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Nonoral Routes of Estrogen Administration

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There are about 40 million women in the United States who no longer have ovarian function and for whom hormone replacement may be appropriate. Recent estimates by the FDA indicate that only about 4 million women are currently using replacement therapy. Thus, approximately 90 per cent of American women who might benefit from replacement therapy have chosen not to use it. There are several explanations for this, including ignorance about its benefit, contraindications to its use, fear of its risk, development of side effects, and lack of menopausal symptoms. Thus, the challenge in this field is to develop methods that retain the benefits of hormone replacement while reducing or eliminating its side effects and risks.

ORAL ESTROGEN: RISK VERSUS BENEFIT

Estrogens, particularly those administered by mouth, have effects on the gastrointestinal system. These include the symptoms of nausea, vomiting, and abdominal bloating. Estrogens also affect hepatic proteins and lipid metabolism. They enhance the production of carrier proteins, such as sex hormone–binding globulin (SHBG), cortisol-binding globulin (CBG), thyroxine-binding globulin (TBG), transferrin, and ceruloplasmin. These changes do not represent a medical hazard but do alter the results of the clinical laboratory tests used to determine serum levels of the substances bound to these carrier proteins.

Estrogens do influence the hepatic synthesis of other proteins that have been incriminated in causing or contributing to the occurrence of certain disease processes. For example, hypertension may occur or be exacerbated in women receiving estrogen replacement therapy.[8] The elevation of blood pressure is usually reversible when

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the therapy is discontinued. The problem is seen less frequently with estrogen replacement than with the use of oral contraceptives. Although increases of blood pressure have been reported, estrogen replacement has not been associated with an enhanced risk of cerebral vascular accidents.[34]

The mechanism responsible for this increase in blood pressure is believed to be related to the renin-angiotensin-aldosterone system.[22] Under physiologic conditions, renin substrate (angiotensinogen) is the rate-limiting step of the renin reaction. Estrogen administration stimulates the hepatic synthesis of this protein. Associated with this are increases of angiotensin I formation and aldosterone secretion.

Although all women who take a sufficient dosage of estrogen have increases in renin substrate levels, only a small percentage develop hypertension. There is also no difference in the absolute circulating levels of renin substrate in women with estrogen-induced hypertension and normotensive women receiving equal doses of the hormone.[12] Thus, the role of renin substrate in the genesis of estrogen-induced hypertension has been questioned. Recent work has shown that estrogen replacement therapy induces the synthesis and release of several forms of renin substrate, which are electrophoretically and immunologically distinct from the predominant form of this plasma protein.[11] A large molecular weight form has a greater affinity for the enzyme renin than the predominant form. Circulating levels of this large form increase in women with estrogen-induced hypertension but not in normotensive subjects on the same dosage of medication. The induction of this large form is particularly profound following the administration of ethinyl estradiol.[39] These data suggest that induction of the synthesis of particular forms of renin substrate by estrogen may play an important role in the development of hypertension in some women on this form of therapy.

The administration of oral contraceptives increases the risk of overt venous thromboembolic disease and the occurrence of subclinical thrombosis extensive enough to be detected by laboratory procedures, such as ^{125}I fibrinogen uptake[37] and plasma fibrinogen chromatography.[1] In uncontrolled studies, thrombophlebitis has been reported with estrogen replacement therapy, whereas this association has not been present in controlled experiments.[5]

Both procoagulant and anticoagulant factors are present in blood to maintain its fluidity while permitting hemostasis with vascular injury. Estrogen exerts several effects on the clotting mechanism, which may contribute to or be responsible for a generalized hypercoaguable state. Some of these effects are exerted through the action of estrogen on hepatic function. For example, the levels of clotting factors VII, IX, X, and X complex are increased with estrogen administration.[4,44] These factors are hepatic in origin. Estrogen replacement therapy can also lower levels of anticoagulant factors such as antithrombin III and anti Xa.[4] The former is of particular interest. It is hepatic in origin and inactivates thrombin, activated factor X, and other enzymes involved with the generation of thrombin. The

potential importance of a reduction of antithrombin III during estrogen replacement therapy is suggested by the occurrence of thrombophilia (intravascular clotting) in subjects with a congenital deficiency of this factor.[10] A reduction of 20 per cent or more has been found to be highly predictive of the occurrence of subclinical venous thromboembolic disease that is extensive enough to be detected by ^{125}I-fibrinogen uptake.[40]

Estrogen replacement therapy also influences hepatic lipid metabolism. An increased incidence of gallbladder disease has been reported with oral contraceptive usage and estrogen replacement therapy.[5] Because bile saturation of cholesterol is between 75 and 90 per cent, small increases in bile can initiate precipitation leading to stone formation. Increased amounts of cholesterol in bile are a common finding in gallbladder disease. Estrogen replacement increases the cholesterol fraction of bile. Proposed mechanisms for this include increased turnover of body cholesterol and increased hepatic cholesterol synthesis. Cholesterol synthesis is regulated by the enzyme hydroxy-methyl-glutanyl-CoA-reductase, which is inhibited by chenodeoxycholate. A decrease of chenodeoxycholate and an increase of cholate are present in bile of women on estrogen replacement. These changes in bile acids provide a plausible explanation for the presence of increased cholesterol in this fluid.

