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Neonatal Hypothyroidism Causes Delayed Sertoli Cell Maturation in Rats Treated With Propylthiouracil: Evidence That the Sertoli Cell Controls Testis Growth

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ABSTRACT ***Background:*** **The testes of rats treated neonatally with propylthiouracil (PTU) grow to almost twice their normal size. The cause of testicular enlargement has been suggested to be the result of delayed maturation of Sertoli cells, allowing Sertoli cell division to occur beyond the 15th postnatal day, the commonly recognized cutoff date for Sertoli cell divisions. It has been shown that an increased population of Sertoli cells in postnatal development supports increased numbers of germ cells in adult animals. After examining developing rats treated neonatally with PTU, we hypothesized that an approximate 10-day delay in maturation was occurring and proceeded to test this hypothesis experimentally. Thus the purpose of this report was to determine if a 10-day delay in maturation could explain the increased numbers of Sertoli cells and increased testis size in PTU-treated animals.**

Methods: **Both control animals and animals treated neonatally with PTU N = 5/group were sacrificed at 15 and 25 days of age and prepared for electron microscopy.**

Results: **Micrographs show and morphometric ultrastructural analysis of numerous parameters demonstrated at the 95% probability level that Sertoli cells from 25-day-old PTU animals are not different in size and most constituents (volume and surface area) from 15-day-old control animals and are less mature than 25-day-old control animals. Mitosis of Sertoli cells was observed in PTU-treated animals in 25-day-old animals but not in age-matched controls. The number of Sertoli cells in 25-day-old PTU-treated animals is significantly increased over age-matched controls. Micrographs show the presence of immature Sertoli cell nuclei in 25-day-old animals receiving PTU as well as increased germ cell degeneration in this group. Sertoli cell tight junction formation is also delayed in PTU-treated animals as compared with controls.**

Conclusions: **Together, the data show that delayed maturation of Sertoli cells occurs in treated animals that corresponds to a minimum of 10 developmental days. In the immature state, Sertoli cells continue to divide. Data presented herein and published data related to PTU treatment indicate that delayed maturation of the Sertoli cell results in delayed maturation and proliferation of other testicular cell types. From this and from published data, the hypothesis is presented that *the Sertoli cell is responsible for the overall control of testis development.***

Key words: Sertoli cell, Testis, Morphometry, PTU, Rat

Until recently, little was known about the factors that govern testis size. Smaller than normal testes can be produced in rats if mitosis of Sertoli cells is experimentally inhibited during the neonatal period (Orth et

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al., 1988). These data suggest that the population of Sertoli cells can regulate the numbers of germ cells in the testes and that decreases in both Sertoli and associated germ cells contribute to a smaller testis. More recently, neonatal administration of 6-propyl-2-thiouracil (PTU) to rats caused testes to develop that were ~80% larger than normal and produced ~140% more sperm than normal (Cooke, 1991; Cooke and Meisami, 1991). These testes possessed a greater number of Sertoli cells than controls. Reported increases ranged from 80% at 30 days (Van Haaster et al., 1992) to 157% at 90 days of age (Hess et al., 1993). In mice, a smaller increase in adult testis size following neonatal PTU treatment was reported (29%; Joyce, 1993). Bunick et al. (1994) have shown, based on mRNA analysis of Sertoli cell secreted products, that Sertoli cell maturation is delayed in PTU-treated animals as compared with control animals.

In studies employing PTU, a transient hypothyroidism was produced due to lowered triiodothyronine (T_3). T_3 has been shown in vitro to be an inhibitor of Sertoli cell mitosis and a promoter of Sertoli cell differentiation (Cooke et al., 1994). T_3 receptors are known to be present on Sertoli cells (Palmero et al., 1988). These data suggest that the enlarged testis is due to a transient neonatal hypothyroidism producing decreased T_3 levels, a condition that maintains the immature state of the Sertoli cells and promotes their proliferation.

The mechanism by which Sertoli cell numbers are increased in PTU-treated animals has been suggested to be due to a delay in maturation of Sertoli cells (Van Haaster et al., 1992), allowing Sertoli cells to divide beyond the 15th postnatal day—the generally recognized normal cutoff period for cell divisions (Bortolussi et al., 1990; Steinberger and Steinberger, 1971). Increased populations of Sertoli cells and the germ cells that eventually come to accompany them would then result in increased testis size (Hess et al., 1993). In addition, two reports have suggested that Leydig cells are increased in number subsequent to PTU treatment (Hardy et al., 1993; Mendis-Handagama and Sharma, 1994).

In their study, van Haaster et al. (1992) performed limited light microscope studies on Sertoli cell nuclear parameters and from these data have suggested that the Sertoli cell remains immature during pubertal development in neonatally PTU-treated animals as compared with control animals. This "delayed-maturation hypothesis" was tested in the present study using ultrastructural morphometric techniques. A previous morphometric study of immature Sertoli cells has demonstrated logical increases in volume and surface areas of cell components during pubertal maturation (Russell et al., 1991), allowing the structural parameters of the Sertoli cell to be examined in an objective manner.

The results of the present study extend the results of van Haaster et al. (1992) to show clearly that there is a delay in Sertoli cell maturation in PTU-treated animals and that Sertoli cells continue division beyond the expected period. From data presented herein and from other published studies, a hypothesis is presented that places the Sertoli cell as the "controller" of testis size.

MATERIALS AND METHODS

Animals, Treatments, and Tissue Preparation

Sprague-Dawley rats derived from parental stock purchased from Sasco (Lincoln, NE) were bred and maintained as described previously (Cooke and Meisami, 1991; Cooke et al., 1992). After birth, pups were sexed and weighed. Only male pups were retained, and litter size was adjusted to 6–8. Pups were 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. For treated litters, 0.006% (w/v) propylthiouracil (PTU; Sigma, St Louis, MO) was added to the mother's water beginning immediately after birth. PTU ingested by the mothers is transferred to the pups through the milk, resulting in hypothyroidism in the pups (Cooke et al., 1993). The 0.006% dose used in the present experiment suppress thyroxine, but perhaps not maximally, during the treatment period (Cooke et al., 1993) and leads to increases in adult testis size and sperm production at 90 days, which are equal to that seen with the higher 0.1% PTU dose originally used in these studies (Cooke, 1991; Cooke and Meisami, 1991; Cooke et al., 1993).

For treated mothers, food and the PTU-containing water were offered ad libitum. PTU has a bitter taste, so diet cherry Kool-Aid was added to the PTU-containing water to increase palatability (Cooke and Meisami, 1991). Maternal water consumption by PTU-treated dams was monitored daily from birth to day 25 postnatal to insure adequate water intake and to prevent dehydration of the dam and pups (Cooke et al., 1993).

