

# Effects of Caudal Elevation on Testicular Function in Rats

## Separation of Effects on Spermatogenesis and Steroidogenesis

D. R. DEEVER,\* R. P. AMANN,† R. H. HAMMERSTEDT,‡ R. BALL,‡ D. N. R. VEERAMACHANENI,† AND X. J. MUSACCHIA§

From the \*Department of Dairy and Animal Science, Pennsylvania State University, University Park, Pennsylvania; †Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, Colorado; the ‡Department of Molecular and Cell Biology, Pennsylvania State University, University Park, Pennsylvania; and the §Department of Physiology and Biophysics, School of Medicine, University of Louisville, Louisville, Kentucky.

**ABSTRACT:** A variety of biologic processes are perturbed when exposed to microgravity (space flight) for more than 7 days, including testicular function. Suspension of rats in a special harness (caudal elevation) to induce thoracic pooling of blood fluids and remove the support function of the hind limbs is used to mimic, on earth, the effects of microgravity encountered during space flight. Typically, this induces cryptorchidism in male rats. Three experiments were conducted to differentiate the effects of caudal elevation (30° angle) and anatomic location of testes on spermatogenesis and steroidogenesis. Rats were subjected to caudal elevation for 7 days using either a tail harness (experiments 1 and 2) or a whole-body harness (experiment 3). Testes of rats fell into the abdominal cavity when a tail harness was used, but ligation of the inguinal canal prevented this repositioning. For rats with abdominal testes, testicular weight was reduced ( $P < 0.05$ ) and histology of testes was abnormal; the number of spermatids per gram parenchyma was lower ( $P < 0.05$ ) in tail-suspended rats compared with control rats. In con-

trast, spermatogenesis was not affected by caudal elevation in most rats in which the inguinal canal was ligated or in rats elevated by whole-body harness. Concentrations of testosterone in serum and testicular interstitial fluid were lower ( $P < 0.05$ ) in suspended rats, regardless of the method used for caudal elevation or anatomic location of testes. Concentrations of luteinizing hormone in serum were elevated ( $P < 0.05$ ) in rats with intra-abdominal testes. The authors conclude that caudal elevation of rats using either a whole-body harness or a tail harness after ligation of the inguinal canal results in altered steroidogenesis but normal spermatogenesis. Because this is similar to what occurs in the testes of male rats exposed to space flight, caudal elevation can be a useful model system to study the mechanisms responsible for reduced testosterone secretion during exposure to microgravity.

Key words: Hind-limb unloading, testicular function, testosterone, spermatogenesis, microgravity, rats.

J Androl 1992;13:224-231.

During space flight, humans and laboratory animals experience microgravity. A variety of biologic processes are perturbed after exposure to microgravity for more than 7 days, including changes in calcium homeostasis (Whedon et al, 1976), bone growth (Patterson-Buckendahl et al, 1987), muscle mass (Fast et al, 1985), and secretion of growth hormone (Grindeland et al, 1987). Space flight has been reported to affect both the exocrine and endocrine functions of testes. Slight changes occurred in the histology of the seminiferous tubules in testes after a 7-day flight on Space Lab-3 (Phillpott et al, 1985), and the number of B-spermatogonia per tubular cross section was reduced in

rats flown for 14 days on Cosmos 1887 (Sapp et al, 1990). Serova et al (1989), however, concluded that spermatogenesis was not affected in rats flown 7 or 13 days in Cosmos-1667 or Cosmos-1887, respectively. Serum and testicular concentrations of testosterone were lower in rats after return from space flight (Amann et al, 1992; Grindeland et al, 1990). Thus, endocrine function of the testes probably is altered during exposure to microgravity.

Animal model systems can be used to mimic certain effects of space flight and study mechanisms by which microgravity alters biological systems. A caudal-elevation rat model, in which the hind limbs are unloaded by use of a tail suspension harness (Morey-Holton and Wronski, 1981), or a whole body harness (Musacchia et al, 1980), frequently is used. Muscle atrophy and loss of calcium from bone occur during a 7- to 14-day period of suspension (Roer and Dillaman, 1990), and these changes are similar in many respects to those observed during space flights of similar duration. In rats and rabbits, as contrasted with humans, the inguinal canal remains open throughout life, so that the

This work was supported by funds provided by the Pennsylvania State University, Center for Cell Research and NASA Grants #N-AGW-1196 and NAG 2-386.

Correspondence to: Dr. Daniel R. Deaver, Department of Dairy and Animal Science, Penn State University, University Park, Pennsylvania 16802.

Received for publication June 26, 1991; accepted for publication November 4, 1991.



testes are held in the scrotum by a combination of gravity and intra-abdominal pressure, except when retracted by contraction of the external cremaster muscle. When a caudal-elevation model is obtained using a whole-body harness, the testes remain in the scrotum (Musacchia and Dombrowski, personal communication). When male rats are elevated using a tail harness, however, their testes fall into and remain in the abdominal cavity, resulting in destruction of the seminiferous epithelium and decreased testicular weight (Serova et al, 1989). Because spermatogenesis was stopped and testicular weight was reduced in caudal-elevation rats but remained essentially normal in flight rats, it was concluded (Serova et al, 1989) that the caudal-elevation rat model obtained with a tail-suspension harness was not appropriate for studying the effects of microgravity on testicular function.

It must be assumed that translocation of the testes to the abdominal cavity has confounded all studies in which a tail harness was used to provide caudal elevation. In a preliminary study, we found that placement of appropriately loose ligatures around the inguinal canal constricted this passageway so that the testes could not fall or be retracted into the abdominal cavity, but did not constrict blood flow or lymphatic drainage; the testes remained in the scrotum when the rats were elevated. Herein we report the results of three experiments on caudal-elevation rats designed to differentiate testicular changes associated with retraction of the testes into the abdominal cavity (cryptorchidism and heat) from those induced by repositioning the testes above the heart and by altered fluid flow.

