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Neonatal Goitrogen Treatment Increases Adult Testis Size and Sperm Production in the Mouse

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ABSTRACT: Male rats made hypothyroid during neonatal life show unprecedented increases in adult testis size and daily sperm production (DSP). To determine if this effect was unique to the rat or could also be demonstrated in other species, we examined the effects of neonatal treatment with the reversible goitrogen 6-propyl-2-thiouracil (PTU) on adult testis size and function in the mouse. Male Swiss-Webster mice were untreated (control) or given PTU by adding 0.1% (w/v) to their mother's water from birth to day 25 postpartum. All pups were then weaned and given no further treatment. Sertoli cell proliferation was examined using tritiated thymidine autoradiography in some control and treated mice at 0, 5, 10, 15, 20, and 25 days, while the remainder were killed at 90 days to determine a variety of reproductive parameters. Neonatal PTU treatment decreased growth; body weight of treated mice at 4 weeks of age was 57% less than controls. Treated mice grew rapidly following cessation of PTU treatment, although their weights never equalled controls, remaining 17% smaller at 90 days of age. At 90 days of age, testis weight and DSP were increased by approximately 30% and 50%, respectively, in PTU-treated mice compared to controls. Despite the increased testis weight and function, serum testosterone concentrations were not different in control and treated mice. Testicular and epididymal histology in treated mice was similar to controls, while epididymal sperm in treated mice were motile and morphologically normal. Sertoli cell proliferation was altered in treated mice. The normal decrease in proliferative rate seen during early postnatal life was slowed, and by day 10 postnatal, the labeling index of treated Sertoli cells was about fourfold greater than that of controls. Furthermore, Sertoli cells in treated mice proliferated until day 25, whereas proliferation ceased in controls by day 15. In summary, neonatal PTU treatment increases testis weight, DSP, and the efficiency of sperm production (DSP/g testis) in the mouse, indicating that the PTU effect on testis development clearly occurs in other species. Furthermore, increased Sertoli cell proliferation appears to be the critical event for the development of this phenomenon in mice, as it is in rats. The existence of unique mutations that affect testicular development make the mouse an advantageous model for determining the mechanism of this effect. This technique may also be useful for increasing testicular size, sperm production, and fertility in various mutant mouse strains and transgenic mice in which these parameters are reduced.

Key words: Testis, propylthiouracil, Sertoli cells, hypothyroidism, sperm.

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A novel rat model system has recently been developed for studying the role of thyroid hormones in testis development. In this system, transient hypothyroidism during early postnatal development, induced by adding the goitrogen 6-propyl-2-thiouracil (0.1%; PTU) to the mother's water from birth until day 25, results in increased adult testis size and sperm production (Cooke, 1991; Cooke and Meisami, 1991; Cooke et al, 1991). Testis weights and daily sperm production (DSP) are increased by 40% and 83%, respectively, at 90 days of age in PTU-treated rats (Cooke and Meisami, 1991; Cooke et al, 1991, 1992), and maximal increases in these parameters are obtained at 160 days of age, when testis weight and DSP are about 80% and 140% greater than normal, respectively (Cooke, 1991; Cooke and Meisami, 1991; Cooke et al, 1991). The increases obtained in both of these parameters with this system are unprecedented.

The stimulation of testis growth seen in PTU-treated rats results from increases in both the seminiferous tubule and interstitial components of the testis (Hess et al, 1993) and is accompanied by large increases in the neonatal proliferation and adult numbers of Sertoli cells (van Haaster et al, 1992; Hess et al, 1993), germ cells (Hess et al, 1993), Leydig cells (Hardy et al, 1993), and peritubular cells (Cooke et al, unpublished data). There are also morphological alterations in the seminiferous tubules of PTU-treated rats (e.g., increased diameter and length of the seminiferous tubules) that result from the increased numbers of Sertoli and germ cells in the seminiferous epithelium (Hess et al, 1993). Testosterone (T) levels are normal in adult PTU-treated rats (Cooke and Meisami, 1991; Kirby et al, 1992) while the levels of gonadotropins, thought to be the major regulators of testicular develop-

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ment and function, are low both during PTU treatment and even after the rat returns to euthyroidism (Kirby et al, 1992).

