CHAPTER 1
BIOSTACK—A STUDY OF THE BIOLOGICAL EFFECTS OF HZE GALACTIC COSMIC RADIATION

by

Professor Horst Bücker

University of Frankfurt, Federal Republic of Germany

Introduction

The high atomic number–high energy particle component (HZE particles) of galactic cosmic radiation was discovered in 1948 and radiobiologists soon became concerned as to the effect this new type of ionizing radiation might have upon living systems exposed to it. Soon after discovery of the HZE particles, Tobias in 1952 predicted that a visual light flash sensation could be experienced by individuals exposed to these particles. There followed direct experimental evidence of the character and effectiveness of HZE particles. Chase (1954) describes graying of hair in balloon-borne black mice; Eugster (1955) demonstrated cellular death by single hits of heavy ions on *Artemia Salina* eggs; and similar effects were reported by Brustad (1961) on maize embryos. Brain injury studies were attempted by Yagoda and co-workers (1963) and by Haymaker and co-workers (1970) in balloon-borne mice and monkeys, respectively.

Very high local concentration of absorbed energy produced by an HZE particle can cause serious biological effects upon an organism since complete cells can be damaged or destroyed. The ultimate consequence of such damage is dependent upon the organism's ability to repair or replace the affected cell. The destruction of cells in the central nervous system is of serious concern since these cells cannot regenerate.

Although the potential hazards to living systems from the heavy nucleii component of galactic cosmic radiation was recognized, very little active research was conducted until the crews of Apollo 11 and subsequent Apollo missions reported experiencing a visual light flash phenomenon. The primary reason for the inactivity in this field was an inability to generate particles with comparable charge and energy with existing accelerators. The light flashes experienced by the astronauts provided an increased impetus for radiobiological experimentation by direct exposure to the HZE particles in space. Exposure to HZE particles during a spaceflight mission offers several unique advantages, principally, exposure to the primary spectra modified only by the interactions in the relatively lightly shielded space vehicle. Balloon-borne exposures were

limited to a spectrum significantly modified by the shielding of the remaining atmosphere and by the geomagnetic field.

The Biostack experiment was designed to study the effect of individual heavy nucleii of the cosmic radiation environment upon biological systems during actual space flight. Since there were no means by which the Biostack experiment could be insulated from other spaceflight factors, such as null gravity, the experiment must be considered one of studying the combined effects of cosmic radiation and other spaceflight factors.

The objectives of the Biostack experiments were to study, in a direct manner, the biological effects of individual heavy nucleii with high energy loss (HZE); to obtain as much information as possible on the mechanisms of biological damage by HZE particles; to measure the charge and energy spectra of cosmic radiation within the Apollo Command Module; and to provide data to allow an estimate of the hazard to man from space radiation.

It was of great importance to place this experiment on the last two Apollo flights, since both were lunar missions. Apollo 16 and 17 would leave the Earth's magnetic field and enter a region of space where the galactic cosmic ray flux was modulated only by the solar magnetic field. Very little is known concerning the radiation environment outside the geomagnetic field, and HZE particles are of special interest. At the time of Apollo 16 HZE particles were not available on Earth, and only a limited capability to generate them has been achieved since the completion of the Apollo lunar missions.

Accomplishment of these objectives of the Biostack experiment required considerable ingenuity. The experiment design had to meet several criteria in order to take advantage of the two remaining flight opportunities. The design had to be simple enough to be implemented and qualified for spaceflight in a relative short time. The package had to be compact, lightweight, and require minimal changes in the spacecraft to enable stowage. Most importantly, the experiment design could not draw power from the spacecraft or impact the astronauts' activities.

Procedures

The objectives of the experiment and the constraints imposed upon it were met by a design that allowed the study of the combined action of individual heavy nucleii of cosmic radiation and spaceflight factors in biological systems in a state of rest. Detailed information on the particle incidence, energy loss, and spectra were essential information to be obtained. The Biostack experiment package contained a series of monolayers of selected biological objects fixed in position and interleaved with physical track detectors (figure 1). This arrangement permitted evaluation of individual tracks, and allowed identification of each penetrating particle and determination of its relationship to possible effects on biological matter in its path.

