Measurement of Human Luteinizing Hormone in Plasma by Radioimmunoassay

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ABSTRACT The recent isolation of highly purified human pituitary luteinizing hormone (LH) has permitted the development of a sensitive and specific radioimmunoassay for this hormone in plasma. Results of this immunoassay system employing anti-LH serum agree closely with previous reports for the measurement of plasma LH in which immunoassays employing cross-reactive antisera to human chorionic gonadotropin were used. The immunoassay and bioassay of LH in several crude and partially purified pituitary and urinary extracts show acceptable agreement. The sensitivity of the LH immunoassay (0.2 mμg/ml) is adequate to measure LH levels in almost half of all prepuberal children and in all but a few normal adults. A small, but significant, rise in plasma LH level occurs at pubescence in both boys and girls. In women, plasma LH level varies with both age and the phase of the menstrual cycle. The mean LH concentration in nine normal women during the follicular phase (1.2 mμg/ml was found to be significantly higher than during the luteal phase (1.0 mμg/ml). At midcycle, the mean peak LH level was 10.2 mμg/ml. In a large group of normal women, the mean plasma LH concentration rose significantly at menopause to a level of 5.8 mμg/ml during the fifth decade and 10.5 mμg/ml during the seventh decade. A small, but significant, rise in plasma LH concentration also occurred in men from the third and fourth decades (0.7 mμg/ml to the seventh and eighth decades (1.7 mμg/ml). Both estrogen and testosterone suppress plasma LH levels, but marked variation in response exists. The immunoassay serves as a useful diagnostic tool in evaluating men with gonadal failure, amenorrheic women of reproductive age, and postmenopausal women suspected of hypopituitarism. From the half-time disappearance of LH-^{131}I in plasma (mean 69 min) and the calculated volume of distribution (2.5–2.8 liters) it has been determined that approximately 30 μg of LH is secreted per day in men, and in women except at midcycle, at which time the release of LH is estimated to be 10–15 times this basal rate.

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INTRODUCTION

Until recently, the secretion of human luteinizing hormone (LH) could be measured only indirectly by bioassay of urinary extracts (1–4). Bioassay techniques have also been applied, to a limited extent, to extracts of large volumes of plasma for the determination of LH levels in plasma pooled from several individuals (5–8). The demonstrated immunologic similarity of LH and human chorionic gonadotropin (HCG) (9), and the recent isolation and purification of pituitary LH by Hartree, Butt, and Kirkham (10), and Parlow, Condliffe, Reichert, and Wilhelmi (11) has permitted the development of sensitive immunoassays utilizing standard hemagglutination inhibition

methods (12, 13) to measure this hormone in urine, and radioimmunoassay techniques (14–17) to measure LH levels in plasma. All previous workers (14–17) have employed antisera directed against HCG in their radioimmunoassay procedures. The purpose of this communication is to describe a double antibody radioimmunoassay for LH using antiserum directed against purified pituitary LH and to report the concentrations of this hormone found in the plasma of normal subjects under physiologic and experimental conditions, and in patients with various pituitary and gonadal disorders. A preliminary report of some of these studies has been published previously in abstract form (18).

METHODS

Hormone preparations. Two preparations of human pituitary LH prepared by methods previously described (11) were available for the development of this assay. Partially purified preparation "A" (bioassay potency determined by ovarian ascorbic acid depletion (OAAD): 1.0 times NIH-LH-S1) was employed for the generation of antisera to LH (anti-LH). Highly purified preparation "B" (bioassay potency: 5.1 times NIH-LH-S1) was used for iodination and standards.

Highly purified hormone preparations used for the study of the immunologic specificity of the LH assay were: human follicle-stimulating hormone (FSH, preparation NIH-R-780), bioassay potency 53.2 times NIH-FSH-S1 (prepared by Dr. L. E. Reichert and furnished by Dr. C. M. Cargille); human growth hormone (HGH), potency 1.0 IU/mg [prepared by Dr. M. S. Raben (19) and furnished by Dr. M. L. Parker]; human thyroid-stimulating hormone (TSH), potency 20 U/mg, contaminated approximately 5% by weight with LH [prepared by Dr. P. G. Condliffe (20) and furnished by Dr. R. D. Utiger]; porcine ACTH, potency 120 IU/mg (prepared by Dr. S. H. Eppstein and furnished by Dr. J. W. Hinman); human chorionic gonadotropin, potency of 8700 IU/mg (obtained from the Ortho Research Foundation, Raritan, N. J., through the courtesy of Dr. A. M. Reiss).

Preparation of antisera. Anti-LH of high potency was generated in two guinea pigs after three 1-mg immunization doses of preparation "A." For purpose of immunization, this preparation of LH was dissolved in 0.85% NaCl solution (4 mg/ml), frozen at −20°C, and aliquots were thawed and homogenized with an equal volume of complete Freund's adjuvant[1] just before immunization. The initial injection of antigen was given into the four footpads, and subsequent injections were given subcutaneously every 2 wk. Animals were bled under ether anesthesia by intracardiac puncture 10 days after the third and subsequent immunizations. For the second antibody reaction, antisera to guinea pig gamma globulin (anti-GPGG) was obtained from adult albino rabbits after repeated immunizations with 2-mg doses of guinea pig gamma globulin.[2] The same immunization procedure was used as for the production of anti-LH except that the doses of GPGG were administered monthly instead of biweekly. Batches of anti-GPGG were individually titered to determine the requisite amount of this antiserum to assure complete precipitation of all GPGG in the system.