It is not known if the stimulatory effects of early treatment with PTU or other goitrogens such as methimazole (Cooke et al, 1993) on adult testes occur only in the rat, or if this treatment can be successfully used in other species to increase testis size and function. If this technique is effective in other species, one of the most potentially important animals for this type of work is the mouse. A large number of mutant strains of mice in which there are abnormalities in various aspects of testicular differentiation and development have been identified (Chubb, 1989). These unique mutations are potentially useful for determining the mechanism of PTU action, and thus the mouse has clear advantages as a model system for studying this effect. Furthermore, numerous strains of mutant or transgenic mice have been described in which testis size is reduced and sperm production and/or fecundity is low (Chubb and Nolan, 1985; Chubb, 1989, 1992). A system for increasing sperm production in the mouse, in addition to its possible applications for mechanistic studies of this phenomenon, may have practical applications in increasing reproductive efficiency in strains in which male sperm production is reduced.

To determine if neonatal goitrogen treatment can increase testis size and function in other species, we examined the effects of early PTU treatment on testicular development and adult testis weight and sperm production in the mouse. Our results indicate that neonatal PTU treatment induces large increases in adult testis size and function, and that the mechanism of this effect in the mouse may be similar to that observed in the rat.

Materials and Methods

Animals and Treatments

Midpregnant Swiss-Webster mice were purchased from SASCO (Omaha, Nebraska) and housed in our animal colony under standard controlled temperature (22°C) and lighting (14 hours light, 10 hours darkness). Mice were given Purina rat chow and water *ad libitum* until birth. Following birth, only male pups were retained, and litter size was adjusted to four to six. Pups were weighed weekly and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Some litters were randomly designated as controls; control mothers and their pups were untreated and received food and water *ad libitum*. Other litters were made hypothyroid by the addition of 0.1% (w/v) PTU (Sigma, St. Louis, Missouri) to the mother's water from birth until day 25 postpartum (Cooke and Meisami, 1991; Cooke et al, 1992). PTU, a reversible goitrogen that inhibits thyroxine (T4) synthesis and also inhibits peripheral deiodination of T4 to triiodothyronine (T3), is transferred through the milk and produces severe hypothyroidism in pups (Kirby et al, 1992). For treated litters, food and PTU-containing water were offered *ad libitum*. PTU has a bitter taste and at this concentration can compromise maternal water consumption (Cooke et al, 1993), so diet cherry Kool-Aid was added to the PTU-containing water to increase palatability (Cooke and Meisami, 1991). Diet cherry Kool-Aid was not added to the control's water; previous work has shown that addition of this substance alone does not affect testis growth of control rats (Cooke et al, unpublished results). At 25 days of age, PTU treatment was discontinued and pups were weaned and housed three to four per cage. Pups were given tap water and food *ad libitum* thereafter.

Sertoli Cell Proliferation

The pattern of Sertoli cell proliferation in testes from control and PTU-treated mice ($n = 4$ for each group and timepoint) was determined at 5-day intervals from birth to day 25 by tritiated thymidine autoradiography. The technique used for autoradiography has been described in detail previously (Cooke et al, 1986). Briefly, testes were removed and cut into 2-mm^3 pieces and incubated in Dulbecco's modified Eagle's medium containing 5 μCi of ^{3}H-thymidine (specific activity = 80 Ci/mmol; Amersham). After 2 hours, the testes were fixed in 10% neutral buffered formalin and embedded in glycol methacrylate. Sections (2 μm thick) were dipped in Kodak NTB-2 emulsion and stored at 4°C for 2–3 weeks until sufficient labeling could be detected. Cells were judged to be labeled when at least six grains were visible above the nucleus. The autoradiograms were developed by standard techniques (Cooke et al, 1986); then the slides were stained with hematoxylin and eosin.

The labeling index (LI; labeled cells/total cells) for Sertoli cells was determined by counting at least 1,000 cells per group and timepoint. Each analysis involved two trials in which at least 500 Sertoli cells were scored. In each trial, 125–175 Sertoli cells/testis were scored from contiguous seminiferous tubules located in the periphery of the testis sample (which have the strongest labeling when the tissue is exposed to tritiated thymidine *in vitro*). All Sertoli cells in selected tubules were scored, and the fields used in each trial were nonoverlapping to insure that different areas of the tissue were counted during each trial.

