosensitivity due to the alteration of physiologic state is part of the explanation. However, this does not explain the observed effects fully, and it is likely that genuine postirradiation DNA repair, and perhaps rejoining of chromosomes, takes place as in other organisms. Such repair processes depend on metabolism and use of repair enzymes. It seems conceivable that repair processes function less efficiently in the weightless state than at normal gravity; this would account for the higher percent of wing abnormalities observed after spaceflight.

There appeared to be a greater number of dominant lethals induced in females receiving preirradiation on Earth and gamma irradiation

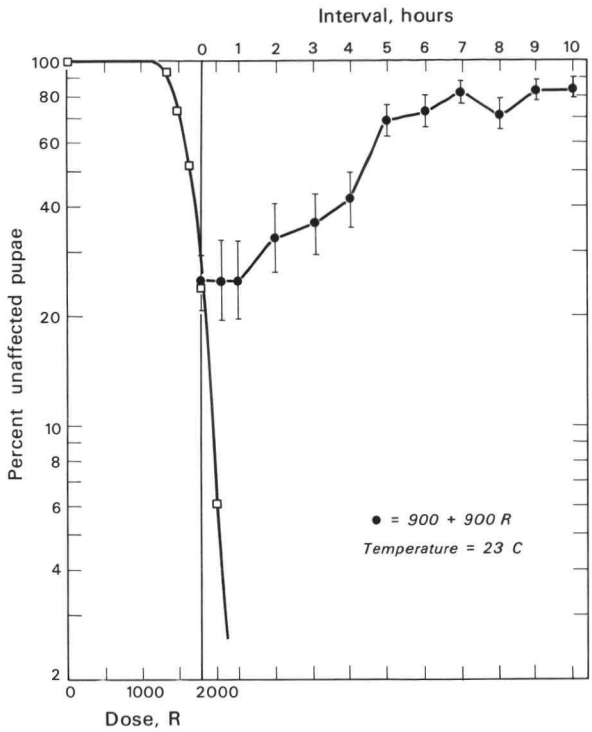

FIGURE 14.— Split-dose response for induced wing abnormalities.

TABLE 10. — Percentage of Dominant Lethals in F_1 From Vibration-Acceleration Test

Parent sample	Dominant lethals, %[a]	
	Forward (~950 R)+preirrad. (1350 R)	Aft not preirrad.
Control:		
Females	86.6 (127)	19.6 (204)
Males	61.3 (862)	27.6 (507)
Vibrated and accelerated:		
Females	79.8 (84)	
Males	59.0 (874)	

[a] Number of eggs scored for hatchability in parentheses.

in weightlessness, in the same qualitative direction as the wing abnormalities. Vibration controls did not seem to explain this effect either; however, because of the relatively small numbers, the effect has borderline significance only. It is interesting to note that 60 percent of the flight females produced no eggs at all in the particular group.

Several avenues of research suggest themselves.

(1) The isolation of repairless mutants might be useful in future tests of the effects of radiation and weightlessness, particularly since these may pinpoint the mechanisms involved.

(2) The present work, as well as that of others, appears to suggest that development of animals has considerable internal compensation for changes in the gravitational field. Since plants usually relate themselves to an existing gravitational environment, it becomes of interest to search for gravity-compensating mechanisms in animals. Some of the answers might be in molecular specificity of cell surfaces and in cell-to-cell communication. Radiation appears to be an interesting tool to use in this connection since irradiated organisms often lose sense of their organization and, in some instances, their response to gravity is lost. It is particularly clear that radiation-induced neoplasms often show unorganized growth independent of gravity.

(3) It seems important to expose organisms to weightlessness for a much more extended time than has been possible so far. Studies for a whole population of *Tribolium* over a number of generations seem entirely feasible.

(4) Another approach to this problem would be to allow organisms to develop during weightlessness while under additional environmental stresses, as, e.g., those of temperature and atmospheric conditions.

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Experiments With Plants

Radiobiologic Studies of *Tradescantia* Plants Orbited in Biosatellite II

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The Biosatellite II *Tradescantia* experiment was designed to determine the effect of weightlessness and other spacecraft environmental conditions on spontaneous and radiation-induced somatic mutation rates and on selected cytologic changes. The plan was to compare data obtained from irradiated and nonirradiated plants flown in Biosatellite II with data from nonflight irradiated and nonirradiated plants maintained under conditions essentially similar to those of the flight material. Preliminary, as well as detailed, reports on these comparisons have been published (Marimuthu et al., 1970a and b; Schairer et al., 1970; Sparrow et al., 1968).

The Biosatellite experiments, in general, were undertaken with three objectives in mind: First, the study of possible interactions in orbit between irradiation and spacecraft environmental stresses would contribute to our basic understanding of the effects of ionizing radiation on biologic systems; second, that the unirradiated plants in the satellite would offer an opportunity to study the effects of weightlessness on various biologic processes; third, that information on genetic or cellular damage would contribute to a better assessment of the possible hazards to living systems of a new spectrum of stresses encountered during orbital flight.

EXPERIMENT DESIGN AND METHODS

Biologic Material

The plant used in the Brookhaven National Laboratory (BNL) experiment was a special clone (02) of *Tradescantia* (fig. 1) that was heterozygous for flower color and hence useful for easy detection of somatic mutations. These plants are characterized by 12 large somatic chromosomes that are well suited for detailed cytologic analyses. Flowering axillary cuttings are readily obtained and easily rooted, thus providing both root tip and floral tissues for study. When cuttings are placed in nutrient tubes, they form small, light, compact units suitable for use in the confines of a crowded Biosatellite.

The stamen hair system in *Tradescantia* has been used by several investigators to study both somatic mutation rates and loss of reproductive integrity and is described in detail elsewhere (Alvarez and Sparrow, 1965; Davies, 1963; Ichikawa and Sparrow, 1967a, 1967b, and 1968; Ichikawa et al., 1969; Mericle and Mericle, 1967; Nayar and Sparrow, 1967). The hairs develop in acropetal succession from the base of the filament to the top. They increase in length primarily by successive divisions of terminal and subterminal cells, but all cell division in the hairs stops about 4 or 5 days prior to anthesis. At maturity each of the six stamens per flower normally possesses about 50 hairs, but the number is usually smaller in older inflorescences. In normal untreated plants the hairs are shorter at the apex of the filament than at the base and range from 16 to 21 cells in length, but the number of cells per hair varies widely in irradiated material.

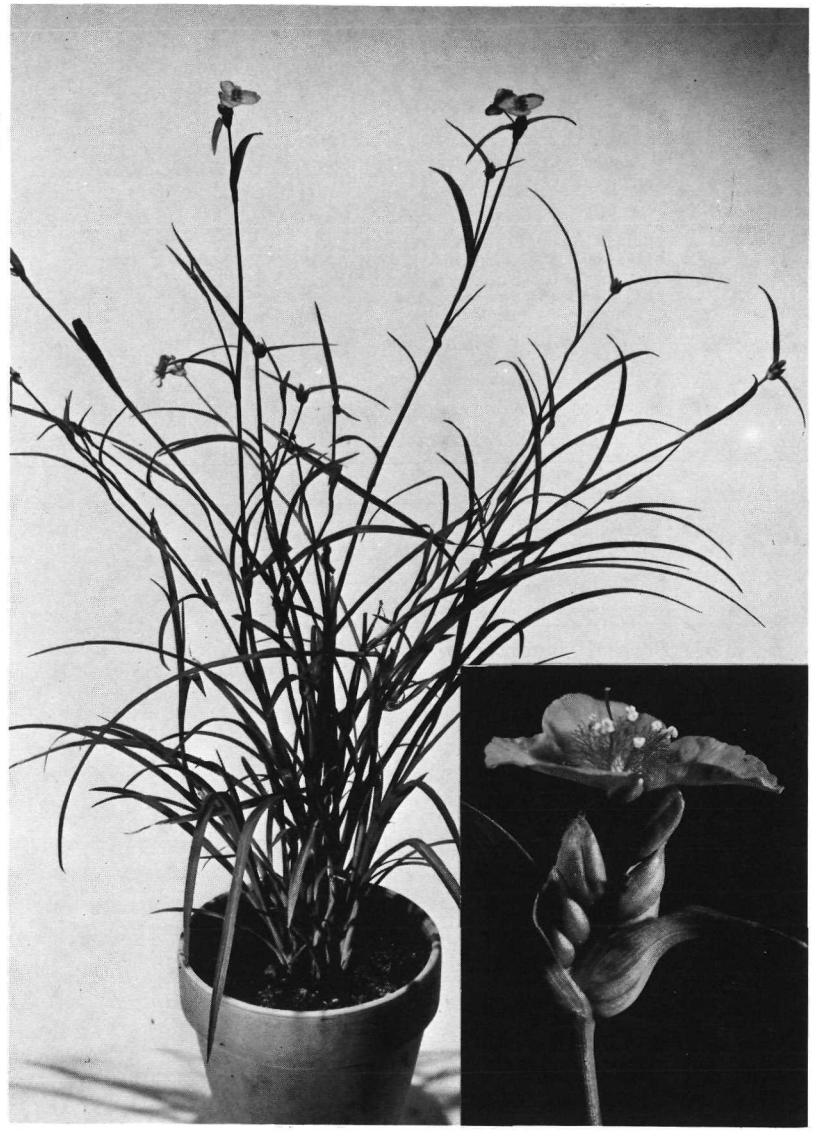

FIGURE 1.— Mature plant of *Tradescantia* clone (02) with several flower stalks at a suitable stage for treatment. The young inflorescence (lower right) shows the arrangement of flower buds at different stages of development from premeiosis (within brackets) to anthesis.

Each mature inflorescence has a complement of buds covering stages from premeiotic to mature pollen in the anthers and premeiotic to mature embryo sacs in the ovaries (figs. 1 and 2). The various stages of bud development have the following designations: (1) A–4, buds at 4 to 6 days before anthesis, a stage at which the microspore has a large vacuole and a late interphase or early prophase nucleus (Sax and Edmonds, 1933). (2) A–3, a stage with microspores in various stages of mitotic division or just past division (fig. 2 *(a)*). Any deviation from the normal orientation of the chromosomes at anaphase through late telophase in A–3 buds is thought to reflect a disturbance of normal spindle behavior. (3) A–2, the stage with one of the daughter nuclei near the heavy dorsal wall elongating and differentiating to form the generative nucleus. (4) A–1, the elongation of the generative nucleus is complete and the bud will open the next day (fig. 2 *(e)*). (5) A, the bud is open (anthesis).

In *Tradescantia,* the ovary is tricarpallary and syncarpous and contains six orthotropous ovules. The embryo sac is monosporic and 8-nucleate (fig. 3) and is similar to that of the *Polygonum* type (Maheshwari, 1950). A single megaspore mother cell divides meiotically about 12 to 17 days prior to anthesis to form four haploid megaspores. Only

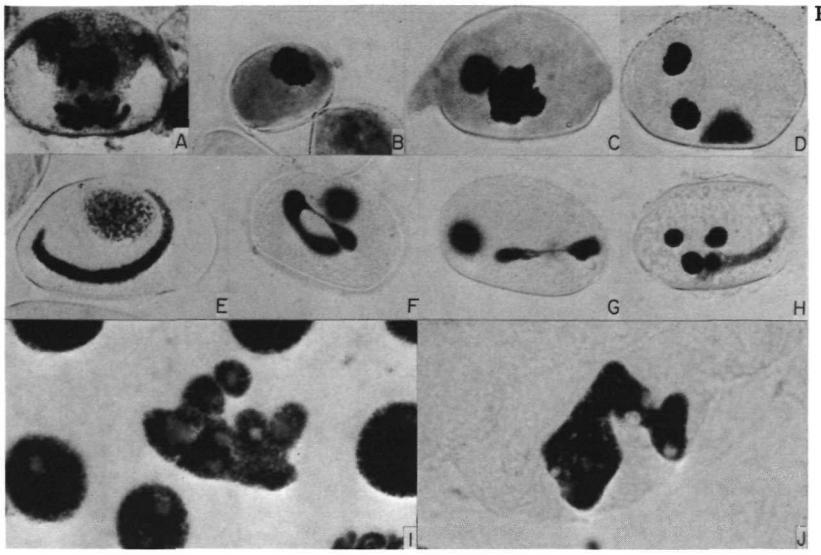

FIGURE 2.— *Tradescantia* microspores, pollen, and root tip cells from the Biosatellite II experiment. (*a*) Normal microspore anaphase from nonflight control. (*b*) to (*d*) Abnormal microspore mitosis from flight control. (*e*) Normal pollen from nonflight control. (*f*) to (*h*) Abnormal pollen from flight control sample. (*i*) Root tip cell showing effects of spindle malfunction; multinucleate cell. (*j*) Root tip cell showing effects of spindle malfunction; lobed nucleus suggests the position of scattered chromosomes. Magnifications: (*a*) to (*h*), 550 ×; (*i*) and (*j*), 1000 ×.

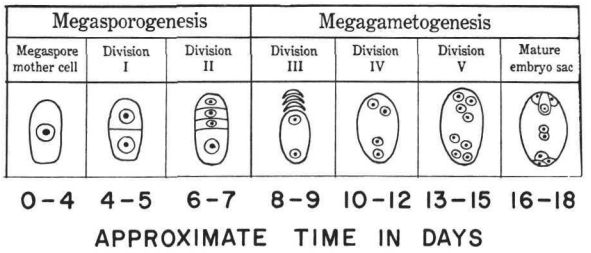

FIGURE 3.— Diagrammatic sketch of *Tradescantia* embryology showing typical numbers and locations of nuclei in the developing embryo sac (Maheshwari, 1950) and the approximate timing for the various stages.

one megaspore functions; it undergoes three mitotic divisions about 5 to 11 days prior to anthesis to form the 8-nucleate embryo sac at maturity (fig. 18(*a*)). The embryo sac contains two synergids and one ovum at the micropylar end; two polar nuclei in the middle, either partially or, occasionally, completely united; and three antipodals at the chalazal end. However, the disintegration of either one, two, or all three of the antipodals in mature embryo sacs is not uncommon.

Experimental Procedures

In the experiments, the clone (02) inflorescences were arranged so that they received a uniform 2-day exposure totaling about 220 R from an ^{85}Sr gamma source. Stamen hairs and petals were scored for spontaneous and radiation-induced effects appearing as changes in cell color and cell shape in the flowers that opened every day for 22 days after retrieval and periodically for another 32 days. The color changes (somatic mutations) are caused by genetic alterations in meristematic cells. When the normal or wild-type blue color gene is lost or mutated the recessive pink allele can be expressed. Successive divisions of the cell with the mutant gene produce a row or cluster of pink or colorless cells; each cluster is considered to be the consequence of one mutational event. Single cell mutants also occur. The size of the mutant sector or the location of the abnormal cells reflects the stage of development of the hair at the time the mutant event is produced. The number of mutant clusters can be counted easily with the aid of a stereoscope, and, from these counts, a mutation rate can be calculated. Changes in size and shape of stamen hair cells also were observed and compared with those of the controls in a similar manner.

The frequency of these changes in color, size, and shape of stamen hair cells is dose dependent and approximates a linear relationship up to at least 350 R under our experimental conditions (fig. 4). An exposure-response series was not feasible in the flower bud zone because of the limited volume in the radiation compartment of the satellite. Therefore, a single 3-day exposure of 300 R was chosen as high enough to induce a significant level of mutation and yet be within a range which would permit expressions of either enhanced or reduced effects of spaceflight factors.

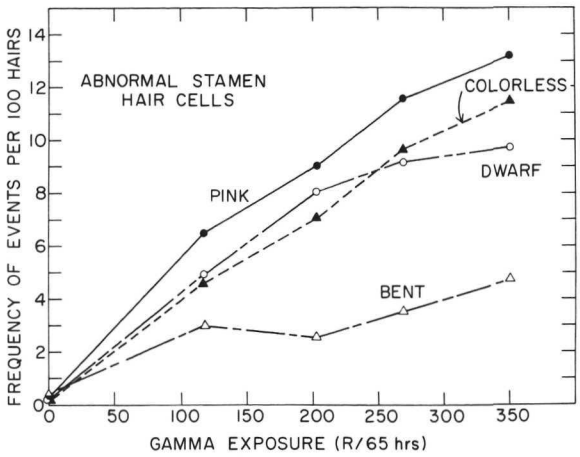

FIGURE 4.— Exposure-response curves based on computed peak mutation rates for various *Tradescantia* end points. Data are from a preflight experiment utilizing a 65-hour exposure to ^{137}Cs gamma rays.

The loss or partial loss of reproductive integrity (stamen hair stunting) was established by counting the number of cells in each hair. A hair was scored as stunted when it had less than 75 percent of the established number of cells occurring in unaffected hairs in nonirradiated control plants. Since the normal number of cells per hair varies from base to apex of the filament, hairs in each third of the filament require a different definition for stunting. In the apical section, for example, the normal average number is 16, so hairs with 11 cells or less were scored as stunted.

In order to facilitate computer analysis of the stamen hair data a special scoring technique was evolved. The purpose of this scoring method was to "map" or record the frequency and location of all mutations or combinations of mutations, morphologic abnormalities, and/or losses of reproduc-

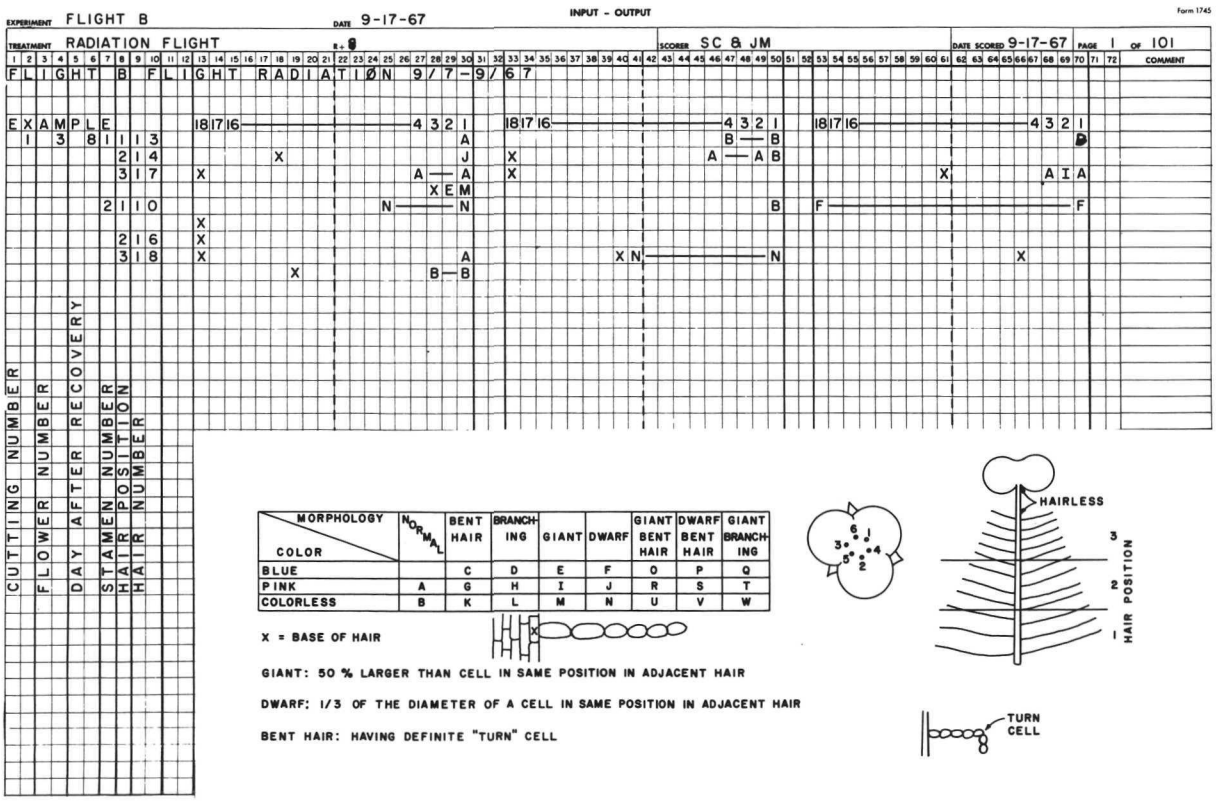

FIGURE 5.— Sample of a typical computer input page describing the method of mapping abnormal cells in *Tradescantia* stamen hairs. Columns 1 to 10 identify the flower and treatment. Columns 11 to 70 represent the coded mutations in the terminal 18 cells of each hair. The insert (lower right) gives the coding and general scoring definitions.

tive integrity. The format used and the code for the various combinations of abnormalities are shown in the sample computer data-input page (fig. 5). The scoring was done by two-man teams with one person observing and dictating the abnormalities and the second person recording that data on computer input sheets. These data were then transferred to punchcards and processed by a CDC 6600 computer. Various abnormalities or combinations of abnormalities were analyzed and the data printed out as shown in the typical summary of figure 6. Other analyses compared the frequencies of mutations or mutant events of different lengths, at different times, and in various positions in the hairs.

Microspores at various developmental stages during the orbital flight were fixed periodically within the first 48 hours after retrieval and were observed in Feulgen-stained smears. The stage of microsporogenesis during the flight could be established from knowledge of division cycle times, growth rates (Beatty and Beatty, 1953; Parchman, 1964; Sax and Edmonds, 1933; and Taylor, 1950), and the time of postflight fixation (see above). The young microspores were used to study the effects of spaceflight factors on micronuclei frequency and on the spindle mechanism in irradiated and nonirradiated cells. For this study eight buds from each package, selected as A–3 buds based on morphologic characters, were fixed after flight immediately upon disassembly. For other cytologic studies, A–2, A–1, and A flowers were fixed 24 and 48 hours after retrieval.

Mature pollen grains collected each day as the flowers opened were used to study the rate of pollen abortion and other development abnormalities.

FLIGHT E 9/7-9/67 RED FLIGHT IRRADIATION A GHIJ RST

DAY	MUT CLS	MUT RUN	STA	HAIRS	HAIRS EST	MUT CLS /STAMEN	SE	MUT CLS /HAIR	SE	MUT CLS /100 CLS	SE	MUT RUNS /STAMEN	SE	MUT RUNS /HAIR	SE
8	2	1	4	240	240	.5000	.5000	.0083	.0083	.0515	.0364	.2500	.2500	.0042	.0042
9	181	118	100	4979	4979	1.8100	.2549	.0364	.0053	.2269	.0168	1.1800	.1149	.0237	.0022
10	122	90	65	3135	3135	1.8769	.2743	.0389	.0046	.2472	.0224	1.3846	.1920	.0287	.0031
11	161	107	48	2047	2047	3.3542	.5606	.0787	.0101	.5593	.0440	2.2292	.3025	.0523	.0052
12	53	26	13	676	676	4.0769	1.4388	.0784	.0207	.5107	.0700	2.0000	.4529	.0385	.0080
13	161	60	24	1163	1163	6.7083	1.4933	.1384	.0261	.9159	.0718	2.5000	.4127	.0516	.0070
14	109	33	18	722	722	6.0556	1.8104	.1510	.0369	1.1736	.1117	1.8333	.3981	.0457	.0083
15	221	73	24	921	921	9.2083	1.6822	.2400	.0437	1.6164	.1079	3.0417	.4908	.0793	.0117
16	26	6	6	252	252	4.3333	2.3617	.1032	.0662	.6900	.1349	1.0000	.2582	.0238	.0111
17	43	17	12	520	520	3.5833	1.1772	.0827	.0283	.5725	.0871	1.4167	.4840	.0327	.0078
18	147	29	24	839	839	6.1250	2.0495	.1752	.0532	1.0970	.0900	1.2083	.2885	.0346	.0074
19	237	35	30	852	852	7.9000	1.7560	.2782	.0639	1.7346	.1117	1.1667	.2095	.0411	.0076
20	448	95	114	3591	3591	3.9298	.6755	.1248	.0194	.7663	.0361	.8333	.1245	.0265	.0035
22	379	59	72	2702	2702	5.2639	1.1311	.1403	.0262	.8238	.0421	.8194	.1444	.0218	.0037
24	240	51	66	2568	2568	3.6364	1.0915	.0935	.0210	.5536	.0356	.7727	.1791	.0199	.0036
26	259	42	72	3144	3144	3.5972	1.0512	.0824	.0186	.4866	.0302	.5833	.1247	.0134	.0023
29	220	23	60	2663	2663	3.6667	1.6120	.0826	.0207	.4949	.0333	.3833	.1282	.0086	.0019
TOTALS	3009	865	792	31014	31014										

FLIGHT E 9/7-9/67 RED FLIGHT IRRADIATION B KLMN UVW

DAY	MUT CLS	MUT RUN	STA	HAIRS	HAIRS EST	MUT CLS /STAMEN	SE	MUT CLS /HAIR	SE	MUT CLS /100 CLS	SE	MUT RUNS /STAMEN	SE	MUT RUNS /HAIR	SE
8	0	0	4	240	240	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
9	67	49	100	4979	4979	.6700	.1280	.0135	.0025	.0840	.0103	.4900	.0823	.0098	.0015
10	79	56	65	3135	3135	1.2154	.3271	.0252	.0056	.1601	.0180	.8615	.1624	.0179	.0024
11	97	70	48	2047	2047	2.0208	.4608	.0474	.0074	.3370	.0342	1.4583	.2679	.0342	.0041
12	61	47	13	676	676	4.6923	.9432	.0902	.0156	.5878	.0750	3.6154	.7385	.0695	.0111
13	270	166	24	1163	1163	11.2500	2.6411	.2322	.0308	1.5359	.0928	6.9167	1.7382	.1427	.0127
14	95	43	18	722	722	5.2778	1.7672	.1316	.0365	1.0228	.1044	2.3889	.4511	.0596	.0092
15	282	159	24	921	921	11.7500	1.7808	.3062	.0354	2.0626	.1216	6.6250	1.1306	.1726	.0157
16	120	21	6	252	252	20.0000	8.0581	.4762	.1543	3.1847	.2861	3.5000	.6708	.0833	.0183
17	149	87	12	520	520	12.4167	3.0238	.2865	.0464	1.9838	.1609	7.2500	1.8551	.1673	.0226
18	99	52	24	839	839	4.1250	1.1426	.1180	.0285	.7388	.0740	2.1667	.4068	.0620	.0102
19	150	55	30	852	852	5.0000	1.3374	.1761	.0404	1.0979	.0891	1.8333	.3493	.0646	.0112
20	465	221	114	3591	3591	4.0789	.4934	.1295	.0137	.7954	.0367	1.9386	.2159	.0615	.0051
22	167	89	72	2702	2702	2.3194	.4453	.0618	.0106	.3630	.0280	1.2361	.1923	.0329	.0044
24	94	59	66	2568	2568	1.4242	.2985	.0366	.0078	.2168	.0223	.8939	.1559	.0230	.0039
26	82	46	72	3144	3144	1.1389	.3393	.0261	.0065	.1541	.0170	.6389	.1484	.0146	.0028
29	74	23	60	2663	2663	1.2333	.5296	.0278	.0098	.1665	.0193	.3833	.1117	.0086	.0023
TOTALS	2351	1243	792	31014	31014										

FIGURE 6.—Sample of computer analysis with typical summaries of data from daily scorings of hair mutations for all types of pink events (A GHIJ RST, upper table) and colorless cells (B KLMN UVW, lower table). "Red" was the code word used to enable sample identification during blind scoring.

The percentage of pollen grains unstained by cotton blue provided a measure of the abortion rate of pollen treated during various stages of development. The frequencies of micronuclei and development abnormalities were analyzed in the mature pollen after propiono-carmine staining.

After the daily removal of the stamens, mature ovaries were fixed for microtome serial sectioning, stained with safranin and fast green, and analyzed for the rate of embryo sac abortion and other development abnormalities. When an embryo sac failed to develop, or when all the nuclei in a partially or fully developed embryo sac had disintegrated, it was considered to be aborted. Any major abnormality in the arrangement of nuclei in the mature embryo sacs producing a fusion, clumping, or scattering of the nuclei was scored as a spindle malfunction.

Root tips were pretreated in 0.1 percent colchicine for 4.5 or 6 hours and fixed in LaCour's 2BD solution at various intervals after disassembly. The location of the roots within the package permitted an exposure-response determination for chromosome aberrations. Total gamma exposures in the 2-day flight ranged from 125 R to 287 R in the root zone (see the discussion of dosimetry later in this paper). For the exposure-response study, the roots were fixed simultaneously at the launch and recovery sites 20.5 hours after retrieval of the Biosatellite II capsule at Hawaii.

Various methods were employed to present and analyze that data from the numerous *Tradescantia* end points. Data for chromosome aberration, spindle effects, microspore abortion, and microspore development abnormalities were derived from material from a single postflight collection. Hence, data from these single collections from the various packages or treatments were averaged and compared directly. However, data for all other end points were collected daily, as flowers opened, for at least 22 days after the flight. These data were generally presented as a plot of frequency against time in days after flight (see, e.g., fig. 11). Since considerable daily variation occurs in data for some end points, two techniques were devised to facilitate a more meaningful comparison between test samples. When the end points were clearly time or stage sensitive, a computer was programmed to (1) fit a smooth curve to the data; (2) compute the peak rate of mutation or aberration; and (3) compute the time at which the peak rate occurred. Thus the peak mutation frequencies and rates of development could be compared among the various samples. The second curve-smoothing technique was to add data for each day to the sum of the data from all preceding days. This procedure results in a measure of the cumulative effects with time and effectively permits the comparison of the areas under each curve or, after a simple division, an average value per day for various samples. This technique is particularly useful in comparing data of end points which have either little or no dose dependency or have sensitive stages of extended duration as in the end points of flower production, megaspore spindle abnormalities, bud blasting, and embryo sac abortion. Analyses of variance and/or χ^2 tests were used to establish the statistical significance of differences.

Hardware Design and Preflight and Postflight Tests

The packages designed to hold the *Tradescantia* plants during treatment were constructed of polypropylene so as to minimize absorption and scattering of gamma rays. The packages were somewhat boomerang-shaped plastic housings 42 cm long, 4.7 cm wide, and 4.7 cm deep (fig. 7). Each package or housing held 32 plants with the roots sealed in plastic tubes filled with Hoagland's nutrient solution. The comblike retainer prevented the nutrient tubes from moving as a result of vibration, centrifugation, etc. The buds (inflorescences) were restrained in a single narrow (1.2-cm) tier (left side, fig. 7) for more uniform gamma exposure unobstructed by other packages. Holes in the cover permitted air exchange and hence better temperature control. Temperature was monitored by the use of a thermistor inserted through the wall of the housing near the inflorescence zone. The plastic fibers were placed around loose buds or stems during the loading process as shock-absorbing packing.

A large number of nonflight experiments were conducted both preflight and postflight. The preflight tests conducted at BNL were designed to demonstrate the flight worthiness of the *Tradescantia* plant material as well as to facilitate the design of suitable hardware. BNL ^{137}Cs gamma

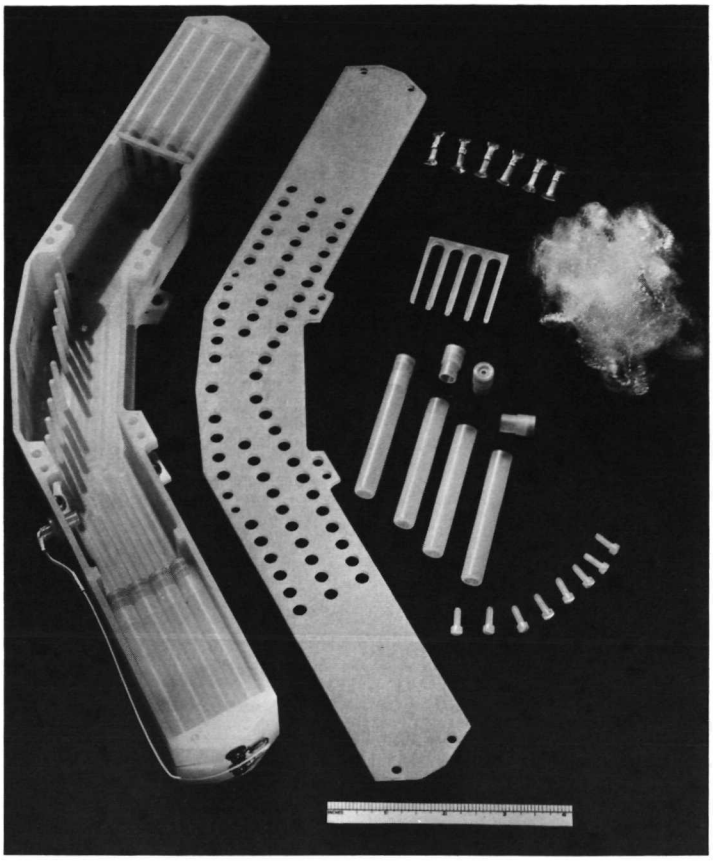

FIGURE 7.— *Tradescantia* package assembly with the polypropylene housing and cover at left and, from top to bottom, six lithium fluoride dosimeters, shock-absorbing plastic fibers, nutrient tube retainer, and nutrient tubes with caps and screws for securing cover. Scale is in inches.

sources were used for these tests and M-2 reach-in chambers [1] were used to control light, temperature, and relative humidity before, during, and after the tests. A similar chamber was used at Cape Kennedy for maintaining plants used in preflight or actual flight experiments.

Preflight biocompatibility and qualification tests of the flight hardware were conducted at the NASA Ames Research Center, Moffet Field, California (ARC) and at General Electric Co., Re-entry Systems Dept., Philadelphia, Pennsylvania (GE). The usefulness of these tests for the *Tradescantia* experiment was compromised by the lack of final flight hardware for the ARC biocompatibility test on June 17 to 20, 1966, and by the lack of gamma radiation in the GE hardware qualification tests during the period from June to September 1966. Other preflight tests were conducted at the Cape Kennedy Space Center just prior to the launch of Biosatellite II. These tests served to provide training for personnel under the critical timing of launch conditions as well as to provide more baseline data for comparison with subsequent flight data.

The final integrated nonflight experiments were conducted at ARC after the flight of Biosatellite II under the designations phase A, B, and C. These postflight tests were extremely important because they provided a means of comparing dosimetry, biocompatibility, and baseline data obtained from the flight vehicle (301) previously flown and the nonflight Earth control vehicle (201) as they were operated side by side under similar conditions. In all instances, matched ^{85}Sr gamma sources were used.

In the phase A dosimetry test, either simulated or no biologic samples were used in most other experiment packages. However, since the *Tradescantia* package was large and contained a fairly dense mass of plant material, live plants were used to give a more accurate measure of direct and scattered radiation.

[1] Purchased from Environmental Growth Chambers, Chagrin Falls, Ohio.

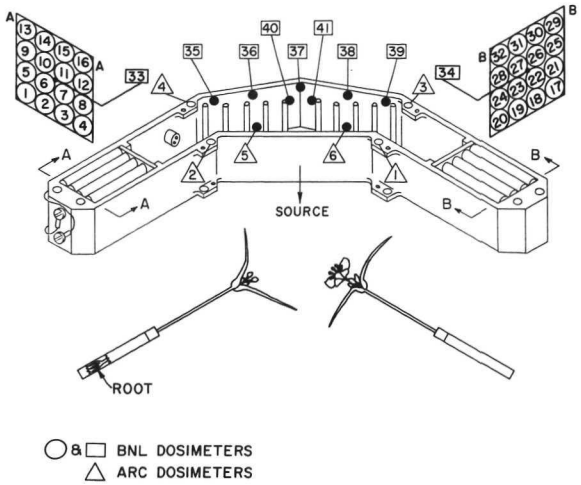

FIGURE 8.—Drawing showing the number designation and location of all lithium fluoride dosimeters used in the *Tradescantia* package. Dosimeters other than those indicated by the triangular symbols were prepared and processed at Brookhaven National Laboratory. Those indicated by the triangular symbols were provided by the NASA Ames Research Center. Tube numbers used are shown in sections *A* and *B* at top left and right and are the same as plant number designations.

The determination of the gamma exposure to the various parts of the plant package was difficult because of the short source-to-target distance and the complex arrangement of experiment packages in the limited space surrounding the gamma source. The dosimetric procedures had three objectives: (1) To "map" the radiation levels throughout the *Tradescantia* package; (2) to determine the percentage of scattered radiation below 100 keV; and (3) to cross-check the BNL and ARC dosimetry (Hewitt, 1968).

The gamma exposure in the root and flower bud zones was measured with lithium fluoride (LiF) powder dosimeters. These dosimeters were placed in the nutrient tubes and along the bud zone as indicated in figure 8. Data from the phase A test and eight other experiments using ^{85}Sr gamma sources (including the flight) were averaged to establish the final dosimetry used in subsequent radiobiologic analyses.

Determination of the amount of low-energy radiation (less than 100 keV) was made by Edgerton, Germeshausen and Grier, Inc., of Goleda, California. Differential energy sensitivity of LiF and CaF_2 dosimeters was utilized to estimate the percentage of scattered radiation. Calculations showed that an insignificant amount of scattered radiation (<2 percent of the total exposure) was present throughout the *Tradescantia* package.[2]

The phase B experiment was designed to compare the biologic and radiobiologic effects of the environment within the flight vehicle (301) and nonflight vehicle (201) on the various end points analyzed. This test was conducted with a full complement of live biologic material loaded and maintained according to flight specifications for temperature, humidity, etc. The 301 and 201 vehicles were operated side by side with material being exposed to matched ^{85}Sr sources for the same period as in the flight. The major differences in treatment of the flight and nonflight vehicles are outlined in table 1. In all three tests (flight II, phase B, and phase C) the 201 vehicle used conditioned room air circulated through the vehicle at a flow rate the same as that of the air flow in the 301 vehicle. However, the 301 vehicle was completely sealed with recirculating capsule air maintained at a normal 1 atmosphere of pressure. It was not possible to have duplicate functional gas management systems.

The phase C test was conducted in the same manner as phase B except that simulated dynamic flight factors of longitudinal axis vibration, shock, acceleration, etc. were imposed upon the 301 vehicle at the levels recorded during the actual flight. It was expected that the effect of these dynamic flight factors alone, if found, could be separated from the effects observed following actual flight and that any differences could be attributed to weightlessness in the actual flight.

In the postflight vibration test at ARC, *Tradescantia* packages (components) were loaded with plants and then subjected to random vibration (along the longitudinal axis only) at levels approximating those recorded during various phases of the Biosatellite II flight. The actual flight levels of vibration and the method by which they were measured are described elsewhere (Look, 1968), but a summary of those levels of vibrations is given below. The vibrations occurred primarily during three phases of the Biosatellite II flight. The lift-off phase, with an overall level of acceleration of 0.7 *g* (root mean square), a duration of 6 sec, and

[2] Personal communication received in 1968 from A. C. Lucas.

TABLE 1. – Summary of Environmental Factors in Biosatellite II and Various Postflight Experiments

Experiment	Vehicle	Gravity, *g*	Dynamic factors[a]	Environment[b]
Biosatellite II:				
Flight	301	10^{-5}	Yes	Closed
Nonflight	201	1	No	Open
Phase B:				
Nonflight	301	1	No	Closed
Nonflight	201	1	No	Open
Phase C:				
Nonflight	301	1	Yes[c]	Closed
Nonflight	201	1	No	Open
Vibration: Nonflight	None	1	Vibration	Open
Clinostat: Nonflight	None	(d)	No	Open

[a]Includes vibration, shock, centrifugation, etc.
[b]In the 301 vehicle the same capsule air was recycled (3.5 cm per minute) for humidity and temperature control. In the 201 vehicle, conditioned room air was circulated at the same rate of flow as that in the 301 vehicle.
[c]Simulated.
[d]Gravity compensated.

a frequency range of 3 to 100 Hz, had a maximum power spectral density of 0.02 g^2/Hz at 15 to 16 Hz. The powered flight phase, with an overall level of acceleration of 1.22 *g* (rms), a duration of 10 sec, and a frequency range of 3 to 100 Hz, had a power spectral density greater than 0.2 g^2/Hz at 19 to 22 Hz. The aerial retrieval phase, with an overall level of acceleration of 0.42 *g* (rms), a duration of 6 minutes, and a frequency range of 3 to 100 Hz, had a power spectral density greater than 0.001 g^2/Hz at about 12 to 14 Hz and again at about 80 Hz. All of these vibrations were simulated as closely as possible on a Ling Model A300–B Shaker at ARC. No radiation was used in the vibration experiment in an effort to isolate any effects on the *Tradescantia* plants induced by flight-level vibration alone.

Clinostat experiments were performed at BNL to determine whether gravity compensation approximating the 10^{-5} *g* attained during orbital flight would have any effect on the various *Tradescantia* end points scored. In this experiment the plants were rotated at 2 rpm with and without ^{137}Cs gamma radiation (Marimuthu et al., 1970b). The rotation rate of 2 rpm was a rate used to insure that a gravitational force was not exerted in any single direction for a period greater than the minimum presentation time (Larsen, 1962) and yet not fast enough to develop a centrifugal force greater than the 10^{-3} *g* "threshold" for geotropic response (Gordon and Shen-Miller, 1966). The axis of rotation was a styrofoam cylinder with a 1-inch diameter, which was large enough to accommodate 10 inflorescences along its perimeter and to produce a range of centrifugal accelerations from approximately 5.7×10^{-5} to 7.4×10^{-5} times that due to Earth's gravitational attraction (1 *g*) from the inner to the outer flower buds, respectively. The clinostats were constructed in the BNL shops from a design furnished by Dr. Solon Gordon (Gordon and Shen-Miller, 1970).

RESULTS

The 3-hour delay in launch of Biosatellite II was not long enough to affect the *Tradescantia* experiment, but early calldown resulted in the loss of about one-third of the total expected gamma exposure. This reduced exposure resulted in lower levels of response (mutation rate, stamen hair stunting, etc.) which made the determination and substantiation of significant flight effects more difficult.

The physical characteristics of the spacecraft environment were, on the whole, adequately monitored and controlled within the limits required for the *Tradescantia* experiment. The temperatures were maintained within specification (70 ± 5 F), with the exception of the first few orbits when the temperature of the flight control package dropped slightly below 65 F (fig. 9).

When the packages were opened, the plants from both flight and nonflight experiments ap-

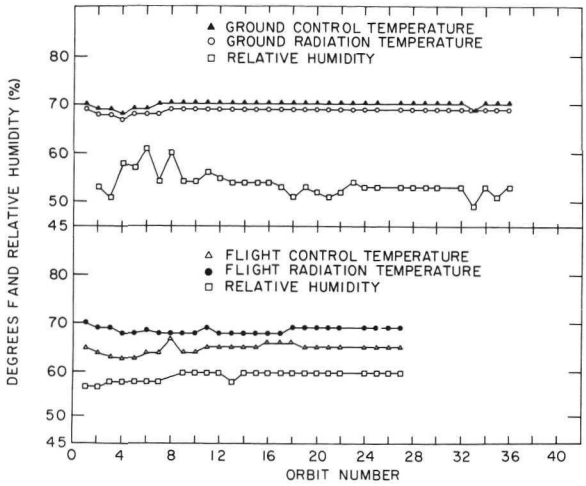

FIGURE 9.—Relative humidity and temperature in the *Tradescantia* package monitored once per orbit in both flight and nonflight vehicles.

peared to be in good condition. A reasonable level of flower production was maintained over the entire period of postflight scoring (fig. 10). However, in the samples loaded in the 301 vehicle in the actual flight and in all the nonflight tests, no flowers opened until 8 or 9 days after retrieval of the vehicle or the end of the test. The buds that normally should have bloomed during this period aborted and hence there was more bud blasting than in either their irradiated or control counterparts in the 201 vehicle (table 2). In the flight and postflight experiments during the period from retrieval *(R)* + 9 to *R* + 26 days, a consistent increase in the total cumulative flower production (table 2) was observed in all the samples from the 301 flight vehicle (468, 508, and 450 flowers) over that of the respective 201 samples (297, 368, and 410 flowers). This increase was due to a

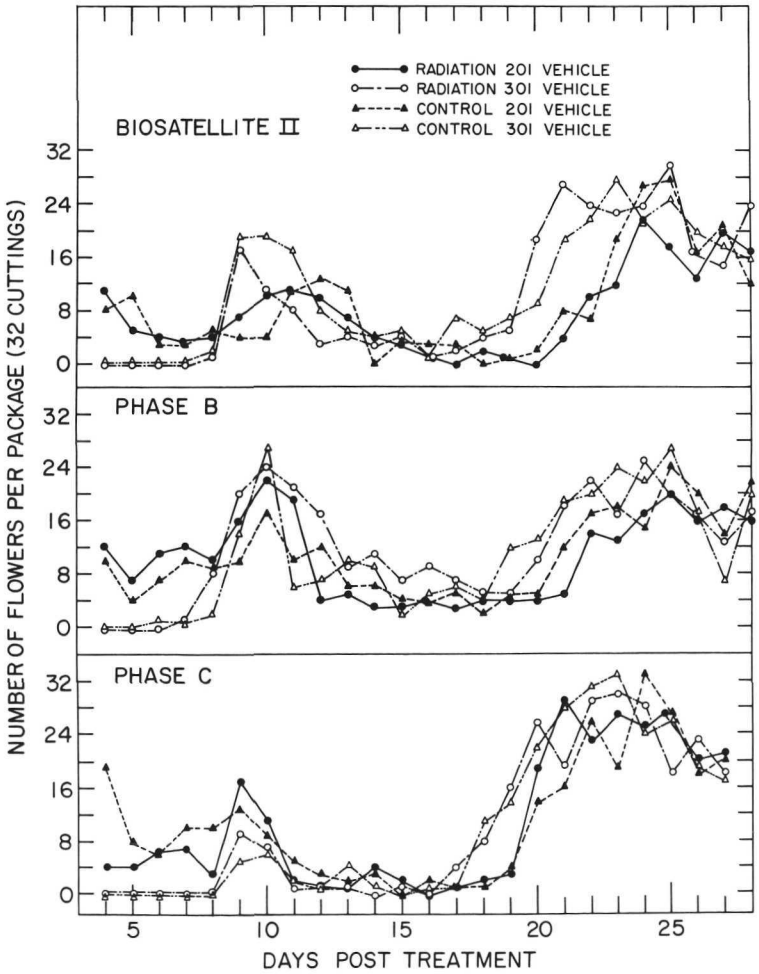

FIGURE 10.—Postflight flower production for plants in flight (301) and nonflight (201) vehicles and for phase B and C tests.

surge in flower production in days $R + 9$ to 11 and early recovery in flowering in 301 samples on about days $R + 18$ or 19 (fig. 10).

The total number of flowers produced throughout the entire period of $R + 1$ to $R + 26$ days (table 2) showed no significant difference between the 301 and 201 samples of the postflight tests. However, flower production in the flight 301 samples (471 flowers) was significantly greater (at the 1-percent level of confidence) than in the nonflight 201 control (353 flowers), which indicated that flight conditions somehow had stimulated flower production. In the clinostat experiment, no difference either in the pattern of flower production or in the total number of flowers produced during the period of $R + 1$ to 26 days was observed between the plants treated on the clinostat and their controls (table 2).

TABLE 2. – Postflight Flower Production and Bud Blasting in Orbited and Nonorbited *Tradescantia* Plants

Experiment	Vehicle	Total number of flowers (32 cuttings)[a]			Average number of buds blasted per inflorescence ±S.E.
		R+1 to R+8 days	R+9 to R+26 days	R+1 to R+26 days	
Biosatellite II:					*Recorded on* R+*52 days*
Flight radiation	301	1	226	227	5.75±0.39
Flight control	301	2	242	244	5.31±0.27
Total		3	468	471	11.06±0.47
Nonflight radiation	201	27	135	162	4.16±0.29
Nonflight control	201	29	162	191	5.07±0.33
Total		56	297	353	9.23±0.44
Phase B:					*Recorded on* R+*27 days*
Radiation	301	9	264	273	6.16±0.38
Control	301	4	244	248	6.91±0.46
Total		13	508	521	13.07±0.60
Radiation	201	58	176	234	5.22±0.39
Control	201	81	192	273	5.03±0.37
Total		139	368	507	10.25± 0.54
Phase C:					*Recorded on* R+*25 days*
Radiation	301	0	221	221	8.19±0.39
Control	301	0	229	229	9.16±0.39
Total		0	450	450	17.35±0.55
Radiation	201	29	214	243	6.94±0.75
Control	201	65	196	261	5.47±0.44
Total		94	410	504	12.41±0.87
Clinostat:					
Clinostat+radiation		134	199	333	
Clinostat+no radiation		118	212	330	
Radiation		140	188	328	
Control		124	188	312	

[a] Flower production for the clinostat experiment was based on 30 cuttings.

The dosimetry data from the flight showed an exposure range of about 125 to 287 R in the root zone and average exposures in the front of the bud zone of 233.5 and 236 R for the nonflight and flight samples, respectively (table 3). Agreement was very good between gamma sources for the flight and the two postflight tests. Since the gamma sources used in the three major tests differed less than 3 percent and the radiation levels of each location in all three tests were so similar, one com-

TABLE 3. – Exposure Distribution Throughout *Tradescantia* Package Measured in Biosatellite II and Averaged Over All Treatments[a]

Location of dosimeters[b]	Dosimeter No.	Biosatellite II		Average of all tests	
		Nonflight radiation, R	Flight radiation, R	Radiation normalized to bud zone, R	Average exposure, R
Root zone, sec. A	1	186	170	0.77	180
	2	211	208	.90	212
	3	246	237	1.02	241
	4	284	284	1.24	291
	5	181	191	.80	188
	6	206	213	.91	215
	7	239	247	1.03	243
	8	272	287	1.20	282
	9	176	195	.82	194
	10	206	219	.92	217
	11	230	248	1.03	244
	12	280	285	1.19	280
	13	195	185	.83	195
	14	.....	222	.94	221
	15	245	249	1.07	251
	16	258	282	1.19	280
Root zone, sec. B	17	127	136	.49	116
	18	132	125	.53	125
	19	138	146	.61	144
	20	167	166	.74	174
	21	148	140	.63	149
	22	135	147	.65	153
	23	147	190	.82	194
	24	191	221	.94	222
	25	161	159	.69	162
	26	196	176	.79	186
	27	220	212	.92	216
	28	.....	246	1.05	247
	29	166	174	.74	174
	30	186	187	.80	188
	31	206	214	.90	213
	32	241	249	1.03	243
Dosimeters between tubes	33	234	249	.....	.....
	34	223	219	.....	.....
Flower bud zone	35	215	201	.89	211
Back	36	215	197	.90	214
	37	212	201	.89	212
	38	211	206	.89	212
	39	212	203	.88	210
Front	40	236	233	1.00	236
	41	231	239		

[a]Control flight and nonflight packages, <0.4R.
[b]See fig. 8.

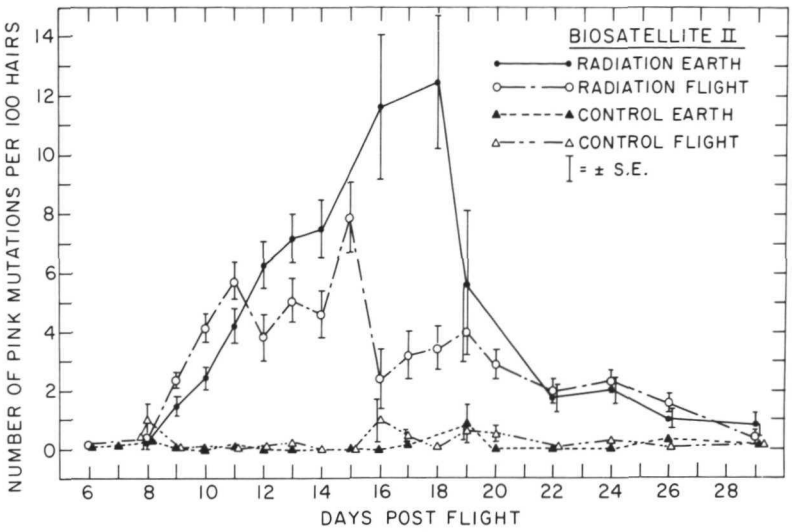

FIGURE 11.— Frequencies of pink mutations per 100 stamen hairs for all four treatments versus time in days after flight.

mon set of averaged dosimetry values was used in all analyses (table 3, last column). Flight control material received 0.24 R and nonflight control, 0.37 R during the experimental period (Hewitt, 1968).

The frequency of pink mutations per 100 hairs is given in figure 11 and table 4. The radiation-induced frequencies increased with time from day 8 to about day 16 postflight and by 28 days dropped to frequencies approaching the control values. Although the data are quite variable from day to day, a statistical analysis showed the peak nonflight radiation mutation rate (8.21 ± 0.39) to be significantly higher than that of the flight radiation (4.89 ± 0.27). The simultaneous rise of mutation rates of irradiated nonflight and flight material from 8 to 12 days postflight (fig. 11) indicates no significant alteration in the rates of bud development after the flight. In the phase B postflight experiment utilizing the flight (301) and nonflight control (201) vehicles a similar significant increase in mutation rate was observed in the plants irradiated in the nonflight vehicle (9.95 ± 0.54) when compared with data from irradiated plants in the flight vehicle (6.13 ± 0.19). This consistent reduction in pink mutation rate in the "sealed" 301 vehicle used in the flight, phase B, and phase C is also shown graphically in figure 12. This figure indicates that all data from 301 samples are not only lower than those of their respective 201 counterparts but also considerably lower than the comparable points on the exposure-response curve. This

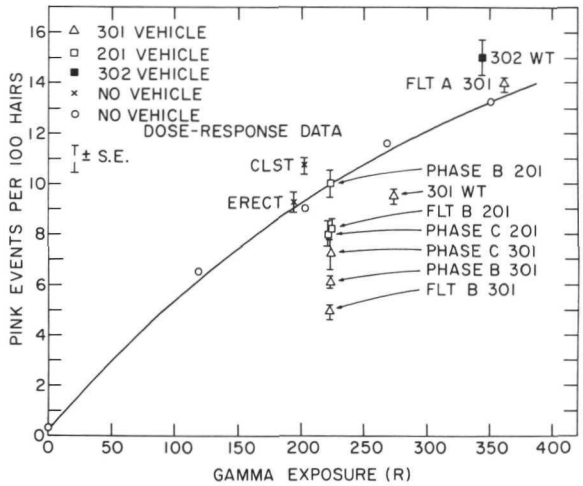

FIGURE 12.— Computed peak rate of pink-celled stamen hair mutations for the various experiments indicated. Exposure-response curve (see fig. 4) is included for reference. Note the consistently low flight, phase B, and phase C mutation rates in the 301 vehicle.

significant difference may therefore be attributable to some *unknown characteristic* of the 301 vehicle and not necessarily be the result of flight factors.

The mutation rates in all unirradiated controls (flight and postflight tests) were consistently low throughout the experiments, with no significant differences between those for the flight and the nonflight samples (fig. 11 and table 4).

The numbers of pink mutant sectors per petal from flight material are given in figure 13. The

TABLE 4. – Summary of Computed Peak Aberration Rates ± Standard Error for Various *Tradescantia* End Points From Biosatellite II and Nonflight Tests Showing Effects Indicated

End points	Biosatellite II				Simulated flight (phase C)				Component test	
	Flight (301)		Nonflight (201)		301 vehicle		201 vehicle		Clinostat	Erect
	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Non-irradiated	Irradiated	Irradiated
No significant effect										
Pink/petal	16.9±0.85	[a]1.37±0.17	13.3±0.63	[a]1.10±0.20	18.7±0.66	[a]1.47±0.48	18.7±0.7	[a]2.22±0.28	23.1±0.80	20.8±0.7
Colorless/100 stamen hairs	10.7±0.83	[a].132±0.1	11.1±1.8	[a]0.32±0.26	5.88±1.1	[a]0.09±0.09	5.11±0.59	[a]0.26±0.16	6.21±0.37	5.5±0.8
Dwarf cells/100 hairs	11.2±0.65	[a]3.05±0.83	13.1±1.1	[a]3.44±1.2	3.84±0.39	[a]0.53±0.27	[b]2.47±0.97	[a]0.72±0.26	4.29±0.30	4.1±0.3
Giant cells/100 hairs	5.69±0.46	.....	4.96±0.19	.....	1.83±0.42	[a]0.59±0.50	1.95±0.38	[a]0.65±0.34	1.4±0.1	1.4±0.1
Chromosome aberrations/cell in roots[c] (194 R)	0.53±0.09	0.04±0.02	0.48±0.10	0.02±0.01	.....	.....	.....	.....	.....	.....
Enhanced flight effects										
Loss of reproductive integrity (stunting)/100 hairs	26.9±1.3	[a]9.60±3.8	11.6±0.73	[a]7.26±0.70	[d]14.8±1.5	[d]13.2±1.5	[d]18.9±1.8	[d]10.7±1.4	11.1±1.6	.....
Pollen abortion, %	69.5±2.4	[a]36.5±2.2	49.6±2.5	[a]41.0±2.5	69.2±2.5	47.0±2.1	58.8±3.0	47.1±1.9	90.0±2.7	83.8±3.1
Micronuclei/100 pollen	24.1±1.4	4.47±0.44	12.1±1.1	3.0±0.3	[b]33.3±15.6	.....	[b]22.9±10.0	.....	.....	.....
Flower production, 26-day total	227	244	162	191	221	229	243	261	333	328
Disturbed spindles, % cells:										
Roots[c]	0.55±0.08	0.25±0.05	0.06±0.03	0	0.08±0.03	0	0	0	0	0
Microspores[c]	All aborted	27.5±0.92	0.3±0.09	0.18±0.07	0.93±0.21	0.50±0.11	0.15±0.06	0.12±0.05	.....	.....
Megaspores[b]	6.2 ±1.3	4.48±0.9	1.2 ±0.68	1.94±0.86	1.39±0.79	0.50±0.50	1.54±0.88	.....	.....	.....
Enhanced effects attributed to internal vehicle environment										
Pink/100 stamen hairs	4.89±0.27	[a]0.31±0.24	8.21±0.39	[a]0.20±0.10	[d]6.13±0.19	[a]0.11±0.08	[d]9.95±0.54	[a]0.13±0.09	10.7±0.33	9.3±0.3
Microspore developmental abnormalities, % cells[c]	.....	3.28±0.28	.....	0.43±0.10	.....	4.73±0.34	.....	0.33±0.09	.....	.....
Microspore death, % buds[c]	100	100	16.7	33.3	100	100	25	12.5	.....	.....
Bud blasting/plant[b]	5.75±0.39	5.31±0.27	4.16±0.29	4.94±0.33	8.19±0.39	9.16±0.39	6.94±0.75	5.47±0.44	.....	.....
Embryo sac abortion, %[b]	52.1±5.0	17.6±2.8	35.0±6.1	19.0±6.0	50.1±5.1	13.8±3.6	35.9±5.9	14.3±4.4	.....	.....

[a]Average of 4 days around peak day of corresponding irradiated sample.
[b]Average of daily observations over extended postflight scoring period (no distinct peak rate).
[c]Observations of single postflight tissue collection.
[d]Average of daily observations over extended posttreatment scoring period of phase B test.

curves for the pink-celled mutation data from the irradiated flight and nonflight control petals are nearly superimposed as were the curves for three other Earth-based postflight tests. Where differences did occur around days 16 to 18 posttreatment, the standard errors are too large to conclude that this difference is real. Even the slight increase in mutation rate in the flight irradiated sample is the reverse of the effect observed in the stamen hair data in figure 11. All control samples had consistently low mutation rates and showed no difference between flight and nonflight samples (fig. 13 and table 4).

The frequency of radiation-induced colorless mutations could be scored only in the stamen hairs and these data are shown in figure 14. Although

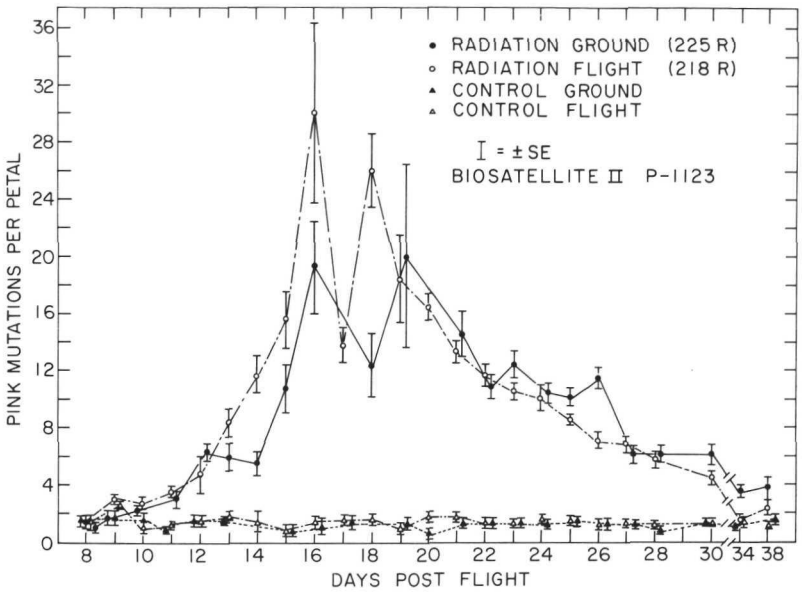

FIGURE 13.— Frequencies of pink mutations in *Tradescantia* petals for all four treatments versus time in days after flight.

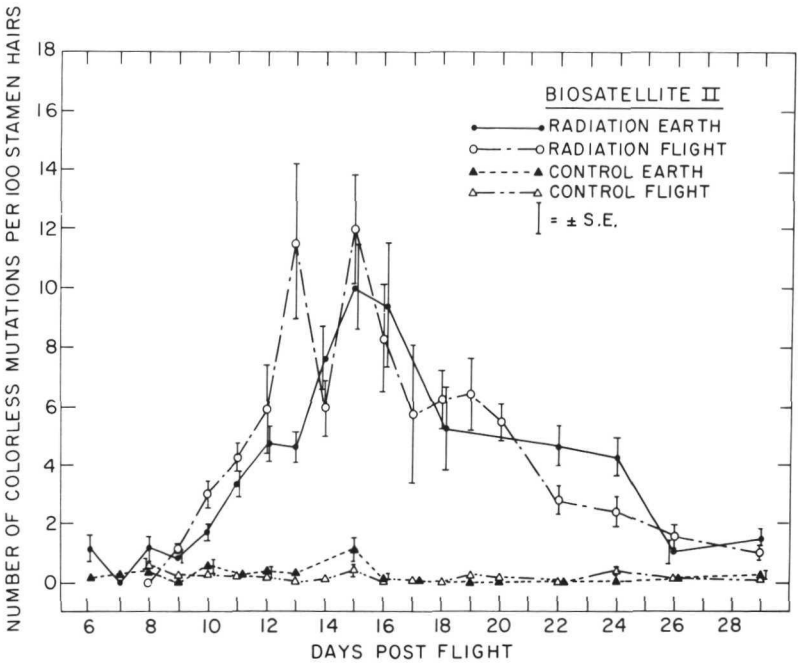

FIGURE 14.— Number of colorless mutations per 100 hairs for the four treatments versus time in days after flight.

a slightly higher mutation frequency is suggested in data from the irradiated flight sample, this difference is not significant in view of the relatively large standard errors. Postflight tests in the 201 and 301 vehicles also showed little or no differences in mutation rates (table 4). At the control level, again, the results are consistent and no difference exists between data from the flight and those from the nonflight samples.

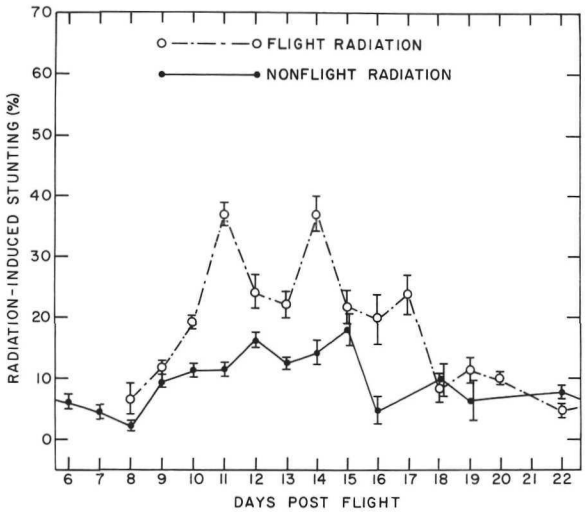

FIGURE 15.— Percentage of stunted *Tradescantia* stamen hairs for irradiated flight and nonflight samples against time in days after flight. Hairs were scored in distal third of filament.

The frequency of dwarf cells increased following gamma exposure, but there were no significant differences in the peak values of flight and nonflight irradiated samples (table 4).

The loss or partial loss of reproductive integrity of terminal or subterminal hair cells can result in a short or stunted hair (Ichikawa et al., 1969). Hair lengths normally vary somewhat, but the incidence of stunted hairs increases with increasing exposure and postirradiation time. Figure 15 shows that throughout the period between 9 and 17 days after retrieval the percentage of stunted hairs was higher in the flight than in the nonflight irradiated samples. Many more very short hairs (5 cells or less) were observed in the irradiated flight sample than in the comparable nonflight sample, indicating that the same exposure produced a greater deleterious effect on cell division in the Biosatellite II than in the nonflight plants.

Mature pollen was collected daily, and the percentages of aborted pollen were determined for all four treatments (fig. 16). It should be emphasized that this clone of *Tradescantia* is characterized by an unusually high rate of spontaneous pollen abortion (average control levels over the entire scoring period were 36 and 41 percent for flight and nonflight samples, respectively). These data show a consistently higher abortion rate in the flight-irradiated sample than in the nonflight-irradiated sample with one point (day 14) being significantly

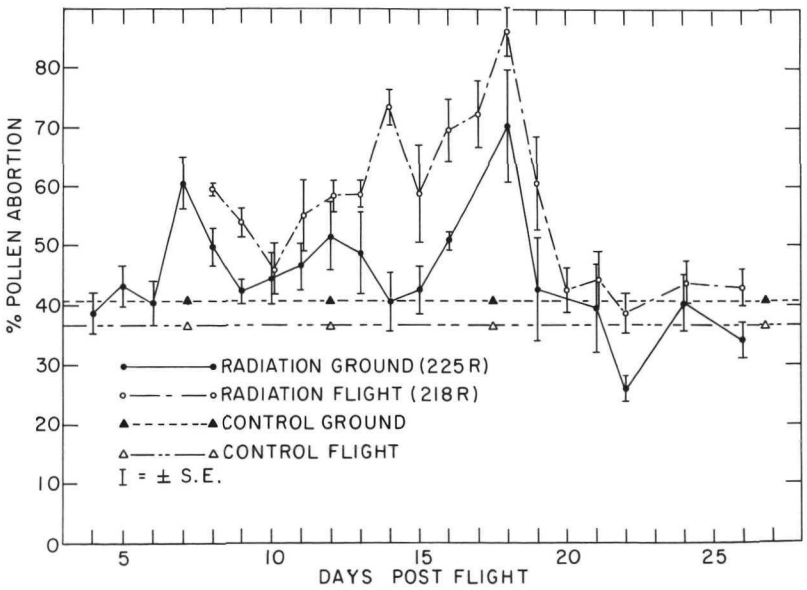

FIGURE 16.— Percentage of pollen abortion in flight and nonflight buds. Unirradiated control lines are averages of combined daily observations.

higher at about the 1 percent level. Comparable postflight tests showed considerable variability, but, in general, data from the 301 and 201 samples did not show the same significant differences as those in the actual flight (table 4).

A portion of the mature pollen that was collected each day was also stained with propiono-carmine for subsequent scoring of micronuclei. Although the analysis of these data has not been completed for all experiments, the computed curve and peak values (fig. 17) indicate a twofold increase in frequency of micronuclei in the flight-irradiated sample over the nonflight-irradiated sample. The peak response on days 17 to 18 after retrieval agrees with that observed for pollen abortion and further indicates the greater sensitiviy of the cells orbited during meiotic stages occurring 14 to 19 days prior to anthesis.

Each day as the flowers opened, mature ovaries were also collected and fixed for histologic analyses (fig. 18). The frequencies of embryo sac abortion (fig. 18(*d*)) during the peak exposure-response days after the treatment ($R+14$ to $R+19$) in the flight and phase C experiments are given in table 4. A significant increase was observed in the abortion rate in the irradiated samples loaded in 301 vehicles in both the flight and phase C experiments over that of their irradiated 201 controls. However, there was no difference observed between the 301 and 201 nonirradiated controls.

The frequencies of embryo sacs with the nuclei fused, clumped, or scattered (figs. 18(b) and 18(c)), presumably because of the malfunctioning of the spindle, are given in table 4 for the flight and phase C experiments. Significant increases were observed both in the irradiated and in the nonirradiated flight samples over their respective nonflight controls at the 1 and 8 percent levels, respectively. No such differences were observed between 301 and 201 samples in the phase C experiment.

Data for the end points described above are summarized in table 4. These data are either the computed peak values based on data from all days

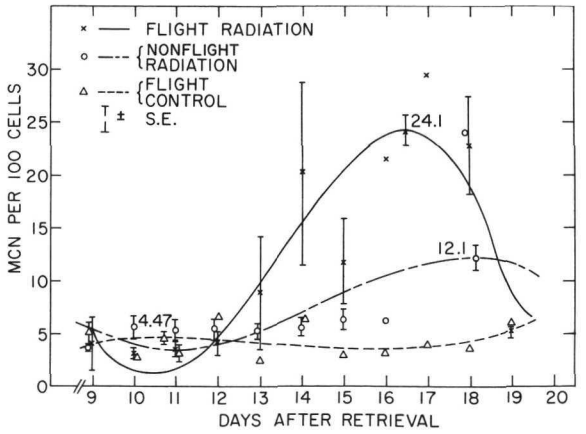

FIGURE 17.— Frequency of micronuclei (MCN) observed in carmine-stained pollen from *Tradescantia* flowers maturing on various days after retrieval. Smooth curves are based on computer program utilizing coefficients of third-degree polynomial and the MCN frequencies of 4.47, 12.1, and 24.1 represent the computed peak values for the treatments indicated.

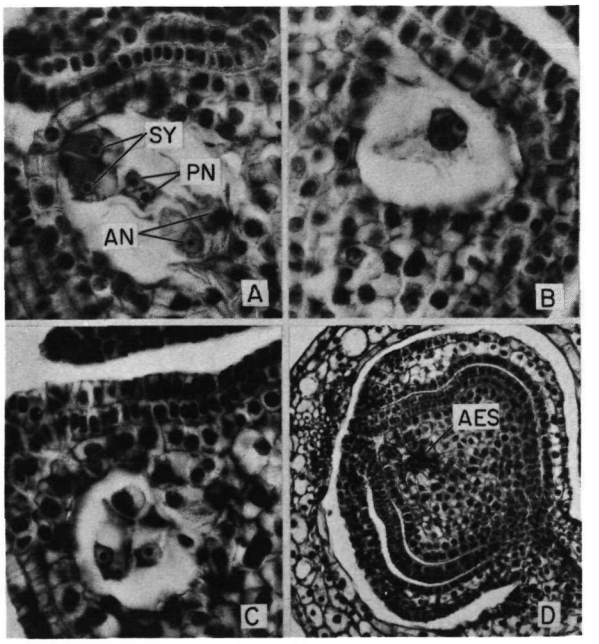

FIGURE 18.— Longitudinal sections of mature *Tradescantia* ovules. (*a*): Normal fully developed embryo sac showing typical orientation of two synergid (SY), two polar nuclei (PN), and two antipodals (AN). The egg and other antipodal, not shown, were in adjacent sections. (*b*) and (*c*): Embryo sacs from nonirradiated flight sample, fixed after anthesis. Normal development was interrupted, presumably by spindle malfunction; and the nuclei were clumped and scattered in (*b*) and (*c*), respectively. (*d*): Section of mature ovule showing an aborted embryo sac (AES) which resulted when the embryo sac failed to develop. Magnifications of (*a*), (*b*), and (*c*) 200 ×; (*d*), 50 ×.

or represent the average of the data from about a 5-day period showing the highest mutation or cell-aberration response. The data for the remaining end points are also summarized in table 4, but are average values based on material from a single postflight collection as opposed to the daily scoring described above.

In the flight material all the A–3 buds had a microspore abortion rate of more than 95 percent. For comparison, a postflight experiment was conducted at Brookhaven National Laboratory using 40 A–3 buds from plants treated in flight-type packages, but not in the vehicle. Only 28 percent of the buds had a microspore abortion rate of more

TABLE 5. – The Chromosomal Aberration Frequencies in Metaphase Cells of *Tradescantia* Clone (02) Induced by Various Treatments

Exposure, R	Number of plants scored	Number of cells scored	Number of aberrations per cell	Number of gaps per cell	Number of breaks per cell	Number of exchanges per cell
Flight material (Biosatellite II)						
116.0	1	53	0.32±0.08	0.06±0.04	0.13±0.05	0.06±0.03
125.4	1	32	.41±0.12	.06±0.04	.19±0.08	.16±0.07
143.0	1	129	.57±0.07	.12±0.03	.33±0.06	.10±0.03
177.1	2	178	.63±0.07	.17±0.04	.28±0.04	.16±0.03
187.2	3	133	.44±0.06	.09±0.02	.18±0.04	.09±0.02
194.2	2	112	.53±0.09	.23±0.06	.19±0.04	.07±0.03
215.5	2	315	.62±0.05	.19±0.03	.27±0.03	.12±0.02
244.3	2	169	.85±0.08	.26±0.05	.33±0.04	.21±0.03
0.0	3	259	.04±0.02	.01±0.01	.01±0.01	0±0
Nonflight material (pooled data from Biosatellite II and phase B)						
116.0	2	178	0.23±0.04	0.07±0.02	0.08±0.02	0.03+0.01
125.4	1	120	.38±0.06	.12±0.03	.12±0.03	.07±0.02
143.0	3	175	.41±0.05	.10±0.03	.20±0.04	.07±0.02
173.9	4	238	.50±0.06	.19±0.04	.20±0.03	.08±0.02
187.2	2	136	.53±0.07	.13±0.04	.27±0.05	.13±0.03
194.2	1	80	.48±0.10	.08±0.03	.25±0.05	.10±0.05
215.5	2	145	.79±0.09	.34±0.06	.33±0.06	.11±0.03
244.4	2	118	.62±0.09	.15±0.04	.20±0.05	.08±0.03
0.0	3	225	.02±0.01	.01±0.01	0±0	0±0
Radiation with clinostat						
197.0	4	270	1.14±0.08	0.47±0.10	0.40±0.09	0.13±0.02
Radiation without clinostat						
197.0	3	200	1.40±0.11	0.68±0.03	0.42±0.03	0.26±0.03
Component random vibration						
0.0	2	200	0.03±0.01	0.02±0.01	0±0	0±0

than 95 percent. The aborted microspores were of two kinds: (1) Those containing no Feulgen-staining material (i.e., they appeared completely empty) and (2) those containing Feulgen-positive material throughout the cytoplasm which presumably had diffused from the vegetative or generative nucleus or both (Tobias et al., 1967). Subsequent nonflight tests in the flight vehicle (301) produced consistently high microspore death rates similar to those of the orbited material (table 4). This effect may be due, therefore, to some environmental variable in the 301 vehicle rather than to flight conditions.

In the surviving fraction (<5 percent) of the microspores, abnormalities reflecting disturbances in the spindle mechanism (figs. 2*(b)* to 2*(d)*) were observed at a much higher frequency in the flight control samples (27.54 percent) than in the nonflight control samples (0.18 percent) (table 4).

The frequency of development abnormalities observed in the generative and vegetative nuclei of the older buds (binucleate pollen) on plants from the various experiments are presented in table 4 and figures 2*(f)* to 2*(h)*. The frequencies of these abnormalities are significantly higher in 301 samples than in the 201 samples both in Biosatellite II and phase C simulated flight experiments. Furthermore, the frequencies of these abnormalities in the vibration and clinostat experiments (conducted without the use of the vehicle) are not different from those of the nonflight 201 control.

Evidence of a disturbance in the spindle mechanism was seen in root tips of flight material in the form of multinucleate cells and nuclei of abnormal shape. If the spindle completely failed to function, the somatic chromosome complement should change from 12 to 24, i.e., becomes tetraploid, and the shapes of the resulting nuclei would reflect, for a short time, the position of the chromosomes as they had been scattered throughout the cytoplasm. Root tip cells showing abnormal nuclei resulting from disturbed spindles in flight control materials are shown in figures 2*(i)* and 2*(j)*. Table 4 shows that the percentage of cells with such abnormalities in both the flight irradiated (0.55±0.08) and nonirradiated (0.25±0.05) samples were significantly higher than in all other tests either with or without radiation (<0.08±0.03). The percentages are small when expressed as a percent of all cells scored, but they would be much larger if only cells at metaphase to late telophase stages were scored. However, it is clear that not all cells in division showed evidence of spindle inhibition or malfunction.

Chromosome aberrations in root tip metaphases were scored in all the treatments and exposure-response data are given in table 5. No significant differences were found in the frequency of total aberrations, gaps, breaks, and exchanges between irradiated flight and nonflight samples (figs. 19 and 20).

DISCUSSION

The *Tradescantia* Biosatellite II experiment was designed to provide data from two levels of stress (with and without gamma irradiation) for evaluating the effects of weightlessness and the spacecraft environment. The two nonirradiated samples provide an indicator of the effects or interaction of spaceflight factors on spontaneous aberration and mutation rates, whereas the irradiated flight and nonflight samples provide material with which to

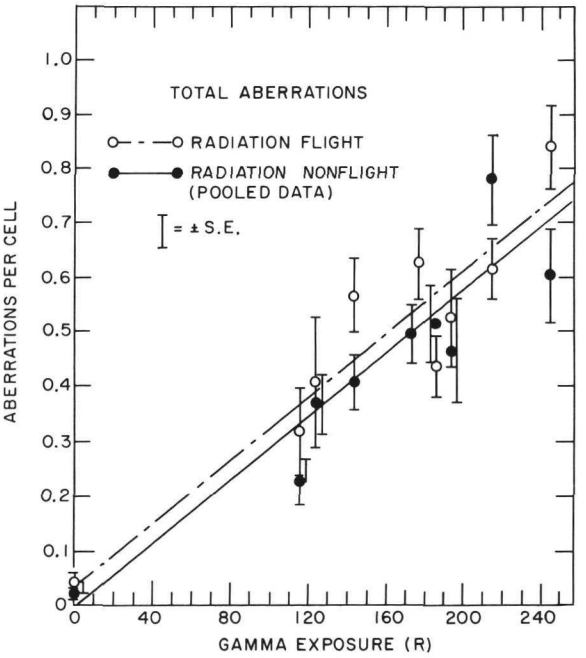

FIGURE 19.— Exposure-response regression curves for chromosomal total aberrations (table 5) in root tip cells from flight and nonflight material. Nonflight curve represents pooled data from Biosatellite II and phase B tests.

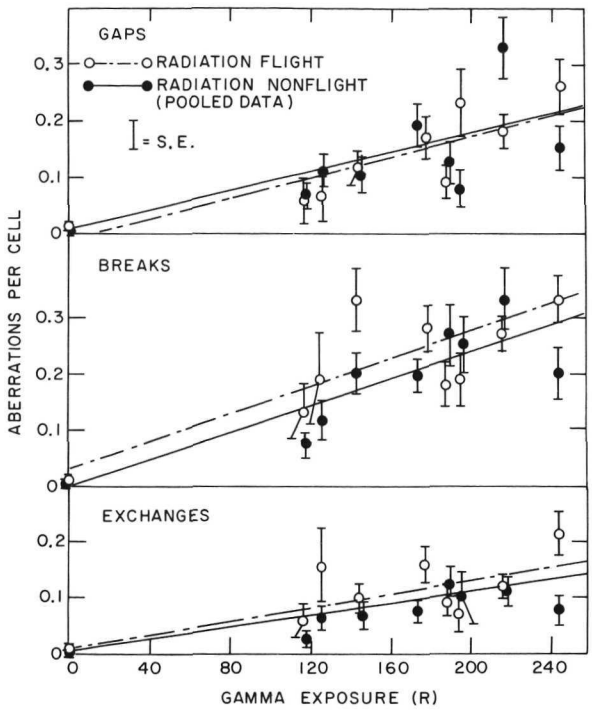

FIGURE 20.— Exposure-response regression curves for types of chromosomal aberrations indicated. Exchanges are totals of intrachanges and interchanges. Nonflight curves represent pooled data from Biosatellite II and phase B tests (table 5).

determine if there is any interaction with a predetermined exposure of gamma radiation.

In the flight experiment the total number of flowers produced in the flight 301 samples during the period of $R+1$ to $R+26$ days was significantly larger than in the nonflight 201 samples. No such difference was observed between 301 and 201 samples in the subsequent nonflight experiments. These observations rule out the possibility of either a 301 vehicle effect alone or a vehicle effect combined with the effects of simulated dynamic flight factors as the cause for increased flower production observed in the flight samples. The data presented suggest, then, that this effect is largely attributable either to weightlessness or to weightlessness preceded by dynamic factors.

At present there is no suitable explanation for the apparent stimulation in flower production. The probability of an effect of weightlessness on auxin gradients in the orbited *Tradescantia* inflorescences is high considering the effects noted in other plants flown in Biosatellite II (Gray and Edwards, 1968; Johnson and Tibbitts, 1968; Lyon, 1968). Even though altered auxin levels may enhance flower bud initiation (Clark and Kern, 1942), such an enhancement could not explain these data because flower bud initiation in *Tradescantia* plants grown under the conditions of our experiment takes place at least 26 days before anthesis (Mericle and Mericle, 1967). Therefore, the flowers opening within the 26-day postflight scoring period had already been initiated by the time of the flight. Further, if this increase in flower production is due to an alteration of auxin levels due to weightlessness, then it is reasonable to expect such an alteration in auxin levels and, as a consequence, an increase in flower production in the clinostat experiment also (Gray and Edwards, 1968; Johnson and Tibbitts, 1968; Lyon, 1968). However, no such increase in flower production was observed in our clinostat experiment.

An alternative explanation for the significant differences in flower production between flight and nonflight samples of the Biosatellite II experiment might be that the nonflight samples had an unusually low rate of flowering due to unknown causes. This explanation, too, is improbable, since all *Tradescantia* cuttings were randomized prior to loading the packages, preflight and postflight handling was carefully controlled within required specifications, and any suspected vehicle effect (discussed later) would tend to cause greater injury to the 301 flight samples and not to the 201 nonflight control samples. Despite our inability to present a logical explanation for the enhanced flower production following the flight of Biosatellite II, the fact remains that a statistically significant increase was observed.

In the control material the genetic loci controlling flower color have average mutation rates of about 0.15 (pink) and 0.20 (colorless) mutation per 100 stamen hairs. The extreme sensitivity of these loci to low levels of ionizing radiation, changes in light intensity, and changes in temperature has been demonstrated by various experimenters (Mericle and Mericle, 1965, 1966, and 1967; Sparrow et al., 1968). Hence, the effects of any potential mutagen should be readily detected. It is also conceivable that interaction between the spacecraft environment and irradiation could modify the expected effect. The pink-cell mutations in the petals

and the colorless mutations in the hairs showed no significant differences between flight and nonflight irradiated samples. The pink-cell mutations in the stamen hairs, however, did show a decrease in mutation rate in the flight sample. The computed peak mutation rate for the nonflight sample was 8.21 mutations per 100 hairs, which is in good agreement with established values for a 225 R exposure, and the decrease noted here was found to be significant at the 1-percent level. However, a similar reduction in pink-cell mutation frequency was observed in one of the postflight tests (phase B, 301), thus indicating no strict correlation with flight factors such as weightlessness. This interpretation is further supported in that none of the other somatic mutation end points showed a decreased rate in flight plants. Such a decrease in mutant cells could be induced by some vehicle effect of an undetermined nature.

The data of Nayar and Sparrow (1967) suggest that a 225 R, 16-hour gamma exposure would cause stunting in about 20 percent of the hairs as compared with the desired stunting rate of 35 to 40 percent if the full 3-day exposure of about 330 R had been attained. The loss of reproductive integrity (stunting) of hairs observed in the Biosatellite II experimental material when expressed as computed peak values was 11.6 and 26.9 percent for nonflight and flight irradiated samples, respectively, a difference which is significant at the $<$1-percent level. The fact that the flight radiation sample had significantly more hairs stunted than did the nonflight radiation sample at the same time and that no difference was observed between the flight and nonflight unirradiated samples indicates an enhanced interaction between irradiation and spaceflight factors. This conclusion was further supported by data from postflight experiments which were consistent among all nonflight irradiated samples in both the 301 and 201 vehicles.

Evidence of disturbed meiotic and/or mitotic spindle function in microspores, megaspores, and roots in both flight samples suggests that similar effects might also occur in the meristematic hair cells and result in abnormal cell division and perhaps cell death. Radiation is known to damage or inactivate spindles in several different materials and in both somatic and meiotic cells (Carlson, 1954; Koller, 1943; Marquardt, 1938a and 1938b; Sparrow and Sparrow, 1954).

A similar enhanced interaction apparently exists in the production of aborted pollen. Again, no significant difference was observed between the unirradiated samples, but the irradiated flight sample had a computed peak pollen abortion rate of about 70 percent as compared with 50 percent in the nonflight radiation sample. Although the flight radiation rates were higher throughout the postflight scoring period, the greatest effect was seen 14 to 19 days after flight. Assuming that the rate of microspore development was not affected by flight conditions, this period of high abortion rate would indicate the enhanced interaction of radiation and weightlessness during meiosis. Meiosis is known to be a highly sensitive stage and irradiation of certain stages is well known to lead to high rates of pollen abortion (Yamakawa and Sparrow, 1966). Although the spontaneous pollen abortion rate is high (about 40 percent) and variable from day to day, the postflight tests did not show the same consistent increase throughout the scoring period for the sample irradiated in the 301 flight vehicle as for the flight irradiated sample. Therefore a vehicle effect can be ruled out as a cause of this result.

A very high death rate ($>$95 percent) of microspores was observed in A-3 buds of the flight material fixed immediately after disassembly. However, microspore death, embryo sac abortion, and bud blasting in material from flight and postflight tests showed consistent increases in all samples loaded in the 301 flight vehicle. One possible cause under consideration is the fact that the 301 vehicle was a closed environment system while the 201 control vehicle used circulated room air. The airflow rates were at the same velocity in both vehicles, but the closed system could lead to the accumulation of volatile substances such as ethylene. Preliminary experiments with ethylene suggest that exposure to this gas at the same concentration as that measured in the phase C 301 vehicle (0.9 ppm) (NASA ARC, 1968) can cause an amount of bud blasting, microspore death, and, perhaps, development abnormalities in microspores comparable to that observed in flight samples. Ethylene alone, however, did not affect the pink-cell mutation rate in stamen hairs.

Enough healthy roots were obtained from the flight radiation plants to construct a valid exposure-response curve. In the nonflight radiation treatment, plants at several of the high exposures did

not produce enough healthy roots to give reliable values on the curve. The reason for this poor growth in not known. Since data from the Biosatellite II nonflight irradiated roots were insufficient, the results of postflight tests were pooled for comparison with flight radiation data for each exposure (table 5). No significant increase in chromosome aberration rate was observed at any exposure level in the orbited material. This negative effect was evident whether one considered the total aberration rate or the gaps, breaks, and exchanges separately. There is good agreement then between the lack of a flight effect on somatic mutation rates and on chromosome aberration rates.

The surviving microspores of the A–3 buds from orbited material showed various kinds of nuclear abnormalities indicating a disturbance in the spindle mechanism which was not observed in any of the nonflight tests. Similar abnormalities in *Tradescantia* microspores have been observed by Delone et al. (1964 and 1968). Furthermore, these Soviet experiments showed that the frequency of these abnormalities increased with time in orbit in samples fixed after 1.5, 76, and 120 hours after launch, thus suggesting a direct correlation with weightlessness rather than spacecraft dynamic factors.

More evidence for the malfunctioning of the spindle due to weightlessness was obtained from the abnormal arrangement of nuclei in the embryo sacs obtained from the flight material. In these abnormal embryo sacs the nuclei were either clumped together or scattered throughout the embryo sac.

Root tip cells with one to five nuclei varying in size and shape were observed in flight radiation roots in 0.55 percent of the cells as compared with only 0.06 percent in the comparable nonflight sample. They also occurred in unirradiated flight samples. These nuclei resemble those which would result from treatment with spindle-destroying chemicals such as colchicine; so the peculiarly-shaped nuclei observed in the orbited roots were also considered as resulting from a disturbed spindle mechanism. The possibility that the spindle disturbance might have resulted from the colchicine pretreatment was ruled out for three reasons: (1) The same colchicine treatment was used in all flight and nonflight tests, and no such increase in spindle disturbance was observed in nonflight samples; (2) a nonflight experiment showed that even after a 6-hour colchicine pretreatment of roots only a slight increase in spindle effect appeared; and (3) the microtome sections of root tips from the flight control, fixed at the zero hour and not pretreated with any chemicals, also showed similar abnormal nuclei at an average rate of about 4.2 cells per root. However, from these results we cannot rule out the possibility that colchicine might have enhanced the effects of some factor or factors associated with the Biosatellite flight that produced the abnormal spindles, and that this effect is further enhanced by exposure to gamma radiation. The probability of flight-level vibrations contributing to these effects is low (Knepton, 1966), and in our experiments no significant response was observed when *Tradescantia* plants were subjected either to system (whole capsule) or component (single package) vibration stresses. Weightlessness, then, is the most probable spaceflight factor responsible for these effects.

The *Tradescantia* Biosatellite II experiment has given results indicating either enhanced, antagonistic, or unchanged effect with or without ionizing radiation depending on the *Tradescantia* tissue or end point used. Variable responses also have been observed in other organisms flown in Biosatellite II and are described elsewhere (Saunders, 1968).

SUMMARY AND CONCLUSIONS

The Brookhaven experiment on board Biosatellite II was designed to determine the effects of weightlessness and other spacecraft environmental conditions on spontaneous and radiation-induced mutation rates and on cytologic changes in the higher plant, *Tradescantia* clone (02). This hybrid clone of *Tradescantia* has a $2n$ chromosome number of 12; it is heterozygous for flower color; and it has a high mutation rate of the loci determining flower color. During the 2-day Biosatellite II flight, 32 young plants were arranged in a plastic housing so that the flower buds were exposed to 223 R of gamma rays and the roots, immersed in nutrient solution, were exposed to radiation levels from about 116 to 285 R. Thirty-two flight control plants were flown in a package in the spacecraft behind the radiation shield and identical nonflight control packages (with and without irradiation) were maintained at the Cape Kennedy launch site. The spacecraft environment was rigorously controlled

and its conditions were monitored throughout the flight with similar conditions maintained in a vehicle held at the launch site. The temperatures were between 65 and 70 F in the *Tradescantia* packages and the relative humidity was about 55 percent. The gravitational force was maintained at about 10^{-5} *g* during the irradiation phase of the 30-orbit flight. Telemetered and onboard records were made of levels of vibration, shock, acceleration, etc. for use in subsequent flights and nonflight tests. Immediately after retrieval of the spacecraft near Hawaii, samples of root tip, ovary, and stamen tissues were collected. These and the intact plants were flown to Brookhaven for observations on the following end points: (1) Somatic mutation (blue to pink or colorless cells); (2) cell size (giant and dwarf condition); (3) loss of reproductive integrity (cell death and stunting in stamen hair growth); (4) pollen grain mortality (early and late stages); (5) megaspore development; (6) abnormal cell divisions; and (7) chromosome aberrations. Results to date are summarized in table 4 and indicate no effect of spaceflight factors on spontaneous levels of somatic mutation, pollen abortion, stamen hair stunting, embryo sac abortion, and chromosome aberration. An enhanced deleterious effect in flight samples attributed to weightlessness was noted, however, in the mitotic spindle mechanism in microspores, megaspores, and root tip cells. This latter observation of an effect associated with a spindle malfunction concurs with the Soviet scientists' findings in orbited *Tradescantia* microspores. Interaction of ionizing radiation with spaceflight factors was expressed in various patterns. Established nonflight radiation-induced somatic mutation rates in general were unaffected by flight factors, with the exception of the pink stamen hair cell mutation which exhibited an antagonistic response to flight factors. This discrepancy is at present unexplained, but is not considered a valid spaceflight effect. Enhanced interactions between radiation and spaceflight factors were observed in pollen abortion, micronuclei frequency in pollen, and stamen hair stunting. These observations suggest increased injury during the more sensitive stages of meiosis and mitosis.

Clearly differences exist between flight and nonflight samples both with and without irradiation. The significance and possible mechanisms for these effects are being considered further in continuing nonflight tests in an effort to assess better the possible hazards to living systems of the new spectrum of stresses encountered during orbital or free flight.

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The Effect of Weightlessness on the Growth and Orientation of Roots and Shoots on Monocotyledonous Seedlings

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The force of gravity is inescapable because of the attraction between the Earth and objects on or near its surface. Gravity is unique among factors of the biologic environment in that its force may be increased in the laboratory but cannot be decreased for any appreciable length of time. Only the development of spaceflight with the obvious problems of chronic acceleration of launch, the free fall of orbital flight, and the fractional *g* forces of the Moon and other planets has given impetus to studies of the physiologic response to gravity or its absence. (The varied attempts to simulate weightlessness were reviewed by Wunder, Duling, and Bengele (1968a).)

Although it was not possible to eliminate gravity before the development of spaceflight, it has been possible to nullify in the laboratory some of its effects on living organisms. Slow rotation in a horizontal axis compensates for the response to gravity in any single direction so that over a period of time the pull of gravity acts equally in all directions about the axis of rotation. An instrument for thus nullifying gravity is known as a clinostat (Sachs, 1882) and has been widely employed by botanists since 1806 (Knight, 1806).

The history of the use of the clinostat in plant physiology has been reviewed by Larsen (1962). It has rarely been employed by zoologists since its use would require restraint of the animal, and restraint itself is an important physiologic stimulus. The clinostat has an important difference from true free fall in that the ever-changing direction of gravitational pull imparts a rotational movement to all fluid components of the organism including the cytoplasm and the organelles of the cell. This "stirring action" of rotation means that the clinostat does not simulate free fall at the subcellular level. One of the important objectives of the flight of Biosatellite II was to determine whether free fall or weightlessness is adequately simulated by the clinostat.

Accelerative forces of almost any magnitude can be produced easily in the laboratory by means of a centrifuge. Such forces have been applied widely to the analysis of cell and tissue components. Much more rarely, chronic acceleration has been used to alter the normal biologic environment of living organisms. Up to 1955 when Wunder at State University of Iowa and Gray and Edwards at Emory University began to work with the physiologic responses in animals and plants, respectively, only sporadic papers had appeared on the subject. Early work in this field has been reviewed by Wunder, Lutherer, and Dodge (1963), Gray and Edwards (1965), and Wunder, Duling, and Bengele (1968b).

The clinostat and the centrifuge have been used chiefly as tools for the study of specific physiologic processes rather than for the study of weightlessness or increased gravity *per se*. Biosatellite II was the first organized attempt to provide a controlled, weightless environment for a wide variety of living

organisms. The flights of Biosatellite II and its successors mark the beginning of several new fields for biologic study.

When the possibility of placing germinating wheat seedlings in the weightlessness of an orbiting satellite was first proposed in 1963, the following hypotheses were established on the basis of our earlier work with wheat seedlings subjected to supranormal gravity (fig. 1).

(1) The true weightlessness of orbital flight would alter the growth and shape of wheat seedling organs so that: (a) shoots and perhaps roots would grow faster than at normal gravity; (b) coleoptiles would become more elliptic in cross section; (c) orientation of shoots and roots would become random in the absence of geotropism; and (d) dry weight would decrease.

(2) These changes would be reflected at the cellular level by alterations in: (a) The position of statolith starch grains; (b) the pattern of mitosis and cell elongation; (c) the localization of enzymes in cells and tissues; and (d) the biochemistry of the seedlings.

(3) The true weightlessness of orbital flight is not identical with the simulated weightlessness of the clinostat.

The evolution of the actual flight plan caused changes in evaluations of the hypotheses as originally proposed. It proved impractical to grow the seedlings in a substrate of sand or vermiculite, hence only indirect comparison could be made of growth of roots and shoots with our own earlier

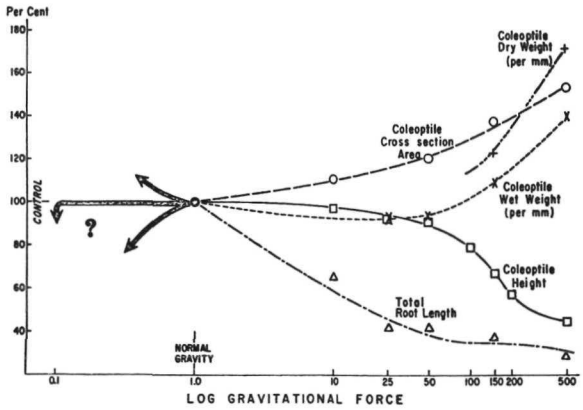


FIGURE 1.—Responses of wheat seedlings to changes in gravity.

experiments. Also, no seedlings could be spared for dry and wet weight determinations if other requirements were to be met.

Certain other inescapable characteristics of the flight became apparent early in the project. Seeds would have to be planted well before launch. They would thus become oriented to Earth's gravity for about 12 hours (15 hours in actual flight) before reaching the weightlessness of orbital flight. Recovery would require about 5 hours from deorbit to actual measurement in the laboratory. During this time the living plants would be subject to normal Earth gravity. Launch of Biosatellite II entailed considerable vibration, the effects of which were entirely unknown before the flight. No experience or literature existed to prepare us for the effects of this aspect of flight. It was not considered in the original hypotheses, but proved to be an important factor in the growth and development of the seedlings.

EXPERIMENT DESIGN AND METHODS

The Wheat Seeds

Wheat seedlings were used in this experiment because of their convenient size, their rapid and consistent germination, and their measurable geotropic response. In addition, the authors had previously determined their response to chronic acceleration up to 500 *g* (Gray and Edwards, 1955; Edwards and Gray, 1956) and grass seedlings have been used in plant hormone research. The regularity of the numbers and orientation of seedling roots of wheat was especially desirable for another of the experimenters, Dr. Charles J. Lyon (see paper by Lyon, p. 167). Figure 2 shows the typical appearance of a germinating wheat seedling.

The specific wheat used in all of the tests and on the flight itself was a hexaploid, soft, red, winter wheat, *Triticum vulgare* (Vill.) Host, hort. var. Georgia 1123. It may also be designated as *Triticum aestivum* L. em. Thell. subsp. *vulgare* (Vill.) Host (MacKey, 1954). Georgia 1123 was developed at the Georgia Agricultural Experiment Station (Gore and Stacy, 1961). Seeds of the 1964, 1965, and 1966 crops were used in the tests; 1966 seeds were used for the flight and its Earth controls. They were grown in Jefferson County, Georgia, by Mr. W. M. Pritchard. The untreated seeds were stored in bulk at 45 F at Emory University until needed.

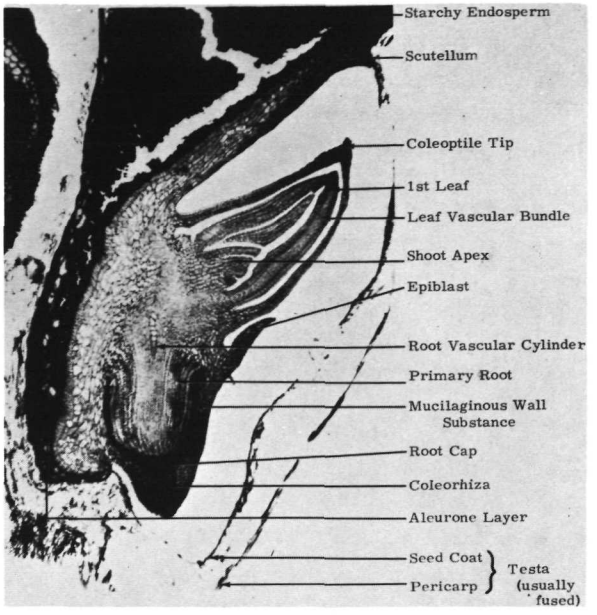


FIGURE 2.—Embryo of wheat (*Triticum vulgare* L., variety Georgia 1123) 9 hours following initiation of soaking period lasting 3 hours.

The Flight Package

The flight package and associated hardware were designed by the experimenters in collaboration with engineers at NASA Ames Research Center and North American Rockwell Corp. (formerly North American Aviation Co.). The fabrication of the packages was by North American Rockwell Corp. The flight package consisted of three cylindrical, nontoxic plastic chambers about 3 inches in diameter and 6½ inches in height, each containing a seed-holding stalk placed in its long axis. A fourth, larger chamber contained three similar seed-holding stalks. The chambers were fixed to a common base and had individual lids with gaskets to close them (fig. 3). Each had an insulating blanket to eliminate light and minimize heat loss. In order to identify each seedling adequately, the chambers were numbered as in figure 4. The polycarbonate plastic stalks were hollow with angled side arms spirally arranged at 120° angles so that each seed, when affixed, had the maximum amount of space in which to grow without its roots touch-

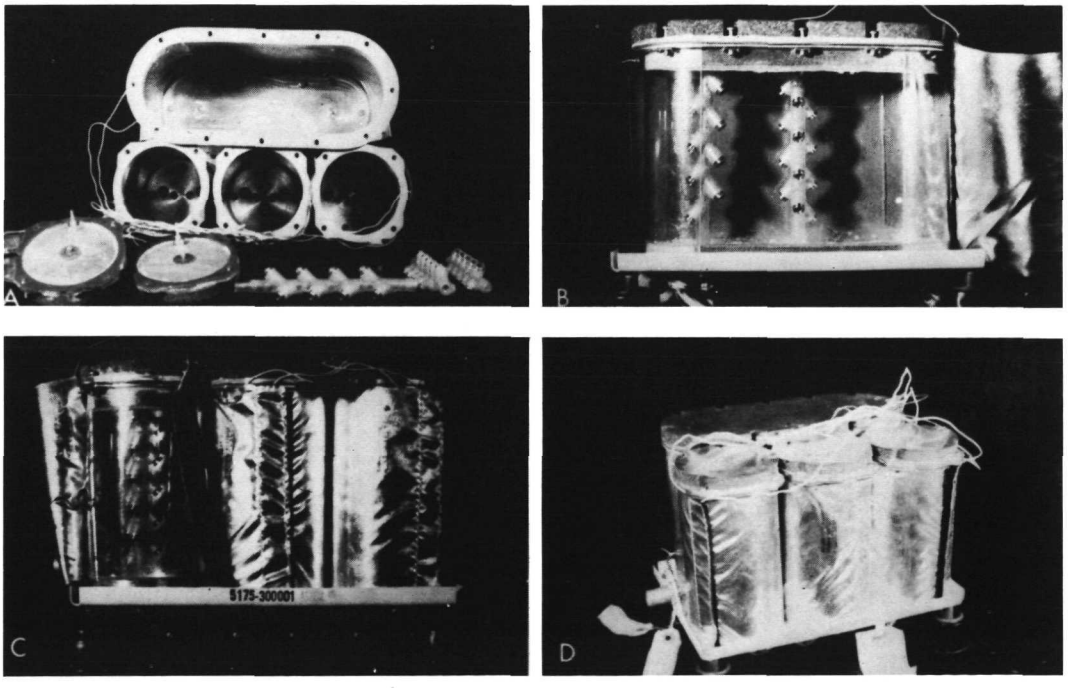


FIGURE 3.—Wheat seedling package (prototype). (*a*) Open package viewed from above with seed stalks. (*b*) Side view of larger chamber with insulating blanket peeled back to show stalks in place. (*c*) Side view showing small chambers. Blanket removed from chamber II. (*d*) Package with lids in place ready for delivery to engineers.