## Materials and Methods

In experiments 1 and 2, a harness around the base of the tail (Morey-Holton and Wronski, 1981) was used to provide caudal elevation of about 30°. Animal phases of these experiments were conducted at Penn State University. In experiment 3, however, a whole-body harness (Musacchia et al, 1980) was used to provide 30° elevation. Animal phases of this experiment were conducted at the University of Louisville.

### Experiment 1

Sprague-Dawley rats (Harlan; 56 days old) were randomly assigned to one of three groups: 1) control with sham surgery (CON,  $n = 10$ ); 2) caudal elevation by tail harness (CET,  $n = 9$ ); or 3) caudal elevation with a tail harness after ligation of both inguinal canals (CET-L,  $n = 15$ ; Fig 1). Before and during the 7-day study, rats were maintained on a 14-hour light: 10-hour dark cycle and given free access to ground Purina Rat Chow #5010 (Ralston-Purina, St. Louis, MO) and water. For sham surgery or ligation of the inguinal canal, rats were anesthetized with 1:1 mixture of ketamine/rompun. Each inguinal canal was ligated using a purse-string stitch with 0000 braided silk suture on a cutting P-3 needle (Ethicon Suture Laboratories, Somerville, NJ); rats were suspended 2 to 6 hours after surgery. After 7 days, rats were removed

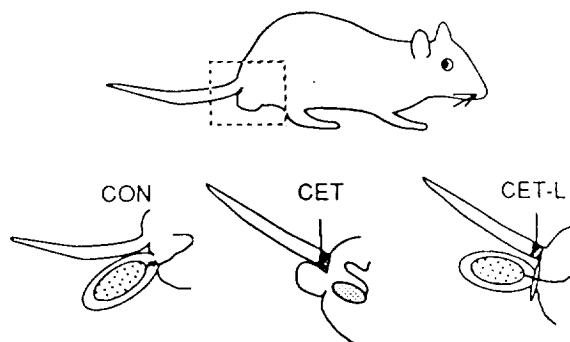


FIG. 1. Placement of ligatures and location of the testes during the experimental period.

from the suspension harness and were decapitated within 30 seconds (between 9:00 and 11:00 AM). Trunk blood, adrenal glands, testes, and epididymides were collected. Each testis was inspected, separated from the spermatic cord and epididymis, and weighed. One testis, generally the right, was processed as previously described (Turner et al, 1985) to collect interstitial (TIF) and seminiferous tubule fluid (SNF). The other testis was processed for histologic evaluation at Colorado State University. Both ends of the testis were cut off using a razor blade, and the major portion of the testis was placed into Bouin-Hollande fixative (Humanson, 1979) for 2 to 3 days, passed through graded ethanol, and imbedded in Paraplast (Sherwood Medical Industries, St. Louis, MO). Sections (5  $\mu$ m) were prepared and stained with periodic acid-Schiff plus hematoxylin. Sections representative of each testis were independently evaluated by two observers (RPA and DNRV) experienced in evaluation of testicular histology. Sections were classified as normal, subnormal, or abnormal. For testes not considered to be normal, subjective descriptions of defects were noted. To enable statistical analysis, subjective evaluations of testicular histology were coded as: 1.0, normal; 1.2, subnormal; or 3.0, abnormal. In addition, minor tubule and luminal diameters of 15 essentially round cross sections through seminiferous tubules were measured for each testis, using a BioQuant (R & M Biometrics, Nashville, TN) with a resolution of <1.0  $\mu$ m.

### Experiment 2

Forty 56-day-old male Sprague-Dawley (Harlan) rats were randomly assigned to one of four groups ( $n = 8$ /group): 1) control with sham surgery (CON), 2) control with both inguinal canals ligated (CON-L), 3) caudal elevation with a tail-harness (CET), and 4) caudal elevation with a tail-harness after ligation of both inguinal canals (CET-L). Ligation of the inguinal canal, housing, procedure for caudal elevation, and procedures for collection of fluids and tissues were undertaken as in experiment 1, except that both testes were processed to obtain TIF and SNF. After fluids were collected, testicular parenchyma was frozen at  $-80^{\circ}\text{C}$  until homogenized for determination of daily sperm production using a modification of a procedure previously described (Robb et al, 1978), rather than examined histologically. In brief, frozen tissue was thawed, weighed, and minced using razor blades. Tissue was transferred quantitatively using 2.0 mL buffer (0.5 mol/L TRIS-HCl, 0.23 mol/L sucrose, 5 mmol/L  $\text{MgCl}_2$ , and 4 mmol/L  $\text{NaN}_3$ )



into a 7-mL Dounce homogenizer (Wheaton, Millville, NJ), and subjected to 10 strokes with pestle-B followed by 10 strokes with pestle-A. Homogenate was centrifuged (800g for 10 minutes) at 4°C. Supernatant was removed and the pellet was resuspended in 2 mL buffer and homogenized a second time. After centrifugation, the pellet was resuspended in 5 to 10 mL 0.145 mol/L NaCl containing NaN<sub>3</sub> and 0.05% (vol/vol) Triton X-100; concentration of spermatid nuclei in the suspension was determined by cytometry. The resulting values, expressed on a per gram of testis basis, should provide a sensitive measure of the efficiency of spermatogenesis. Because the procedure for tissue preparation is different from that originally described (Robb et al, 1978), absolute values are not comparable with those reported by others (Robb et al, 1978; Amann and Berndtson, 1986).

