8. Artom, C., *J. Biol. Chem.*, 1953, v205, 101.
9. Olivecrona, T., *Kinetics of Fatty Acid Transport*, Thesis, Lund, 1962.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

Effects of Stilbestrol on Pituitary-Adrenal Function in Male and Female Rats.* (28138)

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Female rats respond to stress or to ACTH injection with higher peripheral plasma levels of corticosterone (Cpd. B) than do male rats(1). Additionally, a higher concentration of steroid obtains in adrenal vein blood of female rats, as well as a shorter biological half-life of Cpd. B *in vivo* and more rapid hepatic metabolism of the steroid *in vitro* when compared to male animals. The present study was undertaken to determine the effects of stilbestrol on pituitary and adrenal function in adult rats and to explore the possibility of a sex difference following its administration.

Materials and methods. Adult male and female rats of the Sherman strain were maintained in a constant temperature room on a diet of Purina Laboratory Chow and water *ad libitum*. Stilbestrol was administered by subcutaneous implantation of a 5 mg pellet. Control animals were either untreated or given a comparable pellet of cholesterol. Preliminary experiments revealed no effects attributable to control pellet implantation. All experiments were conducted 14 days later immediately following sacrifice by decapitation. Pituitary ACTH content was determined by an *in vitro* technic described previously(2). Pituitary and adrenal RNA and DNA contents were determined by the methods of Webb(3) and Webb and Levy(4) respectively. Adrenal secretory capacity *in vitro* was evaluated by preparation of pools of adrenal slices as used in the ACTH bioassay (2). All other methods have been described (1).

Results. The effects of stilbestrol pellet implantation on body, pituitary and adrenal weights are summarized in Table I. Body weight losses and increased pituitary and adrenal weights were demonstrated in both sexes. The mean adrenal weight in the female controls significantly exceeded that in their male counterparts ($P<.01$). All these findings are consistent with many published reports.

Pituitary changes. Although pituitary weight was increased markedly by stilbestrol administration in male rats, no change was observed in pituitary ACTH content. Multiple assays were performed on pooled glands; the control pool served as the standard with an assigned potency of 100%. In contrast, ACTH content increased 2.4-fold in stilbestrol-treated female rats and was associated with a proportionately lesser increment in pituitary weight. Here, also, multiple assays were performed with an arbitrary assignment of potency of 100% to the control standard pool. No significant difference in pituitary ACTH content was observed between female and male controls.

The increases in pituitary weight produced by stilbestrol were evaluated in greater detail by measurement of changes in RNA and DNA content as indices of cytoplasmic volume and nuclear mass respectively. Despite the significant increment in pituitary weight in the male animals given stilbestrol, no changes were found in RNA or DNA content. The effects of stilbestrol must be attributed, therefore, to an unmeasured factor, such as an increase in intra- or extra-cellular water or, perhaps, an increase in vascularization. In contrast in female rats, stilbestrol produced an increased pituitary RNA content without change in DNA, *i.e.*, hypertrophy without hyperplasia. The sex differ-

* Supported by a grant from Nat. Inst. Health.

TABLE I. Effects of Stilbestrol on Body Weight and Adrenal and Pituitary Glands.

	Male		Female	
	Control (12)	Stilbestrol (12)	Control (12)	Stilbestrol (12)
Body wt (g)	222 ± 9	159 ± 4†	175 ± 6	134 ± 4†
Pituitary				
Wt (mg)	4.0 ± 0.3	7.3 ± 0.7†	5.7 ± 0.3	8.4 ± 0.7†
ACTH (%)*	100	105 (60–160)	100	240 (180–330)
RNA (μg)	18.3 ± 3.8	17.8 ± 3.7	21.7 ± 3.6	40.9 ± 6.1†
DNA (μg)	13.0 ± 1.6	12.0 ± 1.0	18.6 ± 2.3	22.2 ± 1.3
Adrenal				
Wt (mg)	33.2 ± 1.8	48.9 ± 3.2†	53.2 ± 2.0	64.4 ± 3.9‡
RNA (μg)	89.2 ± 6.8	186.0 ± 28.2†	140.5 ± 7.2	177.6 ± 9.9†
DNA (μg)	46.8 ± 3.5	47.5 ± 8.3	82.8 ± 8.5	57.2 ± 6.8‡

Entries represent means ± standard error of mean.
* ACTH potencies presented as % of control content with 95% confidence limits in parentheses.
† $P < .01$ ‡ $P < .05$

ence in the pituitary RNA response to stilbestrol may be related to the changes observed in ACTH content.

