Studies on the Metabolic Clearance Rate and Production Rate of Human Luteinizing Hormone and on the Initial Half-Time of Its Subunits in Man

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ABSTRACT The metabolic clearance rate (MCR) of human luteinizing hormone (hLH) has been determined in 10 normal men, 3 normal women, and in 12 women with ovulatory disorders resulting in oligomenorrhea or amenorrhea. The MCR was determined by the constant infusion technique using either iodinated or unlabeled highly purified hLH, and these results were compared to MCR determined by using crude pituitary preparations containing both follicle-stimulating hormone and hLH. Both preparations produced essentially similar results for the MCR of hLH and virtually identical results were obtained when complete or incomplete immunoprecipitation of the infused material was achieved. The MCR/body surface area of hLH was significantly greater in normal men (25.6±3.6 ml/min·m^2) than in normal premenopausal (19.2±0.9 ml/min·m^2) or postmenopausal women (17.4±1.9 ml/min·m^2). No difference was noted in the MCR of hLH in women with oligomenorrhea or amenorrhea. Production rates (PRs) were calculated by using a pituitary standard, the values being 85.1±21.5 IU/24 h in normal men, 39.9±12.6 IU/24 h in normal premenopausal women, and 294.6±61.9 IU/24 h in normal postmenopausal women. The initial half-times of disappearance of the α- and β-subunits of hLH were measured in two normal men and found to be 15–18 min, respectively. The half-time of intact hLH was twice as great.

INTRODUCTION

Although many studies on the physiology of human pituitary luteinizing hormone (hLH)[1] have been published in the past decade (1), very little attention has been given to the measurement of the secretion rate of this hormone or to the metabolism of its subunits. Kohler, Ross, and Odell (2) determined metabolic clearance rates (MCR) and production rates (PR) in pre- and postmenopausal women, using the technique of constant infusion of [^{125}I]hLH. For calculation of PR they used the results of radioimmunological measurement of endogenous hLH concentrations in single blood samples taken before [^{125}I]hLH infusion and expressed the results in terms of the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) used directly as the immunoassay standard. No allowance for the fluctuations in plasma hLH levels (3) in the calculation of PR was made in their subjects.

This report presents the results of the determination of MCR and PR of hLH in normal men and women and in patients with disturbances of gonadal function. It includes the first preliminary data on the half-time of disappearance of the α- and β-subunits of hLH in normal men. In the calculation of PR, the mean of a number of basal plasma hLH determinations was used to overcome the effect of episodic fluctuations in plasma hLH concentrations. Furthermore, data concerning the fraction of the PR of hLH excreted in urine is presented. The study examines the necessity for quantitative immunological precipitation of the hormone preparation infused in the calculation of MCR and the effects of varying this parameter. It aims to provide base-line data for subsequent investigations of the role of kidney and liver function in the clearance and metabolism of hLH.

METHODS

Subjects. Studies were performed in four groups of subjects, all of whom had given informed consent after careful explanation of the procedure and possible side effects.

(*a*) 12 normal men aged 19–45 yr.

(*b*) One man, aged 27, with the syndrome of germinal cell aplasia.

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[1] *Abbreviations used in this paper:* FSH, follicle-stimulating hormone; hLH, human luteinizing hormone; hPG, human pituitary gonadotropin; 2nd IRP-HMG, the Second International Reference Preparation of Human Menopausal Gonadotropin; MCR, metabolic clearance rate; PR, production rate; TCA, trichloroacetic acid.

(*c*) Three normal women aged 20, 21, and 44 yr, respectively. The first two were studied on day 7 of the cycle, and the third, who had had a hysterectomy previously but still had ovarian function as assessed by cyclical changes in her basal body temperature, was studied in the mid-follicular phase.

(*d*) 12 anovulatory women. Four aged 54–71 yr were postmenopausal; two aged 19 and 20 yr had primary amenorrhea; five aged 25–36 yr had secondary amenorrhea of greater than 12-mo duration; and one, aged 29, had oligomenorrhea with a cycle length of 3–4 mo (see Table I).

In 20 of the 26 patients studied, a 24-h urine specimen was collected on the day before the study and in the 8 patients with primary amenorrhea, secondary amenorrhea, and oligomenorrhea, the 24-h collections were continued for a further 6 days. All patients were resting in bed and ate normal diets during the study. Those patients given ^{125}I-labeled hLH received 5 drops of Lugol's iodine every 8 h for 24 h before and 48 h after the study in order to block thyroid trapping of radioactive iodine.

hLH preparations administered. Three different preparations were employed in the studies, two being highly purified and the other heavily contaminated with follicle-stimulating hormone (FSH): (*a*) Highly purified hLH (LER 1533 B76-93) with an immunologic potency of 3,000 IU hLH and 2 IU FSH per mg and a biopotency (ventral prostate assay) of 4,170 IU hLH per mg assayed in terms of 2nd IRP-HMG.[2] (*b*) Highly purified hLH (Hennen Pr O1), with an immunologic potency of 789 IU hLH and 6 IU FSH per mg and a biopotency (ovarian ascorbic acid depletion assay) of 2–3 U NIH hLH Sl.[3] (*c*) Human pituitary gonadotropin (hPG batch 025, Commonwealth Serum Laboratories, Melbourne, Australia) with an immunopotency of 39.0 IU FSH and 102.5 IU hLH per ampoule, each ampoule being equivalent to the gonadotropin content extracted from half a pituitary gland.

