approximately 1/50th of the subcutaneous unit, while the intravaginal unit of estradiol monobenzoate is 1/100-1/200th of the subcutaneous unit. And whereas estradiol monobenzoate is about 10 times as potent as estrone when tested subcutaneously in rats, it is approximately 30 times as potent by the intravaginal method.

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Experimental Uterine Bleeding in Monkeys Following a Single Injection of Various Estrogens.*†

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Uterine bleeding in the rhesus monkey occurs after withdrawal of an adequate estrogenic stimulus. In routine treatments of 100 to 400 I.U. per day for 10 days, bleeding occurs in from 5 to 16 days after the last injection. In our experience, the most frequent day for bleeding of estrone withdrawal is day 7, or 17 days after the first injection.

The present series of experiments was performed with adult female macacus monkeys all of which were previously castrated. A single massive injection of an estrogen was given and the day on which uterine bleeding occurred was noted. Vaginal lavages were made daily in each animal from the time of the maximal sex skin response.

Treatment with estradiol benzoate oleosum (Progynon B Schering). A single injection of 150,000 I.U. (15 mg) of estradiol benzoate in oil was made. The site of injection in 2 monkeys was into the uterine cervix. By means of a vaginal speculum and adequate lighting 1 cc of the preparation was injected into the dense, fibromuscular tissue of the vaginal portion of the cervix with a number 25 hypodermic needle. The site was cleansed before and after injection with alcohol. Due to the great density of the tissue it was necessary to make 5 or 6 separate injections into the cervix around the

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external os, the total dose of 1 cc thus being deposited in several pockets forced into the tissue by the pressure applied. If considerable resistance to injection does not occur, the needle is withdrawn, as a gland or the cervical canal has been entered. The purpose was to place the oily solution only in the fibromuscular coat of the cervix.

The greatest estrogenic response of the sex skin, color and edema, occurred between days 9 and 15 after the injection, after which it underwent a progressive involution. Bleeding occurred in one animal from 25 to 36 days (in 8 instances) after treatment and in the other from 21 to 49 days (Table I). The longer intervals between injection and bleeding occurred when the solution was most favorably placed in the fibromuscular stroma of the cervix.

Treatment of these animals is being continued in order to observe the effect of this type of stimulation and trauma on the uterine cervix.

The synovial bursa of the knee was used as the site of injection of 1 cc, 150,000 I.U., in 3 instances. Injection into the bursa is not difficult, although it is not possible to be certain that the entire amount is left in the bursa. The response of the sex skin was similar to that found in the intracervical injections. Bleeding occurred on days 22, 27, and 31 after the single injection.

Intraperitoneal injections caused a very slight response of the sex skin in 3 instances. Bleeding occurred on days 15 and 20, but in the third no bleeding occurred.

A single injection of estradiol benzoate will permit the typical uterine bleeding of estrone withdrawal, although the interval between the single injection and bleeding is considerably greater than that between the bleeding and the last injection in a frequently injected animal.

With this standard single injection it is clear that the rate of absorption of the hormone is a factor in regulating the length of the interval between the injection and bleeding. The site of injection giving the least satisfactory response was the intraperitoneal route, while the intrasynovial and intracervical gave the greatest interval.

Treatment with estrabioldipropionate Ciba. This substance is reported to cause greatly prolonged estrous smears in the female rat.[1] The response of the sex skin of the monkey is similar to that induced by other estrogens. A single injection of 1 mg of estradioldipropionate is adequate to cause the usual sex skin response and the appearance of bleeding on day 17. With 5 mg the bleeding occurred in 2 instances on days 21 and 26, while with single injec-

[1] Unpublished data from Ciba Corporation, by courtesy of R. C. Mautner.

TABLE I.

