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Gonadotropin-Steroid Interrelationships*

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THE use of radioligand methods for the measurement of insulin by Yalow and Berson in 1959 (113), for thyroxin by Ekins in 1960 (22) and for cortisol by Murphy *et al.* in 1963 (73) antedated by several years their use for the determination of gonadotropins and gonadal hormones. Radioimmunoassays to measure gonadotropins in human serum or plasma have been available since 1966 (table 1). For the luteinizing hormone (LH) assay, either LH itself or human chorionic gonadotropin (HCG) may be used as an antigen to generate antibodies since there is cross reaction between anti-LH and anti-HCG. In most of the available methods, the separation of free and antibody-complexed LH is obtained by precipitation by the second antibody technique but several other approaches are in use. The antibody and its complex may be absorbed to special plastic discs (table 1, method 14) while other variations of solid phase radioimmunoassay have been designed more recently with immuno-absorbing Sephadex (methods 20, 22) or cyanobromide-activated microcrystalline cellulose (method 21). In still other methods, the antibody complex is precipitated by dioxane (method 16) or the free and bound LH are separated by chromatoelectrophoresis (method 17). Charcoal has also been used as an adsorbent for free LH (method 9). Most recently, methods were designed to use, instead of antibodies, receptor proteins of rat testes homogenates (11, 52).

Antisera suitable for radioimmunoassay of follicle stimulating hormone (FSH) can be raised by immunizing animals to purified human FSH. Such antisera usually show considerable cross reaction with LH and HCG and can be used for assay purposes only after adsorption with these compounds. Available methods to assay FSH by radioimmunoassay techniques are listed in table 2. Details pertaining to the radioimmunoassay of gonadotropins and of the steroid hormones may be found in the reviews of Lunenfeld and Eshkol (58) and of Stevenson and Loraine (94) and in the proceedings of several workshop conferences and symposia (16, 17, 48, 76, 82).

Techniques to measure progesterone and related compounds are listed in table 3. Progesterone competes effectively with corticosterone bound to corticosteroid binding globulin (CBG) of human and animal blood and this approach was initially used for the measurement of this steroid. This earlier method still has the advantage of being simple and reliable; however, its sensitivity does not extend beyond the nanogram range. When higher sensitivity is required, such as in studies involving males, or females in the preovulatory phase, a radioimmunoassay is the method of choice.

Radioligand methods to measure estradiol and estrone are listed in table 4. In most clinical situations only radioimmunoassays will give sufficient sensitivity but other methods with uterine cytosol preparation and the estradiol binding globulin (SH-BG) of blood have contributed valuable information. The most frequently used antibody is made by immunization with estradiol-17β-hemisuccinate-bovine serum albumin (BSA) (53) and cross reacts rather completely with estrone and only to a minor extent with estriol, but the degree of cross

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TABLE 1

Radioimmunoassay of luteinizing hormone (LH)

1.	Midgley and Jaffe, 1966 (65)
2.	Midgley, 1966 (63)
3.	Odell *et al.*, 1966 (80)
4, 5.	Franchimont, 1966 (26, 27)
6.	Wide and Porath, 1966 (110)
7, 8.	Odell *et al.*, 1967 (79, 81)
9.	Neill *et al.*, 1967 (74)
10.	Faiman and Ryan, 1967 (25)
11.	Aono *et al.*, 1967 (5)
12.	Franchimont, 1968 (28)
13.	Donini *et al.*, 1968 (20)
14.	Catt *et al.*, 1968 (12)
15.	Dolais *et al.*, 1968 (19)
16.	Thomas and Ferin, 1968 (100)
17.	Saxena *et al.*, 1968 (91)
18.	Yen *et al.*, 1968 (114)
19.	Jaffe and Midgley, 1969 (43)
20.	Miyata *et al.*, 1971 (69)
21.	Franchimont, 1971 (29)
22.	Goldstein *et al.*, 1972 (34)

TABLE 2

Radioimmunoassay of follicle stimulating hormone (FSH)

1.	Franchimont, 1966 (27)
2, 3.	Faiman and Ryan, 1967 (24, 25)
4.	Midgley, 1967 (64)
5.	Odell *et al.*, 1967 (79)
6.	Rosselin and Dolais, 1967 (88)
7.	Odell *et al.*, 1968 (78)
8.	Thomas and Ferin, 1968 (100)
9.	Butt and Lynch, 1968 (10)
10.	Saxena *et al.*, 1968 (91)
11.	Aono and Taymor, 1968 (4)
12.	Franchimont, 1968 (28)
13.	Yen *et al.*, 1970 (115)
14.	Rosselin *et al.*, 1971 (89)
15.	Franchimont, 1971 (29)

reaction will vary considerably between antisera. In an attempt to obtain antibodies specific for estradiol, antigens in which coupling between protein and estradiol is to the sixth position of estradiol (51) have been used (table 4, method 13).

A number of radioligand methods have been designed for measuring serum androgens (table 5). So far, these have not been extensively applied to the study of the menstrual cycle. In contrast to the radioligand methods for protein hormones for progestins, estrogens and androgens, it is necessary to pretreat plasma samples (extraction, defatting, chromatography) before they are subjected to the radioligand procedure. Since these manipulations influence blank values as well as parallelism of standard curves, great attention should be given to these steps in the determination of steroids by radioimmunoassay. Although the motions involved in these techniques are relatively few and simple and do not require real chemical skill of the technician, it should be emphasized that the conduction of reliable assays requires a great deal of judgment. The same factors which make for the extreme sensitivity of the assays also bring about a very undesirable variability of the assay systems. Most of the solutions contain protein: the antibody, gelatin, γ-globulins, serum proteins, or others, and these undergo constant, slight changes even when bacteriostatic agents are added. Solutions must be carefully dated and their "lifetime" must be rigidly adhered to. Some of the problems connected with the protein nature of the hormone to be measured will be dealt with in this symposium. The other very sensitive component is the radioisotopically-labeled hormone, be this radioiodinated gonadotropin or tritiated steroid. Small deteriorations in this component can drastically alter the standard curves. Steroids are best purified in small portions about once monthly and may be kept in ethanol-benzene mixtures. But once they are in a buffer solution, it is best not to use them for longer than 5 days.