Radioiodinated LH (LH-^{125}I) and standard solutions. Highly purified LH preparation "B" was dissolved in 1.0 ml of 0.01 M phosphate buffer, pH 7.5, and immediately divided into a number of aliquots of 25 μg each, sealed in glass ampules, frozen, and stored at −20°C. Aliquots of this material were thawed just before use, both for iodination and for preparation of standard solutions. 10 μg samples of LH were radioiodinated at intervals of 3–4 months according to the procedure of Greenwood, Hunter, and Glover (21), and approximately 0.75 mc of Na-^{125}I[3] was used to achieve specific activities of 25–50 μc/μg. LH-^{125}I was separated from unreacted inorganic ^{125}I by passing the reaction mixture through a Sephadex G-50 column (1.0 × 14 cm) and collecting 0.5 ml fractions into 0.1 ml of a 30% solution of bovine serum albumin (BSA)[4] dissolved in barbital buffer, pH 8.6. The most immunologically competent LH-^{125}I was consistently found to be in the eluate fraction of the protein peak which contained the most radioactivity. This fraction was further diluted with 1.0 ml of 5% BSA barbital solution (pH 8.6, 0.1 ionic strength), immediately divided into 0.05 ml aliquots, and frozen. Approximately 85–95% of the LH-^{125}I was precipitable initially in the presence of excess anti-LH; this labeled preparation can be used for 3–4 months with only a slight increase (10–15%) in damage due to storage. 10 μg of LH-^{125}I is sufficient to assay approximately 9000 standard or plasma samples.

The LH-^{131}I used for the disappearance studies was prepared in the same manner as the LH-^{125}I except that 3 mc of Na-^{131}I were used for iodination of 10 μg of LH, resulting in specific activities of approximately 50–100 μc/μg of LH.

Standard solutions of LH were prepared in 1% BSA 0.01 M phosphate solution, pH 7.5, and were found to be stable for at least a year when stored at −20°C.

Radioimmunoassay procedure. This procedure is similar to the double antibody insulin immunoassay of Morgan and Lazarow (22) with modifications as previously reported (23, 24). A 5% BSA barbital buffer, pH 8.6, was used for all dilutions, and reagents were added to 10 × 75 mm disposable glass tubes in the following order: (*a*) 0.5 ml of buffer; (*b*) either 0.05 ml of a standard solution and an equal amount of plasma from a patient with documented panhypopituitarism or 0.05 ml of unknown plasma; (*c*) 0.1 ml of anti-LH appropriately diluted (1:50,000) to precipitate 30–40% of the LH-^{125}I in the system with no

[1] Difco Laboratories, Detroit, Mich.

[2] Pentex, Inc., Kankakee, Ill.

[3] Isoserve, Inc., Cambridge, Mass.

[4] Armour Pharmaceutical Co., Chicago, Ill.

unlabeled LH present; and (*d*) 0.05 ml of LH-^{125}I (approximately 0.03 mμg). These reagents were incubated at 4°C for 60–72 hr and the total ^{125}I in each tube was counted using an auto-gamma spectrometer.[5] The following reagents were then added: (*e*) 0.1 ml of suitably diluted (1:5) anti-GPGG to insure complete precipitation of all GPGG in the system; and (*f*) 0.1 ml of normal guinea pig serum (NGPS) diluted 1:200, to serve as carrier protein. After a second incubation period (12–24 hr) the tubes were centrifuged for 20 min at 2000 rpm and the supernatants removed by aspiration. The precipitates were washed with 0.5 ml of distilled water and recentrifuged and the supernatants aspirated. The precipitated LH-^{125}I was counted and expressed as the per cent of the total radioactivity added to each tube. A standard curve was constructed with each assay, covering the range of 0–16 mμg of LH/ml. From the percent of LH-^{125}I precipitated in unknown plasma samples, the concentration of LH could be read directly from the standard curve.

Bioassay procedure. The bioassay methods of Parlow (25, 26) and Steelman and Pohley (27) as modified by Parlow and Reichert (28) were used to measure the concentrations of biologically active LH and FSH respectively in several crude and partially purified pituitary and urinary fractions in which LH was also determined by immunoassay.

Clinical studies. The normal subjects employed in these investigations included volunteers from the professional and technical staff of the Strong Memorial Hospital, college students, ambulatory residents at the Monroe County Home, and patients convalescing from acute nonendocrine diseases. There was no history of endocrine or metabolic disorder in any of the normal subjects. Patients with pituitary or gonadal disorders were studied on the general medical, obstetric and gynecology, and pediatric services, or seen in the endocrine out-patient clinic. All subjects were informed as to the nature of these studies and any attendant risk which might be associated with them. Most bloods were centrifuged within 30 min after collection, and all plasmas were immediately frozen and kept at −20°C until assayed.

RESULTS

Radioimmunoassay system. A typical LH standard curve is illustrated in Fig. 1. 50 μl of plasma from an individual with known complete gonadotropin deficiency was routinely added to the standard tubes as a control for damage of LH-^{125}I which results during incubation in the presence of plasma. On repetitive studies, this damage has been found to be small, since there was only a 3.5% drop in the total immunoprecipitability of the LH-^{125}I in the presence of plasma during 96 hr of incubation. The sensitivity of the LH radioimmunoassay is 0.2 mμg/ml of standard solution or plasma sample when the volume employed in the assay system is 50 μl of sample being assayed. The average per cent variation of duplicate samples from the mean LH value is 7.6 ± 1.3% (± SE) over the entire range of the standard curve.

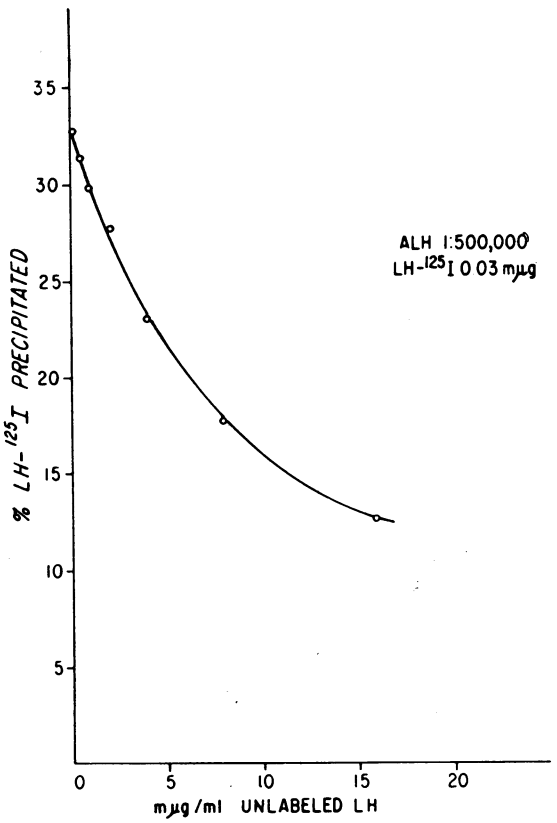

FIGURE 1 Luteinizing hormone (LH) standard curve. Each point is the average of duplicate determinations. ALH is anti-LH serum.

