# A Metabolic Cage for the Hindlimb Suspended Rat

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## Introduction

Hindlimb suspension has been successfully used to simulate the effects of microgravity in rats. The cage and suspension system developed by Holton (ref. 1) is designed to produce a headward shift of fluid and unload the hindlimbs in rodents, causing changes in bone and muscle similar to those in animals and humans exposed to spaceflight. While the Holton suspension system simulates many of the conditions observed in the spaceflight animal, it does not provide for the collection of urine and feces needed to monitor some metabolic activities. As a result, only limited information has been gathered on the nutritional status, and the gastrointestinal and renal function of animals using that model.

Although commercial metabolic cages are available, they are usually cylindrical and require a centrally located suspension system and thus, do not readily permit movement of the rats. The limited floor space of commercial cages may affect comparisons with studies using the Holton model which has more than twice the living space of most commercially available cages. To take advantage of the extra living space and extensive data base that has been developed with the Holton model, Holton's cage was modified to make urine and fecal collections possible.

#### Background

Astronauts undergo various physiological changes as their bodies adjust to weightlessness. Since the first manned spaceflights in the 1960s, abnormalities in mineral metabolism (ref. 2) and disturbances in fluid and electrolyte balances (ref. 3) have been observed in astronauts. It is important to monitor these changes using balanced diet studies to determine if the changes are directly related to microgravity exposure or are diet-related.

The expense and limited access to spaceflight opportunities has led to the development of numerous ground based models to simulate weightlessness on Earth.

In humans, head down bedrest and water immersion have been used to study the effects of the headward shift of fluids and the unloading of bone and muscle that occurs in spaceflight. However, the use of invasive techniques such as bone and muscle biopsies in human subjects is limited. As a result, animal models have been developed and are widely used to simulate the physiological effects of microgravity.

Methods for simulating the effects of weightlessness in rats include restraint, immobilization, and suspension. Immobilized and restrained animals show some effects of a weightless environment, but are unable to move about and groom themselves normally. The tail suspension model developed by Holton (ref. 1) simulates the changes associated with spaceflight, by unloading the hind limbs causing a headward shift of fluids. To suspend the rat, the base of the tail is attached to an overhead pulley system using orthopedic tape, a plastic clip, and a fishline swivel which allows 360° of rotation. The height of the cage sides can be adjusted vertically to compensate for the size and weight of the animal. Holton's model is unique; the animal is suspended head down to simulate the headward fluid shift observed in spaceflight but can move about the entire cage by pulling itself along the plastic mesh floor with the front paws. The hind limbs are unloaded but not restrained.

Holton's model allows the rat to eat, drink, groom itself, exercise, and move about the cage. These "normal" activities provide a less stressful environment than restraint or immobilization.

#### Description

#### **Original Cage**

As shown in figure 1, the Holton tail suspension cage was used to develop the metabolic system. The clear pieces illustrate modifications while the shaded pieces indicate Holton's original design. The suspension system (A) is shown in detail in appendix B.

The original cage dimensions were maintained but the material was changed from acrylic to polycarbonate for added durability during repeated handling and washing. All joints are welded together using IPS 4 Weld-on acrylic cement. The usable floor space in this model is 144 in.<sup>2</sup> more than double the 70 in.<sup>2</sup> recommended by the National Institutes of Health (ref. 4).

The cage floor (G) is made from 1/2 in. thick, 1/2 in.  $\times$  1/2 in.<sup>2</sup> fluorescent lighting diffuser material.

#### Modifications (Figures 1 and 2)

The water bottle (D) is standard. Its location was moved slightly from Holton's centered location to the left edge to allow room for the tunnel and food cup. The cage sides (C) were extended beyond the cage face to allow the suspended animal to reach into the food cup. The cage sides can be adjusted vertically to obtain the height required to unload the hindlimbs.

Major changes include: (1) relocating and redesigning the food cup, (2) the addition of a tunnel that provides access to the food cup, (3) adding a funnel and metabolic collection-separator devices, and (4) the addition of a larger base to support the cage, funnel and separator.

#### Food Cup and Tunnel (Figures 3 and 4)

The food cup (F), patent pending 08/217,909, was relocated from a recess in the cage floor to the face of the cage. The front of the cage has a 1.75 in. dia. hole, centered 3.125 in. from the bottom of the cage face, to provide access to the tunnel.

The rat enters a short tunnel to gain access to the food cup. The tunnel (E) is a  $1.5 \times 1.75$  in. ID acrylic tube with a grid floor of the same material as the cage floor. This tunnel allows the animal access to the food cup, while the open floor prevents spilled food from entering the cage and contaminating the urine and fecal collections.

