ADRENAL ESTROGENS
IN RELATION TO OVARIAN FUNCTION

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Abstract—The effect of gonadotropins and ovarian hormones on adrenal estrogens
in female rats was examined in relation to the ovarian function. Pregnant mare serum
gonadotropin (PMS) increased adrenal estradiol level in intact rats but did not alter
estrone levels. Human chorionic gonadotropin (HCG) with PMS did not alter the
estrone and estradiol levels. Neither PMS or HCG with PMS affected these steroid
levels in ovariectomized rats. Injection of hexestrol to intact rats decreased the estrone
level. Further decrease in the estrone level resulted after administration of hexestrol
with progesterone. In a developmental study, the estrone level at 35 days of age was
higher than that at 50 and 80 days of age. The estrone level gradually decreased with
adrenal development after ovariectomy performed at 21 days of age. When adrenal
slices were incubated with either 3H-estrone or 3H-estradiol, higher radioactivity of
estrone accumulated in the adrenal gland of immature rats than in that of adult rats.
There was a larger conversion of 3H-estradiol to 3H-estrone than the reverse process
in both immature and adult rats. These results indicate that the adrenal estrogens
were influenced by ovarian function.

The interaction between the adrenal glands and gonads has been suggested from findings
of fluctuation in adrenal estradiol at proestrus (1). From the point of the close histological
and biochemical relationship, there may be an interaction between adrenal glands and
gonads. With respect to the physiological role of ovarian estradiol in the adrenal gland,
it has been reported that estradiol enhanced corticosterone production and inhibited the
activity of 5α-reductase in rat adrenal gland (2, 3). Recently, evidence has been reported
for cellular and nuclear receptors of estradiol in the adrenal gland of rats and mice (4, 5).
As little is known of the relationship between adrenal estrogens and the ovarian function,
the effect of gonadotropins and ovarian hormones on adrenal estrogens was examined in
relation to the ovarian function.

MATERIALS AND METHODS

Animals: Female Wistar rats weighing approx. 200 g, or immature rats (21–35 days)
were used. The rats were housed in a room kept at 24 ± 1°C on a lighting schedule of
12-hr light (6:00 h to 18:00 h); 12-hr darkness with free access to food and water. Vaginal
smear was recorded daily for each rat. Puberty was determined by daily inspection for
vaginal opening. Ovariectomy was performed by a dorsal approach with the rats under
ether anesthesia. Ovariectomy was also performed in immature rats at 21 days of age.
The surgically treated rats were sacrificed 15, 30, and 60 days after the operation and the
intact animals served as controls.

**Injection of gonadotropins:** Pregnant mare serum gonadotropin (PMS) and human chorionic gonadotropin (HCG) were purchased from Teikoku Zoki Co. Ltd. In the first group, 50 IU of PMS in 0.1 ml of saline was given s.c. In the second group, 50 IU of HCG in 0.1 ml of saline was also injected 48 hr after PMS treatment. Control animals were not given any injection. Animals were decapitated 48 hr after PMS and 72 hr after HCG.

**Injection of hexestrol and progesterone:** In the first group, 2 μg of hexestrol in 0.1 ml of sesame oil was given s.c. daily × 3. The second group was also given 2 μg of hexestrol with progesterone in 0.1 ml of sesame oil. The controls were given 0.1 ml of sesame oil.

**In vitro experiment:** [6, 7-3H] estrone (specific activity, 60 Ci/m mole) and [6, 7-3H] estradiol (specific activity, 57 Ci/m mole) were obtained from New England Nuclear Co., U.S.A. Tissue slices of the adrenal gland from adult and immature rats (35 days) were incubated with 0.05 μCi of either 3H-estrone or 3H-estradiol in 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) at 37°C for 2 hr in an atmosphere of 95% oxygen and 5% carbon dioxide. The reaction was terminated by sudden chilling and the tissue slices were homogenized with 4 ml of ethanol:acetone (1:1) after the addition of 20 μg of nonradioactive estrone and estradiol as a carrier. The supernatant obtained after centrifugation was decanted and the residue was extracted twice with the same solvent. Combined extracts were evaporated under N₂ gas. The chromatographic procedure followed the method of Sharma and Venkitasubramanian (6). After the spots corresponding to estrone and estradiol were identified, the radioactivity in the separated steroids was measured with a Aloka liquid scintillation spectrometer.

