Serum Follicle-Stimulating Hormone (FSH) in Normal and Androgenized Male and Female Rats[1] (35847)

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Sexual differentiation of the hypothalamic–hypophyseal gonadotropin control mechanisms in the rat has received a great deal of attention (1, 2). The most widely accepted view assigns to testicular androgen the role of masculinizing the hypothalamic–hypophyseal axis so that a male pattern of gonadotropin output results. This pattern has not been clearly defined except in the sense of a noncyclic release of luteinizing hormone (LH). This noncyclic function prevents ovulation, or corpora lutea formation, in ovaries implanted into normal males. Females treated with androgen (androgenization), or any of several other steroids (1), shortly after birth display this same lack of cyclicity and therefore have been assumed to have the male pattern of gonadotropin output.

A male pattern for follicle-stimulating hormone (FSH) release has not been established, but we do know that the pituitary (3) and plasma (4) content of this hormone in males is far greater than it is in females. This high circulating level of FSH no doubt plays some role in the ease with which ovarian implants take and grow in males (5). The questions arise: Is a high concentration of FSH in the plasma part of the male gonadotropin pattern, and does early exposure to androgen establish this pattern? When ovarian weight changes in hypophysectomized parabiotic partners of intact males or females were used to evaluate plasma FSH (6) no differences between androgenized and normal females could be detected: both had much less FSH than normal males. In contrast to those findings, Barraclough (1) published data showing that androgenized females had very high blood levels of FSH as determined by bioassay. In the present study, radioimmunoassayable FSH was examined in sera from males and females in an attempt to resolve this controversy. The results support the conclusion that neonatal treatment with a small dose of androgen does not induce the male pattern of high serum FSH in the female.

Material and Methods. Albino rats of the Holtzman strain, kept in temperature (24 ± 2°) and light (14 hr light between 6 a.m. and 8 p.m.) controlled quarters were used. They had free access to Purina laboratory chow and tap water. Both males and females received 50 μg of testosterone propionate (androgenization) dissolved in 0.05 ml of sesame seed oil, on the fifth postpartum day. Litters were reduced to 8 pups at the time of this injection. Controls received the vehicle only. The litters were weaned, and the sexes were separated when 21 days old.

Animals were randomly selected from various storage cages for sacrifice. They were anesthetized with ether and quickly bled via the abdominal aorta. The blood was allowed to clot at 4° and centrifuged in the cold. The serum was stored frozen until assayed. The reproductive tract in each animal was examined with particular attention given to the presence of corpora lutea in the ovaries. The gonads and sex accessory organs were dissected out, blotted on paper towel, and weighed on a torsion balance.

The FSH was assayed using the radioimmunoassay kit supplied by the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Program. The directions supplied with the kit were generally followed, the only significant exception being that a 1

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M phosphate buffer was used for the iodination procedure. The details and performance of this assay kit have recently been published (7). In most cases two doses of serum, and always several replicates at each dose, were assayed and the amount of FSH was determined from a standard curve which was constructed using rat FSH (RP-1; 2.1 X NIH-FSH-S1 by bioassay) in doses of 10 to 500 ng.

Results. The andogenization treatment used did not alter body weights in the females. Ovarian weights up until day 33 were also not different from those of controls, but of course, normal ovaries increased considerably in weight after ovulation had occurred. Corpora lutea were not found in the ovaries of any of the androgenized females.

Androgenized females characteristically undergo precocious puberty, which is first manifested by early vaginal opening. This occurred by day 25 in all of the females of this study. Increased uterine weight, indicative of increased estrogen production, was apparent in the animals autopsied on day 33: 76.1 ± 12 mg in four animals on day 30 and 187 ± 15 mg in four killed on day 33. However, 2 of the females killed on day 29 and also on day 30, had enlarged uteri.

