Effect of Ethinyl Estradiol on Plasma 17-Hydroxycorticosteroids, ACTH Responsiveness and Hydrocortisone Clearance in Man.* (23369)

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Studies of the effect of estrogens on adrenal size and function in the experimental animal have yielded conflicting results. Administration of natural and synthetic estrogenic preparations has been followed by pituitary and adrenal hypertrophy in the intact rat(1,2,3), a phenomenon not seen in the hypophysectomized animal. In male rats, Gemzell(4) has reported an increase in plasma ACTH after administration of estradiol monobenzoate but Vogt(5) found that hexoestrol appeared to lower concentration of corticosterone in adrenal vein blood. Recently, Carter(6) was unable to demonstrate significant increases in adrenal weight in rats treated with estradiol dipropionate.

In guinea pigs, Zondek and Burstein(7) have reported marked increases in urinary corticosteroid excretion during estrone and stilbesterol treatment. Chesterman, Franks, Knudsen and Williams(8) have recently described a picture highly suggestive of primary aldosteronism following long term stilbesterol therapy in the same species. In the rabbit, estrogens appear to have no effect on adrenal histology or corticosterone secretion(5).

Little is known in man of the effect of estrogens on adrenal secretory activity. In one study(9), although estradiol caused a decrease in total neutral 17-ketosteroids in the urine of male subjects, increases in daily excretion of dehydroisoandrosterone suggested an augmented adrenocortical secretion. More recently, Taliaferro, Cobey and Leone(10) reported elevated levels of plasma 17-hydroxycorticosteroids in patients with carcinoma of the prostate who were receiving large doses of stilbesterol. Such a rise did not occur in 1 male patient with Addison's disease.

The present study was undertaken to evaluate adrenocortical function in castrate and post-menopausal females before and after administration of ethinyl estradiol.

Materials and methods. The subjects included 17 females who had had a spontaneous menopause 10 or more years before, 1 female 4 months post-surgical castration, 3 female patients with documented hypopituitarism due either to pituitary tumor or necrosis and a male with established Addison's disease. These patients received either 0.1 or 0.5 mg ethinyl estradiol in one oral daily dose.

Plasma 17 - hydroxycorticosteroids (17 - OHCS) were determined using the method of Silber and Porter as modified by Peterson (11). Normal control levels range from 4-30 μg/100 ml of plasma in this laboratory.

The adrenocortical response to ACTH was measured by the effect of 25 U.S.P. units of ACTH in 500 ml 5% glucose in water given intravenously over 6 hours on plasma 17-OHCS levels. Control samples were obtained and infusions begun between 8 a.m. and 9 a.m.

Plasma hydrocortisone clearances were studied by administering 100 mg of hydrocortisone sodium succinate in one rapid intravenous injection and determining plasma 17-OHCS levels before and at 4, 6, and 8 hours following injection. Hydrocortisone administered in this fashion is cleared from the plasma of normal subjects within 6 hours(12).

Plasma samples were analyzed before and at 2, 4, and 6 hours after the ingestion of a single dose of 0.5 mg of ethinyl estradiol by mouth in 4 patients in order to determine whether this compound could cause a direct or artifactual elevation of plasma 17-OHCS.

Results. I. *Effect of ethinyl estradiol on plasma 17-hydroxycorticosteroids and on*

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TABLE I. Effect of Ethinyl Estradiol on Plasma 17-Hydroxycorticosteroids and on Their Response to ACTH.

Patient	Age	Description	Pre-ethinyl estradiol Plasma 17-OHCS, μg/100 ml Pre-ACTH	Post-ACTH	Ethinyl estradiol mg per day p.o.	No. of days	Post-ethinyl estradiol Plasma 17-OHCS, μg/100 ml Pre-ACTH	Post-ACTH
N.K.	61	10 yr post-menopause	12.4	35.6	.5	21	62.2	116.5
J.W.	66	10 " "	11.1	38.6	.5	21	94.4	156.2
L.F.	63	20 " "	20.4	48.4	.5	21	73.3	174.4
M.P.	85	30 " "	11.8	40.5	.5	21	79.3	116.7
A.G.	78	30 " "	19.6	43.5	.5	16	43.6	64.5
E.S.	74	30 " "	14.7	35.5	.5	16	64.7	103.1
R.H.	78	30 " "	22.8	48.3	.5	16	58.4	113.2
A.R.	65	16 " "	12.9	52.2	.5	16	50.6	102.3
M.P.$_1$	64	20 " "	10.6	39.3	.5	16	57.4	113.7
M.P.$_2$	76	30 " "	30.0	52.1	.1	11	62.5	—
M.J.	73	20 " "	20.2	—	.1	10	40.9	—
H.C.	75	25 " "	20.3	41.2	.1	17	54.3	89.7
J.N.	23	Pituitary tumor	3.8	12.1	.5	10	4.7	13.3
						30	5.5	—
C.G.	31	Post-partum pituitary necrosis	3.8	27.3	.5	12	18.1	64.2
						33	22.9	—
G.G.	36	17 yr postop. craniopharyngioma	8.4	30.0	.1	24	16.6	—
			2.0	—				
W.H.	37	Addison's disease	7.4	6.2	.5	14	6.1	—
						30	2.6	—

