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SURVEY OF CELL BIOLOGY EXPERIMENTS IN REDUCED GRAVITY

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ABSTRACT

Although single cells are generally considered to be less vulnerable than higher organisms to variations in gravitational forces, many cell experiments have been conducted in the reduced gravity of space. Studies involving isolated viruses, bacteria, yeasts, filamertous fungi, protozoans, and cells in small groups (such as tissue cultures and early embryos) are reviewed to illustrate the variety of species examined. Early studies, conducted with high altitude balloons, sounding rockets, and primitive orbital satellites, demonstrated the capability of cells to survive the space flight environment. These results revived interest in Panspermia and demonstrated the possible requirement for sterilization of all spacecraft landing on foreign heavenly bodies.

Because space vehicles, equipment, and passengers are not sterilized before flight, it has been important to study the effects, if any, of spaceflight on terrestrial cell systems. With some important exceptions, which are discussed, static cell systems carried aboard USA and USSR space flights have failed to reveal space-related anomalies. Some sophisticated devices which have been developed for viewing directly, or continuously recording, the growth of cells, tissue cultures and eggs in flight, are described and the results summarized. The unique presence of high energy, multicharged (HZE) particles and full-range ultraviolet irradiation in space has prompted several investigators to evaluate the response of single cells to these factors.

Summary results and general conclusions are presented. Potential areas of research in future space flights are identified.

INTRODUCTION

Experimental evidence that organisms are affected by gravitational forces was first obtained in 1806 by Knight who demonstrated, with the

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aid of a water-driver centrifuge, that orientation in plants was determined by the gravitational vector (1). The dependence of animals upon gravity was first observed in 1883 by Pfluger with demonstrations that the development of frog eggs in an inverted position resulted in a high rate of abnormalities (2). Many studies, designed to evaluate the effects of resultant forces in excess of one gravitational unit, issued from these beginnings. In addition, some investigators have observed organisms in devices which compensate for, or oppose, the Earth's gravitational attraction. The neutral bouyancy tank which provides a flexible lift equal to the mass of the test object, and the rotating clinostat which continually alters the direction of the gravitational vector, are the two most widely used devices (3).

With the advent of the space age it became possible to reduce the total force upon test systems to less than one gravitational unit by removing them from the Earth. This opportunity was recognized by many investigators who conducted experiments on a large variety of different types of living test systems. Those systems which involve single cells or small groups of cells (such as blastulas or tissue culture) are reviewed and summarized in Tables I through VI, in an effort to demonstrate the variety of tests that have been conducted in space. In addition, general conclusions are presented and areas potentially worthy of future space research are identified.

REVIEW

Survival Of Cells In Space

Prepatory to studies on orbital spaceflights, several microbial species were exposed to altitudes up to 1900 Km in balloon and sounding rocket flights (Tables I, II, III, and IV). These exposures, which were initiated in 1935 (4), were conducted to determine if microorganisms could survive high altitude flight and have been thoroughly reviewed (5, 6, 7, 8). Although rudimentary, these studies permitted the investigators to observe that a large percentage of fungal spores and dormant vegetative cells could survive short-duration direct exposure to the space environment at these altitudes (9,10).

Beginning with the USSR recoverable Sputnik 5 flight in 1960 (11) and the USA Gemini/Agena missions in 1963, the requirement to sterilize space vehicles destined to land on other heavenly bodies has been studied (8). In a typical example, a variety of microbial species (*Penicillium roqueforti*, *Bacillus subtilis* spores, Tobacco Mosiac Virus, and T_1 coliphage) were carried aboard the Gemini 9A and Gemini 12 spacecraft (9). Viable representatives of all species were recovered following nearly 17 hours of "direct exposure" to space conditions. These same species, when protected from direct solar irradiation, survived 4 months of exposure on

the Agena 8 orbiter (10). Similar tests on the Soviet Cosmos 368 Earth-orbital satellite, and the Zond 8 automatic lunar station, revealed that Hydrogenomonas eutropha, Saccharomyces ellipsoides, Zygosaccharomyces baili, and Escherichia coli, cells were all able to survive spaceflight (12,13).

