# Production Rates and Metabolic Clearance Rates of Human Follicle–Stimulating Hormone in Premenopausal and Postmenopausal Women

YANK D. COBLE, JR., PETER O. KOHLER, CHARLES M. CARGILLE, and GRIFF T. ROSS

*From the Endocrinology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014*

ABSTRACT The production rates (PR) and the metabolic clearance rates (MCR) of human follicle-stimulating hormone (HFSH) were determined in six pre- and five postmenopausal women. Human FSH (PER-780) labeled with $^{131}I$ to specific activities of 50–150 $\mu c/\mu g$ was used as a tracer. Both double antibody and trichloroacetic acid (TCA) precipitation techniques were used to determine HFSH-$^{131}I$ levels in infusate and plasma. In four of the subjects MCRs measured by both constant infusion and single injection techniques were the same. By constant infusion, plasma HFSH-$^{131}I$ levels reached equilibrium between 4–5 hr.

MCRs in six premenopausal women were 14.2 ±1.1 (mean ±SE) ml/min. MCRs in five postmenopausal women were 12.6 ±1.1 ml/min. Simultaneous HFSH and human luteinizing hormone (HLH) MCRs were determined in a single patient using HFSH-$^{125}I$ and HLH-$^{131}I$ as tracers by both constant infusion and single injection methods. These studies showed that the MCR of HFSH was 10.8–11.1 ml/min, and the MCR of HLH was 18.5–19.4 ml/min. From these data and previous MCR and PR studies of HLH from this laboratory, it appears that the MCR of HFSH is about one-half that of HLH.

Endogenous HFSH and HLH levels were measured by radioimmunoassay. The PRs of HFSH, calculated by the product of endogenous level and MCR, were 146 ±27mU/min in the premenopausal women and 2141 ±264 mM/min in the postmenopausal women. 24-hr PRs, based on these results, compared with reports of 24-hr urinary excretions of biologically active HFSH indicate that 3–5% of production is found in urine in biologically active form. After our single injections of HFSH-$^{131}I$, 8–29% was recovered in urine over 24 hr.

This work was presented in part to the American Federation of Clinical Research, Endocrine Section, Atlantic City, 5 May 1968.

Dr. Coble is a member of the Nutrition Program, National Center for Chronic Disease Control, Bureau of Disease Prevention and Environmental Control, Bethesda, Md. His present address is Veterans Administration Hospital, Nashville, Tenn. 37203. Dr. Kohler's present address is the Endocrinology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014.

*Received for publication 19 July 1968 and in revised form 9 September 1968.*

## INTRODUCTION

Circulating plasma levels of human follicle-stimulating hormone (HFSH) measured at any single point in time are the resultant of both the rate at which the hormone is secreted into the blood by the pituitary and the rate at which the hormone is degraded or cleared from the blood by target glands or other organs. Changes in plasma HFSH levels may reflect changes in rates of either or both of these processes. Conclusions concerning the relative contributions of entry and exit of the hormone from the blood depend upon measurements of metabolic clearance rates (MCR) in steady states associated with different plasma hormone concentrations.

The purpose of the present study was to determine the MCRs of HFSH in premenopausal and postmenopausal women by the constant infusion of tracer hormone and to estimate the production rates (PR) from these MCRs and the endogenous HFSH levels determined by radioimmunoassay. We have also compared the single injection and constant infusion techniques for estimating MCRs in several patients. Simultaneus MCRs of HFSH and human luteinizing hormone (HLH) were measured in a single patient by both methods.

## METHODS

*Subjects.* Studies were performed on seven premenopausal and six postmenopausal women (Table I). Six of the

premenopausal subjects were normal volunteers studied at random times during their menstrual cycles. The seventh premenopausal subject had infertility associated with amenorrhea and galactorrhea (No. 7). Four of the postmenopausal women had carcinoma of the breast with metastases (Nos. 9, and 11–13). One of these four had had a hypophysectomy (No. 13). None had evidence of metastases to the liver or abnormal liver fucntion tests. All subjects were active but were at bed rest and received clear liquid diets during infusions. Saturated potassium iodide, six drops every 6 hr, was administered for 24 hr preceding and 24 hr after initiation of the study in order to block thyroid trapping of radioactive iodine.

