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Morphometric Studies on Hamster Testes in Gonadally Active and Inactive States: Light Microscope Findings[1]

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ABSTRACT

This study provides quantitative information on the testes of seasonally breeding golden hamsters during active and regressed states of gonadal activity. Seminiferous tubules occupied 92.5% of testis volume in adult gonadally active animals. Leydig cells constituted 1.4% of the testicular volume. The mean volume of an individual Leydig cell was 1092 μm^3, and each testis contained about 25.4 million Leydig cells. The volume of an average Sertoli cell nucleus during stage VII–VIII of the cycle was 502 μm^3. A gram of hamster testis during the active state of gonadal activity contained 44.5 million Sertoli cells, and the entire testis contained approximately 73.8 million Sertoli cells. Testes of the hamsters exposed to short photoperiods for 12–13 wk displayed a 90% reduction in testis volume that was associated with a decrease in the volume of seminiferous tubules (90.8% reduction), tubular lumena (98.8%), interstitium (72.7%), Leydig cell compartment (79.3%), individual Leydig cells (69.7%), Leydig cell nuclei (50.0%), blood vessels (85.5%), macrophages (68.9%), and Sertoli cell nuclei (34.1%). The diameter (61.1%) and the length (36.8%) of the seminiferous tubules were also decreased. Although the number of Leydig cells per testis was significantly lower ($p<0.02$) after short-photoperiod exposure, the number of Sertoli cells per testis remained unchanged. The individual Sertoli cell in gonadally active hamsters accommodated, on the average, 2.27 pre-leptotene spermatocytes, 2.46 pachytene spermatocytes, and 8.17 round spermatids; the corresponding numbers in the regressed testes were 0.96, 0.20, and 0.04, respectively. The striking differences in the testicular structure between the active and regressed states of gonadal activity follow photoperiod-induced changes in endocrine function and suggest that the golden hamster may be used as a model to study structure-function relationships in the testis.

INTRODUCTION

The golden (Syrian) hamster (*Mesocricetus auratus*) is a seasonal breeder. In the wild, gonads are functional during the long days of spring and summer and atrophic during the shorter fall and winter days. This seasonal reproductive rhythm can be elicited in a laboratory population of this species at any time of year by artificial alteration in photoperiod (for reviews, see Bartke, 1985; Reiter, 1985). Exposure of adult male hamsters to a short photoperiod (<12.5 h light/day) produces testicular atrophy (Gaston and Menaker, 1967; Reiter, 1980). Reproductive function, however, undergoes spontaneous recovery (recrudescence) after approximately 25 wk or can be restored much earlier by returning the animals to a long photoperiod (Matt and Stetson, 1979; Stetson and Tate-Ostroff, 1981; Nelson and Zucker, 1987). Short photoperiod-induced testicular atrophy is usually accompanied by a marked decrease in serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and testosterone (Berndtson and Desjardins, 1974; Turek et al., 1975; Bex et al., 1978) and in the number of LH, FSH, and PRL receptors in the testis (Bex and Bartke, 1977; Bartke et al., 1987; Klemcke et al., 1987). Thus, golden hamsters are distinguished from most of the commonly used laboratory rodents by their marked seasonal testicular cycle of spermatogenic activity and the ability to "turn-off" or "turn-on" spermatogenesis in the laboratory by exposing animals to short (inhibitory) or long (stimulatory) photoperiods. Numerous studies concerning the endocrine aspects of photoperiod-induced reproductive cyclicity in hamsters are now available (for reviews, see Bartke, 1985; Reiter, 1985). A few studies describing the selected morphological changes in hamster testis following optic enucleation or light deprivation are

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also available (Reiter, 1968; Desjardins et al., 1971; Gravis, 1977; Gravis and Weaker, 1977). By contrast, morphometric studies on hamster testis at various phases of gonadal activity have received surprisingly little attention (Berndtson and Desjardins, 1974; Hardy et al., 1987; Johnson et al., 1987). Spermatogenic activity has been assessed in paraffin sections by counting the number of germ cell and Sertoli cell nuclei in round cross-sections of seminiferous tubules at stage VII of the seminiferous epithelial cycle during photoperiod-induced regression and recrudescence of the hamster testis (Berndtson and Desjardins, 1974). Studies by Hardy et al. (1987) and Johnson et al. (1987) have focused on Leydig cells; but even with limited focus, the results have been conflicting as to the numerical stability of Leydig cells in hamsters at different phases of gonadal activity.

We have recently described major cellular and subcellular changes in Leydig cells, germ cells, and Sertoli cells in hamsters after exposure to inhibitory photoperiods (Sinha Hikim et al., 1988). On the basis of our findings and those of others (Desjardins et al., 1971; Berndtson and Desjardins, 1974; Gravis and Weaker, 1977), we have suggested that the hamster could be employed as a model to study structure-function relationships in various stages of gonadal activity. Recent studies on another species of hamster, the Djungarian hamster (*Phodopus sungorus*) also support our contention (Bergmann, 1987). In the present study, accepted stereological methods—emphasizing serial section reconstruction to obtain nuclear volumes—were employed to obtain quantitative information on Leydig cells, germ cells, and Sertoli cells of the seasonally breeding golden hamster exposed to stimulatory and inhibitory photoperiods. The morphometric data obtained are the most comprehensive, to date, for most aspects of the hamster testis and show dramatic changes in various compartments, cells, and cell components within the testis.

MATERIALS AND METHODS

Animals

Adult, male golden (Syrian) hamsters of the Lak:LVG(SYR) strain, obtained from Charles River Breeding Laboratories (Wilmington, MA) were housed in a photoperiod-regulated facility and provided with food and water ad libitum. Hamsters were divided into two groups and were exposed for 12–13 wk to either a long photoperiod (n = 5) consisting of 16L:8D or to a short photoperiod (n = 5) consisting of 6L:18D.

