Proceedings of the
NATIONAL ACADEMY OF SCIENCES

Volume 60 • Number 2 • June 15, 1968

BIOSATELLITE II EXPERIMENTS: PRELIMINARY RESULTS

Report of a symposium convened at the National Academy of Sciences, February 23–24, 1968, and organized by the N.A.S. Space Science Board and the National Aeronautics and Space Administration

By KENNETH V. THIMANN, *Symposium Chairman*

UNIVERSITY OF CALIFORNIA (SANTA CRUZ)

For a number of years, biological experiments have been carried out in an essentially casual way in space, both by the U.S.S.R. and by the U.S.A. Organisms have been inserted into balloons or orbiting vehicles designed for other purposes and have been subsequently observed. Some effects have appeared but it was not known for certain which of several phenomena might be the cause. Controls were mostly far from adequate. The Space Science Board of the National Academy of Sciences therefore decided about five years ago to ask NASA to consider preparing a properly equipped satellite with selected specific experiments and good controls on earth to settle some of the problems of space biology.

The selection of experiments, following customary NASA procedure, was in the hands of the Associate Administrator for Space Science and Applications, who was advised by specially organized scientific committees. Now that the experiments have been completed and most of the results are available, it is appropriate to present them to the public and to draw such conclusions as are warranted. What follows is a necessarily brief account of the first successful flight, its predecessor, Biosatellite I, having been lost. The experiments will be presented here in summary form only, since they will be published in detail elsewhere.[1]

The Flight.—Biosatellite II (1967 83A) was launched from Cape Kennedy, Florida, on September 7, 1967, at 18.04 EDT and controlled from Goddard Space Flight Center. The orbit chosen was a nearly circular one, with apogee 313 km and perigee 300 km, inclined 33.49° to the equator. The satellite entered orbit at 18.13 EDT and was supposed to remain in orbit for 69 hours, or 46 orbits. However, early on the second day, the tropical storm Sarah was observed to be moving towards the recovery area southwest by south of the Hawaiian Islands. This was confirmed by pictures of the recovery area that were received from the ATS weather satellite and made available several times daily—a remarkable instance of one satellite helping another. Because of this storm and a continuing problem of communication with the spacecraft, the decision was made to shorten the flight to 30 orbits. The final command to "deorbit" was sent at 11.21 EDT,

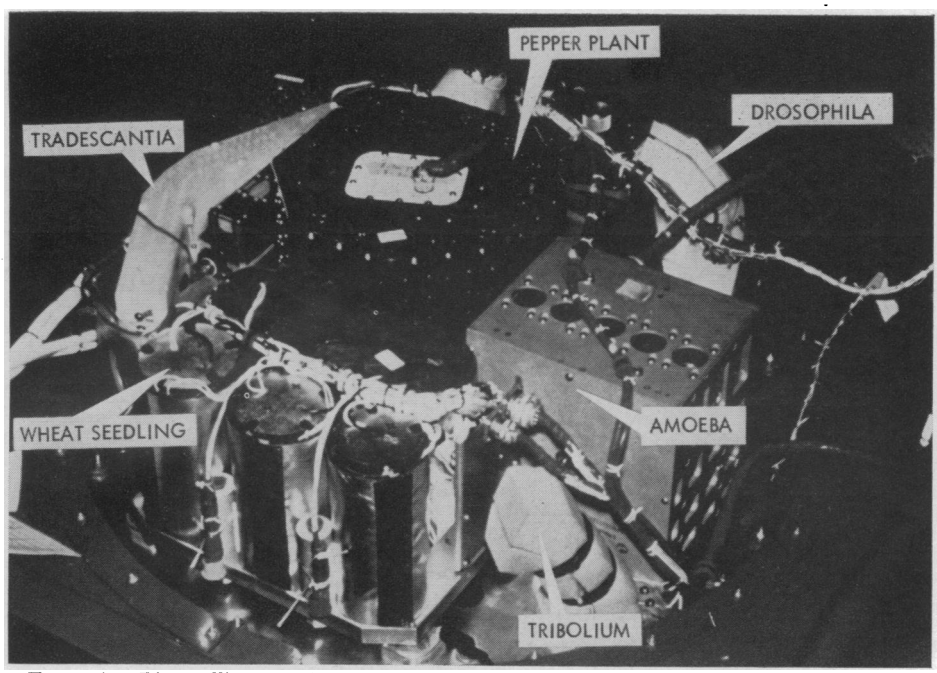

FIG. 1*A*.—Biosatellite experiments payload assembly (behind radiation shield). Arrangement of containers for experiments on effects of flight alone, in afterpart of capsule.

