THE URINARY EXCRETION OF OESTROGENS, PREGNANEDIOL AND GONADOTROPHINS DURING THE MENSTRUAL CYCLE

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SUMMARY

1. The urinary excretion of oestrogens, pregnanediol and pituitary gonadotrophins has been studied throughout nine ovulatory menstrual cycles.
2. The pattern of hormone excretion was relatively constant from one individual to another, but the actual amounts excreted varied considerably in different individuals.
3. In none of the subjects studied did the mid-cycle peak in gonadotrophin precede the oestrogen peak.
4. The increase in urinary pregnanediol during the luteal phase occurred at the same time as or just before the rise in basal temperature and 1–4 days after the oestrogen peak.
5. There was no correlation between the amounts of oestrogens and pregnanediol excreted during the luteal phase of the cycle.
6. When gonadotrophin assays were conducted by the mouse uterus test and that depending on the prostate of the hypophysectomized rat, the results obtained agreed very closely at all stages of the cycle.
7. In one subject a marked rise in gonadotrophin output was observed as early as 9 days after a successful artificial insemination.

In the last two decades many investigators have studied the excretion of oestrogens, pregnanediol and gonadotrophins throughout the menstrual cycle. Such studies have been published by Gustavson, Mason, Hays, Wood & D’Amour [1938], Smith, Smith & Pincus [1938], D’Amour [1943], Heller, Farney, Morgan & Myers [1944], Pedersen-Bjergaard & Tønnesen [1948], de Watteville [1951], Brown [1955a], Borth, Lunenfeld & de Watteville [1957], Buchholz [1957] and Klopper [1957]. It is generally agreed that peaks of oestrogen and of gonadotrophin excretion occur about the middle of the cycle, and that during the luteal phase the pregnanediol output rises and a second oestrogen peak occurs. Main, Cox, O’Neal & Stoeckel [1943] have suggested that in some women a second gonadotrophin peak can be demonstrated before the onset of menstruation.

Some doubt remains about the relationship in time between the various cyclical changes which have been described. There are two reasons for this. First, many of the assay methods employed have not been sufficiently sensitive or convenient for daily estimations and second, parallel determinations of oestrogens, pregnanediol and gonadotrophins have seldom been attempted.

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In the present investigation the hormone excretion has been studied in nine ovulatory menstrual cycles. Assay methods of proved reliability have been used. An attempt has been made to correlate the cyclical changes in the excretion of one hormone with those of the others. Most subjects kept basal temperature records and, where possible, any change in basal temperature has been correlated with the pattern of hormone excretion.

**MATERIALS AND METHODS**

Complete 24 hr urine specimens were collected throughout one menstrual cycle in each of the nine subjects. Collections were usually started a few days before the expected onset of menstruation and were continued until the end of the next menstruation. The day on which bleeding commenced was designated day 1. Oral temperatures were recorded each morning on awakening.

Seven of the nine subjects were healthy women, normally active; one of these (D.H., Fig. 7) was artificially inseminated with her husband’s semen and became pregnant while under observation. The remaining two patients (W.N. and M.R., Figs. 3 and 5) were patients in hospital suffering from carcinoma of the breast; one of these (M.R.) was treated by ovarian irradiation during the period of study. There was no history of menstrual abnormalities in any of the subjects investigated.

Urine samples were collected without preservative and were stored in a refrigerator at 4°C. When the 24 hr output was <1200 ml. the specimen was made up to this volume with distilled water before the assays were done. One half of each 24 hr collection was used for oestrogen and pregnanediol determinations; the other half was used for gonadotrophin assays, being combined usually as 3-day pools during the follicular and luteal phases of the cycle and as 2-day pools at the middle of the cycle. In some assays involving the hypophysectomized rat prostate (H.R.P.) test, pooled urine collected over longer periods was used.

**METHODS OF ESTIMATION**

1. **Oestrogens.** The method of Brown [1955b] was used. This measures urinary oestriol, oestrone and oestradiol-17β, but not the more recently discovered urinary oestrogens such as 16-epioestriol [Marrian & Bauld, 1955], 16α-hydroxyoestriol [Marrian, Loke, Watson & Panattoni, 1957] and 2-methoxyoestriol [Kraychy & Gallagher, 1957]. The reliability of Brown’s method has been recently reviewed by Brown, Bulbrook & Greenwood [1957]. It would appear that this method is suitable for the present purpose.