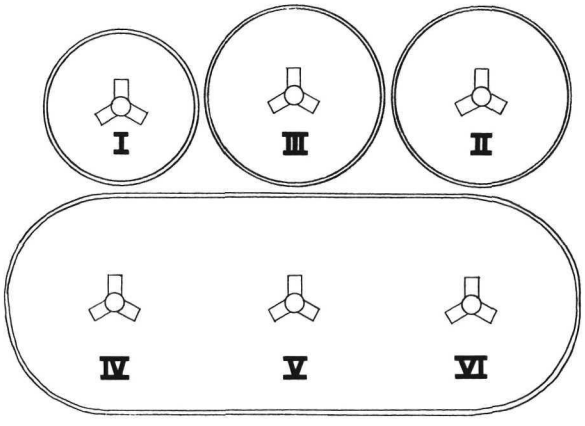


FIGURE 4.— Diagram of chambers showing position and numbering of stalks. Note that chambers I and II held 15-arm stalks and were the chambers into which fixative was sprayed on command at 58 hours.

ing another seedling or the chamber wall (fig. 5). Fifteen seeds each were carried on two of the seed stalks, and 12 seeds each on the other four stalks. The seedlings each were numbered according to their position on the stalk. Two of the small chambers were fitted with cylinders below containing fixative to be released in flight. Each chamber contained a thermistor for recording temperature. All growth took place in darkness. Five such packages and one prototype package were used for the flight of Biosatellite II and the Earth controls.

Selection, Sterilization, and Soaking of Seeds

Dry seeds were selected for size (38 to 39 mg), plumpness of the embryo, and absence of visible injury. They were rinsed for 30 seconds in a solution of 0.05 percent mercuric chloride and then soaked in deionized water at 90 F for 3 hours. During this time air was bubbled through the water by means of an aquarium pump adjusted so that the seeds were agitated gently by the stream of bubbles. At the end of 3 hours, the soaked seeds were again selected for quality under a magnifying lens and set out in petri dishes for planting.

Planting Procedures

PREPARATION OF THE STALKS

Prior to planting, the seed-holding stalks were cleaned and dried and each arm was capped with rubber dam (Davol 1350 Bandage Gum) secured by dental ligatures (S. S. White number 241). The stalks were then filled with Zonolite vermiculite (Zonolite Division, W. R. Grace Co., Atlanta, Ga.) which had been screened to pass a number 40 mesh sieve but to be retained by a number 80 mesh sieve. Slits to receive the seeds were burned in the rubber diaphragm with a modified leather burning tool. The stalks were then injected with deionized water so that the vermiculite was wetted and there were no visible air pockets. In order to insure thorough wetting, the prepared stalks were then immersed in deionized water for 24 hours before the seeds were inserted. Planting was accomplished by in-

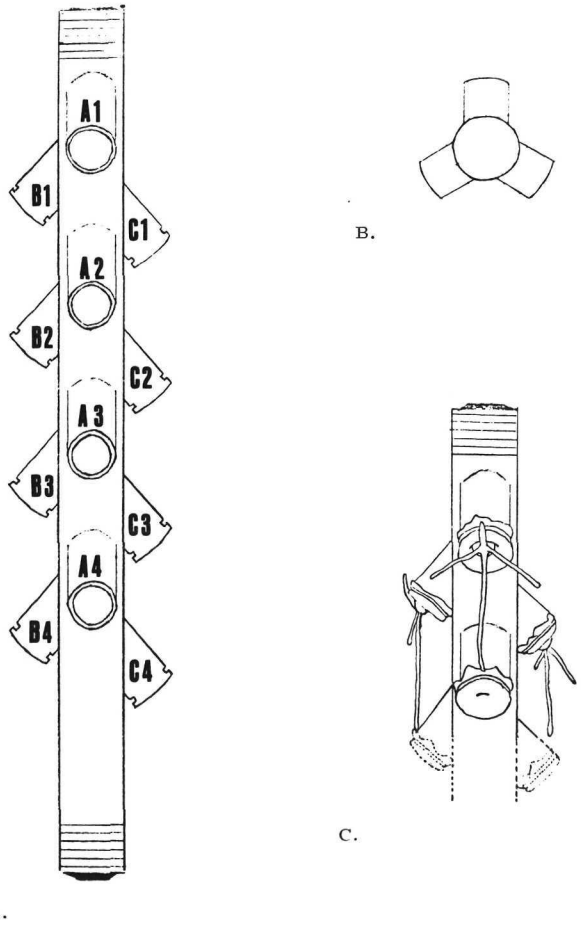


FIGURE 5.— Wheat seedling stalk. (*a*) Stalk with 12 arms showing positions numbered to illustrate method of identifying seedlings. (*b*) Stalk viewed from above showing position of arms at 120° angles (*c*) Diagram of seedlings held in rubber diaphragms covering stalk arms.

serting the previously soaked seeds into the slits in the rubber diaphragm with the embryo to the outside (fig. 5). The seeds were sealed to the rubber by applying a warm mixture of lanolin and beeswax at the crease of the seed.

CLOSURE OF THE PACKAGE

Planted stalks were placed in the package chambers, and, in order to insure moist air, free water was added to the chambers (2 ml to chambers I, II, and III, and 5 ml to the large chamber). The atmosphere was that of sea level at Cape Kennedy. The chambers were sealed by screws through the lids.

The flight package and two control packages were prepared simultaneously. Forty-five minutes elapsed between the start of planting and closure of the packages. Two more control packages were similarly planted 4 hours later than the first group.

Methods of Fixation and Measurement of Seedlings

METHOD OF FIXATION IN FLIGHT

Chambers I and II of the flight package were equipped to fix the seedlings on orbit upon telemetered command. Confirmation of fixation was reported. Earth control packages, similarly equipped, were actuated directly. Two cylinders, each with a capacity of 25 ml, were arranged to discharge their contents through nozzles in the floor of the chamber to which they were attached so that a fine spray of fixative bathed the seedlings. The firing of a squib activated a plunger which discharged the fixative at high presure through the nozzles. Seedlings in the other chambers were returned from flight unfixed and alive. Although fixatives containing mercuric chloride gave good results, they produced severe corrosion of the stainless-steel fixative cylinders and their fittings. The fixative which finally evolved was a formalin/acetic-acid/alcohol mixture (FAA) containing dimethyl sulfoxide (DMSO) to increase penetration of tissues. The formulas consisted of 50 ml 95 percent alcohol, 7.0 ml glacial acetic acid, 10.0 ml neutral formalin, 0.3 ml DMSO, and 33.0 ml distilled water.

MEASUREMENTS

Measurements of coleoptile height and root length were taken without removing the seedlings from the seed stalk. Measurements were made to the nearest half millimeter (coleoptile) or the nearest millimeter (roots) by an experienced operator using a steel rule graduated to half millimeters.

Coleoptile diameters could not be measured directly because of time limitations. They were measured by an ocular micrometer on front and side views from photographs of the seedlings taken by Dr. Charles J. Lyon (see p. 167).

POSTRECOVERY PROCEDURES

Because of the many constraints existing, rapid and accurate teamwork involving three groups of experimenters was required at recovery of the flight seedlings at the Hickam Field laboratory and of the control seedlings at Cape Kennedy. The seedlings were photographed and measured, and, in addition, the following problems were considered:

(1.) Dehydration. The seedlings were sprayed with water while they were exposed to room air.

(2.) Position. Living seedlings were fixed as soon as possible after removal from their experimental position.

(3.) Light. Exposure to light was kept to a minimum.

(4.) Preservation. Seedlings were subject to various preservation techniques with the least possible delay.

The required conditions were met by virtue of long practice over several years of preflight testing, during which the time required for recovery procedures was reduced from several hours to about 30 minutes for each package.

Postrecovery Disposition of Seedlings

We were allotted 19 of the seedlings fixed in flight and 14 of the seedlings returned alive. Matching seedlings were selected from each of the control packages so that comparisons of seedlings of the same coleoptile height could be made. A total of 171 seedlings was available to our Emory University team from the flight and the Earth controls.

These seedlings were distributed as follows:

Group I — Seedlings spray-fixed in orbit with FAA plus DMSO were immersed for several hours in FAA, then postfixed for 24 hours in chromium trioxide/acetic-acid/formalin (CRAF).

Group II — Seedlings recovered alive, fixed by immersion in FAA plus DMSO, then postfixed in CRAF.

Group III — Seedlings recovered alive and fixed for 24 hours in Lewitsky's fixative to preserve lipids.

Group IV — Seedlings recovered alive, embedded in OCT Lab-Tek embedding medium (−15 to −30 C), and frozen in dry ice for histochemistry and electrophoresis.

Group V — Seedlings recovered alive, planted in potting soil, and grown to maturity.

Earth Controls and Baseline Testing

Control and baseline data were accumulated over several years at Emory University and during tests at NASA Ames Research Center, Moffett Field, California; North American Rockwell Corp., Downey, California; General Electric Co., Philadelphia, Pennsylvania; and Cape Kennedy, Florida. Many useful baseline data were obtained from hardware qualification tests at these laboratories. Development work at Emory University over 4 years was devoted to establishing standards for fixation, staining, histochemistry, and electrophoresis of seedlings, as well as to perfecting planting and recovery methods.

POSTFLIGHT TESTS

Three postflight tests were performed at the Ames Research Center in order to simulate conditions which could be determined only after the Biosatellite II flight. These were: Phase A, radiation profile, February 1968; phase B, temperature profile, March 1968; and phase C, vibration profile, May 1968. We did not participate in the phase A test and only the phase C test yielded information useful for our experiment.

BIOSATELLITE II EARTH CONTROLS

The simultaneous Earth controls for the flight of Biosatellite II consisted of four packages, identical with the flight package, grown at Cape Kennedy and one prototype package grown at Hickam Field. (The Hawaii erect control package provided erect control seedlings of matching size to freeze in the same blocks with seedlings from the flight package for histochemical study. It was not otherwise used for control data.)

Germination and Survival

An unknown environment at once suggests the possibility of lethal factors. None were encountered in the Biosatellite II flight. Three seeds in the flight package failed to germinate, and three seeds in each of the Earth control packages similarly failed to grow (table 1). The germination rate of 96.15 percent is comparable with that obtained in preflight tests using the 1966 seeds.

Three seedlings recovered from the flight packages were planted in potting soil in Hawaii and brought back to the Georgia Agricultural Experiment Station where the strain Georgia 1123 was first developed. They were repotted and grown by Dr. U. R. Gore in a greenhouse under light and temperature conditions optimal for heading. Al-

FIGURE 6.— Three flight seedlings 131 days after launch. These seedlings were grown to maturity at the Georgia Agricultural Experiment Station where the strain (Georgia 1123) was developed.

TABLE 1. – Germination of Flight and Earth Control Seedlings

Package	Germination, %		
	Stalks I and II	Stalks III and VI	Total
Flight	27/30=90.00	48/48=100.00	96.15
Erect control	55/60=91.67	95/96= 98.96	96.15
Clinostat control	58/60=96.67	92/96= 95.83	96.15

though germination had taken place at markedly higher temperatures than usual and the season of the year was wrong, the three plants tillered out and headed about 9 months later. Seeds harvested from these normal-appearing plants will be planted to be sure that they have sustained no genetic injury. Figure 6 shows the mature plants, and figure 7 shows the seed head produced.

Changes in Gross Morphology

CHANGES IN ORIENTATION OF SEEDLING ORGANS

The changes in orientation of roots and shoots when seedlings are confronted with the free-fall state is covered by the report of Dr. Charles J. Lyon who was specifically concerned with this aspect of the experiment (see p. 167).

PATTERN OF SEEDLING MALFORMATIONS

The most frequent malformation is the absence of one or both lateral roots. Coleoptiles and primary roots are less often absent. Only four seedlings in the flight package were missing one or more of the four normal seedling organs. Three were missing one lateral root, and one had a primary root but no coleoptile or lateral roots. This amounted to 5.3 percent of the 75 seedlings which germinated. In contrast, there were 12 percent malformed seedlings among the erect control plants and 10 percent among the clinostat controls. The malformations encountered in each package are shown in table 2.

Seedling Growth

CONSISTENCY OF GROWTH IN DIFFERENT CHAMBERS

A cursory examination of the plants recovered alive from the flight and Earth control packages suggested that the shortest coleoptiles were those grown in chamber III and the tallest were those grown in stalk V in the large chamber. To verify this, growth in the four stalks of each chamber was ranked; 1 was assigned to the stalk with the greatest average growth and 4 to the stalk with the least average growth. The distribution in each package by rank is shown in table 3. The differences which appear are not statistically significant. Nevertheless, they may represent real variations in the experimental conditions.

FIGURE 7.– Wheat seed heads from flight seedling. Mature heads show normal seed formation.

TABLE 2. – Incidence of Developmental Failure of Seedlings

Package	Number failing to grow	Number malformed									Total malformed
Flight	3		2	1						1	4
Erect control:											
I	3	1	1	2	3		2				9
III E	3	3		3		1		1		1	9
Clinostat control:											
II	3	1	1						1		3
III C	3	4	3		1	2				2	12

TABLE 3. – Growth Rank in Relation to Position of Seed Stalk Within the Package; $\chi^2=3.600$; $P<0.30$

Package	Chamber III, Stalk III	Large chamber		
		Stalk IV	Stalk V	Stalk VI
Flight	3	4	1	2
Control:				
I E	4	1	2	3
II C	4	1	3	2
III E	3	4	1	2
III C	3	4	1	2
Sum of ranks	17	14	8	11

From analysis of the gas in the chambers after recovery, oxygen consumption per seedling was 0.43 cc in chamber III and 0.75 cc in the large chamber (Conrad, 1968). The volume of air per plant was 77 ml in chamber III and 102 ml in the large chamber, hence a possible decrease in the partial pressure of oxygen was considered (see table below). There is no evidence that the plants in chamber III were deprived of oxygen. Why they consumed 57 percent less oxygen than and grew only 96.7 percent as tall as plants in the large chamber is not known.

	Chamber III	Large chamber
Volume of air per plant, ml	77.00	102.00
Volume of O_2 per plant (21%), ml	16.17	21.42
O_2 consumed per plant, ml	—.43	—.75
Residual O_2 volume per plant, ml	15.74	20.67
Residual O_2, %	20.44	20.26

CORRECTION OF MEASUREMENT FOR TIME

Measurements of flight and control plants were taken from seedlings fixed in flight or on the Earth at approximately 58 hours after soaking was started (43 hours of flight) and at the end of the flight approximately 60 and 65 hours after soaking. By determining the rate of growth between the actual times of measurement, the sizes of seedling organs were corrected to 58 and 65 hours. In the case of control II the correction amounted to nearly 5 hours and the resulting corrected size cannot be considered to be valid. In other cases the corrections were small. The computations for correction to 65 hours are shown in table 4.

TABLE 4. – Correction to 65 Hours[a]

Package	Time, min	Rate, mm/hr	Amount to be corrected, mm
		Coleoptile height	
Flight	+4	0.1513	+0.01
Control:			
I	−2	.1156	−.004
II	+285	[b].1396	[b]+.66
III E	−81	.1102	−.15
III C	−7	.1740	−.02
		Primary root length	
Flight	+4	1.9031	+0.13
Control:			
I	−2	1.8587	−.062
II	+285	[b]4.4138	[b]+20.97
III E	−81	1.7235	−3.68
III C	−7	1.9463	−.23

[a]During the period involved, lateral root growth is not constant with time. Corrections for time cannot be made by the above method.

[b]Rate calculated on 58 = to 60 = hour measurements. Extrapolation is manifestly absurd in the case of the primary root.

TABLE 5. – Specimens Excluded From Means

Package	Specimens excluded[a]	
	At 58 hr	At 65 hr
	Coleoptiles	
Flight	IA2, B3, B5; IIA5	None
Control:		
I	IB1, C3; IIA4	IIIA1; IVC3
II	None	VA1, C1; VIA1, B4
III E	IA2, C3; IIB5	IIIC4
III C	IA4; IIB4, C1	IVC2
	Primary roots	
Flight	IB3, B5; IIA5	None
Control:		
I	IC3; IIA4, B1	IIIA1, C2; IVC1
II	None	VA1, C1; VIA1, A4, B1, B4
III E	IA2, C3; IIB5	IIIB3, C4
III C	IA3, A4; IIC1	IIIB4, IVC2

[a]Roman numeral, stalk; letter, row; arabic numeral, position of seed.

CORRECTION FOR DEFECTIVE SEEDLINGS

There is no evidence that orbital flight or the clinostat produced any increase in seedling mortality; hence, seeds which failed to grow were not averaged with live seedlings. If a coleoptile or a root was absent, the plant was excluded when the average size of that organ was computed. Measurements of growth are thus based on data for organs which actually grew. The specimens excluded from the averages are shown in table 5.

DEFINITIVE MEASUREMENTS OF SEEDLING ORGANS

Coleoptile and Primary Root

After adjustment for time of measurement from table 4 and the exclusion of plants with defective growth listed in table 5, the definitive organ measurements and their standard deviations were calculated. Coleoptile heights at 58 and 65 hours of age are shown in tables 6 and 7. Primary root lengths for the same times are shown in tables 8 and 9.

TABLE 6. – Coleoptile Height at 58 Hours

Package	Age from soaking, hr:min	Number of plants	Mean measurement, mm	Mean corrected to 58 hr, mm	Standard deviation
Flight	57:52	26	3.08	3.10	±0.743
Erect control:					
I	58:02	27	2.78	2.77	±0.497
III	57:53	27	2.91	2.92	±0.721
Clinostat control:					
III	57:55	26	2.67	2.69	±0.619
II	58:00	30	3.30	3.30	±0.702

TABLE 7. – Coleoptile Height at 65 Hours

Package	Age from soaking, hr:min	Number of plants	Mean measurement, mm	Mean corrected to 65 hr, mm	Standard deviation
Flight	64:56	48	4.15	4.16	±0.871
Erect control:					
I	65:02	46	3.59	3.58	±0.874
III	66:21	47	3.84	3.69	±1.008
Clinostat control:					
III	65:07	47	3.93	3.91	±0.943
II	60:15	44	3.61	(4.28)	±0.986

TABLE 8. – Primary Root Length at 58 Hours

Package	Age from soaking, hr:min	Number of plants	Mean length, mm	Mean length corrected to 58 hr, mm	Standard deviation
Flight	57:52	27	8.09	8.35	±3.230
Erect control:					
I	58:02	27	9.11	9.05	±3.797
III	57:53	27	10.41	10.61	±4.544
Clinostat control:					
III	57:55	27	7.26	7.42	±3.089
II	58:00	29	11.38	11.38	±2.352

TABLE 9. – Primary Root Length at 65 Hours

Package	Age from soaking, hr:min	Number of plants	Mean length, mm	Mean length corrected to 65 hr, mm	Standard deviation
Flight	64:56	48	21.54	21.67	±6.673
Erect control:					
I	65:02	45	22.12	22.06	±6.673
III	66:21	46	25.00	21.32	±6.471
Clinostat control:					
III	65:07	46	21.27	21.05	±5.442
II	60:15	42	21.31	(42.26)	±8.212

Lateral Roots

The two lateral roots of the wheat seedlings do not follow the growth pattern of the coleoptile and the primary root. Their elongation starts some time after their appearance so that the distribution of their lengths is skewed to the left. The picture of their growth is best described by the percentage which have started to elongate and the mean length attained by them, rather than by using a simple average length (table 10).

In both flight and clinostat control III seedlings only about half of the lateral roots had begun to elongate at 58 hours, whereas in erect control III more than three-fourths had elongated, and the elongation was proportionately greater. In erect control I the lateral root growth resembled that seen in the clinostat and flight packages rather than that seen in erect control III.

TABLE 10. – Lateral Root Length at 58 Hours

Package	Percent elongating	Mean length, mm
Flight	50.0	3.89
Erect control:		
I	59.2	2.76
III	86.7	5.45
Clinostat control:		
III	43.3	2.73
II	79.7	4.56

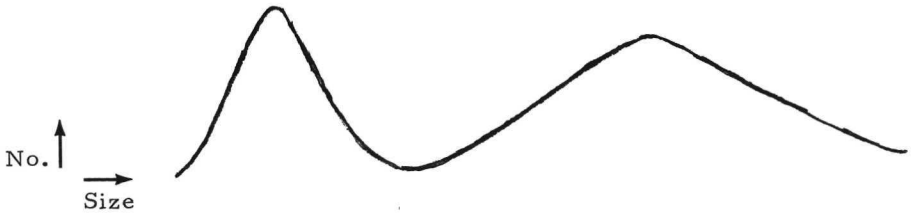
By 65 hours the lateral roots of seedlings in all packages show a bimodal distribution of lengths. The smaller mode is at 1 to 2 mm, and the larger is at 13 to 16 mm. With such a distribution an average root length is meaningless and these lengths cannot readily be corrected for differing times of measurements. With increasing age there is a decrease in the number of roots in the smaller mode, whereas more root lengths appear in the larger mode. These relationships are shown in table 11.

Although roots of flight seedlings which have appreciably elongated are longer than similar roots in the control packages, the differences are statistically significant only between flight and erect control III seedlings. This difference must be discounted by the fact that the latter are 85 minutes older than the flight seedlings.

Coleoptile Diameters

Coleoptile diameters were measured with an ocular micrometer in a dissecting microscope at a specific level of the coleoptile from photographs of the seedlings taken by Dr. Charles J. Lyon. The coleoptile is elliptic in cross section; both large and small diameters were measured. At the age of 58 hours, no consistent differences in the diameters or in their ratios could be observed. Clinostat control II seedlings had diameters larger than did the others. Table 12 shows the average value obtained. Both large and small diameters of the coleoptile had increased by 65 hours of age. No difference could be observed between coleoptiles from the flight package and the two erect control packages. Coleoptiles from both the clinostat packages were larger in both diameters and were more elliptic in cross section than were coleoptiles from flight and erect control packages (table 13).

TABLE 11. – Size Distribution of Lateral Roots at 65 Hours



Package	0 to 4 mm		4.5 mm and over		Number of roots
	Mode, mm	Percent in mode	Mode, mm	Percent in mode	
Flight	1	30.2	16	69.8	96
Erect control:					
I	2	30.8	13	69.2	94
III[a]	1	10.5	13	89.5	95
Clinostat control:					
III	2	24.2	13.5	75.8	95
II[b]	1	24.5	15	75.5	94

[a]66:21 hr old.
[b]60:15 hr old.

TABLE 12. – Coleoptile Diameters at 58 Hours

Package	Average large diameter, mm	Average small diameter, mm	Ratio of average diameters
Flight	1.31	0.94	0.71
Erect control:			
I	1.32	.89	.68
III	1.28	.92	.72
Clinostat control:			
III	1.27	.90	.69
II	1.42	.96	.68

TABLE 13. – Coleoptile Diameters at 65 Hours

Package	Average large diameter, mm	Average small diameter, mm	Ratio of average diameters
Flight	1.37	0.98	0.71
Erect control:			
I	1.33	.95	.71
III	1.38	.98	.71
Clinostat control:			
III	1.41	.96	.69
II	1.43	.97	.68

TABLE 14.— Coleoptile Height at 58 Hours: Significance of Differences Between Flight and Control Packages

Values of *P*

Package	Flight (26 plants)	Erect control I	Erect control III	Clinostat control III
Erect control I (27 plants)	0.10			
Erect control III (27 plants)	0.40	0.50		
Clinostat control III (26 plants)	0.05	0.95	0.30	
Clinostat control II (30 plants)	0.30	0.005	0.05	0.005

TABLE 15.— Coleoptile Height at 65 Hours: Significance of Differences Between Flight and Control Packages

Values of *P*

Package	Flight (48 plants)	Erect control I (46 plants)	Erect control III (47 plants)
Erect control I (46 plants)	0.005		
Erect control I (48 plants)	0.001		
Erect control III (47 plants)	0.02	0.50	
Erect control III (48 plants)	0.01		
Erect controls I + III (93 plants)	0.005		
Clinostat control III (47 plants)	0.02	0.025	0.30

TABLE 16.— Primary Root Length at 58 Hours: Significance of Differences Between Flight and Control Packages

Values of *P*

Package	Flight (27 plants)	Erect control I	Erect control III
Erect control I (27 plants)	0.50		
Erect control III (27 plants)	0.05	0.20	
Clinostat control III (27 plants)	0.30	0.10	0.005
Clinostat control II (29 plants)	0.001	0.01	0.50

TABLE 17.— Primary Root Length at 65 Hours: Significance of Differences Between Flight and Control Packages

Values of *P*

Package	Flight (48 plants)	Erect control I	Erect control III
Erect control I (45 plants)	0.80		
Erect control III (46 plants)	0.80	0.60	
Clinostat control III (46 plants)	0.70	0.50	0.90

SIGNIFICANCE OF THE DIFFERENCES IN MEASUREMENTS

Statistical Methods [1]

The significance of the differences between measurements of seedling organs was calculated using the student t test and the value of $2P$ obtained from the table in Documentia, Geigy (1962). The P values for differences in coleoptile height are shown in tables 14 and 15, and values for differences in primary root length are shown in tables 16 and 17. Values of $P < 0.005$ are heavily shaded and are considered significant. Values of $P < 0.05$ and $P > 0.01$ are lightly shaded and are significant at a lower level. Unshaded blocks indicate no significant difference.

The lateral roots required slightly different treatment since their lengths were distributed bimodally. The significance χ^2 was determined by the Kolmogorov-Smirnov (1 tailed) test:

$$\chi^2 = 4D^2 \frac{(N_1N_2)}{N_1 + N_2}$$

where D is the greatest difference $(y_1 - y_2)$ at any value of x. The P values were determined as before. The values obtained are shown in table 18.

Changes in Variance With Age

Variance of measurements of seedlings in a given package might be expected to increase with the passage of time if individual seedlings grew at different rates. If the rates were similar, but some seedlings started to grow sooner than others, the variance might be expected to remain constant. If seedlings which were slightly delayed in starting their growth grew faster, or if seedlings growing at different rates are reaching the same maximum size, then variance would be expected to decrease. Variance (the standard deviation divided by the mean) of the five packages is shown in table 19.

Variance of the coleoptiles is similar in all of the packages and increases with age in all but the flight package, but this difference may not be significant. Variance of the primary roots decreases with age. This is probably because elongation of the primary root slows down at about 65 hours, and those roots which started growing later have begun to overtake those which started sooner. Differences between primary roots in the different packages also decreased between 58 and 65 hours (compare tables 8 and 9). Variance of primary roots in control II, the 60-hour clinostat package, appears to have increased markedly in only 2 hours, whereas after 7 hours the variance of all the other packages had decreased. Whether this is due to the slightly higher temperature experienced by this package during the 15 hours before launch or to the specific time of measurement, we do not know.

Seedling Response to Vibration and/or Flight

EXPERIMENTAL GROUPS

In addition to data from the flight Biosatellite II and its Earth controls, data were available from three preflight vibration tests performed at Philadelphia in 1966 and from the postflight vibration test at NASA Ames Research Center in 1968. Seedlings were exposed to simulated launch vibration at various times after the start of germination and were subsequently grown erect or on a clinostat. Seedlings from the flight and from these tests

TABLE 18.—Lateral Roots at 65 Hours (Nominal): Significance of Differences Between Flight and Control Packages[a]

Package	Values of P: Flight (93 roots)
Erect control I (92 roots)	0.20
Erect control III (93 roots)	0.01
Clinostat control III (93 roots)	0.50
Clinostat control II (87 roots)	0.10

[a] χ^2 determined by Kolmogorov-Smirnov (1-tailed) test.

[1] We are indebted to Dr. Kenneth V. Anderson of the Anatomy Department, Emory University, for advice on statistical methods and their interpretation.

TABLE 19. – Changes in Variance with Age of Seedlings

Package	Variance for age—		Percent increase or decrease
	58 hrs	65 hrs	
Coleoptile			
Flight	0.241	0.210	−12.9
Erect control:			
I	.179	.243	+35.8
III	.248	.263	+ 6.0
Clinostat control:			
III	.232	.240	+ 3.4
II	.213	[a].273	+28.0
Primary root			
Flight	0.400	0.310	−22.5
Erect control:			
I	.417	.302	−27.6
III	.437	.259	−40.7
Clinostat control:			
III	.425	.256	−39.8
II	.207	[a].385	+86.0

[a]Age, 60 hr.

could be divided into the following experimental groups:

(1) *Biosatellite II flight* — Seedlings grown in orbiting Biosatellite II for 45 hours.

(2) *Vibrated clinostat* — Seedlings subjected to vibration followed by growth on a horizontal clinostat rotating at 6 rph.

(3) *Nonvibrated clinostat* — Seedlings grown on a horizontal clinostat rotating at 6 rph. (Normal clinostat controls.)

(4) *Vibrated erect* — Seedlings subjected to vibration followed by growth in the erect position.

(5) *Nonvibrated erect* — Seedlings grown in erect position. (Normal erect controls.)

GROWTH FAILURE

Carefully selected seeds of Georgia 1123 wheat have a germination rate of 96 to 98 percent. The seeds which fail to grow may be dead or merely dormant. There was no significant difference between the number of such seeds among plants in the five experimental groups. Growth failure is relatively constant and is unaffected by vibration or weightlessness (table 20).

MALFORMATIONS

During germination the wheat seedling normally produces four easily identifiable seedling organs: a coleoptile, a primary root, and two lateral roots. Failure of any one of these organs to develop will be termed "absence" of the organ, although, since the primordium is present in the embryo, dormancy or aplasia are more correct terms.

There are 16 possible configurations of the wheat seedling including the presence of all organs (normal) and the absence of all organs (no growth). Of the 14 possible malformations, five are common (more than one percent of seedlings each), five are rare, and four were not encountered in our material. All are diagrammed in table 21, which shows the distribution of malformations by types under experimental conditions.

The figures in tables 21 and 22 were obtained by combining the results of five tests grown at different times. Table 23 shows the number of malformed plants at each separate test, and table 24 shows the number of missing lateral roots at each test. Tables 21 to 24 demonstrate that in *each test* the vibrated erect group shows more malformations than do the nonvibrated erect controls. The *relative* number of malformed plants is consistent between tests although the absolute number varies.

Effects of vibration on seedling growth in the vibration tests can only be estimated since tests were performed at different temperatures and measurements were made at different ages in each test. In spite of these difficulties, the data indicate that seedlings vibrated and then grown erect showed poorer growth than did seedlings in the other experimental groups. The greatest effect was produced when seedlings were over 24 hours of age at the time of vibration (table 25).

As the ages of the seedlings differed at the time of their exposure to vibration in these tests, it is tempting to relate this age to the number of malformations produced. The amount of vibration received was not constant for all of the tests, therefore this approach may not be significant.

Histology

GENERAL HISTOLOGIC STRUCTURE

The general histology of the germinating wheat embryo was shown in figure 2. By 58 to 66 hours, the primordia of root and shoot have emerged from the seed coat and greatly elongated. The characteristic external appearance of flight, erect, and clinostat seedlings at 65 hours is shown in figure 8. The histologic structure of organs from such seedlings forms the basis of our report on this phase.

TABLE 20. – Incidence of Seedlings Showing No Growth

[201, 301, and 302 vibration tests and phase C postflight test.]

Experimental condition	Number of seeds	Number failing to grow	Percent failing to grow
Flight	78	3	3.85
Nonvibrated:			
Erect	351	14	[a]3.99
Clinostat	434	10	[a]2.30
Vibrated:			
Erect	195	5	2.56
Clinostat	195	5	2.56

[a]Significance of the difference: $\chi^2 = 1.858$; $P < 0.20$.

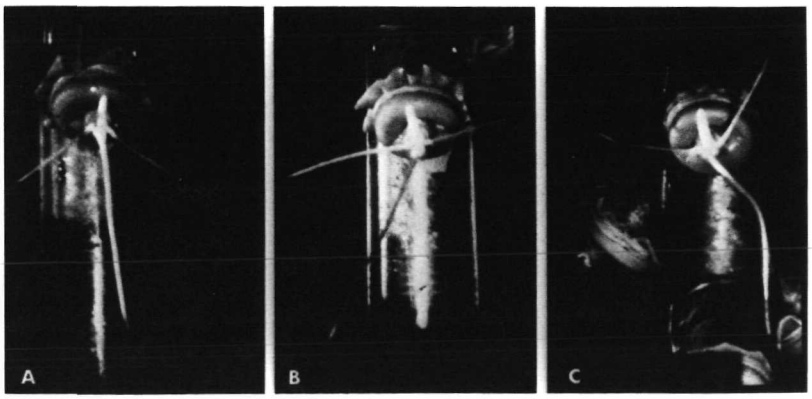


FIGURE 8.—Typical wheat seedlings from Biosatellite II flight and control specimens at 65 hours. Front view of side arms of seed stalks showing characteristic orientation of seedling organs. (*a*) Erect control EV-C4. (*b*) Flight FV-C4. (*c*) Clinostat control KVI-A1.

TABLE 21. – The Effect of Vibration and Weightlessness on the Incidence of Malformations

Experimental condition	Number alive	Percent normal	Percent malformed	Percent of specific malformation										
Flight	75	94.7	5.3	...	2.7	1.3	...	...	1.3	...	...	...	...	...
Nonvibrated:														
Erect	337	89.0	11.0	3.3	1.8	1.8	1.5	1.5	0.3	0.6	...	0.3	...	...
Clinostat	424	91.3	8.7	2.4	2.4	0.7	1.2	.9	.5	.2	0.2	...	0.2	...
Vibrated:														
Erect	190	78.4	21.6	10.5	5.3	2.1	2.6	...	1.1	...	...	...	...	...
Clinostat	195	94.7	5.3	1.6	.5	1.6	.5	.5	.5	...	...	...	...	...

Differences:

Experimental condition	χ^2	P
Erect, vibrated clinostat	4.8871	<0.05
Erect, vibrated erect	7.8386	~ 0.005

Coleoptile

The obvious shoot is the outer surface of the oval coleoptile, a sheath covering the leaves within it. The coleoptile outer epidermis is definitely outlined by an outer cell wall thickening and generally longer cells. Stomata occur in the epidermis near the coleoptile tip, and there is a small slit on the surface of this tip on the side away from the seed attachment. (Here the leaves emerge from the coleoptile at more than 85 hours of age; none had emerged in our younger seedlings.) The parenchymal layer, immediately under the epidermis, appears to be more basophilic and more active than the other layers. Within the coleoptile are two vascular bundles, one at each side. The coleoptile parenchyma varies from two to six cells in

TABLE 22. – Number and Percent of Organs Absent

Experimental condition	Number of plants alive	Lateral roots absent		Primary roots absent		Coleoptiles absent	
		No.	Percent	No.	Percent	No.	Percent
Flight	75	5	[a]3.33	0	0	1	1.33
Nonvibrated:							
Erect	337	47	[bc]6.97	10	2.97	4	1.19
Clinostat	424	46	5.42	11	2.59	4	.94
Vibrated:							
Erect	190	[b]58	15.26	5	2.63	2	1.05
Clinostat	190	[ac]14	3.68	2	1.05	1	.53

[a]$\chi^2 = 0.38$, $P>0.80$.
[b]$\chi^2 = 18.62$, $P<0.001$.
[c]$\chi^2 = 4.82$, $P>0.05$.

TABLE 23. – Number and Percent of Malformed Plants

Experimental condition	201 vibration test	Phase C postflight test	Biosatellite II flight and controls	301 vibration test	302 vibration test	Total
Flight:						
Number live seeds			75			75
Number malformed			4			4
Percent malformed			5.33			*5.33*
Nonvibrated erect:						
Number live seeds	35	76	150	38	38	337
Number malformed	9	4	18	5	1	37
Percent malformed	25.71	5.26	12.00	13.16	2.63	*10.98*
Nonvibrated clinostat:						
Number live seeds	42	153	150	40	39	424
Number malformed	7	8	15	6	1	37
Percent malformed	16.67	5.23	10.00	15.00	2.56	*8.73*
Vibrated erect:						
Number live seeds	38	77		37	38	190
Number malformed	16	10		8	7	41
Percent malformed	42.11	12.99		21.62	18.42	*21.58*
Vibrated clinostat:						
Number live seeds	39	74		39	38	190
Number malformed	1	2		4	3	10
Percent malformed	2.56	2.70		10.26	7.89	*5.26*

thickness, except that it may be thicker around the vascular elements and at the base. The thinner endodermis shows less obvious differences from the coleoptile parenchymal cells.

Leaves

From the meristematic area around the scutellar node, two or more leaf primordia rise within the sheath of the coleoptile. The first leaf has a prominent midvein bundle with usually five provascular bundles on either side. The epidermis of the leaf is distinguished by slightly thicker cell walls, although the cells are the same size in cross section as are the parenchymal cells.

Scutellum

The scutellum does not increase in size and is visible only after the seedling is removed from the seed. It is already well developed and functions to transmit nutrients from the endosperm to the growing parts of the seedling. The scutellum has an epithelium closely applied to the endosperm; its parenchymal cells closely underlie this epithelium and surround the scutellar bundle which courses

TABLE 24. – Number and Percent of Absent Lateral Roots[a]

Experimental condition	201 vibration test	Phase C postflight test	Biosatellite II flight and controls	301 vibration test	302 vibration test	Total
Flight:						
Number live seeds	.....	.....	75	.....	.....	75
Number roots absent	.....	.....	5	.....	.....	5
Percent absent	.....	.....	3.33	.....	.....	*3.33*
Nonvibrated erect:						
Number live seeds	35	76	150	38	38	337
Number roots absent	15	7	19	5	1	47
Percent absent	21.43	4.61	6.33	6.58	1.32	*6.97*
Nonvibrated clinostat:						
Number live seeds	42	153	150	40	39	424
Number roots absent	11	9	22	3	1	46
Percent absent	13.10	2.94	7.33	3.75	1.28	*5.42*
Vibrated erect:						
Number live seeds	38	77	.....	37	38	190
Number roots absent	25	13	.....	11	9	58
Percent absent	32.89	8.44	.....	14.86	11.84	*15.26*
Vibrated clinostat:						
Number live seeds	39	74	.....	39	38	190
Number roots absent	2	4	.....	6	2	14
Percent absent	2.56	2.70	.....	7.69	2.63	*3.68*

[a]Two lateral roots assumed for each seedling.

TABLE 25. – Effect of Age at Vibration on Size of Coleoptiles and Primary Roots

Seedling age at vibration, hr	Test or vehicle	Percent difference, vibrated erect from nonvibrated erect	
		Coleoptile	Primary root
11:40	201	−12.06	−12.54
13:30 to 14:30	Phase C	− 2.01	− 1.64
18:45 to 19:00	301	−45.67	−24.79
27:00	302	−64.84	−22.55

upward within the flattened scutellum (see fig. 2). The scutellar bundle passes through the scutellar nodes to become the midvein of the first foliage leaf and gives off bundles to leaf and coleoptile on either side.

Roots

The primary root and two lower, secondary roots on either side have usually developed. The upper lateral root primordia show clearly in sections, although they have not yet emerged from the coleorhiza layers which cover all roots in the germ. Each root tip is covered with a root cap of specialized cells. Within the primary root the epidermis, cortex, and pericycle can be distinguished, as well as early provascular elements and the apical meristem at the root tip.

These, then, are the morphologic elements which are present and which could be altered by experimental conditions or with flight. Our comments on histology and histochemistry will, therefore, refer to them. The various structures within flight seedlings have always been studied and compared with those of all controls. Erect and clinostat grown control seedlings from preflight tests, from the Biosatellite II flight, and from postflight tests are included in references to staining results. Unless specifically stated, statements relative to clinostat material will be assumed for all seedlings.

We are particularly indebted to the excellent monograph of Bradbury, Cull, and McMasters (1956) for an understanding of the microscopic anatomy of the wheat seedling. Our appreciation of coleoptile histology was increased by several papers of O'Brien and Thimann (1965 and 1967). The root tissues have been well described by Avers' excellent work (1959 a and b, 1961, and 1963) and that of Jensen (1963).

FIXATIVES

Seedlings were fixed for general histology as noted earlier and as shown in tables 26 and 27. Seedlings were spray-fixed at 58 hours within chambers I and II of each package with FAA plus DMSO fixative and were postfixed by immersion in CRAF fixative upon recovery. Some of the seedlings recovered alive at 65 hours were fixed by immersion for several hours in the same fixative as that contained in the package cylinders and similarly postfixed in CRAF. The seedlings were then stored in their individual labeled jars in 70 percent ethanol for holding until they could be mounted in paraffin blocks for sectioning. Others were fixed in Lewitsky's fixative preserving lipids which would be destroyed by the alcohol and acid of the other fixatives. Seedlings fixed by Lewitsky's method showed the best starch grain morphology, although the lipid stains were not entirely successful.

Great care was taken to choose seedlings of similar height in each experimental category for cutting in long, frontal, or cross section. Each coleoptile, with its base, scutellum, and base of roots, was carefully oriented in its paraffin block; its root tips were similarly oriented in a separate block, with the primary root tip and its cap left appreciably longer so as to be identifiable. (This

TABLE 26. — Fixation Schedule A

Program	Group I: 58 hr FAA spray then CRAF	Group II: 65 hr FAA immersion then CRAF	Group III: 65 hr Lewitsky	Group IV: 65 hr $KMnO_4$
Flight	19	2	3	3
Erect control:				
I	18	4	2	2
III	18	5	2	3
Clinostat control:				
III	18	4	2	2
II	18	[a]3	[a]3	[a]2
Total	91	18	12	12

[a]60-hr fixation.

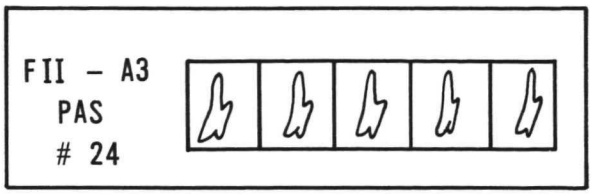
precaution proved to be very helpful since we could be sure that differences in lateral and primary root tips were, indeed, due to their relative position.)

Postflight test material was fixed exactly as were the control and flight seedlings of Biosatellite II, except that some of the phase B test seedlings were not postfixed in CRAF (see table 27). This change was made after we noted that differences existed between the appearance of amyloplasts (starch granules) of periodic acid Schiff (PAS) stained seedlings fixed in Lewitsky's fluid and those fixed in FAA plus CRAF. It was decided to fix some seedlings in FAA alone to determine if it was the chromic acid of CRAF which had affected these organelles.

One other group of seedlings in each category was immersed in potassium permanganate ($KMnO_4$) which was recommended as a good fixative for plant electron microscopy. We are still hopeful that we will be able to have our specimens sectioned and photographed.

STAINING PROCEDURES

Serial sections were cut of all seedlings. Coleoptiles were cut in long, transverse, or frontal section. Roots were cut in long or cross section. Five to eight sections were mounted on each slide, which was marked in sequence with the seedling identification as follows:



This slide can be identified as part of the seedling from:

Flight, chamber *II*

Stalk row *A*, arm position *3*

Stained with PAS (periodic acid Schiff)

*24*th slide in the series

After it had been sectioned, each seedling was stained according to the schedule shown in table 28, schedule A or B, with each slide bearing a notation as to the stain employed on it. The purpose of each particular staining technique is noted be-

TABLE 27. – Fixation Schedule B

Program	Group I: 58 hr		Group II: 60 to 65 hr		Group III: 60 to 65 hr	Group IV: 60 to 65 hr
	FAA spray then CRAF	FAA only	FAA immersion then CRAF	FAA only	Lewitsky	$KMnO_4$
Postflight test, phase B:						
Erect (65 hr)	6	4	11	5	3	1
Clinostat control:						
III (65 hr)	5	6	3	3	3	1
II (60 hr)	8	7	10	5	4	1
Total	19	17	24	13	10	3
Postflight test, phase C:						
Nonvibrated:						
Erect control (65 hr)	3	3	3	5	2	.....
Clinostat control:						
III (65 hr)	3	3	3	5	2	.....
II (60 hr)	6	5	3	4	3	.....
Vibrated:						
Erect control (65 hr)	3	3	3	5	2	.....
Clinostat control (65 hr)	3	3	3	4	2	.....
Total	18	17	15	23	11	.....

TABLE 28. – Schedule of Histologic Stains

Slide	Stain and counterstain[a]	Designed to show—
	Schedule A: FAA fixative (formalin, acetic acid, and alcohol plus 0.3% DMSO)	
1	Periodic acid-Schiff/fast green (PAS/FG)	Total carbohydrate content: polysaccharides and starch
2	Hematoxylin/fast green	Nuclear detail
3	Methyl green/pyronin	DNA/RNA
4	Toluidine blue O	Lignin
5	(Blank)	
6	Methyl blue/eosin	Nuclear/cytoplasmic detail (to compare with 2, 11)
7	Altmann's acid fuchsin	Mitochondria (to compare with 11)
8	Feulgen/fast green	DNA (quantitative)
9	Control (or blank)	
10	PAS/FG	See 1 above
11	Ferric hematoxylin/eosin	Mitochondria and chromatin
12	Azure B	DNA/RNA (check on 3)
13	Toluidine blue O	See 4 above
14	(Blank)	
15	Alloxan-Schiff	Total protein
16	(Blank)	
17	Feulgen/FG	See 8 above
18	Control:	
	Iodine	
	PAS/diastase	Controls for 1, 10
	Minus pectin	Cell wall constituents control for 1, 4
19[b]		
	Schedule B: Lewitsky's fixative (chromium trioxide and neutral formalin)	
1	Periodic acid-Schiff/fast green (PAS/FG)	Total carbohydrate content: polysaccharides and starch
2	Hematoxylin/fast green	Nuclear detail
3	Methyl green/pyronin	DNA/RNA
4	Toluidine blue O	Lignin
5	Controls for:	
	Acid hematein	
	Orange G	
	PAS	
6	Acid hematein	Phospholipid localization
7	Orange G	Phospholipid localization
8	Feulgen/fast green	DNA
9	Nile blue	Total lipid localization
10	PAS/FG	See 1 above
11	Ferric hematoxylin/eosin	Mitochondria and chromatin
12	Azure B	DNA/RNA
13	Toluidine blue O	See 4 above
14	Control	
15	Acid hematein	See 6 above
16	Orange G	See 7 above
17	Feulgen	See 8 above
18	(Blank)	

[a]Blanks to be saved for additional controls or new techniques for special structures.
[b]Begin repeat as above 18 slides.

side it in table 28. In general, we used nuclear stains to try to determine if the nuclear division process had been affected by weightlessness and cytoplasmic stains to determine if other organelles show effects. Apparently our fixatives adversely affected the mitochondria, as these do not show up well even if ferric hematoxylin or acid fuchsin stains, which are specifically for them, were used. The PAS technique, designed to demonstrate both polysaccharides and starch, proved the most revealing of the techniques we had chosen.

In order to be sure that any differences in staining reaction were due to experimental differences and not to different batches of stain, for any particular series all of the PAS sections were stained at the same time on seedlings grown in flight, on a clinostat, and erect. This same precaution was taken with other more routine stains also.

APPEARANCE OF TISSUES WITH DIFFERENT STAINS

Nucleus

With the several nuclear stains used, we were unable to determine any differences between the nuclei of flight and control plants. In both, there are many mitotic figures in the rapidly growing root tips and leaf shoots, and all of the mitoses appear to be normal with respect to chromosomal and cytoplasmic detail. The tip of the coleoptile and the tipmost area of the leaf almost never show mitotic figures; these appear to be areas of cell elongation only. The scutellum of all seedlings is also devoid of mitotic figures, since, however active this area may be, it is not growing.

A few mitochondria were visualized near the forming cell plate in dividing cells, but no differences could be determined between the experimental categories.

Deoxyribonucleic Acid and Ribonucleic Acid (DNA and RNA)

In the flight seedlings, the coleoptile seemed to contain much less DNA in its individual cells than was present in the cells of the leaves, but this difference also existed in control seedlings and probably indicates simply the relative activity of these tissues. The rapidly dividing cells of the cortex of the roots in all seedlings showed an intense Feulgen reaction, but the differences between flight and control seedlings were not consistent nor measureable quantitatively.

Cytoplasmic RNA is much more evident in the layer of cells immediately beneath the outer epidermis of the coleoptile and the layer of cells immediately surrounding the coleoptilar vascular bundles than in the other parenchymal cells of the coleoptile. All the parenchymal cells of the leaf stain very deeply with this stain, as one would expect with these rapidly dividing cells. No differences in these tissues were seen between flight and control seedlings, but the difference in various areas of the scutellum was emphasized by the azure blue. The upper and lower portions of this organ appear to be much more deeply stained than the area at the center opposite the meristematic level. This suggests that this central portion is less active, or has already depleted its store of RNA in the release of substances from the endosperm through the scutellum. The endosperm seems more dispersed and less dense under this middle area. We were able to show only that the coleoptile of flight seedlings seemed to contain less RNA than do its leaf tissues, although the relative concentration of RNA in the cytoplasm of subepidermal parenchymal cells was typical in flight as in other coleoptiles. Erect seedlings showed greater RNA in this layer than flight or clinostat seedlings. The RNA and DNA seemed to be increased in vibrated seedlings of all types from postflight tests. Differences were observed in upper, middle, and lower areas of the scutellar epithelium, with the upper area showing the greatest RNA content in all seedlings.

Lipids

The subscutellar cells beneath the epidermal layer contain varying amounts of aleurone material and phospholipids, as well as cytoplasmic RNA. For discussion, the scutellum should be divided into an upper part (toward the leaf and above the meristem), a lower part (beneath the meristem and near the root), and a middle portion opposite the meristem. There appears to be a sharper line between the epidermal layer and the subepidermal cells in the clinostat seedlings than in the erect control or the flight seedlings, which is less apparent in the pyridine-extracted material.

Cell Walls

We had hoped to discover some differences in lignification between our experimental categories by the use of the stains shown in table 28. In

some instances we felt that clinostat seedlings showed slightly thicker, greener cell walls in coleoptile tips and in the scutellar parenchyma, but it was not consistent enough to state as a real difference. We were unable to detect differences in thickness, number, or spiral pattern of wall thickening of the xylem or protoxylem. The thickening of the outer cell wall of root and coleoptile is greater and more variable in erect controls than in either flight or clinostat control seedlings. We have been unable to arrive at an explanation for this difference.

Plastids

Several sizes of starch granules are visible in all of the wheat seedling tissues. The differences between flight and control seedlings are in the starch granules themselves and in their localization within the tissues of the shoot and the root. There are more starch grains present in the 65-hour seedlings than in the 58-hour seedlings. This is apparent in both coleoptile and meristematic tissues. There are more starch grains present in clinostat-grown seedlings than in flight- or erect-grown seedlings. The starch which has accumulated in certain cells at the base of the coleoptile and in the meristematic area of the scutellar node is shown in figure 9. The increase in the amount of starch in the flight and especially in the clinostat control seedlings is apparent. Since this difference is seen in seedlings at both ages, it does not seem to be an age-influenced factor. The distribution of the starch grains is also typical. Much smaller amyloplasts occur in the coleoptile epidermis. They were more frequent in parenchymal cells, although some small plastids are present in all cells. Small plastids are characteristic of the leaves and are often found around the nucleus within the leaf cell or in cells of the coleoptile epidermis. They were more frequent in the clinostat grown and the flight seedlings than in the erect control plants. The major differences in amyloplast distribution are found in the sedimentation of certain large granules in two particular areas of the seedling: in root cap cells and certain cells of the coleoptile. These granules are found in the bottom of the cells with relation to gravity in all erect controls, but are distributed at random in similar cells of seedlings from the flight package or those grown in the clinostat.

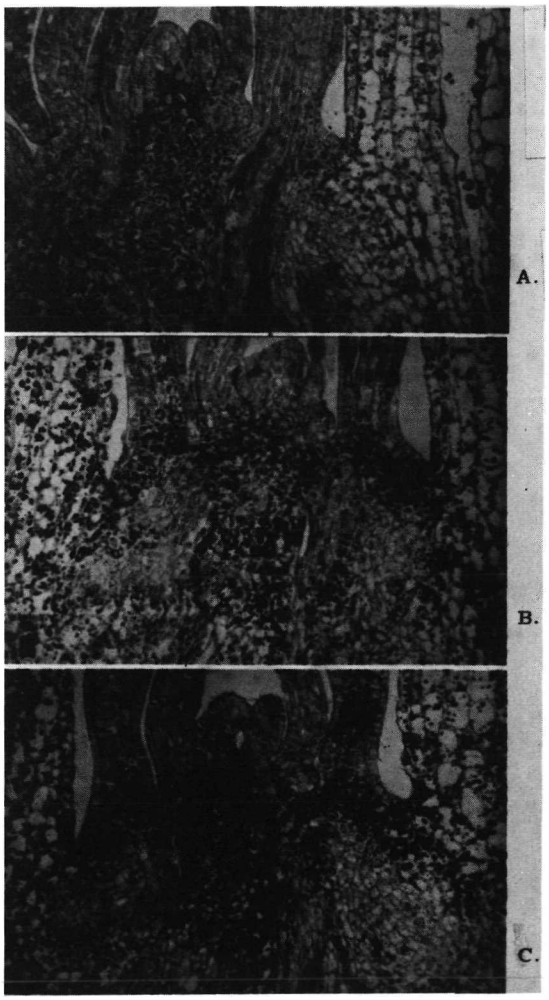


FIGURE 9.— Base of coleoptile and leaf; PAS reaction; 40 ×. Longitudinal sections from seedlings showing typical amyloplast distribution. (*a*) Erect control. (*b*) Flight. (*c*) Clinostat control.

Certain cells in the parenchyma of the coleoptile tip of erect-grown seedlings show an accumulation of large starch grains at the bottom with respect to gravity (fig. 10 (*a*), top row). Similar sedimentation is seen in a single layer of cells surrounding the coleoptile vascular bundle extending approximately one-third of the distance from the tip (fig. 10(*d*), bottom row). In comparable cells of flight seedlings, the starch grains tend to be located at random while those of clinostat control seedlings are located at random or toward the center of the cells. Figure 11 shows the same tissues at higher magnifications so that the cellular detail is clearer.

In the cells of the primary root cap, similar sedimentation in erect control plants or lack of it in flight and clinostat control plants may be

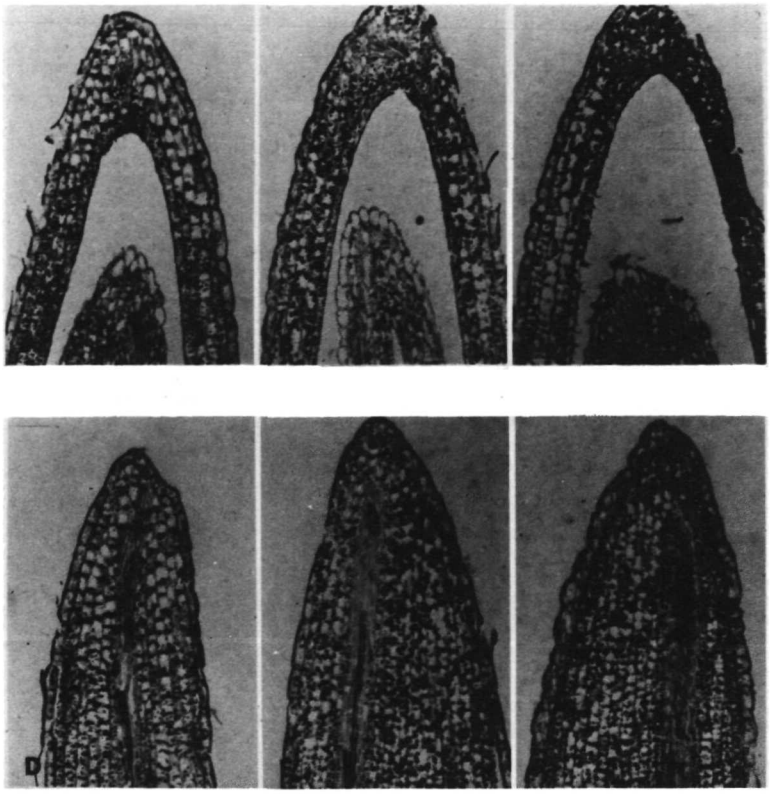


FIGURE 10.— Coleoptile tips showing typical distribution of amyloplasts; PAS and Fast Green; 40 ×. Top row: Longitudinal section of coleoptile sheath with leaf inside; bottom row: Lateral section through vascular bundle of coleoptile. (*a*), (*d*) Erect control. (*b*), (*e*) Flight. (*c*), (*f*) Clinostat control.

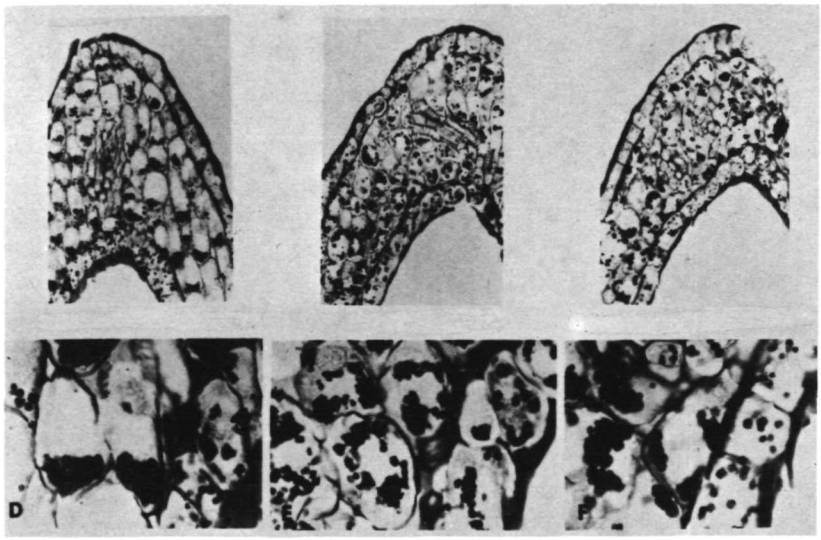


FIGURE 11.— Coleoptile tips; median longitudinal sections; PAS and fast green; 100 × and 400 ×. (*a*), (*d*) Erect control. (*b*), (*e*) Flight. (*c*), (*f*) Clinostat control.

observed. Figures 12 and 13 show the apical meristem and the root cap of seedlings in each gravitational condition; it is apparent that the granules are at the bottom of the cells in the erect control seedlings but are distributed at random in flight and clinostat seedling roots. The bottom row of figure 12 shows the interesting sedimentation at an approximate 45° angle across the root cap cells of secondary roots from erect-grown seedlings. (These roots grow out at this characteristic angle from the vertical as shown in figure 8(*a*).) This illustrates the gravity-dependent sedimentation

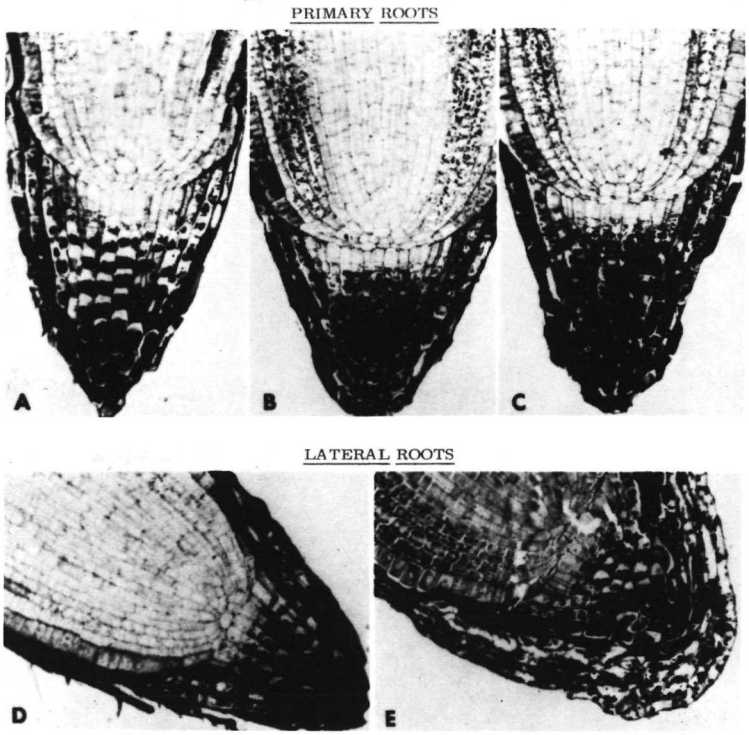


FIGURE 12.— Root tips with caps; PAS reaction; 64 ×. Longitudinal center sections from 58-hour seedlings showing characteristic distribution of amyloplasts. (*a*) Erect control. (*b*) Flight. (*c*) Clinostat control. (*d*) Erect control; 7.0-mm root. (*e*) Erect control; 1.0-mm root still within coleorhiza.

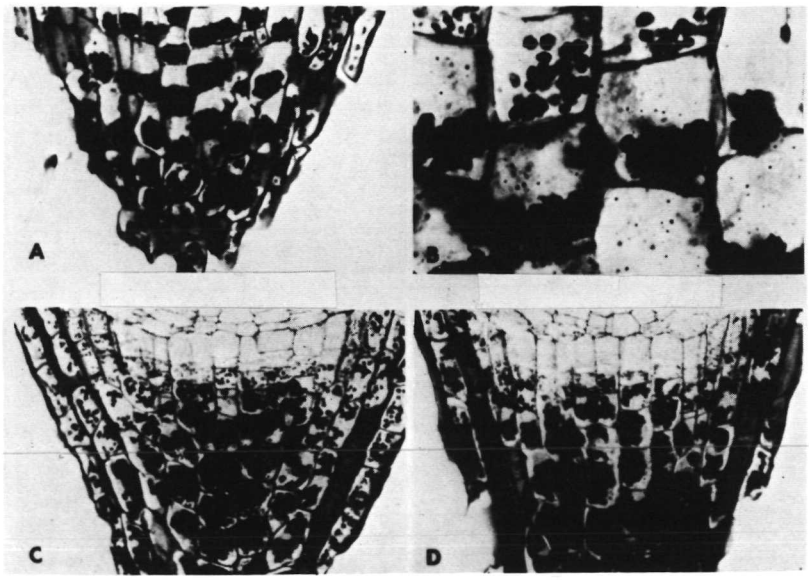


FIGURE 13.— Primary root caps; PAS reaction. (*a*) Erect control; 128 ×. (*b*) Erect control; 400 ×. (*c*) Flight; 128 ×. (*d*) Clinostat control; 128 ×.

of these large starch granules, and in our judgment warrants their designation as "statolith starch."

The characteristic distribution in the cells of amyloplasts remained the same regardless of fixative. Those of the 65-hour clinostat control seemed to have returned to the erect condition during the simulated recovery period, that is, during the period between the removal of the package from the clinostat at 60 hours and fixation at 65 hours.

As usual, there seem to be more amyloplasts present in all clinostat plants than in those grown erect. The chromic acid fixative appeared to affect primarily the large statolith starch granules of the parenchyma of the coleoptile tip and base and often causes them to be disrupted and dispersed, whereas the starch granules of the outer and inner layers of the coleoptile epidermis are not similarly affected by the fixative. We noticed no difference in other cytoplasmic features between the fixatives.

TABLE 29. – Distribution of Frozen Seedlings

Program	Electrophoresis	Histochemistry
Biosatellite II:		
Flight	2	5
Erect control:		
I	2	6
III	4	4
Clinostat control:		
III	2	6
II	4	6
Total	14	27
Postflight, phase B:		
Erect control	2	4
Clinostat control:		
III		4
II	2	5
Total	4	13
Postflight, phase C:		
Nonvibrated:		
Erect control	2	2
Clinostat control:		
III	3	2
II	2	3
Vibrated:		
Erect control	1	4
Clinostat control	3	2
Total	11	13

Histochemistry

Those seedlings selected for histochemical treatment comprise group IV (see section entitled "Postrecovery Disposition of Seedlings") and the numbers chosen from each experimental group are listed in table 29. Tissues were quick-frozen immediately after removal alive from the experimental environment to preserve the protein structure characteristic of that environment or of flight. All of these seedlings were 60 to 66 hours old, since the younger seedlings in chambers I and II had been spray-fixed on command in orbit or within the control packages. Seedlings from the postflight tests were frozen at ages corresponding to those of Biosatellite II seedlings and their controls.

Each seedling was frozen whole in dry ice with the roots and shoots surrounded by a gelatin mixture (O.C.T. LabTek medium). Each was kept under Dry-Ice refrigeration until sectioned. All three root tips of a seedling were cut in a single block; the coleoptile with its base and scutellum was sectioned separately. Care was taken to freeze and to cut sections of seedlings of similar size. Serial sections were cut at 16 to 20 micra on an International Equipment Company (IEC) cryostat, one or two sections being placed on each coverslip. The coverslips were placed in the appropriate substrate, with the flight and control sections simultaneously treated in the same container so as to insure reliable results. The histochemical procedures which we used are listed in table 30.

TABLE 30. – Histochemical Procedures

Substance demonstrated	Technique described by—
General protein	Ornstein (1964)
Glucose-6-phosphatase	Avers and Grimm (1959)
Succinic dehydrogenase	Nachlas, Tsow, DeSouza, Cheng, and Seligman (1957)
Acid phosphatase	Burstone (1962)
Peroxidase	Van Fleet (1959)
Cytochrome oxidase	Burstone (1960), Culling (1963)

GENERAL PROTEIN

All seedlings showed a marked reaction in the aleurone material within scutellar parenchymal cells and in the rapidly growing leaf tissues within the coleoptile sheath. Electrophoretic gels showed the greatest protein reactivity in the midportion of the coleoptile, probably because the rapidly growing leaf fills the space within the coleoptile.

GLUCOSE-6-PHOSPHATASE

The glucose-6-phosphatase was found associated with the amyloplasts in all tissues. The strongest reaction to glucose-6-phosphatase was found within the scutellar parenchyma and around the scutellar node. Since this enzyme is associated with conversion of starch to glucose, it is not surprising that the reaction appeared greater in flight and clinostat control seedlings which have more starch granules in this area than do erect seedlings. The distribution in the root tissues was principally in a layer several cells wide just under the epidermis, which showed up much more in the erect than in the clinostat or flight seedling roots.

SUCCINIC DEHYDROGENASE

The typical blue granular reaction of succinic dehydrogenase was not consistently successful, possibly because this enzyme may be harmed by freezing. Nevertheless, the distribution of the enzyme was characteristic within tissues known to be actively dividing, such as the young leaves, and the apical meristems of coleoptile and root. This reaction is heaviest in meristematic tissue in seedlings of all categories. The coleoptile tip of the erect seedling was more reactive than those of the flight or clinostat. The leaf within the coleoptile was always more reactive than the coleoptile, regardless of the gravitational environment. The root caps of all roots showed a heavy succinic reaction, but the clinostat seedling root apical meristem had many more granules than had those of erect or flight seedling roots.

The cells of the scutellar epithelium showed heavy granules throughout their length in flight seedlings, in their tips only in erect seedlings, and were relatively few in the scutellar epithelium of the clinostat control. However, the scutellar parenchyma of the clinostat seedling scutellum showed a very different reaction pattern since its parenchymal cells were markedly reactive, in contrast with the pale epithelium, just the reverse being the case in both the flight and erect scutella.

If an enhanced respiratory rate (Dedolph, 1967) is the result of growth on the clinostat, then respiratory enzymes, such as succinic dehydrogenase, should show differences between erect- and clinostat-grown seedlings, and these are seen in our sections.

ACID PHOSPHATASE

Using the technique of Burstone (1962) we have obtained excellent results with acid phosphatase. The acid phosphatase reaction was strongest in the vascular bundles and more pronounced in the subepidermal cells and the epidermis of the coleoptile and of the root in all seedlings. The flight seedlings showed considerably greater response than either clinostat or erect controls, and the parenchymal cells of the erect control coleoptile showed more of the red acid phosphatase granules than did the same cells in other seedlings. The acid phosphatase reaction in the scutellum varied considerably between erect, flight, and clinostat seedlings, as seen in figure 14. The epithelium of the scutellum showed a concentration in the tips of the cells in the erect control seedlings; this was intensified in the clinostat control seedlings and entirely lacking in the epithelium of the flight seedling. Yet, the subepithelial cells of the flight seedling scutellum showed a greater concentration of granules in the cytoplasm, particularly close to the cell walls.

Among the postflight test phase C seedlings, the vibrated clinostat seedlings showed a much heavier reaction in the scutellum, both in the epithelium and subepithelial cells. On the other hand, the coleoptile and leaf of the vibrated erect seedlings showed a marked increase in reactivity. The strength of the reaction in the vascular bundles and the epithelial cells did not differ. The differences noted may be explained by surmising that the vibration of flight or the phase C test caused greater response to this enzyme. Further tests should be made before such an assumption could be verified.

Our localization of acid phosphatase bears out the theory of Czernik and Avers (1964) that differential enzyme activities are associated with specific cell types in relation to their position. Our results are consistent with those published by Avers (1961), by Benes et al. (1961) and by Glick and

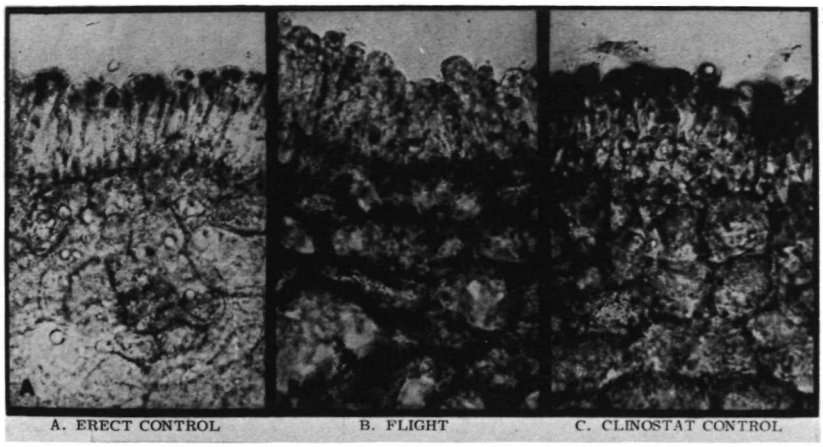


FIGURE 14.— Scutellum. Longitudinal section through epithelium and parenchyma; acid phosphatase reaction; 160 ×. (*a*) Erect control. (*b*) Flight. (*c*) Clinostat control.

Fischer (1946). Glick and Fischer (1946) showed in wheat roots that the cortex and nuclei also react to glycerophosphate substrate. In scutellar cells the cytoplasm showed a strongly positive reaction, as did the vascular bundles and the cell walls. The scutellar epithelium was negative, but positive cytoplasmic granules were found in these cells at certain areas. This localization of acid phosphatase is quite similar to that which we have observed in our material.

PEROXIDASE

The older cells of the scutellum, both epithelium and parenchyma, show the presence of peroxidase principally in the cell walls. The epidermis and the vascular bundles of the roots of the seedlings are well outlined by this reaction to this substrate. The epidermis of coleoptiles also is very reactive and appears to vary from one side to another, although we did not notice differences in the two sides of any root. The epidermis of the scutellum is slightly more reactive in the clinostat control than in the erect or flight seedling, but in no instance did the scutellum seem as reactive as the epidermis of the coleoptile or the root tip. The base of clinostat and flight seedlings showed a heavier reaction than did the erect base. The small epiblast opposite the scutellum is particularly reactive to this enzyme in all types of seedlings, an observation difficult to interpret since this structure has no known function.

From the postflight phase C vibration test, those seedlings which were vibrated and then grown erect had roots much more reactive to peroxidase than those found in the erect control for this test or Biosatellite II. The striking difference was accentuated by the fact that clinostat roots from the same test showed very little reaction. The epithelium of the vibrated erect coleoptile showed greater reactivity on one side than the other, and its leaf was only slightly more reactive than those of the erect control or clinostat. The greater effect of vibration on root tissue seems to be corroborated by this finding.

Electrophoretic Studies

Frozen seedlings of the same height were chosen for electrophoretic studies. For each test approximately 3 mm of the tip of each coleoptile was macerated to impregnate a filter paper disk; approximately 2 mm of the meristematic area of the base and 3 mm of root tip tissue were similarly macerated and used. These saturated disks were then placed in contact with freshly mixed poly-

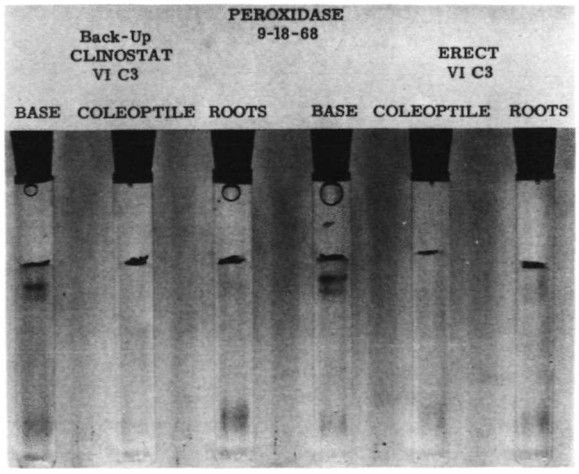


FIGURE 15.— Disk electrophoresis gels; peroxidase reaction.

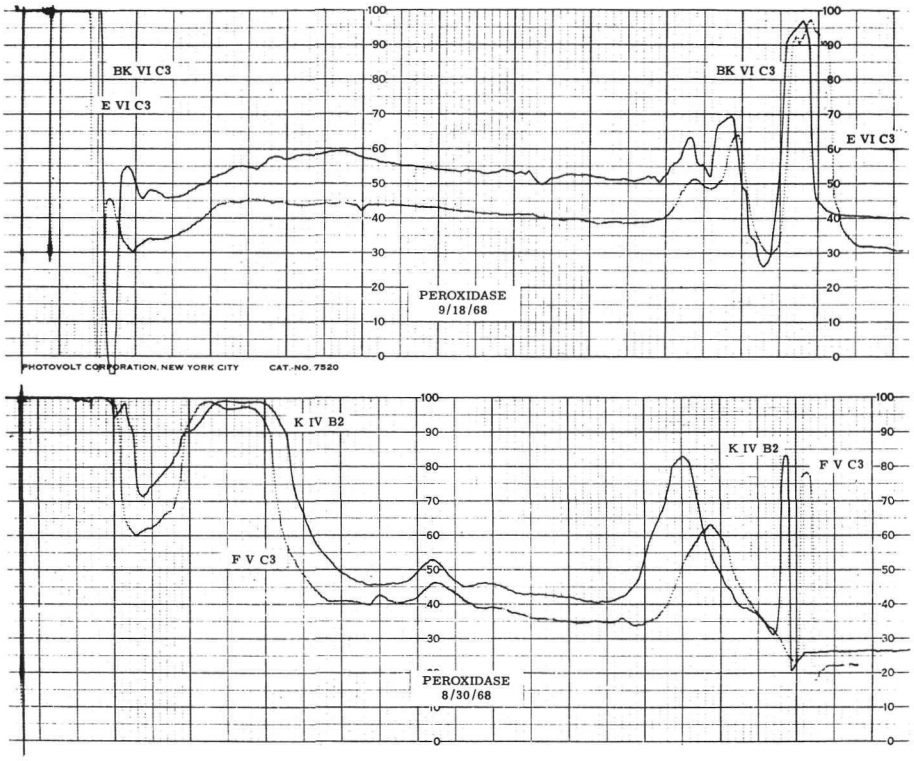


FIGURE 16.— Peroxidase reaction of tissue from base of wheat seedlings. Densitometer tracing from disk electrophoresis gels containing tissue extract from basal area of wheat seedlings grown under different environment conditions during the flight of Biosatellite II, September 7 to 9, 1967.

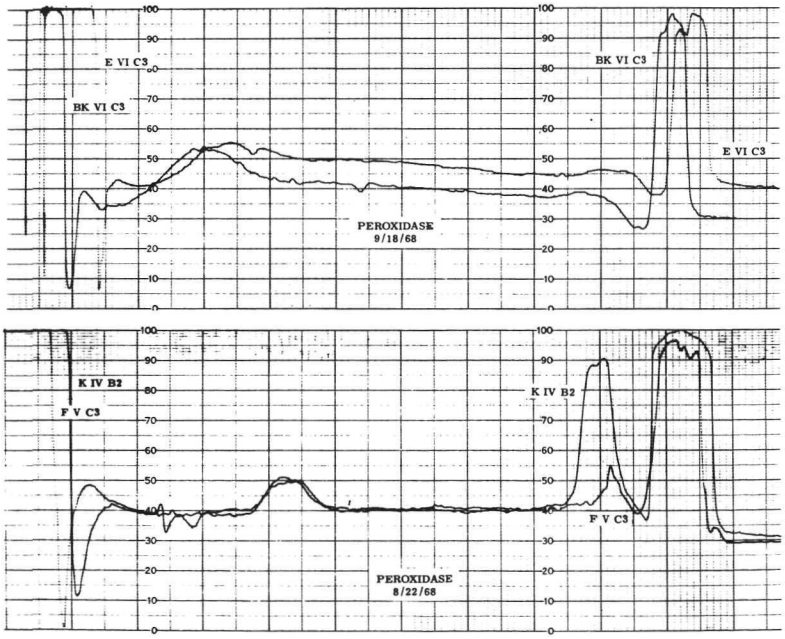


FIGURE 17.— Peroxidase reaction of tissue from root tips of wheat seedlings. Densitometer tracing from disk electrophoresis gels containing tissue extract from root-tip area of wheat seedlings grown under different environment conditions during the flight of Biosatellite II, September 7 to 9, 1967.

acrylamide gels as a conducting matrix and a potential applied across its length, thus setting up a potential gradient along the matrix. (Canalco Disc Electrophoresis apparatus from the Canal Industrial Corp. was used.)

Following electrophoresis, the gels were stained for the presence of specific proteins, similar to histochemical localization. In this way, we compared seedlings from flight with erect and clinostat controls for peroxidase, acid phosphatase, and general protein. Figure 15 shows typical gels and comparative activity of the base and tip of clinostat and erect control seedlings stained for peroxidase. The greater reactivity of the base and roots of the clinostat seedlings is apparent in darker, wider bands of greater optical density, but the coleoptile tip of the erect-grown seedling shows more reaction than does that of the clinostat-grown seedling. A similar pattern of density occurs for the acid phosphatase reaction. Electrophoretic studies of flight seedlings showed gel patterns more like those of clinostat control seedlings than like those of seedlings grown erect.

Densitometer tracings of similar gels (figs. 16 and 17) show the possibility of quantitating the difference in the reactivity of tissue from different types of seedlings. These optical density curves identify homologous protein bands among spectra with discrepant migration velocities. Figure 18 is an expansion of an area from figure 17. It will be noted that when the lower graph of figure 17 is expanded on a larger scale there is considerable variation in peroxidase isozymes between flight- and clinostat-grown root tips. This difference is most noticeable in those components which diffused readily and progressed almost to the base of the gel.

Our studies thus demonstrate that the flight seedlings had recovered less than had the clinostat control seedlings; that is, they had not resumed the metabolic state characteristic of erect-grown seedlings. This is in accordance with the position of statolith starch granules in the coleoptile tip of clinostat control II seedlings (see figs. 10 and 11). It is also in agreement with Dr. Johnson's results with the pepper plants: the leaves of his flight plants had not returned to the normal configuration of erect-grown plants after 5 hours, whereas the leaves of seedlings removed from the clinostat had returned to the typical erect-grown position (Johnson and Tibbitts, 1968).

Cytology

MITOTIC RATE AND CELL ELONGATION

Dividing cells in the terminal millimeter of the tips of both primary and lateral roots were counted and classified by five stages of mitosis. From 1000 to 3000 cells were counted for both primary and lateral roots at each time of fixation for each experimental condition. A total of 37 388 cells with nuclei were counted in flight and Earth control plants.

Significantly fewer cells were dividing in the root tips of flight plants than in those of erect or clinostat control seedlings. The chief difference appears in the number of cells in early prophase. We can

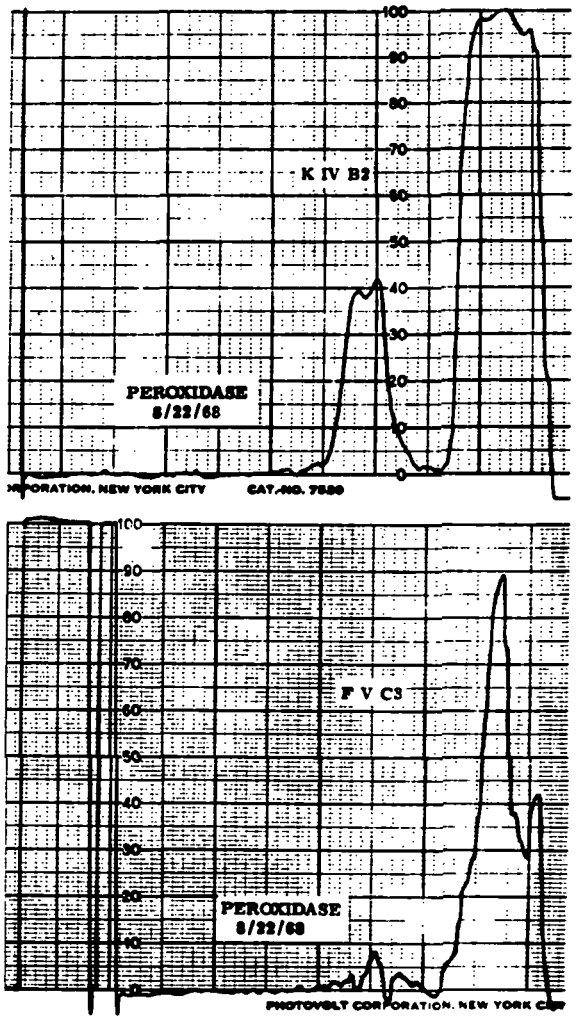


FIGURE 18.— Root-tip peroxidase densitometer tracing; sections of figure 17 expanded to larger scale.

not be sure of this until an analysis of variance has been run on the data, and this is not yet complete. The raw data are shown in figure 19.

As root lengths of flight and control seedlings did not differ significantly, an increase in cell elongation might have compensated for the reduced mitotic activity in flight seedling roots. To check this, at least 20 cells in the proximal 20 percent of the terminal millimeter of at least five root tips were measured. These cells in flight seedlings at 58 hours were 25 percent longer than similar cells in the control seedlings. At 65 hours of age cells of the flight root tips were still 13.5 percent longer than cells of control root tips. The clinostat had no effect on cell length. The data are shown in figure 20.

It is evident from the above findings that, although mitotic rates are depressed in weightlessness, greater elongation of the cells actually produced serves to maintain the normal root length of the seedling.

NUCLEAR VOLUME

The volume of interphase nuclei of cells in the upper part of the terminal millimeter of coleoptiles and roots was computed from measurements of nuclear diameter. Fifty nuclei were measured in each root and 150 were measured in each coleoptile. A total of 730 nuclei in flight and control plants was measured.

Nuclei of flight seedlings were from 13 to 26 percent larger than those of erect control seedlings. Clinostat control seedlings showed a similar increase in volume only in the primary root at 58 hours. The volume of the nuclei in the other organs remained the same as that in the erect control plants.

It is our impression from the data in tables 31 and 32 that the increase in nuclear volume is not a continuous variable, but that volume increases about 20 percent or not at all. We do not yet have a biologic explanation for this.

DISCUSSION

The two major conditions of the Biosatellite II flight were weightlessness and launch vibration. The fact that fewer malformed seedlings appeared in the flight package than in the ground control packages suggested that either weightlessness, vibration, or both had an influence on the normal development of the seedlings. The typical conformation of wheat seedlings (fig. 8) shows how the orientation of lateral roots changes under different gravitational environments. In addition to these changes there are differences in organ growth, in gross morphology, and in cellular morphology.

The Growth Response to Flight

COLEOPTILE HEIGHT

By the age of 58 hours, 43 of which were spent on orbit, coleoptiles of the flight seedlings were slightly larger than those of the erect control and

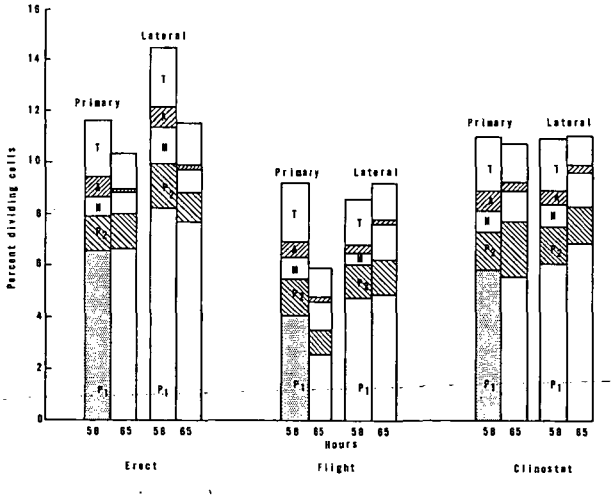


FIGURE 19.— Mitotic stages in wheat seedling roots.

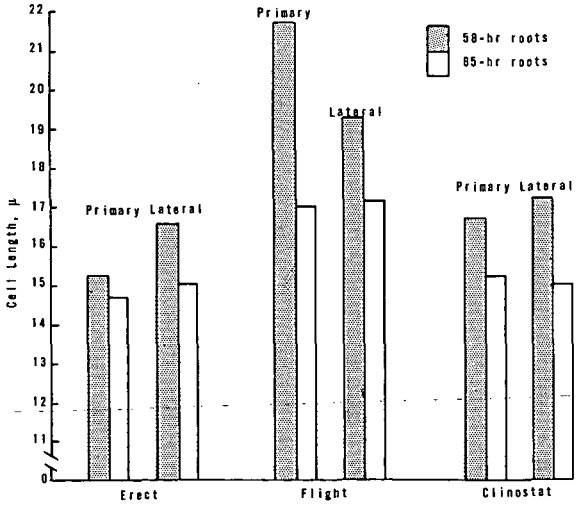


FIGURE 20.— Cell length in wheat seedling roots.

clinostat control III seedlings (table 6). Clinostat control II coleoptiles were taller than those of the flight, but they had been subjected to higher temperatures for much of the time. Seven hours later, at 65 hours of age, the flight coleoptiles were significantly taller than were the erect Earth control coleoptiles (table 7). Table 30 shows that the rate of growth of both flight and clinostat control cole-

TABLE 31. – Effect of Weightlessness on Nuclear Volume

Organ	Volume of erect, 1 *g*, μ^3	Variation from erect, percent	
		Flight, true weightlessness	Clinostat, simulated weightlessness
58- hr seedlings			
Coleoptile, tip	578	[a]+13	+3
Primary root	535	[a]+26	[a]+21
Lateral roots	582	[a]+18	+5
65-hr seedlings			
Coleoptile, tip	550	[a]+26	−2
Primary root	649	[a]+19	−3
Lateral roots	621	[a]+20	−6

[a]Significant to $P<0.05$.

TABLE 32. – Effect of Vibration on Nuclear Volume for Phase C Postflight Test, Simulated Vibration of Biosatellite II

Organ	Volume of erect, 1 g, μ^3	Variation from erect, percent		
		Erect Vibrated	Clinostat	
			Vibrated	Controls
58-hr seedlings				
Coleoptile, tip	527	[a]−15	+0.3	−0.9
Primary root	553	+13	[a]+21	[a]+26
Lateral roots	586	[a]+17	+6	+8
65-hr seedlings				
Coleoptile, tip	474	−2	+3	+6
Primary root	570	+15	[a]+24	+9
Lateral roots	550	[a]+30	[a]+16	[a]+14

[a]Significant to $P<0.05$.

optiles exceeded that of erect control coleoptiles even though weightlessness, true or simulated, was no longer operating during 5 of the 7 hours. Indeed, the major increase in growth rate may have occurred after normal gravity was restored.

From the data available two interpretations are possible: (1) Increased growth rate is produced by weightlessness and persists for several hours after its removal; or (2) the return to normal gravity is itself a stimulus to growth. Each of these hypotheses is illustrated by the same data in figure 21.

In favor of the first interpretation is the fact that the depression of growth produced by greatly increased gravity manifests itself for several hours after the seedling is returned to normal gravity (Edwards and Gray, 1956). In favor of the second interpretation is evidence that a small increase in gravity over normal (about 10 *g*) results in growth stimulation (Gray and Edwards, 1955). Wunder, Briney, et al. (1960) found a similar stimulation of the growth of *Drosophila* larvae. We incline toward the second interpretation and suggest that the increased growth seen at 65 hours in flight and clinostat seedlings may be a response to the return to normal gravity rather than to weightlessness *per se*. If we assume under hypothesis (1) that growth of flight and Earth control plants had continued at the rates shown in table 33 for an additional 15 hours (80 hours of age) coleoptile height of flight and clinostat seedlings would have been very close together as would the height in the two erect control packages. The difference between the two groups, assuming a consistent change in variance, would be significant to less than $P<0.001$.

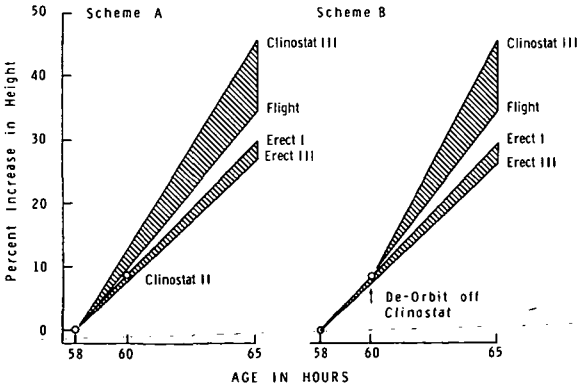


FIGURE 21.— Growth of coleoptile of wheat seedlings between 58 and 65 hours.

ROOT LENGTH

The same effect operating on the coleoptiles may be operating on the primary roots. Although those of the flight and clinostat control plants were shorter than those of the erect control seedlings at 58 hours (table 8) the difference had disappeared by 65 hours (table 9) because of the increased growth rate of flight and clinostat roots (table 33). More lateral roots have started to elongate by 58 hours in erect control plants than in plants of the flight or clinostat control III (table 10). By 65 hours there is little difference between the packages (table 11). In both cases it appears that weightlessness, whether true or simulated, inhibits root growth and that the return to normal gravity stimulates growth.

ROOT/SHOOT RATIOS

When total root length of a seedling is divided by coleoptile height, the greater coleoptile growth of the flight plants is evident from the resulting ratios. In seedlings with a total root length of more than 20.5 mm the difference is about 13 percent and is statistically significant ($P<0.001$). The ratios at various stages of growth are summarized in table 34.

It is surprising that the roots of flight and clinostat control plants were shorter relative to their coleoptiles than were those of erect control plants. Higher-than-normal gravity produces the same differential inhibition of root growth. The effects may differ in origin, however. Weightless seedlings have roots of normal length and taller coleoptiles.

TABLE 33. — Rate of Growth Between 58 and 65 Hours

Package	Rate of growth, mm/hr, of—	
	Coleoptile	Primary root
Flight	0.151	1.903
Erect control:		
I	.116	1.859
III	.110	1.724
Clinostat control: III	.174	1.946

Centrifuged seedlings have inhibited root growth. In the latter case, the roots grew in sand rather than in the open air (Gray and Edwards, 1955).

The question of stimulus or inhibition as the result of horizontal rotation on a clinostat or of weightlessness in orbit has also occupied other scientists. Hoshizaki and his coworkers (1964) showed the rotation on a horizontal clinostat at 15 rph slowed elongation in stems of *Xanthium*. Since this effect was decreased or corrected by the application of gibberellic acid or IAA, they concluded that the dwarfing effects of rotation may have been due to change in the availability of auxin or gibberellins in rotated plants. Later work by this group (Hoshizaki et al., 1966) showed that plants rotated in two axes at a calculated zero gravity did not show geotropic response. Their coleoptiles appeared to be oriented by position of seed, while root direction was affected by proximity of other roots.

Russian biologic experiments have accompanied a number of their manned spaceflights. At the Committee on Space Research (COSPAR) meeting in Tokyo, 1968, the Soviet scientists (Parin, Grigorev, et al., 1968) presented data from the flight of Cosmos 110 in which wheat seeds, among other organisms, had received low doses of radiation and subsequently showed slower growth, but only a slightly lower germination rate, than control plants. It was impossible to ascertain the radiation dose received by the seeds, or whether radiation, vibration, or weightlessness was responsible for the observed effects. It is hard to reconcile these data with those reported in 1966 from the Vostok 5 and 6 flights (Il'ina et al., 1966) in which weightlessness and radiation dose equivalents of 1500 R had no retarding effect on growth and development of wheat plants. We are inclined to think that, since the radiation doses were administered to dry seeds which were subsequently planted, it is not surprising that there were no radiation effects. Our earlier work (Edwards, 1963) showed that it took more than 10 000 R of X-irradiation on germinating seeds to produce very noticeable effects on germination and growth.

Regarding the growth of roots, Dedolph et al. (1966) maintained that root growth is enhanced under zero gravity conditions. They denied that this is caused by modified auxin production or distribution, but believed it to be the result of enhanced respiration rates and the modified distribution of active particulate inclusions. This enhanced respiratory metabolism seemed to be revealed in our sections which were treated for succinic dehydrogenase in which the clinostat control seedlings showed increased reactivity, especially in the root tips.

Growth Responses Reflected in Histochemical Data

There are a few general statements which are possible regarding the responses of our seedlings as reflected in the localization of starch and enzymes. We agree with Conrad (1968) that the area of greatest enzyme activity is the apical meristerm in the root and in the coleoptile. We believe that only coleoptiles of equivalent length should be compared, since the activity of various enzymes varies with the distance from the tip of the coleoptile or from the scutellar node. We noted, as did Glick and Fischer (1946) that the reaction in the columnar epithelial cells of the scutellum is highly

TABLE 34. – Ratio of Total Root Length to Coleoptile Height

Package	Ratio of total root length to coleoptile height of —		
	58-hr seedlings	65-hr seedlings	
		Root length less than 55 mm	Root length 55 mm and longer
Flight	3.30	8.93	12.82
Erect controls	4.35	11.24	14.93
Clinostat control III	3.80	8.62	12.05

variable; perhaps this reflects its changing physiologic state. They had observed that with all substrates the coleoptile showed a stronger enzyme reaction than did the inner leaves. In our tissues, only the coleoptile tip had more pronounced reactions.

The typical enzyme distribution in seedlings grown erect or on the clinostat was not altered by vibration preceding their growth, as seen in tissues from the phase C postflight test, although the reactions were accentuated in certain tissues of vibrated seedlings. The starch content of vibrated seedlings seemed greater than that of those not vibrated; this effect was also observed in seedlings recovered from flight.

Zalokar (1960) reported that the following enzymes were restricted to mitochondria in the hyphae of *Neurospora*: Succinic dehydrogenase, cytochrome oxidase, peroxidase, β-galactosidase, and acid phosphatase. Since most of these enzymes in our wheat seedling tissues were prominent in the dividing cells of leaf and the cortex cells of the root meristem, it is possible that they also are associated with mitochondria.

Wright (1963) reported that the final cell number is attained in the wheat coleoptile by approximately 60 hours and does not increase after 65 hours; we may assume therefore that the total cell number would be present in our coleoptiles. Those enzymes related to cell division, such as peroxidase, would have been most active during the cell division phase of growth prior to 60 hours. Later the metabolic processes related to cell elongation and cytoplasmic volume increase would become more prominent, and different enzyme patterns might become apparent. The period from 48 to 72 hours is the period of maximum increase in weight of coleoptile and in volume of cells (Wright, 1963). Although the rate of increase declines after 40 hours, weight and cell volume continue to rise up to approximately 90 hours of age. Earlier tests in which seedlings grew about 85 hours not only produced longer organs but demonstrated greater differences in certain enzyme activities such as were reported after the 1967 test preceding the launch of Biosatellite II. Had the Biosatellite II continued in orbit during this period of rapid growth from 48 to 72 hours, more striking differences in enzyme activity might have appeared.

Other Responses to Vibration and/or Flight

COLEOPTILE DIAMETERS

Under increased gravity, the large and small diameters of the wheat coleoptile increase and the coleoptile becomes more circular in cross section. A similar change in the cross-sectional shape of the mouse femur under increased gravity has been reported by Wunder and his colleagues (1960b). We expected, therefore, to find that weightless coleoptiles become more elliptic than normal. Such a change took place in the coleoptiles of the clinostat control plants, but this did not happen in the flight plants. The fact that the flight seedlings were subject to launch vibration, while the clinostat control seedlings were not, led to measurement of seedlings from the vibration experiments.

Figure 22 shows that both diameters of the coleoptile increase with height, the large diameter (slope 0.155) slightly faster than the small diameter (slope 0.125). Both diameters of clinostat control coleoptiles are slightly larger than those of erect control coleoptiles. Vibrated erect coleoptiles show little difference. Both diameters in flight plants

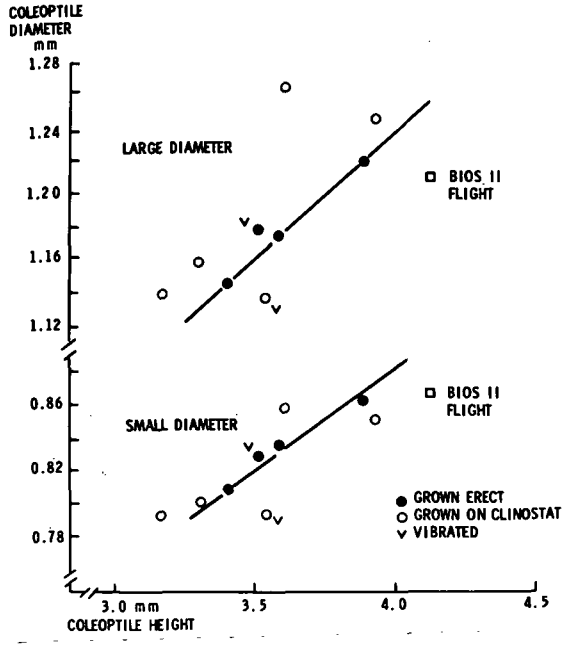


FIGURE 22.— Relation between coleoptile height and diameters. Large and small diameters of coleoptiles are related to coleoptile height in Biosatellite II flight and control seedlings and postflight (phase C) vibrated and control seedlings.

and vibrated clinostat plants are smaller than would be expected for their coleptile height. It appears that the observed difference in diameters between flight and clinostat control coleoptiles is the result of launch vibration on the flight seedlings and not of weightlessness alone.

MALFORMATIONS

Since each of the four seedling organs can be absent, either alone or in combination with other organs, all four organs might be simultaneously affected, resulting in a seedling designated "no growth." In figure 23 the frequency of one, two, three, and four absent organs has been plotted for each experimental condition. An extrapolation of the curve thus produced for the erect control seedlings suggests that the incidence of four absent organs should be of the order of 0.5 percent. Among the vibrated clinostat seedlings it should be about 1.0 percent. As the actual number of seedlings in which no organs grew is of the order of 3 to 4 percent, it is apparent that most of these seeds are defective for causes other than those producing the malformations under discussion. The probable cause is natural dormancy of a relatively constant percentage of seeds of this strain of wheat.

If we separate these malformations into the particular organs affected, we find that most of the differences observed in the number of malformations lie in the number of lateral roots affected. Table 22 and figure 24 show that the number of missing lateral roots was more than doubled in the vibrated erect plants compared with the non-vibrated erect control plants. Among the vibrated clinostat plants and the Biosatellite II flight plants,

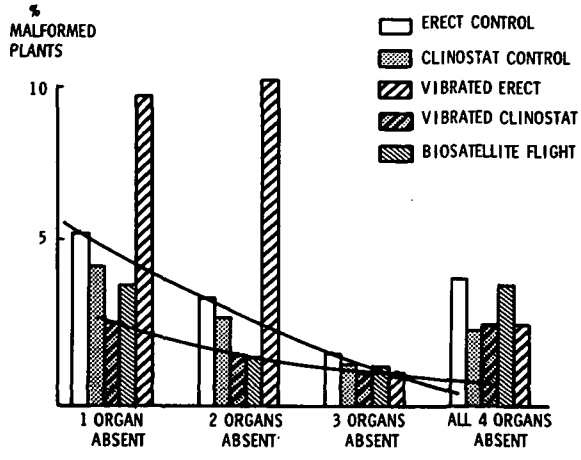


FIGURE 23.— Relation between single and multiple malformations in seedlings exposed to vibration and weightlessness.