The introduction of radioligand methods has made it possible to move the measurement of gonadotropins and steroids out of the microgram range into the range of the nanogram and picogram. The significance of the contribution of these new analytical methods to the better understanding of reproductive function becomes apparent when the progress made in the last 5 years is contrasted to the evolution in this field up to the 1960's. It is interesting to note how

TABLE 3

Assay of progesterone and related compounds

Steroid	Protein	Separation	Sensitivity in PG	Reference
1. Progesterone	CBG*	Florisil	200	Neill *et al.* (74)
2. Progesterone	CBG	Sephadex G-25	100	Yoshimi and Lipsett (118)
3. Progestins	CBG	Florisil	200	Johansson (44)
4. Progesterone	CBG	Florisil	100	Johansson (45)
5. Progesterone	CBG	Florisil	200	Reeves *et al.* (83)
6. Progesterone	CBG	Florisil	100	Stone *et al.* (96)
7. Progesterone	Anti-P	Ammonium sulfate	25	Furuyama and Nugent (31)
8. Progesterone	Anti-S†	Charcoal	25	Abraham *et al.* (3)
9. Progesterone	Anti-P‡			Monroe *et al.* (70)
10. 17α-Hydroxyprogesterone	CBG	Florisil	250	Strott and Lipsett (97)
11. 17α-Hydroxyprogesterone	CBG	Non-polar Sephadex	200	Holmdahl and Sjövall (38)
12. 17α-Hydroxyprogesterone	CBG	Florisil	500	Stewart-Bentley and Horton (95)
13. 17α-Hydroxyprogesterone	Anti-S†	Charcoal	50	Abraham *et al.* (2)
14. 20α-Dihydroprogesterone and progesterone	CBG	Florisil	200	Rubin *et al.* (90)
15. Δ^5-Pregnenolone	CBG	Florisil	300	Bermudez *et al.* (9)
16. 17α-Hydroxy-Δ^5-pregnenolone	CBG	Florisil	100	Strott *et al.* (98)
17. 17α-Hydroxy-Δ^5-pregnenolone	Anti-D§	Charcoal	200	Loriaux and Lipsett (56)

* CBG, corticosteroid binding globulin of the blood.

† Antiserum raised against 11-desoxycortisol-21-monosuccinate coupled to human serum albumin.

‡ Antiserum raised against progesterone-11-albumin. Tracer is ^{125}I labeled progesterone-11-tyrosine-methyl ester.

§ Antiserum raised against dehydroisoandrosterone-17(O-carboxymethyl) oxime coupled to serum albumin.

each new phase in our understanding of reproductive endocrinology started with a methodological breakthrough. What may be termed the modern period of ovarian hormone research started in the early 1920's when the development of suitable bioassays made it first possible to isolate and to elucidate the structures of estrone, estradiol, estriol, pregnendiol, and progesterone. The introduction of chromatographic and radioisotope methods led to the possibility of measuring the urinary metabolites of the ovarian hormones and to establishing patterns of their fluctuations during the normal menstrual cycle. A typical example of studies belonging to this era is shown in figure 1. Estrogens are low at the time of menses and reach a first peak at the time of midcycle followed by a second peak during the luteal phase. Pregnendiol, the main metabolite of progesterone is excreted in very small amounts during the preovulatory phase, rises at the time of ovulation to reach a peak coincident with the luteal peak in the excretion of estrogens. There was much less certainty about the evolvement of the gonadotropins. Most studies reported a midcycle peak for LH, but there was considerable controversy about the fluctuations in FSH.

Changes in the level of the urinary metabolites reflect only very incompletely changes in the secretory processes since several hormonal precursors may contribute to the same urinary metabolite: pregnendiol, for instance, derives from the secretion of pregnenolone by the adrenal as well as from the secretion of progesterone by the ovaries;

TABLE 4

Estrogen assays

Sterod	Protein	Separation	Sensitivity in PG	Reference
1. Estradiol, estrone	Uterine cytosol	Charcoal	20	Korenman (49)
2. Estradiol	Uterine cytosol	Charcoal	20	Korenman *et al.* (50)
3. Estradiol	Uterine cytosol	Charcoal, Sephadex	100	Shutt (93)
4. Estradiol	SH-BG*	Ammonium sulfate	250	Mayes and Nugent (62)
5. Estradiol, estrone	Anti-E_2†	Solid phase	10	Abraham (1)
6. Estradiol	Anti-E_2	Double antibody	100	Midgley *et al.* (67)
7. Estrone	Uterine cytosol	Charcoal	25	Tulchinsky and Korenman (105)
8. Estradiol	Uterine cytosol	Charcoal	4	Corker and Exley (15)
9. Estradiol	SH-BG	Ammonium sulfate	250	Mayes and Nugent (61)
10. Estradiol	SH-BG	Charcoal	50	Dufau *et al.* (21)
11. Estradiol, estrone	Anti-E_2†	Antibody polymerization	50	Mikhail *et al.* (68)
12. Estradiol	Anti-E_2†	Charcoal	5	Hotchkiss *et al.* (40)
13. Estradiol	Anti-E_2	Solid phase		Exley *et al.* (23)
14. Estradiol	Anti-E_2	Charcoal	30	Shaikh (92)
15. Estradiol, estrone	Anti-E_2†	Charcoal	20	Wu and Lundy (112)
16. Estradiol	Anti-E_2	Solid phase	75	Moore and Axelrod (71)
17. 2-Hydroxyestrone	Anti-2.OH.E_1	Charcoal	4	Yoshizawa and Fishman (119)
18. Estrone sulfate	Anti-E_2	Charcoal	100	Loriaux *et al.* (57)
19. Estriol	Anti-E_3‡	Ammonium sulfate	200	Gurpide *et al.* (35)
20. Estriol	Anti-E_3§	Charcoal	25	Tulchinsky and Abraham (104)
21. Estriol	Uterine cytosol	Charcoal	100	Tulchinsky *et al.* (106)
22. Estetrol¶	Anti-E_3‡	Ammonium sulfate	200	Giebenhain *et al.* (33)

* SH-BG, sex hormone binding globulin of plasma which binds both estradiol and testosterone. The usual source for this protein is last trimester pregnancy plasma.

† Antiserum raised against estradiol-17β-hemisuccinate-BSA.

‡ Antiserum raised against estriol-16,17-disuccinyl-BSA.

§ Antiserum raised against estriol hemisuccinate-HSA.

¶ 1,3,5(10)-Estratrien-3,15,16,17-tetrol.

urinary estrone derives not only from estrone and estradiol secreted by the ovary, but also *via* peripheral conversion from Δ^4-androstenedione secreted either by the ovary or by the adrenal. Additional uncertainties are brought into the picture by the fact that some metabolites are cleared more rapidly than others making deductions about timing of secretions of their precursors difficult. As far as gonadotropins are concerned, it was impossible at this time to study short-time fluctuations because of the insensitivity of the available bioassay methods. It was necessary to use the extract of several days urine to have precise results making short-time studies impossible.

In the 1950's, the interest of many investigators turned away from the problems of steroid metabolism and steroid excretion to that of steroid dynamics. Interest lay in the measurement of secretory and production rates of the hormones and of their rates of interconversion. Such studies became possible after the pioneering work of Tait (99) at the Worcester Foundation and of Gurpide *et al.* (36), then at Columbia, whose theoretical studies led to the development of new experimental approaches. The fundamental question, namely, that of the control of the menstrual cycle, could, however, not be approached until the advent of the radioimmunoassays. By their sensitivity these assays finally made it possible to measure simultaneously, and at hourly or even