### Experiment 3

Sprague-Dawley rats (Charles River; 85 days old) were conditioned for 7 days and adjusted to eating powdered food and drinking from a sipper tube (Musacchia et al, 1980). Rats were randomly assigned to one of two groups: 1) control (CON, n = 5) and 2) caudal elevation (CEB, n = 6).

Control rats were maintained in metabolism cages. Suspended rats were placed in a denim harness and attached to an aluminum fixture connected to a bracket and a swivel attached to the roof of the cage (Musacchia et al, 1980). The entire torso was inclined about 30° from the horizontal, and design of the harness minimized thermal stress. After 7 days, each rat was lightly anesthetized immediately after removal from the harness, and blood was taken by cardiac puncture; rats then were killed. The left testis and epididymis were trimmed and weighed. Blood serum was harvested, frozen, and shipped to the Pennsylvania State University for analysis of testosterone. The testis and epididymis were fixed as for experiment 1 and shipped to Colorado State University for further processing and evaluation.

### Radioimmunoassays

Concentrations of testosterone in serum, TIF, and SNF were determined using immunoreagents obtained from ICN (Carson, CA). For each experiment, all samples were evaluated in a single assay. Intra-assay and interassay coefficients of variation were less than 10%. Serum concentrations of luteinizing hormone (LH) were

determined using immunoreagents obtained from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Iodogen was used to iodinate NIDDK-rLH-I-7. Values for LH are expressed relative to NIH-rLH-RP3. Samples for each experiment were analyzed in a single assay, and intra-assay and interassay coefficients of variation were less than 10%.

### Statistical Analyses

For experiment 1, data for body weight, left or right testis weight, paired testis weight, testicular histology, seminiferous tubule diameter, and testosterone were subjected to one-way analysis of variance using the general-linear models procedure of SAS to test for differences among the three treatment groups. When the F-ratio was significant, a Student-Newman-Keuls test was used to partition the three treatment means. For experiment 2, data were subjected to two-way analysis of variance using the general linear model procedure of SAS to test for significance. Effects of position, ligation, and the interaction of position x ligation were included in the model. Because of the episodic nature of LH release, data for LH in experiments 1 and 2 were pooled (to increase the number of observations) and subjected to one-way analysis of variance using the general-linear models procedure of SAS as described for experiment 1. Data for experiment 3 were analyzed by *t* tests.

## Results

### Experiment 1

Body weight for both CET and CET-L rats was depressed ( $P < 0.05$ ) compared with CON rats (Table 1). Weight of the paired adrenal glands was similar for all three groups of rats. Weights of the left or right testes, or both testes together, however, were similar for the CET-L and CON rats; values for CET rats were about two-thirds those for the other two groups. Seminiferous tubule and lumen diameters also were low ( $P < 0.05$ ) for the CET rats when compared with CET-L and CON rats.

Subjective evaluations of testicular histology showed

Table 1. Characteristics of caudal elevation rats and control rats in Experiment 1

	CON (n = 10)	CET (n = 9)	CET-L* (n = 15)	Pooled SEM
Body weight (g)	303 <sup>a</sup>	278 <sup>b</sup>	268 <sup>b</sup>	8
Paired adrenal weight (g)	58.0 <sup>a</sup>	55.3 <sup>a</sup>	60.6 <sup>a</sup>	2.50
Left testis weight (g)	1.57 <sup>a</sup>	1.03 <sup>b</sup>	1.47 <sup>a</sup>	0.06
Right testis weight (g)	1.56 <sup>a</sup>	1.01 <sup>b</sup>	1.44 <sup>a</sup>	0.07
Paired testes weight (g)	3.13 <sup>a</sup>	2.04 <sup>b</sup>	2.91 <sup>a</sup>	0.12
Seminiferous tubule diameter (μm)	234 <sup>a</sup>	197 <sup>b</sup>	223 <sup>a</sup>	5.5
Lumen diameter (μm)	88 <sup>a</sup>	71 <sup>b</sup>	88 <sup>a</sup>	3.2
Histologic results	1.03 <sup>a</sup>	3.00 <sup>b</sup>	1.35 <sup>a</sup>	0.07
Testosterone (ng/ml)				
Serum	8.4 <sup>a</sup>	3.7 <sup>b</sup>	2.2 <sup>b</sup>	0.69
SNF	217.8 <sup>a</sup>	370.3 <sup>a</sup>	253.2 <sup>a</sup>	53.1
TIF	406.9 <sup>b</sup>	142.5 <sup>c</sup>	127.3 <sup>c</sup>	40.6

Means with different superscripts differ ( $P < 0.05$ ).

\* Sutures were placed loosely around both inguinal canals to prevent movement of testes into the abdominal cavity.