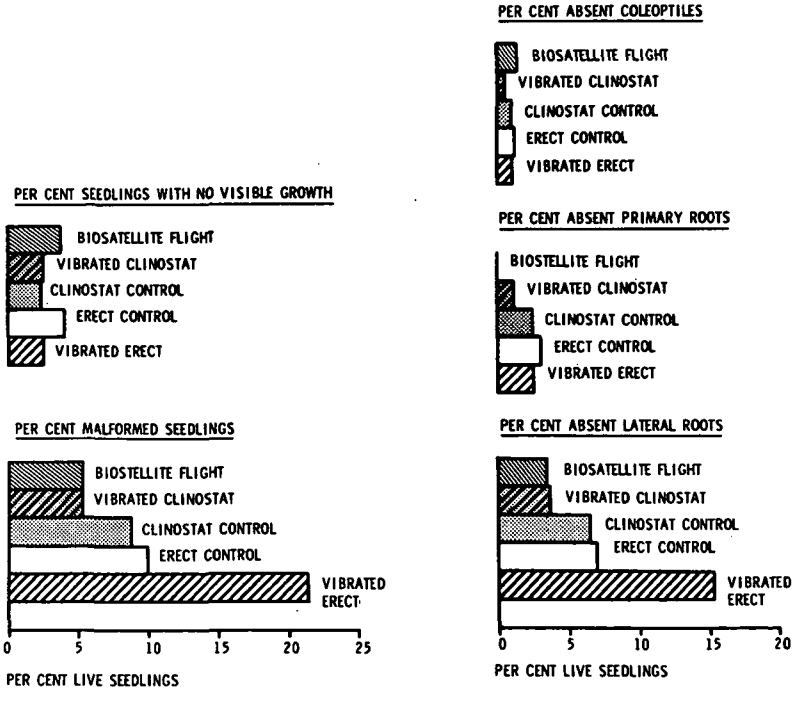


FIGURE 24.— Effect of vibration and weightlessness on the incidence of malformations in wheat seedlings.

the number of missing lateral roots was almost halved. Figure 24 shows graphically the agreement between the flight control plants and the vibration test control plants as well as the striking increase in lateral root defects to be found in the seedlings held erect after vibration. It emphasizes the similarity of the Biosatellite II flight seedlings with those grown on the clinostat following vibration. The percentage differences, shown in figure 24, between absent primary roots and absent coleoptiles in the five groups are small and not so significant as those between lateral roots. Nevertheless, there are the least number of primary root defects in the flight and vibrated clinostat groups.

Three facts are apparent from inspection of table 21 and figure 24: (1) There is a marked *increase* in the number of malformations in the vibrated erect group and a *decrease* in the number of malformations in the vibrated clinostat group. (2) The Biosatellite II flight seedlings most closely resemble the vibrated clinostat group in the number of malformations displayed. (3) There is no difference between the *types* of malformations encountered in the four groups.

From figure 24 it is apparent that Biosatellite II flight seedlings resemble the vibrated clinostat seedlings in the number of malformations produced. Vibration, comparable to that experienced at launch, increases the number of organs failing to grow if the vibrated plants are subsequently grown in the erect position, but decreases the number of malformations if subsequent growth is in orbital weightlessness or on a clinostat. Weightlessness alone seems to have little effect. There is, thus, a definite relation between a deleterious effect of vibration at the level experienced in the Biosatellite II launch and a recovery effect obtained only by subsequent weightlessness. It is surprising that vibration with subsequent weightlessness results in even fewer malformations than occur normally in nonvibrated plants grown at normal gravity.

Very little pertinent literature on the effects of vibration exists. Pokrovskaya and his colleagues in Russia (1965) found a decrease in mitotic activity in the bone marrow of mice exposed to 35 to 70 Hz at 4-mm amplitude for as little as 15 minutes. They reported chromosome disruption, separated fragments, and adhesions. Frequency was not critical. Short, Newsom, and Brady (1967) found no synergistic effects of borderline lethal radiation and vibration at 20 Hz 0.5-inch double amplitude for 10 minutes. Some ultrasonic vibrations have been applied to plant material. These resulted in stimulation of potato sprouts (Bazhanova, 1958) and increased tillering of wheat (Weinberger and Measures, 1968).

The marked increase in malformations observed in seedlings subjected to acute vibration and grown erect may result from two possible types of injury. First, injury to the cells of the meristematic region of the organ may occur so that division and subsequent elongation of the cells are suppressed. Second, injury may occur to the vascular bundles supplying nourishment to the organ so that material necessary for growth is withheld. If only the former type of injury occurred, one might expect a random distribution of absent organs quite different from that which appears spontaneously among erect grown seedlings not subjected to vibration. As is seen in table 21, this is not the case. The most frequent spontaneous defect, absence of both lateral roots, is the most frequent defect among the vibrated seedlings. It appears that the increase in specific defects after vibration is proportional to the spontaneous frequency of these defects. This suggests that vibration is intensifying the spontaneous defect pattern and not introducing a wholly new and different factor.

There may, of course, be a differential sensitivity to injury among the wheat seedling organs such that the apical meristem of the lateral roots is more easily injured by all developmental vicissitudes and hence even more easily injured by chronic vibration than the similar tissues of other organs. If we apply this idea of differential sensitivity to the transport system of the various organs rather than to their meristems, the second possible site of injury must be considered. All nutriment of our seedlings must come from the endosperm of the seed. It is absorbed through the scutellum and moves through a fan of vascular strands which unite to form the main scutellar trace. This trace passes directly to the coleoptile and to the nodal plate of the first node. From the nodal plate arises the bundle of the primary root and separate bundles for the two lateral roots as well as the median bundle for the first foliage leaf (McCall, 1934). It is probable

that for any organ there is a point at which blockage of the vascular bundle could interfere with nutrition. It is suggested that blockage of these bundles may sometimes occur spontaneously, in bundles of the secondary roots most frequently, and less so in bundles of the coleoptile and primary root. Vibration may simply increase the frequency of blockage of the lateral root bundles above that which occurs spontaneously.

We are presently searching for histologic evidence for either of the possibilities suggested. Starch depletion of root primordia which have failed to evolve, actual interruption or maldevelopment of vascular bundles, and visible injury to apical meristem tissues are the areas in which the search is being conducted.

We are not prepared at this time to discuss the extremely interesting question of how weightlessness can cancel out the obvious injurious effects of vibration. There is little literature on the effects of vibration, and almost none on plants. At this time we can only suggest that the disruption of cells, and perhaps tissues, is more readily repaired in weightlessness than in normal gravity and that even spontaneous defects correct themselves more readily if all mechanical stresses are removed.

Response to Gravity and Its Perception

One of the most important facets of the problem of the growth response to gravity is the mechanism within plants for the perception of gravity. In higher vertebrates the otolith mechanism of the inner ear has been proved to serve this function, but there has been considerable controversy over graviperception in plants. According to Wilkins (1966), the means whereby a plant organ perceives its orientation in a gravitational field is most probably the movement of one or more of its component parts. The identity of these components is an intriguing problem (Haupt, 1965). Certain starch grains, which sediment in response to gravity, have been proposed as the mechanism of graviperception in most plants by Audus (1962 and 1964) and others (summarized by Larsen, 1965). There is evidence for this hypothesis from the experimental manipulation of starch by Larsen (1965), by Dedolph and others (1967) working with clinostat-grown plants, and by Dennison (1961) with centrifuged material. Our seedlings grown under different gravitational conditions are particularly well suited to demonstrate histologic features pertaining to the gravity perception mechanism. Data from the Biosatellite II flight have given additional evidence that there are changes in the position of certain starch grains in coleoptile and root tips and in the nodal meristematic area at the base of coleoptile and leaf. We have shown that erect-grown seedlings show a characteristic sedimented arrangement of starch grains in certain cells, and that this arrangement is different from that seen in flight or clinostat control material. Plants growing in a centrifuge show the erect pattern of starch grain distribution accentuated by the increased gravity.

Our clinostat results have been corroborated by Dedolph and his colleagues (1967) who reported that cellular starch particles are more uniformly distributed as the unidirectional gravitational force is diminished by rotation on a horizontal clinostat. They published photographs of amyloplasts in *Avena* coleoptile tips from clinostat and erect seedlings similar to ours. They state that gravity sensing in plants is broadly based on the physicochemical relationship between particle distribution and the expression of respiration metabolism as growth.

In the seedlings grown in Biosatellite II, both root cap and coleoptile tip showed a random distribution of those starch grains which normally respond to gravity changes. Even after exposure to Earth's gravity for 5 hours this distribution persisted, although a similar distribution seen in clinostat control plants at 60 hours had almost returned to normal by 65 hours. We suggest, therefore, that the flight seedlings grown in the absence of gravity "perceived" this condition. Since their statoliths were randomly distributed in the cells of root and shoot, these organs were randomly oriented, as shown by Dr. Lyon's photographs (Lyon, 1968) and figure 8.

Thimann (1966) expressed the view that tropisms are due to the lateral transport of auxin, although the mechanism of such transport remained to be studied. He stated: "for geotropism, the heavy starch grains or statoliths may well modify auxin transport through the walls on which they lie." Later, Pickard and Thimann (1966) suggested that the weight of the cytoplasm which rests on one side of the cell (rather than the statoliths)

might compress membranes and lead to small differences between upper and lower sides of the cell. These deformations might be translated into biochemical action. Since Wilkins (1966) has related a change in electric potential to lateral transport of auxin, and Graham and Hertz (1964) have related change in electric potential to change in starch grain distribution, it is possible that the combination of starch grain location and membrane changes of the cell are indeed responsible for gravity perception. Electron microscopy of flight seedlings and erect control seedlings may corroborate this theory first suggested by Audus in 1962. We have material suitably fixed for this purpose.

SUMMARY AND CONCLUSIONS

Because the flight of Biosatellite II was called down prematurely after only 45 hours on orbit, the results are less conclusive than if the organisms had experienced the weightless state for the projected 72 hours. Nevertheless, the results of our experiments indicate that short periods of spaceflight do not disorganize the normal processes of the growing wheat seedling. Only small deviations from normal physiology or behavior were observed, most of them returning to normal after several hours. The results may be summarized as follows:

(1) *Mortality* — Germinating wheat seedlings carried in orbital flight aboard Biosatellite II displayed no greater mortality than did seedlings grown under similar conditions as Earth controls. Wheat seedlings recovered from flight and grown to maturity tillered, headed, and produced seeds normally.

(2) *Coleoptiles* of flight seedlings are taller than those of erect-grown Earth control seedlings at 65 hours of age. It is possible that the return to normal gravity at the end of orbital flight was the stimulus for increased growth.

(3) *Root Growth* of flight seedlings is slightly retarded at 58 hours of age, but is comparable to that of roots of erect control seedlings by 65 hours. The ratio of shoot height to root length is thus shifted so that coleoptiles of flight seedlings are taller than those of erect control seedlings having roots of the same length.

(4) *Diameters* — Flight coleoptiles and those of seedlings subjected to both vibration and simulated weightlessness were smaller in diameter than were those of seedlings subjected to vibration or weightlessness alone or those of normal erect-grown controls.

(5) *Malformations* — Flight seedlings and seedlings exposed to simulated launch vibration followed by simulated weightlessness had fewer gross malformations than did seedlings exposed to vibration or to weightlessness separately. Vibration followed by erect growth greatly increases the number of malformations, especially among lateral roots.

(6) *Starch Grains* — The most obvious cytologic changes caused by flight conditions occurred in the starch grains. Starch granules were distributed at random in the cells of flight and clinostat control seedlings instead of being clumped on the lower cell wall as in erect-grown seedlings. Both flight and clinostat control seedlings appear to have more starch granules than do erect control seedlings.

(7) *Statoliths* — Because the distribution of the starch amyloplasts in certain cells of the coleoptile and root cap is characteristic for each different gravitational condition, we agree with workers such as Audus (1962, 1964), Larsen (1965), and Iversen et al. (1968) that these starch grain amlyoplasts should be considered the "statoliths" or gravity receptors of the wheat seedling. The size of the statoliths (approximately 3.15 microns) in our wheat is within the dimensions cited by Audus (1964) as possible for observed geotropic reaction times.

(8) *Metabolic Rate* — Histochemical studies show increased respiratory enzyme activity in clinostat and in flight seedlings. This appears to corroborate the view of Dedolph (1967) that increased metabolic rate is evident in clinostat-grown plant tissues.

(9) *Cell Division and Elongation* — There are no striking mitotic aberrations in the dividing cells of seedlings grown aboard Biosatellite II. Fewer cells were undergoing mitosis in root tips of flight seedlings than in those of control seedlings. Early prophase seems to be the stage most affected. This appears to be an effect of true weightlessness since seedlings grown on the clinostat, with or without simulated launch vibration, do not show a comparable reduction in mitoses. Cells in the root tips of flight plants have elongated more than those of erect and clinostat control plants.

(10) *Nuclear Size* — The nuclei of primary and

lateral roots and coleoptiles of flight seedlings are significantly larger (20 percent) than those of erect and clinostat control seedlings. Although secondary root nuclei of vibrated seedlings show significant differences from those of erect controls, they are unlike the flight. The meaning of this difference in size is not understood.

(11) *Clinostat Usefulness* — In most characteristics, flight seedlings resemble clinostat control seedlings much more than they resemble erect-grown Earth control seedlings, especially those which have also been subjected to simulated launch vibration. This suggests that the Earth-bound clinostat *may be* a tool for predicting some of the responses to weightlessness in suitable organisms.

Finally, Biosatellite II with its 3-hour prelaunch hold and its early calldown left some of our hypotheses answered inconclusively. More prolonged weightlessness than was achieved in Biosatellite II may permit certain of the observed deviations to exceed homeostatic limits and produce irreversible changes leading to an altered morphology, or even death, of the organism or some of its parts.

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We wish to acknowledge the help of our own staff, without whose loyal cooperation the project could never have reached a successful conclusion. Miss Jane Boyd Lee and Mr. John G. Wood worked with us on most of the tests and at the launch and recovery sites during the flight of Biosatellite II. Mrs. Mary Kerkhoff arranged and rearranged transportation and coped with the tremendous volume of paper work generated by the Biosatellites. Mr. Lawrence Wilson, Miss Nancy Perry, and James Sunshine composed the crew at Cape Kennedy for the recovery and processing of the Earth control packages for Biosatellite II.

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Growth Physiology of the Wheat Seedling in Space

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The growing of wheat seedlings in Biosatellite II was an experiment designed to determine whether seeds would produce normal seedlings when they germinated in the absence of significant gravitational force. Would the rate of germination be affected, and would the shoots emerge from the rooting medium when the surface was not "up"? Such a study, combined with analyses of the effects of weightlessness or free fall on the anatomy and biochemical processes of a wheat seedling, provided the opportunity to seek details of the germination steps, the growth rates of seedling organs, and the orientation of each as evidence for growth physiology during orbital flight. The area of growth regulators was particularly promising, because auxin, the universal hormone in the higher plants, is known to be moved by gravity within seedling organs (Boysen-Jensen, 1936; Gillespie and Thimann, 1961; Goldsmith and Wilkins, 1964). The limitation of the multiple experiment, however, to the use of a grass-type seedling forced an emphasis on the seedling roots. They develop early whereas the shoot remains a short, atypical organ at the end of 3 days of germination, even under the best of conditions.

The use of seeds buried in a moist medium like soil could not be considered because rates of growth would be difficult to measure and the orientation of all organs would be disturbed by contacts with solid particles. The experiment team was obliged, therefore, to provide for growth of the seedlings with both roots and coleoptiles in air. This method would also keep the organs free of chemical contamination and avoid possible distortions of internal morphology.

From our earlier work with the shoots of leafy plants grown for long periods on horizontal clinostats, it was hypothesized that the general form of a 3-day seedling developed on orbit would be unlike that of a seedling grown erect to the Earth's surface. The negative geotropism of the coleoptile and the positive geotropism of the roots would be eliminated during orbital flight. The seedling organs could be expected to show alterations in growth form and orientation comparable to those produced in the axes, branches, and leaves of vascular shoots as they develop on horizontal clinostats (Lyon, 1962; Lyon, 1963a and 1963b). In spite of the limitation of growth to 3 days, measurable differences in coleoptile and primary root orientation should appear in the absence of the even distribution of auxin in these organs by reason of the downward transport of and equalizing action on the auxin by the force of gravity.

The entire morphogenetic development of a wheat seedling might also be greatly altered in the absence of gravity through the lack of polarity effects on cell division and organ-forming regulators. If the plane of cell and nuclear division in the axial tissues of the germinating embryo were determined at least in part by the vector of gravitational force along the axis, irregularities in tissues might well produce atypical organs.

We also considered the possibility that the basic metabolism of growth might be disturbed at critical points of energy release and transfer within meristematic cells. A lack of sedimentation and other physical effects of gravity on the organelles could affect the energetics of germination (Dedolph, 1967; Dedolph et al., 1967). Some oxidative enzymes are known to be associated with mitochondria which migrate at certain times to the cell plate zone of meristematic cells (Hepler and Jackson, 1967). Failure of these enzyme carriers to move

normally to the sites of critical growth processes would affect at least the rates of growth processes. The complex process of plant growth in the absence of gravitational force had never been tested.

EXPERIMENT DESIGN AND METHODS

Seed Supply

The choice of wheat as the test plant was determined by factors of size, rapidity and reliability of germination, and previous work with wheat seedlings subjected to strong centrifugal forces (Edwards and Gray, 1956). Seeds for all tests of experimental systems were obtained from a certified stock of *Triticum aestivum* L. (*T. vulgare* Vill.) hort. var. Georgia 1123 (Gore and Stacy, 1961). Supplies were procured in late summer from disease-free stocks with very high percentages of germination, but the 1966 crop used for the actual flight and Earth control experiments proved deficient in rate of germination and seedling growth in comparison with the 1964 and 1965 crops.

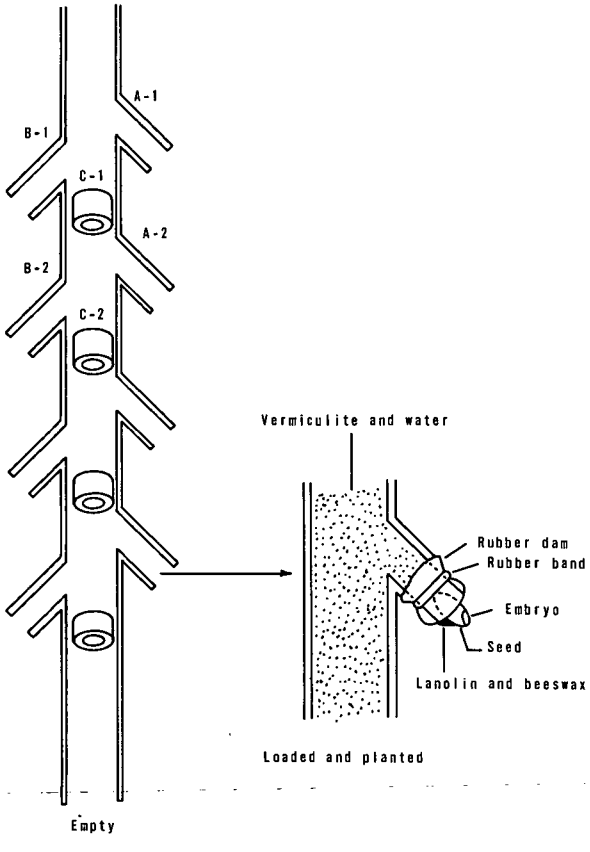

FIGURE 1.— Plastic holder system for culture of grass-type seedlings in moist air of a sealed chamber.

The special value of wheat seedlings for our study of the orientation of coleoptile and roots in the absence of tropistic effects of gravity was shown by comparative tests of wheat, rye, barley, and oats. When seedlings were grown in the special seed holders designed for our experiment (figs. 1 and 2 and table 1), the regularity of the root system in

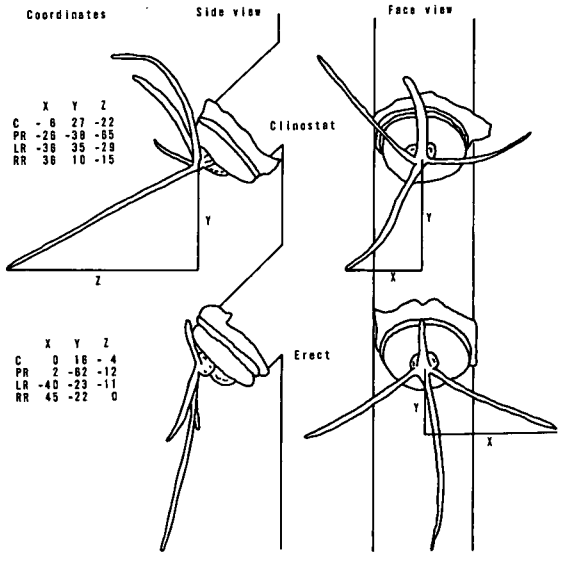

FIGURE 2.— Tracings of projected pictures of seedlings plus lines to show method of measuring *x*, *y*, and *z* coordinates for computing angular positions of organ tips. In data tables, C=coleoptile, PR=primary root, LR=left root, RR=right root.

wheat was found to provide for quantitative work which could not be carried out with any other cereal grain. Only wheat has a pattern of two lateral (seminal) roots which grow at characteristic, predictable angles to the primary root both in our seed holders and when grown in soil (cf. table 1). The plagiotropic orientation of the lateral roots proved to be particularly valuable in providing criteria for describing the effects of orbital flight on the physiology of the growth hormone in roots.

Culture Method

In order to grow seedlings without contacts between their roots and solid matter that would introduce the effects of thigmotropism or traumatropism, it was necessary to devise a method which would allow the roots to grow in all possible directions from the embryo without touching each other or

TABLE 1. – Mean Sizes and Orientations of Seedling Organs

Seedling	Age, hr	Number of seedlings	Coleoptile		Primary root		Left root		Right root	
			Length, mm	Face, deg	Length, mm	Face, deg	Length, mm	Face, deg	Length, mm	Face, deg
Wheat, erect	72	71	6.1	3.6	29.3	182.2	17.3	233.1	17.0	122.5
	72	[a]30	25.8	4.5	65.4	184.1	41.9	229.4	43.1	127.9
Wheat, clinostat	72	322	6.9	10.5	31.2	([b])	18.9	285.4	19.7	74.6
Barley, erect	48	24	3.3	4.6	17.4	180.2	11.6	192.1	11.5	156.4
Barley, clinostat	48	15	7.3	10.0	23.9	([b])	18.9	257.7	21.9	79.5
Rye, erect	48	20	3.7	3.6	30.4	182.9	15.5	193.6	14.6	164.5
Rye, clinostat	48	14	6.7	15.4	34.2	([b])	25.0	254.3	23.9	129.5

[a]Seeds planted 1 cm deep in soil.
[b]Mean angle not significant.

any other objects. A high relative humidity, like that found within the pores of an arable soil, was also essential. Since the volume of water needed for growth of an embryo into a seedling cannot be obtained by diffusion from the water vapor in moist air, the development of a large seedling during a 3-day experiment required delivery of the water to the embryo by way of the endosperm. This part of the seed was also the only available region for holding the seed and later the seedling while the young plant grew in moist air of normal composition for root environment and at a favorable temperature for rapid development.

These cultural requirements were met by an original method of suspending seeds in sets of 12 or 15 within a moist chamber, with a large part of the endosperm of each held firmly in contact with films of water. To further insure rapid germination after planting, the hydration process was started by immersing selected surface-sterilized seeds in aerated water for 3 hours before setting them in special seed holders.

Seed Holder

The design of a successful holder was worked out in cooperation with the other wheat-seedling experimenters. Details of the system and its use for terrestrial experiments were described by Lyon and Yokoyama (1966). We started with a branching system of plastic tubes filled with a slurry of finely ground vermiculite and water and provided with hollow, cloth wicks on side arms. The endosperm end of a soaked seed was inserted into the outer end of the wick and in contact with the wet vermiculite. Later we had the technical assistance of engineers at the NASA Ames Research Center and at the laboratories of North American Aviation Co. (now North American Rockwell Corp.) where practical models were finally designed, fabricated, and tested for biologic compatibility and capacity to withstand the stresses of launch and reentry of the satellite capsule.

The model used in the flight-type hardware was a system of plastic tubes in which a central stalk had 12 or 15 side arms, each set at an angle of 45° with one end of the central reservoir, as shown diagrammatically in figure 1. The short side tubes were arranged in four or five sets of three each, with the vertical spacing determined by the lengths and forms of seedling organs which developed with earlier models that were inserted in moist chambers and rotated on a horizontal clinostat. When the holder was loaded for a growth test, the central tube was sealed at each end with an Allen screw after all air bubbles had been expelled.

The enlarged detail of one arm in figure 1 shows how the seed was planted by inserting the brush end through a small hole in a rubber membrane which formed a cap over the side arm. A rubber band forced the rubber dam into a groove in the side-arm wall near the end of the arm. Leakage of water from the side arm by way of the groove in the seed on the side opposite the embryo was prevented by applying a blended mixture of lanolin and beeswax (4:1 by weight) after the seed had been planted as illustrated. A small drop of the mixture,

just warm enough to liquefy, was placed as indicated. The drop solidified rapidly, adhered to the rubber and seed, and thereby aided in holding each seed in place with proper orientation of the embryo (figs. 1 and 2). During the orbital experiment, this seal also helped to hold the seed during launch and the seedling during reentry. The rubber membrane was very effective in gripping the seed and served the important function of damping some of the vibration during launch. No seed was ever dislodged by launch stresses or reentry forces in either pretests or actual flight.

Flight-Type Package

Four seed holders of the type shown in figure 1 were included in each of the experimental packages used in the flight experiment and the four ground controls. Each package also included two holders of 15 seeds each, giving a total of 78 seeds planted for each experiment. The seedlings from these two sets of seeds were killed and fixed by chemical spray shortly before deorbit of the capsule.

Three of the sets of 12 seeds each were installed in the large moist chamber of the package while the fourth 12-unit seed holder was included in the middle of the three small growth cylinders. Conditions for germination and growth were alike in all sections of the package except for the provision to spray-fix part of the seedlings while they were still in orbit.

Experimental Procedures

The first step in conducting a growth experiment was to load each of the six seed holders with fine vermiculite, burn a hole in each rubber cap, inject the air spaces of the core with distilled water, insert a properly oriented seed about halfway through the hole in each cap, seal the groove of the seed with the mixture of lanolin and wax, and insert the planted holder at the axis of a moist chamber of the package. A small excess of water in each chamber kept its air nearly saturated with vapor (fig. 3).

The seedling organs are produced more slowly than when the roots are in contact with films of water but the form of the young plant is the same as that of a covered seedling. The germination stage and orientation of the organs are first recorded on color film. The length of each organ at the end of its growth period is then measured without removing the plant from its attachment.

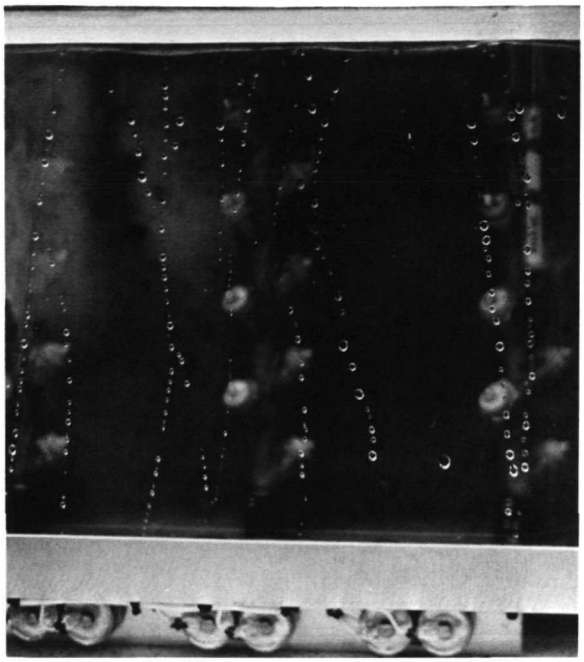

FIGURE 3.— Wheat seedling package (inverted) with three planted seed holders in position of launch and reentry. Seeds and excess water are visible.

The photography is done rapidly in a high-humidity room by means of a special camera rack and stage which includes a 45° mirror at the left side of the seed holder position. This mirror records the side view of each seedling in a row that is set to face the camera (figs. 3 and 4). An electronic flash beside the camera and coupled to the shutter release provides identical, correct exposures and a brief, intense lighting that does not injure even the root hairs on the seedling roots. The three exposures for the set of 12 or 15 plants on a holder are used as lantern slides with consequent high accuracy in measurements made from sharp, enlarged projections.

A second rack and camera system was also used for a second picture with the mirror on the right side of the seedlings in all important experiments. The tip of the right-hand lateral root is sometimes hidden behind the side arm if only a mirror view of the left side of the plant and arm is available. The second picture on a second film also served as insurance against loss of any one film during processing or in some other way.

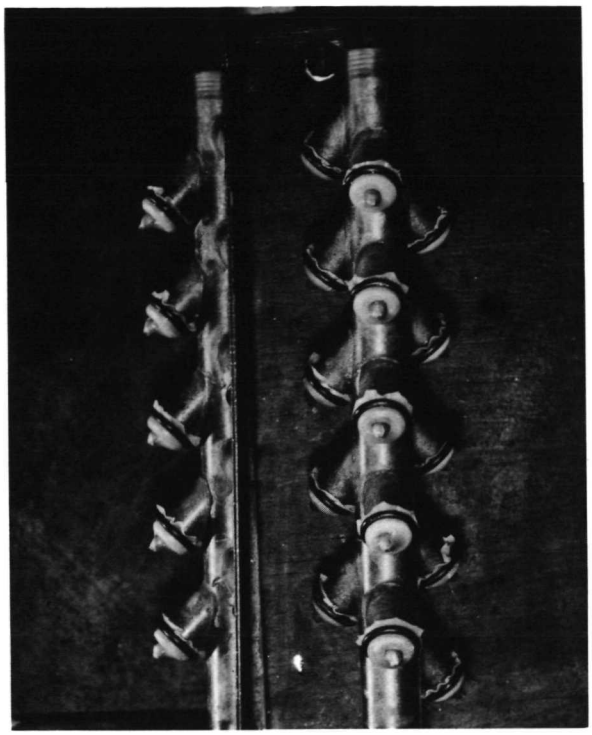

FIGURE 4.— Postflight record of typical swollen but ungerminated seeds at launch time of 15 hours after initial contact with water.

Orientation Data

The orientation of a seedling organ is described in quantitative terms by the combination of face-view and side-view angles that are made by the intersection of a line from the tip to the base of a root or coleoptile with a vertical line through the axis of the seedling (Lyon and Yokoyama, 1966). The data for computing these angles are easily obtained from the projection of each color slide on a large sheet of graph paper that is fastened to a smooth wall about 8 feet from the projector. With the vertical lines of the paper oriented with the seed-holder axis in the picture projection, the x and y coordinates for lateral organ displacement are measured with dividers and scale from the face-view image and recorded, with minus signs as needed, on a tally sheet beside the projection. The measurement for the z coordinate is made and recorded in the same way from the side-view image on the wall. The method and sample sets of data are illustrated in figure 2 by the tracings and recorded measurements from pictures of two seedlings, each grown for about 3 days on separate holders. The measurements of organ lengths in millimeters are entered on the tally sheet immediately after the photographs are taken with the mirror on the left side only. The data for the three coordinates were read in tenths of an inch and reflect the use of projected images which are larger than the actual seedling parts by a factor of about 4.8.

Each of the two orientation angles for the position of an organ tip was obtained from an X/Y or Z/Y ratio as the tangent of the desired angle or one from which the descriptive angle could be derived by suitable addition or subtraction. The computations were made with the aid of computer programs. The language is the simple BASIC devised for use with the GE 625 computer at Dartmouth College and made available to us on a time-sharing and program-storage plan by way of a teletype in our building.

The angle measurements for face-view orientation are reported to tenths of a degree on the arc clockwise from the erect 0° position. Side-view angles are measured as displacements of organ tips from the 0–180° line and recorded as negative if the curvature of the organ is not outward from the axis of the seed and seed holder. All data for orientation angles are entered on a record sheet and there used for calculations of means and their standard errors.

Early Baseline Data

With the plastic seed holders and general culture system proved acceptable for use, we began, in 1965, to compile baseline data for the lengths and orientation of seedling organs as they would develop in Earth control experiments during the period of the projected 3-day flight experiment. We were using seeds from the 1964 crop and a temperature of about 77 F for the germination and growth period of 72 hours after soaking the seeds. The planted seed holders were installed in plastic growth cylinders of a dismantled prototype package. The cylinders were placed in a dark, constant-temperature room that could be provided with high humidity for photographic records of the test results.

Since there could be no geotropic growth responses of seedling organs that might grow during weightlessness, the baseline data for determining

other possible effects of the absence of gravitational force on growth on orbit had to be obtained from seedlings grown on a horizontal clinostat (Lyon, 1965a and 1967). A rotation rate of 1 rph was used during most of the growth tests in our laboratory and later for systems tests in Philadelphia to check on possible effects of vibration on the developing roots (Lyon, 1961). Special rotators with capacity to turn an entire seedling package at the rate of 6 rph were built for the later clinostat control experiments in the launch laboratory and for the postflight tests at the Ames Research Center. A full report of the differences in orientation of seedling organs grown erect to gravity or with geotropic responses eliminated by rotation of the plants in vertical planes was published in 1966 (Lyon and Yokoyama) on the basis of the baseline data obtained in 1965 with an excellent lot of seeds.

A simplified summary of these early test results has been entered in table 1. There were no significant differences in the lengths of roots and coleoptiles which grew in the seed holders under the two gravity environments (1*g* and clinostat). The notable differences in organ orientation shown in table 1 are the curvatures in the wheat coleoptiles that developed on a clinostat and the difference of about 50° in the mean face-view angles of the lateral roots. These differences in root orientation caused the root tips to grow into the quadrants adjoining the 0° position rather than at an angle of about 53° with the 180° orientation line of a primary root with perfect response to positive geotropism. This proof of root epinasty in the lateral roots of wheat established an excellent criterion for using the baseline data from clinostat experiments in evaluating the results of seedling growth on orbit.

Wheat in Soil

The data in line 2 of table 1 were obtained from wheat seeds planted in soil as a check on the validity of orientation of roots and coleoptiles grown in our seed holders. At the end of 72 hours at the same temperature, the soil was washed from the roots which were allowed to spread naturally in shallow water for measurements of orientation angles with a goniometer. Although the seedling organs grew much faster and longer with the roots in contact with soil water, their mean face-view angles were not significantly different from the corresponding angles for organs formed with the seeds held in the special holders. Side-view angles could not be measured accurately but they appeared to be much like those observed for wheat seedlings grown in our seed holders.

Other Cereal Seedlings

The results of a few tests with barley and rye seedlings grown in the same holders for 48 hours are included in table 1. Strict comparisons with wheat seedlings could not be made because only wheat has a real primary root and a pair of plagiotropic laterals. The three longest roots, however, with orientations approximately similar to those of wheat, showed the presence of root epinasty to about the same degree in both rye and barley. The data for coleoptile form also show comparable changes in the face-view angles of wheat, rye, and barley when they are grown on clinostats rather than erect to gravity. The side-view angles of all four organs were much greater when the tropistic effects of gravity were eliminated.

This limited study of other grass-type seedlings made it clear that wheat is the best test plant available among the monocotyledons. The differences in growth form of their seedlings in experiments, with and without the directional effects of gravity, are not peculiar to wheat. The supplies and distribution of growth regulators within these grass seedlings appear to be much the same between genera. Other seed-holder tests with oat (*Avena*) seedlings showed similar results for the coleoptile but there were such weak geotropic responses in the roots that the data indicate quantitative differences in the amount and mobility of their growth hormone.

Spacecraft Tests at Philadelphia

Preflight tests of operation of the wheat seedling package within the spacecraft capsule during simulated orbit for 3 days were conducted at the General Electric Co. facility in Philadelphia during the summer of 1966. All spacecraft systems were tested for environmental controls, command activities, communications, and engineering records. Experimental packages with live organisms were used just as planned for actual flight.

The critical points for the operation of the wheat seed package included seed germination and growth of seedlings while the capsule was sealed in the

thermal-vacuum chamber for about 72 hours and subjected to the planned profile of temperature and other environmental factors of orbit. The wheat was to be planted, placed within the payload capsule, provided with the specified conditions for germination, and recovered in seedling form, and the results were to be used for Earth control data if they were acceptable as evidence of satisfactory spacecraft performance.

The first three of these tests failed to produce good wheat seedlings in spite of an otherwise generally acceptable performance of the spacecraft systems and the other experiments. The poor results from our package can be illustrated by the schedule and experimental data for the test of June 12 to 16, 1966. The period of simulated orbit was June 13 at 1000 hours to June 16, same time, preceded by about 12 hours of prelaunch hydration of seeds and followed by about 13 hours of disassembly before return of the package to the experimenters. A control set of planted seed holders was kept in shaded plastic cylinders for the same period at a slightly higher temperature, with seed embryos held erect to gravity as in the thermal-vacuum test package. The seedlings in chambers I and II of both test and control packages were spray-fixed as indicated in table 2.

The contrasting poor and good results, respectively, for the test and control sets of seedling growth are evident from table 2. Germination was initiated for all seeds but was severely retarded in the spacecraft by some nontoxic, inhibitory factor. Low temperature in the test package was at once considered to be a possible cause for the very slow growth in spite of thermistor records which were within the specifications for the 3-day test. Similar unsatisfactory growth results were obtained with a second test in the same capsule and with the corresponding test in a capsule being prepared for launch.

The results of a subsequent intensive study of the problem by means of special tests for temperature gradients and possible deficiencies led to our discovery that seedling growth had been retarded by the use of the heater blankets to raise the temperature in the growth chambers of the package to several degrees above that of the capsule atmosphere. The exact cause of the depressant effect was then unknown but the evidence was the basis for the decision to disconnect the electric circuits in the blankets for the fourth thermal-vacuum test in September 1966. The result was acceptable growth of the seedlings, limited only by the suboptimum temperature of the spacecraft. In later experiments in November, the seeds were heated to 76 F with a hot-water blanket around the sides of the moist chamber in place of the electric blanket and the ends of the chamber were held at a constant room temperature of 70 F. We found that the growth retardation in the test packages had been due to distillation of water from the warm seeds to the colder lids and bases of the growth chambers.

The measurements of seedling organ lengths from the successful September systems test could not be used for baseline data of the flight experiment 1 year later. The profile of temperature and growth period was entirely different and no 1964 seeds were available for the experiment launched and recovered in September 1967. Aside from proof of the biocompatibility of using our package without the supplementary heat, the most impor-

TABLE 2. – Mean Lengths of Organs From Capsule Test of First Thermal-Vacuum Experiment (June 1966)

Package	Chamber number	Number of seedlings	Age, hr	Coleoptile, length mm	Primary root length, mm	Lateral root length, mm
Test	I	15	[a]60	0.40	0.37	0
	II	15	[a]72	.90	.57	.13
	III–VI	48	97	1.03	1.95	.47
Control	I	15	[a]60	2.40	18.30	4.60
	II	15	[a]72	3.70	25.50	11.20
	III-VI	48	96	4.26	22.14	14.67

[a]Spray-fixed at indicated germination ages.

tant result of the thermal-vacuum tests was the development of the timetables and laboratory equipment needed to deliver the planted package to the capsule assembly at the proper time, and to process the results of seedling growth within the flight-type packages of the orbital and Earth control experiments.

Preflight Vibration Tests

It was essential to our seedling experiment to determine, preferably in advance of the orbital growth test, that possible differences observed in the growth physiology of seedlings produced on orbit and on clinostats were not due to the vibration stress of launch. The danger of interference of this factor in our experiment was greatly reduced by the damping effect of the rubber membrane around the seeds and by the absence of seedling organs at the time of launch. It was impractical to pretest all of the vibration stress of launch and impossible to combine them with the elements of the acceleration forces. The choice was made to expose the seeds to the most severe component of launch vibration — the longitudinal vector which was tested for random-type vibration which produced an applied force much greater than that experienced during actual launch. The stress on the damped seeds could not be measured in either test or launch but the effects were directly comparable for the two situations.

Of the Philadelphia pretests with the qualifying spacecraft capsule and the two vehicles prepared for orbit, only the first was carried out with good simulation of the 4 minutes of vibration when the seeds were in the stage of hydration projected for launch. One test was made after a 7-hour hold preceding two periods of vibration. In the other test stress was applied after a 15-hour hold and with seeds in unknown stages of retarded germination caused by the desiccation effect of the heater blankets which had been activated at least 15 hours preceding the vibration operation. In spite of these irrgularities, no ill effects of the launch stresses could be seen in dislodgment of seeds from the holders or in the subsequent growth of the seedlings for about 72 hours after simulated launch.

The successful test of the qualification capsule was sufficient of itself to establish the absence of effects of launch vibration on the growth and growth regulator processes of wheat seedlings. The force of the longitudinal stresses was intentionally raised to 50 percent above that expected at launch and the vibrations were applied only ½ hour later than scheduled. Delays in returning the vibrated package to the experimenters resulted in the seeds having just started to germinate at age 25½ hours from initial hydration after exposure to an unknown temperature profile for most of the germination period. Seeds in the nonvibrated control package had germinated to a slightly greater extent at 74±1 F when they were transferred to grow-out chambers from initial hydration at experimental age 23 hours.

These grow-out chambers were used to simulate a 72-hour growth period of orbit and capsule recovery after launch of the test package. They were plastic cylinders with black paper jackets; half of the chambers were erect to gravity and the other six were turned on horizontal clinostats until the simulated flight test (and Earth control) at 74 F was concluded with measurements and photographs at seedling age of about 82 hours. The vibrated and nonvibrated seeds were distributed in sets evenly between the clinostat and erect grow-out chambers, as shown by the arrangement of data in tables 3 and 4 for the sizes and orientation angles of seedling organs from the test and control packages. (In the tables n indicates the number of seedlings for which results are given.)

The measurements of seedling organs (table 3) show that both vibrated and nonvibrated seeds grew faster on clinostats than erect, a result that may be due in part to an even distribution of the water supply only on clinostats by reason of rotation of the horizontal seed stalks. Some of the stalks from both growth conditions were found by later inspection to be unevenly filled with vermiculite and therefore unable to maintain contacts of all seed coats with the water in the erect seed holders. Another contributing factor to the higher mean lengths for the clinostat seedling organs was the contact of the tips of a few primary roots with excess water along the walls of the horizontal cylinders, resulting in abnormally long organs.

That the seedling organs from vibrated seeds were slightly smaller than those from nonvibrated seeds with the same growth conditions after vibra-

TABLE 3. – Mean Lengths of Seedling Organs of Vibration Test in Qualification Spacecraft

[Pairs of means in any column are not significantly different statistically if coded with the same letter]

Treatment	Coleoptile		Primary root		Lateral roots	
	n	Length, mm±S.E.	*n*	Length, mm±S.E.	*n*	Length, mm±S.E.
Vibrated:						
Clinostat	36	4.9±0.5	36	25.0±1.7 a	72	14.7±0.9 a
Erect	38	3.4±0.2 b	38	19.1±1.6 b	76	8.5±0.9 b
Nonvibrated:						
Clinostat	40	6.9±0.5	40	29.3±2.2 a	80	17.6±1.0 a
Erect	32	4.0±0.4 b	32	24.0±1.6 b	64	9.3±1.1 b

TABLE 4. – Mean Organ Orientation Measured About 3 Days After Vibration Test in Qualification Spacecraft

[Pairs of means in any column are not significantly different statistically if coded with the same letter]

Treatment	Coleoptile			Primary root			Left root			Right root		
	n	Face angle[a]	Side angle	*n*	Face angle[a]	Side angle	*n*	Face angle	Side angle	*n*	Face angle	Side angle
Clinostat vibrated	36	9.1 a	9.2 a	34	20.5 a	32.1 a	29	282.4 a	106.9 a	31	86.0 a	97.4 a
Clinostat nonvibrated	40	13.8 a	12.8 a	38	20.4 a	31.9 a	34	278.9 a	106.4 a	34	73.2 a	115.3 a
Erect vibrated	37	3.7 b	4.1 b	31	8.5 b	15.6 b	23	231.3	21.4 b	23	125.1 b	15.5 b
Erect nonvibrated	32	4.4 b	7.3 b	28	9.4 b	17.2 b	20	242.9	24.0 b	20	124.3 b	18.0 b

[a] Angle measures deviation from the 0° to 180° line, left or right.

tion may have been due to loss of some water from a few stalk arms during vibration. With one exception, the differences in mean lengths were not statistically significant. The desiccation effect of the activated heater blankets while the test package was held in the capsule would also have delayed the germination of the vibrated seeds. The retardation was noted when the test package was opened for transfer of the seed stalks to the grow-out chambers, but the specific effect of the heater blankets was unknown at that time.

Possible effects of launch vibration on the orientation of seedling organs through changes in the amounts or transport of their growth hormones are shown in table 4 to have been absent in this preflight test. The displacement of organs produced on clinostats was characteristically different from their positions on erect seedlings in both vibrated and nonvibrated plants. There was only one case of significant difference in the means for orientation angles of comparable organs on the two sets of plants when they were grown either erect or on clinostats after vibration.

Postflight Vibration Tests

The successful launch of the orbital experiments and their recovery, together with a good record of the temperatures and stresses for the curtailed growth period of about 65 hours, made it possible to conduct a second set of Earth control experiments which could be subjected to vibration and acceleration stresses at the physiologic stage of actual launch. The flight profile of time and temperature could also be used in the interests of possible improvement in the baseline data for terrestrial experiments.

The postflight tests, with vibrated and nonvibrated packages, were carried out in May 1968

at the Ames Research Center, Moffett Field, California. The materials, hardware, and procedures were essentially identical with those used for the flight and Earth control experiments in September 1967. The seed supply came from the same 1966 crop and had been stored in refrigerators since its purchase, but the seeds were about 8½ months older than the seeds used for the 1967 experiments.

Control tests included two tests with seedlings grown in holders erect to gravity, either vibrated (and centrifuged) or nonvibrated (tables 5 and 6). There were three tests with germination and growth of plants on clinostats for use in determining the baseline data for organ orientation (table 6) in the absence of the effect of gravity, with and without launch stresses on the seeds. As shown in table 5, test 5 was closed at a seedling age of 60 hours in order to obtain data on the extent of seedling growth at the time of deorbit of the flight experiment.

A possible effect of launch stresses on the growth rates of seedling organs in these tests can be seen to be entirely lacking (table 5). The few cases of significant differences are between organs developed on clinostats rather than erect. These differences probably reflect the irregularities in water supply that influenced greatly the growth of seedlings from the overaged 1966 seeds. Delivery of water to the seeds in our holders is favored by their rotation in the clinostat experiments, a factor which has proved to be insignificant for seedlings making vigorous growth. This lot of 1966 seeds has never produced seedlings under our culture system as rapidly as did seeds from the 1964 and 1965 crops of the same strain. We suspect subtle differences in the permeability of the seed coats to

TABLE 5. – Mean Lengths of Organs From Postflight Tests With Comparative Data From Flight Experiment

[Comparable pairs of data are not significantly different statistically if coded with the same letter]

Test	Treatment	Coleoptile		Primary root		Lateral roots	
		n	Length, mm	*n*	Length, mm	*n*	Length, mm
			58 hr				
	Flight	25	3.2	25	8.6	50	2.4 ab
1	Erect vibrated	29	2.0 a	29	14.5 a	54	1.8 a
2	Erect nonvibrated	26	2.3 ab	26	15.3 a	52	3.8 bc
3	Clinostat vibrated	27	2.5 b	27	17.4 a	50	5.3 c
4, 5	Clinostat nonvibrated	59	2.7 b	59	14.3 a	116	3.9 c
			65 hr				
	Flight	48	4.1 a	48	21.5 a	96	10.1 a
1	Erect vibrated	47	3.5 ab	47	21.4 a	94	4.8 b
2	Erect nonvibrated	48	3.4 b	48	21.2 a	92	6.1 bc
3	Clinostat vibrated	47	3.5 b	47	20.5 a	94	7.8 ac
4	Clinostat nonvibrated	45	3.3 b	45	22.1 a	90	7.9 ac
			60 hr				
5	Clinostat nonvibrated	48	3.2	48	16.4	96	4.9

water. Percentage of germination of 1966 seeds has always been high but growth of organs is commonly very slow in some seeds of a set, as though it were restricted by slow entry of water through the surfaces in contact with the wet vermiculite. In these postflight tests the coleoptiles and lateral roots seem to have shown a different pattern of growth, generally slower, from that shown by the comparable organs from the younger seeds used in the flight experiment. This conclusion is justified in part by the good agreement (see table 7) between sizes of organs produced in flight and in the

TABLE 6. – Mean Organ Orientation After Postflight Test Profile of Temperature and Vibration

[Pairs of means in any column are not significantly different statistically if coded with the same letter]

Treatment	Coleoptile		Primary root			Left root			Right root		
	n	Face angle[a] +side angle	*n*	Face angle[a]	Side angle	*n*	Face angle	Side angle	*n*	Face angle	Side angle
Flight	73	15.2	71	21.2	34.7 a	45	279.7 a	135.8 c	51	79.7 a	112.8 ac
Clinostat vibrated	74	9.5 a	74	32.4 a	34.6 a	47	275.0 a	111.3 a	45	84.3 a	100.4 a
Clinostat nonvibrated	69	10.3 a	73	39.1 a	41.1 a	46	278.1 a	116.0 ac	44	80.4 a	117.2 ac
Clinostat nonvibrated	75	9.6 a	72	33.1 a	41.2 a	38	277.8 a	109.1 a	39	82.0 a	123.5 c
Erect vibrated	74	8.7 a	75	6.2 b	11.8 b	20	240.1 b	15.6 b	27	119.0 b	16.9 b
Erect nonvibrated	72	7.7 a	71	9.1 b	11.4 b	40	243.7 b	13.1 b	37	117.2 b	18.1 b

[a] Angle measures deviation from the 0° to 180° line, left or right.

TABLE 7. – Mean Lengths of Organs in Control Experiments With Data for Flight Experiment Also Shown

[Comparable pairs of means are significantly different statistically only if coded with the same letter]

Experiment	Temp., F	Position	Coleoptile		Primary root		Lateral roots	
			n	Length, mm	*n*	Length, mm	*n*	Length, mm
58 hr								
Flight	67.5	Free fall	25	3.2	25	8.6	50	2.4 a
Control IIIC	68.6	Horiz.	25	2.7 a	25	7.7 a	50	2.0 cd
Control II	70.7	Horiz.	30	3.3 a	30	11.0 a	60	3.8 ade
Control IIIE	67.7	Erect	27	2.9	28	10.4	56	4.0 bc
Control I	70.0	Erect	27	2.8	27	8.8	54	1.8 be
65 hr								
Flight	67.5	Free fall	48	4.1 a	48	21.5	96	10.1 a
Control IIIC	68.6	Horiz.	46	4.0	46	21.3	92	10.6
Control II[a]	70.7	Horiz.	43	3.6	43	20.9	84	11.7
Control IIIE[b]	67.7	Erect	46	3.9	46	25.0	92	13.8 ab
Control I	70.0	Erect	45	3.6 a	46	21.5	92	9.7 b

[a] Organs measured at deorbit time, age 60 hr.
[b] Organs measured 75 min. after those of control IIIC, age 66.3 hr.

Earth control experiments conducted simultaneously during the launch and flight of the orbital experiment in September 1967.

The simulated launch vibration and acceleration forces during the minutes between lift-off and injection into orbit are shown (table 6) to have been without effect on the physiology of organ orientation. The mean positions of all roots grown on clinostats were unquestionably different from those found on erect seedlings. The roots that developed from vibrated seeds grew at angles which were on the average alike when compared with those measured for nonvibrated experiments, whether germination took place on erect seed holders in spacecraft capsules or on rotating clinostats. There were no significant differences in the total displacements of coleoptiles from the original seed axis whether germination took place on erect or horizontal seed holders and whether the seeds were vibrated. This is contrary to our previous experience and must be due to the slower growth of coleoptiles from the aging 1966 seeds. The coleoptile curvatures amounted to even less than those in the short coleoptiles of the curtailed flight experiment.

The lack of growth vigor in these postflight seedlings can be seen also in the data for orientation of lateral roots. The differences between their mean face- and side-view angles and the means for the corresponding angles of the flight experiment are not statistically significant. There is a tendency for weaker root epinasty in the postflight clinostat seedlings than in the seedlings developed from the younger seeds of the flight experiment. Even with their reduced vigor, the roots of the postflight seedlings provided good criteria for possible effects of launch stresses on growth regulators. No effects can be seen in our data for orientation from either preflight (table 4) or postflight experiments (table 6).

Seed Hydration Test

The delay of 3 hours in the launch of Biosatellite II meant that the processes of seed hydration had progressed for 12 hours beyond the time of their removal from immersion in water before they were planted. This raised the question of the degree to which they had absorbed water by imbibition and osmosis at critical points before and after launch of the flight experiment. The extra hydration time provided by the 3-hour delay was somewhat beneficial for the curtailed period of orbital growth, but it was desirable to know that the seeds had been sent into orbit while germination was still in the stage of absorbing water, prior to complete liquefaction of the endosperm and availability of water for the enlargement phase of root initials.

The entry of water into sets of 15 selected seeds under the temperature and moisture conditions of the launched experiment is shown graphically in figure 5 as determined by a special postflight study.

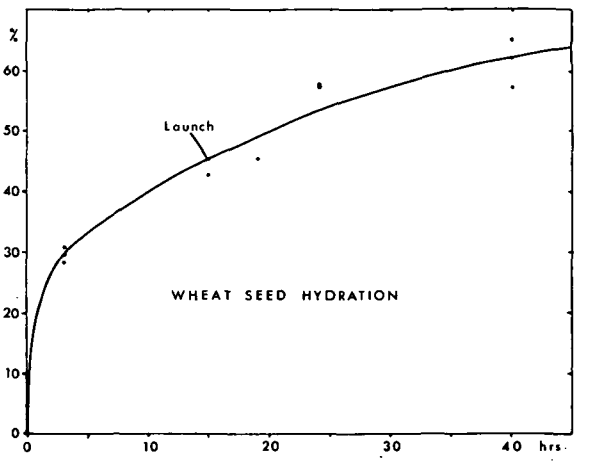

FIGURE 5.— Postflight record of seed hydration before and after launch time. Percentages show gains in weight of seeds immersed in water for 3 hours, then planted in holders until weighed.

Each set of seeds was weighed before contact with water and at certain times later as indicated on the plot. The increases in weight due to absorption of water are shown as percentages of the original seed weight. Water was absorbed rapidly by imbibition to the extent of about 30 percent during the 3-hour immersion in water at 90 F before the seeds were planted as usual in the seed holders. Water from the wet core of the holder continued to enter before and after the launch time of 15 hours when the water content of the seeds had reached about 70 percent of capacity.

The plotted curve for three sets of seeds was analyzed by a computer method and found to have the simple exponential form of imbibition for the first 19 hours. Soon after that the curve changed to a new form (cf. fig. 5), probably because the observed organ formation shortly before the 24-

hour stage of hydration had begun to draw on the supply of water entering through the endosperm. Seed hydration at launch time was therefore shown to be so incomplete that the six sets of seeds in the package had presumably been placed in orbit before germination had reached the stage of organ elongation.

Germination at 15-Hour Stage

Postflight experiments to obtain visual evidence for this absence of organ elongation at 15 hours were conducted with the same flight-type seed holders, plastic moist chambers around the seeds, planting procedures, and mean temperature as for launch of the orbital experiment. As illustrated in figure 4, which shows face and mirrored side views (at the left) of seeds with maximum germination results observed at 15 hours, no roots or coleoptiles of measurable length were present. Of 180 seeds examined at this germination age, none had progressed beyond a condition of ruptured seed coats over the swollen radicle end of the embryo. When the seedling organs from this series of tests were measured at the 65-hour stage, normal germination and seedling growth had taken place.

Earth Controls for Flight Experiments

As provided in the schedule for preparation of the flight experiment and delivery of the sealed package to the spacecraft payload assembly, four duplicate test packages were prepared at the same time. One served as a backup package and all were used for control experiments that were conducted in the launch laboratory at Cape Kennedy. By using procedures carefully timed to provide operations essentially simultaneous with the events of the orbiting experiment, these control tests were used to expose the developing seedlings to the conditions listed in table 8. Control I was maintained at a constant temperature in a mockup of the spacecraft capsule, with the seedlings growing erect to gravity and its package in place among the other experiments and radiation source from which it was screened. The backup package was used for control II and held with the seed holders erect until the package was placed on a horizontal clinostat in a growth chamber when the satellite was inserted into orbit. This experiment was removed from the clinostat and the contents were processed upon notification that the flight experiment had been removed from orbit. The packages for controls IIIC and IIIE were prepared 4 hours after the other three units so that they could be provided with the temperature profile as reported by telemetry from thermistor records in the orbiting package. These control packages were placed in a growth chamber with temperature programmed to follow that reported from the orbited capsule. All events were 4 hours later than those of the orbited experiment. Control IIIC was attached to a clinostat at simulated entry into orbit, removed to the erect position about 45 hours later, and processed 4 hours after the orbited package had been opened in the Hickam Field laboratory. Control IIIE was left with the seed holders erect to gravity and processed after control IIIC as indicated in table 8. The small difference in mean temperature recorded from thermistors within the two packages was due to a temperature gradient in the growth chamber, which was perhaps due in part to radiant heat emitted by the clinostat motor.

TABLE 8. – Ground Controls for Flight Experiment

Control	Seedling position	Hours (approx)	Mean temp., F	Seedling age (unfixed), hr
I	Erect	65	70.0	65
II	Erect	15		
	Clinostat	45	70.7	60
IIIE	Erect	66.3	67.7	66.3
IIIC	Erect	15		
	Clinostat	45		
	Erect	5	68.6	65
Flight	Erect	15		
	Free fall	45		
	Erect	5	67.5	65

The germination percentage of about 96 percent varied little among the four control tests and was no higher with the higher temperature of control II. This temperature increased the growth rates of seedling organs enough to produce coleoptiles and roots of mean length as great at 60 hours (cf. table 7) as those of other controls closed at 65 hours. The result was loss of a good record of probable organ lengths in the flight experiment at the time of deorbit, the object of closing this control with seedlings only 60 hours old.

The mean lengths or organs from the four control (and flight) experiments are shown in table 7. The agreement in elongation is good among data for comparable pairs of these controls. The significant differences for 58-hour seedlings occur chiefly in comparisons involving control II with its higher temperature and in pairs of data for lateral roots; they showed great variability in their elongation at this stage of germination.

When measured at the close of each control experiment, there were no significant differences in mean lengths of comparable pairs of organs with the exception of the lateral roots on the 66.3-hour seedlings of control IIIE in comparison with those of younger seedlings in control I. The higher temperature effect of making longer roots in control II was offset by measurement of the roots at the age of 60 hours. The small differences in temperature among the other control packages had no significant effects on the comparative elongation of the seedling organs.

This matter of slight differences in temperature came to be an important point in view of the unsatisfactory growth of the overage seeds in the postflight controls, each with the temperature profile of the flight experiment. The Earth controls at Cape Kennedy have proved to be reliable for organ length except for the accelerated growth in control II. Our measurements of orientation angles of the roots and coleoptiles of the Earth control seedlings at Cape Kennedy also proved to provide good baseline data for the flight experiment. As shown in table 9, the orientation of the organs of the clinostat controls (IIIC and II) agreed almost perfectly between the two sets of measurements. There was complete agreement between the comparable pairs of data for orientation of the erect controls (IIIE and I) and in only one case was the mean of angles for erect seedlings not significantly different from the corresponding angles for organs developed on clinostats.

The roots grew rapidly enough to show strong epinastic curvatures in both face and side views of clinostat plants. The total displacements of the tips of the coleoptiles, however, were significantly different for the erect and clinostat conditions only for those with the greater curvatures of control II. This is understandable as reflecting the faster growth of their warmer coleoptiles. The effect of the absence of the gravity vector for basipetal transport of auxin in the control IIIC seedlings was offset by the opportunity for the diffusion of auxin to equalize its distribution laterally in the slow-

TABLE 9. – Mean Orientation Angles of Organs in Control Experiments With Data for Flight Experiment Also Shown

[Pairs of means in any column are not significantly different statistically if coded with the same subscript letter]

Experiment	Coleoptile		Primary root			Left root			Right root		
	n	Face angle[a] +side angle	*n*	Face angle[a]	Side angle	*n*	Face angle	Side angle	*n*	Face angle	Side angle
Flight	73	15.2 a	71	21.2 a	34.7 ac	45	279.7 a	135.8 a	51	79.7 a	112.8 a
Control IIIC	71	13.1 ab	69	20.2 a	26.1 a	47	272.1 a	94.5 c	50	83.1 a	102.6 a
Control II	72	15.7 a	72	28.0 a	43.8 c	61	277.9 a	114.2 ac	58	76.7 a	118.3 a
Control IIIE	73	11.1 b	71	8.5 b	10.3 b	63	239.8 b	21.1 b	64	116.8 b	16.5 b
Control I	67	10.6 b	70	7.8 b	12.5 b	48	242.0 b	18.1 b	53	116.4 b	16.7 b

[a] Angle measures deviation from the 0° to 180° line, left or right.

growing coleoptiles. Except for this trivial difference, we shall see that the orientation of organs in the control seedlings supplied excellent criteria for judging the effects of seedling growth in orbit.

RESULTS

General Appearance of Seedlings

Inspection and photographs of the six sets of seedlings in the package returned to the Hickam Field laboratory, after 45 hours on orbit and five hours of growth in the recovery airplane and disassembly trailer, gave good evidence that the plants had grown as much as could be expected for the growth period and temperature profile. The general form, origin, and color of the roots and coleoptiles showed no peculiarities for wheat seedlings. The germination of about 96 percent of the seeds was the same as in earlier tests and there were no greater irregularities in morphology and aborted roots than had been observed previously with the 1966 seeds. The first impression was of a close resemblance to seedlings freed of the directional effects of gravity by growth on a horizontal clinostat (Lyon and Yokoyama, 1966).

The two sets of plants from the fixation chambers had very short roots and shoots, with the primary root most conspicuous as illustrated in figure 6. Even at germination age 58 hours, the longest of the lateral roots usually showed strong epinasty in both face and side views. The orientation of most primary roots reflected their epinastic tendency as seen in side view. The absence of the geotropic effects of gravity was clearly the most pronounced effect of seedling development in weightlessness for slightly less than 43 hours. The four sets of live plants in the other two chambers of the package had grown about 2 hours longer in orbit and 5 hours with the seed holders erect before the seedlings could be seen and photographed. The primary roots were conspicuously longer than those on the fixed plants, and many of the lateral roots were much longer and oriented as on clinostat seedlings, but there were many lateral roots only ½ to 4 mm long. Seedlings with fully developed roots, roothairs, and characteristic orientation are shown in figures 7 and 8. The measurements of all roots, however, were included

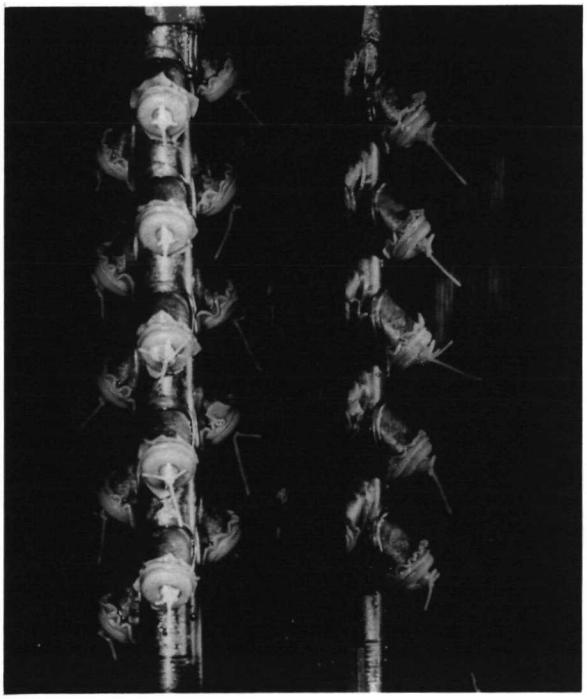

FIGURE 6.— Set of seedlings that grew for about 43 hours in orbit until spray-fixed by ground command at age 58 hours. Side views of five plants appear in 45° mirror at right of their face views.

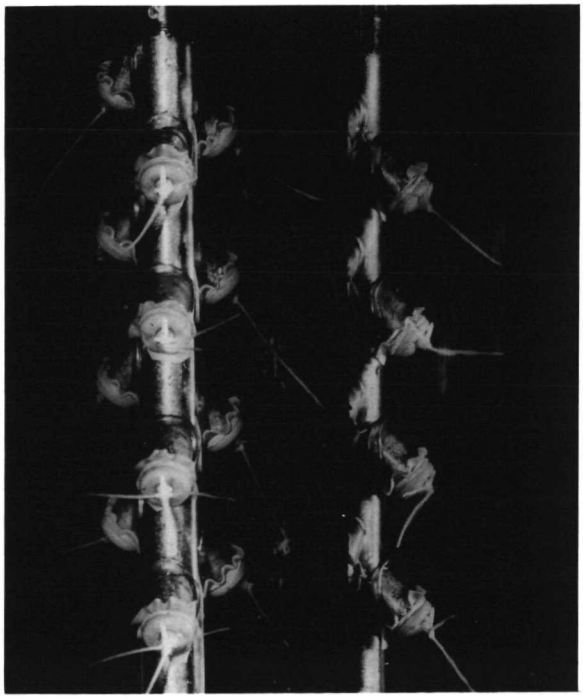

FIGURE 7.— Live seedlings that grew for about 45 hours in orbit and 5 hours during recovery operation. Side views of four plants appear in 45° mirror at right of their face views.

in the data for mean lengths of seedling organs produced after about 65 hours of germination. The occasional absence of a root was recorded as zero, but ungerminated seeds were omitted from computations. One seedling was so small and defective that it was considered too irregular for use as an indicator of growth reaction.

Lengths of Seedling Organs

The large variation in the entries for length of lateral roots reflects the unplanned close of the experiment at the height of their growth activity. The seedlings from stalks V and VI came nearest to reaching a balance of growth of all organs but even for them the mean length of coleoptiles was not much greater than for the seedlings fixed about 7 hours earlier in chambers I and II. For these younger seedlings, most of the lateral roots were still in the arrested stage that follows an initial elongation of less than 3 mm.

FIGURE 8.— Set of 12 live seedlings that grew for about 45 hours in orbit and 5 hours during recovery operation. Side views of four plants appear in 45° mirror at right of their face views.

There were considerable differences in the growth rates of seedlings borne on different seed stalks, as shown statistically by the data of table 10. The seedlings fixed at age 58 hours in chamber I were smaller, on the average, than those of the same growth age in chamber II, yet the variance for each set was so great that there is no significant difference between their mean lengths. A notable case, however, of a different rate of germination in one set of seeds is the retardation of lateral root growth on stalk IV. Since these seeds were located in the same large chamber with those of stalks V and VI, the cause of the retarded growth is an unknown factor in the seed holder (probably an irregularity in water supply to its 12 seeds). The delay in germination of some seeds was not great enough to affect the mean lengths of primary roots and coleoptiles on the same seedlings. The growth responses in the four sets of unfixed seedlings and two sets of fixed plants can best be considered as two combined data units in comparing them with corresponding baseline data of Earth control experiments.

These means for flight organ length at 58 and 65 hours will be found as entries in table 7 and are

TABLE 10. — Mean Lengths of Flight Seedling Organs

[Comparable pairs of data in any column are not significantly different statistically if coded with the same letter]

Stalk	Age, hr	Mean lengths, mm ± S.E.			
		Coleoptile	Primary root	Left root	Right root
I	58	3.0±0.2 a	8.1±0.9 a	1.9±0.6 a	1.7±0.4 a
II	58	3.3±0.2 a	9.1±0.7 a	2.8±0.6 ac	2.9±0.5 a
III	65	4.0±0.2 b	19.8±1.9 b	9.9±2.5 b	8.8±2.3 bc
IV	65	3.9±0.3 b	21.4±1.2 b	4.3±1.5 c	6.3±1.5 b
V	65	4.3±0.1 b	21.0±2.6 b	10.3±2.0 b	12.4±1.3 c
VI	65	4.3±0.3 b	23.9±1.9 b	14.8±1.3 b	13.7±1.4 c

displayed there for convenient comparison with the data for growth of Earth control seedlings for the same periods of time in the launch laboratory at Cape Kennedy. There is close agreement between sizes of organs in the flight and control seedlings, as indicated by the coding for comparison of lengths. The statistical analysis of the data was directed specifically toward significant differences at the 1-percent level of confidence. Our computer program for means, variance, and T-ratio was based on the Kurtz (1963) method for pairs with possible unequal variance. The exact probability was computed by the Veldman (1967) two-tail formula for use of known T-ratio and degrees of freedom.

Significant differences between organ growth in flight and control seedlings were limited to slightly shorter coleoptiles at 65 hours in an erect control, longer lateral roots at 58 hours in control II with its mean temperature 3 degrees higher, and longer lateral roots in control IIIE for which the organs were measured when they had grown 75 minutes longer than those of the flight experiment. The roots and coleoptiles which developed without an action of gravity except near the close of the flight experiment were, on the average, neither longer nor shorter than the organs which grew either erect to gravity or on a rotating horizontal clinostat in the Earth control laboratory.

Organ Orientation

To compare the orientation of organs in flight and Earth control experiments, it was unnecessary to separate the data on the basis of age of seedlings. The means of orientation angles for sets of flight seedlings were much alike for the organs fixed at 58 hours and for those which grew until the chambers were opened at 65 hours. The variance of these means, however, was so great among data for the six sets of seedlings in each package of the flight and control experiments that it was impractical to use the six sets of means in the analysis of the effects of weightlessness on organ orientation. It was less confusing and more effective to combine the six sets of data for the face- and side-view angles of each organ in the flight experiment and in each of the four control experiments.

These data for orientation angles formed the basis for table 9. Orientation data were not obtained for roots shorter than 2 mm in either flight or control seedlings, since the initial angles for all roots are determined by factors unrelated to gravity and auxin transport. The statistical methods used for analysis of comparable data were the same as those used for length of organs. The number of organs involved in computing each mean angle of table 9 depended on percentage germination, aborted organs, and, particularly, the presence of many lateral roots too short to register differences in orientation due to the gravity environment.

The critical test for possible special effects from seed germination during weightlessness is comparison of orientation angles of organs in the flight and clinostat control seedlings. The data for these angles in table 9 show close agreement of the mean values for all organs, as indicated by the coding of statistically nonsignificant differences. There are very few differences among the pairs of comparable angles which describe the orientation of organs that developed without a geotropic effect of gravity. Also without exception, the angles formed during flight (and exposure to gravity for the last 5 hours of the experiment) are very different on the average from the corresponding mean values for seedlings held erect to gravity throughout their growth period.

DISCUSSION

Growth Processes

With the molecular forces of imbibition and osmosis acting to move water into the seeds for 15 hours before launch, these early steps in germination could be expected to continue on orbit. Certain cells of the embryo would enlarge as the seed swelled by hydration. Digestion of starch and protein could be initiated by their hydrolytic enzymes before other metabolic processes were available to supply energy for orderly growth. Analysis of the hydration curve in figure 5 has shown that the exponential function associated with the initial absorption of water by imbibition held for about 20 hours before other processes started to participate in the movement of water through the endosperm.

The development of normal wheat seedlings during the subsequent hours of weightlessness is evidence that the absence of gravitational force within the organs of a grass-type seedling has no serious effect on the basic growth processes of metabolism and cell division. The lack of disturb-

ance of such enzymes as oxidases and dehydrogenases in the metabolic pathways, which provide energy for growth and control of the rates of meristematic activities in developing embryos, could not be predicted with confidence. The limited use of wheat seeds in Russian satellites (Il'ina et al., 1966) did not touch on the point because the seeds were not tested for germination until after their return to Earth. An increased activity of peroxidase in growth curvatures of wheat seedling organs has recently been found by Conrad [1] but not yet assigned a specific role in their growth. Some of the enzymes in the cycles of energy transfer are associated with mitochondria which move into the equatorial plate region of a dividing cell and there furnish the energy required to build the cell plate (Hepler and Jackson, 1967). Elimination of the gravity factor in the wheat seedling organs seems not to have caused an abnormal distribution of such mitochondria and the enzymes essential for supplying energy for cell division.

Morphogenesis

The preservation of normal anatomic structure and organography in the orbited seedlings was also not completely predictable. The organization of tissues in the morphogenesis of terrestrial plants has evolved in a polarized field of force that might have introduced an active controlling factor. An eminent morphologist has written that the "growth pattern of a plant body is a specific reaction to gravity" (Sinnott, 1960, p. 354). The weight effect of gravity has been said to act in part by producing "tension and compression of tissues with consequent effects on cell division and expansion" (Sinnott, 1960, p. 355). The "differential distribution of auxin" is also thought to control symmetry in plant organs. Since the distribution of auxin is definitely controlled in part by gravity, absence of this force might have affected some aspects of morphology in seedling roots and coleoptiles. None have been observed in the simple structures of this young plant as grown in weightlessness.

Auxin Supply

The possibility of disturbances in the physiology of auxin synthesis and transport as the seedlings developed on orbit was tested at several points in growth of the organs. They had all been formed on orbit with their auxin produced in or near the apices. If present, auxin was mobile by its endogenous transport mechanism and by the effect of gravity (Lyon and Yokoyama, 1966) after deorbit, with predictable results on orientation of an elongating organ. The change from orbit to an erect position of each seedling for 5 hours just previous to the end of the flight experiment provided an opportunity to look for changes in direction of growth of the root tips. The primary roots, however, had passed their peak of rapid elongation and did not develop positive geotropic curvatures but some were found near the ends of a few lateral roots; they were still elongating rapidly enough to respond to the 5-hour action of gravity. These growth curvatures in lateral roots were also seen to an equal degree in the photographs of clinostat control IIIC, thus providing good evidence for the presence of auxin in both sets of root tips during that period. The strong epinastic curvatures in roots in the absence of a gravity vector (cf. figures 6 to 8) had already demonstrated the normal synthesis and peculiar lateral transport of auxin, particularly in the lateral roots of wheat.

Coleoptile Curvatures

Comparisons of the orientation angles of seedling organs produced in the flight and Earth control experiments provide quantitative criteria for determining possible effects of orbital growth on the balance of auxin transport into growth zones. Imbalances in distribution result in growth curvatures which are described by the angles of displacement from the seedling axis.

The record sheets for the flight and control experiments show that most of the coleoptiles were less than 5 mm long but did exhibit measurable curvatures in at least one direction. In order to obtain significant data for the orientation of these short, slow-growing organs, it was necessary to combine the two angles for each coleoptile and to use this measure of total curvature in the analysis of experimental results.

The data in table 9 for these mean total curvatures show close agreement between the orientation measurements for the flight and clinostat control experiments only. There are no significant differences among them. Under the otherwise same growth conditions, the mean curvatures for coleoptiles of the erect controls (I and IIIE) are

[1] Personal letter from H. M. Conrad received in 1968.

significantly smaller (at the 1-percent level) than those of the flight experiment. These small displacements of coleoptiles from the vertical are due largely to irregularities in growth through ruptures in seed coats combined with slightly imperfect orientation of the embryos when the seeds were planted in the holders. These same factors apply to the initial growth of coleoptiles in the flight and clinostat experiments, but the displacements would have been corrected to a larger extent in the erect controls if the organs had grown longer.

The imbalances in coleoptile growth without a tropistic effect of gravity are directly comparable to the axial curvatures of leafy plants on clinostats and of the pepper plant stems in the liminal angle experiment on Biosatellite II (cf. paper by Johnson, p. 213). The apical ends of the young pepper plants grew during orbit so much faster on one side than on the other that some of them were bent sidewise in spite of attempts to prevent bending by tying the axis to a stake in each pot. Such growth curvatures in negatively geotropic stems have been shown (Lyon, 1965b) to be caused by unequalized basipetal transport of their auxin in the absence of a gravity vector. The wheat coleoptile appears to depend so much on gravity for its almost vertical growth on Earth that larger growth displacements from the seedling axis are formed even in short coleoptiles when they lack the usual action of gravity.

Primary Root Epinasty

The mean deviation of the primary root from growing straight down in erect seedlings or parallel to the seedling axis in other experiments is measured by two columns of data in table 9. From the mean face-view angles it is clear that the deviations were much greater when the positive geotropic response was eliminated either by use of the clinostat or by a flight experiment. The two methods were equally effective in allowing an uneven distribution of auxin between the two sides of the root in the face-view plane.

In the plane of the side-view angles (fig. 2) the deviations from the axis line were again much greater when the gravity vector was eliminated in weightlessness or made ineffective on a rotary clinostat. The curvatures were usually away from the endosperm side of the seed and were smaller on the average when the seed germinated with the embryo erect to gravity. Curvatures toward the endosperm side were often associated with atypical roots, as illustrated in figure 8 by seedling number A2 with a hairy root that indicates an excess of endogenous ethylene (Chadwick and Burg, 1967).

This nonvertical orientation of the primary root of wheat was reported by Rufelt (1957) and confirmed by Lyon and Yokoyama (1966), who added the observation of greater outward curvature of the root when it developed on a clinostat. This identified the uneven growth as an epinastic curvature produced in the absence of the gravity effect. The flight experiment has produced the same increased curvature (cf. figs. 7 and 8) to the same degree and thereby given evidence from the primary root for an undisturbed physiology of root epinasty.

Lateral Root Epinasty

The mean orientation angles for the face views of the lateral roots are in excellent agreement in measuring the epinastic curvatures as they developed in these roots in the flight and clinostat control experiments. The left and right roots were, on the average, oriented at about 61° and 63°, respectively, from the 180° end of the seedling axis when grown erect to gravity in controls I and IIIE. The mean face-view angles were then 241° and 117°. These angles were changed to about 280° and 80° when the roots were formed on orbit. For both roots there were no significant differences in the mean epinastic curvatures of 38±1° induced by growth on a clinostat or during weightlessness.

Additional evidence from this use of mean orientation angles to evaluate the auxin physiology of lateral roots during orbit was provided by the measurements of the 1968 postflight test in which the 1966 seeds proved to germinate more slowly than they had at the time of the flight experiment. The data for mean orientation angles of that test (and flight experiment) were displayed in table 6. The agreement between the measurements of angles for the two sets of Earth controls is so close that they reinforce the evidence for no disturbance of the growth physiology of lateral roots during their growth on orbit.

The value of these criteria for judging the effects of orbital flight on the wheat seedling is further supported by the evidence that the orientation of the lateral roots in the presence or absence of a

gravity vector during germination is a characteristic of a particular crop of seeds. The data of table I were derived from the use of 1964 seeds of the same strain of wheat grown in the same section of Georgia. Comparison of the face-view angles of these 1964 seedlings with those of seedlings grown by the same method from 1966 seeds reveals a considerable difference in mean face-view orientation angles for both erect and clinostat seedlings.

The position of the average lateral root from 1966 seeds was 6° or 7° nearer the horizontal for erect seedlings. When grown on a clinostat, the average change in angle by epinastic growth effect was about 50° for the 1964 seeds as opposed to only 38° for 1966 seeds. This agrees with other evidence of less vigor in the 1966 seeds. These differences in orientation behavior also support the decision to use the results of the launch laboratory Earth controls as the source of the most reliable baseline data for scoring the results of the flight experiment in the area of growth physiology.

The evidence from side-view angle data for lateral roots in table 9 is less precise for the comparable effects from flight and clinostat control experiments. The mean side-view curvatures of the left and right roots on orbit or on a clinostat were much larger than the mean displacement of 17° to 20° in the erect controls. The symmetry of the increased mean displacement on a clinostat was less perfect, but the distribution of growth imbalances in these roots was essentially the same for the two methods of eliminating the auxin transport action of gravity.

Clinostat Confirmation

A secondary but valuable result from this experiment was confirmation of the rotary horizontal clinostat as a reliable device for terrestrial studies of geotropism and related aspects of growth physiology. For none of the several criteria used to compare seedlings grown in weightlessness with those from the clinostat Earth controls was there a quantitative difference in the results of eliminating the effects of gravity by the two methods. The clinostat has been the basic tool for nearly a century in the analysis of all aspects of geotropism in plants but this was the first opportunity to test its accuracy by a control test with acceleration forces of the environment reduced to a level below the sensitivity of geotropic receptors. Validation of experimental results obtained by proper use of the clinostat in the past has come at an opportune time. Recent discoveries in subcellular anatomy and cell biology have caused a few informed students of tropisms (Dedolph, 1967; Gordon and Shen-Miller, 1966) to challenge work done with some models of the clinostat. Establishment of the validity of results with the relatively slow rotation of our instruments has emphasized the reliability of the device over a range of rotation rates that now needs to be delimited carefully by appropriate experimentation.

Experiment Sufficiency

Many of the results from this single experiment are based on sufficient quantitative data to warrant the statement of a few conclusions. A duplicate experiment to be conducted according to the original 3-day plan would be welcomed by the scientific community. The difficulties are understood by all. The question of immediate concern is the applicability of the conclusions from growth of the wheat seedlings in weightlessness to the biology of other plants in relation to gravity. The apparent lack of disturbance of the basic metabolic and regulatory processes in this plant body and meristematic centers in a grass-type seedling, however, leaves some uncertainty about morphogenesis as it relates to the gravity factor in growth regulation of organs more complex than a tubular coleoptile and an unbranched root.

This experiment has provided no evidence for the development in space of leaves, stems, and typical roots. Much of the clinostat work of recent years (Dedolph et al., 1967) has been done with monocotyledonous seedlings. The important area of morphogenesis in dicotyledonous plants has received a minimum of attention from workers with clinostats in spite of the interesting reports of changes in anatomy (Brain, 1926 and 1939; Lyon, 1965b). Until more evidence is available from these more complex plants with dorsiventral organs and special relations between gravity and their growth hormones, it is impossible to predict with confidence the growth behavior of such plants in the absence of gravity.

SUMMARY AND CONCLUSIONS

An original culture method developed for germination of seeds of wheat (*Triticum aestivum* L.) with the seedlings suspended in sealed moist chambers was successful in producing young plants with

organ orientation like that of seedlings rooted in soil.

The stresses of launch acceleration and vibration had no measurable effects on ungerminated wheat seeds, as determined by the physiology of their subsequent germination and seedling growth during orbital flight of about 45 hours. Growth physiology of wheat seed germination and the development of wheat seedlings in their early stages was not disturbed enough by the absence of gravitational force to be reflected in the growth rates or external morphology of roots and coleoptiles. The apparent lack of disturbance of the basic metabolic processes which supply the energy for normal growth indicates the independence from gravitational force of certain organelles which carry key enzymes to the sites of energy release and use. The absence of gravitational force within wheat seedling organs had no measurable effects on the endogenous mechanisms for production and distribution of auxin, the growth hormone that is known to be transported in part in seedlings by the effects of acceleration forces.

The wheat seedling proved to be an excellent choice of a small test plant for an orbital experiment in the growth and orientation of plant organs known to be geosensitive. The development and performance of the experiment led to the discovery of root epinasty, previously unknown to plant physiologists, as a factor in the orientation of plagiotropic roots.

To the extent that the results of a single, curtailed experiment can be accepted without confirmation, the essential indentity of growth responses of wheat seedling organs to the elimination of tropistic effects of gravity, as the organs developed either during the weightlessness in Earth orbit or during their rotation in vertical planes about the axis of a horizontal clinostat, has confirmed the reliability of the rotary clinostat as an instrument for terrestrial studies of geotropism and related aspects of growth physiology in plants.

The lack of typical shoot and root system in the wheat plant makes it impossible to predict with complete confidence the results of growing plants with more complex organs and organization in the absence of gravitational force.

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A Study of the Effect of Weightlessness on the Biochemical Response of a Monocotyledonous Seedling

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The characteristic fashion in which plants respond to gravity has been known for centuries. Yet the recognizable effects of gravity can be nullified in either a mature leafy plant or a germinating seedling merely by rotating it about its horizontal axis (Ruhland et al., 1962). When the Biosatellite program was conceived, however, it was not known whether plants grown in weightlessness would respond with epinastic curvatures and biochemical activities identical to those of plants grown on a clinostat on Earth.

The bulk of the evidence used to explain the mechanism governing the epinastic response of a plant to a gravity-nullified environment is based upon the translocation of auxin (Boysen-Jensen, 1936; Gillespie-Pickard and Thimann, 1966; and Lyon, 1962). Yet, the basic biochemical phenomena within the plant that accompany the movement of the hormone were and still are not known. A physiologic "penalty" results from biologic work in all living systems. It seems inconceivable, therefore, that the profound disorientation of the plant organs typical of growth on a clinostat could occur without a concomitant conservation or utilization of energy.

The experimental program was designed to: (1) Relate the anticipated response to metabolic and energetic reactions throughout the length of roots and shoots; (2) determine whether changes in metabolism which occur on the clinostat would also be found in space-grown seedlings; and (3) compare the biochemistry of epinastic curvature with that of geotropic curvatures. The experimental protocol called for measuring the activity of key enzymes associated with some of the pathways of intermediary metabolism and energetics. These enzymes were examined for structural integrity because of speculation over the possible weakening of the tertiary forces which hold protein molecules together in a characteristic highly ordered manner.

The present report describes the results of the extensive biochemical analyses performed on the space-grown seedlings, their corresponding Earth controls, and postflight verification experiments. It will be shown that growth of seedlings on the clinostat simulates growth in a weightless environment. However, the biochemical parameters measured indicate that only auxin-mediated reactions are simulated by growth on the horizontal clinostat. One enzyme, peroxidase, showed evidence of increased activity at or near the site of epinastic curvature. Another enzyme, transaminase, also shows this tendency, but the data are less consistent and therefore less conclusive. The increased activity was found to be physiologic and not due to structural changes in the enzyme while the seedling is in the weightless state. The kinetic studies indicate that spaceflight does not affect the affinity of an enzyme for its substrate.

EXPERIMENT DESIGN AND METHODS

The primary objective of this study is to correlate changes in metabolism and energetics with the reorientation of plant organs expected, and indeed

observed, during weightlessness. The biochemical studies, which constitute our major effort, utilized the same specimens and experimental hardware as those of our coinvestigators studying other aspects of the developing wheat seedling in the weightless environment (see papers by Gray and Edwards, p. 123, and by Lyon, p. 167). The experimental design required that 78 seeds be planted in the flight package and in each of the Earth controls. The presoaked seeds were grown in a specially designed holder (see p. 168) filled with fully wetted ground vermiculite which permitted the organs to grow unhampered in water-saturated air. By allowing the seedlings to grow in this unhindered fashion, it was possible to observe changes in root orientation as well as in shoot curvature. The protocol called for postflight photographing of all the seedlings in order to determine the extent of root displacement and coleoptile curvature before the seedlings were divided for subsequent biochemical analyses.

The enzymes chosen for study in this program were selected to represent the biosynthetic activity of several pathways of energetics and intermediary metabolism. Figure 1 illustrates the logic used for selecting the key enzymes which catalyze the energy-yielding reactions in germinating seedlings. The relationships between the selected enzymes and the various metabolic functions are given in figure 2.

Materials and Methods

The plants examined in this study were seedlings *Triticum vulgare,* Georgia strain 1123. The unsoaked seeds were weighed, and only those weighing from 38 to 39 mg were used for flight and Earth control experiments. Additional selection was made on the basis of appearance (no fractures, etc.). The seeds were then surface-sterilized in 0.05 percent $HgCl_2$, washed in running distilled water, and soaked for 3 hours in aerated distilled water at 95 F.

The presoaked seeds were then inserted into the rubber diaphragm of the nontoxic polycarbonate stalks containing thoroughly wetted ground vermiculite. Each seed was inserted about half-way through the hole burned in each cap and then water was injected into the air spaces of the core. Water loss was prevented by applying a lanolin beeswax mixture at the point of contact between the rubber diaphragm and the seed. The stalks were then placed in the experiment package; 2 ml

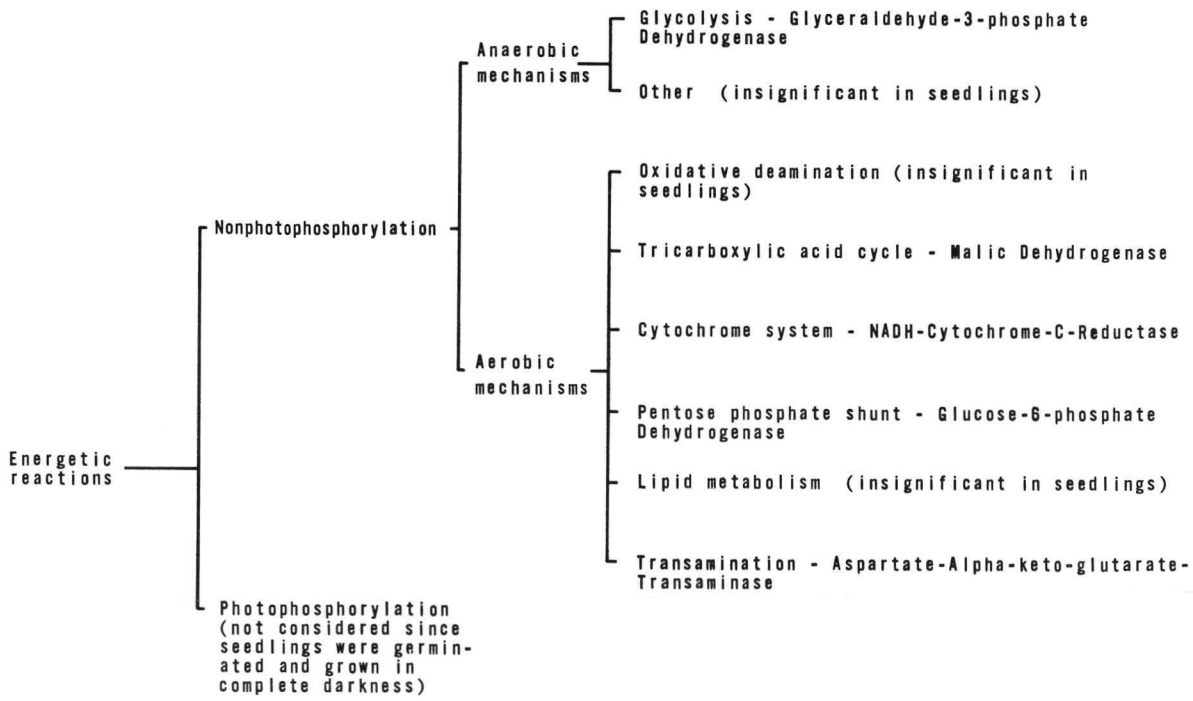


FIGURE 1.— Correlation of enzymes with energy-yielding reactions.

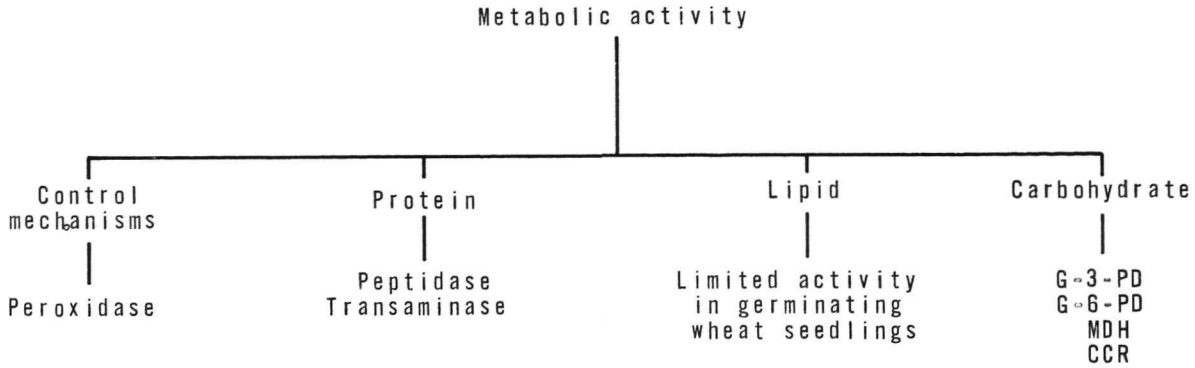


FIGURE 2.— Correlation of enzymes studied with metabolic functions.

of distilled water were added to each of the three small chambers and 5 ml, to the large chamber. Gas samples were taken and the lids were tightly sealed.

The biochemical studies were planned so that the information acquired could be directly correlated with the results of the morphologic and histologic investigations. The shoots were sectioned into 200-micron slices and the enzymatic analysis was performed on each slice. For convenience, meristematic mass was analyzed together with the shoot. Since the diameter of the root tissue is considerably less than that of the coleoptile, the primary root was sectioned into 1 mm slices to provide a sufficient concentration of enzymes for analysis. In the region of the root tip where enzymatic activity is greatest, the size of the slices was reduced to 0.5 mm in order to obtain more complete information. This procedure allowed a plot graph to be drawn which illustrates the enzymatic activity along the length of the tissue. The advantage of this particular approach is that the biochemical findings in each individual tissue can then be composed with its morphological response to orbital flight and, later, correlated with histologic and histochemical findings of similarly treated tissues. In this manner, any alteration in the physiologic processes resulting from weightlessness could be located within a confined area of the tissue and perhaps identified with a specific type of tissue or even a locus within the group of cells.

The unfixed seedling organs from the flight and Earth control packages were gathered by lifting the roots and the shoots as a unit from each seed. The roots were cut away and, for convenience, the scutella were left attached to the shoot. Each part was then inserted into a prepared agar slug, sealed with melted agar at 107.6 F, frozen first on Dry Ice to prevent cracking of the agar, and then preserved under liquid nitrogen until analyzed. The seedlings at several stages of processing are illustrated in figure 3. The organs from those seedlings fixed during the course of the experiment were treated differently. The organs were removed as a unit from the endosperm as described above, separated into three groups based upon organ length, and resuspended in FAA/DMSO fixative of the same formulation as that used for the original fixation without the organs being separated. These tissues were kept in fixative until they were

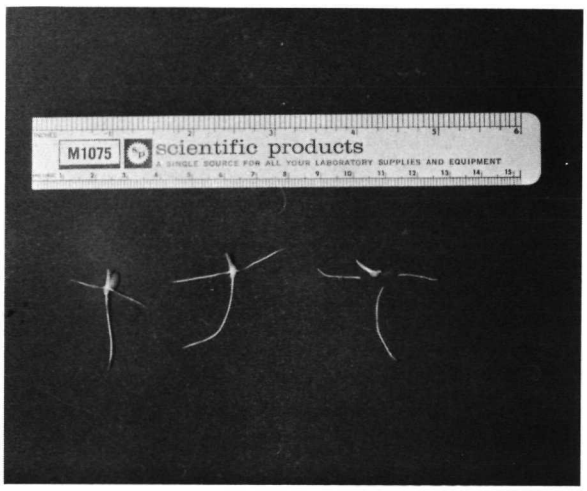


FIGURE 3.— Dissection of wheat seedlings in the first and last stages of harvesting. Roots and shoot removed as a unit from endosperm and roots trimmed away from shoot leaving the bulbous scutellum attached to the coleoptile.

analyzed. Confirmatory experiments were also performed on green leafy plants. For this purpose, immature 45-day-old pepper plants, *Capsicum annuum,* were grown in soil under continuous fluorescent daylight (Johnson and Tibbits, 1968).

Analytic Procedures

The gas content of each air sample was determined by standard gas chromatography techniques (Szymanski, 1964). The following enzymes were analyzed.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G–6–PD)

The analytical procedure employed for the studies of G–6–PD was a modification of the techniques described by Kornberg and Horecker (1962). Initial work established the pH optima for the reaction at 8.0, in 0.05 *M* Tris buffer, and the optimum substrate and cofactor concentration for the quantity of tissue present in the system. The activity in 200-micron slices of 65-hour coleoptiles and 1-mm slices of primary roots (except for the first 3 mm from the root tip where the slice size was reduced to 500 microns) was determined. At the conclusion of the analysis, each tissue was vacuum-dried at room temperature and weighed by using a Cahn RG Electrobalance.

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (G–3–PD)

The procedure used for G–3–PD was a modification of that described by Racker (1965). Frozen tissue of 200-micron sections was used. At the conclusion of the assay, the sections were removed from the reaction vessel, placed on an aluminum pan, and dried under vacuum in a desiccator at room temperature for at least 6 days, and the dry weights were measured as before.

MALIC DEHYDROGENASE (MDH)

A modification of the method described by Siegel and England (1961) was used on each 200-micron section.

TRANSAMINASE

Transaminase was assayed according to the procedures described by Henry (1963) on each 200-micron slice of tissue.

CYTOCHROME C REDUCTASE (CCR)

Each tissue slice was added to 0.30 ml of a reaction mixture, at 32 F, comprised of 0.03 *M* Tris/Cl buffer at pH 7.85, 4.87×10^{-4} *M* DPNH, and 8.33×10^{-6} *M* cytochrome C. After each slice was added to the reaction mixture, the reaction flasks were incubated for 30 minutes at 98.6 F and then cooled again to 32 F, and the absorption was measured at 550 mμ and compared with that of suitable blanks.

PEROXIDASE

Peroxidase activity was measured in the homogenates by using a modification of the method described by Aurand et al. (1965). The determination is based upon the oxidation of a hydrogen donor (*p*-phenylenediamine dihydrochloride) in the presence of hydrogen peroxide to produce a colored compound. The shoots were sectioned while frozen into 200-micron slices. The protein concentration of each slice was determined spectrophotometrically according to procedures described by Murphy and Kies (1960).

The electrophoretic separation of peroxidase isozymes was performed in the following manner. A 2 percent agar solution was made by using 0.10 *M* borate buffer of pH 9.0. The hot solution was poured on a glass plate into 10- by 1-in. strips to a thickness of 3½ to 4 mm. The same buffer solution was poured into the electrode compartments of the electrophoresis cell. The tissue was dissected and the portions to be examined were placed on a 3- by 8-mm piece of filter paper on a glass plate. The tissue was crushed with a stirring rod and the tissue fragments were removed from the filter paper. Liquid-saturated filter paper was then inserted in slits cut into the agar slab.

A potential of 200 volts was applied for 90 minutes. After electrophoresis, the isozymes were visualized by flooding the agar surface with a solution that was 5×10^{-3} *M* in guaicol, 5×10^{-3} *M* in hydrogen peroxide, and 0.50 *M* phosphate buffer at pH 5.8.

PEPTIDASE

Peptidase activity was determined by using the titrimetric method of Linderstrom-Lang and Holter (1932). Each slice was incubated for 4 hours with alanylglycine at 104 F, and the liberated ammonia

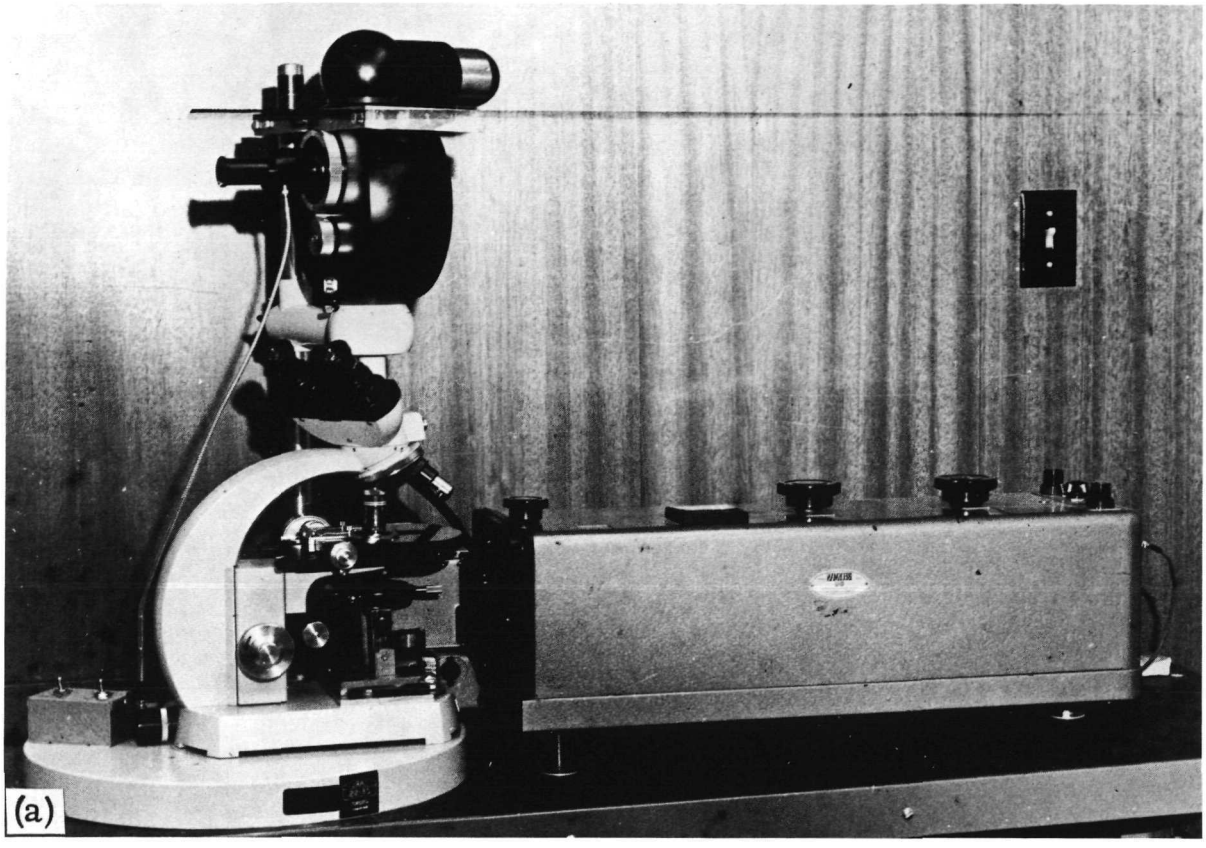
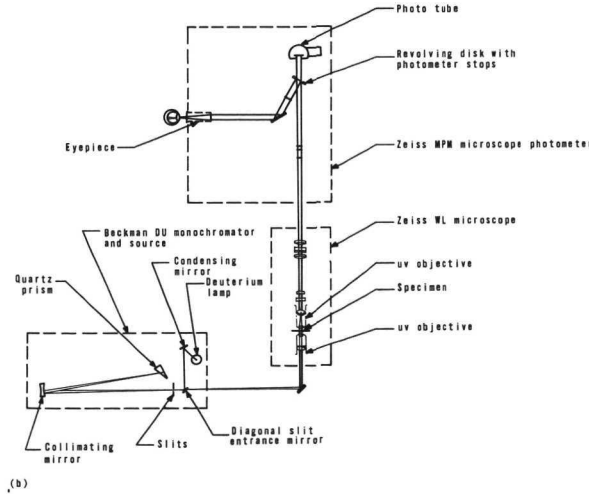


FIGURE 4.—Cytospectrophotometer. (*a*) Photograph. (*b*) Optical diagram.



was titrated with 0.05 *N* HCl. Each slice was then recovered and the dry weight was obtained.

Enzyme kinetics were employed in order to obtain Michaelis constants and the maximum reaction velocities. The calculations were made from plots of the reciprocals of velocity against substrate concentration, as described by Lineweaver and Burke (1934). The tissues used for the kinetic studies consisted only of lateral roots.