TABLE 5

Androgen assays

Steroid	Protein	Separation	Sensitivity in PG	Reference
1. Testosterone	SH-BG*	Charcoal	(5 ng)	Horton *et al.* (39)
2. Testosterone	SH-BG	Sephadex G-25	250	Kato and Horton (47)
3. Testosterone	SH-BG	Ammonium sulfate	250	Mayes and Nugent (60)
4. Testosterone	SH-BG	Florisil	100	Halberg *et al.* (37)
5. Testosterone	SH-BG	Charcoal	250	Rosenfield *et al.* (86)
6. Testosterone	SH-BG	Ammonium sulfate	200	Maeda *et al.* (59)
7. Testosterone	SH-BG	Florisil	(3 ng)	Frick and Kincl (30)
8. Testosterone	SH-BG	Charcoal	300	August *et al.* (6)
9. Testosterone	SH-BG	Sephadex G-25	500	Uettwiller (107)
10. Testosterone	SH-BG	Ammonium sulfate	120	Winters and Grant (111)
11. Testosterone	SH-BG			Concolino and Marocchi (14)
12. Testosterone	SH-BG	Ammonium sulfate	200	Benraad *et al.* (8)
13. Testosterone	Anti-T†	Ammonium sulfate	10	Furuyama *et al.* (32)
14. Testosterone	Anti-T‡	Ammonium sulfate	10	Ismail *et al.* (41)
15. Testosterone	Anti-T†	Charcoal	10	Collins *et al.* (13)
16. Δ^4-Androstenedione (*via* testosterone)	SH-BG	Charcoal	250	Rosenfield (84)
17. Δ^4-Androstenedione (*via* testosterone)	SH-BG	Charcoal	250	Moshang *et al.* (72)
18. Dihydrotestosterone§	SH-BG	Sephadex G-25	200	Ito and Horton (42)
19. Dihydrotestosterone§	SH-BG	Florisil	200	Tremblay *et al.* (101)
20. Dehydroisoandrosterone and its sulfate	SH-BG	Charcoal	60	Rosenfield (85)
21. Dehydroisoandrosterone and its sulfate	Anti-D	Charcoal	100	Nieschlag *et al.* (75)
22. Δ^5-Androstenediol	Anti-D	Charcoal	200	Loriaux and Lipsett (56)

* SH-BG, sex hormone binding globulin of plasma which binds both estradiol and testosterone.

† Antiserum raised against testosterone-3-carboxymethyloxime-BSA.

‡ Antiserum raised against testosterone-3-oxime-BSA.

§ Antiserum raised against dehydroisoandrosterone coupled to BSA.

¶ 5α-Androstan-17β-ol-3-one.

shorter intervals, the levels of most of the gonadal hormones and of the gonadotropins. In this fashion the temporal relationship between the changes of all these hormones could be precisely determined and deductions about causal relationships made. The radioimmunoassays beyond their great sensitivity have the other advantage that they can be conducted with considerably less work and skill than the classical chemical methods, an advantage that becomes critical when time-series experiments involving hundreds of samples are carried out.

In figure 2 are illustrated the changes in plasma gonadotropins, estrogens and progesterone in two normal cycles, together with the basal body temperature curves of the women used in the study. Each cycle presents a very characteristic midcycle peak of luteinizing hormone which occurs in M.G. on day 12 and in A.E. on day 14 of the cycle. In both instances, the peak in LH occurs at the nadir of the basal body temperature curve. Plasma estrogens remain at base levels for approximately 1 week, then increase to a midcycle peak, the ascending limb of which coincides with the rise in LH. The second (luteal) maximum of the estrogens parallels the maximum of plasma progesterone. Figure 3 illustrates a study of Ross *et al.* (87) in which 17α-hydroxyprogesterone was measured together with the gonadotropins and

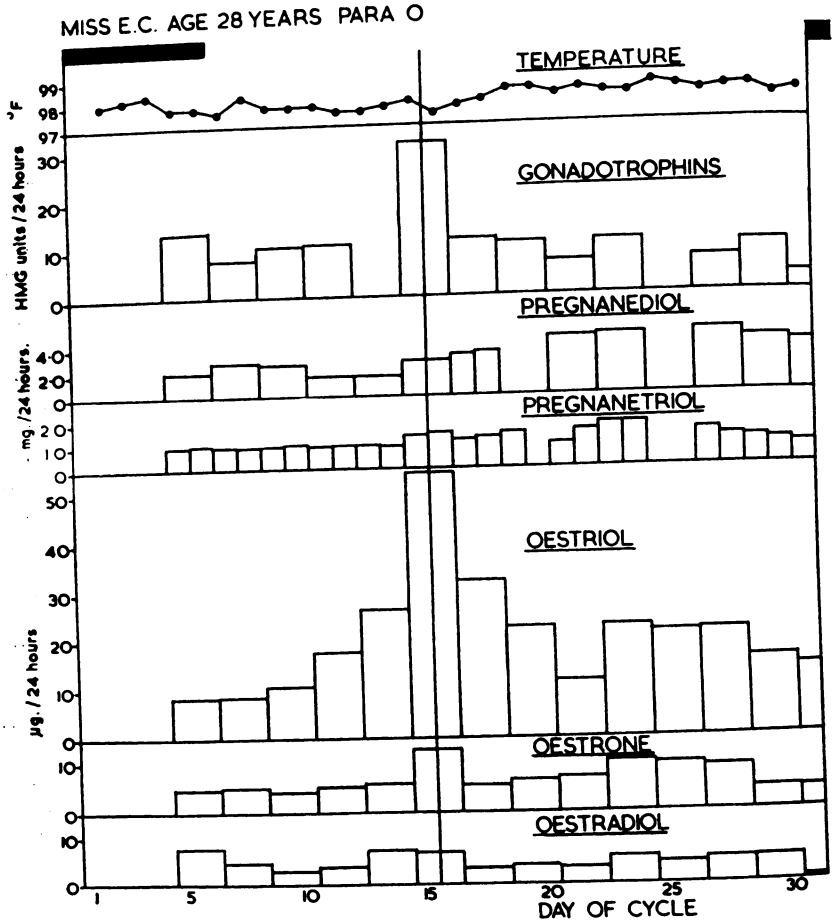

FIG. 1. Urinary hormone excretion during the normal menstrual cycle. (From J. A. Loraine and E. T. Bell: Hormone excretion during the normal menstrual cycle. Lancet 1: 1340–1343, 1963.)

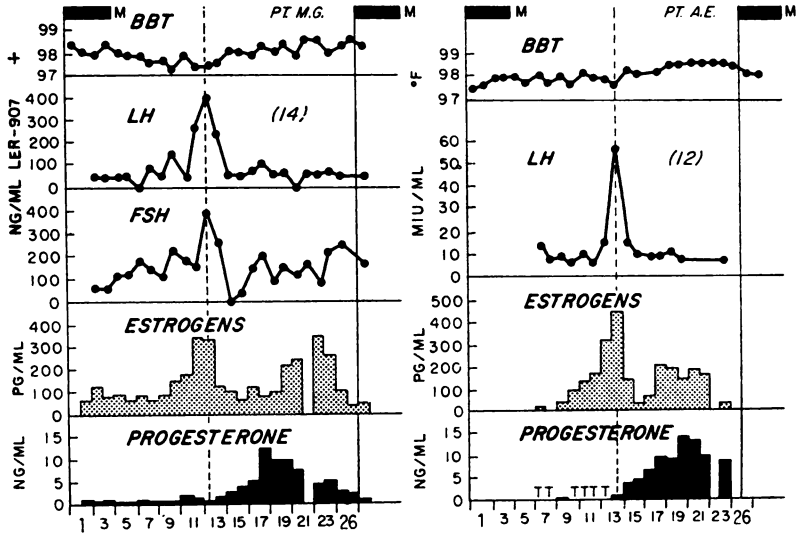

FIG. 2. Patterns of ovarian and pituitary hormones in the plasma of two healthy women during normal ovulatory cycles. Menses are indicated on top of the graph by black horizontal bars. The bracketed figure in the upper right of each pattern gives the number of days between the LH peak and onset of menses.

