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Experimental Cryptorchism: Effect on Serum LH and FSH in the Rat *

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Summary. The effects of cryptorchism on testis weight and histology, ventral prostate weight (VPW), and serum LH and FSH were assessed in rats 49 days of age. Rats 49 days of age have elevated FSH levels which fall to adult range when mature testis weight is achieved. Bilateral crytorchism produced: 1. histological changes in the testes by 3 days and a final reduction of testis weight of 75%; 2. a reduction of VPW of 40%; and 3. elevations of both serum LH and FSH by 4 days; serum FSH did not increase to castrate levels. It is concluded: 1. because serum FSH levels correlate with testicular maturation, some product of the germinal epithelium may selectively regulate FSH secretion; 2. because cryptorchism destroys the germinal epithelium but does not elevate FSH to the castrate range, spermatogenic activity is not the sole regulator of FSH; and 3. because cryptorchism damages both the gametogenic and androgenic functions of the testes, it will not provide a model for studies of selective FSH regulation.

Key words: Testis, spermatogenesis, anterior pituitary, gonadotropins, cryptorchism.

Testicular function is regulated by two gonadotropic hormones: luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH stimulates the Leydig cell secretion of testosterone and testosterone acts to suppress further LH release. (9) Although it is well known that FSH acts on the seminiferous tubules and is necessary for the initiation of mature spermatogenesis,(8) the gonadal substance responsible for the feedback control of FSH is not certain. Because isolated germinal damage in men, produced by either irradiation (6) or chemotherapy,(12) produces an increase in serum FSH but not LH, it has been suggested that a product of the germinal epithelium mediates the selective release of FSH. However, the nature of this substance remains unclear.

It is widely assumed that cryptorchism produces injury only to the seminiferous tubules, leaving Leydig cell function intact. If so, then cryptorchism in animals should provide an ideal experimental model for investigating the feedback regulation of FSH. In this study, the effects of cryptorchism on serum LH and FSH, testis weight and histology, and ventral prostate weight were tested in rats.

Methods and Materials

Ninety-six 7 week old male Wistar rats weighing 250 grams were kept in a controlled 12-hour (7:00 AM - 7:00 PM) light and 12-hour dark environment throughout the studies and fed routine laboratory chow. One day after they were received, cryptorchism was produced through an abdominal incision; the testes were delivered into the abdomen and the internal inguinal ring closed with a pursestring suture. In control rats, only an abdominal incision was made. Thereafter, at daily inter-

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vals for 5 days and then weekly for 3 weeks, rats in groups of 6 were anesthetized with ether, weighed, and exsanguinated by aortic puncture. The testes and ventral prostate were dissected free and weighed. The testes were placed in Bouin's fixative and stained with hematoxylin and eosin for histological examination. Blood samples were allowed to clot at room temperature for 2 h before being centrifuged; the serum was removed and stored at -20° C until assayed. Thirty other rats in groups of 5 underwent either: unilateral castration, unilateral abdominal placement of one testis, bilateral placement of both testes, bilateral castration, or sham operation. After 2 weeks, they were sacrificed and organ weights and serum samples were collected utilizing the same protocol.

Serum LH and FSH were measured by radioimmunoassay using techniques previously described.(10) The within-assay coefficient of variation for samples with similar hormone content was 8.0% for the FSH assay and 3.7% for the LH assay.

Results

A. Effect of bilateral cryptorchism on testis weight and histology (Fig. 1-2):

In control rats, testis weight increased by 30%, reaching full adult weight during the 3 weeks of observation. Cryptorchism produced an abrupt fall in testis weight after 3 days, with a maximal 75% reduction of testis weight reached by 2 weeks. Histological examination of the cryptorchid testes at 3 days demonstrated uninucleated hypertypic and multinucleated giant cells and a decrease in the number of mature sperm in the tubules. By 5 days, these changes were more pronouced with cellular debris in the lumen of some tubules, and by 14 days, the tubules were shrunken and vacuolated with only Sertoli cells and a few spermatogonia remaining.

