

Pituitary-Testicular Responsiveness in Male Hypogonadotropic Hypogonadism

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ABSTRACT An isolated deficiency of pituitary gonadotropins was demonstrated in six 46 XY males, 22 to 36 years of age, with and without anosmia. Undetectable or low levels of serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) clearly separated hypogonadotropic from normal adult males. Chronic (8–12 wk) administration of clomiphene citrate caused no increase in serum FSH or LH in gonadotropin-deficient subjects. However, the administration of synthetic luteinizing hormone releasing factor (LRF) resulted in the appearance of serum LH and, to a lesser degree, serum FSH in three subjects tested. While levels of plasma testosterone were significantly lower in gonadotropin-deficient subjects, plasma androstenedione and dehydroepiandrosterone were in a range similar to that of age-matched normal men. Treatment with human chorionic gonadotropin (HCG) increased levels of plasma testosterone to normal adult male values in all gonadotropin-deficient subjects. Cessation of treatment with HCG resulted in the return of plasma testosterone to low, pretreatment levels. That HCG therapy with resultant normal levels of plasma testosterone may somehow stimulate endogenous gonadotropin secretion in gonadotropin-deficient subjects was not evident. The adult male levels of serum FSH and LH after LRF, and plasma testosterone after HCG, confirm pituitary and Leydig cell responsiveness in these subjects.

INTRODUCTION

An isolated deficiency of gonadotropin secretion has been noted for many years (1–16). Despite this knowledge, few studies are available which have examined blood levels of androgens and gonadotropins before and after treatment with clomiphene citrate and human

chorionic gonadotropin (HCG)¹ (17). We have studied six males with hypogonadism due to an isolated deficiency of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and have quantitated levels of pituitary gonadotropins, testosterone,² androstenedione, and dehydroepiandrosterone before and during treatment with clomiphene citrate and HCG. Additionally, we have achieved normal levels of serum FSH and LH with the administration of luteinizing hormone releasing factor (LRF). These results point to a deficiency of hypothalamic releasing factor as an explanation of the isolated deficiency of gonadotropins found in these subjects.

METHODS

Urinary 17-ketosteroids were measured by the procedure of Vestergaard (18). Plasma cortisol and 11-deoxycortisol were measured by double-isotope derivative techniques (19). Plasma testosterone, dehydroepiandrosterone, and androstenedione were measured by a modification of the double-isotope derivative technique combined with gas-liquid chromatography, as described by Kliman and Briefer (20). Preliminary separation of testosterone, dehydroepiandrosterone, and androstenedione was accomplished in the paper chromatographic system: hexane 100: methanol 90: water 10. Androstenedione was converted to testosterone with sodium borohydride (Sigma Chemical Co., St. Louis, Mo.); dehydroepiandrosterone was converted to testosterone with aluminum tertiary butoxide (K & K Laboratories, Inc.,

¹Abbreviations used in this paper: FSH, follicle-stimulating hormone; HCG, human chorionic gonadotropin; 2nd IRP-HMG, second International Reference Preparation of Human Menopausal Gonadotropin; LH, luteinizing hormone; LRF, luteinizing hormone releasing factor; NIAMD, National Institute of Arthritis and Metabolic Diseases; NIH, National Institutes of Health, Bethesda, Md.; NPA, National Pituitary Agency; PBS, 0.01 M phosphate buffer, pH 7.8, with 0.15 M sodium chloride; RIA, radioimmunoassay; SU-4885, metyrapone.

²Systematic nomenclature for trivial names in this paper: androstenedione, 4-androstene-3, 17-dione; dehydroepiandrosterone, 3 β -hydroxy-5-androstene-17-one; testosterone, 17 β -hydroxy-4-androstene-3-one.

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Hollywood, Calif.) and sodium borohydride, respectively (21). Both steroids were quantitated as testosterone. Serum growth hormone was measured by radioimmunoassay (RIA) (22).

