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# Hormonal Regulation of Spermatogenesis in the Hypophysectomized Rat: FSH Maintenance of Cellular Viability During Pubertal Spermatogenesis

LONNIE D. RUSSELL, MIKE KERSHAW, KURT E. BORG,* AHMED EL SHENNAWY, SUSANA S. RULLI,† ROBERT J. GATES,‡ AND RICARDO S. CALANDRA†

*From the Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, Illinois.*

**ABSTRACT:** The potential for follicle-stimulating hormone (FSH) to promote germ-cell survival and the cellular sites of FSH action were studied using a gonadally maturing (pubertal), hypophysectomized (Hx) rat model in which residual testosterone (T) activity was blocked by injections of an androgen-receptor antagonist, flutamide. Recombinant human FSH was given to androgen-deprived and androgen-blocked male rats at 27 days of age to determine maintenance of individual germ-cell types at 35 days of age. Follicle-stimulating hormone significantly increased testis weights and tubular diameters as compared with Hx and Hx-flutamide controls, although testis weights in FSH-treated animals were significantly lower than in pituitary-intact animals. Morphometric assays to determine ratios of germ cells to Sertoli cells and to determine the number of germ cells present per hour of development showed that the population of type A spermatogonia in the early stages of the cycle was not responsive to FSH. Follicle-stimulating hormone had a marked ability to maintain cell viability in the rapid, successive divisions that begin in the latter part of the cycle and that continue through the next cycle (i.e., from type $A_1$ to $A_4$ and from intermediate spermatogonia to type B spermatogonia to preleptotene spermatocytes to leptotene/zygotene spermatocytes to young pachytene spermatocytes). The data also suggest T responsiveness of these cell types since the Hx-FSH-flutamide group showed lower cell viability at the aforementioned steps when compared with the Hx-FSH group. Too few cell types were present at subsequent phases of spermatogenesis to allow a sensitive determination of FSH activity in the maintenance of cell viability. The data show the potential of FSH in the absence or relative absence of T activity to maintain cell viability. These data support the concept of overlapping and synergistic (or additive) effects of T and FSH in the immature rat and identify the cellular sites of FSH action.

Key words: Testis, testosterone, hypohysectomy, cell viability.
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The role of follicle-stimulating hormone (FSH) in the maintenance and reinitiation of spermatogenesis in the adult animal has been the subject of considerable debate. What would seem a relatively easy issue to resolve turns out to be complex (reviewed by Zirkin et al, 1994). The first wave of spermatogenesis, or what has been termed "pubertal spermatogenesis" (Russell et al, 1987), may not be regulated in a manner similar to adult spermatogenesis. Nevertheless, there is currently more information to substantiate a role for FSH in stimulation of pubertal spermatogenesis than there is for FSH in adult spermatogenesis (Raj et al, 1976; Chemes et al, 1979a,b; Russell et al, 1987; Niklowitz et al, 1989).

One approach to studying pubertal spermatogenesis has been to analyze degenerating germ cells (Russell et al, 1987). Data from these kinds of experiments support a role for both FSH and luteinizing hormone (LH) that is independent and also synergistic during pubertal spermatogenesis through the prevention of cellular degeneration. We note that although cellular degeneration is one approach to studying and understanding hormonal control, it is only valid when the initial hormonally induced loss of cells takes place. Quantitation of cell degenerations over a long period does not reflect the impact of cell loss on spermatogenesis since degenerating cells are short-lived.

In searching for better methodologies and a better model to study FSH action in the testis, we focused on using improved methodologies. To selectively test the role of FSH, it was necessary to eliminate or reduce the influence of the other major supporting hormone, testosterone (T). We did this in hypophysectomized (Hx) animals by simultaneously administering FSH and flutamide, a compound known to bind specifically to androgen receptors

Correspondence to: Dr. Lonnie D. Russell, Laboratory of Structural Biology, Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, Illinois 62901-6512.

*Present address: Department of Pharmacology and Toxicology, University of North Dakota School of Medicine and Health Science Center, 501 North Columbia Road, Grand Forks, North Dakota 58202-9037.

†Present address: Instituto de Biologia y Medicina Experimental, Obligado 2490, 1428 Buenos Aires, Argentina.

‡Present address: Cooperative Wildlife Research Laboratory and Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901-6504.

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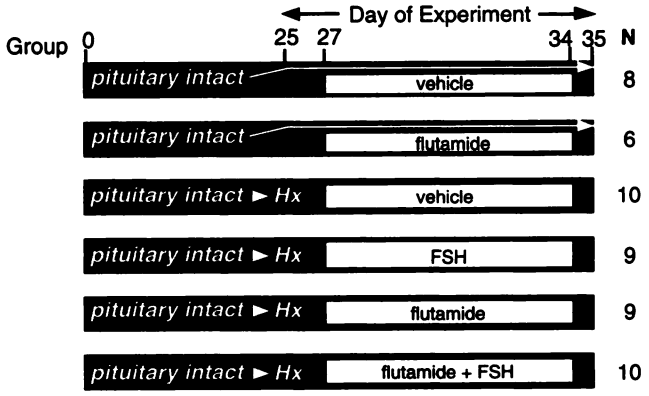


**FIG. 1.** Diagramatic scheme of the experimental protocol.

(Peets et al, 1974), thereby minimizing and/or eliminating residual T action (Sharpe, 1994). To prevent confounding results by using partially purified hormone preparations, we chose to use a pure preparation of human recombinant FSH. Finally, we developed a method for quantification of viable germ cells to assess the conversion of cells from one phase of spermatogenesis to another in control and experimental groups. The objective of this report was to examine the role of FSH in maintaining specific germ-cell viability in the developing rat, specifically by using an antagonist of T action such that the role of FSH could be examined.

