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## Effect of Tranquilizers and Other Agents on Sexual Cycle of Mice.* (24031)

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Some agents which act on the central nervous system have been shown to affect the reproductive system of animals. Daily subcutaneous doses of reserpine have been shown to interfere with estrus in mice(1) and to suppress ovulation and menstruation in monkeys (2). Chlorpromazine may delay ovulation and menstruation in women(3). Reserpine, chlorpromazine, pentobarbital, morphine, atropine, dibenamine and SKF 501, given as single doses prior to ovulation, can all block release of ovulating hormone in rats(4,5). Our work shows that some of these and other centrally acting agents interfere with estrus cycles in mice when given more or less continuously by drug-diet administration.†

*Methods.* Vaginal smears were obtained by saline lavage once daily from ZBC mice‡ before, during and after treatment. Only animals with regular estrus cycles before therapy were used. Mice were housed in individual cages. Control diet was Purina laboratory chow. Drugs were given by drug-diet administration *ad libitum* with all drugs except alcohol, which was administered in drinking water *ad libitum*. Drugs, other than alcohol, were carefully powdered and thoroughly mixed with control food in the desired concentrations. The number of estrous cycles originating during treatment was compared with the number of cycles originating during the previous control period over the same number of days, usually 20. Thus mice served as their own controls. Cycles originating during the first 3 days of treatment were excluded.

*Results.* Table I gives results obtained with the various agents used. Reserpine (0.00075 and 0.001%), chlorpromazine HCl (0.3%), promethazine HCl (0.3%), phenaglycodol (1.0%), meprobamate (3.0%), phenobarbital (0.2%), ethyl alcohol (20%), atropine $SO_4$ (0.1 and 0.2%) and SKF 501 (1.0%)§

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‡ Mice were obtained from Dr. J. J. Bittner. ZBC mice are back cross Z($C_3$H) mice bred by mating AxZ $F_1$ hybrid females with Z males.

§ SKF 501, or N-(9-fluorenyl)-N-ethyl-B-chloroethylamine hydrochloride, is an adrenolytic agent.

TABLE I. Effect of Drugs on Estrous Cycle of Mice.

| Drug | Conc. in %* | No. of mice | % decrease in No. of estrous cycles† | No. mice with no estrus after day 3 | % change in No. of cycles in post-treat. period against No. in pre-treat. period | Change in body wt in g due to therapy |
|---|---|---|---|---|---|---|
| Reserpine | .001 | 20 | 83.6 (20) | 12 | −21.9 | −1.7 |
| | .00075 | 9 | 74.1 (18) | 5 | | − .9 |
| | .0005 | 9 | 48.3 (20) | 1 | | + .1 |
| Chlorpromazine‡ | .3 | 10 | 70.0 (20) | 4 | +17.1 | −1.4 |
| | .2 | 10 | 46.6 (20) | 0 | | − .8 |
| Promazine§ | .2 | 8 | 78.1 (20) | 3 | − 6.2 | −5.6 |
| | .1 | 10 | 48.8 (20) | 1 | 0 | −4.0 |
| Promethazine | .4 | 10 | 100.0 (20) | 10 | −25.7 | −6.0 |
| | .3 | 10 | 71.1 (20) | 5 | 0 | +1.3 |
| Phenaglycodol | 1.0 | 10 | 100.0 (13) | 10 | −25.0 | −1.5 |
| | 1.0 | 9 | 88.8 (14) | 8 | −23.5 | −4.4 |
| | .75 | 10 | 38.7 (17) | 1 | −25.0 | −1.6 |
| | .5 | 10 | 10.7 (15) | 0 | −14.3 | +1.3 |
| Meprobamate | 3.0 | 10 | 88.8 (20) | 7 | −33.3 | − .9 |
| | 2.0 | 10 | 61.1 (20) | 6 | + 2.8 | −1.4 |
| | 1.0 | 10 | 0 (20) | 0 | +11.4 | +1.5 |
| Phenobarbital§ | .2 | 9 | 100.0 (20) | 9 | −10.3 | − .8 |
| | .1 | 9 | 37.9 (20) | 0 | | − .8 |
| Ethyl alcohol | 20.0 | 9 | 92.0 (20) | 7 | −20.0 | − .4 |
| | 10.0 | 9 | 28.0 (20) | 3 | | + .7 |
| Atropine | .2 | 10 | 67.6 (20) | 4 | −17.7 | +1.3 |
| | .1 | 10 | 73.5 (20) | 5 | | −1.7 |
| SKF 501 | 1.0 | 10 | 88.6 (20) | 7 | −17.1 | − .3 |
| | .4 | 10 | 37.1 (20) | 1 | | + .3 |
| | .1 | 10 | 20.0 (20) | 1 | | + .4 |

* Drug-diet conc. or, with alcohol, conc. in drinking water.
† No. in parentheses are No. of days used to determine % decrease in estrus, excluding first 3 days of diet administration.
‡ .4% conc. was tried but discontinued due to toxicity.
§ .3% *Idem*

all caused marked decreases in number of estrus cycles (68 to 100%) in doses causing no severe loss in body weight. Promazine HCl was less effective inasmuch as the decrease in estrus was associated with greater weight loss and fewer mice developed persistent diestrus.