Studies have been carried out to characterize the highly purified preparation of LH used in this radioimmunoassay, both from the standpoint of its biological potency and of its immunological similarity to standard preparations of pituitary and chorionic gonadotropins. A comparison of the LH values obtained by immunoassay and bioassay of various crude and partially purified pituitary and urinary fractions is shown in Table I. The agreement between the values obtained by the two methods is acceptable except in the case of preparation "F" in which the FSH/LH ratio is extremely high. For the other four preparations, the LH concentration determined by immunoassay is within or slightly greater than the fiducial limits for LH determined by bioassay.

[5] Packard Instrument Co., Downers Grove, Ill.

TABLE I

Comparison of the LH Potency of Various Human Pituitary and Postmenopausal Urinary Gonadotropin Preparations Obtained by Radioimmunoassay and Bioassay

Preparation	LH potency* radioimmunoassay	LH potency* bioassay	FSH potency‡ bioassay	FSH/LH ratio bioassay
A (pituitary)	1.3	1.0 (0.8–1.3)	0.05	0.05
C (pituitary)	0.27	0.14 (0.1–0.2)	17.50	125.00
D (pituitary)	0.12	0.08 (0.05–0.12)	0.69	8.60
E (urinary)	0.019	0.013 (0.009–0.017)	0.25	19.20
F (urinary)	0.0060	0.016 (0.01–0.025)	2.20	1375

* Expressed in terms of NIH-LH-S1. Although radioimmunoassay potency estimates were obtained in terms of the highly purified "standard" LH preparation "B," conversion of these potency estimates, in terms of NIH-LH-S1, was achieved by utilizing the biopotency estimate of 5.1 times NIH-LH-S1 for this preparation.
‡ Expressed in terms of NIH-FSH-S1.

Using the immunoassay system, we compared an inhibition curve for highly purified LH with that of the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) and dilutions of a plasma sample obtained from a normal woman at midcycle (Fig. 2). It can be seen that the slopes of these curves are similar, thereby establishing the close immunologic similarity of these antigens. From these data, one can calculate that the amount of LH in the 2nd IRP-HMG is equivalent to approximately 0.08% of the total weight of this material.

It has been demonstrated (10, 11) that LH preparations frequently contain small amounts of both FSH and TSH. Studies carried out to determine the extent of the contamination of the puri-

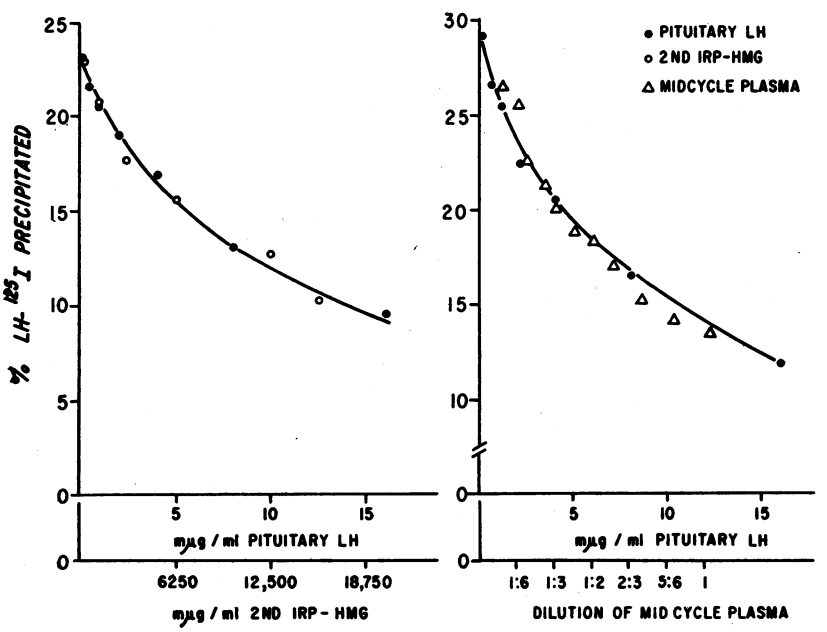

FIGURE 2 Comparison of the immunologic cross-reactivity of pituitary LH, postmenopausal urinary gonadotropin, and dilutions of midcycle plasma LH. The slopes of the inhibition curves in which the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) and dilutions of midcycle plasma LH were used are virtually the same as those in which purified pituitary LH was used. 1 mμg purified pituitary LH is equivalent to approximately 10 mU 2nd IRP-HMG.

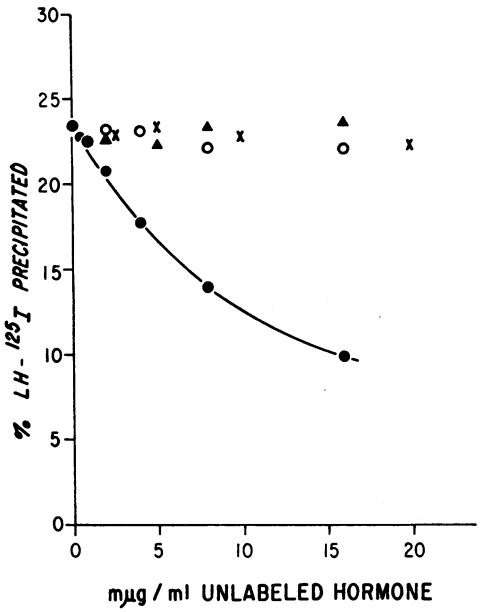

FIGURE 3 LH standard curve. No cross-reactivity of human growth hormone (HGH) (×), human follicle-stimulating hormone (FSH) (○), or adrenocorticotropic hormone (ACTH) (▲) with anti-LH serum could be demonstrated. The concentration of the various unlabeled hormones are measured along the abscissa in mμg/ml.