Radioimmunoassay of FSH and LH. Human pituitary FSH (LER 869-2; 2,782 IU/mg bioassay) and human pituitary LH (LER 960; 923 IU/mg bioassay) supplied by Dr. Leo E. Reichert and distributed by the National Pituitary Agency (NPA) and the National Institute of Arthritis and Metabolic Diseases (NIAMD), National Institutes of Health (NIH), Bethesda, Md., were used for radioiodination. Rabbit anti-human FSH antiserum (batch #3) was supplied by the NPA and NIAMD, NIH. Rabbit anti-human HCG antiserum was supplied by the Sylvania Chemical Co., Milburn, N. J. The second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) containing 40 IU of FSH and 40 IU of LH, supplied by Dr. D. R. Bangham, Medical Research Council, National Institute for Medical Research, London, was used as the reference standard. 1 µg of the Human Pituitary Extract Reference Preparation LER-907, supplied by the NPA and NIAMD, contains 68 mIU of the 2nd IRP-HMG for FSH and 441 mIU of the 2nd IRP-HMG for LH in this laboratory.

Radioiodination was performed by a modification of the method of Hunter and Greenwood (23). Two 1-µg samples of FSH (LER 869-2) and one 1-µg sample of LH (LER-960) were used for radioiodination.

Both FSH and LH radioimmunoassays were performed over a 6-day period; antiserum was added on day 1, whereas the radioactive hormone was added on day 4. Sheep anti-rabbit globulin serum and normal human serum were added on day 6 to precipitate antibody-bound hormone. The diluent was 0.01 M phosphate buffer, pH 7.8, with 0.15 M sodium chloride (PBS) containing 1% bovine serum albumin and 0.01 M EDTA. All assays were calculated with the aid of a computer. All samples were counted in a Packard Auto Gamma Spectromometer (Packard Instrument Co., Inc., Downers Grove, Ill.) at a preset count of 5,000 cpm.

Tests

Hypothalamic-pituitary responsiveness. Insulin, 0.1 U/kg, was given intravenously and blood was obtained at 0,

30, 60, 90, and 120 min. In this laboratory plasma growth hormone in normal men increased to at least 10 ng/ml when the blood sugar decreased by 50% of the initial concentration.

Metyrapone (SU-4885), 750 mg, was given orally every 4 h for six doses. A normal response is defined as a fall in plasma cortisol (50% or greater) accompanied by a rise in plasma 11-deoxycortisol concentration to 10 µg/100 ml or more (19). Plasma 11-deoxycortisol was not measured before SU-4885 since its concentration is negligible (< 0.5 µg/100 ml).

Clomiphene citrate, 200 mg, was given orally for 7 days, and blood was obtained at 8 A.M. on days 6 and 7. A normal response in this laboratory in normal men, ages 20-40 years, is an increase of at least 100% in serum levels of FSH and LH, respectively. Additionally, clomiphene citrate, 25-200 mg daily, was given for 8-12 wk, and serial measurements of serum FSH, LH, and plasma testosterone were determined.

Highly purified, synthetic LRF supplied by Dr. R. Guillemin, La Jolla, Calif., and synthesized by Dr. J. Rivier (24) was utilized in the present study. It has full biological activity in the various in vitro and in vivo assay systems used to characterize LRF (25). Millipore-filtered, pyrogen-free LRF, diluted in 0.5 g/100 ml human serum albumin in distilled water, was stored at 4°C until used. At the time of this study, 1.0-ml (100 µg/ml) quantities were rapidly injected intravenously. Blood was withdrawn from a peripheral venous catheter before administration of LRF (time 0) and 5, 10, 15, 30, 45, 60, 90, 120, 240, and 480 min later. Samples were analyzed for FSH and LH.

Leydig cell responsiveness. HCG (APL, Ayerst Laboratories, New York), 5,000 U intramuscularly, was given daily for 4 days, then three times per week. Levels of plasma androstenedione, dehydroepiandrosterone, testosterone, and serum LH (HCG) were measured before, during, and after HCG administration. All gonadotropin-deficient subjects had undetectable (<1 mIU/ml) or low (<2 mIU/ml) levels of LH before therapy. During HCG administration, serum levels of HCG, and not LH, were measured because of cross-reactivity of the antibody.

