Long-term (6-wk) hindlimb suspension inhibits spermatogenesis in adult male rats

JOSEPH S. TASH,1 DONALD C. JOHNSON,1 AND GEORGE C. ENDERS2
1Department of Molecular and Integrative Physiology and 2Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, Kansas 66160

Received 10 September 2001; accepted in final form 31 October 2001

Tash, Joseph S., Donald C. Johnson, and George C. Enders. Long-term (6-wk) hindlimb suspension inhibits spermatogenesis in adult male rats. J Appl Physiol 92: 1191–1198, 2002. The International Space Station will allow extended habitation in space and long-term exposure to microgravity (μG). A concern is the impact of long-term μG exposure on the ability of species to reproduce. The model often used to simulate μG is rat hindlimb suspension (HLS), where the hindlimbs are elevated above the cage floor with a tail harness. Experiments described here are the first to examine the effect of long-term HLS on testicular function in adult male rats. Free-roaming (controls), animals with only the tail harnessed but hindlimbs in contact with the cage floor (TO), and HLS animals were tested for 6 wk. Cryptorchidism was prevented in TO and HLS animals by partial constriction of the inguinal canal with sutures. All parameters were compared at the end of the 6-wk experiment. Testicular weights and spermatogenesis were significantly reduced by HLS, such that no spermatogenic cells beyond round spermatids were present and epididymides were devoid of mature sperm. In many tubules, loss of all germ cells, except a few spermatagonia, resulting in histopathology similar to the Sertoli cell, was observed. Spermatogenesis appeared unaffected in control and TO animals. Sertoli and Leydig cell appearance, testosterone, luteinizing hormone, and follicle-stimulating hormone levels, and epididymal and seminal vesicle weight were unchanged by HLS. Cortisone was not elevated by HLS; thus stress may not be a factor. These results demonstrate that spermatogenesis is severely inhibited by long-term HLS, whereas testicular androgen production is not. These results have significant implications regarding serious effects of long-term exposure to μG on the reproductive capability of scrotal mammals, including humans.

Testis; microgravity; simulation; spaceflight; fertility

AS ASSEMBLY OF THE International Space Station proceeds and long-term habitation in microgravity (μG) is realized, the National Aeronautics and Space Administration has expressed the need to determine whether the ability of organisms to reproduce is impacted in the μG environment. With regard to the male, the capacity to reproduce is determined by 1) the ability of the testis to produce, in sufficient numbers, spermatocytes with normal morphology and complement of haploid DNA and 2) whether these sperm can mature, become motile, and ultimately bind to and fertilize the egg.

In ground-based studies, hindlimb suspension (HLS) has been widely used as a model for mimicking the physiological changes (loss of bone and muscle mass) that occur in μG and employed as a ground control for μG flight experiments (6, 10, 30, 36, 41, 46, 48, 49). With regard to an impact of μG on the testis and spermatogenesis, a rapid decline in circulating testosterone has been consistently observed in rats (in biosatellite experiments) and astronauts during Space Shuttle flights (3, 15, 28, 47). The decline in testosterone has been observed, even in the absence of increased cortisol, suggesting that a corticosteroid-associated stress response is unlikely to be responsible for the androgen decline and the associated bone loss in such animals (28). However, the stress response appears to be variable in different studies. Some studies found increased cortisol in μG or during HLS, whereas other studies showed no significant change (14, 20, 21, 25, 33, 45, 47, 50).