Animals were perfused via the descending aorta using a perfusion pump (Hess and Moore, 1993). Prior to perfusion, the animal was anesthetized with sodium pentobarbital (4 mg/100 g body weight). After fixation, the testes were removed and weighed. For subsequent morphometric calculations, the specific gravity of testis tissue was considered to be 1.0. The tissue was postfixed and embedded as previously described (Russell and Burguet, 1977).

Morphometry of the Testis

Five animals each from control and PTU-treated groups were utilized. The general plan for morphometry of the testis has been previously described (Sinha Hikim et al., 1988) and is presented in brief below. Initially, the volumes of individual Sertoli cell nuclei were determined by light microscope serial section reconstruction of five nuclei from each animal used in the study. The volume of Sertoli cell was determined by employing the following formula applied to point counting data:

$$\text{Sertoli cell volume} = \frac{\text{points over cell}}{\text{points over nucleus}} \times \text{nuclear volume},$$

where the points for the counts were obtained by transmission electron microscopy (×4,500)

The number of Sertoli cells in the testis was determined by the formula:

$$N_v = \frac{V_v \times 10^{12}}{V} \times \text{testis weight},$$

where N_v is the numerical volume of Sertoli cell nuclei, V_v is the volume density of Sertoli cell nuclei within the testis as determined by point counting under the light microscope (11,000 points/animal), V is the volume of an individual Sertoli cell nucleus, and 10^{12} is a conversion factor from cm^3 to μm^3. (Testis volume and weight are roughly equivalent given that the specific gravity is near 1.0.)

Since there were few identification criteria for determination of the stage of the spermatogenic cycle in PTU-treated animals due to the paucity of viable germ cells (the result of cell degeneration), tubules selected for morphometry were those containing preleptotene spermatocytes. Blocks were trimmed around the first round seminiferous tubule noted that fit the criteria of this cell association. Sections showing silver-gold interference colors were made and placed on 200-mesh grids.

Pie-shape (Bugge and Plöen, 1986) montages made from negatives taken at $\times 9{,}000$ times magnification and enlarged to 22,500 times original size of round or near round tubular profiles were constructed from individual electron micrographs to provide proportional sampling (Ye et al., 1993) of the Sertoli cell.

The volume densities of organelles were determined in high magnification montages by point counting methodology using a multipurpose grid. Over 2,500 points were used to sample the Sertoli cell in each montage. The volume of each organelle was determined as the product of the cell volume and the volume density of a particular organelle.

Morphometry for surface area measurements was conducted using a multipurpose grid overlay on the pie-shaped montages following standard stereological methods (Weibel, 1973). To make surface area determinations, both points over the Sertoli cell and intersections with the Sertoli cell plasma membrane and with Sertoli cell organelles were counted and recorded. The surface density (S_v) of particular subsections of the plasma membrane and organelle membrane were determined using the following formula:

$$S_v = \frac{4 \times I}{P_t \times Z},$$

where I is the number of intersections on the Sertoli cell membrane parameter, P_t is the number of points over the Sertoli cell, and Z is the length of the line between points in terms of the magnification of the micrograph. The surface (S) area of the Sertoli cell was the product of its surface density and its volume

Means from each group were analyzed by ANOVA and comparisons made using the Student-Newman-Keuls test of significance at a level of $P < 0.05$.

RESULTS

Descriptive Results

Mitotic Sertoli cells were identified by published criteria (Russell et al., 1995), which include the morphological appearance of mitochondria and the presence of phagocytozed germ cells within the boundaries of Sertoli cells. Mitotic Sertoli cells were not seen in either the 15-day-old or the 25-day-old control group. They were, however, commonly seen in both the 15-day-old and to a lesser extent in the 25-day-old PTU-treated group (Figs. 1, 2). Mitotic Sertoli cells commonly displayed phagocytozed cytoplasm of germ cells contained within their boundaries (Fig. 1A). Sertoli cell divisions in 25-day-old animals occasionally showed areas of ectoplasmic specialization (Fig. 2A,B) and evidence of intact Sertoli-Sertoli junctions (Fig. 2B), although such tight junctional regions were not as extensive as in control animals.

The appearance of Sertoli cell nuclei in three of four groups studied were similar. Only the 25-day-old control animals showed evidence that Sertoli cell nuclei were maturing like that expected in developing animals (Gondos and Berndtson, 1993). Figures 3 and 4 show a portion of a tubule from each of the four groups studied. Sertoli cells from 15-day control (Fig. 3A), 15-day PTU-treated (Fig. 3B) and from 25-day PTU-treated animals (Fig. 4B) show nuclei that were ovoid with an uneven layer of chromatin along the inner aspect of the nuclear envelope. In these groups, nucleoli were small and not in association with satellite DNA. Nuclei of 25-day-old control animals (Fig. 4A) were irregularly rounded and possessed little continuous chromatin along the nuclear envelope, except for occasional large masses. Their nucleoli were large and rounded and frequently seen in association with satellite DNA. In addition, indentations of the nuclear envelope, a sign of maturity, were more common in this group than in other groups.

The peritubular cell layers in 25-day PTU-treated animals (see Fig. 6) appeared immature like that of the 15-day control animals (Fig. 5A) in that multiple cell layers were present. In 25-day-old control animals (Fig. 5B), the peritubular cell layer consisted of two or in some areas three cell layers characteristic of the adult testis (Russell et al., 1995).

Germ cell degeneration was common in all groups studied. Cell degeneration was more common in the 25-day PTU-treated animals when compared with age-matched control groups (Fig. 7). A count of 20 randomly selected tubules for each animal for the 25-day-old group revealed that the mean level of cell degeneration was 11 times greater in the PTU-treated group (Fig. 7) than in the control group. Approximately 80% of controls tubules showed tubular lumens whereas no lumens were seen in 25-day-old PTU treated animals. However, in the 15-day PTU-treated group, there were few degenerating cells compared with age-matched controls. In this treated group there were very few viable cells present from which cell degeneration could occur.

Quantitative Results

Testes weights increased ~ threefold in control animals in the period from 15 to 25 days of age. However, testes in PTU-treated animals at 25 days of age were not significantly different than 15-day-old control animals. They were significantly smaller than testis weights of 25-day-old control animals (Table 1).