In the ensuing years, viability measurements have generally been included in all space cell biology studies. As a result it has been established that microorganisms in and on interplanetary spacecraft may be capable of surviving to contaminate extraterrestrial bodies (8, 9, 10, 12, 13, 14, 15, 16, 17). The record for viability in space was reported for Streptococcus mitis which was recovered from internal components of a Surveyor III television camera that had resided on the surface of the Moon for 2.5 years (18). Even though the possibility of survival in space has been repeatedly proved, it was considered operationally non-feasible to sterilize space vehicles, equipment, and passengers before flight. Accordingly it became important to evaluate the effects, if any, of spaceflight on terrestrial cell systems.

Although interested in the same objective, the American and Soviet space programs proceeded differently to evaluate these effects. This difference is outlined by Jenkins (6) who demonstrated that in the first decade of orbital flight, Soviet scientists evaluated 56 different species (or preparations) including viruses, bacteria, yeasts, fungi, plants, animals, and tissue cultures. During the same period the USA evaluated only 35 different species and cellular preparations. More importantly, several of the Soviet satellites were flown primarily to obtain biological data to qualify man for spaceflight. In contrast, the early American biology studies were operated on a non-interference basis and no successful, dedicated biology satellite was flown until the launch of Biosatellite II in September 1967 (6).

Effect Of Spaceflight On Growing Cultures

In addition to the previously mentioned viability tests which involved static or dormant cells, spores, or cysts, some important studies, outlined in Table VII, have been conducted on growing cells. Inflight microbial growth was first monitored during the flights of Sputnik 5 (19) and other, non-recovered Soviet satellites (20), with the aid of an automated device known as "Bioelements". This device was designed to measure the rate of gas production in actively growing Clostridium butyricum cultures and to relay these data to earth. Data from this test, and from Vostok 1 and 2 where a modified "Bioelements" was used, showed gas production rates indistinguishable from ground controls.

Growing and reproducing protozoans have been variously studied. Planel et al. (21) have reported an increase in cellular growth rate for Paramecium aurelia exposed to high-altitude balloon flight for 6 hours. Additionally, amoebae were observed following the 45 hour flight of Biosatellite II. There were no significant differences between flight cells

and ground controls, but Ekberg et al. (22) reported a "trend" towards a higher division rate during flight. It is well known that amoebae require gravity (or some force vector) to attach to substrates. Although this attachment is generally considered to be required for locomotion and feeding, these organisms survived the flight and fed, assimilated food, grew, and performed all other measured functions in a manner indistinguishable from the ground controls (23). These results generally confirm data obtained from earlier simulation studies aboard C-131 aircraft in Keplerian trajectory (24).

Another test system, which was unusually refined for automated satellite studies, was designed to study the developing frog egg under reduced gravity conditions. This series, flown aboard the Gemini 8, Gemini 12, and Biosatellite II spacecraft, provided for inflight growth and differentiation of fertile eggs from the 2 cell stage. Developing frog eggs on Earth exhibit a marked sensitivity to disorientation with respect to the normal gravity vector, with the early embryo (up to the eight-cell stage) being the most sensitive (25). In spite of this known sensitivity no differences could be determined between flight and ground controls. The authors point out that, to complete this line of research, frog eggs should be fertilized after launch and maintained for a longer time in the reduced gravity state (25, 26).

In a similar manner, young Killifish eggs (Fundulus heteroclitus), were allowed to develop and hatch during the 56-day Skylab, the 20-day Cosmos 782, and the 10-day Apollo Soyuz Test Project (ASTP) flights. In all cases space-hatched fry exhibited no observable tendency toward disoriented swimming activity (27, 28) although dependence on visual orientation cues aboard the Skylab and following return to Earth suggested the possible absence of vestibular input (28).

One of the most elegant and complex growth studies to date was conducted with Wistar-38 human embryonic lung cells in tissue culture aboard the middle Skylab flight (Skylab 3). Continuous cultures were maintained at 36° C and photographed with time-lapse motion picture cameras, through phase-contrast microscopes at 20X and 40X magnification, for 28 days (29). Many parameters were evaluated, including growth curves, mitotic indices, cell migration rates, vacuole formation, cell size, nuclear size and location, nucleolar size and number, and G- and C-band patterns in chromosomes. Although the experiment operated according to plan, no differences were detected between flight cells and suitable ground controls (30).

