Intratesticular Assay of Follicle-Stimulating Hormone in Hypophysectomized Rats. (30620)

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Several investigators have reported FSH (hypophysial follicle-stimulating hormone) assay methods using ovarian weight(1,2) and follicle growth as the end points(3,4). In a recent paper, Igarashi and McCann(5) reported their method for FSH assay using the mouse uterine weight method. Their method was reportedly 10 to 30 times more sensitive than the follicular development test.

Greep *et al*(6), and Greep(7) suggested that the gross testis enlargement of the immature hypophysectomized rat was usable as an assay for FSH. Recent work by this author(8) using intratesticular injections of FSH in immature hypophysectomized rats, suggests a more sensitive assay method for FSH than has been previously reported.

Materials and methods. 46 Male rats of the Long-Evans strain were hypophysectomized, *via* the parapharyngeal approach(9), when 21 days of age. Intratesticular injections(8) of 2 μg doses of NIH-FSH-S-2,* in not more than 10 μl of 2% butanol, were started on day 22, and continued every other day for different periods of time. Animals were sacrificed 1, 3, 5, 7, 9, 11, and 15 days after the first injection and 24 hours after the last.

At autopsy, the testes and seminal vesicles were removed, weighed, and fixed in Bouin's fluid. The sella turcica was examined for remnants of pituitary tissue. The following day, the tissues were dehydrated in the usual manner, processed in nitrocellulose, and sectioned at 5 μ. All tissue sections were stained with periodic acid-Schiff's reagent with hematoxylin and orange G.

Results. The effect of intratesticular injections of NIH-FSH-S-2 was evident *one* day after a single 2 μg dose of FSH (Fig. 1-2).

TABLE I. Hypophysectomized Rats Injected with Different Levels of NIH-FSH-S-2.*

Days after first inj	Total dose of FSH (μg)	No. of rats	Testis wt (mg)		Seminal ves. (mg)	Seminiferous tubule diameter (μ)†		Sertoli count‡	
			Right	Left		Right	Left	Right	Left
1	2	5	95.4 ± 1.1§	79.2 ± 1.0	9.6 ± .5	99.6 ± 1.2	94.4 ± .7	36.2 ± .6	32.3 ± .5
3	4	5	93.4 ± 3.9	75.8 ± 1.6	8.2 ± .3	94.2 ± 2.4	86.5 ± .5	38.8 ± .7	33.9 ± .8
5	6	5	97.0 ± .5	74.4 ± 1.0	8.2 ± .4	95.0 ± 1.4	88.4 ± .7	41.3 ± .4	34.5 ± .4
7	8	5	90.6 ± 4.0	70.2 ± 2.5	7.4 ± .2	92.0 ± 3.3	87.0 ± 1.7	40.1 ± .6	33.1 ± .5
9	10	5	84.0 ± 1.5	62.2 ± 1.0	6.0 ± .0	87.4 ± 1.3	82.4 ± .2	40.3 ± .4	32.6 ± .6
11	12	5	86.6 ± 4.5	61.8 ± 1.9	6.0 ± .5	73.2 ± 1.3	81.4 ± .7	38.5 ± .5	32.4 ± .5
15	16	5	100.1 ± 3.0	64.4 ± 2.2	6.0 ± .2	88.0 ± 1.9	79.0 ± 2.1	38.5 ± .4	31.5 ± .4

* Rats were hypophysectomized day 21 and injected intratesticularly (right side only) with 2 μg FSH, thrice weekly. Rats were killed at noted intervals after the first injection.
† Avg of 20 tubules measured in micra.
‡ Avg of 20 tubules counted.
§ Mean ± standard error.