CYTOSPECTROSCOPY

Specimens for analysis with the cytospectrophotometer (fig. 4) were fixed with FAA and then dehydrated with a *t*-Butanol series and vacuum-infiltrated with a paraffin oil-paraffin series. The methods used were those of Johansen (1940). Sections 7 microns thick were floated on a bath with no adherent and picked up on clean, uncoated quartz slides. After they had dried for several hours the wax was removed and the tissue rehydrated with a xylene-ethanol-water series. A drop

of glycerine was placed on the section, which was then covered with a quartz cover slip. The cover slip was held in place by running a bead of paraffin around the edge.

The motor drive for the mechanical stage of the Zeiss Model WL microscope moved slides at a rate of 0.30 mm/min parallel to their length. Since comparison of the sides of the coleoptiles was desired, specimens were floated onto the slides with the coleoptile perpendicular to the length of the slide.

Before the adsorption was measured, the specimen was photographed with a low-power objective and a print was made. The specimen was then examined visually to determine a series of paths for measurement that appeared to be typical of the specimen and that avoided traces of paraffin, bubbles, and other contaminants. The stage was set to track one of these paths and the specimen was brought into focus with the high-power quartz objective. The field of view was moved off the specimen, the monochromator was set at the desired wavelength, either 260 mμ or 280 mμ, and the instrument was set to read 100 percent transmission. The recorder was then set for 0 and 100 percent transmission, and the mechanical stage drive and the recorder were started simultaneously. Care was taken to run a substantial distance before and after tracking across the specimen to insure that the portion of the slide was typical for 100 percent transmission for a given run.

After one or more runs were made on a given track, the wavelength was returned to the visible spectrum and the location of the track was plotted on the photograph of the specimen.

RESULTS

Growth and Morphology

The flight specimens appear to have grown normally. The average lengths of the roots and shoots under all conditions of growth are presented in table 1. No significant differences due to orbital flight were observed in organ length.

The orientation of the tissues of flight resembles that of those grown on the horizontal clinostat. Precise measurements of organ angles from the photographs of the seedlings recovered from space have already been reported (Lyon, 1968).

An apparent difference in the weight of meri-

TABLE 1. – Length of Organs of Seedlings From Both Flight of Biosatellite II and Earth Controls

Package	Coleoptile				Tap root				Lateral roots			
	65 hours		58 hours (fixed tissues)		65 hours		58 hours (fixed tissues)		65 hours		58 hours (fixed tissues)	
	Average length, mm	Standard deviation	Average length, mm	Standard deviation	Average length, mm	Standard deviation	Average length, mm	Standard deviation	Average length, mm	Standard deviation	Average length, mm	Standard deviation
Flight	4.14	0.90	2.78	1.18	21.54	6.73	7.12	4.05	10.04	6.76	2.04	1.96
Control I	3.44	1.13	2.52	.98	20.75	8.33	8.20	4.60	9.33	6.36	1.65	1.55
Control II[a], clinostat	3.30	1.39	3.30	.71	18.66	10.50	11.01	3.95	10.77	7.37	3.78	2.50
Control III, erect	3.76	1.15	2.72	1.00	23.98	8.09	9.37	5.32	13.29	6.13	3.64	3.53
Control III, clinostat	3.84	1.10	2.42	.84	20.38	6.71	6.53	3.97	10.02	5.99	1.43	1.47

[a] Tissue recovered at time of deorbit (60 hours).

stematic tissue between the space-grown seedlings and their comparable Earth controls was observed. This difference in the weight of this tissue was not found in either of the horizontal clinostat controls or the stationary erect Earth controls. In each instance, the dry weight of the meristematic tissue of the seedlings grown in space was less than that measured in corresponding Earth controls. These differences can be observed from the data presented in figure 5. No significant differences were found in slice weight along the shoot in the flight or Earth control specimens. Each curve represents the average of results for four specimens. These data compare favorably with protein measurements performed in a similar manner (fig. 6).

Epinastic Threshold

An area of particular interest to the wheat-seedling experimenters is the threshold of the epinastic response. The mission profile called for the spacecraft to provide 10^{-5} *g* for 95 percent of the flight. Extensive testing of the seedlings grown on a horizontal clinostat at an effective gravitational level of 10^{-5} *g* provided a high degree of confidence in the anticipated response of the seedlings to spaceflight. What would the response of the seedlings be if the craft failed to provide the desired *g* level?

In one study the effect of various continuous transverse accelerations below 1 *g* on the reorientation of roots and shoots of oat seedlings was measured (Dedolph, Gordon, and Oemick, 1966). The investigators inferred that there was no discrete threshold for gravity perception, although no accelerations between 10^{-2} and 0 *g* were studied. In a subsequent study (Shen-Miller, Hinchman, and Gordon, 1967) the threshold for georesponse to acceleration in gravity-compensated *Avena* seedlings was reported to be 1.4×10^{-3} *g* for shoots and 1.3×10^{-4} *g* for roots. The georesponses were measured between 65 and 70 hours after imposition of the centrifugal force.

A comprehensive investigation was conducted for measuring the onset of geosensitivity and

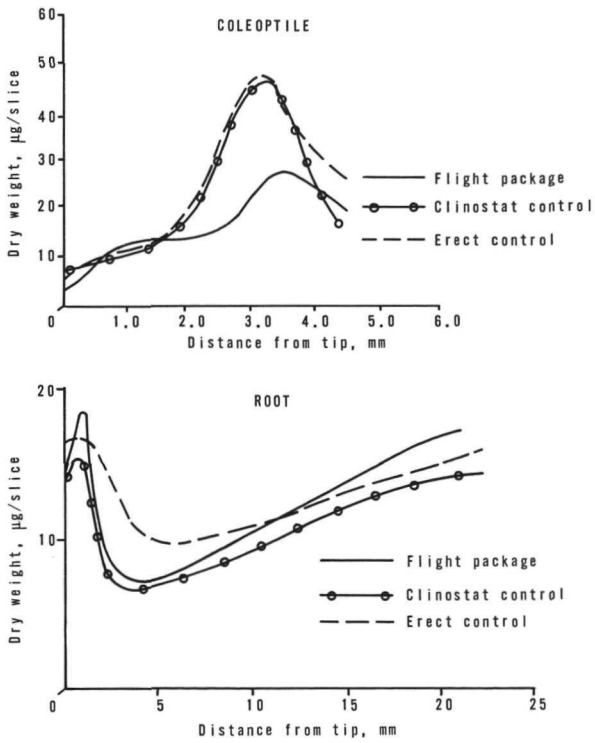


FIGURE 5.—Comparison of the dry weight of coleoptile and root sections of flight and Earth-bound control specimens.

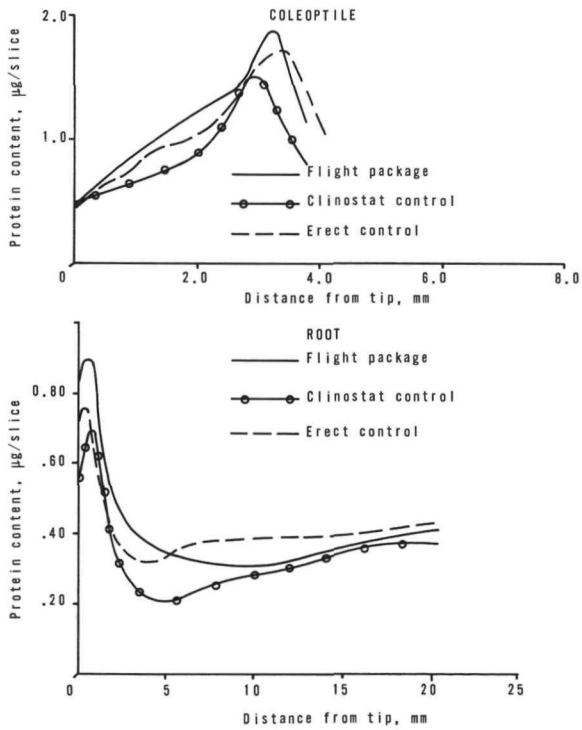


FIGURE 6.— Comparison of the protein content of coleoptile and root sections of flight and Earth control specimens.

thereby determining the threshold for epinasty in wheat seedlings. The *Avena* studies cited above were conducted with an experiment design differing from the protocol established by the wheat-seedling investigators. Thus, it was important to determine the epinastic threshold for the plants used in these experiments. Varying hypogravity environments were provided by adjusting the angle formed between the clinostat and the level bench top. Previous calculations (Ruhland, 1962) have shown that the sine of this angle is equivalent to the effective gravitational force experienced by the plants.

The results of this study (fig. 7) indicate that the onset of geosensitivity occurs at approximately 1/6 *g*. Capsicum and other green leafy plants, on the other hand, exhibit an epinastic threshold at approximately 2/3 *g* (fig. 8). These are well above the effective gravitational force predicted for, and indeed observed in, Biosatellite II. We can only conclude, therefore, that the response of the seedlings to growth in the orbiting spacecraft was within the sensitive range at all times. These studies have been amplified and reported elsewhere in greater detail (Conrad and Yokoyama, 1969 and 1970). The growth processes following deorbit until the tissues were dissected and frozen must be assumed to follow normal 1 *g* patterns.

Enzymatic Activity

The spatial distribution of enzymatic activities was determined in each of four separate shoots and roots from each condition of growth. No significant differences were revealed between the two sets of erect Earth controls (I and III–E) or the two sets of clinostat controls (II and III–C). Shoots ranging in length from 3 to 4 mm were selected for these analyses to minimize variations due to differing growth rates. Comparing data from one tissue with another appeared to be a difficult task because of the differences observed in organ length. Several shoots of different lengths were examined, therefore, in order to determine the enzyme distribution patterns. If a consistent pattern could be observed, it would then be possible to have a basis for comparison and thereby to obtain more meaningful results. Experiments were conducted in order to determine how enzyme distribution patterns differ in shoots of varying lengths. Peroxidase, G–6–PD, and G–3–PD activities were determined on shoots ranging from 3 to 18 mm in length. Initially the seedlings were grown in wrapped petri dishes on thoroughly moistened filter

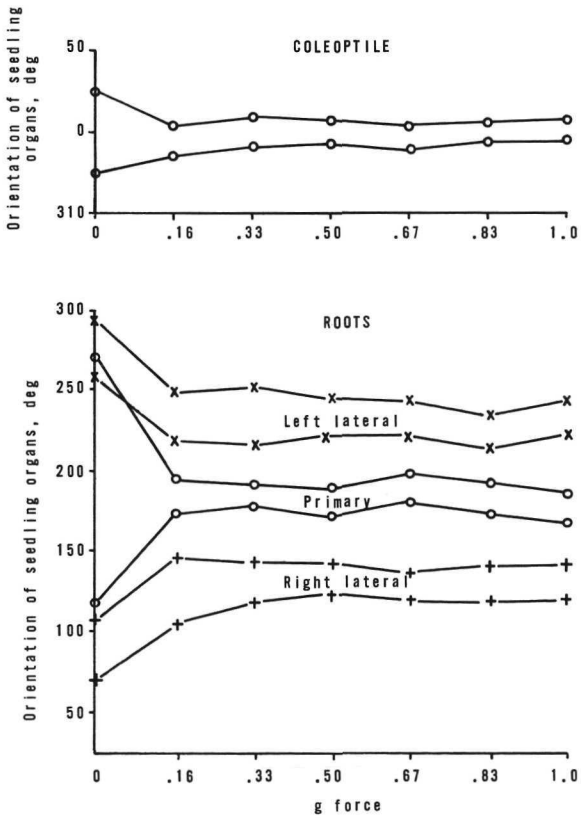


FIGURE 7.— Response of wheat seedling organs to a hypogravity gradient.

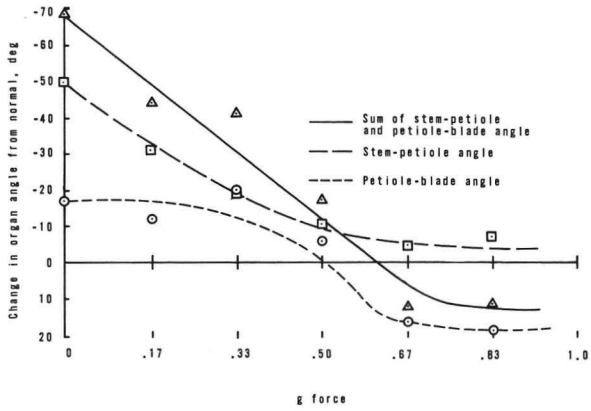


FIGURE 8.—Response of *Capsicum* to various levels of hypogravity.

paper. The findings were then confirmed by performing the same measurements on seedlings grown on the clinostat under simulated flight conditions. Proteins and dry weights were not measured, because our major effort was to relate enzyme activity to the kind of tissue examined.

This study indicates that the area of greatest enzymatic activity resides in the meristematic region (fig. 9). The activity along the shoot appears to remain relatively constant when the shoot length is 3 mm or longer. We now believe that meaningful comparisons between shoots can be made by identifying the meristematic region and adjusting the data to equivalent coleoptile length. Since there is little apparent difference in enzyme activity along the length of the shoot, adjusting the data to equal organ length is justifiable.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

The data illustrated in figure 10 indicate greater activity in the space-grown shoots than in the clinostat or erect-grown tissues. Although these differences appear to be relatively small, they are consistent. No differences in G–6–PD activity were found between the roots grown in space and those in the corresponding Earth controls.

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE

In figure 11, the specific activities of G–3–PD found in the space-grown shoots are slightly greater than those in comparably grown Earth controls. Values are averages for all tissue examined.

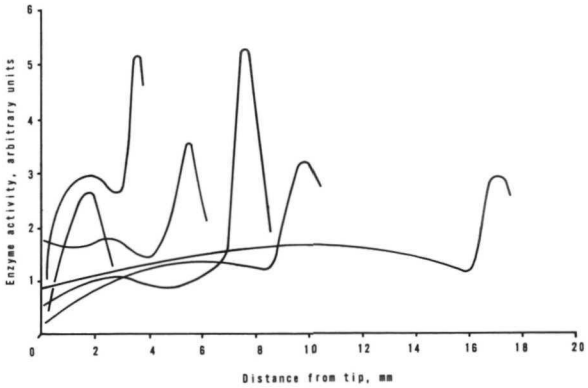


FIGURE 9.—Typical enzyme activity distribution in coleoptiles as a function of length.

MALIC ACID DEHYDROGENASE

The spatial distribution of MDH along the length of the shoots was determined (fig. 12). No significant alterations in the activity of this enzyme resulted from growth in a weightless environment.

CYTOCHROME C REDUCTASE

The analyses performed to date indicate no alteration in cytochrome C reductase activity due to weightlessness. The range of specific activities (fig. 13) falls well within the normal range of results found during our previous studies. As expected, the greatest relative activity was observed in the area containing the greatest bulk of tissue. The area containing greatest specific activity resides in the leaf primordium. No apparent differences could be observed between the two unfixed flight chambers.

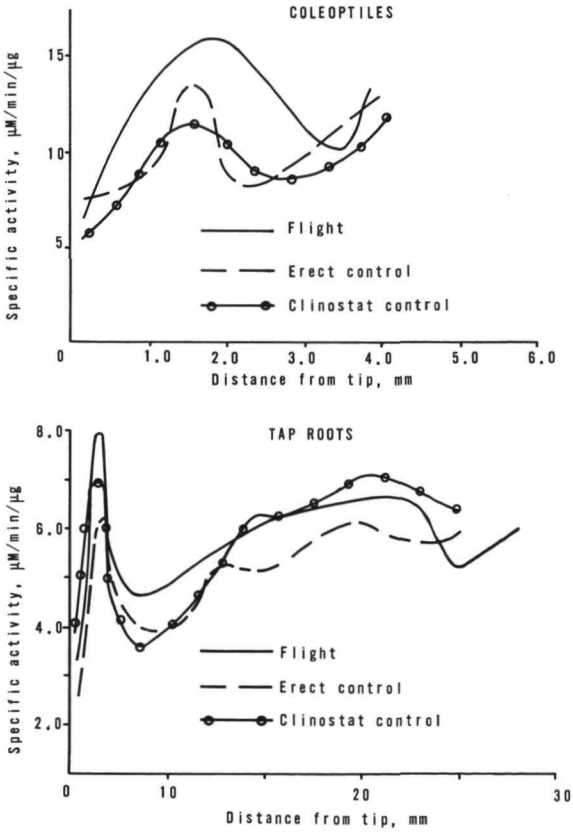


FIGURE 10.—Comparison of the specific activity of glucose-6-phosphate dehydrogenase in root and shoot sections from flight and Earth control specimens.

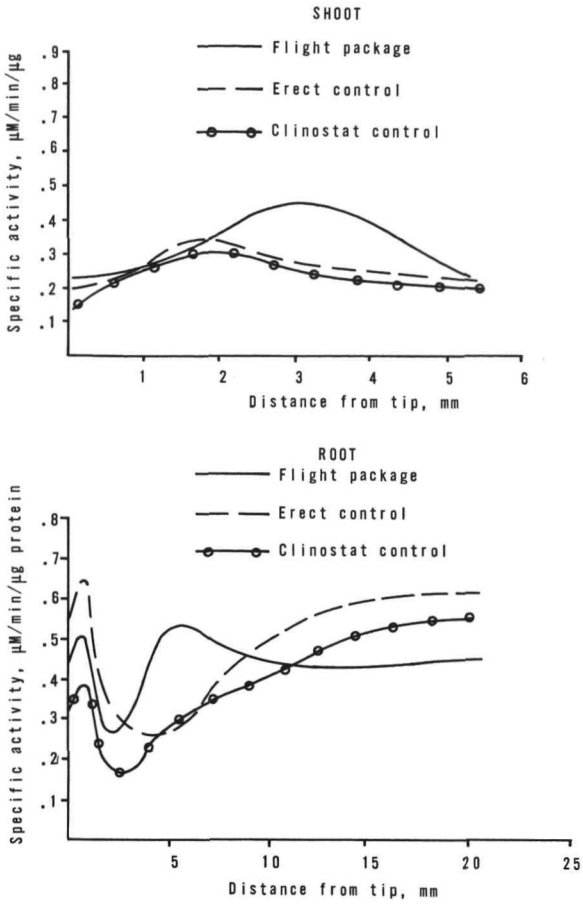


FIGURE 11.— Comparison of the specific activity of glyceraldehyde-3-phosphate dehydrogenase in root and shoot sections from flight and Earth control specimens.

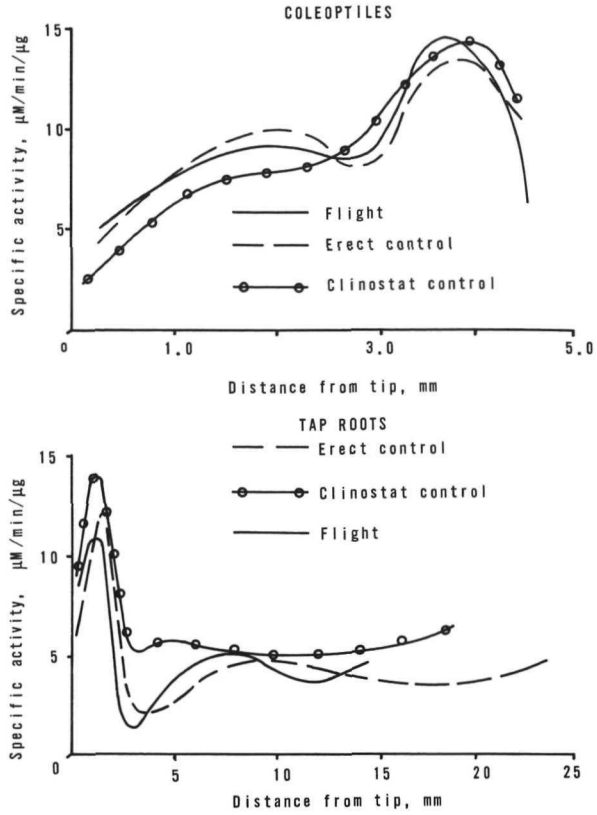


FIGURE 12.— Comparison of the specific activity of malic dehydrogenase in root and shoot sections from flight and Earth control specimens.

PEPTIDASE

The activity values of peptidase measured in the roots and shoots of the flight seedlings is comparable with the values obtained for the corresponding earth controls (fig. 14). No unusual patterns were found in any of the tissues set aside for the peptidase assay.

PEROXIDASE

The specific peroxidase activity is slightly higher near the site of epinastic curvature. This finding was observed in those tissues grown in weightlessness and on the horizontal clinostat. Erect earth controls not exhibiting epinasty show a relatively constant peroxidase activity. Furthermore, the increased activity appears to be related to the intensity of curvature (figs. 15 and 16) with greater activity found with more pronounced curvature. Subsequent attempts to determine whether the peroxidase activity varies between the convex and concave half of the curved tissue were inconclusive.

Since peroxidase activity appeared to be related to epinastic curvatures, it became necessary to perform two additional critical experiments. We wanted to learn whether peroxidase activity was affected preferentially on the concave or convex half of the curved tissue. We also thought it necessary to determine whether one particular isozyme was preferentially affected by gravity or whether each was equally affected.

Sectioning the primary root of 3-day-old wheat seedlings into two equal longitudinal halves proved to be impractical. Pea seedlings were grown therefore in the seed stalks in the same manner used for

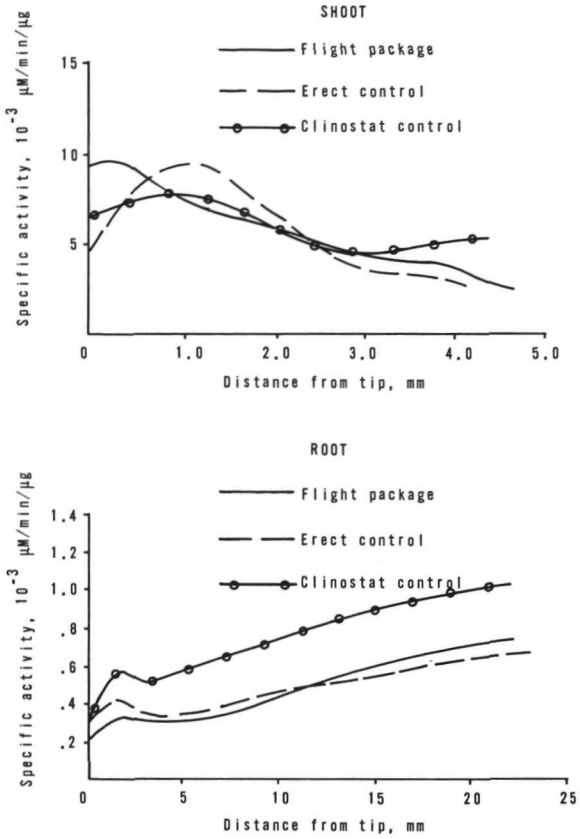


FIGURE 13.— Comparison of the specific activity of cytochrome C reductase in root and shoot sections from flight and Earth control specimens.

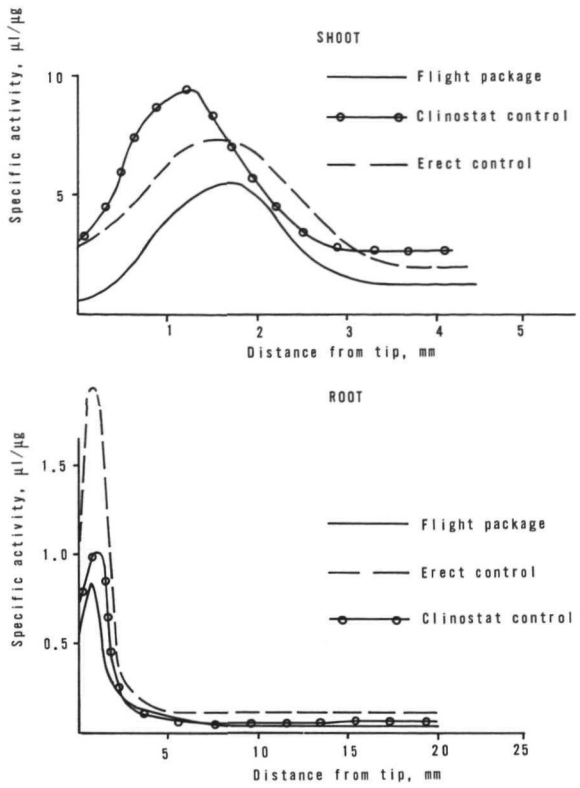


FIGURE 14.— Comparison of the specific activity of peptidase in root and shoot sections from flight and Earth control specimens.

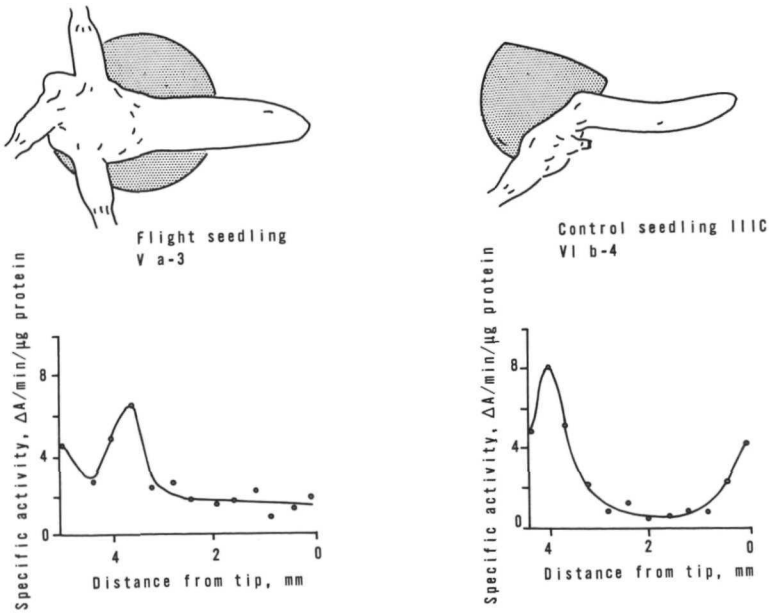


FIGURE 15.— The relationship of peroxidase activity to shoot curvature.

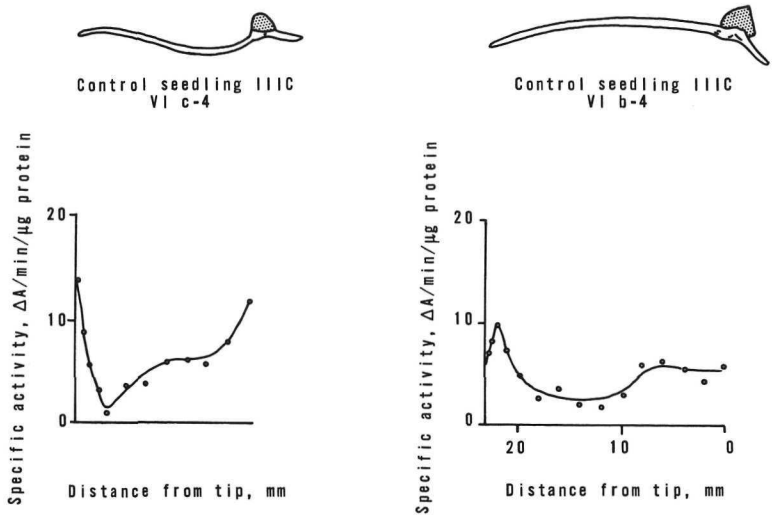


FIGURE 16.—The relationship of peroxidase activity to root curvature.

TABLE 2. – Peroxidase Activity in Convex and Concave Portions of Pea Roots

Growth conditions	Peroxidase activity o.d. 485/min/μg protein		Difference, %
	Convex	Concave	
Horizontal clinostat	12.7	11.1	14.4
Inverted, not rotated	9.2	9.1	Nil

wheat throughout the duration of this project. The pea root is considerably thicker, is easier to manipulate, and responds to rotation on a clinostat in the same manner as does the wheat. Consequently, all subsequent studies leading to the clarification of peroxidase involvement with gravity-dependent reactions in roots were performed upon pea root sections.

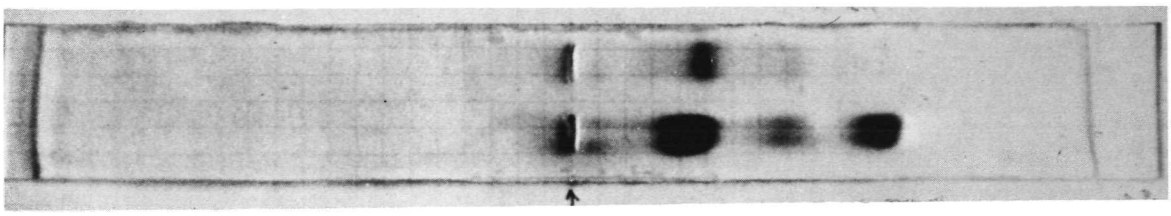
Phorograms of the peroxidase isozymes are presented in figure 17. It is quite evident that the techniques employed effectively separate the various isozymes of peroxidase. It is also clear that the root contains a greater number of isozymes and in more abundant quantities than do the coleoptiles. It is apparent that both light and gravity influence peroxidase activity. These phorograms confirm our decision to use peas for subsequent root studies, since the peroxidase isozymes appear to be similar in roots and shoots.

A comparison of total peroxidase activity between the convex and concave portions of both epinastic and geotropic curved tissues is given in table 2. A difference between geotropically induced curvatures and clinostat-induced curvatures was found. Peroxidase activity was found to be approximately 14 percent greater on the convex section of pea roots than on the concave or inner portion of the curved tissue. Geotropic curvatures induced by inverting the tissues did not show significant differences in peroxidase activity.

TRANSAMINASE

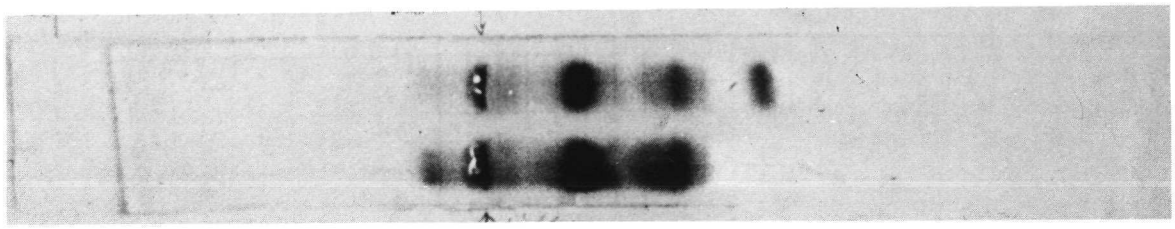
When transaminase was measured along the roots and shoots of space-grown and Earth controls, little difference could be found in the activities between specimens (fig. 18). We suspect, however, that some correlation between transaminase activity and curvature exists, but we have not accumulated sufficient evidence to form any definite conclusions.

Shoot



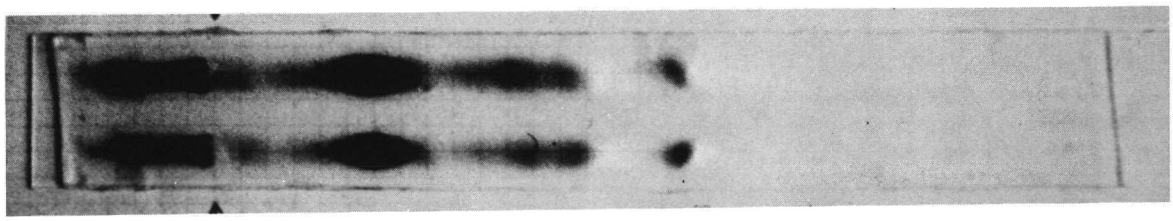
Root

Dark Grown



Light Grown

Convex



Concave

FIGURE 17.— Phorograms of peroxidase isozymes isolated from the roots and shoots of wheat.

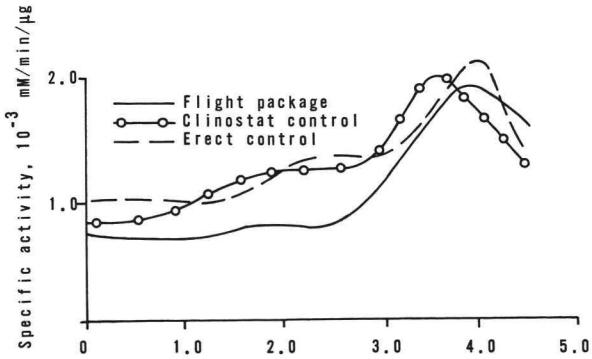


FIGURE 18.—Comparison of the specific activity of transaminase in coleoptile sections from flight and Earth control specimens.

TABLE 3. – Oxygen Measurement From Flight and Earth-Control Packages

Package	Oxygen, %		
	Before	After	Net change
Flight	20.7	20.9	+0.2
Control I, erect	20.8	20.8	None
Control II, clinostat	20.8	20.7	−.1
Control III–C, clinostat	20.7	20.5	−.2
Control III–E, erect	20.8		

Gas Analyses

RESPIRATION

The effects of spaceflight upon respiration of wheat seedlings were examined after the recovery of the spacecraft. Gas samples were obtained from the flight and from each of the control packages at the beginning and again at the conclusion of the experiment. Oxygen was measured by using the Sholander technique (Sholander, 1947). The analyses were performed for us by the Life Science Department of North American Rockwell Corp. From the data in table 3 we concluded originally that neither weightlessness nor the gravity-nullified environment provided by the horizontal clinostat affected respiration.

We now have reason to believe that our original conclusions might have been incorrect. Subsequent measurements (table 4) using the more sophisticated gas chromatography techniques at the Air Pollution Research Center at the University of California, Riverside, indicate that respiration is indeed influenced by gravity. Seedlings grown on the horizontal clinostat show evidence of increased respiration when compared with their comparably grown erect Earth controls. These new data compare favorably with those previously reported (Dedolph et al, 1966; Curtis, 1967).

ETHYLENE BIOSYNTHESIS

Several investigators have postulated the involvement of ethylene in the epinastic response. Small concentrations of ethylene in the air surrounding certain plants cause a triple response of (1) leaf epinasty, (2) stem swelling, and (3) inhibition of extensive growth of the 202 species and varieties tested; 89 showed ethylene-induced epinasty (Crocker et al., 1932). As little as 0.01 ppm of ethylene produced leaf epinasty in tomato plants. They also found that acetylene and propylene induced epinasty, but the concentration required to produce this response was 500 times greater than that required for ethylene. Our studies, using standard gas chromatographic techniques (Stephens et al., 1967), indicate that ethylene is not produced by wheat seedlings which germinate and grow in the dark (table 5). Ethylene, therefore, cannot be responsible for the root epinasty and shoot curvature exhibited by wheat seedlings under

TABLE 4. – The Effect of Light and Balanced Gravity on Respiration of Wheat Seedlings

Sample source	O_2, %	CO_2, %
Before growth	23.7	0.05
Light grown, rotated vertically	22.6	.78
Light grown, horizontal clinostat	21.9	1.53
Dark grown, horizontal clinostat	20.5	2.04

TABLE 5. – The Effect of Light and Balanced Gravity on Ethylene Biosynthesis and Utilization of Two Carbon Fragments

Sample source	Ethylene, ppb	Ethane, ppb	Acetylene, ppb
Before growth	56.0	232.0	59.0
Light grown, rotated vertically	79.0	236.0	58.1
Light grown, horizontal clinostat	100.0	158.0	32.5
Dark grown, horizontal clinostat	53.0	99.5	30.0

the gravity-nullified conditions of our experiment. In the presence of light, however, ethylene biosynthesis is stimulated and the epinasty of plant organs becomes more pronounced.

Cytospectrophotometry

The fixed seedlings from the flight experiment and the postflight controls were examined cytospectrophotometrically. Each slide was photographed and enlarged prints were made. The area scanned was then recorded on the photograph for future reference. An example of the recording technique is provided in figure 19. The results of a typical analysis of flight, erect, clinostat, and inverted coleoptiles are presented in figures 20 to 23. It is apparent that the fixed seedlings were too young to exhibit differential growth rates in the coleoptiles recovered from the flight or their corresponding Earth controls. The major finding as a result of this analytic technique is that when growing seedlings are inverted and geotropic curvatures are induced in the coleoptile the growth rate of the cells in the concave portion is reduced whereas the growth rate in the cells of the convex half continues to increase at a normal rate.

Postflight Controls

Extensive postflights studies were conducted in order to establish whether the physiologic alterations observed in tissues grown in space were caused by balanced gravity or such other physical factors associated with spaceflight as vibration, acceleration, and shock. These tests included growing wheat seedlings in the same experiment hardware used during Biosatellite II and examining the effect of vibration and acceleration upon the same physiologic parameters measured in the tissues recovered from Biosatellite II. The purpose of this exercise was to factor out those physical conditions, other than weightlessness, which were responsible for the physiologic aberrations observed in the wheat seedlings grown in space.

With the possible exception of G–6–PD, vibration did not appear to influence any of the biochemical parameters measured. Neither respiration, enzyme activity, nor growth appeared to be significantly different in those seedlings which were vibrated at Biosatellite II levels when compared with equivalent nonvibrated controls. It is quite evident from these studies that the reorientation of seedling organs, with the concomitant biochemical and physiologic aberrations observed in the tissues recovered from space, was the result of balanced gravity (weightlessness) and not due to the accompanying physical factors.

DISCUSSION

The conditions for growth in this experiment were unique. The seeds remained on the Earth for the first 15 hours of the experiment. During this time imbibition of water occurred. The seeds were then subject to approximately 45 hours of weightlessness, during which time imbibition presumably continued and growth of the roots and

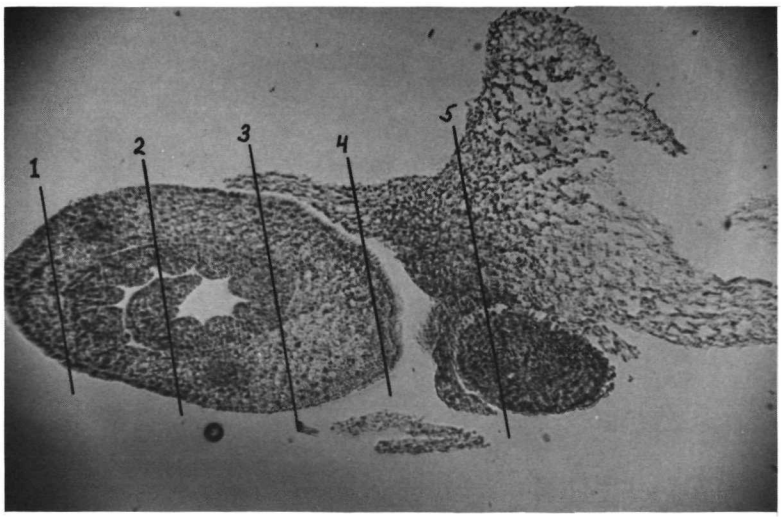
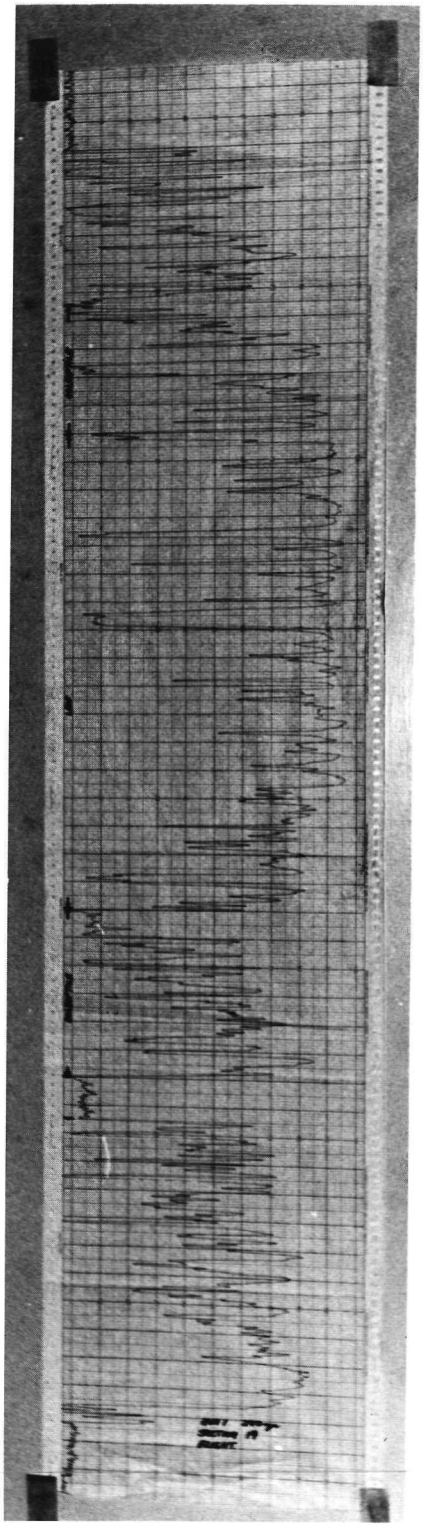


FIGURE 19.—Typical series of cytospectrophotometric scans through the coleoptile and scutella of a fixed seedling.



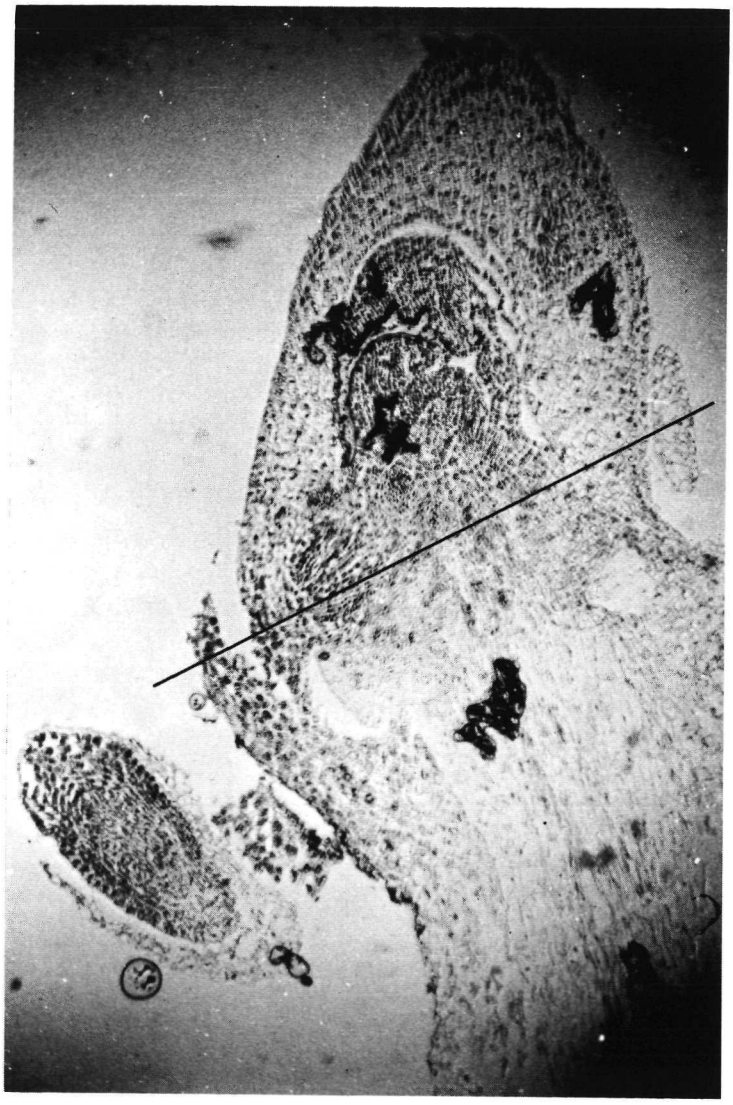


FIGURE 20.— Typical cytospectrophotometric scan of a fixed flight tissue.

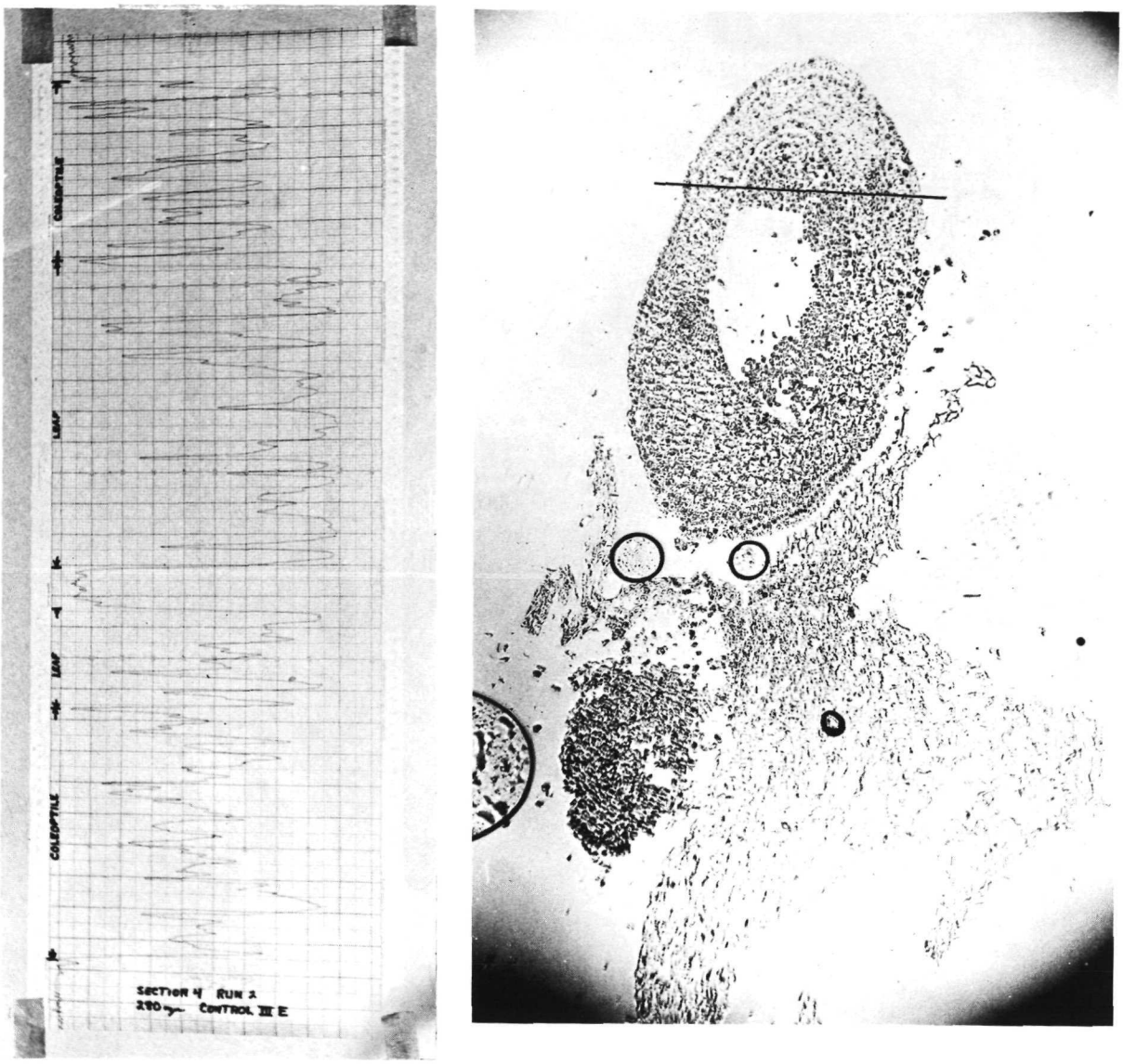


FIGURE 21.— Typical cytospectrophotometric scan of a fixed erect Earth control tissue.

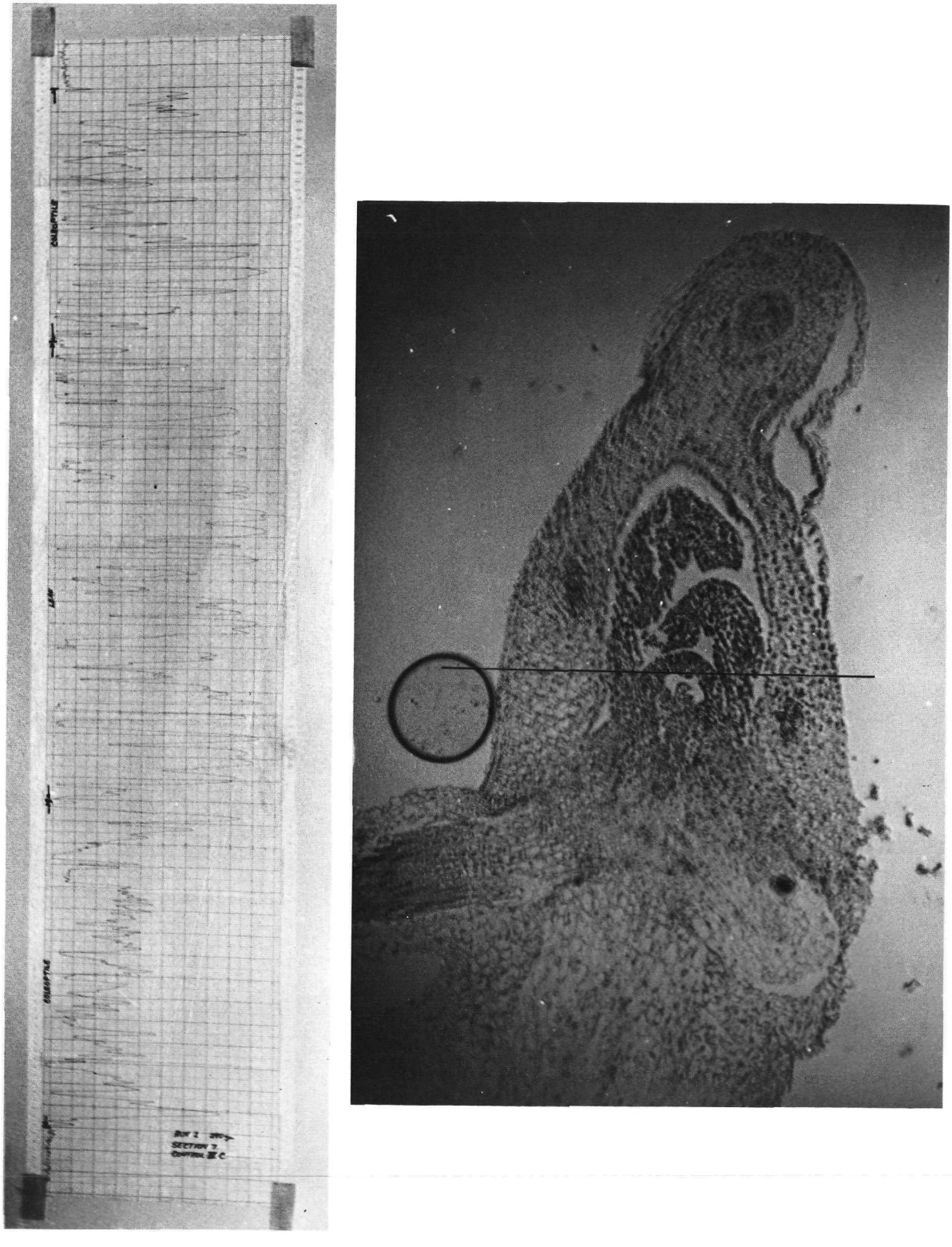


FIGURE 22.— Typical cytospectrophotometric scan of a fixed clinostat control tissue.

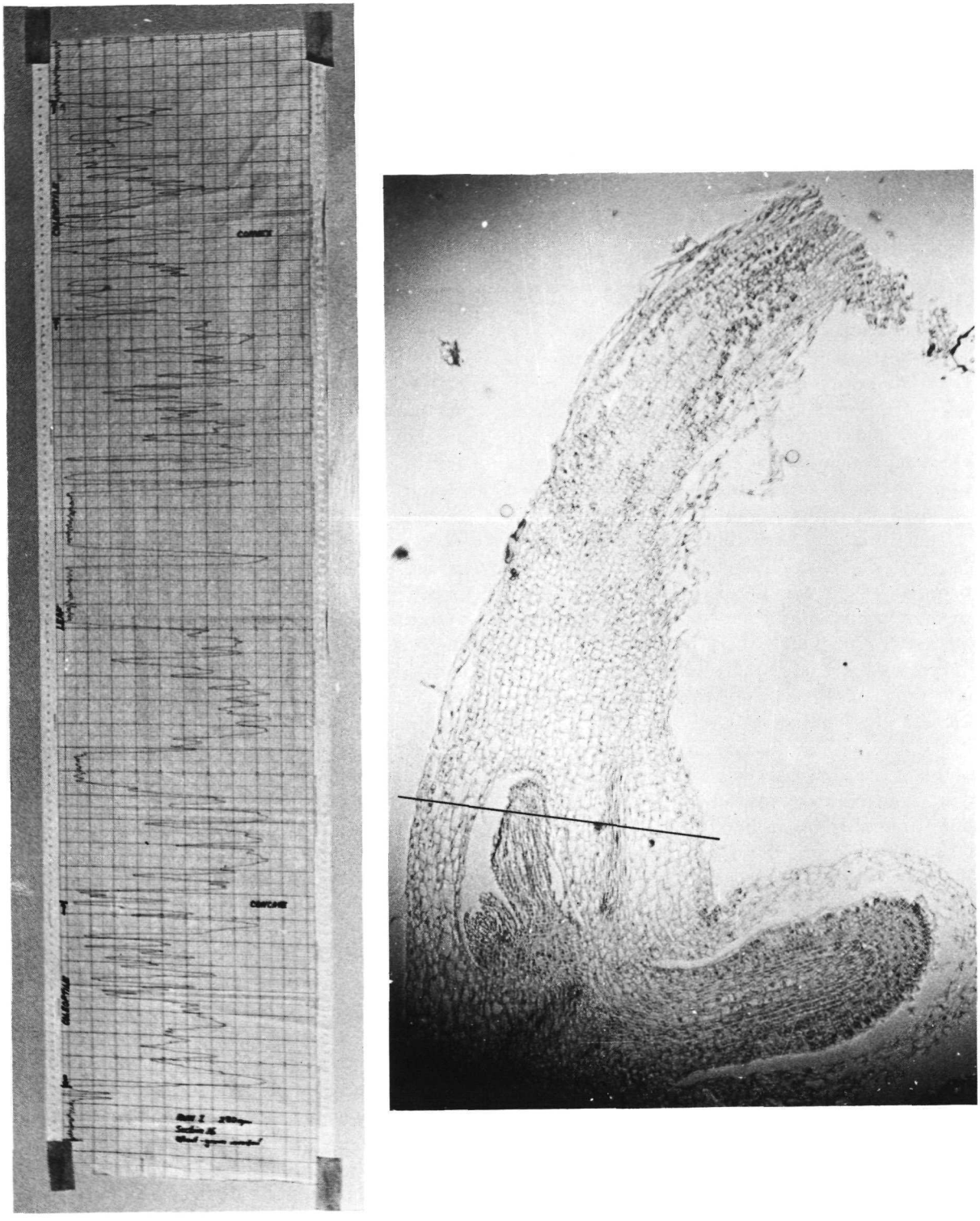


FIGURE 23.— Typical cytospectrophotometric scan of a fixed inverted tissue in which geotropic curvature was induced.

shoots was evident. A number of the specimens were fixed in space after approximately 42.5 hours of weightlessness, or about 58 hours of elapsed experiment time. The remainder of the seedlings grew for a total of roughly 44.5 hours in weightlessness. Growth continued for an additional 5 hours at 1 *g* before the package was opened for inspection and analyses.

It appears from the data presented in table 1 that the germination and growth of the wheat seedlings were normal during the mission of Biosatellite II. There does not appear to be any significant difference in the length of the roots and shoots between seedlings flown in space and their Earth controls. The overall growth and appearance of the seedlings grown in the space environment or under the gravity-nullified conditions provided on the horizontal clinostat are no different from the growth and appearance of seedlings grown on erect stalks normal to Earth's gravity. The normal variability in seedling growth far exceeds the small differences in organ lengths observed between the flight and Earth control seedlings and, thereby, contraindicates the temptation to report altered growth rates.

Profound effects were observed, however, in the orientation of seedling organs grown aboard Biosatellite II. The lateral roots grew, in the main, in the first and fourth quadrant rather than in the normally occurring second and third quadrants. The primary root, instead of growing straight down, appeared to grow off at an oblique angle. The orientation and displacement of the roots and shoots of the seedlings grown in space were similar to those obtained only from growth on a horizontal clinostat under similar conditions of time and temperature. From visual inspection alone, it was not possible to distinguish the weightless seedlings from their comparable Earth controls. The gross morphology of the seedlings grown in space is similar to that obtained on the horizontal clinostat (Lyon and Yokoyama, 1966).

The description which best characterizes seedling growth in space is that the roots grow in a manner more random than is normal. On Earth, root growth is highly ordered and occurs along a regular path. In space and on the clinostat, however, the highly ordered direction of root growth is replaced with increased randomness. This may be attributed to the loss of normal auxin control during weightlessness, but the biochemical reactions associated with such auxin control remain unsettled. Biochemical measurements performed upon the seedlings recovered from orbital flight indicate that metabolic changes are also associated with growth in a weightless environment. The metabolic functions affected appear to be respiration, carbohydrate metabolism, and an as yet unidentified mechanism in which peroxidase is intimately involved.

Although oxygen and carbon dioxide measurements of the air in the plant growth chambers indicated no changes in the respiration of the space-grown or Earth control seedlings (table 2), new evidence has been acquired which questions that original observation. The titrimetric method of analysis originally used was found to be less accurate than the gas chromatographic methods used in the carefully controlled postflight experiments. It now appears that seedling growth on a clinostat results in increased respiration (table 4). These newer findings are in close agreement with earlier published reports by other investigators (Dedolph et al., 1966; Curtis, 1967). Since the overall physiology of the seedlings grown on the clinostat resembles that of space-grown seedlings, it is likely that respiration did increase during weightless growth. This conclusion can only be confirmed through subsequent space experiments.

Carbohydrate metabolism also appeared to have been affected in the wheat seedlings grown during the flight of Biosatellite II. Glucose-6-phosphate dehydrogenase appeared more active per unit weight in the shoots of orbited plants than in the shoots of the others. This difference occurred in the coleoptile rather than in the leaf (Conrad, 1968). Furthermore, this one area stands out as the only example of a metabolic difference between space-grown and clinostat-grown seedlings. A critical examination of the tissues from the postflight controls indicates that the vibration profile may have increased the activity of this enzyme in vivo. If vibration and clinostat data are grouped together, the enzyme activity appears to differ from that of the stationary erect controls. Since Gray and Edwards (1969) reported cytologic similarities between clinostat and vibrated seedlings, the rationale for grouping the data in this fashion may be valid. No conclusive evidence has been forthcoming despite the large number of analyses performed on this enzyme. Because of the importance

of this enzyme in the growth processes of all living creatures, the effects of weightlessness on this enzyme should be critically examined on tissues from future space experiments.

Extrapolation of these data indicates that the energy for plant epinasty could be generated through altered carbohydrate metabolism. The increase in G–6–PD activity, together with increased respiration, suggests such a possibility. This relationship, if substantiated, carries enormous implications to all life in space, since almost all forms of life, both in the plant and animal kingdoms, respire in a uniform manner.

The effect of spaceflight on glyceraldehyde-3-phosphate dehydrogenase activity is more ambiguous. Activity measurements in the organs of seedlings grown in the large chamber indicate greater G–3–PD activity than in those of the seedlings grown in the small chamber. Although this finding occurred in each of the two seedlings examined, the total number of seeds taken from each chamber was small and the results may have been fortuitous. There appears to be no explanation capable of withstanding severe scrutiny which can establish the role of chamber size (within the limits of the experiment) to the growth processes. Since the differences in activity observed represent the extreme values within the range of variability of a large number of tissues, it must be concluded that spaceflight does not affect the reaction catalyzed by this enzyme.

Malic acid dehydrogenase, cytochrome C reductase, and peptidase activities were normal in all of the tissues examined. No differences were observed in the activity of these enzymes between the orbited and Earth control seedlings. Earlier ambiguities, particularly with MDH, have been clarified through additional analyses. Although the curve for the erect Earth control is now modified from the preliminary report (Conrad, 1968), the conclusion remains unchanged that there is no peculiar effect of weightlessness or the balanced gravity of the horizontal clinostat upon any of these enzymes.

The one enzyme most strikingly influenced during growth in a hypogravity environment is peroxidase. The activity of this enzyme increases dramatically in the region of epinastic curvature whether produced through growth on orbit or on the horizontal clinostat (fig. 11). No peaks of activity were found, however, in tissues which did not demonstrate epinasty (fig. 12). Transaminase activity also appears to follow this pattern, but the variability of this response dictates further study before a proper conclusion can be made. We have shown that peroxidase activity is stimulated under weightlessness. Although the exact biologic function of this versatile enzyme is not known (Saunders et al., 1964; Galston and Davis, 1969), morphogenic roles are suggested by its action in producing auxin (Riddle and Mazelis, 1964) as well as in activating auxin (Galston et al., 1953), in converting hydroxyphenylpropanes, such as coniferyl alcohol, to ligninlike materials (Siegel, 1955), and also in oxidizing such important metabolic compounds as NAD and NADP (Akazawa and Conn, 1958).

When normal genotypes are stunted by compounds which inhibit gibberellin biosynthesis, the peroxidase activity rises markedly (Gaspar and Lacoppe, 1968). The application of exogenous gibberellic acid depresses peroxidase synthesis and speeds growth, whereas gibberellin antagonists stimulate peroxide synthesis and retard growth. The evaluation of peroxidase in gibberellin-deficient plants is apparently helpful in oxidizing exogenous auxin. Ethylene production is stimulated by auxin, and ethylene causes an increase in peroxidase activity as well (Hall and Morgan, 1964). Auxin represses and kinetin promotes the synthesis of peroxidase in geranium (Galston et al., 1969). It appears, therefore, that these two growth factors also interact in the control of peroxidase activity as they do in the control of growth. Thus it appears that four different plant growth factors, auxin, gibberellin, cytokinin, and ethylene, can control the level of peroxidase activity in different tissues; and through a peroxidase-mediated mechanism they can cause the plant to react to reduced gravity with typical epinastic curvatures.

It has also been proposed that the response of pea roots to gravity is due to the ethylene production and, therefore, to growth inhibition on the lower sides of the roots caused by concentrations of auxin greater than the optimum for growth promotion (Chadwick and Burg, 1967). While it is evident that, as in stems, applied auxin elicits ethylene production and that this gives a growth inhibition, the theory has been criticized on the basis that growth responses in pea roots caused by supraoptimal auxin concentrations are different from those caused by ethylene (Andreae et al.,

1968). Auxin appears to reduce growth more rapidly than does ethylene. The auxin effect is reversible whereas that caused by ethylene is not, and even in the presence of ethylene the addition of high concentrations of auxin (10 to 200 micromoles per liter) further inhibits growth. The enhanced ethylene production might be contributory to the auxin inhibition of root growth (calculations indicated that 20 to 30 percent of the inhibition could be caused by ethylene), but it is evident that in roots, as in coleoptiles, the growth inhibition by high concentrations of auxin cannot be entirely explained by ethylene. This opinion (Galson and Davies, 1969) appears to have been confirmed by data returned from the Biosatellite II experiments, since no ethylene (0.1 ppb or less) was found in the wheat seedling growth chambers (Conrad, 1968).

These experiments have always been conducted in complete darkness in order to separate geotropic reactions from phototropic responses, both of which are controlled by auxin. If light is required for the biosynthesis of ethylene, it would be unlikely that this compound contributes to epinasty. Additional experiments were conducted, therefore, to determine the effect of light upon epinasty and ethylene biosynthesis in seedlings grown on the horizontal clinostat. The results of this study (table 5) indicate that ethylene biosynthesis is indeed a light-requiring reaction. Moreover, ethylene biosynthesis increases significantly when the seedlings are grown on a horizontal clinostat in the light. It has already been shown that ethylene interferes with the transport of auxin in seedlings as well as in leafy plants. It would also follow that epinasty in seedlings would be exaggerated by growth on a clinostat in the presence of light. Leafy plants, on the other hand, should respond to rotation on a clinostat in the dark with reduced epinasty.

This hypothesis was confirmed by experimental observations. Further reduction in epinasty occurs in 45-day-old pepper plants by preconditioning the plants to complete darkness for 24 to 48 hours. These experiments have been described elsewhere (Conrad and Yokoyama, 1970) and later confirmed by Lyon (1970). It appears therefore, that ethylene might have been partially responsible for the epinasty observed in the pepper plant experiment but not in the wheat seedling experiment. If the controlling mechanism functions in a similar manner in both plants, it then seems unlikely that ethylene biosynthesis was responsible for the epinasty observed in the space-grown seedlings.

Peroxidase is known to function in several biochemical reactions in plant tissues. Whether one specific pathway is preferentially affected by growth in balanced gravity still remains to be demonstrated conclusively. Certainly, continued study in this area is indicated by the findings obtained during the course of this program.

Another finding of interest is the increased utilization of two carbon fragments by seedlings growing on a clinostat over their erect controls. Both ethane and acetylene appear to have been metabolized more rapidly under these conditions (table 5). The significance of this finding and the ultimate fate of these compounds are still not clear. Because of the implications of altered metabolic processes during weightlessness, pursuit of this observation could prove to be of considerable interest in future space research.

The kinetic studies performed during this program (table 6) indicate that balanced gravity does not result in structural changes to the enzyme molecule which may affect its ability to catalyze biochemical reactions. The conclusions drawn from biochemical measurements, therefore, indicate that weightlessness does indeed influence specific physiologic and biochemical functions of the plant which are ultimately manifested by altered configurations of organs. These biochemical changes were not due to changes in the tertiary structure of the enzyme protein molecule, as some investigators had prophesied.

SUMMARY AND CONCLUSIONS

Profound effects of spaceflight have been demonstrated upon the biochemistry and physiology of wheat seedlings. These effects are, for the most part, duplicated on Earth by growth on the horizontal clinostat. The visible manifestations of growth in a balanced gravity environment (clinostat) are short curvature and root epinasty. Root growth is random when compared with the highly ordered pattern observed in control seedlings grown in the normal erect position. The growth rate does not appear to be affected, however.

The altered orientation of seedling organs is believed to be caused by the translocation of auxin and its concomitant biochemical reactions. The

TABLE 6. – Enzyme Kinetic Studies Using Lateral Roots of Wheat Seedlings Under 3 Conditions of Growth

Enzyme	K_m, M			V_{max}, mM/min./μg protein		
	Flight	Clinostat	Erect	Flight	Clinostat	Erect
Peroxidase	1.81×10^{-3}	2.86×10^{-3}	2.45×10^{-3}	.....	.....	.....
Glucose-6-phosphate dehydrogenase	4.00×10^{-5}	4.01×10^{-5}	4.02×10^{-5}	4.55×10^{-3}	4.37×10^{-3}	4.95×10^{-3}
Glyceraldehyde-3-phosphate dehydrogenase	2×10^{-3}	1.8×10^{-3}	1.8×10^{-3}	8.3×10^{-3}	7.2×10^{-3}	4.8×10^{-3}
Malic dehydrogenase	2.3×10^{-2}	2.3×10^{-2}	2.3×10^{-2}	1.1×10^{-2}	2.9×10^{-2}	7.7×10^{-2}
Transaminase	5.5×10^{-4}	2.9×10^{-4}	5.5×10^{-4}	1.0×10^{-2}	9.7×10^{-3}	8.9×10^{-3}
Cytochrome C reductase	4.3×10^{-6}	5.3×10^{-6}	2.8×10^{-6}	3.4×10^{-5}	6.9×10^{-5}	4.1×10^{-5}

biochemical parameters affected by growth in space are increased respiration, increased peroxidase activity at the site of epinastic curvature, and a possible influence upon carbohydrate metabolism. The latter effect may have resulted from the vibration incurred during lift-off or reentry.

Ethylene was not found in any of the seedling growth chambers. Since the sensitivity of these measurements is 0.1 ppb, it is doubtful that the compound was responsible for the altered orientation of seedling organs observed.

The physiologic effects observed were caused by alteration in the metabolism of the plant. Kinetic studies rule out the possibility of structural changes in the protein affecting the ability of the enzymes to catalyze biochemical reactions.

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Biochemical Changes in the Endosperm of Wheat Seedlings in the Weightless State[1]

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The biochemical mechanisms responsible for the tropic responses in plants have interested scientists for a number of years. The role of the plant hormone, indoleacetic acid (IAA), in the production of epinastic curvatures has been reasonably well established. The Cholodny-Went theory states that growth curvatures, whether they be induced by internal or external factors, are due to an unequal distribution of auxin between the two sides of the curving organ. In the tropisms induced by light and gravity, the unequal auxin distribution is brought about by a transverse polarization of the cells which results in a lateral transport of auxin (Went and Thimann, 1937). This theory was based on the early work of Cholodny (1924) and Went (1927) in the study of growth-accelerating substances. In recent years it has been demonstrated by the use of ^{14}C-labeled IAA solutions that tropically induced curvatures are the result of an unequal distribution of the hormone (Ching and Fang, 1958; Gillespie and Thimann, 1961; and Lyon, 1963).

The exact mechanism of the mediation by the hormone of induction of movement and curvatures is less well understood. The role of carbohydrates has received some interest, although the role of amino acids is even less well understood. It was reported by Warner (1928) that sugars accumulate on the convex side of the curving dahlia shoots. It has been observed that the statenchyma or statoliths change position in the plant cells in response to geoinduction in plants (Sievers, 1965). Further, subjecting seedlings to lower temperature reduces the size, number, and starch content of the statoliths; these alterations show a close correlation with the reduced sensitivity to geoinduction (Hawker, 1932 and 1933). However, the exact role of the starch grains on the epinastic response to gravity remains a matter of controversy.

The role of soluble sugars and the amino acids in plant tropisms has been pursued in a number of studies. Direct evidence of the participation of these compounds in epinastic curvature in pepper plants was demonstrated in this laboratory by Finn (cited in Johnson and Tibbits, 1968). It was shown that the direct application of solutions of sucrose or amino acids to the leaves of pepper plants resulted in a downward curvature of approximately 11 degrees in the upright plant at the end of ½ hour, with a return to normal within an hour. After 24 hours of rotation on the horizontal clinostat, it was found that a significant decrease in leaf starch occurred with a concurrent increase in leaf glucose and sucrose as compared with those of an upright control. Furthermore, the amount of sucrose was positively correlated with the degree

[1] The experiments discussed in this report were coordinated by Dr. Johnson with related contracts performed by Dr. C. Lyon of Dartmouth College, Dr. S. Gray and Dr. B. Edwards of Emory University, and Dr. H. Conrad of Resources Planning and Control Corp.

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of curvature of the leaf. These preliminary results suggested that carbohydrates play an important and direct role in leaf epinasty (Johnson and Tibbits, 1968).

In the embryo of the monocotyledonous plants, the principal enzymes secreted are hemicellulase, which results in the dissolution of the endosperm cell walls, and diastase, which participates in the digestion of starch (Curtis and Clark, 1950).

It has been found that excised embryos will not grow unless a wide spectrum of preformed amino acids are present in the support media (Oaks and Beavers, 1964). The more important amino acids appear to be the basic and neutral acids, while the ones closely related to the tricarboxylic cycles appear to be produced through transamination (Falkes and Yemmn, 1956; Wilson et al., 1954).

It was hypothesized that the weightlessness encountered during 72 hours of orbital flight might retard the growth of the root and shoot because of a modification of protein synthesis and the incorporation of carbohydrates into cell wall constituents. This retardation of growth would be expressed as an excess of free amino acids and soluble carbohydrates in the endosperms as compared with those in the controls. The growth of root and shoot would not be retarded in response to the absence of gravity, so there would be a normal transport mechanism. The incorporation of carbohydrates and amino acids into the cellular substance and the distribution of free entities would proceed normally. Growth of roots and shoots would be accelerated in the weightless environment, with the result of an increased length in both the roots and shoots. The transport mechanisms would be enhanced. A smaller concentration of protein and carbohydrates would be found in the endosperm of the seedlings following the orbital flight than would be found in the control plants.

EXPERIMENT DESIGN AND METHODS

Hexaploid winter-wheat seeds were selected for the experiments from the Georgia 1123, 1964 harvests and Pritchard Farms 1966 harvest. One thousand seeds from each lot were selected visually for intact embryos and weighed individually for a uniformity of between 38 and 39 mg each.

Seventy-eight growing wheat seedlings were subjected to weightlessness during the flight of Biosatellite II. The flight package consisted of six cylindrical chambers, two of which contained 15 seedlings each, a similar chamber with 12 seedlings, and a larger complex of chambers with three sets of 12 seedlings each. The seedlings were affixed to branched stalks within the chambers so that both their shoots and roots grew free in water-saturated air. In order to prevent any phototropic response, all growth took place in the dark. The seedlings in two chambers were subjected to fixation by a formalin/acetic-acid/alcohol spray on command; hence, their endosperms were not used in this study. Seedling endosperms in the remaining chambers were obtained for the investigation.

Four identical packages served as Earth controls during flight. Two packages were maintained erect throughout the experiment and two packages were rotated on horizontal clinostats at 6 rph during the flight period only.

The control plants were subjected to a temperature profile the same as that present in the flight package. The temperatures of the flight packages for 5 hours while they were on the recovery aircraft are not known. The packages were designated:

(1) Control I erect
(2) Control II clinostat
(3) Control III erect
(4) Control III clinostat

The seedlings in the control II clinostat were fixed following notification of recovery of the flight capsule. The remaining controls were maintained throughout the flight and recovery period (12 hours prelaunch, 45 hours orbit, and 5 hours recovery).

In addition to these controls, 48 seeds were affixed to the experiment seed stalks and germinated in darkened cylinders at 72 F until launch, at which time the seeds were removed and the embryos were discarded. These seeds became the *T*—0 control endosperms. Details of the experiment seed stalk and procedures for mounting the seed for germination and observation have been presented by Lyon and Yokoyama (1966).

Laboratory Chemical Analyses

Approximately 45 hours following launch, the experiment was recovered on descent by aircraft near Hawaii. It was delivered to the investigators

in the Hickam Field Laboratory approximately 5 hours later. Disassembly proceeded without incident and within 1 hour the endosperms were separated from the rest of the seedlings, divided into two groups with respect to coleoptile length, and quick-frozen on dry ice.

PLANT PREPARATION

The endosperms were separated into two groups and distributed according to coleoptile lengths. This division was performed for two reasons: (1) The amount of amino acids and carbohydrates present in the endosperm is related to the growth of the coleoptile and roots for both flight and controls; and (2) in addition to the effect of growth of coleoptile and roots, there is a direct correlation between the degree of geotropic response and length of the organ involved. Previously, it was determined that a correlation at the 0.01 level of confidence existed between the length of the coleoptile and the total length of roots. The two groups were coded by coleoptile length as: A, 3.0 to 3.9 mm; and B, 4.0 to 4.9 mm. After recovery the endosperms were promptly placed on dry ice and transported to the North American Rockwell Corp. Laboratory in El Segundo, California. On arrival, the endosperms were removed from the dry-ice container and oven-dried to a constant weight.

CARBOHYDRATES

Samples of the oven-dried endosperms weighing 100 mg were extracted in hot 95 percent ethanol. The supernatant liquid was heated on a steam bath until it was dry. Neutral lead acetate was added to precipitate the proteins. After centrifugation, the sample was made up to volume with distilled water. Glucose was determined by the glucostat reagent method (Worthington Biochemical Corp., 1963). Invert sugars were determined by hydrolysis with invertase (Sigma grade VI) and were further hydrolyzed at room temperature by concentrated HCl. The amount of glucose present was determined by the glucostat method. The total sucrose content was determined by subtracting the initial weight of glucose from the total glucose content of the hydrolysate and multiplying by the factor of 1.9. The residue from the alcoholic extraction was incubated for 12 hours with Taka-diastase and then hydrolyzed with HCl and made to volume with distilled water. The amount of starch present in the residue was determined by the Folin-Wu copper reduction method.

AMINO ACIDS

Samples of the oven-dried endosperms weighing 200 mg were extracted several times in 80 percent ethanol and processed on the Autotechnicon amino acid analyzer according to instructions in the manual for that instrument.

NITROGEN

The total amount of nitrogen was determined by standard procedures with a Coleman Nitrogen Analyzer. Measured quantities of the endosperm material were used in this determination.

The nonprotein nitrogen fraction was obtained by extracting 50-mg samples of the oven-dried endosperms with 10 percent NaCl. The proteins were precipitated with sodium tungstate. The extract was centrifuged to remove all solids, the Folin-Wu digestion mixture was used to free the nitrogen, and the amount was determined by Nesslerization. The protein nitrogen was determined by the difference between the total and nonprotein nitrogen fractions.

Postflight Test

In an effort to elucidate further the effects of the flight dynamics and the shortened flight time, the procedures were repeated by subjecting seedlings to the vibration and acceleration profile recorded for the actual flight. The increased hold prior to launch and temperature profiles of the flight were simulated.

RESULTS

The flight of Biosatellite II was terminated approximately 45 hours after launch. Growth of the seedlings was considerably less than that that would have occurred in the 72-hour flight originally planned. Previous work indicated that during a 72-hour period the seedlings would have growth ranges allowing the samples to be divided into four groups of coleoptile lengths ranging from 3.0 to 6.9 mm. Instead, only two groups, corresponding to coleoptile lengths of 3.0 to 3.9 (A) and 4.0 to 4.9 (B), were obtained. The number of endosperms per group for each control and the flight package is shown in table 1. Correlation values r of coleoptile length versus total root length (primary root plus two lateral roots) were determined for each control

and the flight unit. These values are shown in table 2. The highly significant correlation between coleoptile and total root length indicates that the methods used to select the seeds (visual examination after soaking and using single lots of weighed seeds) produced a singularly uniform group of wheat seedlings for analysis. It had previously been determined that the length of the coleoptile could not be correlated with the total length of the roots when the seeds were selected at random and without regard to weight.

The average dry weights of the endosperms in each of the two groups and the weight differences between the groups are shown in table 3. The percent decrease in starch content in comparison with that present before the start of the test (T—0) for each of the groups and the differences in percent decrease between the groups are shown in table 4. In each case the average dry weights of the endosperms of group B were less than those of group A, and, with the exception of the control III clinostat specimens, the endosperms of group B showed a greater percent decrease in starch content than did those of group A. The discrepancy exhibited by the control III clinostat specimens (group B specimens had a percent decrease in starch 0.2 less than that of group A) may be explained by the variability in starch content of the seeds in the seed lot and the small difference in the average dry weights of the endosperms between the two groups (0.34 mg).

Chemical Analyses

Preliminary baseline laboratory analyses of control seedlings indicated that horizontal rotation on the clinostat did not significantly alter the carbohydrate distribution in the endosperm as compared with that of upright control plants. The results of these tests are presented in table 5. However, control studies indicated that there was a general reduction in the concentration of the free amino acids following both 72 and 96 hours of rotation on the horizontal clinostat as compared with that of the stationary upright controls. These data are presented in table 6.

The analyses of the carbohydrate distribution

TABLE 1. – Number of Endosperms in Groups A and B

Package	Number of endosperms	
	Group A	Group B
Control I erect	27	15
Control II clinostat	26	19
Control III erect	24	23
Control III clinostat	20	24
Flight	8	30

TABLE 2. – Correlation Values r Between Coleoptile Length and Total Root Length

Package	Number of seedlings, n	r values[a]
Control I erect	45	0.472
Control II clinostat	42	.834
Control III erect	46	.478
Control III clinostat	47	.698
Flight	48	.436

[a] $r_{01}=0.372$ when $n=45$; $r_{05}=0.288$ when $n=45$.

TABLE 3. – Average Dry Weights of Endosperms in Groups A and B

Package	Average dry weight of endosperms, mg		
	Group A	Group B	Difference
Control I erect	31.76	31.10	−0.66
Control II clinostat	31.51	30.88	− .63
Control III erect	31.70	31.17	− .53
Control III clinostat	32.09	31.75	− .34
Flight	31.83	31.23	− .60

of the endosperms of the control and flight wheat seedlings are presented in table 7. It will be noted that there are no significant differences between results for the various test configurations.

The effects of the control procedures and the weightlessness of flight are presented in table 8. The endosperms were divided into groups A and B on the basis of coleoptile length. It will be noted that there are no significant differences between any combinations of test conditions. It was postulated that this lack of difference was due to the reduced length of the orbital period (compare table 6).

On the basis of this thesis and preliminary baseline data, the postflight tests were conducted. There was a greater variability in the free amino-acid concentrations, but the pattern was irregular and without statistical significance. For this reason, the data are not reproduced here. There was no variation in starch, sucrose, glucose, total, or protein nitrogen.

DISCUSSION

Although the results of the analysis of carbohydrates, amino acids, and nitrogen fractions of the endosperms do not show an effect due to weightlessness, it was postulated that the orbital flight was not of sufficient duration to elicit a response. The postflight tests did not resolve this question.

TABLE 4. – Percent Decrease in Starch of the 2 Endosperm Groups in Comparison with *T*–0 Controls

Package	Percent decrease in starch		
	Group A	Group B	Difference
Control I erect	19.6	20.0	0.4
Control II clinostat	18.8	20.9	2.1
Control III erect	19.1	21.2	2.1
Control III clinostat	18.8	18.6	– .2
Flight	19.8	21.4	1.6

TABLE 5. – Baseline Studies of the Carbohydrate Content of 50 Wheat Seedling Endosperms Following 24, 48, 72 and 96 Hours of Rotation on the Horizontal Clinostat

Experiment	Content, mg/100 mg dry weight, of —		
	Glucose	Invert sugars expressed as glucose	Starch
Nongerminated	0.11	0.36	58.0
24 hours:			
Stationary erect	.19	.39	55.2
Horizontal clinostat	.19	.36	55.1
48 hours:			
Stationary erect	.80	.42	49.9
Horizontal clinostat	.90	.40	49.7
72 hours:			
Stationary erect	1.79	.48	46.8
Horizontal clinostat	1.69	.54	45.9
96 hours:			
Stationary erect	2.68	.53	42.5
Horizontal clinostat	2.24	.59	43.5

TABLE 6. – Baseline Studies of the Amino Acid Concentration in the Endosperm of Wheat Seedlings Following 24, 48, 72, and 96 Hours of Rotation on the Horizontal Clinostat

Experiment	Concentration, μ mole per gram dry weight, of —																
	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Cystine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine
Control[a]	0.77	0.60	0.33	0.50	0.16	0.20	0.37	([b])	0.13	([b])	0.07	0.08	0.09	0.06	0.05	0.37	0.15
24 hours:																	
Upright	.48	.67	.82	.83	.74	.44	1.22	([b])	.58	.14	.36	.85	.34	.47	.15	.75	.30
Rotated horizontally	.62	.65	.81	.86	.73	.43	1.18	([b])	.57	.13	.39	.85	.40	.52	.16	.88	.33
48 hours:																	
Upright, stationary	.79	1.13	1.68	.97	1.97	.75	1.78	.31	1.43	.26	1.06	2.05	1.08	1.45	.37	.92	.88
Rotated horizontally	.78	.97	1.61	1.03	1.91	.83	1.78	.41	1.44	.25	1.01	1.78	1.03	1.36	.36	.82	.90
72 hours:																	
Upright, stationary	1.32	1.92	2.46	1.44	3.61	.99	2.49	.73	2.57	.42	2.24	3.37	2.06	2.91	.59	1.52	1.72
Rotated horizontally	1.05	1.39	1.68	1.53	3.08	.93	2.23	.33	2.18	.42	1.90	2.45	1.76	2.38	.48	1.34	1.40
96 hours:																	
Upright, stationary	1.43	2.54	3.03	1.47	4.37	1.35	3.86	.74	4.00	.54	2.71	4.76	2.99	4.18	.73	1.56	2.30
Rotated horizontally	1.33	1.74	2.07	1.27	3.64	1.11	2.66	.39	3.83	.41	1.96	2.98	2.18	3.96	.60	1.53	2.16

[a] Seed soaked 2 hours in distilled water.
[b] Trace.

The lack of statistically valid differences or a definite pattern with the postflight tests was due to the stresses of test delays and other procedural problems.

In preliminary experiments it was found that a general decrease in amino acid content occurred following 72 and 96 hours of rotation on the horizontal clinostat. At 24 and 48 hours, no differences were noted. Carbohydrate differences did not occur at 24, 48, 72, or 96 hours in either the baseline studies or the present flight program.

In these experiments, two lots of intact seeds were selected at random. One lot was germinated in a normal position so that the coleoptile grew upward. The other lot was germinated while being rotated on the horizontal clinostat. At the end of each experiment, those seeds treated in the same manner were pooled and analyzed.

As a refinement in technique, four sets of endosperms divided into two groups according to coleoptile length were specified for the analysis of endosperms from the controls and flight units of Biosatellite II. This change in technique was based on the reports of Hawker (1932), who showed that the geotropic sensitivity of various plant roots increased with increasing length of root up to a certain point. Further, Warner (1928) showed that the accumulation of sugars increased with time in the convex side of geoinduced dahlia stems. The greatest accumulation occurred 24 hours after geoinduction. It was hoped that, as a minimum, the longer coleoptiles, representing a higher degree of sensitivity to geoinduction, would demonstrate a change in metabolism of the endosperm attributable to weightlessness.

If weightlessness did show an effect on metabolism of the endosperm, the effect would theoretically be one of decreased starch and increased glucose and sucrose (or sucrose alone as was found in pepper-plant leaves and stems). In addition, a change in the pattern of amino acids would occur, as demonstrated by either an increase or decrease in concentration. The data of these studies indicate that there is a change in the metabolic products of the wheat seedling, but the altered distribution of these materials, with time, could not be detected in response to changes in gravitational alterations.

The changes in metabolism of a plant organ undergoing geoinduction appear to be universal. These changes occur in leaf, stem, hypocotyl, coleoptile, and roots of plants and appear in the form of an increased amount of soluble sugars and amino acids. This mobilization of soluble sugars and amino acids in the curving parts of plant organs undoubtedly induces cell elongation and enlargement on the convex side of the curving part through increasing osmotic pressure of these cells. It would be of interest if a storage organ like the endosperm could be shown to be affected in a similar manner since this organ is not known to be responsive to geoinduction.

TABLE 7. – Carbohydrate Content of Control and Flight Wheat Seedling Endosperms

Program	Content, mg/100 mg dry weight, of —							
	Total soluble sugars as glucose		Glucose		Sucrose		Starch	
	Group							
	A	B	A	B	A	B	A	B
Control I erect	7.67	6.60	0.65	0.71	0.74	0.73	52.2	53.3
Control II clinostat	6.20	7.70	.59	.75	.66	.68	53.1	52.7
Control III erect	6.40	7.75	.60	.71	.70	.70	52.7	52.7
Control III clinostat	6.13	7.33	.57	.60	.70	.78	52.2	53.3
Flight	6.28	7.57	.56	.63	.68	.76	51.8	51.9

TABLE 8. – Amino Acid Content of Control and Flight Wheat Seedling Endosperms

Experiment	Content, μ mole per gram dry weight, of —																
	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	Alanine	Valine	Cystine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine	Lysine	Histidine	Arginine
T–0 control	0.77	0.24	0.52	1.41	0.47	0.25	0.84	0.23	([a])	([a])	0.15	0.26	0.18	0.18	0.14	0.95	0.28
Control I erect:																	
Group A	1.11	.97	1.88	2.18	3.32	.73	2.00	1.50	([a])	0.31	1.04	2.09	1.27	1.69	.38	([b])	1.19
Group B	1.12	1.11	2.08	2.17	3.91	.79	2.02	1.66	([a])	.30	1.17	2.37	1.40	1.95	.38	1.42	1.14
Control II clinostat:																	
Group A	.89	.74	1.33	2.02	2.55	.59	1.83	1.09	([a])	.24	.79	1.60	.93	1.22	.29	1.27	.81
Group B	.97	.86	1.49	1.73	3.04	.63	1.74	1.28	([a])	.23	.85	1.88	1.13	1.50	.29	1.37	.96
Control III erect:																	
Group A	.94	.89	1.50	2.08	2.80	.60	1.53	1.27	([a])	.24	.87	1.78	1.03	1.44	.29	1.30	.81
Group B	.97	.99	1.75	1.65	3.21	.63	1.59	1.41	([a])	.27	1.01	2.03	1.18	1.66	.29	([b])	.91
Control III clinostat:																	
Group A	.88	.74	1.37	1.88	2.42	.55	1.53	1.22	([a])	.23	.76	1.61	.99	1.26	.27	1.41	.68
Group B	.88	.93	1.41	1.42	2.92	.54	1.57	1.19	([a])	.23	.82	1.65	1.02	1.38	.20	([b])	.82
Flight:																	
Group A	1.23	.88	1.66	2.32	2.85	.66	1.65	1.35	([a])	.24	.95	1.81	1.12	1.44	.35	1.48	.96
Group B	1.01	.88	1.64	1.62	2.94	.56	1.44	1.51	([a])	.27	.92	1.87	1.06	1.48	.26	([b])	.94

[a] Trace.
[b] Sample lost.

SUMMARY AND CONCLUSIONS

The biochemical analyses of wheat seedling endosperms exposed to 45 hours of weightlessness during the flight of Biosatellite II did not reveal any differences in the concentrations of carbohydrates, amino acids, and nitrogen fractions from those in the control samples. Postflight tests designed to verify the results of the flight revealed differences in the concentration of individual amino acids, but the pattern was irregular and not statistically significant.

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The Liminal Angle of a Plagiogeotropic Organ Under Weightlessness[1]

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The present experiment was designed to evaluate alterations in the behavior of the pepper plant, *Capsicum annuum,* in response to the weightless environment. The mode of geotropic reaction of plants is specific for each organ. The main and lateral roots carry out a positive or downward response to gravity. The coleoptile, stem, lateral branches, and leaves respond with a negative reaction or in a direction opposite to the gravitational field. In the nomenclature of Larsen (1962), orthogeotropic organs of a plant are those which normally orient their axes parallel to a plumbline in either the positive or negative direction. Examples are the main roots, stems, and coleoptiles. Plagiogeotropic organs, such as the lateral roots, branches, and leaves, are those that orient at an angle to the plumbline under normal conditions. The plagiogeotropic organs of interest in this report are the leaf and petiole. The measurement of epinasty, or downward curvature of the leaf, concerns the angle formed by the leaf and petiole with the main stem (plumbline.)

The response of plants to the weightless environment of space is of interest from several viewpoints. One is whether the classic epinastic response of plants exposed to horizontal rotation on the clinostat would be duplicated by the subgravity environment of orbital flight. The clinostat is a device developed by Sachs in the 1880's to study geotropic responses in plants (Sachs, 1887). This device has provided a means of subjecting plants to essentially omnilateral geotropic stimulation along the major axis. It consists of a motor-driven rotating plate that can be oriented in either a vertical or a horizontal plane. In an experiment a potted plant is attached to the plate so that the plant axis may be oriented horizontally or parallel to the Earth (fig. 1). The clinostat is rotated at a rate of one revolution every 10 minutes. As the plant rotates, it is alternately subjected to gravity force in opposing directions so that a mathematical summation of the gravity vector forces equals zero.

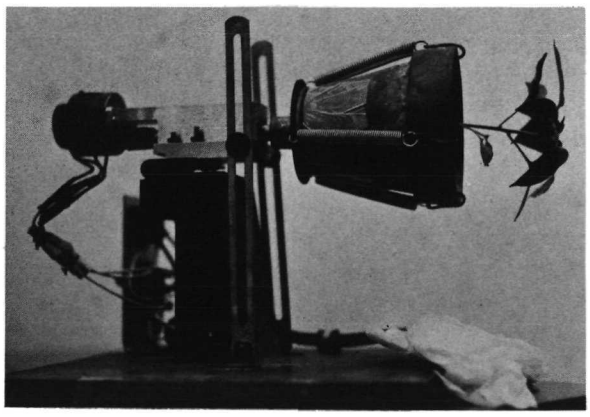


FIGURE 1.— Response of pepper plant to horizontal rotation on clinostat.

The total response of the plants to the weightless environment is not only of interest from the view-

[1] The present report was prepared by Dr. J. A. Green with the assistance of D. K. Chapman.

point of basic plant physiology but provides more complete knowledge of plant growth dynamics for future long-duration spaceflights. The quest for a physiologic basis for epinastic curvature of leaves began with a report by DeVries (1872). Freitag (1930) suggested that the controlling factor in the responses of plants was related to an asymmetric distribution of growth regulators. Although it was known that the application of auxin to leaves and petioles of plants produced an epinastic curvature, the in vivo demonstration of the actual participation of auxins was first demonstrated by Lyon (1963). By utilizing ^{14}C-labeled indole acetic acid (IAA–^{14}C) in the growth substrate, a higher percentage of radioactivity was found in the upper side of the petiole than in the lower portion after geoinduction of epinasty on the clinostat.

The role of carbohydrates and amino acids in the process of plant movement has received very little attention. It was known that the isolation of plants in the dark for prolonged periods eliminates the response on the clinostat, presumably because of a reduction of carbohydrate stores. However, it was demonstrated in this laboratory that when 1.0 M sucrose or a 0.1 percent amino acid solution was applied to the leaves following the dark exposure the plants responded to rotation on the clinostat. Warner (1928) reported that sugars accumulated on the convex side of dahlia shoots following several hours of geoinduction. The difference amounted to a 30- to 50-percent increase in soluble sugars. Because of the number of hours between the beginning of geoinduction and the appearance of measurable differences in sugar distribution, most workers postulated that the effect was the end product of auxin activity. It was demonstrated in this laboratory that the application of either the sucrose or amino acid solution to the leaves of the pepper plants in the upright position (without rotation) resulted in a curvature of approximately 11°. These data indicate that carbohydrates and the related transport mechanisms are involved in the geotropic response of epinasty in plants. A portion of the data reported herein has been published (Johnson and Tibbits, 1968).

The flight experiment was based upon the hypothesis that the low level of gravity during orbital flight would produce results similar to those noted in response to rotation on the horizontal clinostat. This hypothesis states that the liminal angle will be decreased and accompanied by a differential mobilization of carbohydrates and amino acids in the various plant organs.

EXPERIMENT DESIGN AND METHODS

The procedures for this program may be divided generally into operations performed in selecting a plant species for use in the various experimental conditions, evaluation of the environment and geostimulation on the selected specimens, selection of specimens for use in the actual flight experiment, manipulation of the plant materials, and various chemical analyses related to the experimental conditions.

Plant Species Selection

Several species of plants were considered for their potential use in the spaceflight package. The plant species were evaluated on the basis of (1) compactness, (2) leaf spread, (3) response to geotropic stimulation on the clinostat, and (4) ability to withstand the anticipated dynamics of flight during launch and reentry.

Xanthium pennsylvanicum, utilized extensively in prior ground-based investigations of plagiogeotropic organ response, was rejected because of size. The large-size seedling required to exhibit a significant response to geostimulation would not fit the space allotted in the flight vehicle. (The leaf spread of the experimental plants at the time of flight was required to be less than 3 inches because of the spatial limitations of the package.) Holly, oleander, and olive plants were found to be sturdy enough to pass a part of the flight qualification tests but failed to show significant response to rotation on a horizontal clinostat. Coleus plants were evaluated but rejected because of their delicate nature and tendency to wilt readily.

The plant selected for the experiment was *Capsicum annuum,* the bell pepper, commonly called the Yolo Wonder. This plant is a determinate cultivar in which 80 percent of the leaves are opposite. It responds with a marked reduction in the petiole liminal angle when subjected to rotation on a horizontal clinostat. This species also has a flexible stem, which enables it to withstand the acceleration and vibration levels associated with launch and orbital insertion and the deceleration and impact forces of reentry.

Plant Characteristics

Initially, it was believed that a four-leaf plant would provide maximum data return. It was discovered that, because of interference between leaves and obscuring of the stem, a number of the angles formed by the leaves and petioles were not readable. Hence, the decision was made to utilize a plant reduced to two leaves. A study was conducted to determine which of the leaves were most responsive to geoinduction by rotation on the horizontal clinostat. In preliminary screening, it was found that a pepper plant approximately 44 days after planting would satisfy the requirements for size, sturdiness, and response. The plants have three pairs of expanding leaves at this age. On the basis of the results of this study, leaves 3 and 4 were retained for the flight experiment and leaves 1, 2, 5, and 6 were removed. This modification was performed at least 72 hours prior to utilizing the plants experimentally in order to minimize the effects of shock.

Since most plants exhibit optimum periods of geotropic response to the horizontal clinostat during leaf development, plants were evaluated for petiolar epinasty between the ages of 25 and 41 days.

Considerable variability in epinasty of the individual leaves was observed when the stem of the plant developed a marked curvature at the petiole node. The stem characteristically bent toward leaf 3 in most instances. When the stem bent toward the third true leaf, the degree of petiole curvature of that leaf decreased whereas the petiole curvature of the opposite, or fourth, leaf increased significantly. The petiole of leaf 4 began to curve downward initially and then increased in angle as the stem bent toward leaf 3.

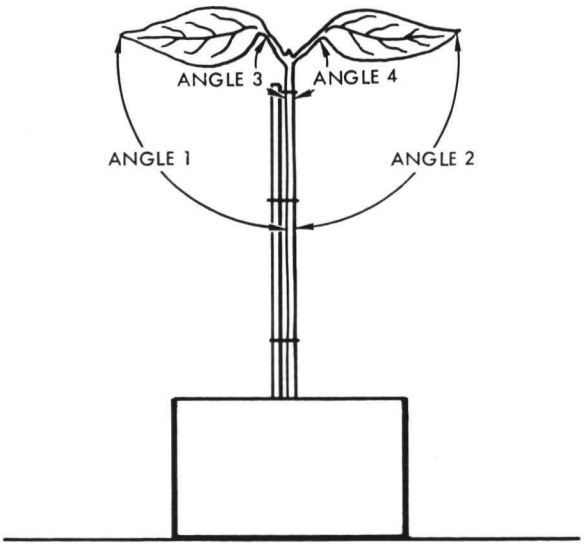


FIGURE 2.— Data acquisition points for liminal-angle determination.

Attempts were made to eliminate stem bending in the experimental plants with limited success. Securing the stems just below the leaf nodes to a rigid stake minimized the effect, but the phenomenon still occurred. The selection of plants in which the third and fourth true leaves were more nearly the same size and positioned on the stem in direct opposition to each other reduced the number of plants that exhibited stem bending when they were rotated on a horizontal clinostat by about one-half. The angles measured and the stem support are illustrated in figure 2.

Environmental Factors

TEMPERATURE AND HUMIDITY

In view of the temperature ranges expected during the Biosatellite flight and reentry, experiments were conducted with *Capsicum annuum* plants to determine the optimum temperature range that would produce the maximum response and recovery following variations in geostimulation on the clinostat. *Capsicum* plants were pruned to the first four leaves above the cotyledons. They were then placed on horizontal clinostats in an environment chamber and subjected to various temperatures to determine the effect on liminal-angle response and recovery. The relative humidity was maintained at 50 percent with a continuous illumination of 200 foot-candles provided by incandescent lamps. The petiole liminal angles of all leaves were measured and averaged together in the determination of response and recovery at various temperatures.

In order to evaluate the effects of various relative-humidity levels on *Capsicum annuum,* plants approximately 6 weeks of age were pruned to four leaves and rotated on the horizontal clinostat at a constant temperature of 77 F with the relative humidity varied. At the end of 12 hours, epinastic responses were determined by measuring the liminal angle of the petioles.

ILLUMINATION

It was hypothesized that the low light intensity in the Biosatellite, coupled with a possible 8- to 20-hour dark hold period, might exhaust the starch supply within the plants. In this case, the liminal-angle response and the carbohydrate activity associated with this response would be affected. Tests were made to evaluate the effects of low light intensity. One test was designed to determine the effect of the dark hold period on the liminal-angle response. In this test, *Capsicum* plants were kept in a lightproof container at 77 F and 55 percent relative humidity for various periods. After the dark hold periods, the plants were placed on a horizontal clinostat for 24 hours and evaluated for epinastic response. The liminal angles were measured before and after each exposure on the clinostat. The dark exposure reduced the liminal angle response from 7° to complete inhibition of response, the amount depending on the length of the dark hold.

An attempt was made to build up the starch reserves in the plants by subjecting them to high light intensity (850 foot-candles) for 24, 38, and 72 hours prior to evaluating the effect of the dark hold. After the exposure to the high light intensities, the plants were placed in the lightproof container at 77 F and 55 percent relative humidity for 24 hours. Subsequently, they were placed on horizontal clinostats and exposed to 200 foot-candles of intermittent incandescent illumination (5 seconds every 10 minutes) for 24 hours. A control group of plants received neither high light nor dark hold but was subjected to 24 hours of horizontal rotation in intermittent light.

Growing Procedures

Although the total number of plants used in the Biosatellite flight experiment was limited, it was necessary to develop culture methods that would produce a relatively large group of plants of the same physiologic age and size from which a representative sample could be selected for use in various control procedures as well as in the flight experiment.

A commercial planter mix[2] was supplemented with Hoagland's nutrient solution in distilled water (Bonner and Galston, 1952) and the pH was adjusted to approximately 6.5 with calcium hydroxide. Seeds[3] were placed in rows ¼-inch deep, spaced 2 inches apart, covered with soil, and incubated under 850 foot-candles of fluorescent light. Seedlings of uniform size were transplanted into individual plastic pots when they were 11 days old and maintained under 850 foot-candles of light for one additional week, at which time the illumination was reduced to 200 foot-candles of continuous light. The moisture level in the individual pots was controlled to obtain uniform growing condition. Each pot contained approximately 180 grams of moist soil as the transplanting medium. After the seedlings were transplanted, 1000 ml of distilled water were added to each flat of 36 pots. The seedlings were watered twice a week thereafter. Two liters of water were added to each flat and permitted to infiltrate the pots for 1 hour, at which time the excess water was removed. The room temperature was maintained continuously at 70±2 F and the relative humidity, at 55±5 percent.

Plants growing in soil might incur serious damage to the root system because of shifting of the medium during exposure to the spaceflight environment conditions (vibration, acceleration, and impact). Therefore, several synthetic growth substrates were investigated to determine a suitable medium for sustaining the plants in flight. A synthetic sponge fortified with a nutrient solution, Nutrifoam,[4] was selected for use in the experiment. The foam could be shaped to fit the desired plant container, saturated with a prescribed amount of distilled water, and sealed with a plastic compound,[5] so that the pepper plant would be provided with sufficient water and nutrients to last for 10 days.

The correct amount of water in the sealed plant container was predetermined by saturating the foam at various levels, sealing the container, and observing growth under controlled light and temperature conditions. The plants performed best when the foam was between 40- and 60-percent saturated. Water levels above 60-percent saturation resulted in root damage, and those below 40-percent saturation resulted in excessive wilting. In order to assure a 60-percent saturation of the

[2] Sears Cross-Country planter mix.

