Induction of Estrus in Mice: Hypophyseal-Adrenal Effects

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P-Chlorophenylalanine (p-ChPhe) was found to induce estrus in estrogen-primed ovariectomized C57BL/6J and DBA/2J mice but not in adrenalectomized females. α-Methyltyrosine had no such effects. It was concluded that serotonergic pathways are involved in reserpine- or p-ChPhe-induced estrus. Both p-ChPhe and reserpine were found to be ineffective in induction of estrus in hypophysectomized female mice. It appears that pituitary ACTH released by these drugs causes release of an adrenal hormone (probably progesterone) which is of functional significance in estrus.

The induction of estrus in intact or ovariectomized female rats and mice by administration of exogenous estrogen and progesterone has become a standard laboratory procedure (Beach and Whalen, 1959; McGill, 1962). Under certain conditions, estrogen alone is effective in inducing estrus in laboratory rodents (Davidson et al., 1968), but with the injection regimen used with mice in this laboratory, estrogen alone is relatively ineffective. Several investigators have, however, reported effective substitutes for progesterone.

Myerson (1964a,b) found that reserpine would induce sexual receptivity in estrogen-primed rats, but not in estrogen-primed hamsters (1970). Uphouse, Wilson, and Schlesinger (1970) reported that reserpine induced estrus in estrogen-primed inbred mice unless they had been adrenalectomized. Several interpretations of this effect have been proposed. Myerson (1964a,b) hypothesized that serotonergic inhibitory centers in the brain inhibit presumed excitatory centers which control sexual receptivity. Since reserpine lowers levels of 5-hydroxytryptamine (5-HT, serotonin) and norepinephrine (NE) in

1This research was supported by Research Grant GM-14547 from the National Institute of General Medical Science, and by Training Grant MH-11167 from the National Institute of Mental Health.

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brain and other tissue (Goodman and Gilman, 1970), it is possible that reserpine-induced estrus is mediated by the effect of this drug on these inhibitory centers in the brain. Myerson discounted the importance of the adrenal glands in mediating this effect. Uphouse et al. (1970) found that the adrenal glands are critical in mediating the effects of reserpine since the effect of reserpine in induction of estrus in mice was eliminated in adrenalectomized animals. They speculated that the 5-HT and NE released by reserpine acts on the hypothalamic-hypophyseal system to stimulate ACTH secretion which, in turn, leads to the release of adrenal progesterone. Thus, the observed response in reserpine-treated mice would still be based upon the synergistic effects of estrogen and progesterone.

The use of reserpine in these experiments leads to two difficulties of interpretation. The first is due to the lack of tissue specificity of reserpine; its action may be due to effects on brain, liver, adrenals, or elsewhere (Goodman and Gilman, 1970). The second difficulty is that reserpine depresses levels of both 5-HT and NE, making it impossible to determine whether adrenergic or serotonergic pathways are involved. Use of more specific inhibitors of these presumed neurotransmitters would eliminate this difficulty; for example, \( \alpha \)-methyltyrosine (\( \alpha \)-MT) can be used to inhibit NE (Kopin, 1968), and \( p \)-chlorophenylalanine (\( p \)-ChPhe) or PCPA can be used to inhibit 5-HT (Bloom and Giarman, 1968). Injection of 320 mg/kg \( p \)-ChPhe 3 days before assay has been shown to reduce 5-HT levels by approximately 51% in C57BL/6J and DBA/2J mice; no effects on NE were noted (Schlesinger et al., 1969). Injection of 80 mg/kg \( \alpha \)-MT 4 hr before assay has been shown to lower NE levels by approximately 40% in DBA/2J mice; no significant effects on 5-HT were observed (Schlesinger, Boggan, and Freedman, 1970).

The experiments reported below were designed to explore further the contribution of the adrenal glands to endocrine events underlying estrus in mice by: (1) ascertaining whether adrenergic or serotonergic pathways are involved, and (2) determining whether the adrenal effect is mediated via the pituitary.

**METHODS**

*Subjects*

A total of 130 inbred female mice (C3H/2CrI, C57BL/6J, and DBA/2J) and 70 genetically heterogeneous mice were used as subjects in these experiments. The origin and degree of inbreeding of these mice has been described (Jay, 1967). The heterogeneous mice were descendants of an eight-way cross among the A, AKR, BALB/c, C3H/2, C57BL, DBA/2, Is/Bi, and RIII (all CrI) inbred strains (McClearn, Wilson, and Meredith, 1970).
These mice are maintained in this laboratory as a genetically heterogeneous stock (HS/Ibg). All animals were maintained with free access to food and tap water, at a temperature of $76\pm3^\circ F$. They were placed in a reversed light-cycle room for 3 weeks prior to testing and were maintained there for the duration of the experiments. Adrenalectomized mice were given 1% NaCl solution as drinking water. Ages of the animals used in various experiments are given below.

