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EFFECT OF ESTRIOL ADMINISTRATION ON THE HYPOGONADAL WOMAN

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To study the biologic effects of estriol, 14 hypogonadal patients received either vaginal or oral estriol. The vaginal administration of 0.5 mg of estriol to five patients resulted in increases of serum unconjugated and conjugated estriol levels which were maximal at 1 and 2 hours following treatment (161 ± 59 [SE] pg/ml and 762 ± 146 pg/ml, respectively). Significant suppression of luteinizing hormone (LH) to 84% and 81% of base line levels was observed at 2 and 3 hours following treatment, respectively ($P < 0.05$), and significant suppression of follicle-stimulating hormone (FSH) was reached only at 5 hours ($P < 0.05$). The ingestion of 8 mg of estriol orally caused a minimal increase in the serum unconjugated estriol concentration (from ≤ 25 pg/ml to 75.3 ± 17.4 pg/ml) and a dramatic increase of 600-fold in the serum conjugated estriol concentration (from 0.1 ± 0.01 ng/ml to 62.4 ± 13.8 ng/ml). This was associated with no significant decrease in serum FSH levels and with only a 10% to 15% decrease in LH ($P < 0.05$). The continued daily ingestion of 8 mg of estriol for 30 days was associated with a further increase in unconjugated estriol concentrations (to 132 ± 28 pg/ml) and a further decrease of serum FSH and LH to 66% and 83% of pretreatment levels, respectively ($P < 0.05$). A shift to the right in the vaginal maturation index ($P < 0.05$) was noted at the end of the treatment course and suggested an estrogenic effect on the vaginal epithelium.

Estriol is a weak estrogen. Rapid conjugation of the orally administered estriol renders it a weakly potent estrogen. Estriol administered vaginally is conjugated less rapidly than orally administered estriol, thus rendering it more biologically potent. Fertil Steril 30:278, 1978

Suggestions that estrogen replacement therapy may promote endometrial cancer have led to a continuous search for an agent that will relieve climacteric symptoms without adding to the existing risk of cancer.

One of the agents which should be considered is estriol, a steroid with weak estrogenic properties.[1, 2] Estriol is known to have little stimulating effect on the endometrium,[3] and it may possibly have beneficial effects in relieving menopausal symptoms[4] and preventing vaginal atrophy.[5, 6] The protective effect of high estriol excretion against the development of breast cancer has been suggested by some and denied by others.[7, 8] Moreover, while some studies in experimental animals have suggested that estriol administration will reduce the incidence of breast cancer,[9] others have shown that estriol can stimulate the growth of breast cancer.[8, 10]

Some of the controversy concerning the biologic potency of estriol may be related to the route, dose, and duration of its administration.[11, 12] Our study, therefore, was designed to determine the biologic potency of orally and vaginally administered estriol. The rate of estriol absorption and conjugation following a single dose of vaginal es-

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triol was compared with that of oral estriol. The biologic effect of estriol was assessed by monitoring changes in serum gonadotropin concentration and by determining the vaginal maturation index before and following estriol treatment.

MATERIALS AND METHODS

Patients. Fourteen healthy postmenopausal or castrated women ranging in age from 35 to 66 volunteered to participate in the study. All had symptoms associated with vaginal atrophy or vasomotor flushes and none had received estrogens for at least 2 months prior to being studied. Each patient signed an informed consent approved by the research advisory committee of the Boston Hospital for Women.

The purity of the estriol administered (obtained from Dr. R. Kolli, Carnrick Laboratories, Cedar Knolls, N. J.) was confirmed by dissolving the estriol in ethanol and subjecting it to paper chromatography. This revealed a single compound which corresponded to authentic estriol. Moreover, no changes in serum estrone and estradiol concentrations were detected in patients after 1 month of treatment with oral estriol.

The first group of patients consisted of five women who received 0.5 mg of estriol vaginally. This dose was selected to allow comparison of the vaginal absorption of estriol with that of estrone and estradiol previously reported.[13] Three blood samples were obtained from each patient at 20-minute intervals. Thereafter, 0.5 mg of estriol dissolved in 2 ml of saline was applied to the posterior fornix of the vagina. The patients remained recumbent for the 1st hour after treatment, after which they were allowed to move about. Blood samples were obtained at 30 and 60 minutes after the vaginal estriol was applied, and again 60 minutes thereafter for 4 more hours.

