# CHAPTER 4
# THE APOLLO 17 POCKET MOUSE EXPERIMENT (BIOCORE)*

*by*

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## Introduction

Travel outside the protective atmosphere of Earth can expose a spacecraft and its occupants to potentially dangerous regions of radiation. Missions conducted to date, including those of Apollo, have been fortunate since radiation doses received by astronauts have been low and of no clinical significance. However, as space missions increase in duration and move beyond the moon, the danger from radiation will become more serious.

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*A full report of this experiment (BIOCORE M 212: Biological Cosmic Ray Experiment) is given in the April 1975, Special Issue of Aerospace Medicine. The present paper represents an amplification of Paper I of the report. Permission for use of that paper was granted by Aerospace Medicine.

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In order to gain a better understanding of radiation hazards, the Biocore Experiment was flown on Apollo 17. This experiment attempted to assess the degree to which exposure to cosmic ray particle radiation might present a risk to astronauts. In this study, five pocket mice, with plastic dosimeters implanted beneath the scalp, were flown in a sealed canister. The objective was to determine whether microscopically visible lesions, attributable to particle radiation, could be found in brain, eye, and other tissues in these animals.

Particular interest in the effects of particle radiation on tissue arises from the markedly different character of high energy (HZE) particle radiation as compared with that of electromagnetic (E-M) radiation (X-rays, $\gamma$–rays). The energy deposition (dosage) in E–M irradiation *decreases* exponentially with penetration depth into the target. In contrast, the energy deposition by a particle can *increase* as the particle penetrates the target and decelerates, the maximum energy loss per unit path length (LET: linear energy transfer) occurring near the stopping point (Bragg peak) (figure 1). Most of the energy deposition from particle radiation occurs in a very narrow cylinder around the trajectory, within which there is intense ionization of the target's atoms. While the concept of dosage is not strictly meaningful in assessing the radiobiological effects of HZE particle radiation, perspective on the potential destructive character is obtained by noting that the "dosage" (energy deposition per gram) in the immediate vicinity of the particle trajectory can be on the order of megarads or higher.

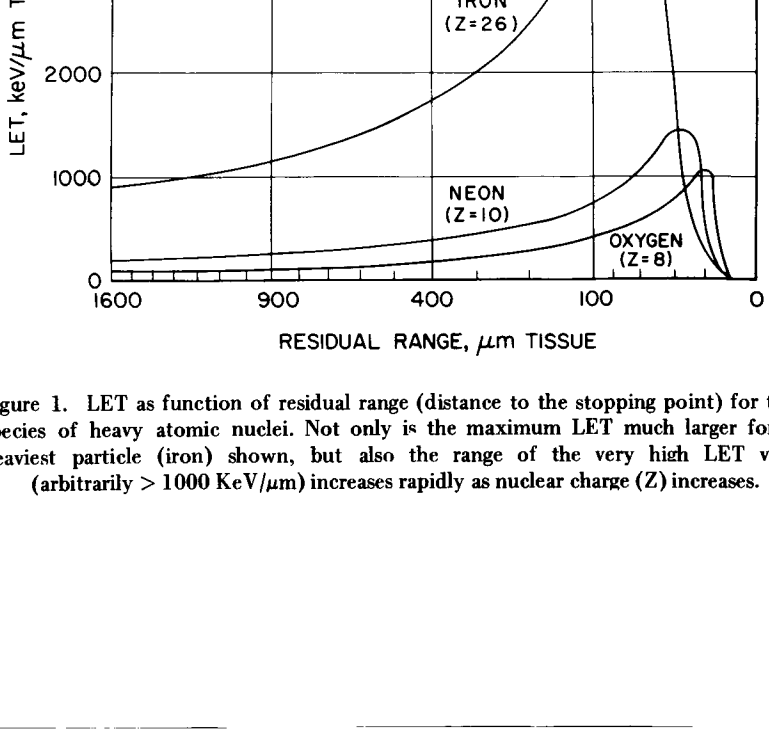


Figure 1. LET as function of residual range (distance to the stopping point) for three species of heavy atomic nuclei. Not only is the maximum LET much larger for the heaviest particle (iron) shown, but also the range of the very high LET values (arbitrarily > 1000 KeV/$\mu$m) increases rapidly as nuclear charge (Z) increases.

For a given incident energy, a charged particle will penetrate a target to a relatively well-defined depth that is a function of the particle's charge. Collaterally, the LET of a particle at any point along its trajectory is a function of the particle's charge and distance from the stopping point. In the present experiment, use was made of this last property, that is, measurement of the LET, where the LET of each HZE particle was determined from measurements on the particle's track in the subscalp detector. Charge and distance to the particle's stopping point were calculated from the detector data.

## Plan of the Experiment

The primary objective of this experiment was to determine whether a specific portion of the high Z – high energy (HZE) galactic cosmic ray particle spectrum, especially particles with $Z \geqslant 6$, can produce microscopically visible injury of the brain and eye. Pocket mice *(Perognathus longimembris)* obtained from the California desert were selected as the biological target (figure 2). Five of these mice were flown on Apollo 17. Not only the brain and eyes, but also many other tissues of these animals were studied for evidence of cosmic ray particle damage.

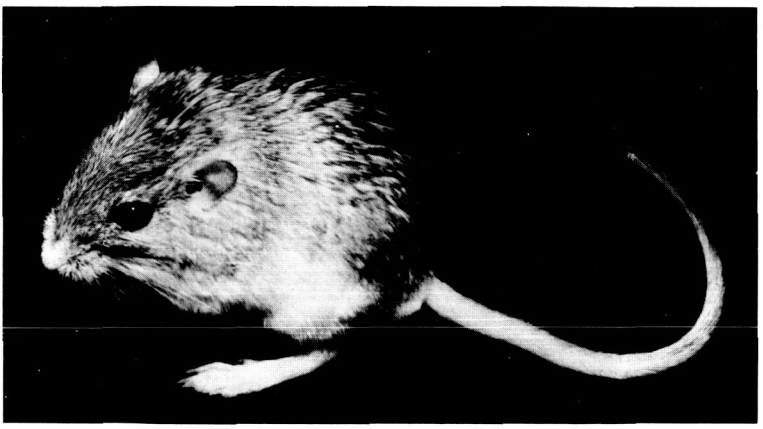

Figure 2. The Little Pocket Mouse, *Perognathus Longimembris.*

In order to correlate any observed tissue damage in the heads of the flight mice with the passage of HZE cosmic ray particles, it was necessary to record the trajectories of as many of the particles passing through the heads during the flight as possible. To monitor the primary targets – the brain and, to some extent, the eyes – a particle detector composed of four layers of plastic (two of Lexan polycarbonate and two of cellulose nitrate), sealed into a unit and coated with Paralene C for protection against tissue fixatives, was developed. The dosimeter, designed to cover the entire brain from the olfactory bulbs anteriorly to the cerebellum posteriorly, was mounted on a Silastic elastomer platform, the underside of which was contoured to the skull (figure 3). The assembly was implanted beneath the mouse scalp, where scalp tension fixed its position

with respect to the skull. No deleterious effects in the mice due to the presence of the subscalp assembly were observed, even several months after implantation.

