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Precipitation of Menstrual Bleeding in Monkeys by a Folic Acid Antagonist[1]

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Failure of estrogens to induce growth of the female reproductive tract in the absence of folic acid was first described for the chick (*4*). Similar results have been obtained with the frog (*2*), the rat (*5*), and the monkey (*3*). In 1948, Hertz (*3*) reported that six out of eight sexually immature monkeys on a folic acid-free diet failed to show cornification of the vaginal mucosa, development of the external genitalia, or growth of the sex skin after treatment with estradiol benzoate. Data presented in this report show that maintenance of the uterine endometrium of monkeys by estradiol[3] is also affected by a lack of folic acid.

Five castrated female monkeys (*Macaca mulatta*) were injected subcutaneously with 10 μg of estradiol daily throughout the experiment. This dosage of estrogen is sufficient to maintain the endometrium indefinitely without bleeding. After 20 days' pretreatment with estradiol, the monkeys were given daily injections of a folic acid antagonist, aminopterin,[4] along with the estrogen. The susceptibility of monkeys to a lack of folic acid and individual differences of tolerance to aminopterin made it advisable to start the treatment with a low dose of the antagonist and to increase the amount, about every 10 days, until the animals menstruated or were prostrate due to the vitamin deficiency. This schedule was adhered to for all the animals, with two exceptions. Monkey 181 was kept on 100 μg of aminopterin throughout the experiment, and monkey 175 was continued on 500 μg of aminopterin when that level was reached (Table 1).

All monkeys were examined daily for menstrual bleeding, and blood counts were made at frequent intervals. Complete post-mortem examinations were performed on the animals when menstruation was noted or when the animals were in a state of collapse and near death.

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[3] Estradiol was obtained through the courtesy of Schering Corporation, Bloomfield, New Jersey.

[4] Aminopterin (4-aminopteroylglutamic acid) was obtained through the courtesy of Dr. E. L. R. Stokstad, Lederle Laboratories, Pearl River, New York.

Menstruation occurred in three of the five monkeys treated with estradiol and aminopterin (Table 1). These animals had received the antagonist for a total of 23–48 days, while the two monkeys that failed to menstruate had been under treatment for 22 days. It is apparent that neither length of treatment nor amount of antagonist given can explain the failure to produce menstruation in these two monkeys. A possible explanation may be found in the extreme variability in length of time required to produce a folic acid deficiency in monkeys (*1*), and the difficulty in keeping them alive once the deficient state is attained. Apparently, in order to produce menstruation in monkeys during estrogen treatment, it is not only necessary to produce a folic acid-deficient condition, but it is also necessary to keep the animals alive long enough for menstruation to occur.

TABLE 1

PRECIPITATION OF MENSTRUATION BY AMINOPTERIN IN MONKEYS UNDER DAILY TREATMENT WITH ESTRADIOL*

Monkey No.	Body wt in kg	Days of treatment with aminopterin				Total No. days of treatment	Results
		100 μg	200 μg	500 μg	1 mg		
169	4.52	10	10	9	9	38	Menstruation
175	3.36	10	10	28	—	48	Menstruation
179	3.77	10	10	2	—	22	No menstruation
180	3.69	10	12	—	—	22	No menstruation
181	3.52	23	—	—	—	23	Menstruation

* Castrated monkeys under daily treatment with 10 μg of estradiol alone for several months to a year failed to menstruate during the course of treatment.

Previous work in this laboratory has shown that the daily dose of estradiol used in these experiments will induce maximal growth of the endometrium in monkeys within 20–30 days, and that menstruation does not occur when such treatment is continued for several months. Therefore, our results indicate that in the absence of folic acid, estrogen cannot maintain the uterine endometrium or prevent menstrual bleeding.

Menstruation failed to occur in three castrated monkeys that were first given 10 μg of estradiol for 20 days and continued on daily injections of 10 μg of estradiol, 2 mg of progesterone and aminopterin. Also, three castrated animals given 2 mg of progesterone and aminopterin daily, following a 20-day pretreatment with estrogen, did not menstruate. Thus it would appear that estrogen and progesterone act through different metabolic systems and only estrogen is inhibited sufficiently to precipitate menstruation.

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