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## Precipitation of Menstruation in Castrated Monkeys with Progesterone in The Presence of Estrogen.\* (28710)

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Much is known about the physiological conditions under which menstruation occurs in monkeys but its exact cause is yet quite obscure. It has been found that uterine bleeding in a castrated animal usually follows a series of injections of either estrogen or progesterone and that one or more injections of progesterone during an estrogen treatment may be followed by a menstruation. Also, when the 2 hormones are given separately, a minimal dosage is required to prevent "breakthrough" bleeding during a treatment. These and other considerations have been the subject of a recent review(1).

We have observed on several occasions when a dosage of estrogen was being given which ordinarily was sufficient to prevent "break-through" bleeding, it did not do so when combined concurrently with a small dosage of progesterone. This effect of progesterone on the action of estrogen seemed of sufficient importance to deserve further study.

Methods and results. Two adult monkeys (Macaca mulatta) no. 260 and 261, weighing 5354 and 5074 g respectively were castrated and after surgical bleeding and a brief rest period were used in experiments of the following nature. Each animal was given (A) a subcutaneous injection of 10  $\mu$ g estradiol-17B dissolved in 0.1 cc propylene glycol daily for 15 to 23 days. This was followed immediately by a treatment (B) in which each received 10  $\mu$ g estradiol-17B and pro-

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gesterone daily, injected at different sites in 0.1 cc propylene glycol, until bleeding occurred or for a time sufficiently long for it to have happened. Vaginal lavages were taken daily, starting soon after initiation of part "B" of each experiment until its conclusion. After this, the animals were given a rest for a few days to several months before being put on another treatment.

It will be noticed that these animals received 10  $\mu$ g estradiol daily for the duration of each experiment. It has been found that this dosage of estrogen is sufficient to maintain the endometrium without bleeding for an indefinite period in the great majority of animals. In fact, the 2 monkeys used in these experiments were given 10  $\mu$ g estradiol daily for 51 days (3-5-60) and uterine bleeding did not occur but did take place on the second day in one and the third day in the other after the injections were discontinued.

The daily dosage of progesterone that will prevent bleeding following discontinuance of 10  $\mu$ g estradiol daily for about 20 days is approximately 1.0 mg(2). Therefore, the dosages of progesterone used in these experiments were less than this amount, ranging from 0.062 to 0.75 mg. Also, it should be noted that the experiments were distributed over a period of about 4 years, and the animals were used for no other purpose.

Examination of the data shows that bleeding did not occur when 0.75 mg of progesterone was given with 10  $\mu$ g estradiol, and once in 3 instances when 0.062 mg was given, thus indicating upper and lower limits of dosage required for the reaction. Bleeding with cer-

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	Α	В		
		Estradiol, $\mu g/$	Bleeding	
	Estradiol	day + proges-	after	
Treatments	$10 \ \mu g/day$	terone, mg/day	start of B	Remarks
			Monkey 260	0
11 - 24 - 57	15	10 + .25	17	
12 - 31 - 57	15	10 + .25	12	
2 - 1 - 58	` 16	10 + .25	9	Also, 3000 GPU Relaxin during B.
3- 4-58	<b>27</b>	10 + .125	14	,
9 - 29 - 58	20	10 + .25	14	
11- 2-58	15	10 + .125	27	
12 - 14 - 58	17	10 + .50	<b>21</b>	
2 - 20 - 59	16	10 + .062	(24)	
4 - 20 - 59	21	10 + .75	(24)	Estrogen continued. Bled 9 days later.
10- 1-59	<b>23</b>	10 + .062	<b>`</b> 14´	
11 - 1 - 59	19	10 + .75	(37)	Estrogen continued. Bled 4th day.
3- 5-60	51		Ò Ó	Stopped. Bled 3rd day.
10-30-61	20	10 + .25	25	
2-5-62	20	10 + .25	21	
4 - 16 - 62	20	10 + .125	(28)	
10-19-62	20	10 + .25	16	
			Monkey 26	1
11-21-57	16	10 + .25	20	Treatment continued 38 days without 2nd bleeding. Stopped. Bled 3rd day.
2 - 10 - 58	17	10 + .125	27	0 II ·
9 - 29 - 58	20	10 + .25	20	
11 - 9 - 58	15	10 + .125	31	
12 - 30 - 58	15	10 + .50	17	
2 - 20 - 59	15	10 + .062	(24)	
4 - 20 - 59	21	10 + .75	(24)	
10-1-59	23	10 + .125	`15	
11 - 18 - 59	19	10 + .75	(25)	Estrogen continued. Bled 3rd day.
3- 5-60	51	·	0	Stopped. Bled 3rd day.
*10-30-61	20	10 + .25	(29)	
* 2- 5-62	20	10 + .25	(25)	Continued progesterone. Bled 4th day.
4 - 16 - 62	20	10 + .125	(28)	
10 - 19 - 62	20	10 + .25	22	