*Endogenous hormone measurement.* Plasma HFSH and HLH levels were determined by double antibody radioimmunoassays expressed as milliinternational units of International Reference Preparation No. 2 human menopausal gonadotropin (mU of IRP No. 2 HMG) (footnotes 1 and 2 and references 1 and 2) which was used as standard in both assays. In the HFSH assay, preparation R-780[3] was labeled as trace hormone. A minimal detectable quantity was 1 mU or less. Within assay precision had 95% confidence limits of ±1.6–1.9 mU/ml for values in the range seen in young normal adult women. Studies for specificity revealed less than 1% cross-reactivity for HLH, human growth hormone (HGH), human chorionic gonadotrophin (HCG), and adrenocorticotrophic hormone (ACTH) when compared to HFSH on a weight basis.[4] Thyroid-stimulating hormone (TSH) cross-reactivity was 3.5% at the doses tested.

*Preparation of tracer hormone.* Highly purified HFSH, R-780,[3] and Hartree HLH (3) were used in this study. Both hormones were labeled with $^{131}I$ to specific activities of 50–150 $\mu c/\mu g$, and HFSH was labeled with $^{125}I$ to 25–50 $\mu c/\mu g$ after the method of Greenwood, Hunter, and Glover (4). The HFSH-$^{131}I$ was used for most studies; HFSH-$^{125}I$ was utilized only in instances where $^{131}I$ was used simultaneously. Immediately after iodination, the reaction mixture was passed through a Sephadex G-75 column to remove damaged hormone and free iodide. The labeled hormones were used for studies within 1 wk of iodination and after Millipore filtration, culture, and pyrogen testing (5).

*Constant infusion studies* (6, 7). Venous blood was obtained between 6 and 8 a.m. for determinations of endogenous hormone levels before administration of tracer hormones. A total dose of 4–22 $\mu c$ of HFSH-$^{131}I$ or HFSH-$^{125}I$ and HLH-$^{131}I$ was used. One-fifth was given intravenously as a primer dose. 10 min later the remaining four-fifths, dissolved in saline containing 1% human albumin, was continuously mechanically infused intravenously for 6–10 hr at a rate of 0.97–0.99 ml/min. Antibody precipitable labeled hormone concentrations remained constant in the infusion system throughout the study.

Heparinized venous blood samples were drawn hourly after the 3rd hr of infusion and every ½ hr for the last 3 hr. Four or more samples were used to calculate the mean plasma labeled hormone concentration at equilibrium. The concentration in each sample was expressed as a per cent of that in the last and examined to assure that equilibrium had been reached. Urine was collected for 48 hr after the priming dose.

Plasma MCRs, the volume of blood cleared of labeled hormone per unit time, were calculated by the method of Tait (6): antibody precipitable radioactivity infused per minute divided by antibody precipitable radioactivity per milliliter of plasma at equilibrium.

$$\text{MCR} = \frac{\text{FSH-}^{131}\text{I infused/minute}}{\text{FSH-}^{131}\text{I/ml plasma at equilibrium}}$$

*Disappearance studies* (6–8). After a venous blood sample the total dose of tracer hormones was obtained, 4–22 $\mu c$ diluted to 2.5–5.0 ml in saline was injected intravenously. Heparinized venous blood samples were obtained at intervals for 24 or 48 hr after the injection and urine collected for 48 hr.

Disappearance curves for both HFSH and HLH appeared multiexponential. Thus, plasma MCRs were calculated according to the formula for any system of pools (7, 8):

$$\text{MCR} = \frac{R}{\int_0^\infty x' \cdot dt}$$

where $R$ is the total labeled hormone injected as a single dose, $x'$ is the concentration of antibody precipitable radioactivity, and t is time. The integral $\int_0^\infty x' \cdot dt$ was determined by numerically measuring the area under the disappearance curve generated when the antibody precipitable plasma labeled hormone concentrations extrapolated to zero were plotted, against time after administration, on Cartesian coordinates (8).

*Counting of tracer hormone.* Total radioactivity in 1-ml aliquots of infusate, plasma, and urine was determined in quadruplicate by well-type scintillation counting. Precipitable radioactivity was determined by trichloroacetic acid (TCA) and double antibody methods. Duplicate samples were precipitated with TCA in a final concentration of 20%. The samples were centrifuged, the supernatants removed, and the precipitates counted. In separate duplicate samples excess rabbit anti-FSH and anti-LH were incubated at 4°C for 24 hr. Excess sheep anti-rabbit serum was added and incubation continued 24 hr. These samples were then centrifuged, the supernatants removed by aspiration, and the precipitates counted.