The food cup (F) is vacuum formed from 0.09375 in. thick clear polycarbonate. Flanges on the cup slide into rails on the inclined end of the tunnel which allows easy removal of the food cup for weighing, cleaning, and replacement with a clean food cup. The food cup is inclined  $30^\circ$ , to keep the food in the cup, preventing it from falling into the funnel and contaminating urine and fecal collections. When food intake data is not critical, the food cup can be positioned on the cage wall in place of the tunnel. The sliding gate on the food cup is made from 0.0625 in. thick aluminum sheet and fastened to the cup with a screw knob. The height of the gate is adjusted for the size of the animal to minimize scattering of food.

A 3 in.  $\times$  3 in. plastic weighboat is positioned under the tunnel to serve as a "spill tray." It is held in place by two, 2.5 in. long springs that attach into holes on each side of the weighboat and to a small tab on top of the food cup. Food spilled in the tunnel can be returned to the food cup from this tray before weighing to accurately determine consumption.

#### Base (Figure 5)

The base (H) is constructed of two 12.5 in.  $\times$  17.9 in. solid sides of 0.375 in. clear polycarbonate sheet which are held in place at the bottom, front and back by 1 in.  $\times$  14.25 in. strips of the same material. Similar strips 0.5 in. wide attach the sides together across the top of the two side pieces. The rails that hold the cage floor and funnel are made of 0.5 in. strips welded along the top of the base side pieces.

#### Funnel (Figure 6)

The 12 in.  $\times$  12 in. open funnel (I) is vacuum formed from 0.125 in. ABS plastic (RAL Plastics). A tab welded on the funnel spout attaches the urine-feces separator (J, K) (Maryland Plastics).

#### **Collection System (Figure 7)**

The urine-feces separator (Maryland Plastics) separates and collects the urine and feces into screw top centrifuge tubes (30 mm dia.) with a 30 or 50 ml capacity. Urine flows down the funnel, into the narrow channels of the diffuser (K) which lead to a collection tube. The feces roll down the funnel, over the channels and into a second tube. Collection tubes (L) screw into the separator, allowing easy removal and replacement without disturbing the animal.

# Evaluation

To evaluate the metabolic cages, body weight, food and water consumption and the excretions of 30 mature rats  $(453 \pm 20g)$  were monitored for 30 days. This experiment was later repeated with smaller (200g) animals (ref. 5). Double distilled water was provided ad libitum, and water consumption was determined daily by weighing the bottles. Powdered AIN-76 purified diet was provided ad libitum and changed daily. Food consumption was monitored by weighing each animal's food cup and the food in the spill tray. Metabolic samples as well as food and water consumption data, were collected and weighed at the same time each day to control for diurnal variations. Suspended and control animals had similar growth rates after a short period of adaption to suspension (ref. 5). The rats appeared healthy and able to groom normally.

In earlier studies, the rats had chewed on the cage and cage floors. To minimize this damage, each rat was provided with a wooden dowel (hardwood 1.5 in.  $\times$  1 in. dia.). While some rats continued to chew on the cage floor, most chewed on the dowels and the damage to the floor was not extensive. The shavings from the dowels fell

through the cage floor and into the feces collection tubes, where they were easily identified and separated from the feces before fecal weights were determined.

Urine samples were visually inspected and found to be free from contamination of food, feces and wood shavings. Urine volumes were similar to those of rats housed in commercial metabolic cages (ref. 6).

The original cages made of acrylic proved durable; none were replaced during the study. However, some of the cages were broken during transport between buildings. Newer cages are made of clear polycarbonate to increase cage strength and durability for shipment and storage. A few of the separator tabs broke off of the funnels during the daily washings and were repaired. No problems were found with the separators, diffusers, food cups, or tunnel assemblies.

In the first 30 day study with mature animals 7 of the 450 water bottles leaked, a 1.5% collection error. In the second study with young rats, the water bottles were filled and observed for leaks before the study started, thus eliminating water contamination of the urine (ref. 5).

# Conclusions

The metabolic additions to Holton's tail suspension cage allow uncontaminated urine and fecal samples to be collected using a well established spaceflight model. Holton's suspension cage design has almost double the usable floor space as well as higher sides than commercial metabolic cages allowing the investigator more flexibility in animal age and size.

This new cage design can be used to study the effects of simulated microgravity on many metabolic and physiological systems, including bone, muscle, and the endocrine system. This cage will also enable investigators to study the effects of diet, gastrointestinal, and renal function.