Vaginal opening was first noted on day 34 in the normal females, but one of the 6 animals killed on day 36 still had a closed vagina. Corpora lutea were found in 2 of the 6 animals killed on day 35 and in 4 of the 6 killed on day 36. Uterine weight increase was clearly evident on day 34: 79.8 ± 17 mg in 7 animals on day 33 and 134 ± 14 mg in 9 animals killed on day 34. Therefore, for this particular group of females, and at this time, puberty occurred between days 34 and 37. This is about 2 days earlier than Moore (8) found for the same strain a few years ago.

Nine groups of androgenized and 7 groups of normal males were used. They were not killed on the same days, and therefore, statistical comparisons between body and organ weights were not made. Testicular and sex accessory organ development was not retarded, however, in the androgenized males which is consistent with the findings of Kincl *et al.* (9) who found that rather large doses of androgen were necessary before development was altered.

Serum FSH in androgenized females was relatively high on day 20, but quickly, and steadily, declined to reach undetectable levels on day 30 (Fig. 1). There was a slight increase after this nadir, but the level was only 67 ± 10 ng/ml on day 45. The values for two groups of androgenized females killed at 60 days and at about 1 year, but not shown in Fig. 1 were 152 ± 16 and 201 ± 39 ng/ml, respectively.

Normal female serum FSH values varied from day to day and no distinct pattern was discernible (Fig. 1). A large decrease was found on day 33 and this may have some physiologic significance. The concentration on day 32 was 208 ± 17 ng/ml, while on day 33 it was 62.4 ± 9 ng/ml, and on day 34 it returned to 177 ± 46 ng/ml. Every animal in the group of 12 killed on day 33 had serum FSH less than 100 ng/ml. Changes in FSH were not followed after puberty in the normal females since cyclic changes are known to occur, and therefore, careful checking of the stage of the estrous cycle would be required.

Very high concentrations of FSH were found in the serum of both normal and androgenized males until about 30 days of age and then they declined steadily until day 60 (Fig. 2). Not enough samples of the normal males were examined to make statistical analyses meaningful. The pattern of changes and the magnitude of FSH in the serum was obviously similar in the two types of males. Even at the low point of 602 ± 44 ng/ml in androgenized males on day 57, the level was about three times that of females.

Discussion. The question of whether early androgen treatment of females induces a male pattern in FSH release was answered: it does not. While young androgenized females had relatively high concentrations of FSH, they quickly fell to essentially zero by day 30, just about the time that the uterine enlargement indicated the presence of increased amounts of estrogen. Possibly, the production of estrogen by the ovaries was responsible for the drop via a negative feedback effect upon the release of FSH. The decline