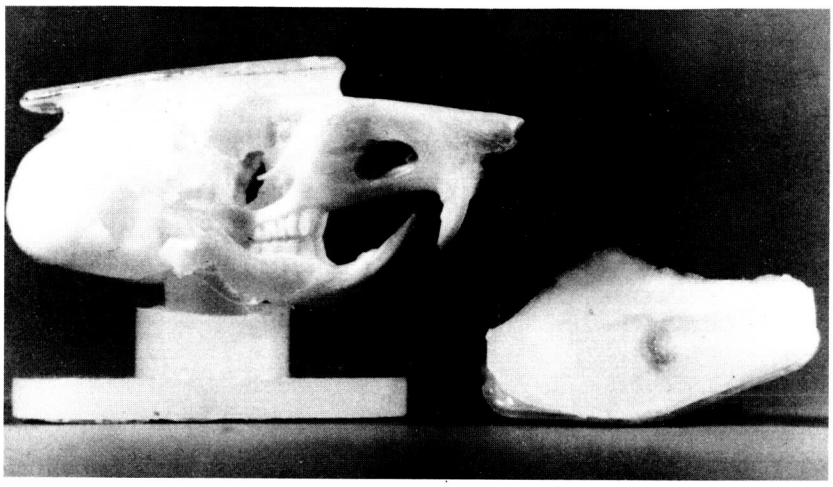

Figure 3. Monitor assembly on skull of pocket mouse. At right is an assembly viewed from below; the Silastic elastomer is molded to fit the skull.

## Flight Experiment Preparation

To house the five mice during the Apollo mission, a closed, self-sustaining system was developed in which potassium superoxide ($KO_2$) served as the oxygen source and as the carbon dioxide absorber. The system was perfected to the point that the well-being of the mice over their projected 13-day flight would be reasonably assured. The major problem was to house the mice and the $KO_2$ in a canister 35.6 cm long and 17.8 cm in diameter (14 in. and 7 in.), in such a manner that the mice could feed and move about despite the tendency to free float. A water supply system was unnecessary since the mice produce water metabolically from their food. Each mouse was housed in a metal tube having a diameter [2.54 cm (1 in.)] slightly larger than the mouse that would allow it to turn about. Each tube ran the full length of the canister. The $KO_2$ tube of a larger diameter, centrally located, ran the full length of the canister (figure 4).

There was concern whether the mice would experience excess fatigue from negotiating in the weightless state and lose their appetite. To explore this possibility, 14-day clinostat tests were carried out. The mice together with their seeds, housed in plastic boxes, were rotated at 1/4 RPM. During the revolutions of the boxes, cascading seeds inundated the mice, and when they could no longer stay on top of the seeds, the mice would become torpid and roll with the seeds. At intervals, the mice became active again and ate. It was expected that the cylindrical shape and small bore of the tubes in the flight canister would minimize tumbling in the zero-g environment, that the mice

would not have difficulty in moving about within their tubes, and that they would be able to consume an adequate number of seeds to survive.

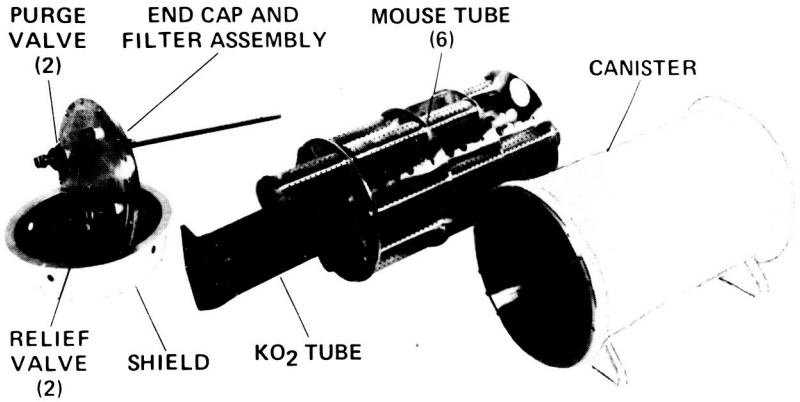


Figure 4. Components of flight package, partially assembled. The $KO_2$ tube and the mouse tubes can be removed from the supporting spool for cleaning and for reloading the $KO_2$. The purge tube attached to the end cap carries the oxygen to the closed end of the canister to assure ample purging of the air in the canister during experiment startup.

Four aspects of the environment within the canister needed to be investigated in order to determine whether the mice would be taking the trip under survivable conditions: the oxygen partial pressure, the carbon dioxide partial pressure, the temperature, and the relative humidity.

During the many tests (about 60) that were performed under ambient temperature conditions approximating those of the Apollo flight, the oxygen partial pressure within the canister frequently rose to as high as $83 \times 10^3$ N/m$^2$ (12 psi), and occasionally higher. Consequently, a separate study was conducted in which 28 mice were individually exposed in an environmental chamber to oxygen at a partial pressure of $83 \times 10^3$ N/m$^2$, at 297°K (24°C), and to a relative humidity of 20 percent over a period of seven days. All survived the test.

The KOH generated by the interaction of $KO_2$ and respiratory $H_2O$ appeared to be an ample absorbent, but nonetheless the tolerance of the mice to carbon dioxide buildup needed to be determined. To this end, six mice were sealed in a chamber in which the initial oxygen partial pressure was $33 \times 10^3$ N/m$^2$ (4.8 psi). The mice withstood an atmosphere in which the partial pressure of carbon dioxide rose to $19 \times 10^3$ N/m$^2$ (2.8 psi), while the oxygen partial pressure fell to $13 \times 10^3$N/m$^2$ (1.87 psi) in a four-hour test.

The other aspects of the canister environment requiring investigation were (1) temperature, and (2) relative humidity (R.H.). Too high a temperature would be prejudicial to the animals' well-being and potentiate the toxic effects of oxygen. Too low a relative humidity would dehydrate both the animals and the seeds.

Prior studies determined that pocket mice in a sea-level atmosphere can easily tolerate an ambient temperature of 308°K (35°C) for one month. A calculated temperature profile anticipated a temperature of 300°K (27°C) during part of the flight (figure 5), and was the cause of some concern, since free convection does not occur in zero g, and the heat generated by the $KO_2$ and by the mice in the canister would have to be dissipated by conduction and radiation. Accordingly, the heat dissipated from the canister, including heat loss at the canister–Command Module interfaces, was investigated through studies conducted on the canister in a vacuum environment. It was established that heat dissipation at the interfaces would probably maintain the temperature in the mouse tubes at no higher than 301°K (28°C).

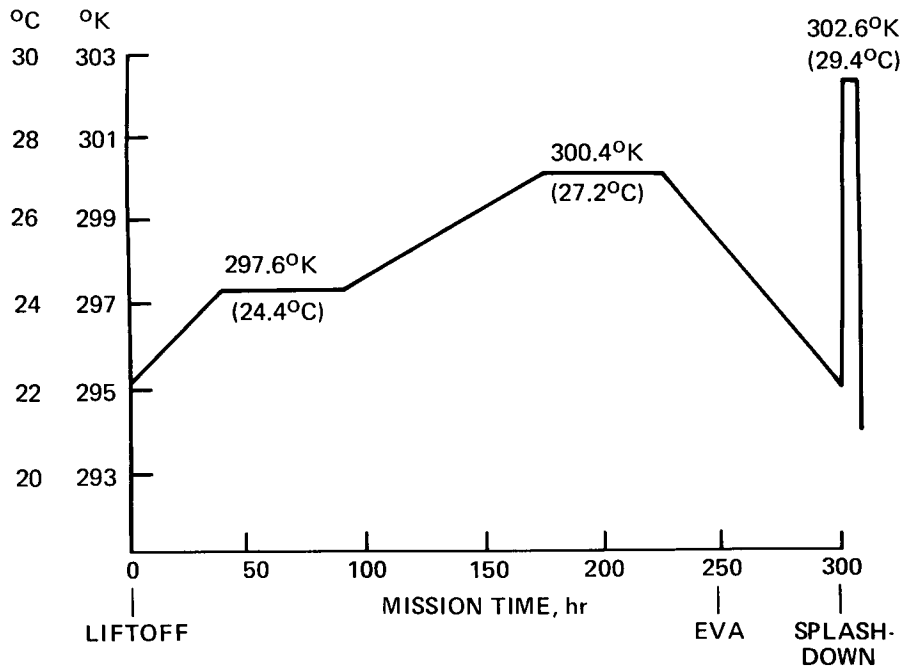


Figure 5. Approximate temperature profile in the Command Module in the region where the canister was to be located, as calculated for the Apollo 17 flight.

