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Research Article

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Radioimmunoassay for Human Follicle-Stimulating Hormone: Physiological Studies

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ABSTRACT Most of the information concerning secretion changes in follicle-stimulating hormone (FSH) in humans has been gained with relatively insensitive bioassays of concentrates of pools of urine. We have developed a sensitive and specific radioimmunoassay for FSH that is 500–1000 times more sensitive than the rat ovarian-weight augmentation assay and which is capable of quantifying FSH in small volumes of serum. Anti-FSH was prepared by immunizing rabbits with an impure FSH preparation. The majority of antisera showed complete inability to distinguish LH, TSH, and FSH, illustrating the immunological similarities of these hormones. One antiserum was specific when used in a radioimmunoassay. Potency estimates by bioassay were in good agreement, with a single exception, with those obtained with the radioimmunoassay for 10 FSH-containing preparations. Highly purified LH gave a higher potency by immunoassay than by bioassay.

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constant until mid-cycle; in the majority of women a mid-cycle rise of FSH occurs coincidentally to the mid-cycle LH ovulatory peak; during the luteal phase FSH levels are relatively constant and lower than during the follicular phase. Nonsequential oral contraceptives containing estrogen and progestogen abolish these changes and FSH concentrations remain low throughout treatment. Treatment of castrate men and castrate or postmenopausal women with high doses of oral estrogens results in a fall of FSH to levels found in eugonadal men or women, but not to undetectable levels. Children less than 5 yr of age had undetectable FSH (< 5 mIU/ml).

INTRODUCTION

Most of the information available concerning the fluctuations of follicle-stimulating hormone (HFSH) in human subjects has been gained by bioassay of concentrates of pools of urine. Sensitive, specific, and precise radioimmunoassays for human luteinizing hormone (HLH) have been developed (1–5) which are capable of quantifying this hormone in unextracted serum, and this assay has been utilized in a variety of physiological studies concerning HLH (6–8). We wish now to present details of a radioimmunoassay for HFSH and results of a number of studies performed with this assay. These studies have previously been presented in abstract form (9–12). Midgley (13) and Faiman and Ryan (14) have previously published preliminary reports of a radioimmunoassay for HFSH, and Franchimont (4) has published

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development and use of such an assay in some physiological studies.

METHODS

Preparation of antisera. New Zealand white rabbits of either sex were immunized with impure preparations from pituitary or urinary sources. Generally, about 450 IU¹ of HFSH were administered in two subcutaneous sites every 10 days. Animals were bled 10 days after either the second or third injection. A total of 60 antisera from 30 animals have been studied. The antiserum selected to develop the HFSH assay described was obtained after three injections of an impure preparation² from pituitary sources. Each injection contained 3½ mg (832 IU of HFSH per mg) of this preparation dissolved in 2 ml of 0.9% saline, and homogenized in 2 ml of complete Freund's adjuvant. After a fourth injection the antiserum from this animal was not specific for HFSH, since it showed almost complete cross-reaction with HLH and HFSH.

Radioiodinated HFSH. For most of the studies, purified HFSH containing 1415 IU/mg by bioassay³ was labeled to specific activities of 200–700 mc/mg with ¹²⁵I. In the early studies another purified HFSH⁴ containing 6046 IU/mg by bioassay was labeled. No significant differences were noted when both labeled preparations were used and compared in one assay. The method of radioiodination was a modification of that reported by Greenwood, Hunter, and Glover (15) and the same as previously reported for HLH (2).

Performance of the radioimmunoassay. For radioimmunoassay of HFSH we have employed a modification of the method previously developed for HLH (2). This simplified, partially automated method is also used in our laboratory for human thyroid-stimulating hormone (HTSH), HLH, and human growth hormone (16). Reagents were added to 1 × 15 cm disposable glass tubes in the following order:

- (a) Buffer⁵ (sufficient to make final volume 1 ml).
- (b) 100 μl of 0.1 M ethylenediaminetetraacetate (EDTA).

¹Units of the Second International Reference Preparation of Human Menopausal Gonadotropin (IRP-HMG No. 2) distributed by the National Institute for Medical Research, Mill Hill, London, N.W. 7, England. This standard is impure, contains both HLH and HFSH, and serves as a reference preparation for both.

²Obtained through the National Pituitary Agency.

³R-790 prepared and kindly supplied through the National Pituitary Agency by Dr. Leo Reichert, Department of Biochemistry, Emory University, Atlanta, Ga.

⁴Prepared and kindly supplied by Dr. William Peckham, Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

⁵All buffer used to set up the assay and dilute constituents was 0.01 M PO₄, 0.15 M NaCl, 2% normal rabbit serum, and 0.1% sodium azide, pH 7.8.

- (c) Serum sample to be assayed, or in tubes containing known or standard amounts of HFSH, 300 or 400 μl of dog serum. (This is discussed later.)
- (d) 100 μl containing 0.05–0.1 mμg of HFSH-¹²⁵I and approximately 15,000 cpm, diluted in buffer.
- (e) 100 μl of anti-HFSH in a dilution of 1:400 in the buffer. (Final dilution = 1:4000.)

After 5 days of incubation at 4°C, 50–100 μl of a potent sheep or goat anti-rabbit serum (volume depended on titer of antiserum) was added to each tube. The tubes were incubated 24 more hr at 4°C and centrifuged 20 min at 500 g. Total counts were assessed in each tenth tube to assure or assess uniformity of total counts in each tube. The supernatant was aspirated by continuous suction and the counts precipitated determined in an automatic gamma spectrometer. The machine settings and time of counting was determined as previously published (16).

