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Hemiorchidectomy Leads to Dramatic and Immediate Alterations in Pituitary Follicle-Stimulating Hormone Secretion and the Functional Activity of the Remaining Testis in the Adult Male Bonnet Monkey (*Macaca radiata*)[1]

R. MEDHAMURTHY,[2] G.R. ARAVINDAN, and N.R. MOUDGAL

Primate Research Laboratory, Centre for Reproductive Biology & Molecular Endocrinology
Indian Institute of Science, Bangalore 560 012, India

ABSTRACT

The aim of the present study was to examine the effect of hemiorchidectomy (HO) on serum FSH, LH, testosterone (T), and inhibin (INH) concentrations as well as on the testicular volume (TV) and on changes in the kinetics of germ cell turnovers in the remaining testis of adult male bonnet monkeys. Blood samples collected at 2200 h at various times before and after HO and testicular biopsies obtained at different periods were subjected to hormone analysis and DNA flow cytometry.

Though serum T levels were lowered ($p < 0.05$) at 12 h after HO, T levels rapidly returned to intact control concentrations by Day 5. While serum LH remained unaltered, serum FSH increased markedly within 2 days of HO and remained significantly ($p < 0.05$) elevated over the next 90 days. Though serum INH showed a significant decrease ($p < 0.05$) by 15 min of HO, it returned to ~80% of intact levels within one week. The TV of the remaining testis showed maximal increment by Day 30 ($p < 0.05$) of HO. DNA flow cytometric analysis 24 days after HO showed increases ($p < 0.05$) in spermatogonia (2C) and primary spermatocytes (4C). These cell types by Day 45 had transformed to round (1C) and elongate (HC) (by 38%, $p < 0.001$) spermatids. Overall spermatogenesis (conversion of 2C to 1C and HC) showed significant enhancement at Days 110 and 175, suggesting that the spurt in spermatogenic activity is not confined to a single spermatogenic cycle.

Hemiorchidectomy in the adult male bonnet monkey thus leads to 1) rapid compensation in serum T and FSH concentrations and 2) increased TV of the remaining testis. In keeping with these alterations, the kinetics of germ cell turnover revealed dynamic changes.

INTRODUCTION

Hemiorchidectomy (HO) in farm animals either before or after onset of puberty is known to lead to a selective hypersecretion of FSH [1–9]. Most studies have failed to find concordant increases in basal LH levels [2, 4–7]. Secretion of testosterone (T), however, appears to be restored in these animals to precastration levels within days or weeks [1–9]. Furthermore, some of these studies revealed alterations in testicular weight and the kinetics of spermatogenesis in the remaining testis [4, 5]. Since the seminiferous tubules that comprise the major portion of the testis are packed mainly with germ cells (the Leydig, Sertoli, and other non-germ cells constituting a very small population) [10], the changes in testicular weight that follow HO might reflect increased activation of seminiferous tubules leading to proliferation of germ cells. The purpose of the present study was to describe the endocrine changes that follow HO and to examine the kinetics of germ cell turnover via DNA flow cytometry in the remaining testis of the hemiorchidectomized adult male bonnet monkey (*Macaca radiata*). Results of such studies in the adult male primate have hitherto not been reported.

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[2]Correspondence. FAX: 91-080-341683.

MATERIALS AND METHODS

General Methodology

Fourteen intact adult male monkeys 9–12 yr of age were used in the present study. At the time of surgery, their body weights ranged from 7.4 to 9.5 kg. The general maintenance of such animals under a controlled photoperiod (12L:12D) has been described previously [11]. This experiment was cleared by the local ethical committee for use of laboratory animals for biomedical research. Before initiation of the study, all monkeys were screened for the existence of normal diurnal T rhythms and normal germ cell populations as estimated by DNA flow cytometry. Only 9 of the 14 monkeys were subjected to the HO and frequent blood sampling protocol. The other 5 monkeys, which served as intact controls, were neither subjected to sham HO nor bled to monitor endocrine hormone changes. However, testicular biopsies were performed on all 14 monkeys at the same periodic interval for DNA flow cytometry of germ cells.