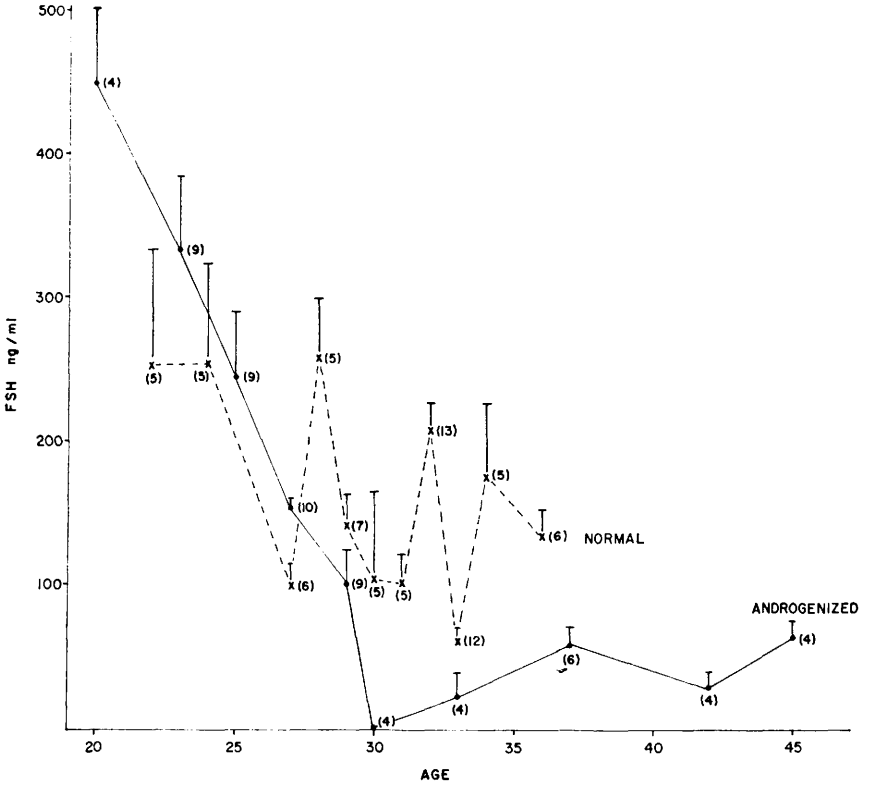

FIG. 1. Changes in serum FSH in normal and androgenized females with age: Values are in terms of Rat-FSH-RP-1 with standard errors indicated by vertical lines. Number of animals used at each age is given in parentheses.

in FSH was greater in androgenized than in normal females at this time and may be related to an increased sensitivity to estrogen. They certainly have an increased sensitivity to androgen feedback effects upon LH (10). This sensitivity could also account for the low FSH levels maintained until day 45, but this could also be explained by a lack of a positive feedback effect of estrogen on FSH. A single dose of estradiol benzoate to immature normal females causes an increase in circulating FSH (11), but this does not occur in androgenized females (Johnson and Naqvi, unpublished data).

The FSH concentration of normal females also tended to decline at the time of puberty, but variability made this difficult to establish. The level was lowest just prior to the first outward indications of estrogen increases; vaginal opening and increased uterine weight. As mentioned above, we cannot determine whether these changes were due to a negative or positive feedback effect of the estrogen, or both. An increase in FSH at the time of vaginal opening would be consistent with the findings for the other two gonadotropins. Voogt *et al.* (12) found that prolactin increased sharply on the day of vaginal opening and cited a communication from Midgley indicating that LH also increased at this time. LH and prolactin were not systematically examined in the present study but a few assays were done, and they substantiate the report of Voogt *et al.* (12). In normal females LH increased from 21.9 ± 2.1 ng/ml (Rat-LH-RP-1) on day 32 to 46.7 ± 3.7 ng/ml on day 33 (12 samples), while prolactin went from 11.0 ± 0.6 ng/ml (Rat prolactin-1) on day 31 to 25.8 ± 4.2 ng/ml on day 32 and to 44.7 ± 12 ng/ml on day 35. On day 36, the prolactin concentration was 70.5 ± 28 ng/ml. In contrast to the changes in