hPG batch 025 was used unlabeled whereas LER 1533 was used unlabeled and after iodination with ^{125}I to a specific activity of 100–150 μCi/μg by the method of Greenwood, Hunter, and Glover (4). Separation of the ^{125}I-labeled hLH from free ^{125}I was achieved by filtration on a 10-cm column of cellulose, the elution buffer being 5% human serum albumin-Veronal at pH 8.6. The fraction selected for infusion showed essentially no damaged [^{125}I]hLH or free ^{125}I by chromatoelectrophoretic analysis (5) and 90–95% of added counts were immunoprecipitable with excess antibody under conditions described below. Hennen hLH was labeled and assessed in the same way. All preparations were sterilized by Millipore filtration and cultured before use, the labeled material being stored at 4°C and used as soon as possible after bacteriological assessment.

Subunit preparations administered. The α- and β-subunits from two different preparations of hLH were utilized. Drs. G. Hennen and R. Lequin kindly provided α-hLH (biologic potency 0.05 U NIH hLH Sl/mg, OAAD assay) and β-hLH (0.01 U Sl/mg) prepared as previously described (6). Dr. L. Reichert generously provided α-hLH (LER 1756-2) and β-hLH (LER 1756-1) (7).

Measurement of endogenous hLH. Plasma hLH was determined in a specific double antibody radioimmunoassay described previously from this laboratory (8). A laboratory preparation of human pituitary gonadotropin (hPG) of bioassay potency 40 IU/mg FSH and 144 IU/mg hLH was employed as standard and the results of the assay were expressed in mIU of the 2nd IRP-HMG. In this assay system, 1 μg LER 907 is equivalent to 40 mIU hLH. The sensitivity of the assay was 0.1 mIU/ml and the precision, expressed as the coefficient of variation as calculated by the method of Burger, Lee, and Rennie (9), was less than 6% for values in the range seen in young adult men and women. Interassay variation of duplicate plasma samples in 20 consecutive assays was 10.4%.

This assay system was used to measure hLH levels after infusions or injections of unlabeled hLH for half-time or MCR determinations.

Urinary hLH. Urinary hLH was measured by a modification of the method described by Baghdassarian, Guyda, Johanson, Migeon, and Blizzard (10). 5 vol of ethanol rather than acetone were used and two extractions were performed. The standard used was the 2nd IRP-HMG. The sensitivity of the assay was 0.5 mIU/ml urine and the precision, as defined above, was less than 5% for values in the range seen in young adult men and women. Interassay variation of measurements made on duplicate urine extracts in 15 consecutive assays was 11.0%.

Plasma hLH disappearance studies. Disappearance studies were performed in one subject with both labeled and unlabeled hLH (LER 1533 B76-93) and with hPG 025. Because of the multiexponential nature of the disappearance curves obtained, the initial plasma half-time of each preparation was defined as the time at which the plasma hormone concentration had fallen to 50% of the peak value achieved after intravenous injection, plasma samples being collected every minute for 5 min and then at frequent intervals for 6 h.

MCR determination. The constant infusion technique of Tait (11) was employed in all subjects, one subject also having his MCR determined by measuring the area under the disappearance curve after a single intravenous injection. The plasma volume was determined by the ^{131}I-labeled albumin technique of Wagner (12).

To allow subsequent calculation of hormone PR, plasma samples were collected every 15 min for 1–3 h before each study for determination of endogenous hormone levels. For the purposes of these experiments, it was assumed that

TABLE I

Details of Patients with Anovulation

No.*	Category	Urinary estrogens	Other comments
		μg/24 h	
19	Primary amenorrhea	0.2	Untreated
20	" "	3.0	"
21	Secondary amenorrhea	–	Posthypophysectomy
22	" "	8.0	Post-OC‡, 2 yr
23	" "	8.0	Post-OC (galactorrhea)
24	" "	11.0	Post-OC
25	" "	9.0	Galactorrhea and amenorrhea, 14 yr
26	Oligomenorrhea	1.0	4-mo cycles

* See Table IX.

‡ OC = oral contraceptives.

[2] Reichert, L. Personal communication.

[3] Hennen, G. Personal communication.

TABLE II

Precipitation of Labeled hLH (LER 1533 B76-93) and FSH (LER 1563) by Antisera Used

Antibody	Final dilution	Percentage precipitation of [^{125}I]hLH	Percentage precipitation of [^{125}I]hFSH
Rab 12	1/800	90.4	58.9
Rab 13	1/800	89.2	60.2
Rab 2	1/60,000	76.0	34.0
	1/1,800,000	33.0	2.3

these levels did not change significantly during the infusion.

The hormone preparation infused was dissolved in 100 ml of 0.9% saline containing 1% human albumin and 20 ml was given intravenously as a priming dose. 20 min later, the constant infusion was commenced and the remaining 80 ml was usually given over 6–7 h; in two subjects the infusion was continued for a total of 12 h. The effects of varying the loading dose and the time interval between it and the commencement of the constant infusion were evaluated in three subjects.

Heparinized venous blood samples were drawn every 30 min after the infusion had been in progress for 3 h. In the initial studies, blood samples were drawn after the priming dose and every 30 min for the first 3 h also. It was assumed that equilibrium had been reached if the hormone levels measured in at least five consecutive plasma samples fluctuated around the mean value and differed from it by no more than 6%, this being the coefficient of variation for the routine hLH assay quoted previously. The intra-assay variation of 10 duplicate specimens of the same labeled plateau plasma sample was less than 2%.