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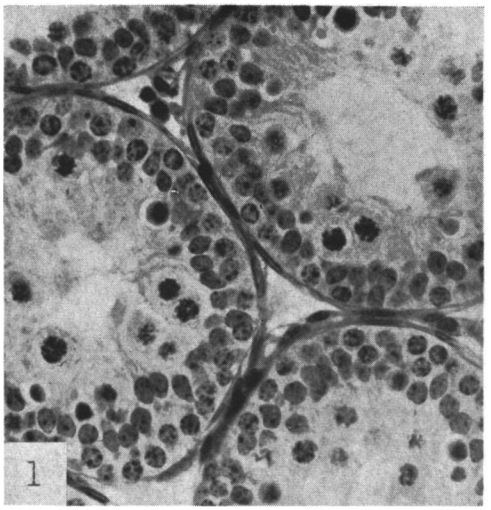
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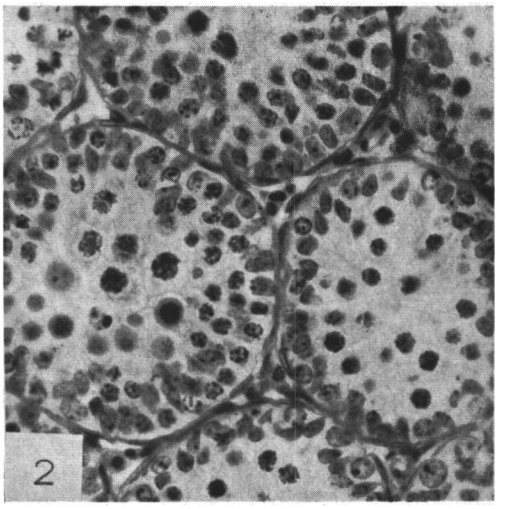
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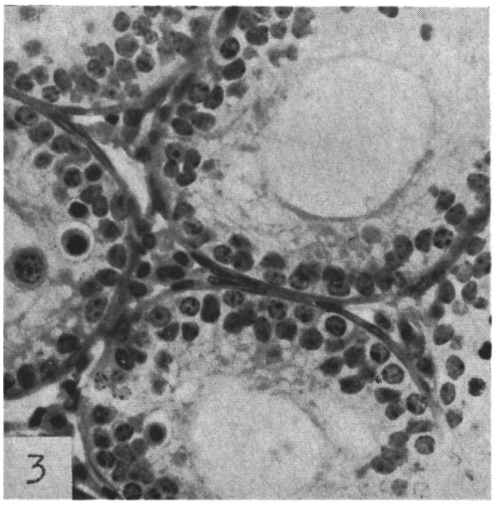
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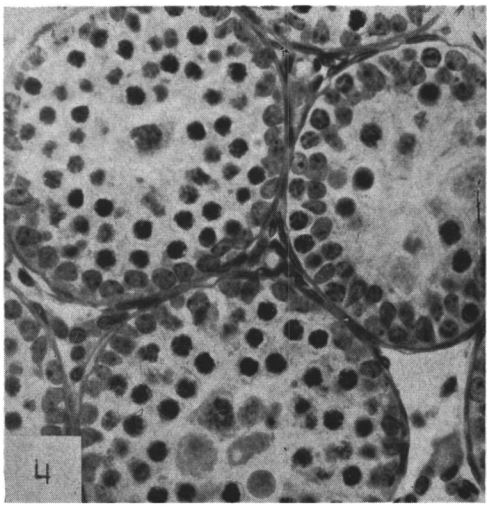
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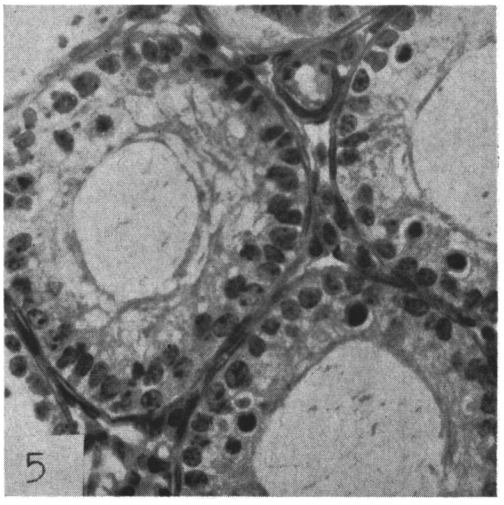
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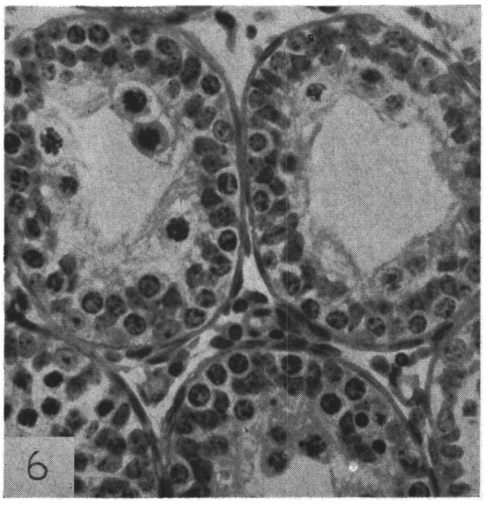
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TABLE II. Hypophysectomized Control Rats* Operated Day 21 and Sacrificed at Known Time Intervals.