The volume of the Sertoli cell, its nucleus, and its cytoplasm was significantly greater in 25-day-old control animals compared with 15-day-old control animals. There were no significant difference in these parameters when 25-day-old PTU-treated animals were compared with 15-day-old control animals, but all parameters were significantly lower in 25-day-old PTU-

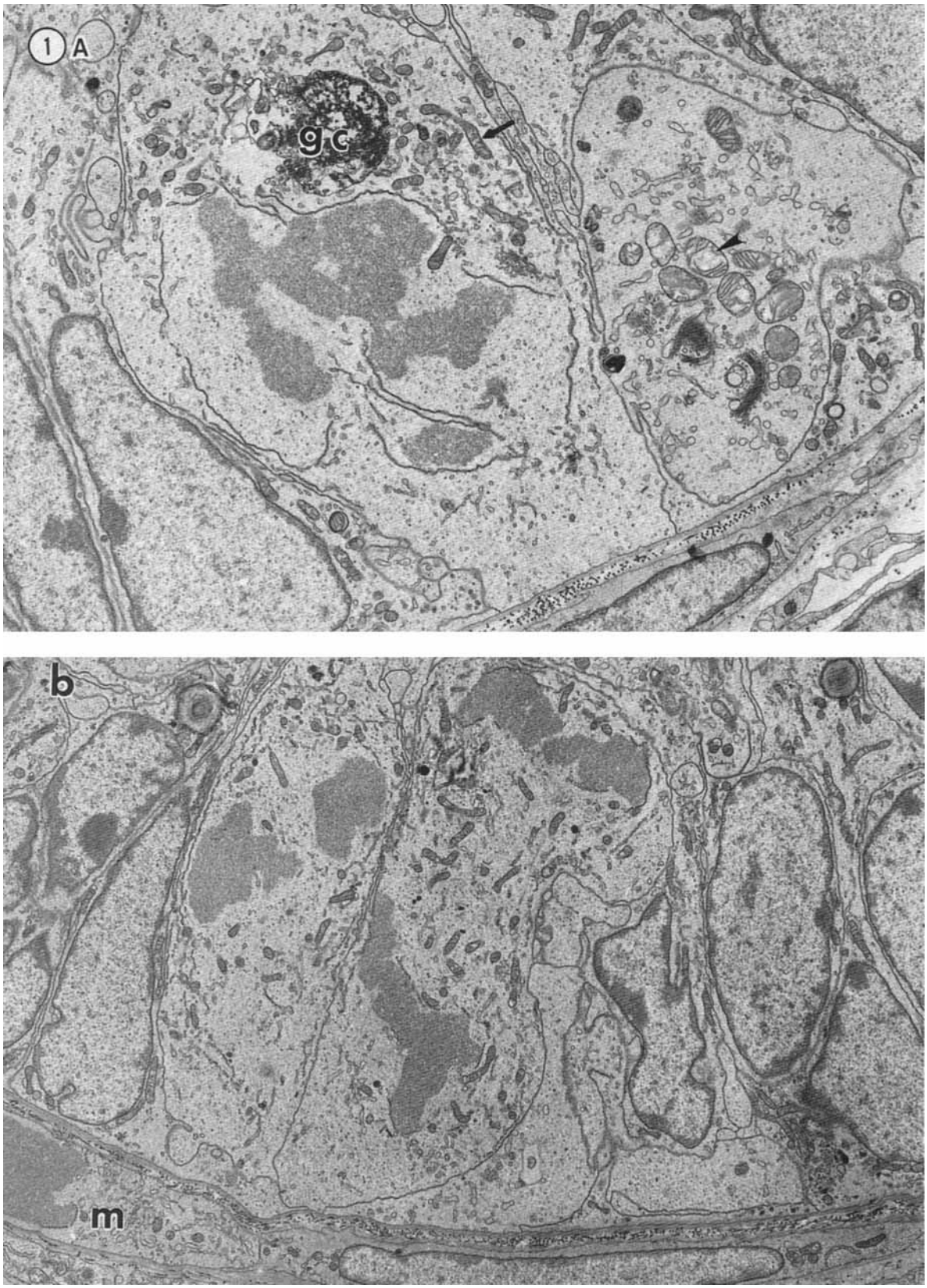

Fig. 1. Mitotic Sertoli cells from 25-day-old, PTU-treated animals. In **A,** a prophase Sertoli cell is shown containing a phagocytozed germ cell (gc). In **B,** two adjacent Sertoli cells are in division as is a myoid cell (m). In both A and B, rod-shape mitochondria are noted (arrow) that characterize the cell as a dividing Sertoli cell and not a germ cell (arrowhead indicates rounded germ cell mitochondria). × 6,000; × 6,000, respectively.