Hemiorchidectomy was performed through a scrotal incision following ketamine (Ketmin-50; Themis Chemicals Limited, Baroda, India) anesthesia administered initially at a dose of 10–15 mg/kg BW. Anesthesia was maintained by administration of ketamine at doses of 5 mg/kg BW as required. Each monkey was treated with 100 000 IU of penicillin G sodium and 250 mg of streptomycin (Dicrysticin-S; Sarabhai Chemicals Limited, Ahmedabad, India) i.m. as a postoperative prophylaxis daily for 3 days after surgery. In

addition, each monkey was given 500 mg of Analgin (Novalgin; Hoechst, Bombay, India), an analgesic, daily for 3 days.

Hormone Radioimmunoassays

Testosterone was assayed in duplicate 10–20-μl aliquots of serum without chromatography as previously described [12]. The sensitivity of the assay was 10 pg/tube (0.5–1 ng/ml). The inter- and intraassay coefficients of variation of the assay were 11.6% and 10%, respectively.

FSH was measured in 400-μl aliquots of the serum by a solid-phase RIA system as reported previously [13]. This system employs human FSH (hFSH-AFP-4822B) both for iodination and as standard; the antibody used was an antiserum raised in an adult monkey to ovine FSH. The sensitivity of the assay was 0.4 ng equivalent of hFSH-AFP-4822B/tube. The inter- and intraassay coefficients of variation were 14.1% and 10.8%, respectively.

LH was estimated in 100-μl aliquots of the serum by use of a sensitive radioreceptor assay standardized recently in this laboratory [14]. Human LH (NIH-AFP-4745B) was used for iodination and as standard. The source of the receptor (30 μl of an appropriate diluted receptor preparation/tube) was a crude particulate preparation made from sheep corpus luteum membrane. The sensitivity of the assay was 0.77 ng equivalent of hLH-NIH-AFP-4745B/tube. The inter- and intraassay coefficients of variation were 13.4% and 10.2%, respectively.

Serum inhibin (INH) concentrations were measured by use of an NIH RIA kit distributed by the Contraceptive Development Branch, NICHHD, Bethesda, MD. It consists of a bovine (b) INH (bINH-I-90/1): anti-bINH (#1989) RIA system that utilizes the recombinant human (rh) INH (rhINH-R-90/1) as standard. In brief, bINH was iodinated with iodogen as the oxidizing agent and the tracer was separated from free ^{125}I on a Sephadex G-25 column. The tracer was further purified sequentially on matrix Gel Red A and then Sephadex G-25 columns. The specific binding of the tracer (10 000 cpm) to the antiserum at a final dilution of 1:12 000 ranged from 21.5 to 31.4%; nonspecific binding was < 5%. Addition of castrated-monkey serum significantly reduced the percentage of nonspecific binding but did not have appreciable effect on the standards. Castrated-monkey serum (100 μl/tube) was, therefore, included with all the standard doses. Serum from an intact adult male bonnet monkey produced a dose-response curve that paralleled the standard curve. Assays were performed with a 100-μl volume of serum. The sensitivity of the assay was 0.125 ng equivalent of rhINH-R-90/1 per tube. The inter- and intraassay coefficients of variation (n = 5) of the assay were 8.2% and 4.6%, respectively.