For the radioactive studies, the MCR was calculated after the method of Tait and Tait and Burstein (11, 13): antibody-precipitable radioactivity infused per minute divided by antibody-precipitable radioactivity per milliliter of plasma at equilibrium: MCR = ([^{125}I]hLH infused/minute)/([^{125}I]-hLH/milliliter plasma at equilibrium).

For the studies using unlabeled hormone, account was taken of the endogenous levels in calculating the MCR: MCR = (hLH infused/minute)/(Plateau hLH − basal hLH).

Estimation of hLH infused and hLH at plateau. In the studies using unlabeled hormone, the volumes of infusate delivered in two consecutive 5-min samples immediately after the end of the study were determined. To determine the concentration of hLH infused, serial dilutions of the infusate were added to plasma obtained before the infusion, this plasma being subsequently diluted 1:8 in 1% bovine serum albumin in phosphate-buffered saline. Plasma samples obtained at plateau were similarly diluted 1:8 and all samples from each patient were analyzed in the same assay. Serial dilutions of both the infusate and the patients' plasma at plateau produced displacement of labeled antigen from hLH antibody parallel to that obtained with the assay standard.

In the studies using radioactive hormone, the percent counts precipitable by antibody remained constant in the infusion system throughout the study and two consecutive 5-min samples were collected at the end of the study to determine the infusion rate. The infused material was diluted in the patient's basal plasma to concentrations of 1/100, 1/200, and 1/400 and then, together with duplicate specimens of the plateau samples, subjected to double antibody precipitation. Excess rabbit anti-hLH antibody was added to 0.4 ml of duplicate plasma samples, allowed to incubate at 4°C for 24 h, and then goat anti-rabbit gamma-globulin serum added. After a further 16 h, the samples were centrifuged, the supernate removed, and the precipitates counted.

TABLE III

Characteristics of Antisera Used for Subunit Studies

Tracer	Percent of Tracer Precipitated by Antiserum		
	Rab 12	Anti-α-hLH*	Anti-β-hLH*
α-hLH (Hennen)	57.1	78.2	69.9
β-hLH "	58.9	2.3	44.1
hLH "	63.4	47.4	13.7
α-hLH (Reichert)	95.3	87.4	1.1
β-hLH "	46.9	1.2	42.5

* A gift from Dr. R. Leguin, Nijmegen, Holland.

The hLH antisera were raised in rabbits against pituitary hLH prepared in our laboratory by the method of Stockell-Hartree (14) and precipitated varying amounts of hLH and FSH (Table II). All three antisera were employed in some experiments but for the majority of studies of MCR, the Rab 12 antiserum[4] was used. In two studies, the effect of varying the concentration of the anti-hLH antiserum, used for immunoprecipitation, on the calculation of MCR was evaluated. Furthermore, in another study, duplicate plasma samples were treated with trichloroacetic acid (TCA) in a final concentration of 20%, and the precipitated counts so achieved used to calculate the MCR; this result was compared with the MCR obtained by using immunoprecipitable counts obtained with the three different antisera to hLH.

PRs. PRs were determined from the MCRs from constant infusion and the level of endogenous hormone (i) per milliliter of plasma, this latter value being calculated as the mean of the samples taken every 15 min before the commencement of the study: PR = MCR × i.

Disappearance studies of hLH subunits. Disappearance studies, similar to those described for hLH above, were performed in two subjects by using the Hennen and Reichert subunit preparations. All subunits were labeled with ^{125}I as described above. Three antisera were employed for immunoprecipitation and their characteristics are summarized in Table III.

RESULTS

Validation of methods used. Initial studies using [^{125}I]hLH confirmed the findings of Kohler et al. (2) that plateau was reached after 3 h of constant infusion, a priming dose of 20% of the total given being injected 20 min before the commencement of the infusion. When the priming dose was omitted, plateau had not been achieved after 5 h of constant infusion. Commencement of the infusion immediately, or 10 min after the priming dose, did not reduce the time taken to reach equilibrium. Ingestion of food had no consistent effect and, in two prolonged infusions of 9 and 12 h, respectively, no sig-

[4] Refers to local nomenclature for animals immunized with various gonadotropin preparations.

