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STUDIES ON THE MECHANISM OF THE PLASMA 17-HYDROXY-CORTICOSTEROID ELEVATION INDUCED IN MAN BY ESTROGENS *

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The administration of estrogens to man produces sustained elevation above normal levels of plasma 17-hydroxycorticosteroids (17-OHCS) (1, 2). The elevated levels of plasma 17-OHCS are associated with an augmented response of plasma levels of these steroids to exogenous adrenocorticotrophic hormone (ACTH) and with a marked delay in the rate of clearance of exogenous cortisol from plasma (2). The last trimester of pregnancy is associated with similar changes in levels of plasma 17-OHCS and in their response to exogenous ACTH (3-9). In the last trimester of pregnancy there is a variable increase in urinary 17-OHCS excretion, a decrease in the rate of clearance of exogenous cortisol from plasma (9-11), a decrease in the rate of clearance of exogenous tetrahydrocortisone from plasma (11) and an increase in corticosteroid-binding protein (12). The apparently normal adrenal status of pregnant women and of patients treated with estrogens for long periods of time suggests that the administration of estrogens to man elevates plasma 17-OHCS levels by altering the normal metabolism of endogenous cortisol by a mechanism similar to that seen in pregnancy. Studies have been made of urinary 17-OHCS excretion, of clearance of tetrahydrocortisone from plasma, and of plasma corticosteroid-binding protein after the administration of estrogens to man.

MATERIALS AND METHODS

The patients studied were primarily adult females ranging in age from 37 to 75 years, all of whom either had been surgically castrated or were at least 3 years past a spontaneous menopause. A number of these subjects were patients with metastatic carcinoma of the

breast. One male was studied during recovery from a myocardial infarction.

After appropriate control studies, the patients were treated for periods of 2 weeks to 6 months with either oral ethinyl estradiol (Estinyl), diethylstilbestrol or conjugated equine estrogens (Premarin). Only doses of these preparations which uniformly elevated plasma 17-OHCS levels above normal were used in the studies (Table I). Ethinyl estradiol 0.1 and 0.5 mg, diethylstilbestrol 15 mg, and Premarin 10 mg, daily by mouth, all caused significant increases in the levels of plasma 17-OHCS.

The effect of oral ethinyl estradiol, 0.5 mg daily, on urinary excretion of free and total 17-OHCS and of 17-ketosteroids was studied. Twenty-four hour urinary excretion of steroids was determined in each patient before estrogen therapy and again after plasma 17-OHCS levels had become elevated. All determinations were performed on 24-hour urines collected under refrigeration. Urinary creatinine determinations were made on all specimens by the method of Bonsnes and Tausky (13). Urinary 17-ketosteroids were measured by the Gibson and Norymberski modification (14) of the method of Dreker and associates. Urinary 17-OHCS were determined before (free 17-hydroxycorticosteroids) and after (total 17-hydroxycorticosteroids) hydrolysis with β -glucuronidase,¹ by the modification of the Silber-Porter technique of Peterson and colleagues (15).

TABLE I
Effect of various estrogen preparations on plasma 17-hydroxycorticosteroids

Therapy	Dose p.o.	Subjects	Plasma 17-OHCS	
			Control	After therapy
	<i>mg/day</i>	<i>no.</i>	<i>μg/100 ml</i>	
Ethinyl estradiol	0.1	5	23 ± 5.0*	46 ± 12.2
Ethinyl estradiol	0.5	18	15 ± 6.0	58 ± 18.1
Stilbestrol	15.0	8	19 ± 4.1	54 ± 10.1
Conjugated estrogens (Premarin)	10.0	5	24 ± 4.8	42 ± 5.0

* Mean ± standard deviation.

¹ Ketodase, Warner-Chilcott.