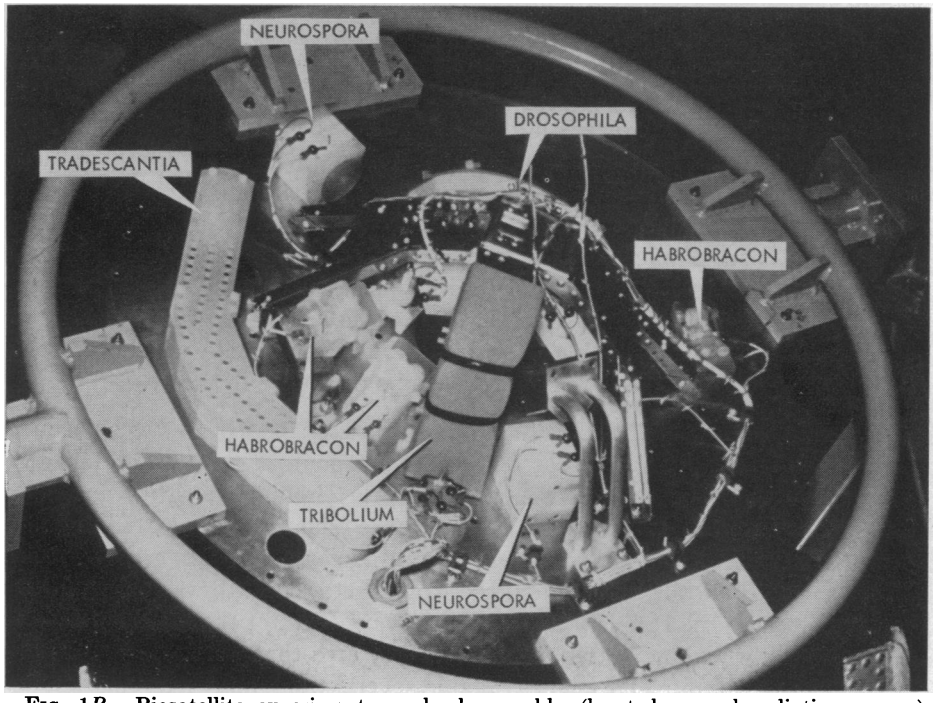

FIG. 1*B*.—Biosatellite experiments payload assembly (located around radiation source). Arrangement of containers for experiments on interaction between irradiation and flight conditions, in forward part of capsule.

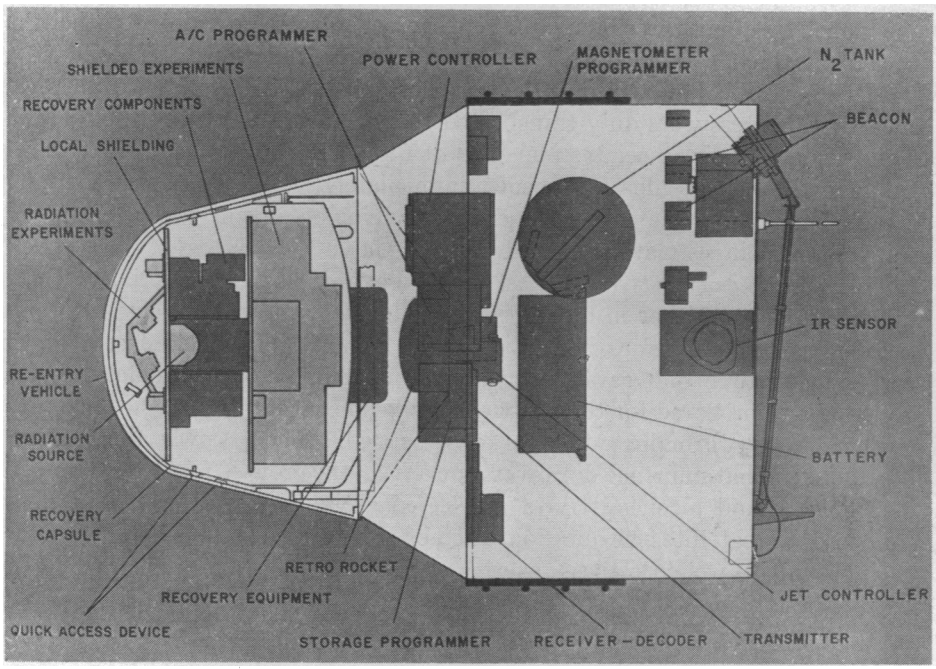

FIG. 1C.—General layout of components in the spacecraft.

and the parachuting capsule was successfully recovered in the air at 15.15 EDT on September 9. It was flown directly to Hickam Field, Honolulu, arriving there about $3^1/_2$ hours later.

The vehicle was a two-stage Delta; the spacecraft weighed 435 kg and measured 2.5 m long by a maximum of 1.4 m wide. The reentry capsule with all the experiments, weighing 127 kg, is shown in Figure 1.

The success of the flight called for an unprecedented amount of cooperation between the design engineers, the flight controllers, the recovery crews, and the biologists. The NASA organization worked smoothly.

Biological Preparation and Facilities.—The experiments selected (see below) had been under study for periods up to three years. Each had been reduced to the minimum acceptable volume, automated where necessary, and provided with several controls. A laboratory was set up adjacent to the hangar at Cape Kennedy, and the installation of the experimental material into its prepared containers began about 12 hours before flight. Preparations had been elaborately rehearsed and as a result went almost without a hitch. The earlier preparations for Biosatellite I had, of course, provided invaluable rehearsals too. So far as is known, the only actual mistake made was that a mounting bracket under one of the several containers of *Habrobracon* was installed backwards, so that the radiation received by this package was only about 0.8 of that intended.

After the experiments had all been prepared and installed in the capsule, and the ground controls similarly set up, a technical delay occurred that postponed

the launch by about three hours. Thereafter, there were no problems other than the storm which, combined with command difficulty, shortened the flight.

At Hickam Field, a laboratory had been established in a group of trailers, and the experimenters were duly transported there to await arrival of the material, while some of their associates remained at Cape Kennedy to tend the controls. On arrival, the capsule was opened immediately, the material measured or sampled as appropriate, and the fixed or surviving organisms were subsequently shipped to the investigators' laboratories for study.

The Experiments and Aims.—As noted above, biological material had been taken into space, either in high-altitude balloons or in orbiting satellites, on a number of occasions before, both by U.S. and U.S.S.R. personnel. However, this was the first time that carefully controlled experiments, in a vehicle specially designed and prepared for the purpose, had been sent into orbit.