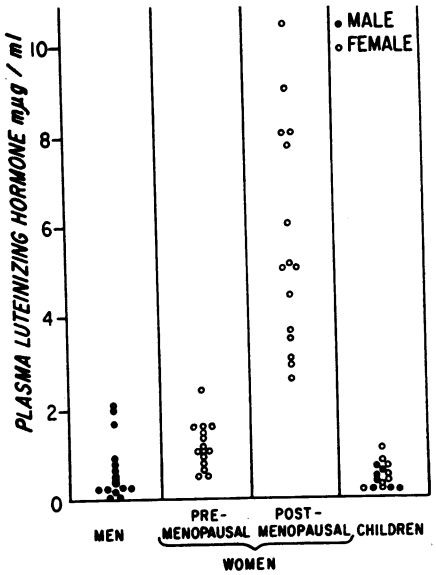

FIGURE 4 Plasma LH levels in normal men, women, and prepuberal children.

fied LH used for iodination and standards have revealed that highly purified FSH (NIH-R-780) does not compete with the LH-^{125}I in this immunoassay system (Fig. 3), and that TSH-^{125}I is bound to the anti-LH serum with only 5% of the affinity with which LH-^{125}I is bound. That this small degree of apparent cross-reactivity does not interfere with the LH immunoassay system is evidenced by the finding that there was no correlation between LH and TSH levels in the plasmas of several patients with thyroid disease (Table II).[6] Neither HGH nor porcine ACTH, which is known to cross-react with human ACTH (29), shows any cross-reactivity with LH-^{125}I in this system (Fig. 3).

TABLE II
Plasma LH and TSH Levels in Patients with Primary Thyroid Disease

Subject	Age/Sex	Condition	LH	TSH
			mμg/ml	
M. B.	54/F	Hypothyroid	8.0	6.0
V. C.	40/F	Hypothyroid	1.8	13.4
H. L.	32/M	Hypothyroid	1.7	13.8
E. M.	58/F	Hypothyroid	6.2	5.2
L. O.	58/M	Hypothyroid	1.0	15.0
B. H.	34/F	Hyperthyroid	1.2	<0.4
L. M.	29/F	Hyperthyroid	<0.5	<0.6
R. M.	15/M	Hyperthyroid	<0.5	<0.6
W. S.	40/F	Hyperthyroid	7.8	<0.5
A. W.	46/F	Hyperthyroid	<0.5	<0.6

Clinical studies: physiologic conditions. Plasma LH levels have been measured in normal men, women, and prepuberal children (Fig. 4). Care was taken to obtain blood samples during either the pre- or postovulatory phases of the menstrual cycle for women in the reproductive age. When daily plasma LH levels were determined in three normal men and nine normal women, it was found that the day-to-day variation in LH concentration was small in both men (0.3 mμg/ml) and women (0.5 mμg/ml) when the midcycle rise was excluded. Plasma LH concentrations were markedly elevated in postmenopausal women as compared to men, premenopausal women, and children.

Plasma LH was not detectable (< 0.2 mμg/ml) in 48% of a group of 61 prepuberal children under 10 yr of age. Of these children, the mean value for measurable LH levels in girls (0.5 ± 0.1 mμg/ml) was not significantly different ($P > 0.25$) from that in boys (0.6 ± 0.1 mμg/ml).

[6] Studies of TSH-^{125}I binding and TSH immunoassays were very kindly performed by Dr. R. D. Utiger, Washington University School of Medicine, St. Louis, Mo.

After puberty, the mean plasma LH level increased slightly, but significantly, according to the chi-square test ($P < 0.01$) to 0.9 ± 0.1 mμg/ml in girls and 0.8 ± 01 mμg/ml in boys. These observations have been presented in greater detail elsewhere (30).

In women, plasma LH concentration varies markedly both with age (Fig. 5) and with phases of the menstrual cycle (Fig. 6). Mean nonovulatory phase LH levels were virtually the same during the second to fourth decades. By the fifth decade, at which time all of the women chosen for the study had entered menopause, the mean plasma LH concentration was significantly elevated ($P < 0.005$), and it continued to rise until the seventh decade. After puberty, plasma LH was consistently lower in men than in women throughout life ($P < 0.005$), but there appeared to be a corresponding, though considerably smaller, increase in the concentration of this hormone in older men. The mean level of LH in men during the seventh and eighth decades (1.7 ± 0.4 mμg/ml) was significantly higher ($P < 0.02$) than the mean level

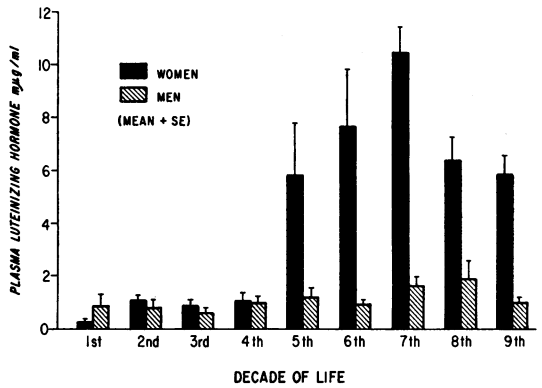

FIGURE 5 Plasma LH levels in normal males and females in relation to age. Each group consists of between 5 and 12 normal subjects. All women in the fifth decade were postmenopausal.

