

Plasma Progesterone Levels and the Menstrual Cycle of the Monkey.*† (17616)

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Present concepts of the humoral environment of the endometrium through the menstrual cycle are almost entirely deductions from observations of the effects of artificially supplied steroid hormones, from studies of the structure of the endometrium and ovaries during the cycle, and from measurements of urinary steroids during the various phases of the cycle. With respect to progesterone, apparently the only reported attempts to ascertain blood levels have been those of Clauberg(1), Bloch(2), and de Allende(3). Clauberg was unable to detect progestational activity in extracts of human menstrual cycle blood and of human pregnancy serum and blood. Bloch found less than 1 Rabbit Unit of progesterone in 8 to 12 liters of human pregnancy blood. De Allende reported that blood samples taken at 3-day intervals throughout several menstrual cycles of monkeys assayed at from 0.06-0.12 μg to 0.25-2.5 $\mu\text{g}/\text{cc}$ (McPhail scale). A method for the bioassay of progesterone has been developed(4) primarily with physiological measurements of this nature in mind. The method involves injection of serial dilutions of the material to be tested into the lumina of uterine segments of ovariectomized mice. The method regularly

detects 0.0002 μg of progesterone, is apparently specific for progesterone(5), and can be applied to measurements of blood levels(6).

The present study was undertaken solely as a preliminary survey and with reference to several questions: is blood progesterone present in the pre-ovulatory phase of the cycle; what is the magnitude of blood level of progesterone in the luteal phase of the cycle; is the level uniform throughout the luteal phase; is the onset of menstruation preceded by a significant decline in the level of progesterone in the blood?

Each of 2 adult rhesus monkeys was studied through a cycle extending from the middle of May until mid-June. The 2 animals had exhibited cycles averaging 27.8 days (range, 26-29 days; monkey A42) and 28.6 days (range, 25-32 days; monkey A49), respectively, as revealed by daily vaginal smears through the several months preceding the assays of the blood. The dates when blood was obtained are shown in the chart. In each monkey the study began toward the end of one cycle and continued to approximately the same day in the succeeding cycle. The lengths of the cycles during the study are indicated on the chart. Points indicating assay values have been connected with straight lines solely to aid visualization; it should not be inferred that the lines indicate progesterone levels obtaining on the days on which blood samples were not taken. In each instance a one ml sample of blood was drawn from a saphenous vein with sodium citrate serving as the anticoagulant. The sample was promptly centrifuged, and the plasma progesterone was separated into the free and bound fractions as described earlier(6). (It has already been shown that progesterone is present in plasma

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† Dr. F. E. Bryans at the Harvard Biological Laboratories has independently completed a similar and more extensive study which will appear soon.

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2. Bloch, P. W., *Endocrinology*, 1936, v20, 307.

3. Allende, I. L. C. de, *Proc. Soc. Exp. Biol. AND MED.*, 1940, v44, 534.

4. Hooker, C. W., and Forbes, T. R., *Endocrinology*, 1947, v41, 158.

5. Hooker, C. W., and Forbes, T. R., *Endocrinology*, 1949, v45, 71.

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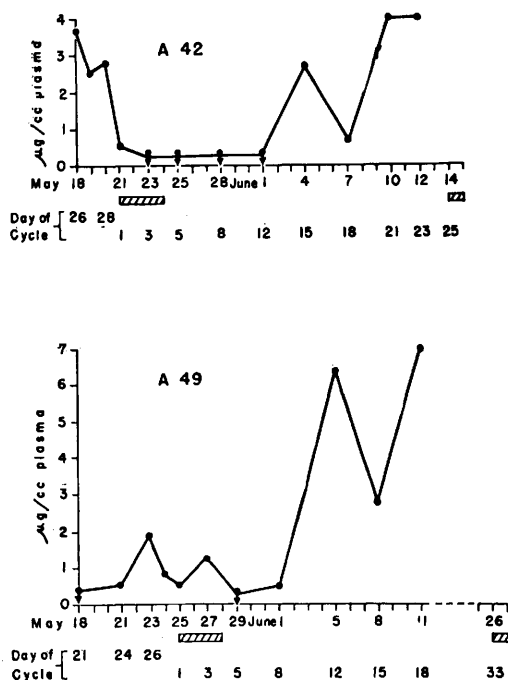


FIG. 1.

Free plasma progesterone levels in 2 monkeys through a menstrual cycle. In each instance the day of the cycle and the date are shown. The arrows signify that the values thus indicated are not known, but are less than those indicated by the solid circles.

and not in the blood cells)(6). The two fractions were incorporated in sesame oil, and dilutions were prepared so that the assay values obtained are believed to be accurate within a fraction of a μg per ml of original plasma.

