Sertoli Cell Stimulation Following Intratesticular Injections of FSH in the Hypophysectomized Rat.* (30080)

H. D. MURPHY (Introduced by W. R. Lyons)
Department of Anatomy, University of California Medical Center, San Francisco

The separate and synergizing role of follicle-stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) on the testis has not been completely clarified chiefly because of residual impurities present in the hormones tested. Smith(1) showed that spermatogenesis was abolished by hypophysectomy, thus providing a new method of studying the effects of hormones on the developing tubular epithelium. Early workers found that androgens could maintain spermatogenesis even in the absence of pituitary hormones(2). Others(3) suggested that the androgen acted directly on the seminiferous tubules.

The influence of FSH and ICSH on tubule epithelium and spermatogenesis has been extensively studied(4-11). Recent work by Lostroh(9) in long term hypophysectomized rats reported that both ICSH and FSH must be present for tubular repair and that FSH alone can produce substantial repair when 1 μg, or less, of ICSH is present.

Route of administration of the hormone is important. Squire *et al*(8) showed that intratesticular injections of ICSH or human chorionic gonadotropin (HCG) stimulate the Leydig cells at dose levels about one-tenth of those required by the systemic route. In the following experiments the effects of small intratesticular injections of FSH on the various testicular components were studied.

Materials and methods. Long-Evans rats were hypophysectomized on day 21. Intratesticular injections were started on the following day and continued thrice weekly for 3 weeks. Eleven dose levels of FSH in 2% butanol were studied: 0.007, 0.03, 0.12, 0.5, 2, 5, 10, 15, 25, 50, and 100 μg. In all cases, the right testis was used for injection while the left was the control. The right testis of the control animals was injected intratesticularly with bovine serum albumin.

Injections were carried out with microliter syringes† with a 30-gauge needle. The animal was lightly anesthetized with ether and the scrotum shaved and cleansed with 80% ethanol. The right testis was immobilized by light pressure between the thumb and first finger and the needle inserted into the middle of the testis. Total volume injected was never greater than 10 μl. Reflux was prevented by pausing briefly after pushing in the plunger and before the needle was withdrawn.

At autopsy the testes, seminal vesicles, prostate, and adrenals were removed and fixed in Bouin's fluid. The sella turcica was examined for pituitary remnants with the aid of a dissecting microscope. Animals were considered completely hypophysectomized on the basis of body weight change, appearance of pellage, examination of the sella turcica, and adrenal histology.

All tissues were weighed after fixation, washed in 80% ethanol, dehydrated in the usual manner, processed in nitrocellulose and sectioned at 5 μ. Two stains were used, hematoxylin alone, and the periodic acid-Schiff stain with hematoxylin and Orange G.

Results. Rats injected with 100 μg FSH showed a tripling in testis weight over the corresponding controls. However, average tubular diameter and number of Sertoli cells per cross section of tubule were not greater than those with 2 μg FSH. Between 0.5 and 0.007 μg FSH, tubular diameter dropped to control levels.

Injections of 2-100 μg FSH stimulated approximately a 40% increase in Sertoli cells over the average number observed in hypophysectomized controls. These differences were found to be significant ($p<0.01$). Levels of 0.5-0.03 μg FSH maintained the number of Sertoli cells at a level slightly above

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† #705, Hamilton Co., Inc., Whittier, Calif.

that of the controls. Animals receiving 0.007 μg FSH had tubule diameters and Sertoli cell counts below those of the controls.

Injection of bovine serum albumin had no effect on tubular width or upon the number of Sertoli cells. Tubular diameter and Sertoli cell counts were below those of controls. No evidence of edema or other indications of testicular damage were found microscopically following injection of the albumin.

Following FSH injections, cytoplasm of the Sertoli cells showed many vacuoles toward the luminal side (Fig. 2 and 3). Sertoli cytoplasm was filamentous and in many tubules was concentrically compressed giving the impression of an increased fluid secretion. Developing germinal elements were less evident in the FSH injected testis.

Seminal vesicle and prostate weight remained at the hypophysectomized control level at all doses of FSH tested (Table I). Leydig cells were atrophic in both right and left testes at all dose levels below 100 μg (Fig. 3 and 4). At the 100 μg level the injected testes showed a few Leydig cells that appeared less atrophic, apparently because of the 0.06% contamination of the FSH with ICSH.

Discussion. These results cannot be accurately compared with recently published work of Lostroh(9), Steinberger *et al*(10), and Delsol *et al*(11), because of the doses of FSH used, route of administration and interval of time following hypophysectomy. The term FSH has no meaning in the male except vague suggestions in the past that the hormone so designated seems to synergize with ICSH in the attainment of completely normal testicular function.

