PERIPHERAL PLASMA LEVELS OF OESTRONE, OESTRADIOL-17\beta AND PROGESTERONE DURING OVULATORY MENSTRUAL CYCLES IN THE Rhesus Monkey WITH SPECIAL REFERENCE TO THE ONSET OF MENSTRUATION

By

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ABSTRACT

The levels of oestrone (E₁)*, oestradiol-17\beta (E₂) and progesterone (P) were determined simultaneously by radioimmunoassay in serial peripheral plasma samples from 11 rhesus monkeys (Macaca mulatta) during ovulatory menstrual cycles. In 3 other monkeys luteectomy was performed on day 20 or 21 of the menstrual cycle. The mean plasma levels of oestradiol-17\beta predominated over the mean E₁ during the follicular phase, but during the luteal phase the E₁ and E₂ patterns varied widely. Before menstruation, increased or unchanged levels of E₁ and E₂ were mostly found. The decrease found in a few monkeys never reached the low levels found during the days after the midcycle peak. The plasma levels of progesterone increased from less than 0.2 ng/ml during the follicular phase to maximal values within 3 to 4 days after the midcycle peak of oestradiol-17\beta. The plasma levels of progesterone decreased to less than 1 ng/ml and remained low for 3 to 5 days before menstruation. In the luteectomized monkeys the plasma levels of P, E₁ and E₂ fell dramatically within 24 h and vaginal bleeding was detected within 72 h post-operatively. In 2 of the 3 luteectomized monkeys the levels of E₁ and E₂ increased before the bleeding. The ratio of oestrogens (oestradiol-17\beta and oestrone) over progesterone changed markedly in favour of the oestrogens

* Oestrone (E₁): 3-hydroxy-1,3,5(10)-oestratrien-17-one.
Oestradiol-17\beta (E₂): 1,3,5(10)-oestratriene-3,17\beta-diol.
during the pre-menstrual period. It is concluded that the patterns of pro-
gesterone and oestrogens are divergent during the luteal phase. The maxi-
mal levels of progesterone are reached early while the maximal levels of
oestrogens are reached later during the luteal phase.

The recent introduction of radioimmunoassay techniques for the estimation of
oestrogens (Hotchkiss et al. 1971) and progesterone (Thorneycroft & Stone
1972) has made it possible to determine picogram concentrations of these
steroids in small volumes of biological fluids. The peripheral plasma levels of
oestradiol-17β and total immunoreactive oestrogens have been determined by
radioimmunoassay techniques during the menstrual cycle of the rhesus monkey
(Hotchkiss et al. 1971; Bosu et al. 1972). However, the information on the ratios
of oestradiol-17β to oestrone in peripheral plasma during the various phases
of the menstrual cycle in the rhesus monkey is scanty. The purpose of this study
was to utilize radioimmunoassay techniques for the concurrent determination
of the concentrations of oestradiol-17β, oestrone and progesterone in aliquots
of the same plasma samples obtained daily during ovulatory menstrual cycles
in the rhesus monkey. Special attention has been given to the plasma levels
and the relationships of these three steroids during the days before menstrua-
tion, in the normal cycles and after luteectomies.

MATERIAL AND METHODS

Blood samples were collected daily from 14 mature regularly menstruating rhesus
monkeys (Macaca mulatta) during one ovulatory menstrual cycle. Blood sampling was
started from the day of the onset of the menstrual flow until and including the day
of onset of the next menstrual flow. The maintenance of the animals and dating of
cycles have been previously described (Bosu et al. 1972). Ovulations were confirmed by
the presence of recent corpora lutea in the ovaries at laparoscopy. In 3 monkeys
luteectomies were performed on day 20 or 21 of the menstrual cycle.