A fairly prompt return of cycles occurred after cessation of therapy in most mice. This is indicated by figures in column 6, Table I, which compares the number of estrus cycles during a control period of 20 days immediately following drug administration with the control period preceding therapy.

Pregnant mare serum (Gonadogen), given subcutaneously to mice while still on diet, induced estrus in most mice on all diets (Table II). Doses of one or 2 international units (I.U.) were used. In mice on control diet, who were in persistent diestrus for no known reason, one I.U. induced estrus in 6 of 10 mice and 2 I.U. did so in 5 of 6 mice.

That restriction in food intake sufficient to

TABLE II. Effect of Pregnant Mare Serum in Diestrous Mice.

| Drug | Conc. in % | I.U. of PMS* | Estrous/diestrous mice† |
|---|---|---|---|
| Reserpine | .001 | 1 | 4/6 |
| | .001 | 2 | 8/9 |
| Chlorpromazine | .3 | 1 | 8/8 |
| Promazine | .2 | 2 | 2/3 |
| Promethazine | .3 | 2 | 6/7 |
| Phenaglycodol | 1.0 | 2 | 8/8 |
| Meprobamate | 3.0 | 2 | 9/9 |
| Phenobarbital | .2 | 2 | 9/9 |
| Ethyl alcohol | 20.0 | 1 | 6/8 |
| Atropine | .2 | 2 | 4/7 |
| SKF 501 | 1.0 | 2 | 7/8 |

* International units of pregnant mare serum.
† Ratio gives No. of mice developing estrus out of total No. in persistent diestrus.

TABLE III. Effect of Diet Restriction for 23 Days on Estrous Cycles and Body Weight.

| Food given, g/day | Body wt lost, g | % decrease in No. of estrous cycles | No. mice |
|---|---|---|---|
| *ad lib.* | .3 | 0 | 6 |
| 4.0 | 1.8 | 21.6 | 10 |
| 3.5 | 3.5 | 40.0 | 9 |
| 3.0 | 4.5 | 56.4 | 10 |
| 2.5 | 6.9 | 67.6 | 10 |
| 2.0 | 9.9 | 91.7 | 10 |

cause severe weight loss will disturb the sexual cycle is well-known. Table III shows the effects of varying intakes of control ground food on body weight and estrus cycles of ZBC mice in our laboratory. A graph (not included) of these results gives essentially a straight line and shows that a 10% decrease in number of estrus cycles occurred/gram of body weight lost. Thus, decrease in incidence of estrus in mice on drug therapy (Table I) cannot be accounted for by weight loss, except possibly in the case of promazine. Also, no correlation between development of persistent diestrus in individual mice and their changes in body weight could be found. Mice in persistent diestrus frequently lost no weight or less weight than those with continuing cycles.

Mice were not handled except to do daily vaginal smears so only subjective impressions regarding any tranquilizing effect were obtained. Ptosis was observed in mice on reserpine. A mild sedation seemed to be present in mice on reserpine and phenobarbital. No definite effect was discernible with chlorpromazine, promethazine, phenaglycodol, meprobamate, alcohol, atropine or SKF 501. Promazine appeared to increase activity slightly.

*Discussion.* Interference with estrus can conceivably occur by many mechanisms. These experiments do not pin-point a site of action for the agents studied. However, induction of estrus by administration of pregnant mare serum suggests that a decrease in secretion of gonadotropin occurred. Such an effect could be explained by the theory of a hypothalamic regulation of gonadotropin secretion(5,6) in which both an adrenergic and a cholinergic link is involved(7).

Inhibition of estrus in mice by sedative and tranquilizing drugs could have its counterpart clinically for drug-diet administration simulates repeated oral administration of drugs. Disturbances in sexual cycles in the human do not appear to be a frequent finding but closer observation and questioning may reveal some changes.

*Summary.* Five tranquilizing drugs (reserpine, chlorpromazine, promethazine, phenaglycodol and meprobamate), 2 sedative drugs (phenobarbital and ethyl alcohol), an adrenolytic agent (SKF 401) and an anticholinergic drug (atropine) have been shown to cause persistent diestrus in mice when administered in food or water.

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