Estrogenic Properties of Quinestrol and Estradiol-17β in the Ovariectomized Rhesus Monkey (35508)

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(Introduced by H. H. Freedman)
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Castrated monkeys have been employed as an experimental model for investigating estrogen effects on the primate uterus (1–3). Studies have shown that subcutaneous treatments of 10 μg of estradiol for 20 days will cause bleeding approximately 7–9 days after cessation of treatment (1–2). The synthesis of quinestrol,[4] the 3-cyclopentyl ether of ethynylestradiol, results in a compound with prolonged oral estrogenic activity in both rats and women (4, 5). This prolonged oral activity is attributed to storage in, and a subsequent release from, body fat after administration (6, 7).

This report describes the activity of quinestrol compared with estradiol-17β on the pattern of estrogen withdrawal bleeding in the castrated rhesus monkey.

Materials and Methods. Adult ovariectomized female rhesus monkeys (*Macaca mulatta*) (Nos. 316, 317, 318, and 319, weighing respectively, 3635, 4610, 3325, and 3820 g were employed in this study. When given subcutaneously, both compounds were dissolved in sesame oil. Quinestrol, when given orally, was formulated as 5-, 10-, and 20-μg tablets and administered to the monkeys in either a raisin or grape. Each monkey was trained to accept raisins or grapes with placebo tablets to establish the feasibility of this procedure. If necessary, more than one tablet was given to provide the required dose.

Two experimental designs were employed:

1. Treatment for 20 days and observation of the time interval between cessation of treatment and the onset of withdrawal bleeding.

2. A single treatment with observation of time interval to onset of withdrawal bleeding.

Dose levels are shown in Tables I and II.

Results. Table I presents data on latent period to withdrawal bleeding after cessation of daily estrogen treatments. Estradiol (10 μg) given subcutaneously resulted in a time to bleeding, averaging 7–8 days. Quinestrol administered subcutaneously (10 μg) or orally (5 or 10 μg) produced a delay in withdrawal bleeding averaging about 12 days. Thus, quinestrol had a significantly greater ($p \leq 0.001$) time to bleeding compared with the standard, estradiol. Neither route of administration nor amount of the oral dose had an effect on the results. Treatment dates also revealed no correlation between sequence of treatments and results.

Time to withdrawal bleeding after a single treatment is presented in Table II. Estradiol-17β (50 or 200 μg) failed to produce any withdrawal bleeding. Quinestrol at an oral dose of 50, 100, or 200 μg produced a delay, averaging 22 days, with no indication of a dose–time relationship in 7 of 8 animals.

Discussion. These data clearly demonstrate that the synthetic estrogen, quinestrol, can produce sufficient endometrial stimulation in the ovariectomized monkey to precipitate withdrawal bleeding. Quinestrol had a significant ($p \leq 0.001$) greater delay to bleeding after 20 daily treatments, with an average of 12

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[4] 3-Cyclopentyloxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17β-ol.

TABLE I. Days to Withdrawal Bleeding in Castrated Rhesus Monkeys After 20 Days of Estrogen Treatment.

Treatment	Dose (μg)	Monkey no.	Route of treatment	Starting dates of treatment		Time to bleeding: Days	Time to bleeding: Av $\pm$ SE
Estradiol-17β	10	316	sc	2/19/68	9/23/68	7, 8	7.5 $\pm$ 0.3
	10	317	sc	3/26/68	9/23/68	7, 7	
	10	318	sc	2/19/68	9/23/68	7, 9	
	10	319	sc	3/26/68	9/23/68	7, 8	
Quinestrol	10	316	sc	3/26/68	5/7/68	11, 11	11.5 $\pm$ 0.3[a]
	10	317	sc		5/7/68	11	
	10	318	sc	3/26/68	5/7/68	12, 13	
	10	319	sc		5/7/68	11	
	10	316	po	1/6/69		13	12.0 $\pm$ 0.9[a]
	10	317	po	1/6/69		14	
	10	318	po	10/29/68		11	
	10	319	po	10/29/68		10	
	5	316	po	10/29/68		14	12.0 $\pm$ 0.7[a]
	5	317	po	10/29/68		11	
	5	318	po	1/6/69		11	
	5	319	po	1/6/69		12	

[a] Significantly different from estradiol ($p \leq 0.001$).

days, compared to 7.5 days with estradiol. A single oral treatment of quinestrol caused bleeding on the average of 22 days after dosage; whereas estradiol injections at the same doses were without effect.