## Methods

### Overview of Experimental Design

The experiment employed Hx to eliminate gonadotropins and utilized flutamide (Eulexin; Schering Canada, Ltd., Quebec, Canada) as a blocker of residual T action at the receptor level. Flutamide competes with T for receptor occupancy (Peets et al, 1974). Recombinant human FSH (National Institutes of Health National Pituitary Program, Bethesda, Maryland), free of LH, was utilized to determine FSH effects in gonadotropin- and androgen-deprived animals. The following groups of animals were employed (also see Fig. 1).

1) Pituitary-intact animals were given a saline vehicle from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 8).
2) Pituitary-intact animals were given flutamide from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 6).
3) Animals Hx at 25 days of age were given a saline vehicle from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 10).
4) Animals Hx at 25 days of age were given FSH from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 9).
5) Animals Hx at 25 days of age were given flutamide from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 9).
6) Animals Hx at 25 days of age were given both flutamide and FSH from 27 days through 34 days of age and were sacrificed at 35 days of age ($n$ = 10).

Endpoints were testis weights, tubular diameters, and two types of assays of viable cells at various stages or stage groupings of the spermatogenic cycle. In addition to the above, serum T was measured for all animals.

### Animal Treatments

Male Sprague-Dawley rats from Harlan Labs (Indianapolis, Indiana) were used for the study. Hypophysectomies were performed by the supplier via the transauricular route with a stereotaxic apparatus on animals of 25 days of age. After surgery, animals were provided continuous water containing 5% sucrose and solid food pellets *ad libitum*. Animals were shipped to Southern Illinois University and were housed in the vivarium where the lighting regimen was programmed at 14 hours of light to 10 hours of dark, and administration of 5% sucrose was continued.

Flutamide was administered twice daily using 2.5 mg, for a total of 5 mg/day, as a suspension in 0.9% sodium chloride and 1% gelatin. Follicle-stimulating hormone was administered at 12 IU per day in 50% polyvinylpyrrolidone dissolved in saline. Injections were initiated 2 days post-Hx (in animals of 27 days of age), and animals were sacrificed at 35 days of age. The rationale for sacrificing rats at 35 days of age was that, at this relatively advanced state of testis development, many germ-cell types are present and identifiable. The treatment time with FSH was limited to 7 days, a sufficiently short duration to avoid a marked immunological response to the injected FSH.

### Tissue Preparation and Tubular Diameter Measurements

Tissues were prepared according to the methods of El Shennawy et al (1998; this volume). Tubular diameters were measured using an ocular micrometer calibrated with a stage micrometer. Profiles of 50 round or near-round tubules were measured at random for each animal, and mean values were calculated. Only the short axis of each tubule was measured.

### Quantitative Methods

Two methods were used to quantify spermatogenesis. One method, the ratio method, quantified only very immature germ-cell types. A second method, point counting, quantified some young germ-cell types as well as more advanced germ-cell types. The ratio method provided the sampling needed when germ-cell numbers in tubules were low; the point counting method was found to be more suitable for quantification of large populations of germ cells and their expression as absolute values.

*Assay by the Point Counting Method*—A method devised for determining the numbers of viable germ cells per unit period of time was utilized (El Shennawy et al, 1998; this volume). Tissue sections were examined under an oil immersion lens at ×1,000 magnification on a binocular Nikon Microphot-FX (Nikon, Garden City, New York) microscope. Since the cells commonly used to characterize the cycle (Russell et al, 1990) were not present

Table 1. *Body weight, paired testis weight, seminiferous tubule diameter, and serum testosterone*

| Group | Body weight† (g) | Paired testis weight (g) | Seminiferous tubule diameter (μm) | Serum T (ng/ml) |
|---|---|---|---|---|
| Intact | 152.2 ± 1.7*[a] | 1.419 ± 0.027[a] | 226 ± 2[a] | 0.620 ± 0.049[a] |
| Intact-flutamide | 114.0 ± 1.7[b] | 1.142 ± 0.040[b] | 210 ± 3[b] | 2.384 ± 0.110[b] |
| Hx-FSH | 69.8 ± 2.6[c] | 0.608 ± 0.047[c] | 168 ± 5[c] | 0.312 ± 0.042[c] |
| Hx-FSH-flutamide | 65.1 ± 1.4[c] | 0.415 ± 0.017[d] | 152 ± 2[d] | 0.298 ± 0.037[c] |
| Hx | 72.5 ± 3.0[c] | 0.156 ± 0.007[e] | 103 ± 4[e] | 0.254 ± 0.017[c] |
| Hx-flutamide | 56.0 ± 1.4[d] | 0.134 ± 0.006[f] | 96 ± 2[e] | 0.239 ± 0.025[c] |

T, testosterone; Hx, hypophysectomy; FSH, follicle-stimulating hormone.
* Values are indicated ± standard error.
† Different superscripts within groups indicate significant differences.

in all treatment groups, it was necessary to utilize cells near the basal lamina to identify stage groupings, as has been previously shown (Clermont and Perey 1957; Russell and Frank, 1978). The four stage groupings were as follows. Stage group I was identified by the presence of intermediate spermatogonia. Stage group II was identified by the presence of type B spermatogonia. Stage group III was identified by the presence of preleptotene spermatocytes. Stage group IV was distinguished by the presence of type A spermatogonia in the absence of intermediate or type B spermatogonia or preleptotene spermatocytes. Stages in which basal compartment mitotic cells were present were not quantified. Furthermore, since germ cells were present for different periods of time, it was necessary to express their number per unit of time of development so that their developmental kinetics could be approximated.

*Assay by the Ratio Method*—The ratio method utilized relies on the principle that the number of Sertoli cells remains relatively constant throughout the life of the rat after the onset of puberty. Sertoli cells are not known to be lost after Hx (Ghosh et al, 1992a,b). Thus, the germ-cell type of interest was quantified in relation to the Sertoli cells surrounding the periphery of the tubule. The ratios were expressed as numbers of germ-cell nuclei per Sertoli cell nucleus. For each animal, no less than 3,000 Sertoli cells were counted along with associated germ cells in the same tubules. This method allows the comparison of changes in ratios of germ cells between groups.