More recently, growing colonies of Streptomyces lavoris were flown aboard the Soyuz 16 and the Apollo Soyuz Test Project flights (31). The formation of alternating rings of spore-bearing and sterile mycelium allowed continuous analysis of changes in cyclic growth and provided a method for keeping track of certain inflight mutations. A correlation between the cyclic spore formation and spaceflight was not demonstrated. Although analytical data are not yet available it should also be noted that Soviet investigators have reported active observation of cultures of

coliform bacili, fertilized frog eggs and Serian Hamster cell tissue cultures in the "flying oasis" of Soyuz 17 - Salyut 4 (32).

Genetic Studies

Bacteriophage induction has been extensively employed, by Soviet investigators, as a model system for visualizing the effects of spaceflight on the genetic apparatus of microorganisms (Table VIII). *Escherichia coli* K-12 (λ) bacteriophage have been carried aboard most of the flights of the Sputnik series, all six of the manned Vostok flights, Voskhod 1 and 2, the unmanned biosatellite Cosmos 110, and Zond 5 and 7, both of which circled the Moon (19, 33, 34). This system was used as a radiation dosimeter because increases in phage production could be stimulated by as little as 0.3 rad of gamma radiation or by small doses of protons or rapid neutrons (33, 35). Because phage induction involves injury to the genetic apparatus, the lysogenic bacteria system was used to provide information about the potential mutagenic activity of cosmic radiation. It was reported that the spaceflight effect (measured in terms of increased phage production in space as compared to the magnitude of spontaneous phage production in the ground controls) increased with mission duration throughout the Vostok series (7, 35). This relationship is summarized in figure 1. Laboratory studies demonstrated that simulated launch vibration followed by exposure to ^{60}Co gamma radiation resulted in an increased mutation rate which was higher than that obtained by gamma radiation or simulated launch vibration alone (33, 35). This was interpreted as indicating that the Vostok launch vibrations "sensitized" the cells so that they were not susceptible to inflight irradiation.

Two different bacteriophage systems were tested as part of the 45-hour Earth-orbital flight of the American Biosatellite II (36, 37). *Salmonella typhimurium* BS-5 (P-22)/P-22, and *E. coli* C-60 (λ)/λ were tested for alterations in bacterial cell growth and bacterial prophage induction following spaceflight (Table VIII). During the flight, different aliquots of cells were exposed to a total dose of from 265 to 1648 rad of ^{85}Sr gamma radiation with the resulting radiation response curves being compared with appropriate ground control curves. Neither ultrastructural nor viability differences were noted between flight and ground-control *E. coli* systems. However, with the *S. typhimurium* system the authors reported an increased cell density in the space-flown culture fluid indicating increased growth activity. This same result was later duplicated in clinostat studies which supplied a continually shifting gravitation vector, did not allow settling of cells, and kept the growth medium continually agitated. Even though the resultant increase in growth could be simulated in the clinostat the authors speculated that the mechanism was probably different (36, 37).

Testable numbers of phage were not produced with the *E. coli* system because the flight was shorter than had been planned. In the *S. typhimurium* system there was no differences in the free P-22 density of the flight and ground cultures, although the space-flown cells were more resistant to gamma radiation, as indicated by a decrease in phage production. Efforts to reproduce these results with acceleration, vibration, and clinostat tests were unsuccessful. This decrease in phage induction supports the results reported for the *E. coli* system flown on Cosmos 110 but is counter to the results reported for all of the other Russian coliphage studies (34).

Additional spaceflight irradiation studies have been conducted which did not involve phage induction systems (Table IX). A variety of microorganisms, carried aboard the Cosmos 368 earth-orbital satellite, were irradiated with ^{60}Co gamma irradiation before flight and/or after return to earth. There was no evidence that the spaceflight had sensitized these species in a way that altered their viability or mutability (15).

During the flight of Gemini XI, conidia of *Neurospora crassa* were exposed to a ^{32}P beta source, and cells of the same species were exposed to a ^{85}Sr gamma source during the 45-hour Biosatellite II flight (38, 39). For both experiments the assayed system was a genetically marked two-component heterokaryon which was heterozygous for two different genes that control sequential steps in purine biosynthesis. The exposure of ground control and inflight cells to a range of radiation in both tests allowed for comparative analyses of dose-response curves.

Analyses of the Gemini XI samples indicated that neither the survival rate nor the mutation frequency of conidia deposited on membrane filters was altered by 71 hours of orbital flight. However, the flight cells suspended in agar demonstrated higher levels of survival and lower frequencies of induction, indicating that the spaceflight affected a protective influence (39). The authors point out that these data must be considered equivocal since they could have been the result of anoxia caused by high temperatures in the spacecraft. However, when the experiment was repeated 12 months later in the Biosatellite II unmanned orbitor agar suspensions were not used and this portion of the test was never repeated. As in the Gemini XI test, there were no differences between the flight and ground control radiation survival curves or overall induction.