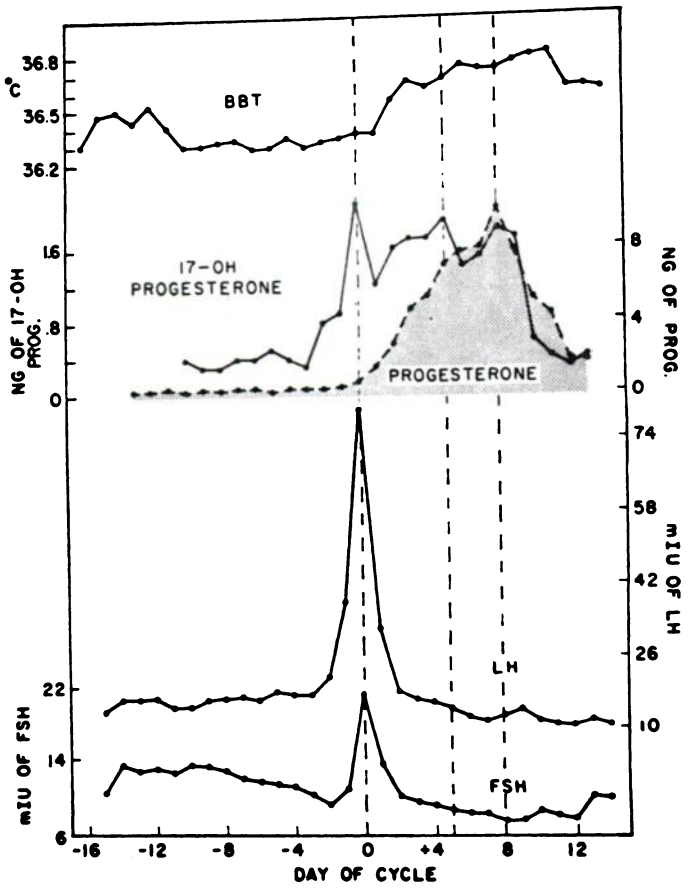

FIG. 3. Plasma levels of 17α-hydroxyprogesterone, progesterone, LH and FSH in normal menstrual cycles. (From G. T. Ross, C. M. Cargille, M. B. Lipsett, P. L. Rayford, J. R. Marshall, C. A. Strott and D. Rodbard: Pituitary and gonadal hormones in women during spontaneous and induced ovulatory cycles. Recent Progr. Hormone Res. 26: 1–48, 1970.)

progesterone and indicates that the course of 17α-hydroxyprogesterone during the menstrual cycle is similar to that of the estrogens.

Although radioimmunoassays are now available for the measurement of testosterone, androstenedione, and dehydroisoandrosterone, up to now there have been no systematic studies of changes in these androgens throughout the menstrual cycle. Lloyd *et al.* (54) have measured by double isotope method the hormonal content of ovarian vein blood collected at different phases of the cycle (fig. 4). Although only in small amounts, the normal ovary clearly secretes testosterone but the main androgen secreted by the ovary is Δ^4-androstenedione. As is the case with the estrogens, androstenedione seems to be secreted at the highest levels during the late proliferative phase and during the midluteal phase.

Figure 5 illustrates a composite of the curves of 40 normal menstrual cycles. The day of the LH peak was considered as day +1 and each day in the 40 cycles was superimposed with this orientation. The features common to all normal cycles appear clearly. The LH pattern is dominated by a midcycle peak but smaller elevations are seen pre- and postovulatory. The FSH levels are highest in the beginning of the cycle and approach a nadir 1 or 2 days before the initiation of a small midcycle peak which coincides with that of LH. The estrogens

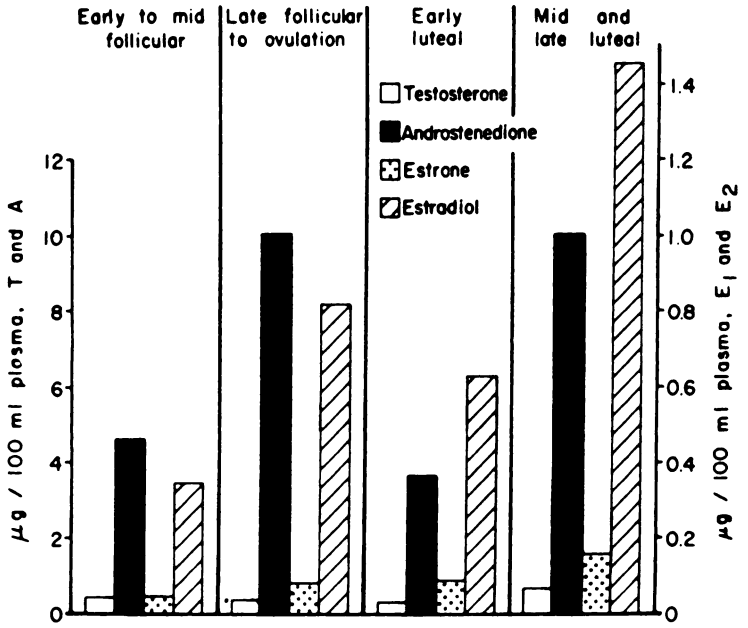

FIG. 4. Steroids in ovarian venous plasma of 18 normal women. (From C. W. Lloyd, J. Lobotsky, D. T. Baird, J. A. McCracken, J. Weisz, M. Pupkin, J. Zarrartu and J. Puga: Concentration of unconjugated estrogens, androgens and gestagens in ovarian and peripheral venous plasma of women: The normal menstrual cycle. J. Clin. Endocrinol. Metab. 32: 155–166, 1971.)

remain low for approximately a week, then begin a gradual rise which reaches its maximum a day before the LH peak. Although this way of plotting is useful in that it brings out the features that are common to all cycles it obliterates individual variations which by themselves provide clues for the understanding of details in the regulatory processes.