TABLE I
Laboratory Findings in Hypogonadotropic Males

Patient	Plasma growth hormone		Cortisol		11-Deoxycortisol		Protein-bound iodine	17-Ketosteroids
	B*	ITT†	B	SU‡	B	SU		
	ng/ml		µg/100 ml		µg/100 ml		µg/100 ml	mg/24 h
J. J.	5	62	4.3	1.7	—	11.0	6.9	3.9
D. J.	3	48	12.5	4.0	—	12.0	5.5	9.1
T. B.	4	27	13.0	2.5	—	14.0	6.9	10.5
L. E.	3	31	12.0	2.0	—	15.0	7.5	14.9
J. S.	4	63	14.4	2.7	—	12.8	5.7	13.4
R. M.	2	22	6.9	1.9	—	20.0	5.9	7.7
Normal range	0-6	>10.0	4-25	>50%	<0.5	>10	3.5-7.5	6-22 mg/24 h

* B, base line.

† ITT, insulin tolerance test.

‡ SU, SU-4885.

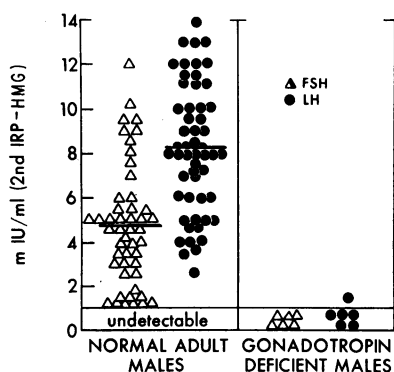


FIGURE 1 Serum FSH (open triangles) and LH (closed circles) in normal adult and gonadotropin-deficient males. FSH was undetectable (<1 mIU/ml) in all gonadotropin-deficient subjects while LH was detectable, but below the normal range, in a single subject, and undetectable in the other five subjects.

CLINICAL SUMMARY

Six gonadotropin-deficient males, ages 22–36 years, were studied. R. M. and J. J. had received 3- and 8-mo courses of HCG, respectively, which were stopped 3 mo before the onset of the present study. D. J. and L. E. had received intermittent testosterone treatment over 10 yr, but no treatment 3 mo before the beginning of the study. J. S. and T. B. received no hormonal therapy before the initiation of this study. All subjects showed retarded sexual development and/or eunuchoid habitus. Testicular size (greatest diameter) was less than 2 cm in all subjects except T. B. (3.0 cm). One subject, L. E., was anosmic and had a congenital absence of the left kidney. Cryptorchidism, midline defects (harelip or cleft palate), skeletal anomalies (short fourth metacarpal), nerve deafness, and colorblindness were absent in all subjects. Two subjects, J. J. and D. J., were brothers, and from a total of four other adult male siblings, only two had achieved puberty and proved fertile. Three sisters had reached menarche, and one was fertile. The other hypogonadal subjects had no siblings and gave no family history of hypogonadism. X-ray examination of the skull, visual field examination, and buccal smear for sex chromatin were negative in all subjects.

RESULTS

Table I: Insulin-induced hypoglycemia resulted in a normal rise in plasma growth hormone in all gonadotropin-deficient subjects. The presence of ACTH reserve was confirmed by the normal rise in plasma 11-deoxycortisol after administration of SU-4885. Tests of thyroid function revealed a normal protein-bound iodine and radioiodine uptake. Urinary excretion of 17-ketosteroids was in the normal range in all gonadotropin-deficient

subjects except J. J., whose value was low. Since the testicular contribution to urinary 17-ketosteroids is about one-third (26), low levels would not be unexpected in some gonadotropin-deficient males.

Serum FSH and LH values in gonadotropin-deficient subjects are compared to normal adult males in Fig. 1. Serum FSH was undetectable (<1 mIU/ml) in all subjects. Similarly, serum LH was undetectable (<1 mIU/ml) in five subjects. D. J. had a serum LH of 1.7 ± 0.31 mIU/ml (mean \pm SD). In contrast, serum FSH ($n = 48$) and LH ($n = 54$) in normal subjects were 4.8 ± 2.8 and 8.3 ± 3.0 mIU/ml (mean \pm SD), respectively. After clomiphene citrate administration, 200 mg daily for 7 days, no rise was observed in the undetectable or low levels of serum FSH and LH in gonadotropin-deficient subjects (Fig. 2). Serum FSH and LH increased 100% or more following clomiphene citrate in all normal subjects.