There were two major questions to be settled. The first was the relatively broad one: What influence does gravity exert on the growth, form, development, morphology, and biochemistry of a selected group of organisms? Put in the context of the satellite, the question asked became: What influence does a period of approximate weightlessness[2] exert on these functions? A cognate question was: Does a period of approximate weightlessness[2] cause any detectable change in these functions that cannot be imitated by gravity-balancing devices on the earth's surface?

The second major question arose from observations made on occasional exposures of organisms to radiation during earlier flights: During the state of approximate weightlessness, are organisms more, or less, sensitive to ionizing radiations than on the ground? To study this question, instead of relying on cosmic radiation, solar flares, etc., the investigators supplied a source of γ-radiation, consisting of Sr^{85}, to a group of experiments. These were mounted in the forward part of the capsule; the afterpart, which contained unirradiated controls as well as the experiments not involving radiation, was not only shielded from the forward part but also separated from it by a zone of electrical and electronic equipment.

The experimental materials selected were as follows.

(a) *For general effects of flight in orbit:*

Wheat (*Triticum aestivum*), seedlings grown in flight, and studied for spatial orientation, morphogenesis and histochemistry, and biochemical changes.

Pepper (*Capsicum annuum*), young plants for study of the orientation of leaves.

Frog (*Rana pipiens*) eggs for study of the rate and location of division during flight, and the morphology and differentiation of the tadpoles.

Amoebae (*Pelomyxa carolinensis*), for studies of feeding and growth, as well as cellular structure.

(b) *For interaction between radiation and flight conditions:*

Tradescantia, hybrid clone 02, plants bearing flower buds.

Parastic wasp (*Habrobracon juglandis*) adults.

Flour beetle (*Tribolium confusum*) pupae.

Vinegar fly (*Drosophila melanogaster*) larvae and adults.

Lysogenic strains of *Salmonella typhimurium* and *Escherichia coli*.

Neurospora crassa, asexual spores.

Controls.—Controls of several kinds were included. For the experiments on orientation and general morphology (*Triticum* and *Capsicum*), ground controls both in the ordinary erect position and rotated on horizontal clinostats at 6 rph were used. Ordinary ground controls served for the other experiments. For the experiments involving radiation, unirradiated controls were flown (in the after compartment), and both irradiated and unirradiated controls were held on the ground.

The temperature settings for the ground controls in most experiments were duplicated, one set being run at constant temperature equal to, or close to, that set for the flight, the other at slightly varying temperatures that followed, with a four-hour lag, the temperatures recorded on board the satellite and telemetered to earth. This precaution, which proved to be of some value in several of the experiments, was taken because absolutely constant temperature could not be maintained on board.

Results.—The experiments, and the names of the experimenters who carried them out, were as follows:

(1) *Growth physiology of wheat seedlings in space* (C. J. Lyon, Dartmouth College, Hanover, N. H.): To supply seedlings with water under "weightless" conditions and to allow freedom for the shoot and the primary and lateral roots to orient themselves in space, special holders were devised in which the wheat seeds were held at the endosperm end and water was supplied there from wet powdered vermiculite. Each seed holder was at the axis of a small moist chamber. Beginning 15 hours before launch, the seeds were first soaked in water for three hours and then were in the initial erect position for 12 hours; at this time, visible organs had not developed. The mean lengths of the organs measured after recovery (Table 1) showed that variation between different controls was generally greater than that between average controls and the plants in flight. Although the maximum age of the plants was only 65 hours, growth rates during that time had not been significantly altered by flight or by rotation on the horizontal clinostat.

The orientation angles of the organs were determined from photographs taken of face view and side view. They showed that the coleoptiles diverged from the vertical by 15.2° in orbit, 14.4° on the clinostat, and 10.8° erect. Primary roots diverged from the 180° position by 21.2°, 24.1°, and 8.1°, respectively. In side

TABLE 1. *Mean lengths (in mm) of wheat seedling organs after 65 hours of growth.*

	Orbited	On horizontal clinostat	Erect
Coleoptile	4.1 ± 0.1	4.0 ± 0.1 3.6 ± 0.2*	3.7 ± 0.1† 3.6 ± 0.1
Primary root	21.5 ± 1.0	21.3 ± 0.8 20.9 ± 1.3*	25.0 ± 1.0† 21.5 ± 1.1
Lateral roots	10.1 ± 1.0	10.6 ± 0.9 11.7 ± 0.7*	13.8 ± 0.6† 9.7 ± 0.6

* Measured at 60 hr.
† Measured at 66¼ hr.

view, the corresponding divergences were 34.7°, 35.0°, and 11.4°. The lateral roots gave similar results, the orbited and clinostated values agreeing well and differing greatly from the erect controls.

(2) *Wheat seedling morphogenesis and histochemistry* (Stephen W. Gray and Betty F. Edwards, Emory University, Atlanta, Ga.): The 78 seeds were supported in holders as described above, with two clinostated controls, one erect control in a mock-up of the flight capsule, and one other erect control in a growth chamber. Each control also held 78 seeds. The timing was as in experiment (1). A group of seedlings was fixed in flight at 58 hours of age, and another group was fixed immediately after recovery. Other seedlings were made available for the biochemical studies below.