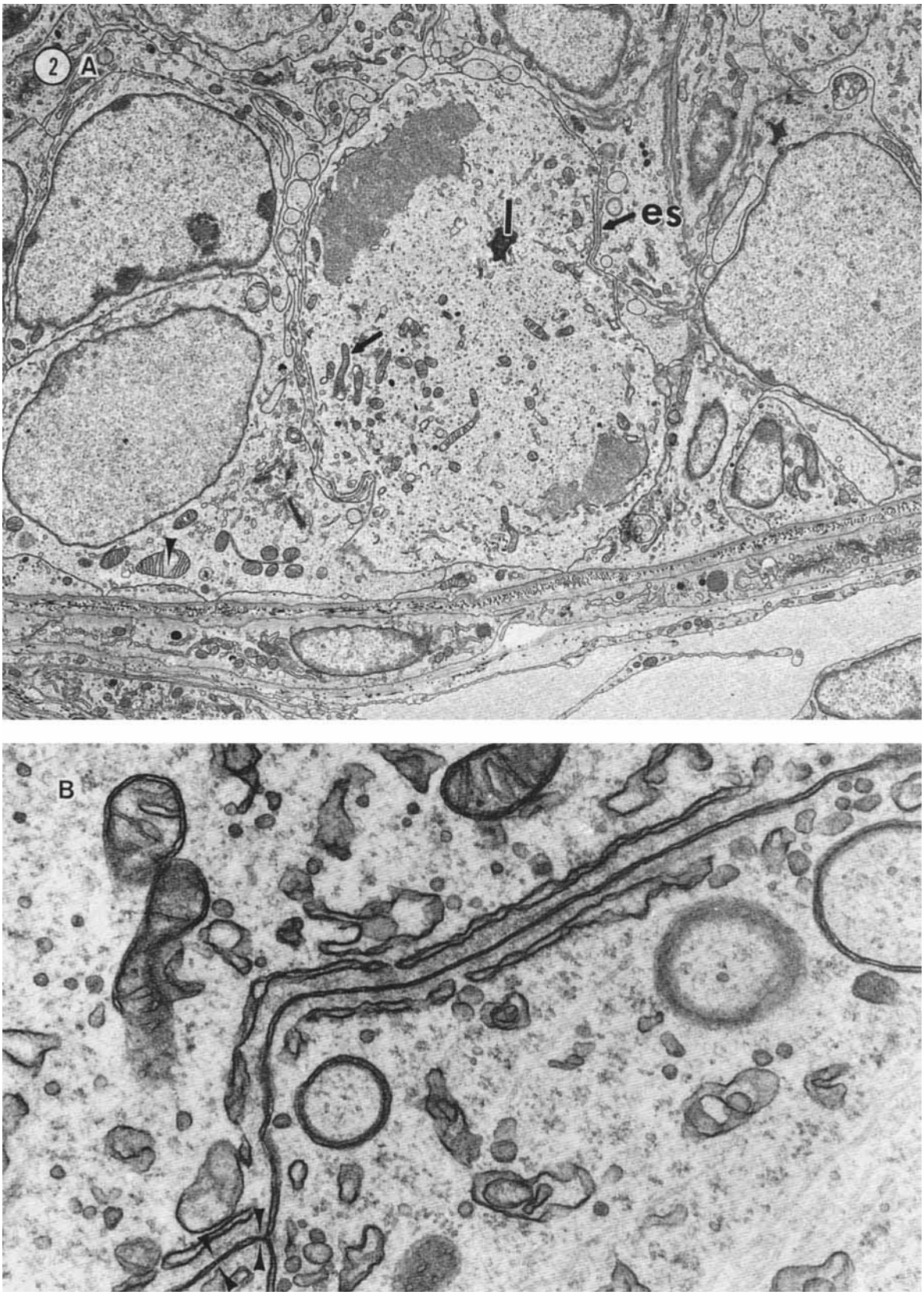

Fig. 2. Dividing Sertoli cells from a 25-day-old, PTU-treated animals. **A.** Indicated is a lipid droplet (l) and ectoplasmic specialization (es). Sertoli cell mitochondria are rod-shaped (arrow) and germ cell mitochondria are larger and more rounded with lamellar cristae (isolated arrowhead). **B** shows the ectoplasmic specialization of A at higher magnification. A translucency in the membranes of dividing cells (apposing arrowheads) indicates that a tight junction is present. × 6,000; × 45,600, respectively.

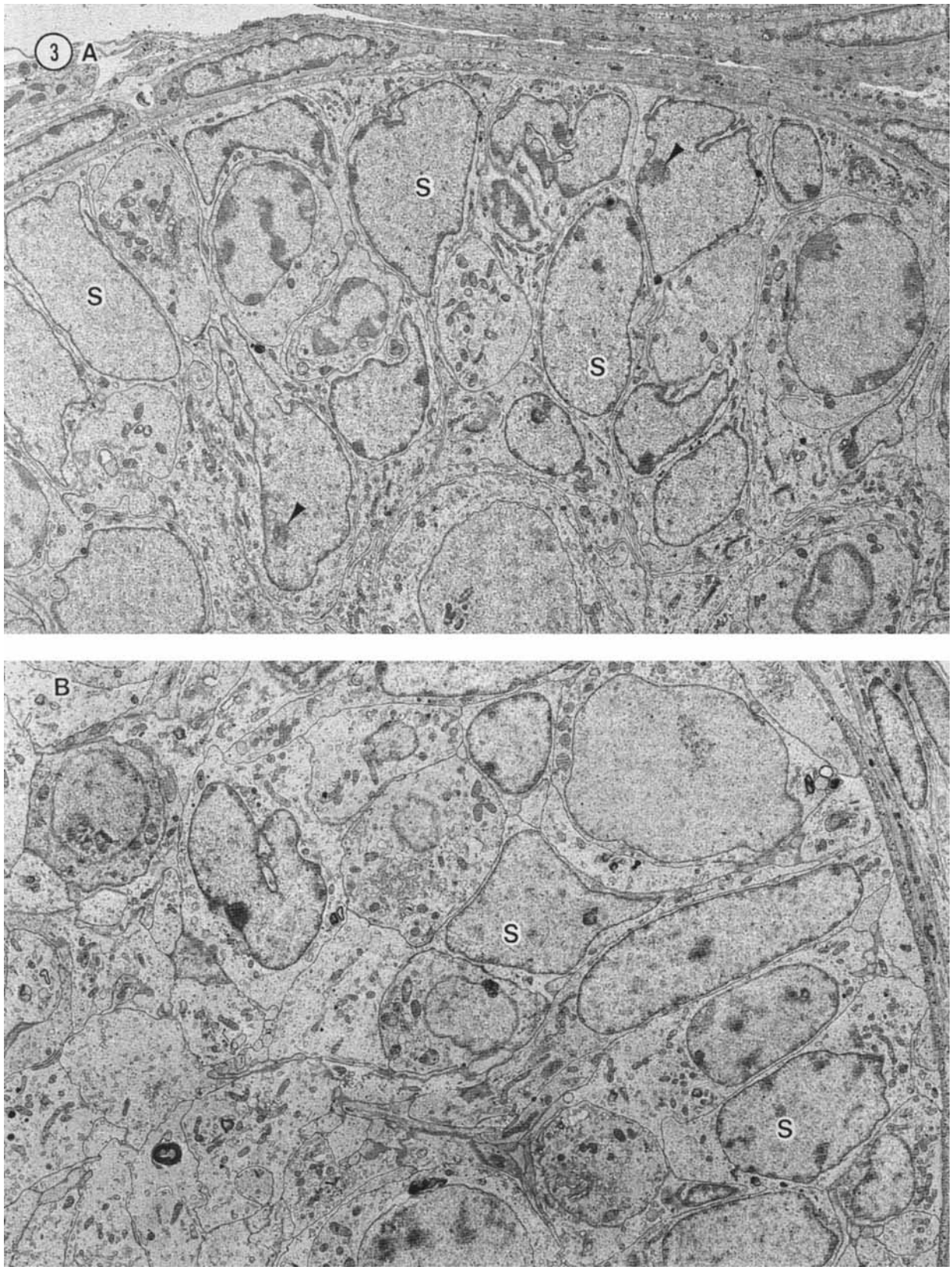

Fig. 3. Seminiferous tubules showing the degree of maturation of the Sertoli cell nuclei (S). **A.** In 15-day control animals, Sertoli cell (S) nuclei are ovoid with dense material associated with the nuclear envelope. Nucleoli (arrowheads) are small. **B.** In 15-day-old, PTU-treated animals, Sertoli cell nuclei appear similar to 15-day-old control animals; however, they show slightly more patchy heterochromatin within the nucleus. × 3,900; × 3,900, respectively.

TABLE 1. Basic morphometric data (mean ± SE) (N = 5)

Parameter	Control 15 Days	PTU-treated 15 days	Control 25 days	PTU-treated 25 days
Testis weight (g)	0.07 ± 0.003[a,c*]	0.06 ± 0.002[a]	0.20 ± 0.01[b]	0.09 ± 0.002[c]
Sertoli cell volume (μm^3)	583 ± 63	434 ± 41	1094 ± 69[a]	534 ± 19
Cytoplasm volume (μm^3)	365 ± 46	257 ± 28	820 ± 72[a]	316 ± 14
Nucleus volume (μm^3)	218 ± 18[a]	177 ± 14[b]	274 ± 12[c]	219 ± 9[a,b]
Number of Sertoli cells/testis ($\times 10^6$)	41.3 ± 3.3[a]	48.9 ± 5.4[a,b]	40 ± 3.6[a]	58.0 ± 4.6[b]

*Means in rows with different superscripts indicate significant differences.

treated animals compared with their age-matched controls (Table 1).