RESULTS

All the antisera tested were capable of binding HFSH-¹²⁵I, and this binding was inhibited by unlabeled HFSH. For most of the antisera, this binding of HFSH-¹²⁵I was also inhibited by unlabeled HLH, and HTSH, and the amounts required of these latter hormones were only slightly greater than for HFSH itself. Such nonspecific antisera were obviously unsuitable for development of an assay for HFSH. *One antiserum* was specific for HFSH. This antiserum was obtained at a single bleeding from a rabbit that had received three injections of an impure HFSH described under Methods. The antiserum was used in a final dilution of only 1:4000, as this dilution in the 1 ml of total reaction mixture gave a starting bound: free ratio of approximately 1.0 (about 7500 cpm bound in 100% tubes described in Methods). It was not necessary to absorb this antiserum with human chorionic gonadotropin (HCG) or with HLH to produce specificity. A semilog dose response curve for the highly purified HFSH (6046 IU/mg) is shown in Fig. 1. Note the curve is encompassed from 1 to 100 mμg and that the steep and usable portion (10–90%) extends from 2 to 10 mμg. Also shown in Fig. 1 are the responses elicited by up to 100 mμg of purified HTSH (containing 9.5 IU of TSH per mg)⁶ of purified HLH (containing 3600 IU/mg by bioassay)⁷

⁶In terms of the HTSH Reference Standard A, distributed by the National Institute for Medical Research, Mill Hill, London, N.W. 7, England.

⁷LER-960 prepared and kindly supplied through the National Pituitary Agency by Dr. Leo Reichert. Bio-

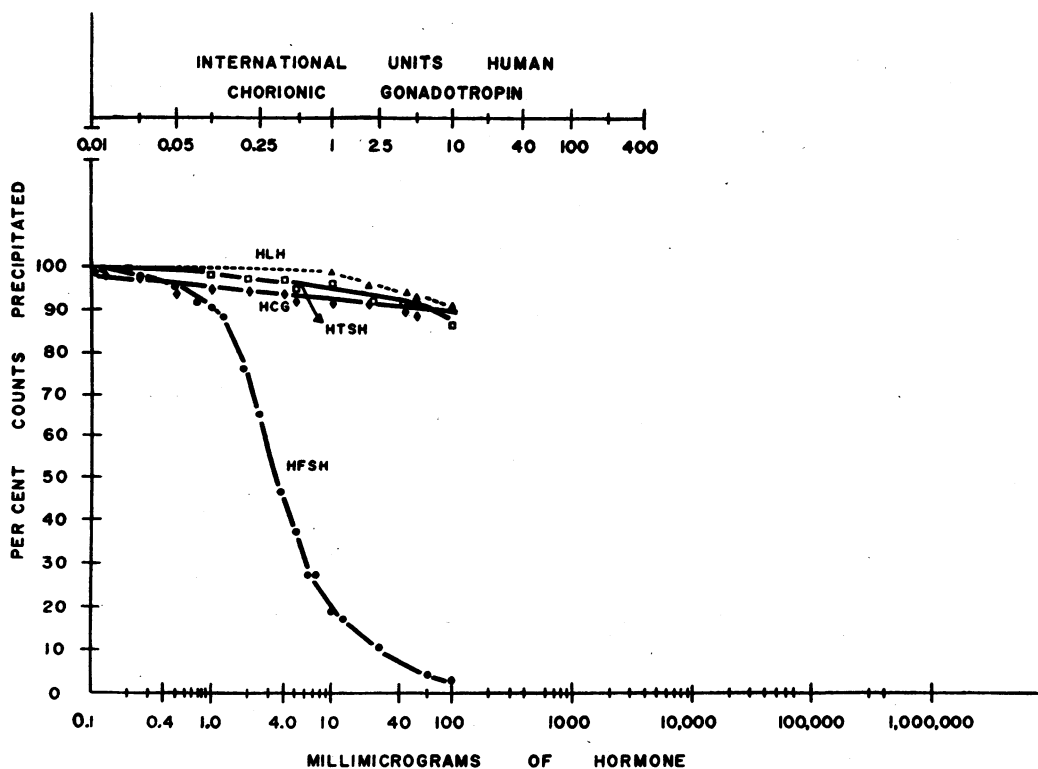


FIGURE 1 Dose response curve for purified human follicle-stimulating hormone (HFSH), human luteinizing hormone (HLH), human thyroid-stimulating hormone (HTSH), and for impure human chorionic gonadotropin (HCG). Note that the steep and usable portion of the HFSH dose response curve is between 20 and 90%, and that this encompasses the dose of 1-10 μg of HFSH. When the response of unknown samples are compared to this standard only values between 20 and 90% are read. This eliminates nonspecific responses outside this range. Note also that up to 100 μg of HLH, HTSH, and 10 IU of HCG do not give significant responses in this assay.

and up to 10 IU⁸ of HCG containing about 3000 IU/mg. Note that displacement of HFSH-¹²⁵I was less than 10% with these amounts of the other carbohydrate-containing polypeptide hormones. No displacement was seen with bovine HFSH, bovine HLH, human growth hormone, or bovine ACTH when added in amounts up to 10,000 times the dose of HFSH used in the assay. However, if the purified HTSH and HCG were added in larger amounts to the assay system, significant displacement occurred. In fact, a dose response curve indistinguishable from HFSH occurred between 1000 and 10,000 μg of HTSH and HLH and between 40 and 4000 IU of HCG.

Table I gives the results of potency estimates for assay by prostate weight increment in hypophysectomized, immature, male rats.

⁸ International units of HCG, purchased from Ayerst Laboratories, New York 10017.

a number of impure gonadotropin preparations containing different HLH:HFSH ratios immunoassayed and bioassayed in terms of the IRP-HMG No. 2. The potency estimate by bioassay is very close to that obtained by immunoassay for all the preparations except highly purified HLH. When highly purified HLH was added to the immunoassay, a higher HFSH potency estimate was obtained by immunoassay than by bioassay. Such high HLH:HFSH ratios are not believed to occur in biological fluids and the assay is highly specific for HFSH when applied to serum or plasma containing both HFSH and HLH. However, it should be pointed out that such ratios may occur in various fractions during purification of pituitary hormones, and the radioimmunoassay should not be used as the only assay to follow such purification.

