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PLASMA 20α-DIHYDROPROGESTERONE PROGESTERONE AND 17-HYDROXYPROGESTERONE: DAILY AND FOUR-HOURLY VARIATIONS DURING THE MENSTRUAL CYCLE

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SUMMARY

Peripheral plasma levels of 20α-dihydroprogesterone (20α-OHP), progesterone (P) and 17-hydroxyprogesterone (17-OHP) were determined daily throughout the menstrual cycle of five healthy women and in four-hourly samples collected during the follicular and luteal phases. LH was determined daily and, taking the LH peak as day 0, the mean concentration for 20α-OHP in ng/ml was 0.46 on day −8; 0.90 on day 0 and rose to 6.20 on day +9. The mean for P in ng/ml on day −8 was 0.40; 1.20 on day 0 and rose to 17.70 on day +7. There was evidence for the presence of two small peaks of 17-OHP on days 0 and +2. In the follicular phase, 20α-OHP and P showed minor fluctuations of a reciprocal nature until day −6 after which the P values increased threefold and the 20α-OHP values twofold. There was clear evidence of a nycterohemeral variation in the concentrations of 17-OHP with maximum values at 8.00. The variations in four-hourly samples for 20α-OHP and P showed reverse patterns—the highest concentration of the former at 20.00 coinciding, in general, with the lowest concentrations of the latter. Changes were observed in these four-hourly variations at different times in the menstrual cycle.

INTRODUCTION

In the early 1950's, several authors reported the presence of unidentified steroids with the 4-ene-3-ketone configuration in extracts of human placenta and cord blood [1–4]. In 1956, Salhanick[5] reported that one of these compounds isolated by Jones *et al.*[4] was 20α-hydroxypregen-4-en-3-one (20α-OHP).

Subsequently, Zander *et al.*[6] isolated 20α-OHP from ripe follicles and corpora lutea and the steroid was identified in cord blood [7]; pregnancy plasma [8]; pooled peripheral plasma collected during the luteal phase of the menstrual cycle [9] and ovarian venous blood [10]. These observations offered an explanation for the discrepancy between the results obtained by physico-chemical and biological methods for the determination of progesterone since the latter also determined 20α-OHP and possibly other progestogens.

In vitro the formation of 20α-OHP from labelled acetate has been demonstrated in ovarian tissue [11] and the transformation of progesterone to 20α-OHP has been shown to occur in human placental tissue, myometrium and endometrium [12–15].

The interconversion of 20α-OHP and P was demonstrated by Weist [16] in rat ovarian tissue and by Zander [13] in the foetoplacental unit. Recently, the conversion of 20α-OHP to P has been demonstrated in human endometrium and there was evidence that this reaction varied with ovarian activity [17].

Although a radioimmunological method has been developed for the determination of 20α-OHP and applied to groups of men and women [18] there is little information upon variations throughout the menstrual cycle [19–21]. Progestogen levels in males [22] and in women during preovulatory and ovulatory phases of the menstrual cycle [23, 31] have been investigated. The present study was undertaken in order to study daily and four-hourly changes in relation to the peak of plasma LH and involving the simultaneous determination of progesterone (P) and 17-hydroxyprogesterone (17-OHP). It was hoped that this might begin to throw light upon the significance of 20α-OHP in human reproductive physiology.

EXPERIMENTAL (WITH METHODOLOGY)

Subject. Five unmarried women volunteers, between 21 and 28 years in apparent good health and on no hormonal medication with cycle length ranging from 27 to 30 days were studied. All the volunteers carried on with their usual activities during the period of study. None were involved with night duties.

Blood was drawn daily at 8.00 a.m. from the cubital vein throughout the menstrual cycle. During these cycles, four hourly blood samples were taken in the follicular and luteal phase. The blood was collected into heparinized tubes and the plasma separated from red cells immediately by centrifugation. The plasma was stored at −15°C until assayed.

Radioimmunoassay. Methods for the radioimmunoassay of 20α-dihydroprogesterone, progesterone, and 17-hydroxyprogesterone in peripheral plasma have already been reported [18, 24, 25]. These steroids were determined simultaneously in the same aliquot of plasma (1 ml in the follicular phase and 0.5 in the luteal phase) after chromatographic separation as previously described [18]. Plasma LH was determined by a double antibody radioimmunological method. The assay system contained HCG labelled with I^{125} and an antiserum to HCG. (Wellcome Foundation Ref. No. R.D.01/02). Wellcome anti-rabbit precipitating serum (Ref. No. R.D.17) was used as second antibody and LER-907. (Nat. Pituitary Agency, Bethesda, U.S.A.) as standard.

RESULTS

Daily determination. The average values for the plasma concentrations of 20α-OHP, P and 17-OHP from four menstrual cycles are shown in Fig. 1. The LH peak was designated as day 0 and the results were plotted with reference to this point.