The effects of the combined temperature-oxygen pressure stress were investigated next. Eighteen mice (while in canisters) were exposed to an oxygen partial pressure of $83 \times 10^3$ N/m$^2$ (12 psi) in a room with a temperature of 305°K (32°C). The relative humidity was maintained at 22 percent during the test. Six additional mice were exposed to the same temperature, but in a sea-level atmosphere, to serve as test controls. All of these mice had undergone earlier oxygen tolerance testing. Most of the heavier mice survived the test in satisfactory condition, while four of the lighter mice and one heavy mouse (weighing 10 gm) died; all control mice in heat alone survived, indicating that relatively heavy mice (mice weighing 9.5 gm or more) were the animals of choice.

The problem of relative humidity (R.H.) as it affected the pocket mice was considered. In an open-system, oxygen flow-through experiment, with an oxygen partial pressure of 28 x $10^3$ to 34 x $10^3$ N/$m^2$ (4 to 5 psi) and ambient temperature of 302°K (29°C), it was shown that the mice could withstand a relative humidity of 90 to 100 percent over a period of five days. Furthermore, in test runs in which the R.H. was rather low – 23.4 percent R.H. or lower – the animals survived in apparent good condition despite a loss in weight.

From the results of these and other studies it was evident that the pocket mouse is exceptionally hardy and can survive wide variations in its environment. Moreover, histological studies performed on many mice subjected to testing in canister oxygen environments revealed no change in the brains or eyes of the animals, and relatively little change in the lungs.

The primary criteria in the selection of the mice to be carried on Apollo 17 were weight (9.5 gm or more), the general state and behavior, condition of the scalp over the dosimeter, the presence or absence of nasal discharge, the appearance of the pelage, and the activity of the animal and its housekeeping habits.

## Test Procedures

Of the animals used as the major controls for the flight animals, some were non-experimental controls, while others had been subjected to $KO_2$ oxygen tests as controls against the oxygen partial pressures anticipated in the canister during flight. But the most appropriate controls for the flight animals were the five mice taken to NASA Kennedy Space Center (KSC) a few days prior to launch. Two canisters were loaded with five mice each at KSC; one was chosen to fly, and the other to serve as flight backup. The flight backup canister was flown back to NASA Ames Research Center (ARC), where the mice were subjected to all stresses anticipated for the flight mice that could be carried out on the ground. They were perfused with fixing fluid on the same day (December 19) as the flight animals. Four of these mice were used as flight controls.

A week or two prior to the time of anticipated spacecraft splashdown, 12 control animals were perfused at the University of Hawaii in Honolulu (during the time the engineers and pathologists were stationed there to process the flight animals in the event of a mission abort), and an additional 17 animals were perfused at Pago Pago. Four of the latter served as flight controls. The others were used as controls for subsequent histological studies.

Two flight acceptance tests were run to qualify the hardware for flight. The two tests were run concurrently (November 5 through 22, 1972). In these tests as well as in preparation for flight, the initial step after the animals had been sealed in the canisters was to flush the canisters with 100 percent oxygen for 15 or 25 minutes, a procedure that left little residual nitrogen in the canisters. In the acceptance tests, the oxygen partial pressure fell to a minimum of 17 x $10^3$ N/$m^2$ (2.4 psi) and rose steadily thereafter. On day 15, the pressure reached peaks of 81 x $10^3$ and 84 x $10^3$ N/$m^2$ (11.7 and 12.2 psi), but fell to about 34 x $10^3$ N/$m^2$ (5 psi) at the start of the simulated EVA maneuver. Figure 6 shows the test profile.

| MOUSE NO. | MOUSE WEIGHT (gm) | | FOOD LEFT (gm)[a] |
|---|---|---|---|
| | START | END | |
| A-3372[b] | 11.7 | 10.06 | 10.60 |
| A-3377[b] | 10.6 | 9.15 | 10.21 |
| A-3475[b] | 9.9 | 9.78 | 11.50 |
| A-3488 | 10.7 | 10.18 | 8.77 |
| A-3557 | 10.1 | 9.84 | 3.90 |

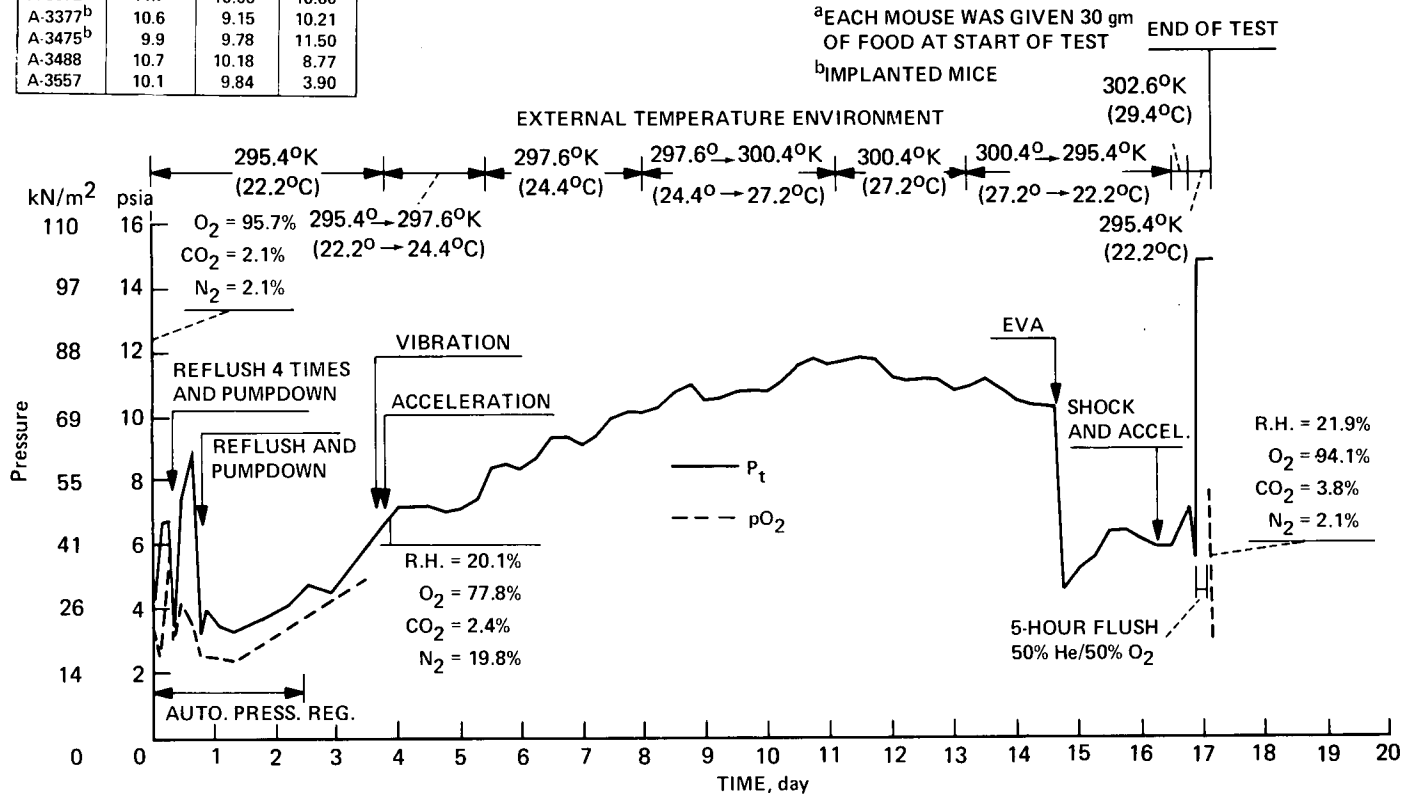


Figure 6. $KO_2$ Expt. No. 64, an acceptance test of the S/N 05 hardware. The tube containing the $KO_2$ was of stainless steel. A total of 520 gm of 40% catalyzed/60% noncatalyzed $KO_2$ mixture was used.