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L. Collection Tubes

Figure 1. Metabolic suspension cage illustration. Modifications are clear while the shaded portions indicate Holton's original design.



Figure 2. Metabolic suspension cage allows the collection of urine and fecal samples from animals in a simulated weightless environment.



Figure 3. Close-up of the tunnel and food cup assembly.



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Figure 5. Collection funnel and base; a small tab connects the funnel to the separator.



Figure 6. Standard 50 ml tubes attach to the separator housing.



Figure 7. Separator housing and linear diffuser.

# Appendix A

# Cage Requirements, Vendors/Sources

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No. pieces	Thickness	Size	
-		English	Metric
1	3/16 in. (0.177)	12 in. × 13 in.	304.8 × 330.2
1		12 in. × 12 in.	$304.8 \times 304.8$
2		12 in. × 12 3/8 in.	304.8 × 314.32
2		12 7/8 in. × 8 1/2 in.	327.02 × 215.9
2	3/8 in. (0.375)	12 1/2 in. × 17 15/16 in.	317.5 × 455.61
2	•	$1 \text{ in.} \times 14 1/4 \text{ in.}$	25.4 × 361.95
1		$1/2$ in. $\times$ 14 1/4 in.	12.7 × 361.95
2		$1/2$ in. $\times$ 12 1/2 in.	12.7 × 317.5
4		7/16 in. × 3 in.	11.11 × 76.2
1	1/8 in. (0.125)	3 in. × 3 5/8 in.	76.2 × 92.07
1	· · ·	$3 \text{ in.} \times 3 \text{ in.}$	$76.2 \times 76.2$
4	1/2 in. (0.500)	$1/2$ in. $\times$ 12 1/2 in.	12.7 × 317.05
1		2 3/16 in. × 14 1/4 in.	55.56 × 361.95
Tubing	Acrylic	2 in. OD	
		1/8 in. wall	
		1 3/4 in. ID	
Egg Crate Louvers			
3		12 1/4 in. × 13 1/4 in.	

#### Metabolic Suspension Cage Plastic Requirements Dimensions (pieces/cage) All pieces are Clear Polycarbonate

# **Cage Materials**

### Plastic

See polycarbonate requirements, available from a variety of sources AIN Plastics, Inc. 3380 Keller Street Santa Clara, CA 95054 (408) 988-6611 or TAP Plastics 312 Castro Street Mountain View, CA 94041 (415) 962-8430

#### Plastic Glue IPS WELD-ON no. 4

AIN Plastics, Inc. 3380 Keller Street Santa Clara, CA 95054 (408) 988-6611 or TAP Plastics 312 Castro Street Mountain View, CA 94041 (415) 962-8430

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#### **Cage Floor**

Egg crate louvers (white) 12 1/4 in. × 13 1/4 in. 1/2 × 1/2 × 1/2 Fluorescent lighting diffuser material AIN Plastics, Inc. 3380 Keller Stree Santa Clara, CA 95054 (408) 988-6611 or TAP Plastics 312 Castro Street Mountain View, CA 94041 (415) 962-8430

## Acrylic Tubing (for tunnel)

2 in. OD, 1/8 in. wall × 1 3/4 in. ID AIN Plastics, Inc. 3380 Keller Street Santa Clara, CA 95054 (408) 988-6611 or TAP Plastics 312 Castro Street Mountain View, CA 94041 (415) 962-8430

# Bolts/Washers/Wing nuts (cage sides)

Carriage bolts Steel, zinc 1/4 -20 in. × 3/4 in. Cat no. 25C100CARZZ

Flat washers 1/4 in. SAE flat Steel, zinc Cat no. 37NSAEZZ

Wing nuts 1/4 in.-20 Steel, zinc Cat no. 25CWNTZZ

> Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

#### Water Bottle Springs

3/16 O.D. × 5 in. long 0.020 wire dia. Utility extension spring Cat. no. C.201 Available from any hardware store

#### Water Bottle Spring Screws

6-32 × 3/16 in. Round head, cross recess drive Steel, cadium plated Available from hardware supply stores

#### **Funnels and Tabs**

ABS Plastic Molded 1/8 in. RAL Plastic Forming 1390 Bayport Ave. San Carlos, CA (415) 592-6767 (415) 595-0366 FAX Contact Juli Evans or Jerry Mulenburg for molds