Plasma dehydroepiandrosterone concentrations (mean 0.33 ± 0.17 μ g/100 ml) in gonadotropin-deficient subjects were similar to age-matched normal adult males (mean 0.55 ± 0.37 μ g/100 ml) and increased after 4 days (mean 0.37 ± 0.21 μ g/100 ml) and 4 wk (mean 0.49 ± 0.27 μ g/100

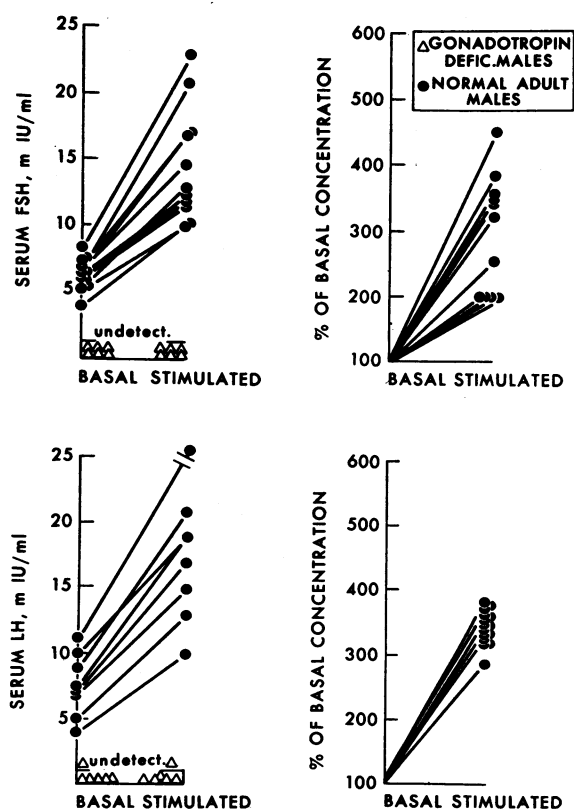


FIGURE 2 Response in serum FSH and LH (mIU/ml and percent of basal concentration) in gonadotropin-deficient (open triangles) and normal (closed circles) adult males after 7 days of clomiphene citrate, 200 mg/day.

ml) of HCG administration. Likewise, plasma androstenedione concentrations in gonadotropin-deficient subjects (mean $0.08 \pm 0.02 \mu\text{g}/100 \text{ ml}$) and age-matched normal adult males (mean $0.11 \pm 0.04 \mu\text{g}/100 \text{ ml}$) were similar. No increase in androstenedione concentrations was observed after 4 wk of HCG administration.

Plasma concentrations of testosterone in gonadotropin-deficient males (mean $0.03 \pm 0.03 \mu\text{g}/100 \text{ ml}$) were significantly less than age-matched normal males (mean $0.62 \pm 0.29 \mu\text{g}/100 \text{ ml}$) ($P < 0.01$) (Fig. 3). After 4 days of HCG administration (mean $0.14 \pm 0.09 \mu\text{g}/100 \text{ ml}$) plasma testosterone increased compared to pretreatment levels ($P < 0.05$). After 2 wk (mean $0.45 \pm 0.37 \mu\text{g}/100 \text{ ml}$) and 4 wk (mean $0.60 \pm 0.37 \mu\text{g}/100 \text{ ml}$) concentrations increased significantly ($P < 0.01$) and were similar to adult male values (Fig. 3).

D. J., the single gonadotropin-deficient subject whose plasma testosterone ($0.08 \mu\text{g}/100 \text{ ml}$) was above the female range ($< 0.06 \mu\text{g}/100 \text{ ml}$) was the only gonadotropin-deficient subject whose serum LH (1.7 mIU/ml) was detectable.

That normal concentrations of plasma testosterone were achieved in all gonadotropin-deficient subjects with HCG administration clearly indicated the presence of Leydig cell responsiveness. Thus, it seemed pertinent to show whether onset of gonadotropin secretion would occur after (a) withdrawal of HCG treatment, (b) chronic treatment with clomiphene citrate, and (c) administration of LRF.