Testicular Biopsies and DNA Flow Cytometry

At each biopsy, testicular parenchyma was collected through use of a sterile needle and trochar (i.d. 3.0 mm, 90 mm length; Silverman's liver biopsy needle, adult size). Testicular biopsy was performed under ketamine anesthesia. Testicular biopsies were obtained from the same testis of 5 control and 9 experimental monkeys on Days 0, 24, 45, 70, 110, 175, and 285 post-hemiorchidectomy. The methods employed for obtaining testicular biopsies and preparing single-cell suspensions of testicular germ cells have been described earlier [13]. The retrieved germ cells were fixed in 70% ethanol and stored at 4°C until analysis. The fixed cells were washed once in Dulbecco's PBS, pH 7.4 (DPBS; Himedia, Bombay, India) and treated with 0.5% pepsin (Serrafein Biochemica, Heidelberg, Germany) at pH 2.0 for 1 h at 37°C. After centrifugation at 800 × g for 10 min, the cells were resuspended in the staining solution containing 25 μg/ml of ethidium bromide (Sigma Chemical Co., St. Louis, MO), 40 μg/ml of ribonuclease A (Sigma), and 0.3% Nonidet P-40 (Sigma) in DPBS for 20 min at 4°C before analysis in a flow cytometer (FACScan; Becton Dickinson, San Jose, CA) equipped with an argon ion laser for fluorescence excitation at 488 nm. At least 10 000 cells were acquired during analysis using Consort-30 software (Becton Dickinson). Monkey peripheral blood leukocytes were used as the diploid standard to ascertain peak positions and to identify germ cell populations in the flow cytograms. The different cell populations quantitated on the basis of their DNA contents were expressed as "C" values. Essentially five distinct populations of germ cells could be determined: spermatogonia and other diploid cells (2C), the cells in S-phase, primary spermatocytes (4C), round spermatids (1C), and elongate spermatids (HC). The 1C and HC cells contain the same amount of DNA, but because they differ in the state of condensation of chromatin, they bind quantifiably different amounts of the fluorochrome; this facilitates discrimination of these two populations.

Statistical Analyses

The data are expressed as mean ± SEM. The significance of differences for mean hormone concentrations between days was determined by use of two-way analysis of variance followed by least significant difference test [15]. The means of germ cell flow cytometric data on percentages of germ cell populations and ratios were statistically analyzed via Student's t-test [16]. The correlation coefficients between different testicular germ cell populations and ratios were calculated by use of the Lotus 1–2–3 software package and the level of significance read from the table of critical values for correlation coefficients [17].

Experimental Protocol

Hemiorchidectomy was performed between 0900 and 1200 h. In 4 of the 9 monkeys, the left testis was removed; in the remaining 5 monkeys, the right testis was removed. Testicular volume (TV) was measured by means of Prader beads [18] immediately before and at 15, 30, 60, and 90 days

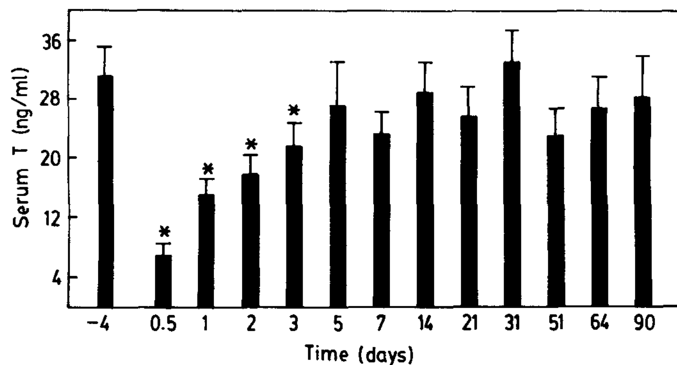

FIG. 1. Mean ± SEM concentrations of circulating T in adult male bonnet monkeys before and at various time intervals after hemiorchidectomy. For the days shown, blood samples were collected between 2100 and 2200 h. *Values differ ($p < 0.05$) from before, and from other days after, HO.

after HO. Blood samples were collected 4 days before and at 0.5, 1, 3, 5, 7, 14, 21, 31, 50, 64, and 90 days after HO. Additional blood samples were collected in 5 of the 9 monkeys one day before and 15 min before HO, and at 15, 30, and 120 min after HO for measurement of INH. Blood was collected through use of a sterile vacutainer blood collecting system via femoral vein/arterial punctures. To collect blood between 2100 and 2200 h, the 24-h L:D cycle was briefly interupted with the aid of a 2-cell (1.5 volt) torch. A nocturnal sampling was selected because the T secretion is maximal at this point in the 24-h cycle of the male bonnet monkey [19].