With this information about the hormonal changes has come new insight in the mechanisms controlling the phases of the cycle. The midcycle period has been studied most extensively since it comprises the time immediately before and after ovulation. The LH surge has been found by all investigators to precede follicular rupture and expulsion of the ovum. In fact, the recent studies of Yussman *et al.* (120) indicate an interval from 24 to 40 hr between the initial rise of LH and the rupture of the follicle. Much support is now available for the thesis that the estrogens act as a trigger for the LH peak. In all instances, the LH peak is preceded by a rise in the estrogens and in cases where estrogen levels have been measured at short intervals, there is always a peak or a spike in the estrogen pattern before the peak of LH (fig. 6). Furthermore, there have been several studies which clearly indicate that estrogens have a positive feedback effect upon the secretion of LH (70, 102, 103, 108, 116). Figure 7 shows such a study in which Premarin was given to four amenorrheic women, a treatment which in each instance produced a significant peak in LH. Under certain experimental conditions progesterone will also produce a rise in LH and this observation has led to the suggestion that progesterone rather than estrogens is the trigger of the ovulatory LH surge (77). However, careful analysis of the temporal relationship of the estrogens, progesterone, and LH rises indicate that in the normal cycle, the LH rise always precedes the progesterone rise. This point is clearly illustrated in figure 8 which is derived from the work of Johansson and Wide (46). These

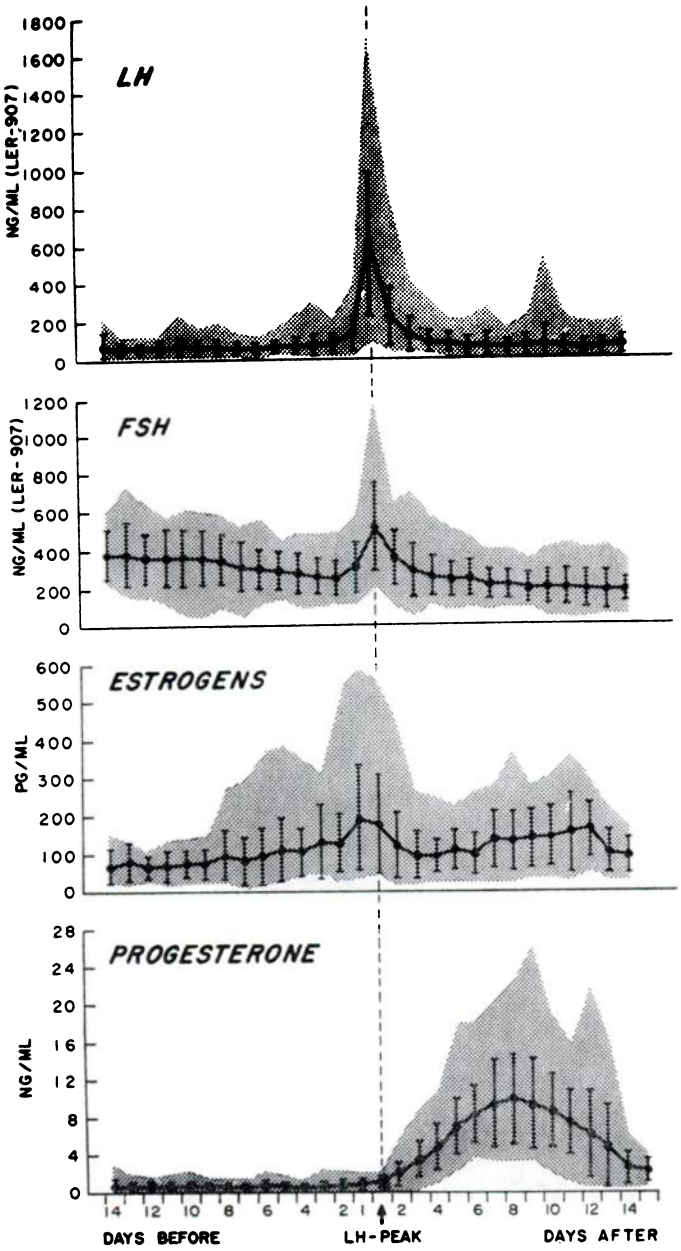

FIG. 5. Ovarian and pituitary hormones in the plasma of 40 women during normal menstrual cycles. Mean ± S.D. Shaded background indicates entire range of observations.

authors measured progesterone and LH simultaneously at 6 hourly intervals and found that the LH rise antedated the progesterone rise by 12 hr.

Recent studies in which LH was measured at hourly, or even shorter, intervals have led to the important demonstration that the LH secretion (and to a smaller degree the FSH secretion) is oscillatory in nature (18, 66, 117). A good example of such an oscillatory pattern is illustrated in figure 9 which derives from a study by Midgley and Jaffe

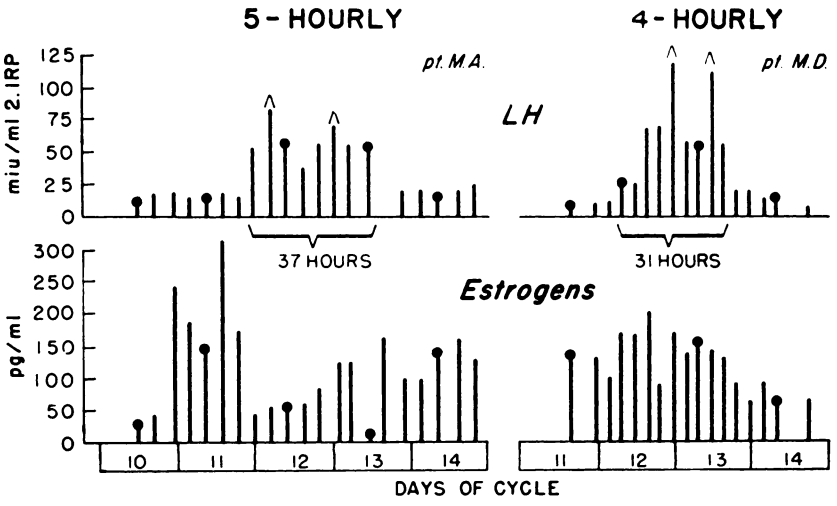

FIG. 6. Four- and five-hourly determinations of plasma LH and estrogens in two women with normal menstrual cycles at midcycle. (From K. Thomas, J. Ferin, I. Dyrenfurth and R. L. Vande Wiele, to be published.)

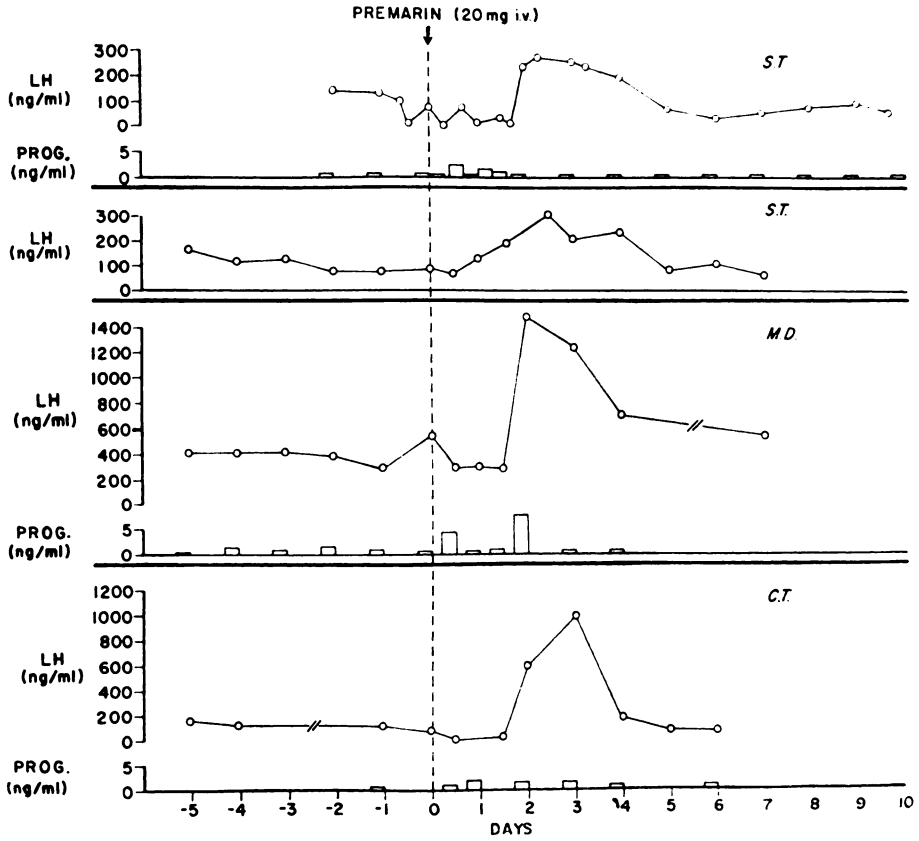

FIG. 7. Plasma levels of LH and progesterone after intravenous administration of 20 mg of Premarin to anovulatory women.