## ABS glue (for funnel tabs)

IPS WELD-ON plastic pipe cement no. 773 for ABS Medium bodied, fast set RAL Plastic Forming 1390 Bayport Ave. San Carlos, CA (415) 592-6767 (415) 595-0366 FAX

#### Separator/Linear Diffuser

Separator housing cat. no. E1309 Linear diffuser cat. no. E1304 Maryland Plastics, Inc. Scientific Division 251 East Central Avenue Federalsburg, MD 21632 (410) 754-5566 (410) 754-8882 FAX or Fisher Scientific 2170 Martin Avenue Santa Clara, CA 95050 (408) 727-0660

#### Food Cups

RAL Plastic Forming 1390 Bayport Ave. San Carlos, CA (415) 592-6767 (415) 595-0366 FAX Contact Juli Evans or Jerry Mulenburg for molds

## **Metal Gate**

2024 T3 aluminum sheet 0.63 in. thick

#### Knobs

Knurled torque knob BI 10-32 in. × 5/16 in. Cat no. 116-307

Soc set cup, stainless  $10-32 \times 7/8$  in. Cat no. 10F87SSCS

> Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

## **Disposables**

Collection Tubes (50 ml)

(408) 378-2013

Polypropylene centrifuge tubes, conical bottom, nonsterile with separate caps, 30 mm dia. Cat no. 611531 E & K Scientific Products PO Box 882 Saratoga, CA 95070

#### Collection Tubes (30 ml)

30 ml conocal tubes with caps, non-sterile bulk packed, 30 mm dia. Cat no. 300-3532-G0 Cat no. 200-3928-030 (use both numbers when ordering) Evergreen PO Box 58248 2300 East 49th Street Los Angeles, CA 90058-0248 (800) 372-7300

#### Weighboats

Square, polystyrene Cat no. B580053 E & K Scientific PO Box 822 Saratoga, CA 95070 (408) 378-2013

#### **Wooden Dowels**

36 in. hardwood cut into 1 1/2 in. lengths 1 in. dia. Available from any hardware store .

# Appendix B

# Suspension Procedure, Supplies, Hardware and Vendor/Sources

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#### **Suspension Procedure**

A normal alert animal is loosely restrained in a towel. The tail is cleaned with gauze soaked in 70% ethanol to remove dead skin and dirt. Tincture of Benzoin is sprayed on the tail and dried with a blow dryer on low heat or allowed to air dry until tacky. Benzoin protects the tail from the adhesives in the skin trac (orthopedic tape). The skin trac is folded over the suspension clip and applied laterally to both sides of the tail, starting from the base of the tail, just distal to the hair line. The tip of the tail should stick out about one inch. A strip of stockinette is wrapped around the skin trac and secured by placing fibrous packing tape just below the suspension clip and at the base of the tail. The tape should be snug but not interfere with the normal blood flow. The suspension clip is then attached to a pulley system by the fishhook swivel. The system is mounted onto an acrylic cage (ref. 1). The pulley system and the height of the cage sides are adjusted to keep the hind limbs just above the cage floor (figs. B-1 through B-4). The animals hind toes should not touch the floor when the leg is fully extended. The pulley system can be adjusted as needed to maintain unloading of the hindlimbs.

This system allows the rat to move about the cage using its front paws to pull. The pulley system moves both vertically and horizontally across the cage top, allowing the rat to utilize all available floor space. The hind limbs are unloaded, and unrestrained. The animals are able to eat, drink and groom normally.

## Suspension Supplies

#### **Tincture of Benzoin**

8 oz. can Cat no. 23450-008 Baxter Scientific Products 31353 Huntwood Ave. Hayward, CA 94540 (510) 471-7122

#### Stockinette

2 in. × 50 yd. Cat no. 23652-020 Baxter Scientific Products 31353 Huntwood Ave. Hayward, CA 94540 (510) 471-7122

#### Skin Trac Tape

2 in. × 24 in. (cut to size) Cat no. 3874-11 Zimmer Patient Care 320 Turtle Creek Court San Jose, CA 95125 (408) 293-0103 (800) 227-7198

#### Alcohol, Ethanol 70%

Available from a variety of sources (drug stores and medical suppliers)

#### Gauze

2 in. × 2 in. squares Available from a variety of sources (drug stores and medical suppliers)

#### Hair Dryer

Any model with a cool or warm setting

#### Towel

Used to restrain the animal

#### **Fiber Tape**

Filament reinforced Commonly used for packing shipments Available at stationery and office supply stores

## **Suspension Hardware**

#### Pulleys

Arvan, Inc. 14083 S. Normandie Ave. PO Box 1326 Gardena, CA 90249 (213) 770-3700 Cat no. 29965 or Federal Military Supply S9C 3020-00-775-6831

#### **Suspension Brackets**

Aluminum alloy 2024 Temper T4 or T351 Flat (usually sold in 12 ft lengths) 0.250 in. × 1 in.