2. **Pregnanediol.** Estimations of urinary pregnanediol were made by the method of Klopper, Michie & Brown [1955]. This procedure has been critically examined by Coyle, Mitchell & Russell [1956] and by Huis in’t Veld, Dijkstra-van Katwijk & Jonkman [1957]. They have found it to be reliable for the assay of urinary pregnanediol during the menstrual cycle.

3. **Gonadotrophins.** Gonadotrophins were extracted from urine by the method of Loraine & Brown [1956a, b]. The main steps are adsorption on kaolin, elution from kaolin, acetone precipitation and purification of the crude kaolin acetone powders by treatment with tricalcium phosphate.
Bioassays were done by (a) the uterine weight test in intact immature mice, and (b) the ventral prostatic weight test in hypophysectomized immature rats. The former method is not specific for either follicle stimulating hormone (FSH) or interstitial cell stimulating hormone (ICSH), but probably provides a useful measure of ‘total gonadotrophic activity’; the latter test is generally assumed to be specific for ICSH activity. The reliability criteria of the two methods have been discussed elsewhere [Loraine & Brown, 1954; Loraine, 1956, 1958]. Estimations by the mouse uterus (m.u.) test were made in all nine subjects; in three patients (E.B., A.D. and W.N., Figs. 1–3, respectively), parallel assays were made by both tests on the same urine samples. Assay results were calculated in terms of a standard prepared from the urine of menopausal and postmenopausal women (HMG-20A) and were expressed as HMG u./24 hr.

It should be noted that assays were not performed by a method specific for FSH activity. Until such determinations have been made it is obviously not possible to draw any final conclusions regarding the ratio of urinary FSH to urinary ICSH during the human menstrual cycle.

RESULTS

The results in individual subjects are shown graphically in Figs. 1–9. In each figure a solid vertical line has been drawn to indicate the day on which the peak of oestrone and oestradiol excretion occurred. This illustrates more clearly the time relationship between the mid-cycle oestrogen peak, the gonadotrophin peak and the start of the luteal phase rise in pregnanediol excretion and in basal temperature.

A notable feature of the results is the constancy of the hormonal excretion pattern from one individual to another, although the actual quantities excreted vary considerably in different subjects. The amounts of oestrogens and pregnanediol are very similar to those previously reported by Brown [1955a] and by Klopper [1957].

(1) Gonadotrophin determinations during the menstrual cycle

In all nine subjects in whom the urinary gonadotrophin excretion was estimated by the m.u. test the output was low during the follicular phase of the cycle, being generally <10 HMG u./24 hr. A definite rise in gonadotrophin excretion ranging from 13 to 40 HMG u./24 hr was observed at approximately mid-cycle in seven of the nine cases studied. In one individual (H.S., Fig. 8) the rise was absent; in the other (K.K., Fig. 9) it is possible (but unlikely) that the peak might have been missed due to the loss of two 24 hr urine samples. In five of the seven cases the increased output of gonadotrophins was confined to a single pooled sample collected over 48 hr; in the remaining two the peak was more diffuse, being spread over 6 days (W.N., Fig. 3) and over 9 days (A.D., Fig. 2). During the luteal phase of the cycle and menstruation itself the gonadotrophin levels were again low, usually being <10 HMG u./24 hr. None of the subjects showed a pre-menstrual rise in gonadotrophin excretion.

In one subject with mammary carcinoma (M.R., Fig. 5) the gonadotrophin excretion was studied first during a menstrual cycle and then subsequent to ovarian irradiation. No cyclical changes in oestrogen and pregnanediol excretion were observed after radiotherapy. The gonadotrophin excretion values rose steeply and
Figs. 1–9. The daily excretion of gonadotrophins, pregnanediol, oestriol, oestrone and oestradiol-17β, and also the variations in basal temperature. Gonadotrophin assays:  — , results by the mouse uterus (M.U.) test; ····, results by the M.U. test in which the reading is actually 'less than' the figure shown; ·····, results by the hypophysectomized rat prostate (H.R.P.) test; ····, results by the H.R.P. test in which the reading is actually 'less than' the figure shown. The vertical line indicates the day of the mid-cycle peak of excretion of oestrone and oestradiol-17β. ■ menstrual period. The subjects' ages and parities are indicated at the top of each figure.
V.N. Age 41. Para 0