Monkey No.	Weight, g	Substance	Site of injection	Date of injection	Uterine bleeding days after injection
428	5700	Progynon Benzoate	Intracervical	11/ 8/37	32
		150,000 I.U.	,,	12/14/37	36
		(15 mg, 1 cc)	,,	1/24/38	27
			,,	3/ 1/38	27
			,,	4/ 4/38	28 (trace only)
			,,	5/23/38	25
			,,	7/ 8/38	No bleeding in 60 days Slight sex skin response
			,,	9/27/38	31
429	6000	Progynon Benzoate	,,	10/ 7/37	15
		75,000 I.U.	,,	11/ 8/37	25
		150,000 I.U.	,,	12/14/37	32
			,,	1/24/38	32
			,,	3/ 1/38	21
			,,	4/ 4/38	No bleeding in 49 days
			,,	5/23/38	23
			,,	7/ 8/38	49
			,,	9/27/38	36
450	3700	Progynon Benzoate, 150,000 I.U.	Intrasynovial	12/22/37	27
452	3750	,, ,, ,, ,,	,,	1/24/38	31
480	6000	,, ,, ,, ,,	,,	4/ 4/38	22
450	3700	,, ,, ,, ,,	Intraperitoneal	1/24/38	20
462	4350	,, ,, ,, ,,	,,	4/ 4/38	15
452	3750	,, ,, ,, ,,	,,	12/22/37	No bleeding in 34 days
463	5250	,, ,, ,, ,,	Subcutan., no massage	1/24/38	16
450	3700	,, ,, ,, ,,	Subcutan., ,, ,,	4/ 4/38	25

462	4350	Estradioldipropionate Ciba (3190A), 1 mg (1 cc)	Intramuscular	3/ 6/38	17
450	3700	Estradioldipropionate Ciba (3190A), 5 mg (5 cc)	,,	3/ 2/38	26
481	5200	Estradioldipropionate Ciba (3190A), 5 mg (5 cc)	,,	6/ 5/38	21
480	6000	Estradioldipropionate Ciba (3190A), 10 mg (10 cc)	,,	3/ 6/38	30
481	5200	Estradioldipropionate Ciba (3190A), 10 mg (10 cc)	,,	4/ 2/38	30
481	5200	Triphenylethylene ¼ g (1.56 cc-oil)	,,	9/28/38	22
462	4350	Triphenylethylene ½ g (3.12 cc-oil)	,,	9/28/38	No bleeding in 55 days
505	4450	Triphenylethylene 1 g (6.25 cc-oil)	,,	9/28/38	33
480	6000	Triphenylethylene 2 g (12.5 cc-oil)	,,	7/11/38	51
504	4750	Triphenylethylene 2 g (12.5 cc-oil)	,,	9/ 3/38	67
504	4750	Triphenylethylene 4 g (25 cc-oil)	,,	6/16/38	67
			2 g (12.5 cc-oil) at each injection	6/20/38	

tions of 10 mg the bleeding occurred in 2 animals on day 30, being similar to the interval obtained with 15 mg of the benzoic acid ester of estradiol (Schering), although the amount of oil was 10 times as great in the dipropionate preparation.‡

Treatment with Triphenylethylene. Dodds and his associates have been responsible for the development of numerous synthetic estrogenic substances, the most active of which are derivatives of stilbene.[2, 3, 4] Of these substances, triphenylethylene was used by Robson,[5] who secured typical estrogenic sex skin response and proliferative endometrium in the monkey. These reports led to the use of triphenylethylene to determine its effect on the interval between a single injection and bleeding time. A total dosage of 3.4 g was used by Robson on one monkey to produce a proliferative endometrium. One gram of triphenylethylene permits bleeding on the 33rd day, the time interval being comparable to that of 15 mg of the Progynon B. (Table I.) Two grams at a single injection delayed bleeding for 51 and 67 days respectively. Four grams also resulted in bleeding on day 67. In these instances a large amount of oil was necessary as a conveying medium. There was no local reaction to the medium. It is worthy of note that in delayed bleedings, as at 51 or 67 days, the sex skin response was maintained at a high level for about 30 days.

In this group, as with the group receiving Progynon B, recession of the sex skin took place in one instance without subsequent bleeding, a phenomenon previously related to a very slow diminution of the available estrogen.

‡ Subsequently used with 5 mg per 1 cc.

2 Dodds, E. C., and Lawson, W., *Nature,* 1937, **139**, 627.

3 Dodds, E. C., Fitzgerald, M. E. H., and Lawson, W., *Nature,* 1937, **140**, 772.

4 Dodds, E. C., Goldberg, L., Lawson, W., and Robinson, R., *Nature,* 1938, **141**, 247.

5 Robson, J. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 153.