TABLE I
Correlation of Bioassay and Immunoassay
Potency Estimates for HFSH

| Preparation | Bioassay potency | Immunoassay potency | I:B * |
|--|------------------|---------------------|-------|
| Pergonal-PR2075† | 84.0 | 88.2 | 1.05 |
| Pergonal-PR2128† | 75.0 | 72.8 | 0.97 |
| Pergonal-PR2119† | 82.0 | 64.8 | 0.79 |
| Humagon-No. 525577† | 77.0 | 76.2 | 0.99 |
| HMG-No. 2167† | 72.8 | 58.2 | 0.80 |
| Kaolin extract of urine‡ | 0.42 | 0.53 | 1.30 |
| Human pituitary powder§ | 2.11 | 3.17 | 1.5 |
| Partially purified pituitary fraction§ | 124.1 | 187.4 | 1.5 |
| Purified HFSH | 6046.0 | 7568.0 | 1.3 |
| Purified HLH§ | 1.32 | 31.7 | 24.0 |

HFSH, human follicle-stimulating hormone; HLH, human luteinizing hormone; HMG, human menopausal gonadotropin.

Bioassay by ovarian weight augmentation (21).

* Immunoassay potency:bioassay potency.

† As IU/vial.

§ As IU/mg.

Fig. 2 demonstrates the dose response curve for the highly purified HFSH, for a less pure preparation of HFSH, and for the IRP-HMG No. 2 (an impure preparation containing both HLH and HFSH and used as standard for both). Note the identical shape of these curves, a fact which further supports the specificity of the assay. Cross-reacting substances often but not always give dose response curves different from the standard purified hormone. (See, for example, reference 17.)

Serum samples known to be free of HFSH (for example, bovine, or canine serum) gave a small displacement of HFSH-¹²⁵I amounting to about 15% with 300 μ l when compared to standards in buffer. To eliminate this nonspecific interference by serum, dog serum was added to each of the tubes containing standard or known amounts of hormone. Under these conditions unknown serum samples gave dose response curves identical with the standard curve. Fig. 3 illustrates these points. Generally, 300 or 400 μ l of dog serum was added to each tube containing standard, since unknown

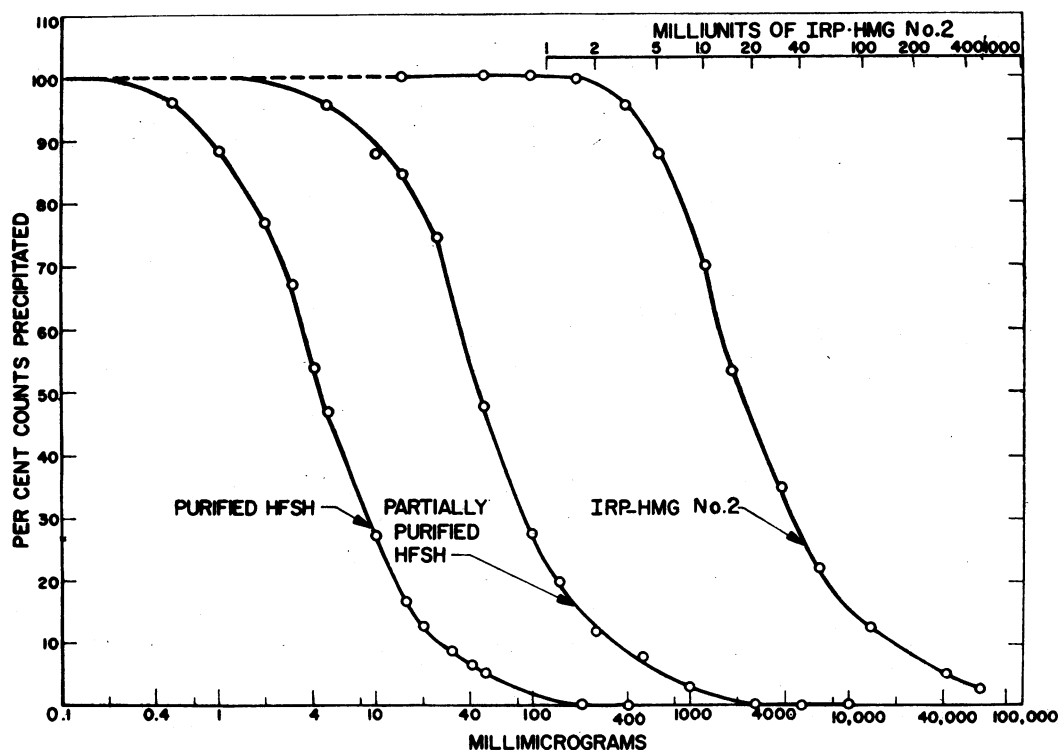


FIGURE 2 Dose response curves for purified HFSH, for a partially purified HFSH preparation, and for the Second International Reference Preparation of Human Menopausal Gonadotropin (IRP-HMG No. 2). Note the similarity in shape of the dose response curve for these three preparations of quite different degrees of purity. For the IRP-HMG No. 2 both dosage as weight of material and as milliunits are given.

