Effect of Chorionic Gonadotropin on Corpus Luteum of the Monkey (37721)

ALI H. SURVE, F. EUGENE HARRINGTON, AND RICHARD L. ELTON
Research Department, Endocrine Section, Sandoz-Wander, Inc., E. Hanover, New Jersey 07936

Hisaw in 1944 (1) reported that the onset of menstruation was delayed considerably in monkeys treated chronically with large doses of human chorionic gonadotropin (HCG) starting at various times during the luteal phase of the menstrual cycle. Recently, Neill and Knobil (2) observed that small doses of HCG administered to monkeys at a time when the corpus luteum (CL) was waning resulted in a dramatic rise in levels of peripheral plasma progesterone within 24 hr. However, the increase in progesterone was not sustained as basal values were observed, in spite of continued treatment, prior to the menstruation which was delayed. These investigators (2) speculated that the delay in the onset of menstruation may be attributed to the sustained high level of estrogen secreted by the stimulated CL.

Such an abrupt and nonsustained increase in plasma progesterone has also been reported (3) in pregnant monkeys approximately 9–11 days after ovulation. Implantation occurs about this time resulting in low levels of chorionic gonadotropin, perhaps secreted by the developing syncytiotrophoblast, in peripheral blood (4).

The purpose of the present investigation was to study changes in estrogen and progestin in peripheral blood plasma in response to small doses of HCG administered for 10 days in cycling monkeys. Treatment was initiated about the time of implantation had the female been pregnant and overlapping the period when the ovaries would be required for maintaining pregnancy in the monkey (5).

Materials and Methods. Seven normally cycling rhesus monkeys (*Macaca mulatta*) weighing 4.1–7.3 kg were used in this study. Previous cycle lengths of these animals ranged from 26 to 32 days. The animals were housed year-round in a controlled environment (temperature 22°, humidity 50–55%, and 12 hr light). They were fed monkey chow once a day, and water was provided *ad libitum*. The diet was supplemented with multivitamin tablets.

All females were examined daily for evidence of menstruation. When blood was observed in the cage, a vaginal smear was obtained to verify menstruation. Day 1 of the cycle was the day on which menstrual flow was observed for the first time.

On day 5 of the cycle, blood samples were collected from the saphenous vein in heparinized syringes, and, with the exception of one animal, ♀ 83, sampling was continued on a daily basis until each animal menstruated again. Female 83 menstruated on day 59 of the cycle; however, samples were collected only for the first 41 days. Plasma was separated by centrifugation, transferred to glass vials, and stored at −20° until assayed.

Five of the seven females received a series of 10 im injections of 50 IU HCG (Antuitrin "S", Parke Davis) in saline beginning on the tenth day following the midcycle estrogen peak. Two females served as controls and were treated with saline during the same period.

Estrogen concentrations were measured by radioimmunoassay of ether extracts of 200 μl plasma using the method described by Hotchkiss *et al.* (6). The antibody used was kindly provided by Dr. B. V. Caldwell. Standard curves were obtained using estradiol-17β. It should be pointed out that the antibody used in these assays cross-reacts with other estrogens. However, when the reactivity with estradiol-17β is taken as 100, the corresponding values for estradiol-17α, estrone, and estriol are 80, 33, and 9, respectively. All estrogen values are, therefore, to be considered as picograms of estradiol-17β equivalents per ml of plasma. Plasma aliquots from ovariectomized monkeys were included in each assay to estimate blank values and recoveries of added nonlabeled estradiol-17β. Using 1:90,000 dilution of the antibody and dextran-coated charcoal for separation of bound from free estrogen, the mean and standard error for blanks was 6.8 ± 0.3 pg ($N = 51$), whereas, of the 20.0 pg estradiol-17β added, 17.7 ± 0.3 pg ($N = 51$) were recovered.

Blood plasma progestin was estimated by a rapid competitive protein-binding assay as described by Johansson *et al.* (7). Crude petroleum ether extracts of 200 μl plasma were assayed employing corticoid-binding globulin obtained from blood plasma of a metyrapone-treated male dog. In each assay, two aliquots of human male plasma containing 2.0 ng of added crystalline progesterone were analyzed. The mean recovery of progesterone in 65 assays was 1.84 ± 0.03 (SE) ng; the mean blank value was 0.15 ± 0.006 (SE) ng. The values reported for blood plasma estrogen and progestin were not corrected either for blank or procedural losses.

