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Effects of Oophorectomy and Various Doses of Estradiol-17β on Corticosterone Production by Rat Adrenal Slices.* (30483)

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Production of corticosterone by rat adrenal slices *in vitro* was decreased by prepubertal oophorectomy and increased after replacement with a single injection of a depot preparation of estradiol-17β(1). These observations were made at only one time interval after replacement therapy (2 weeks) and only one large dose of estradiol (2 mg/100 g body weight) was used. The present study was undertaken to obtain additional data concerning adrenal production of corticosterone in female rats and to evaluate the effects of oophorectomy and estrogen administration in greater detail.

Materials and methods. Female rats of the Sherman strain obtained from Camm Research Institute, Wayne, N. J., were maintained under standardized conditions of light (14 hr/24 hr) and temperature (75 ± 1°F) on a diet consisting of Purina Laboratory Chow and water *ad lib.* Unless otherwise specified, oophorectomy was performed at 30 days of age. Six weeks later, estrogenic hormone replacement was administered as a single, subcutaneous injection of polyestradiol phosphate (Estradurin, Ayerst). Control rats were uninjected, because preliminary experiments revealed no effects attributable to a single injection of the vehicle, distilled water, alone. Animals were rapidly decapitated, and adrenal slices were prepared and incubated according to a technique previously described(2). ACTH was added *in vitro* in doses (mU per 100 mg adrenal weight) indicated for each experiment. Corticosterone was measured by a fluorometric technique(3).

TABLE I. Corticosterone Production by Adrenal Slices from Intact, Untreated Female Rats of Selected Body Weights (μg/100 mg Adrenal Wt/2 Hr).

Body wt (g)	Adrenal wt (mg)	Corticosterone produced	
		5 mU ACTH	200 mU ACTH
116 ± 1	30.6 ± 1.1	9.6 ± .3	26.4 ± 1.7
133 ± 1	33.9 ± 1.6	9.2 ±1.1	.24.8 ± 3.7
174 ± 4	53.8 ± 2.6	6.3 ± .4	15.8 ± 1.2*
203 ± 2	64.6 ± 5.9	8.1 ± .2	21.4 ± .2
230 ± 2	67.2 ± 4.7	11.6 ± .7	24.1 ± 2.1

Entries represent means ± S.E. of 4 observations.
* Value differs from all others in column at $P < .01$.

Results. Untreated female rats were divided into groups with homogeneous body weights (Table I). Adrenal glands from animals with a mean body weight of 174 g (168-181 g) produced significantly less corticosterone *in vitro* than did adrenal tissue from rats of any other body weight tested. This difference was most clearly manifested when 200 mU of ACTH per 100 mg adrenal weight was added to the incubation medium. Significant differences with the 5 mU dose of ACTH were obtained only upon comparison with steroid yields from rats of the lowest

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TABLE II. Duration of Effect of a Depot Dose of Estradiol-17β on Corticosterone Production by Adrenal Slices from Rats Oophorectomized Prior to Puberty (μg/100 mg Adrenal Wt/2 Hr).

Interval after inj (wk)	Body wt when inj (g)	Corticosterone produced 5 mU ACTH	200 mU ACTH
Control (0)	—	3.2 ± .4	8.6 ± .2
1	220 ± 4	5.2 ± .3*	13.0 ± .6†
2	207 ± 8	7.4 ± .7†	19.2 ± 1.3†
3	166 ± 2	4.7 ± .4*	11.4 ± .7*
4	154 ± 4	3.0 ± .2	6.8 ± .5
5	130 ± 3	3.2 ± .3	8.7 ± .7

Entries represent means ± S.E. of 4 observations.
* Value differs from corresponding control at $P < .05$.
† Value differs from corresponding control at $P < .01$.

and highest body weights. Similar results were obtained in 2 additional experiments performed several months apart.

Duration of effect of a depot dose of estradiol-17β. Rats oophorectomized at 30 days of age were divided into 5 groups. The first group was given a single injection of 30 μg of polyestradiol phosphate one week later (Table II). Thereafter, successive groups were injected once each at weekly intervals. All the rats were sacrificed together 6 weeks after oophorectomy, including an uninjected control group with a mean body weight of 225 ± 7 g. The mean body weight of all the injected rats at that time was 221 ± 4 g. Significant increases in corticosterone production were observed at 1, 2 and 3 weeks after the injection of estradiol, with the peak effect noted at 2 weeks.