Lengths and ellipticities of the coleoptiles showed no significant differences between orbited and control plants, although the former were 12 per cent taller than the erect controls. The primary roots of the orbited plants were about 10 per cent shorter than those of the erect controls at 58 hours, but at 65 hours the differences were insignificant. There was evidence that growth was accelerated after normal gravity had been restored.

Nuclear stains showed no differences among groups, and all mitoses appeared normal. Staining for DNA and phospholipids likewise showed no differences. The periodic acid-Schiff procedure showed starch grains in the erect plants at the bottom of the cells in the root cap, coleoptile tip, and also in a single cell layer around the coleoptile vascular bundles. In the orbited and clinostated plants, the grains were located at random or near the center of the cells. In the clinostated plants, more starch grains appeared to be present than in the other two groups, for no obvious reason. The epidermis of the scutellum showed more peroxidase, and the scutellum more glucose-6-phosphatase, in the clinostated plants than in the other two groups. On the other hand, the scutellum of the orbited plants showed the most acid phosphatase.

(3a) *Biochemical changes in the developing wheat seedling* (Herbert M. Conrad, Resources Planning and Control Corp., El Segundo, Calif.): Immediately after recovery, the coleoptile with enclosed leaf and attached scutellum was frozen and maintained in liquid nitrogen and sectioned transversely into 200-μ slices; then the slices were assayed for six enzymes, dry weights, protein contents, oxygen consumption, CO_2 evolution, and ethylene production. Lateral roots were similarly cut into 1-mm sections.

Glucose-6-phosphate dehydrogenase appeared more active per unit weight in the shoots of the orbited plants than in the shoots of the others; this difference, if real, occurred in the coleoptile rather than in the leaf. Peroxidase was three times as active (per unit weight of protein) in the coleoptile as in the leaf, and in the orbited and clinostated plants was some 30 per cent higher than in the erect controls. Malic dehydrogenase, transaminase, and cytochrome *c* reductase showed no significant differences, while peroxidase appeared more active in the scutella of the orbited plants than in those of the other plants. Calculations of K_m and V_{max} for four of the enzymes in the roots showed no differences. Some further analyses are still to come. It must be noted that only

four orbited plants and eight from each of the controls were analyzed for each enzyme.

(3b) *Endosperm analysis of the developing wheat seedling* (Samuel P. Johnson, North American Rockwell Corp., El Segundo, Calif.): Separate analyses were also made of the endosperms. There were no significant differences in dry weight, glucose, sucrose, starch, or nitrogen contents between the orbited group and the four control groups. There were some differences in individual amino acids, but the pattern was irregular, the orbited plants sometimes giving values nearer to the erect plants, sometimes nearer to those clinostated.

(4) *The interaxial angle of the pepper plant* (Samuel P. Johnson, North American Rockwell Corp., El Segundo, Calif.): For this experiment, 25-day-old *Capsicum annuum* plants were reduced to two fully expanded leaves and transplanted to small plastic cups. Ten days later, they were flown for the 45 hours in orbit, plus the 5 hours spent in recovery and transfer to the receiving laboratory. During this period, the interaxial angle between the petioles and the main stem was photographed by means of a ten-second flash of white light (200 ft-c) every ten minutes. Subsequent measurements of the photographs revealed that all leaves showed epinasty, the interaxial angle decreasing by 20° to 60°. The plants on the horizontal clinostat behaved comparably, the decreases ranging from 20° to 70° in the same time period. Since there were only four plants in each group and since the behavior of the leaves was not identical within a group, a precise comparison cannot be made. On returning to vertical, the clinostated plants made somewhat better recovery than the orbited plants.

Mobilization of starch from the leaves to produce sucrose in the stems occurred during orbit and clinostating, but not in the erect controls.

(5) *The dividing egg of Rana pipiens* (Richard S. Young, NASA, and John W. Tremor, Ames Research Center, Calif.): Ever since the 1894 experiments of Schultze, it has been known that, on inverting the frog egg from its normal position so that the yolk-rich vegetal pole is uppermost, twin-headed monsters and other abnormal forms develop. The experiment was therefore designed to study the influence of flight conditions (primarily weightlessness) on division, differentiation, and subsequent development. A previous experiment, on a Gemini flight, had shown that weightlessness did not cause the same effects as did inversion on the ground. However, there were some anomalies in the Gemini experiment.

Frogs were maintained throughout the year in a reproductive state by being kept at low temperature and treated with antibiotics. Beginning three days before launch, the females were injected with three to five pituitary glands each to induce ovulation; then the eggs were stripped and fertilized 12.5 hours before the scheduled launch. They were transferred to the special plastic vessels and held at 6°C in the hangar laboratory and also within the spacecraft by a specially attached coolant line. After launch, strip heaters raised the temperature to 21°C, and thereafter the eggs developed at ambient spacecraft temperatures. Ground controls were (1) held at 21°C in a model of the capsule, (2) held at 21°C in an incubator, and (3) subjected to flight temperatures with a lag of four hours (see *Controls* above). In addition, a group of eggs was centrifuged at 3 *g*.

Samples of the eggs from all treatments were fixed in 2.5 per cent glutaraldehyde in sucrose-phosphate buffer at 0, 1, 2, 3, 32, and 40 hours after launch, and on recovery.

Unfortunately the three-hour delay in launching allowed the mid-first cleavage (the stage most sensitive to inversion) to appear before launch. Later stages developed in flight, however. The embryos fixed in flight showed only slight differences from the controls in their stages of development, and these could be attributed to the differences in temperature. The types of abnormalities developing were the same in orbited and control eggs; the Cape laboratory controls had a larger percentage of abnormalities, but this may be because they were more slowly fertilized, those fertilized first having been selected (because of the time factor) for the flight. The larger, apparently heavy, yolk granules were located at the vegetal end in both orbited and control eggs. No differences in development could be detected; mitotic figures were normal, and mitochondria, yolk platelets, and nuclear and cellular membranes showed no differences. The live embryos developed just as did those of the controls.

