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PROGRESS REPORT

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1 July through 30 September 1964

J. J. Gambino
R. G. Lindberg
PRINCIPAL INVESTIGATORS

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NORTHROP SPACE LABORATORIES
3401 WEST BROADWAY
HAWTHORNE, CALIFORNIA 90250

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# Investigation of Perognathus as an Experimental Organism for Research In Space Biology (Contract NASw-812)

Fourth Quarterly Progress Report
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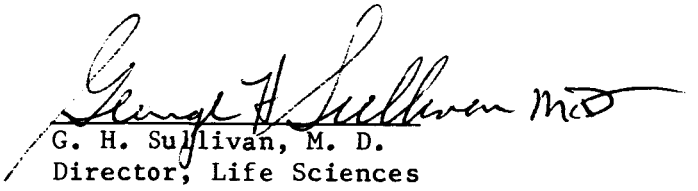

G. H. Sullivan, M. D.
Director, Life Sciences

Northrop Space Laboratories
3401 West Broadway
Hawthorne, California 90250

I. Changes in Contract Scope

By June of 1964 our success in demonstrating the adaptability of pocket mice to space biology research had reached the point of justifying a request for supplementary funds to provide for development of a specific orbital experiment.

In cooperation with Dr. Colin S. Pittendrigh of Princeton University, we submitted to Dr. Reynolds for presentation before the Biosciences Subcommittee a proposed experiment to study the effects of prolonged orbital flight on the circadian rhythm of pocket mice. We also solicited from NASA/OSSA additional funding to (1) substantiate the reliability of the rhythmic free-running period in Perognathus, (2) continued development of a temperature and activity telemeter, and, (3) design an experiment on circadian rhythms in pocket mice. It was the original intent that these latter objectives be pursued concurrently with the radiobiological studies during the last quarter of the original twelve month contract. The final negotiation however, resulted in a three month extension of the contract.

Since we had already begun some aspects of the Biorhythms studies in August 1964 we decided to take advantage of the time extension and manage the funding in a way to permit additional radiobiology studies during that period. At present both radiobiological studies and biorhythms studies are progressing simultaneously and will be summarized in the final report to be completed by 30 December 1964.

II. Mechanism of Radiation Resistance in Perognathus longimembris

A. Response of P. longimembris to Massive Gamma Irradiation

Three groups of pocket mice P. longimembris were administered massive doses of gamma-irradiation to determine whether pocket mice follow the pattern of CNS death familiar in other mammals. Ten animals were used in each irradiation group and one group of ten was kept as controls.

In order to obtain massive irradiation, the animals were lowered into a 5,000 curie $Co^{60}$ source composed of 12 eight inch long $Co^{60}$

needles arranged to form a cylindrical basket twelve inches in diameter. The dose rate within the source is 5.2 krads per minute. Because the mechanism for raising and lowering samples into the source is slow, a transit dose of 8.5 krads is accumulated during exposure. Total dose is determined by adding dose received while within the source to transit dose.

Of the three groups exposed, one received the transit dose of 8.5 krads only. A second group received the transit dose plus one minute exposure within the $Co^{60}$ source accumulating a total of 13.7 krads. The third group was administered 24.1 krads during a 3 minute exposure within the $Co^{60}$ source.

RESULTS

The animals administered 8.5 krads exhibited no locomotor or other behavioral effects either immediately post-irradiation or anytime following until death. Mean survival time in this group was 7.8 days with all of the deaths occurring between the 5th and 10th days.

Animals administered 13.7 krads and 24.1 krads all showed early locomotor disturbances. Ataxia was noticed as soon as the animals were replaced into their individual cages from the exposure cages. Some showed extreme sensitivity to handling or other stimuli. For example, they reacted violently to prodding with a pencil. Others showed uncommon aggressive behavior in the first six hours after irradiation. Behavioral effects were most pronounced in the highest dose group (24.1 krads). This group had a mean survival time of about one day. All of the animals died before 21 hours post-irradiation, except one which survived an additional 24 hours.

Mean survival time in the group receiving 13.7 krads was 7.9 days. Eight of the ten died between the 7th and the 11th day. Two died earlier; one on the 1st day and the other on the 4th day post-irradiation.

No controls died during the course of this experiment.