While the steroids measured were not isolated before estimation in the assay systems used, it is reasonable to believe that the values and the patterns obtained are real. Besides estradiol-17β, estradiol-17α, and their common analogs, the estrogen antibody does not bind significantly with any other steroid normally present in blood (8). On the other hand, it has been documented (7) that the petroleum ether extract used for the competitive protein-binding assay provides reliable estimates of plasma progesterone.

Results. During the pretreatment period, all animals had normal estrogen and progestin values during the follicular phase of the menstrual cycle (Figs. 1 and 2). At midcycle, increases in both steroids were observed. Injection of physiological saline in animals 88 and 144, starting 10 days after the midcycle estrogen peak, had no demonstrable effect on levels of plasma estrogen and progestin, and these animals menstruated before the regimen of the ten injections was completed (Fig. 1). Since blood sampling and treatment was stopped on the day of menstruation, females 88 and 144 received, respectively, 6 and 8 injections of saline. The duration of luteal phase in these animals was 16 and 18 days, respectively.

Four of five animals treated with HCG responded to the stimulatory effect of HCG. Values for steroids from these four animals were combined and normalized, using the start of treatment as the point of reference (Fig. 2). On the day treatment was initiated, progestin levels were declining, whereas estrogen levels were rising. Twenty-four hours after the first injection of HCG, progestin levels reached a peak and declined steadily thereafter despite continued treatment. Estrogen, on the other hand, increased slowly, reaching highest levels sometime prior to the sixth injection and then, like progestin, dropped steadily in spite of continued treatment. The animals menstruated within 72 hr after the last injection even though the steroid levels were basal for several days.

Normal progestin levels, despite a midcycle estrogen surge, were not observed in female 83. There was a slight increase in both estrogen and progestin 24 hr after the first injection of HCG, but this may be coincidental since subsequent patterns of steroid secretions were different from those of the other treated animals (Fig. 3). Menstruation in this female was not observed within the 72-hr period following treatment withdrawal as was the case in the four remaining treated monkeys. The duration of the menstrual cycle in this animal was 59 days.

Discussion. Handling and im injections had no effect on the menstrual cycle of the animals in this study since saline-treated animals demonstrated normal steroid patterns (Fig. 1) with cycle lengths within the normal range.

The data presented in Fig. 2 clearly show, as far as we know for the first time, the rise in peripheral plasma estrogen levels of monkeys treated with HCG during the luteal phase of the cycle. These results confirm the

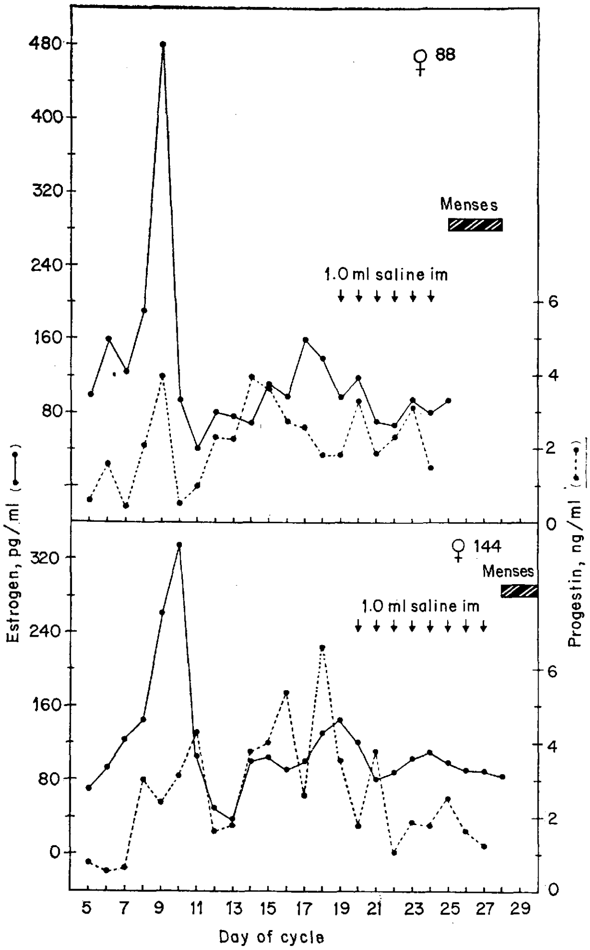

FIG. 1. Daily changes in blood plasma estrogen and progestin in saline-treated animals.