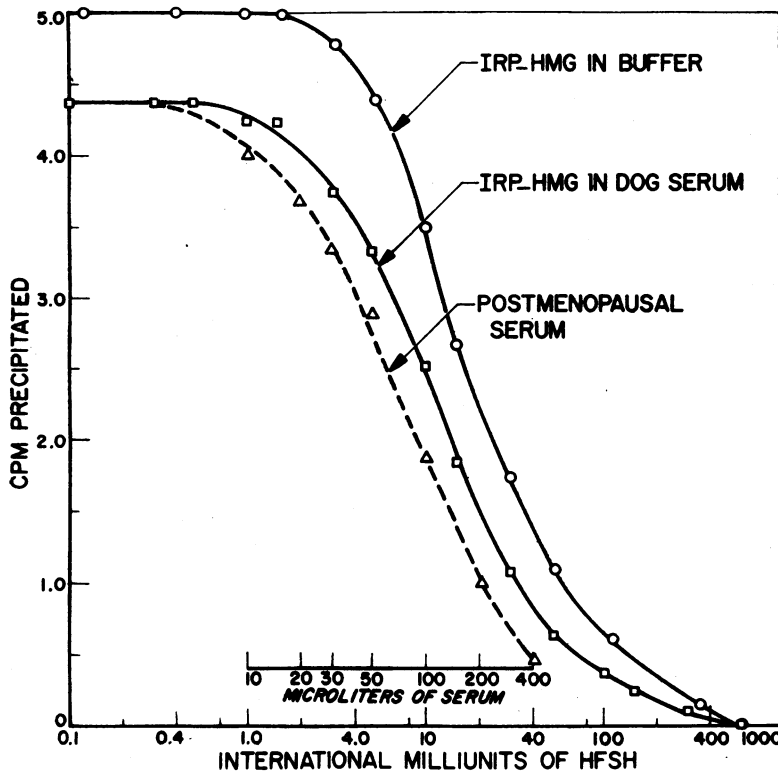


FIGURE 3 Dose response curves of IRP-HMG No. 2 in buffer and in 300 μ l of dog serum. Note the non-specific reaction caused by the dog serum. Also shown is a dose response curve of postmenopausal serum demonstrating the identity to the IRP-HMG No. 2 with dog serum added, and the difference of these from the IRP-HMG No. 2 in buffer. See Results for further details. Ordinate in 1000's of cpm.

samples were generally added in a volume of 300 or 400 μ l. If less than 300 or 400 μ l of unknown sera were used, it was necessary to add sufficient dog serum so that the total serum in all tubes (standards and unknowns) was identical. The minimal detectable quantity of HFSH with this assay technique was usually 5-7 mIU/ml of serum. Samples giving responses of over 90% were reported as undetectable.

Table II gives the HFSH concentrations obtained from eugonadal men, eugonadal women outside the mid-cycle, postmenopausal women, and children under age 7. The eugonadal men and women had similar values, ranging from 5 to 25 mIU/ml.⁹ Serum from postmenopausal women, or castrate men or women, contained between 30 and 250 mIU/ml. A single serum HFSH determination was usually sufficient to differentiate between patients with primary gonadal failure and patients with hypogonadotropic hypogonadism or eugonadal subjects.

Fig. 4 depicts the serum HLH and HFSH levels in a 58 yr old castrate woman. After 3 control days with no treatment, 0.4 mg/day of ethinyl

⁹ Milliinternational units per ml.

estradiol were administered orally. Both HLH and HFSH fell progressively. It is interesting that the rate of fall of these hormones was relatively slow, requiring for HLH about 4 days to reach a fairly constant level, and for HFSH about 7 days. We have observed this pattern for decline for HFSH and HLH in eight other patients. In one patient given a large dose (15 mg) of diethylstilbestrol

TABLE II
HFSH Concentrations

| Sex | Gonadal status | HFSH mIU/ml* |
|---------------------------|-------------------------------------|-----------------|
| Male | Eugonadal | 5-25 |
| | Castrate | >30 |
| Female | Follicular phase menstrual cycle | 7-25 |
| | Luteal phase menstrual cycle | 5-15 |
| | Postmenopausal | 40-250 |
| Children, boys & girls | Less than 7 yr | <5 |

* Milliinternational units per milliliter.

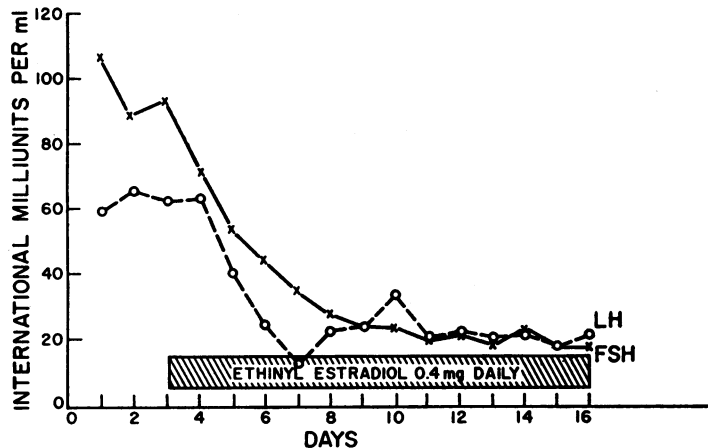


FIGURE 4 Decrease in HFSH (FSH) and HLH (LH) in serum from a 58 yr old castrate woman during 3 control days and during treatment with 0.4 mg of ethinyl estradiol daily. Note the fall in FSH over 8 days. Also note that LH and FSH are both detectable on this dose of estrogen.

orally, HLH levels fell from 55 to 20 mIU/ml in 6 hours, but HFSH fell relatively slowly as in the study depicted in Fig. 4. The cessation of pituitary secretion of HLH and HFSH upon administration of large doses of estrogen appears to be slower than cessation of thyrotropin secretion after large doses of thyroxin (17) or of growth hormone after elevation of blood glucose (18). The half-times of disappearance of thyrotropin (19) and HLH (reference 20 and footnote 10) are similar, but the half-time of HFSH in humans has not yet been determined. Note also that the levels of HFSH and HLH did not become undetectable on these large doses of oral estrogens. In 12 other castrate or postmenopausal women treated with either 0.4 mg of ethinyl estradiol or 15 mg of diethylstilbestrol daily the serum levels of HFSH and HLH fell to the range found in eugonadal men or women outside the mid-cycle.