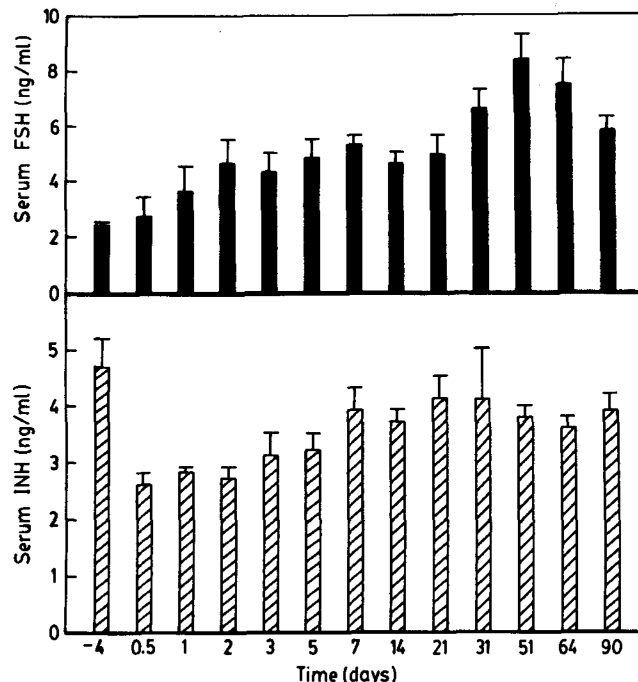

FIG. 2. Mean ± SEM concentrations of circulating FSH (top) and INH (bottom) in adult male bonnet monkeys before and at various time intervals after hemiorchidectomy. Blood samples were collected between 2100 and 2200 h. HO was performed between 0900 and 1200 h. Two-way ANOVA followed by least significant difference test indicated that mean FSH concentrations were significantly ($p < 0.05$) higher on all days beginning 2 days after HO as compared to precastration. Mean INH concentrations were significantly ($p < 0.05$) lower on Days 0.5 and 1 of HO as compared to precastration.

RESULTS

Effect on Serum T and LH Concentrations

The nocturnal T secretion was high in all monkeys before HO (Fig. 1). Circulating mean T concentrations decreased significantly ($p < 0.05$) within 12 h of HO. However, serum T recovered rapidly, and by Day 5 of HO, mean T concentrations were not significantly ($p > 0.05$) different from those in intact controls. Thereafter, mean T concentrations in serum collected at various time intervals were not significantly different ($p > 0.05$) from concentrations observed prior to HO (Fig. 1).

Circulating serum LH concentrations were 27.1 ± 2.1, 19.6 ± 1.2, 27.2 ± 1.1, 28.3 ± 1.7, 27.4 ± 1.8, 26.5 ± 2.1, and 28.2 ± 2.1 ng/ml on Day −4 and Days 0.5, 1, 2, 3, 5, and 7 after HO, respectively.

Effect on Serum FSH and INH Concentrations

The circulating mean FSH concentration was low (2.4 ± 0.1 ng/ml) 4 days prior to HO (Fig. 2, top). This progressively increased to a level 100% greater (4.6 ± 0.2 ng/ml FSH; $p < 0.05$), reached by Day 2 after HO. Mean serum FSH concentrations continued to be elevated, and by Day 51 of HO were 4-fold greater ($p < 0.05$) than the pre-HO values (Fig. 2). Thereafter, serum FSH concentrations appeared to show a marginal decline but the levels at Days 64 and 90 were still significantly ($p < 0.05$) higher than the pre-HO levels.

The circulating mean serum INH concentration 4 days before HO was 4.7 ± 0.5 ng/ml (Fig. 2, bottom). In 5 of the monkeys that were subjected to additional sampling, mean serum INH concentrations 15 min before HO were 4.4 ± 0.1 ng/ml; they decreased significantly ($p < 0.05$) to 2.4 ± 0.1, 2.4 ± 0.5, and 2.5 ± 0.6 ng/ml at 15, 30, and 120 min after HO, respectively. Mean serum INH concentrations in all 9 monkeys were ~60% of intact control concentrations 12 h after HO, but progressively returned toward normal (~80% of intact control concentrations) within a wk of HO (Fig. 2). The INH concentrations remained at this level throughout the remainder of the study (Fig. 2).