Some degree of caution is recommended in interpreting the data from the cell ratio method and in determining yields from one cell type to another since ratios obtained are based on counts of section profiles where the sizes and shapes of different germ cells are not similar. Given that size and shapes change somewhat during the period of cell development utilized for quantitation by the ratio method (França et al, 1995; Russell and França 1995), the expected yields may vary slightly between the ratio and the point counting methods.

### Serum T Assay

Serum samples were extracted twice with diethyl ether. The extracts were resuspended in 0.05 M phosphate buffer containing 0.1% (w/v) gelatin, 0.05% (w/v) sodium azide, and 0.15 M sodium chloride. Testosterone was measured by radioimmunoassay, as described (Suescun et al, 1985), using an antibody (T-7 = FD- butyrate-BSA), which cross-reacts 35% with dihydrotestosterone, and 1,2-$^3$H-testosterone (New England Nuclear, Boston, Massachusetts). The specific activity was 60 Ci/mmol. The sensitivity of the assay was 12.5 pg/tube. The within assay coefficient of variation was less than 10%. All of the samples were measured in a single assay.

### Statistical Analysis

Data analysis was conducted using the methodology presented in El Shennawy et al (1998; this volume). The accepted cutoff for significance was $P < 0.05$.

## Results

### Body Weights, Testis Weights, and Seminiferous Tubule Diameters

Mean body weights in the pituitary-intact groups were significantly greater than in all Hx groups. The mean body weight for Hx animals given FSH was not significantly different from that of the Hx group but was significantly greater than that of the Hx-flutamide group (Table 1).

Paired testis weights were significantly higher in pituitary-intact groups as compared with all Hx groups. The mean paired testis weight in the untreated Hx group was significantly greater than that of the Hx-flutamide–treated group. In both Hx groups where FSH was administered, animal testes weighed significantly more than in the Hx or the Hx-flutamide group. The Hx-FSH group given flutamide showed a significantly lower mean testis weight as compared with the Hx-FSH group not receiving flutamide.

The mean seminiferous tubule diameter differed significantly among all groups, except there was no difference between the Hx and the Hx-flutamide groups. Flutamide given to pituitary-intact animals significantly depressed the mean seminiferous tubule diameter as compared to pituitary-intact animals not receiving flutamide. Hypophysectomy resulted in over 50% depression in seminiferous tubule diameter as compared with pituitary-intact animals. Treatment with FSH significantly increased seminiferous tubule diameter as compared with the Hx control. The FSH-treated Hx group showed a significantly

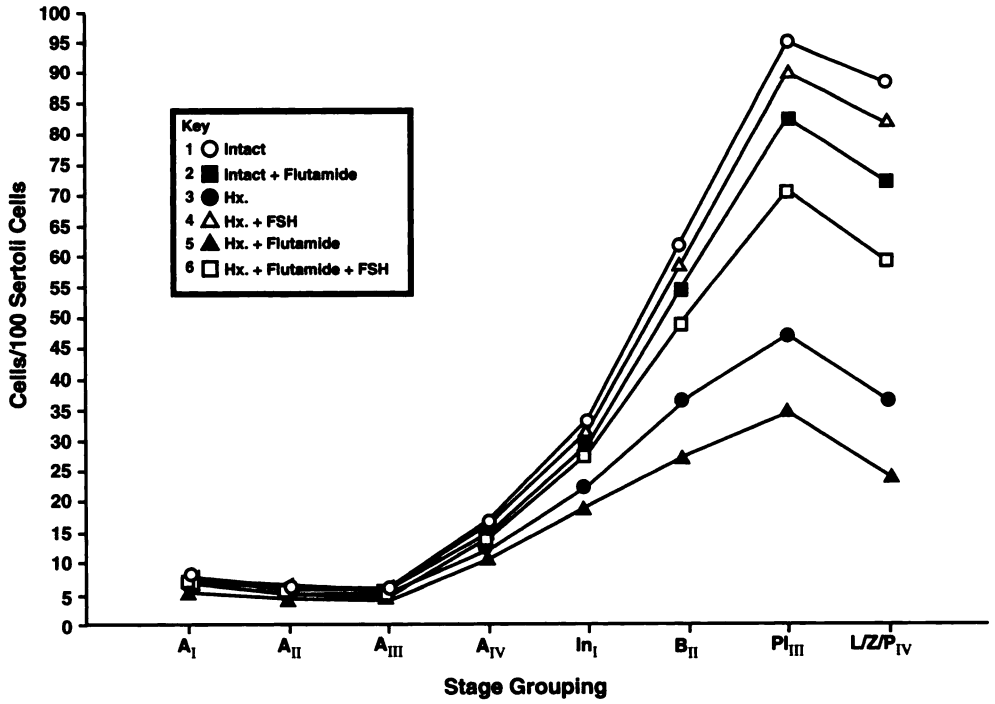


**FIG. 2.** A line graph showing the population of specific germ-cell types expressed as a ratio to Sertoli cells. Abbreviations used are $A_I$, $A_{II}$, $A_{III}$, $A_{IV}$ for type A cells in groups I through IV; In for intermediate spermatogonia in group I; B for type B spermatogonia in group II; PI for preleptotene spermatocytes in group III; and L/Z/P for leptotene, zygotene, and pachytene cells as seen in group IV. All group definitions are provided in Methods and in Figure 1. Error bars were not included in this figure so as to not obscure data points. Standard errors were approximately 10% of the mean.

greater mean tubular diameter than the Hx-FSH-flutamide–treated group.

### *Serum T*

All groups showed serum T levels that were significantly different from the pituitary-intact control (Table 1). Pituitary-intact animals given flutamide showed a mean serum T level approximately fourfold greater than pituitary-intact controls. In all Hx groups, the mean T levels were approximately 50% of the pituitary-intact controls and differed significantly from the controls. The T levels among Hx groups were statistically indistinguishable from each other.