Specimen Preparation

Fifteen minutes before animals were killed, they were injected i.p. with heparin (130 IU/100 g body weight; Russell et al., 1986). Subsequently, the animals were anesthetized with Nembutal®, a blood sample was withdrawn by cardiac puncture, and the left testis was surgically removed, weighed, and its volume measured by water displacement (Steer, 1981). The specific gravity was then determined by dividing the testicular weight by its volume (Steer, 1981). Blood plasma and testicular tissue were stored frozen for hormonal analysis and receptor studies, which are currently in progress. The right testis was perfused-fixed via the cardiac route with 5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) preceded by a brief saline wash. After perfusion, the right testis was removed and weighed. The volume and the specific gravity were determined (Steer, 1981) and the testis was sliced into ~1-mm-thick transverse slabs. One slab each from the proximal and distal poles and one from the middle region of the testis were then cut into smaller slabs with approximate dimensions of 1 × 2 × 2 mm and were immersed in the same fixative solution for an additional hour. The rationale for making slabs was that the tops and bottoms of the tissue slabs (2 mm) are easily differentiated from the sides (1 mm), allowing blocks to be oriented at the time of embedding. After glutaraldehyde fixation, the tissues were washed at 4°C in cacodylate buffer overnight and post-fixed in 1% osmium:1.25% potassium ferrocyanide mixture (Russell and Burguet, 1977), dehydrated in a graded series of ethanols, and embedded in Araldite. Tissue blocks were oriented so that seminiferous tubules could be sectioned transversely. To correct for shrinkage, two testicular cross-sectional slabs were used for each animal to obtain an index of shrinkage during dehydration and through embedding. The index of shrinkage in one dimension (linear) was derived as the ratio of the length of one axis in resin to the length of the same axis before secondary fixation, dehydration, and embedding. The value obtained was 0.952 ± 0.015 (SEM), or a reduction of 4.8% in linear dimension. Since there was no change in volume during primary fixation (see Results), no correction factor was introduced for this step. A 4.8%

change in linear dimension during tissue processing translated into a volume reduction of the embedded tissue of 13.7% ($0.952^3 = 0.863$) as compared with the fresh testis. A factor of 1.158 was applied to all volumetric measurements to correct for shrinkage.

Embedded tissue blocks were sectioned with a Sorval MT-1 ultramicrotome at microtome setting indicating 1 μm (after bypasses) for serial section reconstruction and routine morphometric analyses, or at 10 μm for diameter measurement of round germ cell nuclei (see below for determination of section thickness). All sections were stained with 1% toluidine blue. Sections cut at 10 μm thickness were stained longer to allow stain to penetrate the sections.

Morphometric Procedures

Volume density determinations. The volume density of the testicular components, including seminiferous tubules, tubular lumena, interstitium, Leydig cells, blood vessels, macrophages, and connective tissue cells, was obtained by point counting (see Weibel, 1979; Mori and Christensen, 1980). Ten randomly selected sections per animal in each group were examined under a binocular Microphot-FX microscope (Nikon, Garden City, NY) equipped with a bright-field condenser with a 40X objective and a 10X eyepiece fitted with a square lattice containing 441 intersections. The number of intersections ("hits") on pertinent structures over the entire tissue section was counted by predetermined and systematic movement of sections across the grid without overlap. Volume density (V_v) of each testicular component (which is the volume of a given testicular component per unit volume of testis tissue) was obtained by dividing the sum of points falling on each structure (Pi) by the total number of points over the tissue (Pt). The results were expressed as a percentage of the testis volume (V_v%) obtained by multiplying the volume density of each testicular component by 100. The volume fraction of the testicular capsule (%) was obtained by subtracting the volume fraction (%) of the seminiferous tubules and of the interstitium from 100. The present data on volume fraction of testicular components were based on at least 12,000 points per testis for each animal in each group. Absolute volume of each of the testicular components (V) was then determined by multiplying its volume density (V_v) by fresh testis volume (V_T): $V = V_v \times V_T$.

Seminiferous Tubule Length and Diameter

The diameters of 20 randomly selected transverse sections of the seminiferous tubules were measured for each animal across the minor axis of their cross-sectioned profiles (Wing and Christensen, 1982) with an ocular micrometer calibrated by means of a stage micrometer, and a 10X objective. The total length of the seminiferous tubule per unit volume of the testis (L) was calculated by the standard equation ($L = \frac{V_v}{\pi r^2}$) for a tube model (used by Wing and Christensen, 1982), where V_v is the tubular volume per unit volume of the testis tissue as measured above by point counting and r is the mean radius as determined from the diameter. Absolute values of tubular length were then calculated by multiplying the value obtained by the fresh testicular volume (V_T).

Leydig and Sertoli Cell Volumes and Numerical Densities

The absolute volumes of Sertoli cell and Leydig cell nuclei were determined by serial section reconstruction of five randomly selected nuclei of each kind per animal (total = 50) for each group. Calculations used for these volumes were based on a mean for each animal. From each animal, 20 serial sections were utilized. The tenth section was viewed at 1000X, and a region was identified in which Sertoli and Leydig nuclei appeared to be sectioned in their central region. The nuclear area for each of the five selected Sertoli cells (taken from stages VII or VIII only) and five Leydig cell nuclei was drawn by using a camera lucida attachment. This procedure was repeated for all sections containing the cells being drawn. The actual magnification at which the drawings were made was determined by projecting the stage micrometer onto the drawing paper. The individual nuclear volume (V_n) was then obtained by summing individual volumes of nuclear profiles (V_{ni}) in serial sections. The volumes of individual nuclear profiles (V_{ni}) were determined as follows:

$$V_{ni}\ (\mu m^3) = \frac{\text{nuclear area } (\mu m^2)}{(\text{magnification})^2} \times \text{mean section thickness } (\mu m)$$

The nuclear area was determined with a Numonics (Model 1224) electronic digitizer. The digitizer was checked for accuracy periodically by using graphs of known areas. To determine the mean section thickness

of light microscope serial sections, twenty-five round spermatid nuclei at stage VII of the cycle were reconstructed first by obtaining the mean diameter of the nucleus by direct measurements of its diameter using ~10-μm-thick sections where the focal plane revealed the greatest width of the cell. This approach has been utilized to yield the actual diameter (Christensen and Peacock, 1980; Mori and Christensen, 1980). The mean number of sections needed to traverse the mean diameter predicted the mean section thickness. Mean section thickness by this method was found to be 0.919 ± 0.017 (SE) μm. To verify this estimate, five thick sections were reembedded in Araldite, oriented perpendicular to the plane of the original sectioning, and thin-sectioned with a Reichert ultramicrotome. Electron micrographs were taken at 21,000× and section thickness was measured by this method. The mean section thickness was determined to be 0.922 μm (figure utilized was 0.92 μm). The average coefficients of variation for volume determination for Leydig and Sertoli cell nuclei between means from animals in each group were 11.7% and 11.9%, respectively. Leydig cell volume (V_{Lc}) was calculated from the nucleus cell ratio (Pc/Pn) multiplied by the volume of the nucleus (V_{Ln}). The nucleus:cell ratio was obtained by point counting (Sinha Hikim et al., 1986) from the sums of points falling on the nuclei (Pn) and on whole cells (Pc). Point counting was performed with a 441 intersection grid at 1000× magnification. The total number of grid fields counted for each animal varied from 80 to 100. The cytoplasmic volume of the Leydig cells (V_{Lcy}) was determined by subtracting the nuclear volume (V_{Ln}) from the cell volume (V_{Lc}).