In some HLS studies, the experimental design did not account for the anatomy of the rat that allows the testes to enter the abdomen through the inguinal canal when the hindlimb is elevated (37, 54). In such experiments, it is likely that changes in testicular function can be, to a large extent, attributed to changes resulting from hyperthermic cryptorchid testes. For example, in the HLS ground controls for COSMOS-1667 and COSMOS-1887, the inguinal canal was not partially ligated to prevent cryptorchidism in the rats (42). Failure to ligate the inguinal canal in HLS animals clearly allows the testes to become abdominal and causes marked and rapid reduction in the population of spermatogenic cells (3). In later studies, the potential of cryptorchidism was eliminated by partial ligation of the inguinal canal. In such experiments, Amann et al. (3) demonstrated a marked drop in circulating testosterone and an increase in luteinizing hormone (LH) after 7 days of HLS in 56-day-old rats. In 27% of these HLS animals, there were reductions in the diameter of

Address for reprint requests and other correspondence: J. S. Tash, Dept. of Molecular and Integrative Physiology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160-7401 (E-mail: jtash@kumc.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org 8750-7587/02 $5.00 Copyright © 2002 the American Physiological Society

1191
the seminiferous tubules and some losses of spermatogenic cells. In animals exposed to μG for 14 days, circulating testosterone and seminiferous tubule diameter were significantly reduced and the number of germ cells per tubule cross section decreased compared with the HLS ground controls for COSMOS-2044 (3). After a 22-day exposure to μG on COSMOS-605, rats showed an increase in relative testicular weight, calculated as testicular weight ÷ body weight (34). However, if the actual mean testicular weights are recalculated from their data, then a decrease in testicular weights relative to controls was observed in μG. Although the data for the HLS animals (simulated flight controls) for COSMOS-1887 cannot be used (no inguinal ligation was performed), there were significant reductions in circulating testosterone levels, testis weight, and numbers of spermatogonia in the flight animals vs. the free-roaming controls (15, 39). Similar results were obtained for male rats flown on the 7-day Space Shuttle Space Lab 3 (STS-51B) mission (32).

On the basis of the above observations, the hypothesis for these studies is that long-term HLS of adult male rats inhibits spermatogenesis. The studies summarized above have focused on simulating μG exposure times similar to the duration of most Space Shuttle flights (7–16 days). Until the study reported here, no investigations have been published concerning effects of μG or HLS on testicular function under long-term conditions similar to that experienced on the International Space Station or planned for long-term spaceflights such as the manned Mission to Mars. We report here that extending HLS to 6 wk in mature adult rats yielded much more dramatic and seriously negative changes in spermatogenesis. These new findings support the suggestion of Anann et al. (3) that exposure to μG for >2 wk might result in additional negative changes in spermatogenesis and testicular function. These results are particularly important if similar results are obtained in μG. If this proves to be the case, then astronauts returning to Earth after long exposure to μG may be infertile.

MATERIALS AND METHODS

Materials. All reagents were American Chemical Society grade or better unless otherwise specified. Water was MilliQ (Millipore, Bedford, MA) deionized to $\geq 18.2$ MΩ.

Rat HLS and control groups. HLS cages were constructed following designs supplied by Dr. Emily Holton (30, 56) with modifications published by Park and Schultz (31). Briefly, a harness consisting of strips of Skintrac (Zimmer, Lenexa, KS) is fastened to the tail with tincture of benzoin aerosol spray (Professional Packaging, Aurora, IL) and filament tape and then attached to a suspension device above specially designed cages. The suspension device elevates the hindlimbs above the cage floor at an angle of 30° but allows free movement of the animals with the forelimbs while preventing the hindlimbs from touching the cage floor or walls. All animals (Fisher 344, National Institutes of Health) were 13- to 15-mo-old retired breeders of proven fertility and were handled frequently for 1 wk before the start of experiments. A second group of animals (tail-only (TO) controls) was also tail-harnessed, sutured at the inguinal canal, and attached to the suspension device, but the hindlimbs were allowed to continue to have full contact with the cage floor. A purse-string stitch around the inguinal canal sufficiently tight to keep the testes in the scrotum but not restrict blood flow (3) was applied to the HLS and TO groups. The TO control group was important to discriminate effects of HLS from harnessing and ligation. The same type of cage used for the HLS and TO groups was used to house the third group of animals, free-roaming controls. All groups consisted of at least six animals. The animal care protocol for rat tail-harnessed HLS utilized previously (30) was followed in this study (7, 19, 51, 56). All animals were housed in individual cages with water provided ad libitum. HLS rats were fed ad libitum. Because of differences in food consumption by HLS animals, the control groups (TO and free roaming) were fed the mean of the food consumption of the corresponding HLS group so that all groups had the same caloric intake. Animals were maintained on a 12:12-h light-dark cycle and at 24°C, a metabolically neutral temperature where rats neither gain nor lose heat.