Reproductive Organ Weights, Analysis of Sperm Production, and Motility

At 90 days of age, all mice were killed by decapitation. Blood was collected from the cervical stump, and the serum was separated and stored at −20°C for subsequent T assay. Testes, kidneys, epididymides, ventral prostate, seminal vesicles, and coagulating glands were removed from all animals and weighed. Some testes were frozen for subsequent determination of sperm content, while others were used for histological analysis as described below. Epididymides were either used for histology or for analysis of sperm motility.

Testicular sperm content and DSP were determined by the procedure of Robb et al (1978), with a slight modification described previously (Cooke et al, 1991). Briefly, testes were removed, weighed, and homogenized for 3 minutes in 25 ml of physiological saline containing 0.05% (v/v) Triton X-100 (Sigma) using a semimicro Waring blender (Robb et al, 1978). Step

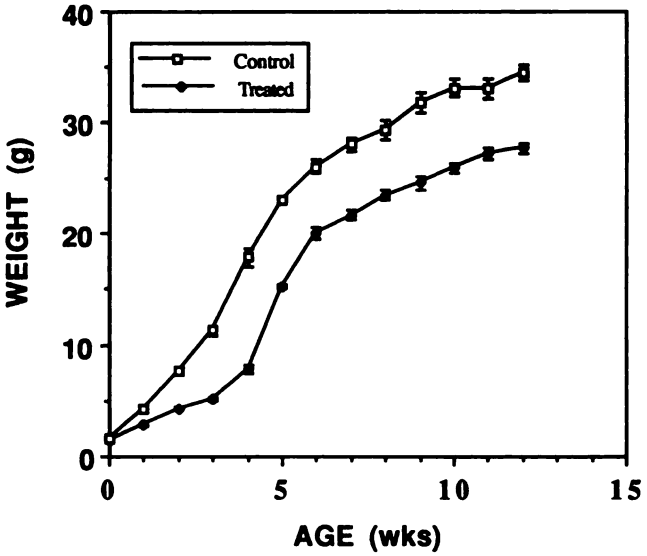

FIG. 1. Growth of mice that were untreated (control) compared to those given 0.1% PTU from birth to day 25. Data points are mean ± SEM. At least four litters were used for all points. Body weights of PTU-treated mice were significantly less than controls ($P < 0.05$) beginning at 2 weeks of age.

14–16 spermatids (stages II–VIII) survive this homogenization and their nuclei can then be counted using a hemacytometer. To count the spermatids, a 200-μl sample of homogenate was diluted with 300 μl of saline and 500 μl of 4% trypan blue, which stains spermatids and facilitates counting (Cooke et al, 1991). Sample aliquots of 5.5 μl were placed on the hemacytometer and counted twice under a microscope to determine average number of spermatids per sample. These values were used to obtain total number of spermatids per testis, then divided by the testis weight to give spermatids per gram of testis. Developing spermatids spend 4.84 days in steps 14–16 during spermatogenesis in the mouse (Oakberg, 1956a,b). Thus, the values for the number of spermatids per testis and spermatids per gram testis were divided by 4.84 to obtain DSP and efficiency of sperm production (DSP/g testis), respectively (Robb et al, 1978).

Sperm motility was assessed as previously described (Cooke et al, 1991). Briefly, fluid from the caudal epididymis was drawn from the epididymis into a warmed pipette tip as soon as the animal was killed, then placed into warmed Ham's F-12 medium, and immediately examined under the microscope.

Histology

For light microscopy, testes and epididymides from control and treated 90-day-old mice were fixed as above. Tissues were processed, embedded in glycol methacrylate, then sectioned at 2 μm, and stained with hematoxylin and eosin. Epididymides were sectioned longitudinally throughout their length to allow analysis of all regions of this organ.

Testosterone Assays

Testosterone (T) was measured with a solid-phase ^{125}I radioimmunoassay (Diagnostic Procedures, Los Angeles, California) using unextracted serum, as described previously (Cooke and Meisami, 1991). Due to the small volume of serum obtained from each animal, sera from four mice were combined, and the T concentrations of the resultant pooled samples were determined in a single assay. Each sample was assayed in triplicate. The assay sensitivity was 0.04 ng/ml, with an intraassay coefficient of variation of 3.0%.

Statistical Analysis

Testes weights, sperm production, and T in control and PTU-treated mice were compared using Student's *t*-test. Factorial analysis of variance in arcsin-transformed values for the Sertoli labeling index and body weights during development in the control and PTU-treated rats was used to determine treatment and time effects in these variables. Differences between various groups were considered significant when $P < 0.05$. All data were expressed as mean ± SEM.