Effect on Testicular Volume

In 7 of the 9 monkeys, TV increased significantly ($p < 0.05$) within 15 days of HO (Fig. 3), becoming maximal by Day 30 after HO ($p < 0.05$). The TV remained at this level throughout the duration of the study. The data from the remaining two monkeys were excluded from computation since the TV prior to HO in those animals was 25 cc and

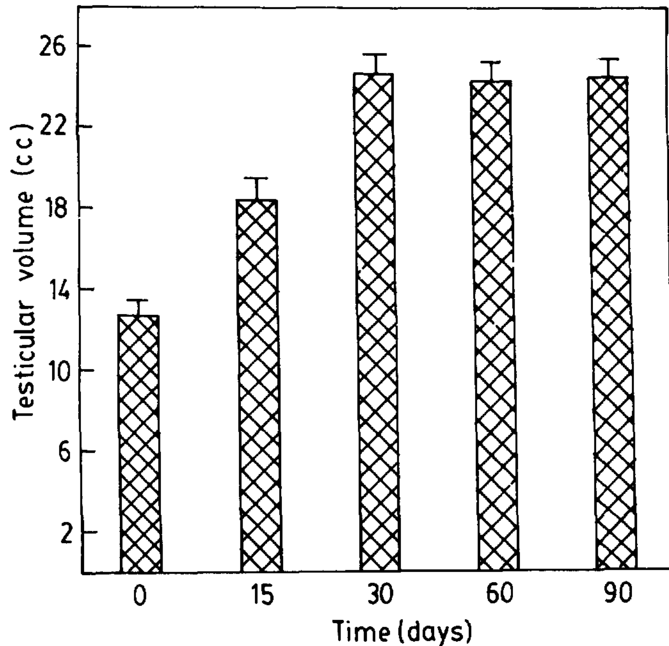

FIG. 3. Testicular volume (mean ± SEM) of a single testis of adult male bonnet monkeys before and at different days after HO. TV was significantly higher ($p < 0.05$) after HO.

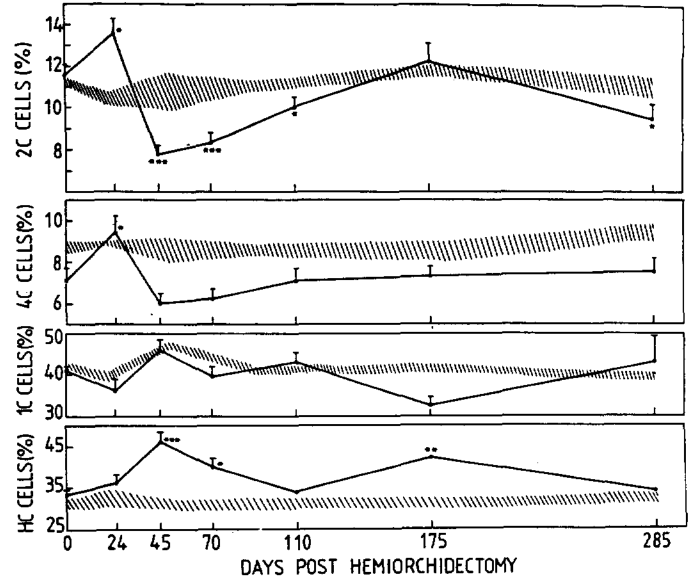

FIG. 4. Changes in percentages of germ cell populations as a consequence of testicular hypertrophy following hemiorchidectomy of nine bonnet monkeys over a period of 285 days. The hatched area represents mean ± SEM of percentages of germ cell populations obtained from five normal monkeys for the same period. 2C, spermatogonia; 4C, primary spermatocytes; 1C, round spermatids; HC, elongate spermatids. The germ cells were quantitated by quantitative ethidium bromide (EB) binding to germ cell DNA followed by flow cytometry. The ability of HC to bind a relatively lesser amount of EB, in spite of possessing an equal amount of DNA, permitted differential quantitation of the two populations. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

increments over this volume could not be measured with accuracy because of limitation in the size of Prader beads available.