The plasma levels of E₁ and E₂ were measured by a radioimmunoassay technique.
A detailed description of the method has been published (Edqvist & Johansson 1972).
Volumes of plasma of 200 to 400 µl were used for extraction. The dried ether extracts
were applied to Sephadex LH-20 columns, after which the E₁ and E₂ fractions collected
were dried and subjected to radioimmunoassay using the antiserum against E₂ (which
cross-reacts with E₁) at dilutions of 1:150 000. The results reported here were not
corrected for procedural losses since the extraction and column losses were insignificant
(Edqvist & Johansson 1972). The column separation system gave a distinct separation
of oestrone and oestradiol. Dilution studies of the oestradiol fractions from the columns
indicated that the oestradiol fractions in the plasma from rhesus monkeys were mainly
oestradiol-17β (Bosu et al. 1972).

Plasma levels of progesterone were determined by radioimmunoassay. The method
used was essentially that described by Thorneycroft & Stone (1972) with slight modi-
fications. The antibody (IHT-R11-1516-1 a gift from Dr. Thorneycroft) was used
at a dilution of 1:3000; petroleum ether was used for extraction and 0.5 ml dextran
coated charcoal was employed for adsorbing the free steroid.

Volumes of plasma 100 to 200 µl aliquots during the follicular phase of the menstrual
cycle and 10 to 100 µl during the luteal phase were extracted once with 10 volumes of petroleum ether by vigorous handshaking for 60 sec. The petroleum ether used was never less than 1 ml. The dried petroleum ether extracts were subjected to RIA and the levels of progesterone in the samples were estimated from the progesterone standard curve (range 0 to 200 pg) and the results expressed as ng/ml plasma.

The mean recovery of tritiated progesterone added to different volumes of ovariectomized monkey plasma and extracted once with petroleum ether was 82 ± 2.7 (sd) (n = 40). The plasma progesterone concentrations reported have been corrected for extraction losses.

The accuracy of the method was determined by a comparison between the measured and added amount of progesterone in 25 µl of ovariectomized monkey plasma. The endogenous progesterone in the plasma was subtracted and the results corrected for extraction losses. When 10, 25, 50, 100 and 150 pg of progesterone were added to ovariectomized monkey plasma, 7.9 ± 1.5 pg (s) (n = 6); 25.1 ± 2 pg (s) (n = 6); 44.1 ± 6 (s) (n = 10); 99 ± 7 pg (s) (n = 10) and 146 ± 22 (s) (n = 8) were estimated.

The precision of the method was determined by comparing duplicate estimations of plasma samples obtained during the menstrual cycle. When the concentrations of progesterone in the extracted plasma volumes were between 0 and 25 pg the coefficient of variation was 18.4 % (n = 24); 26 and 50 pg, 14 % (n = 18); 51 and 100 pg, 12 % (n = 12) and over 100 pg 12 % (n = 12).

**RESULTS**

All the menstrual cycles studied were considered ovulatory based on the progesterone patterns in plasma after the midcycle oestradiol-17β peaks and the presence of recent corpora lutea in the ovaries at laparoscopic inspection.

The plasma levels of E2 were low during the first nine days of the cycle. This was followed by a period of gradual increase culminating in a peak around day 14 of the menstrual cycles. The levels of E2 in plasma decreased thereafter for two to three days followed by a subsequent small increase during the second half of the cycle. The plasma levels of E1 were two to five times smaller than E2 during the follicular phase. Midcycle peaks of E1 (50 to 200 pg/ml) occurred coincident with the E2 peaks. During the luteal phase the levels of E1 and E2 in relation to each other varied (Figs. 2, 3, 4 and 6). A trend towards an increase in plasma concentration of E1 and E2 was observed in most of the cycles studied during the pre-menstrual period (Figs. 4–7).

The mean concentrations of progesterone in plasma were less than the 0.2 ng/ml in the first twelve days of the cycle. A small but gradual increase occurred between days 13 and 15. Maximal levels were recorded between days 16 to 25 of the cycle after which the mean progesterone concentration decreased to less than 1 ng/ml in the pre-menstrual period (Fig. 1).