Results

Effect of PTU Treatment on Body Growth

PTU treatment decreased pup growth (Fig. 1). Body weights (BWs) in PTU-treated mice were marginally reduced by 1 week of age ($P = 0.05$) and were significantly reduced ($P < 0.05$) compared to controls at all subsequent ages up to 90 days. The reduction in BW in treated mice was most pronounced during and immediately after the period of PTU treatment. For example, at 4 weeks of age, BW in PTU-treated mice was reduced by 57% ($P < 0.05$) compared to controls. Approximately 1 week after PTU treatment was stopped, treated pups began growing rapidly and their subsequent growth rates paralleled that of controls, although BWs of treated animals remained 17% less ($P < 0.05$) than controls at day 90 (Fig. 1).

Reproductive and Nonreproductive Organ Weights

In contrast to the decreases seen in BW, testis weight was 111 ± 4 mg in treated animals at 90 days of age (Fig. 2), an increase of 29% ($P < 0.05$) compared to control testis weights (88 ± 1 mg). Relative testis weight (mg testis/g BW) showed an even more substantial increase due to the lower BWs in the PTU-treated mice and was increased 51% ($P < 0.05$) in these animals at 90 days of age (Fig. 3). Testes from treated mice were morphologically normal and exhibited no gross pathologies.

Like overall BW, adult weights of nonreproductive organs were reduced following neonatal PTU administration. For example, at 90 days of age, kidney weight in control mice was 294 ± 6 mg but was reduced 26% ($P < 0.05$) to 218 ± 5 mg in treated mice of the same age. Despite the large increase in testis size at 90 days in treated mice, weights of other reproductive organs including epididymis, seminal vesicle, coagulating, and ventral prostate were not increased in treated mice (data not shown). For example, epididymal weights in control and treated

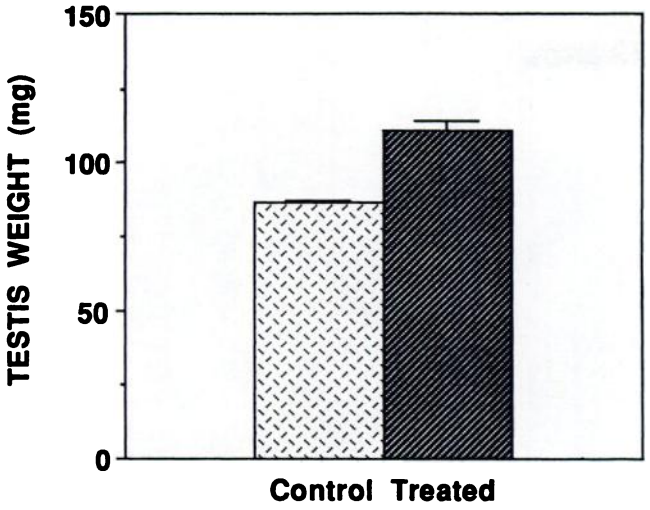

FIG. 2. Wet weight of testes from 90-day-old control mice and mice treated with PTU from birth to day 25. Bars represent mean ± SEM weights of single testes; n = 36 and 22 for control and treated testes, respectively. Testis weights in the PTU-treated group were 29% (P < 0.05) greater than that of controls.

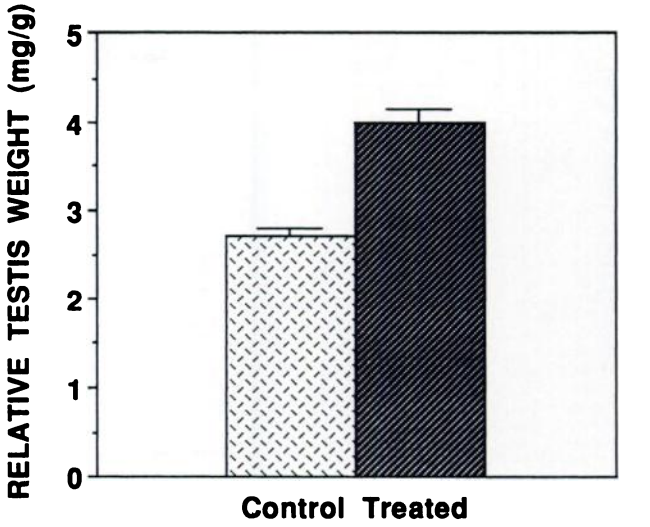

FIG. 3. Relative testis weights (mg testis/g body weight) in 90-day-old control mice and PTU-treated mice. Bars represent mean ± SEM weights of single testes; n values were as in Figure 2. Relative testes weights in the PTU-treated group were 51% (P < 0.05) greater than that of controls.

mice were 31 ± 1 mg and 31 ± 1 mg, respectively, at 90 days of age.