Effect of HO on the Kinetics of Testicular Germ Cell Turnovers

The percentages of testicular germ cell populations for the 9 hemicastrated and 5 normal monkeys were determined by DNA flow cytometry over a 285-day period (Fig. 4). The germ cell percentages of controls remained relatively constant over consecutive biopsies taken at different times of the 285-day experimental period, indicating steady-state spermatogenesis. At 24 days following HO, significant increments ($p < 0.05$) in the premeiotic cell types, particularly spermatogonia (2C) and primary spermatocytes (4C), were observed. By Day 45, however, these cell populations showed significant reductions from the Day 24 values (2C by 43%, $p < 0.001$; 4C by 36%, $p < 0.01$); there was then a gradual increase over the next 130 days (Fig. 4). The percentage of 2C cells was positively related to the percentage of 4C cells and was negatively related to the percentage of HC cells ($r = 0.5$ and -0.4 respectively, $p < 0.02$; n = 54).

The kinetics of germ cell turnovers from one cell type to another, represented as ratios, are depicted in Table 1. While the 1C and HC populations remained virtually unaltered at Day 24, the 2C and 4C populations showed a marked increment, resulting in an apparent reduction in 1C:2C, HC:2C, and 1C:4C turnover ratios at this time. However, the 1C:2C and HC:2C ratios, which essentially represent overall spermatogenic rate, were significantly ($p < 0.01$) increased at Days 45 and 70 over the 24-day values, and these ratios also showed a secondary set of increments at Day 285. In contrast to the 4C:2C and 1C:2C ratios in controls, which were in steady state with no correlatable changes, the changes in the 4C:2C ratio in animals after hemiorchidectomy were correlatable to those in the 1C:2C ($p < 0.01$; $r = 0.4$) as well as the HC:2C ($p < 0.01$; $r = 0.35$) ratio.

DISCUSSION

The present study demonstrates that hemiorchidectomy in the adult bonnet monkey unleashes immediate and profound changes in the endocrine activity of the pituitary-testicular axis. Furthermore, the results document changes in the kinetics of germ cell turnover following HO in this primate species. The surprising finding that T secretion, which had halved following HO, returned to intact control concentrations within 5 days of HO—without concomitant measurable changes in peripheral serum LH secretion—indicated that mechanism(s) other than increment in serum LH may be responsible for the increased ability of the remaining testis to virtually double its output of T. It is unlikely that the increase in serum T level is due to changes in metabolism of T after HO, as it has been shown that in the monkey, peripheral T concentrations reflect de novo synthesis from the gonad [20]. Serum T concentrations have also been observed to increase following HO of GnRH-

TABLE 1. Ratios of testicular germ cells.[a]

	Kinetics of germ cell turnover during				
Days	Spermatogonia to Spermatocyte 4C:2C	Meiosis 1C:4C	Spermatocytogenesis 1C:2C	Spermiogenesis HC:1C	Overall spermatogenesis HC:2C
0	0.61 ± 0.05	6.07 ± 0.58	3.72 ± 0.18	0.78 ± 0.05	2.9 ± 0.30
24	0.69 ± 0.07**	3.81 ± 0.27	2.63 ± 0.30**	1.00 ± 0.21	2.63 ± 0.33
45	0.77 ± 0.04**	5.90 ± 0.44	4.54 ± 0.15	1.31 ± 0.17**	5.92 ± 0.68**
70	0.75 ± 0.06**	6.44 ± 0.68	4.80 ± 0.26	1.02 ± 0.13	4.9 ± 0.56*
110	0.71 ± 0.07	6.07 ± 0.70	4.31 ± 0.42	0.78 ± 0.14	3.37 ± 0.30
175	0.60 ± 0.05*	4.45 ± 0.37	2.66 ± 0.34*	1.31 ± 0.17**	3.5 ± 0.42
285	0.79 ± 0.04	5.77 ± 0.80	4.46 ± 0.41	0.79 ± 0.04	3.59 ± 0.42

[a]2C: spermatogonia; 4C: primarily spermatocytes; 1C: round spermatids; HC: elongate spermatids.
*$p < 0.05$; **$p < 0.01$, significantly different from Day 0 values. The germ cell ratios of treated monkeys on Day 0 was no different from that obtained for untreated control monkeys.