Age in days	No. of rats	Testis wt (mg) Right	Left	Seminal ves. (mg)	Seminif. tubule diameter (μ)† Right	Left	Sertoli count‡ Right	Left
24	5	61.8 ± 1.5§	63.2 ± 2.2	7.4 ± .3	86.6 ± 1.4	84.8 ± .8	24.3 ± .2	24.0 ± .2
28	5	47.0 ± 1.1	47.6 ± 1.2	7.0 ± .2	70.0 ± 1.5	63.6 ± 1.4	23.6 ± .0	23.5 ± .3
32	5	43.8 ± 1.9	44.4 ± 1.6	7.2 ± .5	69.8 ± .86	65.4 ± .8	21.7 ± .0	21.8 ± .2
36	5	40.0 ± .6	40.0 ± .3	7.4 ± .4	63.2 ± 1.1	62.6 ± 1.0	19.7 ± .2	19.4 ± .2

* Rats were hypophysectomized day 21 and sacrificed on day indicated.
† Avg of 20 tubules measured in micra.
‡ Avg of 20 tubules counted.
§ Mean ± standard error.

The injected right testis showed loss of germ cells and evidence of fluid secretion by the Sertoli cells. The contralateral testis was not visibly affected by the FSH administration.

After two 2 μg doses of NIH-FSH-S-2, (Fig. 3-4), the germ cells were more depleted. There was a definite vacuolation and concentric flattening of the Sertoli cytoplasm which was indicative of fluid secretion by the Sertoli cells and tubular accumulation of fluid. The control, left testis remained unaffected.

After three 2 μg doses of NIH-FSH-S-2, further loss of germ cells from the injected right testis was evident (Fig. 5-6). The left testis now showed loss of some germinal elements and fluid accumulation which was suggestive of systemic absorption of the hormone. Tests performed after four to eight 2 μg doses of FSH were of no greater value than the 3-dose assay.

The FSH-injected testis weights in all groups were significantly increased in comparison with those of contralateral controls and non-injected hypophysectomized controls (Tables I, II). There was a decreased weight of both testes between days 7 to 9. By day 15, the injected testis remained significantly heavier in weight than its control.

Much of the testis weight increase was caused by the sudden early increase, and accumulation of fluid secreted by the Sertoli cells. Measurements of the seminiferous tubule diameters showed that early fluid accumulation occurred after a single injection of the hormone. Tubular distention, however, did not increase proportionately with number of injections.

Sertoli cell counts in the FSH injected testis reached 41 per tubule cross section after three 2 μg injections, in contrast with 34 per tubule for the uninjected testes. With an increase in the number of doses of FSH the Sertoli cells remained the same, but were significantly increased in comparison with hypophysectomized controls throughout the experiment (Tables I, II). Leydig cells were

Effects of single and multiple, 2 μg doses of ovine follicle-stimulating hormone, NIH-FSH-S-2, injected intratesticularly in hypophysectomized rats. Rats were sacrificed one day after the stated number of 2 μg injections. Nitrocellulose sections, 5 μ in thickness, were stained with P.A.S. hematoxylin and orange G. All Figures 350 × magnification.

FIG. 1. *Right* testis from a rat that received one, 2 μg dose of FSH. Note: fewer germ cells than in the left testis, Fig. 2; large Sertoli cells with large and pale staining nuclei.

FIG. 2. *Left* uninjected testis from the same rat in Fig. 1. Note greater number of germinal cells than is present in injected testis.

FIG. 3. *Right* testis from a rat that received two, 2 μg injections of FSH. Note beginning of tubular secretion as evidenced by presence of many small vacuoles and granules in the Sertoli cytoplasm and by concentric flattening of cytoplasm. Note fewer germ cells than seen in Fig. 1.

FIG. 4. *Left* uninjected testis from the same rat in Fig. 3. The large number of germinal cells seen here suggests that insufficient FSH was absorbed and distributed systemically to this testis.

FIG. 5. *Right* testis from a rat that received three 2 μg injections of FSH. Note presence of granules and many small vacuoles in Sertoli cytoplasm, and luminal fluid accumulation.

FIG. 6. *Left* uninjected testis from the same rat in Fig. 5. Germ cells are depleted because of systemic absorption of the FSH. Large spermatocytes appeared to have been sloughed first; then the smaller cells. This control testis is histologically similar to that shown in Fig. 1; but the tubular diameter is smaller here. Note fluid accumulation in lumen of tubules.

not stimulated because of the low amount of ICSH present as a contaminant in the NIH-FSH-S-2(10). Seminal vesicles were not stimulated.