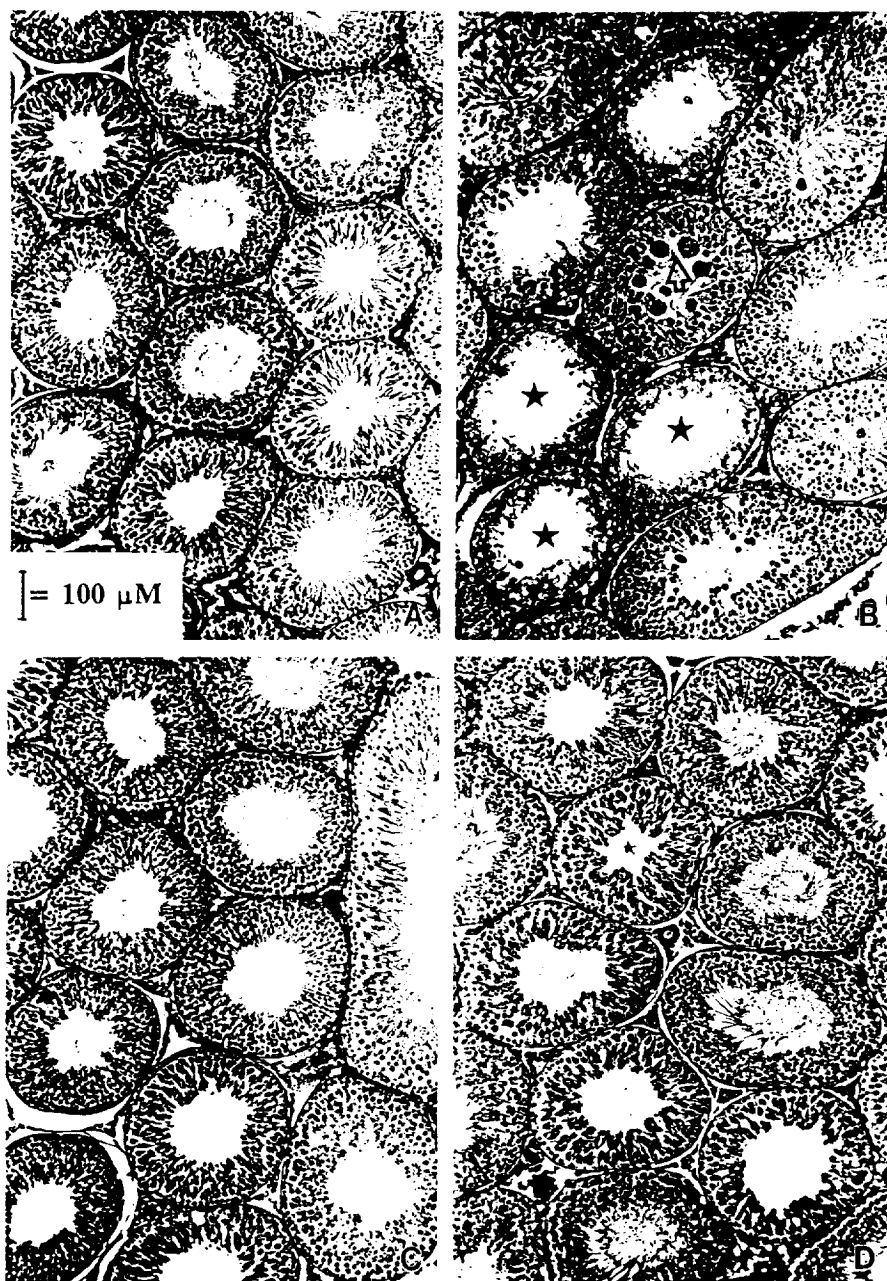
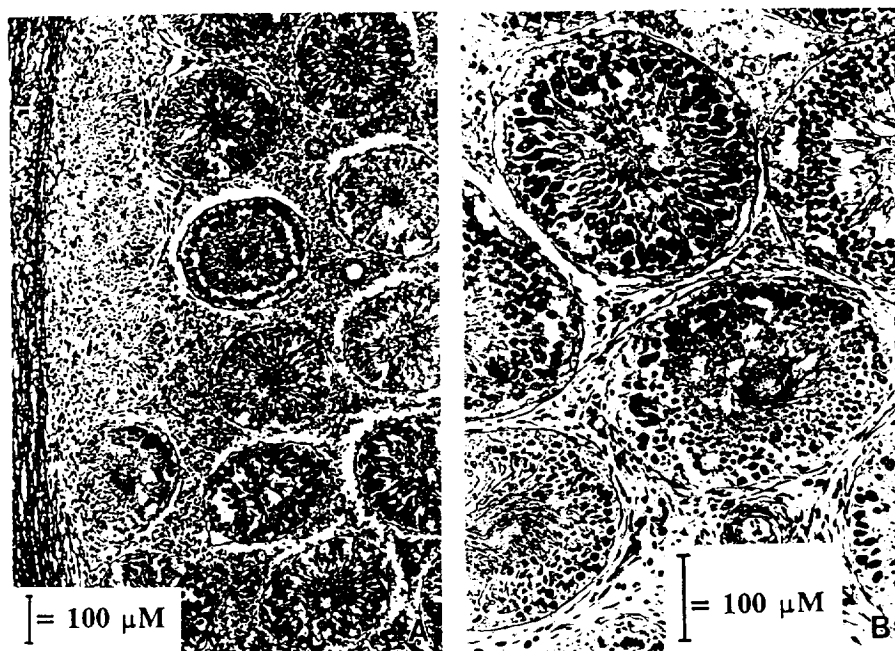


FIG. 2. Testicular tissue from rats representing control (A), caudal elevation by means of a tail harness (B), caudal elevation after bilateral ligation of the inguinal canals (C), and caudal elevation by means of a whole-body harness (D). In control rats and rats undergoing caudal elevation by means of a whole-body harness or after bilateral ligation, the seminiferous epithelium is normal and tubules are in different stages of the cycle of the seminiferous epithelium (A, C, and D). In testis of rats undergoing caudal elevation by means of a tail harness (B), various changes typical of heat-induced degeneration, such as pyknotic cell nuclei, desquamated germinal cells, and multinucleated giant cells (arrow) are evident; other seminiferous tubules (star) contain only vacuolated Sertoli cells and a few spermatogonia (periodic acid-Schiff and hematoxylin; original magnification  $\times 75$ ).

that the testis from each CON rat was normal, whereas the testis from each CET rat was abnormal (Fig 2). The latter testes contained seminiferous tubules in various degrees of degeneration (Fig 2B), characteristic of a heat-induced lesion (Niemi and Kormano, 1965; Steinberger and Dixon, 1959). In many seminiferous tubules, virtually all germinal cells were absent, and the seminiferous epithelium was characterized by the presence of Sertoli cells and spermatogonia (star in Fig 2B). In other tubule cross sections, some primary spermatocytes or spermatids persisted, but frequently they were present as coalesced, multinucleate primary spermatocytes or multinucleate spermatids (arrow in Fig 2B).

