DEVELOPMENT OF LIFE SCIENCES EQUIPMENT FOR
MICROGRAVITY AND HYPERGRAVITY SIMULATION

G.M. Mulenburg, J. Evans, M. Vasques, D.P. Gundo
NASA Ames Research Center

J.B. Griffith, J. Harper, T. Skundberg
Bionetics Corporation

INTRODUCTION

The mission of the Life Science Division at the NASA Ames Research Center is to
investigate the effects of gravity on living systems in the spectrum from cells to humans. The
range of these investigations is from microgravity, as experienced in space, to Earth's gravity, and
hypergravity.

Exposure to microgravity causes many physiological changes in humans and other
mammals including a headward shift of body fluids, atrophy of muscles - especially the large
muscles of the legs - and changes in bone and mineral metabolism. The high cost and limited
opportunity for research experiments in space create a need to perform ground based simulation
experiments on Earth. Models that simulate microgravity are used to help identify and quantify
these changes, to investigate the mechanisms causing these changes and, in some cases, to
develop countermeasures.
Hypergravity likewise creates physiological changes of fluids, muscle, and bone. Hypergravity experiments can therefore add substantially to the knowledge of how gravity, in this case greater than 1G, affects living systems. It has been hypothesized that on a long space voyage, exposure to hypergravity for short periods, rather than to 1G for longer periods, may beneficially counteract the deleterious effects of microgravity. Hypergravity experiments, using centrifugation in ground based facilities adds to the knowledge base of gravitational effects on living systems.

This paper describes two unique pieces of research equipment developed by the Life Science Division of NASA's Ames Research Center to study the effects of simulated microgravity and hypergravity: 1) a new metabolic cage for use in simulated microgravity studies in rats, and 2) a paste feeder that was used recently in a two week continuous 2G hypergravity study.

DEVELOPMENT OF A METABOLIC CAGE TO SIMULATE MICROGRAVITY IN A LABORATORY ENVIRONMENT

Hindlimb suspension of rats is a well established model used to simulate the effects of microgravity. The suspension apparatus includes a light weight foam tape that is attached to the length of the rat's tail and to a pulley system. The hind limbs are raised off of the ground and the rat can move about the cage freely on its forepaws. The animal can eat, drink and groom normally. This model is widely used and has been shown to cause no undue stress to the animal. This suspension model, developed by Wronski & Morey-Holton (1), simulates the effects of microgravity during spaceflight by unloading the hindlimbs and simultaneously producing a headward shift of fluids similar to that seen in a weightlessness environment. For most systems studied, this model accurately mimics microgravity. However, obtaining quantitative data on the metabolic status of suspended rats for nutritional, metabolic and renal function studies has not been possible until now.
Using Holton's tail suspension cage as a starting point, a new metabolic suspension cage, Figure 1, was developed. This new cage, effectively collects and separates urine and fecal samples for quantitative analysis. The added information from these samples, when coupled with food and water intake data, gives investigators an opportunity to examine the effects of microgravity on many complex body systems.

**Requirements**

The requirements to provide the Holton cage with this new capability and how the new metabolic suspension cage was developed follows:

1) Make the new cage interchangeable with standard Holton cage parts. This requirement was based on results using Holton's cage (a large amount of documented research exists using this cage) and the large floor space available to the animal (144 in. sq.). Clear plastic allows the animal to be observed easily.

2) Provide a means to easily and efficiently separate and collect urine and feces with minimal cross contamination. This requirement is essential to perform accurate analyses of the samples collected.

3) Ensure the ability to efficiently remove samples daily for measurement, storage, and analysis.

4) Ensure the ability to easily and quickly replace the collection equipment with clean units daily, to easily clean the removed equipment, and also, to easily remove animals for daily weighing, inspection or manipulation.

5) Ensure the ability to prevent contamination of the urine and fecal collections with food spilled by the animal.