Concentrations of serum LH (HCG) and plasma testosterone were measured during a 12-wk period of HCG administration (Fig. 4). During the period of treatment, serum LH (HCG) levels ranged between 50 and 175 mIU/ml . Plasma testosterone was in the normal adult male range in all subjects during HCG administration. 6 wk after HCG was discontinued, serum LH and

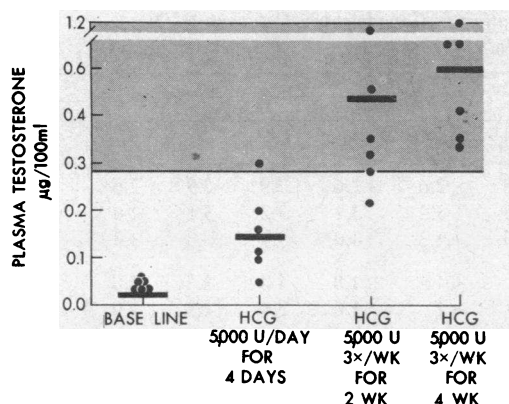


FIGURE 3 Plasma concentrations ($\mu\text{g}/100 \text{ ml}$) of testosterone in gonadotropin-deficient males, with the normal range (hatched area) and mean (solid line) after 4 days, 2 wk and 4 wk of HCG.

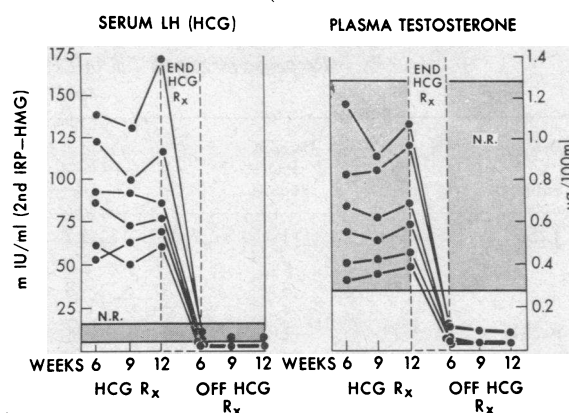


FIGURE 4 Concentrations of serum LH (HCG) and plasma testosterone during and after 12 wk of HCG, 5000 U three times a week, in gonadotropin-deficient males. The normal adult range is shown by the hatched area.

plasma testosterone had returned to undetectable or low pretreatment values and remained at those levels 12 wk after all medication had been discontinued.

Clomiphene citrate, 100 mg/day for 6 wk and 200 mg/day for 6 wk, was administered to D. J., J. S., L. E., and R. M., whereas T. B. received 25 mg/day for 4 wk and 50 mg/day for 4 wk. In all gonadotropin-deficient subjects, concentrations of serum FSH and LH remained low or undetectable, unresponsive to clomiphene administration.

After LRF administration in gonadotropin-deficient males concentrations of LH (Table II) rapidly reached detectable levels (5 min). Similarly, in normal and gonadotropin-deficient subjects, serum LH values were maximal between 15 and 30 min. In normals, this maximal rise represented an approximate 2.5–3-fold increase over pretreatment levels. The relative increases in FSH in all subjects were much smaller than those observed for LH (Table III). In gonadotropin-deficient males, detectable levels of FSH occurred at 45 min in R. M., 60 min in L. E., and 5 min in J. S. Maximal values were reached between 30 and 90 min in R. M. and J. S.; however, the peak concentration of FSH in L. E. occurred 4 h after LRF. The maximal rise in normal subjects occurred between 30 and 60 min and was approximately 40% increased over pretreatment values.