(6) *Nutrition and growth of the amoeba* (Donald R. Ekberg, General Electric Company, and Richard W. Price, Colorado State University): The giant multinucleate amoeba *Pelomyxa carolinensis* may require a gravitational force to attach to a substrate for locomotion or feeding. Thus it seemed likely that these large cells would show the effects of weightlessness on the manner and rate of reproduction. The amoebae, with carefully counted numbers of *Paramecium* for food, were placed in 5-ml chambers, 24 of which were mounted in the flight package. Some of the amoebae were starved for one day, others for two or three days, before flight. Some of the chambers were automatically fixed in buffered glutaraldehyde for subsequent examination at 12, 24, 43, and 43.5 hours after launch. Three ground controls and a backup group were used. The latter was flown to Hawaii upon recovery for comparative analysis.

Survival of the starved and of the continuously fed amoebae during preparation and launch was approximately equal. Most of the amoebae that were starved during flight did not divide. Those that were well fed divided about once. Their rate of reproduction was in general about the same in orbit as on the ground, though the cells fixed in flight were seen to have divided a little faster than the controls at first, and then a little slower. In the postflight five hours, the orbited cells divided faster than the backup group but slower than the controls. Temperature differences as well as possible minor differences in treatment between the flight and backup organisms in Hawaii and the controls at Cape Kennedy may have been effective here. In the cells fixed in flight, one prophase and one early interphase were identified. Both appeared normal and the several nuclei within each cell were synchronized with each other. Thus the acceleration and vibration in this case do not appear to have inhibited cell division. Numerous electron micrographs of the amoebae revealed no major abnormalities. Feeding, as indicated by the numbers and stages of food vacuoles, was essentially the same as in controls.

(7) *Genetics and cytology of Tradescantia irradiated during flight* (Arnold H. Sparrow, Lloyd A. Schairer, and K. M. Marimuthu, Brookhaven National

Laboratory, N.Y.): Small flowering cuttings of a well-studied clone of *Tradescantia* heterozygous for flower color were rooted and mounted with roots in nutrient solution. They were set up so as to receive a total two-day exposure of 220 r from the Sr^{85} source. After recovery, the stamen hairs and petals were scored for loss or mutation of the blue color gene to yield pink or colorless cells, for incidence of cell division (by counting the number of cells in each hair), and for rate of pollen abortion and frequency of chromosome aberration. Two packages, each of 32 plants, were flown, only one being irradiated, and the ground controls comprised a similar pair of packages. In earlier ground experiments, only very small effects on the above responses had been observed.

The numbers of mutations, cell divisions, pollen abnormalities, etc., in the unirradiated plants were the same in orbit as on the ground, with two exceptions: disturbed spindle function and percentage of buds in which microspores were more than 95 per cent aborted. These percentages were 100 in the orbited group and only 28 on the ground. (The flowers of this clone have an unusually large tendency to abort the pollen.) The results for the irradiated plants are shown in Table 2. Flight decreased the action of radiation on the blue-to-pink mutation and very significantly increased (difference between the two columns 13.5 $\pm$ 3.2) its action in inhibiting cell division in the hairs. Pollen abortion was unusually high in this clone, and it was significantly greater in the flight-irradiated group than in the others. Thus there was an apparent synergism between flight conditions and irradiation on the cell-division process. Also, disturbed spindle function and other cytological aberrations were noted more frequently after flight than in controls.

TABLE 2. *Effects of irradiation in flight and on the ground on Tradescantia flower buds.*

	Flight (218 r)	Ground (225 r)
Mutation blue to pink in petals	17.3 $\pm$ 2.0	14.3 $\pm$ 2.3
Mutation blue to pink in hairs (per 100 hairs)	4.4 $\pm$ 0.8	7.3 $\pm$ 1.3
Mutation blue to colorless in hairs	8.6 $\pm$ 1.1	9.2 $\pm$ 1.3
Stunting of hairs (per 100 hairs)	26.6 $\pm$ 2.7	12.9 $\pm$ 1.7
Dwarf cells (per 100 hairs)	9.9 $\pm$ 1.1	11.8 $\pm$ 1.4
Pollen abortion (per 100 grains)	66.0 $\pm$ 5.3	47.7 $\pm$ 6.8
Abortion of microspores above 95% (per 100 buds)	100	14.2
Disturbed spindle (per 100 cells)	0.55	0.05

(8) *Mutational response of Habrobracon to combined γ-radiation and weightlessness* (R. C. von Borstel and Roger H. Smith, Oak Ridge National Laboratory, Tenn., and D. S. Crasch, N. C. State University): This experiment was designed to cover radiation effects on mature sperm and on several stages of oögenesis. Males and females of selected mutants were placed in five polypropylene packages (to receive five radiation dosages) for flight, and another three sets were prepared for ground controls, one to be irradiated in imitation of the capsule, one unirradiated at constant temperature, and one unirradiated at temperatures following within four hours the temperatures in flight (see *Controls* above). The irradiations received were approximately: in the spacecraft, 2400 r, 1200 r, 600 r and 530 r, 350 r, and 0.5 r; in the controls, 2400 r, 1200 r, 700 r, 360 r, and 1.5 r. Scoring, in the males, was for dominant and recessive lethals and in-

herited partial sterility, and in the females, for total dominant and recessive lethality induced during oögenesis and for oögonial killing and dominant lethality. The numerical data are not yet complete, but some results are clear.