To test the sexual receptivity of the females, indicator males of the same strain or group (HS) were used. Only active copulators were used as indicator males. Within genotype, assignment of males was random.

**Surgical Procedures**

Ovariectomies were performed in a single-stage operation when the subjects were 45-55 days of age. Bilateral adrenalectomies were performed either in conjunction with ovariectomy or 2 weeks after ovariectomy. Hypophysectomies were performed by the technique described by Lostroh and Jordan (1955) 2 weeks after ovariectomy. Anesthesia was induced in DBA/2J mice by ip injection of 50 mg/kg Nembutal; in C57BL/6J mice by 70 mg/kg Nembutal; and in C3H/2Crgl and HS/Ibg mice by ip injection of 18 mg/kg Avertin (Tribromoethanol, Columbia Organic Chemical). The use of Avertin was indicated by the poor survival rate after hypophysectomy under Nembutal anesthesia. Sham operations were performed under the same conditions. All subjects were operated or sham-operated upon. To increase survival rates of hypophysectomized animals, 0.25 mg/mouse hydrocortisone (Solucortef, Upjohn and Co.) was administered immediately after administration of the anesthetic. After the operation, animals were placed in an atmosphere of 95% O₂ and 5% CO₂ until fully awake, at which time they were given 1 mg terramycin and maintained at $80\pm4^\circ F$ for 24 hr. Two days after operation animals received further injections of 1 mg terramycin and 0.25 mg hydrocortisone.

Vaginal smears were taken for 6 days beginning 10 days prior to testing. Any ovariectomized-only female which showed cycling was discarded. Five of the hypophysectomized females showed cycling during this period, so smears were continued until cycling ceased about 3 weeks later. The behavioral tests were then begun. After testing, necropsies were performed. In the few cases in which operation was found to be incomplete, the data were discarded.

**Drug and Hormone Regimens**

The doses of compounds used were as follows: (1) estradiol benzoate (Progynon, Schering Corp.), 12.5 µg/mouse, in oil; (2) progesterone (Towne and Co.), 1.25 mg/mouse, in oil; (3) p-ChPhe (Aldrich Chemical), 320 mg/kg,
TABLE 1
Injection Schedules

<table>
<thead>
<tr>
<th>Day 1</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>0900</td>
<td>1300</td>
</tr>
<tr>
<td>Estrogen</td>
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</tr>
<tr>
<td>Estrogen</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Estrogen</td>
<td>p-ChPhe</td>
</tr>
<tr>
<td>Estrogen</td>
<td>Vehicle</td>
</tr>
</tbody>
</table>

in distilled water; (4) a-MT (Merk, Sharpe, and Dohme), 80 mg/kg, in distilled water; (5) reserpine (Serpasil, Ciba), 1 mg/kg, in distilled water. The volume injected sc for estrogen and progesterone was 0.05 ml/mouse; for p-ChPhe, a-MT, and reserpine, it was 0.02 ml/gm mouse ip. All animals received a total of four injections according to one of the schedules shown in Table 1. All tests were conducted beginning at 1500 on Day 3.

**Testing Procedure**

Testing began either 3 weeks after ovariectomy and adrenalectomy done together, or 2 weeks after adrenalectomy done singly. Beginning of testing for hypophysectomized mice was somewhat variable due to continuation of estrous cycles in some animals. Vaginal smears were taken ½ hr before behavioral testing began. The pipette method (Snell, 1941) as modified by Uphouse et al. (1970) was employed. Slides were judged to be estrus or nonestrus.

The test for behavioral heat was conducted under red light. Indicator males were prepared for the copulatory session by being presented with a single stimulus (nonexperimental) female in which estrus had been induced with injections of estrogen and progesterone. A single intromission was allowed each male before the stimulus female was removed and replaced by an experimental female. Sexual behavior was recorded for 50 min or until the male ejaculated. If the indicator male failed to mount within 10 min, the female was removed and placed with a second indicator male. If the second indicator male failed to mount, the procedure was repeated with a third indicator male. These procedures were made necessary because pilot data had shown that even experienced indicator males often would not or could not mount certain females (especially those treated with a-MT). These females would often station themselves with their rear quarters in a corner of the testing cage and would fend off the male with teeth and forepaws. For inclusion of data from any female, however, she must have been mounted at least twice. This criterion resulted in the exclusion of many females (most
after α-MT treatment). Most females were mounted far more often than twice. As a measure of the sexual receptivity of the females, a lordosis to mount score (L/M) was computed for each by dividing the number of lordosis responses she displayed by the number of male mounts. Use of this measure tends to compensate for strain differences in male mounting frequency.