Morning temperature

Gonadotrophins

Pregnanediol

Oestriol

Oestrone

Oestradiol

Day of cycle

Fig. 6

H.S. Age 18. Para 0

Morning temperature

Gonadotrophins

Pregnanediol

Oestriol

Oestrone

Oestradiol

Day of cycle

Fig. 8

K.K. Age 18. Para 0

Morning temperature

Gonadotrophins

Pregnanediol

Oestriol

Oestrone

Oestradiol

Day of cycle

Fig. 9

Figs. 6, 8 and 9. For explanation see legend Figs. 1–4.
Fig. 5. M.R. aged 41, para 2. Mammary carcinoma. Estimations made during a complete menstrual cycle and following ovarian irradiation. For explanation see legend Figs. 1–4.

Fig. 7. D.H. Aged 30, para 0. Estimations made during a complete menstrual cycle and following artificial insemination. For explanation see legend Figs. 1–4.
approx. 1 month after treatment were some twenty times higher than those found in the follicular and luteal phases of the menstrual cycle.

Parallel assays on the same urine samples by the M.U. and H.R.P. tests were performed in three subjects. At all stages of the menstrual cycle the results obtained by the two methods agreed very closely (Figs. 1–3).

(2) Relationship between oestrogen and gonadotrophin peaks

The first oestrogen peak is a constant feature of the ovulatory menstrual cycle. The rise and fall in urinary oestrone and oestradiol levels parallel one another, but the changes in oestriol excretion tend to lag about 24 hr behind the other two. Brown [1955a, 1957] explained this phenomenon on the basis that oestrone and oestradiol-17β are the primary ovarian hormones and that it takes some time for them to be metabolised and excreted as oestriol. If this view is correct fluctuations in urinary oestrone and oestradiol levels are probably closely related in time to fluctuations in the ovarian secretion of these hormones.

In none of the subjects studied did the gonadotrophin peak precede the oestrogen peak. Indeed in three of them (M.R., V.N. and D.H., second cycle, Figs. 5–7, respectively) it occurred several days after the first oestrogen peak. If it is assumed that gonadotrophins are eliminated in urine as rapidly as oestrogens, then the rise of oestrogen excretion could not have been initiated by the increase in gonadotrophin output.

No correlation was found between the height of the gonadotrophin peak, on the one hand, and the height of the oestrogen peak, on the other. However, the fact that the gonadotrophin excretion at mid-cycle was usually estimated from urine collected over 48 hr may have masked the exact timing and true height of the gonadotrophin peak.

(3) Relationship of urinary pregnanediol excretion to basal temperature and to oestrogen and gonadotrophin peaks

In eight out of the nine subjects the luteal phase rise in pregnanediol coincided approximately with the rise in basal temperature; one patient (A.D., Fig. 2) showed no increase in basal temperature during the luteal phase but followed the usual hormone excretion pattern in all respects. The luteal increase in urinary pregnanediol always occurred after the oestrogen peak, the time interval ranging from 1 to 4 days. In two subjects (M.R. and V.N., Figs. 5 and 6) the rise in pregnanediol output occurred before the gonadotrophin peak.

(4) Relationship between oestrogen and pregnanediol levels during luteal phase of cycle

In the majority of subjects the over-all rise and fall of oestrogen levels paralleled those of pregnanediol. However, there appeared to be no correlation between the actual amounts of oestrogens and of pregnanediol excreted. In other words, subjects excreting the largest quantities of oestrogens do not necessarily excrete the largest amount of urinary pregnanediol.

(5) Relationship of oestrogen peak to onset of menstrual bleeding

The time elapsing between the first oestrogen peak and the commencement of menstruation varied from 11 to 16 days. It may be of some interest that the shortest
intervals were found in the two individuals (H.S. and K.K., Figs. 8 and 9 respectively), in whom the gonadotrophin peak was apparently absent.

(6) Findings subsequent to artificial insemination

One subject was artificially inseminated on the day of the mid-cycle oestrogen peak and became pregnant during the period of study. The pregnancy proceeded uneventfully to term. The first indication of conception was a sharp rise in gonadotrophin excretion 9 days after insemination. This rise might have been due to the presence in urine of human chorionic gonadotrophin (HCG) rather than of pituitary gonadotrophins. The increase in gonadotrophin excretion was followed by a rise in oestrogen output and by maintenance of luteal phase levels of pregnanediol.