The numerical density (N_v)—number of a given cell type per unit volume of testis of Leydig, Sertoli, and germ cells—was obtained by dividing the volume density of a particular cell type or its nucleus (V_v) by the volume of that cell or nucleus (V) using the formula: $N_v = \frac{V_v \times 10^{12}}{V}$, where 10^{12} is the conversion factor from cm^3 to μm^3, V_v is the volume density, and V is the mean absolute volume. The absolute number of these cells was determined by multiplying the value per unit volume of the testis (N_v) by the testicular volume (V_T).

A minimum of ten round seminiferous tubules at stages VII and VIII of the cycle of the seminiferous epithelium (Clermont, 1954) was used to determine the volume density of the Sertoli nuclei and germ cell nuclei by using point counting (441 intersection square lattice) for each seminiferous tubular profile (Wing and Christensen, 1982). The identification of various germ cells and Sertoli cell was based on the description of the cytologic details of these cell types provided by Clermont (1954). However, in gonadally regressed animals, stages VII and VIII (Clermont, 1954) were identified mainly by the appearance of preleptotene spermatocytes, which could also be identified in gonadally active animals. The number of Sertoli cell nuclei (and thereby cells) was then derived by dividing its nuclear volume density by the volume of an individual nucleus (as determined from serial section reconstruction; see above). The figure obtained was multiplied by the volume density of the seminiferous tubule to yield the number of cells per unit volume of the testis.

The number of germ cells (preleptotene and pachytene spermatocytes and round spermatids) per unit volume of seminiferous tubule was also recorded in active and regressed testes. The volume density of each of these cells was obtained by point counting as described above. Since these nuclei are spherical or nearly spherical, the volume of an individual nucleus (V_n) was calculated from the mean diameter (D) of the nucleus with the standard formula: $V_n = 1/6\ \pi D^3$ (Steer, 1981). The mean diameter of the nucleus (D) was obtained by direct measurements of its axis (maximum profile diameter), regardless of focal plane, at 1000× magnification with an occular micrometer from thick (~10 μm) sections. To verify the accuracy of volume determination by this method, volumes of 25 randomly selected round spermatid nuclei were measured from serial section reconstructions. The estimated spermatid nuclear volume (163.33 ± 3.09 μm^3) by serial sectioning was found to agree closely (164.62 ± 8.09 μm^3; figure utilized) with the ones obtained above. The number of germ cells per Sertoli cell (Sertoli:germ cell ratio) in active and regressed testis was then obtained by comparing average cell numbers in a unit volume of the seminiferous tubule.

In morphometric studies, it is customary to express volume in terms of "cm^3." However, for biochemical application of morphometric data, it is generally more useful to have the volume information in terms of "grams." The two values "cm^3" and "grams" are essentially interchangeable, since the specific gravity of fresh testis in hamster is almost near unity (see Table 1 in Results). We have, therefore, presented

some of our morphometric data in terms of "gram per testis," although in a strict sense they should be expressed "per cm^3 of testis tissue." Statistical significance of the differences between active and regressed testes was determined by Student's t-test; p values less than 0.05 were considered to indicate significant differences.

RESULTS

In the present study, the use of perfusion fixation followed by osmium:ferrocyanide post-fixation and plastic embedding resulted in quality structural preservation (Figs. 1 and 2), thus facilitating morphometric analysis. The basic data on hamsters exposed to stimulatory (16L:8D) and inhibitory (6L:18D) photoperiods are summarized in Table 1. The fresh testes of gonadally active hamsters weighed an average of 1.74 g per testis, with an average volume of 1.66 cm^3. The specific gravity of the fresh tissue was found to be 1.037. No change in testicular volume or weight or specific gravity was apparent between glutaraldehyde-fixed and unfixed tissues. While there was no significant change in body weight, a 90% decrease in testicular weight and volume was detected in gonadally regressed animals in comparison to gonadally active animals. Results of morphometric studies of hamster testes during active and regressed states of gonadal activity are given in Tables 2 and 3, and a graphic summary of data on various testicular parameters in regressed testes expressed as percentages of those in active testis is provided in Figure 3.

Volumetric Composition of the Testis

Seminiferous tubules occupied 92.55% of the testis volume in gonadally active animals; 14.37% of the testis volume was tubular lumen. In addition, 6.22% of testis volume was occupied by the interstitial tissue including Leydig cells (1.42%), blood vessels (1.49%), macrophages (0.25%) and connective tissue cells (0.26%), and 1.23% by the capsule (tunica albuginea). The relative volume of the testicular components (% volume as expressed per cm^3 of testis) was significantly altered in hamsters exposed to short photoperiods. Although there was an apparent reduction in % volume of seminiferous tubule, including lumen, the % volumes of the interstitium, Leydig cells, macrophages, and connective tissue cells were significantly increased in regressed testes (Table 2). The % volumes of the blood vessels during active and regressed states of gonadal activity were not significantly different (1.49% and 2.11%, respectively).

When the absolute volumes (V) of these testicular compartments (obtained by multiplying the value per cm^3 of testis by the fresh testicular volume) were compared between gonadally active and regressed testes, a significant reduction was found for each parameter in regressed testes (Table 2 and Fig. 3): seminiferous tubules (90.8% reduction), tubular lumen (98.8%), interstitium (72.7%), total Leydig cells (79.3%), blood vessels (85.5%), macrophages (68.9%), and connective tissue cells (72.0%).