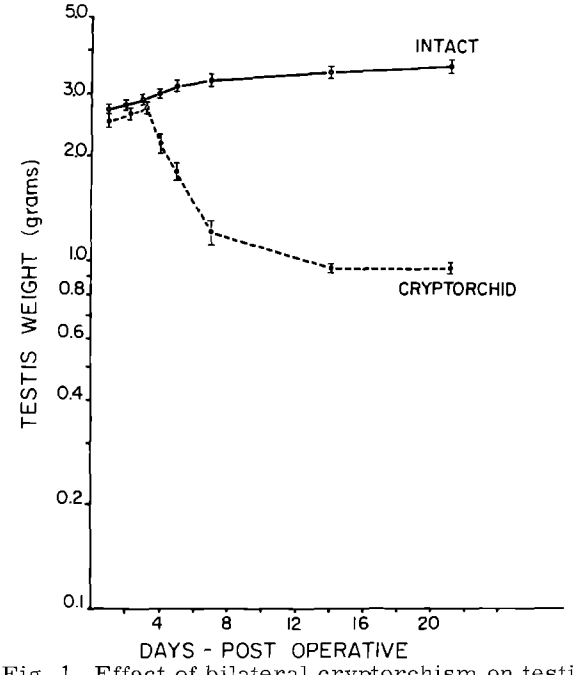

Fig. 1. Effect of bilateral cryptorchism on testis weight. The brackets enclose ± S.E.M.

B. Effect of bilateral cryptorchism on ventral prostate weight (VPW) (Fig. 3):

In control rats, VPW increased 140% during the three weeks of observation. In cryptorchid rats, VPW failed to increase after 7 days and was 40% lighter than control weights at 3 weeks.

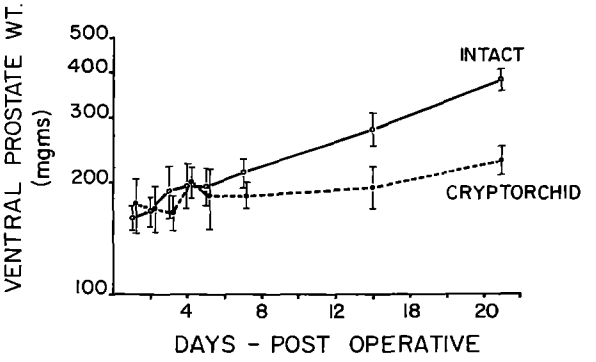

Fig. 3. Effect of bilateral cryptorchism on ventral prostate weight.

C. Effect of bilateral cryptorchism on serum LH and FSH (Fig. 4-5):

In control rats, mean serum LH increased 16%, reaching mature adult levels after one week. Crypt-