RESULTS

Table 2 summarizes the results of the first experiment performed to determine whether adrenergic or serotonergic pathways are involved. An analysis of variance revealed that the treatment groups (hormone or drug)

### TABLE 2

Effects of α-Methyltyrosine, p-Chlorophenylalanine, or Reserpine on the Sexual Responses of Estrogen-Primed Female Laboratory Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surgery</th>
<th>N</th>
<th>Genotype</th>
<th>Lordosis/mount ± 1 SD</th>
<th>% Positive vaginal smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>OV(_{x})(^{a})</td>
<td>5</td>
<td>C3H</td>
<td>.64±.09</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DBA</td>
<td>.77±.08</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C57</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>HS</td>
<td>.59±.16</td>
<td>80</td>
</tr>
<tr>
<td>Progesterone</td>
<td>OV(<em>{x}) + AD(</em>{x})(^{b})</td>
<td>5</td>
<td>C3H</td>
<td>.73±.11</td>
<td>100</td>
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<td></td>
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<td>.66±.17</td>
<td>80</td>
</tr>
<tr>
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<td>C57</td>
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<td>100</td>
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<td></td>
<td></td>
<td></td>
<td>HS</td>
<td>.76±.09</td>
<td>100</td>
</tr>
<tr>
<td>p-ChPhe</td>
<td>OV(_{x})</td>
<td>5</td>
<td>C3H</td>
<td>.59±.12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DBA</td>
<td>.48±.31</td>
<td>80</td>
</tr>
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<td></td>
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<td></td>
<td>C57</td>
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<td></td>
<td></td>
<td></td>
<td>HS</td>
<td>.61±.10</td>
<td>100</td>
</tr>
<tr>
<td>p-ChPhe</td>
<td>OV(<em>{x}) + AD(</em>{x})</td>
<td>5</td>
<td>C3H</td>
<td>.06±.08</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DBA</td>
<td>.00±.00</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>C57</td>
<td>.10±.05</td>
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<td></td>
<td></td>
<td>HS</td>
<td>.08±.16</td>
<td>0</td>
</tr>
<tr>
<td>α-MT</td>
<td>OV(_{x})</td>
<td>5</td>
<td>C3H</td>
<td>.00±.00</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td>C57</td>
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<td></td>
<td></td>
<td>HS</td>
<td>.04±.09</td>
<td>0</td>
</tr>
<tr>
<td>α-MT</td>
<td>OV(<em>{x}) + AD(</em>{x})</td>
<td>5</td>
<td>C3H</td>
<td>.00±.00</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HS</td>
<td>.01±.00</td>
<td>20</td>
</tr>
</tbody>
</table>

\(^{a}\)OV\(_{x}\) is used for ovariectomized.

\(^{b}\)AD\(_{x}\) is used for adrenalectomized.
differed significantly \( F(2,96) = 318.21, p \leq .01 \), the surgical groups differed significantly \( F(1,96) = 66.56, p \leq .01 \), and the treatment by surgery interaction was significant \( F(2,96) = 64.15, p \leq .01 \). A Duncan's Multiple Range Test indicated that the behavior of the estrogen-progesterone groups was different \( p \leq .01 \) from all \( \alpha \)-MT groups and from all other adrenalectomized groups. The behavior of the \( p \)-ChPhe-treated animals was not significantly different from that of the control (estrogen-progesterone) animals. The results from the vaginal smears were consistent with the behavioral scores. Slides were judged to be positive if cornified epithelial cells indicative of estrus were present. Combining the results from the estrogen-progesterone groups with those from the \( p \)-ChPhe (with adrenals) groups, and contrasting them with the adrenalectomized \( p \)-ChPhe animals combined with all the \( \alpha \)-MT groups revealed a significant difference \( \chi^2(1) = 93.6, p \leq .001 \). No strain differences were found.

Table 3 summarizes the results of the experiment performed to determine whether the presence of an intact pituitary is necessary for the induction of estrus with reserpine or \( p \)-ChPhe. The treatment effect (drugs or hormones) was significant \( F(3,64) = 353.75, p \leq .01 \) as were the treatment by strain