Seminiferous Tubule Length and Diameter

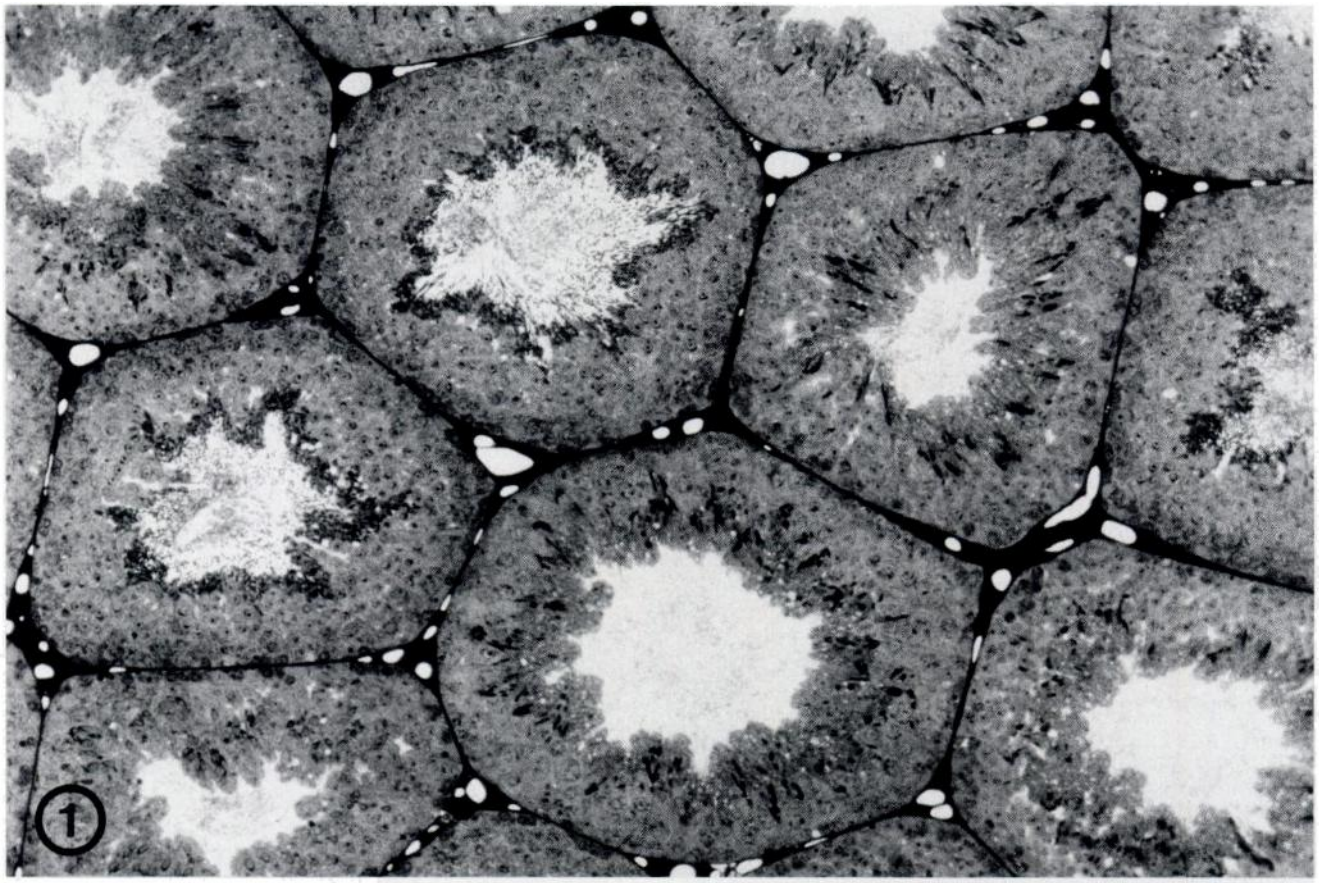
Mean tubular diameter was significantly reduced (61.1%) in regressed testes when compared with that of gonadally active testes. The average length of the seminiferous tubule as expressed per gram of testis and per testis in gonadally active hamsters was found to be 15.61 m and 25.92 m, respectively. The corresponding values for regressed testes were 96.26 m and 16.36 m, respectively. In spite of an apparent increase in the tubular length per gram of testis in

TABLE 1. Basic data on gonadally active and regressed hamsters.

Parameters	Active (n = 5)	Regressed (n = 5)
Body weight (g)	160.400 ± 7.500*	159.300 ± 9.700
Fixed (left) testis weight (g)	1.700 ± 0.110	0.160 ± 0.020[a]
Fixed (left) testis volume (cm^3)	1.630 ± 0.110	0.150 ± 0.020[a]
Specific gravity of fixed testis	1.043 ± 0.005	1.045 ± 0.008
Fresh (right) testis weight (g)	1.740 ± 0.100	0.180 ± 0.020[a]
Fresh (right) testis volume (cm^3)	1.660 ± 0.090	0.170 ± 0.020[a]
Specific gravity of fresh testis	1.037 ± 0.003	1.035 ± 0.008

*Mean ± SE.

[a] $p<0.001$ (p value refers to comparison with gonadally active animals).