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The clearance from plasma of exogenous tetrahydrocortisone was studied in 7 patients in whom plasma 17-OHCS levels had been elevated by estrogen therapy and in 4 control subjects. Seventy-five mg of crystalline tetrahydrocortisone was dissolved in 7 to 8 ml of hot alcohol, added to 80 to 100 ml of normal saline and administered intravenously over a period of 2 to 5 minutes. Plasma samples were obtained prior to and at approximately 20, 40, 60 and 90 minutes after each infusion. Plasma 17-OHCS were determined by the method of Peterson and colleagues (15).

The binding of cortisol to a corticosteroid-binding globulin, transcortin, was kindly determined by Drs. Sandberg and Slaunwhite by the method which they have described (12, 16). Binding was determined by dialyzing 10 ml of diluted plasma (1:5 with physiological saline) against 30 ml of saline, containing approximately 0.3 μg of C^{14} cortisol at 4° C. Binding was also determined by the addition of 1 μg of cortisol to the saline in order to measure the decrease in the binding caused by the addition of cortisol—"transcortin capacity." The binding capacity is inversely related to the decrease in binding caused by the addition of 1 μg of cortisol. Plasma 17-OHCS levels, transcortin-binding and transcortin capacity were measured in 12 patients, 6 of whom previously have been reported (16), before and at 2 to 3 day intervals after the institution of therapy.

RESULTS

1. *Effect of oral ethinyl estradiol (0.5 mg per day) upon urinary excretion of steroids.* Ethinyl estradiol administration for periods of 2 to 4 weeks resulted in a significant decrease ($p < 0.01$) in the mean excretion of total urinary 17-hydroxycorticosteroids in nine patients studied (Table II). Since the excretion of free urinary 17-OHCS was not significantly altered by the administration of estrogens, the major decrease in urinary 17-OHCS appeared to be in the conjugated fraction.

TABLE II
Urinary steroid excretion before and after ethinyl estradiol *

	Patients	Control excretion	Postestrogen excretion	p†
	no.	mg/24 hrs	mg/24 hrs	
Total urinary 17-OHCS	9	4.3 \pm 1.4‡	3.1 \pm 1.5	<0.01
Free urinary 17-OHCS	8	0.51 \pm 0.19	0.67 \pm 0.46	>0.05
Urinary 17-KS	6	8.2 \pm 3.5	7.7 \pm 2.3	>0.05

* Per os, 0.5 mg per day.

† Determined by *t* test.

‡ Mean \pm standard deviation.

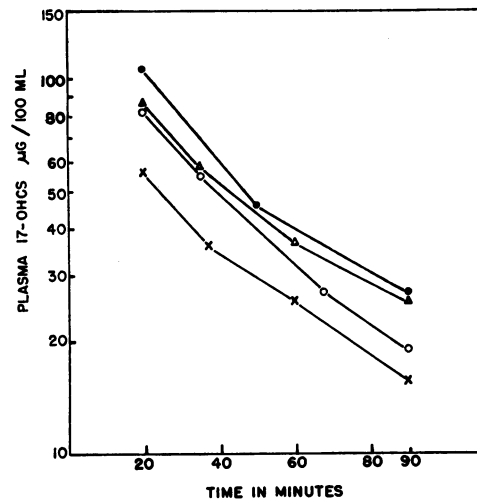


FIG. 1. SEMILOGARITHMIC GRAPH OF RATE OF FALL OF PLASMA 17-HYDROXYCORTICOSTEROIDS AFTER RAPID INTRAVENOUS INJECTION OF TETRAHYDROCORTISONE, 75 MG, IN FOUR NORMAL SUBJECTS.

Mean urinary 17-ketosteroid excretion was unaltered by estrogen therapy ($p > 0.05$).

2. *Effect of the administration of estrogens upon the clearance of exogenous tetrahydrocortisone from plasma.* The rate of disappearance of intravenously administered tetrahydrocortisone from plasma of four control subjects and of seven patients in whom plasma 17-OHCS levels had been elevated by the administration of either diethylstilbestrol or ethinyl estradiol is shown in Figures 1 and 2. The mean decline for each group is shown in Figure 3. The group treated with estrogens cleared intravenously administered tetrahydrocortisone from blood at a significantly ($p < 0.01$) slower rate than did the control group. The decline per minute of plasma 17-OHCS levels was 1.86 and 0.69 per cent in the control and treated groups, respectively.