All animals survived the flight, though the males, but not the females, were disoriented in their mating behavior for two days thereafter. There was no difference between orbited and control animals in regard to the hatchability of eggs fertilized with sperm that had been irradiated; i.e., dominant lethality in sperm was unaffected by flight conditions. However, the total lethality among the oöcytes irradiated during the first meiotic metaphase, and while in early prophase I, showed that the radiation was about twice as effective in flight as on the ground. Oöcytes given 2000 r prior to flight showed higher hatchability than those irradiated during flight, some recovery having no doubt occurred, but the latter also showed more lethality after flight than after being on the ground. Oöcytes irradiated in the diplotene state of meiosis showed only very low lethality in both cases, and their genetic recombination was not affected by the radiation. After a low period in the first week, egg production by females irradiated in flight was actually higher than egg production by controls in the second and third weeks; thus, flight conditions largely eliminated the effects of radiation (see Fig. 2). Also, the orbited females lived significantly longer than the controls. In all, both enhancement and antagonism between irradiation and flight were observed.

(9) *Effect of irradiation during flight on embryonic differentiation in Tribolium pupae* (J. V. Slater, B. Buckhold, and C. A. Tobias, Lawrence Radiation Laboratory, Berkeley, Calif.): *Tribolium confusum* pupae, between 19 and 27 hours old, two thirds of which had been preirradiated with 1350 r of 180 kev X rays, were placed half in the forward compartment exposed to Sr^{85} and half unirradiated in the after compartment. Ground controls with and without Sr^{85} were kept at 30°C. Scoring was for wing abnormalities, which are known to be sensitive to modification by temperature and other parameters, and also for length of the pupal period and for dominant lethals.

The pupal period of the orbited animals was 1.5 hours longer than for ground controls, but this can be ascribed to lower temperatures in flight. Calculated pupal periods corrected for temperature showed no differences. The wing abnormalities in preirradiated animals showed significant synergism, those irradiated in orbit giving *44.8 per cent* abnormalities compared to *29.9 per cent* on the ground. Dominant lethals showed still larger effects (Table 3), the preirradiated females in orbit yielding *two to three times as many* as those on the ground. The data for those not preirradiated, however, show some inconsistencies.

(10) *Mutation in mature reproductive cells of Drosophila irradiated during flight* (L. S. Browning, Rice University, Houston, Tex.): Late third instar larvae, prepupae, males (*sc* $Y.B.^{S}$), and inseminated females (*y* sc^{S1} *In 49* sc^{8}*; dp bw. st* p^{P}) were divided among the capsule and the two types of ground controls; half were unirradiated and half were exposed to Sr^{85} at a dose of about 1400 r. Some of the males had been preirradiated with 4000 r of X rays.

The mature sperm cells in inseminated females irradiated in orbit gave 5.07

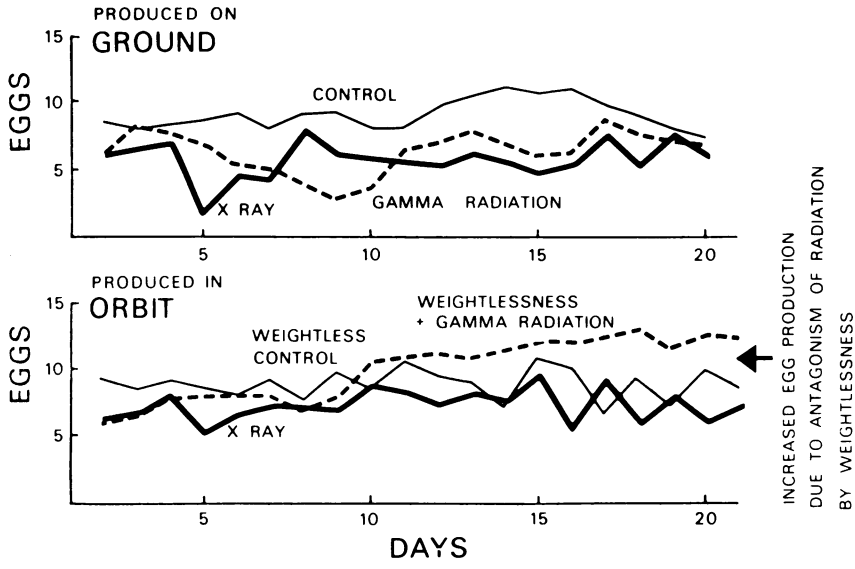

FIG. 2.—Effect of weightlessness and radiation on egg production in *Habrobracon* (from von Borstel, Smith, and Crasch).

± 0.7 per cent recessive lethal mutations in the paternal X chromosome, compared with 3.27 ± 0.55 per cent in irradiated ground controls. The difference is in the 5 per cent region of significance. The unirradiated specimens showed no significant difference due to flight. Of the four recessives studied, that at the *Dumpy* locus showed a highly significant difference ($P < 0.001$), the orbited group giving more than *three times as many* as those on the ground. No significant differences in translocation rates or in the loss of two markers from the Y chromosome occurred. However, in the larvae and prepupae that had pupated just before radiation, the frequency of losses from the Y chromosome was significantly higher ($P = 0.01$) in those orbited than in those on the ground. The largest effect was in the exchange between the B fragment of the Y chromosome and the X chromosome: irradiated in orbit, *42/17,000;* irradiated on the ground, *12/17,000.* Preirradiated males appeared to show opposite effects, the loss of markers from the Y chromosome and the translocations among the Y chromosome and the second and third pairs of autosomes being less in those irradiated during orbit than in those irradiated on the ground. Some synergism in the production of deformed flies is also possible, but not certain.