The three monkeys luteectomized in the afternoon of days 20 or 21 of the menstrual cycles showed vaginal bleeding on the morning of the third day post-operatively (Fig. 8). The cycles were 7, 4 and 5 days shorter than the mean of the three previous cycles in monkey 13, 17 and 114 respectively. The
Composite of mean daily levels of oestradiol-17β, oestrone, progestrone and ratios of $E_2: E_1$ and $E_2 + E_1 : P$ during 11 ovulatory menstrual cycles in rhesus monkeys: mean ± SE. The data were synchronized on the first day of menstrual bleeding in the next cycle.

plasma levels of $E_1$, $E_2$ and $P$ fell dramatically within 24 h after luteectomy in two of the monkeys (13 and 114). In the third monkey (17) where the plasma levels of the steroids were comparatively low before luteectomy, the decrease was not so pronounced (Fig. 8). A slight increase in the oestrogens occurred in these monkeys before menstruation, while the progesterone levels were low. The ratio $E_2 + E_1 : P$ changed in favour of the oestrogens on the second and third day after luteectomy before the onset of menstruation.

A more representative idea of the plasma levels of and the temporal relationships between $E_1$, $E_2$ and $P$ during the second half of the menstrual cycle was obtained by arranging the data according to days before menstruation (Fig. 1). The mean levels of oestradiol-17β were higher than the mean $E_1$
Fig. 2.
Daily plasma concentrations of oestradiol-17\(^\beta\), oestrone and progesterone in an ovulatory menstrual cycle in a rhesus monkey.

Fig. 3.
Daily plasma concentrations of oestradiol-17\(^\beta\), oestrone and progesterone during an ovulatory menstrual cycle in a rhesus monkey.
The peripheral plasma levels of oestradiol-17β, oestrone and progesterone during a 25 day ovulatory menstrual cycle in a rhesus monkey. Note the comparatively high oestradiol-17β levels in plasma throughout the menstrual cycle.

The peripheral plasma levels of oestradiol-17β, oestrone and progesterone during a 28 day ovulatory menstrual cycle in a rhesus monkey. Note the double oestradiol-17β and oestrone surges during the follicular phase of the menstrual cycle. Only one corpus luteum was observed at laparoscopy.
Fig. 6.
The peripheral plasma levels of oestradiol-17β, oestrone and progesterone during a 27 day ovulatory menstrual cycle in a rhesus monkey. Note the increasing oestradiol-17β and oestrone levels in plasma during mid and late luteal phase.

Fig. 7.
The peripheral plasma levels of oestradiol-17β, oestrone and progesterone during a 26 day ovulatory menstrual cycle in a rhesus monkey. Note the increasing oestradiol-17β and oestrone levels in plasma during mid and late luteal phase.
Peripheral plasma levels of oestrone, oestradiol-17β and progesterone, before and after luteectomy in 3 rhesus monkeys.

levels throughout the menstrual cycle. The ratio $E_2 : E_1$ varied between 1.8 and 3.2 in the follicular phase, 3.4 to 5.3 around midcycle, and during the second half of the cycle the ratio was mostly less than one. The ratio $E_1 + E_2 : P$ increased steadily during the first half of the cycle to a height of 1.2 seventeen days before menstruation. Thereafter the ratio fell gradually during the next five days and attained very low levels which were maintained during most of the luteal phase. The ratio began to change in favour of the oestrogens during the last four days before menstruation.

**DISCUSSION**

In the present study considerable individual variations in the steroid levels were observed. However, the values reported here are within the ranges reported earlier (Hotchkiss et al. 1971; Neill et al. 1967; Bosu et al. 1972; Hess & Resko 1973).