Increased seminiferous tubule diameter after FSH has previously been described in the rat(6,12) and rabbit(13). This increase in width need not depend upon cell division but rather upon Sertoli cell hypertrophy and secretion resulting in an accumulation of fluid. Prophase mitotic figures were observed; but it was not ascertained if these were Sertoli cells. Division of Sertoli cells has not been described even with the use of tritiated thymidine(14). That the observations described were probably not due to indifferent protein effect is shown by the control level

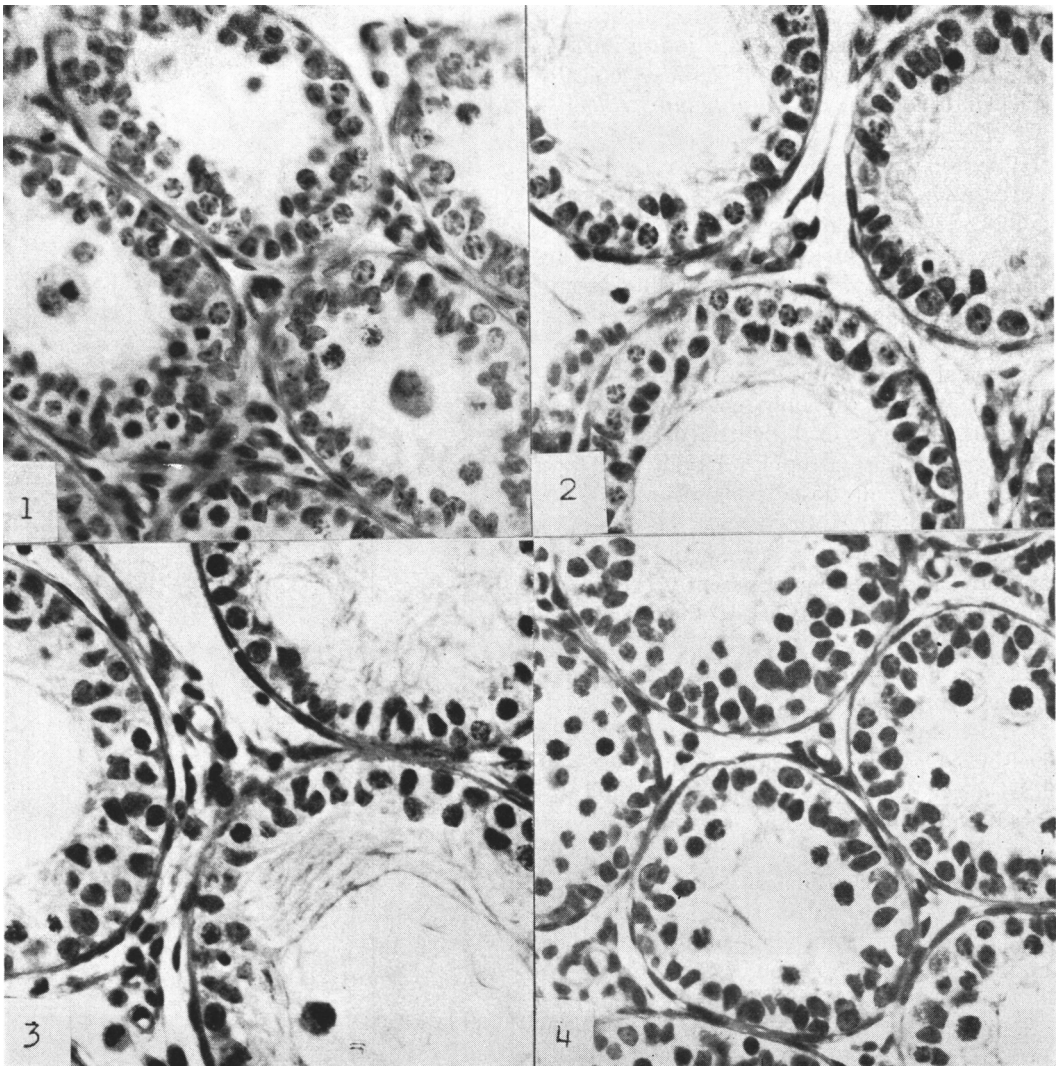
TABLE I. Intratesticular Injections of Follicle-Stimulating Hormone (FSH) in the Right Testis of Hypophysectomized Rats.