The execution of the experiment had two major thrusts; identification and quantitation of the influence of HZE particles on biological systems at the molecular, cellular and organismic levels; and the localization of individual HZE particles and the quantitation of the physical and dosimetric parameters of the particles.

A very sophisticated method must be used to localize precisely the trajectory of a particle relative to the biological objects and to correlate the physical data of the particle relative to the observed biological effects along its path. Special methods were developed

for this purpose (Bücker et al., 1973) in the Biostack experiment. Biological specimens and physical track detectors were selected to achieve the optimum return of information.

The biological systems had to meet the following criteria: (1) the organisms had to survive the period of experimental exposure in the dormant state and yet be viable for the subsequent phases of the experiment; (2) they had to comprise a variety of species to allow evaluation of radiation effects at different levels of biological organization; (3) they had to vary in radiation sensitivity (based on previous radiobiological experimentation with X-ray and other radiations); and (4) based again on previous work, be representative of genetic or somatic radiation damage mechanisms. The biological organisms investigated in the experiment and the responsible investigators are shown in table 1. The radiation effects subsequently studied were changes in cellular and organismic growth, damage to cellular components and induction of mutations leading to genetic changes of biological significance.

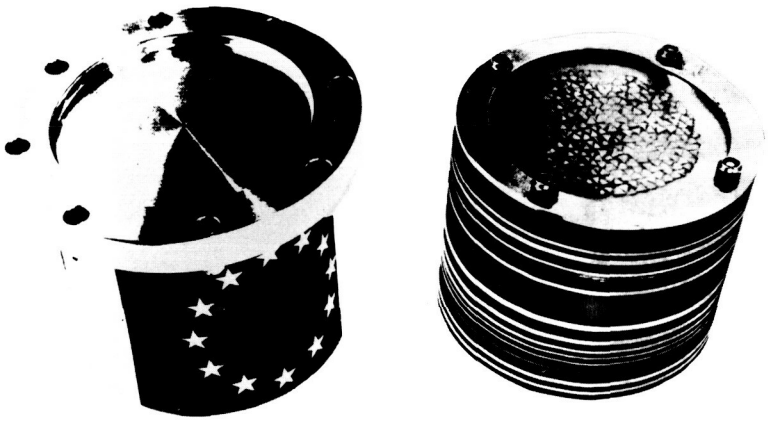

Figure 1. The Biostack experiment package: left, external view of container; right, stack of biological objects in monolayers and physical detectors.

Incident radiation was measured by several different track detectors and an integrating lithium fluoride thermoluminescence dosimeter. The detectors and responsible investigators are listed in table 2. These detectors complemented one another in their recording characteristics of HZE-particles as well as in the localization of the biological region hit.

Special methods were developed for optimal localizing of the point of penetration in the biological layer. The accuracy in determining this penetration point reached $\pm 1\ \mu m$. Therefore, in the case of the animal eggs and seeds, which all exceeded $50\ \mu m$ in diameter, even the hit region inside the biological organisms could be detected. For these objects, a sufficiently high accuracy was obtained with all three types of detectors. In the case of the bacterial spores, however, which were $1.5\ \mu m$ in diameter, several spores usually covered the determined target area, each with a different probability of sustaining a hit.

Table 1
Biological Experiments in the Biostack

Biological System		Investigator	Organization
Monocellular	Spores of Bacillus subtilis	G. Horneck	University of Frankfurt, Germany
	Cysts of Colpoda cucullus	H. Planel, J. P. Soleilhavoup	University of Toulouse, France
Plant	Seeds of Arabidopsis thaliana	E. Reinholz	MPI für Biophysik Frankfurt, Germany
	Radiculae of Vicia faba	W. Scheuermann	T. University of Hannover, Germany
Animal	Eggs of Artemia salina	W. Rüther, E. H. Graul H. Planel, J. P. Soleilhavoup	University of Marburg, Germany University of Toulouse, France
	Eggs of Tribolium castaneum Eggs of Carausius Morosus	W. Rüther	University of Marburg, Germany