Sperm Production and Motility

The DSP in treated mice was 52% greater (P < 0.05) than that of controls at 90 days of age (Fig. 4). The efficiency of sperm production (DSP/g of testis) was also increased in PTU-treated mice compared to controls (47.4 ± 2.8 × 10^6 vs. 37.6 ± 1.2 × 10^6, respectively; P < 0.05). Sperm in the caudal epididymis of treated mice were motile, and no morphological abnormalities in the sperm were evident.

Serum T Concentrations and Testicular and Epididymal Histology

Serum T concentrations were not different in treated and control mice (0.71 ± 0.01 ng/ml and 0.73 ± 0.24 ng/ml, respectively) despite the increased testis weight in treated animals.

The histological appearance of both the testis and epididymis of PTU-treated mice was similar to that of controls (data not shown). There were no pathological changes evident in either organ in treated mice, and morphologically normal sperm were observed in the lumina of the seminiferous tubules and in the caput, corpus, and cauda regions of the epididymis.

Autoradiography of Control and PTU-Treated Testes

Tritiated thymidine labeling of Sertoli cells during development indicated that the proliferative period of these cells was extended in treated mice (Figs. 5, 6). Sertoli cells were proliferating rapidly at birth (LI = 20.0 ± 1.8%), then in control mice their LI decreased rapidly during the postnatal period, and by day 15 essentially no mitogenic activity was detected. Sertoli cell proliferation in treated mice equaled that in controls at 5 days postnatal. In contrast to controls, Sertoli cells in treated mice then showed a less rapid decline in LI during subsequent life, and their LI was significantly (P < 0.05) greater than the control value at 10, 15, and 20 days of age. For example, at 10 days of age, the LI of Sertoli cells in treated testes was 12.0 ± 2.4%, while in controls LI had declined to only

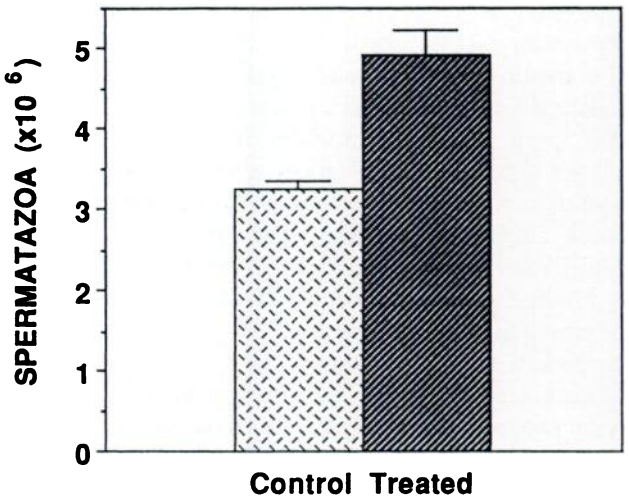

FIG. 4. Daily sperm production (DSP) at 90 days of age in control mice and mice treated with PTU from birth to day 25. Data are presented as mean ± SEM of duplicate determinations from individual testes; n = 18 and 14 for control and treated testes, respectively. DSP was significantly increased (P < 0.05) in the treated group compared to controls.