#### **Brass Rod**

Brass alloy 360 Temper halfhard Round (usually sold in 10–12 ft lengths) Dia. 0.250 in.

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#### Allen Socket Cap Screws

10-32 × 1 1/2 in. stainless Cat no. 10F150SHCS Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

## **Carriage Bolts**

10–24 × 1 1/2 in. Steel, zinc Cat no. 10C150CARZB Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

## Wingnuts

10-24 Thread MS35425 or MS35426 Steel, cadium plated Available from any hardware store

#### Set Screws

10-32 × 1/4 in. Socket set cup stainless Cat no. 10F25SSCS Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

# Spacers

3/16 ID × 5/16 O.D. × 1/4 in. long Round, aluminum Cat no. 10N25RS5U Olanders 144 Commercial Ave. Sunnyvale, CA 94086 (408) 735-1850 (800) 538-1500

#### **Fishhook Swivels**

2/0 brass snap swivels 1700GR Available at fishing supply and sporting goods stores

# **Binder Clips**

Medium size Available at stationery and office supply stores







Figure B-2. Suspension materials: pulley system, orthopedic tape, and suspension clip.



Figure B-3. Suspension apparatus in inches.



Figure B-4. Suspension apparatus in centimeters.

Appendix C

# Exploded View of the Cage

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# Metabolic Suspension Cage Exploded View (Figures C-1 and C-2)

# 1. Suspension Apparatus

- unloads the hind limbs
- simulates the headward shift of fluids that occur in spaceflight
- allows the animal to eat, drink and groom normally
  - 1. Pulley system
    - allows movement in all directions
  - 2. Suspension clip
    - attaches animal to pulley system

## 2. Cage Top

- Living space 144 in.<sup>2</sup>
- Cage cover
  - 1. control animals: use extra cage floor (egg crate louvers) or wire cover
  - 2. suspended animals-none required
- 3. Cage Sides
  - allow the height of the suspension pulley system to be adjusted for the size of the animal
  - support suspension apparatus

#### 4,5,6. Carriage Bolts, Washers, Wingnuts

- tighten to adjust the height of cage sides

#### 7. Water Bottle Assembly

- provides water to the animal
- 8. Springs
  - fasten water bottle to cage

#### 9. Roundhead Screws

fasten spring to cage front

#### 10. Tunnel

- provides access to food cup
- egg crate floor prevents spilled or dropped food from contaminating urine/feces
- 11. Food Cup Assembly
  - provides food to the animal
  - sliding gate allows the investigator to control the size of the food cup opening (access is restricted for smaller animals to prevent them from sleeping in the food cup and scattering the food)
  - spilled food falls through the tunnel floor and is collected in the weighboat, allows quantative recovery of spilled food

#### 12. Cage Base

- supports the cage floor
- provides clearance and support for collection funnel and separator
- urine/feces collected below the cage, the animal is not disturbed

#### 13. Collection Funnel

- directs urine/feces into separator
- a tab on the funnel spout attaches to the separator housing

#### 14. Separator Housing

- holds linear diffuser
- threaded attachment for sample collection tubes

#### 15. Linear Diffuser

- urine runs into grooves, down the trough and into the rear tube
- feces roll over grooves into the front tube

#### 16. Collection Tubes

- standard 50 ml tubes for urine collection
- 30 ml or 50 ml tubes for feces collection
- 30 mm dia.



Figure C-1. Metabolic suspension cage parts with English dimensions. Numbered callouts are detailed in appendix D.

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Figure C-2. Metabolic suspension cage parts with metric dimensions. Numbered callouts are detailed in appendix D.

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# Appendix D

# Cage Specifications, Drawings in English, and Metric Units

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TUNNEL

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All dimensions-inches.



All dimensions-inches.





 4
 POLLEY SUPPORT
 32.70 × 21.59

 5
 GUIDE
 SEE OTHER SHEET FOR DETAIL

 6
 STOP
 SEE OTHER SHEET FOR DETAIL

ALL 0.47 THICK POLYCARBONATE All dimensions-centimeters.

4.00 -

8.01



TUNNEL





All dimensions-centimeters.

STOP

FLOOR

0.31 x 2.54 x 0.63

SEE DETAIL

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2

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6



All dimensions-centimeters.

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