6) Ensure the ability to easily and efficiently remove remaining food for accurate weighing and consumption determination, and replenish with a new, measured, quantity of food.
Development

Figure 2 shows the original Holton suspension cage as the shaded portions with the new metabolic additions as clear. From the figure, it can be seen that the Holton cage design was basically left intact. Letters in parentheses refer to matching numbers in the figure.

Requirement 1) was met by developing a raised base (H) with two solid side walls and an open front and back to support a standard Holton cage as shown in the figure.

Requirement 2) and 3) were met by combining a commercial separator-diffuser (J,K) with a newly designed vacuum formed rectangular funnel (I) to catch and guide the urine and feces to the separator-diffuser. Screw on collection tubes (L), can easily be removed and capped for transportation and storage of samples for later analysis. Installation of new, clean tubes is also quickly accomplished. An open cage top allows easy access to the animal.

Requirement 4) was met by providing for frontal insertion of both the cage floor and the funnel in rails located on the sides of the base walls. A tab was added to the spout of the funnel to insert and support the separator-diffuser. The funnel is made of vacuumed formed ABS plastic and can be easily removed from its supporting rails and cleaned.

Requirement 5) was met through the development of a food cup system (8/217909, patent pending) external to the cage, which allows access to the food by the animal in an area that is not above the collection funnel. This includes a tunnel with an open floor (E) to allow spillage through the floor and not into the funnel. Food is provided to the animal in powdered form to prevent it carrying the food cup back into the cage (and spilling it into the collection funnel). An adjustable metal gate with a rounded cutout on the bottom slides up and down on the food cup itself. The height can be adjusted to limit accessibility to the food for different sized animals.
This is especially important for smaller animals that tend to enter the food cup and scatter the food. Adding this system required shifting the water bottle from its original centered location on the wall to a position slightly off center.

Requirement 6) was met by providing a removable food cup and a spill tray (F) under the access tunnel to collect any spilled food for accurate food consumption measurement.

Discussion

The new cage provided satisfactory collections in both ground based and post-spaceflight studies (2).

DEVELOPMENT OF A PASTE FOOD FEEDER FOR UNINTERRUPTED CENTRIFUGATION STUDIES

Centrifugation has long been used to provide hypergravity for research purposes. Although a complete discussion is beyond the scope of this paper, acceleration due to rotation provides a force that can be substituted for the normal attraction of two masses, such as the Earth and a body on it.

One of the basic questions of gravitational physiology is whether there is a continuum of physiological responses from 0G to 1G and into hyper G. The effects of microgravity (spaceflight) and hypergravity (centrifugation) on living systems have been investigated extensively in independent studies. However, differing experimental conditions (type of rat, diet, etc.) have precluded direct comparison of prior 0G and hyper G studies.
To compare the effects of hypergravity exposure with those of exposure to microgravity in animals flown on the Russian Cosmos 2044 flight, it was critical to continuously centrifuge the animals for 14 days without stopping in order to duplicate the Cosmos 2044 flight length and other environmental parameters.

To duplicate an experiment at hyper G that initially occurred in microgravity all other conditions must be as similar as possible. This included using a paste diet and feeding schedule similar to that used in the Cosmos flight. Stopping the centrifuge for even short periods can create unacceptable changes in research results. Therefore, the feeder must work without stopping the centrifuge for the entire 14 day experimental period.

A specialized paste feeder, Figure 3, was developed to support a hypergravity centrifuge study on the Ames Life Science Division 24 foot diameter centrifuge. The results from this 14 day hypergravity study were compared to the previously flown Russian Cosmos 2044 spaceflight.

Requirements

To accomplish this project, the established requirements were as follows:

1) Provide for 14 day continuous 2G centrifugation with no stops.
2) Provide for feeding the animals the same paste diet (which has the consistency of thick oatmeal) as on the Cosmos 2044 mission. The same amount of food must be dispensed (140g) at the same interval (every 6 hours).
3) Provide on demand water delivery to the rats and a waste collection system to operate throughout the 14 day period.
Development

Unique features that had to be overcome for this experiment included changes in the consistency of the food over time from a thin paste at the start of the experiment to a much thicker consistency toward the end of the 14 day period. Centrifuge and stationary control cages, each containing 10 rats, were fitted with feeders calibrated to provide $140 \pm 2g$ of paste diet every 6 hours.