Autopsies were performed on all animals. There were no grossly observable pathological conditions in the 24.1 krad group. The brain and vital organs of thoracic and abdominal cavity of these animals all appeared normal upon gross examination.

All animals that died between the 5th and 11th days showed signs of severe gastrointestinal bleeding. In most instances the small intestine was full of bloody fluid. There were no signs in any of these animals of cranial hemorrhages as is often seen in pocket mice administered 1500 to 2000 rads.

A dose-survival curve based on these data and data from preceding reports (1, 2) is shown in Figure 1.

DISCUSSION

Previous work has shown the pocket mouse to be resistant to whole body irradiation in the dose range which normally kills other mammals due to hematopoietic or gastrointestinal injury (1, 2). Pocket mice succumb to both these kinds of injuries but it takes almost twice the dose to produce effects comparable to conventional mice.

Data presented here suggests that pocket mice are just as susceptible to high dose irradiation as conventional mice (3). There is even a suggestion that pocket mice may be even slightly more susceptible at the extreme dose levels. Conventional mice and rats survive 24 to 48 hours following doses as high as 20,000 to 30,000 rads; whereas pocket mice survive less than one day. The number of animals employed in this experiment is too small to make a final judgement on this point.

The plateau showing survival in the dose range from 2000 to 14,000 rads is of great interest. In this dose range pocket mice have a mean survival time of 7-8 days, whereas in conventional mice the mean survival time is between 3 and 4 days (3). In this respect pocket mice are similar to germ-free mice which have a mean survival time of 7.2 days (4).

In general, the response of pocket mice to irradiation appears to be modified in the dose ranges where cell renewal systems are important for