80–90% of tracer HFSH in infusate or plasma was precipitable with excess anti-HFSH, and greater than 95% was precipitable with TCA. 24 hr of incubation of HFSH-$^{131}I$ in plasma changed precipitability less than 5%. MCRs calculated by TCA and double antibody precipitation methods were not significantly different and the results given here are from the double antibody method. Because of the variability of antibody precipitation of tracer HFSH and tracer HLH from urine, TCA precipitation was used in estimating urinary excretion of $^{131}I$-labeled protein.

*Production rates.* PRs were determined by the MCRs from constant infusion or disappearance studies and the level of endogenous hormone ($i$) per milliliter of plasma (6).

$$\text{PR} = \text{MCR} \times i$$

[1] Kindly supplied by D. B. Bangham, National Institute for Medical Research, Mill Hill, London, England.

[2] Cargille, C. M., G. T. Ross, and T. Yoshimi. 1968. Daily variations in plasma follicle stimulating hormone, luteinizing hormone, and progesterone in the normal menstrual cycle. *J. Clin. Endocrinol. Metab.* In press.

[3] R-780 HFSH, prepared by Dr. Leo Reichert, Emory University, Atlanta, Ga. The HFSH potency at the time of assay was 61 NIH-FSH-S1 U/mg by the ovarian augmentation assay, although initial potency may have been higher. The HLH potency was 0.28 NIH-LH-S1 U/mg (courtesy of Dr. Leo Reichert).

[4] Reichert 822-2 HLH and Hartree HLH; Ayerst anterior pituitary-like substance (APL), Lot No. AF 8243976; Wilhelmi GH No. HS-475A; Lerner ACTH No. 8B; lyophilized Bates/Condliffe TSH; Reichert 780 HFSH.

TABLE I

*MCRs and PRs in Premenopausal and Postmenopausal Women Studied with the Constant Infusion of Tracer FSH*

| Patient | Age | Weight | Height | Day of menstrual cycle | Precipitable cpm infused | Blood level at equilibrium | Plasma FSH | MCR | PR |
|---|---|---|---|---|---|---|---|---|---|
| | *yr* | *kg* | *cm* | *day* | *cpm/min* | *cpm/ml* | *mU*/ml* | *ml/min* | *mU/min* |
| Premenopausal women | | | | | | | | | |
| 1 | 23 | 82.6 | 155 | 8 | 8430 | 530 ±14 | 14.3 | 15.9 | 227 |
| 2 | 21 | 59.1 | 165 | 5 | 4570 | 273 ±5 | 14.3 | 16.7 | 239 |
| 3 | 22 | 41.6 | 166 | 1 | 7203 | 680 ±21 | 12.5 | 10.6 | 132 |
| 4 | 27 | 57 | 159 | 25 | 5526 | 395 ±6 | 5.5 | 14.0 | 77 |
| 5 | 20 | 77 | 161 | 10 | 8333 | 505 ±7 | 9.0 | 16.5 | 148 |
| 6 | 25 | 56.1 | 168 | 27 | 5790 | 491 ±7 | 4.3 | 11.8 | 51 |
| 7‡ | 23 | 75 | 172 | 68 | 9118 | 656 ±26 | 13.8 | 13.9 | 192 |
| Mean ±SE | | | | | | | 10 ±1.8 | 14.25 ±1.1 | 146 ±27 |
| Postmenopausal women | | | | | | | | | |
| 8 | 55 | 68 | 157 | — | 7305 | 514 ±11 | 220 | 14.2 | 3124 |
| 9 | 55 | 70 | 165 | — | 8590 | 795 ±6 | 252 | 10.8 | 2722 |
| 10 | 56 | 40.5 | 164 | — | 9488 | 871 ±7 | 134 | 10.9 | 1461 |
| 11 | 45 | 80.4 | 172 | — | 6008 | 371 ±8 | 124 | 16.2 | 2009 |
| 12 | 45 | 50.5 | 513 | — | 12124 | 1123 ±29 | 128 | 10.8 | 1382 |
| 13§ | 52 | 52 | 152 | — | 6165 | 576 ±15 | 12 | 10.7 | 128 |
| Mean ±SE | | | | | | | 172 ±24 | 12.6 ±1.1 | 2141 ±264 |

MCR, metabolic clearance rate; PR, production rate; FSH, follicle-stimulating hormone.
Values from patients 7 and 11 are not included in calculation of means and standard errors.
* In terms of 2nd International Reference Preparation of Human Menopausal Gonadotropin.
‡ Patient with infertility associated with amenorrhea and galactorrhea.
§ Patient posthypophysectomy (plasma HFSH, 216 mU/ml before surgery).