The second group consisted of nine patients who received 8 mg of oral estriol daily for 30 days. On the first morning of the study, a smear of the lateral vaginal wall was obtained for assessing the maturation index,[14] and 10 ml of peripheral blood were drawn three times at 20-minute intervals. Each patient in a subgroup of five patients then took her first tablet of 8 mg of estriol. Ten milliliters of blood were obtained 30 and 60 minutes after the oral administration of estriol and again at 60-minute intervals for 4 more hours. All of the patients returned for their second visit after having taken 8 mg of estriol orally daily for 30 days. The last oral estriol tablet was ingested 12 to 18 hours prior to the visit. A vaginal smear was obtained for assessing the maturation index, after which another three blood samples were drawn at 20-minute intervals. All serum samples were frozen and stored at −20° C.

Hormone Determinations. Levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined by radioimmunoassay, using the double-antibody technique.[15] Unconjugated estriol was assayed in serum by a modification of the radioimmunoassay technique previously described.[16] Briefly stated, 1 ml of serum was extracted with ether, which then was dried and reconstituted in 1 ml of water. The water suspension was washed twice with 5 ml of *N*-benzene-hexane (1/1, v/v) to remove other estrogens (e.g., estrone, estradiol). The estriol remaining in the aqueous phase was then extracted with 10 volumes of ether, dried, and assayed, using specific antibodies kindly supplied by Dr. David Watson[16] of the Worcester Foundation. Worcester, Mass. An internal standard was used to monitor all recovery losses. The estriol concentrations in serum from men were always undetectable (<25 pg/ml). The coefficient of variation of estriol determined repeatedly in pooled serum from pregnant women was <12% and the recovery was 84% ± 10 (SD).

After ether extraction, which removed the unconjugated steroids, the serum samples were subjected to overnight enzymatic hydrolysis.[17] ^{3}H-Estriol-16α(β-D-glucuronide) (Amersham Searle Corporation, Arlington Heights, Ill.), specific activity, 40 Ci/mmole. (1000 cpm) was used as the internal standard. The enzyme β-glucuronidase, which is isolated from *Helix pomatia* (Sigma Chemical Co., St. Louis, Mo.) and is capable of hydrolyzing estrogen glucuronide as well as estrogen sulfates, was used. Preliminary experiments using tracer materials had shown that overnight incubation of 5000 units of β-glucuronidase with 1 ml of serum would completely hydrolyze tritiated estriol glucuronide as well as tritiated estrone sulfate. The liberated estriol following hydrolysis was then extracted and assayed by radioimmunoassay using unconjugated estriol as a standard. No attempt was made to correct the results for the differences in molecular weights, and the hydrolyzable (conjugated) estriol was expressed as unconjugated estriol. It can be presumed that a variety of estriol conjugates with different molecular weights were formed following estriol administration. Nevertheless, it can be roughly estimated that the mean molecular weight of the various estriol conjugates in serum would exceed

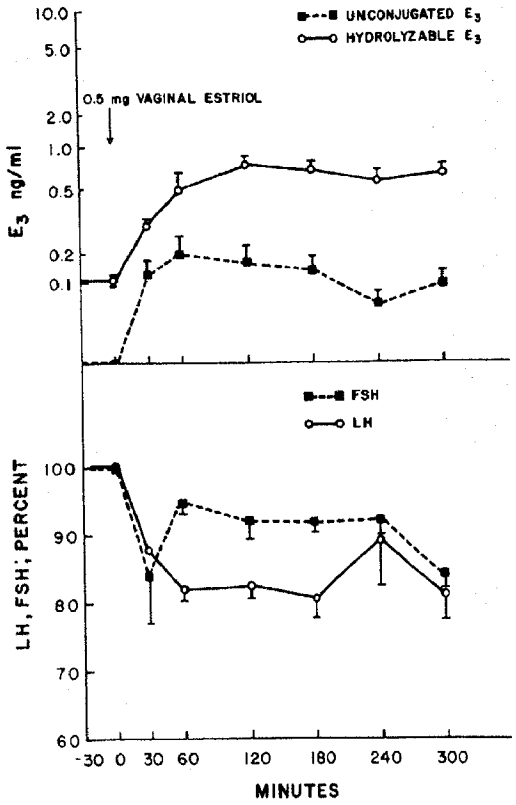

FIG. 1. The concentrations of LH, FSH, and unconjugated and hydrolyzable (conjugated) E_3 following a single 0.5-mg dose of vaginal estriol. Serum LH and FSH concentrations are expressed as percentages of pretreatment levels. The concentrations of the hydrolyzable (conjugated) E_3 are not corrected for their higher molecular weight and are expressed as unconjugated estriol equivalent.

that of unconjugated estriol by approximately 50%. Statistical analysis was carried out by means of the paired Student's *t*-test.

RESULTS

The serum concentrations of LH, FSH, and unconjugated and conjugated estriol following the vaginal application of 0.5 mg of estriol (E_3) are presented in Figure 1. The pretreatment levels of unconjugated estriol of all patients were undetectable (<25 pg/ml). However, at 30 minutes after the treatment they were detectable in four of the five patients. The peak mean serum concentration of unconjugated E_3 (161 ± 59 [SE] pg/ml) was reached at 1 hour following treatment, after which the serum E_3 concentration declined, reaching levels of 96 ± 21 pg/ml at 5 hours.