[3] Uniform lot of seed obtained from the Germain Seed Co.

[4] Obtained from the Tennessee Texas Co., Houston, Texas.

[5] Dow Corning RTV 502.

foam, a weight standard was developed. After the container was sealed, the water was maintained at the desired level by inserting a hypodermic needle to replace water lost through transpiration.

Selection Criteria

The inherent variation of the pepper plants under geotropic stimulation required the development of a method to select a homogeneous group of plants that would respond to, and recover from, simulated weightlessness. The method used was to pretest a group of plants by placing them on the horizontal clinostat for 12 hours, recording the change in petiole liminal angle, and then placing them in an upright position for 24 hours. Plants that exhibited at 20° to 30° change in petiole angle and subsequent recovery to within 10° of their original angle were selected for primary Earth-based controls and flight plants for photography. A similar method was used to screen the smaller auxiliary plants used for the determination of carbohydrate and amino acid alterations.

The flight packages were designed to carry a total of nine plants. Four 35-day-old plants were arranged to permit in-flight photography of the geotropic responses to the weightless environment. Photographic illumination was provided by 15-watt incandescent lamps providing a level of 200 foot-candles for 5 seconds every 10 minutes. The shutter of the camera was synchronized with the periods of illumination. Five additional plants, 25 days of age, were positioned below the level of the mirrors and were used for postflight carbohydrate and amino acid analyses. Because the overall height of the flight package was limited to 10 inches, the maximum allowable height for the plants to be photographed was 4 inches, with a leaf spread of 3 inches. The size of the auxiliary plants was 2 inches high, with a 2-inch leaf spread; hence, the age differences. The total volume of each experimental package was slightly less than 1 cubic foot, with a weight of approximately 12 pounds in the loaded configuration (fig. 3). Four units were constructed for use in the Biosatellite program. These units have been designated as control III erect, control III clinostat, prime, and backup flight packages in this report. The control packages were installed in an environmentally controlled chamber in which the light, temperature, and humidity could be programmed to conform with the actual conditions existing in the flight vehicle, which were telemetered to the laboratory at the Kennedy Space Center.

Dynamic Testing

Upon completion of hardware design and experiment definition, preflight tests were conducted at the General Electric Co. facility in Philadelphia, Pa. Fully loaded flight packages were assembled and subjected to the dynamic environments predicted for the flight vehicle. These tests included vibration and acoustic environments at both the nominal and off-limit levels with respect to amplitude and frequency. After the dynamic exposures, the specimens were inspected visually for damage. Tests were performed subsequently on the clinostat to detect any modification of the geotropic response.

Preflight Procedures

The plants were kept under continuous cool-white fluorescent light of 850 foot-candles from the seedling stage to 18 days of age, at which time the light intensity was reduced to 200 foot-candles. The plants were subjected to 850 foot-candles for 36 to 48 hours prior to flight to insure an adequate supply of carbohydrates for the expression of epinastic responses under weightlessness.

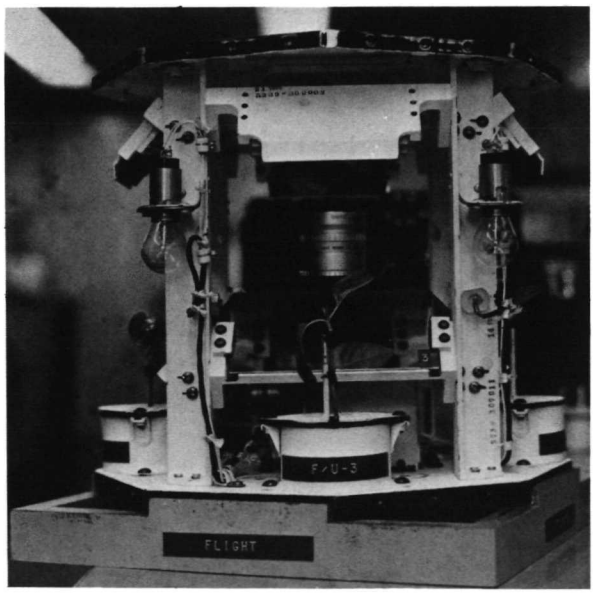


FIGURE 3.— Flight package for the Biosatellite II pepper plant experiment shown immediately after recovery and disassembly of the flight capsule.

Ten days before launch (T—10), 100 plants, 25±1 days old, were selected on the basis of healthy green color, vigorous appearance, and uniformity of size. The specifications were: (1) A leaf spread of the third and fourth true leaves of 2 to 2½ inches, (2) both leaves opposite each other on the stem and of approximately the same size, and (3) a height at the terminal bud of 2 to 2¼ inches. These plants were carefully removed from the 3-inch pots and the soil was washed from the roots. The individual plants were transplanted into precut cylinders of Nutrifoam in plastic cups, saturated with distilled water, and covered with loose-fitting plastic lids. At this time, leaves 1, 2, 5, and 6 were removed (fig. 4).

Five days later (T—5), 100 plants, 18±1 days old, were selected for use as auxiliary plants. Except for size and age, the selection criteria were similar to those used for the prime plants. Final specifications called for: (1) A leaf spread of the first and second true leaves of 1 to 2 inches, and (2) a height at the terminal bud of 1¼ to 2 inches. These specimens were transplanted into Nutrifoam and placed into plastic cups in the same manner as were the experimental plants. The cotyledons were removed at this time.

Fifty of each of the prime and auxiliary plants were selected at T—3.5 days on the basis of vigorous appearance and liminal angles of from 155° to 130°. The edges of the prime plants were trimmed to 3 inches to insure adequate photographic coverage. The amount of water in the plastic foam was adjusted to 60-percent saturation. The pots were then sealed with a 1/16-inch layer of RTV–502 and inverted to check for water leaks.

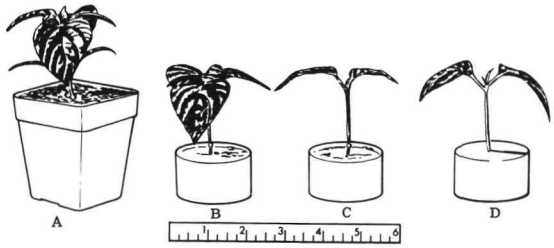


FIGURE 4.— Sequence of plant manipulations prior to insertion into experiment packages. (*a*) Prior to transplant into foam. (*b*) Transplanted into foam substrate. (*c*) Pruned to third and fourth true leaves. (*d*) Sealed in pot with RTV-502.

The plants were pretested at T—2.5 days on the horizontal clinostat for only 12 hours. Plants exhibiting a curvature of the petiole of 10° or more were selected for use as ground-based controls and for flight specimens. All the plants were placed under an 850-foot-candle light to insure adequate carbohydrate reserves.

The prime experimental plants were pretested at T—2 days on the horizontal clinostat for 12 hours. Twenty plants were selected for use in the Earth-based control and flight-unit packages. Twenty-five of the auxiliary plants were selected on the basis of a minimum liminal angle reduction of 10°.

The final group of experimental and auxiliary plants selected at T—12 hours were distributed into five groups by random selection for use as control and flight specimens. Distilled water was injected into the plant containers with a hypodermic needle to adjust to a level of 60-percent saturation. The plants were then placed under 55 foot-candles of illumination until installed in the experiment packages.

The five groups of plants, each composed of four prime plants to be used primarily for in-flight photography, and the five auxiliary plants to be used to augment the chemical analyses were distributed as follows:

(1) The backup package, which became the T—0 control upon installation of the flight package

(2) The flight package, which was installed in the Biosatellite

(3) Control I, which was placed in the dark in the environmental chamber at 72 F with a relative humidity of 55 percent and which served as the radiation mockup

(4) Control III erect, which was installed in a flight package and installed on the clinostat in an upright position in the environmental chamber

(5) Control III horizontal clinostat, which was placed on the clinostat in a horizontal position in the environmental chamber

It should be noted that installation of the control III erect and horizontal clinostat plants was delayed for 4 hours to permit duplication of the temperature and humidity data telemetered from the space vehicle.

Recovery Procedures

At the time of launch, the plants in the flight backup package were removed and designated as

the T—0 controls. The leaves of the prime, or large plants, were removed, pooled, and quick-frozen in dry ice. The younger, or auxiliary, plants were divided into leaves, stems, and growing tips. The separate components were pooled and quick-frozen in dry ice. All specimens were returned to the laboratory for chemical analyses.

Control I plants were processed as follows: On notification of receipt of the flight package at the Hickam Field laboratory, the auxiliary plants were removed and weighed, and the plants were measured for leaf width, length, and height. The plants were separated into leaves, stems, and tips, and each fraction was pooled and frozen in Dry Ice. The cover was replaced, and the large plants were photographed for a period of 24 hours after reentry. At the end of the 24-hour period, the large plants were removed and weighed, and the leaf width and plant length and height were determined. The leaves of the four plants were removed, pooled, and frozen in dry ice for return to the laboratory.

The two control III packages were processed as follows: Four hours following notification that the satellite had reentered the Earth's atmosphere, the horizontal clinostat package was removed from the clinostat and placed in an upright position. On notification of receipt of the flight package at the Hickam Field laboratory, the auxiliary plants were removed, leaf data and plant height were recorded, and the plants were separated as outlined for control I specimens. The cover was replaced, and the primary plants were photographed for 24 hours after notification of reentry. The prime plants were then removed, the leaf data and plant height were recorded, and the plants were maintained at 72 F and 55-percent relative humidity to serve as radiation controls for the flight specimens.

Upon notification of receipt of the flight package at the Hickam Field laboratory, the auxiliary and prime plants of the upright package were processed as outlined for the control III plants. The control and flight films were submitted to the photographic laboratory for developing and printing, and originals were transmitted to NASA Ames Research Center. Copies of the film were used for angular determination by the Telereadex technique. Because of stem bending, some of the angular data were reduced by hand by utilizing stop-frame photography and a protractor.

Chemical Procedures

The samples that were prepared for chemical analyses were composed of the following components from the four control and flight packages: (1) leaves from large or prime plants (T—0 controls only), (2) leaves from small or auxiliary plants, (3) stems from small plants, and (4) growing tips from small plants.

The plant materials were divided into aliquots, purified, and analyzed by standard procedures (Hawk, Oser, and Summerson, 1951).

CARBOHYDRATE AND AMINO ACID ANALYSES

A portion of each sample was placed in 95 percent hot ethanol and filtered to remove the debris. The filtrate was divided into two equal portions, one for carbohydrate analyses and the other for amino acid analyses. The sample allocated for carbohydrate analyses was evaporated on a steam bath, reconstituted to a known volume, and treated with neutral lead acetate to precipitate proteins and interfering compounds. The starch and soluble sugar fractions were subjected to acid hydrolysis and analyzed for total reducing sugars by the method of Folin-Wu. Sucrose analyses were performed by utilizing purified sucrose, and true glucose was determined by utilizing the glucostat reagent method developed by the Worthington Chemical Co. The distribution of the individual carbohydrates was obtained by differences between analyses of hydrolyzed and nonhydrolyzed samples.

The second aliquot of the alcohol extract was tagged with a standard solution of norleucine to serve as an internal standard in the amino acid analyses. The samples were then evaporated with dry nitrogen gas, diluted with a 12.5-percent solution of sucrose, and processed on an Autotechnicon amino acid analyzer.

NITROGEN ANALYSES

Total protein expressed as nitrogen was determined by a combination of micro-Kjeldahl digestion and Nesslerization.

RESULTS

Experimental Criteria Development

The consideration of physical characteristics and physiologic responses of the pepper plant, *Capsicum annuum,* governed the final selection of this plant for use in the experimental evaluation of the response of a plagiogeotropic organ to loss of gravi-

tational stimuli during weightlessness. While establishing optimum conditions for obtaining an epinastic response of the leaves and petioles, it was found that measurement of the liminal angles was not always possible on plants with more than two leaves because of (1) the obscuring of the stem and petiole or (2) physical blockage of leaf movement by the adjacent leaves. It was necessary, therefore, to establish which of the leaves were most responsive to rotation on the horizontal clinostat.

The shock of pruning leaves from the pepper plants resulted in a significant inhibition of epinastic response for the first 48 hours, with full sensitivity returning by 72 hours. The liminal-angle response of alternate pairs of leaves to horizontal rotation in 44-day-old plants is presented in table 1. It was found that the third and fourth true leaves, with regard to chronologic appearance, presented the greatest response with a reasonable area for photographic purposes. Because total leaf spread had to be confined within 3 inches in the flight package to insure adequate photographic coverage by the camera, the effect of leaf trimming on epinastic response was evaluated. The leaves were

TABLE 1. – Effect of Leaf Position and Size on Petiole Liminal-Angle Response to Horizontal Rotation of 44-Day-Old Plants

Leaf No.	Liminal-angle, response, deg.	Area, cm ^{2}
1 (oldest)	50.0	5.2
2	54.7	5.7
3	89.6	7.8
4	92.6	9.2
5	58.0	10.6
6	42.8	11.2

TABLE 2. – Effect of Trimming Leaves on Liminal-Angle Response

Leaf No.	Number of leaves, *n*	Average liminal-angle response
1/4 trimmed		
3	24	76±13
4	24	80±11
1/2 trimmed		
3	25	98±23
4	25	86±16
3/4 trimmed		
3	22	62±21
4	22	67±16

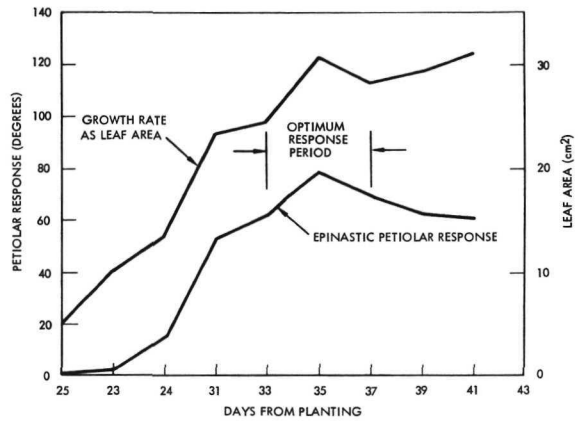


FIGURE 5.– Response of third leaf from 25 to 41 days.

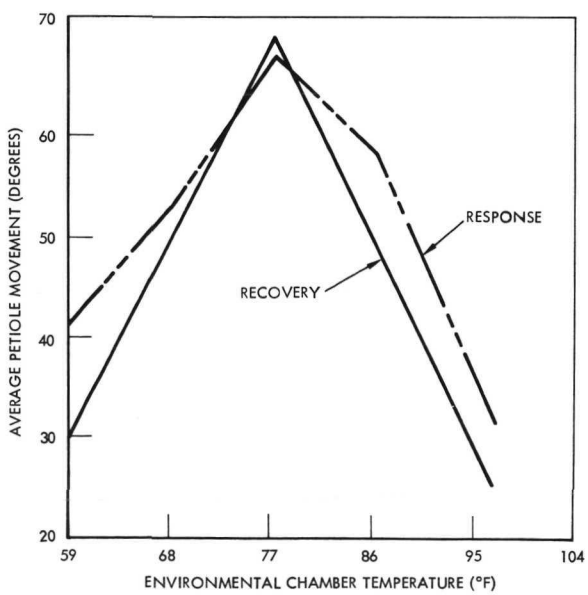


FIGURE 6.– Average petiole movement.

trimmed by removal of one-fourth, one-half, and three-fourths of the distal end. Table 2 indicates that more than 50 percent of the leaf may be removed without reducing the epinastic response to horizontal rotation.

The response of plants to geoinduction is most pronounced at rather limited periods during maturation. The evaluation of the epinastic response and the enlargement of leaf area in *Capsicum* plants between 25 and 41 days after planting are presented in figure 5. It may be noted that the optimum epinastic response occurs between 33 and 37 days; however, the plants still retain sufficient sensitivity to provide reliable data 44 days after planting. A problem at this age is the excessive height and leaf spread of the plants.

Environmental Effects

The effects of variations in the environmental temperature on petiolar movement in response to horizontal rotation on the clinostat are depicted in figure 6. It will be noted that the maximum response occurs between 68 and 86 F with a rather sharp change occurring at a temperature of 77 F. The recovery of the plants from the epinastic induction presents a mirror image of the original response relative to temperature effects.

The effects of varying the relative humidity while plants were rotated at a controlled temperature of 77 F are presented in tables 3 and 4. The response demonstrates rather sharp limits between 50- and 55-percent relative humidity. It is of interest, however, to note that the response between 40- and 80-percent relative humidity is such that substantial variations in temperature control would not negate the validity of the experiment.

Carbohydrate Distribution

Results of the evaluation to test the hypothesis that placing plants in a dark environment would reduce the epinastic response are presented in table 5. The hypothesis states that the dark hold reduces the carbohydrates and, as a consequence, the available energy for the epinastic response. The effect of exposure to high-intensity light, followed by 24 hours of darkness, resulted in restoration of liminal-angle response as presented in table 6. The effect of exposing the plants to 850 foot-candles of high light intensity for 48 hours on total starch content is presented in figure 7. Exposure of the

TABLE 3. – Effect of Humidity on the Epinastic Response of the Petiole of *Capsicum annuum* (Accuracy of Humidity Probe ±2 Percent)

Relative humidity, %	40	50	55	60	70	80
Mean response, deg curvature[a]	77.2	93.9	93.0	78.2	79.3	77.1
Number of plants	11	21	27	21	16	20

[a]The average decrease in liminal angle of leaves 3, 4, 5, and 6 on 44-day-old plants.

TABLE 4. – *t*-Values on Comparison Between Humidities

Relative humidity, %	*t*-value	Relative humidity, %	*t*-value
40 to 50	[a]4.91	50 to 70	[a]4.84
40 to 55	[a]4.61	50 to 80	[a]6.41
40 to 60	.27	55 to 60	[a]4.97
40 to 70	.58	55 to 70	[a]4.49
40 to 80	.03	55 to 80	[a]5.92
50 to 55	.15	60 to 70	.36
50 to 60	[a]5.33	60 to 80	.37
		70 to 80	.77

[a] Significant difference between means.

TABLE 5. – Effect of Intermittent Light[a] and Dark-Hold Periods on Liminal-Angle Responses

Hours in dark	Liminal-angle response on clinostat in intermittent light, deg
None	55.0
4	22.0
8	14.0
12	11.0
16	–.3
24	0

a 200 foot-candles for 5 seconds of every 10 minutes.

TABLE 6. – Effect of Exposure to 850 Foot-Candles of High-Intensity Illumination on Liminal-Angle Response Following 24-Hour Dark Hold

Hours in high light	Response, deg.
Control 1[a]	67
Control 2[b]	0
24	31
48	51
72	12

a No high light; no dark hold.
b No high light; 24-hour dark hold.

plants for an additional 24 hours did not result in a significant increase in energy stores but caused a significant reduction in epinastic response (table 6).

Results of an analysis of changes in the starch distribution in plants subjected to rotation on the clinostat over a 72-hour period are presented in figure 8. The data are presented as a percentage of vertical-control-plant values to insure that any variations in temperature, humidity, light intensity, manipulation, etc. are canceled out by the experimental procedures.

Previous work in this laboratory has established that the rate of liminal-angle change is six times faster during the first 6 hours than during the second 6 hours. It is postulated that the movement of the leaf during the liminal-angle change is work and, consequently, requires energy. In using this thesis, it was proposed that the starch stores of the leaves would decrease during rotation and that the soluble sugar would increase or remain static as the process of catabolism became less than, or equal to, the rate of carbohydrate conversion. Comparison of figures 8 and 9 suggests that the hypothesis is valid. During the first 4 hours, the starch content of the leaves decreased rapidly in association with rapid change in liminal angle. In the following period, the rate of change in liminal angle was greatly reduced, and the plant tended to assimilate starch in a process of partial compensation. The maximum liminal angle change, however, was

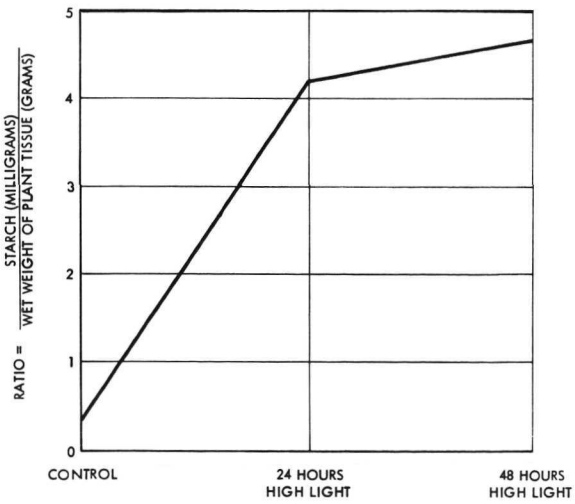


FIGURE 7.– Starch content of *Capsicum annuum* after high light treatment.

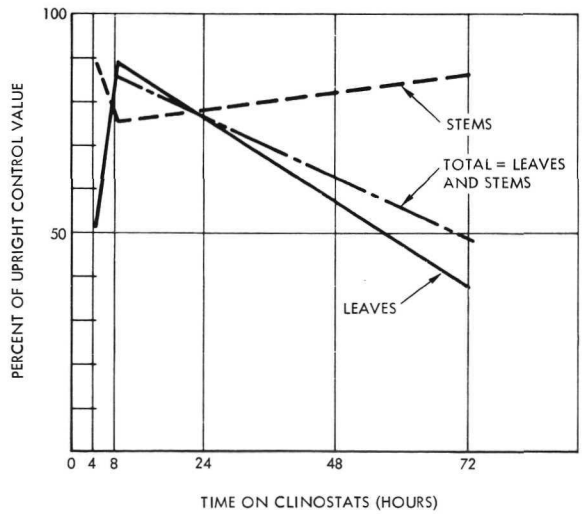


FIGURE 8.– Starch content of plants rotated on horizontal clinostats.

found at approximately 60 to 70 hours in response to geoinduction on the clinostat. The greatest difference in the starch content of the leaf also occurred at this time. Figure 8 shows also that the starch content of the stem changed only slightly. The stem began to modify the total starch ratio somewhat when the starch content of the leaves had reached its minimum equilibrium state, but the activity of the leaves appeared to dictate the shape of the total starch curve.

The foregoing assumptions can be more thoroughly validated by using the data given in figure 9, which presents the changes in soluble sugars during the same periods. These sugars, representing the mobilization products of starch, mainly sucrose, are translocated to energy-requiring areas. After the first few hours, there is more soluble sugar being carried in the stem of the horizontal plant. Within approximately 48 hours, the same relationship holds true for the leaves. By 72 hours, however, the amount of sucrose in the stems and leaves of the horizontally rotated plants exceeds that of the controls.

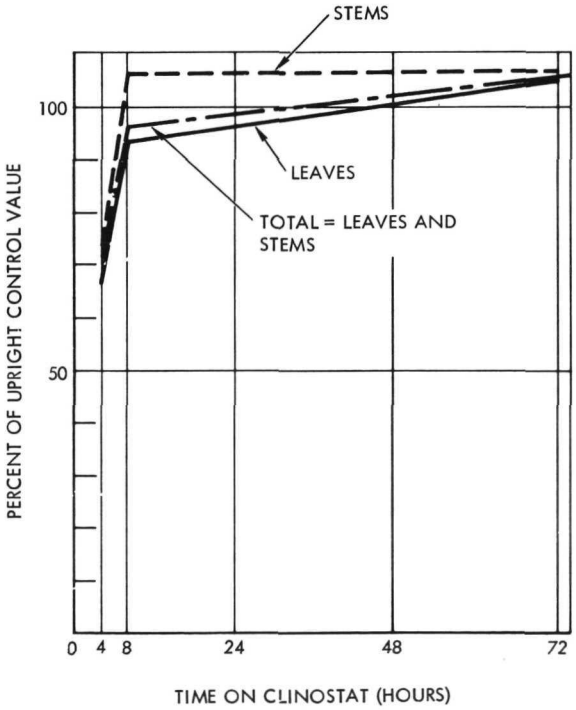


FIGURE 9.— Soluble sugar content (expressed as glucose) of plants rotated on horizontal clinostats.

The previous assumptions necessitate establishing the relationship of energy conversion with the simultaneous translocation of sugars in the closed system. This relationship can be most easily illustrated by using the ratio of total soluble sugars to the total starch present. The data presented in figure 10 indicate that the horizontally rotated plants exhibit a high glucose-to-starch ratio which is greatest at 72 hours.

The results of these tests indicate that *Capsicum annuum,* when rotated on a horizontal clinostat, exhibits a detectable shift in the balance of starch and soluble sugars which is correlated with the epinastic change in liminal angle.

Vibration Exposure

Several vibration tests were conducted to determine the effect of the flight-launch vibration profile on *Capsicum* plants. Three plants from the group were subjected to sinusoidal vibrations ranging from 10 to 2000 cps with as much as 12 *g* of acceleration. They were photographed to determine the greatest stress points in the launch profile. The frequencies of 10, 25, and 50 cps were found to give the greatest resonance.

The remaining plants were placed in four 15-plant groups, each group consisting of five plants for testing each axis of vibration. The groups were

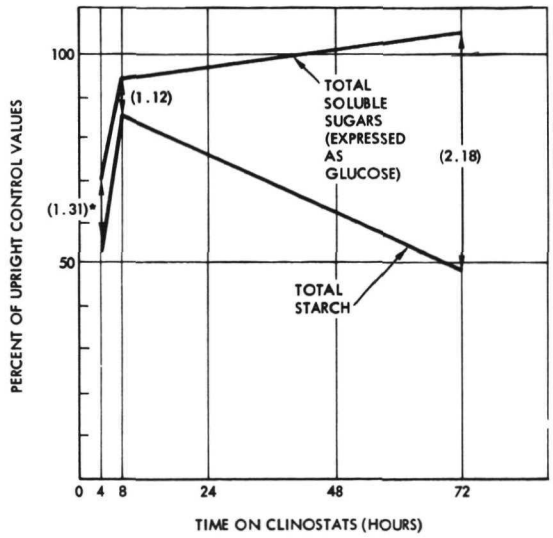


FIGURE 10.— Concentration of soluble sugar and starch in plants rotated on horizontal clinostats. Numbers in parentheses are the ratio of glucose to starch.

subject to the following conditions: Group 1, 10 cps at 2.6 *g* for 4 minutes; group 2, 25 cps at 5 *g* for 4 minutes; group 3, 50 cps at 7.5 *g* for 4 minutes; and group 4 (controls), no vibration. There was no significant difference in liminal-angle response after 24 hours on the clinostats. Later testing at 28 and 55 *g* (well beyond the launch profile stress range) did produce visible plant damage and a significant reduction in liminal-angle response.

Acoustic Tests

Capsicum plants were subjected to acoustic levels that simulated the launch and recovery flight environments to determine the effect on liminal-angle plant response. In each test, four experimental plants were mounted in a preprototype flight package and placed on a stand in an eletrodynamic sound test chamber. The plants displayed no visible damage after acoustic testing. They were then mounted on horizontal clinostats for 24 hours and then placed upright for 24 hours for recovery. Geotropic responses and recovery were not significantly modified by the acoustic environment to which the plants were exposed (table 7).

Additional tests were conducted in an electropneumatic sound test chamber to investigate the effect of higher sound levels (150 dB) on liminal-angle plant response. The plants were mounted in a test fixture and suspended in the chamber by means of a bungee cord to obtain low-frequency isolation. Ten plants were subjected to an 160-dB overall frequency peaking at 200 cps. Because of the visibly discernible excessive damage to the plants, these 10 were not placed on clinostats. A second group of 10 plants showed little visible damage; however, liminal-angle measurements taken following 24 hours on the horizontal clinostat indicated a significant reduction in response.

Flight Experiment

EPINASTIC RESPONSE

Approximately 45 hours after launch from Cape Kennedy, the capsule was recovered during parachute descent near Hawaii. It was delivered to the investigators at the Hickam Field laboratory approximately 4 hours later. After disassembly of the flight package, visual examination indicated that orbital weightlessness had produced significant leaf epinasty in all of the prime 35-day-old plants. Four of the five 25-day-old auxiliary plants exhibited marked epinasty. One plant was found to have a slight epinastic response when compared

TABLE 7. – Effect of Flight Profile Sound Levels on Liminal-Angle Plant Response

Applied noise level, dB	Frequency, cps	Duration, min	Event	Liminal angle, deg	Liminal-angle recovery after test, deg	Liminal-angle recovery, %
132±1	37.5 to 9600	10	Launch	52.5	49.3	94
145±1	37.5 to 9600	2	Recovery	43	47.5	110
Controls	None	None		39	40.3	103

TABLE 8. – Liminal-Angle Response of 25-Day-Old Plants in Biosatellite II Experiment

Leaf	Liminal-angle response, deg		
	Flight[a]	Control III clinostat[b]	Control III erect[b]
3	58.8	16.8	9.3
4	38.8	17.4	−1.3
3 and 4	48.8	17.1	4.0

[a] Average of values for 5 plants.
[b] Average of values for 3 plants.

with the petiole angles recorded at the time the plants were placed in the flight package and mounted in the capsule (table 8). The cameras in the flight and ground control units recorded the recovery of the plants for a period of 17 hours after receipt of the capsule at Hickam Field. After this time, the packages were disassembled, the plants were removed, and the film was submitted to the photographic laboratory for processing.

The prime plants were returned to the North American Rockwell laboratory for observation. They were subsequently maintained at 72±2 F temperature, 55-percent relative humidity, and continuous fluorescent illumination of 200 foot-candles. We had hoped to obtain seed produced by the flowers that were being formed while the plants were in orbit. We were unsuccessful, however; the first flowers formed abscissed, which is common in many plants. The prime flight plants were subsequently taken to the University of California, Davis, for further study and observation by Dr. Paul G. Smith of the Vegetable Crops Department. He has not observed any abnormalities in the growth and development of the plants or of their progeny.[6]

From a visual examination of the films of the control and flight plants, it was clearly discernible that the clinostat and weightlessness produced similar results. However, measurement of the petiole liminal angles of the pepper plants, when plotted by the Telereadex machine, indicated that there were slight differences between the simulated and the weightless environments on epinastic responses. The decrease in the liminal angles was plotted at 1-hour intervals throughout the experimental period (figs. 11 and 12). The data points represent the average of all four plants in each package, plus or minus the standard error of the mean. The first 44 hours of the graphs represent the period of simulated weightlessness. This is followed by a 6½-hour period, which includes the period of reentry, recovery, and transportation to the laboratory in Hawaii. The last portion of the curves presents the change in liminal angle after the packages were placed in an upright position to record the recovery of the plants.

The following comparisons may be made in considering the data. The epinastic response of plants rotated on the horizontal clinostat was less than that of those on orbit. When the epinastic responses of leaves 3 and 4 were combined, the maximum response of the plants on the clinostat was 32° (fig. 11), compared with 38° in the flight plants (fig. 12). Although the epinastic response of the control plants on the clinostat was less than that of the flight plants, they reached their maximum response sooner than those on orbit. The maximum response for the plants on the clinostat occurred between the first and seventh hour at a rate of 4.4° per hour. They reached 88 percent of maximum response by the 12th hour. The maximum response period for the spacecraft plants occurred between the second and 18th hours at a rate of 2.1° per hour. They reached 90 percent of maximum response by the 22nd hour.

The recovery of the leaves is shown in the final 17 hours of the plotted data. The flight plants showed very little recovery during the 6½-hour interval between reentry and return of the capsule to the laboratory at Hickam Field. The recovery of the flight plants at the end of the 17-hour period was significantly less than the recovery of those on the Earth control clinostats. The lack of recovery in the plants in the flight package had been tentatively attributed to the fact that the temperature of the Hickam Field laboratory fell to approximately 55 F for a period of time because of a faulty thermostat. Postflight tests programmed to simulate the Biosatellite II recovery temperature profile indicated, however, that the low temperature experienced by the plants in the flight package was not a significant factor in preventing normal recovery of the leaves. These data suggest that weightlessness produces a slower geotropic response, with a final angular change which is significantly more pronounced than that observed in plants subjected to simulated weightlessness on the horizontal clinostat.

Visual examination of the films from the flight and clinostat controls of Biosatellite II showed that the stems of some plants were bent toward leaf 3. Since all of the angles were read with a plumbline representing the original position of the stem, this bending resulted in a skewing of the angles. It increased markedly the measured curvature of petiole 3 and decreased the measured curvature of the opposite petiole.

[6] Personal communication received September 1968 from Dr. Paul G. Smith.

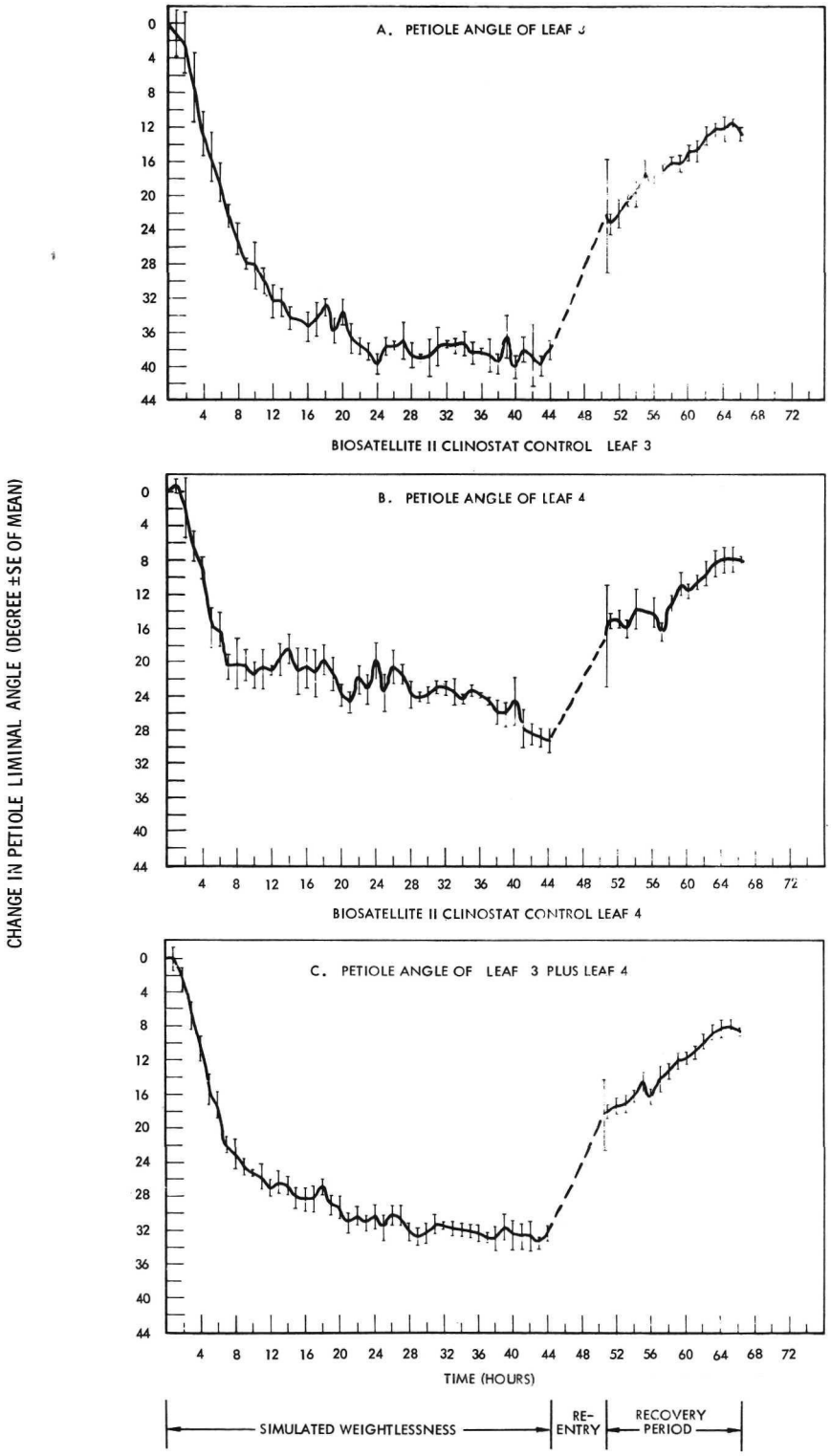


FIGURE 11.— Petiole angles of Biosatellite II checkout control.

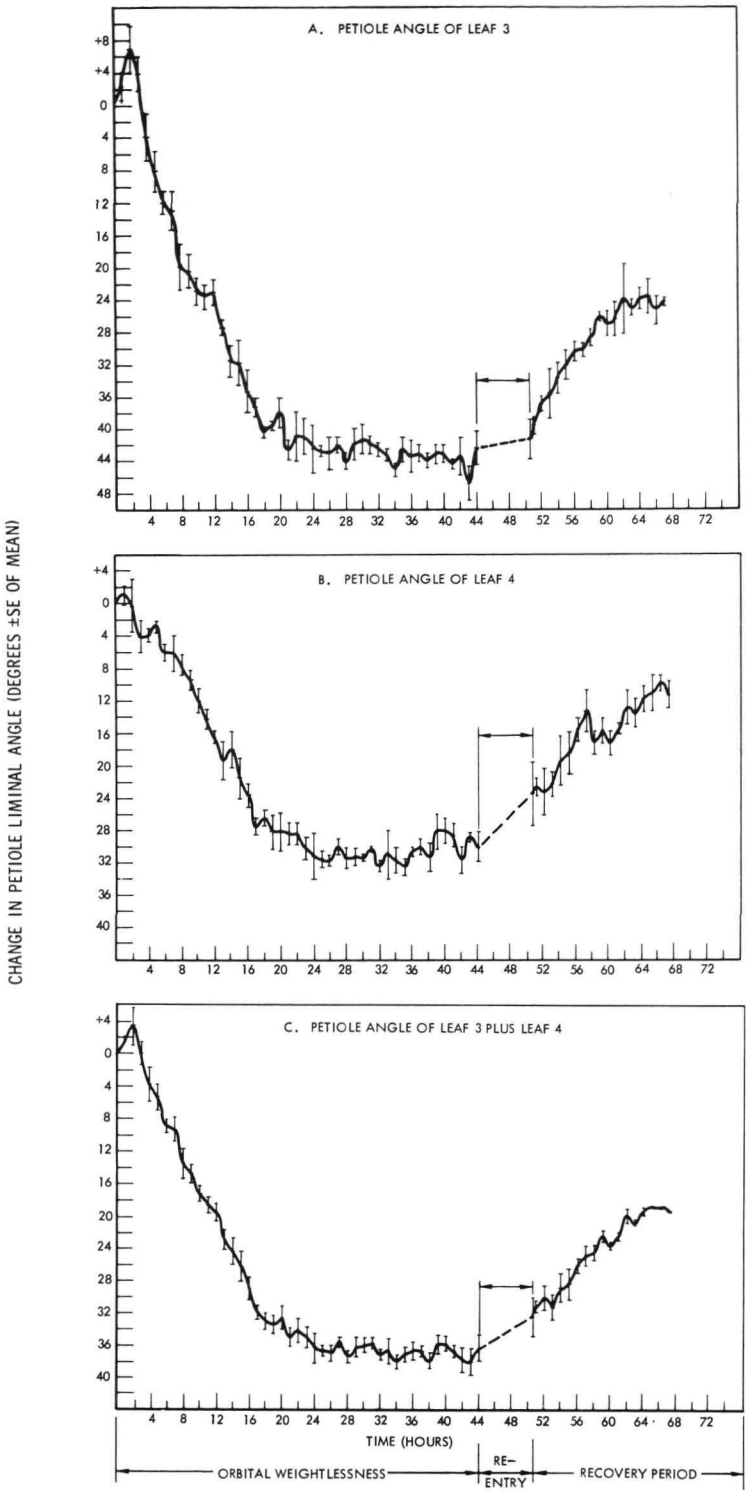
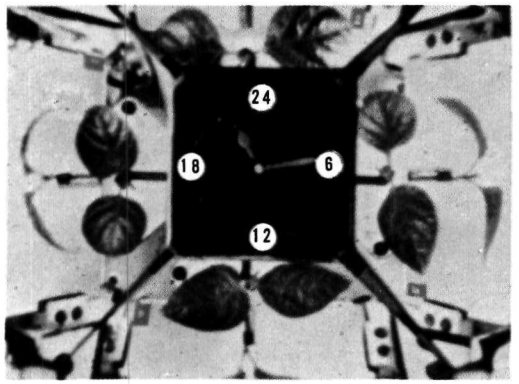


FIGURE 12.— Petiole angles of Biosatellite II flight plants.

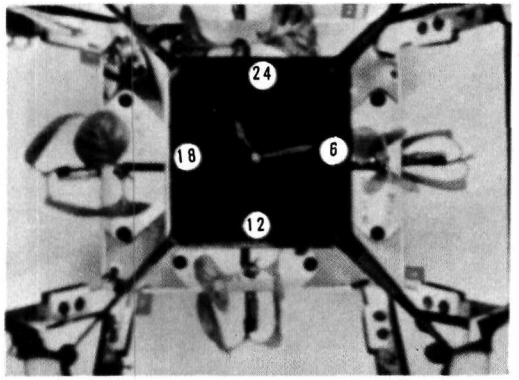
FLIGHT

1



FIRST FLIGHT PHOTO

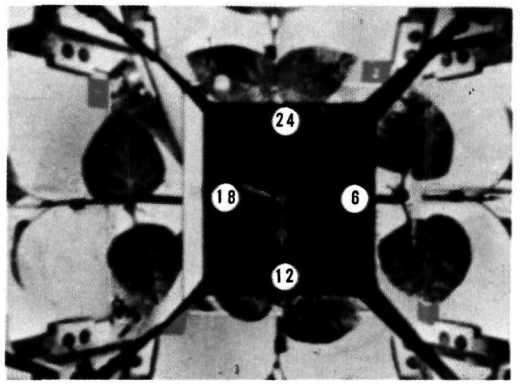
2



24 HOURS AFTER LAUNCH

CLINOSTAT CONTROL

5



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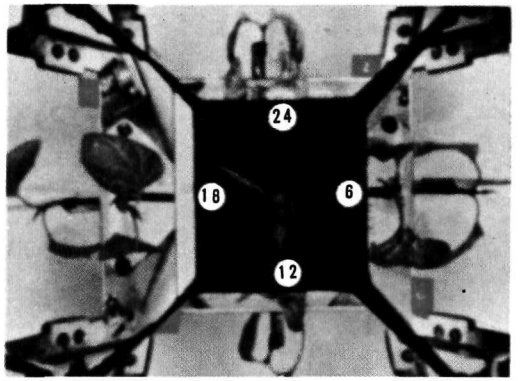
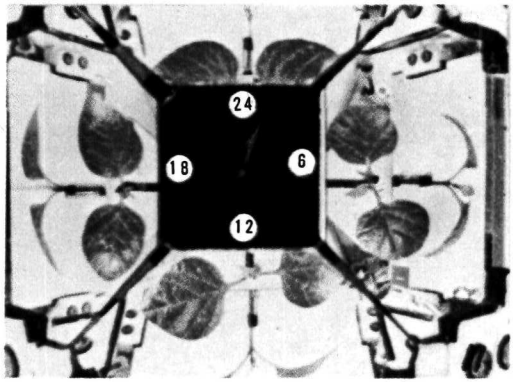


UPRIGHT CONTROL

9



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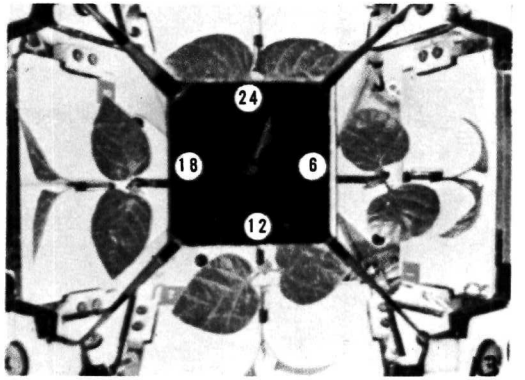

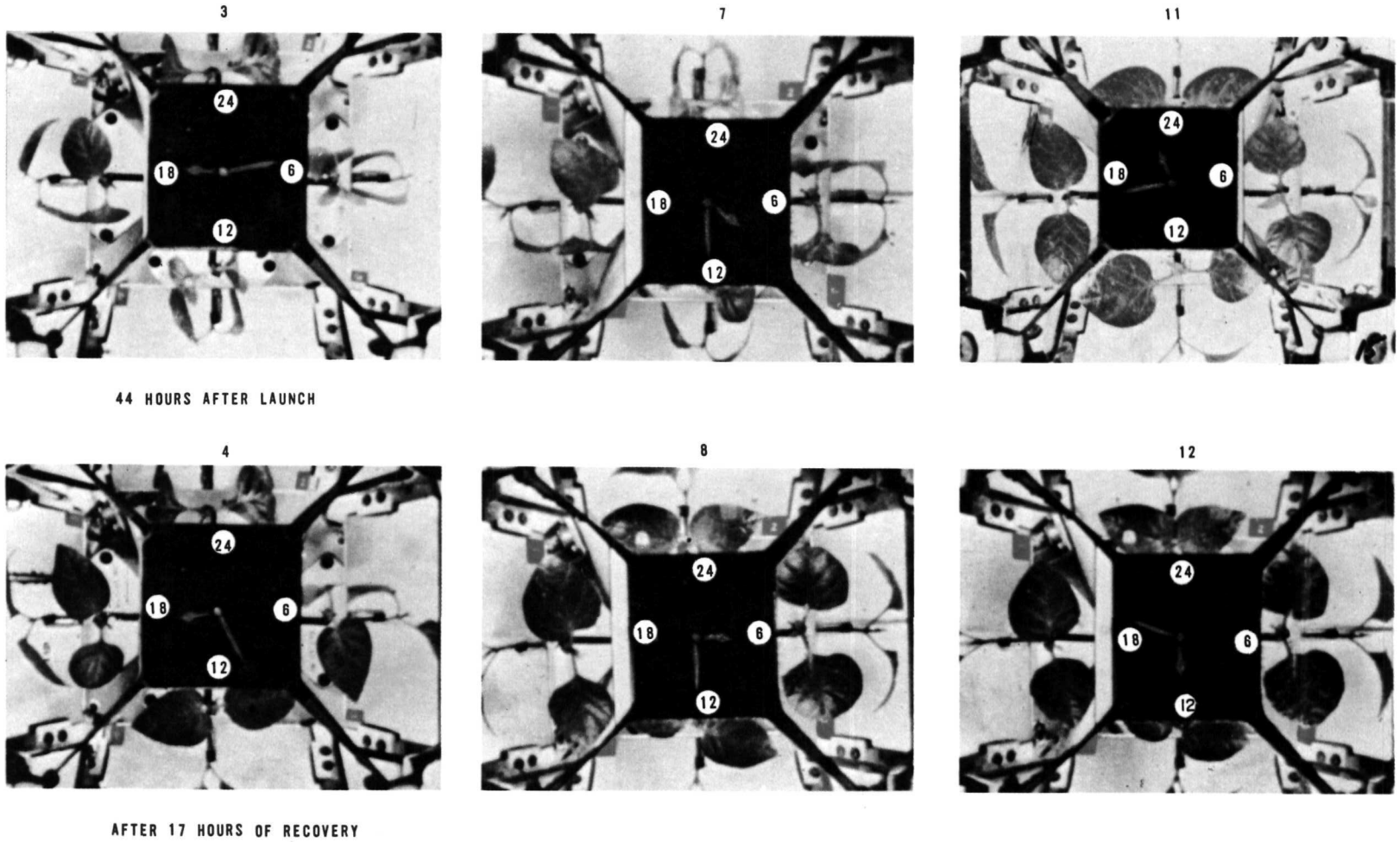

FIGURE 13.— Comparison of flight, clinostat, and upright control plants throughout experimental period of Biosatellite II.

There was a modest amount of movement in the upright controls with a minimum reduction of the liminal angle. This activity was not observed routinely, and there is no explanation available for it in this specific experiment.

Enlargements of single-frame records of the liminal-angle changes of the clinostat controls and the flight plants are presented in figure 13. It can be discerned on the enlargements that the flight plants show more acute folding of the leaves toward the stem than do the control plants. The effect of stem bending on the reduction in the liminal angles of opposing leaves can be seen in figure 13, frame 4.

Laboratory Chemical Analyses

The limited amount of experimental material is demonstrated by the total dry weight materials obtained from the various packages, as presented in table 9. The distribution of carbohydrates obtained from this material is presented in table 10. There is a 17-percent decrease in leaf starch between the $T-0$ and the upright control; this is attributable to the low light levels during the 48-hour experiment period. The reduction of approximately 50 percent of the leaf starch and the almost complete elimination of starch from the stems in the rotated clinostat controls and the flight packages are in accordance with the hypothesis that starch is reduced to sucrose to provide energy for the epinastic response. The flight and clinostat control plants had a slight increase in leaf sucrose in comparison with the upright controls; this is in accordance

TABLE 9. – Dry Weights of Pepper Plant Material Obtained From the Control and Flight Packages

Part	Dry weight, mg			
	$T-0$ control	Control III erect[a]	Control III clinostat	Flight
Leaves	31.6	16.9	27.5	25.0
Stems	2.2	1.9	3.2	4.5
Growing tips	1.4	.8	1.5	.9

[a] Total of values for three plants available.

TABLE 10. – Carbohydrate Analysis of Control and Flight Pepper Plants

Part	Carbohydrate content, mg/gm dry weight		
	Glucose	Sucrose	Starch
Leaves:			
$T-0$ control	0.10	0.47	122.0
Control III erect	.19	.58	103.5
Control III clinostat	.19	.77	53.0
Flight	.16	.61	46.2
Stems:			
$T-0$ control	1.09	1.73	.71
Control III erect	1.27	2.50	.80
Control III clinostat	1.25	10.67	Trace
Flight	1.25	7.92	Trace
Growing tips:			
$T-0$ control	2.18	Trace	Trace
Control III erect	2.00	5.71	Trace
Control III clinostat	2.94	4.55	Trace
Flight	1.33	7.69	Trace

with baseline studies. Glucose levels showed a trend toward increase in all plants used in the experiment when compared with the *T*–0 control. Care must be exercised not to attach great significance to this slight difference. These data support the thesis that mobilization and transportation of carbohydrates did occur as a result of the geotropic stimulus of orbital and simulated weightlessness.

The results of the amino acid analyses of the pepper plant leaves are presented in table 11. These data must be interpreted carefully because of the lack of adequate material for duplication of analyses. When the *T*–0 control is compared with the control III erect, there appears to be little difference in amino acid content except that glycine, phenylalanine, and valine were greatly reduced in concentration in the upright plants. This effect may be related to the reduced illumination for the latter plants. A comparison of the control III erect plants with the control III clinostat and flight plants reveals a significant increase in all the amino acids as a result of rotation on the horizontal clinostat and in response to orbital weightlessness. Comparing the control III clinostat with the flight plants reveals that a significant increase in aspartic acid, threonine plus serine, and glutamic acid resulted from exposure to weightlessness, whereas histidine and leucine were reduced to trace amounts.

Table 12 presents the concentration of amino acids found in the plant stems and growing tips. Because of the small amount of plant material, the stem and tip sections were pooled. Because of the limited resolution of the amino acid analyzer, only 7 of the 14 amino acids could be quantified. Amide interference with threonine and serine did not occur in these determinations. The general increase of amino acids previously noted in the leaves of the clinostat and flight plants did not occur in these samples. However, aspartic acid and glutamic acid increased; serine, glycine, and alanine decreased. Threonine remained unchanged.

Total nitrogen values are shown in table 13. The total nitrogen in the *T*–0 control appears to be somewhat less than that in control III and flight plants. The total nitrogen of the leaves of the flight plants follows a trend similar to that of the total amino acids. These differences may be more apparent than real in that a decrease in dry weight in the control III and flight plant leaf material may have occurred in response to a decrease in total starch content. This loss in dry weight probably occurred as a result of exposure of the plants to light levels below the photosynthetic compensation point for maintaining high levels of carbohydrate stores.

TABLE 11. – Amino Acid Analysis of Control and Flight Pepper Plant Leaves

Acid	Amino acid content, μM/g dry weight[a]			
	T–0 control	Control III erect	Control III clinostat	Flight
Aspartic acid	385.0	308.0	493.0	914.0
Threonine plus serine[b]	121.2	95.1	129.0	275.0
Glutamic acid	456.0	497.0	840.0	1318.0
Glycine	102.0	Trace	21.1	20.7
Alanine	69.8	52.0	90.8	95.6
Valine	95.2	Trace	36.7	37.5
Isoleucine	Trace	Trace	27.2	23.0
Leucine	Trace	Trace	8.5	Trace
Tyrosine	Trace	Trace	24.3	23.7
Phenylalanine	70.6	Trace	55.2	48.0
Lysine	Trace	Trace	26.5	32.4
Histidine	Trace	Trace	38.5	Trace
Arginine	Trace	Trace	28.4	33.7

[a] From one determination only.
[b] Estimated from peaks.

TABLE 12. – Amino Acid Analysis of Control and Flight Pepper Plant Stems and Tips

Acid	Amino acid content, μM/g dry weight[a]			
	$T-0$ control	Control III erect	Control III clinostat	Flight
Aspartic acid	177.0	77.4	183.0	289.0
Threonine	Trace	43.0	Trace	Trace
Serine	291.0	32.6	282.0	107.5
Glutamic acid	418.0	66.0	200.0	270.4
Glycine	114.0	117.0	134.0	48.2
Alanine	132.0	133.0	78.7	53.3
Valine	Trace	108.0	Trace	Trace

[a] The results of single determinations. (The remaining amino acids were not measurable because of limited sample size.)

TABLE 13. – Total Nitrogen Content of Control and Flight Pepper Plants

Plants	Total nitrogen content, mg/g dry weight		
	Leaves	Stems	Growing tips
$T-0$ control	42	12	25
Control III erect	46	16	27
Control III clinostat	54	19	38
Flight	68	20	36

Postflight Test

Subsequent to the flight of Biosatellite II, postflight tests were conducted to simulate the temperature, relative humidity, radiation, acceleration, and vibration environments of the flight capsule in an effort to resolve the cause for certain differences noted in the flight specimens. Four different packages were used, two upright and two horizontal clinostat packages. As programmed for the flight experiment, four 35-day-old and five 25-day-old pepper plants were inserted into each of the four packages. During the experiment, the response of the larger plants was recorded by time-lapse photography.

One upright and one horizontal clinostat package were utilized as dynamic controls. They were subjected to the vibration and acceleration profile recorded during the actual flight. All four packages were transported to the test facility, so that all plants experienced the same environmental profile, except for the programmed dynamic simulation. The packages are referred to as:

(1) Clinostat control A, no vibration or acceleration; (2) clinostat control B, subjected to the vibration and acceleration profile; (3) erect control A, no vibration or acceleration; and (4) erect control B, subjected to the vibration and acceleration profile.

After the dynamic tests were completed, all packages were placed in an environmental chamber. The camera and lights were connected, and the clinostat controls were activated for a period of 44 hours. The temperature and humidity profiles of the flight of Biosatellite II were programmed into the environmental control system. The same sequence of events that prevailed during the flight of Biosatellite II was simulated; this included orbit, reentry, return of the capsule to the investigators, and the period the plants were placed in an upright position to record the recovery of the plants in the 1-*g* field. The low temperature profile that occurred during the recovery period was programmed so as to determine the effect on total recovery.

The changes in petiole liminal angles of the pepper plants plotted by the Telereadex machine at 1-hour intervals are presented in figures 14 and 15. The presentation data points represent the average of all four plants, plus or minus the standard error of the mean. The data indicate that the vibration and acceleration profiles simulating Biosatellite II did not appreciably affect the epinastic response of the plants rotated on horizonal clinostats. The graphs show that the reduction in liminal angle proceeded at about the same rate throughout the simulated weightless period. There was a slightly greater response in the clinostat control A package plants for the first few hours of rotation, but at the end of the 44-hour period the average reduction in liminal angle for both groups of plants was approximately the same. No significant difference occurred in the response of leaves 3 and 4 of any plants in the clinostat packages. The approximately 8° difference between leaves 3 and 4 of the plants in the clinostat control A package, after 44 hours of rotation, was attributed to a slight degree of stem epinasty in two of the plants in that package.

The plants in the upright control B package exhibited significantly more movement that did the upright control A plants, which were not subjected to the dynamic environment. The lack of recovery in the plants of the Biosatellite II flight package had been attributed to the low temperatures experienced in the laboratory at Hickam, since previous clinostat work had shown that low temperatures retarded the recovery of plants. As shown in figures 14 and 15, the recovery of plants in the packages was not particularly inhibited. The plants in clinostat control A recovered to 75 percent of their original liminal-angle position, whereas those in the clinostat control B package recovered to 59 percent of the original liminal-angle position. The reduction in recovery of the plants receiving vibration and acceleration in comparison with that of the clinostat control A plants cannot be explained at this time.

It should be noted that the liminal-angle response of the plants on the clinostat in these tests did not resemble that of the clinostat controls for Biosatellite II. They did not reach a maximum angle reduction, then level out, as did the clinostat controls during Biosatellite flight experiments. Both the rate and the total degree of epinastic response were less for the plants in the postflight tests. These results may be because (1) the plants were transported from El Segundo, California, to Ames Research Center, Moffett Field, California, and (2) they were exposed to 3 days of high light intensity (1000 foot-candles) rather than to 2 days at approximately 850 foot-candles because of delay in starting the test.

DISCUSSION

Preflight and Baseline Studies

The selection of the pepper plant, *Capsicum annuum,* for the Biosatellite II experiment was based upon its size, hardiness, and reliability in responding to alterations in gravitational fields. This plant responds to rotation on the horizontal clinostat over a relatively wide range of temperatures and relative humidities. Furthermore, the period of epinastic response to rotation on the horizontal clinostat is present over plant ages between 3 and 7 weeks; and the plant demonstrates marked resistance to damage during acceleration, vibration, and other dynamic stresses. In addition to these characteristics, the pepper plant responds to topical application of auxins, carbohydrates, and amino acids. This response to chemical entities provides techniques that can be pursued in an effort to elucidate the physiologic basis for the geoinduction of movement in plants.

The hypothesis that the epinastic response of leaves during rotation on the horizontal clinostat is an energy-requiring mechanism has been verified through laboratory investigation. The exact contribution, distribution, or interplay of auxins in the responses were not pursued during these investigations.

In the study of the mechanisms of the response of plagiogeotropic organs, it was demonstrated that the rate of conversion of starch to soluble sugars was greatest during the period of most rapid change in liminal angle. This change occurred in response to horizontal rotation of the plants at a rate of 1 revolution every 10 minutes. It was also demonstrated that the movement of sugars proceeds from the stem to those leaves involved in active positional changes. In the pepper plant, these changes in liminal angle and carbohydrate migration can be demonstrated during the first 30 minutes of rotation. An additional indication of the participation of carbohydrates in leaf movement was demonstrated by the observation that subjecting plants to

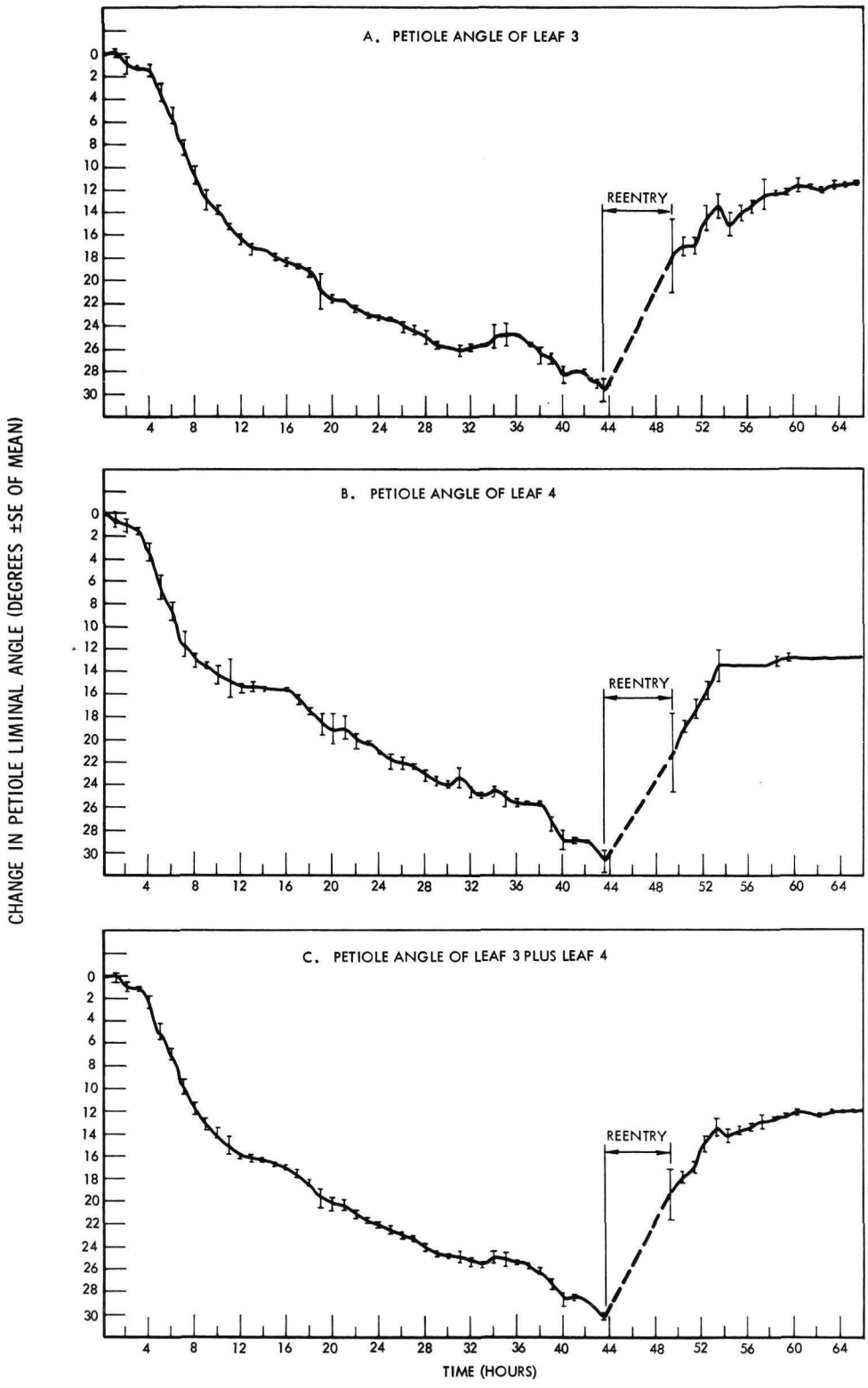

FIGURE 14.— Postflight experiment; clinostat A; dynamic controls.

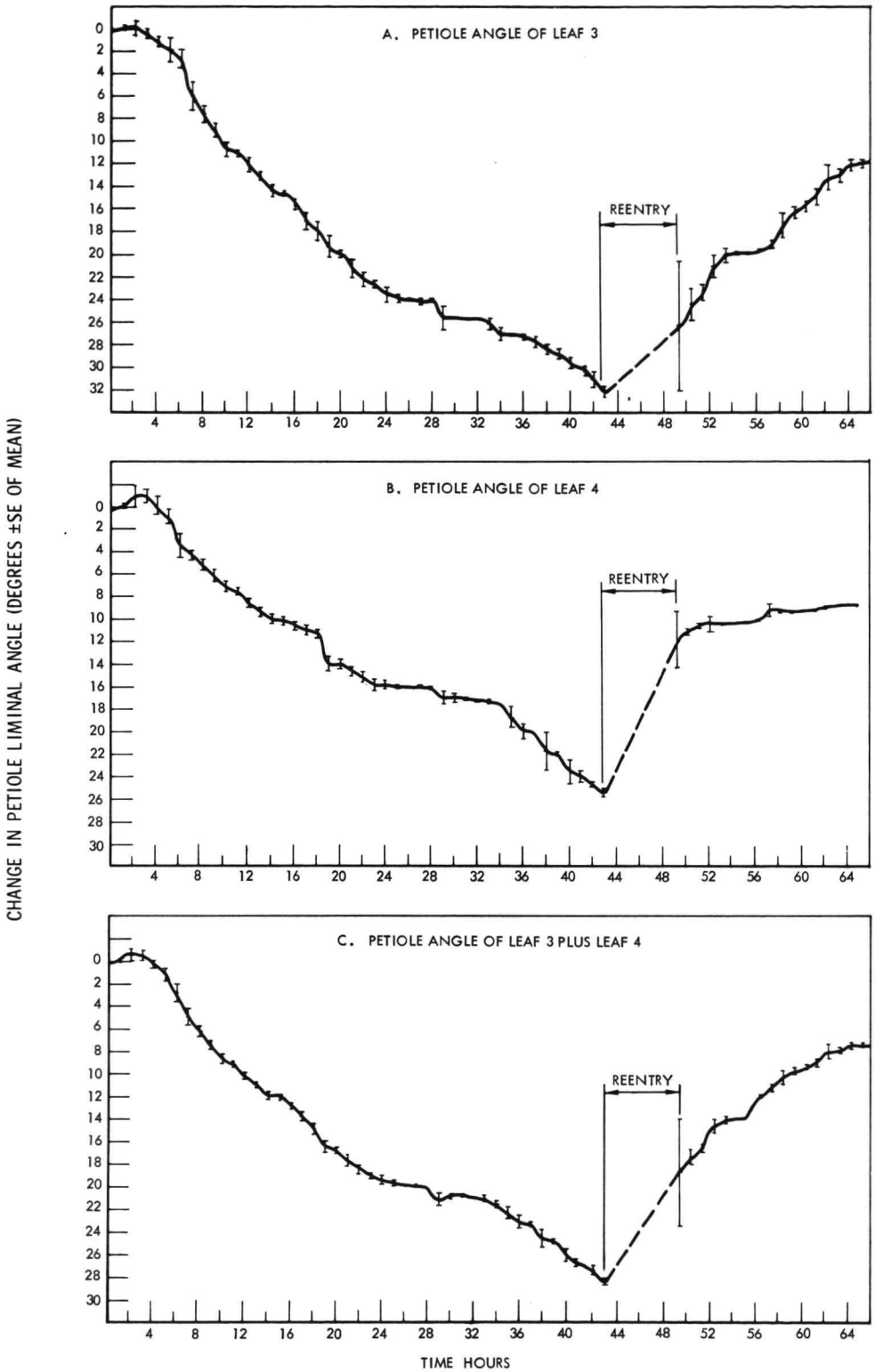

FIGURE 15.— Postflight experiment; clinostat B; dynamic controls.

varying periods of darkness resulted in a reduction of carbohydrate stores roughly proportional to the time of dark exposure. Those plants that had a reduced store of carbohydrates as a result of the dark exposure demonstrated a significant reduction in epinastic response to horizontal rotation on the clinostat. On the other hand, "loading" of the plants with carbohydrates by exposing them to high-intensity light up to 48 hours prevented, or modified, the decrease in epinastic change following prolonged darkness. However, exposure of the plants to high-intensity illumination for 72 hours inhibited the epinastic liminal-angle change. The cause of this inhibitory effect is unknown at the present time.

The exposure of the *Capsicum* plants to vibration and acoustic stresses at levels which did not result in visible plant damage did not inhibit the response of the plants to liminal-angle changes during rotation on the horizontal clinostat. However, the leaves of plants exposed to vibration levels within the ranges calculated for the Biosatellite II flight did not recover as rapidly as did those of the control plants after being returned to the upright position for several hours. This observation, which was reevaluated in the postflight tests, may bear a direct relationship to the failure of the flight plants to return to the preflight configuration following recovery of the vehicle.

Flight Experiment

The results of the flight experiment and the postflight analyses confirm the hypothesis that the response of the pepper plant subjected to the subgravity environment of an orbiting satellite would be similar to the response produced on the horizontal clinostat. Certain significant differences were observed in the liminal-angle responses as well as in the distribution of both carbohydrates and amino acids in comparing the two conditions. The changes in the liminal angles of the plants on orbit proceeded at a slower rate than in those of plants rotated on the horizontal clinostat. However, the movement of the leaves toward the stem lasted longer; the result was a greater reduction in liminal angle. The recovery of the leaves in the flight plants was not so rapid nor so complete as the recovery of the leaves on the clinostat control plants. It appears that, although the effects of weightlessness on plants can be simulated by rotation on a horizontal clinostat, slight but distinct differences occur in the geotropic responses of plants exposed to the two environments.

These differences might be attributable to the slight centrifugal forces encountered in rotation of the pepper plants on the horizontal clinostat. The maximum force exerted on the petioles was 3.42×10^{-6} g. These centrifugal forces were estimated by using the formula $F=4.024\ r/t^2$, where F is the ratio of acceleration of rotation to the acceleration of gravity, t is the time of one revolution in seconds, and r is the distance in meters from the axis of rotation to one-half the length of the leaf petiole and blade. Previous work by Finn (1962) with *Xanthium pennsylvanicum* plants has indicated that approximately 10^{-1} g of centrifugal force was required to have a significant retarding effect on the epinastic response of plants during rotation on a horizontal clinostat. More recently, Shen-Miller, Hinchman, and Gordon (1968) have shown that 10^{-2} to 10^{-3} g is the minimum threshold level for coleoptile bending response in *Avena*. Although the forces generated on the horizontal clinostat were theoretically below the threshold levels, forces of unknown magnitude and direction apparently resulted in the differences in petiolar movement noted in comparison of the control plants with the flight specimens.

The reduction in the rate and degree of recovery in the orbited plants cannot be fully explained. Previous work in this laboratory indicated that the exposure of plants to low environmental temperature retarded recovery. The flight plants were subjected to a period of low temperature at 58 F. However, the conduct of a postflight test utilizing geoinduction by the horizontal clinostat followed by exposure of the plants to low temperature during recovery did not retard the rate or degree of recovery. It was demonstrated that the exposure of the plants to vibrational levels similar to those encountered in the Biosatellite II vehicle resulted in a reduction in the degree of recovery. These results are not conclusive.

Subjecting the plants to rotation on the horizontal clinostat and to the subgravity conditions of orbital flight resulted in a modification in the distribution of carbohydrates and amino acids. It has been suggested that the starch particles suspended in the cytoplasm of the plant cells may act as georeceptors and directly influence the response of

plagiogeotropic organs. Dedolph et al. (1967) found that the distribution of starch granules in the oat coleoptile is directly related to the degree of gravity nullification on the horizontal clinostat. An even distribution of such particles, followed by enzymatic hydrolysis, could account for the reduction in starch and the increase in sucrose.

A number of workers have investigated the role of sugars in geoinduced plant organs. Warner (1928) found that, after a few hours of geoinduction on the horizontal clinostat, the content of reducing sugars was increased in the convex side of the shoot of the dahlia. A further increase in the concentration of sugars was noted in response to increasing geoinduction time. His work has been confirmed by Ziegler (1951), Metzner (1934), and Arslan and Bennet-Clark (1960). However, Warner is of the opinion that the difference in sugar content is a reaction of the plant that is not directly related to the chain of events responsible for the geotropic curvature. Anker (1962) is of the opinion that the increased sugar content of curving plant organs is a result of hydrolysis of starch induced by indole-3-acetic acid and that the hydrolysate is incorporated into cell-wall substances.

The results of the studies presented in this report indicate that carbohydrates and, to some extent, amino acids play a direct role in the response of plants to geoinduction. The carbohydrates presumably provide energy for the accelerated growth and/or elongation of the cells along the convex curvature of the plagiogeotropic organ, thereby resulting in a decrease in the liminal angle.

SUMMARY AND CONCLUSIONS

The effects of rotation on the horizontal clinostat to simulate weightlessness and an orbital flight time of 45 hours were evaluated on 25- and 35-day-old pepper plants, *Capsicum annuum*. It was found that the subgravity condition of 10^{-5} *g* encountered during orbital flight produced reductions in the liminal angle of the petiole with the stem; this was very similar to that produced in the laboratory with the use of the horizontal clinostat. Further, the distribution of carbohydrates and amino acids was altered in the same direction. The carbohydrates were similar in concentration in the control and orbital plants, but the amino acid change was greater in magnitude in the orbital plants. The following differences were observed in addition:

(1) The rate of liminal-angle change was slower in the orbited plants than in Earth control clinostat plants.

(2) The duration of the liminal-angle change in the flight plants was more prolonged than in the clinostat controls, thereby resulting in a greater reduction in angles.

(3) The recovery of the orbital plants toward the preflight liminal angles was not so complete as that observed in the Earth control plants. It was originally postulated that this reaction was due in part to low temperatures experienced in the Hickam Field laboratory. Postflight evaluations, however, indicate that the reduction in degree of recovery may be related to the dynamic environment of launch, especially the vibration levels.

(4) Postflight growth of orbited pepper plants and the cultivation of seed from these plants have not revealed any residual effect of the radiation or weightless environment.

On the basis of these data, it was concluded that the effects of weightlessness are simulated to a remarkable degree by the horizontal clinostat. Nevertheless, additional evaluation of the plagiogeotropic response to weightlessness should be evaluated in future spaceflights, and laboratory investigation of the phenomena should be continued.

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Experiments With Cellular Systems

The Effect of Weightlessness on the Dividing Eggs of *Rana pipiens*

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The developing frog egg exhibits a marked sensitivity to disorientation with respect to the normal gravity vector. The most comprehensive studies in this area, perhaps most closely associated with the early work of Schultze (1894), were those of Penners and Schleip (1928).

Upon fertilization of the egg a gravity gradient is formed along the animal-vegetal axis which brings the heavy, light colored yolk mass lowermost. This "rotation of orientation" occurs without the involvement of the overlying jelly capsule. The symmetry of the embryo is determined by this gradient. Ancel and Vintemberger (1948) have described the effectiveness of fertilization, orientation, and gravity in symmetry determination.

Abnormalities in development, the best studied being that of twinning, can be produced by constraining the egg with vegetal pole uppermost. It has been shown (Schultze, 1894; Penners and Schleip, 1928) that maximum proportions of monsters have been induced by initiating inversion over the early first cell division and through the eight-cell stage.

It was thought that the state of weightlessness achieved by orbital flight might confuse the formation of this gradient early in development and affect differentiation processes. As a corollary, the value of a zero *g* field as a tool of biologic science could be investigated.

EXPERIMENT DESIGN AND METHODS

The eggs of *Rana pipiens,* the grass or leopard frog, were chosen as the experiment material. Normally, these are only seasonally available. In consideration of a seasonally uncertain flight schedule, it became important to devise a method of maintaining the embryologic usefulness of frogs over the year. The technique involved mature frogs at low temperature in a physiologically compatible medium complemented by periodic antibiotic treatment.[1]

The originally conceived experiment was one in which frog eggs were to be fertilized in space. However, by the time of hardware design "freezing," we had not yet achieved an acceptable degree of reliability in sustaining egg fertilizability over the expected launch-pad hold time. It was necessary, therefore, to design an experiment demanding fertilization before launch. This compromise was compatible with the experimental findings, noted above, of gravity sensitivity at the two-cell stage since ground-based studies showed that, after fertilization, maintenance at 42.5 F would adequately inhibit the first division, holding it in check for 14 to 15 hours. The sequence of prelaunch events enforced upon this experiment a 12- to 13-hour hold time, less than that possible but always a source of concern. Temperatures lower than 42.5 F further inhibited division but quite often led to abnormal development, also always a source of concern. Higher temperatures, of course, permitted earlier cleavage.

The effect of weightlessness on the early cleavage planes, on the critical stage of gastrulation, and on

[1] Paper by Tremor now in preparation.

axial development would be of interest. Experiments performed at the specified spacecraft temperature (70±5 F) yielded the time sequence of these stages. The experimental requirements then conditioned the design and use of the supporting hardware.

Frogs, both male and female, were carried from the home laboratory (NASA Ames Research Center) in styrofoam buckets kept cold with frozen ice packs. They were then held at 42.5 F in the Cape Kennedy laboratories until use. Beginning at 3 days before launch, female frogs were injected at 12-hour intervals with 3 to 5 pituitary glands each to induce ovulation. A total of 60 frogs were so injected and maintained at room temperature.

Ripe females were selected at T—24 hours and test fertilizations were made. Over the next 8 hours developing embryos were observed for fertilization and normality of development. From these, two donor frogs were chosen for use in the flight experiment and as Earth controls. Alternate frogs were also identified.

At T—12.5 hours, stripped eggs were exposed for 15 minutes at room temperature to sperm from macerated testes (2 testes/5 cc spring water) and divided into clusters of five. With egg rotation as the criterion of fertilization (about 30 to 40 minutes after initial sperm closure), they were removed in petri plates to the cold room (42.5 F) where all subsequent operations were performed for the reasons noted above.

The hardware (fig. 1) consisted of a package of 16 acrylic modules. Each module is divided into two chambers: a 10-ml egg chamber and a 4-ml fixative chamber. The O-ring-fitted piston separating the chambers was spring loaded and actuable, by module pairs, by program or command. Actuation effected forceful mixing of fixative and egg medium (oxygenated spring water).

A coolant line around the package, with appropriate insulation, was capable of maintaining the experiment at 42.5 F on the launch pad. Temperature readout was effected through four thermistors attached to each of four modules in the package; these read egg-chamber temperatures directly. A fifth thermistor served in switching off strip heaters thermostatically which, immediately postlaunch, were to raise the package temperature from 42.5 to 70 F. At this point, experiment temperature control was to become dependent on ambient spacecraft temperatures.

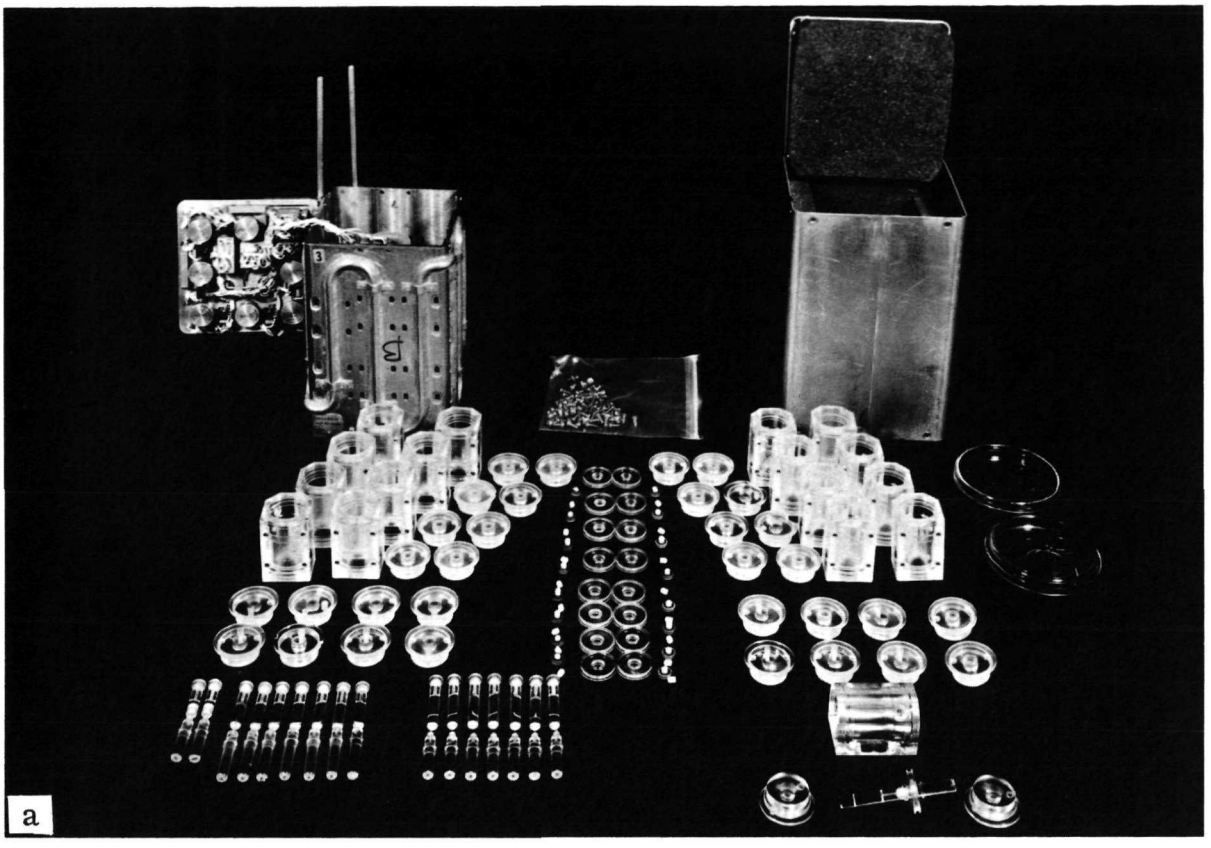
The fertilized eggs were placed in groups of 10 in each of the first eight modules to be actuated, and in groups of five in each of the remaining. Each one of the modules of a pair received the eggs from but one of the two donor frogs. The glutaraldehyde fixative, in osmotically conditioned (sucrose) Sorensen phosphate buffer, yielded after mixing a final concentration of 2.5 percent glutaraldehyde.

The experimental design called for actuations of module pairs at T—0, T+1 hour, T+2 hours, T+3 hours (early cleavage), T+32 hours, T+40 hours (late gastrula), and T+68 hours (tail-bud stage). The last module pair was to return with live embryos.

Three hardware packages were loaded: One for flight; one as a backup for the flight package; and the last as an Earth control. In addition to petri-plate controls, there were three principal groups of controls, all of which were concerned with the loading and actuations of package complement. These were designated controls I, II, and III.

Control I consisted of a fully loaded package placed with all other experiments in an Earth configuration of the flight capsule and its components. This "buttoned-up" capsule was located in an isolated air-conditioned room with a temperature of 70 F and 70 percent relative humidity (the specified nominal spacecraft temperature and humidity). All actuations were to occur at or near the times of flight actuations. Control II consisted of 16 modules, unassembled in hardware, placed in a 70 F constant-temperature box. These were to be actuated manually at or near flight actuation time. Control III was designed to follow the flight temperature and actuation sequence by 4 hours. Sixteen modules, unassembled in hardware and placed in the appropriately controlled box, were to be actuated accordingly. The eggs were fertilized 4 hours later than were the flight and other control eggs.

Once successful launch was accomplished, the flight backup package was to serve as a transportation control, a control suggested primarily by the death soon after recovery of embryos returned by the Gemini 12 mission (Young and Tremor, 1967). The cause of these deaths was never definitely determined. This package then was to be maintained at room temperature with actuations made at or



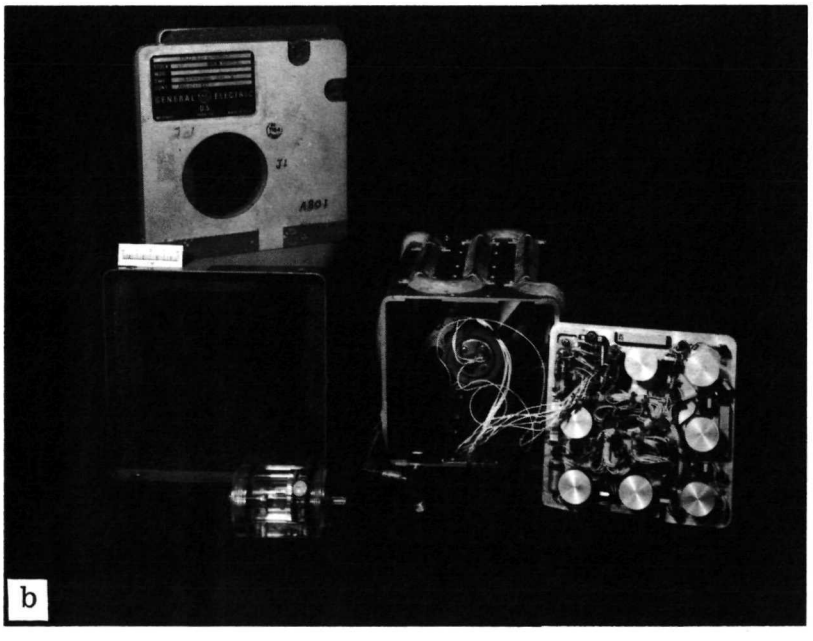


FIGURE 1.—Frog egg hardware. (*a*) Disassembled hardware showing individual modules. (*b*) Actuating and thermal control mechanism.

near flight actuations and transported by air to the recovery site shortly after spacecraft reentry. There it was to be treated as was the flight package as closely in time and method as possible, with special attention paid to the treatment of the live embryos.

Although few background studies had been performed on the effect of long-term centrifugation, it was thought that the implementation of such an additional control might be of value. Accordingly, six modules were loaded with five eggs each, with spring water in the fixative chambers, and mounted on a 24-inch-diameter centrifuge. These eggs were selected from the same batch as those used in control III, and the same time schedule was followed. At T—24 hours, the centrifuge with the modules, which had been static until that time, was removed from 42.5 to 70 F and set in operation. Rotation was controlled so as to yield a relatively low level 3-*g* load, a level selected in consideration of work in this laboratory (Young et al., 1970) which seemed to effect some minimum response in abnormalities with chronic exposure. It was reasoned that possible microscopic changes induced by this relatively mild centrifugation stress might be more comparable to effects induced by weightlessness. The modules were to be disassembled for observation and preparation of the embryos at the end of flight.

RESULTS

At the recovery site, specimens were removed, observed for development, and photographed. Control experiments at Cape Kennedy were treated in a like manner, as closely in time as possible to the recovery operations. Four of the ten unfixed embryos in each hardware package were immediately fixed in 2.5 percent glutaraldehyde. The

TABLE 1. — Comparative Development of Frog Embryos From Biosatellite II

[Numbers in top row are days of September 1967; numbers in second row are times of actuation (fixation) indicated by "Zulu" (Z) time (Greenwich mean time); numbers in third row are stages: St 3, 2 cells; St 4, 4 cells; St 5, 8 cells; St 6, 16 cells; St 12, late gastrula; St 13, neural plate; St 14, neural folds; S5 15, rotation; St 16, neural tube; St 17, tail bud; and St 18, muscular response]

Actuations	Flight	Control III	Control II	Control I	Backup
1	7 2200Z St 3	8 0204Z St 3	7 2210Z St 3	7 2210Z St 3	7 2210Z St 3
2	7 2304Z St 3 late	8 0304Z St 3–4	7 2305Z St 3–4	7 2305Z St 3	7 2305Z St 4 early
3	8 0003Z St 4	8 040Z St 4	8 0005Z St 4–5	8 0005Z St 4	8 0005Z St 4 late
4	8 0104Z St 6	8 0504Z St 5–6	8 0105Z St 5–6	8 0105Z St 4–5	8 0105Z St 5–6
5	9 0635Z St 12	9 1025Z St late 12	9 0628Z St 13	9 0628Z St 13	9 0628Z St 13
6	9 1345Z St 13	9 1810Z St late 13	9 1345Z St 14	9 1345Z St late 13	9 1345Z St 13
7	9 1745Z St late 13	9 2137Z St 14	9 1740Z St 15–16	9 1740Z St 14–15	9 1740Z St late
Nonfixed (alive)	10 [a]0100Z St 15	10 [a]0210Z St 15	9 [a]2330Z St 16–17	10 [a]0100Z St 16-17	10 [a]0800Z St 18

[a] Time of observation.

other living embryos were placed in fresh spring water in an attempt to raise them to maturity.

The results reported here are essentially those published in a previous paper (Young and Tremor, observed for development, and photographed. 1968) but supplemented with experimental data gathered from clinostat studies performed after flight. Temperature curves for flight and control hardware are presented in figure 2. Table 1 summarizes the stages of development reached in flight, Earth control, and backup hardware as influenced by these temperatures and actuation times. Backup package temperatures (fig. 2) were conditioned by a room temperature of 69 F during the 2 days of flight. During transportation from Cape Kennedy to Hawaii, the package temperature peaked at 77 F. The numbered developmental stages (St) are those described by Shumway (1940). Flight package temperatures from separation through recovery, as interpreted from the onboard tape recorder, were a fairly constant 67 F.

With launch occurring 3 hours later than scheduled, the fertilized eggs were held for some 15.5 hours at 42.5 F before exposure to weightlessness. This time was sufficient for the appearance of mid-first cleavage (St 3) on the launch pad. Thus, the stage of development experimentally shown to be most sensitive to the initiation of disorientation (inversion) could not be studied as affected by

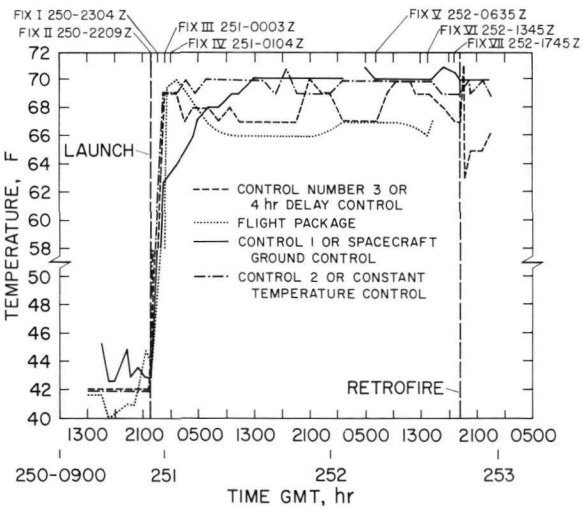


FIGURE 2.— Frog egg experiment; Biosatellite II flight and Earth control temperatures. Times of actuations are given along top margin by day of year (250 is Sept. 7, 1967) and Z-time (Z is "Zulu," Greenwich mean time).

exposure to reduced values of *g*. However, complete first cleavage, as observed in the eggs fixed by the second actuation, was delayed beyond 1 hour postlaunch.

It is evident from table 1 and other information presented above that differences in development stage, when they occur, can be attributable to temperature differences and/or time of observation in the case of the embryos returned alive. An extreme case of the effect of these variables on development observations is that of the stages reached by the flight embryos returned alive, as compared with those reached by living embryos of the backup package. Here the comparatively early stage (St 15) recorded for the flight embryos is accountable to the lower temperature of flight and early time of observation, whereas the later stage (St 18) reached by live embryos from the backup hardware is attributable to higher temperature and later observation.

Table 2 summarizes the quantity and quality of development of the flight, backup, and Earth control embryos. Abnormalities may be any of a wide variety, including asymmetric cleavage planes, localized cytolysis, edema, and exogastrulation. No differences were observed in the types of such abnormalities between flight and Earth control eggs; they all fell well within the range of expected abnormal development encountered in this kind of embryologic study. Although controls I to III were first reported as yielding lower normality rates, a critical reappraisal indicated that these differences were attributable to a difference in criteria applied by observers at Cape Kennedy and in Hawaii. The revised figures show the flight, backup, and control embryos to be essentially the same in normality and rate of development. The number of embryos flown was insufficient to attach significance to the higher rate of abnormality encountered in the flight specimens. It should be mentioned, however, that these three abnormalities were all of the same type, i.e., a collarlike abnormality appearing in three eggs from the same donor frog (H) fixed in stage 12 (fig. 3). The eggs from the other frog (E) fixed later in this same stage did not show this. Further, embryos fixed later or those returned alive showed no evidence of abnormality.

Although this particular anomaly had not been observed in the laboratory before, a similar ringlike abnormality appearing circumferentially in the

TABLE 2. – Frog-Egg Development from Biosatellite II

Program	Type of embryo			Total number of eggs
	Normal	Abnormal	Underveloped	
Flight	114	3	3	120
	95%	2.5%	2.5%	
Backup	116	1	3	120
	96.7%	0.8%	2.5%	
Control I	117	1	2	120
	97.5%	0.8%	1.7%	
Control II	117	1	2	120
	97.5%	0.8%	1.7%	
Control III	118	0	2	120
	98.3%	0.0%	1.7%	
Control mean	97.8%	0.5%	1.7%	

gastrula stage has been occasionally experienced. This has disappeared, leaving no trace, with further development. It is possible that spaceflight contributed, perhaps with other factors, to the production of this transient, minor abnormality. (Embryos in control III donated by the same frog (H) did not show this abnormality when fixed in a late stage 12.)

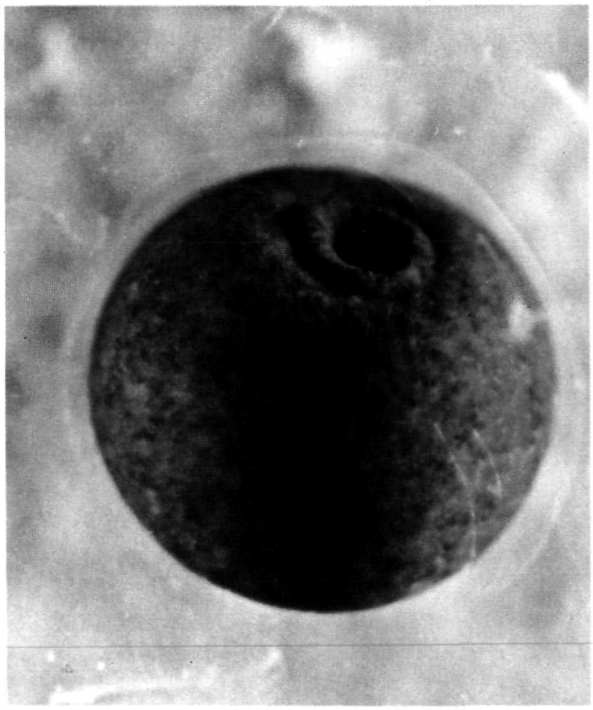


FIGURE 3.– Flight egg with collarlike abnormality; stage 12, fixed at approximately T+32 hours; frog H.

The fixed embryos were either imbedded in Epon for electron microscopy or preserved in 70 percent alcohol for light microscopy. The latter specimens proved to be generally unsuitable for light microscopy. They shattered upon sectioning, apparently because of the comparatively long period of exposure to 2.5 percent glutaraldehyde solution in the hardware. This problem was not unanticipated. Experimentation had shown that exposure to 0.5 percent glutaraldehyde fixation over the anticipated flight time was optimum because concentrations over this lead to brittleness. On the other hand, it was found that 2.5 percent glutaraldehyde solution was best for electron microscopy. Since light-microscope sections of frog eggs flown on previous Gemini flights showed no effects of weightlessness (Young and Tremor, 1967), it was thought advisable to look closely at ultrastructure where perhaps more subtle differences in morphology might be seen if they existed. Therefore, the higher concentration of fixative was chosen.

It was found that very good light-microscope sections (figs. 4 to 12) could be prepared from the plastic-embedded specimens by use of an ammoniac silver hydroxide and formalin stain.[2] Early in egg development in both control and flight specimens, yolk granules are distributed normally; i.e., the larger, more abundant granules are located in the vegetal end. Weightlessness had no apparent effect on the distribution of the granules, and certainly not the extreme effect that inversion has over a

[2] Paper by Corbett now in preparation.

comparable period of time when applied at the same early cleavage stage (Penners and Schleip, 1928). Transverse sections through embryos in later stages of development exhibit no differences between flight and Earth control organisms. Mitotic figures, as delineated by sectioning, appear normal.

Electron micrographs show that the number and width of cristae in mitochondria of flight embryos compare with those of the Earth controls. Flight embryo yolk platelets compare in structure and size with Earth control platelets and those reported elsewhere (Karasaki, 1963); i.e., they exhibit a hexagonal crystalline lattice structure with a periodicity within the net of about 70 Å. Yolk platelets were often found in association with mitochondria in all specimens of these stages (St 15, 16) observed, in confirmation of the reports of others (Karasaki, 1963; Deuchar, 1966). Nuclei and cell membranes are comparable throughout the specimens studied. In short, there appear no ultrastructural differences between the flight and control embryos (figs. 4 to 12).

The live embryos returned from flight and those sampled from the Earth control hardware were maintained, in some instances, up to 4.5 months postflight. They were fed boiled lettuce two to three times a week and kept in spring water at 64.5 F. Losses occurred gradually over this period of time with most deaths occurring at the beginning of metamorphosis. Some reached the forelimb, tail-regeneration stage (figs. 13 to 16). Controls developed and died at a rate indistinguishable from that of the flight embryos. We attribute this inability to reach complete metamorphosis to both the difficulty generally encountered in rearing *Rana pipiens* and our relative inexperience in the effort involved. It should be observed, also, that the continued culture of the organisms was never a primary intention of this or previous studies.

DISCUSSION

The biologic material selected was excellent and uncompromised by the exhaustively tested hardware. Temperatures were maintained at an optimum throughout the flight. The actuations occurred as programmed, except for the last, which was commanded earlier because of the early calldown of the spacecraft. The controls too were prepared and maintained optimally.

As noted above, classic experimentation has demonstrated a high developmental sensitivity (in formation of duplications) to the inversion of the frog egg at the beginning of first cleavage. Sensitivity to the initiation of such exposure decreased through the eight-cell stage (table 3). Seventeen of the 20 eggs fixed on the launch pad were in the middle of stage 3 (the other three were abnormal or underdeveloped); the 20 fixed 1 hour later, with somewhat less than an hour's exposure to weightlessness, were in late stage 3. It would seem that this progress of development, as indicated in table 2, would provide an adequate test if indeed weightlessness were analogous to inversion in its effect. Penners and Schleip (1928) used *Rana fusca* as did Schultze (1894). Indeed, Schultze claimed that inversion after the two-cell stage was ineffective in producing duplications. Others, using different amphibians as well as *R. fusca,* were sometimes unsuccessful in producing duplications in attempts to repeat these experiments. *Rana pipiens* has been exploited comparatively little in this field. Much work remains to be done in exploring the sensitivity of *R. pipiens* to gravity.

TABLE 3. – Results of Inversion Experiments With *Rana fusca* at Different Cleavage Stages

[Penners and Schleip, 1928]

Cleavage stage at which egg was pressed and inverted	Number of normal single embryos	Number of double formations
Second cleavage at the beginning	34	13
Second cleavage encompassing half the egg	40	4
Second cleavage almost or completely finished	76	5
Third cleavage at the beginning	8	4
Third cleavage just finished	6	1
16-cell stage	35	0

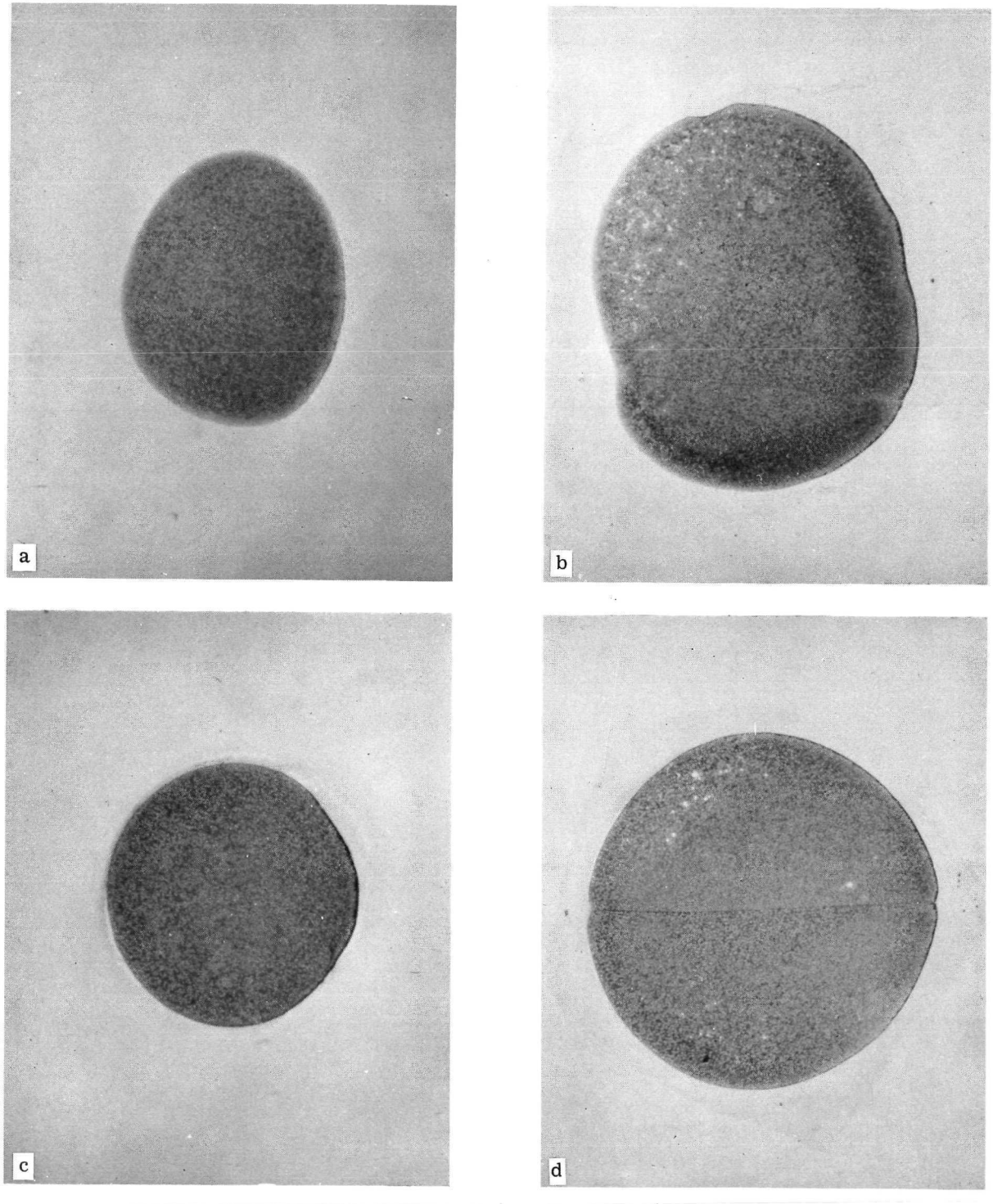


FIGURE 4.— Photomicrographs of early cleavage showing yolk platelet (small light yellow ovoids) polarity, animal pole (pigmented) to right. (*a*) Flight egg; stage 3; sectioned parallel to first cleavage plane; 188 ×. (*b*) Flight egg; stage 4; sectioned obliquely through one cleavage plane; 188 ×. (*c*) Control III egg; stage 3; sectioned parallel to first cleavage plane; 188 ×. (*d*) Control III egg; stage 4; sectioned parallel to one cleavage plane; 188 ×.