For determination of the target area inside the spore layer, plastic detectors of cellulose nitrate (CN) were used. The CN sheet was in fixed contact with the biological layer. This contact was maintained during flight, during postflight etching and track measurements, and during growth studies. Protection of the biological layer against the toxic etching solution resulted in only one etch cone on the side of the CN sheet which was not covered with biological specimens. The trajectory of an HZE particle in the biological layer had to be extrapolated from this etch cone. With silver chloride (AgCl) crystals, on the other hand, the biological layer was not exposed to toxic agents during the development of the particle track images. The nuclear emulsion, attached to some of the biological layers, received the pattern of biological objects by weak optical illumination, during postflight disassembly. The hit biological objects were identified directly from the developed emulsion, which showed the HZE particle track together with faint images of the biological objects and a coordinate grid. Beside identification of the biological area hit, evaluation of the track detectors resulted in extensive information on the flux and angular incidence of the cosmic ray particles, on their absorption by the wall of the spacecraft and the Biostack material, and on the spectral distribution of their charge, energy, and energy loss.

The influence of the factors attendant to space flight (high gravity vectors, null gravity, vibration, and temperature) were assessed by detailed controls made in parallel with the Biostack experiment. For each space flight experiment, four identical Biostacks were built. In each case, three units were delivered to NASA: one prime flight unit, one backup, in case of damage to the prime flight unit, and one ground control to remain in Houston. One laboratory control unit was kept in Frankfurt. Since the primary flight unit was flown in both missions and a backup unit served as ground control, the two

Table 2

Radiation Detectors in the Biostack Experiment

Cosmic Radiation Component	Detector	Range of Information on Z and LET	Threshold	Tissue Equivalence	Background Noise	Investigators	Organization
Ions	Nuclear Emulsion K2, K5	Very broad	No	No	High	R. Kaiser, J. P. Massué, R. Pfohl	LPC – CRN Strasbourg, France
	Plastics Cellulose Nitrate, Poly-carbonate	Medium	Yes	Yes	Low	W. Enge, O. C. Allkofer, K. Bartholomä, R. Beaujean, W. Heinrich, K. Fukui	University of Kiel, Germany
						E. V. Benton	University of San Francisco
	AgCl-Crystals Illuminated	Broad	Yes	No	Medium to Low	E. Schopper, G. Henig, J. U. Schott	University of Frankfurt, Germany
γ-Rays, X-Rays, Protons	LiF Thermo-luminescence Dosimeter	Integrating Dosimeter				H. Francois, G. Portal	CEA, Paris, France

remaining units were available for further investigation. For Biostack I (Apollo 16), a balloon flight at Fort Churchill, Canada, was conducted with one of the remaining units, while the other served as the relevant control. For Biostack II (Apollo 17), one of the remaining units was irradiated at the University of California at Berkeley at the Bevatron with carbon and oxygen ions. The other was subjected to vibration, acceleration, and shock at the Centre National d'Etudes Spatiales (CNES) in Toulouse, France.

Results

The Biostack I experiment was launched with Apollo 16 on April 16, 1972. Splashdown into the Pacific was on April 28. The total mission time was 266 hours. The temperature in the Command Module during the mission ranged between 290°K and 296°K (17°C and 23°C) and the limits of 287°K and 301°K (14°C and 28°C) were never exceeded. The flight data of the Apollo 17 mission, with which the Biostack II was flown, were quite similar to those of Apollo 16. The total mission time of Apollo 17 was 304 hours. In both missions, the Biostack experiment was placed in the R-1 compartment of the Apollo Command Module. Its position relative to the wall of Command Module is shown in figure 2.

The approximate absorption in the four different layers of the wall of the Command Module was about 2.4 gm/cm^2. The bottom of the Biostack container was aluminum 3.00 mm thick with absorption 0.84 gm/cm^2. Since the software of the Biostack itself absorbed radiation, there was a decrease of radiation from outside to inside. Figure 3 shows some data of the physical evaluation of Biostack I as a function of absorption. From this it is evident that the flux of efficient HZE particles behind an absorption screen of 20 gm/cm^2 is still half of the total flux encountered in the mission of Apollo 16. This datum demonstrates the difficulty of shielding the crew of a space vehicle against the HZE particles encountered in deep space.

In each Biostack experiment, several thousand biological objects were hit by an HZE particle. Their response to an HZE particle stopping within the object (an ender) or passing through was studied in detail. The result was a broad spectrum of HZE-particle induced effects in biological matter. This spectrum of biological effects can be categorized as processes (1) insensitive to a hit; (2) moderately sensitive to a hit; and (3) highly sensitive to a hit by an HZE particle.