Fig. 5 illustrates suppression of serum HFSH and HLH in a 72 yr old castrate male treated for metastatic carcinoma of the prostate with 15 mg of diethylstilbestrol daily. As in the women, HLH and HFSH levels fell but *did not become undetectable*. The rate of fall, as discussed previously, was relatively slow. Note also the relative constancy of serum HLH and HFSH levels at the low level.

Serum HFSH and HLH concentrations have

¹⁰ The disappearance of HLH over 24 hr was not linear but curvilinear. We were thus unable to calculate a half-time. However, taking the disappearance during the first 4-6 hr and assuming linearity, we obtained a half-time value similar to that we previously reported for HTSH.

been measured once daily for 5-30 days in 10 eugonadal men. These hormones were constant from day to day, mimicking the levels we have observed in women during the late follicular phase of the menstrual cycle. No elevation peaks of HLH or HFSH levels have been observed in serum from men.

Nineteen normal women taking no medications had HFSH quantified daily throughout the course of a menstrual cycle. These studies are summarized in Fig. 6 and Table III. Each of these women had a sharp mid-cycle peak of HLH, a finding which confirmed that these were normal menstrual cycles. The height of this HLH peak, the day of its occurrence as well as both follicular and luteal phase HLH levels, were similar to those we have previously described for other patients (2, 6). Fig. 6 depicts the range of HLH values obtained

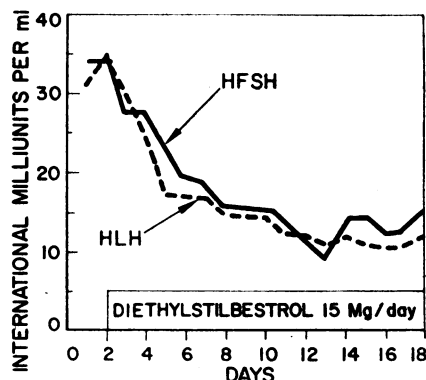


FIGURE 5 Decrease in HFSH and HLH in serum from a 72 yr old castrate man receiving 15 mg of diethylstilbestrol daily. Note that both HLH and HFSH levels fell but both were still detectable after 18 days of treatment with this large dose of estrogen.

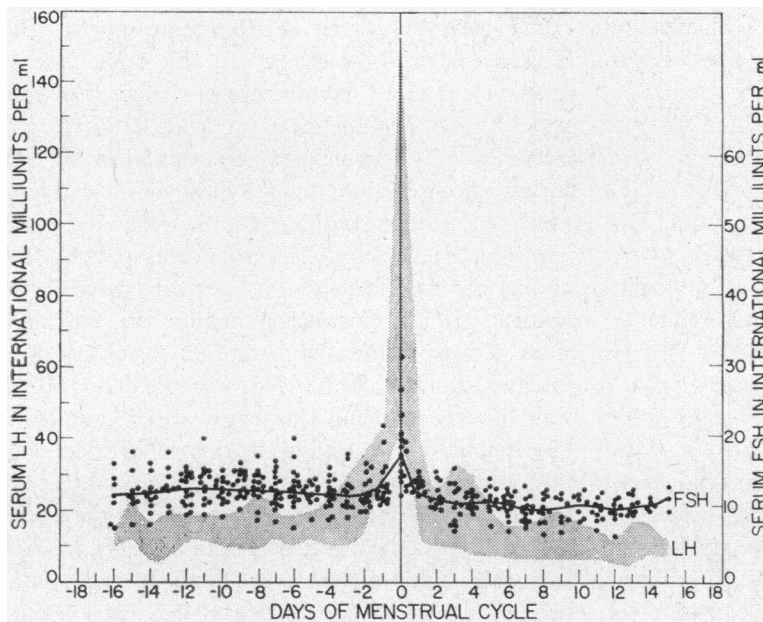


FIGURE 6 Scattergram of serum HFSH values during the menstruation cycle in 19 normal women. Data from all patients were plotted by placing the LH peak on the day 0 line. The shaded area encompasses all of the LH observations. Each dot represents an HFSH determination. The very small rise in HFSH observed early in the follicular phase of the cycle is obscured by this method of plotting. See the Results for further details.

in the 19 women in whom HFSH was simultaneously quantified. These HLH levels ranged from 6 to 26 (mean = 16) mIU/ml during that portion of the follicular phase preceding the HLH peak by more than 5 days, and from 6 to 21 (mean = 12) mIU/ml during the portion of the luteal phase more than 5 days after the HLH peak.

In any one patient the average luteal phase levels were less than follicular phase levels. At mid-cycle a sharp peak of HLH occurred ranging from 40 to 153 mIU/ml. In some patients a smaller HLH peak occurred a day or 2 after the mid-cycle peak (Fig. 9). HFSH levels ranged from 7 to 20 mIU/ml before the HLH

TABLE III
Plasma HFSH Levels in 13 Women Measured Daily during a Menstrual Cycle

| Subject | Cycle length (M ₁ - M ₂) | Day of HLH peak (value*) | Mean† HFSH‡ before HLH peak | Mean HFSH‡ after HLH peak | Highest value of HFSH* | Highest HFSH coincident with HLH peak |
|---------|---|--------------------------|-----------------------------|-----------------------------|------------------------|---------------------------------------|
| 1 | 41 | 33 (111) | 13.0 ± 0.49 | 10.8 ± 0.32 | 17.9 | No (10 days before) |
| 2 | 31 | 16 (54) | 9.4 ± 0.67 | 9.6 ± 0.13 | 14.3 | Yes |
| 3 | 30 | 13 (70) | 10.6 ± 0.61 | 9.8 ± 0.80 | 13.5 | Yes |
| 4 | 29 | 14 (54) | 11.7 ± 0.60 | 11.2 ± 0.49 | 17.9 | Yes |
| 5 | 29 | 16 (45) | 10.7 ± 0.52 | 8.6 ± 1.10 | 27.4 | Yes |
| 6 | 22 | 17 (82) | 7.8 ± 0.72 | 6.7 ± 0.61 | 11.3 | No (12 days before) |
| 7 | 21 | 14 (40) | 12.8 ± 1.40 | 11.3 ± 0.87 | 17.9 | No (6 days before) |
| 8 | 26 | 18 (74) | 9.7 ± 0.60 | 9.6 ± 1.81 | 11.3 | Yes |
| 9 | 26 | 18 (56) | 11.3 ± 0.58 | 9.2 ± 1.20 | 12.2 | No (11 days before) |
| 10 | 33 | 19 (112) | 10.3 ± 0.50 | 9.2 ± 0.51 | 12.6 | Yes |
| 11 | 35 | 22 (153) | 11.1 ± 0.58 | 9.6 ± 0.65 | 17.9 | Yes |
| 12 | 33 | 20 (74) | 12.1 ± 0.67 | 11.0 ± 0.74 | 14.3 | No (1 day after) |
| 13 | 30 | 17 (89) | 12.8 ± 0.68 | 11.6 ± 1.40 | 23.0 | Yes |