In CET-L rats, the results were somewhat variable. For nine of these 15 rats, the testis examined was indistinguishable from those of CON rats (compare Fig 2C and 2A). In four rats, subtle alterations were noted. These testes were characterized by the presence of slight congestion and sloughing of the seminiferous epithelium in a few peripheral tubules (Fig 3A); however, most of the tubules were normal. The observers concluded that some seminiferous tubules contained a reduced number of primary spermatocytes, or the presence of spermatocytes or spermatids with pyknotic nuclei. For each of these animals, the weight of each testis was normal. For two of the 15 rats, the seminiferous epithelium was very abnormal. For one of these





**FIG. 3.** Testicular tissue from caudal elevation rats after bilateral ligation of the inguinal canals in which the ligation procedure induced infarction and inflammation. Coagulative necrosis was evident in all seminiferous tubules (A). The testicular capsule and interstitium were severely infiltrated by leukocytes (chemotaxis), predominately neutrophils, indicating an acute inflammatory process (B). The seminiferous epithelium was completely necrotic, although architecture of the seminiferous tubules remained discernible. Nuclear staining was virtually lacking (B); only pyknotic nuclei in some early germinal cells (lower left) or condensed nuclei in elongated spermatids stained with hematoxylin (hematoxylin-eosin; original magnifications: A,  $\times 75$ ; B,  $\times 150$ ).

CET-L rats, both testes were small (0.73 and 0.94 g), and the animal technician noted that the testes had disappeared into the abdominal cavity after caudal elevation; the histologic appearance was as for a CET rat. For the other CET-L rat with abnormal spermatogenesis, the testis appeared "odd" at necropsy. Histologic examination (Fig 3B) showed congestion, extensive degeneration, and some coalesced multinucleate germ cells; a few seminiferous tubules were normal. The contralateral testis from this individual (not fixed for histologic evaluation) was small (0.98 vs 1.69 g) and presumably had been retracted into the abdominal cavity. Thus, it was concluded that, except for rats where the ligatures failed to maintain proper positioning of the testes outside the abdominal cavity, spermatogenesis was normal in CET-L rats. Conversely, in CET rats the testes fell into the abdominal cavity shortly after elevation and histology of the seminiferous epithelium was characteristic of that expected for an adult rat made cryptorchid.

Concentrations of testosterone in serum and TIF were lower ( $P < 0.01$ ) in CET and CET-L rats than in CON rats (Table 1). Concentration of testosterone in SNF was similar ( $P > 0.1$ ) among all three groups (Table 1). Thus, ligating

the inguinal canal prevented destruction of the seminiferous epithelium in most rats, but not the reduction in testosterone content of serum or TIF.

### Experiment 2

Body weight of caudal elevation rats was lower ( $P < 0.05$ ) than that of CON rats. There was a significant interaction (caudal elevation  $\times$  ligature) for testicular weight and numbers of homogenization-resistant spermatids. Ligatures around the inguinal canal prevented displacement of testes into the abdominal cavity, and testicular weights were similar for CON, CON-L, and CET-L rats, but greater than that for CET rats (Table 2). Concentrations of testosterone in serum and TIF were lower in CET and CET-L rats compared with the CON and CON-L groups (Table 2).

### Serum Concentrations of Testosterone and LH

Dislocation of testes into the abdominal cavity was associated with a decrease in serum testosterone, and an increase in serum LH (Fig 4). Serum from CET-L rats had a con-

Table 2. Data for rats used in Experiment 2\*

Variable	CON (n = 8)	CON-L (n = 8)	CET (n = 8)	CET-L (n = 8)	Pooled SEM
Body weight (g)	326 <sup>a</sup>	313 <sup>a</sup>	288 <sup>b</sup>	285 <sup>b</sup>	8
Testicular weight (g)	3.29 <sup>a</sup>	3.24 <sup>a</sup>	2.05 <sup>b</sup>	3.24 <sup>a</sup>	0.06
Spermatids†	57.6 <sup>a</sup>	52.2 <sup>a</sup>	20.6 <sup>b</sup>	52.0 <sup>a</sup>	5.1
Serum testosterone (ng/ml)	7.9 <sup>a</sup>	6.5 <sup>a</sup>	3.7 <sup>b</sup>	2.9 <sup>b</sup>	0.56
TIF testosterone (ng/ml)	641.1 <sup>a</sup>	507.0 <sup>a</sup>	274.7 <sup>b</sup>	216.4 <sup>b</sup>	22.6

\* Means with different superscripts are different ( $P < 0.05$ ).

† Values are expressed as millions of homogenization-resistant spermatids per pair of testes.