**Flight Backup Test Carried Out Concurrently With the Apollo Flight**

The initial pumpdown period of this test lasted 37 minutes. The minimum oxygen partial pressure reached during autoregulation was $17 \times 10^3$ N/m$^2$ (2.5 psi). About 12 hours after the launch of Apollo 17, the package was flown from the Kennedy Space Center to the Ames Research Center, causing a gap of 20 hours in the pressure data for the time period starting from preparation for transport of the animal package at the Kennedy Space Center until its installation in a test chamber at the Ames Research Center. During those 20 hours the total pressure rose from $37 \times 10^3$ to $64 \times 10^3$ N/m$^2$ (5.4 to 9.3 psia). All five animals survived the test in excellent condition.

The flight backup canister experienced the same ambient temperature except during the flight from the Kennedy Space Center to the Ames Research Center. The flight backup and two other control canisters were flushed with a mixture of 50 percent helium/50 percent oxygen toward the end of the test period, a procedure to be carried out on the flight canister following splashdown. Moreover, the mice in all three canisters were subjected to certain other stressful situations that were expected to be imposed on them aboard Apollo 17: vibration following Apollo lift-off, launch acceleration with a peak of 5 G soon after lift-off and a second peak of 2.5 G at second stage burnout, peaks of 6.8 G and 4 G during reentry of the spacecraft into the atmosphere, and 37 G on splashdown. These were test levels; the values were in excess of those anticipated on the flight of Apollo 17. The mice tolerated the vibration and the G stresses without apparent ill effects.

The data on the experiment package flown on Apollo 17 are given in figure 7. The animals were placed in the canister on December 2, 1972. The initial pumpdown was performed in 36 minutes. The minimum oxygen partial pressure reached during autoregulation was $19 \times 10^3$ N/m$^2$ (2.8 psi). The Apollo was launched on December 7. In the extravehicular activity (EVA) preparation during the flight, the Command Module (CM) was emptied of its atmosphere and exposed to the vacuum of space in about eight minutes, and the EVA was accomplished in about one hour. Hence, the rapidity of the decompression of the mice in the CM (to $34 \times 10^3$ N/m$^2$, 5 psia) can be assumed to have been approximately the same for the mice in the two flight acceptance canisters on the ground and for the mice in the flight backup canisters as well. It can also be assumed that the pressure in the flight canister rose slowly after the EVA maneuver. The rate of recompression of the CM had no effect on the pressure in the flight. Splashdown in the Pacific occurred on December 19, with the package received on the recovery ship on day 13 of the flight (day 17 from the time the animals had been placed in the canister), where it was flushed with $He/O_2$ gas mixture. The flushing was continued during transport by plane to Pago Pago.

Upon arrival at Pago Pago the flight package was taken to a laboratory at the Lyndon B. Johnson Tropical Medical Center. On opening the canister about seven hours after splashdown, four of the five mice were found alive, while the fifth (A-3352) was dead. Two of the surviving mice (A-3305 and A-3356) were active and in excellent condition when released from their tubes into a container for observation. The other two surviving mice (A-3326 and A-3400), when first examined, were docile and hunched up, as though exhausted or arousing from torpor. They moved forward only a few steps when prodded.

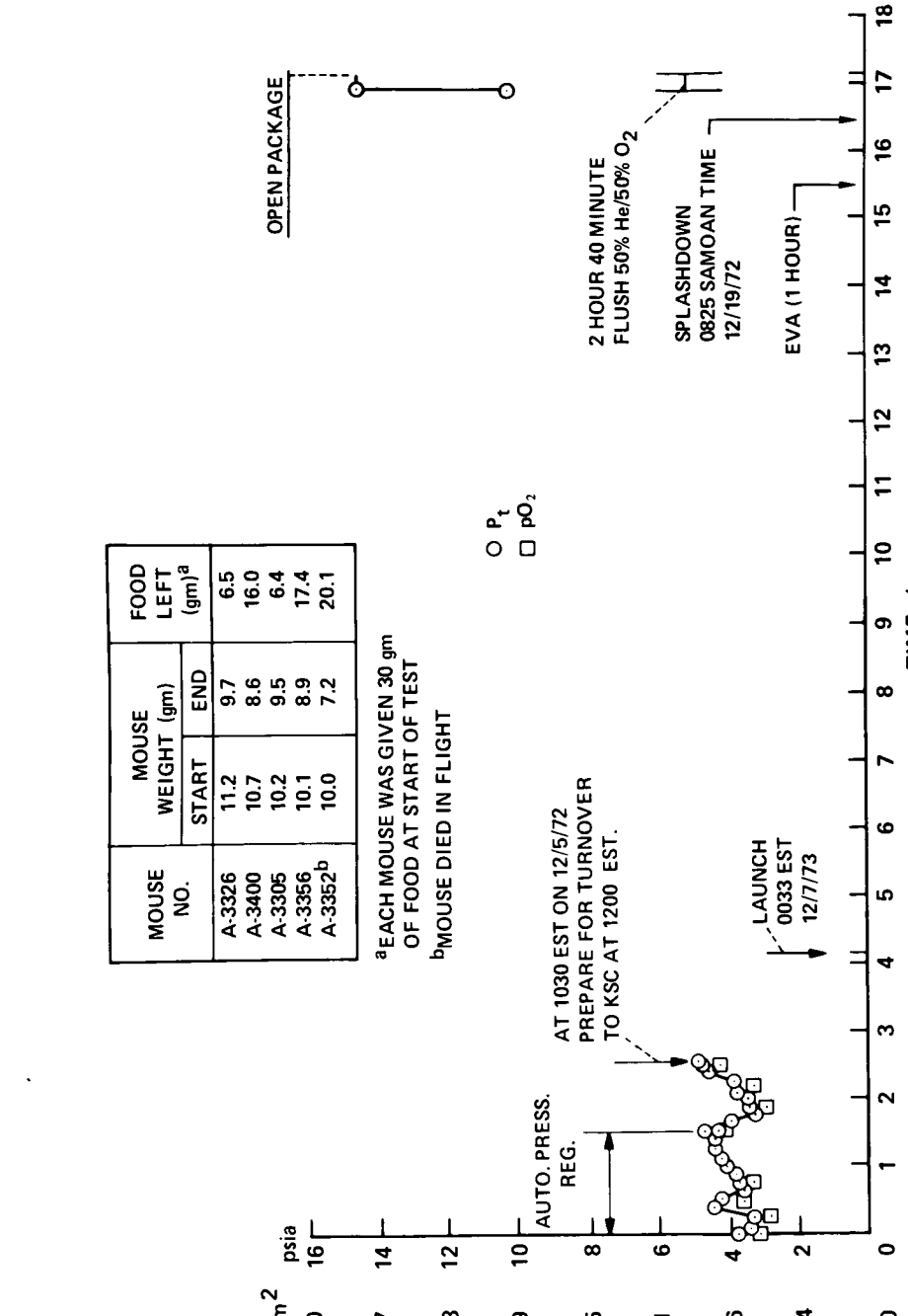


Figure 7. Data on the Apollo 17 flight package (S/N 03). The tube containing $KO_2$ was of stainless steel. A mixture of 40% catalyzed/60% noncatalyzed $KO_2$ was used.

A-3326, the female of the group and the most subdued, was uncoordinated on walking and would fall to one side or the other when it attempted to sit up on its hind quarters. Later, on histological examination, it was found that severe hemorrhage had occurred into its middle ear cavities during the flight. This could easily have accounted for the incoordination.

After all the animals had been examined and their weights recorded, the four live animals were anesthetized with Metofane and perfused with a fixing fluid, FAM (FAM: formaldehyde, 1 part; acetic acid, 1 part; methyl alcohol, 8 parts). The perfusion was carried out via the heart by means of a Harvard apparatus. The brain of the mouse (A-3352) that did not survive the flight was fixed by introducing FAM into the subarachnoid space via the orbits.