Hormone assays. Blood was collected from each animal in all groups at the time of death at the end of the 6-wk experiment. All hormone assays were performed on serum from each animal in triplicate. Follicle-stimulating hormone (FSH) and LH were assayed by radioimmunoassay with reagents provided by Dr. Paul Terranova (University of Kansas Medical Center). Sensitivities of the hormone assays were 62.5 pg/ml for testosterone, 8.66 ng/ml for FSH, 0.1 ng/ml for LH, and 25 ng/ml for corticosterone. Intra-assay coefficients of variation were 4.5% for testosterone, 12.4% for FSH, 5.0% for LH, and 0.43% for corticosterone. Interassay coefficients of variation were 3.4% for testosterone, 5.4% for FSH, and 4.0% for LH. Corticosterone values were determined in a single assay of triplicates; thus there is no value for interassay coefficient of variation.

Whole body weight, organ weight analysis, testis histology, and morphometry. During the 6-wk experiment, animals were weighed to monitor nutrition and general health. The HLS animals were weighed by using a suspension apparatus on the electronic scale so that the hindlimbs remained suspended during weighing. At the end of 6 wk, after exsanguination by decapitation and collection of blood, the testes were removed, separated from the epididymides, and weighed with the tunica albuginea intact. Epididymal and seminal vesicle (SV) weights were also determined at this time. One testis and one epididymis were fixed in Bouin's fixative and processed in paraffin for sectioning and staining with Gill's hematoxylin and no counterstaining. The other testis from each animal was processed for counting of Triton X-100 homogenization-resistant spermatogenic cell nuclei (elongating spermatids and testicular sperm) by using a fixed ratio of testis weight per milliliter of homogenization solution by the method of Robb et al. (35). Data for sperm count are expressed as number of sperm per milliliter of testis homogenate. Seminiferous tubule and luminal diameters were quantitated on digital images of round cross sections of the tubules collected by using bright-field illumination with a ×40 objective on an inverted microscope (Optiphot, Nikon, New York, NY) and a MagnaFire camera (Optronics, Goleta, CA). Digital image analysis with Optimas version 6.5 (Media Cybernetics, Silver Spring, MD) was used to measure seminiferous tubule and lumen diameters on ~10 randomly cho-
RESULTS

HLS of adult male rats for 6 wk reduces testicular weight and inhibits spermatogenesis. After 6 wk of HLS, rats showed a significant decline in testicular weight compared with the control and TO groups (Table 1). The TO group showed no significant difference from the control group. A significant drop in the number of testicular sperm and elongating spermatids accompanied the decline in testicular weight in the HLS animals as well (Table 1). Elongating spermatids and testicular sperm counts were determined by counting homogenization-resistant spermatogenic cell nuclei (35). A 41% drop in these more mature spermatogenic cells was observed in the TO group vs. the controls. However, a more striking 87% decline in the mature cells occurred in the HLS group relative to the free-roaming controls.

The extensive drop in mature spermatogenic cells in the HLS group was confirmed by histological examination of the testes (Fig. 1). Normal spermatogenesis was observed in free-roaming controls (Fig. 1, A and B) as well as in TO control animals (Fig. 1, C and D). However, in HLS animals, spermatogenesis was severely reduced (Fig. 1, E–H). Most animals showed some spermatogenesis up to but not beyond round spermatids (Fig. 1, E and F). However, many tubules showed only spermatogonia and spermatocytes. In many cases,