Insensitive Processes

In bacterial spores and plant seeds, germination was found to be highly resistant to an HZE particle hit. During germination, the bacterial spores, *Bacillus subtilis*, initially phase bright, became dark. This was probably the result of a change in the refractive index, caused by excretion of dry matter, slight swelling, and redistribution of water within the spore. This process has proved to be highly radiation resistant. Irradiation with X-rays of doses approximately 400 krad, which reduced the surviving fraction of colony formers to about 10^{-4}, did not influence the germination process. Much higher doses, approximately 2000 krad are necessary to induce "pseudogermination," which is correlated with an increased permeability of the cell wall. The germinating fraction of the spores hit was more than 90 percent in the Biostack I experiment and reached nearly 100 percent in the

Biostack II experiment. This fraction did not differ significantly from that of the controls, indicating a high resistance to HZE-particle bombardment. Pseudogermination was not observed on the spores hit. Likewise, the *Arabidopsis thaliana* seeds hit germinated with the same frequency and rate as the controls.

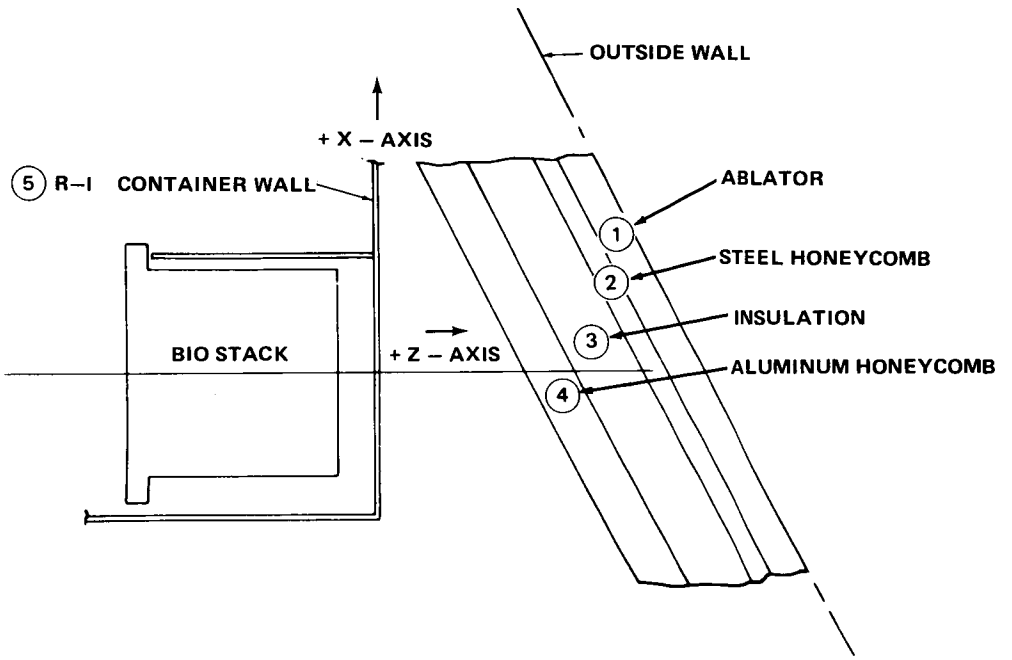

Figure 2. Schematic of stowage and effective shielding of the Biostack experiments in the Apollo Command Module.

① ablator, 1.78 cm thick		absorption 0.914 gm/cm^2
② steel honeycomb, 0.2 mm thick		absorption 0.319 gm/cm^2
③ insulation, 3.175 cm thick		absorption 0.305 gm/cm^2
④ aluminum honeycomb, 0.5 mm thick		absorption 0.139 gm/cm^2
⑤ wall of R1 container, assumed as aluminum, 2.5 mm thick		absorption 0.692 gm/cm^2
	Total	absorption 2.369 gm/cm^2

The growth of *Vicia faba radiculae* also did not differ significantly from that of the controls. It is likely that the surrounding intact cells replaced the destroyed cells, if any destruction occurred. Even cytological investigations dealing with achromasia of the nuclear material or repairability of damages in the nuclear DNA did not reveal any remarkable influence of HZE particles.