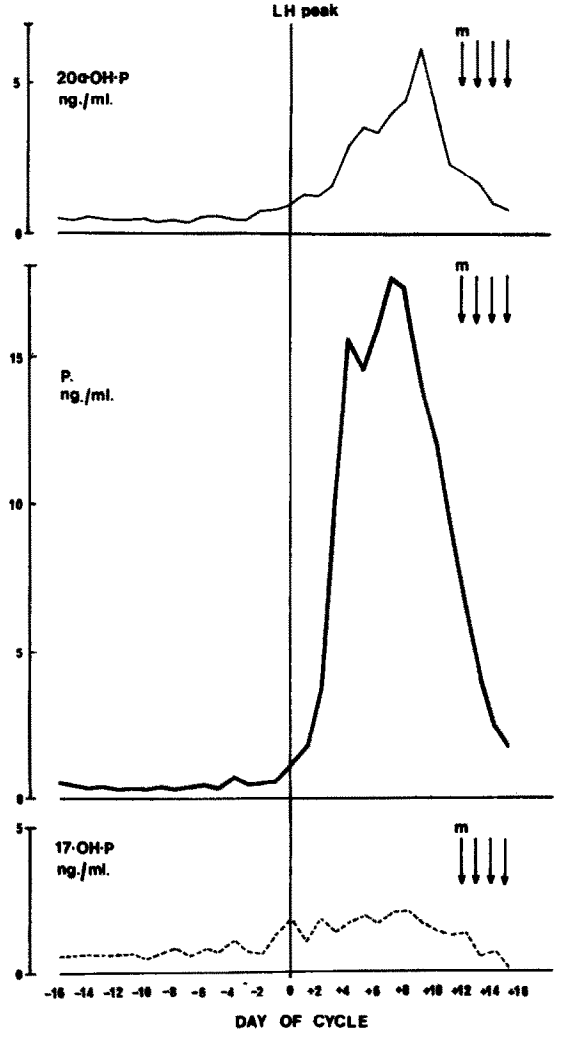

Fig. 1. Average of plasma 20α-OH-P, P and 17-OH.P (ng/ml) Concentrations in daily determinations during four menstrual cycles.

Plasma 20α-OHP concentrations rise slowly from a mean value of 0.4 ± 0.1 ng/ml. (mean ± S.D.) in the early follicular phase until they reach a value of 0.9 ± 0.6 ng/ml at about day +4. Subsequently, there is a rapid increase in the level to reach a maximum of 6.2 ± 4.0 ng/ml on day 9 followed by a rapid decrease towards the end of the cycle. It will be noted that individual variations of from 0.4–1.4 ng/ml occurred towards the onset of menstruation.

The concentration of P in plasma in the early follicular phase had a mean value of 0.4 ng ± 0.1/ml increasing to 1.2 ± 0.2 ng/ml at the LH peak. From day +2, the levels rose sharply to reach plateau between days +4 and +8. The highest level (17.7 ± 5.5 ng/ml was observed on day +7. Following this, there was an abrupt decline to lower concentrations with individual variations of 0.4–1.8 ng/ml on the last day of the cycle.

The level of 17-OHP rises from 0.6 ± 0.2 ng/ml on day −7 to a mean value of 1.8 ± 0.6 ng/ml at the LH peak. Values of this order are maintained until about day +8 and then fall towards menstruation. There is a suggestion of peak at the time of the LH peak followed by several fluctuations during the luteal phase. The premenstrual concentrations were 0.2 and 1.3 ng/ml.

The individual daily variations for the three steroids observed in these four menstrual cycles are shown in Fig. 2. Figure 3 shows the lower values obtained in a fifth patient in whom the LH peak occurred on the 21st day of the cycle followed by a short luteal phase. Reference has already been made to maximum mean concentration of P on day +7 but a study of the individual cycles reveals that the highest value for the hormone may occur between days +5 and +10. Similarly, although the mean value for the peak of 20α-OHP was on day +9, this peak occurred between days +5 and +10 in the individual cycles.

Four-hourly determinations. Individual values, means and S.D. in four-hourly assays of the three steroids in five women during the follicular phase; in two women on day +1 and in three women during the luteal phase of the menstrual cycle are shown in Tables 1, 2 and 3. The average of these values plotted in function of time are shown in Fig. 4. In the follicular phase, P had higher levels at 8.00, at noon and 4.00 followed by a depression of the values between 16.00 and midnight. The lowest concentration was observed at 20.00 with a percentage of variation from the mean (PVM) of −16.9.

In contrast, 20α-OHP had a peak at 20.00 (PVM +17.5) and lower values at 16.00 and midnight (PVM −12.2). The levels of 17-OHP had a marked nycterohemeral variation with the highest values at 8.00 IPVM +54.6) and lower concentrations between noon and midnight. One day after the LH peak (Day +1) the mean levels of P from two women again