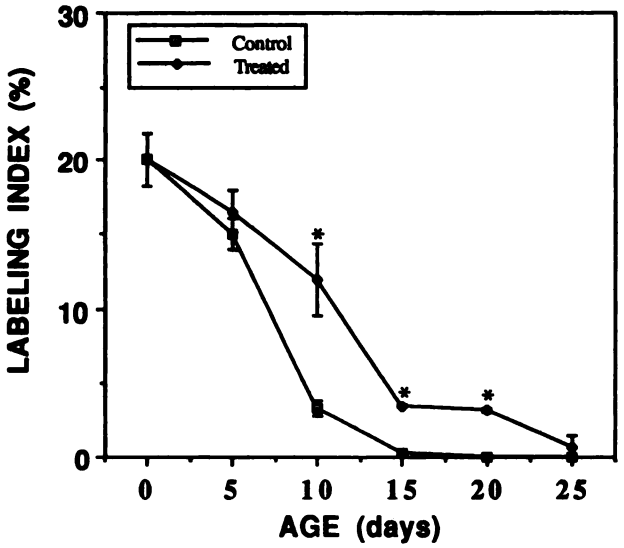

FIG. 5. Sertoli cell proliferation in control and PTU-treated mice. The labeling index (LI) of Sertoli cells is shown at 5-day intervals from birth to day 25 in control and treated mice. The $n \geq 4$ for all groups, and at least 1,000 Sertoli cells were scored per group. Sertoli cells were considered positive when they contained six or more silver grains. *Different from the control at $P < 0.05$.

3.3 ± 0.5% (Fig. 6). The proliferation of Sertoli cells in treated mice continued at a reduced level through days 15 and 20, ages where Sertoli cell mitogenesis could not be detected in the controls. Finally, by day 25, mitogenic activity had essentially ceased in the Sertoli cells of the treated testes.

Discussion

The results of the present study indicate that neonatal PTU treatment of the mouse produces large increases in adult testis weight, relative testis weight (mg of testis/g BW), sperm production, and the efficiency of sperm production (DSP/g testis). The effectiveness of PTU treatment in increasing testis size and sperm production in the mouse, along with the recent observation that this treatment produces similar increases in the hamster (Kirby et al, unpublished data), demonstrate that the PTU effect is not confined to the rat and that this methodology can be successfully applied to other species.

The magnitude of the increases in testis weight and sperm production induced by PTU treatment are less pronounced in the mouse than in the rat. For example, DSP is increased 84% in the 90-day-old PTU-treated rat compared to controls of similar age (Cooke et al, 1991), while DSP is increased 52% in the PTU-treated mouse relative to controls at the same age. The increased DSP in PTU-treated rats results from the multiplicative effects of increased testis size and increased efficiency of sperm production. The magnitude of the increase in efficiency of sperm production in the mouse (25%) is almost identical to that observed in the rat, and it is clearly the smaller increase in testis weight in the PTU-treated mouse com-