primed prepubertal rhesus males, but even there the T levels returned to intact control concentrations within a week of HO [21]. In that study, while bioassayable circulating LH remained unchanged, there was a 30% increase in the immunoreactive LH for the first two days after HO. The results of the present study are in agreement with the previously reported finding that little or no change in either bioassayable or immunoreactive LH occurs after HO in farm animals [4–7]. The mechanism by which the doubling in T output occurs within a short period after HO is currently unknown. It may be due to an actual increase in Leydig cell numbers; or it may be due to increased responsiveness of the existing Leydig cells to unchanging levels of LH because of their exposure to uncharacterized substances from the activated Sertoli cells (due to higher FSH levels). In hypophysectomized prepubertal rats, FSH administration has been reported to increase the transformation of mesenchymal cells to Leydig cells [22]. The authors of that study further suggested that the action of FSH was mediated through Sertoli cell factors, the identity of which is currently unknown [23, 24]. In the present study, serum FSH increased briskly following HO, and it remains to be determined whether elevated FSH was involved in the increased steroidogenetic activity of the remaining testis. Incidentally, it has been reported that administration of exogenous FSH but not LH into prepubertal rats leads to a significant increase in LH receptor concentrations of Leydig cells [25]. Also, an increase in FSH has been reported to be highly correlated with the rise in LH receptor concentration in developing rat testis [26]. Studies in ram lambs [5, 9], however, indicate that HO does not alter testicular gonadotropin receptor concentrations.

The increase in the testicular volume of the remaining testis could be due to either hypertrophy or an increase in testicular parenchyma, or both. Following HO, since FSH increases significantly and T production compensates rapidly to reach to pre-HO levels, it is not clear which of the two (if either) is responsible for the near doubling of TV. In our experience, while chronic FSH neutralization by antibodies does not lead to marked change in the TV [27], neutralization or reduction in LH levels in both the monkey and the rabbit (with consequent reduction in T levels also) leads to marked change in the TV (unpublished observations). On the other hand, it has recently been reported that in crab-eating monkeys the TV, which is normally suppressed following GnRH antagonist treatment, can instead be maintained at control levels by supplementation with exogenous FSH administered immediately after antagonist treatment [28].

Elevated immunoactive and/or bioactive FSH concentrations have been reported to occur after HO in many species [4, 6, 7]. The present study extends this phenomenon to the adult male subhuman primate. It is surprising that serum FSH concentrations remained elevated for more than 2 mo of HO in the present study. There are conflicting reports with regard to the duration of the FSH rise after HO; this rise is known to last from less than two weeks to months after HO in sheep [1, 7]. The role of elevated FSH concentrations in testicular function is not clear, but it becomes important because of its direct trophic effect on Sertoli cell function as well as on spermatogenesis. It has been reported that Sertoli cell numbers increase [1] if HO is performed neonatally, but that only hypertrophy occurs when older animals are HO [29]. Whether there is Sertoli cell hypertrophy or hyperplasia in the adult male monkey subject to HO remains to be clarified.

While INH in the adult male rhesus monkey has been shown to play a major role in regulation of FSH secretion [30], in the rat such negative feedback regulation of FSH has been shown to be operative only prior to the onset of puberty [31]. However, it is surprising that no discernible inverse relationship between FSH and INH secretion could be observed in the present study. The reason for this apparent discrepancy is unclear, but the limitation of the INH RIA employed in this study would have to be considered. Although the heterologous INH RIA of Robertson et al. [32], used in earlier studies in rhesus [33] and cynomologus [34] monkeys as well as in the current study, has been shown not to cross-react with free INH subunits and activin, its cross-reactivity with free INH subunit precursor proteins has

not been ruled out [35]. It has been shown that under situations of exaggerated FSH secretion in humans [36], the free INH alpha subunit precursor protein rather than the whole INH molecule increases. Since FSH has been shown to increase only INH alpha mRNA concentrations [37], it is possible that in the present study the increased FSH secretion may have resulted in increased free INH (alpha) subunit precursor proteins in the circulation.