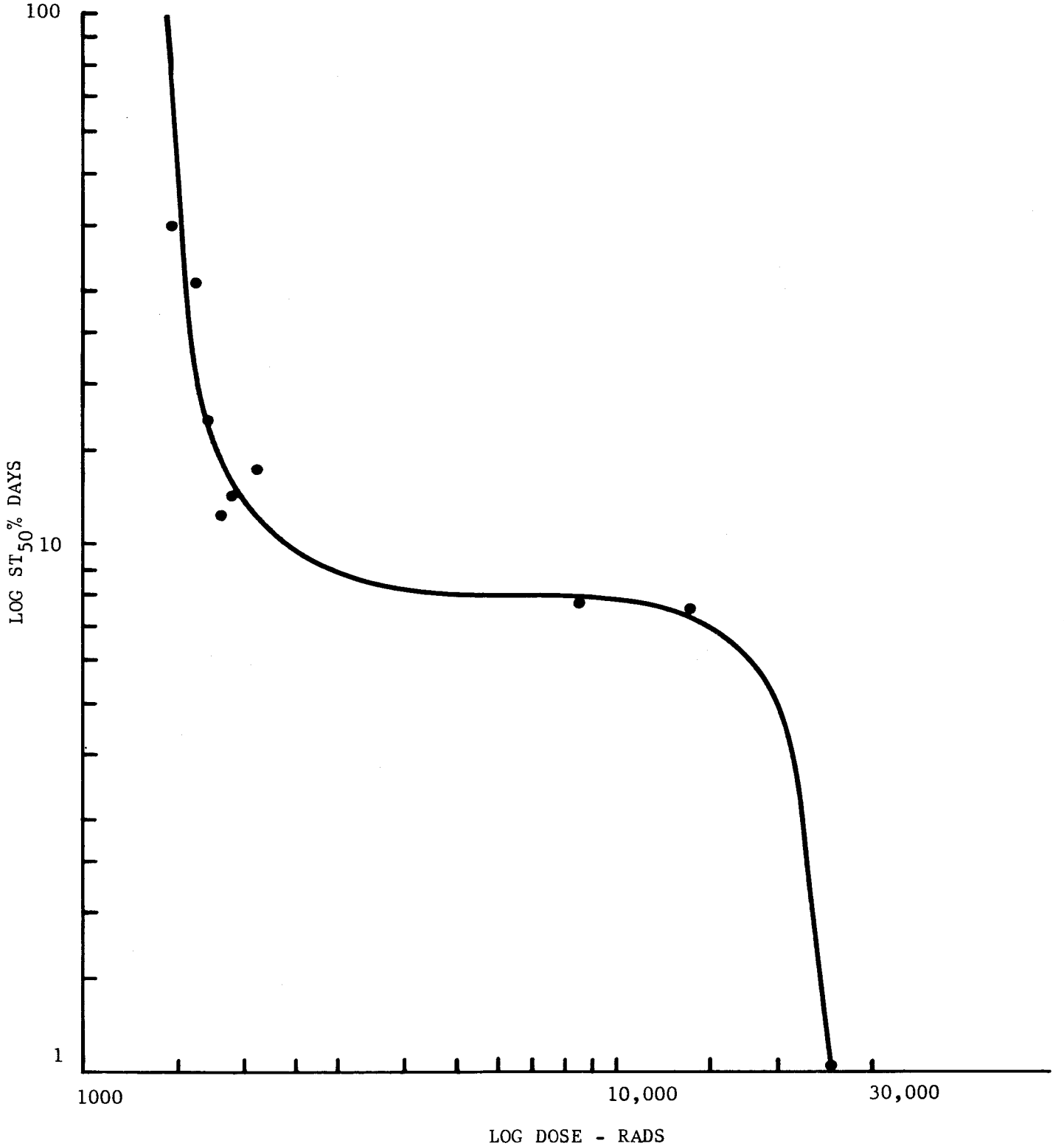


FIGURE 1. Survival ($ST_{50}$%) of pocket mice after various doses of gamma irradiation (curve visually fitted)

survival. Matsuzawa suggests that survival in the "gut death" dose range may be enhanced in germ-free mice by virtue of a prolonged life span of intestinal cells in these animals (4). The course of work in this laboratory is being directed toward elucidating a similar mechanism of radiation resistance in pocket mice.

SUMMARY

Pocket mice exposed to 8.5 and 13.7 krads massive $Co^{60}$ irradiation had a mean survival time of 7 to 8 days. Those exposed to 24.1 krad had an $ST_{50}$% of less than one day.

Animals receiving 13.7 and 24.1 krads exhibited ataxia and other behavioral effects immediately following exposure. Autopsies of the 24.1 krad group revealed no grossly observable pathology. Animals in the 13.7 krad group slowly recover from signs of CNS damage, but like those receiving 8.5 krad, succumb within 5 to 11 days. Animals in these two groups showed typical gastrointestinal injury, including massive gastrointestinal hemorrhage.

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B. Histology and Radiation Histopathology of Pocket Mouse Intestines

Damage to the gastrointestinal mucosa is responsible in a large measure for early deaths in mammals following radiation doses ranging between 1,000 and 10,000 rads. Since the integrity of the gastrointestinal epithelium is contingent upon replacement of cells which are continually being sloughed off, radiation injury incurred at the site of cell renewal is manifested ultimately as villus denudation. The cell renewal system involved, of course, are the cells at the base of the intestinal crypts which are among the most rapidly dividing cells in the body.

Loss of large amounts of body fluid and its consequences such as dehydration and electrolyte imbalance; bleeding into the intestinal lumen; and massive invasion of gut bacteria are among the sequelae of villus denudation.

At high enough doses, complete denudation and death might occur as early as 2 to 4 days following irradiation, or it might be postponed until 8 to 10 or 12 days. Conventional mice and rats, as well as many other mammals tested, have a mean survival time of 2 to 4 days; whereas, germfree mice appear to have a postponed death.

Even though, like germfree mice, pocket mice show prolonged survival after high dose irradiation, normal histology and early changes in the gastrointestinal mucosa of pocket mice have never been studied. For this reason, the following studies have been initiated.

1. Comparative histology

Histological sections of duodenum and jejunum of several species of desert rodents, and of White Swiss mice were prepared. The desert species included were Perognathus longimembris, P. formosus, Dipodomys meriami, Peromyscus penicularis, and Ammospermophilus leucuris. Specimens were fixed in Boiun's and stained with Hematoxylin-Eosin. These slides are being studied to ascertain whether there is an identifiable histological difference

between intestines of pocket mice and other desert forms which may elucidate the radiation response of pocket mice.

