able *in vivo* method of determining the sloughing rate is described.

1. Leblond, C. P., Messier, B., Anat. Rec., 1958, v132, 247.
2. Quastler, H., Sherman, F. G., Exp. Cell Res., 1959, v17, 420.
3. Lipkin, M., Sherlock, P., Bell, B., Gastroenterol., 1963, v45, 721.
4. Golubeva, E. L., Fomina, L. C., Sechenov Physiol. J. USSR, 1957, v43, 156.
5. Fomina, L. C., ibid., 1957, v43, 807.
6. Garen, A., Levinthal, C., Biochim. Biophys. Acta, 1960, v38, 470.
7. Bloch, K., Rittenberg, D., J. Biol. Chem., 1942, v145, 625.
8. Srere, P. A., Chaikoff, I. L., Trietman, S. S., Burstien, L. S., ibid., 1950, v182, 629.
9. Kaltenbach, J. P., Kaltenbach, M. H., Lyons, W. B., Exp. Cell Res., 1958, v15, 112.
10. Miller, D., Crane, R. K., Biochim. Biophys. Acta, 1961, v52, 293.
11. Trier, J. S., Gastroenterol., 1964, v47, 480.
12. Deane, H. W., Padykula, H. A., in Histology, Greep, R. O., ed., McGraw-Hill, New York, 1966, p517.
13. Dahlquist, A., Nordström, C., Biochim. Biophys. Acta, 1966, v113, 624.
14. Dietschy, J. M., Siperstein, M. D., J. Clin. Invest., 1965, v44, 1311.

Received August 25. 1966. P.S.E.B.M., 1967, v124.

Effects of Androgens on Concentrations of LH and FSH in the Male Rat Pituitary.* (31713)

ROBERT J. RYAN AND JOHN E. PHILPOTT
Endocrine Laboratory, Department of Medicine, University of Illinois College of Medicine, Chicago

The relative biologic potency among androgenic steroids is dependent, in part at least, on the end-point used in the assay. The present study was undertaken to compare the ability of several androgenic steroids and their biosynthetic precursors to prevent castration changes in pituitary LH and FSH concentration. An estrogenic steroid was included in the study with the hope that these data would further elucidate the control of gonadotropin secretion in the male rat.

Methods. The experiments were done in two series, the first in the fall of 1963, the second in the summer of 1965. Experimental procedures were made as similar as possible. Two hundred to 250 g male Sprague-Dawley rats were castrated or left intact and segregated into treatment groups. They were fed *ad libitum* with Purina Mouse-Breeder chow and tap water and were housed in a controlled environment of 76°F with 10 hours of light and 14 of darkness.

Subcutaneous injections of the materials to be tested were begun on the day of operation and continued 6 days a week for 30 injections. The volume of material injected was 0.25 ml. The steroids used and the doses employed are given in Tables I and II. Peanut oil (Planters) was used as the vehicle for the steroids and was used unadulterated for the control animals.

The rats were killed with chloroform 24 hours following the last injection. The pituitaries were quickly removed, weighed and soaked in acetone. Body weights were measured. The ventral prostates and seminal vesicles (plus coagulation glands) were dissected, plotted and weighed.

The acetone soaked pituitaries were dried *in vacuo,* homogenized in 0.9% saline and diluted appropriately for assay. Stock solutions were kept frozen at −10°C for reassay, usually within one to two weeks.

The pituitaries were assayed for LH content using Parlow's ovarian ascorbic-acid depletion procedure(1). The first series of assays was done using conditions as previously defined(2).† In the second series, the interval between injection and removal of the ovary for ascorbic acid measurement was in-

* This investigation was supported by USPHS Research Grant A 3758.

† LH assays in both series employed 4 or 5 assay rats at each of the 2 dose levels tested.

creased to 4 hours. FSH assays‡ were performed using the rat ovarian weight augmentation procedure of Steelman and Pohley(3) with the following modifications:

‡ FSH assays were performed using 3 rats at each dose level.