In addition to the studies with ionizing radiation, possible synergistic relationships between spaceflight and solar ultraviolet light have also been tested. The data presented in Table X illustrate that the Tl coliphage, *P. roqueforti*, and tobacco mosiac virus (TMV) particles have been flown on various space vehicles. From these studies, Lorenz et al. (40) concluded that solar ultraviolet irradiation with wavelengths between 200 and 300 nm was the main cause of inflight inactivation of these microorganisms. These data do not differ from the results of the many laboratory UV-response experiments, suggesting that ground-based studies may be used as model systems for preparation of inflight experiments.

In another study, prepared by a group of American and European investigators, eight microbial species were exposed to solar UV and space vacuum outside of the Apollo 16 command module during its return from the Moon (41, 42). The use of various combinations of optical filters to provide exposure of different test aliquots to varying amounts of solar irradiation at peak wavelengths of 254, 280, and 300 nm, allowed for a different dose-response curve at each of these three wavelengths (43). The T-7 bacteriophage preparations of E. coli which were exposed to inflight irradiation were found to be more sensitive to UV light than were irradiated ground controls (44). There were no significant differences reported between postflight survival rates of non-irradiated fungal cells when compared with appropriate ground controls (45) although the survival rate of space-flown Chaetomium globosum, Rhodotorula rubra, and Saccharomyces cerevisiae was slightly depressed and samples of Trichophyton terrestre, and S. cerevisiae demonstrated some sensitivity to inflight solar UV when measured in terms of a loss of cell viability (corresponding ground control data were not reported). No changes in survival rate, mutation rate, or toxin production could be detected with postflight analyses of Bacillus thuringiensis and Aeromonas proteolytica (46). However, it was reported that the combination of solar UV and space vacuum resulted in a greater loss of viability in dried Bacillus subtilis cultures than with UV alone, indicating that the spores were sensitized to UV by the vacuum (17).

Cell Studies With Multicharged, High Energy (HZE), Cosmic Particles

Experiments designed to study the biological effects of individual heavy nuclei of cosmic radiation during space flight outside the magnetosphere of the Earth have been repeatedly conducted by a consortium of European investigators (47, 48). These experiments were housed in the BIOSTACK, a complex package consisting of alternating layers of nuclear track detectors, and biological objects imbedded in polyvinyl alcohol (PVA). Among other species, spores of Bacillus subtilis and cysts of Artemia salina were exposed to HZE particles during the flights of Apollo 16, 17, and the Apollo-Soyuz Test Project (Table XI). Individual cells or cysts in the path of HZE particles were evaluated for germination, outgrowth, and production of abnormals. The first vegetative cells issuing from bacterial spores lying in the path of high energy, multicharged particles were frequently found to be abnormally swollen. Artemia salina cysts, lying along nuclear tracks, showed reduced hatching and larval emergence and an increase in the incidence of developmental anomalies.

In a further attempt to understand the effect of galactic HZE particles upon biological objects, Soviet investigators included the yeast Saccharomyces cerevisiae in the "Bioblock" which was aboard the 2 month Cosmos 613 earth orbital flight. Although many of the colonies did not survive the long storage, a ten-fold increase in the incidence of "radiation damaged cells" was reported (49).

CONCLUSIONS

The above review has illustrated that, whereas a large variety of cell biology studies have been conducted in space, consistent space-mediated alterations have not been identified. Although individual studies often produced equivocal data, evaluation of the aggregate results indicates that cell systems are generally no less stable in space than they are in the Earth-based laboratory. Of course the conditions to which cell systems are exposed in space are usually less well controled (and less controlable), often leading to more variable and erratic results.

It has not yet been demonstrated that the spaceflight environment could be used to affect unique or hitherto unknown cell changes. On the contrary, cell systems appear to remain sufficiently stable to permit experimentation with models which require a fixed cell line. Therefore, taken as a unit, the cell biology studies conducted during the preceding two decades should definitely be considered a success. It is now possible to prepare cell biology experiments for the Space Shuttle era with a reasonable probability that the cells will not react engimatically to the unique environment encountered within the spacecraft.