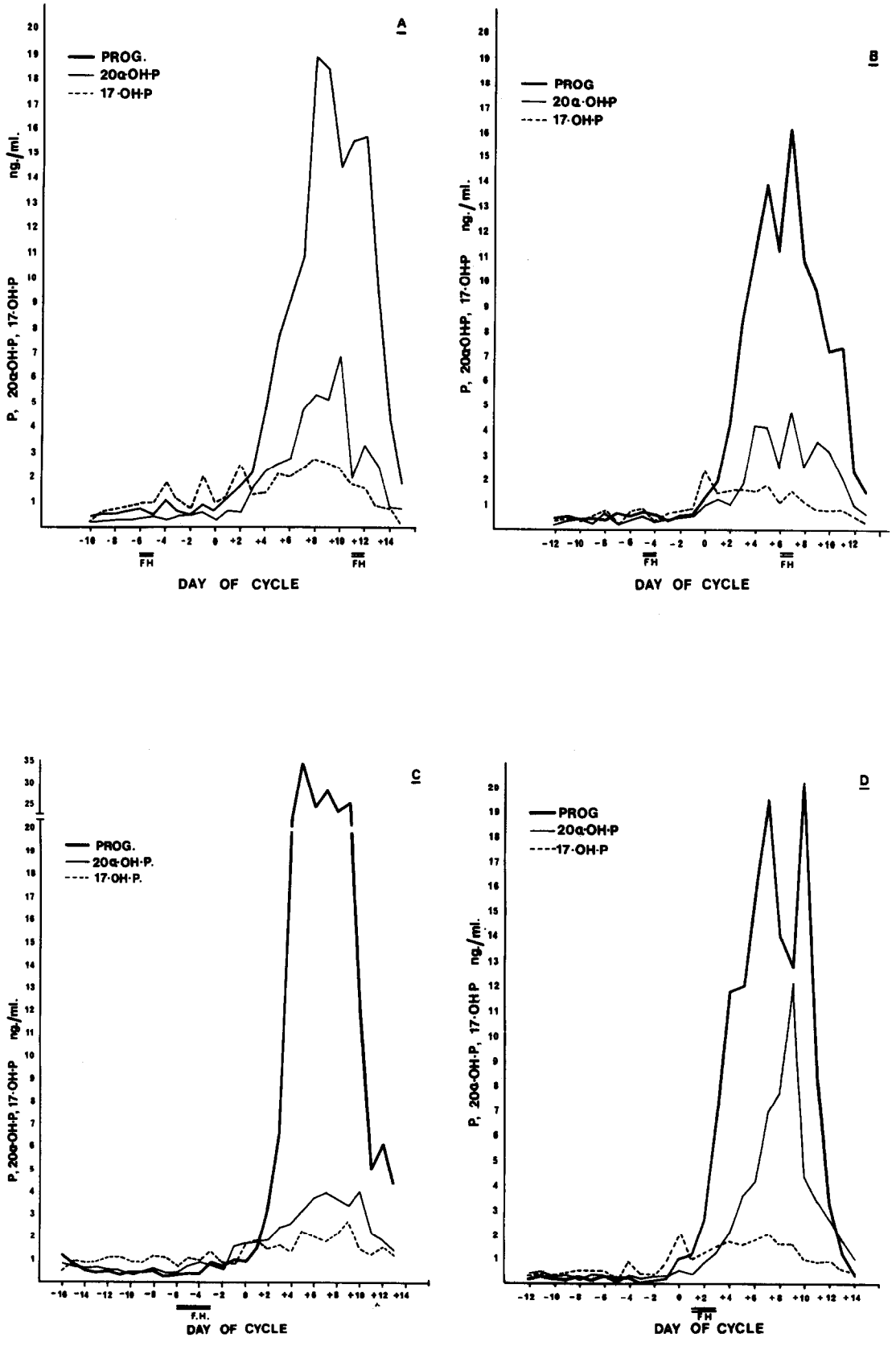

Fig. 2. Daily concentrations of 20α-OH.P, Pand 17 OH.P during the menstrual cycles of the volunteers (A–D) FH, indicates days on which four-hourly samples were taken.

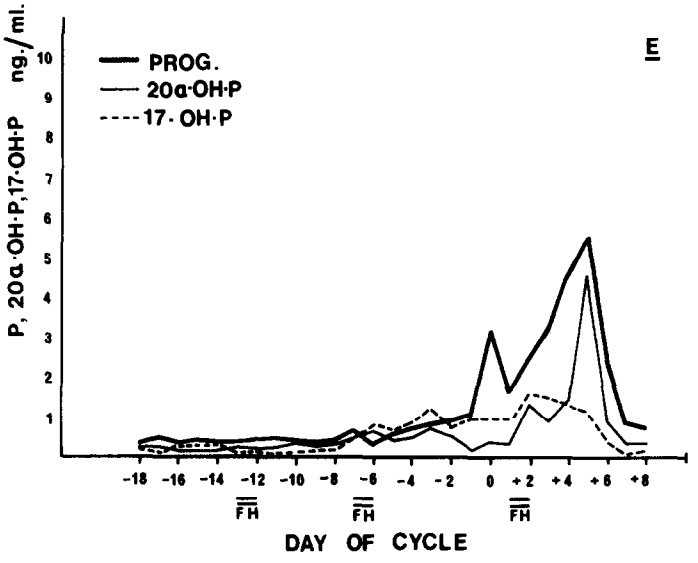

Fig. 3. Daily levels of 20α-OH.P, P and 17-OH.P in volunteer E, in whom the LH peak occurred on the 21st day of the cycle.

had the lowest level at 20.00 (PVM −5.8) and higher concentrations at noon and 4.00 (PVM +14.0). However, the values for 20α-OHP and 17-OHP on Day +1 were different from those in the follicular phase. In the luteal phase, there was a more definite fall at 20.00 in the level of P, whereas 20α-OHP had peak at the same time (PVM +24.6). The nycterohemeral variation of 17-OHP in the luteal phase was less marked than that observed in the follicular phase of the cycle.