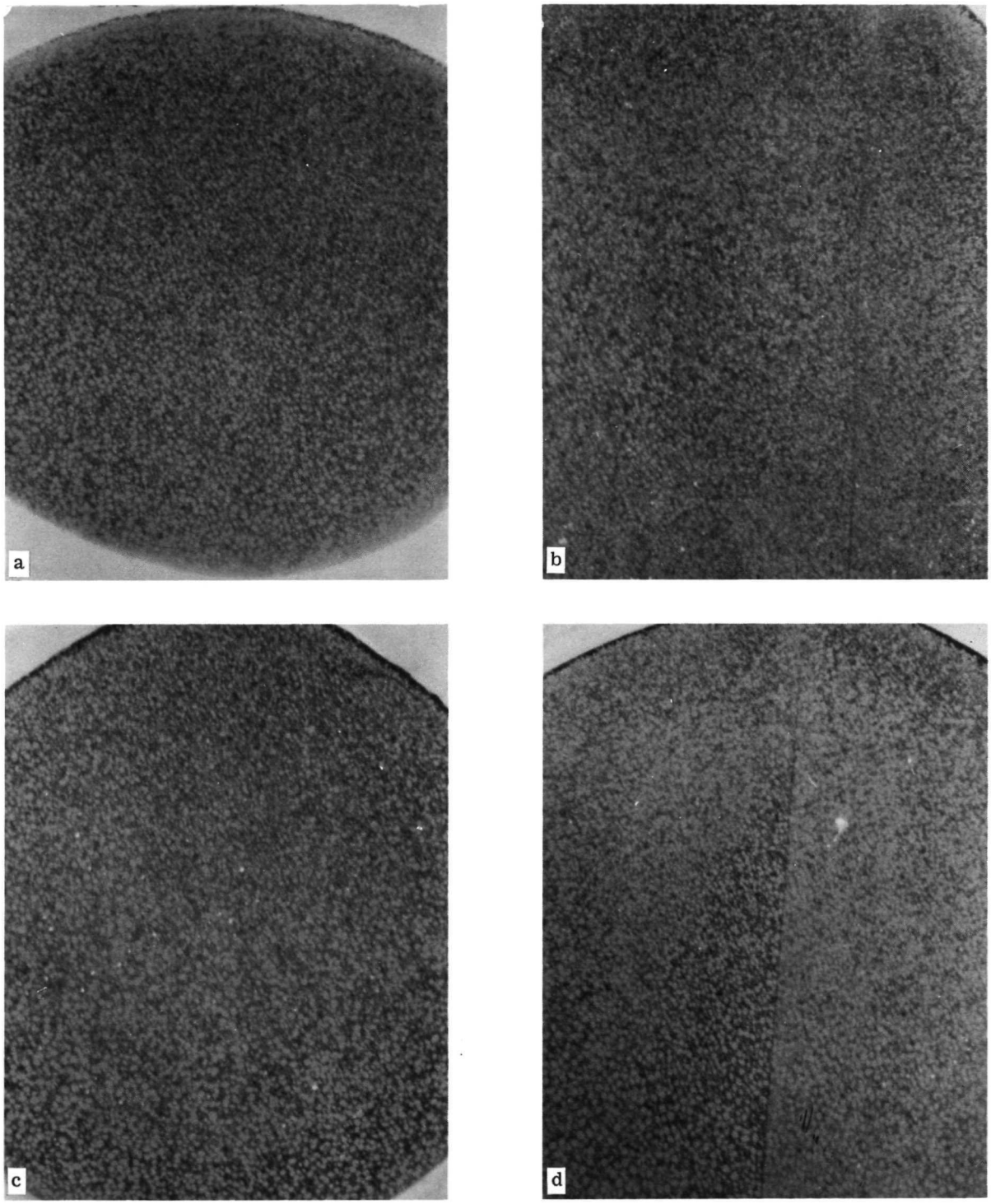


FIGURE 5.— Highly magnified views of specimens shown in figure 4; 470 ×. (*a*) Flight egg; stage 3; specimen shown in figure 4 (*a*). (*b*) Control III egg; stage 3; specimen shown in figure 4 (*c*). (*c*) Flight egg; stage 4; specimen shown in figure 4 (*b*). (*d*) Control III egg; stage 4; specimen shown in figure 4 (*d*).

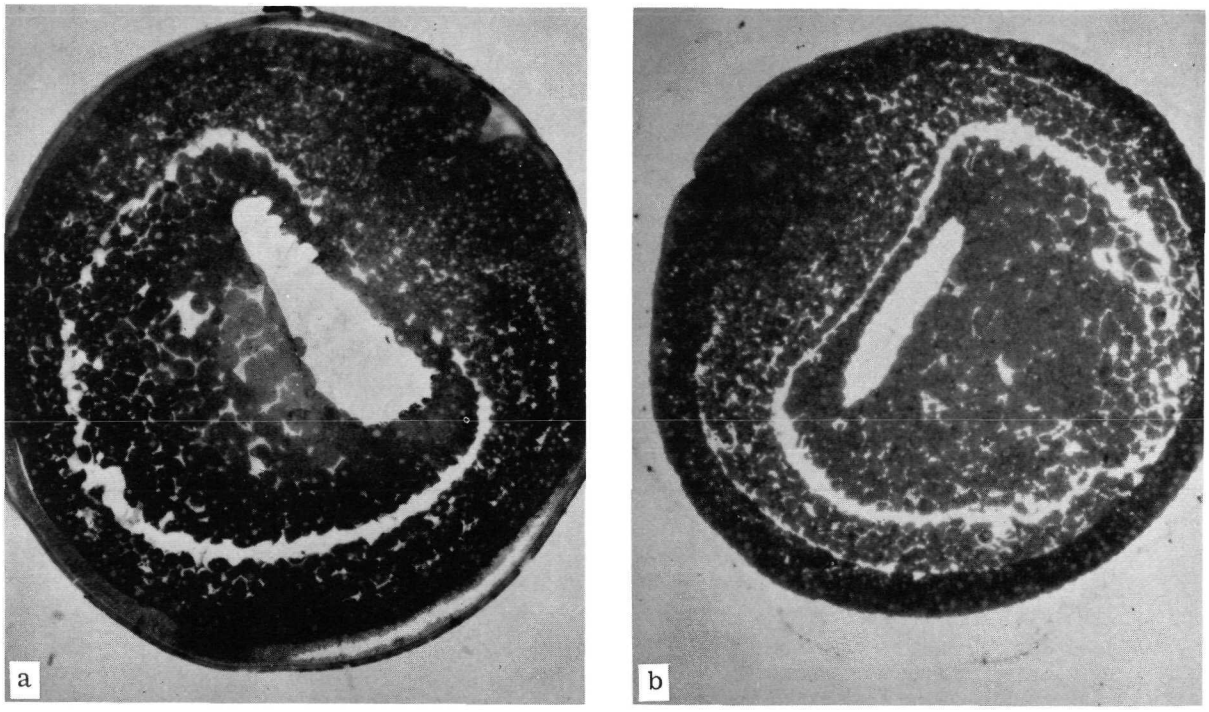


FIGURE 6.— Photomicrographs of stage 13. (*a*) Flight embryo; transverse section; 173 ×. (*b*) Control III embryo; transverse section; 173 ×.

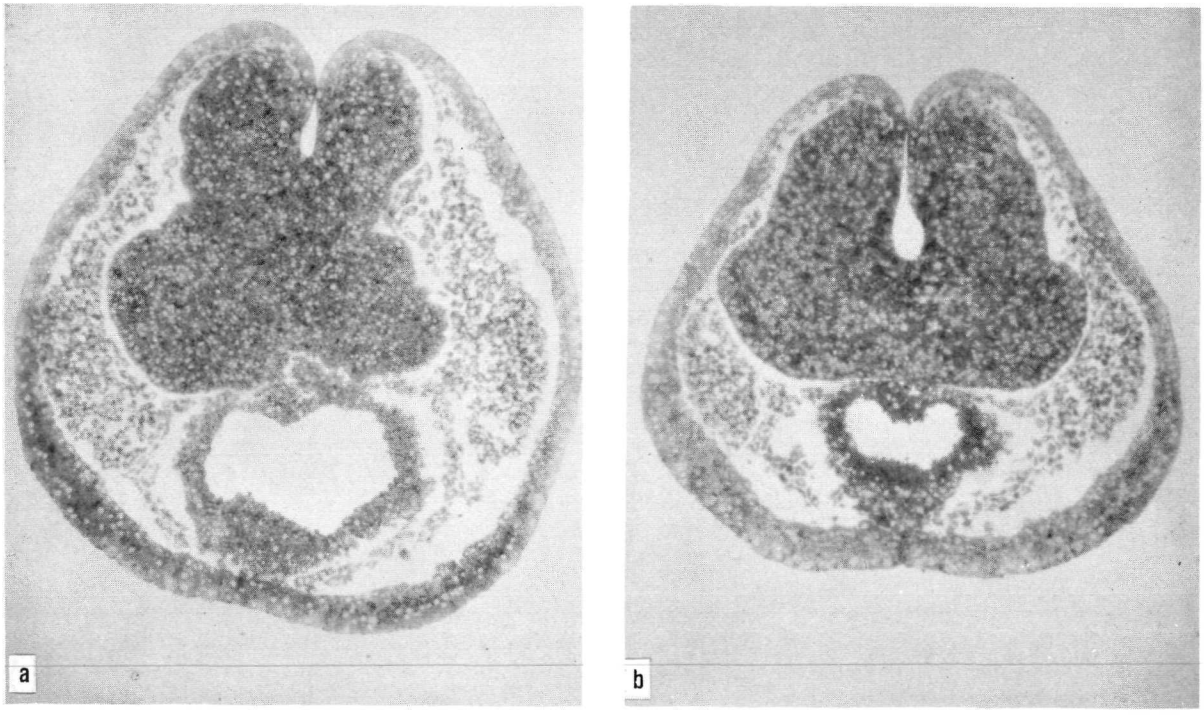
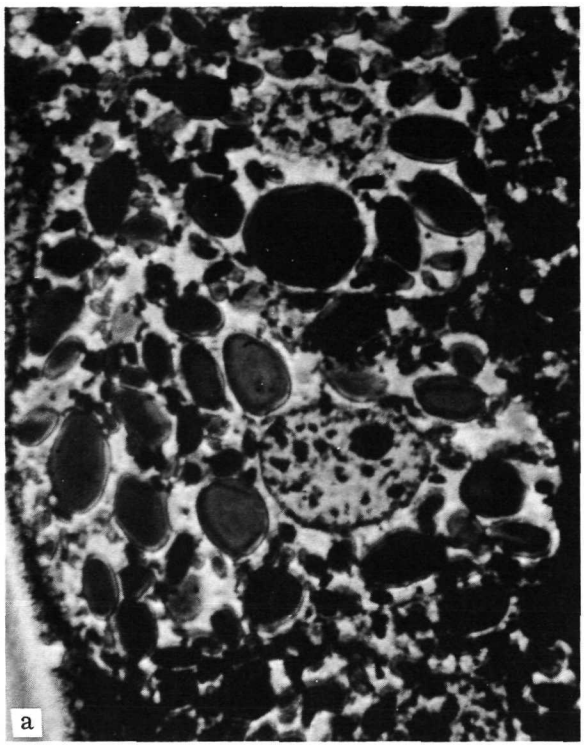
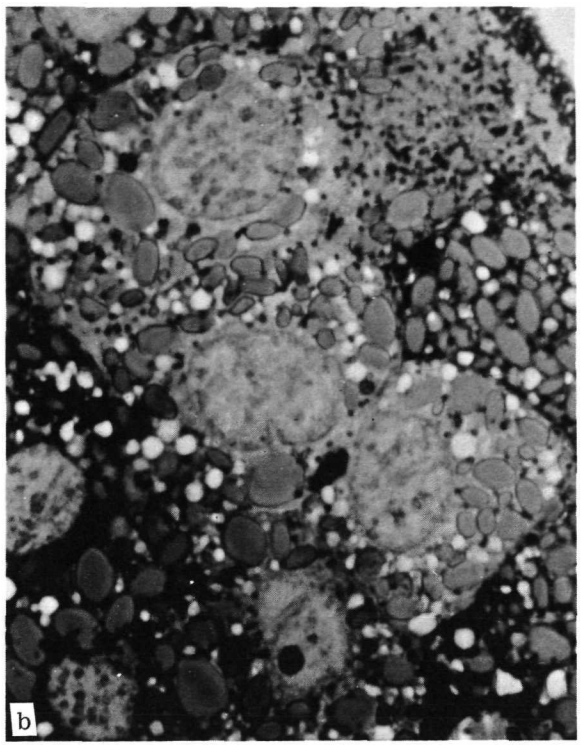


FIGURE 7.— Photomicrographs of stage 15 showing formation of spinal cord from neural fold (right); pharynx lies ventrally (left). (*a*) Flight embryo; section through anterior end; 188 ×. (*b*) Control III; section through anterior end; 188 ×.





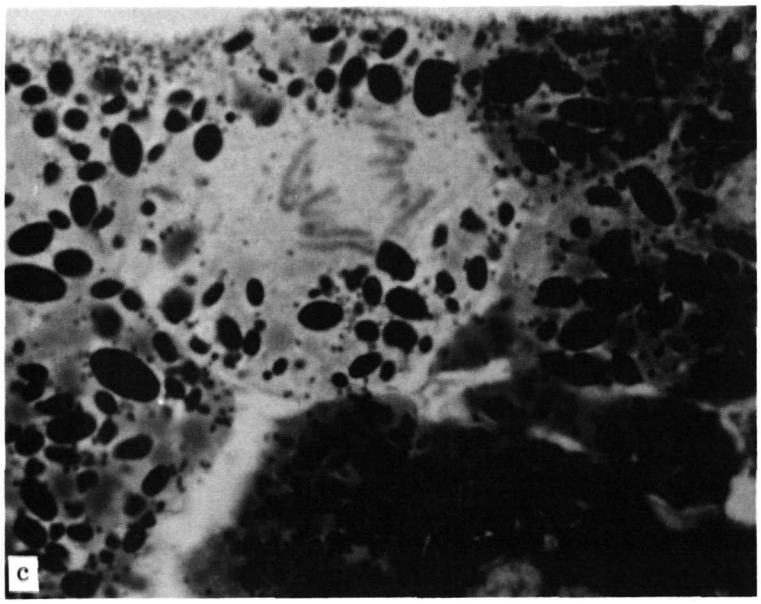
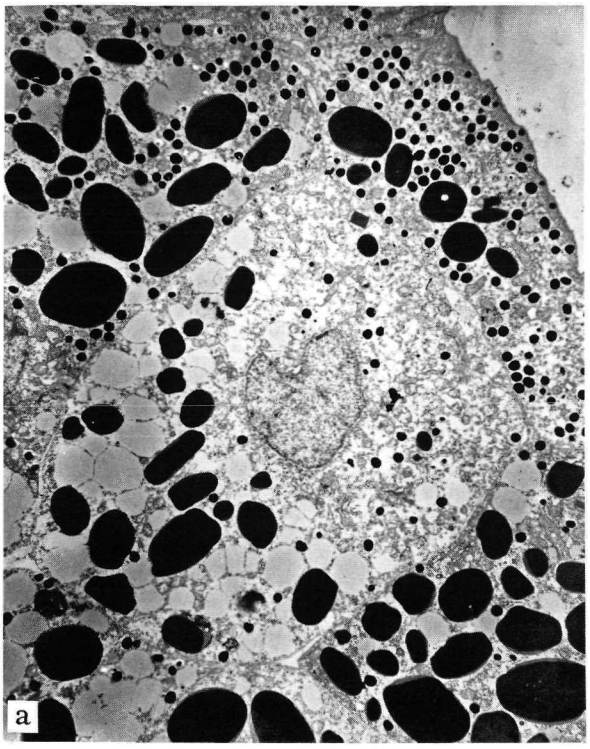
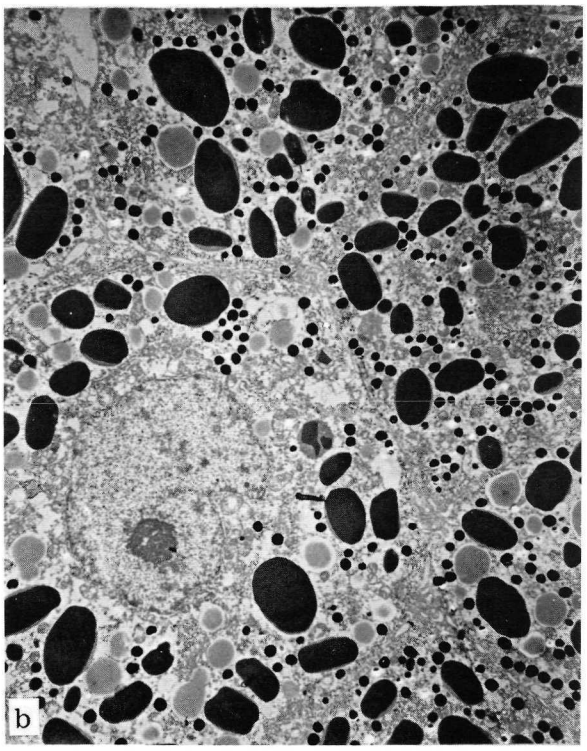


FIGURE 8.— Photomicrographs of individual cells and nuclei. (*a*) Flight embryo; stage 13; section through anterior end. Nuclei (light yellow); nucleoli (darker brown) within nuclei; yolk platelets (light brown or yellow ovoids); 4700 ×. (*b*) Control III embryo; stage 13; section through anterior end. Nuclei (light yellow); nucleoli (darker brown) within nuclei; yolk platelets (light brown or yellow ovoids); 4700 ×. (*c*) Flight embryo; stage 15; section through anterior end; chromosomes in anaphase; 4700 ×.





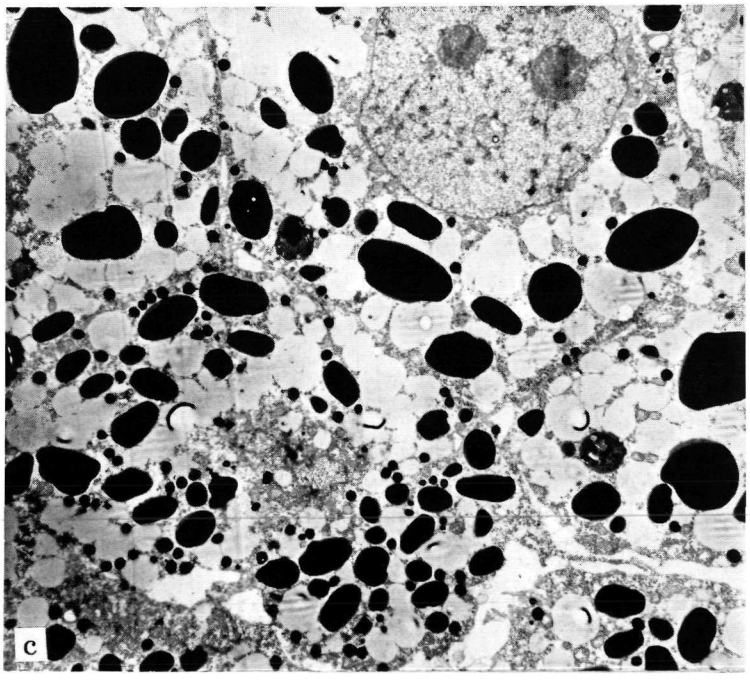


FIGURE 9.— Slightly magnified electron microscopy views of specimens. (*a*) Flight embryo; stage 15; section through anterior end, with cells showing distribution of yolk platelets (large dark ovoids) and pigment granules (smaller dark circles); cell and nuclear membranes; 3335 ×. (*b*) Centrifuge control; stage 16; section through anterior end; 3625 ×. Compare with part (*a*). (*c*) Control III; stage 15; section through anterior end; 3335 ×. Compare with part (*a*).

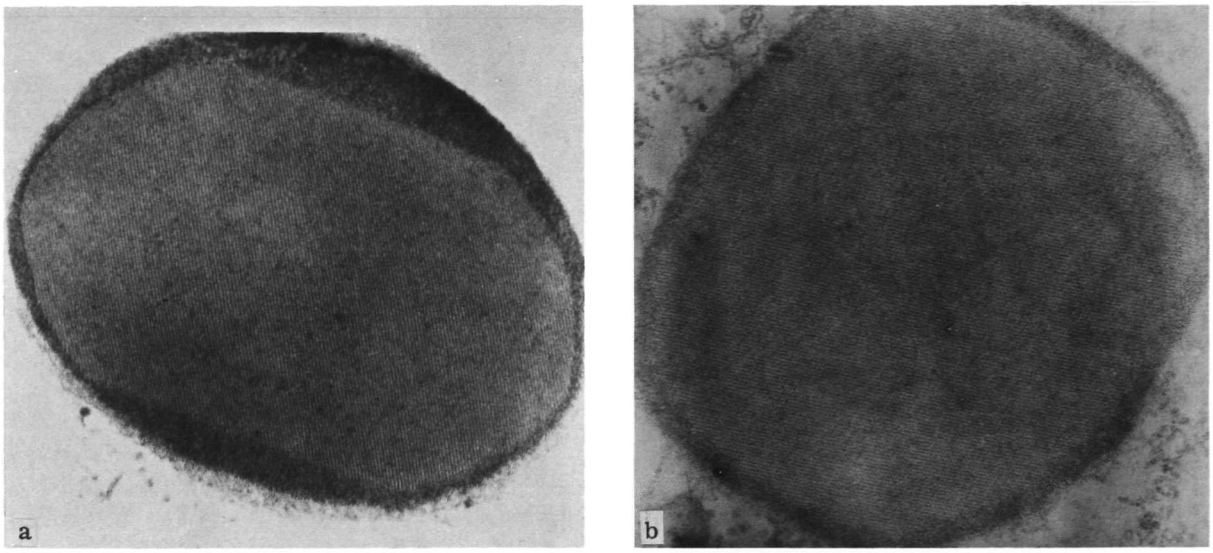


FIGURE 10.—Yolk platelets. (*a*) Flight embryo; stage 15; section through anterior end; single yolk platelet; 71 300 ×. (*b*) Centrifuge control; stage 16; section through anterior end; single yolk platelet; 71 850 ×.

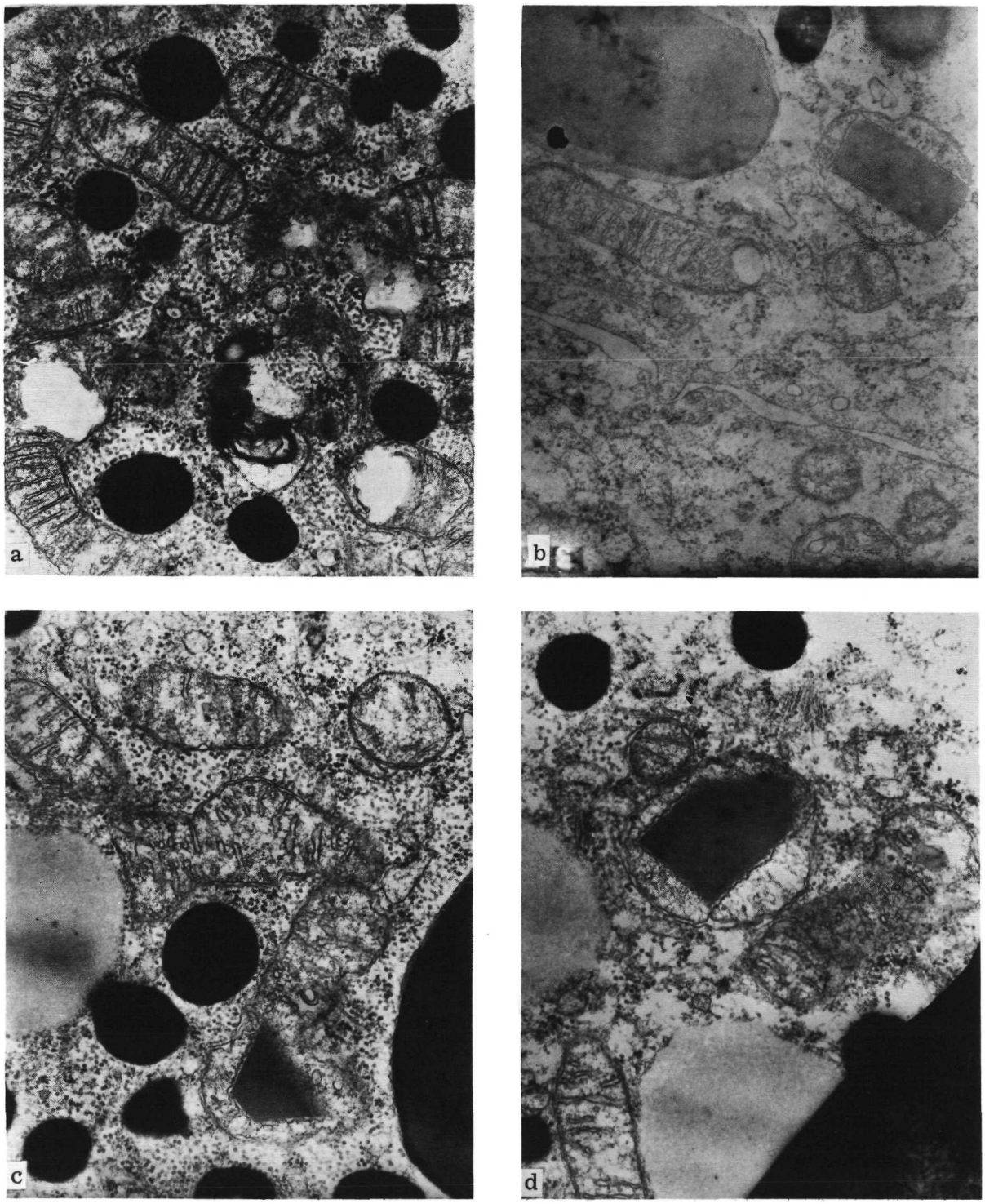


FIGURE 11.— Mitochondria. (*a*) Control III; stage 15; section through anterior end; mitochondria; 30 200 ×. (*b*) Centrifuge control; stage 16; section through anterior end; yolk platelet associated with mitochondrion; 32 600 ×. (*c*) Flight embryo; stage 15; section through anterior end; 31 000 ×. Compare with parts (*a*) and (*b*). (*d*) Flight embryo; stage 15; section through anterior end; 31 000 ×. Compare with parts (*a*) and (*b*).

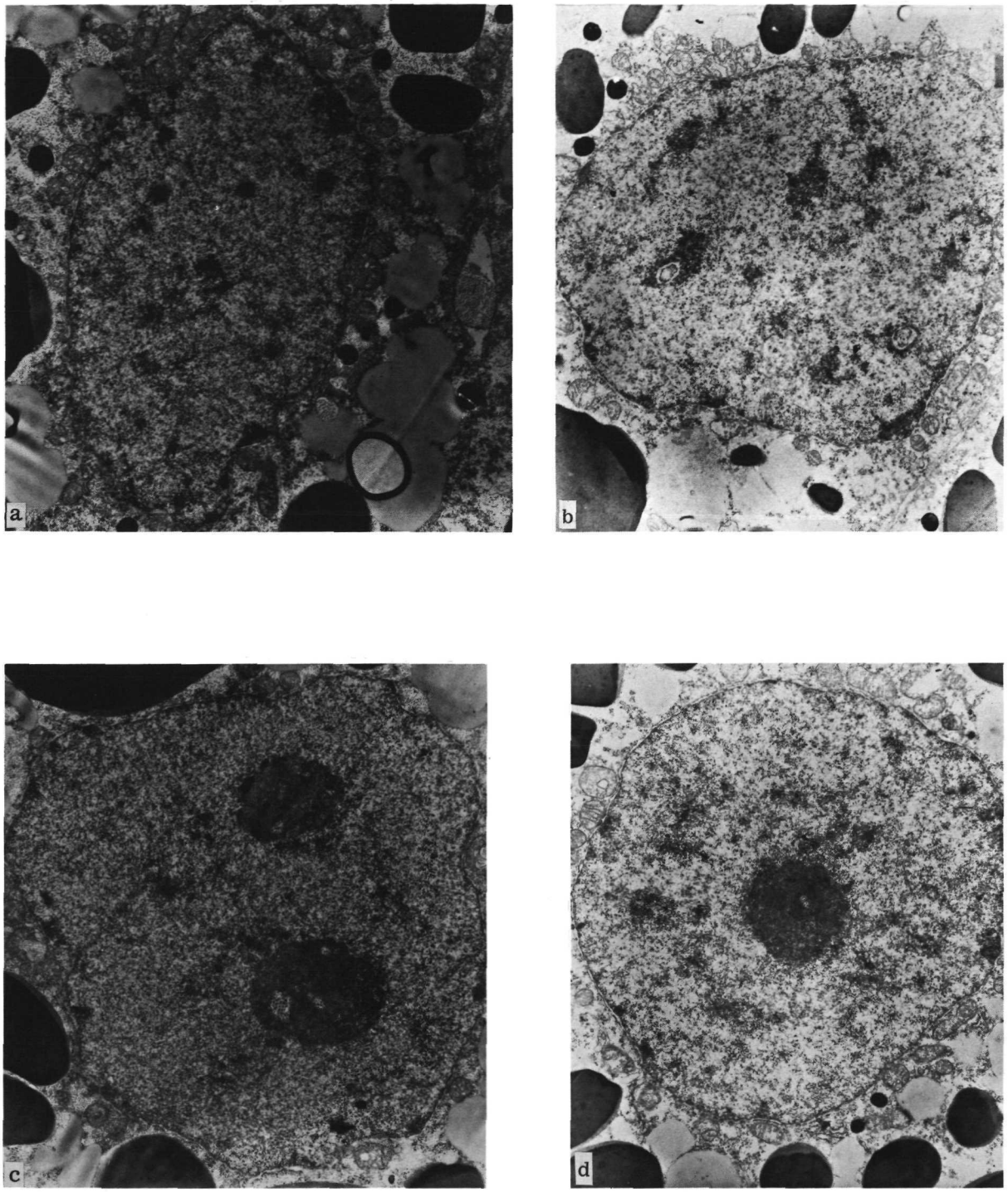


FIGURE 12.— Nuclei. (*a*) Control III; stage 15; section through anterior end; nucleus surrounded by mitochondria; 9700 ×. (*b*) Control III; stage 15; section through anterior end; nucleus surrounded by mitochondria; 8500 ×. (*c*) Flight embryo; stage 15; section through anterior end; 10 400 ×. Compare with parts (*a*) and (*b*). (*d*) Flight embryo; stage 15; section through anterior end; 8500 ×. Compare with parts (*a*) and (b).

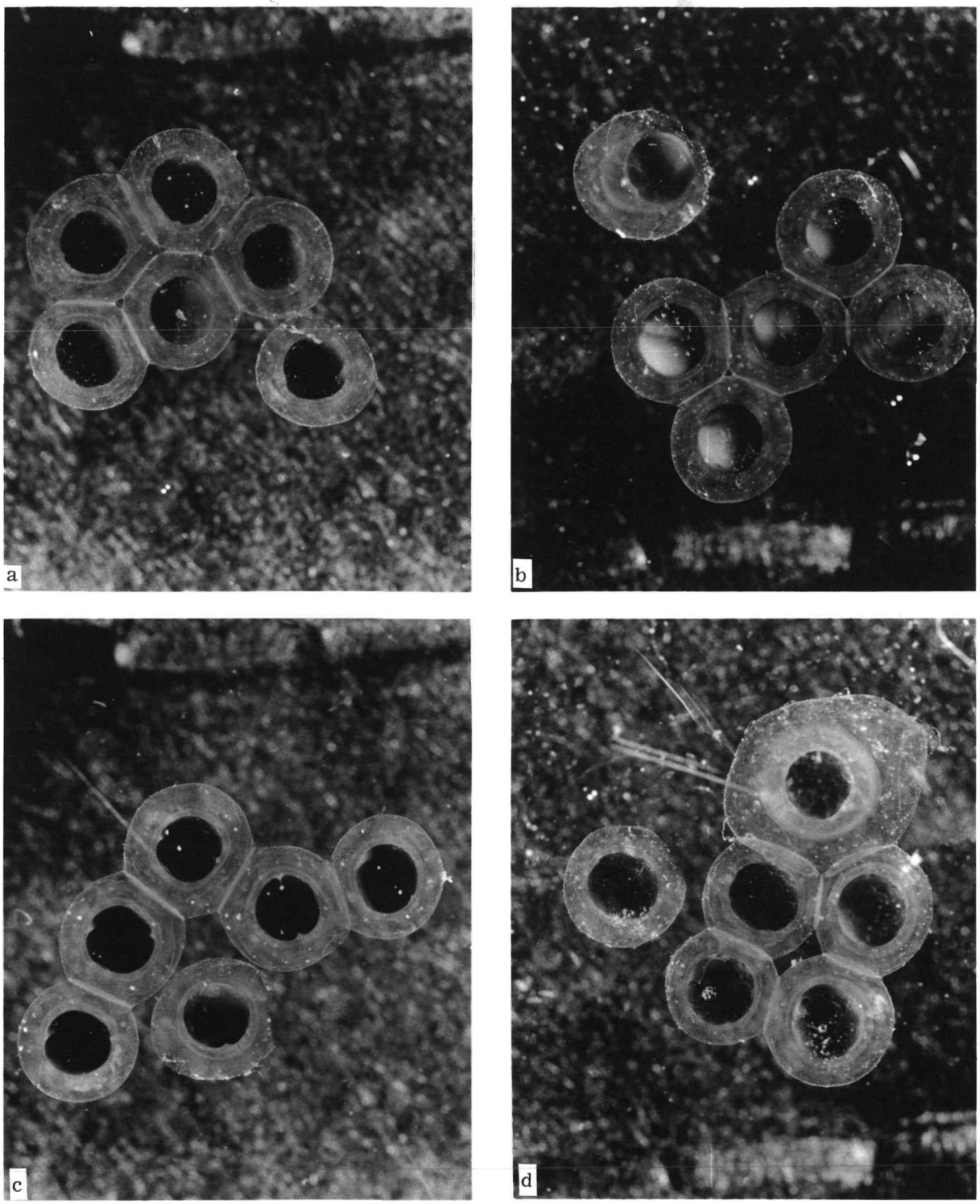
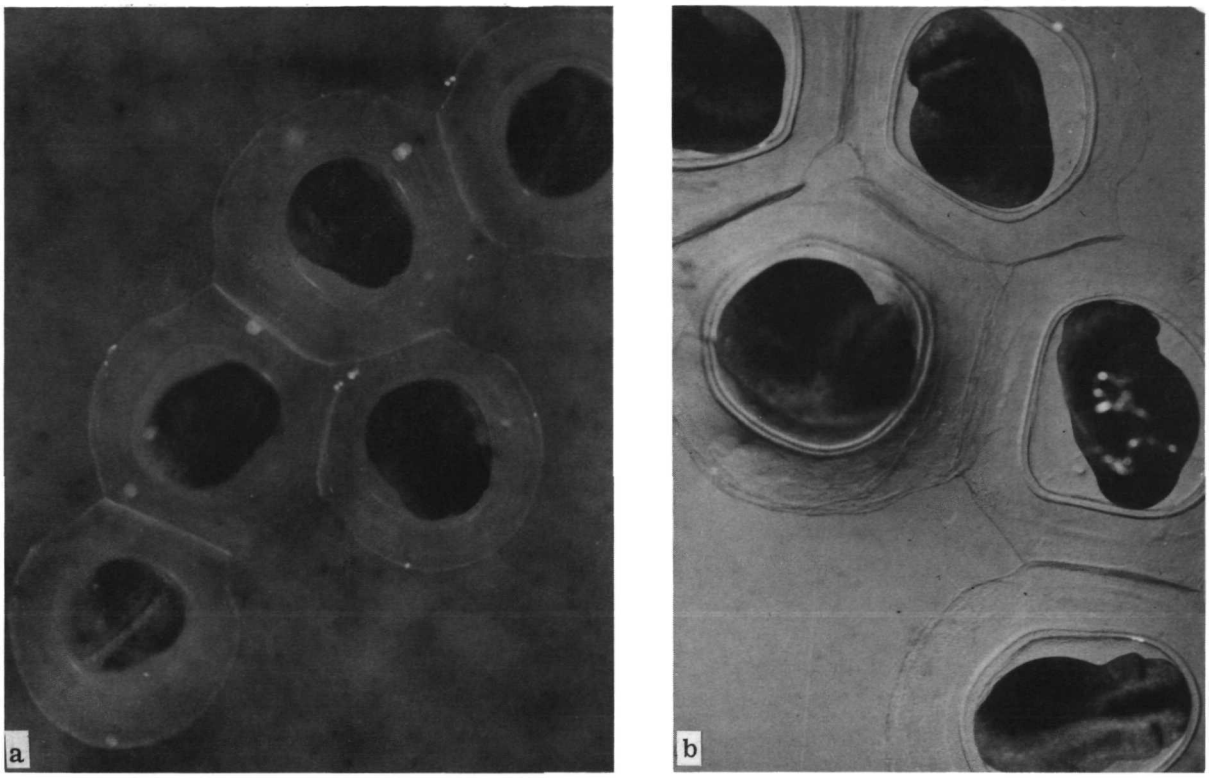


FIGURE 13.— Flight eggs. (*a*) Midstage 3; fixed at approximately $T-1$ minute. (*b*) Late stage 3; fixed at approximately $T+1$ hour. (*c*) Stage 4; fixed at approximately $T+2$ hours. (*d*) Stage 6; fixed at approximately $T+3$ hours.



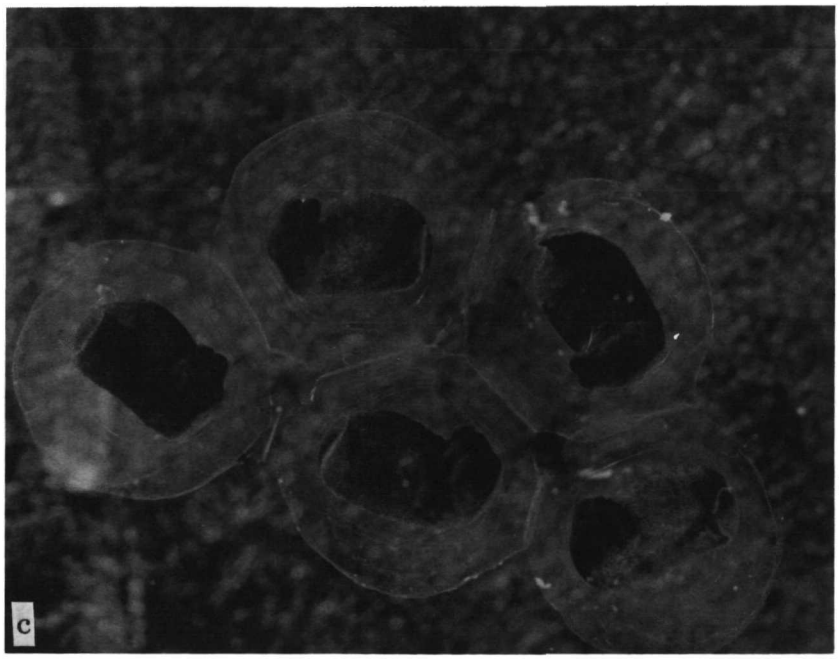


FIGURE 14.—Embryos observed on September 10, 1967. (*a*) Flight embryos; stage 15; observed at 0100Z. (*b*) Control I embryos; stages 16 and 17; observe at 0100Z. (*c*) Backup hardware embryos; stage 18; observed at 0800Z.

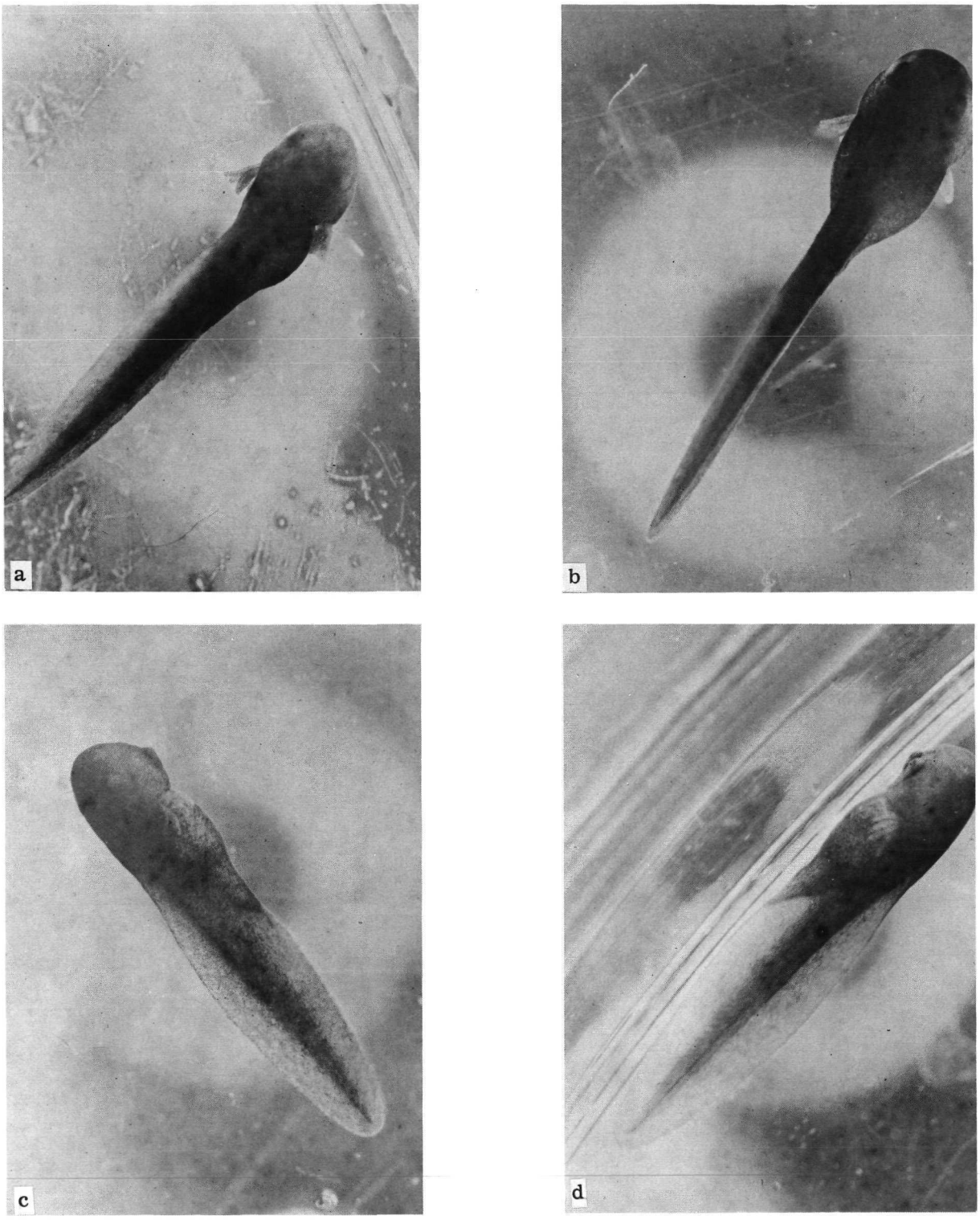


FIGURE 15.— Five-day-old embryos. (*a*) Flight embryo; eggs from frog H. (*b*) Backup hardware embryo; eggs from frog H. (*c*) Flight embryo; eggs from frog E. (*d*) Backup hardware embryo; eggs from frog E.

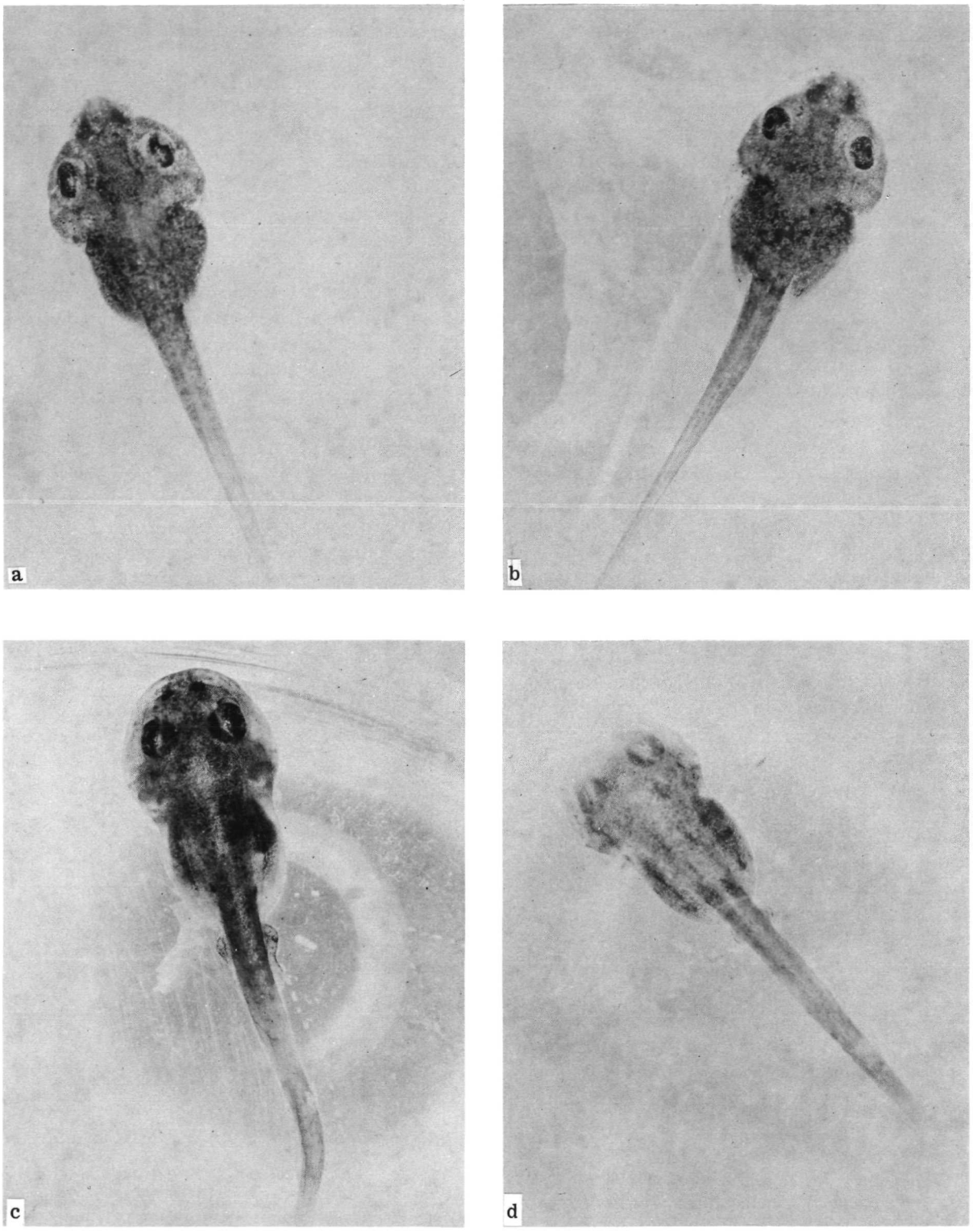


FIGURE 16.— Tadpoles. (*a*) Flight tadpole; 49 days old. (*b*) Control I tadpole; 49 days old. (*c*) Flight tadpole; 127 days old. (*d*) Control I tadpole; 127 days old.

Gross morphology indicates no differences between flight and control organisms other than the transient abnormality occurring in certain flight eggs as noted above. The possibility that this minor effect was flight caused cannot be discounted, but this kind of abnormality can probably be caused by many factors not unique to spaceflight. Light-microscope sections of flight eggs show no rearrangement of yolk materials or other effects of weightlessness. There appear to be no ultrastructural differences between the flight and control embryos observed.

Rates of development (table 1) of flight and Earth control organisms are similar and, where different, explainable principally by temperature variation. Under the given experimental conditions, then, there appears to be no effect of weightlessness on the development of *Rana pipiens* eggs when weightlessness is initially imposed at the presumably sensitive two-cell stage.

The first cleavage, however, represents a stage relatively quite advanced from the critical, gravity-sensitive changes initiated by fertilization. As explained by Willier (1955), the "rotation of orientation" occurring soon after fertilization coincides with the "rotation of symmetrization." The latter is that movement of the pigmented animal cortex which lays bare the gray crescent shortly before first cleavage. The median plane through the gray crescent normally coincides with the axis of the embryo. Inversion of the egg at the two-cell stage does not dislocate the gray crescent where the blastopore, the site of gastrular invagination, determines dorsality. The direction of the invaginations is evidently influenced by the abnormal yolk distribution; this leads to occurrences of the median plane(s) of the embryo at any angle to the original median plane. This finding permits the speculation that the determiner for the site of invagination, and thus dorsality, may reside in the cortex. *It is relatively stable at a stage when the symmetry plane is still labile.*

The preferential site for invagination is determined very early by the rotation of orientation, consequently, by gravity. The distribution of yolk then becomes influential in the determination of bilateral symmetry. Since this study and previous experiments (Young and Tremor, 1967) show no effect of weightlessness when it is imposed at or shortly before the two-cell stage, it would be particularly important now to test the stage before the cortex becomes stable, i.e., with and after fertilization, before the appearance of the gray crescent. Such sensitivity early in development (20 to 30 minutes after fertilization) has been demonstrated in this laboratory where duplications have been induced by centrifugation (Young et al., 1970).

After flight, studies were initiated in exposure of eggs soon after fertilization to the effects of the horizontally rotating clinostat (Tremor and Souza, 1969). The eggs were placed at a maximum distance of ¼ inch from the center of rotation and were exposed, within 5 minutes of fertilization initiation, to various rotational speeds (1/15, ⅛, ¼, 1, 2, 5, and 10 rpm). The eggs remained on the clinostat at 64.5 F until hatching. Since the ¼-rpm speed produced the most abnormalities (fig. 17), that speed alone has since been used in ongoing studies. It is noted that this rate closely approximates the rate of rotation of the fertilized egg when it is inverted and allowed to seek its equilibrium position. Results to date indicate a greater sensitivity to clinostat exposure at a stage prior to the two-cell stage than at or beyond this stage. More quantitation is necessary, however.

SUMMARY AND CONCLUSIONS

The fertilized egg of *Rana pipiens* divided, differentiated, and developed normally in over 2 days

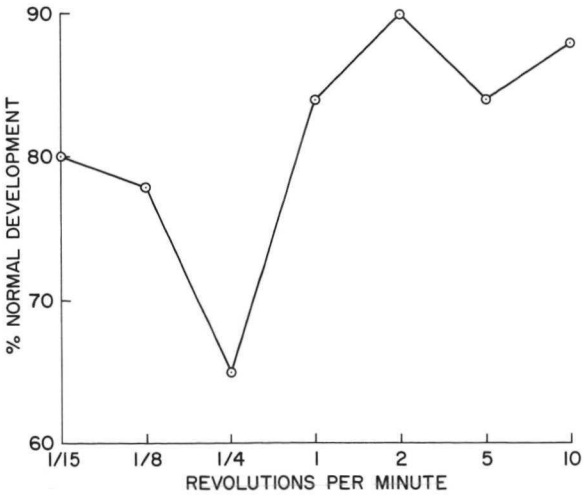


FIGURE 17.— Percent normal development as a function of horizontal clinostat rpm. Each point represents mean of approximately 350 observations (eggs) as adjusted to control (static) values.

of weightlessness despite initiation of exposure at the middle of the two-cell stage. It is possible that flight conditions may have affected a slight, transient abnormality that can probably occur under a variety of conditions. The significance of experimental variables affecting these conclusions has been discussed.

It is thought that earlier exposure of the egg to weightlessness, i.e., with and after fertilization, would complement the findings of Earth-based studies where gravity is implicated as a determiner of normal development.

ACKNOWLEDGMENT

The dedicated assistance of Harry Zabower is gratefully acknowledged.

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Nuclear and Cellular Division in *Pelomyxa carolinensis* During Weightlessness

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Weightlessness may have an effect on nuclear or cellular division, according to the hypothesis of Pollard (1965). The distribution of particles within a cell in a 1-*g* field is determined by the density of the cell and the volume and density of the particles. If this distribution is required for normal cell functions, then alterations in these functions may be expected during weightlessness because this distribution is no longer possible.

In order to study the effects of weightlessness on a single cell, the giant multinucleate amoeba *Pelomyxa carolinensis* was chosen. Although this organism appears to be independent of gravity on Earth, it does require a gravitational force to attach to a substrate for locomotion and feeding. When it is feeding, there is considerable protoplasmic movement which may be independent of gravity fields. During mitosis and cell division, however, there is relatively little motion. Thus, since the cell is large with relatively large nuclei, weightlessness could be expected to alter the manner and rate of reproduction.

EXPERIMENT DESIGN AND METHODS

Since it was desirable to feed and to fix amoebae during weightlessness, a three-compartment chamber was chosen (fig. 1). The number of amoebae which could be maintained for 4 days (estimated time from loading to opening of the chambers for a 3-day flight) had to be determined for each compartment. The oxygen consumption of amoebae and paramecia is approximately 2.5 μl/100 amoebae/hour and 3 μl/2000 paramecia/hour. Thus, the number of organisms placed into a compartment was dependent upon the amount of oxygen present and the time from chamber closure to the time of fixation or live recovery. The concentration of oxygen in the buffer solution and 0.25-ml air bubble is approximately 80 μl. This amount is sufficient for about 15 amoebae and several hundred paramecia for 4 days or for greater numbers of amoebae and paramecia for shorter periods. The CO_2 and waste products which are produced must be sufficiently buffered so that growth is not appreciably hindered within 4 days.

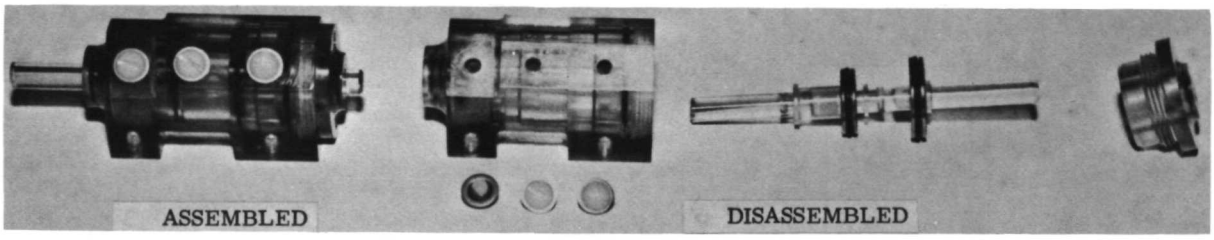

FIGURE 1.— Flight chamber.

It was necessary to obtain at least one amoeba fixed during mitosis. This required about 300 amoebae for the flight package for this part of the experiment. This number was estimated assuming a Poisson distribution as the worst case where the frequency of division per amoeba is one every 2 days, with a mitotic period of 90 minutes. Using 300 amoebae, there was 99-percent probability that an amoeba would be fixed during mitosis, in weightless flight.

TABLE 1. – Flight Loading Plan

Chamber	Compartment 1		Compartment 2		Compartment 3	
	Amoebae	Paramecia	Amoebae	Paramecia		
3-day starved						
1	200	0	0	2000	Fixative	
2	100	0	0	1600	do	
3	60	0	0	1100	do	
4	40	0	0	700	do	
5	30	0	0	500	do	
6[a]	0	500	30	0		
2-day starved						
7	60	0	0	1100	Fixative	
8	60	0	0	700	do	
9	30	0	0	500	do	
10[a]	0	500	30	0		
11	40	0	0	700	do	
12	30	0	0	500	do	
13[a]	0	500	30	0		
1-day starved						
14	30	0	0	500	Fixative	
15[a]	0	500	30	0		
16[a]	0	500	30	0		
Fed						
17	95	1000	95	1000	Fixative	
18	44	800	44	800	do	
19	27	550	27	550	do	
20	15	350	15	350	do	
21	9	250	9	250	do	
					Amoebae	Paramecia
22	10	200	10	200	10	200
23	10	200	10	200	10	200
24	10	200	10	200	10	200

[a]Chamber has only 2 compartments.

The loading plan given in table 1 was devised to provide an adequate number of amoebae for study of nuclear division and growth, yet not to have them so crowded that the oxygen supply would be depleted.

Flight Hardware

Amoebae and paramecia chambers for this experiment were made of polycarbonate resin (Lexan). These chambers were divided into three 5-ml compartments separated by Lexan pistons with ethylene propylene O rings (fig. 1). Initial actuation of a Lexan shaft to which the pistons were attached allowed mixing of the first two compartments which contained amoebae or paramecia. Further actuation mixed the first two compartments with the third compartment which contained the fixative. Twenty-four chambers were mounted on five magnesium plates as shown in figure 2. Front, back, and side magnesium covers were mounted on these plates. The chamber shafts were spring loaded on one end and restrained by a multislotted face cam on the other end. When the cam, which was rotated by a high-torque motor, moved from one position to the next, chamber shafts were pushed into the cam slots to achieve mix or fix chamber actuations. These actuations, in turn, closed microswitches which served as verifications of actuations. Four of the chambers contained thermistors for measuring in-flight temperatures. These data were telemetered to the various ground stations. High-density foam pads were placed between chambers to damp vibration received during powered flight and reentry. The total package, which weighed 15 pounds, was bolted to the aft plate of the Biosatellite II vehicle. A vehicle timer initiated five in-flight actuations as shown in table 2.

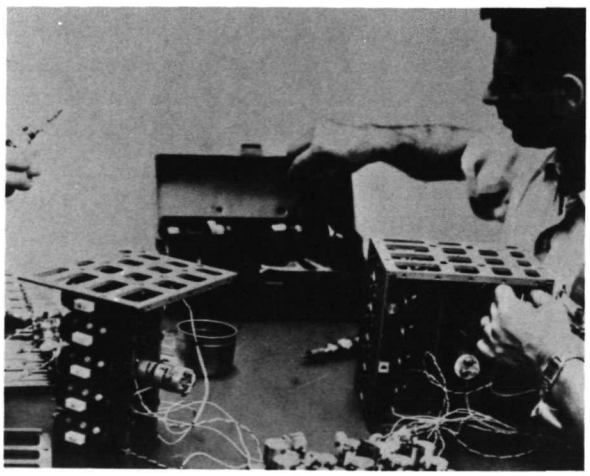

FIGURE 2.— Flight package during assembly.

Preflight Testing

In conducting any biologic space experiment, the first consideration should be the design of a life-support system based upon the environmental requirements of the organism to be maintained in the system. These requirements include not only the physical environments but also details suitable for the organism's metabolism. The experiment itself should be considered in detail only after these factors have been thoroughly investigated, and a system has been designed to keep the organism alive and in good condition for the specific duration of the flight.

Life-support system requirements for the giant amoeba, *Pelomyxa carolinensis,* as determined by extensive study, included temperature, food requirements, waste-product management, oxygen concentration and consumption, magnetic field, aqueous media, and container size and material. With the design and fabrication of the flight hardware additional factors had to be considered. Of major importance were O ring and Lexan toxicity, fixative leakage and detection, and effects of vibration. The latter was studied during the systems qualification tests at General Electric Re-Entry Systems Department, Philadelphia, the vibration testing at NASA Ames Research Center, and the simulated launch at Cape Kennedy, Fla.

EXPERIMENT CONTAINER TOXICITY

One of the largest problems encountered was organism-material compatibility. A suitable plastic had to be selected since glass was impractical because of difficult manufacturing processes, excessive weight, and high costs. After several plastics were tested, Lexan, a polycarbonate resin, was chosen for fabrication of the prototype and flight hardware. Biocompatibility tests with the organisms in flight chambers produced amoeba growth rates considerably lower than those anticipated. This led to numerous attempts to prolong the survivability of the amoebae and to increase their growth rate.

TABLE 2. – In-Flight Actuations

[F, fixed; M, mixed; NF, not fixed (no fixative in chamber)]

Chamber	Time after launch, hours					Time of recovery
	1	12	24	43	43.5	
1	F					
2	M	F				
3	M		F			
4	M			F		
5	M				F	
6	M					NF
7		M	F			
8		M		F		
9		M			F	
10		M				NF
11			M	F		
12			M		F	
13			M			NF
14				M	F	
15				M		NF
16					M	NF
17	F					
18		F				
19			F			
20				F		
21					F	
22						NF
23						NF
24						NF

Various methods of combating chamber toxicity were studied. Testing employed outgassing the chambers at different pressures, temperatures, and time durations and coating the chambers with various chemicals. The paramecia survived in many cases where the amoebae did not; the problem of toxicity therefore appeared to be on the surface of the chambers where the amoebae normally attach themselves. Cleaning the chambers in 0.2 percent $KMnO_4$ in order to oxidize toxic materials on the surface produced far more satisfactory results. After the length of time required to permanganate the chambers was determined, a complete procedure was adopted for preparing the chambers for loading of the organisms and subsequent cleaning.

Nitrile, butyl and ethylene propylene O rings were tested for toxic effects on paramecia. Various cleaning methods were employed including washing the O rings in concentrated HCl, N/10 HCl followed by N/10 NaOH, and N/10 NaOH followed by N/10 HCl. They were tested with and without outgassing. The nitrile and butyl O rings produced definite toxic effects on the paramecia whereas the organisms tested with ethylene propylene O rings showed no detrimental effects. Silicone O rings also were nontoxic, but they were unsatisfactory because of high fixative leakage rates.

AQUEOUS MEDIA

Various buffering agents were added to the buffer of Pace and McCashland (1951) in an additional effort to increase the longevity and growth of the organisms in the Lexan chambers. Unsatisfactory results were obtained with glycine and veronal acetate buffers. The *Pelomyxae* fared better in EDTA, glycyl glycine, and tris (hydroxymethyl) aminomethane (THAM) buffers. After testing several concentrations, a combination of

0.5 mg THAM and 0.1 mg glycyl glycine per ml of Pace and McCashland buffer was selected as the medium of choice.

FIXATIVE LEAKAGE AND DETECTION

Another major problem area was fixative leakage and detection. Alcohol-formalin (HCHO) was originally selected as the fixative to be used in the amoebae experiment. A series of tests was performed to determine the smallest quantity of HCHO that proved fatal to amoebae and paramecia. The study showed the organisms could tolerate up to 2 ppm HCHO for 4 days. Static and dynamic tests with chambers loaded with organisms and fixative were conducted to insure that there would be no leakage of HCHO in concentrations which would be detrimental to the organisms. However, in view of the unsuitability of alcohol-formalin for electron microscopy, it was decided later to use 12 percent glutaraldehyde in 0.1 M phosphate buffer in place of the alcohol-formalin. The leakage of glutaraldehyde was extremely low, and thus no toxicity problem was presented.

PRELAUNCH

The amoebae were cultured in the buffer of Pace and McCashland (1951) in stacking dishes. Subculturing occurred two to three times weekly after which the amoebae were fed paramecia concentrated from beakers of distilled water containing Turtox concentrate for paramecia. These cultures consisted mainly of *Paramecium multimicronucleatum* with some *P. caudatum* and *P. chilomonas.*

The amoebae cultures were starved on three successive days before launch by removing the paramecia. During starvation, the *Pelomyxae* were maintained in THAM buffer (0.5 mg THAM/ml Pace and McCashland buffer). The fed (nonstarved) amoebae received their last subculturing approximately 12 hours prior to chamber loading when they were placed in THAM buffer and amply fed with paramecia. The latter also had been concentrated in the THAM buffer.

Paramecia for loading were concentrated in THAM/glycyl glycine buffer. A count taken immediately before loading showed 1200 paramecia per milliliter. This was determined by pipetting 0.1 ml of paramecia concentrate onto a microagglutination slide with ceramic rings (0.01 ml/ring) and microscopically counting the number of paramecia. After several counts were taken, the number of paramecia per ml was determined. Calibrated Pasteur pipets were used to load the desired number of paramecia into the chambers.

Three days prior to launch, all chambers were rinsed in 2 percent $Na_2S_2O_5$ for 15 minutes and then washed in successive rinses of demineralized water until the resistivity of the rinse water was at least 780 000 ohms/cc. The chambers were then submerged in 0.2 percent $KMnO_4$ for 2 hours and again washed in successive rinses of demineralized water. All final rinse water had a resistivity of at least 920 000 ohms/cc.

A flight, a backup, and three control units were assembled 2 days before launch. Each unit was comprised of 24 chambers containing ethylene propylene O rings which were lightly coated with silicone grease before installation.

CHAMBER LOADING FOR LAUNCH

The chambers which had been filled with THAM/glycyl glycine buffer for preconditioning 9 hours previously were emptied by aspiration immediately prior to loading. Loading of the organisms into the chambers began 18.5 hours before launch and continued for 2 hours. The amoebae were screened microscopically for healthy, nondividing forms and counted as they were selected with Pasteur pipets from the culture dishes into the individual chambers. Table 1 shows the number of organisms loaded per compartment.

After the amoebae and paramecia were loaded, these compartments were filled with THAM/glycyl glycine buffer leaving an air space of approximately 0.25 ml. The fixative compartments contained 12 percent glutaraldehyde in 0.1 M phosphate buffer, pH 6.9. The glutaraldehyde is diluted 3:1 when mixed with the other two compartments. Chamber loading was complete 15 hours before launch. The flight, backup, and control 1 chambers were mounted into experiment packages as described in the section entitled "Flight Hardware."

POSTLAUNCH

As soon as the flight chambers were received at the Hickam Field laboratory, 50.5 hours after launch, the ones containing live organisms were opened and their contents completely flushed out with Pace and McCashland buffer into glass dishes.

After they were counted, the dividing forms were fixed for 2 days in 4 percent glutaraldehyde in 0.1 M phosphate buffer.

Next, eight starved amoebae and 50 percent of the fed amoebae per live chamber were subcultured in separate dishes and amply fed for growth-rate studies. These cultures were maintained in Pace and McCashland buffer in a dark incubator at 70 F for 7 days and microscopically counted twice each day. After 2 days, the fixed dividing amoebae were postfixed for 17 hours in 1 percent OsO_4, dehydrated, and embedded in Epon 812 for electron and light microscopy.

The contents of the starved fixed chambers were removed with 0.1 M phosphate buffer and the amoebae counted. Dividing amoebae were postfixed in 1 percent OsO_4 for 18 hours, dehydrated, and embedded in Epon 812 for electron microscopy.

The fed fixed chambers were emptied with 0.1 M phosphate buffer and the amoeba count was determined. The dividing *Pelomyxae* were selected and treated the same as were the starved fixed dividing forms. Half of the nondividing amoebae also were postfixed. Approximately 25 percent of these were dehydrated and embedded in paraffin. The remaining nondividing amoebae were embedded in Epon 812 along with the other fixed organisms.

After notification of vehicle reentry, the backup unit, which remained in the laboratory at Cape Kennedy, was disassembled. The chambers were placed in an insulated container and hand-carried to Hickam Field where they arrived approximately 8 hours after the flight unit. The amoebae were handled identically with those from the flight chambers.

Organisms from the three control units at Cape Kennedy (flight-simulation control, constant-temperature control, and variable-temperature control) were processed the same as were the flight and backup units.

The maintenance of the amoebae cultures and the preparation of the organisms for chamber loading for the postflight Earth control test was the same as for the Biosatellite II flight. Three days before the test, all chambers were submerged in 0.2 percent $KMnO_4$ for 2 hours, washed 2 hours in running tap water, and rinsed in distilled water. Five units, of 24 chambers each, were assembled the following day. They included two flight-simulation units, designated 301, which received acceleration and vibration, and the qualification vehicle, 201, which received no hard environments. Three other units included a flight-temperature control and two constant-temperature controls. Chamber loading began 20 hours before simulated launch and lasted 2½ hours.

The two flight-simulation units were assembled into amoebae experiment packages, which were mounted on the aft plates of the 301 and 201 capsules. Biosatellite II flight lift-off, reentry, and in-trail (reeling into recovery aircraft) vibration and acceleration profiles were simulated on the 301 capsule (figs. 3 to 8). The 201 capsule was transported along with the 301 capsule but did not undergo the vibration and acceleration.

At the completion of the test, the chambers containing live organisms were opened first. After the chambers were rinsed with Pace and McCashland buffer, amoeba counts were taken. Posttest plasmotomy (cell division) rates were initiated with fed and starved amoebae. The chambers containing fixed organisms were emptied with 0.1 M phosphate buffer and the amoebae were counted. All dividing forms and 50 percent of the fed, fixed amoebae were postfixed 17 hours in 1 percent OsO_4. After dehydration, the amoebae were

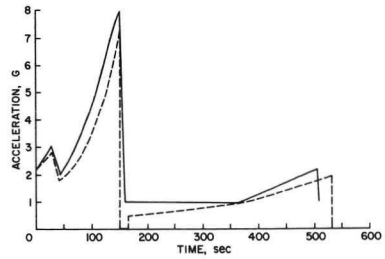

FIGURE 3.— Launch acceleration profile.

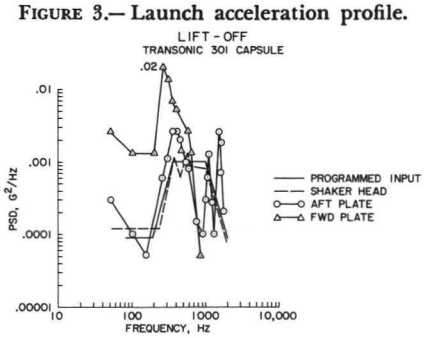

FIGURE 4.—Lift-off; transonic 301 capsule.

embedded in Epon 812 for light and electron microscopy.

Clinostat tests were conducted with three groups of six chambers each loaded with 10 amoebae and approximately 250 paramecia per compartment in THAM/glycyl glycine buffer. One group was mounted onto a horizontally rotating cylinder (clinostat) where the amoebae were approximately 3 inches from the center of rotation. One control group consisted of static chambers, and another of chambers rotated vertically at the same velocity as those on the clinostat. Figure 9 shows the three experimental groups: i.e., the horizontal clinostat, vertical rotator, and static. All testing was conducted in the dark at 71 to 77 F for 3 days. Growth rates Nt/No, where Nt is the number of cells at time t and No is the number of cells at time 0, were determined by counting the number of amoebae. Velocities employed were 6 rph and 1, 4, 10, 20, and 100 rpm.

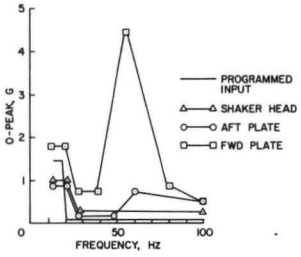

FIGURE 5.— Low-frequency sine sweep; 301 capsule.

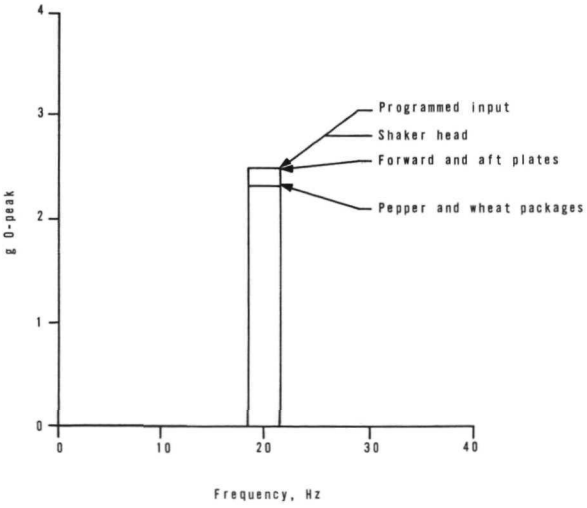

FIGURE 6.— Pogo simulation.

Amoebae undergoing simulated "Pogo" vibration were observed microscopically at 20 Hz, 5 g peak to peak (fig. 10). A stroboscope supplied the light source for visual observation. For high-speed, 16-mm motion pictures, the stroboscope was replaced with a xenon arc lamp (Ekberg, 1967).

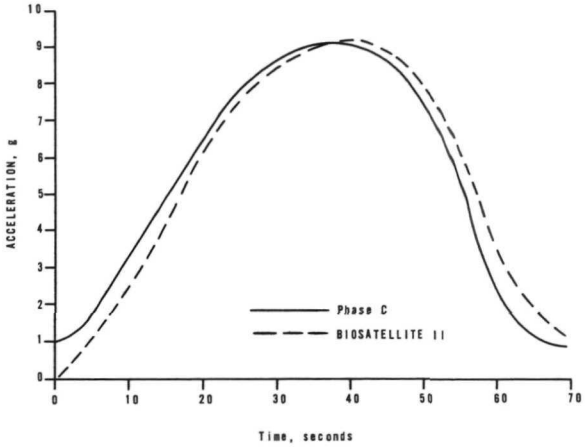

FIGURE 7.— Reentry acceleration profile.

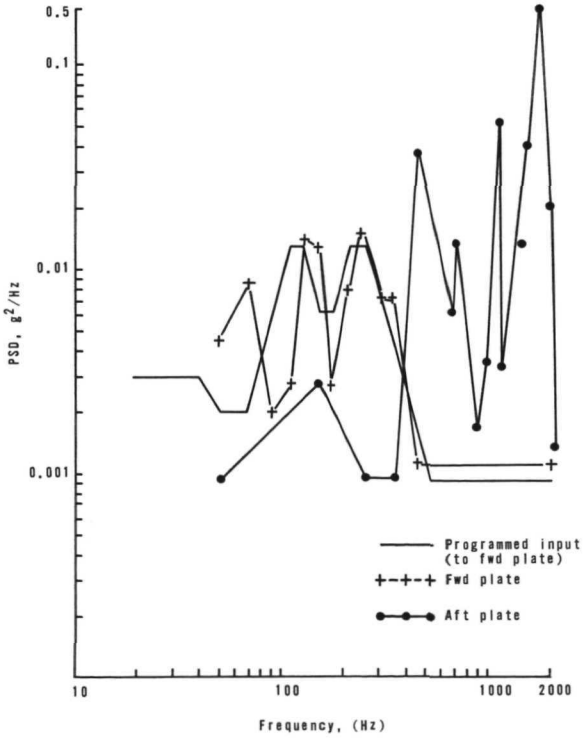

FIGURE 8.— Intrail simulation; 301 capsule.

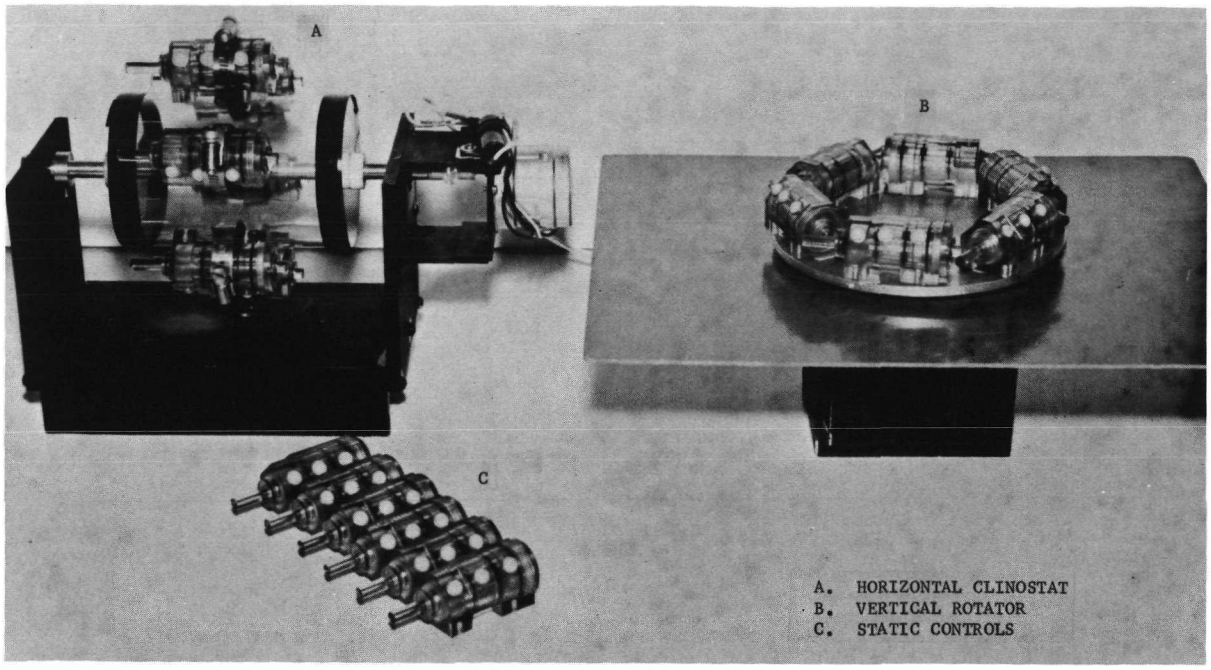

FIGURE 9.— Clinostat apparatus.

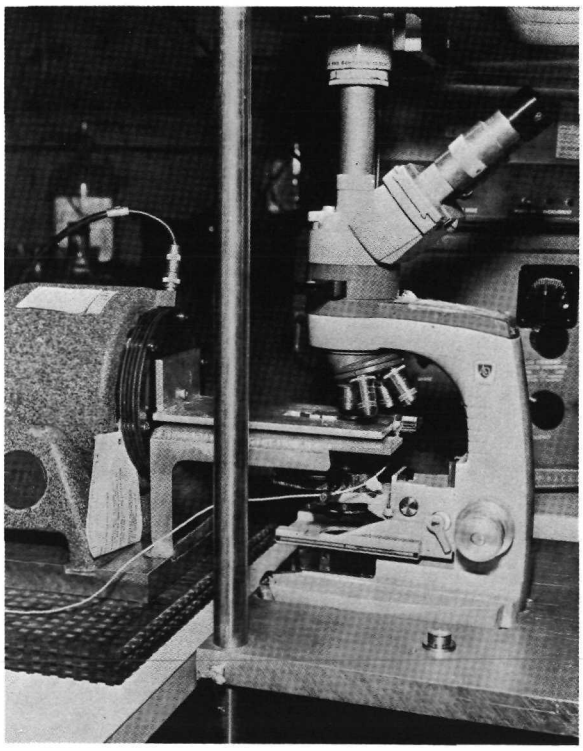

FIGURE 10.— Vibration apparatus.

RESULTS

Temperatures

The temperatures of the flight, backup, and three control packages are given in figure 11. Table 3 compares the Biosatellite II flight temperatures with those of the postflight Earth control test. The results are similar, with the exception of the backup unit, which had no counterpart in the Earth control test.

Plasmotomy Rates

On the basis of the number recovered, the starved amoebae showed no evidence of division during flight (tables 4 and 5). In chamber 6 (nonfixed), slightly more amoebae were returned than were loaded. However, it is impossible to preclude the possibility of division before or after weightless flight.

Table 6 summarizes the results of the fed amoeba group. In the fixed as well as nonfixed (live) amoebae, many cell divisions (plasmotomies) occurred during the flight. No significant difference in the rate of reproduction between the live fed flight amoebae and the controls (including backup)

is apparent. The fixed fed flight amoebae, however, appear to have divided at a greater rate than did the controls, yet to a lesser degree than did the backup (table 6; fig. 12). Table 7 gives a statistical comparison of these groups. The flight group is significantly different from the controls (excluding backup) at less than the 5 percent level. Control 2 (constant temperature) has been deleted from the comparisons because of its poor plasmotomy rate in this group. The fed flight group is not significantly different from the backup and, thus, when the backup is averaged with controls 1 and 3, the comparison with the flight group does not yield a significant difference. If the backup data are reduced to compensate for temperatures higher than those of the flight group (fig. 11), a P value of 0.2 to 0.1 is obtained in comparing the flight group with the controls and the backup. This value indicates no significant difference but suggests a trend toward a higher plasmotomy rate during weightless flight.

The division rates Nt/No of the starved and fed amoebae from the flight and postflight Earth control test appear in table 8. In the Earth control test, results for the 301 and 201 vehicles are similar. There also are no significant differences in the fixed amoebae from any unit. Division rates of the live, starved amoebae show a trend toward higher growth in control 1 than in the 201 vehicle. Control 3 of the live, fed amoebae has a statistically significant higher growth rate than that of control 1. Comparison of the Biosatellite II flight with the Earth control test shows no significant differences.

The postflight division rate of the flight group was somewhat greater than that of the backup, yet less than that of the controls (fig. 13). The long delay in cell division for the backup was probably due to higher previous temperatures (fig. 11) and handling during transport from Cape Kennedy to Hawaii. The differences between the flight group and controls may have been due in part to the fact that the controls were studied at the Cape and the flight and backup in Hawaii. A comparison of the flight group with the controls and backup does not show a significant difference.

Postflight Earth control test cell division rates are shown in figure 14. Comparison with flight data shows similar growth rates (Nt/No). The lag period also was approximately the same in both cases.

TABLE 3. — Flight and Postflight Earth Control Temperatures

Time, hr	Flight temperature, F					Postflight earth control temperature, F				
	Flight	Backup	Control 1	Control 2	Control 3	301	201	Control 1	Control 2	Control 3
$L-14$ to L	68 to 71	70 to 72	71 to 73	69	68 to 69	68 to 73	69 to 73	70	70	70 to 71
L to $L+12$	68 to 69	72 to 73	68 to 71	68 to 70	67 to 69	67 to 70	68 to 70	67 to 69	70	70 to 71
$L+12$ to $L+28$	67 to 68	73	70	69 to 70	67 to 69	66 to 68	66 to 68	67 to 68	70	70 to 71
$L+28$ to $L+44$	67 to 68	72	70	69 to 70	66 to 70	66 to 68	66 to 68	67 to 68	70	70 to 71
$L+44$ to $L+52$	63 to 71	72 to 77	70	70	63 to 71	68 to 69	68 to 69	67 to 68	70	70 to 71

Control 1: Flight simulation
Control 2: Constant temperature
Control 3: Variable temperature

301: Flight simulation with acceleration and vibration
201: Flight simulation without acceleration and vibration
Control 1: Flight temperature
Control 2: Constant temperature
Control 3: Constant temperature

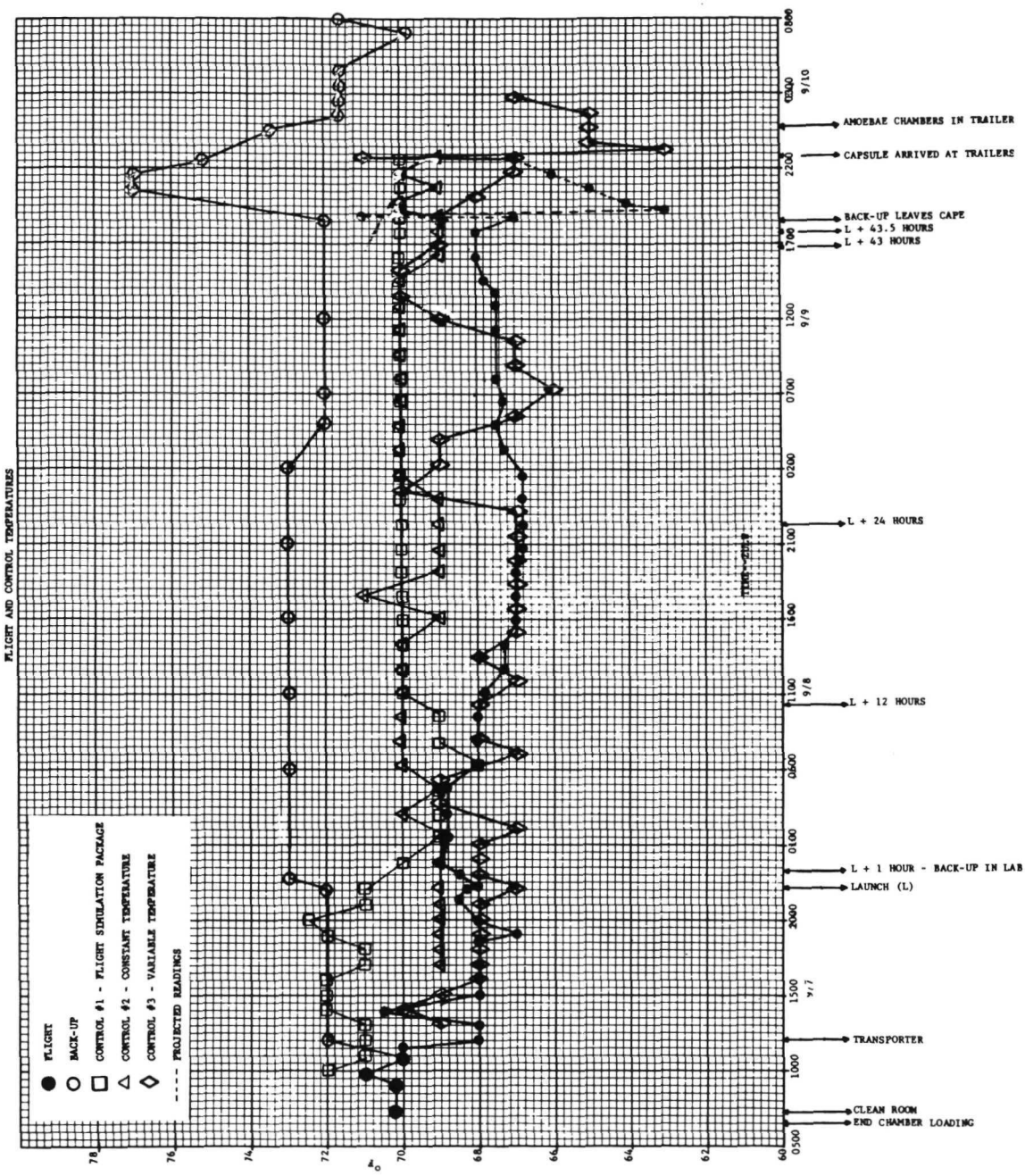

FIGURE 11.— Flight and control temperatures.

TABLE 4. – Fixed Starved Flight Amoebae

Fixed chambers	Feeding to fixation time, hr	Loading to fixation time, hr	Cell division rate, Nt/No					
			Flight	Backup	Control 1	Control 2	Control 3	Average of controls 1, 2, and 3
1	1	18 to 21	0.65	0.76	0.47	0.67	0.63	0.59
14	1.5	60 to 63	.83	1.33	.63	1.33	.77	.91
2	11	29 to 32	.65	.71	.67	.95	.61	.74
7	12	41 to 44	.70	.80	.55	.95	.82	.77
11	18	59 to 62	.63	1.13	.58	1.10	1.03	.90
12	19.5	60 to 63	.77	.97	.53	1.27	.73	.84
3	23	41 to 44	.85	.67	.70	.90	.63	.74
8	30	59 to 62	.70	1.18	.60	1.23	.58	.80
9	31.5	60 to 63	.90	1.30	.73	.73	.77	.74
4	41	59 to 62	.98	1.10	.50	1.45	.68	.88
5	42.5	60 to 63	.60	.97	.53	1.30	.80	.88

TABLE 5. – Live Starved Flight Amoebae

Live chambers	Feeding to unloading time, hr	Loading to unloading time, hr	Cell division rate, Nt/No					
			Flight	Backup	Control 1	Control 2	Control 3	Average of controls 1, 2, and 3
6	50 to 52	68 to 72	1.07		1.03	0.90	1.13	1.02
	58	77		0.77				
10	39 to 41	68 to 72	.87		.63	.90	1.23	.92
	47	77		1.07				
13	27 to 29	68 to 72	.77		.63	.90	.93	.82
	35	77		1.03				
15	9 to 11	68 to 72	.90		1.20	1.20	.70	1.03
	16	77		1.27				
16	8 to 10	68 to 72	.77		.67	.67	.93	.76
	15	77		1.47				
Average total		68 to 72	.87		.83	.91	.99	.91
		77		1.12				

TABLE 6. – Fed Flight Amoebae

Fixed chambers	Loading to fixation time, hr	Cell division rate, *Nt/No*					
		Flight	Backup	Control 1	Control 2	Control 3	Average of controls 1, 2, and 3
17	18 to 21	0.63	0.46	0.62	1.07	0.76	0.82
18	29 to 32	.68	1.18	1.05	.64	.77	.82
19	41 to 44	1.43	1.67	1.13	1.43	1.02	1.19
20	59 to 62	1.67	2.73	1.30	.90	1.33	1.18
21	60 to 63	2.00	1.94	1.50	1.44	1.39	1.44
Live chambers	**Loading to unloading time, hr**						
22	68 to 72	1.53	.....	2.03	2.40	1.87	2.10
	77	.....	1.83	.....	.....	.....	.....
23	68 to 72	1.43	.....	2.07	3.00	1.80	2.29
	77	.....	3.30	.....	.....	.....	.....
24	68 to 72	3.60	.....	2.30	2.43	1.83	2.19
	77	.....	1.97	.....	.....	.....	.....
Average total	68 to 72	2.19	.....	2.13	2.61	1.83	2.19
	77	.....	2.37	.....	.....	.....	.....

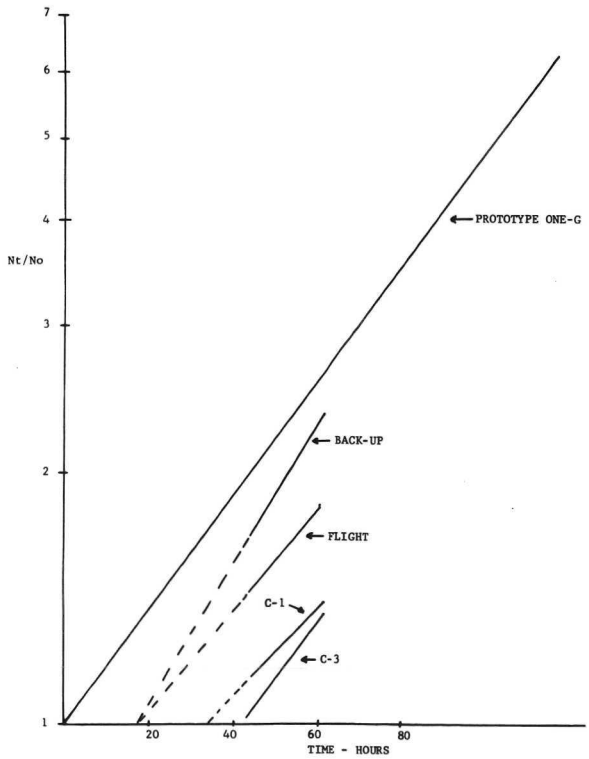

FIGURE 12.– Plasmotomy rates during flight.

TABLE 7. – Statistical Comparison of Fed Flight Fixed Amoebae

[F, flight; B, backup; C1, control 1; C3, control 3]

Chambers 19, 20, and 21	*P* value
F to B	Not sig.
F to $\frac{C1+C3}{2}$	0.025 to 0.01
F to C1	0.05 to 0.025
F to C3	0.025 to 0.01
C1 to C3	Not sig.
F to $\frac{B+C1+C3}{3}$	Not sig.[a]

[a] Backup ×0.9 to compensate for elevated backup temperatures gives *P* value of 0.2 to 0.1.

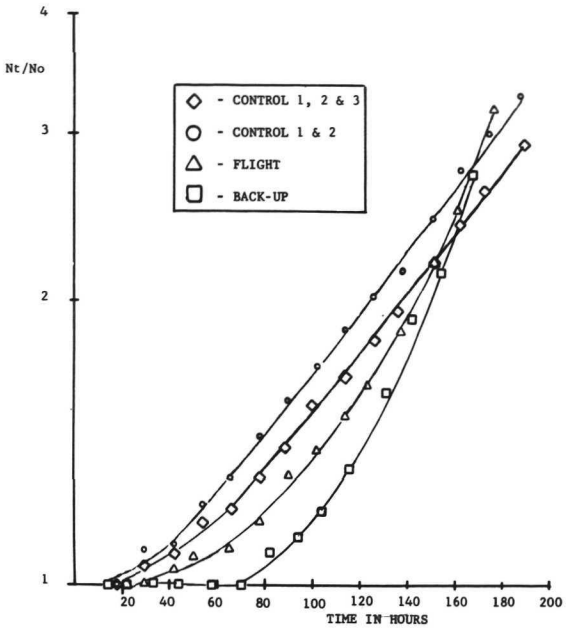

FIGURE 13.— Postflight plasmotomy rates.

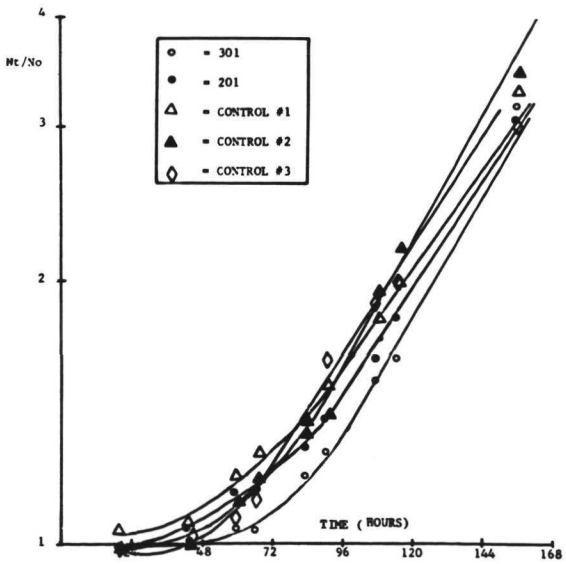

FIGURE 14.— Postflight control plasmotomy rates.

TABLE 8. — Flight and Postflight Earth Control Average Cell Division Rates, *Nt/No*

Amoebae	Flight *Nt/No*					Postflight earth control *Nt/No*				
	Flight	Backup	Control 1	Control 2	Control 3	301	201	Control 1	Control 2	Control 3
Starved, fixed	0.75	0.99	0.59	1.08	0.73	0.72	0.67	0.77	.....	0.88
Starved, live	.87	1.12	.83	.91	.99	.93	.94	1.30	0.99	1.19
Fed, fixed	1.28	1.60	1.12	1.10	1.05	.96	.95	1.03	.....	1.05
(chambers 19, 20, 21)	(1.70)	(2.11)	(1.31)	(1.26)	(1.25)	(1.02)	(1.13)	(1.15)	.....	(1.11)
Fed, live	2.19	2.37	2.13	2.61	1.83	1.80	1.71	2.04	2.48	3.02

Control 1: Flight simulation
Control 2: Constant temperature
Control 3: Variable temperature

301: Flight simulation with acceleration and vibration
201: Flight simulation without acceleration and vibration
Control 1: Flight temperature
Control 2: Constant temperature
Control 3: Constant temperature

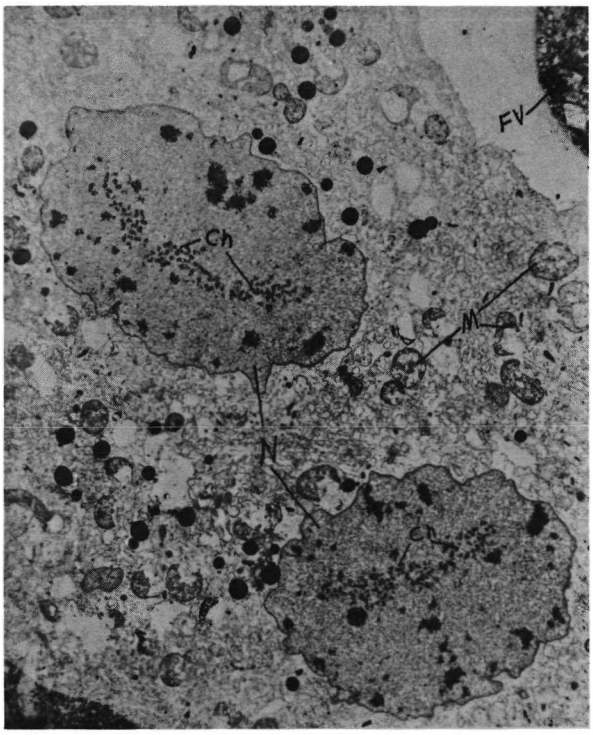

FIGURE 15.— *Pelomyxa* fixed during weightlessness showing prophase nuclei (N), chromosomes (Ch), mitochondria (M), and a partially digested food vacuole (FV).

Nuclear Division

Of those amoebae fixed during weightlessness, one was found to be in prophase (fig. 15) and another was in early interphase (fig. 16). In both of these amoebae, mitosis was in synchrony: i.e., all nuclei were in the same phase of mitosis. This synchrony of nuclear division is always observed under gravity (control) conditions. The flight prophase was found in chamber 8, one of the starved group. Thus, nuclear division did occur in the starved group during flight. The early interphase amoeba was found in chamber 19, one of the fed group. An interphase nucleus from the flight group is shown in figure 17 and one from the control group, in figure 18. Also of interest is the very irregular shape of some of the interphase nuclei (fig. 17) fixed during weightless flight.

Nuclear division was synchronous during the postflight Earth control test, as were the amoebae fixed during flight. Electron photomicrographs taken after the postflight Earth control test also showed irregular nuclear shapes in the control as well as in the 301-unit amoebae which received acceleration and vibration (fig. 19). These irregular nuclei probably were artifacts caused by glutaraldehyde fixation.

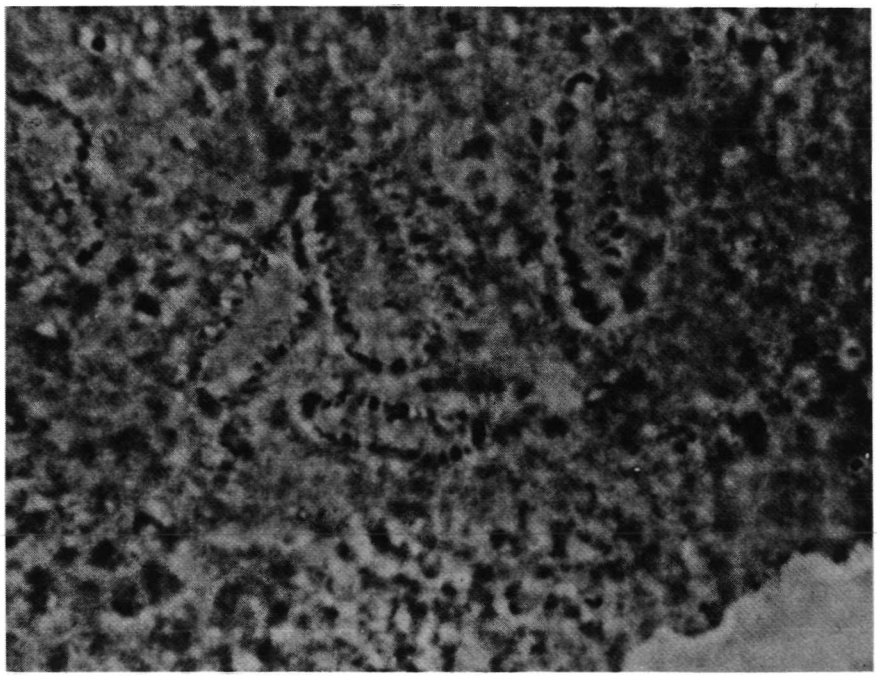

FIGURE 16.— *Pelomyxa* fixed during weightlessness showing early interphase nuclei.

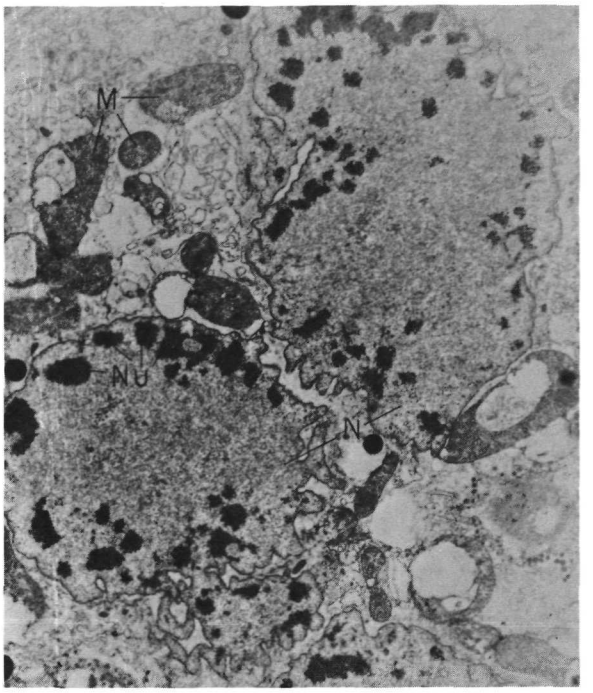

FIGURE 17.— *Pelomyxa* fixed during weightlessness showing interphase nuclei (N), nucleoli (Nu), and mitochondria (M).

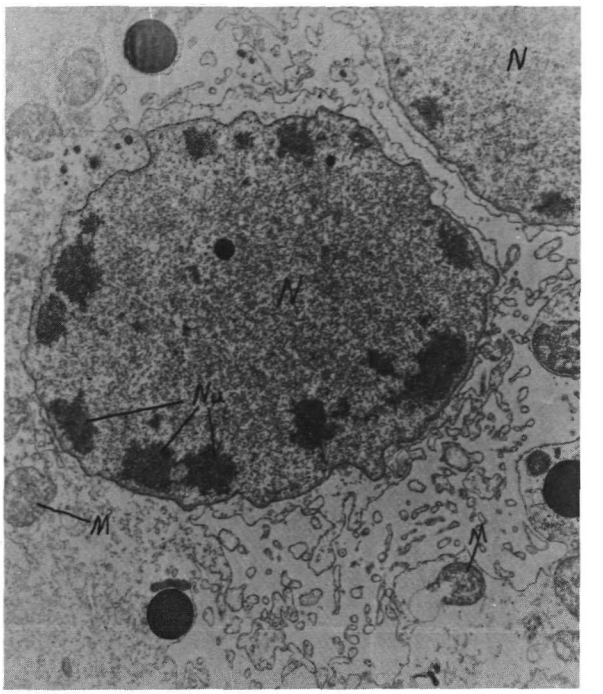

FIGURE 18.— Interphase nuclei from control showing nucleus (N), nucleoli (Nu), and mitochondria (M).

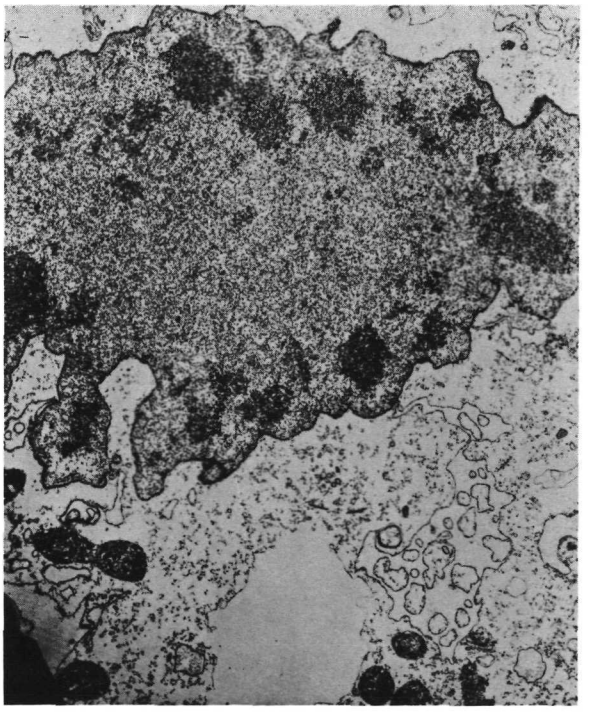

FIGURE 19.— Control test nucleus.