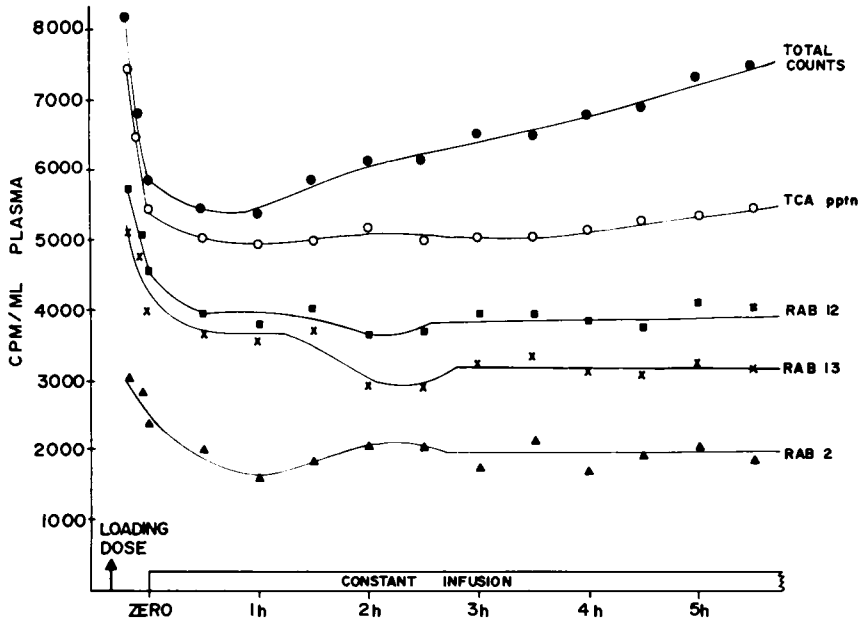

FIGURE 1 Immunoprecipitation of [^{125}I]hLH with antibodies 2, 12, and 13 and precipitation with TCA are compared with the total ^{125}I counts in duplicate samples of plasma collected 5 and 10 min after the priming dose and then every half hour until the end of the infusion.

nificant change was found between the plateau level of the 3–6-h interval and that of the last 3 h of the infusion.

When TCA precipitation was used to assess [^{125}I]-hLH concentration, plateau was not achieved (Fig. 1). However, when the same plasma was assessed by immunoprecipitation, plateau was seen with each antiserum used (Fig. 1). The continuing rise in radioactivity seen when TCA precipitation was used to assess [^{125}I]hLH presumably reflects precipitation of radioactive hormone fragments which are not immunoreactive with the antisera tested. Although the percentage of the infused hormone which was immunoprecipitable varied markedly depending on the antiserum used, the MCRs calculated from the results obtained with each of these antisera did not differ significantly (Table IV).

TABLE IV

Effect of Different Antisera and TCA Precipitation on the Estimation of MCR in a Normal Male Subject

Precipitating agent	Percentage precipitation of infused [^{125}I]hLH	Infusion rate	Plateau level	MCR
		cpm/min	*cpm/ml*	*ml/min ±SD*
Rab 2	33.9	87,853	1,902±162	46.2±3.9
Rab 13	57.2	148,234	3,246± 98	45.7±1.4
Rab 12	65.4*	169,485	3,938±124	43.0±1.4
TCA	97.1	251,637	5,214±160‡	'48.3±1.5§

* The variable precipitation (cf. Table II) is a function of the time elapsed after preparation of [^{125}I]hLH and the concentration of antibody used.
‡ Plateau not achieved. Result quoted represents mean of precipitable counts from 3–5½ h.
§ Approximate result only.

The separate study evaluating the effect of different dilutions of the same first and second antibodies also showed no significant difference in the calculated MCR although the percentage of the infused material which was immunoprecipitable varied between 56.3 and 79.7% (Table V). From these results, complete precipitation of the [^{125}I]hLH does not appear to be essential for the conduct of such studies.

The specificity of the antibodies used in the studies is shown in Table II, the iodinated hormones used to test this specificity being LER 1533 B76-93 for hLH and LER 1563 for hFSH. This latter preparation, although highly purified, does contain 9.3% contamination with hLH as assessed biologically (FSH 2,900 IU/mg, hLH 270 IU/mg)[5] or immunologically (FSH 2,580 IU/mg, hLH 240 IU/mg). Due to limited supplies, Rab. 2 antibody was not used in a concentration greater than 1/60,000. This antiserum is used to measure serum hLH levels routinely at a final dilution of 1/1,800,000 and very little cross-reactivity with FSH was found, the small amount probably being due to labeling of the 9.3% hLH contaminant of the highly purified hFSH. At the concentrations used in the MCR studies however, all the hLH antibodies were nonspecific in that a variable quantity of FSH was precipitated. Because of

[5] Reichert, L. Personal communication.

TABLE V

Effect of Different Dilutions of Rabbit 12 Antibody on the Estimation of MCR in Another Normal Male Subject

Antiserum dilution		Immunoprecipitation of infused [^{125}I]hLH	MCR
First antibody	Second antibody		
		%	*ml/min*
1/400	1/4	79.7	64.2±3.0
1/1,600	1/4	67.5	60.7±2.9
1/6,400	1/16	56.3	65.5±3.2

TABLE VI

Half-Time of Disappearance of hLH and its Subunits in Normal Men

hLH preparation	hLH half-time
	min
LER 1533 B76-93	42.8
LER 1533 B76-93 ^{125}I	43.6
hPG 025	42.1
hLH (Hennen)	31, 33
α-hLH (")	17
β-hLH (")	17
α-hLH (Reichert)	15
β-hLH (")	14

The first three studies were carried out in one normal man, the last five in another.

the almost pure nature of the hLH used in the studies, the nonspecific nature of the antibodies was thought not to be important. Iodination of the 0.067% FSH contaminant of hLH (LER 1533 B76-93) could not achieve the specific activities quoted indicating that most, if not all, of the labeling was to hLH.

The basic assumption which needs to be satisfied when the MCR is determined by using labeled material is that the labeled hormone is a true tracer of endogenous secreted hormone. As it was not possible to find a suitable patient having a hypophysectomy performed in whom the disappearance rate of endogenous hLH could be assessed, labeled and unlabeled purified hLH and hPG were injected into the same patient in successive studies and their hLH half-times compared (Table VI). No significant difference was found and the results obtained were similar to those reported by Schalch, Parlow, Boon, and Reichlin (15), although the latter authors assumed that the disappearance curve, when plotted semilogarithmically, was linear for 2 h; this could account for the slightly longer calculated half-time. Marshall, Anderson, Fraser, and Harsoulis (16) found a considerably longer half-time of about 130 min while Yen, Llerena, Little, and Pearson (17), studying the disappearance of endogenous hormone after hypophysectomy, noted an initial half-time of 21 min.