Table 1. Comparison of male reproductive tract organ and hormone parameters in control, tail-only, and hindlimb-suspended rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Tail Only</th>
<th>Hindlimb Suspended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular wt, g</td>
<td>1.70 ± 0.06</td>
<td>1.60 ± 0.13</td>
<td>1.10 ± 0.11ab</td>
</tr>
<tr>
<td>Sperm count, sperm × 10⁶/ml</td>
<td>7.82 ± 0.46</td>
<td>4.66 ± 1.06c</td>
<td>1.04 ± 0.24a</td>
</tr>
<tr>
<td>Seminiferous tubule lumen diam, μm</td>
<td>89.4 ± 2.6</td>
<td>89.1 ± 3.1</td>
<td>95.2 ± 2.5</td>
</tr>
<tr>
<td>Seminiferous tubule diam, μm</td>
<td>231.2 ± 2.3</td>
<td>239 ± 3.4</td>
<td>188.9 ± 1.9abc</td>
</tr>
<tr>
<td>Serum testosterone, pg/ml</td>
<td>1,099 ± 580</td>
<td>722 ± 127</td>
<td>1,347 ± 156d</td>
</tr>
<tr>
<td>Epididymal wt, g</td>
<td>528 ± 31</td>
<td>596 ± 50</td>
<td>527 ± 58</td>
</tr>
<tr>
<td>Seminal vesicle wt, mg</td>
<td>240 ± 50</td>
<td>156 ± 4</td>
<td>189 ± 13</td>
</tr>
<tr>
<td>Serum LH, ng/ml</td>
<td>162 ± 38</td>
<td>140 ± 33</td>
<td>119 ± 15</td>
</tr>
<tr>
<td>Serum FSH, ng/ml</td>
<td>6.9 ± 1.6</td>
<td>4.8 ± 0.9</td>
<td>7.6 ± 1.0h</td>
</tr>
<tr>
<td>Serum corticosterone, ng/ml</td>
<td>76.0 ± 16.0</td>
<td>76.4 ± 20.0</td>
<td>42.6 ± 12.8</td>
</tr>
</tbody>
</table>

Statistics represent t-test comparisons of the group vs. the control group (C) or the tail-only (TO) group, respectively, as indicated in the parentheses. Values are means ± SE. FSH, follicle-stimulating hormone; LH, luteinizing hormone. aP < 0.01 vs. control; bP < 0.01 vs. tail only; cP < 0.01 vs. control; dP < 0.000001 vs. control; eP < 0.000001 vs. tail only; fP < 0.0001 vs. tail only; gP < 0.01 vs. tail only (t-test).

Fig. 1. Spermatogenesis is blocked after 6 wk of hindlimb suspension (HLS). One testis from each animal was fixed in Bouin’s fixative, embedded in paraffin, sectioned, stained with Gill’s hematoxylin, and examined by light microscopy. Magnification ×4 for A, C, E, and G; scale bar in A, 500 μm. Magnification ×60 for B, D, F, and H; scale bar in B, 50 μm. A and B: testes from free-roaming control animals, where spermatogenesis appeared normal. C and D: tail-only control group; spermatogenesis appeared normal with tails (ST) of the elongating spermatids and testicular sperm similar to those in free-roaming controls. E–H: testes from HLS group. Two major groups of histopathology were observed in the HLS group of animals. In E and F, testes contained tubules showing all stages of spermatogenesis up to round spermatids, including spermatogonia (G), spermatocytes (C), and round spermatids (RT), but no elongating spermatids or testicular sperm. In G–H, testes contained tubules that were nearly devoid of all spermatogenic cells; tubules contained essentially only Sertoli cells (S). Appearance of interstitial cells, including Leydig cells, in all groups was similar to the control group.
seminal tubules lacking all spermatogenic cells and containing only Sertoli cells were found next to tubules with some spermatogenesis. In a very limited number of regions, nearly all tubules within a section contained only Sertoli cells except for very few spermatogonia (Fig. 1, G and H). However, in all the HLS animals, there were regions containing at least spermatogonia. In contrast to the changes observed within the seminal tubules, the interstitial region did not appear to be affected in HLS animals. Although not specifically quantified, the overall appearance and numbers of Leydig cells in HLS animals were similar to those in free-roaming controls and TO animals.