DISCUSSION

In recent years numerous clinical studies describing isolated gonadotropin deficiency in the male have appeared which have called special attention to certain anomalies and conditions that frequently occur with this disorder, its sporadic and familial occurrence, as well as possible modes of inheritance (1–16). The need to critically examine hypothalamic-pituitary and testicular responsive-

TABLE II
Response in Serum LH to LRF Administration in Gonadotropin-Deficient (GD)
and Normal (N) Adult Males

Subject	Diagnosis	Days of treatment	Time, min										
			0	5	10	15	30	45	60	90	120	240	480
J. S.	GD	1	<1	5.7	8.2	10.6	9.5	7.9	6.1	4.3	3.1	1.5	<1
		2	<1	8.0	9.4	11.4	10.3	7.7	5.8	3.8	2.4	1.3	1.2
		3	<1	6.8	12.6	13.7	10.2	8.1	6.6	5.3	4.2	1.2	1.1
R. M.	GD	1	<1	2.4	4.3	6.1	7.5	6.1	4.6	2.9	2.8	1.3	<1
		2	<1	3.2	3.8	6.7	5.3	4.9	2.6	2.0	2.4	1.1	<1
L. E.	GD	1	2.7	4.6	5.3	5.2	4.8	5.1	5.3	5.0	4.2	4.2	4.3
		2	3.9	5.1	7.7	9.5	8.5	7.7	7.0	6.8	6.7	6.8	6.3
		3	2.8	11.9	21.2	23.3	22.8	20.9	20.1	17.4	17.9	14.0	6.4
R. W.	N	1	9.6	12.5	21.3	27.9	27.3	26.0	21.2	17.4	14.8	8.2	8.6
R. A.	N	1	11.6	14.8	22.6	27.0	29.9	25.7	23.9	22.0	16.6	13.0	9.6

ness in affected individuals becomes paramount since the success of newer modes of therapy in this disorder will depend on such responsiveness.

In the present study of gonadotropin-deficient subjects, two were brothers, while the other four subjects represented isolated, sporadic occurrences. In addition to the findings of hypogonadism in all subjects, only L. E. displayed other anomalies, specifically anosmia and unilateral renal agenesis, an association previously reviewed by Nowakowski and Lenz (7).

Although the advent of radioimmunoassay of pituitary gonadotropins provided a means to quantify FSH and LH in blood and easily distinguish between elevated and normal levels, it remained inadequate to quantitatively

separate normal from low levels (17, 27-30). This may be related to methodology, since both precision and sensitivity are less dependable when concentrations of gonadotropins are near the limits of detection by radioimmunoassay (31). A previous study separated gonadotropin-deficient from normal adult males by measuring immunoreactive FSH and LH in urine extracts (17). In the present study, radioimmunoassay of a single blood sample for both FSH and LH permitted us to clearly separate hypogonadal males with deficient gonadotropin secretion from normal adult males. No increase in serum FSH or LH was observed after the 7-day course of clomiphene administration in all gonadotropin-deficient subjects studied; these observations are in agreement with earlier

TABLE III
Response in Serum FSH to LRF Administration in Gonadotropin-Deficient (GD)
and Normal (N) Adult Males

Subject	Diagnosis	Days of treatment	Time, min										
			0	5	10	15	30	45	60	90	120	240	480
J. S.	GD	1	<1	1.2	1.6	2.6	2.6	2.6	2.9	2.9	2.6	2.2	2.1
		2	1.0	1.9	2.7	2.8	3.2	3.2	3.2	3.1	2.8	2.5	2.3
		3	1.9	2.0	2.4	2.9	4.3	4.0	3.7	3.8	3.3	1.3	2.5
R. M.	GD	1	<1	<1	<1	<1	<1	1.8	1.3	1.3	1.0	<1	<1
		2	<1	<1	<1	<1	<1	1.0	2.0	1.5	1.0	<1	<1
L. E.	GD	1	<1	<1	<1	<1	<1	<1	1.2	0.9	1.3	1.8	1.2
		2	<1	<1	<1	<1	2.4	2.1	3.4	3.4	4.3	3.8	2.7
		3	<1	<1	1.6	1.8	3.5	4.2	5.6	3.4	5.4	5.2	2.7
R. W.	N	1	10.3	10.4	10.0	11.9	12.3	13.0	14.0	13.1	12.7	10.9	10.2
R. A.	N	1	4.8	5.3	5.6	6.4	6.7	6.4	6.3	6.3	4.9	4.8	5.6

studies reported by Bardin, Ross, Rifkind, Cargille, and Lipsett (17). The low levels of serum LH (<1 mIU/ml) observed in this study were reflected by prepubertal levels of plasma testosterone. However, the plasma concentrations of the weekly androgenic 17-ketosteroids, androstenedione, and dehydroepiandrosterone, which are derived almost entirely from the adrenal gland, were within the range of values observed in normal adult males. Thus, it appears unlikely that the presence of LH is necessary, acting synergistically with ACTH, to initiate adrenal 17-ketosteroid secretion.