Upon completion of the perfusion procedures, the heads of all the animals were immersed in FAM. The next morning (after about 12 hours' fixation) the heads were transferred to 70 percent methyl alcohol.

**Processing of Tissues for Histological Study, Establishment of Cosmic Ray Particle Trajectories**

Back at NASA Ames Research Center three days after autopsy of the animals at Pago Pago, the first step was to place the head of the animal that died during the flight (A-3352) in a standardized aluminum box, and to secure the head by means of ear bars, a jaw bar, and a nose clamp. The box was then secured on a rotatable stage attached to the platform of a stereotaxic apparatus. Then the scalp was turned back and the position of the dosimeter (with the head still in the box) established by photographs, and the degree of tilt with respect to the stereotaxic apparatus platform established by means of a laser beam. The dosimeter was then removed for analysis at the University of San Francisco. About three weeks later, the same protocol was followed for the other four mice and, in addition, X-rays of the heads in various planes were taken to establish more clearly the position and degree of tilt of the dosimeters.

The five heads, still in the aluminum boxes and immersed in 70 percent methyl alcohol, were transported to Duke University for further processing. The heads were removed from the boxes and each was decalcified. The heads were then returned to their boxes, and alined in exactly the same position as before. The next step was to dehydrate the heads by passing them through alcohols and xylol according to standard methods. Then one end of each box – that near the occiput – was removed and replaced by a microtome chuck, whereupon the boxes were filled with low melting point paraffin. The heads were serially sectioned in the coronal plane, from anterior to posterior, at ten millimicrons. The total number of sections per head came to approximately 1600, of which about 1200 included the brain. All the sections were stained by the PAS-hematoxylin method.

In order to locate the paths of cosmic ray particles through the heads of the flight mice, it was necessary that a procedure be devised whereby the trajectory of each cosmic ray particle registered in the subscalp dosimeters would be extrapolated into and be identifiable in the heads of control mice. Before this procedure could be initiated the dosimeters needed to be analyzed to determine cosmic ray particle trajectories. A total of 80 heavy particle tracks were found in the five dosimeters. The head of each mouse to be used as a

control for a flight mouse was placed in a fixed position in a standardized aluminum box, in the manner just described for the flight mice. The box was then secured on a rotatable stage situated on the platform of a sterotaxic apparatus. Through a painstaking procedure, a manila paper "dosimeter," identical in size and shape to the flight dosimeters, was placed on the head of each mouse in precisely the same position and at the same degree of tilt as had been recorded for each of the flight mice. Fine drills were then directed through the control head by means of the arm of the stereotaxic apparatus, the drills being introduced along the trajectory (within the limits of experimental accuracy) of each of the cosmic ray particles that had penetrated the dosimeter of a flight mouse. Where numerous tracks (up to 20) were found in the subscalp dosimeter of a single flight mouse, the heads of as many as four mice were "tracked," with four to five tracks per head to serve as controls for that flight mouse (figure 8); the number of "tracked" control heads totaled 17. The heads of these animals were carried to Duke University, where they were processed and serially sectioned in the same manner as for the flight mice.

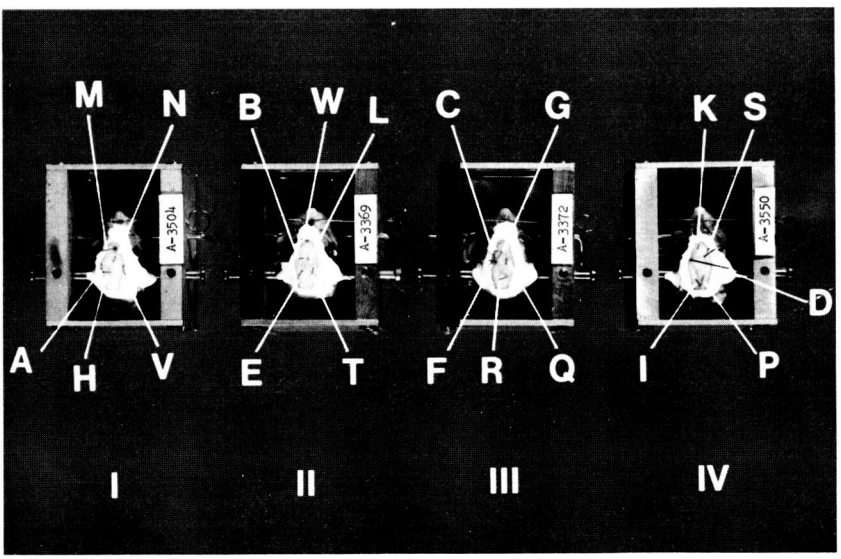


Figure 8. "Tracked" control heads (A-3504, 3369, 3372, and 3550) for flight mouse A-3400, showing drill in place for each trajectory.

The significance of this tracking procedure was that the pathologists could check any lesion found in the brain of a flight animal against the location of the drill tracks in the control brains. If congruity was found between a lesion in a histological section of a flight mouse and a drill core in the corresponding control histological section, and if the lesion was consistent with current concepts of what a cosmic ray-induced lesion should look like – that is linear, or columnar or even spherical – there would be a high probability that the lesion was produced by the cosmic ray particle.

In the meanwhile, the body tissues of the flight mice and many control mice were being processed for study at numerous institutions in the United States as well as at NASA Ames Research Center.

**Results of Analysis of the Subscalp Dosimeters**

For the 80 cosmic ray particles recorded in the five subscalp dosimeters (table 1), the energy loss by the particles per millimicron of dosimeter traversed (LET) ranged from 0.16 to 0.5 MeV/$\mu$m, with only a few of the particles in the very heavy charge group ($Z > 20$). The relatively narrow charge and LET spectra of the registered particles are attributable (1) to attenuation of the frequency of the very high Z component of the free space cosmic ray flux by the shielding of the Apollo spacecraft and of the flight package itself, and (2) to the fact that any high LET particle detector is more likely to register a cosmic ray particle in the high LET ($\leq$ 0.1 MeV/ $\mu$m) range than in the very high LET range ($\geq$ 0.1 MeV/ $\mu$m), since a much smaller portion of the trajectory lies in the very high LET range.

Another set of data listed in table 1 relates to particle thindown direction. Only five of eighty particles were determined with high certainty to have penetrated the dosimeter prior to entering the head, while 41 particles may have passed through the head prior to reaching the dosimeter. The thindown direction of the remaining 32 particles* was not determinable, although statistically, approximately one-half should have thinned down in the direction of the head after traversing the dosimeter. Obviously, particles would have a lower LET in tissue than recorded in the dosimeter if they penetrated the head before reaching the dosimeter. The reverse would be true for the five particles coursing downward into the head after having penetrated the dosimeter; table 2 gives the characteristics of these particles. Two of these particles were classed as in the medium charge group ($Z = 6$ to 9), and three as in the heavy ($Z \geq 10$) category. LETs in the dosimeter for these five particles ranged from 0.24 to 0.32 MeV/$\mu$m. The residual range (distance to the stopping point from the dosimeter) computed for each of the particles is cited in the table. Of the 80 particles recorded in the subscalp dosimeters, these five particles were of paramount interest to the pathologists because their stopping points were calculated to be within or near the brain.

Among the cosmic ray particles whose thindown direction was not determinable were the ten particles of highest charge. These were grouped together as heavy ($Z \geq 10$) and very heavy ($Z > 20$) (H-VH) because of uncertainty as to which of the two charge groups they belonged. All had an LET equal to or greater than 0.5 MeV/$\mu$m at the level of the dosimeter, and their stopping points were more than 1.2 mm beyond the level of the dosimeters. Brain, eyes and other head tissues in areas traversed by these ten particles were given particular attention in the search for lesions because of their heaviness and LET.