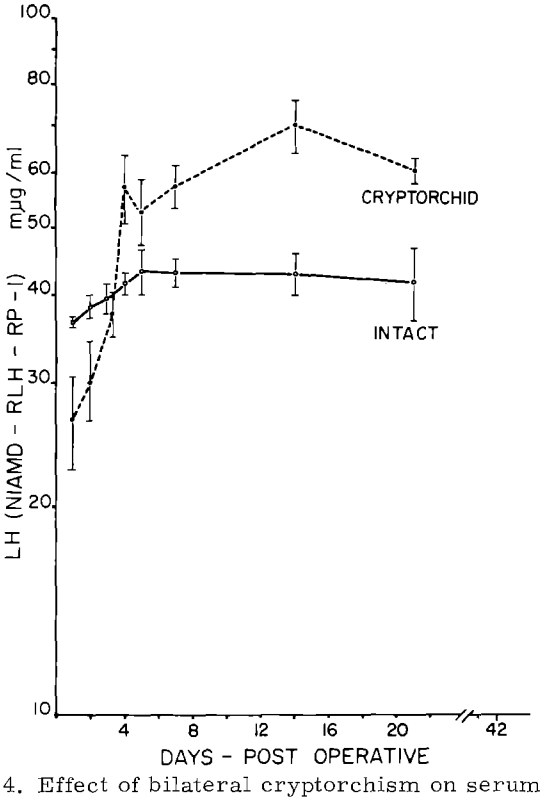

Fig. 4. Effect of bilateral cryptorchism on serum LH

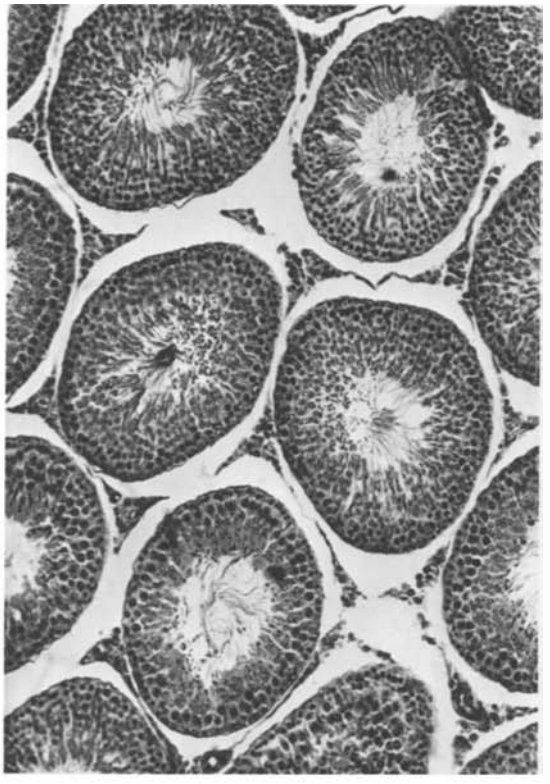

Fig. 2a

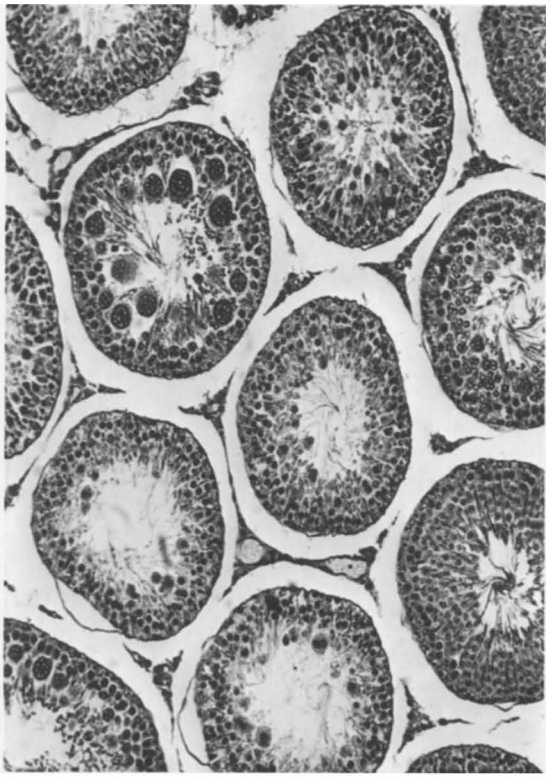

Fig. 2b

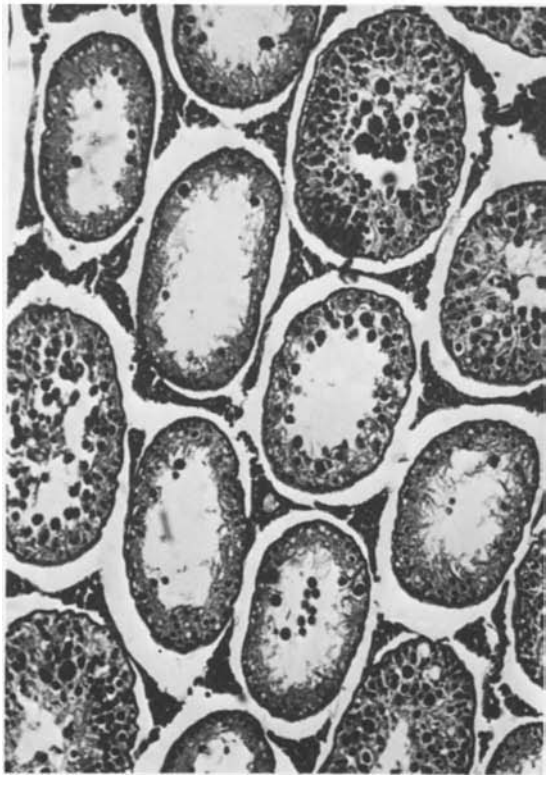

Fig. 2c

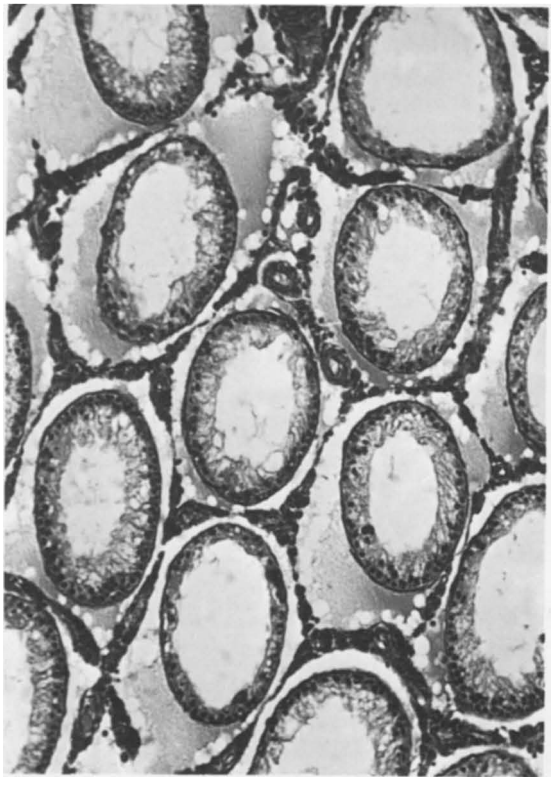

Fig. 2d

Fig. 2a-d. Histological changes in testes produced by cryptorchism: a) control, normal testis; b) at 3 days, giant cell formation has occurred; c) at 5 days, mature sperm are absent; and d) at 2 weeks, marked atrophy of the tubules is present.

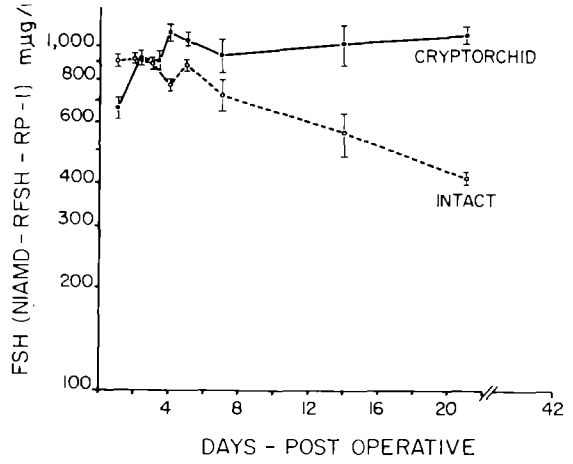

Fig. 5. Effect of bilateral cryptorchism on serum FSH

orchism produced an initial fall in serum LH followed after 3 days by a sustained increase of 40% over intact control levels.

Serum FSH levels in the intact rats were initially high, falling into the adult intact range during the 3 weeks of observation. Cryptorchism increased mean serum FSH levels 30% by 4 days; these high levels were maintained for 3 weeks.

D. Effect of unilateral castration, unilateral cryptorchism, bilateral castration, or bilateral cryptorchism on serum LH and FSH, testis weight, and VPW (Table 1):

Unilateral cryptorchism for 2 weeks did not affect VPW, produced atrophy of the abdominal testis, elevated mean serum LH levels 30%, and increased serum FSH 20%. Unilateral castration did not affect VPW, increased LH 23%, and produced a 28% elevation of FSH. Following bilateral cryptorchism, VPW was decreased 33%, testis weight fell, serum LH increased 58%, and serum FSH was 135% higher. Bilateral castration increased serum LH 700% and FSH 560%.

Discussion

This study demonstrates that cryptorchism produces early injury to both the gametogenic and androgenic functions of the testes. Dramatic changes in testis weight and histology occur within the first 7 days postoperatively: by 2 weeks, VPW is significantly suppressed. The histological alterations occuring after thermal injury have been well documented previously. The multinucleated giant cell formation has been attributed to coalescence of spermatids and the etiology of mononucleated hypertypic giant cells traced to hypertrophied pachytene spermatocytes (4).