In Fig. 5 are shown the individual variations in the follicular phase in four-hourly samples taken from the same volunteer during a period of 60 h, i.e. from day −6 to day −3. The nycterohemeral variation in the concentration of 17-OHP is clearly demonstrated throughout the three days with highest values in the morning and lowest at midnight. The concentrations of both P and 20α-OHP appeared to follow certain trends in that there were peaks of P at approximately 12 h intervals and, in general, the variations of 20α-OHP levels show a trend which is inversely proportional to that of progesterone.

The pattern for the three steroids after the LH surge (days +1 and +2) are shown in Fig. 6. Blood was taken hourly from the same woman from 8.00 (Day +1) to 8.00 (Day +3). The progesterone concentration rose steadily during the study but there was still an indication of lower values at 20.00. Dur-

Table 1. Concentrations in four-hourly samples of plasma 20α-OHP during the menstrual cycle (ng/ml)

Follicular phase									
	Day								
Hour	−12 E*	−6 E	−5 A	−5 B	−5 F	−5 C	−4 C	−3 C	Mean ± S.D.
8	0.33	0.56	0.44	0.59	0.25	0.73	0.81	0.72	0.55 ± 0.20
12	0.20	0.53	0.20	0.44	0.29	0.74	0.58	0.91	0.48 ± 0.25
16	0.32	0.22	0.30	0.40	0.30	0.85	0.55	0.74	0.46 ± 0.23
20	0.35	0.78	0.44	0.60	0.30	0.86	0.79	0.78	0.61 ± 0.22
24	0.29	0.53	0.35	0.23	0.23	0.77	0.80	—	0.46 ± 0.24
4	0.23	1.25	0.41	0.50	0.18	0.68	0.66	—	0.56 ± 0.52
8	0.31	0.60	0.54	0.44	0.30	0.81	0.72	0.50	0.52 ± 0.18

Day + One				Luteal phase				
	Day				Day			
Hour	+1 E	+1 D	Mean	Hour	+2 D	+6 A	+11 B	Mean ± S.D.
8	0.32	0.43	0.37	8	0.90	2.00	2.50	1.80 ± 0.81
12	0.34	0.50	0.42	12	1.22	5.00	3.00	3.07 ± 1.89
16	0.56	0.68	0.62	16	1.23	4.00	3.04	2.77 ± 1.44
20	0.62	0.73	0.67	20	1.72	5.40	3.76	3.62 ± 1.84
24	1.00	0.83	0.91	24	1.11	3.00	4.62	2.91 ± 1.75
4	0.44	—	0.44	4	1.02	3.15	4.00	2.72 ± 1.53
8	1.28	0.90	1.09	8	1.32	3.40	4.70	3.14 ± 1.70

* Volunteers. (F, 6th volunteer in whom only follicular phase was determined).

Table 2. Concentrations in four-hourly samples of plasma progesterone (ng/ml) during the menstrual cycle

Follicular phase									
	Day								
Hour	−12 E*	−6 E	−5 A	−5 B	−5 F	−5 C	−4 C	−3 C	Mean ± S.D.
8	0.73	0.36	0.64	0.71	0.54	0.41	0.37	0.89	0.58 ± 0.19
12	0.52	0.38	0.56	0.66	0.33	0.71	0.68	0.56	0.55 ± 0.13
16	0.62	0.36	0.49	0.79	0.33	0.38	0.34	0.62	0.49 ± 0.17
20	0.35	0.37	0.30	0.62	0.51	0.33	0.43	0.56	0.43 ± 0.12
24	0.64	0.35	0.21	0.69	0.21	0.52	0.35	—	0.42 ± 0.19
4	1.27	0.38	0.34	0.59	0.48	0.34	0.32	—	0.53 ± 0.34
8	0.56	0.44	0.32	0.63	0.46	0.37	0.89	0.76	0.55 ± 0.20

Day + one				Luteal phase				
	Day				Day			
Hour	+1 E	+1 D	Mean	Hour	+2 D*	+6 A	+11 B	Mean ± S.D.
8	1.64	1.27	1.45	8	2.68	15.60	11.20	9.82 ± 6.56
12	2.00	2.17	2.08	12	3.00	14.60	15.80	11.13 ± 7.06
16	1.64	2.25	1.94	16	3.82	15.00	16.60	11.80 ± 6.96
20	1.80	2.02	1.91	20	3.28	10.20	16.70	10.06 ± 6.71
24	1.72	2.18	1.95	24	4.12	14.00	18.80	12.30 ± 7.48
4	2.22	2.39	2.30	4	6.01	16.00	16.40	12.80 ± 5.88
8	2.46	2.68	2.57	8	7.26	15.80	16.20	13.08 ± 5.05

* Subjects (F, 6th volunteer in whom only follicular phase was determined).