Because of the basic similarity in the handling of the hLH preparations shown in Table VI, a comparison was made between the MCR results obtained after constant infusion of labeled and unlabeled hLH in two separate subjects (Table VII). As shown in Table VII, there is a difference in the MCR result obtained depending on whether or not allowance is made for the endogenous hormone secreted during the infusion. In

TABLE VII

MCRs of hLH in Two Normal Men (Subjects 2 and 3) Measured by the Constant Infusion Technique with Different Gonadotropin Preparations

hLH preparation infused	Basal plasma hLH level	Infusion rate	Plateau level	MCR
	mIU/ml			*ml/min ±SD*
Subject 2				
LER 1533 B 76-93 ^{125}I	0.80	73,704±271 cpm/min	1,612±65 cpm/ml	45.7±1.9
LER 1533 B 76-93	0.95	211.9±15.6 mIU/min	5.3±0.3 mIU/ml	40.0±3.7*
				48.5±5.3‡
hPG 025	0.60	381.5±32.6 mIU/min	8.4±0.2 mIU/ml	45.4±4.0*
				48.9±4.4‡
Subject 3				
LER 1533 B 76-93 ^{125}I	0.75	52,900±182 cpm/min	1,118±36 cpm/ml	47.3±1.5
hPG 025	0.75	614±65 mIU/min	13.1±0.5 mIU/ml	46.9±5.3*
				49.7±5.6‡

* Basal plasma level not subtracted from plateau level before calculation of MCR.

‡ "Plateau" level used in calculations = measured plateau level minus basal plasma level.

subsequent studies where a large amount of hLH was infused and the plasma level at plateau was elevated to at least 15 times the basal level, the effect of allowing for changes in endogenous hormone secretion was much less. As shown in Table VIII, a comparison of the MCR of hLH was made in one subject using both the constant infusion and single injection techniques, no significant difference being found in the results obtained. It seems unlikely that the infused unlabeled hLH significantly alters endogenous hLH secretion, although this problem cannot be readily resolved.

Valid determinations of the MCR of hLH can thus be made by using labeled pure hLH, unlabeled pure hLH, or hLH contaminated with FSH (hPG 025).

Results of MCR determinations. The results of the continuous infusion studies in 11 men and 15 women are shown in Table IX. A summary of these results is shown in Table X. The MCR of hLH in normal males of 43.9±8.5 ml/min was significantly greater ($P < 0.001$) than the values obtained for normal premenopausal or postmenopausal women even if allowance is made for surface area. Despite differences in urinary estrogen and pregnanediol excretion between normal premenopausal women and those in the postmenopausal or anovulatory state, no significant difference was seen in their MCR of hLH.

PR results. The calculated PRs in the various groups of subjects are shown in Table IX. It is noteworthy that the PRs in normal men are approximately the same as those in the women with secondary amenorrhea, whilst in postmenopausal women, the PR is 3–4 times higher. The two women with primary amenorrhea had low PRs while the rate in the man with germinal cell aplasia was greater than those of all the normal men studied. The urinary hLH excretion is also shown in Table IX.

Half-times of disappearance of LH subunits. The initial half-times of disappearance of Hennen hLH in the two subjects studied were 31 and 33 min, respectively. In contrast, as shown in Table VI, the subunit half-times were 15–18 min (α-hLH, both Hennen and Reichert) and 14–17 min (β-hLH, both types). No statistically significant difference was seen, whether the Rab 12 or the anti-α-hLH antisera were used in the α-subunit study, nor was there a difference whether Rab 12 or anti-β-hLH was used for the β-subunit study.

DISCUSSION

The present study in general confirmed the findings of Kohler et al. (2) with regard to the MCR of hLH in pre- and postmenopausal women. Those investigators reported MCRs of 24.4±1.8 (mean±SE) ml/min in five premenopausal women and 25.6±4.1 ml/min in four postmenopausal subjects. The corresponding figures in the study reported here are 31.0±2.7 and 29.2±3.8 ml/min, respectively. Furthermore, no significant differences from these figures were seen in women with ovulatory disturbances: the MCR of hLH did not differ from normal in patients with primary and secondary amenorrhea, or in the single patient with oligomenorrhea.

TABLE VIII

MCR of hLH Determined by Two Different Techniques in a Normal Male Subject (Subject 8)

hLH preparation	Technique used	MCR
		ml/min ±SD
LER 1533 B 76-93 ^{125}I	Constant infusion	38.9±0.7
	Disappearance curve	38.6±1.0
LER 1533 B 76-93	Constant infusion	33.4±3.0*
		35.7±3.3‡
hPG 025	Constant infusion	35.0±2.9*

* Basal plasma level not subtracted from plateau level before calculation of MCR.

‡ "Plateau" level used in calculations = measured plateau level minus basal plasma level.

It may therefore be concluded that changes in the peripheral hLH levels in women with intact hepatic and renal function reflect differences in the rates of pituitary secretion rather than differences in disposal rates. Although there is some evidence for uptake and metabolism of hLH by avarian tissue (18), the possible effects of this factor on overall hormone disposal are insufficient to be reflected in mean MCR estimates.