### *Germ-Cell Numbers and Germ-Cell Ratios*

Figure 2 is a line graph of germ-cell numbers expressed per Sertoli cell (ratio method) in the various stage groupings. The percent change in a cell population between one phase of spermatogenesis and another was calculated using the ratio method and is depicted in Figure 3. Figure 4 is a line graph of germ-cell numbers expressed per hour of germ-cell development as determined by the point counting method. The percent change in a cell population for a group as it progresses to the next advanced group is shown in Figure 5. What follows is a partial description of the results that are detailed in both the cited line and bar graphs.

*Type A Spermatogonia*—Only the ratio method was used to quantify type A spermatogonia since too few of these cells were recorded with the point counting method to provide a sensitive measurement of cell numbers. Type A spermatogonia in groups I through III ($A_{I-III}$) were similar in number. However, the line graph revealed that in the cell divisions that form group IV ($A_{IV}$ of Fig. 2) the control and hormone-treated cell populations began to separate from the Hx and the Hx-flutamide groups, in that the latter contained fewer cells. The percent conversion of $A_{III}$ to $A_{IV}$ spermatogonia was significantly lower in the Hx and the Hx-flutamide–treated groups as compared with the other groups. The Hx-flutamide group contained significantly fewer cells than the Hx group.

*Type A Spermatogonia to Intermediate Spermatogonia*—The progression of $A_{IV}$ to intermediate spermatogonia showed a continued separation of groups containing hormones (either endogenous or injected) from the Hx and the Hx-flutamide groups (Fig. 2). Fewer viable cells were seen in the Hx and the Hx-flutamide groups. The percent conversion of cells in these groups showed significantly greater cell loss from $A_{IV}$ to intermediate spermatogonia (Fig. 3) than did the other groups. The Hx-flutamide group contained significantly fewer cells than the Hx group.

*Intermediate Spermatogonia to Type B Spermatogonia*—The trend demonstrated above continued in the di-

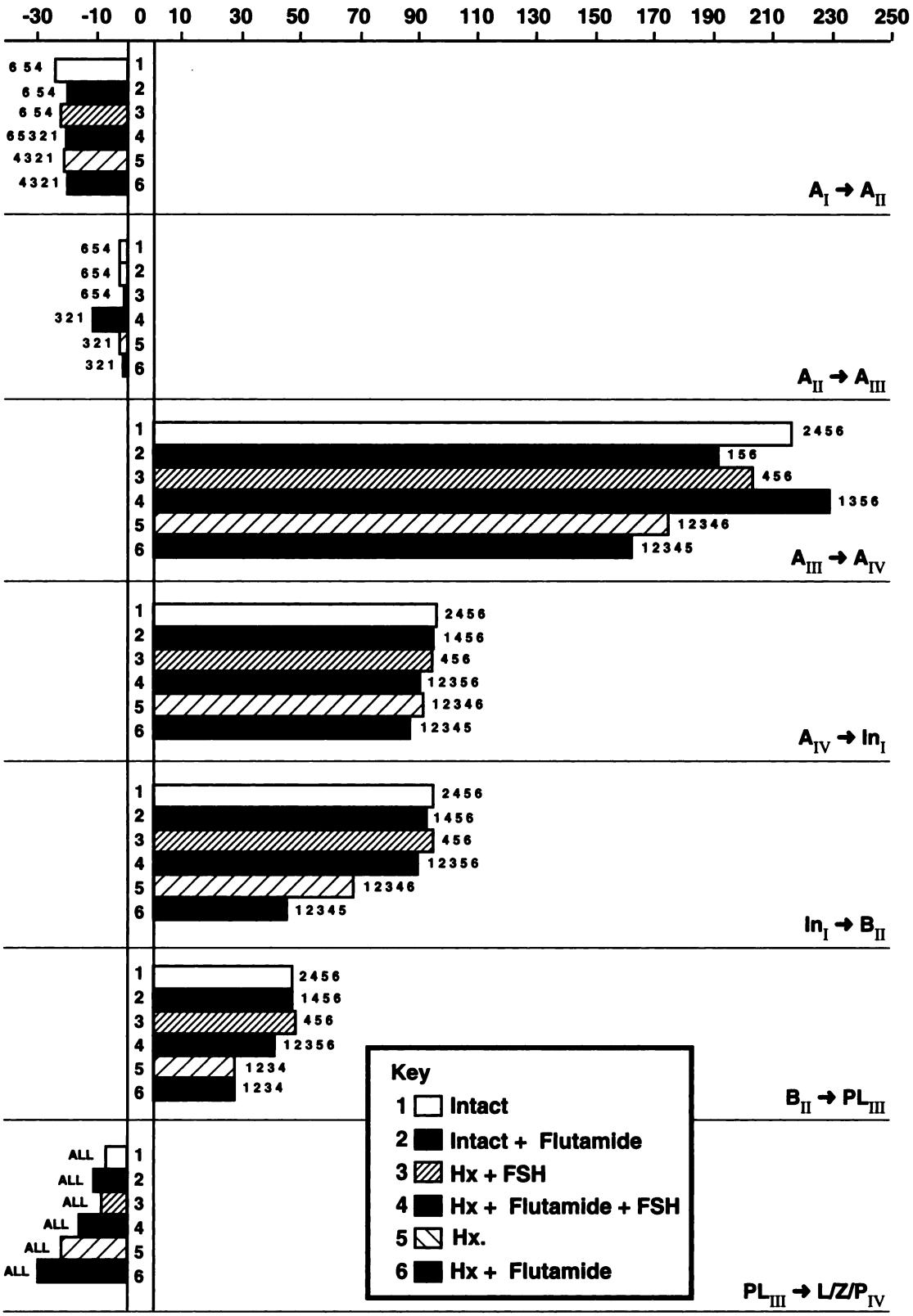


**FIG. 3.** A bar graph showing the percent change in cell populations from one phase of spermatogenesis to another using the ratio method. This change includes cell gain as the result of cell division and cell loss as the result of cell degeneration. The abbreviations used are the same as those detailed in Figure 1. The numbers apical to each bar represent significant difference between the group shown and a specific numbered group.