The testis histology (Fig. 1) suggested that seminal tubule diameters were reduced in HLS testes relative to the control and TO groups. This was confirmed by morphometric measurement using digital image analysis (Table 1). Although the lumen diameter was not significantly different among any of the experimental groups (Table 1), the tubule diameter in the HLS group was highly significantly reduced by 18% relative to the free-roaming controls (Table 1). The HLS seminal tubules, while showing smaller diameters, were not completely collapsed.

Circulating testosterone levels in 6-wk HLS animals are not diminished. Table 1 shows that, after 6 wk of HLS, circulating testosterone levels were not significantly different between the HLS group and the free-roaming controls. The level of testosterone in the TO group was also not significantly different from that in the free-roaming controls. On the other hand, testosterone was reduced (by 46%) in the TO controls relative to the HLS animals.

HLS for 6 wk does not alter epididymal or SV weight. In HLS animals, no change in epididymal weight was observed (Table 1). Figure 2 shows cauda epididymal histology of representative animals from each of the treatment groups. Control and TO animals (Fig. 2, A and B) showed epididymal lumen filled with sperm, whereas the HLS groups (Fig. 2C) contained lumen with cellular debris or vacant space and no evidence of sperm. Only two of the HLS animals showed any evidence of sperm. The few (<50 sperm/animal) that could be obtained by extrusion into capacitation buffer were immotile and decapitated, and the sperm heads were often round or had an abnormal shape. On the other hand, the control and TO control groups had normal mature sperm in the cauda epididymis (Fig. 2) that showed good motility on exposure to capacitation buffer.

The SV are also androgen-dependent organs, critical for accessory fluid production during ejaculation. SV weight was also not diminished in HLS rats (Table 1). The TO control group showed a slight decline in SV weight, but this was not significantly different from the free-roaming control group.

Gonadotropins are not elevated in HLS rats. Circulating levels of FSH and LH were not significantly altered in HLS animals relative to free-roaming controls (Table 1). There was a slight difference in circulating LH between the HLS and TO groups, but neither of these groups was significantly different from the free-roaming control group.

The decline in spermatogenesis in the HLS rats is not due to animal stress or differences in nutrition. Table 1 demonstrates that corticosterone levels were not significantly higher in any of the treatment groups than in the controls. Corticosterone levels were slightly but not significantly lower in the HLS group than in both control groups.

To control for reduced food intake by HLS animals, all other groups were pair fed the mean of the weight of food consumed by the HLS group. Figure 3 summarizes the animal weight changes during the 6-wk experimental period. All groups showed a slight (~10%) reduction in body weight during the 6-wk experiment. At 6 wk, there was no significant difference between the free-roaming control, TO, and HLS groups.

DISCUSSION

The experiments described here are the first to examine the effect of long-term HLS on testicular function in mature adult male rats. After 6 wk of HLS, spermatogenesis is significantly reduced, such that no spermatogenic cells beyond round spermatids were...
LONG-TERM HINDLIMB SUSPENSION INHIBITS SPERMATOGENESIS

Fig. 3. Total body weight determined at weekly intervals during the 6-wk experiment. Weight declined slightly (~10%) in all groups during the 6-wk period, but there were no significant differences (by ANOVA) in the mean body weights in the 3 groups. TO, tail-only control.

The end of the 6-wk experiment. Finally, nutrition as a possible factor in the loss of spermatogenesis (26) was also ruled out because the control and TO groups were pair fed to the HLS group.

A key difference between this study and previous studies is the duration of the experiment. In most previous HLS studies, the duration of treatment was 1–4 wk (3, 12, 18). The ability to detect an effect of a treatment on spermatogenesis is highly dependent on the stage of spermatogenesis affected by the treatment. The earlier the stage of spermatogenesis involved, the longer it takes for the change in spermatogenic cell population to be observed (38). As the histology of our experiment revealed, all populations of spermatogenic cells down to spermatagonia were affected by HLS. Thus it is not surprising that earlier studies of shorter duration of HLS showed more limited effects on spermatogenesis.