Table 3. Concentrations in four-hourly samples of plasma 17-OHP (ng/ml) during the menstrual cycle

Follicular phase

Hour	Day −12 E*	−6 E	−5 A	−5 B	−5 E	−5 C	−4 C	−3 C	Mean ± S.D.
8	1.00	0.23	0.58	0.85	0.33	1.12	0.82	1.42	0.79 ± 0.40
12	0.33	0.13	0.55	0.34	0.14	0.58	0.24	0.30	0.33 ± 0.17
16	0.50	0.17	0.46	0.32	0.11	0.34	0.28	0.43	0.32 ± 0.14
20	0.24	0.25	0.31	0.37	0.85	0.21	0.43	0.55	0.40 ± 0.21
24	0.39	0.15	0.27	0.29	0.72	0.10	0.19	—	0.30 ± 0.21
4	2.66	0.14	0.37	0.46	0.12	0.19	0.21	—	0.59 ± 0.91
8	1.00	0.27	0.79	0.47	0.36	0.82	1.42	0.85	0.75 ± 0.37

Day + one

Hour	Day +1 E*	+1 D	Mean
8	0.90	1.03	0.96
12	1.00	1.14	1.07
16	0.60	1.08	0.84
20	1.34	0.87	1.10
24	1.64	1.07	1.35
4	1.56	—	1.56
8	1.56	1.39	0.97

Luteal phase

Hour	Day +2 D*	+6 A	+11 B	Mean ± S.D.
8	1.39	1.80	1.18	1.45 ± 0.31
12	1.23	0.96	1.30	1.16 ± 0.18
16	0.90	1.28	1.50	1.22 ± 0.30
20	1.11	0.82	1.76	1.23 ± 0.48
24	0.86	1.15	1.80	1.27 ± 0.48
4	0.73	1.80	1.74	1.42 ± 0.60
8	1.52	1.64	1.60	1.58 ± 0.06

* Volunteers (F, 6th volunteer in whom only follicular phase was determined).

ing Day +1, there were no characteristic changes in the concentration of the other two steroids, whereas during the second day of the study the levels of both 20α-OHP and 17-OHP reverted to the type pattern exhibited in the follicular phase with a peak of 20α-OHP coinciding with the lowest value for progesterone.

The plasma concentrations of the three steroids in the follicular phase (days −6 to −5) and luteal phase (days +11 to +12) in the same volunteer are shown in Fig. 7.

The reverse relationship between P and 20α-OHP is again revealed. 17-OHP had the pattern which has been described but this was less marked in the luteal phase. Figure 8 shows the study of four-hourly values on two days of the cycle of the patient in whom the ovulatory peak was on the twenty-first day. Thus the first day covers day −13 to −12 and the second, days −7 to −6. It is interesting to observe changes in the three steroids similar to those described above in the four-hourly samples collected between days −7 and −6. On the other hand, in the samples collected very early in the cycle, these changes are much less apparent.

DISCUSSION

During the early follicular phase, the fluctuations in 20α-OHP and P are in the same range and there is a suggestion that there is an inverse relationship in the daily values of the two steroids, so that small peaks in the plasma concentration of 20α-OHP coincide with small depressions in the level of progesterone. The mean ratio of P/20α-OHP reaches a maximum on day −4 followed by a depression after which the rise in P values begins whereas that of 20α-OHP is post-ovulatory and at a lower level (Fig. 9). The mean levels of the two steroids in the peri-ovulatory phase are shown in Table 4 and Fig. 10 and it may