Numerous organelle volumes (Table 2) and surface area parameters (Table 3) show that 25-day-old PTU animals resembled 15-day-old control animals in tests of significance and were significantly retarded in development compared with age-matched controls. Exceptions were the Golgi apparatus volume and nuclear surface area, which were not different in any group (Golgi) or which increased in 25-day-old PTU-treated group (nuclear surface area).

The number of Sertoli cells in control animals of 15 days of age was similar to historical controls (as listed in Table 7 of Russell, 1993) and was not significantly different from PTU-treated animals of the same age or control animals at 25 days of age. However, there was a significant increase in Sertoli cell number in PTU-treated animals at 25 days of age compared with both control groups (Table 1).

DISCUSSION

This is the first study to examine the ultrastructure of PTU-treated animals and in particular, the developmental aspects of Sertoli cells in the testis of these animals. The results demonstrate that the Sertoli cell in PTU-treated animals remains immature in the period from the 15th to the 25th postnatal day. Virtually all statistical comparisons show that the 25-day-old, PTU-treated rats resemble animals that are at least 10 days younger. The delay in maturation of the Sertoli cell morphology corresponds roughly to the prolongation of expression of early Sertoli cell products and the delay in expression of mRNA for Sertoli cell secreted products that are new secretory products (Bunick et al., 1994). It confirms the data of van Haaster et al. (1992), which suggest Sertoli cell maturation is delayed.

The actual delay in maturation may be slightly longer than the 10 days hypothesized in the current report. For example, van Haaster et al. (1992) have found Sertoli cell mitoses up until day 30, suggesting a possible 15-day delay in maturation. Bunick et al. (1994) show that some Sertoli cell product mRNAs are affected by day 10 of development. The morphometric data presented herein indicate that at day 15 numerous parameters being measured in 15-day, PTU-treated animals are lower, but usually not significantly different than the age-matched control, suggesting that the onset of PTU action on the testis is prior to day 15. Overall, we believe that the delay in maturation begins just prior to day 15 and continues slightly beyond day 25, although the precise bracket for delayed maturation has yet to be determined.

Evidence of active mitosis in Sertoli cells up to 25 days of age and a demonstration of increased numbers show that their delayed maturation allows them to continue through the cell cycle and populate seminiferous tubule in numbers greater than found in normal animals. As Sertoli cells finally mature, germ cells develop in association to Sertoli cells in almost, but not equal numbers (Hess, 1993) to those normally associated with Sertoli cells (Russell and Peterson, 1984; Wing and Christensen, 1982). However, because the Sertoli cell is delayed in maturity in the hypothyroid animals, it is possible that in an animal of ~90 days of age receiving PTU, the testis is still growing and that the full complement of germ cells per Sertoli cell will catch up to control numbers by days *110–120*.

Prior to maturation of the Sertoli cell, degeneration of germ cells in control animals is common (Russell et al., 1987), but in PTU-treated animals it is increased greatly as compared with age-matched controls. Hypothyrodism produced by other means also increases germ cell degeneration (Francavilla et al., 1991; Palmero et. al., 1989; Van Haaster et al., 1992). These data suggest the obvious, that the immature state of the Sertoli cell is deleterious to the development of germ cells. The PTU model may serve to determine the products necessary for germ cell development given that there is selective alteration of Sertoli cell mRNAs subsequent to PTU treatment (Bunick et al., 1994).

Data in the present report and other reports suggest that the role of the Sertoli cell in the development of the testis is paramount. Several studies show that testis development is quantitatively regulated by the Sertoli cell (Berndtson et al., 1987; Hochereau de Reviers et al., 1987, 1990; Orth et al., 1988). For example, the work of Orth et al. (1988) demonstrates that production of fewer than normal Sertoli cells leads to a smaller testis. Studies using the PTU model show that hypothyroidism results in delayed maturation of the Sertoli cell and allows continued division of Sertoli cells, yielding a larger than normal testis. Increased testis size is not only due to an increase in Sertoli cell numbers and an overall larger number of germ cells that accompany them (Hess et al., 1993), but delayed Sertoli cell maturation affects other cell types as well. Leydig cells are increased in number (Hardy et al., 1993; Mendis-Handagama and Sharma, 1994) as are other interstitial cell types (Hardy et al., 1993). The population of Leydig cells responds to a delay in Sertoli cell maturation by also prolonging their proliferative phase (Hardy, pers. comm.). From the present study, it appears that the maturation of the peritubular cells is also delayed. Finally, germ cell viability is not supported after PTU treatment. Thus an apparent primary alteration of Sertoli cell function leads to a quantitative change in nearly all cells comprising the testis