A reduction in blood flow may also contribute to blocking spermatogenesis. A decline in flow rate of 30% caused a significant increase in the number of spermatogonia and spermatocytes showing apoptotic reduction in rats (5). Thus, if reduced blood flow due to the inguinal suture were a major factor, then the TO control would be expected to have produced results more similar to the HLS group than to the free-roaming controls. In addition, in HLS animals the spermatogenic cells that were retained comprised spermatogonia, spermatocytes, and round spermatids, which is the opposite of the results observed with reduced blood flow (5). However, localized changes in blood flow could still be a factor.

Previous HLS studies on testes of male rats can be divided into two categories. First, the experimental design in early studies did not account for the anatomy of the rat that allows the testes to descend into the abdomen when the hindlimb is elevated. In humans, the inguinal canal is normally constricted to prevent the testes from becoming abdominal. In such experiments, it is likely that changes in testicular function can be attributed, to a large extent, to changes that occur as a result of hyperthermic testes similar to that observed in cryptorchidism. For example, in the HLS ground controls for COSMOS-1667 and COSMOS-1887, the inguinal canal was not partially ligated to prevent cryptorchidism in the rats (42). Failure to ligate the inguinal canal in HLS rats clearly allows the testes to become abdominal and causes marked and rapid reduction in the population of spermatogenic cells (12). The second category of HLS experiments accounted for the potential of cryptorchidism by partial ligation of the inguinal canal to prevent abdominal relocation of the testes. However, none of these studies included the nonelevated but still sutured controls that were part of the present study. In such experiments, Deaver et al. (12) demonstrated a marked drop in circulating testosterone and an increase in LH after 7 days of HLS in 56-day-old animals. In most of their animals, testicular morphology was normal. However, in 27% of the HLS animals, there were reductions in the diameter of the seminiferous tubules and some losses of spermatogenic cells. These experiments rep-
resented preliminary ground controls for subsequent μG experiments on COSMOS-2044 (3). In the animals exposed to μG for 14 days, circulating testosterone and seminiferous tubule diameter were significantly reduced and the number of germ cells per tubule cross section decreased compared with HLS ground controls (3). After a 22-day exposure to μG on COSMOS-605, rats showed an increase in relative testicular weight, calculated as testicular weight ÷ body weight (34). However, if the actual mean testicular weights are recalculated from their data, then a decrease in testicular weight relative to controls was observed in μG. Although the data for the HLS animals (simulated flight controls) for COSMOS-1887 cannot be used (no inguinal ligation), there were significant reductions in circulating testosterone levels, testis weight, and numbers of spermatogonia in the flight animals vs. the free-roaming controls (15, 39). Similar results were obtained for male rats flown on the 7-day Space Shuttle Space Lab 3 mission (STS-51B) (32).

One significant difference between the results in this study and the published studies discussed above is the magnitude of the decline in circulating testosterone in shorter HLS-treated animals and in μG. In our experiments, there was no difference in circulating testosterone between the HLS animals and the other control groups at 6 wk. A decline in circulating testosterone is possible during the initial stages of the 6-wk experiment. The fact that LH levels were normal at 6 wk and the fact that the Leydig cell population also appeared morphologically unaffected by 6 wk of HLS are consistent with this conclusion. On the other hand, in the short-term HLS animals, a decline in circulating testosterone was observed in the absence of a compensating increase in LH, except in two of eight animals (12). It was speculated that a reason for this discrepancy was changes in microcirculation and/or subtle changes in testicular temperature analogous to cryptorchidism in μG and HLS (12). In individuals with cryptorchidism, androgen levels are slightly reduced, but within the physiological range, such that a normal male phenotype, including secondary sex characteristics and behavior, is present but spermatogenesis is inhibited. In humans, if the cryptorchidism is bilateral, then these men are infertile and at increased risk for developing testicular cancer. It has been hypothesized since the 1920s (11, 29) that the selective spermatogenic failure is due to exposure of spermatogenesis to abdominal temperatures, which are generally only 2–3°C above scrotal temperature. Testicular hyperthermia has also been proposed as an underlying cause for infertility in cases of varicocele in humans (13, 40, 55, 57).