Earliest effect of a depot dose of estradiol-17β. Oophorectomized rats (mean body weight 230 ± 2 g), given a single injection of estradiol, 100 μg/100 g body weight, were sacrificed at daily intervals and adrenal corticosterone production was measured and compared to that of uninjected, oophorectomized control animals (Table III). No change was observed 1 or 2 days after injection, but significant increments occurred on days 3 and 4.

Minimal effective dose of estradiol-17β. Oophorectomized rats (mean body weight 231 ± 4 g) were given single doses of polyestradiol phosphate ranging from 0.01 to 100 μg/100 g body weight (Table IV). Corticosterone production was measured 2 weeks later. A significant increase first appeared with the dose of 10 μg/100 g body weight. A further increase was obtained with the dose of 100 μg/100 g.

Effects of post-pubertal oophorectomy. The preceding studies were all conducted in rats oophorectomized before puberty. Comparable procedures were also evaluated in sexually mature animals. Female rats (initial body weight 150 ± 4 g) were either sham-operated or oophorectomized. Groups of oophorectomized rats were either uninjected or given various doses of polyestradiol phosphate (Table V). Studies were performed 2 weeks later, at which time a group of pre-pubertally oophorectomized rats was included for comparison. An additional group from the same lot of mature animals was oophorectomized one week prior to study. A significant reduction in corticosterone production was obtained in this last group with no further change noted 2 weeks after oophorectomy. Corti-

TABLE III. Earliest Appearance of Change in Corticosterone Production by Adrenal Slices from Rats Oophorectomized Prior to Puberty Given a Depot Dose of Estradiol-17β (μg/100 mg Adrenal Wt/2 Hr).

	Corticosterone produced—20 mU ACTH 1 day	2 days	3 days	4 days
Control	3.5 ± .4	6.2 ± .3	6.0 ± .4	4.8 ± .2
Estradiol	3.8 ± .3	6.4 ± .4	9.2 ± .3*	11.2 ± .2*

Entries represent means ± S.E. of 4 observations.
* Value differs from corresponding control at $P < .01$.

TABLE IV. Minimal Depot Dose of Estradiol-17β Capable of Increasing Corticosterone Production by Adrenal Slices from Rats Oophorectomized Prior to Puberty (μg/100 mg Adrenal Wt/2 Hr).

Dose of estradiol-17β μg/100 g body wt	μg given	Corticosterone produced 5 mU ACTH	200 mU ACTH
Control	0	2.9 ± .2	8.9 ± .7
.01	.023	2.8 ± .2	9.4 ± .6
.1	.23	3.2 ± .1	10.2 ± .7
1.0	2.3	4.3 ± .4	8.7 ± .2
10.0	23.0	4.7 ± .1*	12.2 ± .7†
100.0	230.0	9.8 ± .2†	22.2 ± .8†

Entries represent means ± S.E. of 4 observations.
* Value differs from corresponding control at $P < .05$.
† Value differs from corresponding control at $P < .01$.

TABLE V. Corticosterone Production by Adrenal Slices from Mature Rats Oophorectomized After Puberty and Given a Depot Dose of Estradiol-17β (μg/100 mg Adrenal Wt/2 Hr).

Treatment	Dose of estradiol-17β (μg/100 g body wt)	Corticosterone produced ACTH 5 mU	Corticosterone produced ACTH 200 mU
Sham operation	0	10.2 ± .4	26.6 ± 1.0
Oophor. 1 wk	0	5.2 ± .3	15.8 ± .5
" 2 wk	0	5.6 ± .4	13.7 ± .2
"	20	5.0 ± .1	13.7 ± 1.3
"	40	13.0 ± .4*	24.1 ± 1.2*
"	100	16.2 ± .9*	42.5 ± 1.8*
Pre-pubertal oophor.	0	3.6 ± .1	9.6 ± .1

Entries represent means ± S.E. of 4 observations.
* Values differ from corresponding untreated oophorectomized responses at $P < .01$.

costerone production was significantly lower ($P < .01$) in the prepubertally oophorectomized rats than in either group gonadectomized after puberty. Estradiol replacement with doses of 40 μg/100 g body weight, or more, increased corticosterone production to a greater degree than did comparable doses in rats oophorectomized prior to puberty. However, the minimal effective dose required was higher in mature rats.