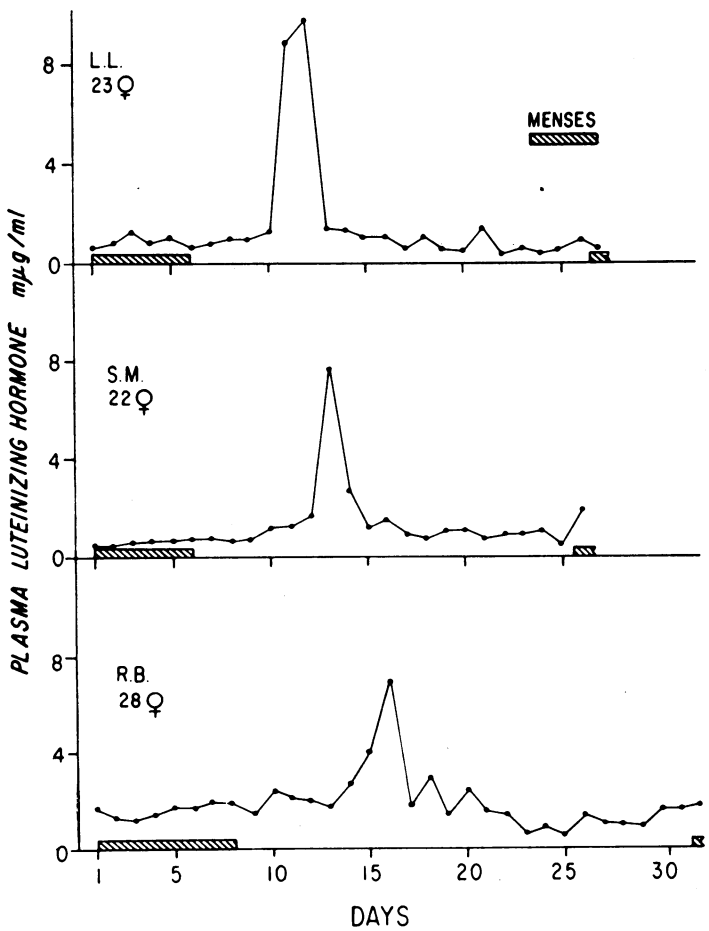

FIGURE 6 Plasma LH levels during normal menstrual cycles in three women.

during the third and fourth decades (0.7 ± 0.2 mμg/ml).

Plasma LH levels have been measured daily in nine healthy women during complete menstrual cycles. The mean LH level during the follicular phase (1.2 ± 0.1 mμg/ml) was very slightly, but significantly, higher ($P < 0.02$) than the mean level during the luteal phase (1.0 ± 0.1 mμg/ml) in these women. Considerable variation was noted in both the length of the two phases of the menstrual cycle and in the peak level of plasma LH at midcycle (Table III). The duration of the follicular phase varied between 10 and 20 days, while the duration of the luteal phase varied between 9 and 16 days. An abrupt rise in plasma LH occurred in all women at midcycle, the mean peak value being 10.2 ± 1.2 mμg/ml. In all but one case, a modest elevation in plasma LH occurred on the day before and (or) after the day of the peak LH level. Typical patterns of plasma LH levels during normal menstrual cycles are seen for three women in Fig. 6. In four of the women studied who experienced Mittelschmerz, the midcycle peak in LH coincided with the day of onset of abdominal pain. The peak LH level also occurred at the time of maximal cervical mucus ferning in the three women who were examined daily, and usually 1 day before the rise in basal body temperature.

Clinical studies: experimental and pathologic conditions. To investigate the effect of oral ovulatory suppressants on plasma LH concentrations, we obtained daily measurements in four normal women both during regular menstrual cycles and during administration of various ovulatory suppressants. These agents contained both estrogen and progestational compounds and were administered either in a combination or sequential manner.[7] In all cases, the midcycle peak was completely abolished during therapy, but it reappeared during the next cycle in the three women who were followed subsequent to the discontinuation of medication (Fig. 7). A similar suppression of LH release was also observed after the administration of an intramuscular preparation of estrogen and progesterone in three normal women.[8]

The effects of both estrogen and testosterone on plasma LH levels in postmenopausal women were also studied. Three women received 3.75 mg of conjugated estrogens a day for 5 days and two other women were given an intramuscular injec-

[7] The combination agents were: Norinyl, Syntex Laboratories, Inc., Palo Alto, Calif., containing 2 mg of norethindrone (17α-ethinyl-17-hydroxy-4-estren-3-one) and 0.1 mg of mestranol (ethinyl estradiol 3-methyl ether), and Provest, The Upjohn Co., Kalamazoo, Mich., containing 10 mg of medroxyprogesterone and 0.1 mg of ethinyl estradiol. The sequential agents were: C-Quens, Eli Lilly & Company, Indianapolis, Ind., containing 0.08 mg of ethinyl estradiol alone and in combination with 2 mg of chlormadinone acetate and Oracon, Mead Johnson & Co., Evansville, Ind., containing 0.1 mg of ethinyl estradiol and in combination with 25 mg of dimethisterone.

[8] Deladroxate (150 mg of dihydroxyprogesterone acetophenone, 10 mg of estradiol enanthate), E. R. Squibb & Sons, New York.

TABLE III

Plasma LH Levels during Normal Menstrual Cycles

Subject	Age	Length of cycle	Plasma LH		
			Follicular phase	Midcycle	Luteal phase
		days		*mμg/ml*	
S. M.	22	26	0.3–1.7 (12)*	7.6	0.5–2.7 (13)
L. L.	23	28	0.6–1.3 (10)	9.7	<0.2–1.3 (16)
J. H.	25	28	0.4–1.2 (13)	15.1	0.3–3.1 (14)
S. H.	24	28	0.9–3.2 (16)	8.4	0.6–1.9 (10)
L. B.	36	28	0.5–2.4 (11)	>16	<0.2–1.6 (15)
A. H.	24	30	<0.2–2.1 (15)	9.6	0.3–2.2 (13)
P. N.	25	30	0.9–2.2 (14)	6.3	0.5–2.3 (15)
R. B.	28	32	1.1–2.6 (14)	6.8	0.5–2.9 (16)
L. G.	24	33	0.3–3.1 (20)	12.1‡	0.4–2.7 (9)

* Duration (days) in parentheses.

‡ The midcycle elevation lasted 4 days.

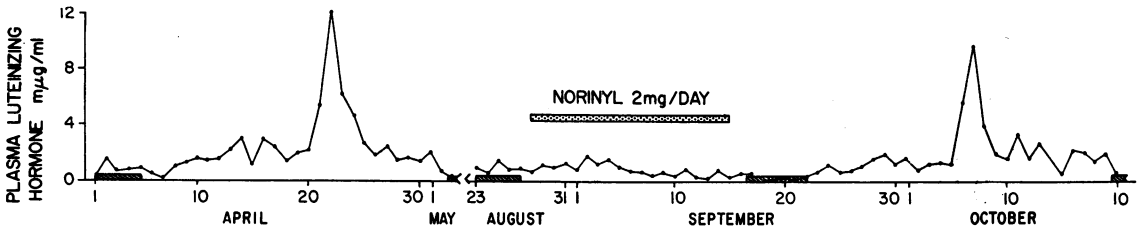

FIGURE 7 Plasma LH levels in response to the administration of an oral ovulatory suppressant. 2 mg of Norinyl (2 mg of norethindrone, 0.1 mg of mestranol) was administered for 20 days. Note the return of the normal midcycle peak in the next cycle after discontinuation of medication. The cross-hatched bars on the abscissa represent the time of menses.

tion of 200 mg of depot testosterone.[9] One of the postmenopausal women had an extraordinarily high plasma LH level (> 16 mμg/ml) which was not suppressed by estrogen administration. However, there was partial suppression of plasma LH in one of the women and complete suppression in the other (Fig. 8). Plasma LH levels were suppressed in both of the postmenopausal women who received intramuscular testosterone (Fig. 9).