We have previously demonstrated that the percentages of individual germ cell types, as well as their ratios quantitated from DNA flow cytograms (FCM) of the testis of the bonnet monkey, remain similar throughout adulthood [13]. In the current 285-day study, testicular biopsies from control monkeys showed that germ cell turnover and, therefore, spermatogenesis is maintained in a steady state. Hemiorchidectomy could be expected to increase TV and possibly sperm production as a result of stimulation of the spermatogenic process by the increased FSH and T. FCM analysis of testicular biopsies of experimental monkeys indeed did show that dynamic alterations in germ cell percentages and ratios occurred throughout the 285-day period studied.

Although the changes in the germ cell population monitored are relative, that this change is not an experimental artifact is evident from the following. A substantial increase in a cell type (e.g., 2C) at a particular time point (e.g., at Day 24) is followed at subsequent time points (e.g., at Day 45) by increase in the transformed cell population (in this case, 1C and 4C), showing that we are truly monitoring germ cell metamorphosis. Although the 2C population monitored could be contaminated with somatic cell types such as Sertoli, Leydig, and other non-germ cells, earlier studies from our laboratory [38] and others [10] have shown this contamination to be less than 3%. The reproducibility of the technique employed here in the preparation and analysis of germ cells is evident from the fact that the germ cell population of control monkeys maintained constancy over a 285-day study period.

The first event observed was the proliferation and transformation of the spermatogonial cells (2C) to primary spermatocytes (4C) at Day 24 of HO (Fig. 4). During this time, the round (1C) as well as elongate (HC) spermatids were not altered, leading to a decrease in 1C:4C and 1C:2C ratios (Table 1). However, by Day 45 there was a marked decrease in 2C and 4C but a significant increase in the spermatid population, particularly that of elongate spermatids (HC). These changes in germ cell population are consistent with stimulation by HO of overall sperm production. From the germ cell flow cytometric profiles it appears that the enhanced testicular activity immediately following hemiorchidectomy gradually returns to normalcy by Day 110, but there appears to be another spurt in spermatogenic activity by Day 175, albeit to a lesser extent than that observed at Day 24. Considering that the duration of spermatogenesis in the monkey approximates 45–48 days [39, 40], it appears that the first change in spermatogenic activity observed following HO is rapid and is concurrent with the acute increase in serum FSH and T production. The second increment in spermatogenic activity, which is apparent only after an additional 130 days (~3 cycles), may be the result of sustained increased levels of FSH and T. Whether such a spurt in spermatogenesis recurs at periodic intervals at later time points too remains to be ascertained.

A lack of significant correlative changes in the 1C:2C and 4C:2C ratios of controls, when considered along with the fact that the kinetics of their turnover showed only minimal and statistically insignificant alterations, indicates that spermatogenesis in the controls was in a steady state. In the experimental monkeys, however, changes in these ratios were positively correlated ($p < 0.01$, $r = 0.4$; $n = 54$), indicating that the first step in the germ cell transformations quantifiable by flow cytometry during spermatogenesis (4C:2C) has contributed significantly to the HO-induced overall changes in the kinetics of germ cell turnovers (1C:2C). From these results, it appears that the premeiotic step in spermatogenesis is the rate-limiting step in facilitating hypertrophy of the testis following HO. We have earlier reported that inhibition of spermatogenesis—brought about either by desensitization of the pituitary gonadotropes (via chronic infusion of a GnRH analog [Buserelin]) or by specific deprivation of endogenous FSH (via active immunization with oFSH)—results in differential inhibition of specific germ cell transformations leading to significant alterations of preceding/succeeding germ cell types for long periods [18, 41]. In the current study, since spermatogenesis was stimulated by HO and germ cell turnovers were not inhibited, accumulation of specific germ cell types was not observed.

In conclusion, the results of the present study demonstrate that in the adult male bonnet monkey, HO leads to rapid compensatory changes in steroidogenesis as well as in the dynamics of the testis germ cell population. The elevated serum FSH and T concentrations following hemiorchidectomy may be the major arbiters of such compensatory changes.

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