Clinostat Studies

Rotation at 6 rph, 1 rpm, and 20 rpm produced no statistically significant differences in growth rate (table 9). At 4 rpm, the growth rate of the horizontal clinostat group was significantly lower than that of the static controls. Repeated use of the polycarbonate chambers probably was responsible for the lower overall growth rates of the 4-rpm tests. Results of the 10-rpm study showed a significant difference in the horizontal clinostat when compared with both the vertical rotator and the static controls. Rotation at 100 rpm produced a highly significant difference when the static group was compared with the horizontal clinostat and the vertical rotator controls.

DISCUSSION

Even though several cell divisions occurred during the loading-to-unloading or fixation time period, there is no direct evidence that these divisions occurred during weightlessness. However, division during weightlessness can be demonstrated indirectly from plasmotomy data by refuting a null hypothesis (no weightless divisions) as shown

TABLE 9. – Clinostat Division Rate Studies with *Pelomyxa carolinensis*

Rotation rate	g	Average 3-day growth rates, Nt/No			Significance	P(a)
		Horizontal clinostat	Vertical rotator	Static		
6 rph	8.6×10^{-7}	4.13	4.51	4.52	Not sig.	
1 rpm	8.6×10^{-5}	3.86	3.94	3.83	Not sig.	
4 rpm	1.37×10^{-3}	2.57	2.79	2.84	Horizontal clinostat to static	<0.025
10 rpm	8.5×10^{-3}	4.18	4.60	4.73	Horizontal clinostat to static	$0.1<P<0.025$
					Horizontal clinostat to vertical rotator	$0.025<P<0.05$
20 rpm	3.43×10^{-2}	3.57	3.82	3.73	Not sig.	
100 rpm	8.57×10^{-1}	3.43	3.68	4.29	Horizontal clinostat to static	<0.001
					Vertical rotator to static	$0.001<P<0.005$
Average		3.62	3.89	3.99		

a At least 70 degrees of freedom.

graphically in figure 20. If it is assumed that the flight fed amoebae divided at a rate equal to the highest rate seen in a control chamber (Nt/No = 3.30) before and after weightless flight and did not divide during weightless flight, the solid growth curve of figure 20 would result. The average of flight amoebae fixed before reentry was higher (1.84 versus 1.35) than that predicted by this null hypothesis. Similarly, the number of flight amoebae recovered alive was greater than that predicted (3.60 versus 1.73). If it is further assumed that the flight amoebae divided at a rate high enough to give an Nt/No of 1.84 before weightless flight (broken line), this rate must have been higher than that ever observed in flight hardware (Nt/No = 8.1 for 96 hours). Even if this had happened before and after weightless flight, the number of live amoebae predicted would have been less than that actually recovered. Thus, this null hypothesis must be discarded in favor of cell division during weightlessness. Because the rate of cell division during weightlessness was at least equal to the rate of the static Earth controls, it does not appear that the acceleration and vibration experienced during powered flight inhibited cell division.

In the postflight Earth control test, the 301 and 201 vehicles experienced identical environments,

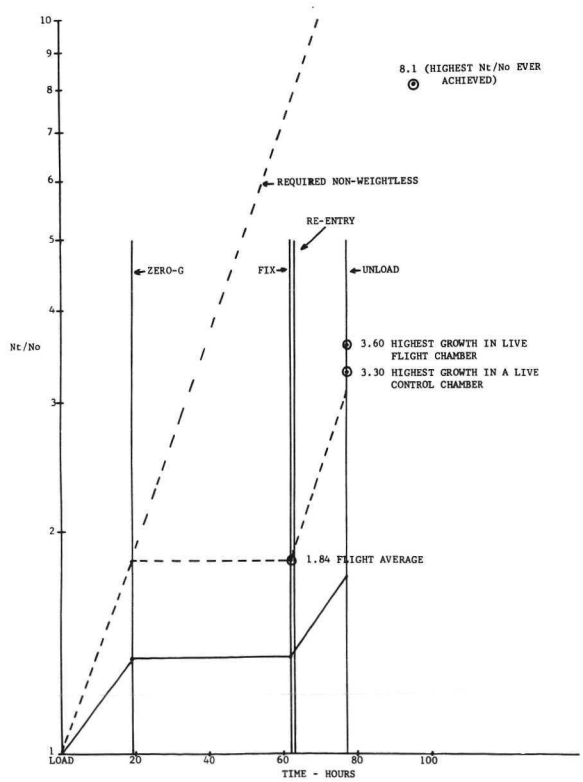

FIGURE 20.– Plasmotomy during weightlessness.

with the exception of vibration and acceleration. Thus, the results from these vehicles are best compared to determine the effects of these dynamic stimuli. Since table 8 shows no differences in the 301 and 201 cell division rates, it appears that Biosatellite II vibration and acceleration levels (figs. 3 to 8) had no effect upon the growth rate of amoebae. Simulation of Pogo vibration, during which no amplification nor cell ruptures were observed, corroborates this evidence. The control 1 profile, which followed the flight temperature profile, may be compared with the 201 profile to determine the effect of handling and transportation experienced by the 201 vehicle. Since the division rates of the live amoebae in control 1 were higher than that in the 201 vehicle, it appears that the handling of the live amoebae inhibited cell division. The significantly higher division rate of the fed live amoebae of control 3, compared with that of control 1, could be due to the higher, constant temperatures of control 3.

The postflight Earth control test starved amoebae had division rates similar to those of the flight starved amoebae. Although the results are not statistically significant, the division rates of the fed flight amoebae were higher than those of the 301 unit in the Earth control test. Growth of the fed amoebae in chambers 19, 20, and 21, which were fixed at different time periods, all exceeded the growth in corresponding chambers in the Earth control 301 unit.

If it is assumed that cellular organelles the size of mitochondria and larger settle within cells (Audus, 1962; Gordon, 1963; and Pollard, 1965), this settling should occur at 1 *g*, but not during weightlessness. A clinostat which continually alters that part of the cell presented to the Earth's *g* vector should also prevent settling and, in this sense, simulate weightlessness. The division rates of amoebae on the clinostat were, in all cases, similar to or lower than those of either static controls or amoebae rotated in a plane normal to the Earth's *g* vector. These data suggest that the growth rate of amoebae during weightless flight would be either similar to or lower than that observed on the Earth's surface. The lack of significance between the division rates of flight and Earth control amoebae tends to corroborate this. Since the division rates in orbital flight were not significantly higher than those of the Earth and clinostat controls, we assume that weightlessness has no stimulating effect. Since the flight amoebae showed a trend toward a faster division rate, additional flight experiments are in order.

SUMMARY AND CONCLUSIONS

No significant differences in division rates between the flight and Earth control groups have been found. There appears to be a trend toward a higher division rate in well fed *Pelomyxae* during weightlessness. Nuclear division during weightlessness is synchronous as in the Earth controls and postflight Earth control test. No difference is apparent in the postflight cell division rates of the flight group as compared with those of the Earth controls. Postflight Earth control test division rates are similar to the postflight results.

Flight vibration and acceleration had no observable effect upon the growth rate of amoebae.

Division rates of the fed flight amoebae were higher than those of the 301 postflight Earth control. The differences are not statistically significant.

Horizontal and vertical rotation of *Pelomyxae* at 6 rph, 1 rpm, and 20 rpm produced no statistically significant differences. Division rates of amoebae horizontally rotated at 4, 10, and 100 rpm were significantly lower than those of static controls. Vertical rotation at 100 rpm produced growth rates significantly lower than those of the static controls.

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Effects of Weightlessness on the Nutrition and Growth of *Pelomyxa carolinensis*

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Life, as we know it, presumably evolved on this Earth at approximately a 1-*g* centrifugal force. This study attempted to answer the question, Can animal cells which are already preformed and viable sustain the delicate balance of life, grow, and divide in the absence or near absence of a gravitational field? Pollard (1965) has suggested that they may not because of calculations which show that the critical mass of some organelles, such as mitochondria and nucleoli, are great enough for them to be irreversibly damaged by prolonged weightlessness.

The carnivorous giant amoeba, *Pelomyxa carolinensis,* was the organism chosen for study because many of the basic physiologic processes of amoebae have been extensively studied and are well understood. Foodstuffs, for example, are taken up either by the processes of pinocytosis or phagocytosis (Christiansen and Marshall, 1965; Wetzel and Korn, 1969; Ulsamer et al., 1969), incorporated into food vacuoles, and broken down by acid and alkaline hydrolases (Chapman-Andresen and Lagunoff, 1966; Müller, 1969; Rothchild, 1967). Digested materials are absorbed by the cell and utilized for cellular anabolism. Undigested materials in old food vacuoles or residual bodies are moved to the cell surface and discarded by a process of exocytosis. The excretion of wastes which accrue from cell metabolism as well as excess water are moved out of the cell by large contractile vacuoles. The distribution and fine structure of mitochondria, nuclei, nucleoli, Golgi apparatus, and endoplasmic reticulum in these organisms also have been described in great detail under a variety of experimental conditions. In this study, the fine structure and distribution of each of these structures were surveyed for changes that may have been induced by weightlessness. Furthermore, an attempt was made to determine if there were a normal growth pattern or a normal progression of food vacuole digestion in amoeba subjected to a weightless environment. This was accomplished by both fixing and feeding in space after variable periods of starvation.

EXPERIMENT DESIGN AND METHODS

The amoebae, *Pelomyxa carolinensis,* were cultured in 1 M Pace and McCashland buffer (1951) in stacking dishes and were subcultured two or three times weekly. Amoebae were placed in 0.05 M tris (hydroxymethyl) aminomethane (THAM) buffer and starved for 1, 2, or 3 days before launch or simulated launch. Fed amoebae were placed in the THAM buffer 12 hours prior to loading and were allowed to feed continuously on paramecia. *Paramecium micronucleatum* were concentrated in 0.075 M THAM glycyl glycine buffer, and the number of paramecia per milliliter was determined.

The details of the methodology for this experiment are described in the paper by Ekberg et al., page 273.

Three days prior to launch or simulated launch, all chambers were rinsed in 2 percent $Na_2S_2O_5$ for 15 minutes, rinsed repeatedly, submerged in 2 percent $KMnO_4$ for 2 hours, and repeatedly rinsed again. Five complete units, each comprised of 24 modules, were assembled 2 days before launch or

simulated launch. All modules were then preconditioned with THAM glycyl glycine buffer for 9 hours prior to loading. Loading of the organisms into the chambers began 18.5 hours before launch and continued for 2 hours. Healthy, nondividing amoebae were selected, counted, and picked up with Pasteur pipets and the correct number were placed into the proper compartment of each module.

The polycarbonate resin (Lexan) modules for this experiment were divided into three 5-cc compartments separated by pistons.

After they were loaded, the compartments were filled with THAM glycyl glycine buffer with a small airspace left. The flight, backup, and control chambers were mounted into experiment packages.

Analysis Procedures

After recovery of the package, the modules with live amoebae were opened, the contents removed, and the chamber gently flushed with buffer. All the live organisms were counted and examined for the presence of mitotic forms. Food vacuoles were counted and graded, and the general external morphology of the cell was described (table 1).

Coinvestigators from General Electric Co. took all the live and fixed amoebae that were dividing, plus one-half of the remaining live amoebae. The latter amoebae were divided into three groups: Group I, approximately 40 percent of the total, was incubated for succinic dehydrogenase (Seligman et al., 1967), using thiocarbamyl-nitro blue tetrazolium (TC–NBT), and fixed in glutaraldehyde for 3 hours. Group II, about 10 percent of the total, was incubated as above, but without substrate. Group III, 50 percent of the total, was fixed for 2 hours in 3 percent glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. All organisms were postfixed in 1 percent osmium tetroxide, buffered in 0.1 M phosphate buffer at pH 7.4, dehydrated, and embedded in Vestopal W or Epon for electron microscopy.

The organisms that were fixed in flight (modules 2 to 5, 7 to 9, 11, 12, 14, and 18 to 21) were placed in a rinsing solution of Pace-McCashland buffer with 1 percent sucrose added, counted, and photographed, and their food vacuoles were counted and graded (tables 1 and 2).

The fixed organisms were divided into five groups. Group I, 20 percent of the total, was incubated for acid phosphatase activity according to a modification of the Gomori technique (Barka and Anderson, 1965). Group II, 10 percent of the total, was incubated in the acid phosphatase me-

TABLE 1. – Growth Rates of Amoeba From the Biosatellite II Flight Experiment and Earth Controls

Experiment duration, hr[a]	Module number	Growth rates									
		Flight	Simulated flight′	Backup control	Backup control′	Control I	Control I′	Control II	Control II′	Control III	Control III′
1	1, 17	0.64	0.60	0.61	0.60	0.54	0.62	0.87	0.64	0.69	0.63
12	2, 18	.66	.90	.93	.95	.85	.83	.80	.70	.69	.89
24	3, 7, 19	.98	.73	1.02	.56	.78	.80	1.08	.81	.82	.81
42	4, 8, 11, 20	.95	.78	1.45	.69	.71	.67	1.19	.68	.87	.67
43	5, 9, 12, 14, 21	.93	.61	1.24	.61	.86	.93	1.19	.87	.84	.....
50	6, 10, 13, 15, 16, 22, 23, 24	1.37	.60	.....	.....	1.32	1.25	1.55	1.27	.....	.....
54	6, 10, 13, 15, 16, 22, 23, 24	.....	.....	1.59	.77	.....	.....	.....	.....	1.30	1.58

[a] Duration of experiment determined from time of launch to fixation.

dium without substrate. Group III, 20 percent of the total, was incubated for either esterase activity (Gomori, 1952) or alkaline phosphatase activity (Hugon and Borgers, 1966). Group IV, 10 percent of the total, was incubated for esterases or alkaline phosphatases without substrate. Group V, approximately 40 percent of the total, was rinsed several times and postfixed for 1 hour in 1 percent osmium tetroxide buffered with 0.1 M phosphate buffer at pH 7.4 and prepared for morphologic studies. After cytochemical incubations, the organisms from the first four groups were rinsed, postfixed in osmium, dehydrated, and flat embedded in Vestopal W. Extensive enzyme inhibitor and substrate control studies were run on Earth-based amoebae to check out the specificity of these reactions.

The backup package of the flight experiment was flown to Hawaii where its amoebae were analyzed and treated identically with those of the flight package. The other three control packages were maintained and disassembled at Cape Kennedy, Fla. The amoebae were counted and their food vacuoles were counted and graded. The live organisms were fixed in buffered 4 percent glutaraldehyde and all organisms were then postfixed in buffered 1 percent osmium, dehydrated, and flat embedded in Vestopal W.

Postflight Experiment

In order to trace more precisely the sequence of events involved in the digestion of food vacuoles in normal amoebae, horseradish peroxidase was added to the paramecia cultures. Amoebae engulf this enzyme along with paramecia. The reaction product derived from peroxidase activity with incubation according to the technique of Graham and Karnovsky (1966) is both a chromophore and osmiophilic. With this product used as a marker, the pattern of digestion in food vacuoles was followed by tracing the progressive loss of peroxidase activity. Amoebae were pulse labeled with peroxidase for 30 minutes, rinsed in fresh media, and then cultured for 4 hours, 2 hours, 1 hour, and ½ hour prior to fixation in glutaraldehyde. The activities of other enzymes were assayed in postflight experiments; the interpretive value of the results obtained was small, however, and is not worth reporting here.

Growth rates and food vacuole data were then carded and prepared for computer analysis. Statistical analysis by tests of variance, standard deviation, and measures of randomness on growth rate and food vacuole data, plus the plotting of histograms, was performed with the aid of a computer program written specifically for these data (Angleton, 1967). Analysis was performed on the CDC 6400 computers at Colorado State University and the University of Colorado computing center.

Subsequently, morphologic studies on the flat embedded organisms were done by cutting alternate groups of 1-micron-thick and ultrathin sections from each amoeba on an LKB and Porter Blum MT2 ultramicrotome. The thick sections were

TABLE 2. – Summary of Biosatellite II Growth Rates of Amoebae From the Flight Experiment and Controls as a Function of the Major Experimental Variables

[The data are shown separately for the backup control carried to the recovery site and controls I, II, III since they were maintained and processed at the launch site. The prelaunch starvation groups were refed in space so that total duration was from 63 to 76 hours.]

Prelaunch feeding (a)	Study condition	Module	Simulated flight′	Flight	All controls′	All controls	Controls I′, II′, III′	Controls I, II, III	Backup control′	Backup control
3 days S....	Fixed	1, 2, 3, 4, 5	0.45	0.75	0.62	0.79	0.64	0.76	0.46	0.84
3 days S....	Alive	6	.53	1.07	.84	.95	1.01	1.02	.50	.77
2 days S....	Fixed	7, 8, 9, 11, 12	.42	.74	.72	.87	.73	.77	.43	1.07
2 days S....	Alive	10, 13	.49	.82	.87	.91	.93	.87	.40	1.05
1 day S.....	Fixed	14	.37	.83	1.63	1.01	1.23	.91	.47	1.33
1 day S.....	Alive	15, 16	.44	.84	1.07	1.01	1.26	.90	.48	1.36
CF.....	Alive	17, 18, 19, 20, 21	1.29	1.28	.98	1.21	.90	1.09	1.13	1.59
CF.....	Alive	22, 23, 24	.83	2.19	1.95	2.23	1.85	2.19	1.30	2.36

[a] S, starved; CF, continuously fed.

mounted on glass slides and stained with a variety of stains such as toluidine blue, PAS, feulgen, and Alcian blue. The plastic was removed, when necessary, from the sectioned amoeba (Lane and Europa, 1965). Thin sections were mounted on copper mesh grids, stained with lead citrate (Reynolds, 1963), or doubly stained with lead citrate and uranyl acetate and viewed under a Siemens Elmiskop 1A from 200 × to 80 000 ×.

RESULTS

Gross Morphology

Immediately after recovery, the live organisms from the flight, simulated flight, and backup packages were counted and then studied under the light microscope for gross morphologic changes. In all control experiments and in the organisms flown in Biosatellite II, the presence of newly ingested paramecia in many amoebae from each module indicated that feeding continued in the continuously fed amoebae and refeeding occurred in the starved amoebae.

No differences in gross morphology between the live flight, the simulated flight, and the live backup control organisms were evident. All the amoebae studied alive appeared to be intact with no fragmentation evident. Cytoplasmic streaming was evident and the amoebae were forming new pseudopodia, but there were relatively few newly formed food vacuoles in the flight organisms recovered alive. In general, the amoebae had maintained their free-form, heteropodal shape. Distinct gross morphologic differences were evident, however, between amoebae in the different modules of the same experiment depending on whether the amoebae were starved for long periods or fed continuously. Hungry amoebae recovered from modules 15 and 16 of the flight and the simulated flight and backup control experiments were generally very elongate with extended pseudopodia, had condensed cytoplasm, and had few food vacuoles. Continuously fed organisms recovered alive from modules 22, 23, and 24 were generally much larger than their starved counterparts, contained numerous contractile vacuoles, were more rounded with lobose pseudopodia, and had a less dense cytoplasm. Organisms from other modules of the live group (modules 6, 10, 13, 15, and 16) contained forms that were transitional between the fed and hungry extremes, and there was a great deal of individual variation in the cellular outlines. In general, the amoebae recovered from any of the packages were not so large and robust as the continuously fed amoebae maintained in the laboratory in finger bowls under constant culture conditions.

Observations of fixed amoebae from the flight and simulated flight and backup controls indicated that the characteristic shape of the amoebae with pseudopodia and newly formed food cups was established within 1 hour after launch or simulated launch. It was maintained throughout the flight period. Although it was possible to distinguish between unfed organisms and those that fed heavily, it was not possible to identify a change in either type that was brought about by weightlessness. There were also no detectable alterations in gross morphology of the amoebae that would indicate a cumulative effect of prolonged weightlessness in the fixed flight amoebae. Amoebae starved from 1 to 43 hours in space apparently refed every time that food was added, since new food vacuoles were observed in every organism from the modules where mixing occurred.

Effects of Weightlessness on Survival and Growth

Growth rates (tables 1 and 3) indicate that growth rates and survival of *P. carolinensis* in flight were similar to the growth rates computed for the flight controls. (The growth rate Nt/No is defined as the total number of amoebae recovered Nt divided by the total number of amoebae loaded No.) There was, however, no significant growth in the simulated flight organisms. The approximate growth rate for all the packages in each experiment (flight and simulated) were similar. The growth rate for the simulated experiment was significantly reduced from that of the flight experiment. (In fact, table 7 shows that the conditions of reentry may have been extremely hard in the simulated flight experiment.)

There was a mean growth rate of 0.70 for amoebae fixed 1 hour after launch. In the simulated flight experiments these two figures were, respectively, 0.62 and 1.08. Growth rates plotted as a function of time of fixation (fig. 1) show that the amoebae entered into the period of most rapid growth at the time of retrofire and reentry in the flight experiment. This was true only for control organisms in the simulated flight. In the flight experiment, the backup control packages had high-

TABLE 3. – Growth Rates of *P. carolinensis* in Each Module for the Biosatellite II Flight Experiment and All Earth Controls

Module	Number of amoebae loaded	Growth rates									
		Flight	Simulated flight′	Backup control	Backup control′	Control I	Control I′	Control II	Control II′	Control III	Control III′
1	200	0.65	0.52	0.76	0.47	0.46	0.58	0.67	0.65	0.62	0.62
2	100	.65	.52	.71	.54	.67	.52	.95	.64	.61	.73
3	60	.85	.38	.67	.43	.70	.65	.90	.47	.63	.58
4	40	.98	.45	1.10	.43	.50	.53	1.45	.28	.67	.83
5	30	.60	.40	.97	.43	.53	.70	1.30	.73	.80	1.07
6	30	1.07	.53	.77	.50	1.03	.93	.90	.97	1.13	1.13
7	60	.70	.38	.80	.35	.55	.87	.95	.73	.82	.85
8	40	.70	.50	1.18	.35	.60	.68	1.23	.50	.57	.98
9	30	.90	.40	1.30	.43	.73	.70	.73	.67	.77	.60
10	30	.87	.50	1.07	.43	.63	.63	.90	1.03	1.23	1.23
11	40	.63	.53	1.12	.60	.57	1.00	1.10	.75	1.02	.75
12	30	.77	.27	.97	.43	.55	.50	1.26	.73	.73	.70
13	30	.77	.47	1.03	.37	.63	.77	.90	.83	.93	1.10
14	30	.83	.37	1.33	.47	.63	1.03	1.33	1.20	.77	1.43
15	30	.90	.40	1.26	.63	1.20	1.17	1.20	1.23	.70	1.00
16	30	.77	.47	1.46	.33	.67	1.13	.67	.97	.93	2.03
17	190	.63	.67	.46	.74	.62	.66	1.07	.63	.76	.63
18	88	.68	1.07	1.18	1.36	1.04	1.08	.64	.75	.77	1.05
19	54	1.42	1.44	1.66	.89	1.13	.87	1.42	1.22	1.02	1.24
20	30	1.66	1.63	2.73	1.37	1.30	.47	.90	1.17	1.33	.13
21	18	2.00	1.63	1.94	1.28	1.50	1.72	1.44	1.00	1.39	.88
22	30	1.53	.33	1.83	1.07	2.03	1.70	2.40	1.70	1.86	1.80
23	30	1.43	1.43	3.30	1.13	1.07	1.83	3.00	1.87	1.80	2.03
24	30	3.60	.73	1.96	1.70	2.30	1.87	2.43	1.57	1.83	2.30

er growth rates throughout most of the experiment period but showed a reduction in growth during the period when the organisms from the other packages began their most rapid growth. There was no such correlation among the organisms from the simulated flight packages. Some time during the period of reentry (42 to 45 hours postlaunch) the growth rates of the starved and refed amoebae (0.7 to 0.9) started to approach the growth rates (1.3+) for the continuously fed controls in the fixed group (fig. 1), but this did not occur in the simulated flight experiment. In all experiments,

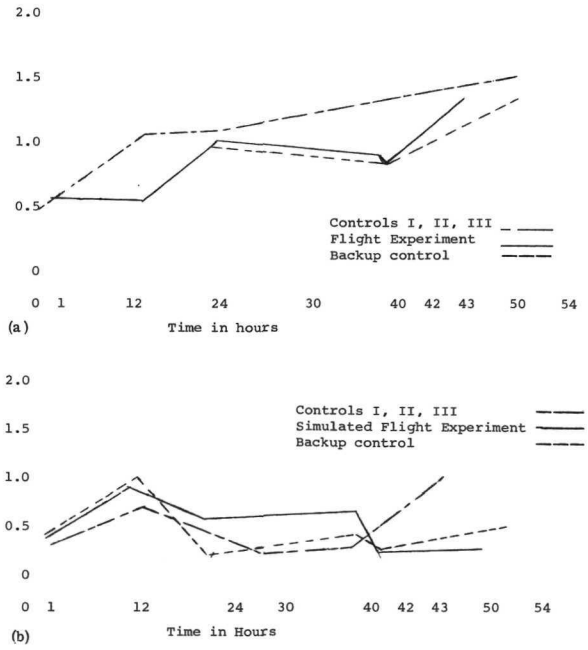

FIGURE 1.– Growth rates of *P. carolinensis*. Amoebae were loaded into experiment modules 18 hours before launch. Numbers of amoebae loaded were the same in all experiments. (*a*) Biosatellite II flight and Earth control experiments during time from loading to fixation. (*b*) Simulated flight and associated Earth control experiments during time of simulated launch.

the growth rate studies have shown that the continuously fed organisms had better growth than did the starved and refed ones and that the dependency of growth on feeding was not altered as a result of weightlessness. Highest growth values were achieved among the flight groups as well as in most controls in the amoebae that were recovered alive.

The growth data (tables 1 to 4) indicate that cell divisions probably took place in many (flight and control) organisms during the time from retrofire to opening of the capsule. Analysis of the growth rates from all of the treatment groups further suggested that *P. carolinensis* retained their ability to divide under weightlessness both in cells fed continuously and in cells refed after short periods of starvation. Starvation periods employed in these experiments did not alter the amoebae's capacity for division when feeding was resumed. This relationship was similar in the flight and in the Earth control experiments.

Counts of food vacuoles performed immediately after recovery (table 5) indicated that the mean number of food vacuoles in flight amoebae that received from 63 to 76 hours of food deprivation was 8.9 vacuoles per cell (range of 1.2 to 17.2), as compared with 6.9 vacuoles per cell (range of 4.0 to 12.1) in the flight amoebae that had been fed continuously. In the backup control experiments, the mean number of food vacuoles per amoeba was 5.2 (range of 2.0 to 10.8) for the starved group and 5.7 (range of 1.7 to 15.9) for the continuously fed amoebae. The three Earth control experiments maintained for the flight period and analyzed at Cape Kennedy showed a range in the numbers of food vacuoles between 4.1 and 19.2 vacuoles per cell in the starved refed group, and between 1.7 and 28.5 in the continuously fed group. Computer analysis of these data (Estabrook, 1967) showed that there was a significant correlation between the feeding and growth data. In other words, those modules containing organisms which were continuously fed and had the highest mean number of food vacuoles had the highest growth rates.

It was stated in the preliminary report on the flight of Biosatellite II (Saunders, 1968) that computer analysis showed that there was a reduction in the rate of food vacuole digestion in organisms from the flight experiment as compared with that from the backup control (figs. 2 and 3). There was no cytologic evidence to back this up and the staff that made these counts was not well enough trained to produce repeatable results. In repeat experiments, we had four members of the team plot the food vacuole progression in the same pack-

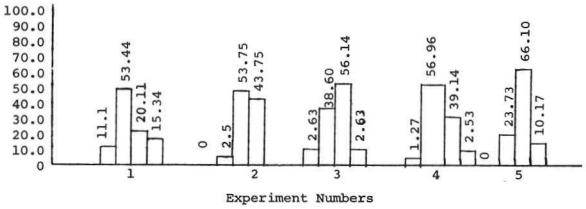

FIGURE 2.— Computer-plotted histogram (Angleton, 1967) comparing the distribution (frequency percent) of the four classes of food vacuole data for module 3 from flight experiment 1 and Earth control experiments 2 to 5.

TABLE 4. — Summary of Growth of *P. carolinensis* From the Biosatellite II Flight Experiment and All Earth Controls

[The same number of amoebae was loaded in each experiment. No data are available for postflight experiments]

Group	Flight	Backup control	Control I	Control II	Control III
Number of amoebae loaded	1280	1280	1280	1280	1280
Number of amoebae recovered	1142	1363	1029	1392	1102
Number of amoebae recovered live	328	381	317	372	313
Number of amoebae recovered fixed	814	982	712	1020	789
Growth rate of amoebae recovered live	1.37	1.59	1.32	1.55	1.30
Growth rate of amoebae recovered fixed	0.636	0.767	0.556	0.797	0.616
Mean growth rate of all amoebae	0.892	1.06	0.804	1.09	0.861

ages so that we could compare their results. The data collected (fig. 4) show that this method of food vacuole analysis cannot be used to draw valid conclusions. In fact, there is good reason to question many of the quantitative aspects of food vacuole counts recorded in earlier reports.

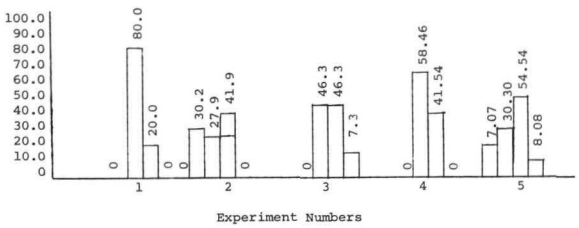

FIGURE 3.—Computer-plotted histogram comparing the distribution (frequency percent) of the four classes of food vacuole data for module 14 from flight experiment 1 and Earth control experiments 2 to 5.

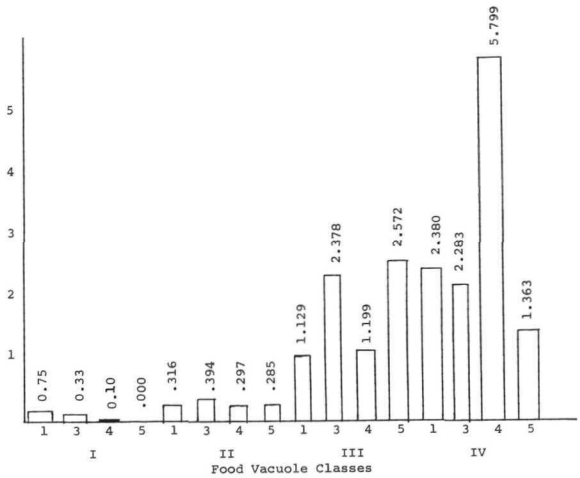

FIGURE 4.—Comparison of observed vacuole frequencies in *Pelomyxa* between the members of the study team for all experiments.

TABLE 5. – Tabulation of Mean Number of Food Vacuoles per Amoeba in Each Module From Biosatellite II Flight Experiment and Earth Controls

[The significance of these data is questionable]

Module	Mean number of food vacuoles				
	Flight	Backup control	Control I	Control II	Control III
1	5.60	3.0	7.8	5.8	5.7
2	11.33	6.0	14.8	16.5	12.1
3	17.2	8.9	11.4	7.9	5.9
4	14.8	4.1	7.7	4.1	14.9
5	9.5	2.2	9.7	6.4	8.2
6	3.6	2.3	11.6	7.4	29.2
7	8.5	10.8	9.6	5.6	8.6
8	10.4	6.5	11.6	7.6	16.0
9	8.7	7.3	11.7	5.3	10.0
10	3.2	2.0	7.0	8.8	10.4
11	10.1	5.1	9.8	6.8	9.9
12	14.5	7.8	7.8	5.9	10.4
13	3.3	4.8	9.4	7.5	10.1
14	5.0	7.2	5.1	6.5	9.9
15	3.8	2.8	9.6	4.8	4.3
16	1.2	2.8	8.7	6.1	6.6
17	12.1	10.6	15.9	8.4	14.7
18	8.5	15.9	11.5	12.0	28.5
19	6.0	6.3	7.2	8.3	7.5
20	6.1	3.3	7.1	6.3	6.6
21	5.2	1.8	8.6	1.8	7.2
22	4.0	3.3	6.7	5.2	5.9
23	6.1	1.7	5.8	5.9	5.4
24	7.9	2.7	6.1	5.6	5.3

Currently, there are substantial data to indicate that: (1) The numbers of food vacuoles formed in flight amoebae were approximately the same as those in the control packages; (2) the rate of digestion of food vacuoles was unaltered in the flight organisms as compared with the various ground controls; (3) the percent of amoebae loaded and the percent recovered, both of continuously fed forms and those receiving some period of starvation, were basically similar for similar treatments in the flight and control packages (table 6); (4) the recovery of organisms from the simulated flight and the simulated Earth-based controls was similar, but there was a dramatic reduction in the total number recovered from this experiment compared with that in the flight experiment; and (5) there was no significant reduction in the size of amoebae exposed to weightlessness.

Cytology

There was great variability in the surface outlines of flight, backup, and control amoebae as viewed in the 1-micron-thick sections. In a single amoeba, some areas of the surface were smoothly rounded and some were pushed into long, relatively smooth-surfaced pseudopodia. Still other areas were extremely tortuous with deep surface indentations and many microvilli. The surface of some pseudopodia even possessed numerous long, dendriticlike processes. Starved amoebae possessed the greatest degree of surface variations, whereas the continuously fed organisms were more rounded with fewer elongated pseudopodia. When they were stained with toluidine blue, the entire surface of all amoebae was outlined with a 0.2- to 0.3-micron-thick blue halo; no metachromasia was evident. No consistent morphologic, quantitative, or colorimetric differences could be noted in this surface between the flight, simulated flight, backup, or Earth control organisms that could not be attributed to the various nutritional states. There were no statistically significant differences in the thickness of the surface coat in any of the organisms.

Analysis of sectioned amoebae indicated that phagocytosis and digestion proceeded normally in all the various experiment packages, since there was a continuous series of newly formed, partially digested, and old food vacuoles within almost all of the continuously fed organisms. The numbers and appearance of food vacuoles from a flight experiment module were similar to those in the Earth controls. In all of the amoebae, including the flight organisms, newly ingested paramecia were found in food vacuoles with large, clear areas surrounding them. Acid phosphatase activity was abundant in most of the large, newly formed vacuoles (fig. 5). Smaller food vacuoles nearer the center of the amoeba contained alkaline phosphatase activity in all cases (figs. 6 and 7) as well as many narrow elongate channels and small vesicles in the peripheral cytoplasm. Food vacuoles observed in the cytoplasm ranged in size from 1 to 10 microns. Partially digested vacuoles contained a thick amorphous and homogeneous material near their surface and concentrated recognizable structures near their center and were also surrounded by a number of channels and micropinocytotic vesicles. Numerous well-organized mitochondria, abundant endo-

TABLE 6. — Relation of Percent of Amoebae Loaded to the Numbers Recovered in the Biosatellite II Flight Experiment and All Earth Controls as a Function of Fixation Time After Launch

Group	Time of fixation, hr					
	1	12	24	42	43	50 to 54
Percent of organisms loaded to be fixed at each time interval	30.3	14.6	13.6	11.7	19.2	.....
Percent of flight organisms recovered	21.7	10.9	13.5	11.4	9.9	28.7
Percent of simulated flight organisms recovered	20.2	13.1	9.2	7.9	6.1	11.8
Percent of all ground control organisms from flight experiment recovered	16.7	12.7	13.2	12.8	11.5	28.4
Percent of ground control organisms from simulated flight experiment recovered	21.1	13.0	9.3	7.0	7.4	30.1

plasmic reticulum, and golgi were observed in the areas of the small food vacuoles (figs. 5 to 20). The histologic details and the sequence of events associated with digestion in food vacuoles were similar in the flight and Earth control organisms. There was also no change in the distribution of hydrolytic enzymes in the flight organism (figs. 5 to 7). In summary, there was absolutely no cytologic evidence to indicate that there was a retardation or alteration in the rate of food vacuole digestion in the flight amoebae.

Residual bodies ranged between 0.1 and 1 micron in diameter, consisted of an electron dense net or dark meshwork of material enclosed by a membrane, and were generally located near the surface of the cell. Contrary to earlier reports (Roth, 1960; Christiansen and Marshall, 1965) a large number of small golgi bodies were evident adjacent to food vacuoles, in areas rich in rough endoplasmic reticulum, and in the cytoplasm near nuclei. The golgi were most prominent in continuously fed amoebae and their ultrastructure was not affected by either the flight or simulated flight conditions.

In order to develop an index on the normal sequence of events in food vacuole digestion, horseradish peroxidase was placed in the amoebae incubation media; it was washed out; and then the amoebae were fixed at various time intervals. After this, peroxidase labeled the extracellular fuzz and lined the numerous pinocytotic channels that arise at the surface of an amoeba (figs. 12 to 13). In newly ingested food vacuoles the peroxidase activity was spread throughout the food vacuole and also in the large channels that formed at its surface. As digestion proceeded, peroxidase activity was progressively lost at the surface of the food vacuole as well as in the associated channels and became restricted more and more to the dense undigested layer in the center of the food vacuole. In the smaller food vacuoles and in the residual bodies peroxidase activity had completely disappeared. This series of events indicates that there is, in fact, a progressive digestion of the material from the outside to the inside of a food vacuole. There is also a close correlation between these observations and the activity of the hydrolytic enzymes. Acid phosphatase activity was prevalent in the large food vacuoles, whereas alkaline phosphatase activity was prevalent in the older, smaller ones (figs. 5 to 7). Alkaline phosphatase activity was not demonstrable in food vacuoles until all of the peroxidase activity had disappeared. Nonspecific esterase activity was localized along the intracellular surface of the plasma membrane and on the cytoplasmic surface of all food vaculoes. Flight conditions did nothing to effect the demonstrability of this. Numerous nucleoli were scattered around the periphery of every nucleus. No differences in the distribution, size, or staining affinities of the nucleoli between the flight and various control amoebae or between the fed and starved forms could be detected. The size and spacial relationships of the nuclear pores in amoebae were similar for all the experimental groups. There is also no correlation between the contortions observed in the outline of the nucleus and the conditions of the flight. In other words, the undulations in the surface of the nucleus were present in both flight and control amoebae. A comparison of the ultrastructural details of nuclei and nucleoli and nuclear pores from the flight and simulated flight and backup Earth controls disclosed no major structural differences as a result of weightlessness or vibration and acceleration.

Plasma filaments were especially abundant in continuously fed cells. They frequently were organized into large bunches that formed near the center of the cell or as small discrete elongate fibrillar bundles at the periphery of the pseudopodia. The large bundles were especially prominent in areas that contained aboundant rough endoplasmic reticulum. The diameter of the filaments ranged from 70 to 200 Å. There was no detectable change in their size or distribution that would indicate that the conditions of flight or simulated flight affected them.

Mitochondria were distributed primarily around nuclei, food vacuoles, and contractile vacuoles (figs. 15 to 17 and 21). They were larger and more abundant in the continuously fed organisms than in the starved forms. Starved amoebae tended to have randomly distributed mitochondria. The size, shape, and structure of the mitochondria were highly variable. There was an outer limiting membrane approximately 60 to 80 Å wide and an inner membrane that was folded into multibranching tubular cristae. The cristae were folded extensively in starved amoebae. The matrices frequently contained numerous ribosomes. Neither weightlessness nor the simulated flight conditions

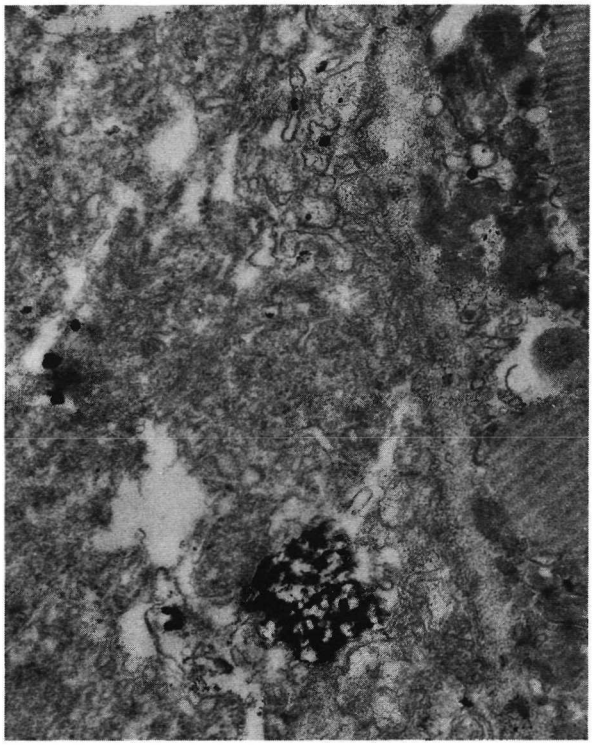

FIGURE 5.

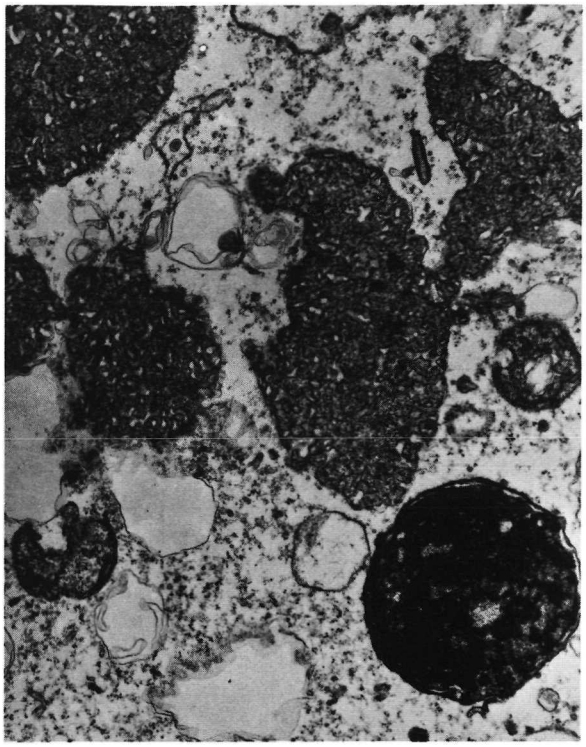

FIGURE 6.

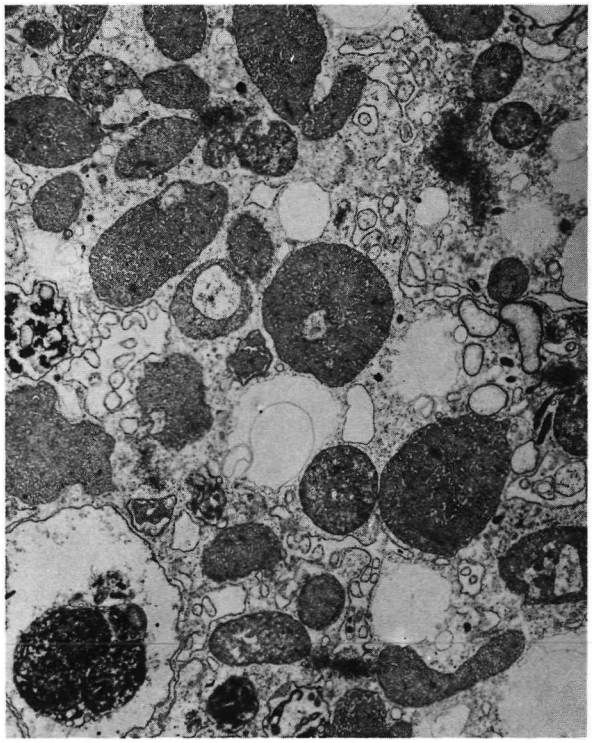

FIGURE 7.

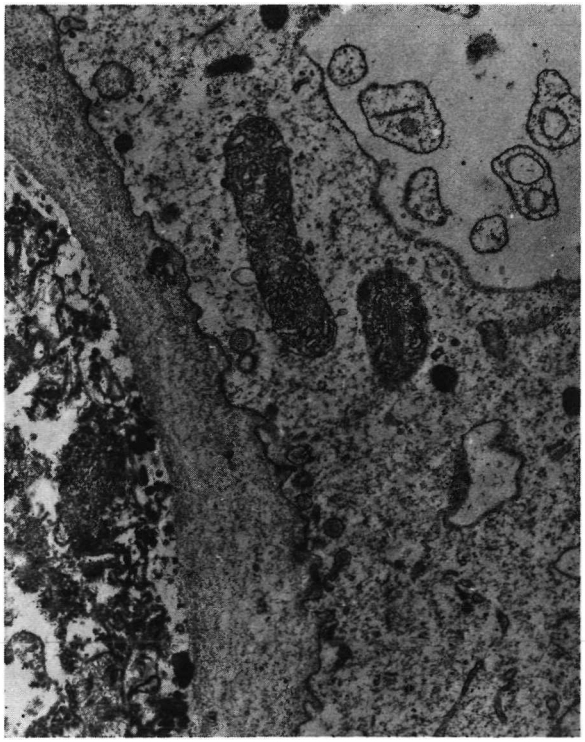

FIGURE 8.

FIGURE 5.— Newly formed food vacuoles; flight module 14; acid phosphatase. Acid phosphatase activity is generally not apparent in newly formed food vacuoles. However, small vesicles with accumulated reaction product begin to appear at their surface. An elaborate array of smooth-surfaced membrane-bounded vesicles and tubules also organize along their surfaces. 22 000 ×.

FIGURE 6.— Portion of a continuously fed amoeba from simulated flight module 20. Numerous mitochondria, abundant smooth and rough endoplasmic reticulum, and packages of golgi surround several small, dense phosphatase positive food vacuoles. Packets of protein and several clumps of plasma filaments are also evident. 9400 ×.

FIGURE 7.— Older food vacuoles from module 24; simulated flight backup; alkaline phosphatase. Older, more compact food vacuoles are generally uniformly reactive for alkaline phosphatase activity. Mitochondria and large amounts of rough and smooth endoplasmic reticulum are also generally well organized around these structures. 17 500 ×.

FIGURE 8.— Amoeba from flight module II; in weightlessness; 11-hour feeding, 12-hour fixation. A corner of a large food vacuole is evident along with a small portion of adjacent cytoplasm. On the left, undigested portions of an ingested paramecium remain intact. The outer border of the food vacuole is lined with a wide band of digested amorphous material and the cytoplasmic surface is coated with membrane-bounded vesicles, mitochondria, and ribosomes. 17 000 ×.

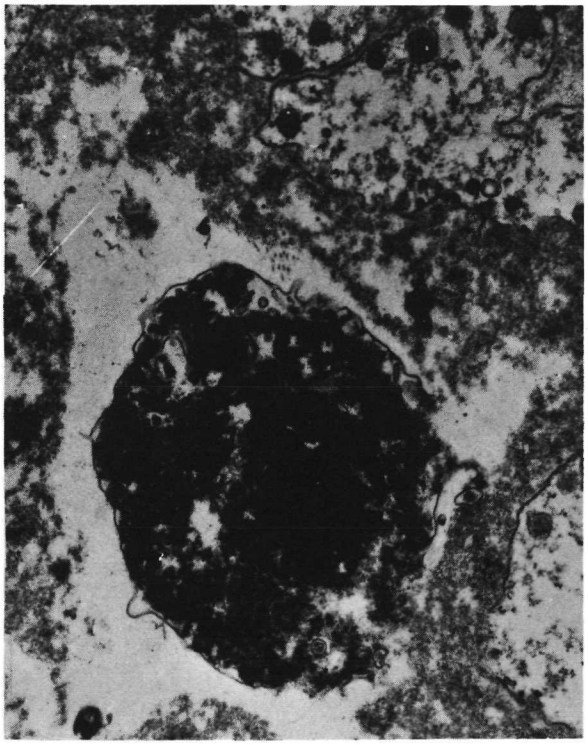

FIGURE 9.— Medium-sized food vacuole; simulated flight organism module 10; fed 31 hours, fixed 42 hours. The reaction product resulting from acid phosphatase activity forms a dense core at the center of the vacuole whereas the perimeter is filled with an unreactive amorphous digested material. Note that this food vacuole appears to be more digested than the one shown in figure 8. Acid phosphatase activity is generally located at the surface of the food vacuole; this one is exceptional since a reaction product occurs at its center. 17 000 ×.

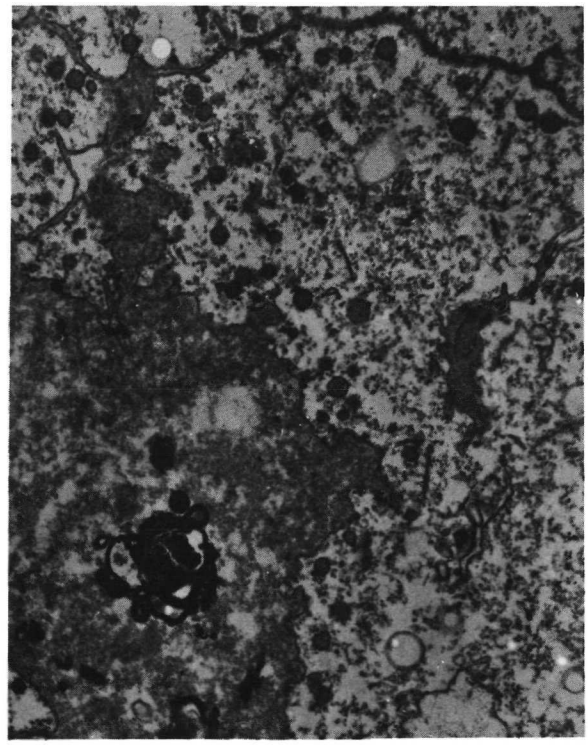

FIGURE 10.— Earth-based control. As the digestion of a food vacuole progresses the core of undigested material is reduced in diameter, whereas the outer amorphous layer increased in thickness. Canaliculi, which extend from the surface of the food vacuole, extend far into the cytoplasm and numerous microvesicles appear all along the surface of the vacuole. Reaction product resulting from peroxidase activity is present at the center of the vacuole, thus indicating that digestion has not proceeded that far yet. Peroxidase was taken into the gullet of this paramecium. The amoeba was sacrificed 4 hours later, just before it was ingested. 14 000 ×.

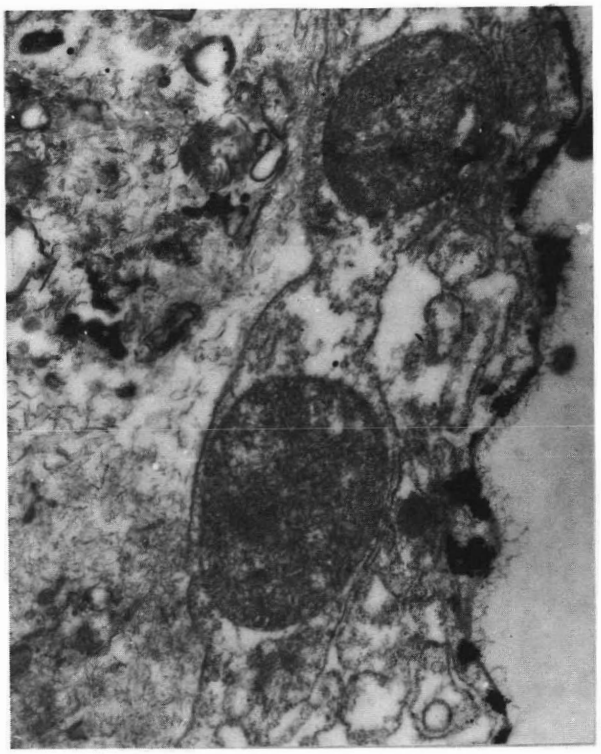

FIGURE 11.

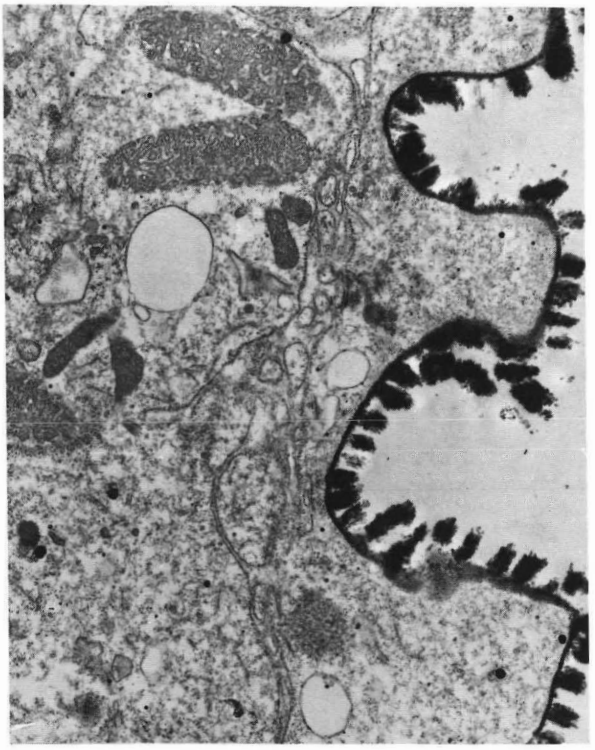

FIGURE 12.

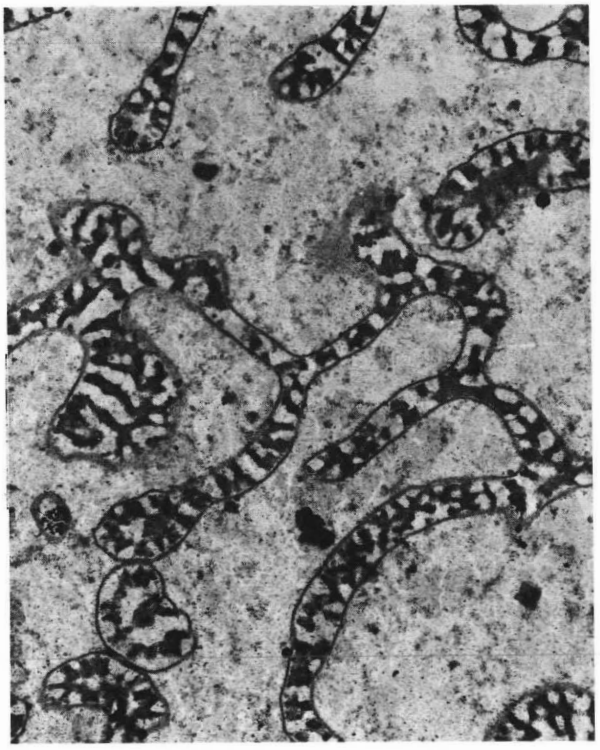

FIGURE 13.

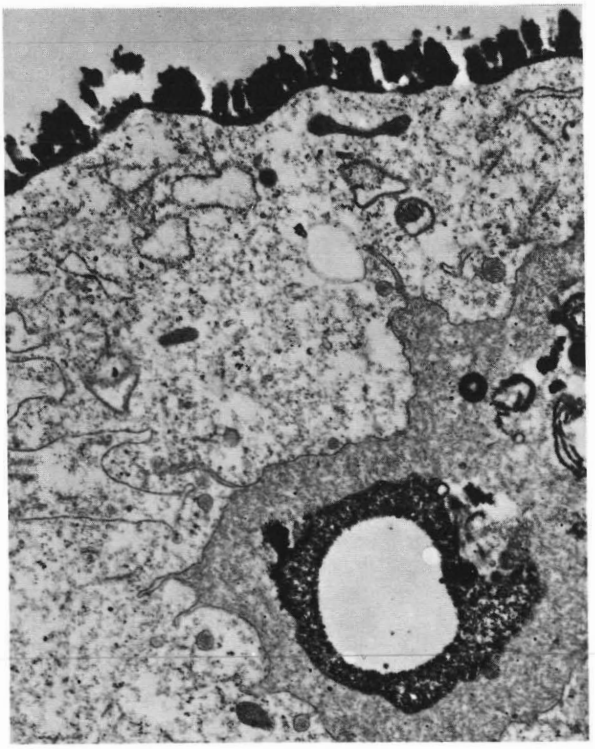

FIGURE 14.

FIGURE 11.— Amoeba from flight module IX. Nonspecific esterase reaction product lines the inner surface of the plasma membrane. Esterase activity was essentially unchanged by the weightless condition since all Earth-based controls were similarly reactive. 18 400 ×.

FIGURE 12.— Based experiment incubated 30 minutes in peroxidase and cultured 30 minutes thereafter. Peroxidase label coats the extracellular fuzzy coat of the amoeba's surface. There is very little intracellular distribution of the enzyme in this micrograph. In the cytoplasm of this cell there are several mitochondria, vesicles of rough endoplasmic reticulum, packages of protein, and one very old food vacuole. 13 500 ×.

FIGURE 13.— After 1 hour of peroxidase treatment large deep channels that are lined with reaction product invaginate from the surface of the organisms toward the center of the cell. The significance of these processes is not yet fully understood but it seems as if they are pinched off and the vesicles fuse with heterolysosomes where the attached enzyme is digested. 13 000 ×.

FIGURE 14.— Section of amoeba peroxidase labeled 30 minutes, cultured 6 hours, and fixed. Peroxidase reaction product is concentrated at the center of the food vacuole in this micrograph. The perimeter of the food vacuole is surrounded by an amorphous material that contains no peroxidase activity. Since peroxidase activity is maintained, it is apparent that the center of the food vacuole is the last part to be digested. Note also the large number of canaliculi and pinocytotic vesicles and the rough endoplasmic reticulum that have developed around this structure. 14 000 ×.

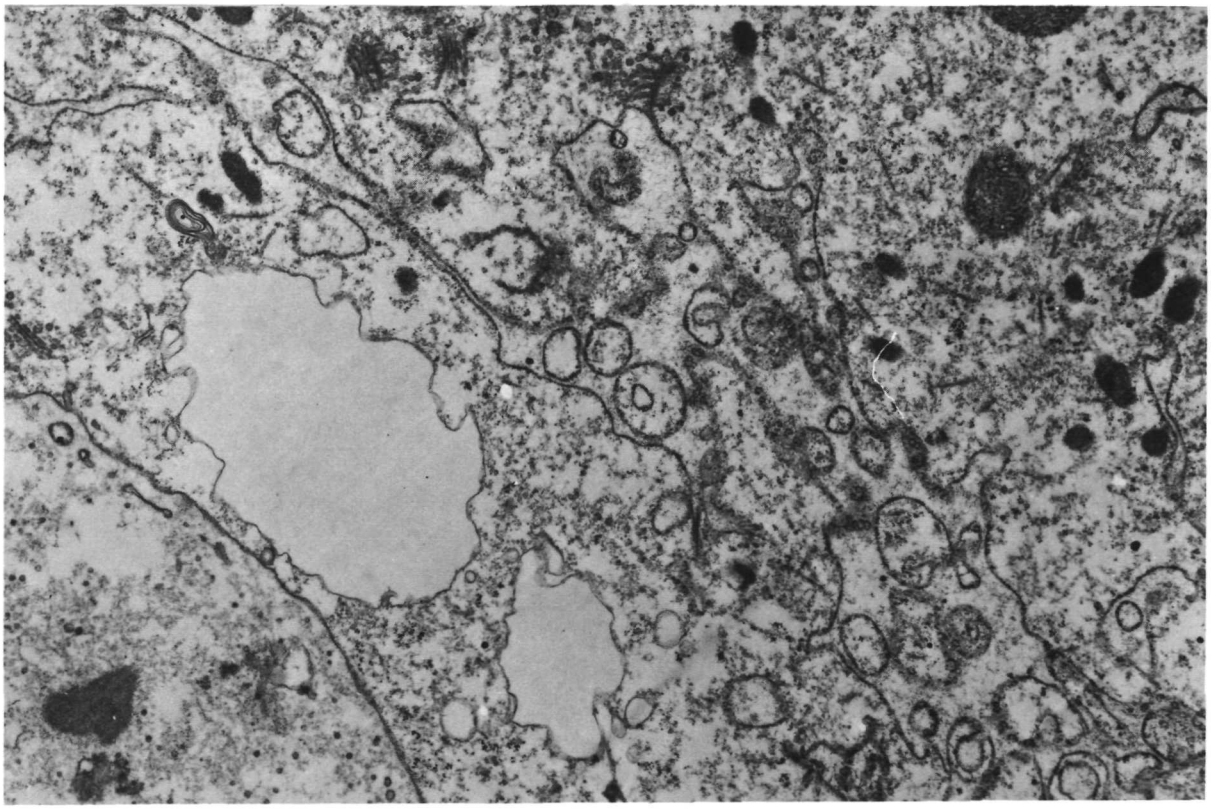

FIGURE 15.— Continuously fed flight organisms; module 21. Figures 15, 16, and 17 were chosen to show the similarities in the fine structure of cytoplasmic organelles in continuously fed organisms. There are essentially no differences in the structure of the golgi, endoplasmic reticulum, and mitochondria or in the appearance of polyribosomes. Furthermore, plasma filaments and dense vesicles are generally scattered throughout the cytoplasm. 13 000 ×.

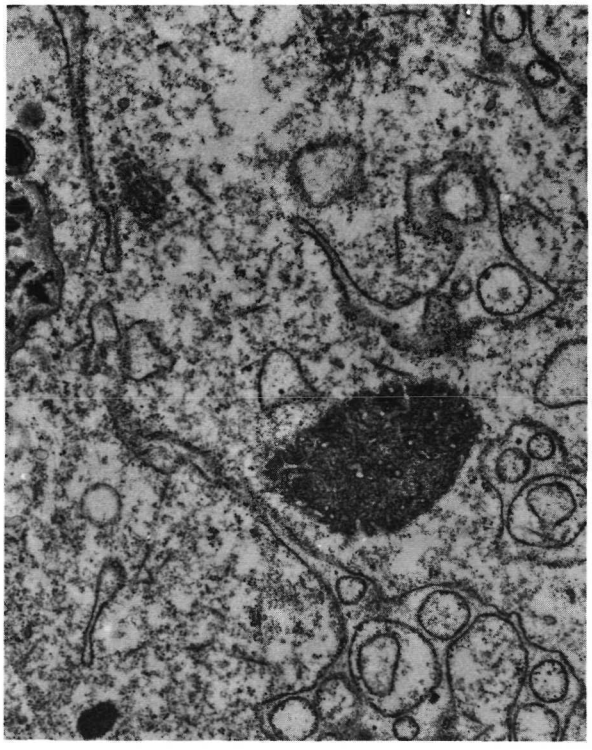

FIGURE 16.

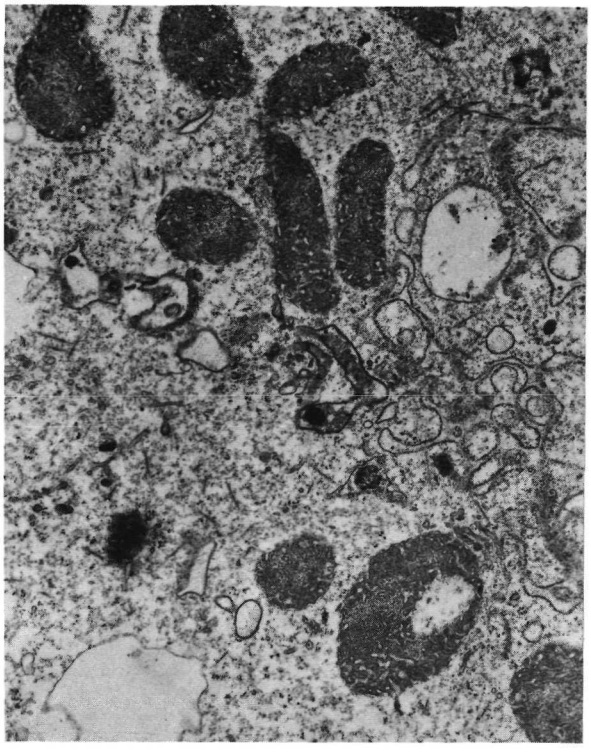

FIGURE 17.

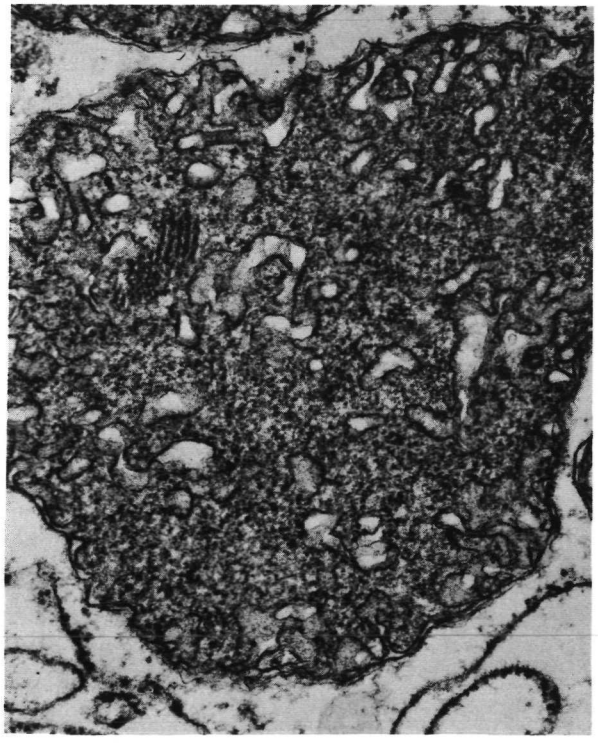

FIGURE 18.

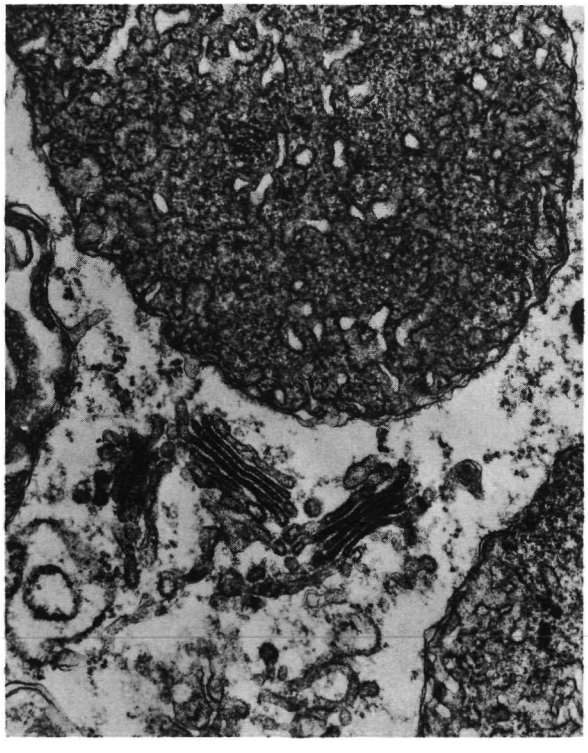

FIGURE 19.

of acceleration and vibration altered the structure of these organelles (figs. 18 and 19) or affected the distribution or activity of succinic dehydrogenase. This enzyme was distributed uniformly within the intracristal space of these organelles.

Contractile vacuoles in various stages of swelling and contraction were present in the fed amoebae, especially in areas where there was a high concentration of food vacuoles. Conversely, contractile vacuoles were not observed with much frequency in the starved forms. The overall outline of the vacuoles was stellate. Numerous small vesicles fused with the membranous surface of these vacuoles giving them numerous microundulations. The peripheral or adjacent cytoplasm was filled with small electron translucent vesicles 150 to 200 microns in diameter and mitochondria (fig. 21). Contractile vacuoles were present in all amoebae. There appeared to be no alteration in these organelles as a result of the flight or simulated flight conditions.

There was a dramatic reduction in the final growth rate of the simulated flight organisms, (tables 1 and 3). The growth rates of the organisms from the simulated flight controls were also retarded, however. Therefore, it is apparent that some introduced variable other than the vibrations and accelerations applied produced the retardation of growth in these organisms.

FIGURE 16.— Continuously fed simulated-flight organisms; module 24. See note with figure 15. 50 000 ×.

FIGURE 17.— Continuously fed Earth control; module 21. See note with figure 15. 15 000 ×.

FIGURE 18.— Continuously fed amoeba fixed 1 hour after launch in flight experiment; module 22. Figures 18 and 19 are presented to compare the fine structural characteristics of mitochondria from the two experiments. Both mitochondria contain anastomosing tubular cristae, a relatively dense matrix with ribosomelike structures, and a continuous outer limiting membrane. 50 000 ×.

FIGURE 19.— Continuously fed amoeba fixed 1 hour after simulated launch in the simulated flight experiment. See note with figure 18. 50 000 ×.

DISCUSSION

Survival and Growth of Amoebae in Space

Amoebae flown in space not only survived the total flight profile but fed, assimilated food, and grew in the weightless environment. A definite correlation between growth of amoebae, the number of food vacuoles present, and the size of amoebae was apparent. Modules containing a predominance of small hungry amoebae with elongated profiles and new food vacuoles tended to have reduced growth rates; as the period of feeding lengthened, the size of the amoebae increased, the number of intracellular food vacuoles increased, and the growth rates began to rise, but only very slowly. Finally, in modules exposed to prolonged feeding periods, the number of new intracellular food vacuoles increased, the amoebae rounded off and became more dense, and the growth rates increased rapidly. There was, in all experiment groups, a normal progression from acid to alkaline hydrolysis as the food vacuoles were progressively digested and reduced in size. The evidence indicates that the conditions of flight or simulated flight did very little to alter normal physiologic processes of these cells or the correlations between the major factors tested, which are food vacuole digestion, growth, and ultrastructural changes in cell organelles.

Digestion of Food Vacuoles

The rate of digestion of the ingested paramecia was determined in the present studies from the percentage distribution of the various ages of food vacuoles (new to old). Comparisons of the results from flight and the various Earth control experiments indicated that neither the rate nor the normal processes of digestion was changed as a result of the flight or simulated flight conditions. Since much of the quantitative data collected were shown to be highly inaccurate, this conclusion is based, mostly, on morphologic observations. The increased feeding of the flight amoebae was not reflected in a difference in growth rates until the time of reentry when the flight amoebae reached their peak of growth. Unfortunately, the period of growth in all of these packages was associated with the period of reentry and any significant measurements of growth rates in space were thus lost.

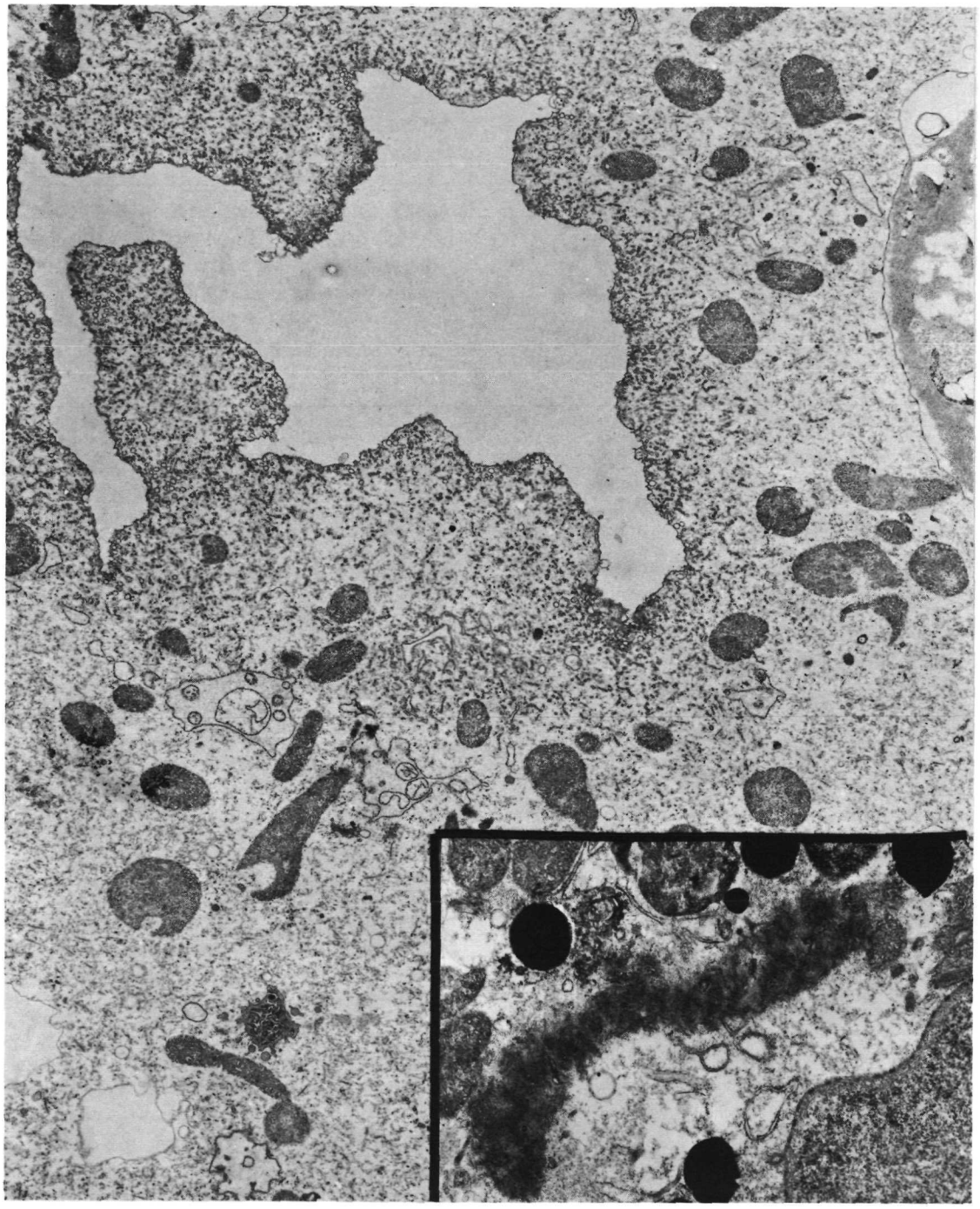

FIGURE 20.—Old food vacuoles; Earth-based control. Packets of microfilaments develop in areas where rough endoplasmic reticulum and golgi accumulate around old food vacuoles. The distribution of these packets was generally unaltered by the experimental conditions employed in this study. 15 000 ×.

FIGURE 21.— Section of a continuously fed flight organism from module 19. A large and completely typical contractile vacuole is apparent. Numerous small vesicles of 50 to 100 mμ accumulate around the periphery of the vacuole, appear to fuse with it, and discharge their contents into its interior. Mitochondria form a perivacuolar ring around the structure. 16 400 ×.

Ultrastructure of Subcellular Organelles

The conditions of the flight or simulated flight produced no detectable changes in the fine structure or distribution of the individual subcellular organelles. The degree of folding in the cristae of the mitochondria changed with the degree of starvation and feeding but was not affected by weightlessness. In all cases there was a normal development of Golgi elements, packages of rough endoplasmic reticulum, and polyribosomal clusters in the areas of the amoebae that contained partially digested food vacuoles. The development of small pinocytotic vesicles and the long channels at the surface of the food vacuoles also proceeded normally in all cases. Although nuclei with undulated surfaces were evident in the flight and simulated flight amoebae, an equal number of nuclei of similar outline were found in the Earth-based controls. Therefore, we have no fine structural evidence that would indicate that weightlessness or the conditions of launch and reentry produced any prolonged adverse effects on the amoebae.

Distribution of Enzymes

Nonspecific esterase activity was localized along the intracellular surface of the plasma membranes and the cytoplasmic surface of the food vacuoles. Activity has not been detected in the residual bodies. Succinic dehydrogenase activity was demonstrable within the intracristal space of mitochondria. Acid phosphatase activity was demonstrable in large food vacuoles whereas alkaline phosphatase was present exclusively in the smaller vacuoles. The conditions of flight or simulated flight did not alter the distribution or cytochemical demonstrability of these enzymes.

Plasma Membrane

Measurements of the thickness of the plasma membrane and its associated fuzzy coat on amoebae from the various experimental groups indicate that it was not altered by any of the tests that were run.

Excretion and Water Balance

Large and small contractile vacuoles were found in the amoebae that were fixed in space and in those that were returned alive and subsequently fixed. There was also a normal distribution of small vesicles and mitochondria at their periphery. From this it is concluded that excretory processes proceeded normally in the weightless environment.

SUMMARY AND CONCLUSIONS

The effects of the space environment, acceleration, and vibration on feeding, growth, and morphology were studied in amoebae that were starved for periods of 27 to 75 hours or were continuously fed. The ratios of amoebae loaded and amoebae recovered were basically similar for all modules of a single experiment regardless of the conditions to which it was subject. The following conclusions are drawn from the analysis of the results of the experiment completed to date.

(1) Survival and growth of *P. carolinensis* were essentially unchanged as a result of weightlessness.

(2) Amoebae subject to weightlessness were not significantly different physiologically and morphologically from the Earth control organisms.

(3) Cytochemical data indicate that little or no difference was produced by weightlessness, acceleration, or vibration on various digestive processes.

(4) Food vacuole data were collected in an improper manner and little, if any, significance can be drawn from them.

(5) Growth rates in flight amoebae, although somewhat reduced during weightlessness, appeared to accelerate around the time of reentry.

Without question, the experiment was cut off 24 hours too soon. If it had continued just 1 day longer, we now could know whether weightlessness had any real effect on the growth of amoebae in space. From the observations made we can conclude only that the weightless environment did not produce any gross irreversible alterations in the normal physiologic processes of the amoebae.

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Induction of Lysogenic Bacteria in the Space Environment

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The Biosatellite II experiment using the lysogenic bacterium *Salmonella typhimurium* BS–5 (P–22)/P–22 was designed to test the hypotheses that weightlessness both with and without gamma irradiation would not affect bacterial cell growth or induction of bacterial prophage P–22. Within constraints of the experiment design, both hypotheses were rejected.

The portion of the experiment dealing with frequency of induction provided an independent test of the earlier results of Zhukov-Verezhnikov (1965, 1968) who used *Escherichia coli* K–12 (λ). These results were not confirmed by our experiment, a discrepancy accountable by several explanations.

EXPERIMENT DESIGN AND METHODS

The variables tested by the Biosatellite II experiment included: (1) Spaceflight, (2) three chronic gamma-irradiation dose levels, and (3) temperature. The design to partition the effect of these variables required nine sets of cultures, each of which consisted of replicate chambers of 1.4±0.3-ml capacity. Two postflight tests were necessary to further partition possible weightlessness effects from other variables of spaceflight. The variables not accounted for by the Biosatellite II experiment itself were: (1) Idiosyncrasies of the hardware systems (experiment as opposed to control modules and spacecraft), and (2) vibration and acceleration. The basic experiment design is given in table 1.

An experiment with *E. coli* C–600 (λ)/λ was employed for the Biosatellite II. The experiment design for this organism was identical to that for *S. typhimurium*. Because the flight duration was shorter than that planned, growth was still in the log phase when the cultures were recovered, unlike the case for *S. typhimurium,* which grew beyond log phase. Thus the densities were too low to have produced testable numbers of phage λ. It was not possible to assay for λ. The attempt to determine whether ultrastructural changes were due to growth under weightlessness, on the other hand, were successful only for the *E. coli* cultures. The results for this portion of the experiment have been reported (Mattoni, 1968). The experiment design was implemented by inoculating each chamber with aliquots of a suspension adjusted to give mean viable density of about 100 cells/ml.

Following the recovery of Biosatellite II, or the other tests, each chamber (fig. 1) was emptied and the contents were assayed according to the procedures detailed earlier (Mattoni, 1968). The parameters estimated included: (1) Total bacterial density by Coulter principle counting; (2) viable bacterial density by colony count on nutrient agar plates; (3) free P–22 bacteriophage titer by plaque count (PFU) using aliquots of substrate as received; and (4) induced P–22 titer by quantifying PFU following dilution and incubation of recovered viable cells. The number of assays are given

in table 1. In addition, the ultrastructural data for *E. coli* were noted; these data showed no detectable differences among experimental and control sets of populations.

The induced and free phage were assayed by plating over a period of 15 days. There is a considerable body of evidence on both phages P–22 and λ to show that there is bacteriophage inactivation with time.[1] Consequently, the phage data from the Biosatellite experiment were corrected for inactivation prior to all analyses. Since it has been our experience that die-off rates for phage are highly variable and dependent on physical condi-

[1] Unpublished work of Mattoni.

TABLE 1. – Design of the Lysogenic Bacteria Biosatellite II Experiment

Program	Number of replicate culture chambers for nominal total γ dose, R, of —			
	Nonirradiated	265	645	1630
Biosatellite II	20	8	8	8
Control I (vehicle at Cape Kennedy)	20	8	8	8
Control III (incubator duplicating Biosatellite II temp.)	20			
Vibration (ARC "C") test:				
Experimental (vibrated-accelerated)	20	8	8	8
Control	20	8	8	8
Hardware (ARC "B") test:				
Flight vehicle and modules	20	8	8	8
Control vehicle and modules	20	8	8	8

Group	Number of dilutions and counts per culture	
	Dilution series	Counts or plates each
Biosatellite		
Total bacteria (Coulter):	6	4 counts
12 nonirradiated chambers	4	4 counts
Viable bacteria:	6	10 plates
12 nonirradiated chambers	4	10 plates
Free bacteriophage	6	10 plates
Induced bacteriophage	6	10 plates
Vibration (ARC "C")		
Total bacteria (Coulter)	4	4 counts
Viable bacteria	2	10 plates
Free bacteriophage	4	10 plates
Induced bacteriophage	4	10 plates
Hardware (ARC "B")		
Total bacteria (Coulter)	2	4 counts
Viable bacteria	2	10 plates
Free bacteriophage	2	10 plates
Induced bacteriophage	2	10 plates

tions, it was decided to use the inactivation rates calculated in each group rather than a standard curve for the correction factors. This use was also indicated by testing several sets of correction factors under the assumption that among a given set of replicate chambers the variance should be minimum when the best correction factor had been found.

The free-phage and induced-phage corrections differed slightly in that for free phage it was possible to calculate an inactivation rate for each experiment group. For the induced phage it was necessary to use an average inactivation ratio across experiment treatments. In subsequent postflight tests the experiment design was modified so that the effect of phage inactivation was blocked out and no corrections were necessary.

No such corrections were necessary for any of the bacterial data.

RESULTS

For the Biosatellite II flight, the actual loading density was about 120 cells/ml, with 102 cells/ml for the subsequent hardware (ARC "B") test and 129 cells/ml for the vibration-acceleration (ARC "C") test. Table 2 summarizes the Biosatellite II data for both *S. typhimurium* and *E. coli* at loading and moment of launch. The latter data were deduced from replicate cultures maintained at the Biosatellite II temperatures and assayed at time of launch. The planned initial densities provided an essential feature of the experiment design: maintenance of log growth through most of the flight duration. Under anaerobiosis at the expected temperatures, growth rates were predicted to produce a stationary phase of growth several hours prior to reentry. Metabolism, as well as virus production by induced cells, is greatly diminished during the stationary phase. Thus, the design circumvented launch and reentry stresses by the flight sets of populations, presumably providing a direct test of weightlessness effects. Every cell of the recovered populations probably would be a descendant of a cell produced, and would itself have been produced, during orbital flight.

The most important reason for anaerobic conditions was the necessity to avoid air and, after growth, gas bubbles in the cultures. Such bubbles would produce physical states of the substrate with unpredictable liquid-gas boundary configurations and distributions. Operation of the Archimedes principle would therefore add an untestable variable, since in the Earth controls such bubbles would rise.

The bacterial data from the Biosatellite II experiment were analyzed by three methods: (1) Analysis of variance; (2) *t*-tests between flight and

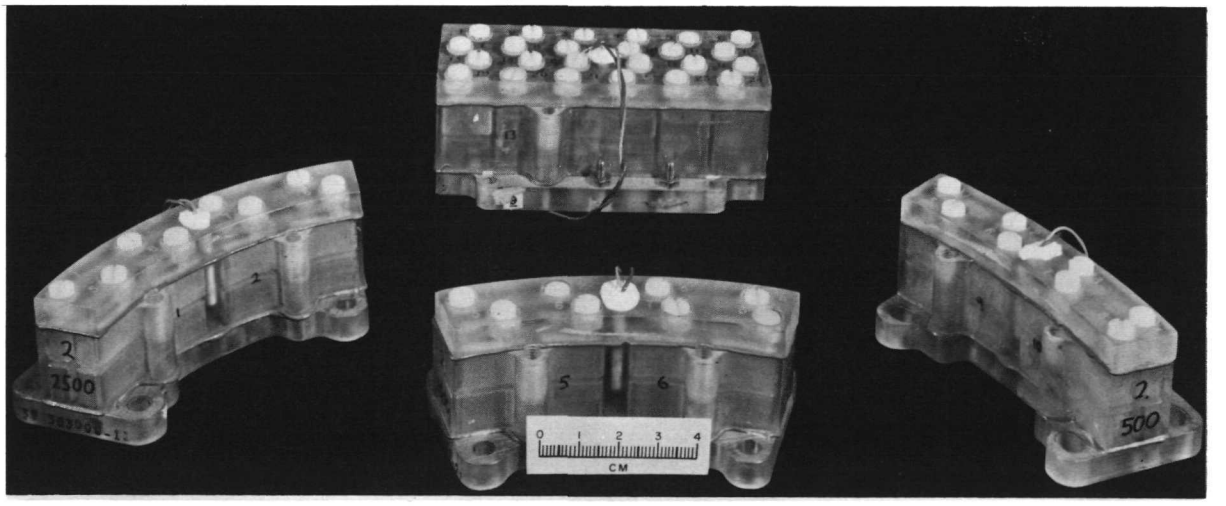

FIGURE 1.— Complete set of four lysogenic bacteria packages. The large square package (upper center) contains 48 1.2-ml chambers, nonirradiated. The three contoured packages contain 16 chambers each, 1.2 to 1.8 ml, and curvature-to-radiation isodose lines of three mean radiation levels.

Earth control data (where t is the percentage difference between experiment and control sets); and (3) graphic presentation. The t-tests and graphic analyses will be discussed first since the interpretation of data presented in this manner is more clearly seen. The analysis of variance confirms the effects seen in tabular and graphic form in addition to adding information on sources of variation in the data and will be discussed in more detail later.

It must be noted here that the t-test used is for a single confidence interval. When more than one t-test is made, the significance level of a given t-value goes down simply because the more t-tests that are made on a given set of data, the greater is the probability that one will turn out to be significant. Thus, some discrepancies will result where the confidence intervals of two means overlap, yet the probability associated with the t-value of the two means indicates they are significantly different. In such a case the significance of the t-value is doubtful; these cases will be pointed out when they arise.

The effect of spaceflight on bacterial growth, quantified by both total and viable final population densities, is clearly shown by figure 2. The

TABLE 2. – Population Densities at Loading and Time of Launch for Biosatellite II Experiment

Time	*Salmonella typhimurium* BS–5 (P–22)/P–22		*Escherichia coli* C–600 (λ)/ λ	
	Chamber	Number of colonies	Chamber	Number of colonies
Preload[a]		126		187
Postload[a]		115		188
	Aft temperature profile incubator			
Launch[b]	1	N.G.	9	131
	2	205	10	82
	3	235	11	102
	4	260	12	81
	5	247	13	87
	6	223	14	141
	7	218	15	87
	8	274	16	110
	Mean count	2 374/ml		1 026/ml
	Forward temperature profile incubator			
	1	1 193	9	213
	2	988	10	94
	3	1 276	11	175
	4	1 047	12	N.G.
	5	1 367	13	103
	6	861	14	200
	7	849	15	150
	8	1 787	16	272
	Mean count	11 710/ml		1 724/ml

[a] Undiluted samples.

[b] Launch densities deduced from populations run under forward and aft temperature profiles. Each value sum of 10 plates made by dispensing 0.1-ml aliquots from a 1.0-ml pipet following tenfold dilution. Means were adjusted for dilution.

confidence intervals given for all figures are calculated from variances of chamber means. The data are presented in tabular form in tables 3 and 4, which also give the percentage differences t and the probability values of the differences being due to chance P. These values are based upon the t-test, where N is the number of chamber means used to make the estimates. The number of replicate cultures assayed from the experimental and control sets are given by N_E and N_C, respectively.

Temperature differences were closely matched (to ±4 percent) by use of the nonirradiation-treatment control III (see table 1). Table 5 presents temperature profiles for Biosatellite II and the vibration-acceleration experiments.

Although the flight populations grew to significantly greater densities for all treatments, the irradiated set of populations was over 80 percent denser than were the Earth controls, whereas the nonirradiated populations were only 30 percent denser. Densities based on total and viable counts correlated closely. In order to look at the effects

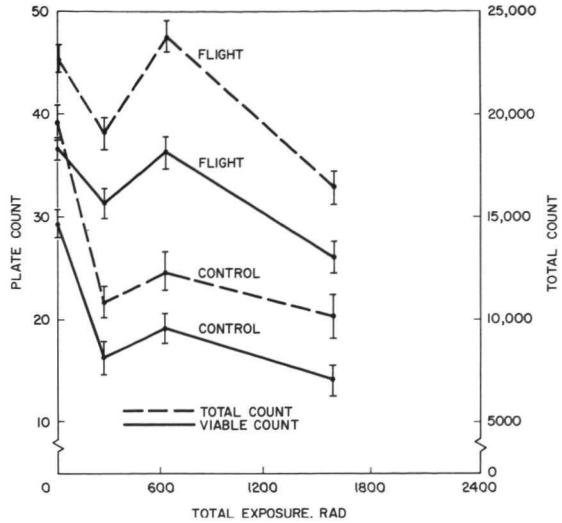

FIGURE 2.— Dose-effect curves for final population densities of *Salmonella typhimurium* BS–5 (P–22)/P–22 Biosatellite II flight and ground controls. Broken line gives mean of Coulter counts (dilution$\times 2\times 10^4$/ml). Solid line shows viable cell counts from colonies formed on agar plates (dilution$\times 10^7$/ml). Vertical bars give 95 percent confidence limits. N=20 for nonirradiated and N=8 for each radiated treatment.

TABLE 3. — Mean Viable Densities of *S. typhimurium* (P–22)/P–22 From Plate Counts; Diluted $\times$ 10^7/ml; Biosatellite II Experiment and Earth Control Sets

N_E	N_C	Total γ radiation, R	Mean viable density, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
20	20	0	37.07 ±1.51	28.48 ±1.53	30.2	4.00	<0.01
8	8	265	31.21 ±1.59	16.36 ±2.91	90.7	4.48	< .01
8	8	645	36.60 ±1.41	19.48 ±2.39	87.8	6.17	< .01
8	8	1630	26.03 ±0.77	14.13 ±2.55	83.5	4.46	< .01

TABLE 4. — Mean Total Density of *S. typhimurium* (P–22)/P–22 Determined by Coulter Counts; Diluted 2 $\times$ 10^4/ml; Biosatellite II Experiment and Control Sets

N_E	N_C	Total γ radiation, R	Mean total density $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
20	20	0	23 104 ±657	20 077 ±970	15.08	2.58	0.01
8	8	265	19 021 ±1061	10 111 ±1990	88.13	3.41	< .01
8	8	645	23 894 ±648	12 376 ±1460	93.07	7.22	< .01
8	8	1630	16 467 ±511	10 176 ±1790	61.84	4.38	< .01

of other variables that might account for the differences seen in flight and Earth control bacterial densities, two types of postflight experiments were performed. The purpose was to determine whether the observed results could be accounted for by (1) differences between the hardware chambers or vehicle systems imparted by some undefined idiosynchrasy, or (2) vibration and acceleration pro-

TABLE 5. – Temperature Profile Summary for Biosatellite II and Vibration-Acceleration Experiments

Elapsed time, hr (a)	Temperature, F/hr above 60 F				
	Biosatellite II				
	Flight (301)		Control I (201)		Control III (incubated)
	Irradiated	Nonirradiated	Irradiated	Nonirradiated	Nonirradiated
0-12	125	65	140	127	69
13-57	326	214	395	515	211
58-63	44	38	64	72	33
Total	495	317	599	714	313
	Vibration-acceleration experiment				
	Vibrated (301)		Control (201)		
	Irradiated	Nonirradiated	Irradiated	Nonirradiated	
0-12	120	103	130	135	
13-57	353	337	314	346	
58-63	53	49	49	51	
Total	526	489	493	532	

[a] 0 to 12 hours, prelaunch time; 13 to 57 hours, orbital flight time; 58 to 63 hours, recovery timespan from deorbit to icing.

TABLE 6. – Mean Total Densities of *S. typhimurium* (P–22)/P–22 Determined by Coulter Counts; Diluted 2 × 10^4/ml; Bacteria Subjected to Acceleration and Vibration Profiles Duplicating Those Stresses Measured on Biosatellite II

N_E	N_C	Total γ radiation, R	Mean total density $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
19	20	0	40 884 ±4824	35 348 ±4069	15.4	3.80	<0.01
8	8	265	37 541 ±2495	36 590 ±1723	2.6	.75	.48
8	8	645	35 260 ±3255	35 977 ±1553	−2.0	.56	.58
8	8	1630	35 028 ±4651	30 614 ±4581	11.2	1.48	.16

files in a single axis duplicating the magnitude of those stresses recorded during the Biosatellite II flight. Population density data from the vibration experiment are graphically presented in figure 3, and tabular data and analyses are given in tables 6 and 7. The vibrated-accelerated sets of populations were consistently denser than were the controls, but the only significant t-value between means is seen in table 7 for the set receiving 1630 R. The inferred difference in means, however, is doubtful, as discussed previously, since the confidence intervals of the two means overlap, as shown in figure 3. Although the trend strongly implies a positive effect, the lack of correlation and considerably smaller percentage differences clearly do not repeat the Biosatellite II results.

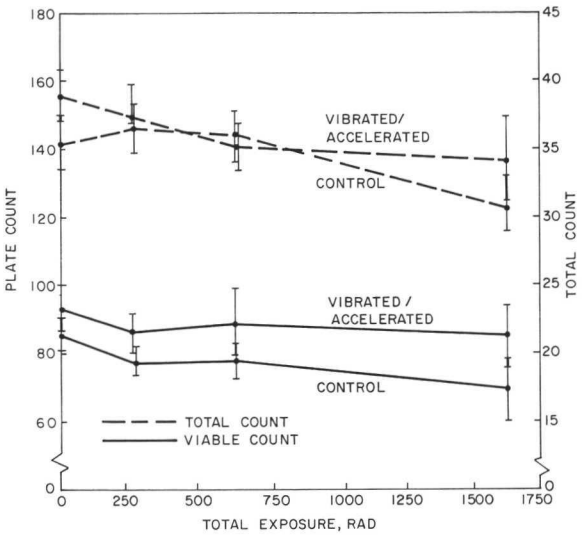

FIGURE 3.— Dose-effect curves for final population densities of *Salmonella typhimurium* BS–5 (P–22) /P–22 for vibrated and accelerated experiments and stationary controls. Broken lines give mean of total Coulter counts (dilution $\times 2 \times 10^7$/ml). Solid line connects viable counts from colonies formed on agar plates (dilution $\times 10^7$/ml). Vertical lines represent 95 percent confidence limits. Experiment conducted at ARC, May 22 to 25, 1968. N=20 for nonirradiated and N=8 for each irradiated treatment.

Experiments to determine whether the hardware itself could account for the results obtained with Biosatellite II were negative. There is no evidence that any chamber used in the flight experiment or postflight tests produced consistently high or low yields of bacteria. On the basis of a correlation test, the differences in chamber means can be assumed to be random errors.

One further step taken in analyzing the data was an analysis of variance tests on the viable bacterial counts from the Biosatellite II data. The analysis, shown in table 8, allowed us to look at the main effects and examine in more detail interaction terms and sources of variation in the experimental data. The ANOV table for *Salmonella* is shown in table 8. One can see from the table that both treatment effects and radiation effects were significant. This is a verification of the results of the Biosatellite II data given elsewhere in graphical and tabular form.

The data on bacterial growth lead to rejection of the null hypotheses: spaceflight produces an increased density of *Salmonella typhimurium* when they are grown in liquid medium, and an interaction to give greater resistance to γ-irradiation at levels between 265- and 1648-R total dose (17 and 103 R per average cell generation). Tests of

TABLE 7. – Mean Viable Densities of *S. typhimurium* (P–22)/P–22 Diluted $\times$ 10^7/ml; Bacteria Subjected to Acceleration and Vibration Profiles Duplicating Those Stresses Measured at Launch and Recovery

N_E	N_C	Total γ radiation, R	Mean viable density, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
19	20	0	93.01 ±3.56	85.55 ±2.81	8.7	1.68	0.10
8	8	265	86.23 ±3.14	77.58 ±2.31	11.2	2.22	.05
8	8	645	88.31 ±5.28	77.98 ±2.75	13.3	1.74	.10
8	8	1630	85.31 ±4.84	69.04 ±4.42	23.6	2.46	.03

other variables eliminate all but weightlessness ($g \cong 10^{-4}$) in accounting for the difference.

Two different estimates of induction of prophage P–22 were made. In order to make one of the estimates possible it was necessary to synthesize a special strain of *S. typhimurium,* BS–5 (P–22)/P–22. The strain is resistant to adsorption by the mature phage. Thus all mature phage P–22 produced would accumulate as free phage in liquid substrate. Otherwise the free phage would be lost by adsorption according to the kinetics of the adsorption coefficient. Data for free P–22 density, by themselves, show no differences for nonirradiated treatments. Significantly greater titers were found at all radiation levels under spaceflight (table 9). However, phage densities have biologic meaning only when expressed as a function of viable bacterial cell density. When so transformed, the statistical significance disappears (table 10 and fig. 4). The expected dose response, an increase in phage yield

TABLE 8. – Analysis of Variance on Viable Bacteria Counts for *S. typhimurium*

Source of variation	Mean square	d.f.	*F*-value	*P*
Treatment	5020.09	1	70.77	< <0.001
Radiation	799.10	3	11.25	<.001
Treatment-radiation interaction	118.22	3	1.66	.20
Chambers	70.93	56	10.57	<.001
Dilution	6.71	192	5.78	<.001
Within (plates)	1.16	2560	.....	.....

TABLE 9. – Mean Densities of Free Bacteriophage P–22 From Lysates Made Following Recovery of Biosatellite II and Concurrent Earth Controls; Determination by Plaque-Forming Units Diluted $\times 10^3$/ml

N_E	N_C	Total γ radiation, *R*	Mean density, $\bar{X} \pm S_{\bar{X}}$			*t*	*P*
			Experiment	Control	Difference, %		
20	20	0	14.83 ±1.74	13.87 ±1.34	6.9	0.44	0.66
8	8	265	34.51 ±2.82	17.82 ±1.55	93.6	5.20	< .01
8	8	645	54.11 ±3.98	26.08 ±3.35	107.4	5.39	< .01
8	8	1630	73.16 ±7.19	46.27 ±4.18	58.1	3.24	< .01

TABLE 10. – Mean Frequency of Free Bacteriophage P–22 per Viable Bacterium Biosatellite II and Concurrent Earth Controls

N_E	N_C	Total γ radiation, *R*	Mean frequency, $\bar{X} \pm S_{\bar{X}}$, $\times 10^{-5}$			*t*	*P*
			Experiment	Control	Difference, %		
20	20	0	4.14 ±0.51	5.23 ±0.59	−20.80	1.40	0.18
8	8	265	11.24 ±0.99	12.04 ±1.25	−6.64	.50	.60
8	8	645	14.91 ±1.20	12.91 ±0.93	15.50	1.31	.20
8	8	1630	28.02 ±2.60	37.80 ±4.55	−25.80	1.87	.09

with increasing γ irradiation, remains. The data for free phage from experiments of both vibration-acceleration and hardware differences likewise showed no significant differences either in free-phage titer or frequency (tables 11 and 12, and fig. 5). There are no effects of factors associated with

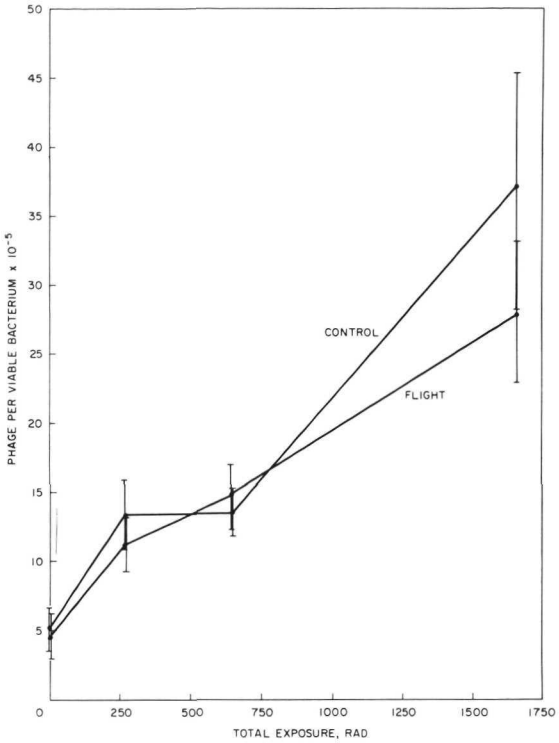

FIGURE 4.— Dose-effect curve for free phage per viable bacterium as recovered from Biosatellite II. Vertical bars represent 95 percent confidence intervals. N=20 for nonirradiated and N=8 for each irradiated treatment.

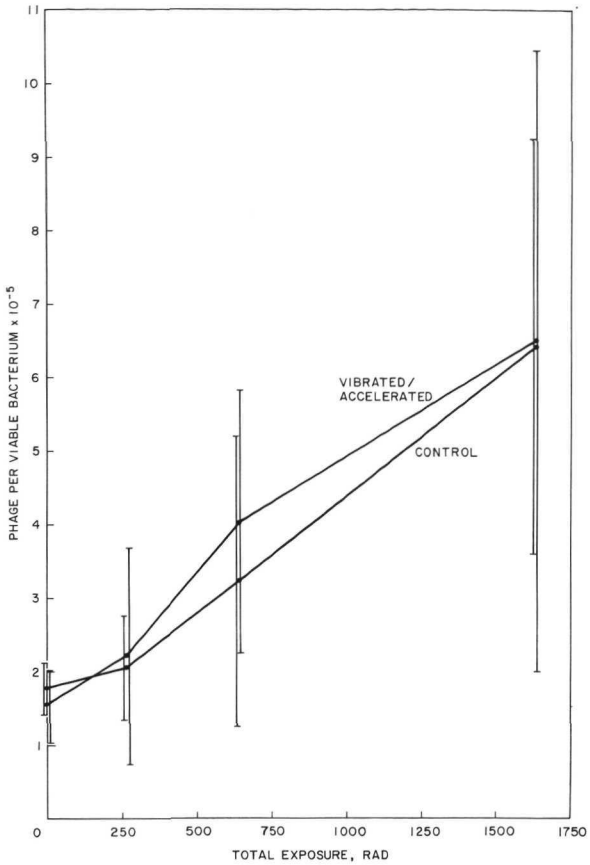

FIGURE 5.— Dose-effect curve for free phage per viable bacterium following vibration-acceleration profile simulating these stresses during Biosatellite II launch and recovery. Experiment conducted at ARC, May 22 to 25, 1968. Vertical bars represent 95 percent confidence intervals. N=20 for nonirradiated and N=8 for each irradiated treatment.