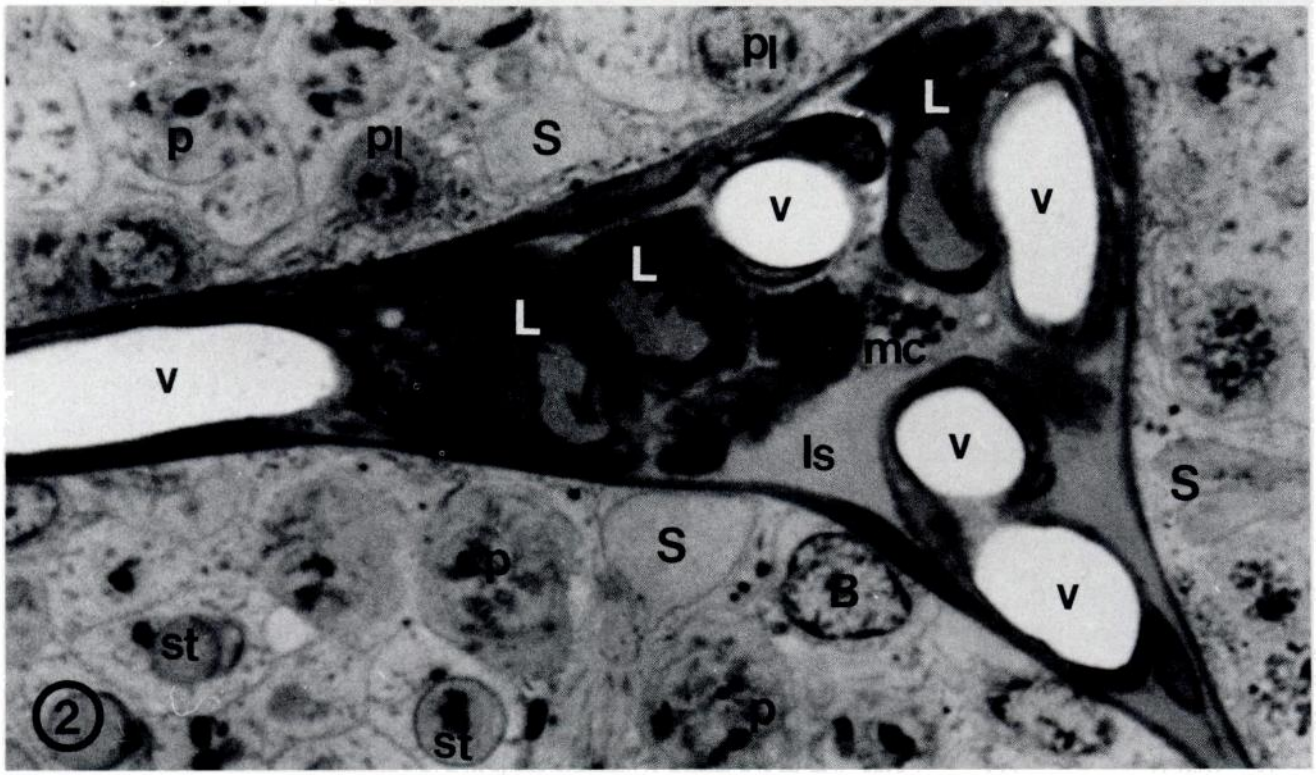


FIG. 1. Light micrograph of a gonadally active hamster testis fixed by perfusion and embedded in Araldite. The seminiferous tubules and interstitial tissue appear well-fixed. ×190.

FIG. 2. Interstitial tissue and adjoining seminiferous tubules at higher magnification exhibit the typical organization and components of interstitium consisting of Leydig cells (*L*), blood vessels (*v*), lymphatic sinusoids (*ls*), and a macrophage (*mc*). Leydig cells are easily distinguished by their characteristic nucleus and dark cytoplasm. Sertoli cells (*S*), B-spermatogonia (*B*), preleptotene spermatocytes (*pl*), pachytene spermatocytes (*p*), and round spermatid (*st*) are indicated in adjacent seminiferous tubules. ×1800.

regressed animals, the absolute length of the seminiferous tubule was decreased by 36.8% during testicular regression.

Size and Number of Leydig Cells

The mean volume of a Leydig cell in gonadally active hamsters was found to be 1092 μm^3. In an average Leydig cell, the nucleus was 197.7 μm^3 (obtained by serial section reconstruction), which was 18.1% of cellular volume. The average number of Leydig cells, expressed per gram of testis and per testis, was found to be 15.32 and 25.43 million, respectively.

In gonadally regressed testes, the Leydig cells were markedly reduced in size. The average values for nucleus, cytoplasm, and whole cell volumes were found to be only 49.9%, 25.9%, and 30.3%, respectively, of the values measured in gonadally active animals. The changes in the volume of the Leydig cell nucleus were less dramatic than the changes taking place in cytoplasmic volume during short photoperiod-induced testicular regression. Although there was an almost sevenfold increase in the average number of Leydig cells per gram of testis in gonadally regressed hamsters as compared to gonadally active animals, the absolute number of these cells (number/testis) declined by 29.3% ($p<0.02$) in the regressed testis.

Size and Number of Sertoli Cell Nuclei

The average volume of an individual Sertoli nucleus, as determined from serial section reconstruction, was

TABLE 2. Morphometric data on testis in hamsters during active and regressed states of gonadal activity following photoperiodic manipulations.

Morphometric parameters	Active (n = 5)	Regressed (n = 5)
Seminiferous tubule diameter (μm)	275.36 ± 5.29 (1.9)*	107.23 ± 6.63 (6.2)[c†]
% Volume of testicular components		
Seminiferous tubule	92.55 ± 0.84 (0.9)	83.33 ± 1.46 (1.7)[c]
Tubular lumen	14.37 ± 0.37 (2.5)	1.66 ± 0.81 (48.7)[c]
Interstitium	6.22 ± 0.27 (4.3)	16.59 ± 1.39 (8.3)[c]
Leydig cell	1.42 ± 0.03 (2.1)	2.88 ± 0.21 (7.3)[c]
Blood vessels	1.49 ± 0.14 (9.3)	2.11 ± 0.30 (14.2)
Macrophages	0.25 ± 0.03 (12.0)	0.76 ± 0.08 (10.5)[c]
Connective tissue cells	0.26 ± 0.03 (11.5)	0.71 ± 0.12 (16.9)[b]
Volume/testis		
Seminiferous tubule (ml)	1.52 ± 0.01 (0.6)	0.14 ± 0.002 (1.4)[c]
Tubular lumen (μl)	238.54 ± 6.14 (2.5)	2.82 ± 1.37 (48.5)[c]
Interstitium (μl)	103.25 ± 4.48 (4.3)	28.20 ± 1.39 (4.9)[c]
Leydig cell (μl)	23.57 ± 0.49 (2.0)	4.89 ± 0.35 (7.1)[c]
Blood vessels (μl)	24.73 ± 2.32 (9.3)	3.58 ± 0.50 (13.9)[c]
Macrophages (μl)	4.15 ± 0.49 (2.0)	1.29 ± 0.13 (10.0)[c]
Connective tissue cell (μl)	4.32 ± 0.49 (11.3)	1.21 ± 0.20 (16.5)[c]
Length of the seminiferous tubule (meters)/g of testis**	15.61 ± 0.37 (2.3)	96.26 ± 10.96 (11.3)[c]
Length of the seminiferous tubule (meters)/testis	25.92 ± 0.63 (2.4)	16.36 ± 1.87 (11.4)[b]
Volume of individual Leydig cell nucleus (fl)	197.71 ± 10.35 (5.2)	98.83 ± 5.24 (5.3)[c]
Volume of cytoplasm per Leydig cell (fl)	894.33 ± 53.29 (5.9)	232.49 ± 13.64 (5.8)[c]
Volume of individual Leydig cell (fl)	1092.04 ± 62.45 (5.7)	331.32 ± 15.57 (4.7)[c]
Leydig cell number ($\times 10^6$)/g of testis	15.32 ± 1.18 (7.7)	105.81 ± 6.79 (6.4)[c]
Leydig cell number ($\times 10^6$)/testis	25.43 ± 1.95 (7.6)	17.98 ± 1.15 (6.4)[a]
Volume of individual Sertoli nucleus (fl)	502.75 ± 26.77 (5.3)	331.09 ± 19.18 (5.8)[c]
Sertoli cell number ($\times 10^6$)/g of testis	44.51 ± 3.22 (7.2)	469.98 ± 80.51 (17.1)[c]
Sertoli cell number ($\times 10^6$)/testis	73.88 ± 5.34 (7.2)	79.89 ± 13.68 (17.1)

*Mean ± SE (SE as % of mean).