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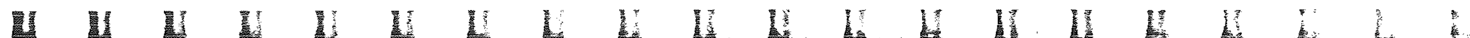

TABLE I. - SPACE-FLOWN ISOLATED VIRUSES

Microorganism	Flight	Condition	Reference	Number
Tobacco mosaic virus	U.S.S.R.	Unknown	Parfenov 1973	7
	Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	Lorenz 1968 Hotchin 1969	10 9
Poliomyelitis virus	U.S. Balloon	34 to 155 km, altitude	Parfenov 1973	7
Vaccinia virus	Gemini XII	Dry	Hotchin 1969	9
Influenza virus	U.S.S.R.	Unknown	Jenkins 1968	6
Influenza (PR-8 strain) Canine hepatitis Infectious bovine Rhinotracheitis	Gemini XII	Dry	Hotchin 1969	9

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TABLE II. - SPACE-FLOWN BACTERIAPHAGE AND HOST

Microorganism	Flight	Condition	Reference	Number
Escherichia coli K-12/K-12	Sputnik 4 and 5	Unknown		
	Vostok 1, 2, 3, 4, 5, 6	Nutrient suspension $^{60}Co-\delta$	Antipov 1967	50
	Cosmos 110	Nutrient suspension $^{60}Co-\delta$	Jenkins 1968	6
Escherichia coli T_1	U.S. Balloon Aerobee Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	Hotchin 1969	9
	Sputnik 5 and 6 Voskhod 1 and 2	Dry	Jenkins 1968	6
Escherichia coli T_4	U.S. Balloon	34 to 155 km, altitude	Parfenov 1973	7
Escherichia coli B/T_2	Vostok 2	Unknown	Zukov-Verezhnikov 1966	35
Escherichia coli $T_{4b}r^+$	ASTP	Dry	Rogers 1976	31
Escherichia coli T_7	Apollo 16	UV Exposure	Spizizen 1975	44
Escherichia coli C-600	Biosatellite II (p-1135)	Growing in liquid $^{85}Sr.-\delta$	Mattoni 1968	36
Salmonella typhimurium BS-5(P-22)/P-22	Biosatellite II (p-1135)	Growing in in liquid $^{85}Sr.-\delta$	DeSerres 1969	39
Aerobacter aerogenes 1321	Vostok 2	Unknown	Parfenov 1973	7

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TABLE III. - SPACE-FLOWN BACTERIA

Microorganism	Flight	Condition	Reference	Number
Escherichia coli	Soyuz 17/ Salyut 4	Growing in nutrient	Apenchenko 1975	32
Escherichia coli K-12 ( ) *Escherichia coli* B *Aerobacter aerogenes* 1321 *Staphylococcus aureus* 0.15	Vostok 1	Agar cultures	Parfenov 1973 Zhukov-Verezhnikov 1966	7 35
Clostridium butyricum	Vostok 1	Spore suspension		
Clostridium sporogenes	Discoverer XVII Discoverer XVIII	Unknown	Parefenov 1973	7
Bacillus brevis	Voskhod 1	Spores		
Bacillus subtilis ATCC 6052	Gemini IXA Gemini X/ Agena VIII Gemini XII	Dry	Lorenz 1968 Hotchin 1969	10 9
Bacillus subtilis 168	Apollo 16 Apollo 17 ASTP	Dry Dry Dry	Bücker 1974 Bücker 1976	47 48
Bacillus subtilis HA101 and HA101 (59) F	Apollo 16	UV Exposure	Spizizen 1975	44
Bacillus thuringiensis	Apollo 16	UV Exposure	Simmonds 1974	46
Aeromonas proteolytica	Apollo 16	UV Exposure	Foster 1973	51
Streptomyces erythraeus 2577 *Streptomyces erythraeus* 8594	Vostok 2	Aqueous spore suspension and liquid mycellium suspension	Glembotskiy 1962	52
Streptomyces streptomycini Kras LS-3	Vostok 2	Unknown	Khvostova 1962	53
Streptomyces aureofaciens LSB 2201	Vostok 4 Vostok 5	Aqueous spore	Glembotskiy 1962	52
Streptomyces levoris	ASTP	Growing colonies	Rogers 1976	31
	Soyuz 16	Growing colonies	Izuvestiya 5 Dec. 1974 p. 5	
Hydrogenomonas eutropha Z-1	Cosmos 368 Zond 8	Cells in aqueous suspension	Grigoryev 1972 Romanova 1971	15 13