(11) *Somatic damage in Drosophila larvae irradiated during flight* (I. I. Oster, Bowling Green State University, Ohio): Larvae were orbited in the capsule, half being unirradiated and half exposed to *ca.* 830 r from the Sr^{85} source. Controls on the ground were similar, those irradiated receiving 820 r in the same time. After recovery, scoring was for mortality, developmental time, and chromosome morphology. Some of the larvae surviving to maturity were bred to test for genetic effects in the germ cells. Since the analysis of the data is incomplete, most of the conclusions are only qualitative.

TABLE 3. *Percentage of dominant lethals in F_1 generation of Tribolium confusum.*

	Irradiation on the ground		Irradiation in orbit	
	0 r	950 r	0 r	950 r
Preirradiated females	13.0	26.7	30.1	78.5
Preirradiated males	27.6	38.1	38.5	38.2
Not preirradiated females	0.0	47.6	19.7	22.8
Not preirradiated males	0.0	8.3	4.6	11.4

TABLE 4. *Population densities of bacteria by Coulter Counter, $\times 10^7$ on recovery (upper part), and bacteriophage P-22 densities (lower part).*

	Salmonella		*Escherichia*	
	Cells $\times 10^7$			
Irradiation dose	Orbit	Ground	Orbit	Ground
0	45.3	40.8	10.5	10.3
265	38.0	21.8	12.7	9.7
645	47.8	24.8	12.3	9.0
1630	33.0	20.4	12.1	8.3
	Phage $\times 10^4$			
0	7.8	10.9	—	—
265	17.4	14.1	—	—
645	34.1	23.4	—	—
1630	50.8	34.2	—	—

Larvae irradiated in orbit showed a somewhat higher mortality than on the ground: out of 480 in each case, 271 survived the irradiation in orbit, and 324 on the ground. Of the unirradiated larvae, about 94 per cent survived in both cases. The orbited larvae, whether irradiated or not, showed some chromosome nondisjunction. Chromosome breakage and bridge formation were higher in orbit than on the ground. Developmental times showed no significant differences among the groups. The incidence of translocations in the reproductive cells of the orbited larvae, both irradiated and unirradiated, appears thus far to be significantly higher than in ground controls.

(12) *Growth and induction in lysogenic bacteria irradiated during flight* (R. H. T. Mattoni, N.U.S. Corporation, Hawthorne, Calif.): Since earlier U.S.S.R. flights had indicated that the frequency with which lysogeny is induced was higher in space than on the ground, the experiment included a test of this using special strains of *Salmonella typhimurium* (BS-5 (P-22)/P-22) and *Escherichia coli* (C-600 (λ)/λ) that do not adsorb the mature phage particles. Population densities of the bacteria were also determined, from which growth rates were inferred. Radiation from Sr^{85} was at average levels of 266, 644, and 1646 r in flight, and 267, 623, and 1650 r on the ground. The temperatures during loading, launch, flight, and reentry averaged 20°C, which is well below the optimum for both species. They also showed some divergence from constancy, which made the control at temperatures reflecting those in flight more reliable than the controls at constant temperature.

The population densities reached in both species were higher in orbit than on the ground (Table 4, *upper part*). The data are considered more significant for *Salmonella* since it is estimated they had reached maximum density; plate counts of these gave values about 25 per cent lower, but the differences between orbited and ground values remained in all cases. The number of free phage particles re-

leased (Table 4, *lower part*) was also higher when irradiated in orbit than on the ground.

In discussion of this paper, the statistical significances calculated were questioned, and the role of vibration and settling was felt to have been critical and to need more experiments.

(13) *Mutagenic effects in Neurospora crassa irradiated in flight* (F. J. de Serres and B. B. Webber, Oak Ridge National Laboratory, Tenn.): Conidia of a two-component heterokaryon were mounted on moist 25-mm Millipore filters in polypropylene holders in the flight capsule and in ground controls. Irradiation doses were 0, 884, 2058, and 3116 r in each group.

The frequencies of spontaneous mutations, induced recessive lethal *ad-3* mutants, and general recessive lethals, in analyses thus far, were the same in orbited cells as in those on the ground. The frequencies of surviving conidia at the different irradiation dosages also showed no effect of flight. It may be noted that the conidia were in a nongrowing and metabolically inactive state.

The characterization of the *ad-3* mutants is as yet incomplete. This analysis provides the most sensitive assay for qualitative differences at the molecular level between the orbited and ground samples.

Scientific Conclusions—The orientation of petioles, coleoptiles, and primary and lateral roots in *Capsicum* and *Triticum* shows no real differences between orbited plants in almost *zero* gravitational force and plants rotated slowly on the horizontal clinostat, i.e., in a *balanced* gravitational field of 1 *g*. The pepper leaves showed a wide variation in angles, but only four plants could be flown; their apparent failure to recover after flight may have been due to temperature differences, or perhaps to traces of ethylene in the air, which would have been effective at levels below those analyzed. The wheat plants showed no difference in growth rates. The distribution of starch grains, known as statoliths ever since the observations of Nemeč and of Haberlandt in 1900, was the same in orbited as in clinostated plants, both being quite different from the gravitational arrangement in erect plants. It must be noted, however, that the "statolith theory," which accords to these grains the role of georeceptors, did not receive a really critical test until the experiments of Pickard and Thimann in 1966, and the theory failed this test. Hence the identity of the georeceptors of plants is still uncertain.