A striking finding in the present study is the demonstration of a sex difference in the handling of a pituitary gonadotropin. The MCR of hLH in the 11 men studied (43.5±8.2, mean±SD ml/min) was significantly greater than in the 15 women (29.7±3.7 ml/min), and the difference remained statistically significant when allowance was made for differences in body surface area. On this basis, the MCR of hLH was 29% higher in men than in women.

The reason for and biologic significance of this sex difference are obscure. Although there is little evidence in favor of the presence of specific serum binding proteins for the protein and polypeptide hormones, Rajaniemi and Vanha-Perttula (19) have reported the binding of hLH and FSH to rat and human serum proteins. Even so, they stated that there were no apparent differences in this binding in sera from normal men, normal menstruating women, pregnant, and postmenopausal women. Thus, it is unlikely that protein binding of hLH is responsible for the sex difference in the MCR.

The method of estimating MCR was validated in several ways, but it must be emphasized that the clearance

TABLE IX

Results of Constant Infusion Studies to Measure MCR and PR of hLH

Subject	Age	Weight	Height	Body surface Area	Radioactive studies: Precipitable cpm infused	Radioactive studies: Plasma level at equilibrium	Nonradioactive studies: Hormone infused	Nonradioactive studies: Plasma level at equilibrium	MCR	Endogenous plasma hLH	PR	Urinary hLH excretion
	yr	*kg*	*cm*	m^2	*cpm/min*	*cpm/ml ±SD*	*mIU/min*	*mIU/ml ±SD*	*ml/min*	*mIU/ml**	*IU/24 h*	*IU/24 h‡*
Normal men												
1	20	83.0	177.0	1.98	42,411	844±18	—	—	50.2	1.70	122.9	—
2	29	79.0	176.1	1.91	73,682	1,612±65	211.9	5.3±0.3	47.1	0.95	64.4	28.4
3	30	73.2	177.6	1.88	52,917	1,118±36	614.0	13.1±0.5	48.5	0.75	52.4	29.4
4	33	73.0	183.0	1.92	90,134	1,419±37	—	—	63.5	1.12	102.4	—
5	19	57.5	177.5	1.70	114,900	2,720±132	—	—	42.2	1.69	102.7	—
6	22	70.2	170.0	1.80	13,598	373±10	—	—	36.5	1.71	89.9	12.2
7	19	75.0	170.0	1.85	169,485	3,938±124	—	—	43.0	1.60	99.1	76.7
8	45	95.4	177.5	2.10	88,077	2,267±41	1,078.0	30.8±1.4	37.7	1.32	71.7	11.3
9	20	68.2	190.5	1.93	226,038	6,088±232	—	—	37.1	1.10	58.8	51.3
10	52	53.0	162.0	1.55	166,532	5,055±139	—	—	42.9	1.82	86.2	35.8
Mean±*SD*									43.9±8.5	1.38±0.36	85.1±21.5	35.0±21.3
Man with Sertoli-cell-only syndrome												
11	27	69.0	183.0	1.88	50,339	1,270±28	—	—	39.6	2.92	166.5	96.8
Normal premenopausal women												
12	44	65.0	167.0	1.71	—	—	189.3	6.5±0.3	34.7	1.05	52.5	—
13	20	54.1	162.4	1.56	51,929	1,725±79	—	—	30.1	—	—	—
14	21	57.3	157.4	1.56	86,844	3,068±142	—	—	28.3	0.67	27.3	15.1
Mean±SD									31.0±2.7	0.86±0.19	39.9±12.6	
Postmenopausal women												
15	68	57.3	161.4	1.59	280,466	10,110±301	—	—	27.7	7.30	291.2	146.4
16	63	70.7	156.3	1.70	350,781	12,191±423	—	—	28.8	5.10	211.5	97.1
17	54	78.5	152.2	1.74	275,110	7,801±341	—	—	35.3	7.60	386.3	258.7
18	71	58.7	168.0	1.66	76,371	3,047±99	—	—	25.1	8.0	289.2	117.2
Mean±SD									29.2±3.8	7.0 ±1.1	294.6±61.9	154.9±62.5
Women with primary amenorrhoea												
19	19	62.1	164.0	1.66	—	—	773.8	31.8±1.4	24.5	0.17	6.0	19.0
20	20	54.2	159.0	1.54	—	—	1,370.4	47.1±1.9	29.9	0.34	14.6	3.6
Mean±SD									27.2±2.7	0.26±0.09	10.3±4.3	11.3± 7.7
Women with secondary amenorrhoea												
21	25	68.3	172.5	1.80	—	—	684.6	24.1±1.1	30.1	1.32	57.2	—
22	27	62.0	170.0	1.70	—	—	1,045.8	47.2±0.8	22.4	0.47	15.2	21.9
23	28	64.5	160.5	1.66	—	—	1,164.8	36.5±1.4	34.6	2.80	139.5	15.0
24	29	67.0	162.2	1.70	—	—	1,446.4	45.4±2.1	33.1	1.72	82.0	21.1
25	36	63.0	165.0	1.69	—	—	1,543.6	50.8±1.1	32.0	2.51	115.7	21.6
Mean±SD									30.4±4.3	1.76±0.84	81.9±43.6	19.9± 2.8
Women with oligomenorrhoea												
26	29	50.0	160.0	1.49	—	—	1,342.1	47.8±2.0	29.0	0.74	30.9	10.4

* In terms of hPG units.