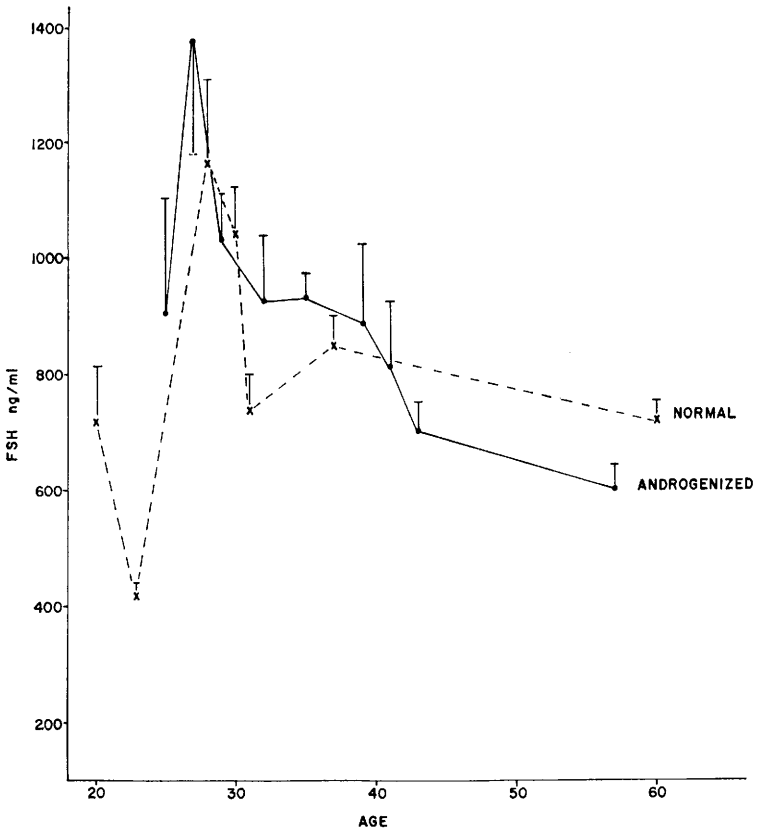

FIG. 2. Serum FSH changes in developing male rats: Rat FSH (RP-1) was used as the standard. Vertical lines indicate standard errors. At least 4 animals were used in each point.

normal females, LH in the androgenized females remained amazingly constant through the time of normal puberty: 40.4 ± 2.7 on day 27; 31.7 ± 4.5 on day 33; 39.4 ± 2.2 on day 37; 35.9 ± 4.1 on day 42. On the other hand, prolactin in androgenized females increased from 40.4 ± 4.7 on day 27 to 98.5 ± 16 on day 37 and to 151 ± 23 on day 42 and was at a level of 152 ± 17 ng/ml on day 60. Since FSH on day 42 was only 29 ng/ml while it was 152 on day 60, there was obviously no correlation between these two gonadotropins. This tempts the speculation that prolactin control mechanisms in the hypothalamus of the androgenized female function normally in response to estrogen (12).

Serum FSH concentrations change cyclically in normal females (13) with the highest levels attained during late proestrus and early estrus. Very few diestrus animals have FSH levels above 200 ng/ml, but values as high as 1000 ng/ml were found in the morning of estrus (13). These high values were only transient and quickly fell to below 200 ng/ml by the afternoon of estrus. A reasonable value for the intact female, therefore, appears to be about 200 ng/ml which is the concentration found in year old androgenized females. We have no information regarding the possibility of cyclic changes in FSH in androgenized females.

Swerdloff *et al.* (7) studied FSH changes in the developing Wistar strain male rat. They found high concentrations in the serum on day 35, but it declined until day 63 with the level then remaining steady until day 77. The same general pattern was found in the present study, but the absolute values for the Holtzman rat were slightly higher (the same assay kit and the same standards were used). In apparent contrast to the results of Swerdloff *et al.* (7) as well as the present

report, parabiotic studies indicated that older males had more FSH than younger animals. This discrepancy can be explained by realizing that the parabiotic technique relies upon a time-integrated response, while the immunoassay indicates the amount of hormone present at a given instant. We do not know whether concentration of FSH is of any physiologic significance to the animal: perhaps the duration of a minimal effective dose is of more value. In the male, the concentration decreases, but the total amount of FSH in the blood increases with age. If we assume that 3% of the body weight is a reasonable estimate for plasma volume, then the immature male has a total of 3000 ng of FSH at the peak concentration. A 60-day-old male would have more than 5000 ng and at 6 months of age his total circulating FSH would amount to more than 7500 ng even if we assumed the concentration went down to about 500 ng/ml.

Summary. Serum FSH was measured by radioimmunoassay in developing normal and androgenized male and female rats. Immature, 30-day-old, as well as mature, 60-day-old males had higher concentrations of this gonadotropin than females. Serum FSH declined in androgenized females (50 μg of testosterone propionate, fifth day of age) between days 20 and 30. The level remained low at 60 days but was at about the level of normal females at 1 year. The results support the conclusion that androgenization in the female does not produce the male pattern of high serum FSH.

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