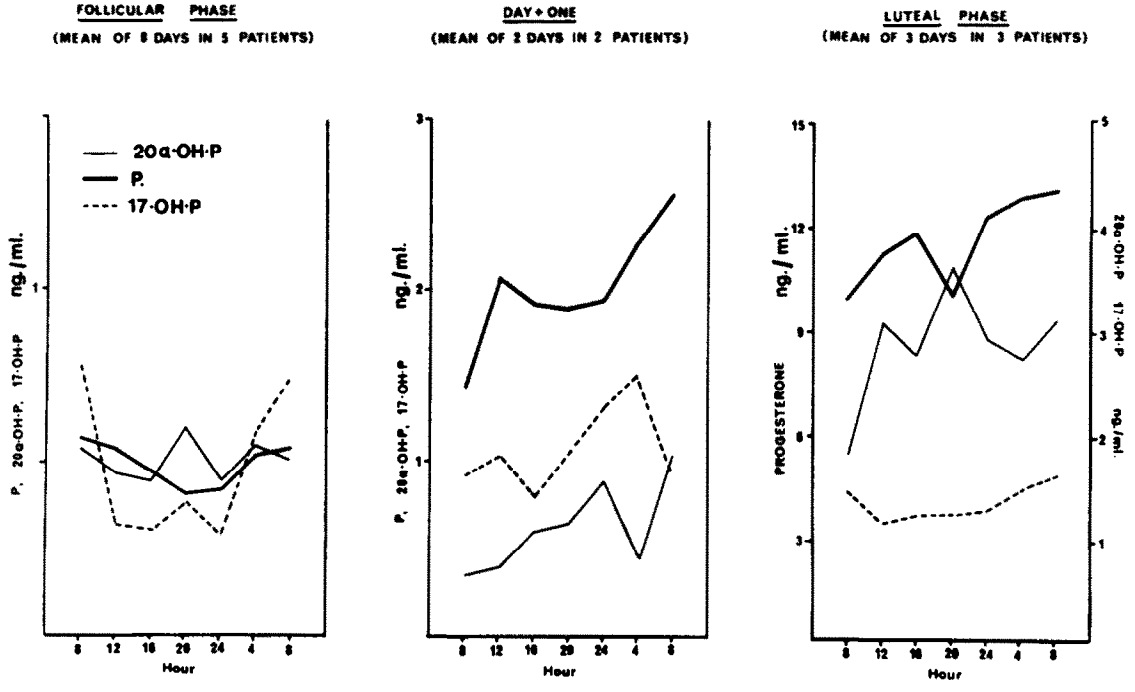

Fig. 4. Four-hourly variations in plasma 20α-OH.P, P and 17-OH.P, (ng/ml) during the follicular phase (mean of 8 days from five subjects); on day +1 (mean of 2 days from two subjects) and in the luteal phase (mean of 3 days from three subjects).

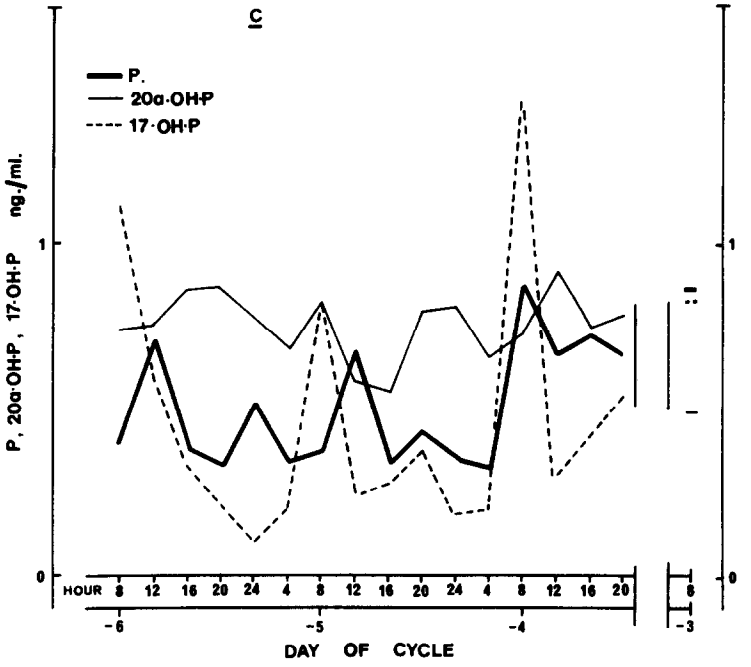

Fig. 5. Four-hourly variations of plasma 20α-OH.P, P and 17-OH.P (ng/ml) in volunteer C during 60 h in the follicular phase (days 6 to 3).

be postulated that variation in the circulating levels of two progestogens of different biological potency may play a part in LH release. Coinciding with the LH peak there is a small peak with daily level of

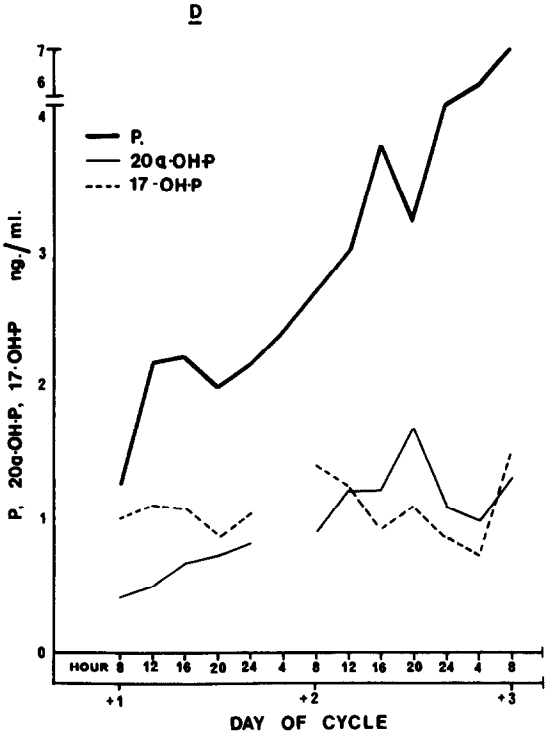

Fig. 6. Four-hourly variations of plasma 20α-OH.P, P and 17-OH.P (ng/ml) in volunteer D, during 48 h after the LH peak (Days +1 to +3).