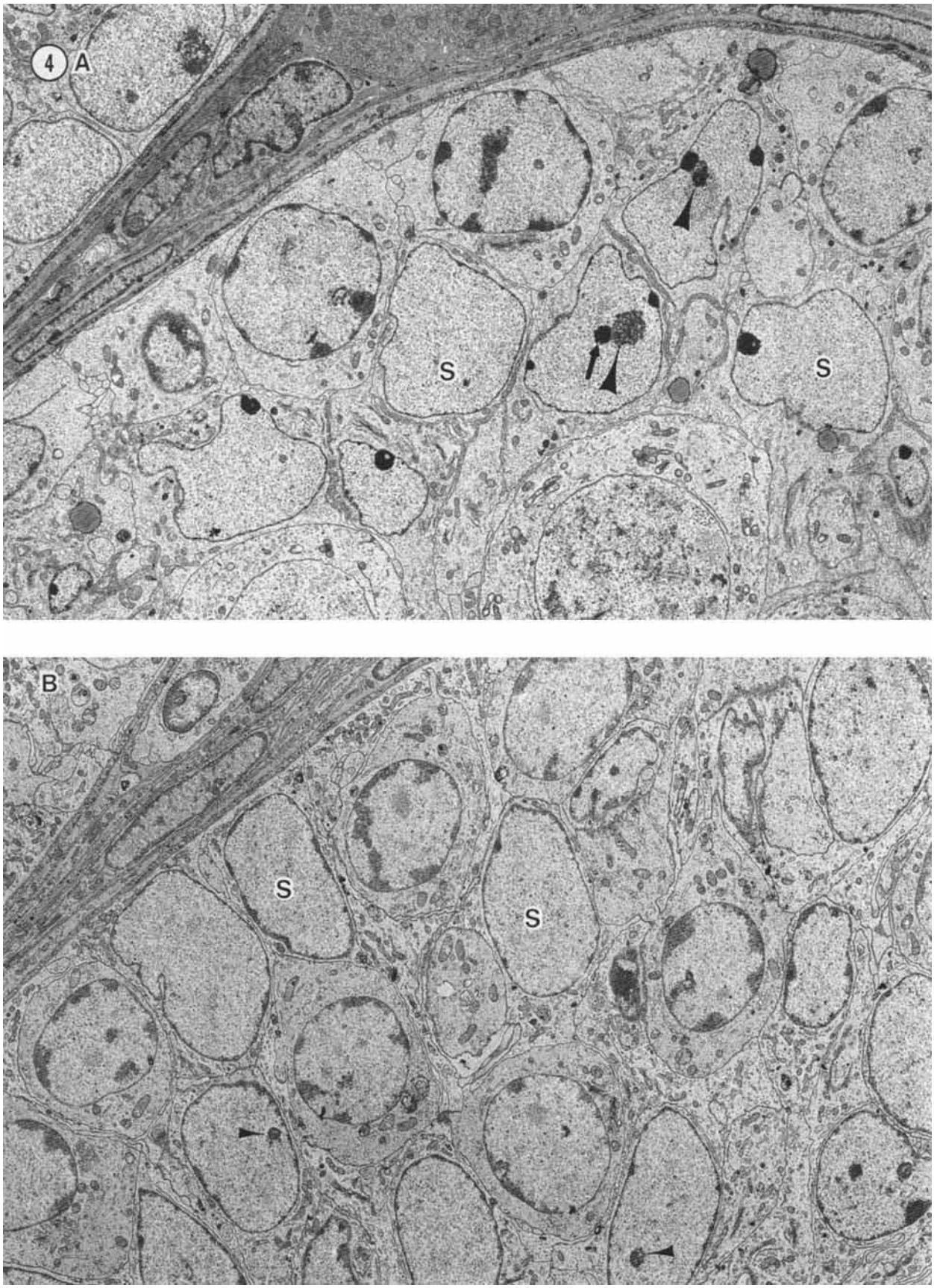

Fig. 4.

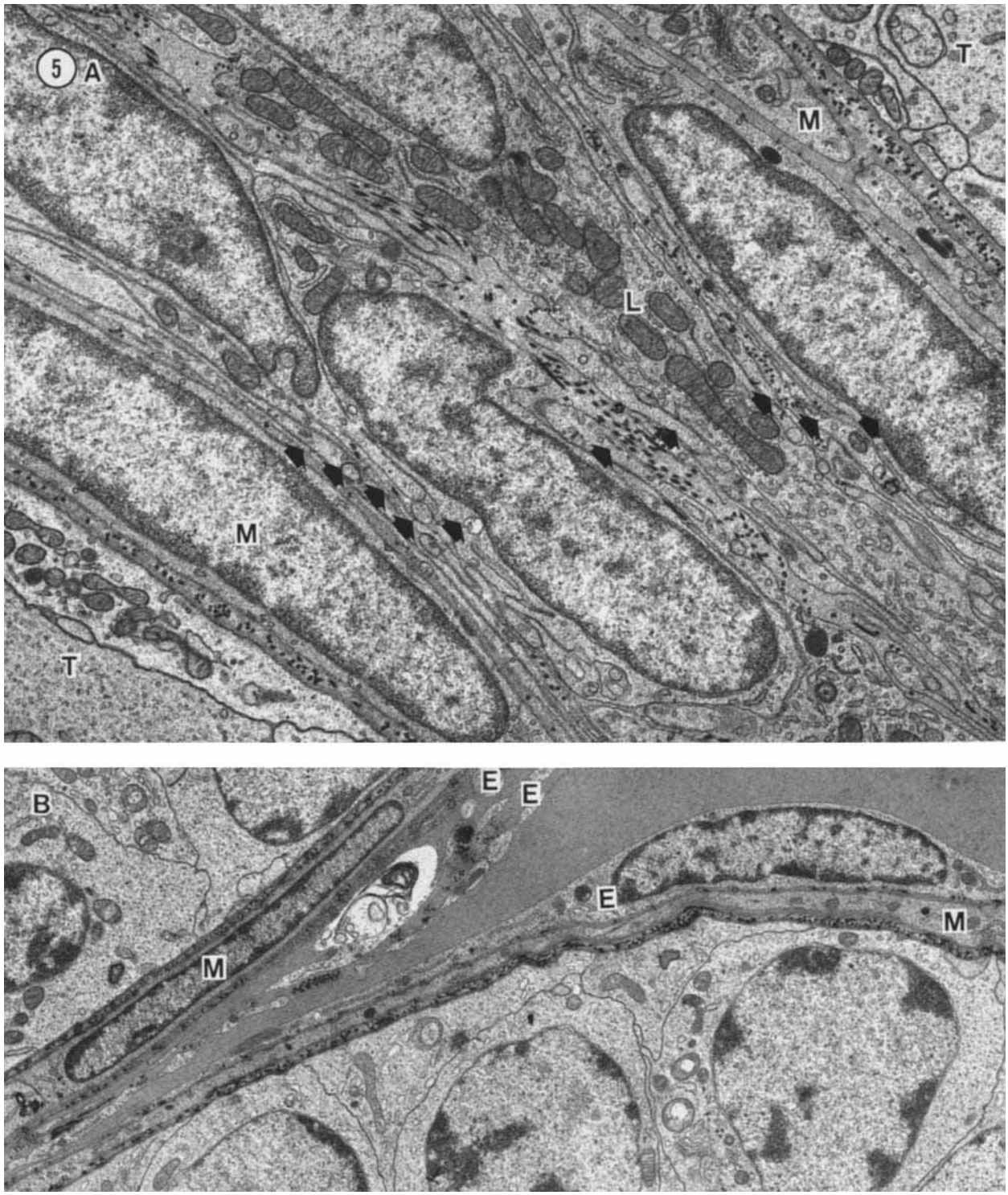

Fig. 5. The peritubular layer in the two control groups studied. **A.** In 15-day-old control animals, the peritubular cells between adjacent tubules (T) is composed of 10 fibroblastic cell (arrows) layers between the myoid cells (M) and sandwiching a Leydig cell (L). Many of these cells appear thick because the particular micrograph shows their nuclear region. **B.** In a 25-day-old control animal, the peritubular layer is composed of a myoid cell (M) and, in most regions, the endothelium of the lymphatics (E) × 12,2000; × 4,800, respectively.

Fig. 4. Seminiferous tubules showing the degree of maturation of the Sertoli cell nuclei (S). **A.** In 25-day control animals, nuclei are rounded or irregularly shaped, showing little patchy chromatin. Their nucleoli are large, densely stained (arrowhead), and reside in association with satellite DNA (arrow). **B.** PTU Sertoli cells, 25 days old, are oval in shape and show dense material along the nuclear envelope similar to the 15-day-old groups. Nucleoli are generally small (arrowheads). × 3,600; × 3,600, respectively.