speculations of other investigators (1, 2) who administered HCG to monkeys but did not quantitate plasma estrogen. Available evidence suggests that the source of estrogen, like progestin, may have been luteal tissue. In this study, peripheral plasma estrogen levels rose gradually to a peak in the four treated females (Fig. 2) in which normal luteal function was observed, as evidenced by the rise in the progestin levels. On the other hand, female 83, which showed no rise in plasma progestin, also had no change in estrogen level when HCG was administered. In the rat, several groups of investigators have demonstrated estrogen secretion by luteinized tissue stimulated with LH (9, 10) or HCG (10).

The sharp increase in plasma progestin within 24 hr of the injection of HCG and the subsequent gradual decline are in agreement with data reported for monkey (2) and man (11). The nonresponsiveness of animal 83 was probably related to very low progestin levels before the start of the treatment (Fig. 3) and suggests that this animal did not have a functional corpus luteum. This observation agrees with the reported data (2) that the rise in peripheral progesterone concentration observed in monkeys following treatment with gonadotropin is due to stimulation of the luteal tissue.

Additional insight into the physiological mechanisms controlling luteal function comes from studies comparing corpora lutea from women during the luteal phase of the menstrual cycle and those from women with

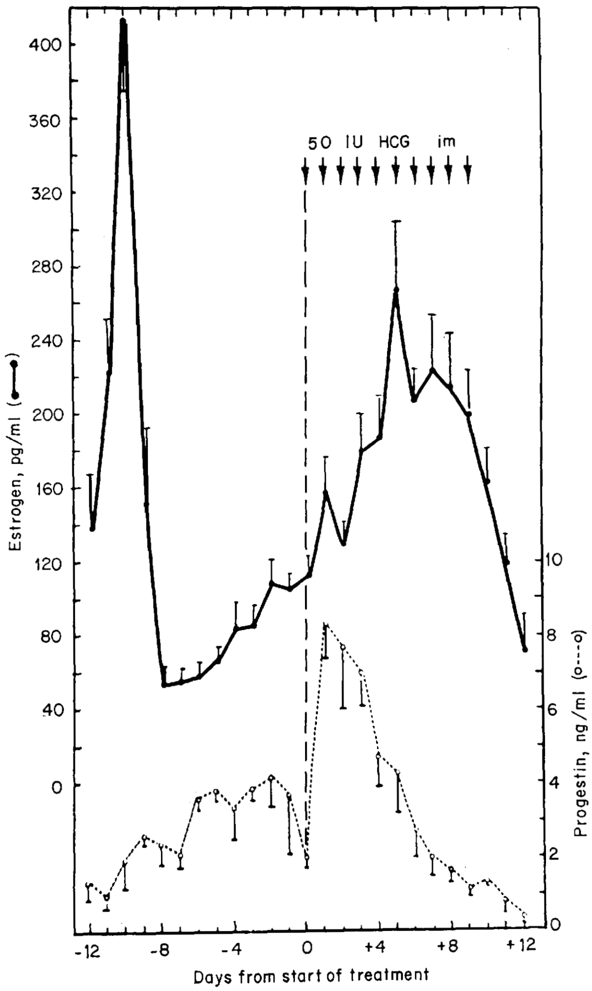

FIG. 2. Mean plasma estrogen (± SE) and progestin (± SE) levels in animals treated with HCG. Abscissa normalized to the day of treatment.

ectopic pregnancy. Rice *et al.* (12) reported that the latter synthesize more estrogen and progestin than the former when they are incubated *in vitro* with human gonadotropins. These investigators attributed this difference in response to the preexposure of the pregnancy CL to the endogenous chorionic gonadotropin. Armstrong (13) speculated that LH has dual effects on rat luteal tissue; it depletes cholesterol storage by increased progestin secretion, and, secondly, it activates enzymes involved in estrogen secretion. That gonadotropins do modify specific enzyme systems involved in steroid biosynthesis has also been reported for rabbit ovary (14). It is feasible, then, that the data obtained in the present studies using HCG reflect a change in the biosynthetic pathway from the Δ^4-3-ketone pathway operative in the monkey CL to Δ^5-3β-ol route. This would favor synthesis of estrogen instead of progesterone. Subsequently, the increased levels of estrogen probably act back on the CL, initiating the process of luteolysis (15) leading to decreases in the levels of estrogen since it is of the luteal origin. This hypothesis, if true, would explain why progestin levels decline while estrogen levels were increasing and also why the estrogen levels were not sustained in spite of continued administration of HCG. *In vitro* incubation of ovarian tissue obtained from a polycystic