TABLE 11. — Mean Densities of Free Bacteriophage P–22 From Lysates Made Following Subjection of Experimental Population to Acceleration-Vibration Stresses Duplicating Those of Biosatellite II; Diluted $\times 10^3$/ml

N_E	N_C	Total γ radiation, R	Mean density, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
19	20	0	14.32 ± 2.48	14.80 ±1.41	−3.0	−0.166	>0.80
8	8	265	18.91 ± 6.41	15.58 ±2.33	21.4	.49	.60
8	8	645	34.63 ± 7.31	23.75 ±6.57	45.8	1.11	.30
8	8	1630	48.25 ±12.94	41.16 ±7.80	17.2	.47	.62

spaceflight on free P–22 bacteriophage production by *S. typhimurium*.

The second estimate of induction was made by determining the frequency of P–22 phage produced per viable bacterium from cells immediately recovered from Biosatellite II. The method required dilution of each culture into fresh broth followed by a 2-hour incubation at 37 C. These conditions permitted the cells to resume active growth. Any cell which had been induced but had not produced mature phage could thereby proceed to complete the lytic cycle. The density of phage recovered by this treatment was about one hundred times greater than that of free P–22, as shown in table 13. Taking the data at face value (table 13), only the nonirradiated set of phage had a significant difference in mean density of phage obtained by induction. Again, however, the data have little meaning

TABLE 12. – Mean Frequency of Free Bacteriophage P–22 per Viable Bacterium from Populations Subjected to Vibration-Acceleration Stresses Duplicating Those of Biosatellite II

N_E	N_C	Total γ radiation, R	Mean frequency, $\bar{X} \pm S_{\bar{X}}$, $\times 10^{-5}$			t	P
			Experiment	Control	Difference, %		
19	20	0	1.57 ±0.28	1.77 ±0.18	−11.3	−0.59	0.60
8	8	265	2.20 ±0.75	2.05 ±0.36	7.3	.18	> .80
8	8	645	4.01 ±0.89	3.22 ±0.99	25.3	.59	.56
8	8	1630	6.44 ±2.22	6.42 ±1.41	.3	< .01	1.00

TABLE 13. – Mean Densities of Bacteriophage P–22 Induced in Cells Incubated and Then Lysed Following Recovery of Biosatellite II; Determination by Plaque-Forming Units; Diluted $\times 10^5$/ml

N_E	N_C	Total γ radiation, R	Mean density $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
20	19	0	15.78 ±1.62	11.73 ± 1.02	34.5	2.04	0.05
8	8	265	17.55 ±2.11	13.91 ± 1.29	26.1	1.47	.16
8	6	645	24.29 ±4.14	20.66 ± 3.84	17.5	.57	.60
8	8	1630	38.27 ±7.18	39.22 ±14.33	−2.4	.059	> .80

TABLE 14. – Mean Frequency of Induced Bacteriophage P–22 Produced per Viable Bacterium; Biosatellite II and Concurrent Earth Controls

N_E	N_C	Total γ radiation, R	Mean frequency, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
20	19	0	4.23 ±0.40	4.67 ±0.50	−9.44	−0.69	0.50
8	8	265	5.68 ±0.74	9.6 ±1.26	−40.8	−2.68	.02
8	5	645	6.54 ±1.06	10.47 ±1.04	−37.6	−2.64	.02
8	8	1630	14.80 ±2.84	24.32 ±3.79	−39.2	−2.01	.06

except when expressed in terms of phage per bacterium.

Table 14 and figure 6 present the data as frequency of P–22 per viable bacterium. The results imply that, although spaceflight by itself does not affect the P–22 inductive system in *S. typhimurium,* under spaceflight the system is less sensitive to radiation. The differences are statistically significant at the 5 percent level for only those populations exposed to total radiations of 265 and 645 R. The magnitude is similar at all levels.

The experiment to determine whether the results could be attributed to vibration and acceleration were negative. The trend is such that were the effects of spaceflight due to vibration and acceleration, the results would be reversed (fig. 7). The qualitative summaries of data are given in tables 15 and 16. The experiments to determine whether the hardware and hardware systems produced these effects in Biosatellite II gave negative results. The remaining variable is weightlessness.

No further analysis beyond the *t*-tests between treatment means at each of the four radiation levels was done on either the free phage per bacterium (table 10) or the induced phage per bacterium (table 14).

In summary, on the basis of the *t*-tests there was no difference in the free phage per bacterium produced under conditions of control and those of flight. The induced bacteriophage per viable bacterium, however, showed significant differences ($P < 0.05$) between flight and control at two radiation levels (265 and 645 R). Further, the flight set had consistently lower yields at all levels of radiation.

The chief difficulty in trying to interpret the phage data, especially the phage per bacterium, is the large coefficient of variation associated with the means. Inspection of the appropriate tabular data clearly shows that the error increases with the mean. A Poisson transformation was tried but did not

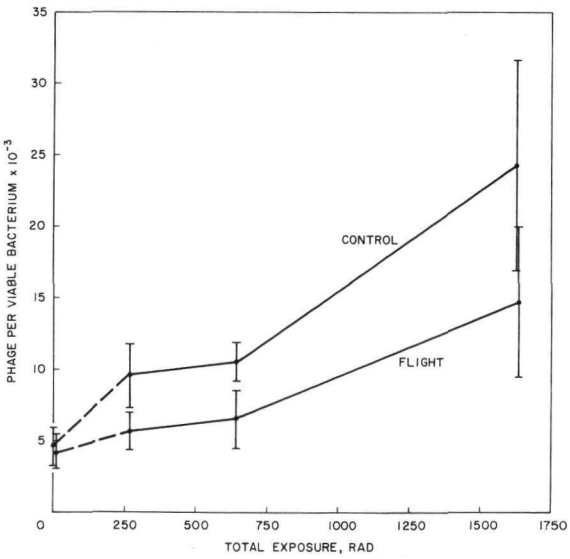

FIGURE 6.— Dose-effect curve for induced P–22 phage from cells recovered from Biosatellite II and permitted to complete a lytic cycle as phage per viable bacterium. Mean and 95 percent confidence intervals given. Broken line connecting the nonirradiated and irradiated population data indicates a temperature difference between these two sets. N=20 for nonirradiated and N=8 for each irradiated treatment.

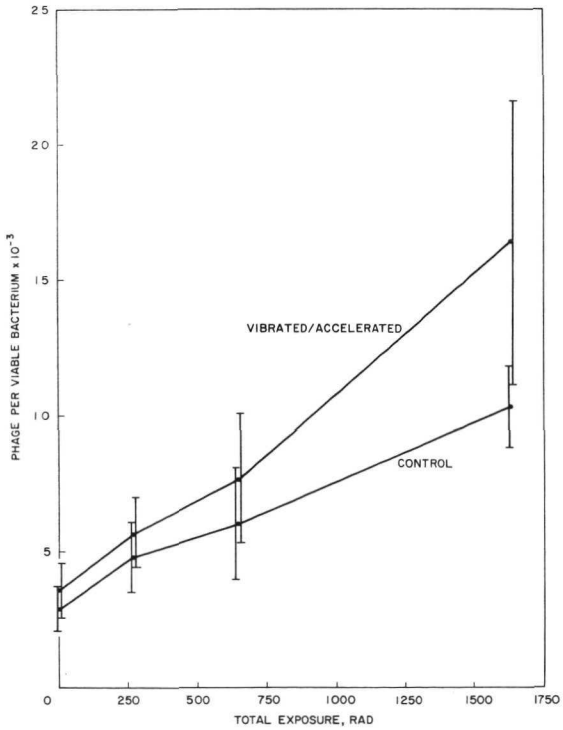

FIGURE 7.— Dose-effect curve for induced P–22 phage from cells recovered from vibration-accelerated experiment and permitted to complete a lytic cycle as phage per viable bacterium. Experiment conducted at ARC, May 22 to 25, 1968. Mean and 95 percent confidence intervals given. N=20 for nonirradiated and N=8 for each irradiated treatment.

solve the problem. Hence, we felt that an ANOV test would have dubious significance.

One problem in estimating the phage per bacterium is that we are looking at the ratio of two random variables. Delbrûck (1945) noted the large variation associated with the estimation of number of phage produced per bacterium. Although he employed a different technique for the purpose of estimating burst size, some of the same sources of variation pertaining to mechanisms of phage production may apply here.

From a statistical standpoint we will propose that factors associated with spaceflight did interact with the lower levels of radiation to retard the induction of bacteriophage. We do suggest, however, that the thesis requires further investigation.

Several experiments have been performed to test whether the weightlessness effects could be reproduced by slow systematic rotation of cultures. Thus far, a clinostat device has been employed only without irradiation. With proper controls the results consistenly show that weightlessness effects on growth can be simulated; i.e., cell densities in the clinostat were significantly higher than those in the stationary control. In addition, it should be noted that in these experiments clinostat effects on induction are negative. Radiation interactions, however, have not been measured. Therefore, clinostat experiments have not been useful in demonstrating whether weightlessness can be duplicated for cellular bacterial systems. The *Salmonella* controls would have achieved stationary growth at about the same time. The extrapolation is reinforced by independent growth-rate determinations at the recorded temperatures. We conclude that *Salmonella* reached a stationary phase prior to reentry and thereby presents a valid set of data on spaceflight effects, probably not influenced by reentry and recovery stress factors.

The *E. coli* failed to achieve stationary phase but were still in the exponential growth phase at reentry, during recovery and return, until icing and preparation for assay. Further extrapolation indicates *E. coli* underwent less than two divisions between reentry and assay. An unfortunate consequence of the lower (7×10^7/ml) than expected

TABLE 15. – Mean Densities of Bacteriophage P–22 Induced in Cells Incubated and Then Lysed Following Acceleration-Vibration Stresses Duplicating Those of Biosatellite II Diluted $\times$ 10^5/ml

N_E	N_C	Total γ radiation, R	Mean density, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
19	20	0	32.56 ± 2.34	24.06 ± 2.42	35.3	2.46	0.02
8	8	265	46.14 ± 6.33	37.85 ± 5.25	21.8	1.00	.32
8	8	645	70.96 ±15.29	47.83 ±10.40	48.3	1.25	.20
8	8	1630	131.24 ±14.17	71.42 ± 6.95	84.0	3.78	< .01

TABLE 16. – Mean Frequency of Induced Bacteriophage P–22 Produced per Viable Bacterium from Populations Subjected to Vibration-Acceleration Stresses Duplicating Those of Biosatellite II

N_E	N_C	Total γ radiation, R	Mean frequency, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
19	20	0	3.62 ±0.32	2.88 ±0.25	25.7	1.81	0.08
8	8	265	5.34 ±0.66	4.83 ±0.63	10.7	.56	.60
8	8	645	6.90 ±1.14	5.99 ±1.07	15.2	.58	.60
8	8	1630	16.38 ±2.63	10.34 ±0.78	58.4	2.20	.05

(3×10^8/ml) densities was insufficient phage production to provide estimates of induction. The bacterial densities are nonetheless enlightening.

The flight and control bacteria population densities, as determined by Coulter counting, are given in table 17. For all radiation levels for both strains, except nonirradiated *E. coli,* the flight populations are significantly greater than those of the Earth controls. Estimates of viable bacterial population densities by plate counting are given in table 18. Except for *E. coli,* which received a 265-R total dose, the flight populations are denser than the control populations and the differences are again statistically significant. These data are shown graphically in figure 8.

The inconsistency between the Coulter counts and the viable plate counts (in particular at the 265-R level)in the *E. coli* data are disturbing. Partial contamination in the viable counts may account for the anomaly but, in fact, we cannot account for the discrepancy other than by believing it to be an error.

Analysis of variance was done on the *E. coli*

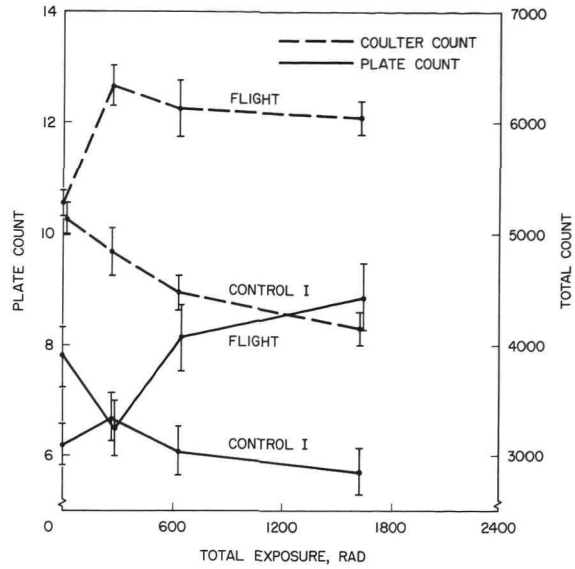

FIGURE 8.— Dose-effect curves for final population density of *E. coli* C–600 (λ) /λ for Biosatellite II flight and ground control I. Broken line gives mean and 99 percent confidence limits of Coulter counts (dilution×2× 10^4/ml). Solid line shows viable cell counts from colonies on agar plates (dilution× 10^7/ml).

TABLE 17. – Mean Total Density of *E. coli* (λ)/λ Determined From Coulter Counts; Diluted 2×10^4/ml; Biosatellite II Experiment and Control Sets

N_E	N_C	Total γ radiation, R	Mean total density $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
18	20	0	5210.0 ±117.5	5197.5 ±198.9	0.2	0.05	>0.90
8	7	5	6344.3 ±197.8	4927.0 ±152.8	28.8	5.54	< .01
8	8	1	6148.8 ±270.7	4436.6 ± 70.7	38.6	6.11	< .01
8	8	2	6059.4 ±112.4	4171.3 ±154.7	45.3	9.87	< .01

TABLE 18. – *E. coli* Viable Bacteria Densities from Plate Counts; Diluted $\times 10^7$/ml; Biosatellite II Experiment and Control Sets

N_E	N_C	Total γ radiation, R	Mean viable density, $\bar{X} \pm S_{\bar{X}}$			t	P
			Experiment	Control	Difference, %		
20	8	0	6.62 ±0.34	5.54 ±0.30	19.4	1.81	0.13
8	8	5	6.71 ±1.03	6.72 ±0.24	.1	.01	> .95
8	8	1	8.13 ±0.81	5.96 ±0.34	36.4	2.48	.02
8	8	2	8.01 ±0.55	5.67 ±0.33	41.2	3.63	< .01

viable bacteria counts using the Poisson transformation to stabilize variance within replicates. The results are shown in table 19.

The treatment effect (flight vs ground control) was significant — a repetition of the results in the *Salmonella* data. The effect of radiation, however, was not significant in the *E. coli,* nor was the treatment-radiation interaction at the commonly accepted 0.05 level. We therefore reject both a radiation effect and a radiation-treatment interaction.

The dilution error within each chamber is significant, but the error from chamber to chamber is the larger error. The latter was used as our estimate of mean bacterial count.

DISCUSSION

Pollard (1965) argued on theoretical grounds that weightlessness or hypogravity will affect the distribution of cellular components only to the extent that the latter possess potential energies greater than the Brownian movement. By calculation, the effect of 1 *g* on the statistical distribution of a single molecule (or chromosome) of bacterial DNA within a cell would be only 0.003.

Kondo (1968), following Pollard's methods, postulated that direct effects of weightlessness on genetic systems of bacteria are impossible. However, Kondo recognized that one element in the equation relating thermal agitation and gravitational orientation, giving the maximum distance between two particles within the system, can be large when bacterial populations rather than single cells are considered in a liquid medium. Thus indirect effects are possible because of intercellular interactions.

The effects of weightlessness on increased bacterial growth (presumably both rate and yield) provide a demonstration of such an indirect action of weightlessness. Although *S. typhimurium* is a motile flagellated bacterium, kinetic experiments on growing cultures clearly demonstrate partial sedimentation when high population densities are reached.[2] In the absence of gravitational ordering, individual cells would be randomly distributed at all population densities and thus not subject to waste and nutrient gradients expected within sedimenting populations. Energy would be conserved and metabolic processes directed to reproduction rather than to locomotion. The conclusion is borne out by experiments performed with systematically rotated clinostat cultures which maintain cells in a random suspension (table 20). Whether the increased radiation resistance of cells themselves under weightlessness is a more complex indirect effect remains to be tested. It is thus seen that the usefulness of the clinostat as a model for reproducing cellular weightlessness effects is not established.

The results indicating increased radiation resistance of the repressor system regulating induction of phage P–22 under weightlessness must then also be due to complex indirect effects. Recent information on the λ lysogen of *E. coli,* a model probably quite similar to *S. typhimurium* (P–22), provides data on the size and relationship of molecules responsible for the repressor sysem. Ptashne (1967) isolated a protein of 30 000 molecular weight which is the repressor material. Echols et al. (1968) showed that the repressor operates by repressing prophage m-RNA synthesis. Size, density, and distance relationships of the molecules involved in information transfer in such a system

[2] Unpublished work by Mattoni.

TABLE 19. — Analysis of Variance on Viable Bacteria Counts for *E. coli*

Source of variation	Mean square	d.f.	*F*-value	*P*
Treatments	195.19	1	10.42	<0.005
Radiations	3.17	3	.17	.70
Treatment-radiation interaction	39.90	3	2.14	.10
Chambers	18.73	56	5.06	<.001
Dilutions	3.70	192	3.77	<.001
Within (plates)	.98	2560	.....	.....

cannot be directly affected by weightlessness. The summary of effects of weightlessness are given in table 21.

In five of seven independent experiments previously reported by Zhukov-Verezhnikov (1965, 1968) it was shown that some environmental factor associated with spaceflight significantly increased the frequency of induction of bacteriophage λ in late logarithmic- or stationary-phase lysogenic *E. coli* K–12 (λ) in broth suspensions (table 22). The amount of increase was shown by other tests not to be attributable to either vibration or low-level radiation. By inference, the results could be due to the weightlessness of orbital spaceflight. Our Biosatellite II experiment results of the non-irradiated cultures indicated, at face value, agree-

TABLE 20. – Clinostat Treatment Compared with Stationary Treatment of Cultures of *S. typhimurium*

N_E	N_C	Type	Density, $\overline{X} \pm S\overline{X}$			t	P
			Clinostat	Stationary control	Difference, %		
16	16	Total cell density, diluted ($\times 2 \times 10^4$/ml)	20 744.40 ±442.00	12 143.3 ±570.7	70.8	11.9	< < 0.01
16	16	Viable cell density, diluted ($\times 10^7$/ml)	40.54 ± 1.22	15.58 ± 1.08	61.6	15.7	< < .01
16	16	Free phage density, diluted ($\times 10^8$/ml)	11.04 ± 1.33	15.15 ± 4.44	−27.6	−.89	.4
16	16	Free phage per viable bacterium ($\times 10^{-4}$)	.271 ± 0.030	.922 ± 0.197	−70.6	02.32	>.05

TABLE 21. – Effects of Weightlessness and the Interaction of Radiation and Weightlessness on Lysogenic Bacteria

Item	Effect[a] for γ radiation of —			
	0R	265R	645R	1630R
Biosatellite II				
Viable bacteria growth	+30.2% <0.001	+90.7% <0.001	+87.8% <0.001	+83.5% <0.001
Virus P-22 produced per bacterium	−9.4% N.S.	−40.8% 0.02	−37.6% 0.02	−39.2% 0.05
Vibration postflight test				
Viable bacteria growth	+8.7% N.S.	+11.2% 0.05	+13.3% N.S.	+23.6% 0.03
Virus P-22 produced per bacterium	+25.7% N.S.	+10.7% N.S.	+15.2% N.S.	+58.4% 0.05

[a] Top value, amount and direction of difference that the experimental sets differ from the controls; bottom value, probability values. N.S., not statistically significant.

TABLE 22. – Phage Yield from Induced *E. coli* K–12(λ) Cells Recovered Following Spaceflight on the Vostok and Kosmos Missions

[From Zhukov-Vereznikov et al., 1965, 1968]

	Vostok No.					
	1	2	3	4	5	6
No. λ experimentals / No. λ ground controls	1.0	1.2	4.60	1.86	3.67	1.70
Significance (*P*, *t* test)	.....	.42	.002	.001	.001	.02
Flight duration, hr.	1.5	25	94	71	119	71

	Kosmos 110		
	New cultures	2nd flight (1st on Vostok II)	Postflight 3000-R X-rays
No. λ experimentals / No. λ ground controls	2.30	1.74	0.47
Significance (*P*, *t* test)	Sign.	Sign.	Sign.
Flight duration, days	22	22	c2

ment. Higher densities of flight-induced phage (1.41 times ground controls) were demonstrated. The difference was negated, however, when the results were computed to reflect phage density per viable bacterial cell.

The discrepancy between our results can be explained by: (1) methodology, (2) strain differences, (3) culture conditions, (4) age of cultures, or (5) misinterpretation.

Two other relevant findings were reported from the Kosmos 110 experiment. The first provides good general agreement with our findings of radiation resistance of induction in that postflight acute 3000-R X-ray irradiation produced a phage frequency from the flight (5.09×10^{-3}/bacterium) half that of the control group (10.96×10^{-3}/bacterium). Zhukov-Verezhnikov et al. (1968) also found that second-flight lysogens, recovered from a previous flight on Vostok II, yielded statistically significant lower phage densities (1.74 experimental/control ratio) than did subcultures flown the first time (2.3).

The latter result, that the spaceflight environment is selective, has perhaps the most profound implication of any space experiment finding to date.

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Mutagenic Effectiveness of Known Doses of Radiation in Combination With Zero Gravity on *Neurospora crassa*

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Successful experiments using *Neurospora* as the test organism have been performed on two different spaceflights. The first experiment was in September 1966 on the Gemini XI mission and the second experiment was in September 1967 on the Biosatellite II mission. In both experiments a study was made of the genetic effects of spaceflight alone and of spaceflight in combination with known doses of radiation. On the Biosatellite II mission an ^{85}Sr gamma-ray source was used; in the Gemini XI mission a ^{32}P beta-ray source was used. In both experiments we used a genetically marked two-component heterokaryon, heterozygous for two different genes that control sequential steps in purine biosynthesis. Mutation at either locus gives rise to a requirement for adenine as well as to an accumulation of a reddish-purple pigment in the vacuoles of the mycelium. This strain was used to study radiation-induced inactivation of heterokaryotic conidia as well as radiation-induced gene mutation at two specific loci. In both experiments a range of radiation exposures was given, so that a comparison could be made between flight and Earth control dose-response curves, rather than between flight and Earth control effects of some single exposure. In the Biosatellite II experiment, *Neurospora* conidia were tested while they were collected on the surface of Millipore filters (figs. 1 and 2). In the Gemini XI experiment they were tested both on the surface of Millipore filters and in a colloidal suspension of agar.

RESULTS

The effects resulting from spaceflight on radiation-induced inactivation and the overall induction of *ad–3* mutations during the Biosatellite II mission have been published (de Serres and Webber, 1968). In brief, there was no difference between the flight and Earth control survival curves (fig. 3) nor between flight and Earth control curves for the overall induction of *ad–3* mutations (fig. 4). The characterization of the *ad–3* mutants recovered in this experiment is complete, and the overall induction curves have been resolved into their different components. These data (de Serres and Webber, 1970) show that there is no difference between the flight and Earth-control curves for point mutations or chromosome deletions (figs. 5 to 8).

The effects of spaceflight on radiation-induced inactivation and the overall induction of *ad–3* mutations on the Gemini XI mission have also been published (de Serres et al., 1969). In brief, these data show that spaceflight affected the samples flown on Millipore filters in the same way as on the Biosatellite II mission; there was no effect on survival or mutation induction (figs. 9 and 10). Quite a different result was found with the *Neurospora* samples flown in suspension. The data showed that the flight samples had higher levels of survival and lower frequencies of mutation induction (figs. 9 and 10). Spaceflight gave protection against both effects of radiation on samples in suspension.

Genetic analysis of the *ad–3* mutants recovered in the Gemini XI experiment is complete, and the overall induction curves have been resolved into their individual components. These data (de

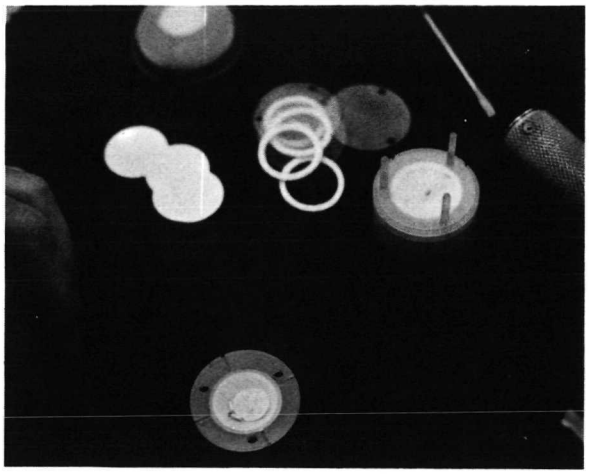

FIGURE 1.— Unassembled sample holder for *Neurospora* experiment. From upper left to right are shown porous screens, porous retaining rings (wetted to keep spore sample from drying), and a stack of polypropylene disks with porous screen in place. Lower disk contains LiF disk dosimeter.

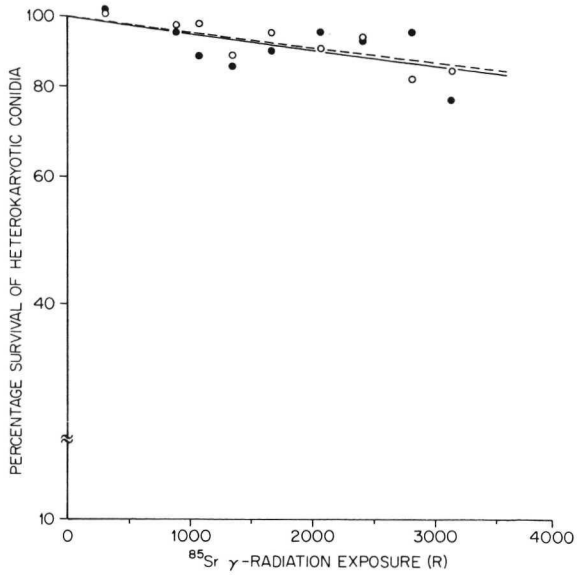

FIGURE 3.— Dose-effect curves for survival on Biosatellite II. —, ● show flight samples; —, ○ show Earth control samples.

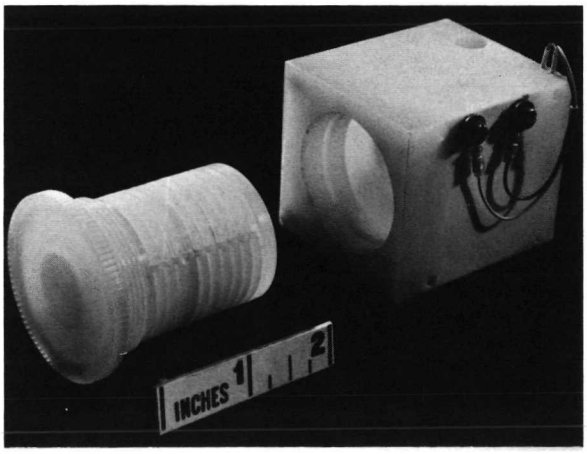

FIGURE 2.— *Neurospora* package components showing (right) housing with thermistor leads and (left) the assembled module.

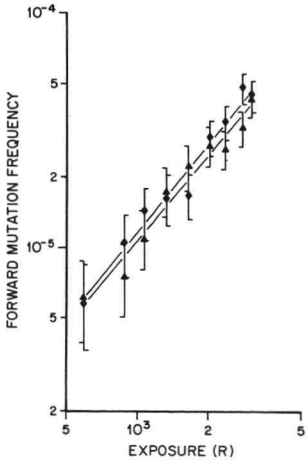

FIGURE 4.—Comparison of overall induction curves for recessive lethal mutations in the *ad*-3 region on Biosatellite II. ▲, flight samples; ◆, Earth control samples.

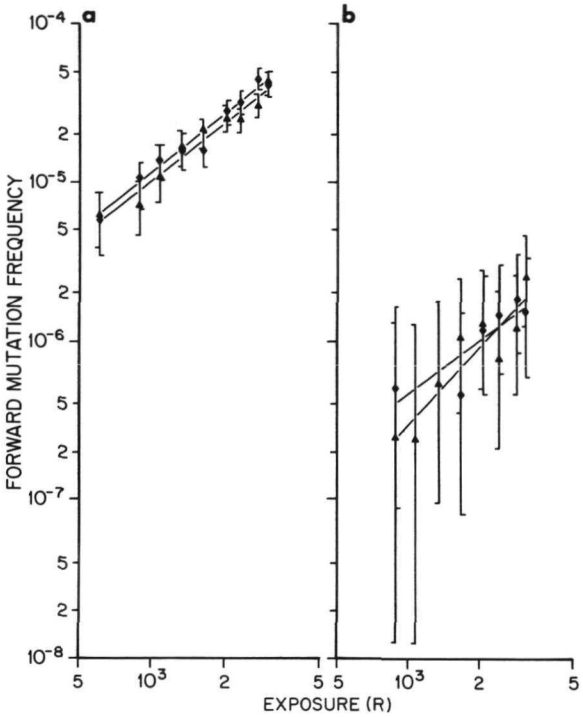

FIGURE 5.— Comparisons of induction curves on Biosatellite II. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3*^{R} mutations. (*b*) *ad-3*^{IR} mutations.

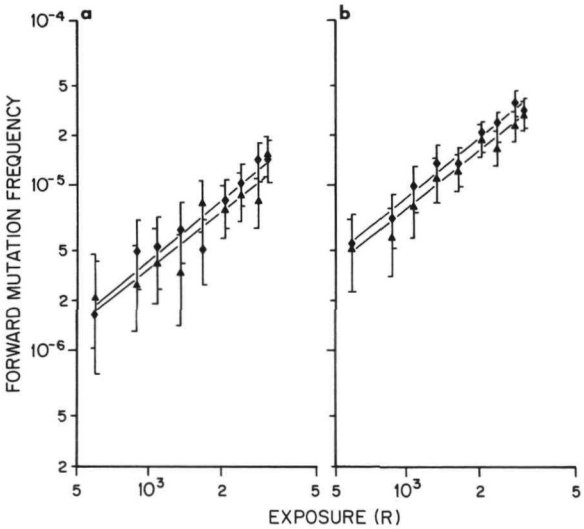

FIGURE 6.— Comparisons of induction curves of Biosatellite II. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3A*^{R} mutations. (*b*) *ad-3B*^{R} mutations.

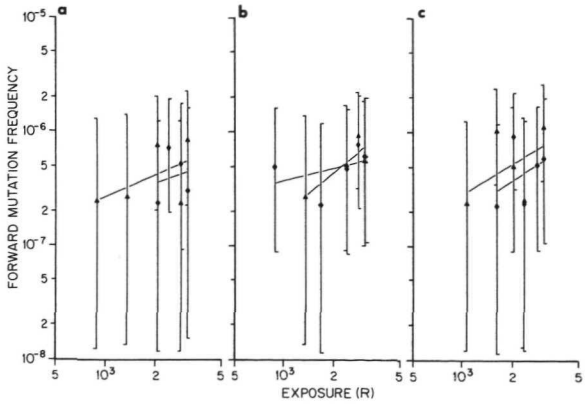

FIGURE 7.— Comparisons of induction curves of Biosatellite II. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3A*^{IR} mutations. (*b*) *ad-3B*^{IR} mutations. (*c*) (*ad-3A ad-3B*)^{IR} mutations.

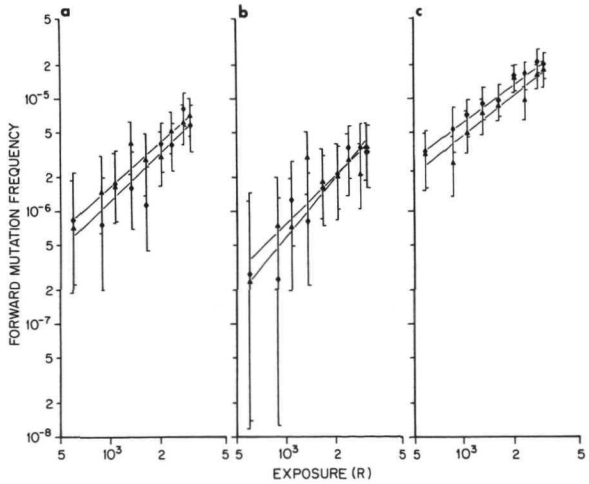

FIGURE 8.— Comparisons of induction curves on Biosatellite II. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3B*$^{R\text{-}NP}$ mutations. (*b*) *ad-3B*$^{R\text{-}P}$ mutations. (*c*) *ad-3B*$^{R\text{-}NC}$ mutations.

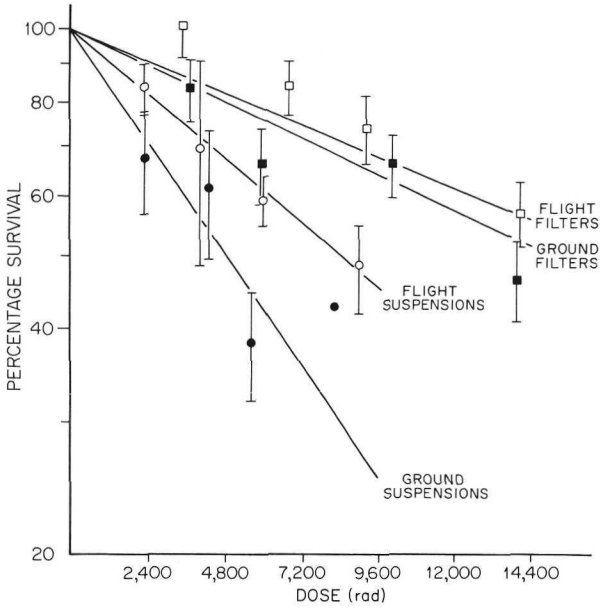

FIGURE 9.— Survival after ^{32}P irradiation on Gemini XI. Conidia were collected on Millipore filters or prepared as suspensions for both flight and Earth control. □, flight filter samples; ■, Earth filter samples; ○, flight suspension samples; ●, Earth suspension samples.

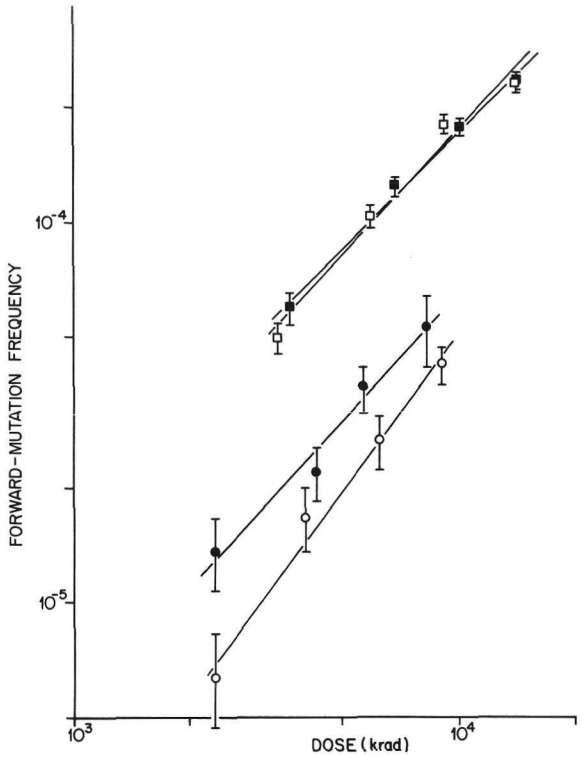

FIGURE 10.— Forward-mutation frequencies for recessive lethal mutations in the *ad-3* region after ^{32}P irradiation on Gemini XI. Conidia were collected on Millipore filters or prepared as suspensions for both flight and Earth control. □, flight filter samples; ■, Earth filter samples; ○, flight suspension samples; ●, Earth suspension samples.

Serres and Smith, 1970) show that the genetic analysis of the *ad–3* mutants in the filter samples gave the same results for the Gemini XI and Biosatellite II experiments. No difference was found between the flight and Earth control curves for point mutations or chromosome deletions (figs. 11 and 12).

The genetic analysis of the *ad–3* mutants recovered from the samples in suspension gave quite different results. These data show that the lower frequencies of mutation induction in the flight samples were due to a specific effect on point mutations and not on chromosome-deletion mutations (figs. 13 to 18).

DISCUSSION

The possibility was considered that the higher levels of survival and the lower forward-mutation frequencies might be due to anoxia because of a difference in the temperature profiles of the spacecraft cabin and the Earth control chamber prior to irradiation. An experiment was performed to study the effects of anoxia on radiation-induced inactivation and induction of mutations.

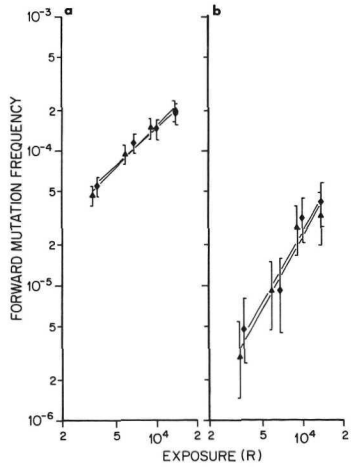

FIGURE 11.— Forward-mutation frequencies for spore samples on Millipore filters in the Gemini XI experiment. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3*^{R} mutations. (*b*) *ad-3*^{IR} mutations.

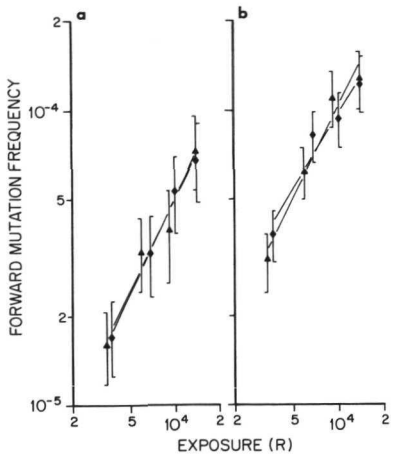

FIGURE 12.— Forward-mutation frequencies for spore samples on Millipore filters in the Gemini XI experiment. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3A*^{R} mutations. (*b*) *ad-3B*^{R} mutations.

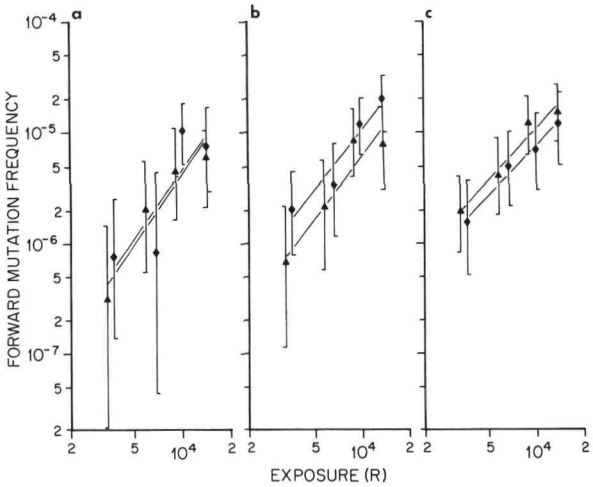

FIGURE 13.— Forward-mutation frequencies for spore samples on Millipore filters in the Gemini XI experiment. ▲, flight samples; ◆, Earth control samples. (*a*) (*ad-3A*^{IR} mutations. (*b*) *ad-3B*^{IR} mutations. (*c*) (*ad-3A ad-3B*)^{IR} mutations.

In contrast to our Gemini XI data, we found that anoxia gives a reduction in the frequencies of both point mutations and chomosome-deletion mutations. In addition, there is a marked difference between the two spectra of point mutations as well as those of chomosome-deletion mutations. With anoxia the effect on chromosome deletions is correlated with size: $(ad\text{–}3A\ ad\text{–}3B)^{IR} > ad\text{–}3B^{IR} > ad\text{–}3A^{IR}$. Tests for allelic complementation

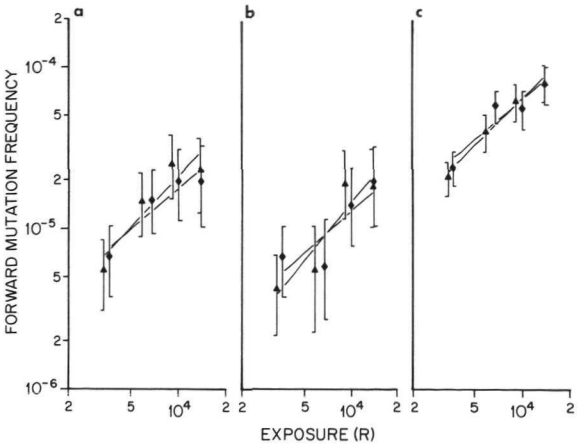

FIGURE 14.— Forward-mutation frequencies of *ad-3B*^{R} mutants as a function of complementation pattern for spore samples on Millipore filters in the Gemini XI experiment. ▲, flight samples; ◆, Earth control samples. (*a*) Nonpolarized, *ad-3B*$^{R\text{-}NP}$ mutations. (*b*) Polarized, *ad-3B*$^{R\text{-}P}$ mutations. (*c*) Noncomplementing, *ad-3B*$^{R\text{-}NC}$ mutations.

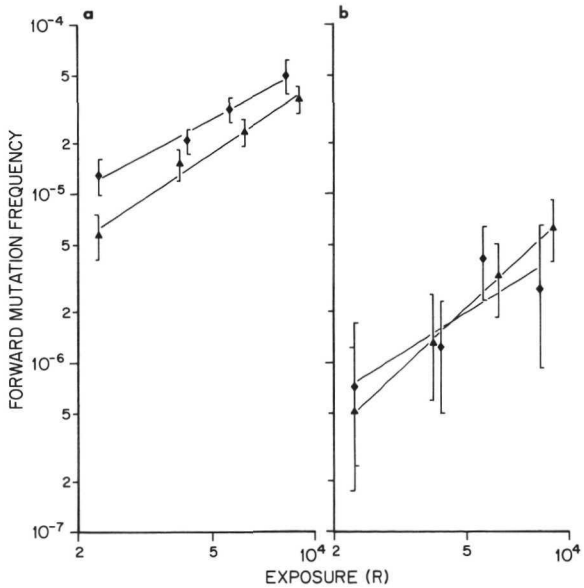

FIGURE 15.— Forward-mutation frequencies for spore samples in suspension on Gemini XI. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3*^{R} mutations. (*b*) *ad-3*^{IR} mutations.

showed that with anoxia there was a different spectrum of complementation patterns. Whereas there was no effect of anoxia on the frequency of mutants with polarized patterns, there was a 2.7-

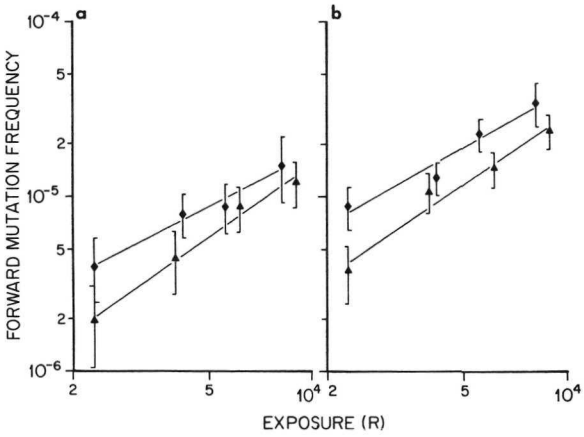

FIGURE 16.—Forward-mutation frequencies for spore samples in suspension on Gemini XI. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3A*^{R} mutations. (*b*) *ad-3B*^{R} mutations.

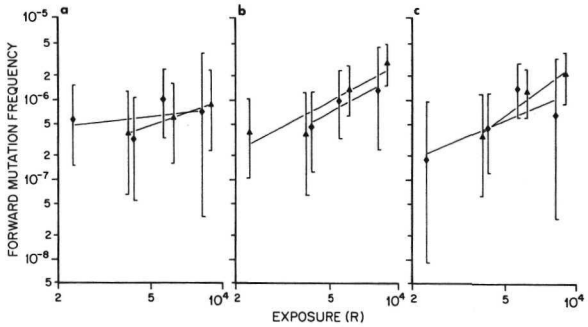

FIGURE 17.— Forward-mutation frequencies for spore samples in suspension on Gemini XI. ▲, flight samples; ◆, Earth control samples. (*a*) *ad-3A*^{IR} mutations. (*b*) *ad-3B*^{IR} mutations. (*c*) (*ad-3A ad-3B*)^{IR} mutations

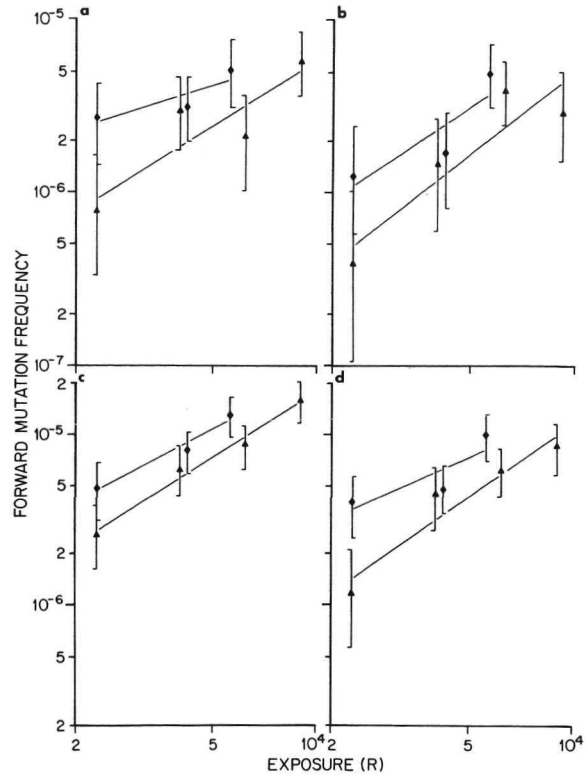

FIGURE 18.— Forward-mutation frequencies *of ad-3B*^{R} mutations as a function of complementation pattern for spore samples in suspension on Gemini XI. ▲, flight samples; ◆, Earth-control samples. (*a*) Nonpolarized, *ad-3B*$^{R\text{-}NP}$, (*b*) Polarized, *ad-3B*$^{R\text{-}P}$, (*c*) Noncomplementing, *ad-3B*$^{R\text{-}NC}$, (*d*) Total complementing *ad-3B*$^{R\text{-}C}$.

fold reduction in the frequency of mutants with nonpolarized patterns and noncomplementing mutants.

The fundamental difference between the two *Neurospora* experiments on the Gemini XI mission is that the conidia on Millipore filters are essentially metabolically inactive, whereas the spores in suspension are metabolically very active. The various experiments in the Biosatellite II mission have shown that the effects of weightlessness on radiation-induced genetic damage are complex; both antagonistic and synergistic effects of radiation have been found. The result depends on the assay system. In most cases, however, the effects are small, twofold to fivefold differences being the usual order of magnitude.

It is important to remember that these experiments were intended as survey experiments to determine whether there were genetic mechanisms on which spaceflight might have an effect. It is only with the proper followup experimentation that we can hope to determine whether the effects that we have found are due to weightlessness and whether twofold to fivefold differences are the maximum level of these effects.

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Radiation Exposures During the Biosatellite II Flight

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Experiments in space are the only way to determine whether identical radiations produce the same effects in biologic organisms on Earth as in the weightless state. One approach to the problem has been to send up biologic materials in a satellite and to measure the radiation to which they have been exposed (Crawford et al., 1962a). Usually the doses were very low and no biologic effect that could be attributed to radiation was seen. It was clear that in order to do an adequate experiment it would be necessary to expose samples to a controlled amount of radiation in flight. The Biosatellite II flight program was developed to meet this need.

Seven of the experiments flown on Biosatellite II were designed to determine the combined effects of radiation and weightlessness on lethality and mutagenesis in a variety of living systems. To accomplish this, they were exposed to an onboard source of radiation during the flight. Several months after the flight was successfully completed, a series of ground tests was initiated. The tests were designed to determine whether the observed effects of interaction could have been due to vibration and acceleration associated with the flight rather than to weightlessness.

EXPERIMENT DESIGN AND METHODS

For the flight, the biologic material from each experiment was divided into four groups. Each group was exposed to one of the following environments:

(1) Aboard Biosatellite II: Group A, radiation and weightlessness; and group B, no radiation and weightlessness

(2) In the Earth control capsule: Group C, radiation and 1 *g*; and group D, no radiation and 1 *g*

Provisions had to be made in the capsule for an essentially radiation-free area for the control experiments and for the nonradiation experiments such as the frog egg and pepper plant experiments. Figure 1 shows the capsule divided into three main sections. The first, or forward, section contains the radiation source and the packages to be irradiated. A tungsten backscatter shield separates this section from the center section, the spacecraft equip-

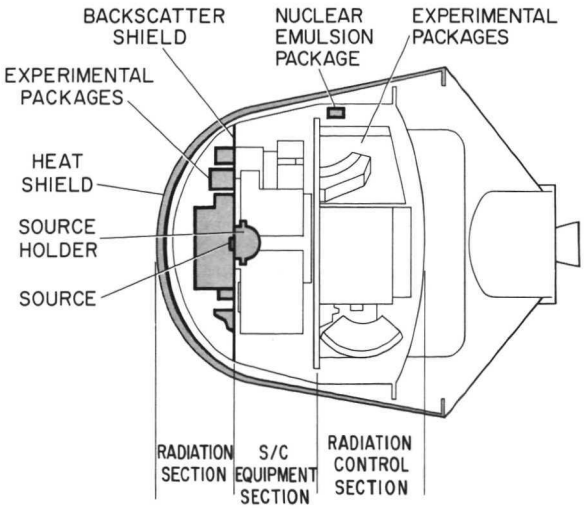

FIGURE 1.— Biosatellite 3-day capsule.

ment section. The third, or aft, section contains the control experiments. It was planned that the radiation exposures in this section should not exceed 1 roentgen (R) per day.

In selecting a radiation source, several factors were considered. It was desirable to irradiate all the packages at the same time, by use of a single source. The source had to be a gamma emitter. In the event that the capsule were lost, a short half-life would reduce the danger of exposure to someone who might accidentally find it. At the same time, the half-life had to be long enough so the source strength would not change appreciably during the planned 3 days of flight of Biosatellite II. The radioisotope finally chosen was 85-strontium, a gamma emitter giving off a single ray of 0.513-MeV energy. It has a half-life of 64 days.

Lithium fluoride (LiF) thermoluminescent powder was selected as the primary dosimeter. Each experiment package was monitored for radiation exposure by tubes of LiF powder attached to the outside of the package. Two pocket ion chambers were placed in the control area for an independent check of the low doses expected there. In addition, several of the experimenters had dosimeters within their packages. These are described in the individual experiment reports. The LiF powder was carefully readout postflight. A dose-response curve was established for the LiF with the use of an ^{85}Sr source. At least three readings were made on each sample and reference powders were read three times per day to detect machine drift.

The space radiation environment was monitored by a nuclear emulsion pack containing Kodak NTA, Ilford K.2, and Ilford G.5 emulsions (fig. 1). Because the emulsions, especially the G.5, are so sensitive to radiation, a tungsten well was made to shield the emulsion from the onboard source. The well was mounted next to the shell of the spacecraft in the control area. Its open side faced outward to accept protons and heavy ions entering the spacecraft.

Prior to flight, two paired radiation sources were received from the manufacturer. The sources, ARC No. 20 and ARC No. 21, were to be placed in the flight capsule and the Earth control capsule, respectively. The strength of each source was measured with a Victoreen R-meter which had been calibrated at the National Bureau of Standards and hand-carried to Cape Kennedy.

Source Capsule

Radioactive ^{85}Sr was produced by irradiating enriched $^{84}Sr(NO_3)_2$ with neutrons. The resulting powder was then poured into a stainless steel capsule, as shown in figure 2. The powder is retained within the capsule volume by welding the base plug in place. The finished capsule is shown in figure 3. Only the portion above the collar contains radioactive powder.

Radiation Source Holder

The purpose of the radiation source holder was to shield the radiation source before and after the exposure period and to shield the control experiment packages in the aft section of the Biosatellite capsule. It consisted of an approximately hemispheric mass of sintered tungsten. The holder is shown in figure 4. A wheel was mounted in the holder; a small section of the wheel projects above the top surface of the holder. In figure 5 the

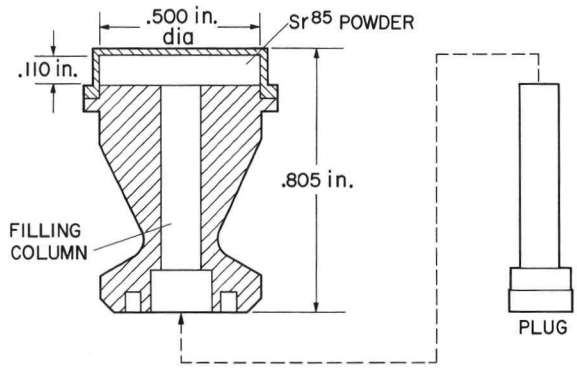

FIGURE 2.— Schematic of radiation source capsule and plug.

FIGURE 3.— Radiation source capsule.

FIGURE 4.—Radiation source holder.

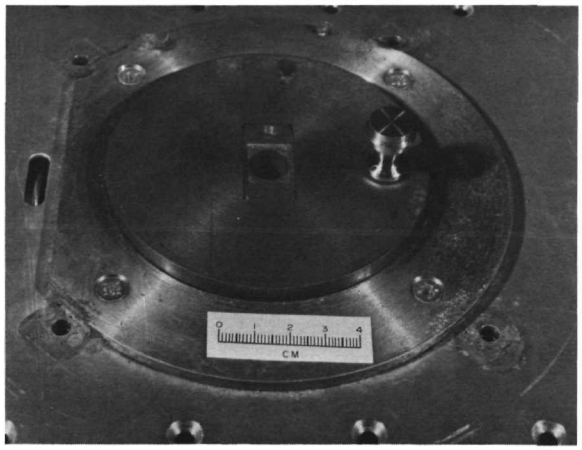

FIGURE 5.— Source capsule on top surface of source holder.

source capsule is shown resting on the top surface of the holder. The hole in the wheel into which the source capsule is inserted is also shown. When the wheel turns, the source is retracted and the intensity at the surface of the holder does not exceed 40 mR/hr.

Location of Experiment Packages

The optimum exposures for the test organisms ranged from 300 R for *Tradescantia* to 6000 R for one of the *Neurospora* samples. The packages were placed at different distances from the source so that each would receive its correct exposure in a nominal 65-hour exposure time. The distances were calculated from the inverse square law with the assumption of a point source of radiation. Figure 6 shows the arrangement of the packages.

Dosimeters

Three dosimetry packages are shown in figure 7. Dosimetry package No. 1 is shown to the right. It was placed in the radiation area and contained film and LiF powder and was expected to receive a nominal 1000 R. The three items in the center of the figure are parts of dosimetry package No. 2. This package was placed in the control area. It contained two pocket ion chambers, one of 5-R capacity and another of 1-R capacity. Dosimetry package No. 3, shown on the left, contained the nuclear emulsion package in a tungsten well.

Postflight Testing

The postflight testing was conducted at NASA Ames Research Center. It was divided into three phases. The phase A test was primarily a dosimetry test designed to determine the amount of scattered radiation received by each package. Several experimenters felt it was necessary to make this determination because low-energy scattered radiation has a greater biologic effect than has high-energy radiation for the same exposure. A second objective of the test was to determine whether, in a series of runs, the difference between the radiation exposures measured in the flight and Earth control capsules would remain constant. There were no biologics in this test. The phase B test served to determine if the biologics would give the same response in each capsule with paired radiation sources and all other factors constant. The phase C test was designed to subject the flight capsule to acceleration and vibration the same as those measured during the flight while the Earth control capsule remained as a test control.

RESULTS

Flight Results

AMES RESEARCH CENTER MEASUREMENTS

The results of source strength measurements before flight are given in table 1. The sources are

TABLE 1. — Source Strength Measurements

Source	Source strength, mR/hr/meter	
	Measured	Average
ARC No. 20 (flight)	360	360
	360	
ARC No. 21 (Earth control)	360	
	354	357

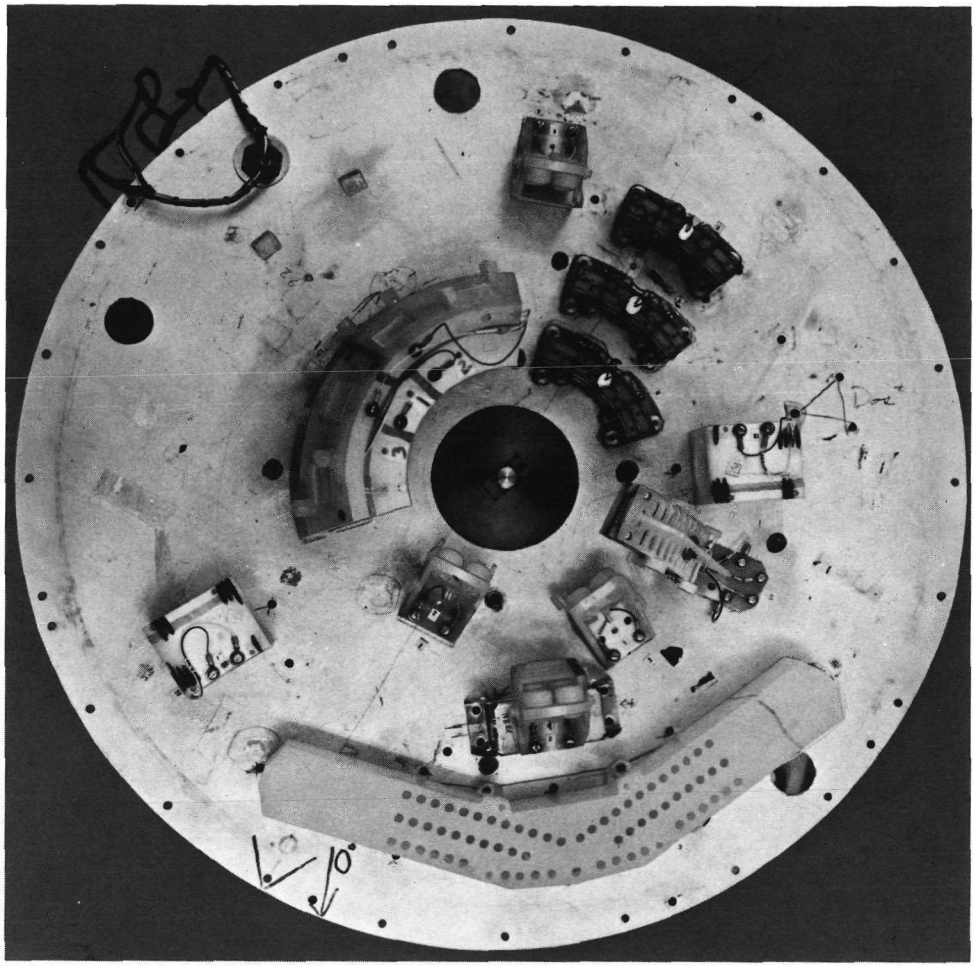

FIGURE 6.— Top view of packages arranged around radiation source. *Neurospora* bracket removed.

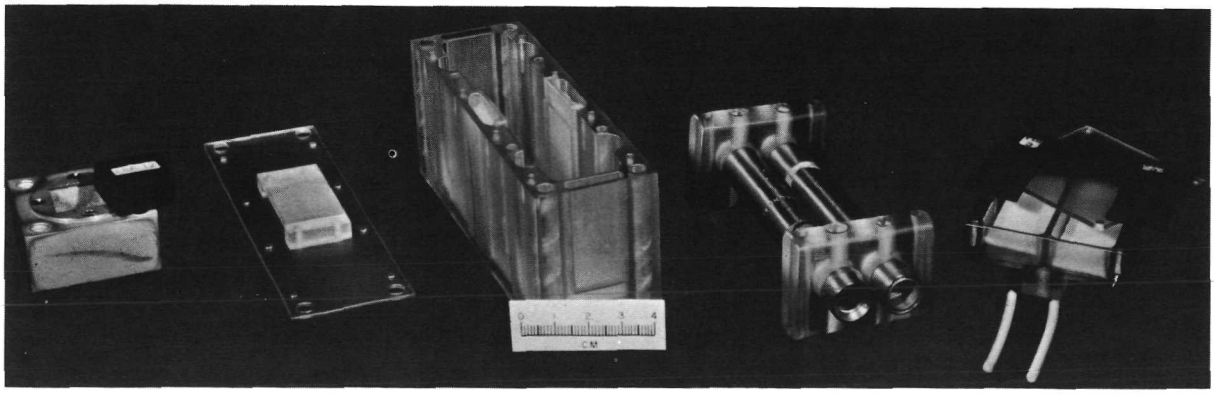

FIGURE 7.— Three dosimeter packages.

paired to within 1 percent. Table 2 gives the measured exposures to the LiF tubes attached to each experiment package. Except for the *Habrobracon* 1000 R package, most of the percent difference figures are within the accuracy of the reading method. The data indicate that for this flight the *Habrobracon* package bracket was reversed. Table 3 presents the radiation exposure of the biologic samples for four of the experiments. These values are calculated from the data in table 2. Table 4 shows that the dose in the control area was very small as measured by both LiF tubes and pocket ion chambers. The range is from 0.15 to 0.77 R.

NUCLEAR EMULSION MEASUREMENTS OF SPACE RADIATION [1]

In the nuclear emulsion flight pack, densitometric doses due to gamma radiation were 43 mR at the top of the tungsten well and 21 mR at the bottom. Whereas the background caused by these doses did reduce the data available in the G.5 emulsion, it did not seriously interfere with the proton ender or heavy nuclei counts.

The flight pack in the tungsten well contained, from top to bottom, the following film sheets: Kodak Type 3 Double Component, 200-micron K.2, 100-micron G.5, 100-micron K.2, 200-micron K.2, NTA, NTA, 100-micron G.5, 200-micron K.2, and Kodak Type 3 Double Component. The 200-micron K.2 emulsion at the top was evaluated for proton enders and the 100-micron G.5 close to the bottom, for heavy nuclei. The enders count, based on a total of 806 identified enders, corresponds to a value of 8.24 enders per square millimeter of emulsion area. As systematic measurements on the last two Mercury and the Gemini missions have shown (Schaefer and Sullivan, 1967a), the enders count reflects, for the energy spectrum of trapped protons in the anomaly at satellite altitudes, the total dose in good first approximation for a given shield configuration. As the shield equivalent becomes lighter, the ratio of enders count to total dose becomes larger, yet the change is a rather weak function. Since the shielding for the radiation pack on Biosatellite II can safely be assumed to be lighter than that for the packs on the Gemini missions, application of the enders count calibration of the Gemini emulsions to the Biosatellite II data will overrate the actual dose. The dose measured is 48 mR and must be considered, for the just-explained reasons, an upper limit estimate for the actual proton exposure on Biosatellite II. It compares with a dose from trapped protons on the 14-day mission of Gemini VII of 230 mR. Expressed in dose rates, the two values correspond to 25.5 and 16.5 mR per 24 hours. Whereas a certain part of the difference is due to the indicated shielding influence on the conversion factor, a sizable part of the difference must be assumed to be real in view of the substantially smaller attenuation from the local hardware on the 940-pound Biosatellite II vehicle as compared with that on the 7-ton Gemini spacecraft. Just how much of the difference is to be attributed to either effect cannot be stated at this time. It is felt that the best estimate for the mission dose from trapped protons at the upper level of the tungsten well is 40 mR. For more heavily shielded locations inside the vehicle, the corresponding doses might have been substantially smaller.

Evaluation of the G.5 emulsion for heavy nuclei was carried out by the method used in Gemini VII (Shaefer and Sullivan, 1967b); i.e., the flux of very heavy nuclei of $Z \geqq 20$ was determined by sorting out heavy tracks under the microscope according to the diameter of the solid silver core and the delta ray aura using a reference track of $Z=20$ in the atlas of Powell, Fowler, and Perkins (1959). The track made by a particle of this size during the flight is shown in figure 8. On the

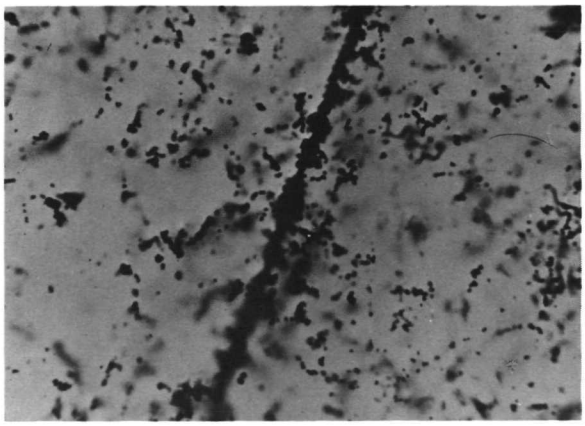

FIGURE 8.—Track of heavy cosmic ray in nuclear emulsion.

[1] The nuclear emulsion report was furnished by Dr. H. J. Schaefer, U.S. Naval Aerospace Medical Institute, Pensacola, Fla.

TABLE 2. – Radiation Doses to LiF Tubes

Package	LiF tube	Exposure, R		% difference, Flight – (Earth control) / Flight
		Flight	Earth control 1	
Habrobracon, 4000 R	1	1440	1470	−2 to 3
	2	3570	3400	+5 to 6
Habrobracon, 2000 R	1	830	830	0
	2	1630	1600	+1 to 2
Habrobracon, 1000 R	1	420	503	−19 to 20
	2	650	825	−26 to 27
Habrobracon, 500 R	1	280	283	−1 to 2
	2	395	400	−1 to 2
Tribolium	1	1080	1070	+0 to 1
	2	1060	1030	+2 to 3
	3	775	770	+0 to 1
	4	750	745	+2 to 3
Drosophila larvae	1	660	655	+0 to 1
	2	655	655	0
	3	640	635	+0 to 1
	4	645	625	+3 to 4
Drosophila adult	1	1580	1505	+4 to 5
	2	1550	1560	−0 to 1
	3	1500	1460	+2 to 3
Tradescantia	1	310	307	+0 to 1
	2	316	312	+1 to 2
	3	205	207	−0 to 1
	4	207	205	+0 to 1
	5	295	288	+2 to 3
	6	300	295	+1 to 2
Lysogenic bacteria 2500 R	1	2250	2230	+0 to 1
	2	2250	2280	−0 to 1
	3	1270	1250	+0 to 1
	4	1260	1220	+3 to 4
Lysogenic bacteria, 1000 R	5	750	695	+7 to 8
	6	730	735	−0 to 1
	7	457	460	−0 to 1
	8	473	480	−1 to 2
Lysogenic bacteria, 500 R	9	254	253	+0 to 1
	10	300	302	−0 to 1
	11	213	206	+3 to 4
	12	215	220	−2 to 3
Neurospora, 6000 R	1	3600	3550	+1 to 2
	2	1160	1180	−1 to 2
	3	3525	3720	−5 to 6
	4	1160	1130	+1 to 2
Neurospora, 2500 R	1	1670	1710	−2 to 3
	2	685	675	+1 to 2
	3	1670	1630	+2 to 3
	4	720	740	−2 to 3
Neurospora, 1000 R	1	645	630	+2 to 3
	2	350	350	0
	3	625	640	−2 to 3
	4	350	350	0
Neurospora, 500 R	1	340	342	−0 to 1
	2	222	220	+0 to 1
	3	335	343	−2 to 3
	4	222	222	0

TABLE 3. – Radiation Doses to Biologics

Package	Exposure, R	
	Flight	Earth control 1
Tribolium, 1200 R	900	882
	877	861
	845	830
Drosophila larvae, 1300 R	832	822
Drosophila adult, 2000 R	1432	1404
Lysogenic bacteria:		
2500 R front chambers	1836	1840
2500 R back chambers	1456	1459
1000 R front chambers	697	674
1000 R back chambers	591	571
500 R front chambers	282	284
500 R back chambers	250	251

TABLE 4. – Radiation Measurements in Control Areas

Package	Radiation, R	
	Biosatellite II Flight	Biosatellite II Earth control 1
Habrobracon	0.38	0.43
	.43	.47
Tribolium	.42	.48
	.45	.49
	.33	.45
	.36	.47
Drosophila adult	.24	.32
	.27	.33
	.20	.28
Tradescantia	.23	.33
	.20	.35
	.25	.40
	.27	.40
Drosophila larvae	.36	.77
	.18	.64
	.32	.71
Neurospora	.60	.28
	.19	.23
	.19	.28
	.15	.19
Lysogenic bacteria	.....	.51
	.43	.46
Ion chambers:		
1.0-R chamber	.22	.25
5.0-R chamber	.28	.34

basis of a total of 25 identified tracks of $Z \geqq 20$, a flux of $Z \geqq 20$ nuclei of 10.1/cm^2 was found. Since this is the total mission flux, it corresponds to a flux of 5.4/cm^2 day. This value matches very closely the theoretical value since it follows from the existing data on the heavy galactic flux. Assuming that the close agreement between the observed flux for $Z \geqq 20$ nuclei and the theoretical value can be extended to the entire galactic flux, one obtains a mission dose of 2.9 mR from galactic radiation.

It must be expressly stated that a substantial fraction of these 2.9 mR are due to heavy nuclei and that the radiobiologic significance of this particular part of the exposure is not adequately expressed in terms of absorbed dose. Investigators with experiments aboard Biosatellite II who wanted to establish hit numbers from passage of individual heavy nuclei through the microstructure of their respective biologic specimens were referred to the value of 10.1 traversals of nuclei of $Z \geqq 20$ through a target area of 1 cm^2 during a time span of 45 hours in orbit. This flux can be assumed to be essentially constant throughout the entire interior of the vehicle assembly. The hit numbers for lighter nuclei have been calculated by using the flux ratios of the $Z \geqq 20$ group to the lighter groups (Schaeffer and Sullivan, 1967b), as presented in table 5.

TABLE 5. – Calculated Fluxes of Galactic Heavy Nuclei

Elements	Z	Flux, nuclei/cm^2/42 hr.
Li, Be, B	3, 4, 5	30.3
C, N, O, F	6, 7, 8, 9	108.0
Ne→K	10→19	27.3
Ca→Ni	20→28	10.1 measured

TABLE 6. – Representative Scattering Measurements

Package	Run 1				Run 2			
	Set	CaF_2	LiF	% <100 keV	Set	CaF_2	LiF	% <100 keV
Tradescantia	1	63.1	62.4	0.7	130	52.3	46.0	1.8
	5	50.4	47.7	.8	134	45.2	40.1	1.7
	10	57.3	53.5	1.0	138	63.5	56.0	1.8
	15	57.4	52.7	1.2	140	48.2	44.1	1.3
	20	65.4	59.4	1.4	142	45.7	44.9	.2
Drosophila adult	21	378	346	1.2	150	380	371	.3
	25	357	344	.5	155	384	342	1.7
	30	422	361	2.3	160	399	358	1.6
	35	384	360	.9	165	390	365	.9
Drosophila larvae	37	243	233	.6	168	255	236	1.1
	40	254	233	1.2	170	242	234	.5
	45	238	235	.2	175	249	225	1.4
	50	233	223	.6	180	235	203	2.1
Tribolium	53	228	212	1.0	.....	.....	.....	.....
	55	252	229	1.4	.....	.....	.....	.....
	57	252	238	.8	.....	.....	.....	.....
Lysogenic bacteria, 2500 R	60	288	397	−.3	190	444	410	1.1
	65	515	440	2.3	195	397	324	3.1
	70	445	447	0	200	378	337	1.6
Lysogenic bacteria, 1000 R	75	181	175	.5	206	178	165	1.1
	80	160	160	0	210	181	183	.2
	85	183	137	4.5	215	164	140	2.3
Lysogenic bacteria, 500 R	91	79.1	73.0	1.1	220	75.9	60.0	3.6
	94	48.5	56.9	−2.0	225	82.5	74.2	1.5
	100	82.8	75.3	1.3	229	79.6	72.3	1.4
Habrobracon	1	756	641	.3	14	159	147	1.1
	10	103	94.2	1.3	20	79.1	73.1	1.1

Postflight Test Results

PHASE A

A measure of the amount of scattered radiation to which the packages were exposed is given in table 6. The primary radiation has an energy of 0.513 MeV. Its energy is reduced by each scattering event. In order to measure that percentage with energy degraded below 100 keV, paired sets of miniature CaF_2 and LiF dosimeters were placed inside the packages. Since the response of CaF_2 increases greatly below this energy and that of LiF does not, it was possible to calculate the percentage from the difference of the two dosimeter readings.[2] In almost all cases the values are less than 2 percent.

Table 7 gives the differences in the dosimeter readings for corresponding locations in the flight (301) spacecraft and the Earth control (201) spacecraft during the three runs. Problems were encountered with unstable LiF during this test. A batch of LiF was received which lost, within a few days, as much as 25 percent of the energy it had absorbed when it was irradiated. This made it impracticable to convert the LiF readings to roent-

[2] These measurements were made by Edgerton, Germeshausen & Grier, Inc., Goleta, Calif.

TABLE 7. – Comparison of LiF Readouts in 301 and 201 Spacecraft, Phase A Tests

Package	LiF tube	Run 1, % difference (301 – 201)/301	Run 2, % difference (301 – 201)/301	Run 3, % difference (301 – 201)/301
Habrobracon:				
4000 R	2	−6.3	−3.3	+2.8
2000 R	2	+.8	+5.4	+3.6
1000 R	2	−28.0	−27.0	+7.4
500 R	2	+3.4	−7.0	−1.1
Tribolium	1	+1.0	−2.5	−8.2
	2	+2.3	−2.3	−5.5
Drosophila larvae	1	−3.8	0	−1.0
	2	−7.0	−8.7	+4.3
	3	−2.1	+4.2	+2.4
	4	−1.2	+2.1	+8.9
Drosophila adult	1	−3.6	−3.6	+2.2
	2	0	0	+1.9
	3	+2.2	−6.4	+1.0
Tradescantia	3	−2.9	+2.9	−5.3
	4	+3.4	0	−4.3
Lysogenic bacteria:				
2500 R	1	+7.0	−11.8	−.7
	2	+.2	−6.7	−7.8
1000 R	1	0	−5.4	+1.9
	2	0	−5.9	−8.5
500 R	1	+2.8	−3.0	+7.0
	2	+1.4	+6.0	0
Neurospora:				
6000 R	1	−3.6	−5.5	+6.0
	3	+2.1	+3.5	0
2500 R	1	+3.6	+5.1	—
	3	−.4	+3.9	0
1000 R	1	+5.1	−2.2	+1.8
	3	+5.1	−2.2	+1.8
500 R	1	0	+4.2	−.4
	3	+3.7	−8.3	+1.2
Net percent difference		+.36	−2.12	+0.01

gen values. However, since the data in table 7 are derived from paired LiF readings, the percent difference values are acceptable. The net differences for each run are small; the large values recorded for the 1000 R *Habrobracon* package in runs 1 and 2 were caused by the bracket of this package being mounted backward as it was in the flight. After this test, the LiF powder was replaced by the manufacturer.

PHASES B AND C

The source strength measurements for the phase B and C tests are given in table 8. The initial calibrations of the phase B sources indicated a difference of 3 percent between source strengths. The manufacturer reported a difference of 1 percent. In order to resolve this discrepancy, another series of runs was made after the test. The results are given in table 8. The values are higher in these runs since they were not made at 1 meter. The relative values of 649 and 653 indicate agreement to within 1 percent. It seems probable that the value of 346 in the original measurements was too low. The strengths of the phase C sources were closely paired; however, the measured intensities are about 4 percent lower than those of the sources used in Biosatellite II and the phase B test.

The *Tribolium, Habrobracon,* and adult *Drosophila* experiments required irradiation of some of the animals with X-ray prior to testing. A GE Maxitron was used for this purpose. The X-ray parameters are given in table 9. They were kept constant for both tests, except for variations of a few percent in dose rate.

The radiation exposures at the LiF tube positions and to the biologics are given in tables 10

TABLE 8. – Source Strength Measurements, Phase B and C Tests

Source	Source Strength, mR/hr/m	
	Measured	Average
Phase B		
ARC No. 27 (201 capsule)	364	.....
	346	354
	353	.....
ARC No. 28 (301 capsule)	367	366
	365	.....
Phase B, repeat (not at 1 m)		
ARC No. 27	649	.....
	652	649
	647	.....
ARC No. 28	655	.....
	654	653
	649	.....
Phase C		
ARC No. 29 (201 capsule)	345	342
	340	.....
ARC No. 30 (301 capsule)	341	345
	349	.....

and 11. For both tests, there is good agreement between the paired positions in the 301 and 201 spacecraft. The net percent differences are +0.35 and −0.15 percent for the phase B and phase C tests, respectively. The large value shown for the *Habrobracon* 1000 R package in the phase C test indicates that its bracket was reversed as it was during the actual flight. As expected, exposures in the control areas were low. The measured values are given in table 12.

DISCUSSION

For the first time, controlled radiobiologic experiments have been conducted in orbital flight. A wide range of biologic material has been flown to determine the effect of weightlessness and radiation on genetic and somatic end points. The use of paired radiation sources permitted the delivery of almost identical radiation exposures to the flight and Earth control packages. This was an essential requirement since it was expected that some of the effects due to weightlessness might be quite small.

The use of ^{85}Sr as a source proved to have disadvantages. The short half-life is troublesome in the face of the uncertainties of launching a satellite. Since the sources were decaying at the rate of 1 percent per day, it was necessary to have a new pair arrive each 5 or 6 days in case the launch was delayed. Consideration should be given to the use of an isotope with a longer half-life, if a similar flight is planned for the future.

The nuclear emulsion measurements verify again that the doses due to space radiations are very low in orbits similar to that of Biosatellite II. It should be pointed out that the radiation exposures from the onboard source were much higher than those one would normally receive from space radiation. For example, the Discoverer XVII flight (Crawford et al., 1962b) took place during a large solar flare. The spacecraft was placed in a polar orbit. During a 50-hour period, an exposure of 30 R inside the vehicle was recorded. Most of the exposure took place as the spacecraft crossed the polar regions where the magnetic field of the Earth offers little shielding. Even if the spacecraft had been located far from the Earth, it is improbable that the exposures would have exceeded the highest doses delivered aboard Biosatellite II.

The postflight tests were extremely valuable to the experimenters in aiding them to distinguish effects which might be due to a vibration and acceleration (centrifuge simulation) rather than to weightlessness.

The results of the dosimetry measurements indicate that it is highly unlikely that any difference found between the biologic responses of the same experiment in the flight and Earth control capsules can be attributed to radiation exposure differences, nor can any unusual results be attributed to the presence in the capsules of a large component of low-energy radiation produced by multiple scattering.

SUMMARY AND CONCLUSIONS

The biologic specimens which were flown in Biosatellite II received the same radiation exposure

TABLE 9. – X-Ray Parameters, Phase B and C Tests

Experiment	kVp	mA	Aluminum filter, in.	Target distance, in.	Phase B		Phase C	
					Dose rate, R/min	Exposure, R	Dose rate, R/min	Exposure, R
Tribolium	180	5	0.042	11.5	102.3	1350	106	1310
Habrobracon[a]	180	10	.092	12.0	120.0	360	124.8	.....
						720	.....	720
						1200	.....	1200
						2000	.....	.....
						2400	.....	2400
								4000
Drosophila adult	190	15	.092	11.5	215	4000	211	4000

[a] 2 mm of plastic were placed over the *Habrobracon* packages to attain electron equilibrium.

TABLE 10. – Radiation Doses to LiF Tubes, Phase B and C Tests

Package	LiF tube	Phase B			Phase C		
		Exposure, R		% difference, (301 – 201)/301	Exposure, R		% difference, (301 – 201)/301
		301 S/C	201 S/C		301 S/C	201 S/C	
Habrobracon, 4000 R	1	1400	1470	−5.0	1490	1480	+0.7
	2	3380	3480	−3.0	3370	3300	+2.1
Habrobracon, 2000 R	1	835	825	+1.2	782	785	−.4
	2	1580	1540	+2.5	1510	1550	−2.6
Habrobracon, 1000 R	1	491	508	−3.5	410	497	−21.2
	2	800	840	−5.0	625	810	−29.6
Habrobracon, 500 R	1	310	285	+8.0	282	294	−4.3
	2	410	397	+3.1	387	390	−.8
Tribolium	1	1100	1100	0	1070	1080	−.9
	2	1080	1080	0	1010	1070	−5.9
	3	812	810	+.2	700	740	−5.7
	4	770	783	−1.7	710	715	−.7
Drosophila larvae	1	704	675	+4.1	625	643	−2.9
	2	684	665	+2.8	610	630	−3.3
	3	635	635	0	603	600	+.5
	4	633	628	+.8	590	590	0
Drosophila adult	1	1480	1490	−.7	1540	1490	+3.2
	2	1530	1520	+.6	1490	1470	+1.3
	3	1530	1490	+2.6	1480	1500	−1.4
Tradescantia	1	315	314	+.3	313	312	+.3
	2	310	315	−1.6	309	308	+.3
	3	207	200	+3.4	200	205	−2.5
	4	213	203	+.5	205	206	−.5
	5	305	305	0	285	286	−.3
	6	288	295	−2.4	282	287	−1.8
Lysogenic bacteria, 2500 R	1	2200	2160	+1.8	2230	2100	+5.8
	2	2030	2070	+2.0	2070	2140	−3.4
	3	1240	1240	0	1180	1210	−2.5
	4	1270	1250	+1.5	1210	1240	−2.5
Lysogenic bacteria, 1000 R	5	767	740	+3.5	687	705	−2.6
	6	778	780	−.3	742	720	+3.0
	7	477	477	0	440	446	−1.4
	8	490	492	−.4	456	460	−.9
Lysogenic bacteria, 500 R	9	276	280	−1.4	265	265	0
	10	310	295	+1.8	300	304	−1.3
	11	215	225	−4.6	206	207	−.5
	12	230	225	+2.2	212	208	+1.9
Neurospora, 6000 R	1	3280	3200	+2.4	3500	3180	+9.1
	2	1080	1130	−4.6	1120	1100	+1.8
	3	3329	3259	+2.6	3220	3250	−.9
	4	1080	1070	+.9	1130	1070	+5.3
Neurospora, 2500 R	1	1630	1600	+1.8	1670	1560	+6.6
	2	720	715	+.7	664	626	+5.7
	3	1650	1600	+3.0	1560	1540	+1.3
	4	740	670	+9.5	660	665	−.8
Neurospora, 1000 R	1	680	665	+2.2	609	627	−3.0
	2	370	383	−3.5	334	328	+1.8
	3	650	665	−2.3	570	602	−5.6
	4	378	387	−2.4	342	333	+2.6
Neurospora, 500 R	1	355	370	−4.2	334	350	−4.8
	2	233	226	+3.0	220	219	+.5
	3	360	360	0	337	332	+1.5
	4	220	230	−4.5	218	215	+1.4
Net percent difference				+0.35			−0.15

TABLE 11. – Radiation Doses to Biologics, Phase B and C Tests

Package	Exposure, R			
	Phase B		Phase C	
	301 S/C	201 S/C	301 S/C	201 S/C
Tribolium, 1200 R	920	920	874	903
	898	898	853	882
	865	865	822	849
Drosophila larvae, 1300 R	850	833	777	787
Drosophila adult, 2000 R	1407	1395	1398	1383
Lysogenic bacteria:				
2500 R front chambers	1685	1726	1754	1730
2500 R back chambers	1336	1368	1391	1372
1000 R front chambers	727	716	674	671
1000 R back chambers	618	608	572	570
500 R front chambers	275	270	265	273
500 R back chambers	243	238	234	241

TABLE 12. – Radiation Measurements in Control Areas, Phase B and C Tests

Package	Radiation, R			
	Phase B		Phase C	
	301 S/C	201 S/C	301 S/C	201 S/C
Habrobracon	0.40	0.39	0.41	0.38
	.42	.40	.46	.44
Tribolium	.36	.47	.....	.....
	.....	.....	.39	.60
	.32	.38	.....	.....
	.....	.....	.32	.35
Drosophila adult	.26	.26	.29	.20
	.29	.29	.30	.42
	.23	.25	.22	.18
Tradescantia	.28	.28	.28	.25
	.26	.27	.28	.28
	.31	.29	.28	.26
	.28	.27	.30	.28
Drosophila larvae	.41	.48	.50	.34
	.21	.22	.19	.18
	.34	.35	.36	.30
	.25	.31	.26	.18
Neurospora	.20	.19	.26	.26
	.19	.19	.18	.19
	.24	.39	.36	.35
	.22	.35	.16	.23
Lysogenic bacteria	.56	.41	.41	.41
	.41	.43	.42	.44
Ion chambers:				
1.0-R chamber	.21	.22	.19	.20
5.0-R chamber	.22	.26	.26	.30

as did the corresponding specimens in the Earth control capsule. This is indicated both by the dosimeter readings and, inferentially, by the close agreement of the strengths of the sources. The delivered doses are in agreement with an exposure time of 42 hours instead of the nominal 65 hours.

The radiation exposures in the control areas were quite low, as determined by LiF readings. Few were above 0.5 R. The ion-chamber measurements independently confirm the low values. Nuclear emulsion measurements were made of the numbers of protons and heavy ions entering the spacecraft during orbital flight. An upper limit dose due to protons is 40 mR for the mission. There were 10.1 traversals of atomic nuclei of $Z \geqq 20$ through each square centimeter during the flight.

Postflight tests have been completed which have established the Earth controls based on vibration and acceleration data obtained during the flight. With respect to the radiation environment, the conditions for a successful determination of the combined effects of weightlessness and radiation have been met.

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The Scientific Conclusions of Biosatellite II

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The experiments of Biosatellite II were designed to obtain information related to three major scientific questions. The first of these concerned the ability of a spectrum of living Earth systems to maintain normal organization and function during exposure to the space environment. The second was related to the biologic effects imposed on gravity-dependent life processes by the absence of Earth's gravity and rotational periodicity. The third question asked whether weightlessness and quantified gamma radiation would act synergistically, antagonistically or not materially affecting biologic processes during space flight. Observations were made on more than 100 end points at the molecular, cellular, and systems level of biologic structure and physiologic function.

In general, the wheat seedling, pepper plant, amoeba, and frog egg experiments were concerned with the effects of weightlessness on (a) alterations of common biochemical pathways; (b) disruption of normal mitotic sequences; (c) changes in morphogenesis, growth, and development; and (d) orientation and locomotion with respect to a hypogravity environment.

Among the hypotheses advanced by the wheat seedling experimenters was that a decrease in gravity will affect the growth of roots and shoots of seedlings, and that the state of weightlessness may destroy the normal orientation of mitosis and alter the direction of cell elongation. It was anticipated that the direction of growth of the roots and shoots of seedlings would be random, thus confirming that the hormonal control of the orientation of these organs is dependent on gravity. It was believed that reduced gravity would not interfere with enzymic function in the gluconeogenic or the photosynthetic pathway of carbohydrate biosynthesis.

No alteration of normal mitotic events, food vacuole formation, and orderly synchronous division of nuclei was expected in space-borne amoebae. Their survival and phagocytic activity was expected to be unaffected. On the other hand, the gravity-sensitive stages of embryologic development in the eggs of *Rana pipiens* might be affected by hypogravity.

The behavior of petioles of nearly mature pepper plant leaves in the state of weightlessness was expected to be equivalent to the marked reduction in the liminal angle of the petioles of leaves rotated on the horizontal clinostat.

The results of the Biosatellite II experiments confirmed many of the hypotheses that had been advanced. Exceptions, however, have been borne out by the spaceflight findings. The results of the pepper plant experiment in flight appeared not to be significantly different from those of the Earth clinostat controls. Yet, there is the vexing consideration of why the flight specimens took five to seven hours to revert to normal geotropism whereas the Earth clinostat control counterparts rebounded immediately when removed from the rotating clinostat. There was no significant difference in the biochemical and physiologic responses of the pepper plants during their exposure to the space environment and the Earth clinostat control.

The wheat seedlings showed an increase in peroxidase activity which seems to be correlated with epinastic curvatures. The diameters of the flight wheat seedling organs are smaller than those of the Earth controls grown on the clinostat. Those of the clinostat-vibrated seedlings were the same.

Flight seedlings and clinostat-vibrated seedlings had fewer gross abnormalities than Earth controls. Fewer cells were seen in mitosis in the flight root tips — the stage most affected was early prophase. The flight root tips, both primary and lateral, showed longer cells. Their interphase nuclei as well as those of the coleoptile were larger in volume. Although clinostat rotation increases nuclear volume in some roots, orbital flight cannot be duplicated by the clinostat at the cellular level, whether or not rotation is preceded by vibration.

No significant differences were observed in the development of frog eggs or in the biochemistry, morphology, and physiologic function of the amoeba. Despite its seeming dependence on gravity during early development, the frog egg does not require a *g* field for normal cell division.

Genetic and somatic changes, meiotic and mitotic aberrancies, longevity, and population dynamics were, in general, the phenomena of experimental concern in the weightlessness-radiation experiments. No effect of a gravity-free field and radiation was expected on dominant lethality, chromosome translocation frequency, and recessive lethality in mature sperm of *Habrobracon*. It was established, however, that since these effects can be precisely quantitated, any unexpected synergistic effect should be detected readily.

Although it was believed that synergism was not likely, the *Drosophila* system allowed for a high probability of detecting relative differences in the mutational spectrum, if synergism did occur. The observation that spatial relationships among broken chromosomes influence the extent to which they undergo irreparable disarrangement as well as the finding that centrifugation, following radiation exposure, enhances radiation-induced chromosome damage suggested that the possibility of synergism between null-gravity and radiation should be investigated very thoroughly.

Certain developmental stages of *Tribolium* had shown that both "gravitational position" and radiation affect normal wing development. Other factors important in modifying wing development act synergistically. Preliminary studies seemed to indicate that position and radiation would act in a nonadditive manner.

Induction of lysogenic cells by radiation in a weightless state could be altered because of changes in the resistance of cells to the induction process or because of changes in the ability of the virus to reproduce itself after induction.

Although no effect of a gravity-free field was expected on cellular inactivation and mutation induced in *Neurospora,* any unexpected biologically synergistic effect should be detected readily.

The assumption in the *Tradescantia* experiment was that data on the biologic interaction of weightlessness and other space environmental factors could provide valuable information for a better assessment of the possible hazards of space travel.

Based on all tests, certain conclusions have been reached. In the larvae of *Drosophila melanogaster* ^{85}Sr-irradiation interacted with some spaceflight factor(s), probably extensively and perhaps exclusively weightlessness, to produce increases in mortality, sex-linked lethal mutations, chromosome breakage followed by the loss or exchange of genetic information, and improper chromosome separation and the formation of chromosome translocation (fig. 1). In the adults of *D. melanogaster,* significantly fewer translocations were found in organisms exposed to postflight Earth control vibration, acceleration, and radiation of the Biosatellite II than were found in the irradiated flight specimens as well as other controls. An increase in frequency of flies with a missing wing and deformed thorax was observed in flies hatched after irradia-

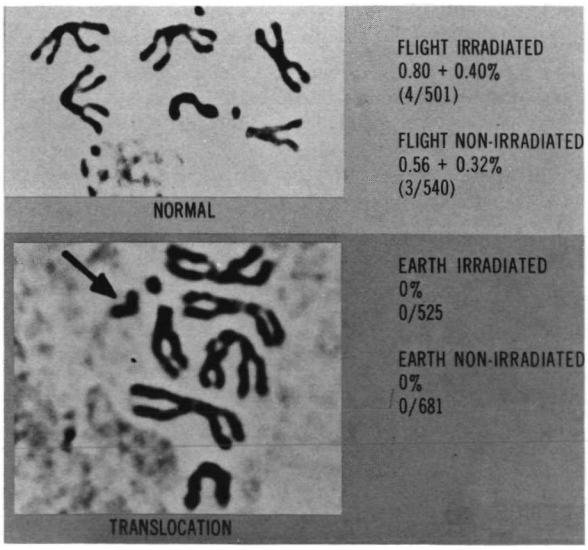

FIGURE 1.—Chromosome translocations in *Drosophila* larvae.

tion in Biosatellite II. Loss of markers from the Y chromosome were fewer in the irradiated flight specimens than in all Earth control specimens.

The experiment with *Habrobracon junglandis* showed that the fertilizing capacity of sperm was enhanced and the fecundity and hatchability of both transitional and primitive oogonia were increased after irradiation during flight. Spaceflight alone as well as irradiation during spaceflight increased the lifespan of females. Insofar as mating behavior was concerned, males from both compartments of Biosatellite II were disoriented up to 48 hours postflight. Hatchability of oocytes in metaphase I and early prophase I was reduced as a result of the flight as well as the flight plus radiation stress.

Wing abnormalities in *Tribolium confusum* due to radiation administered during spaceflight occur with greater frequency than those exposed to radiation administered on Earth in simulated sequences. This effect is not due to vibration, temperature excursions, or altered rate of development in space. There is the suggestion that less dominant lethality occurs (table 1) and that a lowering of fertility results from radiation combined with the factor(s) of spaceflight.

The hybrid clone (02) of *Tradescantia* was subjected to an enhanced deleterious effect in flight samples attributed to weightlessness as noted in the mitotic spindle mechanisms in microspores, megaspores, and root-tip cells. Enhanced interactions between radiation and spaceflight factors were observed in pollen abortion, micronuclei in pollen, and stamen hair stunting, suggesting injury during the more sensitive stages of meiosis and mitosis. Clearly, differences exist between the flight and the nonflight samples, both with and without irradiation, and are indicative of a synergistic effect for the production of aborted pollen, the very high rate of microspore death, and various nuclear abnormalities.

In *Neurospora crassa,* the experiment indicated no effect on the inactivation of spores or the induction of recessive lethal mutations at specific loci in spores contained on Millipore filters. This differs considerably from the effects observed in Gemini 11 wherein spores, flown in suspension, showed a significantly higher survival in the presence of weightlessness while being irradiated with 32-phosphorus.

The growth rate of *Salmonella typhimurium* appeared to be more rapid during spaceflight than that in the Earth controls. In *S. typhimurium* the free phage induction was consistently lower in the flight population versus the Earth controls. The greater bacterial densities (maximum population) are believed to be a function of random cell distribution in the liquid medium under reduced gravity conditions. The *Escherichia coli* experiment was incomplete since the growth phase had not been able to reach maximum because of the early calldown of Biosatellite II.

The results in table 2 show that the wheat seedling experiment revealed a number of statistically significant observations in spaceflight which are not duplicated on the clinostat. A lowered mitotic rate in early prophase, increased cell length, and enlarged nuclear volume were seen in the wheat seedling root tip cells. These are not included in the report entitled "Space Biology" (1970) resulting from a study convened in Santa Cruz, Calif. in 1969 by the Space Science Board of the National Academy of Sciences, where it is stated (page 20): "Biosatellite experiments revealed no sensible difference between the plagiotropic angles of orbiting wheat roots or pepper plants and of clinostated controls on Earth." Not duplicated on the clinostat were death in *Tradescantia* microspores as well as an increase in the frequency of abnormal nuclei (disturbed mitotic spindle) in microspores and megaspores.

In addition, the following results were considered to be significant expressions of a weightless effect at the cellular level of biologic organization. Chromosome translocations occurred in the larvae of *D. melanogaster* at a stage of cell division where translocations are not seen normally (table 2 and fig. 1). Wing abnormalities in the *Drosophila* adults were increased significantly as a result of irradiation during spaceflight. Disorientation of *Habrobracon* males, with respect to mating behavior, was observed for 48 hours postflight. Less malformation, i.e., absence of specific organs, occurred in the spaceborne wheat seedlings. The Biosatellite II wheat seedlings showed decreased respiration, increased peroxidase activity at the site of epinastic curvature, and a possible alteration of carbohydrate metabolism based on an increase in glucose-6-phosphate dehydrogenase activity. The increased G–6–PD activity was not observed in

TABLE 1. — Percent Incidence of Genetic Defects Related to the Interaction of Weightlessness and Radiation[1]

Defect	Organism	$1g$	$1g+R$	$0g$	$0g+R$	Change due to radiation (ΔR)	Change due to $0g$ (Δg)	Prediction due to $\Delta R+0g$	$\frac{0g+R}{\Delta R+\Delta g}$	Effect
Dominant lethality: female	*Tribolium*	0	47.6	19.7	22.8	47.6	19.7	67.3	34%	Antagonism[2]
Blue to pink color	*Tradescantia*	0.2	7.3	0.3	4.4	7.1	0.1	7.2	60%	Antagonism
Dominant lethality: male	*Tribolium*	0	8.3	4.6	11.4	8.3	4.6	12.9	88%	Antagonism
Chromosome translocation	*Drosophila*	0	0	0.56	0.80	0	0.56	0.56	143%	Synergism[3]
Altered nuclei	*Tradescantia*	0	0.06	0.25	0.55	0.06	0.25	0.31	177%	Synergism
Deformed thorax	*Drosophila*	0	1.3	0	2.8	1.3	0	1.3	215%	Synergism
Split wing	*Tribolium*	16.8	29.9	19.0	44.8	13.1	2.2	15.3	293%	Synergism
Pollen abortion	*Tradescantia*	39.0	48.0	37.0	66.0	9.0	−2.0	7.0	944%	Synergism
Stunted hairs	*Tradescantia*	10.5	12.9	10.1	26.6	2.4	−0.4	2.0	1330%	Synergism

R = Radiation $\Delta R+0g$ = Prediction based on additive effects $\frac{0g+R}{\Delta R+\Delta g} = \frac{\text{Observation}}{\text{Prediction}}$

$0g$ = Weightlessness

Calculation: $\frac{0g+R}{\Delta R+\Delta g} = \frac{0g+R}{(1g+R)+(0g-1g)} = 1$

[1] Tabular entries taken directly or recalculated by Dr. A. H. Brown from published sources

[2] Desensitization of a biologic process to radiation-induced damage

[3] Enhancement by weightlessness of the radiation-induced effect

TABLE 2. – Significant Cellular Expressions of Weightless Effects in Biosatellite II

Organism	Abnormalities	Flight		Control		
		Rad	0g	Rad	1g	Clinostat
Drosophila adult	Deformed thorax	2.8%	0%	1.3%	0%	.....
		44/1562	0/2510	95/7625	0/5272	.....
Drosophila larvae	Chromosome translocations	0.80+0.40%	0.56+0.32%	0%	0%	.....
		4/501	3/540	0/525	0/681	.....
Tradescantia	Mitosis (disturbed spindle)	<5%	27.54+0.92%	0.3+0.09%	0.2+.07%	0.16+0.06%
		Survival	656/2382	12/4000	7/4000	8/5000
Tradescantia	Microspore death	100%	100%	16.7%	33.3%	6.26%
Tradescantia	Altered nuclei	0.55+0.0008%	0.25+0.0005%	0.06+0.003%	0%	0%
Wheat seedling	Lowered mitotic rate $N = >1000$	.....	3.9%	.....	6.25%	5.1%
Wheat seedling	Nuclear volume, μ^3:					
	Coleoptile	.....	719	.....	564	565
	Roots	.....	675	.....	579	618

seedlings grown on the clinostat. Xanthine dehydrogenase activity was significantly depressed during flight in *Habrobracon* males and in the male progeny of *Drosophila* flight male adults and flight male larvae.

In all cases, the effects cited above were over and above the flight profile vibration and acceleration energy levels at normal *g*.

With regard to the radiobiologic experiments, the Santa Cruz Summer Study confirmed

> . . . well-designed experiments, using well-known biologic genetic systems to provide for statistical precision of the results. They were exposed to known quantities and qualities of radiation. Adequate controls were provided. Well-defined effects were examined whose dose-effect kinetics were well understood. The systems, furthermore, were sensitive to radiation so that the observed end point would occur at a frequency that would allow statistical precision in the analysis. Entire dose curves were included and homogeneous populations were used.

The report states further:

> Although the evidence is good that the observed effects of spaceflight alone are real, the difficulty and the complexity involved in isolating the variables, and in repeating, directing, and quantifying the stimuli are so great that we believe that this line of study should not be pursued further in spaceflight experiments until its complexities have been thoroughly studied in Earth laboratories.

The experimental evidence from Biosatellite II indicates that radiation and weightlessness exerted significant synergism as well as a desensitization of certain biologic processes to radiation-induced damage (table 1).

If we look at the Biosatellite II results from all views, the radiobiologic experiments, especially, allow us to conclude that Biosatellite II was indeed a scientific success in that it did not experimentally produce any convincing evidence of a radiation hazard to man in Earth orbit during short-duration missions. The biologic information which was obtained from Biosatellite II was unobtainable on Earth because weightlessness cannot be duplicated on Earth for more than a few seconds. The Biosatellite II experiments have restored confidence in the adequacy of the methodology of physical dose estimation for predicting radiation hazards to man. With a degree of credibility, we can say that Biosatellite II contributes to the present impetus being given to the urgency for and the importance of learning more about the heavy particles of galactic cosmic radiation and their biologic effects.

This becomes even more urgent when we consider the significance of the chromosome translocations in *Drosophila* larvae, because cosmic ray primaries cannot be excluded, at this point, as possibly being contributory to the induction of these translocations.

One of the major recommendations resulting from the reviews of the Biosatellite II experiments is that much more Earth-based research is required on selected aspects of the cell and on plant and invertebrate life as a prerequisite to spaceflight experimentation. More emphasis than heretofore should be placed on the study of gravitational biology utilizing the clinostat and the centrifuge. More attention in Earth-based research is needed to unravel the variables associated with spaceflight in order to learn more about their biologic effects either individually or in combination.

This, then, is the biologic legacy of Biosatellite II; and, if adhered to, the potential for exploiting space as a new dimension in biology will become increasingly profitable, scientifically and practically.

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