3. *The effect of administration of estrogens upon the binding of cortisol to transcortin.* The effects of ethinyl estradiol, diethylstilbestrol and Premarin on transcortin-binding of cortisol were identical (Table III). Therapy with these hormones produced an increase in the percentage of cortisol bound to transcortin from mean levels of 90 ± 4.1 per cent cortisol bound before therapy to 97 ± 1.4 per cent bound after therapy when levels of plasma 17-OHCS had become elevated ($p < 0.01$). In addition, estrogen therapy pro-

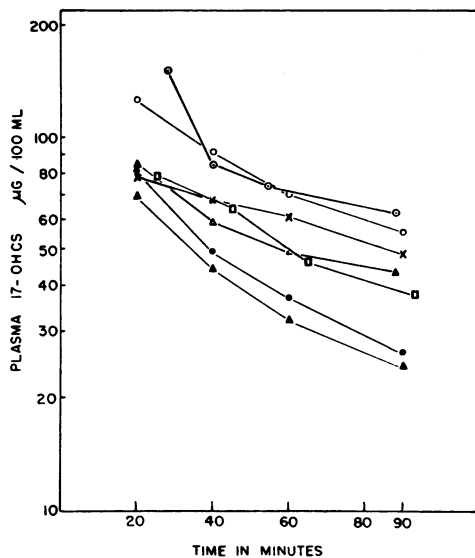


FIG. 2. SEMILOGARITHMIC GRAPH OF RATE OF FALL OF PLASMA 17-HYDROXYCORTICOSTEROIDS AFTER RAPID INTRAVENOUS INJECTION OF TETRAHYDROCORTISONE, 75 MG, IN SEVEN ESTROGEN-TREATED SUBJECTS.

duced a significant increase in transcortin-binding capacity when this was determined by the suppression of binding following the addition of 1 µg of cortisol (F) to the binding system. There was a mean decrease of 23 ± 3.9 per cent bound

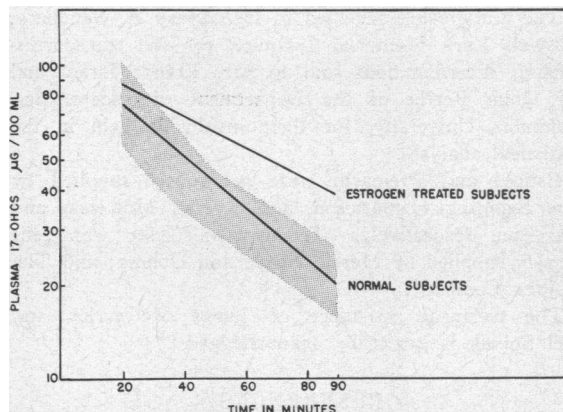


FIG. 3. COMPARISON OF TETRAHYDROCORTISONE CLEARANCE RATES IN CONTROL AND ESTROGEN-TREATED SUBJECTS. Solid lines indicate calculated means for the four control and seven estrogen-treated subjects. Stippled area indicates the extreme limits of variation for the control group. The mean percentage decreases of plasma 17-hydroxycorticosteroid levels per minute for estrogen-treated subjects is significantly less than that for control subjects at the 1 per cent level.

in the control subjects and of 12 ± 2.6 per cent bound in the patients treated with estrogens. The increase in transcortin-binding capacity was significant at the 1 per cent level as determined by the *t* test.

TABLE III
Effect of estrogen therapy on binding of cortisol (F) to transcortin in 12 patients

	Before therapy	During therapy
Plasma 17-OHCS (µg/100 ml)	$20 \pm 5.7^*$	50 ± 11.8
Cortisol binding before 1 µg F (% bound)	90 ± 4.1	97 ± 1.4
Decrease in % bound after 1 µg F	23 ± 3.9	12 ± 2.6

* Mean \pm standard deviation.