‡ In terms of the 2nd IRP-HMG.

rates represent those of immunoreactive hLH, and not necessarily those of biologically active molecules. Similar results were obtained regardless of whether labeled or unlabeled highly purified hLH was used, and there was no difference in the estimate of the clearance of the hLH component of clinical grade pituitary gonadotropin containing relatively large amounts of FSH. Essentially identical results were obtained when the half-lives of labeled and unlabeled exogenous hLH were compared in the same subject, and when MCR was determined by constant infusion of these materials. This is supported by the general similarity of MCR for hLH obtained by Kohler et al. (2), using labeled [^{125}I]hLH, and that shown by Marshall et al. (16), using a different preparation of unlabeled hLH. It must be noted, however, that Kohler et al. studied women, while Marshall et al. studied men, and that in the present study, carried out in both sexes and in the same laboratory, a significant sex difference has been observed. The fact that similar results were seen using a highly specific radioimmunoassay for hLH to measure MCR of unlabeled material, and relatively nonspecific antiserum to precipitate puri-

fied labeled hLH provides further validation of the techniques reported. It is noteworthy that quantitative precipitation of the infused radioactive material was not required to obtain a valid result. The fact that a variety of hLH antibodies was used to give identical results again suggests that the data obtained actually represents the MCR of hLH.

A theoretical criticism of the studies using labeled luteinizing hormone would be that the result might reflect merely the clearance of a labeled subunit. Evidence recently reported (20) indicates that the α-subunit is the one predominantly labeled with ^{125}I, at least as regards the porcine hormone. The data reported here for the first time, that the initial plasma half-times of α- and β-hLH are approximately half that of the intact molecule, provides a strong argument against the above criticism.

A major problem in the reporting of the present data, and its comparison with other reports, concerns the choice of the appropriate standard to be used to express the units of hLH PR. Kohler et al. (2) expressed the plasma concentration of hLH in terms of 2nd IRP-HMG, and used the figures derived for PR to conclude that the pituitary content of hLH turned over once per day. It is now well recognized (21) that a pituitary standard should be used for the estimation of the potency of pituitary gonadotropin preparations, since the use of a urinary standard such as 2nd IRP-HMG will give a relative estimate of potency 5–6 times higher than that of the bioassay using 2nd IRP-HMG as standard. The appropriate standard to be used for estimates of plasma concentration remains a matter of controversy (22), but there has been a general tendency in recent years to employ a pituitary standard (e.g., Medical Research Council Standards, LER 907). In the present study, a pituitary standard has been employed, the units being those of its biological potency in terms of 2nd IRP-HMG. As a result, the range of values obtained for PR of hLH differs significantly from the report of Kohler et al. (2). These authors calculated a PR of 734±170 mU/min (equivalent to 1,060±245 IU/day) in normal women, and 2,400 mU/min (3,450 IU/day) in post-menopausal women. The corresponding figures in the present study were 39.9 IU/day in two normal women and 295 IU/day in postmenopausal women. These striking differences can be attributed almost entirely to differences in the estimated plasma concentrations of endogenous hLH, the differences being due to the use of different immunoassays.

The interpretation of values obtained for urinary excretion of hLH also presents difficulty. From the data presented here, it can be concluded that of the measured PR of hLH in normal men (85.1±21.5 IU/day), approximately 41% is excreted in the urine, when the urinary excretion rate is calculated by means of an assay in which the appropriate urinary reference standard is used. The mean urinary excretion rate of 35.0±21.3 IU/day agrees well with other reports (10). There is, however, a difficulty to be resolved in that, although 70–90% of the radioactivity administered as [^{125}I]hLH is recovered in the urine within 72 h, less than 5% of this was alcohol-extractable (i.e., behaved in this respect like intact hLH).

TABLE X
Summary of Table IX

Clinical diagnosis	No. of subjects	MCR	MCR/body surface area
		ml/min	*ml/min·m²*
Normal men	10	43.9±8.5	25.6±3.6
Man with Sertoli-cell-only syndrome	1	39.6	21.1
Normal women	3	31.0±2.7	19.2±0.9
Postmenopausal women	4	29.2±3.8	17.4±1.9
Primary amenorrhea	2	27.2±2.7	17.1±2.3
Secondary amenorrhea	5	30.4±4.3	17.8±2.7
Oligomenorrhea	1	29.0±3.4	19.5±2.3
All men	11	43.5±8.2	25.1±4.0
All women	15	29.7±3.7	18.0±2.2

Using the same data as quoted by Kohler et al. (2) for the pituitary content of hLH, namely, 725 IU in premenopausal and 1,725 IU in postmenopausal women, the present report indicates a daily turnover of only 17% of the pituitary hLH in postmenopausal women, and approximately 12% in men, if a content similar to that of premenopausal women is assumed.

It seems clear that the use of different standards by investigators for the expression of plasma hLH levels will lead to widely divergent values for the estimation of PR. Consequently, such data can serve only for within laboratory comparisons of PRs in differing reproductive states.

There was a broad correlation between the urinary excretion rate of hLH measured on the day before MCR estimations and the calculated PR: thus, in men urinary hLH averaged 41% of daily PR, while in postmenopausal women this was 53%. There were some notable discrepancies in the estimates of PR and urinary excretion in some subjects: these may reflect day-to-day variability in PR and perhaps in renal handling of the gonadotropin.