**Hormone assay:** Adrenal glands from 2 rats, removed and cleaned from fat, were weighed and immediately homogenized in 4 ml of ice-cold 30% ethanol solution. To the homogenate was added approx. 1000 dpm of both 3H-estrone and 3H-estradiol and the preparation centrifuged at 2000 rpm for 10 min. The supernatant was extracted twice with dichloromethane, and the combined extract was evaporated to dryness under N₂ gas. The residue was chromatographed over Sephadex LH-20 (12 × 0.5) using benzene:methanol (85:15) as an elution solvent for estrone and estradiol. The estrogens were then subjected to radioimmunoassay (RIA) (7). The blood sample was allowed to clot and was then centrifuged at 3000 rpm for 10 min. The serum estrogens were assayed by the same RIA method as above (7). All samples from each experiment were measured within a single assay.

**Statistical analyses:** Experimental results were expressed as mean values ± the standard errors (SE). Statistical comparison between groups was made by Student's t-test.

**RESULTS**

Table 1 shows the effect of gonadotropins on adrenal and serum estrogen levels in intact and adult ovariectomized rats. Injection of 50 IU of PMS resulted in a 4-fold increase in adrenal estradiol level and 14-fold increase in serum estradiol level. In contrast with the increase of adrenal estradiol, estrone levels in the adrenal gland and serum did not change
after PMS treatment. Additional injections of HCG did not produce any significant increase in these hormone levels. In the ovariectomized group, treatment with PMS, HCG and PMS did not increase estrone and estradiol levels.

Table 1. Effect of gonadotropins on adrenal and serum estrogen levels in intact and ovariectomized rats

|                | Adrenal gland | Serum                |
|----------------|---------------|----------------------|
|                | Weight (mg)   | Estrone (pg/mg tissue) | Estradiol (pg/ml) |
| Intact         | 29±1.0        | 1.26±0.26            | 0.42±0.08          | 11±2.1 | 45±5.8 |
| PMS            | 26±0.8*       | 1.43±0.35            | 1.73±0.34**        | 16±1.3 | 619±109* |
| PMS+HCG        | 25±1.1*       | 1.81±0.54            | 0.74±0.20          | 15±1.9 | 60±6.1  |
| Ovx.           | 28±1.1        | 1.09±0.27            | 0.41±0.07          | 14±0.8 | 12±1.2  |
| PMS            | 29±2.4        | 1.09±0.20            | 0.43±0.19          | 12±1.0 | 25±1.6  |
| PMS+HCG        | 26±1.8        | 1.26±0.31            | 0.63±0.19          | 9.2±0.8 | 33±2.1 |

Ovx.: Ovariectomy was performed 2 weeks before experiment. Animals were decapitated 48 hr after PMS and 72 hr after HCG with PMS. Each value represents mean±SE of 10 animals. Significantly different from intact rats at *P<0.05 and **P<0.01.

PMS—Pregnant mare serum gonadotropin
HCG—human chorionic gonadotropin

Figure 1 illustrates the effect of hexestrol and hexestrol with progesterone on adrenal estrogen level. Injection of hexestrol alone lowered the estrone level to 43% of the control. Hexestrol plus progesterone further lowered the level to 34% of the control. On the other hand, the estradiol level was unchanged with both hexestrol, and hexestrol with progesterone treatment.

Change in levels of adrenal estrogen with ovariectomy in immature rats is shown in Fig. 2. Values are expressed in pg/mg tissue for estrogens. In intact rats, the estrone level was highest at 35 days of age, decreased at 50 days of age, and recovered at 80 days. On the other hand, estrone levels in ovariectomized rats decreased gradually with adrenal

![Fig. 1. Effect of hexestrol and progesterone on adrenal estrogen level. Each value represents the mean±SE of 10 animals. Significantly different from control at **P<0.01.](image-url)
development, with no marked change of estrone levels in the intact rats. While the estradiol level was constant in the intact group, these levels in ovariectomized rats decreased gradually with adrenal development. Vaginal opening occurred at 41 days of age on an average in 12 rats, (range 37–44 days).