## RESULTS

Endogenous HFSH levels for the premenopausal women were 10 ±1.8 mU/ml (mean ± SE) (Table I). The two lowest values, found in subject Nos. 4 and 6, were obtained during the late luteal phase of the menstrual cycles. Plasma HFSH levels for the postmenopausal women were 172 ±24 mU/ml. The low HFSH plasma concentration in patient No. 13 was the result of hypophysectomy. Endogenous plasma HFSH determinations at intervals throughout several of the studies showed no effect of either injection or constant infusion of tracer HFSH on endogenous levels.

During constant infusion studies, plasma HFSH-$^{131}$I and HFSH-$^{125}$I appeared to reach equilibrium within 4–5 hr (Fig. 1). MCRs in the six normal premenopausal women were 14.2 ±1.1 ml/min. Five postmenopausal women had MCRs of 12.6 ±1.1 ml/min. MCRs did not correlate with weight or surface area. The MCRs of the two groups did not differ significantly ($P > 0.05$). The premenopausal patient with amenorrhea, infertility, and galactorrhea (No. 7), and th postmenopausal patient with hypopituitarism (No. 13) had MCRs comparable to the normal subjects; values from these two patients were not included in the mean values in Table I.

During simultaneous infusion of HFSH-$^{125}$I and HLH-$^{131}$I, HLH appeared to attain equilibrium within 3 hr, approximately 2 hr faster than HFSH (Fig. 1). The MCR for HLH was 18.5 ml/min and for HFSH was 10.8 ml/min determined with both tracers at equilibrium in this subject.

Disappearance of tracer from plasma after intravenous injection was observed in four subjects previously studied by constant infusion. The difference in clearance of tracer HLH and HFSH from plasma is demonstrated by their disappearance curves after simultaneous injection (Fig. 2). The disappearance curves on semilogarithmic plot had no clearly linear segment, an observation suggesting a multicompartmental system.

The MCRs determined by the single injection technique and the constant infusion method were approximately the same (Table II). The MCR of HLH simultaneously injected with tracer HFSH was comparable to the MCR for HLH found earlier during simultaneous constant infusion studies (18.5 vs. 19.4 ml/min).

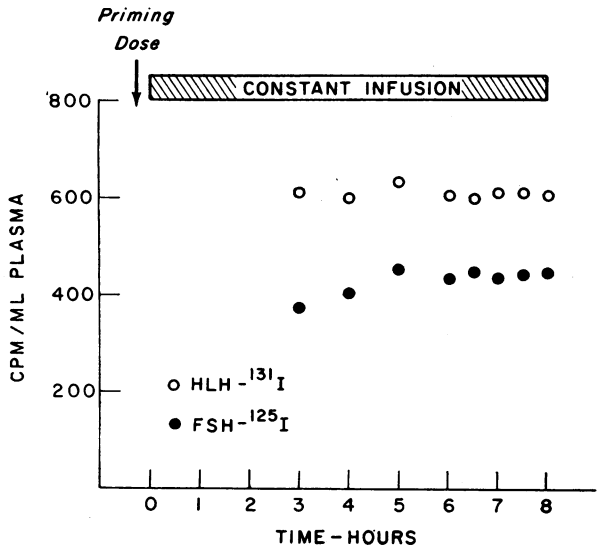


FIGURE 1 Schematic representation of the constant infusion technique. Simultaneously infused $^{125}I$ labeled human follicle-stimulating hormone (HFSH) and $^{131}I$-labeled human luteinizing hormone (HLH) were precipitated from plasma samples and infusate by the double antibody method.

PRs, determined by the product of endogenous hormone levels and MCRs, were 146 ±27 mU/min in the premenopausal women and 2141 ±264 mU/min for the postmenopausal women.

Within 24 hr 57.3% of $^{131}I$ was excreted in the urine. By 48 hr 75% was excreted. 8–29% of $^{131}I$ injected was excreted in the urine in a form precipitable by TCA in 24 hr.