The serum concentration of the total hydrolyzable (conjugated) E_3 prior to treatment was 105 ± 4 pg/ml. It was significantly higher at 30 minutes following treatment ($P < 0.05$), and the highest level (762 ± 146 pg/ml) was reached at 2 hours. In the remaining 3 hours of the study the concentrations of the conjugated E_3 remained relatively unchanged.

The mean serum concentrations of LH at 2 and 3 hours following the treatment, respectively, were 84% and 81% of base line and significantly lower than the pretreatment level of 67 ± 12 mIU/ml ($P < 0.05$) (Fig. 1). However, serum LH concentrations at 4 and 5 hours of the study were not significantly lower than pretreatment concentrations, and a significant decrease of serum FSH was noted only at 5 hours of the study (14% decrease) ($P < 0.05$).

The effect of a single oral administration of 8 mg of E_3 on the serum concentration of LH, FSH, and unconjugated and conjugated E_3 is shown in Figure 2. Serum unconjugated E_3 levels, which were undetectable before treatment, became de-

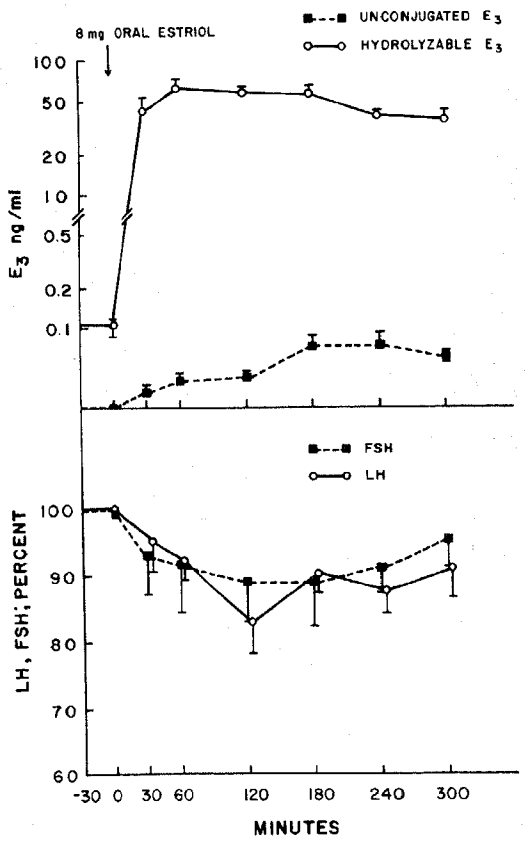

FIG. 2. The concentrations of LH, FSH, and unconjugated and hydrolyzable (conjugated) E_3 following an 8.0-mg dose of oral estriol. Serum LH and FSH concentrations are expressed as percentages of pretreatment levels. The concentrations of serum hydrolyzable (conjugated) E_3 are not corrected for their higher molecular weight and are expressed as unconjugated estriol equivalent.

tectable as early as 30 minutes or as late as 4 hours following the initiation of the treatment. The maximal mean concentration of conjugated E_3, seen at 60 minutes, was 62.4 ± 13.8 ng/ml and was 600-fold higher than pretreatment levels of 0.1 ± 0.015 ng/ml.

The mean serum LH concentration at 60 minutes after treatment was 91% of base line and was significantly ($P < 0.05$) lower than the pretreatment level (53 ± 2 mIU/ml). The decreases at 120 and 180 minutes were 84% and 89% of base line, respectively, and again were statistically significant ($P < 0.05$). However, the lowest FSH levels observed at 120 minutes (53 ± 11 mIU/ml) were not significantly different from pretreatment concentrations (63 ± 2 mIU/ml) ($P > 0.05$).

The serum concentrations of unconjugated and conjugated E_3, LH, and FSH of patients studied after 1 month of daily E_3 administration are shown in Figure 3. Serum concentrations at 12 to 18 hours following the last estriol dose were compared with pretreatment levels.

The mean serum unconjugated E_3 concentration after 1 month of treatment (132 ± 28 pg/ml) was higher than that observed after a single 8-mg dose ($P < 0.05$). However, it was similar to those achieved 3 hours after a single vaginal application of $^1/_{16}$ of the oral dose. After 1 month of treatment and 12 to 18 hours after the last oral dose, the serum conjugated E_3 concentration (58.4 ± 12.5 ng/ml) was similar to that observed 5 hours following a single dose of oral E_3. The mean serum LH concentration of 45 ± 5 (SE) mIU/ml (83% of base line) was significantly lower than the pretreatment value ($P < 0.05$), and a significant decrease of 34% in FSH levels was also observed ($P < 0.05$).