A further point to be made with reference to the monitoring system was that the dosimeters could be expected to record on the average about 50 percent of the cosmic

*This adds up to 78 particles. Two of the particles thought to have traversed the head were found on microscope examination of serial head sections not to have done so.

Table 1

Characteristics of 80 Cosmic Ray Particles
Recorded in the Subscalp Dosimeters of the Five Flight Mice

| Mouse No. | No. of Tracks | LET (MeV/μm) | Charge Group * | | | | | Residual Range (mm) | Thindown Direction † | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | L | M | H | VH | H-VH | | Down § | Up | Not Determinable |
| A-3326 | 13 | 0.16 – 0.30 | 0 | 11 | 2 | 0 | – | > 0.6 – 2.6 | 1 | 9 | 3 |
| A-3400 | 17 | 0.16 – 0.24 | 0 | 15 | 2 | 0 | – | ⩾ 0.6 – 3.6 | 2 | 11 | 4 |
| A-3305 | 18 | 0.16 – 0.27 | 1 | 9 | 8 | 0 | – | > 0.6 – 24 | 0 | 10 | 8 |
| A-3356 | 17 | 0.17 – 0.37 | 0 | 9 | 7 | 1 | – | > 0.5 – 26.5 | 3 | 9 | 5 |
| A-3352 | 5 | 0.20 – 0.27 | 0 | 1 | 4 | 0 | – | 1.1 – 16 | 1 | 2 | 2 |
| H-VH particles for all 5 mice | 10 | ⩾0.5 | – | – | – | – | 10 | > 1.2 | – | – | 10 |

* Light particle, Z 3-5; medium, Z 6-9; heavy Z ⩾ 10; very heavy ⩾ 20.

† Direction: down, through dosimeter into the head; up, through the head before reaching the dosimeter.

§On microscopic examination, the number of particles was corrected from 7 to 5 particles.

Table 2

Data on the Characteristics of Five Cosmic Ray Particles
Which Penetrated the Dosimeter Prior to Reaching the Head

| Flight Animal | Control Head "Tracked" | Track Designation | Charge Group * | LET (MeV/μm) | Residual Range (mm) | Head Structures Penetrated |
|---|---|---|---|---|---|---|
| A-3400 | A-3372 | C | M | 0.24 | ⩾ 0.6 | Left cerebral cortex, hippocampal formation, cerebellum, middle ear cavity |
| A-3400 | A-3369 | E | M | 0.24 | ⩾ 0.6 | Left eye, including retina |
| A-3356 | A-3571 | G | H | 0.32 | 2.2 | Right olfactory bulb, frontal lobe, caudate, putamen, pallidum, int. capsule, optic tract, hippocampal formation, cerebral cortex, bony labyrinth, middle ear cavity |
| A-3356 | A-3554 | T | M | 0.26 | ⩾ 0.6 | Left olfactory bulb, nasal cavity |
| A-3352 | A-3359 | B | H | 0.27 | 3.4 | Left frontal lobe, optic nerve, nasopharynx, roof of mouth |

* M, medium charge group (Z 6-9); H, high charge group (Z ⩾ 10).

ray particle flux through the mouse brain, since particles incident on the mouse came from all directions and the mice were not restrained. As a consequence, some particles could have passed through or terminated in or near the brain without having been registered in the dosimeters. Thus the pathologists were faced with the possibility of observing cosmic ray particle-induced lesions in the brain and other target tissues without the presence of corresponding tracks in the dosimeters.

The pocket mouse heads were exposed to far broader Z and LET spectra of particles than the 80 HZE particles indicated in table 1. However, only the HZE particles, which were registered, were the particles of interest in the present experiment.

The tissues traversed by some of the cosmic ray particles are indicated in table 2. Analysis revealed that one or more head structures of the five flight mice were traversed by particles; the scalp by 76 particles; the eye by 5; the nasal cavity by 15; the middle ear cavity by 23; and the brain by 59 particles (olfactory bulb, 14; cerebellum, 12; hippocampal formation, 11; and hypothalamus, 3).

**Body Tissues**

Study of the body tissues of the four flight animals that survived the flight revealed no changes that could be regarded as due to cosmic particle radiation. Some pertinent observations, however, emerged from the studies. The increased oxygen partial pressure to which the flight animals and control test animals had been exposed depressed erythropoiesis in the bone marrow. The increased oxygen partial pressure did not induce changes in periodontal or other oral tissues. The lungs appeared relatively resistant to oxygen intoxication, attributable in part to the inclusion of nitrogen with the oxygen. Mild pneumonitis was observed in all four flight backup mice, but not in the flight mice. The liver in one flight mouse (A-3305) contained large focal areas of hepatocellular necrosis of undertermined etiology, while those of the other flight mice and the four flight backup mice were normal or virtually normal.

The kidneys of the flight mice were unremarkable. The juxtaglomerular apparatus could not be evaluated because the fixing fluid (FAM) had dissolved the granules from its cells. Assessment of the adrenal cortex according to the method used revealed no significant alterations. A study of certain nuclei of the hypothalamus and of the cell population of the pituitary gland and, to some extent, the adrenal cortex revealed minor enlargement of neuronal nuclei in the supraoptic nucleus as the sole positive finding. This suggested an antidiuretic hormone response.

The thyroid appeared normal in all mice in which it was examined, including the thyroid of three of the flight animals. The same was true for the parathyroids. Soft-tissue calcifications were found in a number of the mice – flight mice and controls alike – and thus the possibility exists that this might be attributable to parathyroid hyperactivity.

Heart muscle showed no ostensible change in any of the animals. Histological changes in skeletal muscle of the flight animals were minimal and were found to occur in the control animals with comparable frequency. This was with the exception of *Sarcocystis* infestation. *Sarcocystis* were not found in any of the flight mice, but they were present in three of the five flight backup mice.

Tissues with continuously replicating cells were given special attention. The lack of abnormalities in bone marrow in the flight mice except for reduced erythropoiesis has

already been mentioned. In the upper small intestine of the flight mice the mitoses in the crypts of Lieberkühn were normal in appearance. The gonads also showed no differences ascribable to the Apollo 17 mission. In two of the three surviving male flight mice (but not in the third) spermatogenesis was advanced to the same degree as in ground control mice at the same season.

**Olfactory Mucosa**

There was another tissue composed of continuously replicating cells, the olfactory epithelium, which was severely damaged in the four surviving flight animals and to a lesser degree in the animal that died. The respiratory epithelium in all these animals was, by contrast, unaltered. The need to assess the changes in the olfactory epithelium in some detail became evident when it was found that the nasal epithelium of the 17 major control animals, of which four were flight backup animals, was entirely free from change.

There were two kinds of pathological change in the olfactory epithelium. One was characterized by disorganization of much of the epithelium, in the sense that the thickness of the layer and the number of its constituent cells varied from area to area in a given strip of olfactory epithelium. The other consisted of multifocal severe lesions originating either in the disarrayed epithelium just mentioned or in intact epithelium. Intermediate stages between these two types of change were sometimes encountered.

The lesions were in various stages of evolution ranging from acute, in which a few cells or masses of cells were being sloughed from the epithelium, to "old," in which newly proliferated cells had replaced the sloughed cells. The acute lesions elicited a conspicuous polymorphonuclear leukocytic response. The proportion of lesions classified as recent, of intermediate duration, and old, was roughly the same in each of the four mice; nor did the lesions vary perceptibly in character from mouse to mouse. Obviously the lesions had been caused throughout the 17-day stay of the mice in the flight canister or throughout the 13 days of flight. The presence of aggregates of polymorphonuclear leukocytes in the tunica submucosa was the chief finding in the olfactory mucosa of the mouse that died during the flight.

