68. Lower Incidence of Nodular Hyperplasia of the Adrenal Cortex after Ovariectomy in Neonatally Estrogenized Mice than in the Controls

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In some strains of mice, adrenocortical tumors frequently occur several months after gonadectomy and secrete estrogens and/or androgens (Franz and Kirschbaum, 1949; Woolley, 1950; Rosner et al., 1966). Since the tumorigenesis is blocked by either continued administration of estrogen (Woolley and Little, 1949) or hypophysectomy (Ferguson and Visscher, 1953) after gonadectomy, the conclusion has been reached that continuous stimulation of the adrenal cortex by excessive amounts of gonadotropins results in the induction of adrenal tumors.

The present study was undertaken to ascertain whether the induction of the cortical tumor would be attenuated by neonatal estrogen treatment which is suggested to bring forth a permanent reduction of the production of gonadotropins in the mouse (Kimura et al., 1967; Mori, 1968; Nishizuka, 1974, 1976). Changes in structure of the cervicovaginal tract, uteri and ovaries in neonatally estrogenized mice will be reported elsewhere.

Materials and methods. Newborn female mice of the F1 hybrids from the crosses between C57BL/6N females and A/HeN males (designated as B6AF1) were divided into 4 groups. Two groups of the mice received daily subcutaneous injections of 20 μg estradiol-17β in 0.02 ml sesame oil for 5 days beginning on the day of birth (Groups 3 and 4), and two other groups either left untouched or given sesame oil injections (Groups 1 and 2) serving as controls. All mice were weaned at 25–27 days of age. Mice of Groups 2 and 4 were ovariec-tomized at 40 days of age.

Animals were autopsied between 491 and 621 days (16–21 months) or between 725 and 971 days (24–32 months) of age. Adrenals were dissected out, fixed in Bouin's fluid, and sections cut at 7 μm in paraffin

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were stained with hematoxylin and eosin. Blood samples were collected between 10:00 and 14:00 h from the carotid artery at autopsy at the age of 16–21 months. Serum was separated and kept frozen at −20°C until assayed for LH and FSH. LH and FSH levels were measured with the NIAMDD radioimmunoassay kits for rat from the Rat Pituitary Hormone Distribution Program, NIAMDD, National Institutes of Health, Bethesda, Md. The assay results were expressed as nanograms of NIH-LH-S1 and NIH-FSH-S1.

**Results. Adrenal nodules.** In non-ovariectomized mice (Groups 1 and 3), the adrenals were generally normal in histology in both the control and the neonatally estrogenized mice, showing no signs of nodular hyperplasia (Table I). However, deposits of varying amounts of ceroid took place in the adrenal cortex, particularly in the circummedullary zone, in all the animals. By contrast, in a majority of ovariectomized controls (Group 2, in 8 of 14) the adrenals were abnormal, bearing cortical nodules in at least one of the adrenals (Fig. 1). The nodules consist mainly of B-type cells of Woolley (1950) with clear cytoplasm like lutein cells (Fig. 2). Besides these, nodules consisting of polyhedral cells surrounded by spindle-shaped cells (A-cell nodules) were sometimes encountered.

Table I. Summary of histological findings of the adrenals in control and neonatally estrogenized B6AF1 mice, intact and ovariectomized

<table>
<thead>
<tr>
<th>Group</th>
<th>Age in months</th>
<th>Total no. of mice</th>
<th>Number of mice bearing nodules</th>
<th>Other lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Control non-ovariectomized</td>
<td>16–21</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24–32</td>
<td>12</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>2 Control ovariectomized</td>
<td>16–21</td>
<td>7</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24–32</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3 Estrogenized non-ovariectomized</td>
<td>16–21</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24–32</td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4 Estrogenized ovariectomized</td>
<td>16–21</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>24–32</td>
<td>4</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* No nodules, but with derangement of cortical cells due to invasion of spindle-shaped cells and small nests of B-cells. Difference in incidence of adrenocortical nodules between Groups 2 and 4 is significant. (The two age groups are taken together. P<0.02, Fisher’s exact probability test.)

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In neonatally estrogenized ovariectomized mice (Group 4), nodules occurred much less frequently (1 of 13 animals) than in the control ovariectomized mice. In a few mice at 24–32 months of age, derangement of cortical cells, proliferation of subcapsular cells and palisade-like invasion of spindle-shaped cells were met with in both neonatally estrogenized and control groups.
Serum LH and FSH. Serum LH and FSH concentrations in the control and the neonatally estrogenized mice at 16–21 months of age are shown in Table II. There was no difference in LH level between the non-ovariectomized control mice and the non-ovariectomized neonatally estrogenized animals (Group 1 vs Group 3). Ovariectomy resulted in elevation of serum LH concentrations in both the control (Group 2) and the estrogenized mice (Group 4), reaching 9.2 and 3.2

Table II. Serum LH and FSH concentrations at 16–21 months of age in control and neonatally estrogenized B6AF1 mice, intact and ovariectomized