* All values in international milliunits per milliliter of Second International Reference Preparation of Human Menopausal Gonadotropin (IRP-HMG No. 2). This preparation is impure and serves as reference standard for both HLH and HFSH.

† Calculated from all HFSH values up to 1 day before HLH peak.

‡ Mean ± SEM. In all 13 patients the mean difference in HFSH before and after the HLH peak is negative (1.25) and significantly different from zero at $P = < 0.01$.

|| Calculated from all HFSH values starting day 1 after HLH peak.

peak (mean = 11) and from 7 to 15 (mean = 9) after the HLH peak. Follicular phase levels of HFSH were significantly ($P = < 0.05$) higher than luteal phase levels in 7 of 13 patients (Table III). If the follicular phase were divided into two equal portions and HFSH levels compared between these two times, the early follicular phase levels were equal to or greater than (but never less than) late follicular phase levels. On an average, first-half follicular phase levels were 8% (range 0-30%) higher than second-half follicular phase levels ($P = < 0.05$). In any one subject such differences were significantly different ($P = < 0.05$) in only 23% of the subjects studied. Inasmuch as the cycle length varied from subject to subject (Table III) a mean curve from all 19 subjects obscures these small differences. Fig. 7 illustrates data from one patient in whom first-half follicular phase levels were significantly ($P = < 0.05$) greater than second-half follicular phase levels. These HFSH levels in the two patients illustrated in Figs. 8 and 9 were not significantly different. A small increase in HFSH activity was seen in all patients near mid-cycle. These mid-cycle levels of HFSH ranged from 11 to 33 mIU/ml. The highest

HFSH level occurred on the same day as the HLH peak in 14 patients. In the other five it occurred up to 12 days before or 1 day after this peak. Fig. 8 depicts the data from a 28 yr old woman. Note the considerable variation in HFSH during the follicular phase with two elevations which were only slightly less than the mid-cycle rise. The HLH peak observed in this patient was among the lowest, and this patient showed the greatest HFSH fluctuation during the follicular phase of any women studied. Fig. 9 depicts data obtained from a 24 yr old women. The HLH peak observed during this cycle was 152 m μ /ml. The height of the mid-cycle rise in HFSH was only slightly higher than that in the subject of Fig. 8, but in the latter subject LH rose to 58 m μ /ml. Thus, the ratio of HLH:HFSH at this time was quite different in these two patients. The physiological significance of this ratio is unknown at present. Typical effects of treatment with a nonsequential oral contraceptive on these serum HLH and HFSH levels are illustrated in Fig. 10. We have previously reported the effects of such treatment on HLH levels (7). Note that the mid-cycle rise in both HFSH and HLH failed to occur.

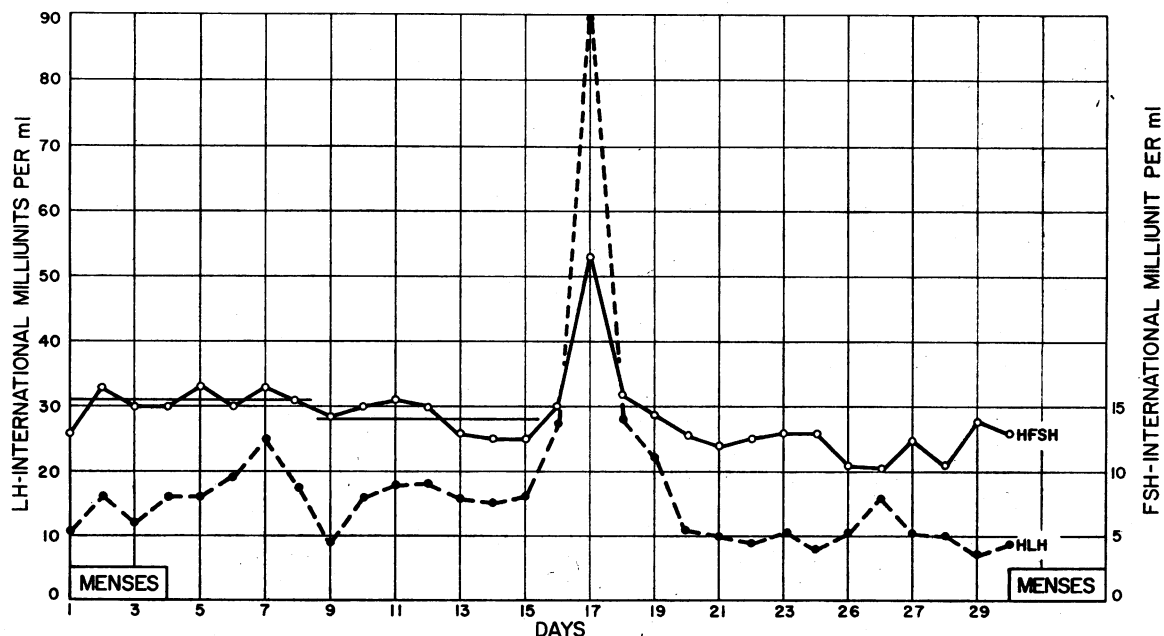


FIGURE 7 Daily serum HLH and HFSH levels in a 24 yr old woman in whom the first-half follicular phase levels were significantly greater than the second-half follicular phase levels. The horizontal lines indicate the means of each half of the follicular phase. See Results for further details.