The lesions in the olfactory epithelium had virtually the same spatial distribution in the epithelium in all these mice. However, their size and configuration in a given animal varied considerably. Most astonishing was their number: at least 51 to 90 lesions per animal. By comparison, the number of high-energy cosmic ray particles $Z \geqslant 6$ traversing the nasal mucosa was calculated to total ten to fourteen particles per animal. Thus, the number of lesions in the olfactory mucosa was at least four to nine times the calculated number of cosmic ray particles $Z \geqslant 6$ that impinged on the mucosa. To determine whether concurrence existed between lesions and cosmic ray particle trajectories, the paths of particles through the nasal cavity were established by tracing the tracks of drills that had been inserted through the heads of the 17 major control mice in the trajectory of each of the particles. A total of 15 particles recorded in the dosimeters were found to have traversed the nasal cavity in the five flight animals (figure 9). Concurrence was usually observed, but since the lesions, which were frequently multifocal and usually relatively large, were also found more or less precisely in the same location in the olfactory mucosa of the contralateral nasal cavity (which presumably had not been

intersected by a cosmic ray particle), implication of the cosmic ray particles under consideration as solely instrumental in lesion production could not be justified.

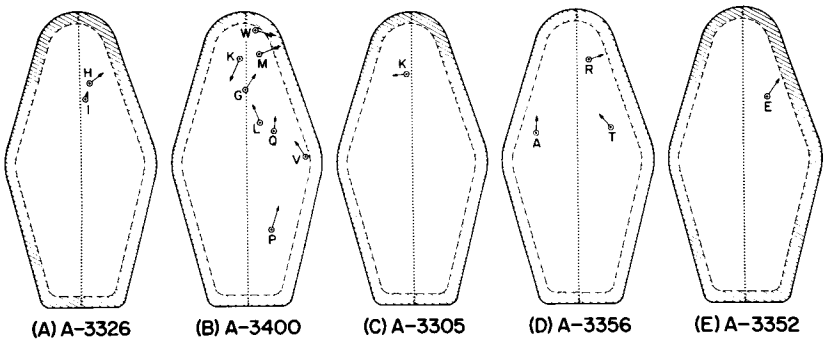


Figure 9. Subscalp dosimeters of the five flight mice, showing the sites at which they were intercepted by cosmic ray particles that penetrated the nasal cavities. The letters are designations of the individual particles. The arrows indicate roughly the projected direction of the particle trajectory through the nasal cavity.

In view of the intensity of olfactory epithelial involvement, the question arose whether the vomeronasal organ was also affected. Examination revealed that the neuroepithelium in two of the four surviving flight mice exhibited alterations analogous to the disarray observed in the olfactory epithelium of the flight mice. In one of these animals the disarray was present in the *left* vomeronasal organ, yet in the "tracked" control animal the drill that had been used intersected the *right* vomeronasal organ. Hence in this animal there was no concurrence between the cosmic ray particle trajectory and the damaged neurosensory cells.

A number of possible causes of damage of the olfactory and vomeronasal epithelium were considered: systemic or regional infection; inhaled particulate material (seed dust); byproducts from the $KO_2$ bed reaching the mice in aerosol or particulate form; gas contaminants originating in the flight package which the animals breathed; volatile substances from the dead mouse reaching the live mice; weightlessness; and cosmic ray particle radiation. Where feasible, studies were conducted in an effort to rule in or rule out some of these potentially causative factors. No definitive conclusions were reached as to the cause of the lesions. One point, however, was evident: whatever the cause, it had to be operative only in the space environment, for the olfactory epithelium in the flight backup animals and the other animals that were used as major flight controls was unaffected. Another point was that if the pathological changes in the olfactory epithelium were cosmic ray particle-induced, then the entire spectrum of cosmic particle radiation (including protons, etc.) would have to be operative, not solely the particles that were recordable in the subscalp dosimeters; and furthermore, it would be necessary that the olfactory mucosa be particularly radiosensitive. Data in support of these possibilities are not available.

## Ear

The status of the finer structure of the inner ear could not be assessed in any of the animals because the perfusion technique used (FAM introduced through the heart) did not provide adequate fixation. Suffice it to say that no changes attributable to factors operative in the space environment were observed.

In all of the flight animals as well as in all of the flight backup animals, hemorrhagic materials were found in the middle ear cavity bilaterally. In the animal that died during flight (A-3352), massive hemorrhage, which was fairly fresh, was found in the middle ear cavity bilaterally. In regard to the four live animals, there was an indication that their condition on recovery after the flight was related to the degree of hemorrhagic materials in their middle ear cavities. Mouse A-3305 was in the best condition when examined: no hemorrhage was found, the only blood constituent in air cells being proteinaceous material, the latter signifying that an alteration in capillary permeability had occurred, not capillary rupture. Mouse A-3326 (the female of the group) was in the worst condition: hemorrhage in its middle ear cavities was severe. Mouse A-3400 was groggy on initial examination: hemorrhage of recent origin was encountered. Mouse A-3356 was in excellent condition: the hemorrhage, which was of moderate degree, had largely been resorbed by the time the mouse was observed.

The occurrence of hemorrhage in the flight and flight backup animals was not unexpected because much the same was noted with considerable frequency during preflight $KO_2$ test runs, presumably as the result of pressure excursions in the canisters in which the mice were housed. The question thus arose: in the space environment would the hemorrhagic materials in the middle ear cavities and the cellular reactions thereto differ from those occurring in the control animals?

In serial sections from the flight mice and flight backup mice, a wide diversity of hemorrhagic materials was found in air cells of the middle ear cavities. To establish a frame of reference whereby possible differences in the reaction of air cell contents to factors in the space environment could be assessed, it was decided that the incidence of polymorphonuclear leukocytes in the hemorrhagic materials (blood clots, plasma, proteinaceous material) would be the sole variable to be taken into account in the evaluation. The results were surprising: air cells that contained proteinaceous material or plasma carried a significantly higher incidence in the flight animals than in the flight backup animals and, moreover, polymorphonuclear leukocytes were encountered in the proteinacous material – sometimes in great number – in the flight animals but not in the flight backup animals. Moreover, leukocyte attraction to resorbing blood clots seemed greatest in the flight animals.

Factors peculiar to the space environment were taken into consideration as instrumental in the greater exudation of blood components into air cells of the flight mice and the greater degree of leukotaxis. No basis was found on which to invoke weightlessness as causative. Analysis of the subscalp dosimeters revealed that 23 cosmic ray particles registered in the dosimeters had traversed the middle ear cavities of the four mice that survived the flight. Concurrence between particle trajectories and aggregates of polymorphonuclear leukocytes in air cells was sometimes observed, but the incidence of the leukocytes along the particle trajectories was no greater than in adjacent air cells

presumed not to have been traversed by cosmic ray particles. Hence some further inquiry was needed.

Ambient atmospheric pressure and air pressure within the middle ear cavities in the pocket mouse are normally kept equalized by means of Eustachian tubes that connect the nasopharynx with these cavities, in much the same way as in the human. It may reasonably be assumed that some factor related to this exchange in the flight animals was different than in the flight backup animals. One outstanding difference in the two groups was the presence of severe lesions in the olfactory mucosa in the flight animals but not in the flight backup animals. This difference may provide the key if it could be assumed that some airborne noxious agent caused not only the olfactory mucosal lesions but also the increased exudation and greater leukotaxis in the middle ear cavities of the flight animals. This could, if the assumption is valid, have been brought about (1) directly upon passage of the agent through the Eustachian tubes, or (2) indirectly through a local effect on the Eustachian tubes that would decrease their patency. The operation of either mechanism could have resulted in greater capillary injury in air cell linings in the flight animals than in the backup animals. However, since no noxious agent within the flight canister was identified as the cause of the lesions in the olfactory mucosa, the actual cause of the greater response in the middle ear cavity remains as open to explanation as was the case for the olfactory mucosal lesions. The presence of exudate in the nasopharynx might have been a factor in Eustachian tube obstruction in some of the flight animals.