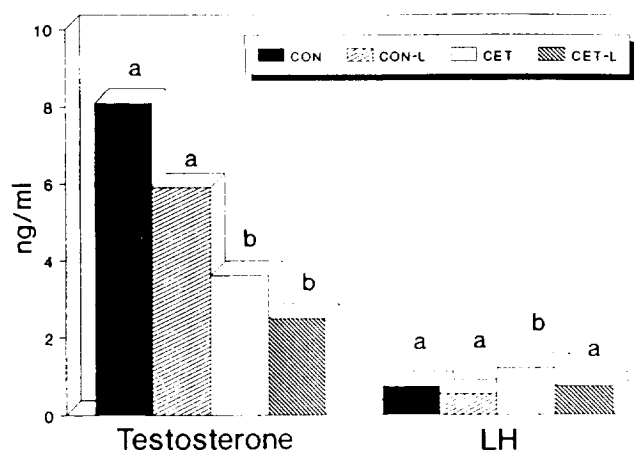


FIG. 4. Concentrations of luteinizing hormone (LH) and testosterone in serum of control rats and rats elevated using a tail harness. Values are presented as means; means with different superscripts differ at  $P < 0.05$ . Data from Experiments 1 and 2 were pooled and means are based on 18, 8, 17, and 21 rats per group for control rats (CON), CON-L, rats elevated using a tail harness (CET), and CET-L, respectively. Serum concentrations of LH were higher in rats elevated using a tail harness than in the other groups. In contrast, testosterone was lower in these rats and CET-L rats than in control rats and CON-L rats.

centration of LH that was similar to values for nonelevated rats (about 0.6 ng/mL), with two exceptions. The concentration of LH was 2.65 and 2.35 ng/mL in the two CET-L rats in experiment 1, with severe lesions of the germinal epithelium.

### Experiment 3

For six CEB rats suspended using a whole-body harness, body weight declined from 372 g to 328 g over 7 days; CON rats weighed 414 g when killed. Left testis weights for CON and CEB rats and other attributes of testis function were similar (Table 3). Concentrations of testosterone in serum were higher in CON rats than in CEB rats ( $2.8 \pm .8$  vs  $0.8 \pm .3$  ng/mL, respectively;  $P < 0.05$ ). Also, the morphology of testes of CEB (Fig 2D) rats was similar to that of the CON group.

Table 3. Data for rats in Experiment 3\*

	CON (n = 5)	CEB (n = 6)
Body weight	414 <sup>a</sup>	329 <sup>b</sup>
Left testis weight (g)	1.69	1.70
Spermatids/g	68.7	85.2
Seminiferous tubule diameter ( $\mu$ m)	244	246
Lumen diameter ( $\mu$ m)	98	101
Serum testosterone (ng/ml)	2.8 <sup>a</sup>	0.8 <sup>b</sup>

\* Means in rows with different superscripts differ ( $P < 0.05$ ).

### Discussion

As an alternative to space flight, investigators have used one of several caudal elevation models to simulate the effects of microgravity on the musculoskeletal system of the hind limbs (Hargens et al, 1984; Morey-Holton and Wronski, 1981; Musacchia et al, 1980; Tsika et al, 1987). Our discussions in 1988, while planning experiments that were to use a tail harness to provide a caudal elevation model to study gonadotropin secretion, led us to question the normalcy of the hypothalamic-pituitary-testicular axis in such rats. It is common knowledge that the inguinal canals in male rats remain patent throughout life, and that contraction of the external cremaster muscles result in retraction of the testes into the abdominal cavity; normally they are pushed back out by the combined action of intra-abdominal pressure and unit gravity. Thus, in normal rats, the testes are not exposed to abdominal temperature ( $38.2^{\circ}\text{C}$ ) for prolonged intervals, and the deleterious effects of prolonged exposure to body temperature greater than  $42^{\circ}\text{C}$  for more than 2 hours (Blackshaw et al, 1973; Niemi and Kormano, 1965; Steinberger and Dixon, 1959; VanDemark and Free, 1970) are avoided.

Serova and co-workers (1989) summarized data for male rats on the COSMOS-1667 and COSMOS-1887 flights of 7 and 13 days duration, respectively. They concluded that exposure to microgravity did not induce a difference in testicular weight or water content, spermatogenesis, or epididymal weight and sperm content. They noted, however, that after 14 days of caudal elevation (a simulation treatment in their study), the testes and epididymides of rats elevated using a tail harness were less than one-half the weights of those in control animals. Testes of caudal-elevation rats contained about 9 million spermatids as compared with 460 million in control rats. Also, other germ cells were reduced in number. The number of Sertoli cells and Leydig cells per testis, however, were only slightly changed. As we had independently postulated, they concluded (Serova et al, 1989) that testicular changes induced in rats by caudal elevation using a tail harness were a consequence of anatomic dislocation of the testes into the abdominal cavity. They speculated that the changes were at least partially attributable to alterations in the blood supply to the testis.

Our observations on spermatogenesis in experiments 1 and 2 confirm those previously reported (Serova et al, 1989), but make an important further observation. Testosterone production is not normal in caudal elevation rats, regardless of whether the testes were in the scrotum (CET-L or CEB rats) or in the abdominal cavity (CET rats). We attribute destruction of the seminiferous epithelium to elevated intratesticular temperature, but altered function of Leydig cells to changes in the fluid distribution within the



body or alterations of blood flow and fluid distribution within each testis, induced by positioning the testes above the heart. There is a wealth of literature on the adverse effects of temperature on spermatogenesis (Clegg, 1963; Chowdhury and Steinberger, 1964), and our observations (Fig 3B) are similar to those reported by others (Steinberger and Dixon, 1959; Niemi and Kormano, 1965). There are no published data for caudal elevation rats concerning blood flow to the testis and in the microcirculation, or concentrations of lipoprotein, oxygen, and glucose in the interstitial fluid bathing Leydig cells and seminiferous tubules. Thus, it is impossible to establish the direct cause for alterations in the function of Leydig cells.