 
 TABLE I. Precipitation of Menstruation by Progesterone in Presence of Estradiol in Castrated Monkeys.

\* Thickening of skin on back.

() Bleeding not observed during Treatment B.

tain variations occurred at all dosages between these 2 limits. Monkey 260 seemed more responsive to the treatments than monkey 261. A possible contributing factor to this difference may have been an unusual thickening of the skin of 261 as a result of the treatments. This became so extensive that it was not always certain that an injection was administered subcutaneously or intradermally. This condition first became a problem in experiment 10-30-61, however, a year later (10-19-62) the condition had improved and the usual response was obtained when 0.25 mg progesterone was introduced into the hormone combination.

Discussion. These results show clearly that surprisingly small doses of progesterone are capable of influencing the action of a maintenance dose of estrogen on the uterine endometrium. Although this interaction between the two hormones is not understood, certain observations may contribute to a better understanding of its nature and probable importance.

The minimal dose of estradiol-17B that will maintain the endometrium indefinitely in a castrated monkey is approximately 10  $\mu$ g and "break-through" bleeding usually occurs when smaller doses are given. The minimal dose of progesterone that will inhibit uterine bleeding following discontinuance of a series of injections of 10  $\mu$ g estradiol is about 1.0 mg, although progestational effects can be seen in the endometrium when 0.5 mg is given concurrently with this amount of estrogen. When 0.75 mg or more of progesterone is combined with 10  $\mu$ g estradiol a synergistic effect on growth of the uterus invariably occurs. It seems remarkable in view of this that such small doses of progesterone (0.125 to 0.5 mg) can induce uterine bleeding in the presence of a maintenance dose of estradiol (10  $\mu$ g).

Circumstantial evidence indicates that the biochemical action of estrogen and progesterone on the uterine endometrium is different. Also, the indications are that the effect produced by progesterone on the endometrium in some way decreases the effectiveness of estrogen, however, when 0.75 mg or more of progesterone is given, a synergistic reaction between the 2 hormones is initiated and consequently the integrity of the endometrium is preserved. When small doses of progesterone (0.125 to 0.5 mg) are given in combination with a minimal maintenance dose of estradiol (10  $\mu$ g) the effectiveness of the estrogen apparently is decreased below that sufficient for sustenance of the endometrium, and at the same time, the dosage of progesterone is too small either to preserve the endometrium by its own action or induce effective synergism, consequently, "break-through" bleeding ensues(2).

The experiments in most instances were spaced sufficiently far apart that it seems unlikely the results were influenced by the effects of a previous treatment (3,4,5). Also, the possibility has been suggested that endogenous progesterone from some extraovarian source may have an influence on uterine bleeding (6,7,8). This may be true, though other explanations seem possible, but even so, if the effects of small doses of progesterone on a minimal maintenance dose of estradiol, as described here, represent normal

physiological relations of the hormones it seems probable that menstruation rarely occurs, even in anovulatory cycles, without the action of progesterone being a contributing cause.

Summary. Two adult castrated monkeys (Macaca mulatta) on a dosage of estradiol-17B (10  $\mu$ g daily), which with rare exceptions maintains the endometrium without "break-through" bleeding, also were given concurrently small doses of progesterone (0.062 to 0.75 mg daily). A total of thirty experiments were performed over a period of about 4 years. "Break-through" bleeding occurred in a great majority of experiments within approximately 2 to 3 weeks when 0.125 to 0.5 mg progesterone was combined with 10 µg estradiol. Bleeding was not observed when 0.75 mg progesterone was given and occurred in one of 3 experiments with 0.062 mg, thus indicating a general upper and lower limit of dosage for the reaction. It is concluded that small doses of progesterone in some way decrease the effectiveness of a minimal maintenance dose of estradiol resulting in precipitation of "break-through" bleeding.

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