TABLE I

Group	Treatment	Dose, mg/day	No. of rats	VP* —mg/100 g ± S.E. or (range)—	SV† —mg/100 g ± S.E. or (range)—	Pituitary LH concentration: Pit., mg ± S.E. or (range)	Pituitary LH concentration: No. assays	Pituitary LH concentration: Mean % cast. (95% CL)
Castrate	Peanut oil		11	7 ± 1	22 ± 1	13.8 ± 0.8	5	100.0
Intact	" "		9	194 ± 17	102 ± 11	11.5 ± 0.4	3	19.1‡(6.2– 58.7)
Castrate	Estrone	.001	3	12 (5– 23)	11 (10– 12)	16.2 (15.8–16.8)	1	89.7 (48.2–166.8)
"	"	.005	3	5 (4– 5)	8 (7– 8)	13.9 (13.8–14.0)	1	7.3 (5.1– 10.4)
"	Testosterone	.05	3	51 (41– 70)	58 (38– 71)	11.3 (10.0–12.6)	1	80.0 (46.0–138.4)
"	"	.5	3	109 (82–150)	117 (106–125)	10.7 (8.2–12.6)	2	31.2§(18.4– 53.0)
"	"	1.0	3	313 (274–390)	139 (115–177)	9.3 (8.6–10.2)	1	12.4 (6.9– 22.2)
"	"	2.0	3	260 (214–304)	288 (252–345)	10.7 (9.4–11.6)	1	14.0 (5.8– 33.6)
"	Progesterone	1.0	3	8 (4– 11)	25 (21– 28)	12.3 (11.0–13.0)	1	48.4 (23.3–100.6)
"	"	10.0	3	10 (5– 16)	21 (20– 21)	12.7 (11.2–15.6)	2	62.2§(41.5– 93.4)
"	Δ_4Androstenedione	.5	3	70 (47– 92)	79 (66– 91)	12.0 (11.8–12.2)	1	166.5 (90.0–308.0)
"	"	2.0	3	222 (149–282)	74 (56– 97)	14.2 (12.8–15.8)	1	85.6 (46.8–156.7)
"	17 α Hydroxyprogesterone	1.0	3	6 (3– 7)	24 (21– 26)	11.1 (9 –12.2)	1	35.3 (18.0– 69.2)
"	"	10.0	3	8 (6– 8)	24 (24– 25)	10.5 (8.4–11.6)	2	62.4§(44.6– 87.4)
"	Pregnenolone	7.5	3	5 (5– 7)	23 (20– 27)	10.8 (8.8–13.2)	2	49.2§(27.0– 89.5)

* Ventral prostate. † Seminal vesicles. ‡ Indicates that assays were heterologous. § Indicates that assays were homogeneous.

TABLE II

Group	Treatment	Dose, mg/day	No. of rats	VP,* mg/100 g ± S.E.	SV,† mg/100 g ± S.E.	Pit., mg ± S.E.	Pituitary LH conc.: No. assays	Pituitary LH conc.: Mean % cast. (95% CL)	Pituitary FSH conc.: No. assays	Pituitary FSH conc.: Mean % cast. (95% CL)
Castrate	Peanut oil		5	10 ± 1	24 ± 2	13.7 ± .4	4	100	4	100
Intact	" "		5	153 ± 10	133 ± 6	9.8 ± .6	3	20.4§(11.9– 34.9)	2	91‡(61–137)
Castrate	Estrone	.001	5	8 ± 1	26 ± 2	14.5 ± .8	2	124.1§(78.1–197.3)	2	109‡(56–210)
"	"	.003	5	9 ± 1	27 ± 2	13.7 ± .9	2	76.3‡(22.7–256.2)	2	76§(61– 94)
"	"	.006	5	9 ± 1	32 ± 1	14.2 ±1.1	2	9.2‡(2.3– 37.3)	2	73§(52–102)
"	Testosterone	.5	4	203 ± 16	167 ± 5	9.7 ± .3	2	9.8§(5.6– 16.9)	2	350§(271–452)
"	"	1.5	5	236 ± 13	204 ± 3	9.1 ± .5	2	9.1§(6.3– 13.1)	—	—
"	"	3.0	4	282 ± 19	291 ±23	8.9 ± .3	1	4.2 (1.5– 12.2)	1	370 (203–673)
"	Progesterone	5.0	5	14 ± 2	26 ± 2	13.1 ±1.1	2	78.9§(57.2–108.9)	1	98 (78–123)
"	"	10.0	5	15 ± 2	28 ± 2	12.8 ± .1	2	35.6‡(10.6–122.2)	2	137‡(78–240)
"	Δ_4Androstenedione	2.0	5	207 ± 26	187 ±20	9.0 ± .3	1	33.3 (21.2– 52.3)	2	229§(178–295)
"	"	4.0	5	246 ± 12	215 ± 4	8.7 ± .3	2	3.3‡(1.2– 9.0)	1	496 (287–858)
"	17 α Hydroxyprogesterone	10.0	5	18 ± 1	32 ± 1	10.5 ± .8	2	127§ (88.2–182.9)	2	212‡(107–422)
"	Pregnenolone	5.0	5	11 ± 1	27 ± 2	13.7 ± .7	2	127.0§(87.6–184.2)	2	149§(123–180)
"	"	10.0	4	12 ± 1	32 ± 2	13.4 ± .4	2	16.1§(12.0– 21.6)	1	204 (121–343)
"	DHA	2.0	5	70 ± 13	62 ± 7	11.5 ± .3	2	211.0§(148–302)	3	222‡(83–593)
"	"	4.0	5	88 ± 7	99 ±10	10.9 ± .5	2	32.0§(19.5– 52.4)	2	232‡(74–731)