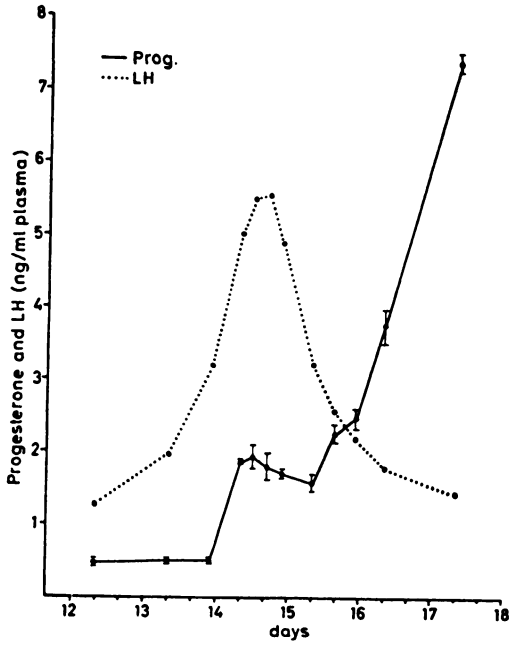

FIG. 8. LH and progesterone at midcycle in a woman with normal menstrual cycles. (From E. D. B. Johansson and L. Wide: Periovulatory levels of plasma progesterone and luteinizing hormone in women. Acta Endocrinol. 62: 82–88, 1969.)

(66). The factors that control these oscillations are at the present time completely unknown.

The availability of radioimmunoassays also contributed greatly to our understanding of the factors controlling the function of the corpus luteum. From the study of normal cycles and even more so from the study of cycles induced in amenorrheic women treated with human menopausal (HMG) and chorionic gonadotropin (HCG) the suggestion had been made that the function of the corpus luteum is autonomous and that once ovulation has occurred, there is no need for further stimulation by pituitary factors (109). Recent studies in which ovulation was induced with human luteinizing hormones (HLH) rather than with HCG have challenged this earlier suggestion of autonomy of the corpus luteum and indeed indicate that after ovulation, normal corpus luteum function is dependent upon further LH secretion. Figure 10 illustrates such a study. After appropriate stimulation of the follicle with HMG, ovulation was in-

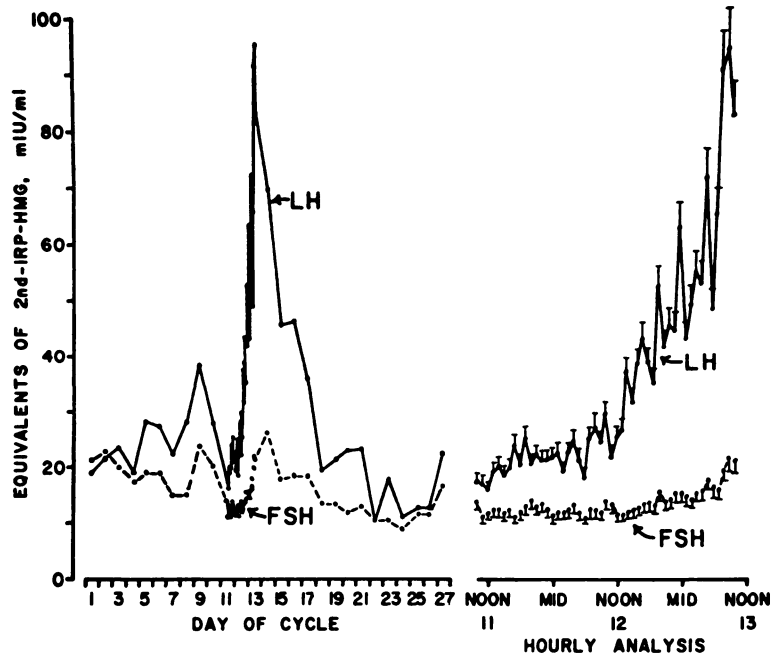

FIG. 9. Hourly analysis of serum LH and FSH at midcycle in a woman with normal menstrual cycles. (From A. R. Midgley and R. B. Jaffe: Regulation of human gonadotropins. X. Episodic fluctuation of LH during the menstrual cycle. J. Clin. Endocrinol. Metab. 33: 962–969, 1971.)

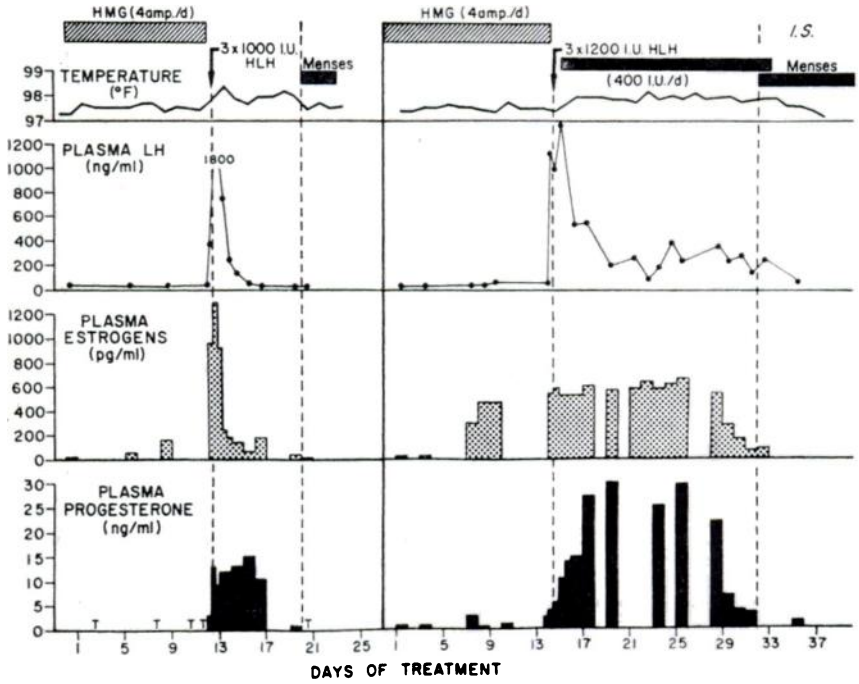

FIG. 10. Induction of ovulation with HMG and human pituitary LH in a patient with secondary amenorrhea.