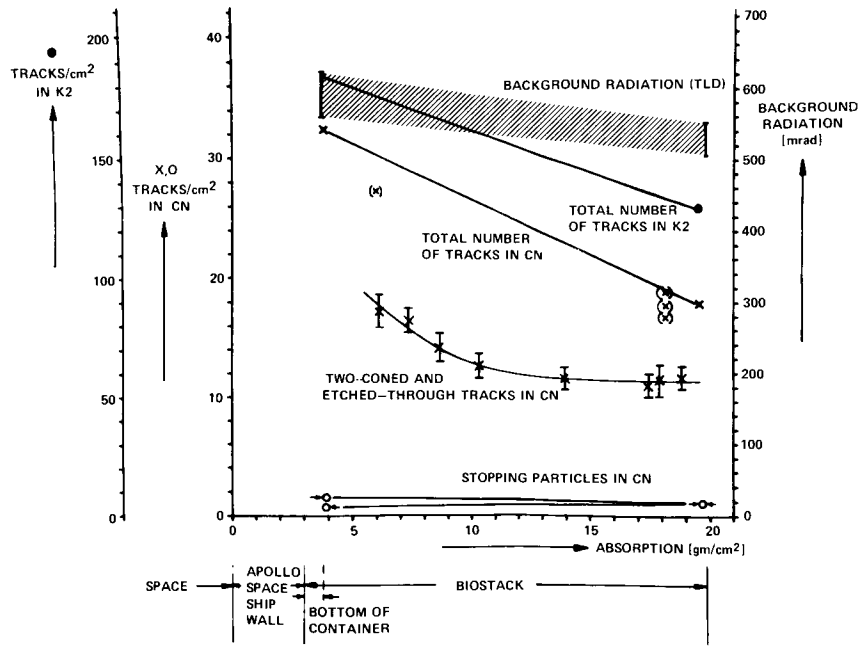

Figure 3. Data from physical detectors in Biostack I, Apollo 16, as a function of absorption.

Moderately Sensitive Processes

The developmental stages, following the germination process, proved to be more sensitive to a hit of an HZE particle. During spore outgrowth, the spore cases rupture and the embryo vegetative cell emerges, to develop into the fully grown vegetative cell. A reduction was noted in the outgrowth of the *Bacillus subtilis* spores hit in Biostack I. After radiation with X-rays, the outgrowing fraction decreased with irradiation dose. A dose of 350 krad produced a surviving fraction of 37 percent of outgrowing cells. Only 45 percent of the spores hit were able to grow out compared to a 72 percent outgrowth of the flight control. The spores that did not grow out simultaneously with the flight control never resumed their development during incubation.

The frequency of multicaulous *Arabidopsis thaliana* plants grown from hit seeds was remarkably increased. This anomaly was not observed in the ground controls and was very rarely observed in the flight controls. Thus it is assumed that the multicaulous forms were developed from seeds, in which cells of the vegetative cone had been destroyed by a penetrating HZE particle.

Only 55 percent of the *Artemia salina* eggs hit were able to pass the first developmental stage, the emergence. During this process, the egg shell cracks open and the nauplius larva emerges, still enclosed in the egg membrane. In most of the eggs hit, no development at all was detected. Clearing the egg shell with antiformin revealed an undeveloped gastrula.

Highly Sensitive Processes

The animal eggs were most sensitive to HZE-particle hits. Whereas irradiation of *Artemia salina* eggs with gamma-rays, neutrons, electrons, and even helium ions (Z=2) resulted in a sigmoidal dose effect curve regarding development to a swimming larva, irradiation with oxygen ions of 160 MeV resulted in a exponential curve. It is assumed that the passage of one single HZE particle may damage a cellular area large enough to disturb embryogenesis. Only ten percent of the *Artemia salina* eggs hit developed to a swimming larvae, compared to 90 percent of the ground controls and 45 percent of the non-hit flight controls. The larvae derived from hit eggs had a high mortality. Only a few reached maturity, and none was completely normal in further growth and behavior. They never reached the normal 12-mm length and pair mating was reached retardedly. Time until deposition of eggs took twice as long as in the case of ground controls. The number of broods varied from none to two and the number of descendants in the F1 generation was reduced. Malformations increased by a factor of ten. Shortened extremities or abnormal thorax or abdomen were most frequently noted.