* Ventral prostate. † Seminal vesicles. ‡ Indicates that assays were heterologous. § Indicates that assays were homogeneous.

TABLE III. A Statistical Analysis of the Relationship of Logarithm of μg of Steroid to Logarithm of Pituitary LH Concentration as Percent of Castrate Control from the Combined Data of Series I and II.

	n	r	b (± S.E.)	Linearity
Estrone	5	−.8881 ($p < .05$)	−1.3979 ± .4177	$F = 11.2$ ($p < .05$)
Testosterone	7	−.8887 ($p < .05$)	− .6910 ± .1314	$F = 22.3$ ($p < .01$)

The assay animals were of the Holtzman strain; injections were given once daily; and, the total dose of HCG was 50 I.U.

All bioassays were performed using a 2 × 2 design. Two assay standards were always employed, the castrate pituitary and the appropriate NIH standard (NIH-LH-S1 or S5 and NIH-FSH-S1). All assays were evaluated statistically by the methods for parallel line assays(4) and are reported only if they fulfilled the criteria of parallelism and a lambda of less than 0.3. Where duplicated assays were performed geometric means were calculated and the 95% confidence limits were derived using the method of Sheps and Moore (5).

Results. Data are presented in Tables I and II. In the first series of experiments the LH concentration of the castrate pituitaries was 9.9 μg of NIH-LH-S1 per mg of wet weight. In the second series of experiments the LH concentration was 9.3 μg of NIH-LH-S1 and the FSH concentration 34.3 μg of NIH-FSH-S1 per mg of wet castrate pituitary. In both series of experiments castration resulted in a 5-fold increase in pituitary LH concentration within 4 to 5 weeks, but there was no increase in pituitary FSH concentration.

Discussion. With respect to LH concentrations in pituitaries of castrated male rats, the data would seem to justify several conclusions. Castration resulted in an increase in pituitary LH concentration as has been noted previously(6,7). Based on administered dose, the C_{18} steroid, estrone, was more effective in preventing the post-castration increase in pituitary LH than any of the C_{19} or C_{21} steroids tested. This is consistent with data previously recorded in the literature(6). The C_{19} steroids were more effective than the C_{21} steroids and among the C_{19} steroids testosterone was several times more potent than Δ 4 Androstenedione or dehydroepiandrosterone. Progesterone, 17 α hydroxyprogesterone, and pregnenolone in doses up to 10 mg per day were either ineffective or showed a modest effect that was not consistently reproducible.

A statistically significant relationship between logarithm of dose of testosterone, or estrone, and logarithm of pituitary LH concentration was demonstrated (Table III). These relationships were linear but were of poor predictive value. Although the dose-response slopes were different for the two steroids the difference did not achieve statistical significance. Thus, on the basis of these data and analyses, it was not possible to detect a difference in the mechanism by which these two steriods affect LH concentration in the pituitary of the castrate male rat.