Requirement 1) was met by the inherent capability of the Ames 24 foot diameter animal centrifuge which has a 3 G maximum capability for extended periods of up to a month or more of continuous operation.

Requirement 2) was the greatest challenge. After attempts to find adequate off the shelf food delivery equipment failed, a new feeder design was developed. Numbers in parentheses refer to matching numbers in Figure 4.

The essence of the design included:

a) Food reservoir (1). A stainless steel funnel was welded to an open bottom 7.6 liter stainless cooking stock pot. A 2.5 cm fitting was welded onto the funnel spout to accept a 2.5 cm diameter threaded valve (2). A sealed cover on the reservoir allowed pressurization of the space above the food to 5 psi with nitrogen (7) to help the food flow through the funnel and into the tubing system. A plate slightly smaller than the inside diameter of the reservoir was placed on top of the food to provide a uniform pressure surface.

b) Tubing system. Stainless steel 2.5 cm diameter tubing (8) provides a path for food to flow from the reservoir to the food tray inside the cage.
c) Electromechanical valves. Two electromechanical valves are electrically sequenced to control the food flow. The first valve (2) at the base of the funnel opens to allow food to flow from the reservoir. When food is ready to be expelled from the delivery tube, the first valve closes and the second valve opens.

d) Piston. A positive displacement piston (9) in the straight section of the tubing is retracted by a dual motor system (4). The stroke length of the piston is controlled by limit switches (10) at each end of its travel.

e) Electrical relay sequencing system and programmable timer. The sequencing and timing system (5) controls valve opening and closing, and motor operation for piston retraction and extension. The piston retracts with valve (2) open and valve (3) closed. This allows food to flow from the reservoir through the curved tube and into the straight tube (8) behind the retracting piston. When valve (2) closes and valve (3) opens the motors reverse and the piston extends to push food down the straight tube and into the food tray. The programmable timer (18) initiates two sequences of operation every 6 hours, delivering 70g each time for a total of 140g per feeding.

Requirement 3). The water supply requirement was met using a 12 liter water storage bottle mounted near the center of the centrifuge about one meter above the cage. Gravity feed, enhanced by centrifugal force, caused the water to flow through plastic tubing from the bottle to two lixits mounted in the side of the cage.

A stainless steel tray with a hole in the center allows animal waste to flow into a waste collection container (14) mounted under the cage.
OPERATION

Operation of the feeder system during the 14 day Cosmos comparison study met all specifications for the experiment.

DISCUSSION

The high cost and rare opportunity for space flight experiments limit accessibility to space. Gravitational research can be conducted utilizing ground based research techniques and specialized equipment. Microgravity simulation using the metabolic suspension cage now offers the capability to gather metabolic data allowing investigators to study the physiological effects of microgravity. Additional details of the metabolic cage design are available in NASA Technical Memorandum 108830.

Hypergravity studies with paste food diets are now possible for continuous periods of centrifugation of up to 14 days. Adaptation of the paste feeder design for longer studies, or more animals, is a straightforward next step. Additional details of the paste feeder design are available in NASA Technical Memorandum 108804.
References


FIGURE 1 ASSEMBLED METABOLIC CAGE
A. Suspension Apparatus  
B. Cage Top  
C. Cage Sides  
D. Water Bottle Assembly  
E. Tunnel  
F. Food Cup  
G. Cage Floor  
H. Cage Base  
I. Collection Funnel  
J. Separator Housing  
K. Linear Diffuser  
L. Collection Tubes

FIGURE 2 METABOLIC CAGE PARTS IDENTIFICATION
FIGURE 3 PASTE FEEDER AND CENTRIFUGE ARRANGEMENT

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FIGURE 4  PASTE FEEDER PARTS IDENTIFICATION