DISCUSSION

There has been increased interest in alterations in the metabolism of 17-hydroxycorticosteroids (17-OHCS) induced by pregnancy and by the administration of estrogens in man. The demonstration that orally administered diethylstilbestrol produced elevations of plasma 17-OHCS levels (1) in man has led to a number of studies designed to define the mechanism of these increases.

The description by Daughaday (17-19) of a specific corticosteroid-binding globulin in human plasma has stimulated investigations of the effects of pregnancy and of estrogen administration upon the protein-binding of plasma 17-OHCS. Slaunwhite and Sandberg (12, 16), using different conditions of dialysis, have reported increases in a corticosteroid-binding protein (transcortin) during the administration of diethylstilbestrol and ethinyl estradiol. In the studies reported here, increases in the percentage of cortisol bound to transcortin and in transcortin-binding capacity are shown to occur with the administration of Premarin as well as with diethylstilbestrol and ethinyl estradiol. Evidence that the elevated Porter-Silber chromogens present after estrogen therapy are cortisol has been reported by Peterson on the basis of isotope dilution and isotope derivative assays of the plasma cortisol (20).

If cortisol bound to corticosteroid-binding protein is physiologically "inactive" and less available for metabolic disposal than is unbound corti-

sol (12), then many of the changes in 17-OHCS metabolism induced by estrogens may be explained by increased binding of plasma cortisol to a specific globulin. Increased levels of plasma 17-OHCS, without clinical evidence of hyper- or hypoadrenocorticism, imply the presence of increased levels of protein-bound cortisol and normal amounts of cortisol not bound to the specific binding protein. The absence of adrenal suppression, as manifested by augmented response of levels of plasma 17-OHCS to ACTH, further suggests that the "active" moiety of circulating cortisol is not present in excess. These findings suggest that the circulating increased levels of plasma 17-OHCS do not suppress the pituitary or adrenal glands.

The quantitatively exaggerated rise in levels of 17-OHCS after corticotropin may be explained, at least in part, by the delay in clearance of cortisol from plasma. The delayed plasma clearance of cortisol is presumably due to the decreased availability of the transcortin-bound fraction of cortisol for metabolism and conjugation by the liver. The decrease in conjugated urinary 17-OHCS may also be explained by such a sequence of events. The unchanged levels of free cortisol in the urine found in this study are compatible with the clinically normal adrenal state of patients receiving estrogens.

The delayed clearance of exogenous tetrahydrocortisone, a steroid not bound in any significant degree by corticosteroid-binding globulin (18), cannot be explained by increases in the binding capacity of this protein. The existence of a protein-binding system for tetrahydrocortisone, as yet undescribed, which may be influenced by the administration of estrogens, should be considered. There is evidence for a diversity of protein-binding systems affected by estrogens in the reported increases in thyroid-binding globulin induced by the administration of diethylstilbestrol (21).

The similarity in changes produced by adequate doses of estrogens and those that occur spontaneously in pregnancy is striking. All of the alterations in steroid metabolism induced by administration of estrogens, except for the decrease in conjugated urinary corticosteroids, occur in the last trimester of pregnancy. The exact physiological significance of the altered corticosteroid me-

tabolism during pregnancy and estrogen administration remains to be defined. Although many of the changes in steroid metabolism noted in both situations can be adequately explained by increases in corticosteroid-binding protein, the data do not exclude the possibility of other contributing mechanisms.

SUMMARY

Estrogen administration to man in adequate dosage produces:

1. Elevation above normal of plasma 17-hydroxycorticosteroid (17-OHCS) levels and excessive rise of these steroids after intravenous ACTH without clinical hyperadrenocorticism.
2. Decreased urinary excretion of conjugated 17-OHCS.
3. Delay in rate of clearance from plasma of exogenous cortisol and tetrahydrocortisone.
4. Increase in cortisol-binding to corticosteroid-binding proteins (transcortin) and in transcortin-binding capacity. This mechanism can explain the reported findings, excepting the diminished rate of clearance from plasma of exogenous tetrahydrocortisone which remains unexplained by the data reported.

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