**Since the specific gravity of fresh testis in the hamster is almost near unity (1.037 ± 0.003), values per gram approximately are interchangeable with values per cm^3.

†Means with a superscript differ significantly from the gonadally active animals: [a]$p<0.02$; [b]$p<0.01$; [c]$p<0.001$.

TABLE 3. Germ cell : Sertoli cell ratios in stages VII–VIII tubules in hamsters during active and regressed state of gonadal activity following photoperiodic manipulations.[a]

Cell types	Active (n = 5)	Regressed (n = 5)
Preleptotene spermatocytes	2.27 ± 0.12[b] (1.76 – 2.51)	0.96 ± 0.07* (0.76 – 1.19)
Pachytene spermatocytes	2.46 ± 0.09 (2.16 – 2.88)	0.20 ± 0.07* (0 – 0.40)
Round spermatids	8.17 ± 0.56 (7.22 – 9.36)	0.04 ± 0.02* (0 – 0.15)

[a]Ratios derived from the cell number per unit volume (cm^3) of the seminiferous tubule.

[b]Mean ± SE (range).

*$p<0.001$ (p value refers to comparison with gonadally active animals).

found to be 502 μm^3 in gonadally active animals. The corresponding value in regressed testes was 331 μm^3, i.e. approximately 65.8% of the value measured in active testes. A gram of testis from a gonadally active hamster contained 44.5 million Sertoli cells and the entire testis contained 73.8 million Sertoli cells. In the average regressed testis, there were 469.9 million Sertoli cells per gram of testis and 79.89 million cells in the testis. The present study, therefore, demonstrates that in spite of significant reduction of Sertoli nuclear volume ($p<0.001$) in regressed testes, their number/testis remained unchanged during active and regressed states of gonadal activity.

Germ Cell : Sertoli Cell Ratio

Table 3 shows the germ cell:Sertoli cell ratios in stages VII–VIII of the hamster testis during active and regressed states of gonadal activity. In active testes, the individual Sertoli cell accommodated an average 2.27 preleptotene spermatocytes, 2.46 pachytene spermatocytes, and 8.17 round spermatids, and the corresponding cell numbers in the regressed testis were 0.96, 0.20, and 0.04, respectively.

DISCUSSION

Studies of structure-function relationships in mammalian testes have frequently used experimentally induced alterations of this system to understand how processes such as spermatogenesis are regulated. Approaches that have been used include hypophysectomy (Ahmad et al., 1975; Chowdhury and Tcholakian, 1979; Buhl et al., 1982), rendering the animals cryptorchid (de Kretser et al., 1980; Risbridger et al.,

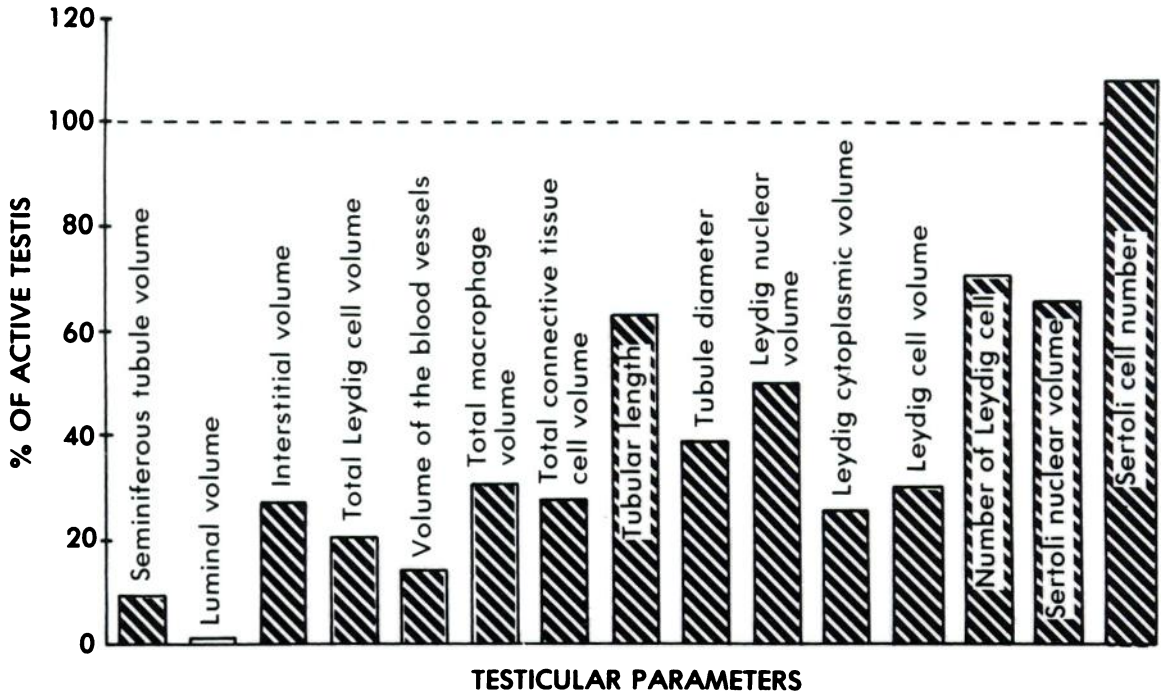


FIG. 3. Graphic summary of morphometric changes during testicular regression in the hamster. Values obtained from the regressed testis are expressed as percentages of those in gonadally active testis.

1981), and exposing the animals to irradiation or to various chemical agents (Setchell et al., 1977; Kerr et al., 1985; Jackson et al., 1986). A major drawback of these procedures is that they may affect multiple systems or cell types in the testis or exert various systemic effects rather than specifically affecting a target site within the testis. The animal model used in the present study, the seasonal breeding hamster, provides a physiological system for studying morphological changes in the testes in relation to changing endocrine states at different phases of gonadal activity (Desjardins et al., 1971; Berndtson and Desjardins, 1974; Stetson and Tate-Ostroff, 1981; Bartke, 1985; Bartke et al., 1987). Gonadal function of this species can be readily suppressed by exposure to a short photoperiod, and restored by return to a long photoperiod at any time of the year in the laboratory (Gaston and Menaker, 1967; Reiter, 1980). In a recent report from our laboratory, light-microscope and ultrastructural changes were described during short photoperiod-induced gonadal regression (Sinha Hikim et al., 1988). In this study, we have combined serial section reconstruction with stereology to obtain detailed morphometric information on hamster testes to substantiate and extend our previous qualitative observations of marked changes in Leydig cells, germ cells, and Sertoli cells in hamsters exposed to short photoperiods. These changes can be correlated with previous physiological and biochemical findings in order to develop a more complete understanding of the controlling factors and structure-function relationships of the various cell types in the testes.