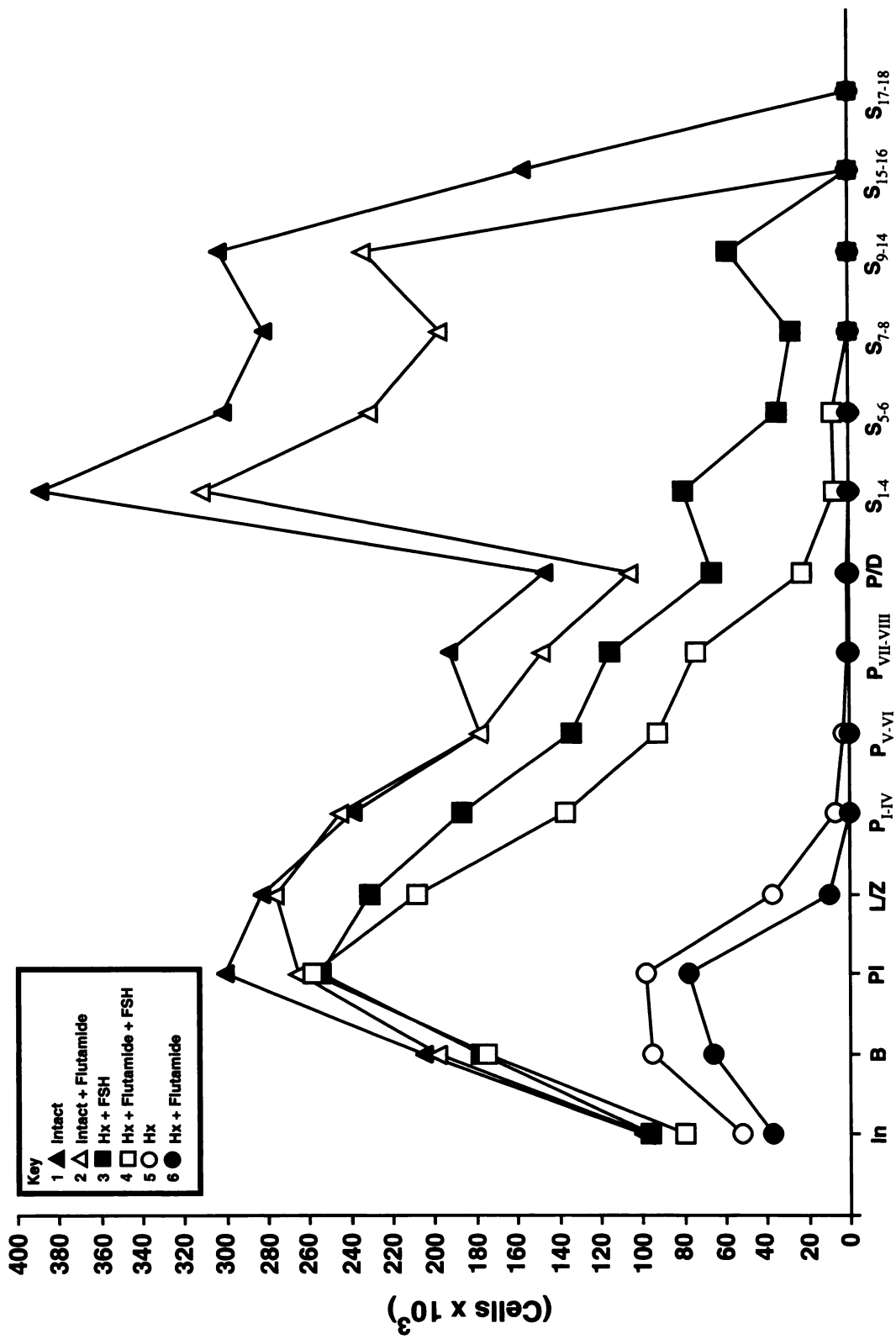


**FIG. 4.** Line graph showing the population of specific germ-cell types expressed per hour of development. Abbreviations used are In for intermediate spermatogonia; B for type B spermatogonia; Pl for preleptotene spermatocytes; L/Z for leptotene/zygotene spermatocytes; $P_{(\text{stage group subscript})}$ for pachytene spermatocytes in their respective groups; P/D for pachytene and diplotene spermatocytes; and S for spermatids in the respective steps of development. Error bars were not included in this figure so as to not obscure data points. Standard errors were approximately 10% of the mean.