17-OHP as previously observed [27]. The present results show clearly that there is a modest rise in 20α-OHP and progesterone in the days before the LH peak in peripheral venous blood. This is especially marked in the case of progesterone which shows a three-fold rise by day 0 accompanied by a two-fold rise in plasma 20α-OHP. This confirms the contention that progesterone is secreted by the Graafian follicle or other components of ovarian tissue before ovulation. It is probable that the extent to which 20α-OHP converted to progesterone varies throughout the menstrual cycle. Thus, in the study of human endometrium '*in vitro*' by Maeyama *et al.* (1973) [17] there was found to be a progressive conversion of 20α-OHP to progesterone in the endometrium from the early proliferative phase to the late luteal phase.

These authors noted high 20α-hydroxysteroid dehydrogenase activity in the secretory endometrium and this may be an interesting aspect of corpus luteum function. The situation may be complex; thus it has been shown in rates that hypophysectomy may lead to an increased formation of 20α-OHP and a decreased production of progesterone [28]. Furthermore, an inter-relationship between 20α-OHP, LH and FSH has been demonstrated in rats [29] and rabbits [30] immediately before ovulation.

The present study reveals a rather consistent fall in plasma progesterone concentration at about 20.00 h. Furthermore, this was usually accompanied by a rise in the plasma level of 20α-OHP at the same hour. This applied to both follicular and luteal phases of the cycle whereas, with regard to 20α-OHP the

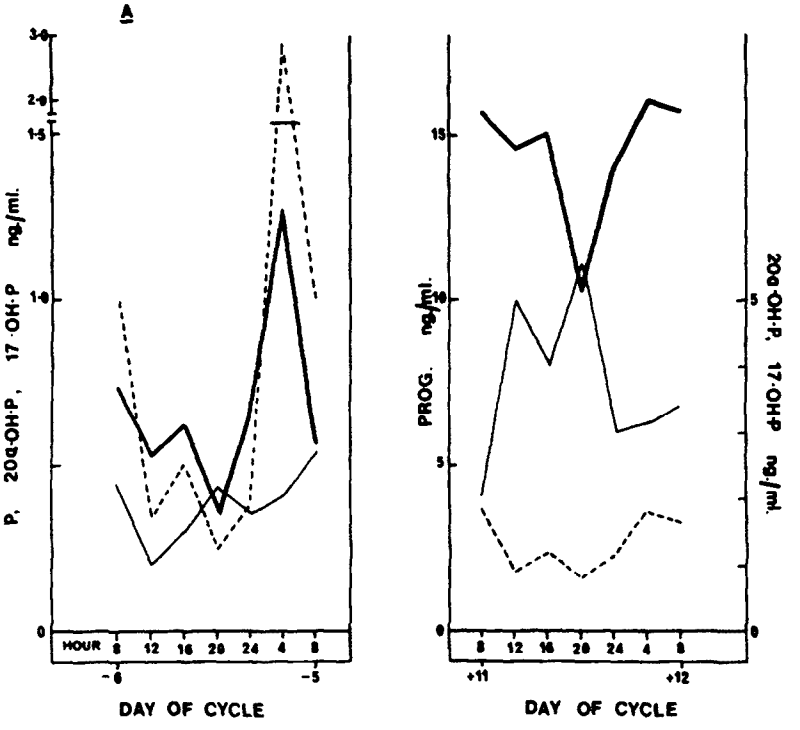

Fig. 7. Four-hourly variations in plasma 20α-OH.P, P and 17-OH.P (ng/ml) in volunteer A, during one day in the follicular phase and one in the luteal phase (Days 6 and +11).