Defective androgen production in the cryptorchid testis, although not so widely known, has also been previously reported. This defect has been attributed to a decreased activity of the testicular Δ^5-3B-hydroxysteroid dehydrogenase enzyme which is necessary for the production of potent androgens (3). Studies utilizing light and electron microscopy, however, have failed to demonstrate any morphological alteration in the Leydig cells (5).

In this study, serum LH levels increased within 4 days postoperatively, responding to the decrease in androgen production. This is in agreement with prior studies in the rat which demonstrated a 50% fall in plasma testosterone levels within the first week of experimental cryptorchism (1). In contrast to this response in mature rats, we noted that when cryptorchism was produced in the rat 21 days of age, serum LH and VPW were not affected for 6 to 8 weeks (10). This suggests that the effect of thermal injury on the Leydig cell may vary with maturation

Table 1. Effect of unilateral castration, unilateral cryptorchism, or bilateral cryptorchism on serum LH and FSH, testis weight, and VPW

	VPW mg ± SE	Testis Weight qm ± SE	LH ng/ml ± SE	FSH ng/ml ± SE
Control	390 ± 53	3.69 ± 0.06	44.6 ± 1.8	421 ± 28
Unilateral Cryptorchid	390 ± 32	1.92 ± 0.02 (scrotal) 0.41 ± 0.02 (abdominal)	58.3 ± 4.3	504 ± 14
Unilateral Castration	419 ± 28	1.92 ± 0.05	54.2 ± 7.9	538 ± 20
Bilateral Cryptorchid	261 ± 51	0.96 ± 0.07	70.3 ± 5.7	1000 ± 130
Bilateral Castration	———	———	351 ± 26	2760 ± 120

of the testis. The same may be true in the human. Zachmann reported that when boys with cryptorchism were injected with human chorionic gonadotropin, older boys (over 7 years of age), but not younger ones, had a borderline insufficient excretion of testosterone glucuronide in their urine; this response appears to be normalized by orchiopexy (15).

Serum FSH levels in the control rats were initially high and did not fall into the adult range until mature adult testis weight was achieved. We have previously reported this observation in younger rats(10). In the rat 35 days of age, before mature spermatozoa appear in the tubules, serum FSH levels are even higher. Serum FSH first decreases when mature sperm appear in the tubules (49 days of age) and finally falls into the adult normal range when mature spermatogenesis and testis weight are achieved (63 days of age). These data suggest that the elevated levels of serum FSH in immature rats are a result of insufficient amounts of FSH inhibiting substance from the immature testes. In support of this concept, when spermatogenesis is inhibited by cryptorchism, FSH returns to these same high levels. In this study, because cryptorchism damaged both the germinal epithelium and function of the Leydig cells, both serum FSH and LH increased. Unilateral cryptorchism again failed to produce a selective increase in FSH and its effect on serum LH and FSH was indistinguisable from unilateral castration.

Cryptorchism produced a marked destruction of the germinal epithelium, but despite this, serum FSH failed to increase to levels as high as those of castrate rats. This indicates that factors other than spermatogenic activity regulate FSH. We have recently demonstrated that androgens primarily influence FSH control. Dihydrotestosterone, an androgen which is not metabolized to estrogenic metabolites, suppresses serum FSH levels in castrate rats (11), and cyproterone (free alcohol), an antiandrogen without antiestrogenic activity, interferes with FSH feedback (14). Estrogen, also, suppresses serum levels of both LH and FSH (2, 13). Consequently, in the cryptorchid rat with destruction of the germinal epithelium, the feedback suppression produced by androgen and/or estrogen prevents serum FSH from reaching castrate levels.

In patients with injury to the germinal epithelium produced by irradiation (6) or chemotherapy (12). and in some patients with idiopathic oligospermia (7), serum levels of FSH, but not LH, are elevated. This suggests that spermatogenic tissue produces a hormone which is responsible for the selective feedback regulation of FSH. Although androgen and estrogen suppress both serum LH and FSH, they fail to preferentially affect FSH (2, 13). It therefore appears that another substance exists, as yet unidentified, which selectively mediates the feedback regulation of FSH. Cryptorchism in rats this age, however, because it affects both the gametogenic and androgenic functions of the testes, will not provide a model for the further investigation of this selective feedback.

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