No. of rats	Treatment	Testis wt (mg)		Seminal vesicles (mg)	Prostate (mg)	Tubule diameter (Avg of 20 tubules) (μ)		Sertoli cells (Avg of 20 tubules)	
		Right	Left			Right	Left	Right	Left
9	Controls	42 ± 1.1	42 ± 1.1	8 ± .78	<3	73 ± .54	73 ± .95	26 ± .17	27 ± .24
9	.007 μg FSH	43 ± 1.3	40 ± 1.3	6 ± .24	*	70 ± 1.2	65 ± 1.9	24 ± .48	25 ± .50
10	.03 "	46 ± 2.6	43 ± 1.8	7 ± .37	*	72 ± 1.4	71 ± 1.6	31 ± .82	27 ± .57
11	.12 "	44 ± 1.8	37 ± 1.1	8 ± .58	*	72 ± 1.5	70 ± .79	33 ± .25	28 ± .45
8	.5 "	46 ± 1.7	40 ± 1.3	8 ± .35	*	77 ± 1.1	70 ± 3.2	32 ± .38	27 ± .38
4	2 "	65 ± 1.6	44 ± .96	7 ± .20	<3	95 ± 1.50	79 ± 1.5	39 ± .91	35 ± .87
5	5 "	87 ± 4.8	50 ± 3.9	7 ± .25	<3	108 ± 5.6	90 ± 3.1	39 ± .37	36 ± .63
5	10 "	76 ± 1.3	47 ± 1.6	6 ± .25	<3	98 ± 2.1	82 ± 1.5	38 ± .74	35 ± .81
5	15 "	103 ± 2.3	55 ± 1.2	5 ± .32	<3	106 ± 1.3	91 ± 1.3	37 ± .55	33 ± .50
7	25 "	117 ± 22.1	57 ± 3.2	9 ± .42	<3	104 ± 1.9	76 ± 1.1	37 ± .73	35 ± .65
8	50 "	106 ± 3.5	66 ± 1.4	8 ± .35	<3	101 ± 3.4	83 ± .83	38 ± .35	36 ± .27
5	100 "	147 ± 2.0	84 ± 3.4	10 ± .25	<3	108 ± 2.1	86 ± 1.2	37 ± .37	35 ± .25
5	100 μg serum albumin (bovine)	34 ± 1.7	34 ± 2.0	7.6 ± .37	*	60 ± 1.3	61 ± 1.4	21 ± .0	21 ± .28

* Prostate too small to be accurately dissected and weighed; less than 2 mg.

tubular diameter and the Sertoli count following bovine albumin injections.

The role of the Sertoli cell as a nurse cell and as a phagocyte(15) has not been fully elucidated. Many workers have described its vacuolated cytoplasm as due to lipid accumulation(16,17). Others suggest that this lipid represents a steroid hormone(15,18,20). In the dog, the Sertoli cell is said to produce estrogen(19,20) but in the human(21,22)



Intratesticular injections of FSH in immature hypophysectomized rats. All animals were hypophysectomized when 21 days of age; injections began on day 22 and continued for 3 wk. Animals were 42 or 43 days of age at autopsy. (Fig. × 320).

FIG. 1. Hypophysectomized terminal control. Right testis was injected with 2% butanol. Uninjected left testis was similar to right.

FIG. 2. Right testis from a rat that received 2 μg FSH. Tubules are larger than those of contralateral testis, and germinal elements are less obvious.

FIG. 3. Right testis from a rat that received 5 μg FSH. Tubules had a 50% greater diameter and 50% more Sertoli cells per cross-section than did terminal control rats. Sertoli cytoplasm and secretory material almost fills the lumen of tubule to upper right.

FIG. 4. Uninjected left testis from rat of Fig. 3. At 5 μg and higher doses some of the FSH was absorbed and distributed systematically to left testis.

there is no conclusive evidence that the lipid represents estrogen. This is also true of the rat.

Under certain experimental conditions used in these experiments, FSH in the doses cited, consistently maintained the Sertoli cells in number, size, and volume above those of corresponding controls. That the NIH-FSH had a low ratio of contamination with ICSH was evidenced by atrophic Leydig tissue, seminal vesicles and prostate(23).

Summary. Intratesticular injections of ovine NIH-FSH in doses ranging from 0.007-100 μg thrice weekly for 3 weeks in immature hypophysectomized rats of the Long-Evans strain produced a secretory hypertrophy of the Sertoli cells. Under the conditions of these experiments the FSH preparations used did not maintain or stimulate either the Leydig or germinal cells. Seminal vesicles and prostate remained atrophic even after injection of the highest doses of FSH; and thus no evidence was obtained suggesting a secretion of androgen by stimulated Sertoli cells.

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