[9] Amnestrogen (conjugated estrogens) and testosterone enanthate, E. R. Squibb & Sons, New York.

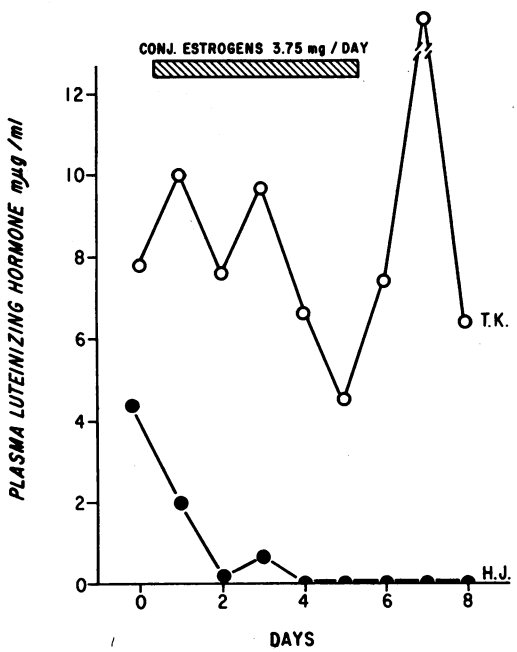

FIGURE 8 Plasma LH levels in postmenopausal women in response to the administration of conjugated estrogens. 3.75 mg of Amnestrogen (conjugated estrogens) was administered for 5 days. Note what appears to be a "rebound" elevation in LH in subject T. K. after discontinuation of medication.

Plasma LH levels have been measured in a number of patients with various pituitary or gonadal disorders (Fig. 10). Detectable levels of LH have been found in the plasma of several of the women with clinical hypopituitarism who have been studied. Plasma LH levels are usually at the lower end of the normal range or undetectable (< 0.2 mμg/ml) in these women. While, therefore, plasma LH levels of patients with amenorrhea secondary to decreased gonadotropin secretion cannot be distinguished from normal levels in many instances, they are readily distinguished from the consistently elevated levels of patients with amenorrhea due to primary ovarian failure.

Elevated plasma LH levels have been found in three patients with Turner's syndrome, one male adolescent with markedly delayed puberty, three young women (18, 20, 33 yr) with premature menopause, and in one of two women with Stein-

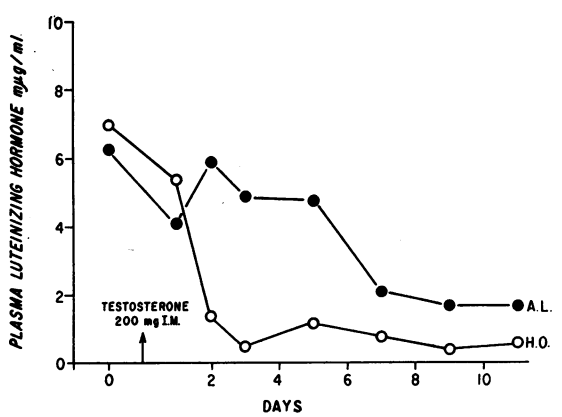

FIGURE 9 Plasma LH levels in postmenopausal women in response to testosterone administration. 200 mg of testosterone enanthate was administered intramuscularly after the base line sample was obtained.

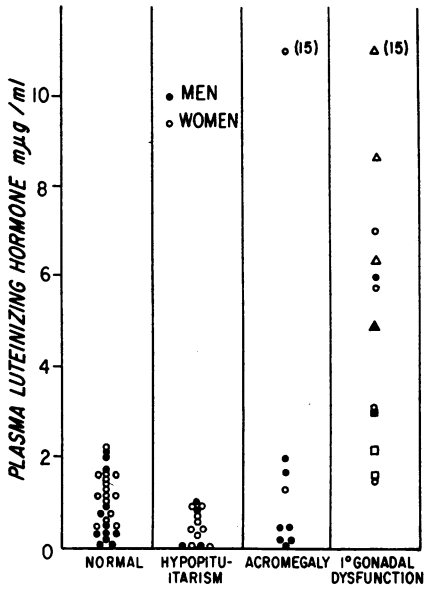

FIGURE 10 Plasma LH levels in normal men and women and in patients with pituitary and gonadal disorders. All of the women with hypopituitarism were amenorrheic though two of them had both normal plasma LH levels and urinary gonadotropin values (by bioassay). The acromegalic men had all been treated by radiation or surgery. The women with primary gonadal dysfunction include three with premature menopause (△), four with Turner's syndrome (○), and two with Stein-Leventhal syndrome (□). A 14 yr-old boy with anorchia (■) and a subject with feminizing testes syndrome (▲) also had elevated LH levels.

Leventhal syndrome. Plasma LH was also moderately elevated in a 14 yr old boy with anorchia, and in an 18 yr old subject with surgically proven feminizing testes syndrome. The elevated LH level in the subject with the feminizing testes was unchanged either by gonadectomy or the intramuscular administration of 200 mg of testosterone enanthate (Fig. 11). However, after the daily administration of 2 mg of norethindrone and 0.1 mg of mestranol for a period of 20 days, there was virtually complete suppression of LH release.

To determine whether other stimuli influence the release of LH, we measured plasma LH after conditions of fasting or feeding, physical stress and exercise, insulin-induced hypoglycemia, or the infusion of 30 g of arginine monohydrochloride. These stimuli had no effect on the release of LH in contrast to their well-known effect in influencing the secretion of HGH and, in some cases, ACTH.