**FIG. 2.** Effect of ovariectomy in immature rats on adrenal estrogen levels. Ovariectomy was performed at 21 days of age. Each value represents mean±SE of 10 animals. Significantly different from intact of 35 days of age at *P<0.05. Significantly different from intact of 50 days of age at $P<0.05.$

**FIG. 3.** Uptake of ³H-estrogens in adrenal gland of immature and adult rats. Either ³H-estrone or ³H-estradiol (0.05 μCi) was incubated for 2 hr. Immature rats at 35 days of age were used. Each value represents the mean±SE of 5 animals. Significantly different from ³H-estrone in immature rats at **P<0.01.
The uptake of $^3$H-estrogens by the adrenal gland of immature and adult rats is shown in Fig. 3. The uptake of $^3$H-estrone in immature rats was 4-fold larger than that in adult rats. On the other hand, the uptake of $^3$H-estradiol in immature rats was only 1.6-fold compared with that in adult rats. An interconversion of $^3$H-estradiol and $^3$H-estrone is shown in Fig. 4. The conversion of $^3$H-estradiol to $^3$H-estrone was significantly higher than the reverse process in the adrenal gland of both immature and adult rats. Moreover, the conversion of $^3$H-estrone to $^3$H-estradiol as well as the reverse process was higher in the adrenal gland of immature rats than the conversion seen in adult rats.

**DISCUSSION**

The present study demonstrated that there was no direct stimulation of the levels of adrenal estrogens after gonadotropin treatment. Some workers proposed that the adrenal gland failed to respond to HCG in ovariectomized women (8, 9), while it has been reported that HCG stimulated adrenal estrogens in ovariectomized Rhesus monkeys (10). The present study indicated that the significant increase in the estradiol level of the adrenal gland by treatment with PMS may be due to a large increase in the peripheral estradiol level originating from the ovary, in intact rats. Increase in adrenal estrone, main adrenal estrogen, was not observed. Increase in corticosterone by treatment with PMS in intact immature rats has also been reported (11).

Hexestrol and hexestrol with progesterone, which were used as potent inhibitors of gonadotropins (12, 13), significantly decreased the adrenal estrone level (Fig. 1). Since treatment with gonadotropins showed no direct increase in adrenal estrone level, as shown in Table 1, these effects of hexestrol and progesterone on estrone can be attributed in part to the direct inhibition of adrenal gland and partly to ACTH suppression (14, 15). Many papers show that adrenal estrogens, mainly estrone, are under the control of ACTH (16-18), similar to the case with corticosteroid.

In a developmental study, the effect of ovariectomy in immature rats on adrenal estrogens was examined in relation to ovarian function. A higher estrone level was detected at 35 days of age than at 50 or 80 days of age in intact rats. Weisz and Gunsalus (19) suggested that serum estrone levels in immature rats were higher than levels in adult female rats in proestrus and that the pattern of change in estrone levels with age probably reflects the primary developmental change in the adrenal cortex. As shown in Fig. 2, the level of estrogens decreased gradually in ovariectomized rats. When ovariectomy was performed after puberty, the level of estrogens was constant with adrenal development (unpublished data). There was no increase in weight of the adrenals after puberty in contrast to that before and during puberty. Although the sudden decrease in adrenal estrone levels at 50 days of age in intact rats may play a significant role in the onset of puberty, the precise role remains unknown.

In an in vitro study, $^3$H-estrone uptake was higher than that of $^3$H-estradiol in both adult and immature rats, particularly immature rats (Fig. 3). A large conversion of $^3$H-estradiol to $^3$H-estrone was detected compared to the reverse process, and the conversion
rate was larger in immature rats (Fig. 4). It was reported that in an in vivo experiment the conversion of $^3$H-estradiol to $^3$H-estrone was about 2-fold greater than the reverse process in the adrenal gland of adult female rats (20). It was also suggested in this same report that liver and adrenals should display either a higher affinity or higher concentration of binding proteins for estrone, in contrast to plasma binding. As a high conversion of estradiol to estrone also occurred in adult rats, it seems likely that the uptake and interconversion of estrogens involved may be why estrone is more responsive than estradiol in the adrenal gland. Increase of corticosteroid production and inhibition of corticosteroid metabolism by estradiol seems to be controlled through the interconversion of adrenal estrogens.

In conclusion, the present results indicate that adrenal estrogens are indeed influenced by ovarian function.

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