The results of the present study provide a basis for further investigations, currently in progress, of the roles of the liver and particularly the kidney, in the overall metabolism and disposal of the pituitary gonadotropins and for an examination of the clearance rates of the hLH subunits.

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REFERENCES

1. Franchimont, P., and H. G. Burger. 1975. Secretion of Growth Hormone and the Gonadotrophins in Health and Disease. Associated Scientific Publishers (A.S.P.), Amsterdam.
2. Kohler, P. O., G. T. Ross, and W. D. Odell. 1968. Metabolic clearance and production rates of human luteinizing hormone in pre- and post-menopausal women. *J. Clin. Invest.* **47**: 38–47.
3. Nankin, H. R., and P. Troen. 1971. Repetitive luteinizing hormone elevations in serum of normal men. *J. Clin. Endocrinol. Metab.* **33**: 558–560.
4. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114–123.
5. Burger, H. G., J. R. Oliver, J. Davis, and K. J. Catt. 1968. Radioimmunoassay for human pituitary luteinising hormone using paper chromatoelectrophoresis. *Aust. J. Exp. Biol. Med. Sci.* **46**: 541–553.
6. Closset, J., G. Hennen, and R. M. Lequin. 1972. Isolation and properties of human luteinising hormone subunits. *FEBS (Fed. Eur. Biochem. Soc.)*. **21**: 325–329.
7. Reichert, L. E., Jr., F. Leidenberger, and C. G. Trowbridge. 1973. Luteinizing hormone and its subunits: development and application of a radioligand receptor assay and properties of the hormone-receptor interaction. *Recent Prog. Horm. Res.* **29**: 497–532.
8. Alford, F. P., H. W. G. Baker, H. G. Burger, D. M. de Kretser, B. Hudson, M. W. Johns, J. P. Masterton, Y. C. Patel, and G. C. Rennie. 1973. Temporal patterns of integrated plasma hormone levels during sleep and wakefulness. II. Follicle stimulating hormone, luteinising hormone, testosterone and estradiol. *J. Clin. Endocrinol. Metab.* **37**: 848–854.
9. Burger, H. G., V. W. K. Lee, and G. C. Rennie. 1972. A generalized computer program for the treatment of data from competitive protein binding assays including radioimmunoassays. *J. Lab. Clin. Med.* **80**: 302–312.
10. Baghdassarian, A., H. Guyda, A. Johanson, C. J. Migeon, and R. M. Blizzard. 1970. Urinary excretion of radioimmunoassayable luteinizing hormone (LH) in normal male children and adults, according to age and stage of sexual development. *J. Clin. Endocrinol. Metab.* **31**: 428–435.
11. Tait, J. F. 1963. Review: the use of isotopic steroids for the measurement of production rates in vivo. *J. Clin. Endocrinol. Metab.* **23**: 1285–1297.
12. Wagner, H. N., Jr. 1968. Principles of Nuclear Medicine. W. B. Saunders Company, Philadelphia. 840.
13. Tait, J. F., and S. Burstein. 1964. In vivo studies of steroid dynamics in man. *In* The Hormones. G. Pincus, K. V. Thimann, and E. B. Astwood, editors. Academic Press, Inc., New York. 5, 441–557.
14. Stockell-Hartree, A. M. 1966. Separation and partial purification of the protein hormones from human pituitary glands. *Biochem. J.* **100**: 754–761.
15. Schalch, D. S., A. F. Parlow, R. C. Boon, and S. Reichlin. 1968. Measurement of human luteinising hormone in plasma by radioimmunoassay. *J. Clin. Invest.* **47**: 665–678.
16. Marshall, J. C., D. C. Anderson, T. R. Fraser, and P. Harsoulis. 1973. Human luteinizing hormone in man: studies of metabolism and biological action. *J. Endocrinol.* **56**: 431–439.
17. Yen, S. S. C., O. Llerena, B. Little, and O. H. Pearson. 1968. Disappearance rates of endogenous luteinizing hormone and chorionic gonadotropin in man. *J. Clin. Endocrinol. Metab.* **28**: 1763–1767.
18. Naftolin, F., D. Espeland, J. A. Tremann, E. A. Dillard, and C. A. Paulsen. 1968. Serum hLH levels in ovarian and systemic vein blood by radioimmunoassay. *In* Gonadotropins. E. Rosemberg, editor. Geron-X, Inc., Los Altos, Calif. 373–379.
19. Rajaniemi, H., and T. Vanha-Perttula. 1973. Evidence for LH and FSH binding protein(s) in human and rat serum. *Horm. Metab. Res.* **5**: 261–266.
20. Combarnous, Y., and G. Maghuin-Rogister. 1974. Luteinizing hormone. 2. Relative reactivities of tyrosyl residues of the porcine hormone towards iodination. *Eur. J. Biochem.* **42**: 13–19.
21. Odell, W. D., L. E. Reichert, and R. S. Swerdloff. 1968. Correlation between bioassay and immunoassay of human luteinizing hormone. *In* Gonadotropins. E. Rosemberg, editor. Geron-X, Inc., Los Altos, Calif. 401–407.
22. Albert, A., E. Rosemberg, G. T. Ross, C. A. Paulsen, and R. J. Ryan. 1968. Report of the National Pituitary Agency collaborative study on the radioimmunoassay of FSH and LH. *J. Clin. Endocrinol. Metab.* **28**: 1214–1219.