TABLE II. Antagonism of the Convulsant and Lethal Effects of Electroshock, Pentylenetetrazol, and Strychnine by Oxanamide and Mephenesin in Mice.

	Oxanamide		Mephenesin	
	No. of mice	ED_{50}, mg/kg	No. of mice	ED_{50}, mg/kg
Electroshock				
Tonic seizure	40	125 (113-139)*	40	139 (121-160)*
Pentylenetetrazol				
Clonic seizure	48	145 (126-167)	40	345 (315-378)
Tonic seizure	56	116 (106-127)	56	140 (123-159)
Death	56	102 (90-117)	56	123 (106-143)
Strychnine				
Tonic seizure	40	445 (390-507)	48	>550
Death	56	236 (203-274)	40	320 (274-374)

* 19/20 confidence limits in parentheses.

prominent for the clonic phase of the seizure. Oxanamide was also more potent in antagonizing the convulsant and lethal effects of strychnine. The 2 compounds were equally effective and potent in protecting mice from electroshock-induced seizures.

Conditioned Avoidance Response. In rats, oxanamide had no effect on the conditioned avoidance-escape response (CR) in doses which did not affect motor ability of the animals. In groups of 12 rats/dose level 30, 100, and 300 mg/kg orally had no effect on the CR. After 500 mg/kg the CR was blocked in 4 animals, and the unconditioned response (UR) blocked in one. However, 1,000 mg/kg blocked both the CR and UR in 2 of 12 rats in the group; therefore, this is not considered a specific response.

Conclusion. Although the exact mechanism of action of oxanamide is not known, its spectrum of pharmacological activity indicates that it is similar to mephenesin and related compounds. Central nervous system depression and muscle relaxation without prior hyperexcitability, attenuation of the pinna reflex before the corneal, and antagonism of the convulsant and lethal effects of strychnine are characteristic of mephenesin-like activity. Furthermore, the first 2 effects differentiate the activity of the centrally acting muscle relaxants from that of barbiturates(8,9). Inhibition of polysynaptic neuronal pathways by oxanamide as judged by its effect on the flexor and linguomandibular reflexes also characterizes it as a member of the muscle-relaxant group of drugs.

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Effect of Estrogen on Steroid Levels in Plasma and Urine.*† (25426)

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Alteration of adrenal steroid metabolism by administration of estrogenic substances has received considerable attention. The conflicting results, chiefly in animals, have suggested

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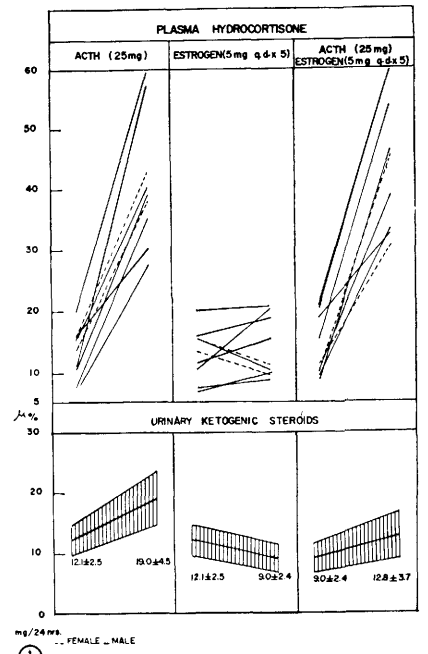
either direct interference with adrenal steroid synthesis, inhibition or stimulation of ACTH (11,12,4). That different parameters of adrenal function, such as adrenal weight, plasma concentration and excretion of Porter-Silber material, 17 ketosteroids, etc., have been studied in various animals and humans and that both synthetic and natural estrogens were used, may have led to these conflicting interpretations. Administration of comparatively large doses of estrogens appears to induce increased levels of plasma corticoids in the human(13,12). However, most authors are hesitant to correlate this with increase in adrenal function. Differences in protein binding of hydrocortisone and in rate of reduction of the molecule have been presented as more attractive explanations(7,13,10). In agreement with this reasoning, it has been shown that total daily production of hydrocortisone by the adrenal is decreased when estrogen is given (9). The present study investigated the effect of approximately physiological levels of naturally occurring estrogenic material on adrenal steroid levels in plasma and urine, and also to determine ACTH responsiveness under such conditions. Through such experiment, more information could be gathered concerning the relation between ovarian and adrenal activity.