TABLE IV. - SPACE-FLOWN YEASTS AND FILAMENTOUS FUNGI

Microorganism	Flight	Condition	Reference	Number
Zygosaccharomyces	Cosmos 368	Cells on Agar	Grigoryev 1972	15
Saccharomyces (Zygosaccharomyces) 40-2587 (haploid)	Vostok 2	Suspensions both unsensitized and sensitized with olic acid	Kovyazin 1962	54
Saccharomyces (diploid) 139-B	Cosmos 368	On agar and in aqueous suspension	Grigoryev 1972	15
	Cosmos 613	Colonies on agar (0.5 - 1.0 mm d.)	Benevolensky 1976	49
	Voskhod 1	Suspensions both unsensitized and sensitized with olic acid	Kovyazin 1962	54
Saccharomyces cerevisiae	Apollo 16	UV Exposure	Volz 1973	45
Penicillium roqueforti	U.S. Balloon	Dry spores (34 km altitude for 6 hrs.)	Parfenov 1973 Hotchin 1969	7 9
	Gemini XII	Dry spores	Hotchin 1969	9
	Gemini IXA Gemini X/ Agena VIII	Dry spores	Hotchin 1969	9
Neurospora crassa	Biosatellite II (p.-1037)	Dry spores ^{85}Sr-	DeSerres 1971	38
Neurospora species	U.S. Balloon	Unknown	Hotchin 1969	9
	Gemini XI	Dry spores phosphorus-32 (^{32}P)-δ - and metabolizing spore suspension ^{32}P-δ	DeSerres 1969	39
	Nerv I	1900 Km altitude for 28 min	Jenkins 1968	6
	Discoverer XVIII	Dry Spores		
Chaetomium globosum Trichophyton terrestre Rhodotorula rubra	Apollo 16	UV Exposure	Volz 1973	45
Candida tropicalis SK-4	Zond 8	On Agar	Romanova 1971	13

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TABLE V. - SPACE-FLOWN PROTOZOANS

Species	Flight	Condition	Reference	Number
Colpoda cucullus	Apollo 17 (Biostack II)	Cysts in monolayers of polyvinyl alcohol	Bücker 1974	47
Pelomyxa carolinensis (giant multinucleate Amoeba)	Biosatellite II	Dividing, Free-feeding cells	Abel 1971 Ekberg 1971	23 22
Amoeba	C-131 Aircraft in Keplerian trajectory	Growing cells	McKinney 1963	24
Paramecium aurelia	USSR Balloon	Growing cultures	Planel 1975	21

TABLE VI. - SPACE-FLOWN CELLS IN SMALL GROUPS

Species	Flight	Condition	Reference	Number
Rana pipiens (Leopard frog)	Biosatellite II	Developing eggs from 2-cell stage	Young 1971	25
Frog Eggs	Gemini 8 Gemini 12	Developing eggs from first cleavage	Young 1968	26
Frog Eggs	Soyuz 10 Soyuz 17/ Salyut 4	Fertile Frog Eggs	Apenchenko 1975	32
Artemia salina (Brine shrimp)	Biosatellite II	Dry Blastocysts	von Borstel 1971	55
	Apollo 16 (Biostack I) Apollo 17 Biostack II) ASTP (Biostack III)	Encysted blastula in monolayers of polyvinyl alcohol	Bücker 1974 Planel 1974 Bücker 1976	47 56 48
Carausius morosus (grasshopper)	Apollo 17 (Biostack II)	Eggs in monolayers of polyvinyl alcohol	Bücker 1974	47
Fundulus heteroclitus (killifish)	ASTP	32-336 hr embryos in sea water	Scheld 1976	28
	Skylab 3	5-day old fertile eggs in sea water		
	Cosmos 782	32-128 hr embryos in sea water		
Danio rerio (fish)	Soyuz 16	Fertilized eggs	Izvestiya 8 Dec. 1974 p. 3	
WI-38 diploid human embryonic lung cells	Skylab 3	Growing cultures from single cells	Montgomery 1974	30
Serian Hamster cells	Soyuz 17/ Salyut 4	Tissue culture	Apenchenko 1975	32
Carrot Tissue culture	Cosmos 782	Crown gall and proembryonic cells	Scheld 1976	28