In contrast, plasma testosterone concentrations were strikingly low and increased markedly after HCG treatment, reaching adult male levels in all subjects. Further, long-term treatment with exogenous testosterone in subjects L. E. and D. J. did not impede Leydig cell responsiveness to subsequent HCG therapy. However, in five of the six (one anosmic) gonadotropin-deficient subjects reported herein, and in six of seven hyposmic patients reported by Bardin et al. (17) following 4 days of HCG, 4,000–5,000 U daily, mean levels of plasma testosterone remained well below the adult male range. These observations suggest a similar Leydig cell response to HCG in gonadotropin-deficient subjects with hyposmia, anosmia, or an intact sense of smell. HCG treatment in normal male children in a stage of pubertal development (testicular size) comparable to these gonadotropin-deficient subjects resulted in similar rises of plasma testosterone (32). Moreover, similar treatment with HCG in some prepubertal males increased levels of plasma testosterone into the range of normal men (33, 34). However, chronic HCG treatment in four out of six hyposmic patients reported by Bardin et al. (17) failed to elicit either a normal adult level of plasma testosterone or clinical evidence of masculinization. This observation led them to conclude that these patients manifested Leydig cell insensitivity to gonadotropin administration as well as impaired gonadotropin secretion. In contrast, all gonadotropin-deficient subjects in this study attained normal adult levels of plasma testosterone within 4 wk of HCG administration, 5,000 U, three times per week, providing clear evidence of Leydig cell responsiveness.

In occasional patients with hypogonadotropic hypogonadism, gonadotropin therapy appears to stimulate endogenous gonadotropin production making further exogenous treatment unnecessary (35). Unfortunately, this event was not observed in any subject in the present study with or without anosmia, since cessation of treatment with HCG resulted in the return of plasma testosterone to low, prepubertal levels, while serum LH was undetectable.

The effectiveness of clomiphene in initiating gonadotropin secretion in anosmic subjects with isolated defi-

ciency of FSH and LH has been debated (36–38). Recently, Santen, Leonard, Sherins, Gandy, and Paulsen observed no significant increments in serum FSH and LH in gonadotropin-deficient males with or without anosmia following 3–4 wk of clomiphene administration (39). Hamilton, Hendin, Weir, and Kliman (40) have recently described responsiveness to clomiphene in two patients with type II hyposmia and one patient with normal olfaction. However, two of the three patients had testosterone concentrations of $0.21 \mu\text{g}/100 \text{ ml}$ and $0.22 \mu\text{g}/100 \text{ ml}$ with measurable levels of serum FSH and LH. In the present study we observed no effect of variable dosage (25–200 mg) or duration (3 mo) of clomiphene citrate administration on gonadotropin secretion. Serum concentrations of FSH and LH and plasma testosterone remained undetectable or extremely low, unchanged from basal levels.

However, the dramatic release of LH and FSH in three of these subjects following administration of LRF clearly localizes the defect to the hypothalamus and confirms the presence of LH and FSH in the pituitary. Two additional reports (41–42) have also demonstrated pituitary responsiveness of LRF administration. Although normal adult levels of serum FSH and LH were achieved, these concentrations were never as great as those in normal subjects despite 3 days of LRF administration. Perhaps pituitary responsiveness, like testicular responsiveness, requires more prolonged stimulation with its respective tropic hormone to achieve normal adult function.

The observations in these studies are particularly meaningful since Leydig cell and pituitary responsiveness must be present before therapeutic modalities such as treatment with combined preparations of HCG and HMG* or gonadotropin releasing factors can be successful in achieving testicular maturation.

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