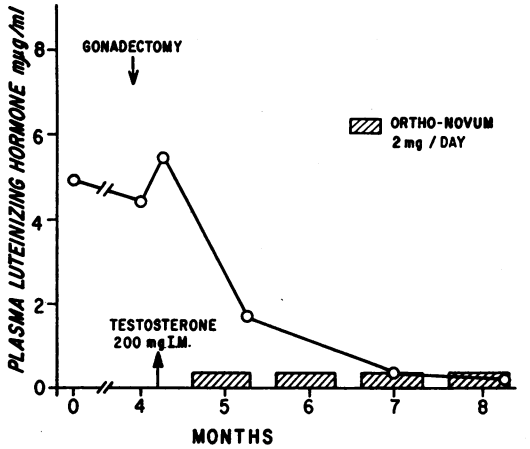

FIGURE 11 Plasma LH levels in an 18 yr-old subject with the feminizing testes syndrome. No change in plasma LH occurred after gonadectomy or testosterone enanthate administration, whereas a marked suppression was observed after cyclic, combined estrogen and progestational therapy.

Metabolism of LH-^{131}I. The disappearance rate and volume of distribution of LH were determined by administering an intravenous dose of approximately 25 μc of a sterile solution of LH-^{131}I to seven subjects, four men and three women. Five of the subjects were normal, one was a mild ma-

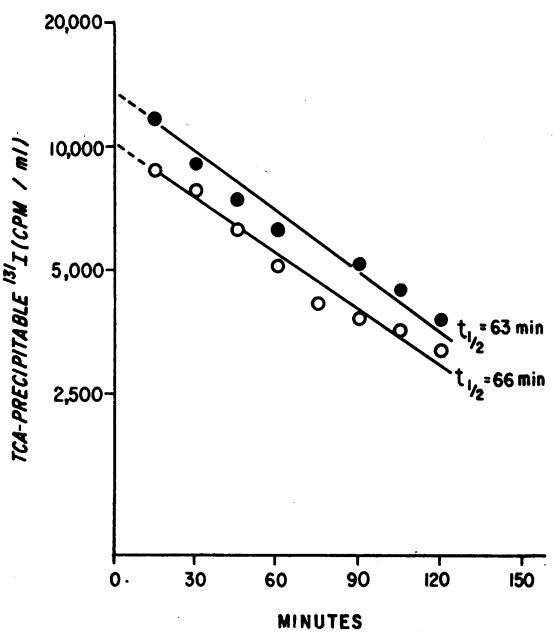

FIGURE 12 Plasma disappearance rate of LH-^{131}I. The disappearance curves for LH-^{131}I for two normal subjects are depicted (J. S., $t_{\frac{1}{2}} = 66$ min; B. T., $t_{\frac{1}{2}} = 63$ min).

TABLE IV
Disappearance Rate of LH-^{131}I

Subject	Age/sex	$t_{\frac{1}{2}}$
		min
P. B.	56/M	76
K. L.	31/M	80
R. R.	21/M	61
J. S.	23/F	66
B. T.	33/F	63
W. H. (diabetic)	56/F	70
F. D. (cirrhotic)	53/M	93*
		Mean 69.3 min

* Not included in calculation of mean value.

turity-onset diabetic, and one had nutritional cirrhosis. The preparation of LH-^{131}I was 98.1% trichloracetic acid (TCA)–precipitable and 97% nondialyzable. Trichloroacetic acid–precipitable ^{131}I in plasma was determined at intervals of 15 min over a 2 hr period after the tracer dose of LH-^{131}I was intravenously introduced. Typical LH-^{131}I disappearance curves for two of the subjects are seen in Fig. 12, and the calculated half time for the disappearance of LH-^{131}I for all seven subjects is given in Table IV. The mean value for the $t_{\frac{1}{2}}$ of LH-^{131}I in the five normal subjects and one mild diabetic was 69.3 min. The $t_{\frac{1}{2}}$ was markedly prolonged in subject F. D., who had moderately severe hepatic cirrhosis. Extrapolating the disappearance curve back to zero time (i.e., at the time of the intravenous injection of LH-^{131}I), the volume of distribution of LH was calculated to be 2.5–2.8 liters. Although this value probably corresponds to the plasma volume in many of these individuals, no correlation with body weight was found. 97% of ^{131}I in plasma was TCA-precipitable initially, and 89.5% was precipitable after 2 hr of circulation. An average of 26.0% of the injected dose of ^{131}I was excreted in the urine within 2 hr after the administration of LH-^{131}I, and of this amount, 54.9% was TCA-precipitable. The plasma renal LH clearance rate was calculated to be 3.4 ml/min.

DISCUSSION

Standard radioimmunoassay techniques employing anti-HCG serum have been applied by Midgley (14), Odell, Ross, and Rayford (15, 31), and Bagshawe, Wilde, and Orr (16) to determine plasma LH concentrations in humans. The immunologic cross-reactivity of LH with anti-HCG serum has been adequately demonstrated (9) and is of particular interest in light of the marked differences in the physicochemical characteristics of the LH and HCG molecules (32). In the present report, in which an anti-LH serum was employed in the immunoassay, the results agree very closely with previous studies and, therefore, serve to validate the data that have been obtained in systems using anti-HCG serum.

Adequate sensitivity of the presently described method is evidenced by the finding of measurable levels of LH in the plasma of all but a very few normal adults and in approximately half of all prepuberal children. The specificity of this method is demonstrated in part by the close agreement between the results of these studies and previous ones in which bioassay techniques were employed. The specificity has also been shown by the lack of cross-reactivity with other purified pituitary protein hormones and by the demonstration that plasma LH levels are not elevated under conditions known to produce high levels of HGH and ACTH (hypoglycemia, physical stress) and TSH (hypothyroidism). Although human prolactin has not yet been purified sufficiently to be employed in an immunoassay system, it has been found that plasma LH levels are not elevated in lactating women, and this observation, therefore, argues against the cross-reactivity of prolactin in this assay.