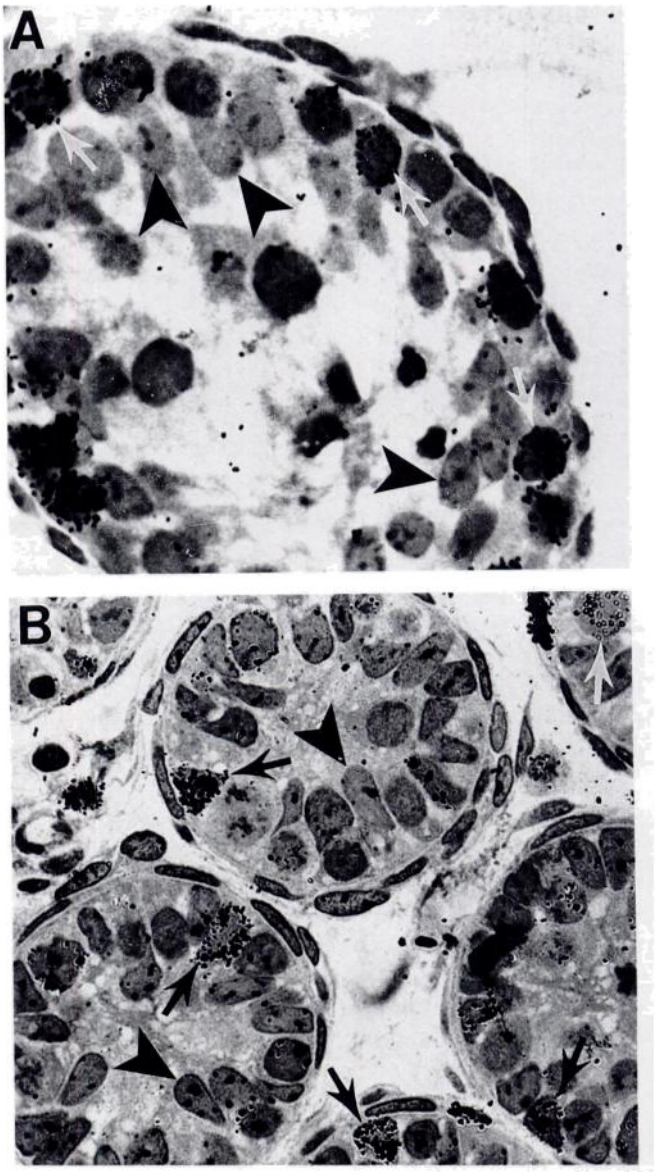

FIG. 6. Autoradiograms of ^{3}H-thymidine labeling in testes from 10-day-old control (A) and PTU-treated (B) mice. Sertoli cells were distinguished by a triangular, droplet, or irregularly shaped nucleus, which was relatively pale staining and by their characteristic position in the seminiferous epithelium. Germ cells were identified by their round nuclear shape and staining behavior. None of the Sertoli cells (black arrowheads) in this section of control testis are labeled. In contrast, several labeled Sertoli cells (black arrows) are seen among the unlabeled Sertoli cells (black arrowheads) in the treated testis. Labeled spermatogonia (white arrows), peritubular and interstitial cells, along with degenerating germ cells were also seen in both control and treated testis. A = 990×; B = 600×.

pared to that seen in the PTU-treated rat that is responsible for the smaller overall increase in DSP.

Despite the smaller effect of PTU on DSP and testis size in the mouse compared to the rat, all facets of the PTU-induced changes in mouse growth, including the magnitude of the decreases in neonatal BW gain, the rapid growth following the cessation of PTU treatment, and the persistence of about a 15% BW decrement in the treated animal at 90 days of age, were similar to the effects of this drug on rat development (Cooke and Meisami, 1991). Furthermore, the similar concentrations of serum T in adult control and treated mice despite the increased testis size in the latter were also in agreement with that observed previously in rats (Cooke and Meisami, 1991; Kirby et al, 1992).

Our previous work using the rat has demonstrated that the magnitude of the DSP increase produced by neonatal PTU treatment is critically dependent on the dose of this drug (Cooke et al, 1993) and the age when it is administered (Cooke et al, 1992). Thus, it may be possible to increase DSP further in the mouse to levels above that reported here by optimizing the dosage regimen for this particular species. Furthermore, PTU delays the plateau of testis weight and DSP in rats from its normal 90–100 days to sometime between day 135 and 160 (Cooke et al, 1991). Given the basic similarity of the testis weight and DSP response in mice compared to rats, it is therefore also possible that the magnitude of the PTU-induced increase in mouse DSP and testis weight may not become maximal until later ages.

The importance of Sertoli cells in controlling many facets of germ cell development and regulating the magnitude of sperm production is well known. PTU treatment is only effective in the rat if begun during the early neonatal period when Sertoli cells proliferate rapidly (Cooke et al, 1992; Meisami et al, 1992). Additionally, PTU treatment extends the period of rat Sertoli cell proliferation (van Haaster et al, 1992). These data, along with the close correlation between the ultimate increases in Sertoli cell numbers and sperm production in this species (Cooke et al, 1991; Hess et al, 1993), strongly suggest that increased neonatal Sertoli cell proliferation is critical for the development of this phenomenon.

Our present results demonstrate that the increased testis growth and sperm production in the PTU-treated mouse are also accompanied by increased Sertoli cell proliferation. Sertoli cells in the mouse are normally proliferating rapidly at birth, then their mitogenic activity decreases steadily during postnatal life, finally ceasing by 15 days of age (Vergouwen et al, 1991). PTU treatment causes a decreased rate of decline in the mitotic activity of Sertoli cells in the treated animals compared to the controls. This results in a marked increase in treated Sertoli cell proliferation at early ages (e.g., at 10 days, the LI of treated Sertoli cells is about fourfold greater than that of controls) and a continuation of proliferation in treated Sertoli cells during ages (15 and 20 days) when control Sertoli cell proliferation has ceased. Although the critical question of what factors cause the increased Sertoli cell proliferation remains, the basic mechanism by which this phenomenon develops, increased Sertoli cell proliferation resulting in eventual increases in germ cell production, appears similar in both species.