The potential for cryptorchidism-like inhibition of spermatogenesis in HLS and μG due to elevated testicular temperature is a possibility that warrants serious consideration. In the HLS model, even though the testes are prevented from descending into the abdomen by partial ligation of the inguinal canal, the testes may spend the bulk of time resting against the body. In μG, the absence of gravitation pull on the testicular mass may cause the testes to be located significantly closer to the abdomen. In both cases, this could elevate testicular temperature, which under long-term conditions could severely impact spermatogenesis (22). Extensive evidence that abdominal temperature is detrimental to spermatogenesis in scrotal mammals has been published by Setchell in a review (43), in which he states, “It is still a mystery why the testes of most mammals appears to need a lower temperature for normal function.” Scrotal temperature is reduced compared with abdominal temperature by a combination of at least three factors: 1) blood vessel structure and function, including countercurrent cooling of testicular arterial blood by the pampiniform plexus of veins and, perhaps, vasomotion, 2) contraction/relaxation of the cremasteric muscles within the scrotal wall, pulling the testicles toward the abdomen at the inguinal canal (or into the inguinal canal in rats), and 3) scrotal sweat glands. The relative importance of these three factors for normal spermatogenesis is unclear (16). It is likely that in μG and the rat HLS model the contribution of the cremasteric muscle to testicular cooling is compromised by the lack of 1-G gravitational pull, keeping the testicles away from the warmer abdominal wall.

In another condition, men suffering spinal cord injuries often show reduced fertility yet present only slightly altered testicular endocrine function (8, 9). In this case, the mechanism underlying reduced spermatogenesis may also be testicular temperatures approaching body temperature as a result of chronic sitting (52). Further evidence that excessive heat disrupts human spermatogenesis comes from studies showing that semen quality of outdoor workers deteriorates during the summer (23). Wang et al. (53) found significant increases in TdT-mediated dUTP nick end labeling-stained apoptotic spermatogenic cells 7, 10, and 14 days after surgically induced cryptorchidism. Studies on supraphysiological heating of the testis show that temperatures up to 43°C for as little as 15 min increase spermatogenic cell apoptosis (24, 43). Recent studies have also specifically examined spermatogenic cell degeneration by in situ TdT-mediated dUTP nick end labeling analysis for apoptotic testicular cells (44, 53).

In summary, these important new observations demonstrate that HLS of mature adult male rats for 6 wk produces a marked blockage of spermatogenesis, such that spermatogenic cells beyond round spermatids are not present. Because the HLS model is widely accepted as a model for μG, it will be critical to determine the mechanisms that underlie the cessation of spermatogenesis. These results also have significant implications regarding potential serious effects of long-term exposure to μG on the ability of mammals, including humans, to reproduce. If this finding holds true in μG, it implies that male astronauts may become infertile after long-term exposure to μG. If the blockage of spermatogenesis proves to be irreversible, then this μG-induced sterility could result.

We acknowledge S. McDonald for excellent technical support, Dr. E. Holton (NASA Ames Research Center) for advice and kindly
LONG-TERM HINDLIMB SUSPENSION INHIBITS SPERMATOGENESIS

supplying the cage plans and animal care protocols for hindlimb suspension, and Dr. P. Terranova (University of Kansas Medical Center) for advise and support. This research was supported by National Aeronautics and Space Administration Grants NAG-2-1016 and NAG-2-1491 and the National Institute of Child Health and Human Development through Cooperative Agreement U54 HD-33994 as part of the Specialized Cooperative Centers Program in Reproduction Research.

REFERENCES