adrenocortical response to a standard ACTH test (Table I). Marked increases in concentration of plasma 17-hydroxycorticosteroids (17-OHCS) were seen in all post-menopausal women receiving ethinyl estradiol. Two of 3 patients with hypopituitarism (C.G. and G.G.) also showed definite increases in levels of plasma 17-OHCS. C.G., a 31-year-old female with post-partum pituitary necrosis, had normal thyroid function (RAI uptake 45% in 24 hours, PBI 9 μg %) and slightly decreased adrenocortical responsiveness (Table I) at the onset of the study. G.G., a 36-year-old female who had had a craniopharyngioma resected 17 years ago, had depressed thyroid function (RAI uptake 2.5% in 24 hours, PBI 2.4 μg %) and moderately impaired adrenocortical secretory capacity (Table I). The third patient with hypopituitarism (J.N.) did not have a rise in plasma 17-OHCS at the end of 30 days of ethinyl estradiol. This patient, a 23-year-old female with x-ray evidence of a pituitary tumor, had low normal thyroid function (RAI uptake 23% in 24 hours, PBI 3.6 μg %) and only a minimal response of urinary and plasma 17-OHCS to ACTH stimulation before administration of ethinyl estradiol (Table I).

A male subject with Addison's disease (W.H.) showed no rise in plasma 17-OHCS after ingestion of 0.5 mg of ethinyl estradiol for 30 days.

Ten post-menopausal patients with normal initial responses to corticotropin (plasma 17-OHCS after stimulation 36-52 μg %) had ACTH tests performed after plasma 17-OHCS levels were elevated during ethinyl estradiol therapy. At this time concentration of 17-OHCS in plasma rose to between 64-174 μg % at the end of the corticotropin infusion.

Two of the patients with hypopituitarism received corticotropin while taking ethinyl estradiol. Patient J.N. whose initial ACTH test revealed markedly depressed adrenocortical responsiveness showed no essential change when the test was repeated after 10 days of ethinyl estradiol. Patient C.G. with only moderate initial depression of adrenocortical function had a distinctly increased response of plasma 17-hydroxycorticosteroids to ACTH on the 12th day of ethinyl estradiol. Neither patient received any other medication during the study.

II. *Effect of ethinyl estradiol administration on plasma clearance of exogenous hydrocortisone* (Table II). Clearance of adminis-

TABLE II. Plasma Clearance of Intravenous Hydrocortisone.

Patient	Age	Description	Ethinyl estradiol mg per day p.o.	Ethinyl estradiol No. of days	Plasma 17-hydroxycorticosteroids, μg/100 ml Control	4 hr	6 hr	8 hr
C.H.	75	20 yr post-menopause	.5	17	48.8	—	141.6	107.8
J.N.	23	Pituitary tumor	.5	30	5.5	140.0	123.3	—
C.G.	31	Post-partum pituitary necrosis	.5	33	22.9	194.6	149.6	—
G.G.	36	Craniopharyngioma, 17 yr post-op.	.1	24	16.6	99.8	77.6	—
D.K.	67	20 yr post-menopause	0		23.8	48.0	32.5	—
A.B.	63	11 " "	0		16.0	39.4	24.4	—

tered hydrocortisone from plasma has been evaluated in 4 patients: one 75-year-old woman who was receiving 0.5 mg ethinyl estradiol each day and the 3 patients who had hypopituitarism. Two of the latter were taking 0.5 mg ethinyl estradiol daily and the other 0.1 mg. In all 4 patients, there was evidence of a marked delay in clearance of the administered dose of Compound F. Two clearance studies in normal post-menopausal patients not receiving estrogens are presented in Table II for comparison.

III. *Effect of ethinyl estradiol on the Porter-Silber Color Reaction.* Administration of a single dose of 0.5 mg of ethinyl estradiol by mouth, failed to produce any increase in Porter-Silber chromogens at 2, 4, and 6 hours after ingestion (Table III). In all instances, results compatible with a normal diurnal variation of plasma 17-OHCS were obtained.