### Table 3

<table>
<thead>
<tr>
<th>Drug or hormone</th>
<th>Surgery</th>
<th>( N )</th>
<th>Genotype</th>
<th>Lordosis/mount ratio</th>
<th>% Positive vaginal smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>( H_x )</td>
<td>5</td>
<td>HS</td>
<td>.67±.09</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>HS</td>
<td>.78±.10</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>( H_x )</td>
<td></td>
<td>C3H</td>
<td>.55±.10</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>C3H</td>
<td>.61±.10</td>
<td>100</td>
</tr>
<tr>
<td>( \alpha )-MT</td>
<td>( H_x )</td>
<td>5</td>
<td>HS</td>
<td>.02±.00</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>HS</td>
<td>.04±.04</td>
<td>00</td>
</tr>
<tr>
<td></td>
<td>( H_x )</td>
<td></td>
<td>C3H</td>
<td>.10±.08</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>C3H</td>
<td>.00±.00</td>
<td>00</td>
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<tr>
<td>Reserpine</td>
<td>( H_x )</td>
<td>5</td>
<td>HS</td>
<td>.00±.00</td>
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<tr>
<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>HS</td>
<td>.06±.07</td>
<td>00</td>
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<tr>
<td></td>
<td>( H_x )</td>
<td></td>
<td>C3H</td>
<td>.11±.07</td>
<td>20</td>
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<tr>
<td></td>
<td>( H_x + O V_x )</td>
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<td>.00±.00</td>
<td>00</td>
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<tr>
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<td>C3H</td>
<td>.05±.07</td>
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<td>( H_x + O V_x )</td>
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<td></td>
<td>( H_x + O V_x )</td>
<td></td>
<td>C3H</td>
<td>.05±.06</td>
<td>00</td>
</tr>
</tbody>
</table>

\( aH_x \) is used for hypophysectomized.

\( bO V_x \) is used for ovariectomized.
[F(3,64) = 6.77, \( p \leq .01 \)] and the strain by surgery [F(1,64) = 10.83, \( p \leq .01 \)] interactions. A Duncan's Multiple Range Test confirmed that the estrogen-progesterone groups differed from all others. This was also true in the vaginal smear data. A Scheffé Test revealed a significant difference [\( F' = 12.39, \ F = 21.05, \ p \leq .01 \)] between the C3H/2 and the HS mice treated with estrogen and progesterone, surgical condition notwithstanding.

DISCUSSION

The major result of the first experiment was the finding that p-ChPhe could function as an effective substitute for progesterone in the induction of estrus in estrogen-primed mice. a-MT did not substitute for progesterone. These results are in good agreement with those reported by Myerson (1964b) in implicating serotonergic rather than adrenergic pathways. Presumably, the positive effects of reserpine in similar experiments have been due to its depletion of 5-HT.

The first experiment also supported the finding by Uphouse et al. (1970) that the adrenal glands can function in endocrine events underlying estrus in mice. The positive effects of p-ChPhe were essentially abolished after adrenalectomy. It remains to be ascertained whether the adrenal contribution to this effect is mediated by progesterone. The work of Feder, Resko, and Goy (1968) and Feder and Ruf (1969) strongly supports this interpretation although this work was performed on animals of different species.

The results of our second experiment clearly implicate the pituitary as a component in reserpine- or p-ChPhe-induced estrus. Very few animals displayed either behavioral heat or vaginal cornification after hypophysectomy unless they were given both estrogen and progesterone. It was confirmed that mice hypophysectomized or hypophysectomized and ovariectomized under the conditions described are physiologically able to display heat behavior, but it was found that they do not do so if progesterone is replaced by either pChPhe or reserpine. The effects of these two drugs must, therefore, be mediated by the pituitary (probably by ACTH). It was not suggested that action of these drugs is directly upon the pituitary, but rather upon higher brain centers, including the hypothalamus, thence the pituitary.

Although these studies have been rather consistent in revealing similar responses by the various genetic stocks employed, it was noted that the C3H/2 and HS mice differed somewhat in their response to exogenous estrogen and progesterone, with the HS mice displaying more estrous responses. The HS are unusually robust animals compared to mice from established inbred strains. A tentative explanation for the differential response found is that the HS females were weakened less by the surgical and drug trauma than were the C3H/2 animals. This view is supported by the different
survival rates of these stocks after hypophysectomy, since 90% of the hypophysectomized-only HS females survived to testing, while only 75% of the C3H group did so. Strain-dependent susceptibility to pituitary loss was reported by Lostroh and Jordan (1955). The present instance of hybrid vigor in the HS animals is typical of their behavior, but remains to be explained.

The results of the present study confirm those of Uphouse et al. (1970) in demonstrating adrenal involvement in induction of estrus in estrogen-primed ovariectomized mice. Further, pituitary control of this adrenal function was found to be necessary. These results are also in good agreement with those of Meyerson (1964a,b) in implicating serotonergic rather than adrenergic pathways mediating these effects. Though we have not yet identified the adrenal factor involved, we suspect it to be the secretion or release of progesterone. This will be the subject of future studies.

REFERENCES


Meyerson, B. J. (1964a). Estrous behavior in spayed rats with estrogen or progesterone treatment in combination with reserpine or tetrabenazine. Psychopharmacologia 6, 210-218.