Five of the enzymes studied showed no differences; peroxidase activity was higher in both the orbited and the clinostated plants than in the erect controls. Oxidation rates gave no certain results because of an unexpected difference related to chamber configuration, which did not occur in ground controls. In general, for these two plants, there is no evidence here that the effects of orbiting in space are any different from those of clinostating on earth. The fact that weightlessness can for biological purposes be so closely imitated by rotation on the horizontal clinostat is most important for all further work.

The frog eggs showed no effects of orbiting. The incidence of abnormalities was even less than on earth, but there is a satisfactory explanation for this. Although it is not certain whether the amoebae were attached or floating during

orbit, they apparently fed normally, showing from 2 to 17 food vacuoles each, and they showed no ill effects after the flight. Although different individuals fed very differently from one another, the same range of behavior was seen in the controls. The fed amoebae divided normally in orbit.

Thus these two nonbotanical objects showed no evident effects of the flight. Chromosome nondisjunction in *Drosophila* larvae, however, did show an increase over that in ground controls. Also, both *Drosophila* and *Habrobracon* showed a significant increase in the frequency of recessive lethal mutations.

The experiments on radiation in orbit seemed to show more positive effects.

In *Tradescantia*, the stunting of hairs (reduced cell division in hair cells) was much greater in orbit than on the ground; pollen abortion (which was high in this strain) and disturbed spindle function were also significantly increased in orbit. Enhancement of radiation effectiveness, or synergism between radiation and flight conditions, can hardly be doubted in this material, even though the mutation from blue to pink showed a smaller but significant antagonism.

The same conclusion can be drawn for the *Habrobracon* wasps; the oöcytes, both in prophase and metaphase, show clear and statistically significant enhancement. Again, however, antagonism shows up in another growth phase, namely is in the oögonia (here flight conditions completely abolish the effect of 2000 r), while no interaction at all appears in regard to dominant lethality in the sperm.

A curious *aftereffect* of flight is shown by *Habrobracon:* egg production of orbited females after the tenth day was higher and life spans after the flight were longer than those of ground controls. As yet, there is no obvious explanation for these favorable effects.

The *Tribolium* data show a clear enhancement, both of wing abnormalities and of dominant lethality, due to flight.

In *Drosophila*, the *Dumpy* wing mutant shows very clear enhancement, but lesser degrees of enhancement, not always fully significant, are shown also in nondisjunction in females, loss of markers from the Y chromosome, total numbers of survivors among larvae, chromosome breakage in larvae, and even in lethals in mature sperm. However, in the last instance, as in several others, there is relatively low sensitivity on the part of nondividing nuclei.

The number of phage particles from *Salmonella* was higher in flight than in the controls, but since the total number of bacteria was also higher, the number of phage particles per cell was somewhat decreased by the flight, and these data are hard to interpret.

Finally, in *Neurospora* conidia on filters (not dividing), five comparisons showed no effect of flight alone and no difference in the genetic effects of radiation due to flight in the analyses completed thus far.

For those phenomena depending on chromosome breakage, it is evident that more detailed controls of the effects of vibration are needed, particularly with the precise frequencies and amplitudes of vibration encountered in launch and recovery. In preflight tests, vibration studies, exceeding the levels encountered in flight by 50 per cent, have not caused the effects observed here. Now that the actual records of the amplitudes of vibration are available, new ground tests will be undertaken. These will include serial exposure to vibration, clinostating, and

irradiation, as well as to linear acceleration. Only if these are fully negative will we have to conclude that weightlessness by itself enhances the effects of irradiation by 50 to 350 per cent. It now remains to be determined whether *balanced gravity*, as on clinostats, has any comparable effect, and this calls for further detailed study on the ground.

When the results are surveyed as a whole, it seems that of the two major questions posed at the outset, one has been answered: Orbiting does not appear to bring about any unusual changes that cannot be imitated by gravity-balancing devices on the ground. The second question has also been answered, though the causal mechanism is not fully clear: Radiation and flight conditions do interact, and when nuclei are dividing, enhancement or synergism occurs in a number of different organisms. The relative roles of weightlessness and vibration (alone and combined) as causes of this interaction need further ground-based experiments. The effect of balanced gravity on sensitivity to radiation also needs exploration in ground-based experiments.

The data offer no good reason to plan for a repetition of the flight of Biosatellite II.

It is a pleasure to acknowledge the cooperation of all the experimenters in reviewing this report. The arrangements for the symposium were admirably made by Dr. H. G. Shepler of the Space Science Board, National Academy of Sciences. The cooperation of Dr. Orr Reynolds of National Aeronautics and Space Administration before and during the symposium, and in a careful review of the manuscript, is most gratefully acknowledged.

[1] *BioScience*, supplement, **18** (July 1968).

[2] The biosatellite is subject to slight aerodynamic drag and some slow rotation so that a very small acceleration was exerted. This was calculated not to exceed 10^{-5} g, except for short periods after separation from the launch vehicle, and before the ignition of the retrorockets causing reentry into the earth's atmosphere. The total fraction of the orbiting time with acceleration above 10^{-5} g was estimated as 11%, with a maximum of 1.4×10^{-4} g. During launch, acceleration up to 8 g was recorded, and during reentry, deceleration lasting 3 min and ranging up to 9 g occurred.