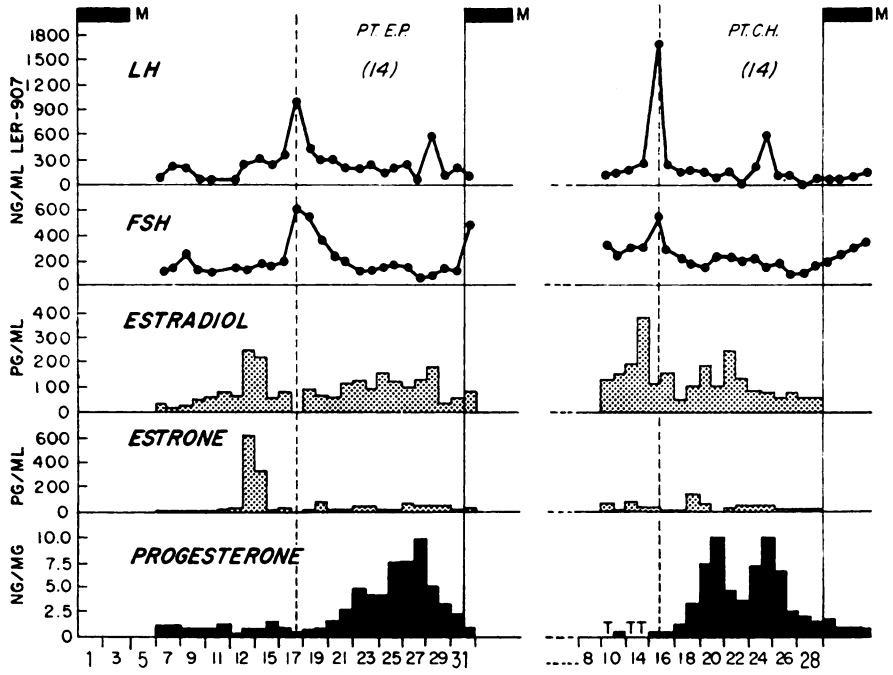

FIG. 11. Patterns of ovarian and pituitary hormones in the plasma of two healthy women during normal ovulatory cycles. Explanations as in figure 2.

duced by the administration of HLH. Although ovulation occurred as evidenced by a rise in the basal body temperature and the levels of progesterone, the corpus luteum was not maintained for the full 14 days and regressed prematurely. Within 7 days after the induction of ovulation, progesterone, estrogens, and the temperature fell and the patient menstruated. In the following cycle the initial treatment was identical but after induction of ovulation with HLH, small amounts of HLH were continued for a total of 17 days. With this modality of treatment, the luteal phase was of normal length as were the patterns of progesterone and estrogen. During the luteal phase of normal cycles, there are often small secondary elevations of LH occurring simultaneously which may coincide with the secondary elevations in progesterone and the estrogens; this suggests a continuous interplay between LH and the corpus luteum during the luteal phase (figs. 5 and 11). In sheep and some other domestic animals, uterine prostaglandins have been demonstrated to have luteolytic activity and to determine the length of the luteal phase. If for instance, a hysterectomy is carried out in sheep during the luteal phase, the corpus luteum will not regress and may persist for several months

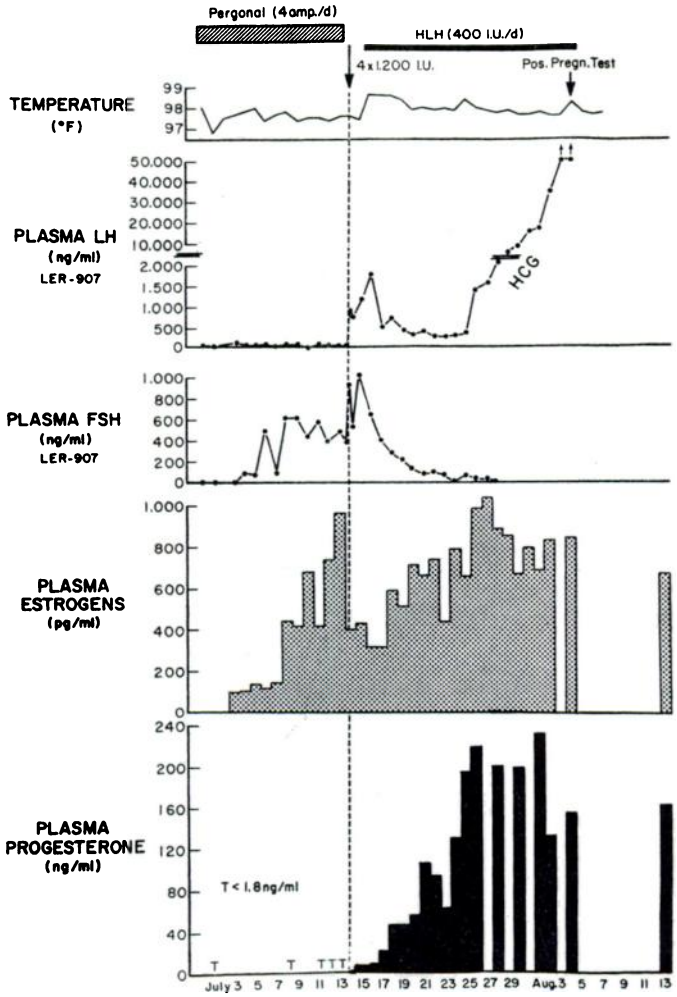

FIG. 12. Induction of ovulation with HMG and human pituitary LH with resulting pregnancy in a pituitary-radiated woman.

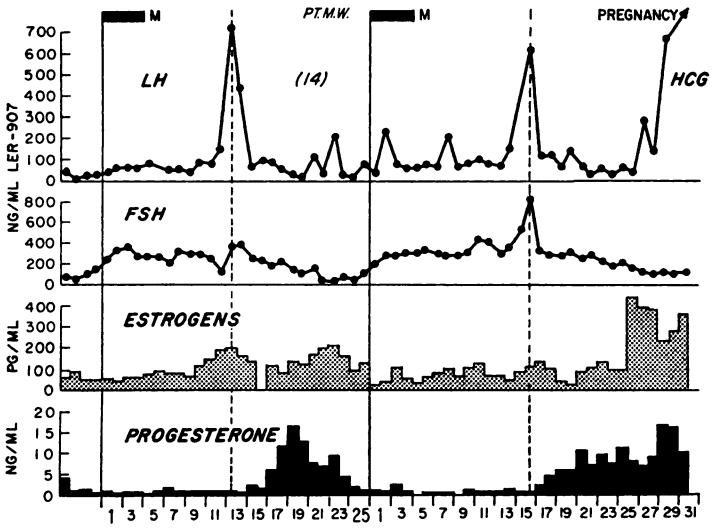

FIG. 13. Patterns of ovarian and pituitary hormones during two subsequent cycles and beginning pregnancy in a healthy woman.