When all of the doses of all of the steroids used in Tables I and II were culled for those producing an increase in prostate size, a significant ($p<0.01$) correlation ($r = -0.67$) was found between logarithm of prostate weight and logarithm of pituitary LH concentration. The following "least squares regression equation" was derived: $\hat{Y} = 3.47587 - 0.98621\ X$. Where:

Y = logarithm of pituitary LH concentration as percentage of castrate controls,

X = logarithm of mg of ventral prostate per 100 grams of rat.

This regression equation did not show significant departure from linearity ($F = 9.96$ $p<0.01$). The standard error of the slope was ± 0.3124.

With respect to FSH, castration did not result in an increase in pituitary concentration. This also agrees with data previously reported(6,7). The C_{19} steroids (testosterone, Δ 4 Androstenedione and dehydroepiandroste-

rone) all increased FSH content and concentration in the castrate pituitary. There was minimal increase in FSH concentration with the administration of 17 α hydroxyprogesterone and pregnenolone but larger doses seemed to be required than with the C_{19} steroids. Progesterone was without effect, and estrone, in the doses employed, produced only a questionable decrease in FSH concentration. Dose-response relationships were not discernible.

In the female rat is is generally held that the mechanisms regulating pituitary LH and FSH are largely if not entirely separate. The dissociation of effects of several steroids, at specific doses, on pituitary LH and FSH concentration in these castrate rats adds further circumstantial evidence that these mechanisms are also separate in the male rat. As a corollary, it appeared justified to conclude that neither testosterone nor estrone alone would be sufficient to control gonadotropin concentration in the pituitary of the male rat.

Summary. Castration of adult male rats resulted in a 5-fold increase in pituitary LH concentration, but no change in pituitary FSH concentration. Estrone, testosterone, Δ 4 Androstenedione and dehydroepiandrosterone inhibited the post-castration increase in pituitary LH and there was a relationship between dose and degree of inhibition. Those steroids which exhibited an androgenic effect increased the pituitary FSH concentration in the castrate pituitary, but a dose-response relationship was not discernible.

1. Parlow, A., Human Pituitary Gonadotropins, A. Albert, ed., Charles C Thomas, Springfield, 1961, p300.

2. Ryan, R. J., J. Clin. Endocrinol. and Metab., 1962, v22, 300.

3. Steelman, S. L., Pohley, F. M., Endocrinology, 1953, v43, 604.

4. Bliss, C. I., Biometrics, 1956, v12, 491.

5. Sheps, M. C., Moore, E. A., J. Pharmacol. Exp. Ther., 1960, v128, 99.

6. Greep, R. O., Sex and Internal Secretions, Vol. I, W. C. Young, ed., Williams & Wilkins, Baltimore, 1961, p240.

7. Parlow, A., Endocrinology, 1964, v73, 1.

Received August 25, 1966. P.S.E.B.M., 1967, v124.

Composition of Plasma Triglyceride Fatty Acids and Free Fatty Acids in Hypothalamic Obese Mice.* (31714)

T. W. REDDING AND A. V. SCHALLY

Endocrine and Polypeptide Laboratory, Veterans Administration Hospital, and Department of Medicine, Tulane University School of Medicine, New Orleans

Administration of gold thioglucose to mice results in an obesity similar to that induced by stereotaxic lesions in the ventromedial nucleus of the hypothalamus in rats and mice. Although early work suggested that little functional damage was done by gold thioglucose other than causing hyperphagia, more recent evidence suggests that other physiological functions may be affected(1). The hypothalamus is well known to contain factors which stimulate or inhibit the release of known anterior pituitary hormones(2). Damage to this area by gold thioglucose may cause, in addition to hyperphagia, an imbalance in the production and release of anterior pituitary hormones. We have recently shown that the pituitary may play an important role in the production of obesity in gold thioglucose treated mice(3).

Using new micromethods for the separation of plasma lipids, followed by gas-liquid chromatography, we have extended our observations to the composition of triglyceride fatty acids and free fatty acids in the plasma of normal mice and in hypophysectomized and non-hypophysectomized gold thioglucose lesioned mice.

Materials and methods. Female mice of

* Supported in part by a grant from Merck and Co., Inc.