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Prolonged oestrogenic activity in rats after single oral administration of ethinyloestradiol-3-cyclopentyl ether

The observation that relief of symptoms in menopausal patients persisted long after oral treatment with the 3-cyclopentyl ether of ethinyloestradiol had been discontinued was interpreted by Bompiani & Bubani (1961) to indicate its storage in and slow release from body depots.

Since then, Meli, Wolff & Honrath (1963) and Meli, Steinetz & others (1965) have shown that the drug is stored in and slowly released from body fat after oral administration to rats. This they considered the mechanism responsible for its increased and prolonged biological activity. Epstein (1967) also found prolonged oestrogenicity in women treated with the drug. Cohen, Bronstein & Leb (1966) showed a uterine-growth stimulating substance to be present in the fat of women taking the drug by mouth. The metabolic fate of the labelled drug in women indicated that it was stored unaltered in body fat depots (Williams, Layne & others, 1967).

While fat storage and prolonged oestrogenic activity occurs in women after the drug had been taken by mouth, no evidence, other than of fat storage, exists for the rat (Meli & others, 1963, 1965).

We now report prolonged oestrogenic activity in the rat given a single oral dose of the drug.

Female rats, 150–180 g, were ovariectomized two weeks before treatment. Groups of 5 animals each received a single oral treatment of ethinyloestradiol or its 3-cyclopentyl ether dissolved in sesame oil at doses of 1, 10, 100 and 1000 μg/animal.

Vaginal smears were taken daily and the animals were killed when vaginal cornification was no longer present. The uteri were then removed and weighed (after pressing out the intra-uterine fluid) to the nearest 0·1 mg on a torsion balance.

In other experiments, 2 groups of ovariectomized rats similarly treated with the 3-cyclopentyl ether at single oral doses of 100 or 1000 μg/animal were killed at 1, 5 and 13 days after the last day of cornified vaginal smear.

On the basis of vaginal cornification, ethinyloestradiol was ineffective at 1 μg dose (Table 1). The effect of the 10 and 100 μg doses lasted only for 24 h whereas that of the 1000 μg dose was effective for 72 h. Vaginal cornification occurred at all doses of the 3-cyclopentyl ether. Duration of action was proportional to the dose given and,

Table 1. *Effects of oral ethinyloestradiol or its 3-cyclopentyl ether on vaginal smear and uterine weight of mature ovariectomized rats*

Treatment	Dose (μg)	No. of rats	Days of cornified vaginal smear	Uterine weight (mg)
Control		5	0	73·3 ± 4·5
Ethinyloestradiol ..	1	5	0	74·9 ± 6·5
	10	5	1	97·7 ± 6·0
	100	5	1	102·1 ± 9·2
	1000	5	3	114·6 ± 9·3
3-Cyclopentyl ether ..	1	4	2	107·7 ± 4·2 ($P < 0·005$)
	10	5	4	125·6 ± 7·2 ($P < 0·025$)
	100	5	8	154·7 ± 13·2 ($P < 0·025$)
	1000	4	13	158·7 ± 16·1 ($P < 0·05$)

Table 2. *Effects of oral 3-cyclopentyl ether of ethinyloestradiol on vaginal smear and uterine weight of mature ovariectomized rats*

Treatment	Dose (μg)	No. of rats	Days of cornified vaginal smear	Days after oestrogenic smear or ovariectomy	Uterine weight (mg)
Control ..		5	0	24	83·8 ± 4·8
		5	0	28	78·8 ± 8·0
		5	0	36	73·3 ± 4·5
Drug	100	5	8	1	163·2 ± 11·3 ($P < 0·001$)
	100	5	8	5	148·4 ± 11·8 ($P < 0·005$)
	100	5	8	13	119·4 ± 16·9 ($P < 0·05$)
Drug	1000	5	13	1	201·2 ± 29·2 ($P < 0·001$)
	1000	5	13	5	157·4 ± 6·0 ($P < 0·001$)
	1000	5	13	13	153·4 ± 21·6 ($P < 0·01$)

after the 1000 μg dose, lasted up to 13 days. At all doses the ether was significantly more effective than the parent drug in stimulating uterine growth. Even 13 days after the last day of positive oestrogenic smear the uteri were still significantly stimulated over those of controls (Table 2).

These results indicate that as in women (Bompiani & Bubani, 1961; Cohen, Bronstein & others, 1966; Epstein, 1967) the rat is capable of releasing fat stored ethinyloestradiol 3-cyclopentyl ether in an active form over a prolonged time.

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January 22, 1969

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