Effects of Caudal Elevation on Testicular Function in Rats
Separation of Effects on Spermatogenesis and Steroidogenesis

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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 contrast, 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.

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 passage-way 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 with 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 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 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-Holland fixative (Humason, 1979) for 2 to 3 days, passed through graded ethanol, and imbedded in Paraplast (Sherwood Medical Industries, St. Louis, MO). Sections (5 μm) were prepared and stained with periodic acid-Schiff plus hematoxylin. Sections representative of each testis were independently evaluated by two observers (RPA and DNRV) 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 μm.

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°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 MgCl2, and 4 mmol/L Na2HPO4)
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 NaN₃ 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 Bermdtson, 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 model 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 model 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*            | 278*            | 268b            | 8               |
| Paired adrenal weight (g)      | 58.0*           | 55.3*           | 60.6*           | 2.50            |
| Left testis weight (g)         | 1.57*           | 1.03b           | 1.47*           | 0.06            |
| Right testis weight (g)        | 1.56*           | 1.01b           | 1.44*           | 0.07            |
| Paired testes weight (g)       | 3.13*           | 2.04b           | 2.91*           | 0.12            |
| Seminiferous tubule diameter (μm) | 234*         | 197b            | 223a            | 5.5             |
| Lumen diameter (μm)            | 88b             | 71b             | 88*             | 3.2             |
| Histologic results             | 1.03*           | 3.00b           | 1.35*           | 0.07            |
| Testosterone (ng/ml) Serum     | 8.4*            | 3.7b            | 2.2a            | 0.69            |
| SNF                            | 217.8*          | 370.3*          | 253.2*          | 53.1            |
| TIF                            | 406.9b          | 142.5c          | 127.3c          | 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.
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
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-
Concentrations 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 ± .8 vs 0.8 ± .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**

<table>
<thead>
<tr>
<th></th>
<th>CON ((n = 5))</th>
<th>CEB ((n = 6))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>414(^a)</td>
<td>329(^b)</td>
</tr>
<tr>
<td>Left testis weight (g)</td>
<td>1.69</td>
<td>1.70</td>
</tr>
<tr>
<td>Spermatids/g</td>
<td>68.7</td>
<td>85.2</td>
</tr>
<tr>
<td>Seminiferous tubule diameter (μm)</td>
<td>244</td>
<td>246</td>
</tr>
<tr>
<td>Lumen diameter (μm)</td>
<td>98</td>
<td>101</td>
</tr>
<tr>
<td>Serum testosterone (ng/ml)</td>
<td>2.8(^a)</td>
<td>0.8(^b)</td>
</tr>
</tbody>
</table>

\(^{a}\) 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 Wronske, 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 greater than 42°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 unloading 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 phenomenon 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-
production of 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.

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Since this manuscript was submitted we have learned of another set of experiments conducted at the Pennsylvania State University using the elicitation 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 ligature 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


Amann RP, Berndtson WE. Assessment of procedures for screening agents for effects on male reproductive function: effects of dibromochloropropane (DBCP) on the rat. Fundam Appl Toxicol. 1986;7:244–255.