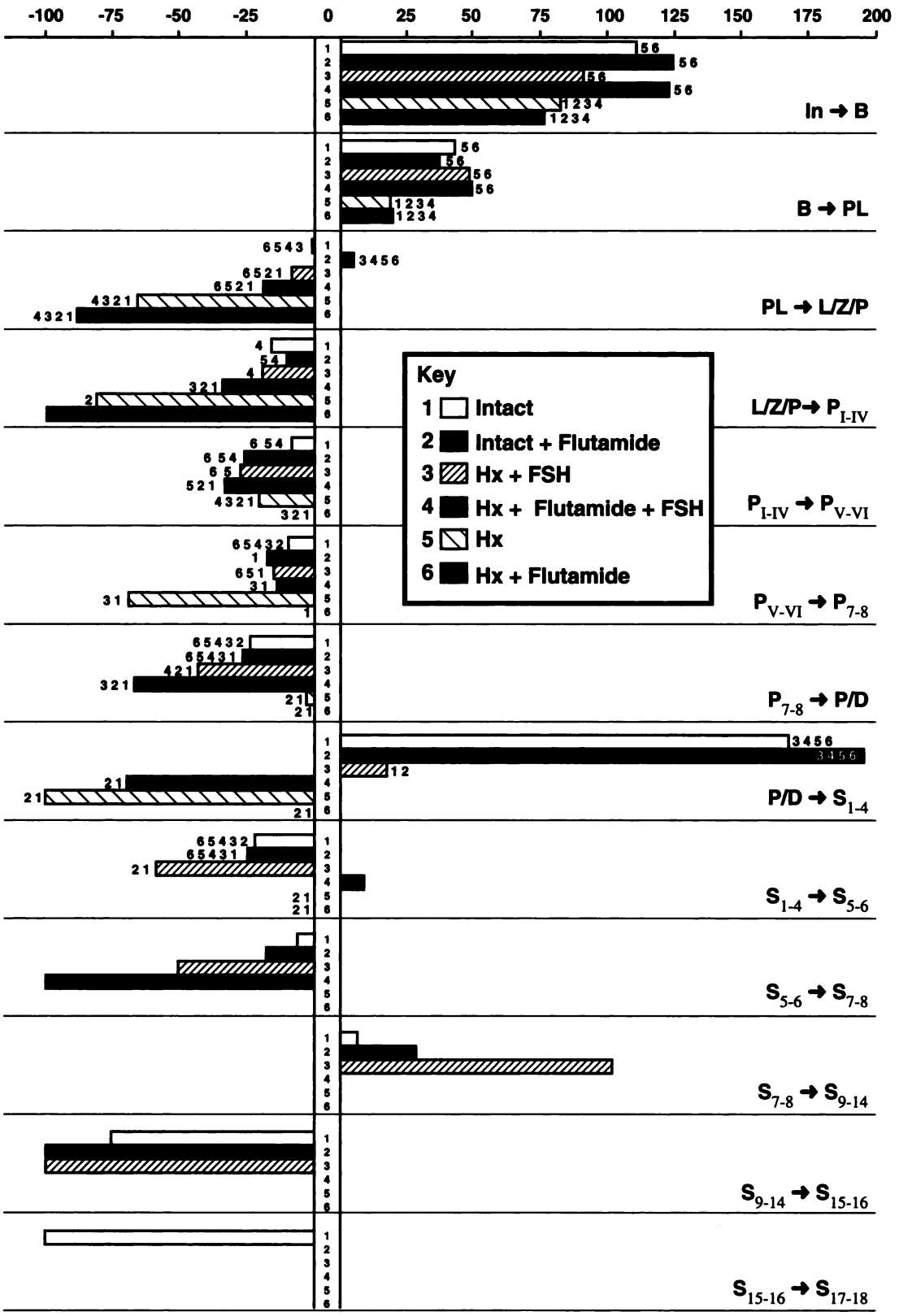


**FIG. 5.** A bar graph showing the percent change in cell populations from one phase of spermatogenesis to another using the point counting method. This change includes cell gain as the result of cell division and cell loss as the result of cell degeneration. The abbreviations used are the same as those used in Figure 3. The numbers apical to each bar represent significant difference between the group shown and a specific numbered group.

vision of intermediate spermatogonia to type B spermatogonia, further separating the two Hx groups without supplemented hormones from all other groups (Figs. 3, 5). The percent change of cells in animals receiving only FSH was not significantly different from that of controls (Figs. 3, 5). Those animals not receiving FSH showed significantly lower cell conversion than control animals (Figs. 3, 5).

*Type B Spermatogonia to Preleptotene Spermatocytes*—Using both the ratio and the point counting methods, a comparison of the numbers of type B spermatogonia converted to preleptotene spermatocytes in the control (pituitary-intact) animals showed that this conversion was inefficient. Control and FSH-administered groups were not significantly different (Fig. 5). Hypophysectomized and Hx-flutamide–treated animals showed significantly lower percent conversion rates than any other group (Figs. 3, 5). The line graphs clearly demonstrated that in animals with an intact pituitary or supplemented FSH cell viability at this phase was maintained as compared to Hx and Hx-flutamide–treated animals (Figs. 2, 4).

*Preleptotene Spermatocytes to Leptotene/zygotene Spermatocytes*—The line graphs showed that minor cell loss was evident in the transition of preleptotene spermatocytes to leptotene/zygotene spermatocytes in control animals (Figs. 2, 4). The bar graphs showed that significantly greater cell loss occurred in animals in the Hx or the Hx-flutamide group when compared with the other groups (Figs. 3, 5). The bar graphs also demonstrated that, while FSH inhibited cell loss, FSH did not maintain cell numbers at control levels.

*Leptotene/zygotene Spermatocytes to Pachytene Spermatocytes*—In the transition of leptotene/zygotene spermatocytes to young pachytene spermatocytes of stage groups I–IV ($P_{I-IV}$ of Fig. 4), cell loss was numerically greater in the Hx groups but was not always significantly different from the control groups (Fig. 5). The mean percent cell viability after FSH treatment more closely resembled pituitary-intact controls than Hx or Hx-flutamide–treated animals.

*Pachytene Spermatocytes to Pachytene and Diplotene Spermatocytes*—After early pachytene ($P_{I-IV}$) through mid-pachytene and into late pachytene and diplotene ($P_{V-VI}$, $P_{VII-VIII}$, and P/D of Fig. 4), the line graph showed that progressive cell loss occurred in control animals. There were too few cells remaining in the Hx and the Hx-flutamide groups for reasonable comparisons of cell loss in this and subsequent periods. The line graph (Fig. 4) indicated that the numbers of viable cells during pachytene were maintained in the Hx-FSH group as compared with the Hx-flutamide-FSH group since the lines in these groups gradually diverged during pachytene.

*Pachytene/diplotene to Step 1 Spermatids*—The control groups showed an almost threefold increase in cell populations during the two meiotic divisions. The Hx-FSH group showed a significantly lower percent cell increase as compared with control groups. The Hx-flutamide-FSH group showed a drop in cell numbers, as compared to a small increase in the Hx-FSH group (Fig. 4), and showed a significantly lower conversion rate in this phase.

*Group 1 Spermatids to Group III Spermatids*—In general, too few germ cells were present to make comparisons of cell viability during spermiogenesis.