Several technical improvements have been utilized in the present study that set this work apart from one or both of the previous stereological studies (Hardy et al., 1987; Johnson et al., 1987) of the golden hamster. For example, we have used perfusion fixation of the testis with buffered glutaraldehyde, post-fixation in osmium:ferrocyanide, and plastic embedding to provide optimal structural preservation. Serial section reconstruction was used at the light-microscope level to determine volume of nonspherical nuclei such as Sertoli cells and Leydig cells. The numerical density of a given cell type was then obtained by dividing its volume density by individual cell or nuclear volume. This method is preferred to the more commonly used stereological methods for counting cells using the Floderus equation (Floderus, 1944), since estimations of real diameter and height of the smallest visible nuclear cap are not required. Estimations of mean nuclear diameters and the cap size for a cell having irregular nuclei are, at best, estimates using conventional stereology (Bertram and Bolender, 1986). The calculation of numerical density by the Floderus equation is only accurate in the strictest sense if the nuclei are spherical (see nonspheroidal nuclei in Fig. 2). Moreover, our data have been corrected for the volume changes that take place during specimen preparation for microscopy, thereby translating values to what might have been present in the fresh tissue.

Quantitative information obtained in the present study confirms and extends earlier findings describing the kinds of morphological alterations seen in hamster testes following short photoperiod-induced testicular regression (Desjardins et al., 1971; Gravis, 1977; Gravis and Weaker, 1977; Sinha Hikim et al., 1988). Exposure to short photoperiod leads to an almost 90% reduction in both testicular weight and volume. The absolute volumes of nearly all testicular compartments (seminiferous tubule, tubular lumen, interstitium, total Leydig cells, blood vessels, macrophages, and connective tissue cells) were significantly decreased in gonadally regressed hamsters in comparison to those in gonadally active animals. Volumetric composition of the average gonadally active testis obtained in the present study is in close agreement with the previously reported values for sexually mature hamsters (Mendis-Handagama et al., 1988).

The Leydig cells were markedly reduced in size in regressed testes, a change that was more a function of the decrease in cytoplasmic than in nuclear volume. There was an almost 70% reduction in average Leydig cell volume. In this respect, our results are in substantial agreement with previous studies on various seasonally breeding animals including hamsters (Neaves, 1973; Hochereau-de Reviers and Lincoln, 1978; Hardy et al., 1987; Johnson et al., 1987). Reduction in Leydig cell cross-sectional area during photoperiod-induced testicular alteration has also been demonstrated in the Soay ram (Hochereau-de Reviers et al., 1985). By contrast, Johnson and Thompson (1986) have not observed significant seasonal fluctuations in the average volume of individual Leydig cells in horses. However, it should be emphasized here that the seasonal or photoperiod-induced changes in testicular structure and function are much more conspicuous in hamsters than in horses, the horse being an animal that shows active spermatogenesis throughout the year, including

the nonbreeding season (Johnson and Thompson, 1983).

Accompanying the reduction in cell size, the absolute number of Leydig cells also declined in the regressed testis (from 25.43 million to 17.98 million) as compared with the active testis. The present findings of decline in Leydig cell number during testicular involution in hamsters exposed to short photoperiod is consistent with the findings of Hardy et al. (1987) but is at variance with those of Johnson et al. (1987), who did not observe significant changes in Leydig cell number between gonadally active and regressed testes of hamsters. The reason for this discrepancy between the findings of Johnson et al. (1987) and those of other studies (Hardy et al., 1987), including the present results, remains unclear. Differences in preparative methods such as use of immersion fixation after slicing the testis in half (Johnson et al., 1987) may partially explain these results.

Quantitative studies of Leydig cells in seasonally breeding animals other than the hamster have provided variable results. In the rock hyrax, absolute number of Leydig cells was reduced from 127 ± 16 (SE) million in breeding to 93 ± 10 million in nonbreeding animals ($p>0.05$; Neaves, 1973). Leydig cell number was significantly reduced by 36% in the nonbreeding stallion (Johnson and Thompson, 1986), whereas no change in cell number was noted in the Soay ram (Hochereau-de Reviers et al., 1985). It is not clear whether there are generalized differences in Leydig cell number in seasonally breeding mammalian species or if the responses are species-specific.

Recent autoradiographic studies demonstrate that Leydig cell renewal occurs during artificially induced testicular recrudescence in hamsters (Hardy et al., 1987; Johnson et al., 1987). The source of additional Leydig cells as noted in the present study in gonadally active hamsters could be explained by the proliferation and subsequent differentiation of interstitial precursor cells. It is widely believed that a pool of connective tissue cells exists in the intertubular tissue that, with appropriate stimulation, can differentiate into Leydig cells (Christensen, 1975; Kerr et al., 1985; Jackson et al., 1986). The present findings of significant increase in the relative volume of connective tissue cells in the regressed testis are consistent with this hypothesis. Detailed ultrastructural and autoradiographic studies using tritiated thymidine are, however, needed to identify putative precursor cells as they undergo differentiation into functional Leydig cells during testicular recrudescence.

In gonadally active hamsters, the length of the seminiferous tubule per unit volume (1 cm^3) of testis and per testis was estimated to be 15.6 m and 25.9 m, respectively. The corresponding values for rats are 12.4 m and 17.4 m, respectively (Wing and Christensen, 1982). Values for tubular length and tubular diameter obtained from regressed animals were decreased about 36.8% and 61.1%, respectively. Significant reduction in tubular length and/or diameter has also been recorded during the nonbreeding season or photoperiod-induced testicular regression in other seasonal breeders such as rock hyrax (Neaves, 1973), Australian bush rat (Hodgson et al., 1979), red deer (Hochereau-de Reviers and Lincoln, 1978), and Soay ram (Hochereau-de Reviers et al., 1985). If not for decreases in tubular length, seminiferous tubules would appear considerably more depleted of germinal elements than suggested by simple examination of cross-sectioned tubules. Thus it is not appropriate to compare germ cell content in short-day animals with long-day animals by simply counting numbers of cells in tubular cross-sections without correction for tubular shrinkage by use of Sertoli correcting factor (Clermont and Morgentaler, 1955) or other acceptable means.