Methods. The subjects were 9 apparently healthy individuals, in third decade of life, 6 males and 3 females. Urine was collected during a 48-hour period and stored in deep freezer until processed. Intravenous ACTH (25 mg) was then administered from 9 to 2 p.m. Blood samples were drawn immediately prior to and following ACTH. Another 24-hour urine pool was collected on day of ACTH infusion, starting at 9 a. m. Subjects were then given 5 mg of estrogen by mouth daily (Premarin 2.5 mg B.I.D.) for 5 days. Urine collections were started again on 3rd day of estrogen periods and on 5th day an ACTH test was carried out, as previously described. The entire experiment was therefore divided into 3 parts. Resting level and normal response to ACTH, resting level and response to estrogen, and response to estrogen plus ACTH. The following determinations were carried out on urine: Pregnanetriol (5) ketogenic steroids(8). 17-ketosteroids



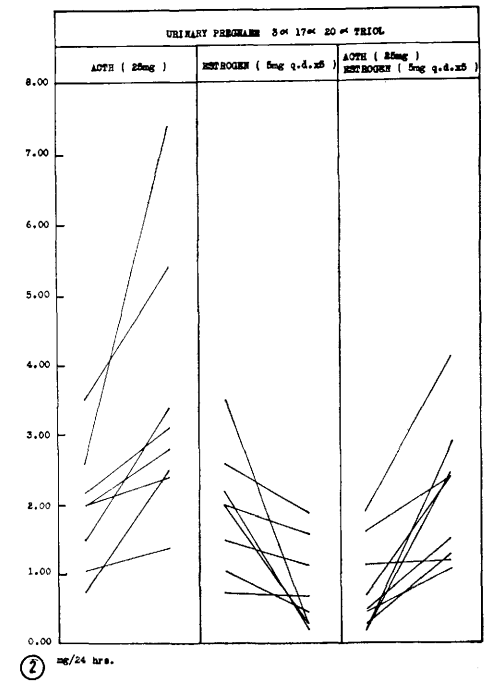


FIG. 1. Response of plasma levels of hydrocortisone (μ/100 ml of plasma) and urinary ketogenic steroids (mg/24 hr ± S.D. of the mean) to ACTH, estrogen and ACTH plus estrogen.

FIG. 2. Excretion of urinary Pregnanetriol (mg /24 hr) before and after ACTH, estrogen and ACTH plus estrogen.