These results show that HZE-particle induced damage in cells of the encysted blastula may be manifest in the gastrula stage, or even in later steps of development of the larva or the adult. This indicates an inability of intact cells to replace the function of destroyed cells.

Similar effects were found during development of hit *Tribolium castaneum* eggs. Hatching frequency was significantly lowered, and, during the first two days after hatching, a high mortality was observed. The frequency of abnormalities was increased from 2.5 percent in controls to 48 percent in the experimental organisms. The most frequent malformations were curved abdomina, fused segments of the abdomen or the antennae, and split or shortened elytra.

Likewise, the hatching of hit *Caurausius morosus* eggs was significantly reduced. Many of the larvae died during the first two weeks after hatching. Curved abdomina and fused segments or shortened legs were the main abnormalities observed in the descendants of the eggs hit. The frequency of malformations was increased from 1.5 to 23 percent.

Discussion

The physical characteristics of the HZE particles are important in regard to biological efficiency. The integral distribution function of the relative energy loss (REL) was obtained from analysis of the plastic detectors of Biostack I. The REL spectrum agrees satisfactorily with that obtained in the MEED experiment (Benton & Henke, 1973 and Section IV, Chapter 3 of this book), which was stowed within the Command Module for nearly the entire mission. However, the personal radiation detectors of the lunar surface crewmen recorded higher fluxes (Benton & Henke, 1972).

In the biological studies of Biostack, special attention was placed on the effects of HZE particles of very high energy. Therefore, it was agreed to restrict the studies of relative energy loss to hits above a threshold of 1.8 GeV/cm^{2}gm. For each particle that hit biological materials, the REL in the biological layer was determined. In cellulose

nitrate detectors, the length of a single etch cone gave the REL of the particle at that point in its path. In hit spores, REL values of 3.1 GeV/cm^{2}gm were determined.

The charge of the HZE particle is another physical characteristic of interest. The charge of each particle that hit was estimated from the relation of the cone length L and the residual range R (figure 4). In the *Bacillus subtilis* spore hit evaluation, charges ranged from $Z \geq 12$ to $Z \geq 24$.

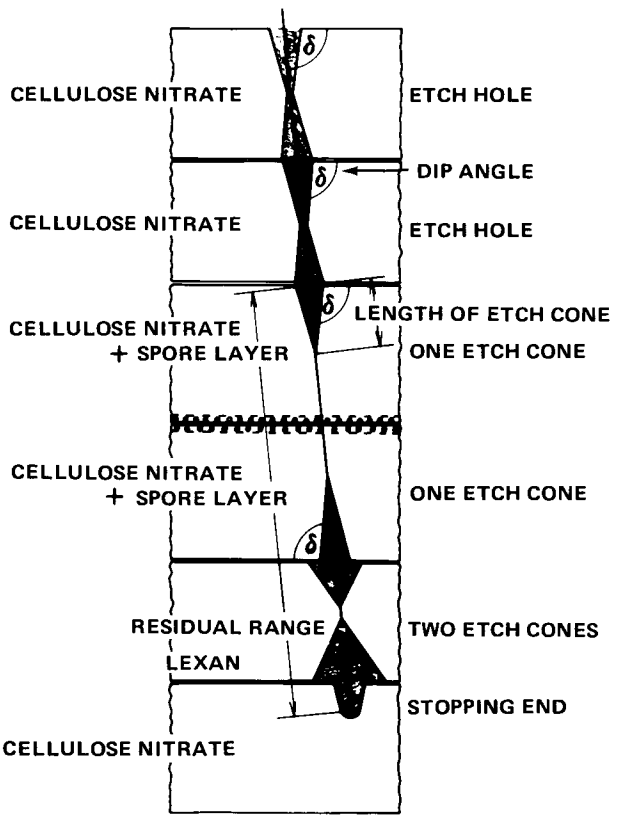

Figure 4. Schematic representation of track of a stopping HZE particle; from *Bacillus subtilis* unit.