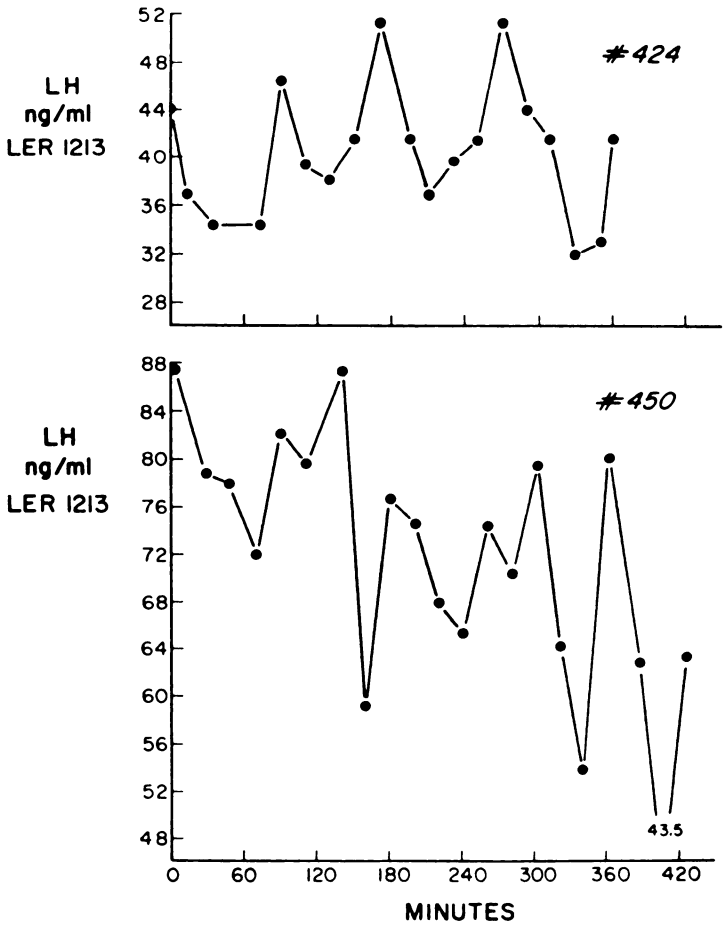

FIG. 14. Oscillatory pattern of plasma LH levels in two castrate rhesus monkeys.

In women, on the other hand, removal of the uterus leaving the ovaries intact does not lead to a prolongation of the luteal phase (7).

As shown in figure 10, the function of the corpus luteum will not be prolonged significantly beyond 14 days even if the patient is given amounts of LH which in the earlier part of the luteal phase were sufficient to produce normal progesterone secretion. Earlier work indicating that human chorionic gonadotropin had a luteotrophic effect led to the suggestion that this was the hormone that was responsible for the prolongation of the lifetime and the function of the corpus luteum during early pregnancy. On the other hand, with the old bioassay methods a significant rise in chorionic gonadotropin could not be demonstrated until several days after the missed period and 3 weeks after ovulation. Since progesterone during the normal luteal phase starts to decline within 7 to 9 days after ovulation, such a late appearance of chorionic gonadotropin casts doubt about the role of chorionic gonadotropin in the earliest phases of pregnancy. By means of radioimmunoassay, however, it has been possible to demonstrate that the actual rise in human chorionic gonadotropin occurs much earlier than was thought from the bioassay data and that the time sequence of the appearance of chorionic gonadotropin and the secondary rise of progesterone in fertile cycles is compatible with the hypothesis that chorionic gonadotropin is the luteotrophic factor. Such a sequence of events is shown in figure 12. The patient who had undergone pituitary radiation conceived during a cycle during which she was treated with gonadotropins. As indicated in figure 12, on the 12th postovulatory day, there was a sharp increase in chorionic gonadotropin (which in the radioimmunoassay used cross reacts completely with LH). Coincident with this rise in chorionic gonadotropin, there was a secondary rise in progesterone which had started to decline on day 10. The same sequence of events is shown in figure 13 which illustrates the sequence of events in a spontaneous normal pregnancy.

Thus far, the preovulatory phase has been much less studied. The growth of a new set of follicles in the early follicular phase can be attributed to the rising levels of FSH (see, for instance, fig. 5). In some cases, the rise in FSH may even antedate the onset of menses (fig. 11). Many factors about the control of events during the first 2 weeks remain to be clarified, however. It is not known, for instance, what controls the beginning of the

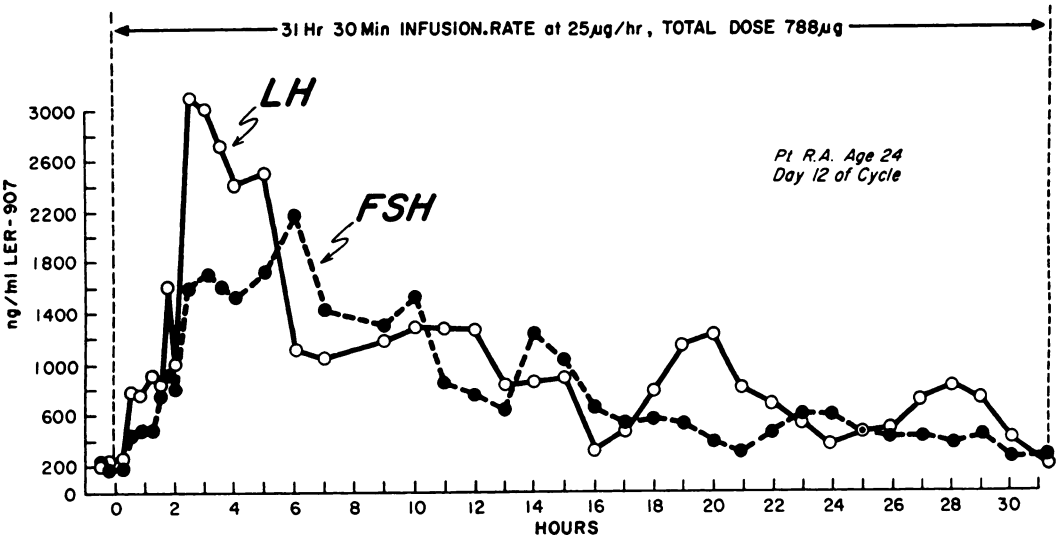

FIG. 15. Serum gonadotropin levels during a 31½-hr infusion of gonadotropin releasing hormone (GnRF) in a healthy woman on day 12 of her cycle.

estrogen secretion by the ovarian follicle, and most importantly, what are the factors which limit the follicles coming to maturity to a single one.

One of the most intriguing developments in this field deals with the control of the oscillatory pattern of the secretion of the gonadotropins. This pulsatile character of the LH secretion can also clearly be demonstrated in menopausal women or in castrated monkeys (fig. 14). Whether these oscillations are dependent upon pituitary mechanisms or on a pulsatile stimulation by the hypothalamic releasing factor cannot be decided at the present time. Since synthetic gonadotropin releasing hormones are now available, it has become possible to study the dynamics of the control of the pituitary by the hypothalamus. Figure 15 illustrates a study in which a continuous infusion of gonadotropin releasing hormone was given to a normal woman on day 12 of her cycle. There was a rapid elevation in both plasma LH and FSH but after about 7 hr, the levels gradually declined, in spite of continuous infusion at a constant rate, to a lower level of equilibrium. Several laboratories are now working actively on the development of a radioimmunoassay for gonadotropin releasing hormone, a development which should open a new phase in the study of hypothalamic function and reproductive processes.

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