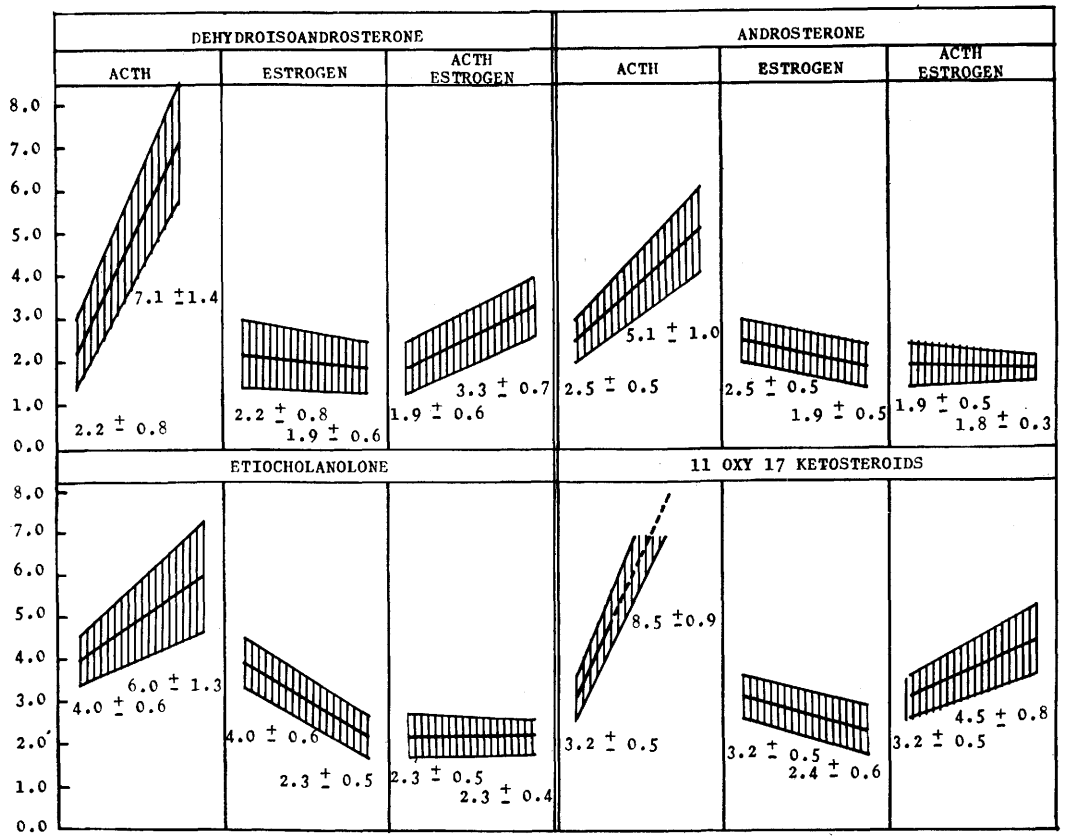


FIG. 3. Excretion of urinary 17 ketosteroids (mg/24 hr ± S.D. of the mean) before and after ACTH, estrogen and ACTH plus estrogen.

were fractionated following β glucuronidase hydrolysis (48 hours at pH 4.5), continuous extraction (at pH 1 and 2 normal for 5 days) and chromatography using alumina column with gradient elution system described by Lakshmanan and Lieberman(6). The binary mixtures appearing in the peaks of the $C_{19}O_2$ compounds were analyzed by the method of Bitman(1). One or 2 peaks in each chromatogram were submitted to infra-red analysis for further identification. Blood samples were tested immediately, by the method of Bondy and Abelson(3) for plasma hydrocortisone.

Results. Hydrocortisone. Plasma levels of hydrocortisone bear some direct relation to adrenal activity. However, under certain physiological and pathological conditions, high values may be found in the absence of proven adrenal hyperactivity. It has also been suggested that patients who have a relative adrenal depression by other standards of adrenal evaluation may still be capable of maintaining a normal plasma level. Results in Fig. 1 must be considered within these limits. There was no significant difference in response to ACTH with or without estrogen. Resting levels did not appear to be different, following estrogen administration.

Urinary Ketogenic Steroids. This method assesses urinary 17 OH-corticoids with the exception of 20-keto-21-methyl steroids. Although it is adequate for clinical purposes, the fact that quantitation is carried out on a crude extract makes it difficult to detect small changes in steroid excretion. This, and the relatively wide spread of normal values, explains the fact that although the observed changes follow the trend of the other urinary steroids, no statistical significance could be attributed to the changes induced in this study. In general, a decreased resting level

was observed following estrogen administration; response to ACTH and estrogen was less than respone to ACTH alone.

Urinary Pregnanetriol. Pregnanetriol is the urinary excretion product of 17-hydroxyprogesterone, precursor of hydrocortisone and perhaps of some C_{19} compounds. Its appearance in urine seems related to adrenal steroid synthesis, as shown by rise of excretion following ACTH and subsequent fall following exogenous cortisone(*2*). In all subjects studied, excretion of the compound diminished following estrogen administration. Adrenal stimulation with ACTH elicited a response of a magnitude comparable to that seen in untreated patients. The final level found after ACTH and estrogen was lower than after ACTH alone (Fig. 2).

The urinary 17-ketosteroids were dehydroisoandrosterone, androsterone, etiocholanolone and 11 oxy-17-ketosteroids. The latter were estimated from the late peaks in the chromatograms, plus calculated amounts of Delta 9 artifacts running with androsterone and etiocholanolone. As in the case of urinary ketogenic steroids and pregnanetriol, estrogen lowered resting levels of the 17 ketosteroids; and values after ACTH and estrogen were lower than those after ACTH alone (Fig. 3).

Discussion. With the present experiment, estrogen did not significantly affect plasma levels of hydrocortisone, nor their response to ACTH infusions, while it significantly lowered excretion of adrenal steroids. These results are in some respects at variance with results reported earlier. It should be noted that doses of estrogen we used were smaller than those used by other authors. Furthermore, the method used for determination of blood corticoids is more specific for plasma hydrocortisone than those used by various workers.

The changes observed in excretion of 17-ketosteroids and pregnanetriol may be explained as results of direct interference with biosynthesis of these compounds or their precursors by the administered estrogen. However, in the presence of normal plasma levels for hydrocortisone, a retarded catabolism resulting in diminished excretion of all adrenal steroids appears as a more attractive explanation. More specifically, this could be caused by increased protein binding in plasma as shown to occur for hydrocortisone. Further studies on plasma levels of 17 ketosteroids under similar conditions are in progress.

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