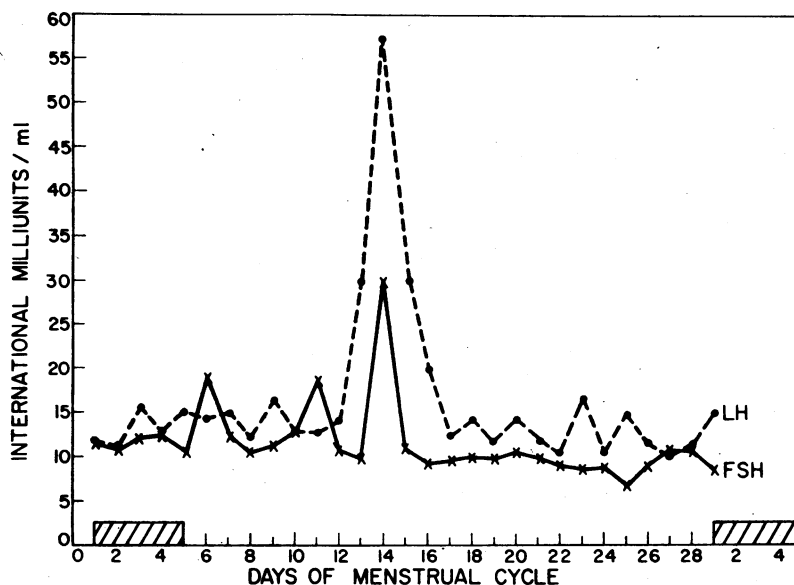


FIGURE 8 Daily serum HLH and HFSH levels in a 28 yr old woman. Shaded areas indicate days of menstrual flow. Note the irregular, smaller rise in HFSH observed in the follicular phase and the difference in HLH:HFSH ratios observed during the mid-cycle ovulatory peak when compared with the data in Fig. 9.

DISCUSSION

Bioassays thought to be specific for HFSH are based on an increase in ovarian or uterine weight in rats or mice pretreated with HCG (21, 22). As originally described (21) the ovarian augmentation assay was relatively insensitive, having a minimal effective dose of about 1.0 IU. By contrast, the radioimmunoassay of HFSH described herein is capable of quantifying as little as 0.001 IU. This radioimmunoassay was shown to be

highly specific for HFSH. Highly purified preparations of HLH and HTSH showed no significant reaction in the assay when added in amounts that for HFSH gave maximal reaction. Furthermore, bioassay and immunoassay of preparations of various degrees of purity and having varying HLH:HFSH ratios were in good agreement unless this ratio was extremely high as in highly purified HLH.

Our previous studies of serum HLH changes

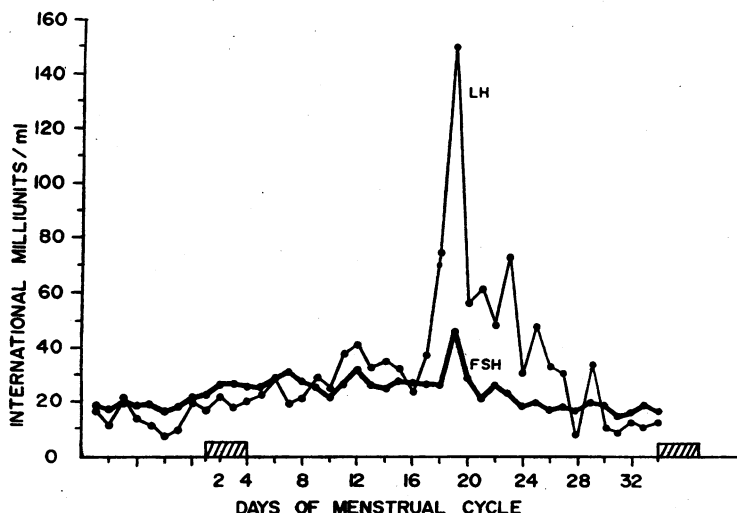


FIGURE 9 Daily HFSH and HLH concentrations from a 24 yr old woman. Shaded areas indicate days of menstrual flow. Note the difference in HLH:HFSH ratios in mid-cycle in the patient depicted in this figure compared with that depicted in Fig. 8.