Studies in monkeys have shown that a threshold dose exists for both multiple and single treatments (3, 8). The latent period between the cessation of multiple treatments and bleeding is reported not to be influenced by the amount of hormone given provided it is above threshold (8). The studies of Engle and Crafts (9) would suggest that, for large single injections of sustained action estrogens, there is a tendency for this latent period to increase with increasing suprathreshold doses. It has been reported that a daily 2-μg estradiol dose is considered threshold (2), hence the dose employed in these studies is probably some 5 times greater than threshold. A potency estimate is not possible since bleeding always occurred, however, it can be stated for quinestrol that threshold levels are no higher than 5 or 10 μg, when given orally or subcutaneously, respectively, and very possibly lower.

Single treatments indicated that quinestrol was at least 4 times more active orally than estradiol given by injection since the former produced bleeding at 50 μg; whereas the latter failed to do so at 200 μg. It has been reported that the threshold for a single intramuscular injection of estradiol was 346 μg (10), thus it is reasonable that a threshold dose for estradiol is probably twofold greater than was employed in this study. It is also interesting that this dose had a latent period of 18 days which is similar to the 22 days seen for quinestrol. It can only be stated that the threshold for a single oral quinestrol treatment was not more than 50 μg. It is not certain if the 22 days to bleeding after a single oral quinestrol treatment denotes a prolongation of estrogenic responses as seen for sustained action injectable steroids (9). Single oral quinestrol treatments in rats and women (4, 11) have shown an increasing prolongation of response as doses are increased; and similar evidence for certain estrogen injections in monkeys is also available (9).

There can be no question that daily quinestrol treatments exhibited a significantly greater latent period when compared to estradiol. The explanation for this increase is unclear and especially uncertain is what role possible fat storage played, since it is not

TABLE II. Days to Withdrawal Bleeding in Castrated Rhesus Monkeys After a Single Estrogen Treatment.

Treatment	Dose (μg)	Monkey no.	Route of treatment	Starting dates of treatment	Time to bleeding	
					Days	Av ± SE
Estradiol-17β	50	316	sc	6/8/69	—[a]	—
	50	317	sc	6/8/69	—[a]	
	200	318	sc	6/8/69	—[a]	—
	200	319	sc	6/8/69	—[a]	
Quinestrol	50	316	po	3/3/69	27	22.6 ± 2.3
	50	317	po	3/3/69	19	
	50	318	po	4/3/69	22	
	50	319	po	4/3/69	—[b]	
	100	318	po	3/3/69	22	23.0 ± 1.0
	100	319	po	3/3/69	24	
	200	316	po	4/3/69	23	21.0 ± 2.0
	200	317	po	4/3/69	19	

[a] Failed to bleed during: 40 days of observation; [b] 54 days of observation.

known if quinestrol is in fact stored in monkey fat. However, evidence is available that major urinary metabolites in the human, after oral administration of quinestrol, are conjugates of ethynylestradiol (11, 12); and unpublished evidence (13) would indicate that the excretory patterns of radioactivity in the urine and feces are similar in both the human and monkey. Jensen *et al.* (14) has suggested that mestranol (ethynylestradiol-3-methyl ether) must be first converted to ethynylestradiol before it can act as a true estrogen by binding with the rat uterine estrogen receptors. Since quinestrol and mestranol are both 3-alkoxy ethers of ethynylestradiol the possibility exists that quinestrol may also need to be converted to ethynylestradiol before it can exert its estrogenic response. Further evidence supporting this concept is the unpublished data (13) from an ongoing research program showing that quinestrol does behave differently from ethynylestradiol in the *in vitro* rat uterine horn estradiol-17β uptake model [procedure described by Jensen *et al.* (15)]. Therefore it is possible that the increased latent period of quinestrol, when compared to estradiol in our experiments, may be a consequence of this metabolic conversion.

Summary. Quinestrol, an orally active synthetic estrogen, was found to cause an estrogen withdrawal bleeding in ovariectomized monkeys. Quinestrol, given for 20 days either by injection or orally, produced bleeding about 12 days after cessation of treatment; whereas, for estradiol, the interval averaged 7.5 days. After a single oral treatment with quinestrol, uterine bleeding resulted 22 days after treatment. Estradiol at similar doses was without effect. Potency estimates after multiple doses were not possible since threshold levels were not established. However, after a single oral treatment, quinestrol was seen to be at least 4 times more active than parenteral estradiol.

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Received Dec. 12, 1970. P.S.E.B.M., 1971, Vol. 137.