### Scalp

The scalps of the flight animals (except that of the mouse that died during flight) were obtained for study at the time that the subscalp dosimeters were removed for analysis. Chronic inflammatory changes attributable to the presence of the dosimeters were observed in all of these scalps. In addition, a total of 13 tiny lesions were found in the epidermis or in hair follicles in three of the flight animals. (In the fourth animal, scarring of the scalp owing to the presence of the dosimeter was too extensive to allow evaluation.) The lesions were characterized by necrosis of epithelial cells, both in the epidermis and the hair follicles, in focal areas measuring up to 100 $\mu$m across. In ten of the thirteen lesions, polymorphonuclear leukocytes were present in varying numbers in the dermis and subcutaneous connective tissue in a columnar distribution extending downward from the sites of the necrotic epidermal cells (scalp thickness, 0.15 to 0.2 mm). It was evident that all the lesions were incurred during the course of the flight inasmuch as leukocyte lifetime in tissues is no more than about five days.

The question was posed whether the epidermal lesions had resulted from scalp contusion during the flight, with the exudation of acute inflammatory cells in the dermis a secondary reactive phenomenon, or whether cosmic ray particles, in traversing the scalp, had in themselves created the lesions. Comparison was made with the scalps of two control animals. In one of the controls (A-3329), in which a dosimeter had been implanted for approximately the same period of time as for the flight animals, the scalp contained two superficial focal epidermal lesions but no polymorphonuclear leukocytes in the dermis or subcutaneous connective tissue. This was in addition to larger areas in the scalp in which chronic reactive changes of moderate degree were observed. The scalp of

the second control animal (A-3494), under which a dosimeter had not been implanted, was free from epidermal-dermal lesions.

If the scalp lesions were indeed attributable to cosmic ray particle "hits," then one would have anticipated that lesions having the same characteristics would be present in the skin of flight animals in areas that had not been subjected to dosimeter implantation. Accordingly, an area of skin from the back of a flight mouse was serially sectioned, then studied. Examination revealed two tiny focal lesions in the epidermis. Beneath one of these lesions the dermis contained a few mononuclear cells and polymorphonuclear leukocytes. A single striated muscle fiber deep to the other epidermal lesion was focally necrotic, and occasional polymorphonuclear leukocytes were found in its vicinity. Moreover, the area contained a few lipid-filled macrophages. In an examination of hundreds of other fields in other sections from the area of skin obtained from this animal no such cells were observed.

Comparison of the 13 lesion sites in the three scalps with the sites of the 76 particle trajectories in the subscalp dosimeters revealed only one possible coincidence between a lesion and a registered particle trajectory. The particle in question ($Z > 10$) passed initially through the mouse head, had an LET of 220 KeV/$\mu$m as it traversed the dosimeter, and stopped in the scalp. Although there was only this one possible coincidence between particle trajectory and lesion, there remains the possibility that some of the lesions were produced by unregistered particles, that is particles with $Z < 6$ and LET $\lesssim$ 150 KeV/$\mu$m. If these lower LET particles were radiobiologically effective, one would have expected that the registered particles would have induced damage. The issue as to whether the focal lesions observed in the scalp of the four flight mice, and in the skin of the back in one of the flight mice, were produced by cosmic ray particles remains unresolved.

### Eyes

Both eyes of two of the mice that survived the flight and one eye each of the other two surviving mice were retained *in situ* and serially sectioned along with the head and examined under the light microscope. After animal perfusion (at Pago Pago), the other two eyes of these flight animals were removed, placed in glutaraldehyde, and subsequently studied by phase contrast and by electron microscopy. One eye of the dead flight mouse was retained *in situ*, whereas the other was not available for study.

Five cosmic ray particles had trajectories that intersected the eyes of the four surviving mice. They were shown to have traversed the retina at varying distances from the optic nerve head. Four of the particles ($Z = 6$ to 9 for three of them, and $Z \geqslant 10$ for the fourth) went through the head before reaching the subscalp dosimeter, while the thindown direction of the fifth ($Z \geqslant 10$) was not determinable. On the average, the particle LET in the retina was $\lesssim$ 200 KeV/$\mu$m. No retinal lesions were observed in the flight animals.

### Calvarium, Brain, Meninges

Preliminary to examining the brain sections of the flight and the flight backup animals, a study was made of the calvaria and related tissues in the region where the

monitor assemblies (dosimeters and their supporting platforms) had been implanted. The objective was to determine whether alterations occurring in these tissues could have created artifacts in the underlying brain tissue. Reference is made to erosion of the very thin calvarium (0.1 mm in thickness) which might allow invasion by an infective agent or in some manner interfere with meningeal blood supply.

Histological examination showed that each of the monitor assemblies had become surrounded by a thin fibrous tissue capsule, in and around which was a mild chronic inflammatory reaction with rare polymorphonuclear leukocytes. Giant cell reaction was surprisingly slight. There was marked atrophy of the calvarium under the monitor assemblies. Fibrosis of the dura mater was slight and was confined to a few small areas. The leptomeninges were virtually unaltered. These findings indicated that tissue reactions to the dosimeters would introduce no complicating factors in the analysis of the brains.

Mitoses in the dentate gyrus of the hippocampal formation were approximately one-third as frequent in the flight mice, and occurred about one-half as often in the flight backup mice as in non-experimental control animals. The significance of these findings is not clear, but it is suggested that the cause may be found in the internal environment of the flight and backup canisters, possibly the oxygen partial pressure. Otherwise no pathological changes were observed in the brain tissue of the flight animals or in the meninges. Special attention was given the meninges in the regions where columns of leukocytes were observed in the overlying scalps. No leukocytes were found in the meninges in these regions. If cosmic ray particles were the cause of the scalp lesions, a difference in vulnerability could be postulated: for mesodermal tissue (scalp), high vulnerability to particle radiation; for neuroectodermal tissue (meninges), low vulnerability.

## Summary and Conclusions

Although detailed studies were performed in an effort to answer the question whether HZE cosmic ray particles are injurious to brain tissue, it should be appreciated that the lack of demonstrable lesions by no means negates this possibility. The lack of lesions or an inflammatory reaction that could be attributed to cosmic ray particle "hits" needs to be evaluated in light of certain limiting factors relative to the recording of particles in the subscalp dosimeters and of the LETs of the particles themselves. A total of 80 particles were registered in the dosimeters of the five mice, nine of which did not pass through the head. Among these 71 particles, only five were known to have had a downward trajectory through the dosimeter, with thindown of the particles within or in the vicinity of the head (table 1). Of the 32 particles of undeterminable thindown direction, ten of which were in the heavy to very heavy charge group (table 2), roughly half must be considered to have also passed through the brain prior to being registered in the dosimeters. Thus, most of the particles had a higher LET in the dosimeter than in the brain. Owing to the attenuation of the very high LET components of the cosmic ray particle flux by the Apollo 17 spacecraft and by the animal package shielding, most of the particles that penetrated the brain were in the lower portion of the high LET range (0.16 to 0.2 MeV/$\mu$m), and of medium to heavy charge. Most of the particles of prime interest

biologically – those with a very high Z (iron group) and an LET in the MeV/$\mu$m range – did not reach the mice.

In summary, the lesions in the scalp can be taken as circumstantial evidence of vulnerability to radiation from cosmic ray particles, but this issue remains unresolved. Also remaining undetermined is the causation of the damage of the olfactory epithelium and the factor responsible for the greater exudation and the greater leukotaxis in the middle ear cavities, as well as the reasons for the difference in frequencies of mitoses encountered in the dentate gyrus of the hippocampal formation in experimental and control groups of animals. The absence of demonstrable lesions in the brain leaves unresolved the degree of vulnerability of brain tissue to this source of radiation. Obviously, substantially less shielded exposures to cosmic ray particles are needed if the effects (or the lack of effects) of the particles on brain tissue and other target structures are to be established.