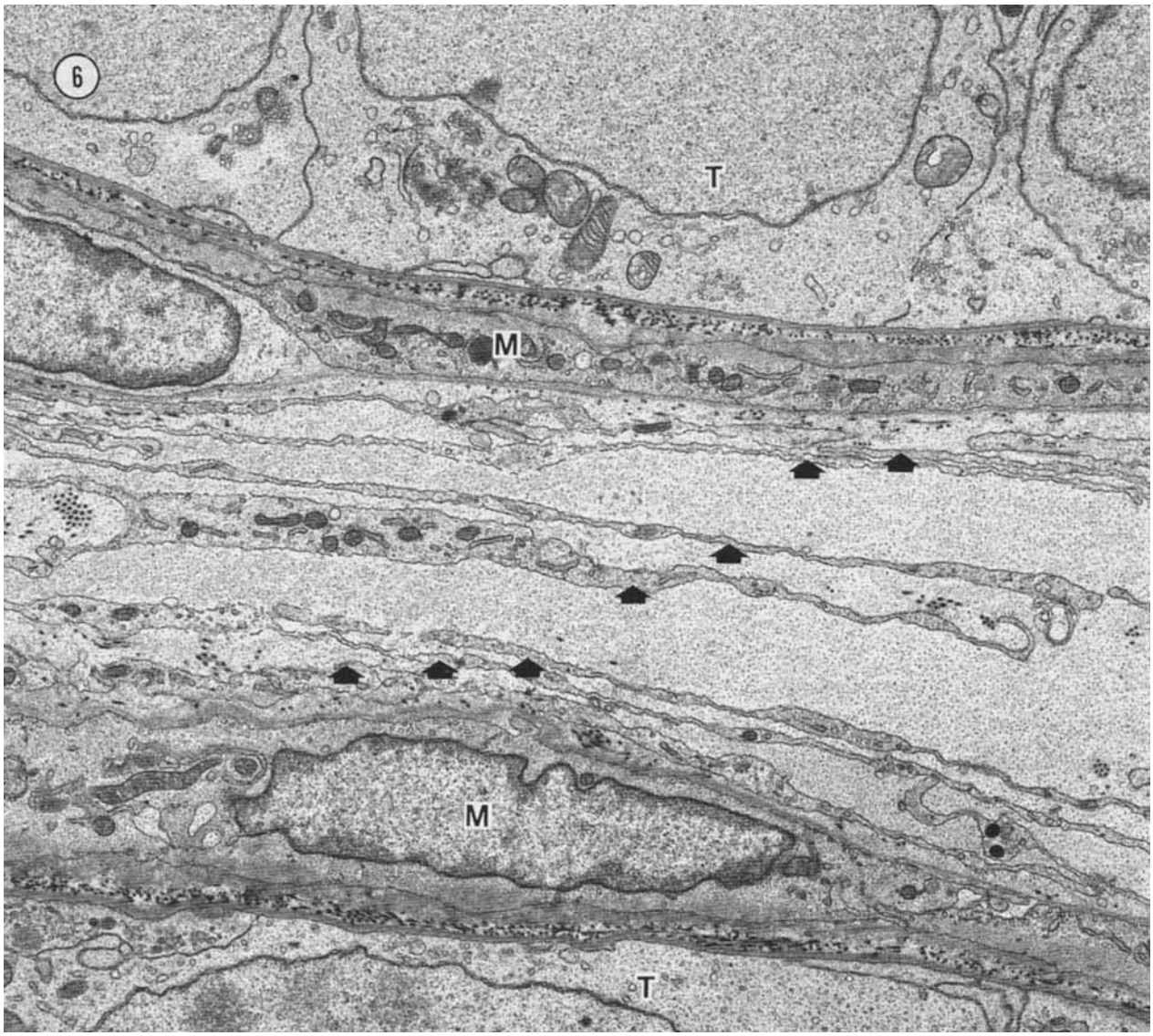

Fig. 6. The peritubular layer of a 25-day-old, PTU-treated animal shows at least seven fibroblastic (arrows) cell layers between the myoid cells (M) of two tubules (T). × 9,000.

resulting in an increase in testis size (Hardy et al., 1993). It not only affects maturation of cells but their rate of maturation. For example, peritubular cells and Leydig cells delay their maturation and germ cells degenerated in PTU treated rats. In other words, *the Sertoli cell is the prime regulator of testis development.* It has been postulated that fetal testis development is under control of the Sertoli cell (Burgoyne, 1988). We extend this postulate to neonatal testis development.

The hypothesis set forth above may be interpreted to suggest that all aspects of testis size are controlled by the Sertoli cell. There are, however, apparently limits to the Sertoli cells ability to regulate testis size and development. For example, the number of germ cells supported by an individual Sertoli cell (Russell and Peterson, 1984) is slightly less than normal in PTU-treated animals (Hess, 1993). This suggests that some factor such as overcrowding within the tubules restricts germ cell development. It would be interesting to determine if the potential to develop is restricted to the spermatogonial stages as the result of density-dependent regulation of germ cells (de Rooij and Janssen, 1987). It was noted by Hess et al. (1993) that Sertoli cell nuclei in PTU-treated adults were more closely spaced than those of control animals, another feature suggesting crowding of the tubule. Observations in the present report suggest that crowding of nuclei in PTU-treated animals of 25 days of age causes them to elongate in the tubule and display increased surface area, although signs of maturity of these nuclei are not evi-