## DISCUSSION

Two major assumptions are necessary for MCR measurements to be valid. First the tracer used is assumed to be cleared from the plasma in a manner identical with that of the endogenous hormone. Secondly, secretion and clearance should be in a steady state during the course of the study. Evidence in support of the first assumption was obtained from studies on subhuman primates. Although we did not have a sufficient quantity of purified HFSH to perform clearance studies of unlabeled hormone in humans, iodinated and unlabeled HFSH had indistinguishable disappearance curves when injected simultaneously into six female Macaca mulatta monkeys.[5] The second assumption, that a steady state existed during the studies, appears justified since endogenous plasma HFSH levels did not vary during three studies. In addition, a steady state must have been reached in regard to clearance because constant plasma HFSH-$^{131}I$ levels were attained during the infusion (6, 7).

[5] Coble, Y. D., P. O. Kohler, W. Tullner, G. T. Ross, and C. M. Cargille. Data in preparation.

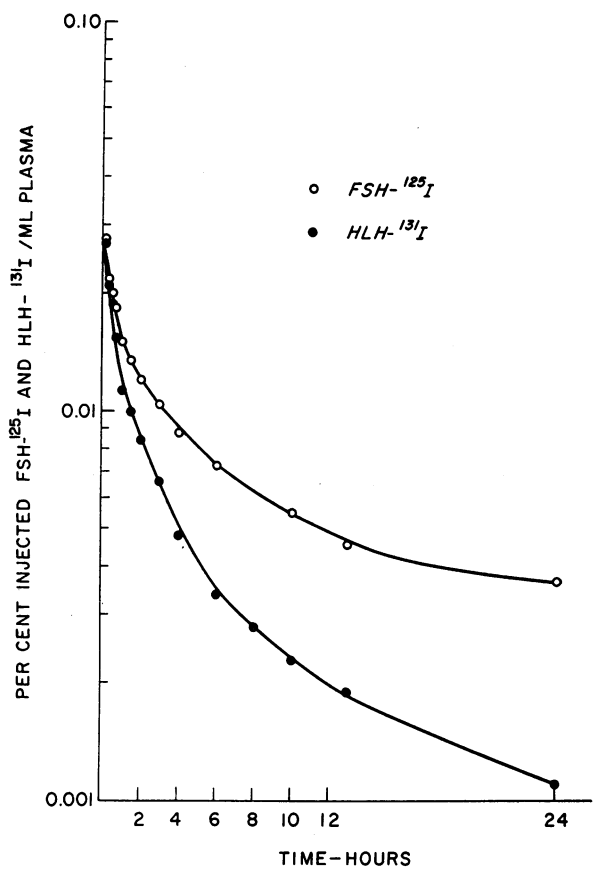


FIGURE 2 Typical disappearance curves of $^{125}I$-labeled HFSH and of $^{131}I$-labeled HLH after a single intravenous injection. The labeled hormones were simultaneously administered to this subject and were precipitated from plasma by the double antibody method.

The multiexponential disappearance curves of HFSH-$^{131}I$ from plasma after a single injection are similar to those previously noted for HLH (7) and suggest that both hormones are distributed in more than one compartment. This suggestion is supported by the nearly

TABLE II

*HFSH MCRs Determined on Four Subjects by Two Methods: Disappearance of Tracer FSH after a Single Injection and Constant Infusion Tracer HFSH to Equilibrium*

| Patient No. | Constant infusion | Single injection |
|---|---|---|
| | *ml/min* | *ml/min* |
| 3 | 10.6 | 9.6 |
| 10 | 10.9 | 11.9 |
| 12 | 10.8 | 11.1 |
| 13 | 10.7 | 8.4 |

HFSH, human follicle-stimulating] hormone; MCR, metabolic clearance rate.

identical values obtained by the constant infusion technique and those calculated by integrating the entire area under the disappearance curve after the single injection of labeled hormone. Calculation of MCRs based on the assumption of a single compartmental distribution of HFSH would disregard a considerable part of the disappearance curve. The constant infusion method for determining MCRs was used in the majority of the studies, because with this technique the area under the disappearance curve is automatically integrated at equilibrium, and calculations are simplified (6). There is also less opportunity for nonspecific damage to the labeled hormone since the duration of exposure of the labeled hormone to plasma is shorter. The major finding of the present investigation is that the MCRs of HFSH are similar in pre- and postmenopausal women. This finding implies that the striking difference in plasma HFSH levels in these two groups are the result of differences in the secretion rate of the hormone by the pituitary rather than differences in the rate of hormone metabolism. The MCRs of HLH are also similar in pre- and postmenopausal women (7). It is of interest that at menopause a 15-fold increase in HFSH PRs occurs, whereas the increase in HLH PRs is only about fivefold.