Adrenal changes. The increased adrenal weight in stilbestrol-treated male rats was associated with increased RNA without change in DNA, or hypertrophy. In female animals, an increase in RNA also occurred but a significant fall in adrenal DNA was observed as well. These changes suggest that stilbestrol administration resulted in loss of cells, or hypoplasia, associated with hypertrophy of the remaining tissue with a net increase in adrenal weight.

Taken together, the changes described suggest several hypotheses concerning the effects of stilbestrol on pituitary and adrenal function. In male animals, the adrenal weight and nucleic acid changes suggest that ACTH secretion was stimulated by stilbestrol. Bransome and Reddy(5) have reported that ACTH administration in the dog increases adrenal RNA without change in DNA. Confirmatory findings have also been obtained in rats given ACTH (unpublished data). The unchanged pituitary ACTH content is not incompatible with this hypothesis in that both increased ACTH synthesis and release might occur without a net change in ACTH storage.

The increases in adrenal weight and RNA observed after stilbestrol in female rats likewise suggest an increase in ACTH secretion but with greater synthesis than release leading to a net increase in ACTH content. A complicating factor is indicated by the fall in adrenal DNA in the female animals suggesting the additional possibility of a toxic or inhibitory effect of stilbestrol on the female adrenal. These hypotheses were explored further in the following experiments.

Plasma corticosterone concentrations. The effects of stilbestrol on plasma Cpd. B were measured at rest and 30 minutes after the stress of ether anesthesia or after ACTH injection (Table II). A 30 minute interval was selected for study since it was found previously to be associated with peak plasma steroid levels in untreated animals of both sexes (1). In conformity with previous findings,

TABLE II. Effects of Stilbestrol on Plasma Cpd. B Concentrations (μg/100 ml plasma).

	Male		Female	
	Control (7)	Stilbestrol (7)	Control (8)	Stilbestrol (8)
Rest	11.6 ± 0.8	13.6 ± 1.4	22.8 ± 6.0	17.8 ± 2.1
Stress	46.4 ± 2.8	41.4 ± 4.3	89.2 ± 2.8	44.4 ± 4.9*
ACTH	39.9 ± 2.4	44.0 ± 7.2	76.9 ± 5.3	42.5 ± 3.4*

Entries represent means ± standard error of mean.
* $P < .01$

TABLE III. Effects of Stilbestrol on Peripheral Metabolism of Cpd. B.

	Male		Female	
	Control	Stilbestrol	Control	Stilbestrol
Biological half life *in vivo* (min)	20.1 ± 1.2 (30)	15.5 ± 1.8† (15)	13.1 ± 1.3 (30)	24.1 ± 2.5† (15)
Hepatic ring A reduction *in vitro* (μg reduced)	37.0 ± 5.1 (10)	56.0 ± 6.1‡ (7)	82.2 ± 2.5 (13)	22.1 ± 5.9† (7)

Entries represent means ± standard error of mean.
Numbers of animals studied indicated in parentheses.
† $P < .01$ ‡ $P < .05$

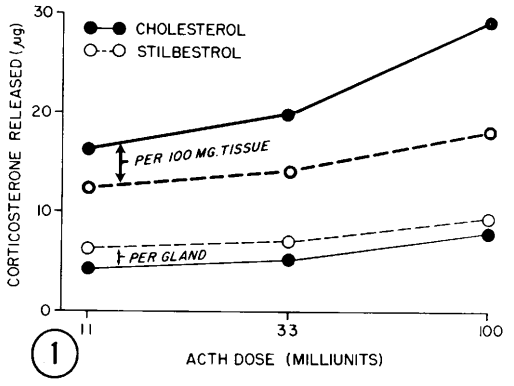
the female controls demonstrated consistently higher plasma Cpd. B levels than did the male controls. No apparent changes were seen after stilbestrol in the male rats, whereas substantial decrements in plasma steroid levels were found after stress or ACTH in female animals so treated.

These data are consistent with those of the preceding experiment in that the inhibitory effect of stilbestrol on adrenal DNA in female rats may be correlated with a marked diminution in plasma Cpd. B concentrations.