Importantly, our data show that degeneration of the seminiferous epithelium induced in rats elevated by a tail harness does not occur in animals elevated by whole-body harness (the testes remain scrotal), or it can be avoided by judiciously restricting the inguinal canals to prevent movement of the testes into the abdominal cavity. Thus, using rats with ligatures around the inguinal canals, it is possible to maintain normal spermatogenesis for at least 7 days of caudal elevation using a tail harness. Because a few of the CET-L rats did not maintain normal spermatogenesis, however, a more efficacious procedure might be to use a whole-body harness. By either approach, spermatogenesis should remain normal despite perturbation of the pituitary-testicular axis. This differential effect was not anticipated, but actually mimics what apparently occurs during space flight (Amann et al, 1991). For normal rats flown 2 weeks on Cosmos-2044, spermatogenesis was essentially normal, although there were significant reductions of both plasma and intratesticular concentrations of testosterone. Certainly these changes must reflect altered function of the Leydig cells, despite the fact that Leydig cells maintained a normal surface density of smooth endoplasmic reticulum, and testicular tissue contained a normal concentration of receptors for LH (Amann et al, 1991). Although the effects of space flight on the secretion of LH in either rats or humans is unknown, ability of the adenohypophysis to secrete LH *in vitro*, after reentry and landing, was not compromised in rats flown on Cosmos-2044. Thus, CET-L responded in a manner similar to that of the flight rats on Cosmos-2044.

Based on other observations (Amann et al, 1991) and data herein, we conclude that when the testes remain within the scrotum, caudal elevation provides a useful model for flight animals. With these model systems, modifications of Leydig cell functions occur that mimic what appears to happen in microgravity. Indeed, if testosterone production, and hence concentrations of testosterone in peripheral blood, did not decrease, the model would be invalid. Only a model depriving tissues of androgenic stimulation mimics what apparently happens in microgravity. Without such a reduction in androgen stimulation, effects of hind-limb un-

loading or disuse of muscle, bone, or other body systems might result in erroneous conclusions. Conversely, as suggested earlier (Amann et al, 1991), beneficial effects of prophylactic steroid therapy on the maintenance of muscle mass or bone density of mammals exposed to microgravity should be considered. Such therapy is beneficial in reducing muscle changes in caudal-elevation rats (Tsika et al, 1987) and has been suggested to alleviate muscle and bone loss in hypogonadal men. Furthermore, estradiol therapy is used routinely for postmenopausal women and might be appropriate for female astronauts, but data on female mammals in microgravity are not available.

Although we postulate that reduction of testosterone production in CET-L or CEB rats was a function of altered fluid distribution or blood flow, this may not be the entire cause for reduced testosterone production in CET rats, in which the testes were repositioned within the abdominal cavity. Concentrations of testosterone in peripheral blood decrease after experimentally induced cryptorchidism (Kerr et al, 1979), and concentrations of LH in blood increase between 7 and 10 days (Amatayakul et al, 1971; Altwein and Gittes, 1972; Walsh and Swerdloff, 1973; Kerr et al, 1979). It has been suggested (Collins et al, 1978; Kerr et al, 1979) that increased secretion of testosterone is secondary to degeneration of the seminiferous epithelium, and that LH increases as a consequence of reduced negative feedback of gonadal steroids. These responses may have occurred in caudal-elevation rats in which the testes fell into the abdominal cavity, but did not occur in CEB rats or most CET-L rats. Similarly, these concepts do not explain the decreased production of testosterone in flight rats (Amann et al, 1991).

Although intratesticular and serum concentrations of testosterone were lower in CET and CEB rats than in CON rats, spermatogenesis was normal. Normal spermatogenesis can occur at intratesticular concentrations of testosterone of about 20 to 25% of those found in normal animals (Zirkin et al, 1989). Thus, intratesticular concentrations of testosterone in the CET-L and CEB rats probably were slightly above the minimum threshold requirement. A similar phenomena likely occurs in rats exposed to microgravity (Amann et al, 1991). It is not known if caudal elevation of rats for 2 to 4 weeks, rather than only 7 days, might result in a further decline in testosterone production and a resultant destruction of the seminiferous epithelium.

Our reason for undertaking this study was to determine if we could differentiate between the effect of caudal elevation *per se* from changes induced by repositioning the testes to the abdominal cavity. Based on our findings, it is clear that disruption of exocrine and endocrine functions of the testes in the typical model, in which caudal elevation is achieved using a tail harness, are due to establishment of a cryptorchid condition. Caudal elevation *per se*, however, by intro-



ducing a postural change and subsequent effects on blood or fluid distribution, also affects testicular function. These latter changes probably are similar to those that occur in microgravity, but the exact causes for changes detected in rats exposed to microgravity or caudal elevation, with retention of the testes in the scrotum, are unknown.

## Acknowledgments

The authors thank J. L. Peters (The Pennsylvania State University) and M. J. Dombrowski (University of Louisville) for their technical assistance, and the NIH Hormone Distribution Program for the immunoreagents used to assay LH.

Since this manuscript was submitted we have learned of another set of experiments conducted at the Pennsylvania State University using the ligation system. This independent team of investigators found no differences in blood or testicular testosterone between sham control and tail-suspended animals (Hadley et al, 1992). Several important differences existed between the studies reported herein and those conducted by Dr. Hall and his colleagues. The following variances in protocols have been identified, and others may exist: 1) personnel involved in the suspension experiments were different; 2) the period from ligation of the inguinal canals and the beginning of the suspension procedure; 3) procedure used for euthanasia; 4) fewer rats per treatment group; and 5) different reagents were used for the testosterone and LH assays.