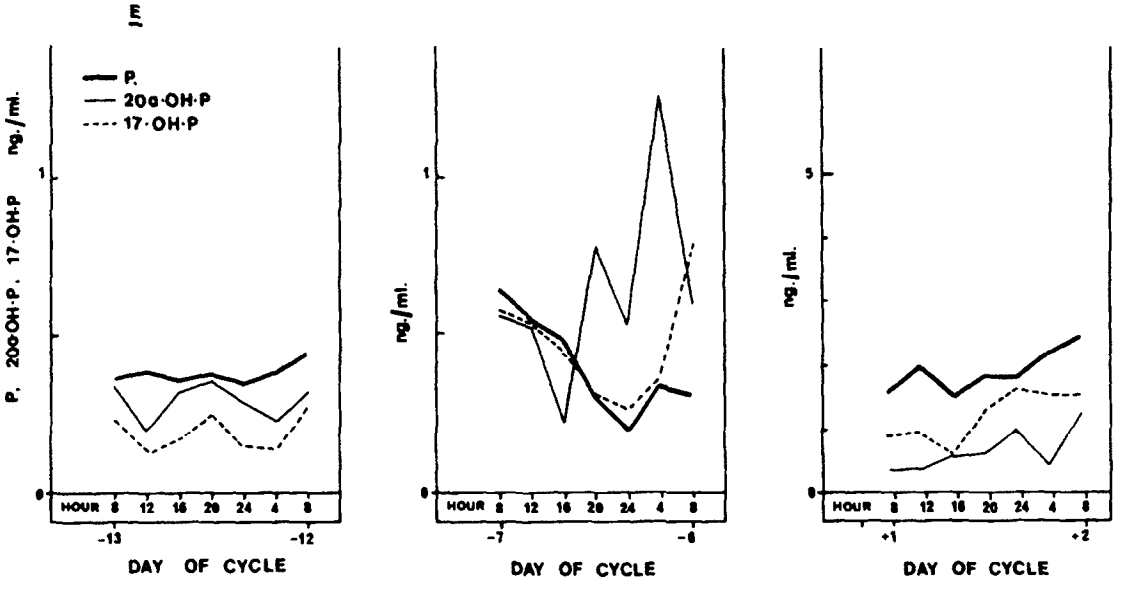

Fig. 8. Four-hourly variations in plasma 20α-OH.P, P and 17-OH.P (ng/ml) in volunteer E during two days in the follicular phase and one day after the LH peak (Days −13, −7 and +1).

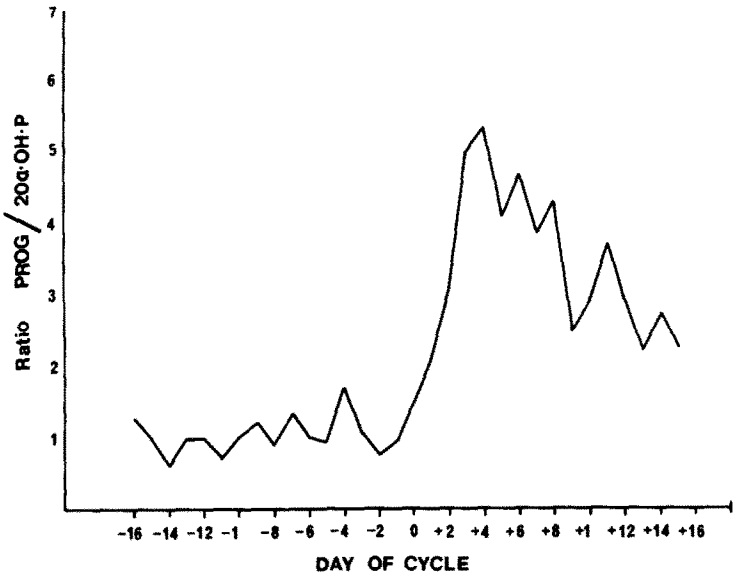

Fig. 9. Ratio P/20α-OH.P of the mean plasma concentrations (ng/ml) of the two steroids in four cycles.

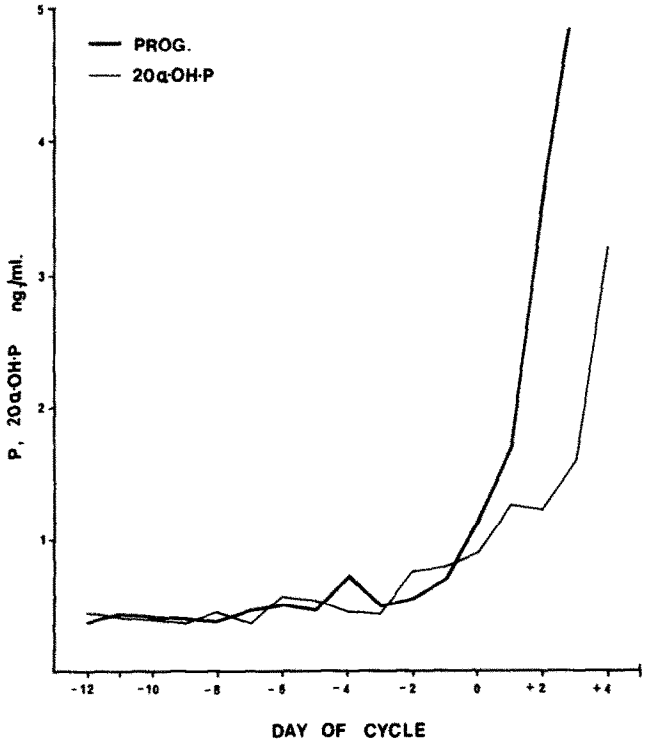

Fig. 10. Periovular variations in the mean levels of P, and 20α-OH.P (ng/ml) in daily samples from subjects (A–D).

Table 4. Periovular variations of the mean-plasma levels of P and 20α-OHP (ng/ml)

Day	P	20α-OHP
	Mean ± S.D.	Mean ± S.D.
−4	0.72 ± 0.34	0.48 ± 0.16
−3	0.51 ± 0.22	0.45 ± 0.10
−2	0.56 ± 0.32	0.78 ± 0.54
−1	0.69 ± 0.33	0.81 ± 0.60
0	1.18 ± 0.34	0.91 ± 0.58
+1	1.73 ± 1.31	1.29 ± 0.45
+2	3.70 ± 2.05	1.24 ± 0.79
+3	10.07 ± 8.63	1.62 ± 0.73
+4	15.51 ± 12.94	2.95 ± 0.94

change was not evident at the time of the LH peak. This again suggests that the interconversion of P and 20α-OHP may constitute an interesting and relatively unexplored aspect of ovarian function.

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