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THE EFFECT OF SOME PHENOTHIAZINE TRANQUILLIZERS ON THE OESTROUS CYCLE OF ALBINO MICE

BY

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The effects of reserpine and of nine phenothiazine compounds on the oestrous cycle of albino mice were tested. With the exception of promethazine (which is not a tranquillizer) all the phenothiazine compounds and reserpine prolonged the oestrous cycle. The mechanism by which these tranquillizers interfere with the oestrous cycle is discussed.

A regular oestrous cycle represents the successful, cyclic, sequential functioning of a number of endocrine glands. The hypothalamus triggers off the secretions of these glands. The tranquillizing drugs, chlorpromazine and reserpine, interfere with oestrus and menstruation in different species. Gaunt, Renzi, Antonchak, Miller & Gillman (1954) found a decreased frequency of cornified vaginal smears in rats treated with 0.05 to 0.1 mg/kg of reserpine, while 0.2 mg/kg suppresses oestrus (Khazan, Sulman & Winnik, 1960). Reserpine (1 mg/kg, subcutaneously) suppresses menstruation in monkeys (DeFeo & Reynolds, 1956 ; Erikson, Reynolds & DeFeo, 1960). Chlorpromazine suppresses oestrus in rats (Das Gupta, 1955) when given intraperitoneally in a dose of 10 mg/kg, and can also interfere with menstruation in women (Ayd, 1959). Since the tranquillizers act on the hypothalamus (Das Gupta, Mukherjee & Werner, 1954 ; Schneider, Plummer, Earl & Gaunt, 1955 ; Tangri & Bhargava, 1960), the possibility was considered that in a sufficient dose all tranquillizers would affect the oestrus as well as the menstrual cycle. In the investigation described here, the effects of a number of phenothiazine tranquillizers were studied on albino mice to test this hypothesis.

METHODS

Female albino mice weighing 18 to 30 g were used. The diet was supplied in the form of a paste and contained wheat (98.5%), yeast (0.5%), salt (0.5%) and cod liver oil (0.5%). The mice were screened for regularity of oestrous cycles, using vaginal smears to assess the stages of oestrus. The tips of a pair of forceps covered with cotton wool soaked in 0.9% saline were introduced into the vagina. After rotating the forceps once, they were withdrawn and the cotton-covered tips were rubbed on a glass slide to obtain a vaginal smear. This was stained with Leishman's stain and examined under a microscope. The criteria used for the

identification of the stages of the oestrous cycle were those of Allen (1922). The stages were distinguished by the presence of: (1) nucleated epithelial cells and/or polymorphonuclear leucocytes in dioestrus; (2) nucleated epithelial cells and a few cornified (non-nucleated) cells but no leucocytes in pro-oestrus; (3) cornified cells only in oestrus; and (4) caked, cornified cells with leucocytes in metoestrus.

Separate smear records were kept for each mouse. Animals which showed regularity of oestrous cycles during the 15 day screening period were selected for the study. Most of the drugs were dissolved in distilled water. Thioproperazine methanesulphonate, pipamazine hydrochloride and thioridazine hydrochloride were dissolved in dilute acetic acid. The drugs were administered subcutaneously. The concentration of the solutions was such that a volume of 0.01 ml./g of body weight was injected into each mouse. Mice of control groups received 0.1 ml. of 0.9% saline subcutaneously.

The doses of the drugs used were selected from the literature, to lie between the dose causing loss of conditioned responses and that causing decrease in locomotor activity. If a dose of a drug proved to be fatal or significantly reduced the weight of mice, the experiment was repeated with a smaller dose of the drug. The drugs were administered for 10 days and the vaginal smears during this period were compared with the smear records of the same mice for the 10 days immediately preceding treatment with the drugs. Each mouse, therefore, served as its own control. Reserpine (Serpasil) (1 mg/kg) and chlorpromazine hydrochloride (10.0 mg/kg) were used as reference drugs; these doses interfere with the oestrous cycle (Gaunt *et al.*, 1954; Das Gupta, 1955). Promethazine hydrochloride, a phenothiazine derivative which is not a tranquillizer, was also used.