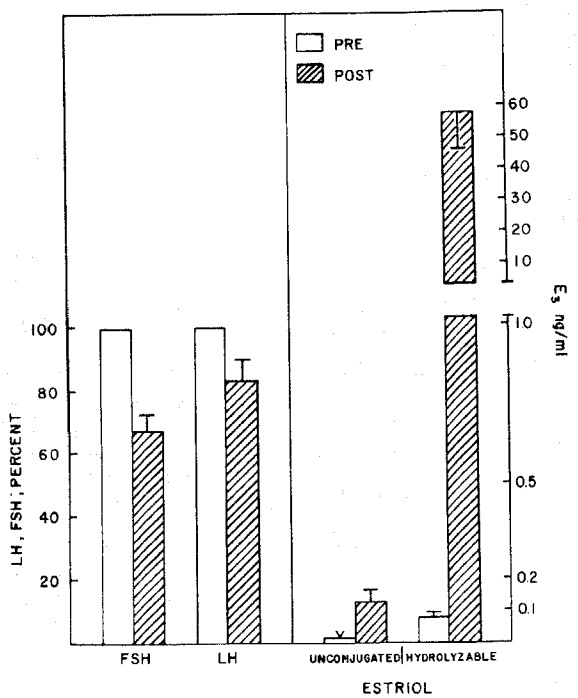

FIG. 3. Changes in FSH, LH, and unconjugated and hydrolyzable (conjugated) E_3 concentrations after 30 days of estriol, 8 mg/day. Post-treatment LH and FSH concentrations are expressed as percentages of pretreatment levels. The serum hydrolyzable (conjugated) E_3 concentrations are expressed as unconjugated E_3 equivalent without correcting for their higher molecular weight.

The effect of estriol on the vaginal maturation index was studied by analyzing the percentage of superficial cells in each cytologic smear. All patients showed a shift to the right in their maturation index, indicating an estrogenic effect. The percentage of superficial cells before treatment (21% ± 12%) rose significantly to 41% ± 15% at the end of 1 month of treatment ($P < 0.05$). Although following treatment many patients reported a reduced number of hot flushes, no attempt was made to quantitate this reduction.

DISCUSSION

This study has demonstrated the estrogenic potency of estriol in the hypogonadal patient. The administration of estriol, either vaginally or orally, resulted in a significant decrease of serum FSH and LH levels. Moreover, an improvement in the estrogenic maturation index of vaginal cells was observed after 30 days of daily estriol treatment. The latter observation is in agreement with previous reports.[5, 6] It also is compatible with the principle of previous studies which suggested that the continuous administration of estriol would have a potent estrogenic effect similar to that of estrone or estradiol.[12, 18]

Several deductions can be made on the estrogenic potency of estriol. First, in our study a single vaginal dose of 0.5 mg of estriol was less effective in suppressing serum LH and FSH levels than an equivalent dose of estrone or estradiol. A single oral dose of 8 mg had even less effect on LH and FSH. This observation may be explained by the rapid dissociation of estriol from its nuclear receptors.[12] Alternatively, the rapid conjugation of estriol observed in this study and the relatively low unconjugated estriol levels achieved may have rendered the administered E_3 relatively impotent.

In our study, the rate of E_3 conjugation following its vaginal administration was much less pronounced than that observed following its oral ad-

ministration. Thus the ratio of conjugated E_3 to unconjugated E_3 (approximately 10) observed following the vaginal administration of E_3 was similar to that observed during pregnancy.[19] In contrast, a ratio of 500 between the two was observed following the oral administration of E_3, resulting in conjugated estriol concentrations in serum which approximated those observed at term pregnancy and an unconjugated estriol concentration in serum which was less than 1% that of term pregnancy. It appears likely that the passage of estriol through the gut has resulted in rapid conjugation of estriol and that the subsequent passage of estriol through the liver has assured conjugation of more than 99.5% of all circulating estriol.

The daily oral administration of 8 mg of estriol for 30 days appears to be more effective than a single oral dose in suppressing gonadotropins. This may be explained in part by the cumulative effect that the repeated administration of estriol had on unconjugated estriol concentrations. It also concurs with previous reports suggesting that the estrogenic potency of estriol would be maximal if it were given for prolonged periods of time rather than as a single dose.[12, 20, 21] The degree of LH and FSH suppression, which ranged between 20% and 35%, and the improvement in the vaginal maturation index observed after 1 month of estriol treatment compared well with that observed after 1 month of Premarin administration (0.625 mg daily).[22] It remains to be seen whether this dose of estriol would cause any degree of endometrial proliferation and whether its effect on relieving vasomotor flushes would be greater than that attributed to placebo.

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