Discussion. The observation of diminished adrenal corticosterone production after oophorectomy in mature, as well as pre-pubertal rats(1), is consistent with the results of a number of investigators(4-11). Others have reported no change(12-13). Although the reasons for the disagreement are not entirely clear, one factor may be the weight (or age) of the animals used. The role of this variable in contributing to differences in adrenal corticosterone production is obscure and merits further study. The differences in steroid secretion associated with body weight in female rats appears to be of sufficient magnitude to warrant consideration also as a possible factor in the failure of some investigators(4,14) to find the sex differences in adrenal steroid production observed by others(15-18). A second factor may be that of differing environmental conditions. Halberg *et al*(7) demonstrated that failure to maintain their animals under standardized conditions of light and temperature obscured the effects of oophorectomy. Other variables include differing time intervals after operation and differing techniques for measurement of adrenal function. For example, with *in vitro* measurements of corticosterone production, decreases have been observed as early as 7 days (present study) to 10 days(4) post-oophorectomy. In contrast, studies of corticosterone secretion in adrenal venous blood *in vivo* revealed no change one week(12) or 2 weeks(13) after oophorectomy in mature rats. Pincus and Hirai(8) found no change at 10 days but observed a significant decrease 20 days post-operatively. Greater decrements were reported 8 to 12 weeks after pre-pubertal oophorectomy(1,9). No change occurred 5 to 8 days after oophorectomy in mature dogs(19).

The data reported here extend earlier observations that estradiol replacement increases corticosterone production by adrenal glands from oophorectomized rats(1). Both the time required for the appearance of effect and the duration of response of the adrenal cortex following a single injection of polyestradiol phosphate are consistent with the observations of Diczfalusy(20) concerning the estrogenic effects of the preparation in oophorectomized mice. Since the compound is inactive immediately after injection, but is hydrolyzed gradually over a period of weeks to free estradiol-17β(21), the effective dose delivered to the animal can only be estimated. Assuming conservatively that complete absorption occurred within 2 weeks, the minimal daily dose effective in increasing adrenal corticosterone production would be about 0.7 μg/100 g body weight. The endogenous secretion rate of estrogen in the rat is unknown. However, this amount is notably lower than those used previously in *in vivo* studies of adrenal function and approximates the amounts of estradiol-17β added per ml in *in vitro* experiments(22).

Few studies have been published concerning the effects of estrogen replacement on adrenal function in oophorectomized animals (1,12,19). A significant increment in adrenal corticosterone output was noted one week after both oophorectomy and unilateral adrenalectomy in rats given estrone, 50 μg/100 g body weight for 6 days(12). Cushman *et al*

(19) found no change in cortisol secretion rate in dogs (oophorectomized 5 to 8 days previously) with isolated adrenal pouches, immediately after a single intra-arterial perfusion with 20 or 100 μg of estradiol-17β. A similar absence of an acute response in the rat has also been observed in this laboratory (unpublished data) consistent with the conclusion that the action of estradiol is not as rapid as that of ACTH.

Summary. Corticosterone production by adrenal tissue *in vitro* from intact female rats weighing 168-181 g was significantly lower than that obtained from animals of higher or lower body weights. A single dose of a depot preparation of estradiol-17β in rats oophorectomized prior to puberty increased adrenal corticosterone production *in vitro* for a period beginning 3 days after injection and lasting 3 weeks. The peak effect was noted 2 weeks after injection. The minimal effective dose was 10 μg/100 g body weight. Rats oophorectomized after puberty demonstrated significant reductions in adrenal corticosterone production at 1 and 2 weeks after operation, but not the same degree as that observed 6 weeks after pre-pubertal oophorectomy. The minimal effective replacement dose of estradiol in mature oophorectomized animals was 40 μg/100 g body weight.

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Effect of Cold Shock, Disease, and Mammalian Corticoids on the Spleen of *Lepomis machrochirus.** (30484)

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Bern(1) has recently shown that the spleen of the cichlid fish, *Tilapia mossambica*, responds differentially to mammalian corticoids, a response not generally shown by teleosts(2, 3). The glucocorticoid, cortisol, caused a marked hypertrophy and hyperplasia of the spleen, accompanied by the proliferation of reticular phagocytes. The mineralocorticoid,

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