Peripheral metabolism of corticosterone. Plasma Cpd. B levels represent a balance between adrenal secretion and clearance of steroid from the circulation. The latter factor was assessed by measurement of both biological half-life *in vivo* and hepatic reduction of ring A of Cpd. B *in vitro* (Table III). The sex differences observed in the control animals have been reported(1). Stilbestrol administration produced opposite effects in the two sexes. The peripheral metabolism of Cpd. B, as measured by both technics, was significantly enhanced in male rats and diminished in females. The data suggest that stilbestrol administration in male rats resulted in increased secretion of both ACTH by the pituitary and Cpd. B by the adrenals with more rapid peripheral clearance of steroid and, thus, no significant increment in plasma steroid levels. In female rats the impairment of peripheral metabolism of steroid would be expected to have produced elevated plasma Cpd. B concentrations. The combination of both decreased plasma steroid levels and decreased clearance indicate even greater inhibition of adrenal secretion than do the plasma values taken alone.

Adrenal secretion in vitro. The multiple factors inherent in the preceding experiments were controlled by examining the effects of stilbestrol upon steroid secretion by adrenal slices *in vitro*. Under such conditions neither endogenous ACTH secretion nor peripheral metabolism of steroid serve as complicating variables. Studies on male adrenal glands (Fig. 1) revealed that, with a range of ACTH concentrations added *in vitro*, there was actually a significant diminution in adrenal secretory capacity when the results were calculated per unit adrenal weight. Increased secretory capacity was demonstrated when steroid output was calculated per gland owing to the marked increase in adrenal weight produced by stilbestrol. Secretory capacity in female adrenal slices (Fig. 2) was reduced as measured by either parameter in complete accord with the preceding experiments. The data in the male animals suggest that the inhibitory effects of stilbestrol observed in the female may also apply, but to a significantly lesser degree, and that they were overcome by increased ACTH stimulation with a resultant increase in adrenal secretory mass.

Massive stilbestrol administration in male animals. Demonstration of sex differences in the effects of stilbestrol suggested the possibility that the greater inhibitory effects observed in female rats might be a dose-dependent phenomenon, *i.e.*, exogenous stilbestrol superimposed upon endogenous estrogen in the female produced a more striking effect than in the male where endogenous estrogen is comparatively negligible. This hypothesis was tested by subcutaneous administration of a water-soluble, readily absorbed preparation of stilbestrol phosphate (Stilphostrol, Ames) to male animals at a dose of 1 mg per day for 3 days. Control animals received comparable



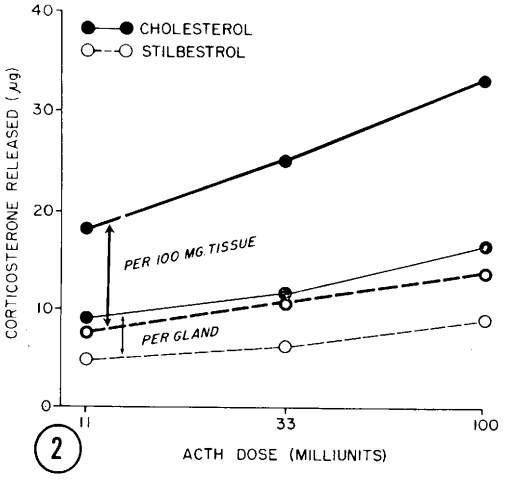


FIG. 1. Effects of stilbestrol on steroidogenesis by male adrenal slices *in vitro*. Each point represents 4 observations.

FIG. 2. Effects of stilbestrol on steroidogenesis by female adrenal slices *in vitro*. Each point represents 4 observations.

injections of the vehicle alone. On the morning of the fourth day, plasma Cpd. B levels at rest and after stress or ACTH were measured and hepatic metabolism of steroid determined as described above (Table IV). Marked impairments of both adrenal secretion and hepatic metabolism observed were consistent with those previously obtained in female animals.

Discussion. Administration of stilbestrol to rats produced both similar and dissimilar effects in the two sexes. Female rats showed impairment of peripheral metabolism of Cpd. B and a decrease in adrenal steroid secretion whereas ACTH secretion was apparently enhanced. Male animals revealed increased peripheral clearance of Cpd. B with a seemingly greater total adrenal secretory capacity. However, this increase appeared related to adrenal hypertrophy, and diminished secretory capacity was actually demonstrated per unit of gland weight. An overall increase in ACTH secretion was suggested in the male animals without the increment in ACTH storage noted in the treated females.

The most definite sex difference produced by stilbestrol was its effects upon peripheral clearance and hepatic metabolism of Cpd. B. The changes noted in male animals are consistent with those observed by Yates *et al.* (6) following estradiol injection in both intact and castrated male rats. No previous studies concerning administration of estrogenic substances to intact female rats are available. Both male and female animals given stilbestrol showed decrements in body weight. Herbst *et al.*(7) have shown that diminished food intake in rats is associated with impairment of hepatic metabolism of Cpd. B. This observation may explain the stilbestrol-induced reduction in steroid clearance in female animals but seems wholly inapplicable to the enhanced clearance produced in males with a greater proportionate loss of body weight.