Although HFSH and HLH are biochemically and immunologically similar, the MCR of HFSH appears to be only approximately one-half that of HLH. This difference in clearance might have been predicted from the findings of previous studies. Parlow injected exogenous HFSH and HLH into humans and followed the disappearance by bioassay. He found the half-time of disappearance for HLH to be approximately 1 hr and for HFSH to be approximately 3 hr (9). Gay and Bogdanove have also found that in rats the disappearance of endogenous LH from plasma was considerably faster than the disappearance of FSH (10).

The mean per cent of radioactivity injected as HFSH-$^{131}$I and recovered in the urine was 57% within 24 hr and 75% within 48 hr. 8–29% of the injected HFSH-$^{131}$I was found in a TCA-precipitable form in urine excreted during the first 24 hr after infusion (mean, 17%), whereas 3–22% was precipitated from aliquots of the same urine (mean, 12%) by a double antibody method. This discrepancy in the two precipitation methods appeared only in urine and not in plasma. It is possible that TCA precipitates some partially degraded hormone not recognized by the specific antibody. Alternately, some factor in urine may interfere with the antigen-antibody reaction.

Becker and Albert found that urinary excretion of biologically active HFSH in premenopausal women ranged from 0.18 to 0.36 mg of NIH-FSH-S1/24 hr, whereas in postmenopausal women it was 3.7 mg/24 hr (11). Converted into units of IRP No. 2 HMG (1 mg of NIH-FSH-S1 = 25 U) (12), urinary excretion would be 4.5–9 U/24 hr for premenopausal women and 92.5 U/24 hr for postmenopausal women. Based on our results, PRs of HFSH are 210 U/24 hr in premenopausal women and 3038 U/24 hr in postmenopausal women. On this basis 24 hr urinary excretion of biologically active gonadotropin represents only 3–5% of the quantity produced in 24 hr. The 11-fold increase in urinary excretion of HFSH at menopause reported by Becker is similar to the 15-fold increase in production at menopause found in this study.

Keller found the renal clearance of HFSH by bioassay to be 0.16–0.7 ml/min (mean 0.58) (13). On the basis of a mean MCR of 13.4 ml/min, urinary excretion would account for less than 5% of the MCR.

## REFERENCES


1. Cargille, C. M., D. Rodbard, and G. T. Ross. 1968. Radioimmunoassay of human follicle stimulating hormone: bias due to antisera. *J. Clin. Endocrinol. Metab.* **28**: 1276.
2. Odell, W. D., G. T. Ross, and P. L. Rayford. 1967. Radioimmunoassay for luteinizing hormone in human plasma or serum: physiological studies. *J. Clin. Invest.* **46**: 248.
3. Hartree, A. S., W. R. Butt, and K. E. Kirkham. 1964. The separation and purification of human luteinizing and thyrotrophic hormones. *J. Endocrinol.* **29**: 61.
4. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of I-131 labelled growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114.
5. Kohler, P. O., J. M. Phang, W. W. Tullner, G. T. Ross, and W. D. Odell. 1968. Effect of iodination on the biological activity and metabolism of human luteinizing hormone. *J. Clin. Endocrinol. Metab.* **28**: 613.
6. 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.
7. Kohler, P. O., G. T. Ross, and W. D. Odell. 1968. Metabolic clearance and production rates of human luteinizing hormone in pre- and postmenopausal women. *J. Clin. Invest.* **47**: 38.
8. Gurpide, E., J. Mann, and E. Sandberg. 1964. Determination of kinetic parameters in a two-pool system by administration of one or more tracers. *Biochemistry.* **3**: 1250.
9. Parlow, A. F. 1965. III. Pituitary hormones. Induction of ovulation with human gonadotropins. *Recent Progr. Hormone Res.* **21**: 201.
10. Bogdanove, E. M., and V. L. Gay. 1967. Endogenous and exogenous FSH and LH clearance rates in the orchidectomized rat. Abstract from the Endocrine Society, June 15–17, Bal Harbour, Fla. 99.
11. Becker, K. L., and A. Albert. 1965. Urinary excretion of follicle stimulating and luteinizing hormones. *J. Clin. Endocrinol. Metab.* **25**: 962.
12. Rosenberg, E., and W. B. Lewis. 1966. Comparative activities of human urinary postmenopausal gonadotrophins. *J. Clin. Endocrinol. Metab.* **26**: 786.
13. Keller, P. J. 1966. The renal clearance of follicle-stimulating and luteinizing hormone in postmenopausal women. *Acta Endocrinol.* **53**: 225.