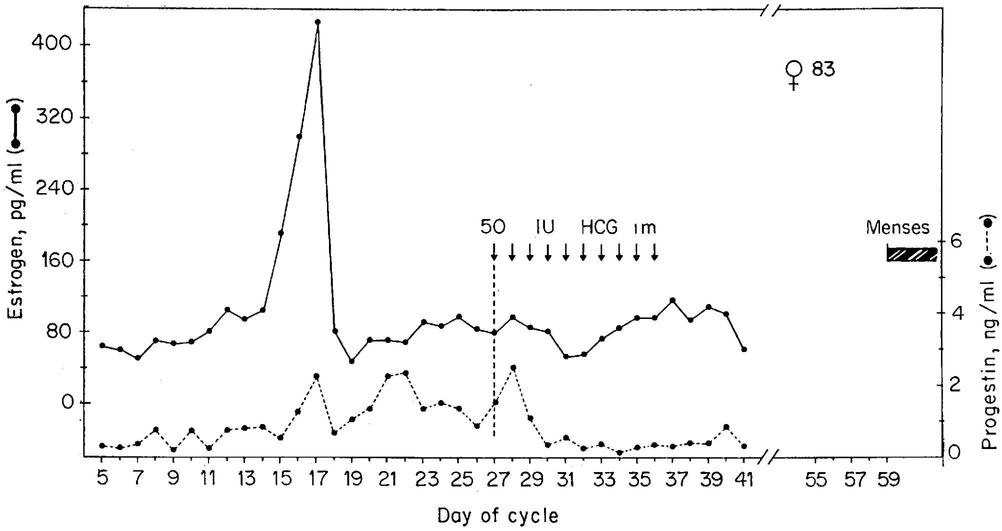

FIG. 3. Daily changes in blood plasma estrogen and progestin in a monkey which did not respond to the HCG treatment.

ovary has demonstrated the presence of such an alternate pathway (16); however, investigation is required to support such a concept under the experimental conditions reported here.

The animals treated with HCG, like those of others (2), did not menstruate for several days after the steroids in the peripheral circulation had fallen to basal level. That menstruation is not a reliable indicator of the ovarian function has also become obvious from the study of summer amenorrhea in rhesus monkeys, where levels of plasma progestin and estrogen remained nondetectable for several weeks before menstruation was observed (unpublished observation).

Finally, it is of interest to note that the patterns of sex steroids in the peripheral circulation during treatment with HCG appear similar to those observed during early pregnancy, from implantation to the time the ovary becomes dispensable (17). It supports the suggestions made for the role of CL in early pregnancy in monkey (2) and human (18)—that the low levels of chorionic gonadotropin released at the time of, and subsequent to, implantation are responsible for extending the life of the CL of the menstrual cycle to maintain pregnancy during the transitional period when the placenta is being established and becomes functional. Such a concept has been questioned by Reinius *et al.* (19), based on a correlation of circulating levels of hormonal changes with the histological examination of an implantation site of one monkey on day 10 of pregnancy. However, as shown in this study, abrupt hormonal changes occur within 24 hr after administration of the gonadotropin, and, therefore, an observation made a few hours earlier in time period could lead to different conclusions.

Summary. During the luteal phase of the menstrual cycle, rhesus monkeys (*M. mulatta*) were treated with 50 IU HCG in saline or saline alone, administered intramuscularly. The ten daily treatments were initiated on the day when implantation would have occurred had the cycle been fertile. Plasma estrogen was determined by radioimmunoassay, and progestin was measured by the competitive protein-binding method. Within 24 hr of the first HCG treatment, progestin levels in the blood reached a peak and then declined gradually despite continued treatment. Estrogen levels also increased but did not reach a peak until the fifth day of HCG administration. Like progestin, the estrogen levels then declined even though treatment was continued. Onset of menstruation in these animals was delayed by several days. The saline-treated animals had normal steroid

patterns and cycle length. It is proposed that the injection of HCG brings about biochemical changes in the corpus luteum similar to those occurring during early pregnancy.

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