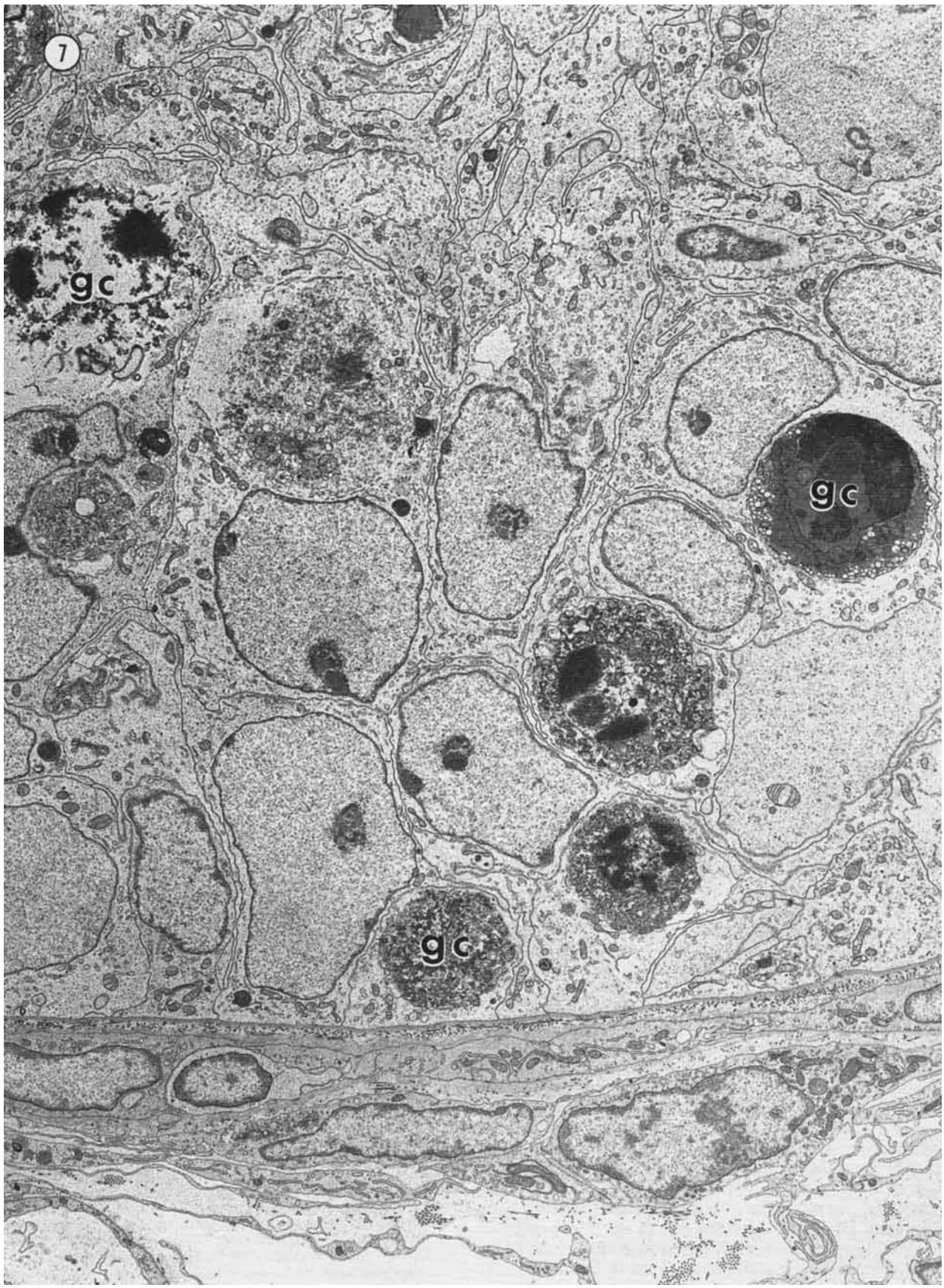

Fig. 7. Micrograph showing extensive germ cell (gc) degeneration in a tubule from a 25-day-old, PTU-treated animal. × 5,000.

TABLE 2. Volume (μm^3) of the Sertoli cell cytoplasm components expressed per cell (mean ± SE) (N = 5)

Parameter	Control 15 days	PTU-treated 15 days	Control 25 days	PTU-treated 25 days
Ground substance	288.3 ± 35	213.8 ± 23	619.5 ± 55[a]*	259.3 ± 14
Mitochondria	26.9 ± 6	17.5 ± 3	52.4 ± 8[a]	21.5 ± 2
Smooth endoplasmic reticulum	43.0 ± 8	20.9 ± 3	113.0 ± 16[a]	25.3 ± 2
Rough endoplasmic reticulum	1.0 ± 0.3	0.6 ± 0.3	6.5 ± 0.7[a]	1.2 ± 0.2
Golgi + associated vesicles	2.3 ± 0.6	1.9 ± 0.3	5.3 ± 1.5	4.6 ± 0.6
Lysosome & multivesicular bodies	2.8 ± 0.8	2.3 ± 0.6	8.1 ± 1.5[a]	2.8 ± 0.6
Lipid	0.7 ± 0.4	0.02 ± 0.002	15.1 ± 2.1[a]	1.1 ± 0.8

*Means in rows with different superscripts indicate significant differences.

TABLE 3. Surface area (μm^2) of the Sertoli cell components expressed per cell (mean ± SE) (N = 5)

Parameter	Control 15 days	PTU-treated 15 days	Control 25 days	PTU-treated 25 days
Plasma membrane (total)	1074 ± 63[a]*	685 ± 111[b]	2533 ± 208[c]	1124 ± 61[a,b]
Associated w/germ cells[1]	290 ± 50	111 ± 19	823 ± 83[a]	148 ± 8
W/ectoplasmic specialization	3 ± 3	2 ± 2	236 ± 32[a]	0 ± 0
Nuclear membrane	176 ± 22	168 ± 16	181 ± 20	259 ± 25[a]
Outer mitochondrial membrane	312 ± 36	202 ± 34	528 ± 91[a]	268 ± 11
Inner mitochondrial membrane	462 ± 68	278 ± 44	845 ± 143[a]	351 ± 15
Smooth endoplasmic reticulum	1129 ± 202	618 ± 91	2877 ± 362[a]	810 ± 81
Rough endoplasmic reticulum	62 ± 17	49 ± 17	526 ± 87[a]	71 ± 18
Golgi + associated vesicles	80 ± 20[a]	72 ± 14[a,c]	229 ± 59[b]	204 ± 35[b,c]

[1]Indented parameters represent a subdivision of main parameter and should not be added to achieve a total plasma membrane surface area.
*Means in rows with different superscripts indicate significant differences.

dent. Finally, prolonged treatment of animals with PTU beyond 25 days of age does not lead to testes that are larger than treatments lasting until day 25 (Cooke et al., 1992).

The present study indicated that the maturation of the Sertoli cell tight junctions was delayed in PTU-treated animals. The 25-day, PTU-treated animals showed the same surface area of the structural complex known as the ectoplasmic specialization (Russell, 1977), a cytoskeletal complex associated with tight junctions (Flickinger and Fawcett, 1967), as did 15-day-old control animals. Although some tight junctions were seen, development of extensive tight junctions was delayed along with numerous other features of the Sertoli cell. It appears that tight junction development naturally accompanies maturation of the Sertoli cell. Junctional development is not dependent on a specific stimulus such as gonadotropins. Gonadotropins influence the development of the tight junctional complex somewhat (Vitale et al., 1973), but are not able to do so in PTU-treated animals. Thus extensive tight junctions characteristic of the adult form only after Sertoli cell division ceases and accompany other features (structural and biochemical) that are associated with maturity of the cell.

The Golgi apparatus appeared to mature in PTU-treated animals, although numerous other organelles showed evidence of immaturity. Bunick et al. (1994) have shown that some proteins, likely processed through the Golgi, appear not be affected by the treatment.

The data presented herein do not rule out the possibility that PTU has a direct effect on germ cells or other cell types. Intuitively, one would expect only one target site for PTU and that appears to be the Sertoli cell as shown in tissue culture studies (Cooke et al., 1994). The possibility that other cell types are effected should be explored in the future.

In summary, we show that the Sertoli cells from animals treated with PTU do not show structural evidence of maturity in the postnatal period between 15 days and 25 days. Sertoli cell divisions continue in this period leading to more adult Sertoli cells and associated germ cells than normal and a larger testis. The present study and other published studies suggest that the Sertoli cell controls testis development both quantitatively and qualitatively.

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