2. Early histopathology of pocket mouse intestine

Two groups of five Perognathus longimembris were exposed to whole-body gamma irradiation, and five non-irradiated P. longimembris were kept as controls. The two irradiated groups received 1,000 and 1,500 rads, respectively. These animals are being sacrificed during the first week post- iradiation and intestinal specimens are being removed for sectioning. Ultimately, these sections will be studied for early post-irradiation changes. Among the early manifestations of radiation damage to gut, which are specifically sought, are mitotic inhibition, cell debris within crypts and intervillus spaces, and villus denudation and size reduction. The kind, degree, and time of appearance of this damage in pocket mice will be compared with that normally appearing in conventional mice.

III. Biorhythms

A. Radiobiology

1. Mitotic activity of intestinal crypt cells

In a continuing effort to relate radiation response to physiological or metabolic states, pocket mice have been sacrificed during their metabolic low period (10 A.M.) and during their metabolic high (10 P.M.). Tissue sections of duodenum and jejumun have been prepared in the standard manner. In addition, intestinal tissue was fixed in ethanol acetic acid (3:1) and stained with Feulgen after the method of Lamerton (1). Crypts so prepared can be teased free of the intestine, placed in 45% acetic acid and squashed under a cover slip. Direct counts of total crypt cells and mitotic figures can thus be made. This work is currently underway.

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2. Day vs night irradiation in P. longimembris

Pizzarello has provided evidence that there are cyclic variations in radiation sensitivity in mice and rats (1,2). The variations appear to be related to the phase of the day-night cycle in which the animal is irradiated. Animals given "almost lethal" doses of whole body irradiation during their inactive period appear to be more radiation resistant than animals administered the same dose during their active period.

Since the high degree of radiation resistance exhibited by pocket mice may be a reflection of this sort of phenomenon, it became necessary to design and perform a day-night radiation experiment using P. longimembris.

One hundred and twenty-five animals were selected from our main colony on the basis of having a metabolic low appearing during the late morning hours. The midpoint of the metabolic low period was 0900 hours and that of the metabolic high was 2300 hours. These animals were divided by random methods into 5 groups of 25 each. Two groups were administered 1500 rads whole body irradiation; one group at 0900 hours and one at 2300 hours. The third group was administered 1000 rads whole body irradiation at 2300 hours. Morning and night non-irradiated control groups were retained.

These groups are currently being observed for signs of radiation injury. Thirty-day mortality data will be used to determine whether night radiation will shift the established $LD_{50}$ of this species.

Since 1000 rads is a sublethal dose in pocket mice administered irradiation in the morning, mortality in the group given 1000 rads during the theoretical radiation sensitive period will serve as a double check on alteration in sensitivity.

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## B. Biosatellite Experiment

### 1. Verification of pocket mice for Biorhythms Research

The objective of this research is to establish the existence of a free-running period in pocket mice. Studies at NSL are concerned with monitoring motor activity and metabolic activity of animals maintained for 21 days in the dark in a constant environment. Both Perognathus longimembris and P. formosus are under study. Preliminary data suggests the existence of a dependable free-running period in both species although some variation does exist between individual animals.

Preparations are being made to monitor both temperature and motor activity in P. longimembris at the Princeton University facilities in cooperation with Dr. Pittendrigh. The automated data handling system at Princeton will permit frequency spectrum analysis of the collected data.

### 2. Instrumentation

A small temperature telemeter suitable for intraperitoneal implantation in pocket mice has been modified to (a) extend the battery life, and, (b) provide a telemeter compatible with the monitoring equipment at Princeton University. The principle modifications are, (a) reduction in size of the transmitter circuitry, and, (b) increase in the pulse repetition rate from one pulse every two seconds to approximately 50 pulses/sec depending upon temperature.

Three systems for monitoring motor activity of pocket mice held in a 1-5/8" diameter tube are under study. One involves the use of banks of capacitor plates mounted along the length of the tube. Another utilizes magnetometers mounted along the tube to detect a small magnet attached to the mouse. The third method utilizes a microwave principle to detect the position of the mouse in the tube at any time.

A logic system is under development to provide digital read-out of both temperature and motor activity at five minute intervals.

3. Life Support

Tests are in progress to determine the optimum length and diameter of the tube which will hold the experimental animals as well as the most desirable construction materials. To this end some attention is being directed to determining the persistence of the metabolic rhythm under conditions of 100% $O_2$ at reduced atmosphere pressure. The primary emphasis is being placed on design of a package for inclusion on the Biosatellite and will utilize the existing environmental control system. Secondary consideration is being given to a self contained package.