The physiological studies reveal the intimate relationship of plasma LH levels to gonadal function under both normal and experimental conditions. Before puberty, plasma LH levels are either low or undetectable in both boys and girls. The present studies confirm the bioassay data of Fitschen and Clayton (33) and the immunoassay data of Odell, Ross, and Rayford (31), that LH can be detected in plasma in many children. However, the finding that 48% of prepuberal children have undetectable plasma LH levels and that a significant rise in plasma LH occurs at the time of puberty in boys and girls when large groups are examined, is not wholly in accord with the speculation of Odell, Ross, and Rayford that in many children puberty is not caused by either the onset or the increase in levels of LH secretion. It is possible that changes in the secretion of FSH are even more important in initiating pubescence,

and the recent development of sensitive radioimmunoassay methods for FSH in plasma (34–36) should contribute immensely to our understanding of this extremely interesting biologic phenomenon. Obviously, before these questions can be adequately resolved, longitudinal studies of both LH and FSH secretion on a large number of children may need to be done.

The negative feedback regulation of gonadotropin secretion by gonadal hormones has been adequately documented in animals (37–40) and man (31, 41–43). The marked increase in plasma LH levels in postmenopausal women associated with a diminution in the secretion of gonadal hormones is evidence for the presence of such a negative feedback system. The present studies also show that a small, but significant, elevation in plasma LH occurs in men during the seventh and eighth decades. This finding suggests that some degree of testicular secretory deficiency analogous to the much more striking changes in ovarian function occurs in older men and is consistent with the observation of decreased plasma testosterone levels in some older men reported by Coppage and Cooner (44).

In normal women, plasma LH rises abruptly at midcycle, confirming older bioassay (1–4) and more recent immunoassay (12–16) data. Considerable variation was found in the length of both the follicular and luteal phases of the menstrual cycle, and in the degree of elevation of LH at midcycle. The variable length of the luteal phase is consistent with the more recent observations of Farris (45) who noted a considerable variation in the relationship of the time of ovulation to the onset of the next menses. The elevation in plasma LH at midcycle extended over a 3 day period for most women and reached a peak value on the 2nd or 3rd day of the midcycle rise in all but one case.

The suppression of ovulation by the administration of cyclic, combined estrogen and progestational therapy has been shown to be secondary to the suppression of gonadotropin release (42, 43). This relationship has now been demonstrated by the measurement of plasma LH levels in several women using a variety of oral ovulatory suppressants and an intramuscular preparation of estrogen and progesterone.

In contrast to the marked reduction in plasma LH levels achieved by Odell, Ross, and Rayford (31) in one postmenopausal women given 0.3 mg of ethinyl estradiol/day for 5 days, in three women given a smaller, more "physiologic" dose of conjugated estrogens, only one had this degree of suppression, and a second had a modest diminution followed by a "rebound" elevation in plasma LH after discontinuation of medication. This variable response of plasma LH to estrogen administration is similar to that previously reported by Rosemberg and Engel (41) for total urinary gonadotropin excretion in postmenopausal women given estrogen therapy.

Two postmenopausal women receiving a depot testosterone preparation showed a marked suppression of plasma LH by the 7th day after testosterone administration. In contrast, plasma LH was unchanged after testosterone administration in a gonadectomized 18 yr old subject with feminizing testes syndrome. The failure of LH to be suppressed in this subject is consistent with the known unresponsiveness of certain target organs to testosterone in such individuals (46), and suggests that the hypothalamic or pituitary receptor feedback sites for testosterone are similarly unresponsive.

From a practical standpoint, the availability of an easy and relatively rapid method of measuring plasma LH offers considerable promise for the resolution of a number of clinical problems regarding the functioning of the hypothalamic-pituitary-gonadal axis. As a clinical diagnostic tool, the assay is of considerable use in evaluating men with gonadal failure, amenorrheic women of reproductive age suspected of gonadal failure, and postmenopausal women suspected of hypopituitarism. In such cases, the distinction between gonadotropin failure and gonadal failure is readily made. On the other hand, normal plasma LH levels in children are so low that the immunoassay does not help in determining the presence of gonadotropin failure, and in some patients with hypopituitarism plasma LH concentrations are not only detectable, but they are indeed within the normal range. Only two patients with Stein-Leventhal syndrome have been studied; the plasma LH level was elevated in one and normal in the other. It should be emphasized, however, that the daily pattern of LH secretion must be studied in order to evaluate these patients properly as has been shown previously in the studies of the daily urinary gonadotropin excretion (47).

The effect of clomiphene citrate in stimulating ovulation under certain conditions is well known (48, 49), and studies using this agent have been conducted in this laboratory and have been reported elsewhere (50). The availability of such a stimulatory test may prove to be of great help in evaluating subjects with suspected diminished pituitary LH reserve.

Studies of plasma levels of any hormone can be used only as an indirect measure of the secretory rate per se, inasmuch as the disappearance rate of the hormone will also play a role in determining the actual plasma concentration at any single moment. If the disappearance rate is constant throughout the day and the volume of distribution is known, some measure of the secretory rate can be calculated. The mean disappearance rate of LH-^{131}I is 0.72%/min in an average individual ($t_{\frac{1}{2}} = 69.3$ min).[10] If we assume a mean plasma LH level of 1 mμg/ml and a volume of distribution of 2.8 liters, the daily secretory rate is calculated to be approximately 30 μg/day, and in women during ovulation probably 10–15 times this basal rate. It is acknowledged that a departure from linearity may have been observed in the disappearance curve of LH-^{131}I if these studies had been continued for more than 2 hr. In this case, some error in the calculation of the secretion rate of LH would certainly exist. If one considers that only 54.9% of secreted LH is excreted in the urine (i.e., TCA-precipitable ^{131}I fraction), the LH excretion rate during an entire menstrual cycle calculated from these data (approximately 3.0 mg) is very similar to that determined previously by bioassay (2.3 mg) (4). Likewise, the mean half-time of disappearance of LH-^{131}I (69.3 min) corresponds closely with the half-time of 60 min determined recently by bioassay (51).

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[10] While this paper was in press, Kohler, Ross, and Odell (*J. Clin. Invest.* 1968. **41**: 38) reported the initial half-time of disappearance of LH-^{131}I in four patients to be 30–60 min. By integrating the disappearance curves over a 24 hr period, these authors estimated the mean daily production rate of LH in five premenopausal women to be 734 mU (2nd IRP-HMG) per min or 1057 U/day (range 541–1823 U/day). The estimated production rate in the present study (30 μg LH/day) is equivalent to approximately 300 U 2nd IRP-HMG per day.

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