The demonstration that PTU treatment can increase testis size and sperm production in the mouse is especially significant as it may be useful in determining the mechanism of this effect and also have practical applications. For example, how PTU increases Sertoli, germ, Leydig, and peritubular cell proliferation (van Haaster et al, 1992; Hardy et al, 1993; Hess et al, 1993; Cooke et al, unpublished data) and eventually causes increased testis size and sperm production is not known (reviewed in Cooke et al, in press). Rat Sertoli cells express thyroid hormone receptors during early development (Jannini et al, 1990) and the temporal expression of these receptors is autoregulated by thyroid hormone (Palmero et al, 1993). The decreased level of thyroid hormone in goitrogen-treated rats may directly inhibit the maturation of Sertoli cells (Palmero et al, 1989, 1990; Francavilla et al, 1991) and prolong their period of neonatal mitogenesis. Alternatively, germ cells also express thyroid hormone receptors (Falcone et al, 1992). Germ cells modulate follicle-stimulating hormone receptor expression in Sertoli cells (Heckert and Griswold, 1991) and also affect the levels of several important secretory products of Sertoli cells such as androgen-binding protein, inhibin, and transferrin (Galdieri et al, 1984; Le Magueresse et al, 1988; Allenby et al, 1991). Therefore, germ cells may be contributory or even essential to the increase in Sertoli cell numbers seen in PTU-treated animals. Mouse mutations in which germ cells are absent and the seminiferous tubules consist of only Sertoli cells (Chubb, 1989) are potentially critical for determining if germ cells are involved in the increase in Sertoli cell number, which occurs with PTU treatment.

The mouse is the preferred animal for gene knockout experiments, in which the expression of a certain gene is eliminated, allowing conclusions about its normal function to be derived from observations of the effects caused by its absence. A number of growth factors are expressed by Sertoli, peritubular, and other cell types during testicular development (Skinner, 1991), which may contribute to normal Sertoli cell proliferation and the stimulated proliferation of these cells seen in PTU-treated rats and mice. Therefore, the gene knockout methodology may also be useful in determining the factor(s) involved in increased Sertoli cell proliferation in PTU-treated mice and further underscores the potential of the successful demonstration of this effect in mice.

The PTU treatment methodology may also be potentially useful for increasing reproductive efficiency in some mouse strains with reduced fertility. Many types of mutant or transgenic mice in which male fertility is reduced or abolished as a result of a large variety of testicular and extratesticular causes have been reported (reviewed in Chubb, 1989); these strains can be difficult or impossible to propagate. PTU treatment would obviously have no beneficial effect in cases of male infertility due to a total lack of germ cells (the Sertoli cell only syndrome), hormonal deficits that preclude normal spermatogenesis, mutations in which germ cells arrest at an immature stage of development, or behavioral abnormalities that disrupt mating (Chubb, 1989). Although mice normally produce severalfold more sperm per ejaculate than are necessary for maximal fertility (Searle and Beachey, 1974), severe decreases in sperm production could impair or abolish fertility even though the remaining sperm produced are viable, morphologically normal, and otherwise capable of fertilization. Therefore, in cases of decreased fertility where males produce normal but decreased numbers of viable sperm, the large increase in sperm production that results from PTU treatment may be a valuable aid in improving reproductive efficiency.

Although the species in which the PTU effect has been demonstrated are all rodents, young boars also express thyroid hormone receptors in their Sertoli cells during early development (Palmero et al, 1992). These results suggest that thyroid hormones may be important regulators of Sertoli cell proliferation in many species and that PTU treatment may increase testis size and function in a wide variety of animals when administered under appropriate conditions. As a cautionary note, recent results indicating that neonatal hemicastration, which produces compensatory hypertrophy in the remaining testis in a large number of animals (reviewed in Thompson and Berndtson, 1993), is not effective in the rabbit (Thompson and Berndtson, 1993) emphasize that substantial species differences may also occur in the PTU response. However, if the PTU system can be successfully used for increasing sperm production in economically important animals such as dairy bulls, where artificial insemination is used extensively, this could potentially lower semen cost while increasing supplies of semen available from bulls with particularly desirable genetic constitutions.

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3rd European Congress of Endocrinology

June 17–24, 1994

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The 3rd European Congress of Endocrinology will be held June 17–24, 1994, in the RAI Congress Centre in Amsterdam, The Netherlands.

Main topics of the scientific programme are:

- Relationship between hormones and cancer in breast, prostatic, and uterine tumors
- Growth factors and oncogenes
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1500–2000 participants are expected to attend the congress.

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