The values for free plasma progesterone are shown in the chart. In general, the 2 animals showed parallel fluctuations in levels of progesterone. During menstruation and in the early days following menstruation the level was low. In each monkey, a relatively high value was present near the middle of the cycle, a decidedly lower level followed, and a high level appeared again in the latter half of the cycle. A low level was present at or preceding the onset of menstruation.

The low level during the first half of the cycle was expected on the basis of the conventional account of the menstrual cycle. The source of the small amounts of progesterone

during this portion of the cycle in monkey A49 is unknown.

Presumably the high level near the middle of the cycle was related to ovulation, although it is not known when ovulation occurred in either animal, or indeed whether ovulation occurred. Moreover, it is not known whether the high level was brief or up to 4 days in duration. The two questions may prove to be highly significant in the analysis of the circumstances at and related to ovulation.

The values in the latter half of the cycle are interesting in relation to the hypothesis that menstruation is provoked by diminution in progesterone. In monkey A42 relatively high levels were found on the 3 days preceding the first menstruation. On the first day of menstruation the level had dropped to a low value. The values in this period of 4 days may indicate a steady decline preceding menstruation. On the other hand, the relatively small day to day differences on the 26th, 27th and 28th days of this cycle may be unrelated to each other or to menstruation. In the succeeding cycle high levels were found 4 and 2 days before a single day of vaginal bleeding. This bleeding, however, cannot be considered to be a typical menstruation inasmuch as a 3-day period of bleeding began 9 days later. Unfortunately, the earlier bleeding had been accepted as menstruation, and the blood levels of progesterone during the next 9 days were not followed. The progesterone levels on May 18 to 20 in this animal seem somewhat at variance with the general observation that experimental menstrual bleeding in ovariectomized monkeys begins approximately 3 days after the last of a series of injections of progesterone(7).

In the second animal, monkey A49, the level was inconstant but relatively low for at least 7 days before menstruation. This circumstance also seems not to be consistent with observations on experimental menstruation, but in the opposite direction. Here, however, it may be that the menstruation followed an

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anovulatory cycle. Hartman(8) has reported that the monkey ovulates infrequently during the summer months, and the menstruation occurred toward the end of May. If the cycle was in fact anovulatory, the source of the progesterone is unknown. The high level of progesterone found in the second cycle in this animal indicates that a corpus luteum must have been present. This cycle, however, was 32 days in length, and menstruation began on June 26, 14 days after the last assay. Consequently, the level of progesterone in relation to the onset of the second menstruation is unknown.

Single values in 3 other intact monkeys may be mentioned. In one animal a level between 7.7 and 8.9 μg per ml plasma was found 10 days before the onset of menstruation; in a second animal the level was 2.8 μg per ml 3 days before menstruation; in a third animal the level was 0.9 μg per ml 2 days before menstruation. It is clearly impossible to say whether these isolated values conform to any pattern shown by the 2 animals studied throughout a cycle. The values for bound plasma progesterone ranged from less than

0.2 to 0.4 μg per ml. The small fluctuations encountered seemed to be quite unrelated to the phase of the cycle.

The uniformly low levels of bound progesterone are consistent with our other experience with this fraction. Its level has been low under all physiologic circumstances in several species(6). It has been high only in animals receiving injections or carrying pellets of progesterone; in the mouse the bound fraction was biologically inert, and binding was a means of hepatic inactivation of progesterone(9).

Summary. Bioassay of the free and bound plasma progesterone at various intervals through a menstrual cycle in each of 2 monkeys indicate the level of free progesterone to be low during and following menstruation, high near the middle of the cycle, lower following this peak, and high in the latter half of the cycle. A low level was present at or preceding menstruation. The levels of the bound fraction were low at all times.

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Survival of *Schistosoma mansoni* *in vitro*.^{*} (17617)

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Information about the survival of schistosomes *in vitro* has been limited to the observation that *Schistosoma japonicum* can be maintained alive in serum for periods of 8 to 83 days(1,2). Similarly, we have found that *Schistosoma mansoni* survives for 14 to 18

days in horse serum. Knowledge about the nutritional requirements of these trematodes could be acquired by investigating the constituents of serum, essential for their survival. Accordingly, studies designed to define these factors were initiated using *Schistosoma mansoni*.

Methods. All experiments were conducted under aseptic conditions. Pairs of schistosomes

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