Discussion. The early, specific loss of germ cells following intratesticular injections of FSH is suggested as an assay method for FSH. As with the intratesticular test for ICSH, Squire *et al*(11), the procedure used for FSH has been a histologic one requiring microscopic study of the target cells—Leydig and Sertoli cells, respectively. Based upon increased secretory functions of these cells, quantitative chemical assay could be developed. Of the various regimens tried, the single injection with autopsy one day later induced measurable differences in the testis weight, tubular diameter, and Sertoli cell count—all of which could be used as objective criteria. However, a microscopist would not necessarily concern himself with such time consuming measurements. Rather, he would use the distinct difference in the appearance and population of the Sertoli and germinal cells in the test gonad and its contralateral control, and determine for preparations to be assayed the minimal dose producing the clearly detectable difference in an adequate number of rats. A note of caution must be sounded against using as an end point only the reduction in number of germinal cells. This might be accomplished by various noxious substances. Emphasis should be placed on the greater prominence of the Sertoli cells due to the increase in their size and secretory function as well as to the obliteration of many of the germ cells. Mitosis and meiosis of the latter cells continue even after hypophysectomy(12); and with them occupying so much of the tubules of the control testis, the Sertoli cells are less easily delineated.

The entire assay, injections and histology, can be done in a few days if one utilizes the rapid nitrocellulose method of Koneff and Lyons(13). Paraffin methods are usable, but give more shrinkage and distortion to the Sertoli cells.

Displaced germ cells were followed in numbers, histologically, as far as the vas deferens. These cells may be crowded out of the tubules because of increased fluid pressure, by increased Sertoli cell cytoplasmic growth, or possibly by a reaction to a poor local hormone environment as suggested by Steinberger *et al* (14). The movement of germ cells along the duct system might be accounted for by tubular smooth muscle and peristalsis(15,16). The large number of intact germ cells found in the rete testis and vas deferens proves, at least, that these cells had escaped Sertoli cell phagocytosis(17-20). The possibility that the degenerating germinal cells and fragments (Fig. 1, 3, 4 and 5) associated with Sertoli cell cytoplasm are actually phagocytosed would have to be proved electronmicroscopically.

Sertoli cell counts are uncorrected(21). The increase in numbers may be satisfactorily explained if one assumes that the tubules contract longitudinally and simultaneously were enlarged in cross sectional diameter by the increased fluid and Sertoli cytoplasm which occurs following FSH injections. Under these conditions more Sertoli cell nuclei would appear in any given tubule cross section because the loss of germ cells would leave space for them.

Summary. The intratesticular injection of a single, 2 μg dose of NIH-FSH-S-2, was detectable histologically, in immature hypophysectomized rats, one day after the injection. FSH induced specific germ cell loss, Sertoli cell hypertrophy, and increased fluid secretion. Germ cell depletion was intensified after 3 days and following two, 2 μg FSH doses. After 5 days, during which time 2 μg FSH was given every other day, systemic absorption of the hormone was evidenced by germ cell loss in contralateral uninjected testes. Days 7 to 15 were histologically similar to day 5. The rapid early changes in germ cell population, and Sertoli cell stimulation following intratesticular injections of FSH in the immature hypophysectomized rat when contrasted with the control status of the uninjected contralateral testis, should provide a basis for a specific assay of this hormone.

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Response of the Hypothalamic-Hypophyseal Adrenal Axis to Stress: Observations in Fetal and Caesarean Newborn Rats.* (30621)

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A response of the hypothalamic-hypophyseal adrenal axis of the fetus to the stress of injected epinephrine has been reported(1,2,3). On the other hand, a lack of response of this axis to the stress of injected histamine has been reported(4).

The experiments to be reported were designed to resolve these conflicting reports. They also were designed to determine whether the hypothalamic-hypophyseal adrenal axis of a premature newborn obtained by Caesarean section is able to respond to the stress of injected epinephrine.

Materials and methods. In rats of the Sprague-Dawley strain (Simonsen Laboratories, Inc.), the adrenals of the experimental rats of a litter (E) were compared with those of an equal number of control rats of that litter (C).

The basic methods for production of stress by injected epinephrine and for determination of the ascorbic acid in the adrenals were as follows. An experimental rat was given 20 μg of epinephrine by subcutaneous injection. One hour later this rat and a control from the same litter were killed, and the adrenals of each rat were removed and cleaned of adherent fat. The adrenals of 2 or 3 rats of a kind were weighed on a Mettler balance with a sensitivity of 0.001 mg and put into a 4% solution of trichloracetic acid, thus making a pool of adrenals for study. The ascorbic acid in a pool of adrenals was determined by Abelson and Baron's modification(5) of the method of Roe and Kuether(6).

In the first experiment, a pregnant rat of the 22nd day was anesthetized with ether and subjected to laparotomy; *via* an incision in left uterine horn, 2 or 3 adjacent fetuses situated near the corpus uteri were removed and killed (Group 1-C, Table I). After the

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