DISCUSSION

The results reported here resemble those of other workers but provide a more detailed picture of the hormonal interrelationships during the menstrual cycle. The most interesting changes are those which occur at mid-cycle and which are presumably related to ovulation. The first indication of increasing ovarian activity following menstrual bleeding is an increase in urinary oestrogen excretion; this rise begins about the 8th day of a 28-day cycle and culminates in a well-defined and characteristic peak some 4 days later. Since the oestrogen peak has been demonstrated in all subjects with a proven ovulatory menstrual cycle [Brown, 1955a, Brown, Kellar & Matthew, to be published], it is probably closely associated with ovulation.

Another characteristic finding which occurs at mid-cycle is the urinary gonadotrophin peak. This peak does not bear a constant relationship in time to the oestrogen peak. In some of our subjects the two peaks occurred simultaneously; in others the gonadotrophin peak followed the oestrogen peak after 1–4 days but in none did the gonadotrophin peak precede the oestrogen peak. In two individuals (H.S. and K.K., Figs. 8 and 9) a gonadotrophin peak was not observed, although all the other evidence indicated that ovulation had occurred. These subjects also showed evidence of poorly functioning corpora lutea, and it is therefore possible that the mid-cycle rise in gonadotrophin output, although not essential to ovulation itself, may have an important effect on the growth and development of the corpus luteum. Further work relating ovulation to the oestrogen peak on the one hand and to the gonadotrophin peak on the other is obviously necessary.

The increase in urinary pregnanediol excretion and the rise in basal temperature generally occurred 2–3 days after the oestrogen peak; in none of the individuals studied did these changes occur before or on the day of the oestrogen peak. In five subjects the rises in urinary pregnanediol and in basal temperature occurred after the gonadotrophin peak; in two individuals (M.R. and V.N., Figs. 5 and 6) the relationships were reversed.

It is generally assumed that the increase in urinary pregnanediol and the rise in basal temperature occur simultaneously, that both are due to an increase in the production of progesterone by the newly formed corpus luteum and that both provide a reliable indication that ovulation has occurred. That these traditional views may have to be modified is suggested by the observations of Dibbelt [1950] and of Klopper
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[1957] who showed that, in some subjects, the increase in urinary pregnanediol may occur before the rise in basal temperature.

The time from the oestrogen peak at mid-cycle to the onset of menstrual bleeding varied in individual subjects from 11 to 16 days, with a mean of 13.9 ± 1.6 (s.d.) days. The corresponding intervals dated from the gonadotrophin peak, the increase in urinary pregnanediol excretion and the rise in basal temperature were respectively 13.2 ± 1.7, 11.7 ± 2.1 and 11.2 ± 2.5 days (± s.d.). The variability of this time interval is least when dated from the oestrogen peak and is greatest when dated from the rise in basal temperatures.

The present investigations and also that of Buchholz [1957] have demonstrated that, when gonadotrophins are extracted from urine by the method of Loraine & Brown [1956a, b] and are assayed by the m.u. and H.R.P. tests, the results obtained by the two methods agree very closely at all stages of the menstrual cycle. It can therefore be stated that throughout the menstrual cycle the mean index of discrimination (uterus/prostate) approximates to unity. (The ‘index of discrimination’ was introduced by Gaddum [1955] as a means of expressing the difference of two substances in their action in two different tests. The index of discrimination (uterus/prostate) can be calculated by dividing the mean potency ratio (standard/unknown) in the m.u. test by the corresponding figure in the H.R.P. test.) It might have been anticipated that varying proportions of urinary FSH to urinary ICSH might have been reflected by the differences in this index. An index in the neighbourhood of unity suggests either that the material prepared by the method of Loraine & Brown [1956a, b] contains a single gonadotrophin with two activities, or that it contains two gonadotrophins which are always present in the same relative proportions. The fact that ICSH activity can usually be demonstrated in urine at all stages of the menstrual cycle conflicts with the generally accepted view that this hormone is secreted only at the time of ovulation and in the early luteal phase of the cycle.

One individual in this series (D.H., Fig. 7) became pregnant subsequent to artificial insemination. The insemination was performed on the day of the cycle on which the peak of excretion of oestrone and oestradiol-17β was expected. It is of interest to note that previous inseminations in this subject performed at the time of the rise in basal temperature had been unsuccessful.

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