## Discussion

In the 9-day post-Hx period, the testis regresses markedly unless hormones intervene to prevent regression. What was being examined in this study was short-term maintenance rather than reinitiation of spermatogenesis. One objective of this study was to determine whether purified FSH has an effect on spermatogenesis in a rat animal model in which FSH and T, or at least T action, have been virtually eliminated. It was shown that FSH stimulates and increases testis weight and tubular diameter and maintains germ-cell numbers. A second objective was to determine whether FSH affected particular phases of spermatogenesis in the immature rat. Follicle-stimulating hormone was shown to act at specific phases of cell development, but FSH action spanned a broad range of the seminiferous epithelial cycle. A third objective was to eliminate most residual T secretion and to determine whether residual T was active in supporting spermatogenesis. It was found that T was active at virtually all of the same cellular sites that FSH was active. Although it could be argued that T was not completely suppressed by flutamide, the relative suppression of T allows the same conclusions to be reached.

The pituitary-intact–flutamide group was employed as a control group to determine if flutamide was active. We hypothesized that flutamide would work at the level of the pituitary gland to prevent negative feedback and, thus, would increase LH secretion and T production, as has been previously shown (Rulli et al, 1995). Administration of flutamide elevated levels of serum T markedly: approximately four times that of pituitary-intact controls. In the pituitary-intact animals that were given flutamide, germ-cell numbers were generally quantitatively similar and followed a parallel pattern to those of the pituitary-intact controls. Therefore, it was postulated that an increase in intratesticular T in the pituitary-intact flutamide-treated animals, as compared to the T level in pituitary-intact control animals, competed effectively for androgen receptors and nullified some or most of the effects of flutamide (Teutsch et al, 1994). Examination of the flutamide-treated group showed that flutamide can work to

some degree in blocking T action, in spite of the extraordinarily high levels of T and the relatively greater affinity of T for its receptor as compared with flutamide (Teutsch et al, 1994). This was also borne out by the testis weights and the tubular diameter measurements of Hx animals given flutamide, in which values were lower than for Hx alone.

The experimental design was such that the role of T could be determined in addition to an examination of the effects of FSH. Given that Hx animals secrete some T (Sharpe and Bartlett, 1985; Kerr et al, 1992), flutamide was used in our groups to block the action of T and to virtually eliminate it from consideration, thereby allowing comparison of animals with some residual T secretion to those where the action of T was eliminated. Although a role for T is suggested below in some steps of spermatogenesis, there can be no definitive conclusion reached about T action since there was no group in which all hormones were eliminated and only T was administered as a replacement. There are numerous studies that indicate that T will support spermatogenesis quantitatively (or nearly so) in the adult animal (Zirkin et al, 1994), so the involvement of T has strong literature support, and it is not unreasonable to propose a role for T.

When interpreting the changes in cell populations (Fig. 4), it should be kept in mind that the animals were immature. In a developing animal, the more mature that germ cells are when examined, the earlier the time in the life of the animal that these cells initiated their development. Since spermatogenesis is progressively less efficient in earlier pubertal development (Russell et al, 1987), there should be progressively fewer germ cells as one examines the more mature germ-cell types from 35-day-old animals. The yield of cells in experimental groups should not be compared with the theoretical yield but with that found in the pituitary-intact group. The data are discussed below with respect to the gain or loss of cells from one stage grouping to the next.

### *Type A Spermatogonia*

The ratios of type A spermatogonia to Sertoli cells in the pituitary-intact group showed cyclic variability (Fig. 3). The number of type A cells reached their peak in stage group IV. The counts performed represented all type A spermatogonia: stem cells, undifferentiated cells, and differentiated cells. The graph represented the total numbers of these spermatogonial types and predominately reflected differentiated cells (Huckins and Oakberg, 1978).

The data from the bar graph showed that there are several areas where type A spermatogonia ratios increase or decrease significantly among stage groups. However, the most dramatic change in cell populations was from stage group III to stage group IV (Fig. 3), where the Hx group and the Hx-flutamide group showed a significant difference from the cell groups that either possessed endogenous hormones or contained FSH. These data strongly suggest that T also is involved in the maintenance of the number of spermatogonia. Testosterone was present in both pituitary-intact groups where spermatogonial numbers were high. Fewer spermatogonia were seen in the Hx group containing flutamide as compared with the Hx control group, also suggesting a role for T. Testosterone also is known as a potent stimulator of spermatogenesis, reinitiating and maintaining spermatogenesis to about 80% of normal in adult animals (Zirkin et al, 1989). In a recent paper by Meachem et al (1997), spermatogonial numbers were found to be lowered by T implants (low levels of T acting to depress pituitary secretion), but the numbers of type A spermatogonia were not restored solely by T alone (high levels of T), suggesting other factors were operative. Flutamide further reduced spermatogonia numbers in T-implanted adult rats, suggesting that residual T secretion from Leydig cells acted on spermatogonia (Meachem et al, 1997).

### *Type A Spermatogonia of Stage Group IV to Preleptotene/leptotene/zygotene Spermatocytes*

The line and the bar graphs representing the point counting method demonstrated a greater population of germ cells in animals with pituitaries or in Hx animals with supplemented hormones as compared with Hx animals or Hx-flutamide–treated animals. The percent conversion of viable cells in the bar graphs showed that cell loss was greater in the latter two groups for virtually all phases of spermatogenesis leading to preleptotene/leptotene/zygotene spermatocytes. The point counting method provided the same results as the ratio method, but the pattern of cell loss extended through pachytene cells of group I. By the leptotene/zygotene/pachytene phase, there were few cells remaining in the Hx and the Hx-flutamide groups.

Evidence from immunoneutralization experiments in gonadally maturing animals suggested that FSH has a role in stimulating cell development prior to the preleptotene phase (Chemes et al, 1979b). Animals that were immunoneutralized to eliminate circulating FSH yielded normal numbers of type A spermatogonia but lower than normal numbers of preleptotene spermatocytes. Other literature suggested that FSH is an important hormone for proliferation of spermatogonia and young spermatocytes in gonadally maturing animals (Vihko et al, 1991; Arslan et al, 1993; Russell et al, 1993; Sinha Hikim and Swerdloff, 1995).