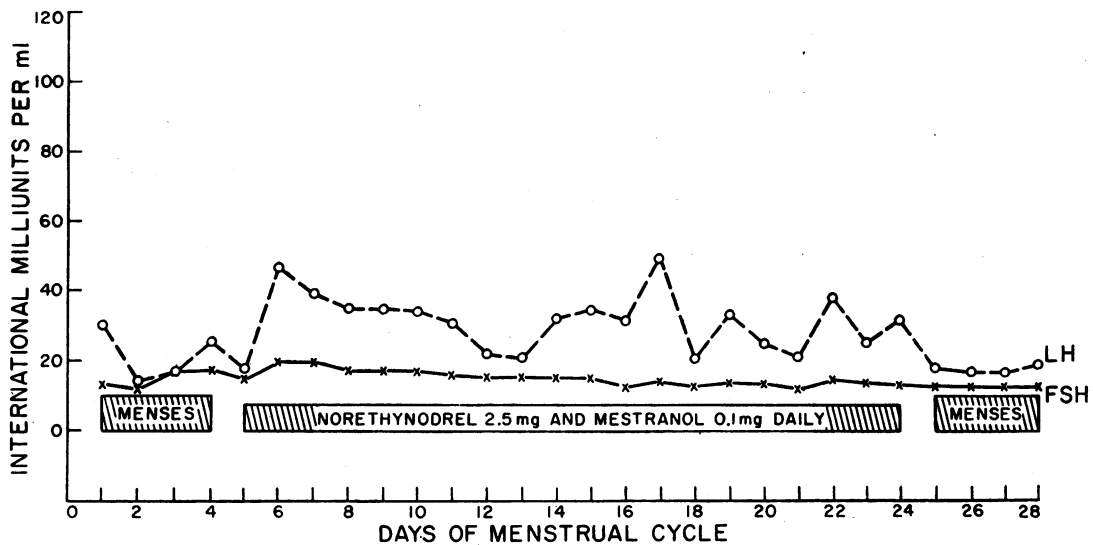


FIGURE 10 Serum HFSH (FSH) and HLH (LH) in a 36 yr old woman receiving norethynodrel, 2.5 mg, and mestranol, 0.1 mg daily. Note that both hormones are detectable but have little fluctuation during the 28 days of the study. No LH or FSH peaks are seen. Compare with Figs. 6-9.

during the menstrual cycle supported the general pattern of changes reported using bioassays or immunoassays of urine concentrates (23-30). However, the changes observed with urine were blunted or less striking than those in blood. Bioassay studies of HFSH on urine concentrates obtained throughout the menstrual cycle have also been reported, but results are conflicting. Fukushima, Stevens, Gantt, and Vorys (23) and Stevens, Vorys, Besch, and Barry (24) for example, reported high HFSH excretion during the late luteal and early follicular phases and no mid-cycle elevations. McArthur, Worcester, and Ingersoll (25), Buchholtz (26), and Rosenberg and Keller (27), and Becker and Albert (30) on the other hand have reported a mid-cycle elevation in the urinary excretion of HFSH. The results reported herein indicate that a very small elevation may occur in the early follicular phase, but no luteal phase elevations were observed. A mid-cycle rise in HFSH was consistently observed.

The values of HFSH we report herein may at this time be compared with a few published by other authors using radioimmunoassay methods. Midgley in a preliminary communication (13) reported values on sera from six adult males to range from 3.5 to 8.6 mIU/ml, and from 11 postmenopausal women to range from 5.3 to 245.0 mIU/ml. Saxena, Demura, Gandy, and Peterson

(31) reported in a recent publication that sera from normal men contained 3.9-42 mIU/ml and postmenopausal women 34.7-217 mIU/ml. Sera from eugonadal women contained 7-27.2 mIU/ml during the luteal phase and 16.8-31.7 during the follicular phase. Faiman and Ryan (14) in a preliminary communication reported sera from normal men contained from < 2.7 to 12.2¹¹ mIU/ml, and from postmenopausal women, 45.2-77.1 mIU/ml. All of these values may be compared to those we found by referral to Table II. The range of values we have observed in normal men is wider than these other authors. This difference is most likely explained by the larger population we have examined with a broader age group. We have found sera from many men over age 40 to contain more HFSH than from men in their 20's. These previous authors evaluated younger men. The values reported by most of these authors for postmenopausal women is in good agreement, except for the lower limit reported by Midgley in his preliminary publication (13). We have never observed values this low in postmenopausal women unless they were taking estrogens. We found HFSH levels during the luteal phase of the menstrual cycle to be generally lower than those dur-

¹¹ Faiman and Ryan (14) gave values in terms of NIH-FSH-S1. We have translated these to the IRP-HMG No. 2 by multiplying by 26.6 IU. (32).

ing the follicular phase. Saxena et al. (31) reported the opposite. The reason for this difference between the data is not presently known.

The relationship of these HFSH and HLH changes to ovarian follicle development, ovulation, and corpus luteum formation is not presently known. However, based on our data the following speculations might be made. Follicular growth occurs in response to low levels of both HFSH and HLH. Early follicular phase HFSH levels are slightly higher than late follicular phase levels, and possibly a small difference is important in early follicle growth. At mid-cycle the large surge of HLH and small rise in HFSH results in ovulation and corpus luteum formation. The cause of the HLH peak at mid-cycle is presently unknown, and it is uncertain whether an ovarian signal is involved in its timing. Once formed, the maintenance of the corpus luteum and the secretion of progesterone in large amounts do not apparently require high levels of HFSH and HLH; in fact, we found luteal phase levels to be lower than follicular phase levels of both of these hormones. Furthermore, cessation of corpus luteum function apparently does not result from a fall in either blood HFSH or HLH concentration, for none was observed. This would suggest that the corpus luteum, once formed, has a finite life of its own, functioning and regressing independent of blood HFSH and HLH concentrations. This picture of corpus luteum function has previously been supported for the sow and ewe (33, 34). One interesting implication of the findings reported herein is that an important aspect of regulation of ovarian function probably resides in the ovary itself. Both follicular growth and maintenance of corpus luteum function do not require either changing or high levels of HFSH and HLH. Another interesting question arising from the observation that HFSH levels are higher at mid-cycle is whether such a rise is required along with the HLH peak to produce ovulation. Luteal phase HLH and HFSH levels are lower than during follicular phase. The reasons for this are not answered by these studies, but of course, one possibility is that progesterone and estrogen secretion (luteal phase) by the ovary is capable of suppressing pituitary secretion of these hormones to a greater extent than estrogen alone (follicular phase). Alternatively, pituitary secretion per se may be slightly

less after the mid-cycle HLH:HFSH peaks because of a decrease in central nervous system releasing factors, independent of correlating gonadal steroid levels.

Prolactin has been shown to have important regulatory actions on corpus luteum function in rats (35, 36). Its role in any endocrine function in humans except lactation is presently unknown. The previously mentioned studies in the sow and ewe (33, 34) indicates that at least in these animals prolactin is not required for corpus luteum function. The role of prolactin in human ovarian control remains unknown.

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