## References

- Altwein JE, Gittes RF. Effect of cryptorchidism and castration on FSH and LH levels in the adult rat. *Invest Urol*. 1972;10:167-180.
- Amann RP, Berndtson WE. Assessment of procedures for screening agents for effects on male reproductive function: effects of dibromochloropropene (DBCP) on the rat. *Fundam Appl Toxicol*. 1986;7:244-255.
- Amann RP, Deaver DR, Zirkin BR, Grills GS, Sapp WJ, Veeramachaneni DNR, Clemens JW, Banerjee SD, Gruppi CM, Wolgemuth DJ, Williams CS, Serova LV. Effects of microgravity or simulated launch on testicular function in rats. *J Appl Physiol*. 1992. In Press.
- Amatayakul R, Ryan R, Uozumi T, Albert A. A reinvestigation of testicular-anterior pituitary relationship in the rat: I. Effects of castration and cryptorchidism. *Endocrinology*. 1971;88:872-880.
- Blackshaw AW, Hamilton D, Massey PF. Effect of scrotal heating on testicular enzymes and spermatogenesis in the rat. *Aust J Biol Sci*. 1973;26:1395-1407.
- Clegg EJ. Studies on artificial cryptorchidism: morphological and quantitative changes in the sertoli cells of the rat testis. *J Endocrinol*. 1963;26:567-574.
- Chowdhury AK, Steinberger E. A quantitative study of the effect of heat on the germinal epithelium. *Am J Anat*. 1964;115:509-524.
- Collins PM, Collins WP, McNeilly AS, Tsang WN. Plasma FSH, LH and testosterone levels in the male rat during degeneration of the germinal epithelium caused by severe heat treatment or ligation of the vasa efferentia. *J Reprod Fertil*. 1978;54:285-291.
- Fast T, Grindeland R, Kraft L, Ruder M, Vasques M, Lundgren P, Scibetta S, Tremor J, Buckendahl P, Keil L, Chee O, Reilly T, Dalton B, Callahan P. Maintenance in the research animal holding facility during the flight of Space Lab 3. *Physiologist*. 1985;28:S187-S188.
- Grindeland R, Hymer WC, Farrington M, Fast T, Hayes C, Motter K, Patil L, Vasques M. Changes in pituitary growth hormone cells prepared from rats flown on Space Lab 3. *Am J Physiol*. 1987;252:R209-R215.
- Grindeland RE, Popova IA, Vasques M, Arnaud SB. Cosmos 1887 mission overview: effects of microgravity on body and adrenal weights and plasma constituents. *FASEB J*. 1990;4:105-109.
- Hadley JA, Hall JC, O'Brien A, Ball R. *J Appl Physiol*. 1992;72:748-759.
- Hargens AR, Steskal J, Johansson C, Tipton CM. Tissue fluid shift, and tail tension in tail-suspended rats. *Physiologist*. 1984;27:S37-S38.
- Humanson GL. *Animal Tissue Techniques*. 4th edition. San Francisco: WH Freeman, 1979.
- Kerr JB, Rich KA, De Kretser DM. Alterations of the fine structure and androgen secretion of the interstitial cells in the experimentally cryptorchid rat testis. *Biol Reprod*. 1979;20:409-422.
- Morey-Holton ER, Wronski TJ. Animal models for simulating weightlessness. *Physiologist*. 1981;24(Suppl):S45-S48.
- Musacchia XJ, Deavers DR, Meininger GA, Davis TP. A model for hypokinesia: effects on muscle atrophy in the rat. *J Appl Physiol*. 1980;48:479-486.
- Niemi M, Korman M. Response of the cycle of the seminiferous epithelium of the rat testis to artificial cryptorchidism. 1965;16:236-242.
- Patterson-Buckendahl P, Arnaud S, Mechanic G, Martin R, Grindel R, Cann C. Fragility and composition of growing rat bone after 1 week in space flight. *Am J Physiol*. 1987;252:R240-R246.
- Phillipott DE, Sapp W, Williams C, Stevenson J, Black S, Corbett R. Reduction of the spermatogonial population in rat testes flown on Space Lab 3. *Physiologist*. 1985;28:S211-S212.
- Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J Reprod Fertil*. 1978;54:103-107.
- Roer RD, Dillaman RM. Bone growth and calcium balance during simulated weightlessness in the rat. *J Appl Physiol*. 1990;68:13-20.
- Sapp WJ, Phillipott DE, Williams CW, Kato K, Stevenson J, Vasques M, Serova LV. *FASEB J*. 1990;4:102-104.
- Serova LV, Denisova LA, Baihova OV. The effect of microgravity on the reproductive function of male rats. *Physiologist*. 1989;32:S29-S30.
- Steinberger E, Dixon WJ. Some observations on the effect of heat on the testicular germinal epithelium. *Fertil Steril*. 1959;10:578-595.
- Tsika RW, Herrick RE, Baldwin KM. Effect of anabolic steroids on overloaded and overloaded suspended skeletal muscle. *J Appl Physiol*. 1987;63:2128-2133.
- Turner TT, Ewing LL, Jones CE, Howards SS, Zegeye B. Androgens in various fluid compartments of the rat testis and epididymis after hypophysectomy and gonadotropin supplementation. *J Androl*. 1985;6:353-358.
- VanDemark NL, Free MJ. Temperature effects. In: Johnson AD, Gomes WR, Vandemark NL, eds. *The Testis*. New York: Academic Press. 1970;3:233-312.
- Walsh PC, Swerdloff RS. Experimental cryptorchidism: effect on serum LH and FSH in the rat. *Urol Res*. 1973;1:22-26.
- Whedon GD, Lutwak L, Rambaut P, Whittle M, Reid J, Smith M, Leach C, Stadler C, Sanford D. Mineral and nitrogen balance study observations: the second manned Skylab mission. *Aviat Space Environ Med*. 1976;47:391-396.
- Zirkin BR, Samulli R, Awoniyi CA, Ewing LL. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentrations within the testis. *Endocrinology*. 1989;124:3043-3049.