All particles that reached the Biostack penetrated the 3 gm/cm^2 shielding of the spacecraft wall. A mean flux of approximately 0.1 particles/cm^2-hr of $Z \geq 4$ was found for Biostack I and II. The flux diminished remarkably from outside to the inside of the Biostack due to absorption of the Biostack material itself, approximately 16 gm/cm^2.

Space flight conditions complicated the radiobiological research of HZE-particle effects. Launch vibration, weightlessness, and ground exposure to cosmic background radiation were the principal factors acting on the biological material. Therefore, specimens flown but not hit were taken as flight controls, in addition to control groups.

Summary and Conclusions

The *Bacillus subtilis* spores were shown not to be influenced by the space flight environment. Germination and outgrowth of the flight controls agreed with that of the ground controls, also the rate of cellular elongation was not different. Likewise, there was no difference in the kinetics of germination of *Arabidopsis thaliana* flight control and ground control seeds. Slight damage, however, was observed in the *Artemia* flight control eggs. The percentage of emergence and hatching was reduced in comparison with the ground controls. Those flight control individuals, those able to hatch, afterwards developed completely in accordance with the ground controls. This slight damage of the flight controls has been assumed to be caused either by vibrational stress or by cosmic background radiation.

The concept of the Biostack experiment made it possible for the first time to examine the relationship between cosmic ray HZE particles and their biological effects. Emphasis should be placed on the fact that the dose causing the biological effects during the Apollo space flights was less than 35 millirem. This dose is much lower than the yearly permissible dose for man on Earth, according to the recommendations of the International Commission on Radiation Protection. At the present time, the question concerning the significance of human HZE-particle exposures in long-duration space flights cannot be answered satisfactorily. Further biophysical experiments will be necessary to establish the upper limit of HZE-particle fluence that can be tolerated inside spacecraft on long-duration missions.

The data of the Biostack I and II experiments confirm the assumption that HZE particle-induced damage might become manifest if a significant number of nonreplaceable cells are destroyed. In manned space flight, the prime candidate in this connection is the central nervous system, which consists of highly differentiated nonreplaceable cells. The question arises as to how many cells might be destroyed by each hit compared to the number of cells that form a functional unit. It is likely that a large number of HZE-particle hits to the same area of the brain would be required to destroy that particular function and that the HZE-particle radiation environment poses no major threat to manned space activities that may be undertaken in the foreseeable future.

References

Benton, E.V.; and Henke, R.P.: The High Energy Multicharged Particle Exposure of the Microbial Ecology Evaluation Device Onboard Apollo 16 Spacecraft. In, Proceedings of the Microbial Response to Space Environment Symposium, G.R. Taylor, ed. NASA TM X-58103, 1973, pp. 179-189.

Benton, E.V.; and Henke, R.P.: Heavy Cosmic Ray Exposure of Apollo 16 Astronauts. USF-TR-72-20, 1972.

Brustad, T.: Molecular and Cellular Effects of Fast Charge Particles. Radiation Research, vol. 15, 1961, p. 139.

Bücker, H.; et al.: Life Sciences and Space Research XI. In, Proceedings of the 16th Plenary Meeting of COSPAR (Konstanz, Germany), May 23 - June 6, 1973.

Chase, H.B.: Cutaneous Effects of Primary Cosmic Radiation. Journal of Aviation Medicine, vol. 25, 1954, p. 388.

Eugster, J.: Weltraumstrahlung. Bern, Switzerland: Medizinsuhen Verlag Hans Huber, 1955.

Haymaker, W.; Bailey, O.T.; Benton, E.V.; Vogel, F.S.; and Zeman, W., in collaboration with others: Brain Study in Balloon-Borne Monkeys Exposed to Cosmic Rays. Aerospace Medicine, vol. 41, 1970, p. 989.

Tobias, C.A.: Radiation Hazards in High Altitude Aviation. Journal of Aviation Medicine, vol. 23, 1952, p. 345.

Yagoda, H.; et al.: Brain Studies of Mice Exposed to Cosmic Rays in the Stratosphere and Report of Nuclear Emulsion Monitoring in Four Balloon Flights from Bemidji, Minnesota, July - August 1960. Military Medicine, vol. 28, 1963, p. 655.