<table>
<thead>
<tr>
<th>Group</th>
<th>LH (ng NIH-LH-S₁/ml)</th>
<th>FSH (ng NIH-FSH-S₁/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.78±0.12 (9)*</td>
<td>783±109 (9)</td>
</tr>
<tr>
<td>2</td>
<td>7.16±1.24 (7)</td>
<td>2962±237 (7)</td>
</tr>
<tr>
<td>3</td>
<td>0.86±0.22 (11)</td>
<td>400±69 (10)b</td>
</tr>
<tr>
<td>4</td>
<td>2.76±0.56 (9)*</td>
<td>1977±120 (9)e</td>
</tr>
</tbody>
</table>

* Mean±standard error. Number of mice is given in parentheses.
Difference between groups (Student’s t-test): (a) Group 2 vs Group 4, 0.01<P<0.02; (b) Group 1 vs Group 3, P<0.001; (c) Group 2 vs Group 4, 0.001<P<0.01.
times that in the non-ovariectomized levels, respectively. The difference in LH concentration between these two ovariectomized groups was statistically significant.

FSH levels were significantly higher in non-ovariectomized control mice than in non-ovariectomized estrogenized mice (Group 1 vs Group 3). Ovariectomy brought about marked elevation of serum FSH levels in both the control (Group 2, 3.8 times) and estrogenized mice (Group 4, 4.9 times). The levels in Group 2 mice were significantly higher than in Group 4 females.

Discussion. The development of adrenocortical nodules after gonadectomy has been reported in several strains of mice, such as Bagg albino, CBA, Ce, C3H, DBA and NH, as well as in rats, hamsters and guinea pigs. The present experiments also indicated that nodular hyperplasia consisting mainly of B-type cells occurred in the adrenal cortex of ovariectomized B6AF1 mice. Cornification of the vaginal epithelium and stimulation of the uterine and vaginal epithelia in such nodule-bearing mice (unpublished observations) suggest the production of estrogenic substances from the nodules. On the basis of their observations that cornification of vaginal smears in long-term ovariectomized rats with adrenocortical tumors disappeared after hypophysectomy and reappeared after gonadotropin administration, Houssay et al. (1955) concluded that the secretion of estrogens by the tumors depends on gonadotropins.

In ovariectomized neonatally estrogenized mice, the incidence of adrenocortical nodules was significantly smaller than in the ovariectomized control mice. Although the vaginal epithelium was persistently stratified and cornified in 10 of the 13 neonatally estrogenized ovariectomized mice (Group 4, unpublished observation), the stimulations of the vaginal epithelium are highly probably estrogen-independent phenomena, well established in female mice given perinatal administration of high doses of estrogen (Takasugi, 1976).

It is well documented that in female mice neonatal treatment with estrogen or androgen permanently alters the hypothalamo-hypophysial axis, resulting in continuous secretion of gonadotropins (Kimura et al., 1967; Mori, 1968; Nishizuka, 1974, 1976). The radioimmunoassay of blood levels of LH and FSH has not yet been carried out in neonatally estrogenized mice. However, Nagasawa et al. (1973) reported that in female rats treated with estrogen for the first 30 days of postnatal life, although serum LH levels were comparable to those in the control rats at proestrus, FSH levels remained intermediate between the values at diestrus and proestrus in the controls. According to Chiappa and Fink (1977), both synthesis and release of LH and FSH were inhibited until about the onset of puberty in
neonatally androgenized female rats. Data for older animals were not given by these authors. The present experiments have demonstrated that, in neonatally estrogenized mice, serum levels of LH were approximately the same as those in the controls, while FSH levels were significantly lower.

Schiavi (1969) reported that pituitary and serum concentrations of both LH and FSH increased markedly in ovariectomized rats which had been given a single neonatal injection of testosterone propionate. Since the increments appeared to be almost the same as those in the ovariectomized controls, he concluded that the hypothalamo-hypophysial axis in the neonatally androgenized rats was capable of responding to the absence of ovarian hormones with a normal increase in synthesis and release of gonadotropins. By contrast, Arai (1964) reported that in female rats given high doses of estrogen neonatally, hypothalamo-hypophysial system was less sensitive to the absence of ovaries, the incidence of castration cells being lowered as compared with the controls. In the present studies, although serum levels of LH and FSH increased about 15–20 months after ovariectomy in both the control and the neonatally estrogenized mice, magnitude of the increase was significantly smaller in the estrogenized mice than in the controls. This difference may be responsible for the difference in incidence of adrenocortical tumors between the two groups of mice. The results in the present experiments lend support to the view proposed by Woolley and Little (1946) and Ferguson and Visscher (1953) that excessive secretion of gonadotropins is required for the induction and promotion of adrenocortical tumors. However, the tumors would eventually acquire the capacity of autonomous growth, independent of gonadotropins (Takasugi et al., 1975).

**Summary.** Incidence of hyperplastic adrenocortical nodules was smaller in neonatally estrogenized mice than in the controls after ovariectomy performed at 40 days of age. The difference may be accounted for by lower serum levels of LH and FSH in the neonatally estrogenized, ovariectomized mice than in the ovariectomized controls.

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