The marked reduction (98.8%) in the seminiferous tubular lumen volume during testicular regression is noteworthy. This suggests almost complete shutdown of Sertoli cell secretion. During testicular regression in the hamster, the amount of seminiferous tubule fluid secreted was decreased compared with that in gonadally active animals as measured by the increase in testicular weight 24 h after ligation of the efferent ducts (Bartke et al., 1987). The small diameter of the seminiferous tubule, the lack of a tubule lumen, and the inhibition of spermatogenesis appear to mimic the effects of hypophysectomy in rats.

This is the first study to utilize serial section reconstruction to obtain average volume of an individual Sertoli cell nucleus of the hamster. The present estimate of Sertoli nucleus volume (502 μm^3) in hamsters during an active state of gonadal activity compared favorably with values reported for other species such as rats (525–591 μm^3; Russell et al., 1988), and humans (512 μm^3; Johnson et al., 1984) but is lower than that reported for horses (693 μm^3; Johnson and Nguyen, 1986). The present study shows that there was a significant reduction (34.1%) in the size (volume) of Sertoli cell nucleus during the transition from an active to a regressed state of gonadal

activity. Reduction in cross-sectional area of Sertoli cell nucleus during photoperiod-induced gonadal regression has been reported in Soay rams (Hochereau-de Reviers et al., 1985). Alterations in morphological parameters of Sertoli cell, although not extensively studied, were expected based upon the reported changes in the responsiveness of the Sertoli cell to testosterone and FSH during testicular regression (see below).

The data regarding Sertoli cells and germinal cells are based on measurements made in stages VII and VIII and may not be generalizable to other stages. An average testis of gonadally active animals contained about 73.8 million Sertoli cells. Thus it appears that hamsters have more Sertoli cells than rats do (54 million, Hochereau-de Reviers and Courot, 1978; or 45.9–53.3 million, Russell et al., 1988), but fewer than most other seasonally breeding animals that had been studied during the gonadally active season, including red deer (463.5 million, Hochereau-de Reviers and Lincoln, 1978), Soay rams (12.9×10^8, Hochereau-de Reviers et al., 1985), and horses (3.68×10^9, Johnson and Nguyen, 1986). However, the hamster has considerably more Sertoli cells per gram of testis (44.5×10^6) in comparison to the red deer (6.55×10^6), Soay ram (12.2×10^6), and horse (24.0 ± 10^6). The differences in total number and packing of Sertoli cells between various animals appears to be species-specific.

Although the number of Leydig cells per testis was significantly lower after short-photoperiod exposure, the number of Sertoli cells remained unchanged. Studies on other seasonal breeders such as the red deer and Soay ram suggest similar findings. Johnson and Thompson (1983) reported seasonal variation in Sertoli cell numbers in horses. Since it has been reported that Sertoli cells neither divide in adult or hypophysectomized and hypophysectomized/hormone-treated animals (Steinberger and Steinberger, 1977), nor degenerate after hypophysectomy (Russell and Clermont, 1977), the mechanism(s) for seasonal loss of Sertoli cells and the source of additional Sertoli cells reported in horses during breeding season remains unclear.

In the present study, the germ cell number in hamsters during active and regressed states of gonadal activity was expressed as a ratio of the number of a particular type of germ cell in stages VII–VIII to the number of Sertoli cells. In active testes, the numbers of germ cells per Sertoli cell were 2.27 for preleptotene spermatocytes, 2.46 for pachytene spermatocytes, and 8.17 for round spermatids, suggesting 17% cell death during the development of cells to the round spermatid phase of development. In comparison to those of gonadally active animals, each Sertoli cell from regressed testes accommodated only 0.96 preleptotene spermatocytes, 0.20 pachytene spermatocytes, and almost no (0.04) round spermatids. Cell loss occurred prior to formation of preleptotene spermatocytes and during each cycle thereafter. With the possible exception of spermatogonia (Orth and Christensen, 1978), the Sertoli cell is generally regarded as the target for both FSH and testosterone (Hansson et al., 1978). The changes in its function are expected to result in lowered germ cell yield such as that seen after hypophysectomy (Clermont and Morgentaler, 1955). Therefore, it seems appropriate to utilize ratios of germ cells to Sertoli cells to express the ability of the Sertoli cell to support germ cells maintained to mid-pachytene and, occasionally, to Step 8 spermatids. Early spermatogenesis is thought to proceed qualitatively without hormones, but quantitative maintenance of early spermatogenesis is not possible in the absence of hormones. Quantitative changes appear to be the result of a hormonal deficit that produces alterations in Sertoli cell function.

We are extending our morphometric observations to the subcellular level in an attempt to understand how the Sertoli cell responds structurally under photoperiodic manipulations that have markedly lowered gonadotropic and steroid hormone levels. Many of the quantitative changes during short photoperiod-induced testicular regression shown in this study presumably relate to functional alterations taking place in the testis. It is well known that exposure of hamsters to short photoperiod leads to a significant reduction in the total content of LH, FSH, and PRL receptors in the testis (Bartke et al., 1987; Klemcke et al., 1987). However, the concentration of LH and, particularly, FSH receptors per unit weight of testis is typically elevated rather than suppressed during testicular regression (Bartke et al., 1987; Klemcke et al., 1987). The present findings of increased numbers of Leydig cells and Sertoli cells per gram of testis in regressed animals in which most, if not all, of the LH (Leydig cells) and the FSH receptors are presumed to be located is consistent with the observations of dissociation of changes in the concentration and in the total content of the receptor during testicular regression.

We also have extended earlier studies in the hamster

by showing changes in parameters not previously recorded. For example, the volume of macrophages is decreased by 68.9% in gonadally regressed hamsters, a feature that suggests that macrophages are responsive either directly or indirectly to changes taking place in the testis during photoperiod manipulations. The role of interstitial macrophages is not known, but it has been reported that in the rat they are responsive to FSH (Yee and Hutson, 1985). Additionally, there was a striking vascular response to change in photoperiod. In gonadally regressed animals, an approximately eightfold reduction in the volume of the vascular compartment was recorded. Thus there is an enormous capacity to lose and/or gain vascular space. The photoperiodically responsive hamsters thus serve as an excellent model to study not only these phenomena, but virtually every testis compartment or cell type (especially Leydig cells, Sertoli cells, and germ cells). Studies are in progress to elucidate the fine structural changes that accompany hormone changes and, specifically, to target the Sertoli cell and the morphological features of this cell that correlate with functional parameters.

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