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TABLE VII. - MAJOR SPACEFLIGHT STUDIES WITH GROWING CELLS

FLIGHT	DEVICE	TEST SYSTEM	RESULTS
Sputnik 5 Vostok 1 & 2	"Bioelements"	Clostridium butyricum	Gas production rate same in flight as for ground controls.
Biosatellite II	Experiment P-1035	Pelomyxa carolinensis (Amoeba)	"Trend" towards higher division rate during flight. No change in survival, food assimilation, growth, etc.
Gemini 8 & 12 Biosatellite II	Experiment P-1047	Rana pipiens Frog eggs in 2-cell stage	No difference between flight and ground control specimens. Authors recommend repeat with inflight fertilization.
Skylab 3 ASTP COSMOS 782	Experiment MA 161	Fundulus heteroclitus (Killifish)	Dependence of hatched fry on visual cues suggestive of absence of vestibular input. No other differences resulting from flight.
SKYLAB 3	Experiment SO 15	Wistar-38 human embryonic lung tissue culture	No differences in growth curves, mitotic indices, cell migration rates, cell size, nuclear size and location, nucleolus size, etc.
Soyuz 16 ASTP	"Biorhythm I"	Streptomyces levoris	No differneces in cyclic spore formation inflight. No biological indications of HZE damage.

TABLE VIII. - BACTERIOPHAGE INDUCTION SYSTEMS TESTED IN SPACE

SYSTEM	FLIGHT	RESULTS
Escherichia coli K-12 λ	Most Sputniks All 6 Vostoks Voskhod 1 & 2 COSMOS 110 ZOND 5 and 7	Number of phages inflight exceeded ground controls. Excess proportional to length of mission. Simulated launch vibration plus ^{60}Co γ irradiation gave increases higher than irradiation alone. No increases from launch vibration alone or after ^{60}Co γ irradiation.
Salmonella typhimurium BS-5 (P-22/ P-22)	Biosatellite II	Increased cell density following 45 hr flight. Space-flown cells more resistant to ^{85}Sr γ irradiation (inflight 265-1648 rads) as indicated by decreased phage production.
Escherichia coli C-60 (λ)/λ	Biosatellite II	No postflight differences in growth when exposed to ^{85}Sr γ inflight. Flight terminated early, no opportunity for phage production.

TABLE IX. - ADDITIONAL SPACEFLIGHT STUDIES WITH RADIATION SOURCES

SOURCE	SYSTEM	FLIGHT	RESULTS
^{60}Co gamma (preflight and postflight)	Hydrogenomonas eutropha Z-1 Saccharomyces ellipsoides (diploid) Zygosaccharomyces baili (haploid)	COSMOS 368	No measurable loss of viability or change in radiosensitivity
^{32}P beta (inflight)	Neurospora crassa conidia	Gemini XI	Neither survival rate or mutation frequency altered for dry cells. Better survival and lower mutation frequency for agar-suspended cells
^{85}Sr gamma (inflight)	Neurospora crassa conidia	Biosatellite II	No inflight effect on dry cells

TABLE X. - INFLIGHT CELL STUDIES WITH ULTRAVIOLET IRRADIATION

FLIGHT	EVENT	TEST SYSTEM	RESULTS
6 sounding rockets 6 balloon flights 3 orbital satellites	Exposed To Direct UV Irradiation	T 1 Coliphage Penicillium roqueforti Tobacco Mosiac Virus	Confirms that UV between 200 and 300 nm is major cause of inflight inactivation.
Apollo 16	Exposed To Direct UV plus Components at 254, 280, and 300 nm	Escherichia coli T-7 bacteriophage	Flight specimens more sensitive to UV than ground controls although shape of dose response curves similar.
		Rhodotorula rubra Saccharomyces cerevisiae Chaetomium globosum Trichophyton terrestre	No evidence of synergism between inflight UV irradiation and reduced gravity
		Bacillus subtilis	No change in survival rate at 1 atm. Combined UV and vacuum resulted in greater loss of viability than UV alone. (Spores sensitized to UV by vacuum)
		Bacillus thuringiensis Aeromonas proteolytica	No change in survival rates. No change in ability to produce toxins