Although oestradiol predominates over oestrone throughout the menstrual cycle (Fig. 1) the decreasing ratio of $E_2 : E_1$ indicates that relatively more $E_1$ is produced in the luteal phase. This observation differs from the results of an earlier report in which oestrone was shown to display the same pattern but at
much lower concentrations than oestradiol throughout the menstrual cycle (Hess & Resko 1973). The relative increase in E1 during the luteal phase may be due to increased biosynthesis of E1. We have observed that after HCG stimulation during the luteal phase of the normal menstrual cycle the plasma levels of E1 are higher than E2 (Fig. 9). The possibility exists that extra ovarian sources contribute to the E1 levels in plasma. Some of the E1 may be of adrenal origin either directly as a secretory product or indirectly as a result of peripheral conversion of adrenal precursors. Another possible source is peripheral conversion of ovarian testosterone. The adrenal gland of the female rhesus monkey secretes androstenedione, testosterone and oestradiol (Resko 1971) and the ovary secretes testosterone (Hess & Resko 1973). However, the adrenal contribution to the peripheral plasma levels of oestrogens in the ovariectomized rhesus monkey is negligible (Resko 1971) and the rate of peripheral conversion of precursors to oestrogen is not well defined.

The gradual increase in the E1 + E2 : P ratio in the pre-menstrual period indicates increased production of oestrogens, most likely by developing ovarian follicles (Koering 1969). Increase in the plasma levels of E1 and E2 in the pre-

![Graph](https://via.placeholder.com/150)

**Fig. 9.**

The peripheral plasma levels of oestrone, oestradiol-17β and progesterone, before and after HCG treatment in the luteal phase of the menstrual cycle in a rhesus monkey.
menstrual period in this study may be a consequence of FSH increase following reduction in progesterone production. Knobil (1973) has reported that FSH increases in the pre-menstrual period and a regulatory role of progesterone on oestrogen secretion in the rhesus monkey has been suggested (Hess & Resko 1973). Though the corpus luteum appears to be the main source of E₁, E₂ and P during the luteal phase as demonstrated by the dramatic fall in the levels of the steroids after luteectomy, the increase in oestrogens observed within 48 h after luteectomy suggests that the ovarian follicles produced E₁ and E₂ in response to gonadotrophin upsurge following luteectomy.

Functional luteolysis and menstruation have been the subjects of several investigations in the rhesus monkey (for review see Knobil 1973). The assumption is that the two are closely related. Knobil and co-workers have recently shown that oestradiol will reduce the plasma levels of progesterone and shorten the luteal phase in the rhesus monkey (Karsch et al. 1973). In women moderate doses of synthetic oestrogens shortened the luteal phase only by one or two days (Johansson & Gemzell 1971) while large doses of diethylstilboestrol produce a dramatic decrease of progesterone levels and of the length of the luteal phase (Johansson 1973). The decline in the plasma progesterone levels seen by Karsch et al. (1973) was not associated with decreases in LH levels suggesting that the luteolytic action of the oestrogens was at the ovarian level. This view was confirmed by Hoffman's observation after the introduction of oestradiol into the ovary containing the corpus luteum in women (Hoffman 1960).

As demonstrated by Knobil et al. (1968) surgical stress will have a clear luteolytic effect in the rhesus monkey. The hormonal factors involved are as yet unknown, but a dramatic reduction in the ovarian blood supply cannot be excluded. The “stress factor” may be central in understanding the regulation of the corpus luteum function. It should be noted that the length of the luteal phase in the monkeys studied by us is slightly longer than that found in other laboratories (Neill et al. 1967) and also by us previously, when the blood samples were taken by femoral punctures. At present the blood samples are taken from leg veins under very calm conditions with the monkey remaining in her cage.

Based on these observations and the results of the present study a working hypothesis of the hormonal events during the normal luteal phase prior to menstruation could be suggested. Progesterone levels start to decline due to increased oestrogen production, inherent aging or changes in the enzyme system of the corpus luteum. The decrease of progesterone will withdraw inhibition of the hypothalamus as demonstrated by Hagino & Goldzieher (1970) in the baboon and the present observations in the luteectomized monkeys. The resultant FSH stimulation of the ovaries will induce increased oestrogen production in the follicles. The oestrogens will cause the release of prostaglandins in the uterus and the prostaglandins in turn will induce myometrial contractions
and menstrual bleeding. Auletta et al. (1972) have recently shown a release of prostaglandin F\(_{2\alpha}\) after oestrogen stimulation in the luteal phase of the menstrual cycle in the rhesus monkey. Maximum prostaglandin rise was subsequent to the fall in progesterone levels in plasma.

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