Although stilbestrol administration increased pituitary and adrenal weights in both sexes, different interpretations were made

TABLE IV. Effects of Stilbestrol Phosphate (Stilphostrol) on Plasma Cpd. B Concentrations and Hepatic Metabolism of Ring A *in vitro* in Male Rats.

Treatment	Plasma Cpd. B (µg/100 ml) Rest	Stress	ACTH	Hepatic ring A reduction *in vitro* (µg reduced)
Control	10.2 ± 2.1	62.0 ± 3.4	69.6 ± 7.2	32.2 ± 6.4
Stilbestrol	18.8 ± 2.7*	29.8 ± 2.8*	35.0 ± 2.7*	11.4 ± 5.0*

Entries represent means ± standard error of mean.
Number of animals studied: 5/group.
* $P < .01$

possible by determination of tissue RNA and DNA contents. Such measurements may help to clarify conflicting experimental findings based on changes in gland weight alone (*e.g.*, 8,9,10).

The impairment of adrenal steroid secretion produced by stilbestrol demonstrated above is consistent with that previously reported by Vogt(11) and Holzbauer(12) with the closely related compound, hexestrol. McKerns and Bell(13) have shown that stilbestrol inhibits adrenal glucose-6-phosphate dehydrogenase activity, an enzyme thought to be essential in steroid biosynthesis.

The pattern of changes induced by stilbestrol is consistent with the conclusion that it produces adrenal inhibition with a resultant impairment of normal feed-back and a secondary increase in pituitary ACTH secretion. On the other hand, stilbestrol may also affect ACTH release directly without adrenal intermediation. In any event, a unitary hypothesis based on a single site of action of stilbestrol does not seem possible in view of its apparently separate actions upon hepatic metabolism of Cpd. B. A sex difference in response to dose and duration of administration may explain the differing effects of stilbestrol in male and female rats. The difference may be due to the presence of endogenous estrogen in the female. Finally, it should be emphasized that stilbestrol is a synthetic estrogen and that its actions with respect to pituitary and adrenal function may not be identical to those of the natural estrogens.

Summary. Stilbestrol administration produced apparently similar increases in pituitary and adrenal weights and losses in body weight upon comparison of male and female rats. Pituitary ACTH content was more than doubled in females and unchanged in male animals. Pituitary RNA and DNA were both unchanged in males, whereas RNA (but not DNA) was increased in females. Adrenal RNA was increased in both sexes while DNA was unchanged in males and decreased in females. Plasma Cpd. B levels after stress or ACTH were unaffected in males and markedly reduced in females. Biological half-life of Cpd. B was shortened and hepatic metabolism of the steroid enhanced in treated males. Opposite changes were observed in female rats. Adrenal steroidogenesis *in vitro* was inhibited in female rats. An increment in steroidogenesis was demonstrated in males per total gland whereas a decrease obtained per unit gland weight. Male responses became similar to those in the female by administration of a more potent dose of stilbestrol.

Acknowledgment is made to Dr. Norman L. Heminway, Ames Co., Dr. Kenneth W. Thompson, Organon, Inc., and Dr. Earl S. Gerard, Upjohn Co., for generous supplies of materials used in these studies.

1. Kitay, J. I., *Endocrinology,* 1961, v68, 818.
2. Kitay, J. I., Holub D. A., Jailer, J. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 165.
3. Webb, J. M., *J. Biol. Chem.,* 1956, v221, 635.
4. Webb, J. M., Levy, H. B., *ibid.,* 1955, v213, 107.
5. Bransome, E. D., Reddy, W. J., *Endocrinology,* 1961, v69, 997.
6. Yates, F. E., Herbst, A. L., Urquhart, J., *ibid.,* 1958, v63, 887.
7. Herbst, A. L., Yates, F. E., Glenister, D. W., Urquhart, J., *ibid.,* 1960, v67, 222.
8. Carter, S. B., *J. Endocrinol.,* 1956, v13, 150.
9. Gompertz, D., *ibid.,* 1958, v17, 107.
10. Segal, S. J., Thompson, D. R., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 623.
11. Vogt, M. J., *J. Physiol.* (London), 1955, v130, 601.
12. Holzbauer, M., *ibid.,* 1957, v139, 306.
13. McKerns, K. W., Bell, P. H., *Recent Progr. in Hormone Res.,* 1960, v16, 97.

Received December 26, 1962. P.S.E.B.M., 1963, v112.