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TABLE XI. - CELL STUDIES WITH COSMIC HZE* PARTICLES

EXPERIMENT	FLIGHT	SPECIES	RESULTS
BIOSTACK (Bücker)	Apollo 16 and 17 ASTP	*Bacillus subtilis* spores	Swelling during growth of first vegetative cells from "hit" spores.
		Artemia salina cysts	Those "hit" by HZE showed reduction in larval emergence and hatching. Incidence of developmental anomalies increased.
BIOBLOCK (Benevolensky)	COSMOS 613	*Saccharomyces cerevisiae* 139-B	Of 1045 colonies, 169 hits with $Z \geq 8$ and 12 hits with $Z \geq 5$ over 2 months. 1.3% of cells demonstrated "radiation damage" compared with 0.15% normally. $2x10^4$ cells damaged per particle.

* HZE = Heavy (high atomic number) high-energy particles

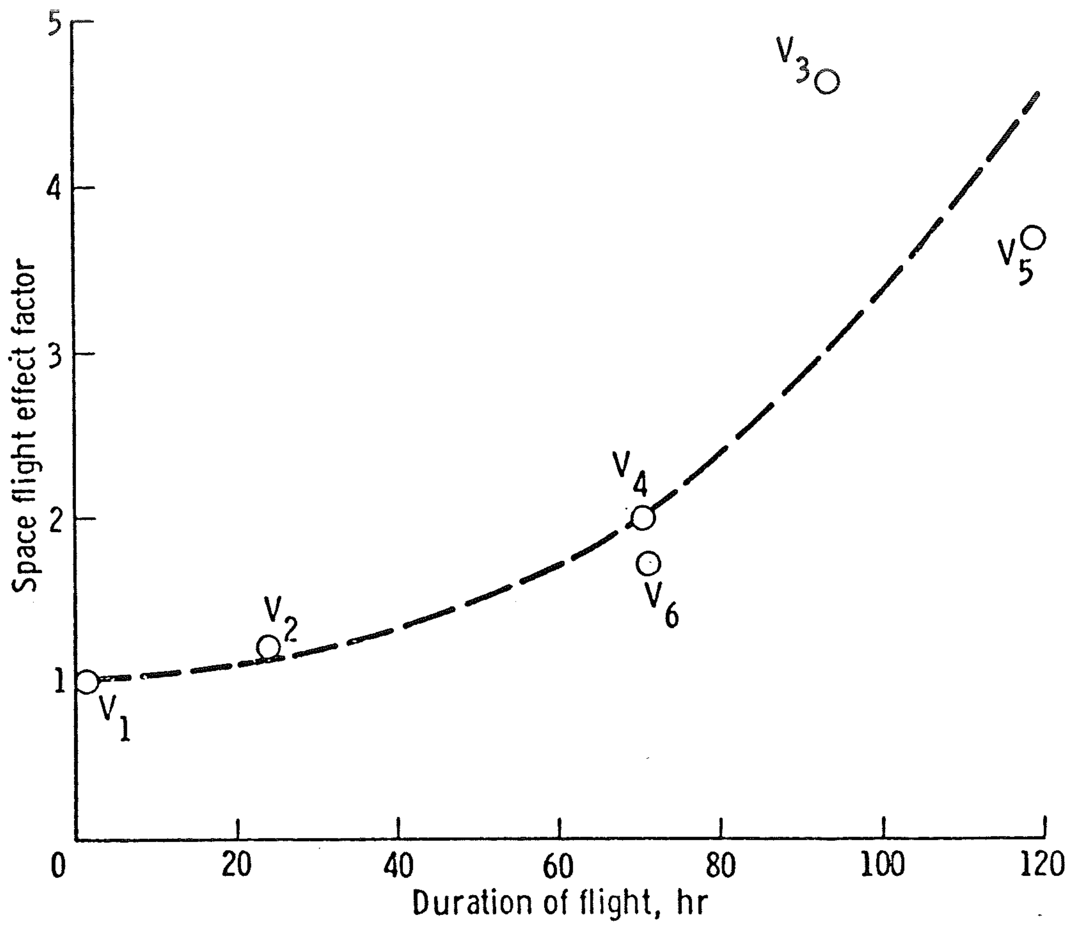

Figure 1.- Effect of duration of Vostok space missions on K-12 (λ) bacteriophage induction in <u>Escherichia coli</u> from data compiled in reference 6. V_1 to V_6 denote Vostok flight number. Space-flight-effect factor = number of bacteriophage particles per ground control cell.