In the aforementioned phases of spermatogenesis, groups containing FSH were generally not different (Fig. 3) from one phase to another with respect to cell survival as compared with control groups. However, the line graph (Fig. 2) showed that from intermediate spermatogonia to pachytene cells of stage group I, the Hx-FSH group was

more successful in maintaining cell survival than the Hx-FSH-flutamide group. Thus, T action was important in the presence of FSH at these phases of spermatogenesis, as was shown using both the ratio and the point counting methods.

### *Stage Group I Pachytene Spermatocytes to Stage Group IV Pachytene/diplotene Spermatocytes*

Numbers of viable cells progressively declined in control animals during pachytene and culminated in diplotene (Fig. 4). The line graph showed that the lines representing populations of germ cells in the two Hx-FSH groups became increasingly separated from the lines representing the populations of germ cells in the pituitary-intact animals, suggesting that FSH alone was not sufficient to maintain cell populations. In a similar manner, the line representing the population of germ cells in the Hx-FSH-flutamide group became increasingly separated from the Hx-FSH group. We interpreted this to mean that the additional T action in the Hx-FSH group supported germ-cell development to a greater extent than did the Hx-FSH-flutamide group, given that T was blocked in the latter. Thus, a role for T is suggested at these steps.

### *Diplotene Spermatocytes to Round Spermatids at Stage Group I*

The divergence of lines representing the pituitary-intact groups and the Hx-FSH–treated groups over the meiotic divisions suggested that FSH marginally promoted meiotic cell survival. The slight increase in cells in the Hx-FSH group, as compared with the Hx-FSH-flutamide–treated group, suggested that T also promotes cell survival. The role of FSH could not be ascertained since too few cells were present in the Hx and the Hx-flutamide groups to make comparisons. There is evidence in adult rats that both T and FSH play a role in the meiotic portion of the cycle (Russell et al, 1993; França et al, 1994), although major effects of T may be at midcycle (Russell and Clermont, 1977; Bartlett et al, 1986).

### *Conversion of Spermatids at Stage Group I to Spermatids at Stage 17–18*

During early spermiogenesis, the line graph showed that spermatid numbers in the control and the FSH groups show the same pattern during spermiogenesis, although the numbers of cells in Hx-FSH–treated rats were substantially lower than in the other groups (Fig. 4). There were too few cells present in the various groups after spermatids reached step 1–4 to draw a valid conclusion as to the efficacy of FSH.

### *General Comments*

Hypophysectomy of animals undergoing puberty is known to increase numbers of total degenerating cells throughout the entire spermatogenic cycle (Russell et al, 1987), a finding that was also supported by the present study. About twice as many total degenerating cells were noted at midcycle (VII–VIII) as at early or late cycle. Midcycle cell loss was also prominent in this study (B to PL; $P_V$ to $P_{VII-VIII}$ and $S_{5-6}$ to $S_{7-8}$), but degenerating cells enumerated in a short-term study and viable cells enumerated in a longer-term study may not provide equivalent results.

The literature is vast when one considers hormonal control of spermatogenesis (Steinberger, 1975; Sharpe, 1994). The literature on adult spermatogenesis supports either the concept that T alone can maintain/reinitiate spermatogenesis (Cunningham and Huckins, 1979; Awoniyi et al, 1989), that FSH is also important in the process (Steinberger, 1975), and/or that FSH and T are both important (Steinberger, 1975; Sharpe, 1994). Information for pubertal spermatogenesis is less abundant than for adult spermatogenesis but suggests a greater role for FSH (Raj et al, 1976; Raj, 1980; Russell et al, 1987; Matsumoto, 1989; Sharpe, 1994). The present study has added to previous studies by showing the potential of FSH to stimulate spermatogenesis and details the specific developmental phases of spermatogenesis promoted by FSH. In addition, data from the present study have suggested a role for T in these same phases of germ-cell development. Thus, as in the adult animal (El Shennawy et al, 1998; this volume), both T and FSH appear to have similar roles.

Whereas it may be easy to explain how hormones can act independently, it is not clear how hormones with vastly different modes of action can promote survival of the same cell types. The molecular mechanisms that may be operative are discussed by El Shennawy and coworkers (El Shennawy et al, 1998; this volume); however, there are other possibilities. One effect of FSH may be to potentiate the effect of T. For example, giving FSH usually results in increased androgen production, although the Leydig cells do not have FSH receptors (Vihko et al, 1991; O'Shaughnessy et al, 1992). In hypogonadal mice (*hpg*), FSH administration will increase T secretion (O'Shaughnessy et al, 1992). The effect of FSH in the adult may also be regulated by the ability of FSH to regulate androgen receptors (Verhoeven and Cailleau, 1988) and/or mRNA concentration, which may in turn increase the effect of T (Blok et al, 1989). The answer may be that, for example, T and FSH have both been shown to regulate cell $Ca^{2+}$ levels in Sertoli cells, a phenomenon that could lead to subsequent common physiological events (Gorczynska and Handelsman, 1991, 1995). There is evidence that FSH can have additive effects with T on differentiation of peritubular cells of the immature rat (Schlatt et al, 1993). In this vein, there is evidence that peritubular cells are obligatory intermediates in the stim-

ulation of Sertoli cells (Skinner, 1993). Data from the hypogonadal mouse (*hpg*) suggest that T alone has a potential to maintain adult spermatogenesis qualitatively (Singh et al, 1995). The ability to maintain quantitative spermatogenesis in the FSH-supplemented rat was not tested in that study. Recent information from the transgenic mouse strain lacking the β-subunit of FSH suggests that FSH is necessary for development of the testis but is not obligatory for any specific step in spermatogenesis since some sperm are produced (Kumar et al, 1997). These data and the present study suggest that FSH may compensate for lowered T levels.

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