Crystalline ethinyl estradiol, 160 μg gave no absorption at 410 mμ when carried through the procedure for estimation of plasma 17-OHCS. This amount exceeds the concentration which could be achieved in the plasma of patients receiving either 0.1 or 0.5 mg of the drug daily.

Discussion. The data indicate that ethinyl estradiol produced elevations in plasma 17-hydroxycorticosteroids in patients with adrenal cortices capable of normal or nearly normal function. Elevated levels were accompanied by an apparent augmentation of responsiveness of the plasma 17-OHCS to a standard infusion of 25 U.S.P. units of ACTH. Both effects were seen in all the post-menopausal females treated with ethinyl estradiol at either of the dose levels used in the study.

Although no rise in plasma 17-hydroxycorticosteroids was obtained in the patient with Addison's disease or in the patient with severe secondary adrenal cortical insufficiency associated with a pituitary tumor, distinct rises were encountered in the 2 other patients with hypopituitarism. Both of these latter patients had only moderately decreased adrenocortical secretory capacity at the onset of the study.

A distinct delay in rate of clearance of exogenous hydrocortisone was found in all 4 patients so tested while receiving ethinyl estradiol. Delayed plasma clearances of infused hydrocortisone have been reported previously only in patients with liver disease(12,13) and with myxedema(13), while normal rates of disappearance of hydrocortisone have been found in both primary and secondary adrenocortical insufficiency(11). Since only one of our patients had any significant degree of hypothyroidism (G.G.), the delayed clearances

TABLE III. Effect of a Single Dose of 0.5 mg Ethinyl Estradiol, p.o., on Plasma 17-Hydroxycorticosteroids.

Patient	Age	Description	Plasma 17-OHCS, μg/100 ml Control	2 hr	4 hr	6 hr
J.M.	91	40 yr post-menopause	33.0	—	27.0	18.4
C.G.	31	Post-partum pituitary necrosis	9.0	3.7	3.0	.6
R.P.	75	25 yr post-menopause	32.9	28.2	19.1	23.8
I.C.	35	4 mo post-total panhysterectomy	20.1	8.9	6.6	8.1

uniformly encountered appear to be related to an alteration in hepatic handling of hydrocortisone in patients treated with ethinyl estradiol.

Hydrocortisone is normally metabolized in large part by a series of steps involving (1) removal from the circulation by the liver, (2) enzymatic reduction to its dihydro and then (3) tetrahydro derivatives, (4) conjugation with glucuronic acid and, finally, (5) reintroduction of the conjugated derivatives into the circulation for excretion by the kidneys. The delay in clearance of infused hydrocortisone from plasma found in this study could reflect alterations in steps 1-4 of this mechanism. The data at hand do not permit more exact localization of the effect of ethinyl estradiol in the liver. Studies are in progress in this laboratory to define more precisely the physiological alteration in hydrocortisone metabolism induced by ethinyl estradiol.

Summary. 1. Ethinyl estradiol in doses of 0.1 and 0.5 mg daily by mouth caused marked elevations in plasma 17-hydroxycorticosteroid levels in 18 post-menopausal and castrate females and in 2 patients whose hypopituitarism was associated with only moderate decreases in adrenocortical secretory capacity. 2. No increases in plasma 17-OHCS levels during ethinyl estradiol therapy were found in a male Addisonian and in a patient with a pituitary tumor and marked secondary adrenocortical insufficiency. 3. Elevations in plasma 17-OHCS were accompanied by augmented responsiveness to a standard ACTH infusion in all 10 of the post-menopausal females studied and in 1 of 2 patients with hypopituitarism. 4. A marked delay in rate of plasma clearance of infused hydrocortisone was present in all 4 of the ethinyl estradiol treated patients so tested. 5. The data suggest that ethinyl estradiol causes an alteration in hydrocortisone metabolism.

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Determination of α-Amino Acid Nitrogen in Urine. (23370)

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The gasometric-ninhydrin method(1,2) is generally conceded to be the most specific technic available for determination of α-amino acid nitrogen in urine, but is rather tedious for routine analyses. The gasometric-nitrous acid(2,3) and the formol titration methods (2,4) are less specific. Folin's photometric technic(5) employing sodium β-naphthoquinone-4-sulfonate reacts with uric acid and ammonia present in urine(6,7). Cagan and coworkers(8) boiled off ammonia and used charcoal to remove uric acid, but did not investigate the specificity of their technic. Pope and Stevens(9) introduced a method wherein copper is bound to the amino group and the bound copper quantitated by iodometric titra-