RESULTS

The results of the study are summarized in Table 1. All the phenothiazine compounds tested, with the single exception of promethazine, caused significant prolongation of the oestrous cycle, as did reserpine.

TABLE 1

THE EFFECTS OF RESERPINE AND PHENOTHIAZINE COMPOUNDS ON THE OESTROUS CYCLE OF ALBINO MICE

Values are means with standard errors. "t," from student's "t" test; *P*, probability

Drug	Dose (mg/kg)	No. of mice	Average duration of oestrous cycle before treatment (days)	Average duration of oestrous cycle during treatment (days)	"t"	*P*
Reserpine	0·1	16	4·58±0·76	8·44±2·4	6·1465	<0·01
Chlorpromazine hydrochloride	.10	12	4·88±0·49	9·17±1·95	7·4093	<0·01
Fluopromazine hydrochloride	2	16	4·58±0·76	8·02±2·67	5·0	<0·01
Trifluoperazine dihydrochloride	2	16	4·58±0·76	9·38±1·71	10·2784	<0·01
Prochlorperazine acid maleate	0·75	16	4·78±1·6	8·44±2·4	5·1189	<0·01
Thioridazine hydrochloride	0·75	16	4·58±0·76	>10	28·5263	<0·01
Pipamazine hydrochloride	0·5	16	4·47±0·75	9·38±1·65	11·6905	<0·01
Thioproperazine methanesulphonate	0·25	15	5·11±1·48	7·67±2·78	3·4545	<0·01
Perphenazine sodium citrate	0·15	16	4·88±1·56	8·64±2·46	5·1370	<0·01
Promethazine hydrochloride	20	12	5·13±1·67	4·57±0·77	1·0566	>0·05

DISCUSSION

A regular oestrous cycle depends on the harmonious functioning of a number of endocrine glands in a regular sequence. A prolongation or suppression of the oestrous cycle indicates interference with this intricate process. In the present study, all the phenothiazines belonging to the group of tranquillizers were active in prolonging the oestrous cycle ; promethazine, a phenothiazine which does not possess marked tranquillizing activity, was inactive in the dose used (20 mg/kg). This result seems to indicate that tranquillizing activity is involved in the action of the phenothiazines on the oestrous cycle.

Decreased serum gonadotrophin levels have been found in post-menopausal women treated with reserpine (Khazan *et al.*, 1960). Further indirect evidence for depression of gonadotrophin secretion by tranquillizers is provided by the finding that equine gonadotrophin prevents testicular atrophy in rats treated with reserpine (Makinen, Lahtinen & Naatanen, 1962).

Chlorpromazine and reserpine increase prolactin secretion in rats (Barraclough & Sawyer, 1959). Prolactin causes gonadal atrophy in fowls and chickens (Bates, Lahr & Riddle, 1935 ; Bates, Riddle & Lahr, 1937). Testicular atrophy in pigeons treated with reserpine (Khazan *et al.*, 1960) and chickens similarly treated (Hagen & Wallace, 1961) again suggests that there is an increased prolactin secretion.

The increased prolactin secretion associated with decreased gonadotrophin secretion indicates that tranquillizers in some way isolate the pituitary gland from the normally present neurogenic influences originating in the hypothalamus, since Everett (1954) has shown that removal of the pituitary gland from nervous influences has an identical effect. This result agrees with the hypothalamic site of action of the tranquillizers.

This mechanism, however, may not be the only one by which tranquillizers affect the oestrous cycle and menstruation. The work of Purshottam (1962) and Hopkins & Pincus (1963) seems to indicate that tranquillizers may render the ovary refractory to the action of gonadotrophins. This action may contribute to the disturbance of the oestrous cycle and of menstruation produced by tranquillizers.

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