Circulating lipids are also influenced by estrogen replacement. Serum lipids are mostly bound to proteins, and the concentrations of the various types of lipoproteins correlate with the risk of heart disease. Low-density and very low-density lipoprotein levels correlate positively with risk of heart disease; high-density lipoprotein concentrations correlate negatively. Large dose estrogen administration replacement is associated with decreases of low-density lipoprotein (LDL) levels, reductions or no changes of cholesterol and very low-density lipoprotein (VLDL) levels, and increases of high-density lipoprotein (HDL) and triglyceride levels. Recently, the heterogeneous nature of HDL has been recognized. The HDL fraction consists of several subfractions. The major ones, defined according to density, are the HDL_2 and HDL_3 subfractions.[6] It has been suggested that the HDL_2 subfraction may be a more effective epidemiologic discriminant of heart disease risk than total HDL. Estrogen has been shown to elevate the HDL_2 subfraction.[15]

In patients with familial defects of lipoprotein metabolism, estrogen replacement therapy has rarely been associated with massive elevations of plasma triglyceride levels, which have led to pancreatitis and other complications.[17] The effects of estrogen on circulating lipids are believed to be related to changes in hepatic synthesis, although altered clearance of these substances may be involved.

The response of the liver to estrogens seems to be greater than other sites of action. This is particularly true of orally administered hormone. Dose response studies using conjugated estrogen given orally showed subphysiologic or physiologic responses of the vaginal epithelium and urinary calcium excretion (an indirect marker of bone

resorption) at estrogen doses that clearly exhibited supraphysiologic responses of hepatic markers of estrogen action.[18] The 0.15-, 0.3-, and 0.625-mg dosages of conjugated estrogen had no measurable effect on the percentage of superficial cells seen with vaginal cytologic analysis (Fig. 1). Only the 1.25-mg dosage significantly increased this marker of estrogen action to a value intermediate between percentages seen in women during the early and late follicular phases of their menstrual cycles. The 0.3-, 0.625-, and 1.25-mg dosages significantly suppressed the fasting urinary calcium:creatinine ratios (Ca:Cr), but only to values comparable to those observed in premenopausal women (Fig. 2). Other investigators have now clearly established that the 0.625-mg dosage prevents bone loss and reduces the occurrence of fractures. These dosages increased the circulating concentrations of renin substrate, SHBG, CBG and TBG to levels in excess of those observed in premenopausal women (Fig. 3). Thus, orally administered doses of conjugated estrogen exerted physiologic actions on nonhepatic but pharmacologic actions on hepatic functions.

Similar responses have been observed with orally administered ethinyl estradiol.[27] A dose response study of 5, 10, 20, and 50 μg of this estrogen showed that all dosages elevated the percentage of superficial cells in the vagina, but even the 50-μg dosage did not raise the percentage higher than that observed in premenopausal women (Fig. 4). Significant suppression of urinary calcium excretion was observed with the 10-, 20-, and 50-μg dosages; however, none of these dosages resulted in urinary calcium values significantly lower than

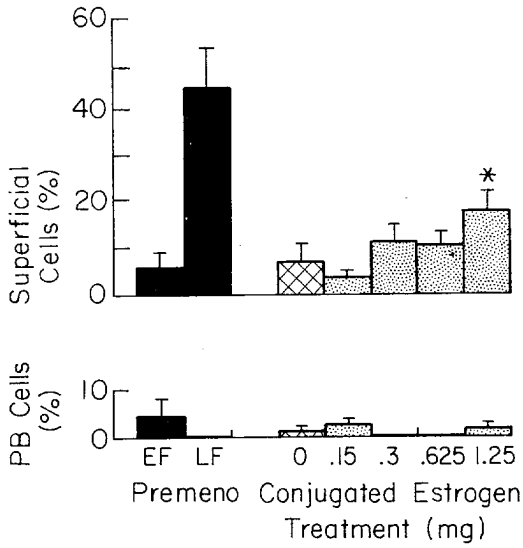

Figure 1. Mean (± S.E.) percentages of vaginal parabasal and superficial cells in postmenopausal women given various dosages of conjugated equine estrogens. The results found in 15 premenopausal women during the early follicular (EF) and late follicular (LF) phases of their menstrual cycles and in 15 additional postmenopausal women studied 6 weeks apart and given no therapy are shown for reference. In all figures, ● = values that are significantly different from premenopausal results, and * = values that are significantly different from baseline results in postmenopausal women. (*From* Geola FL, Fumar AM, Tataryn IV, et al: Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 51:620, 1980; with permission.)

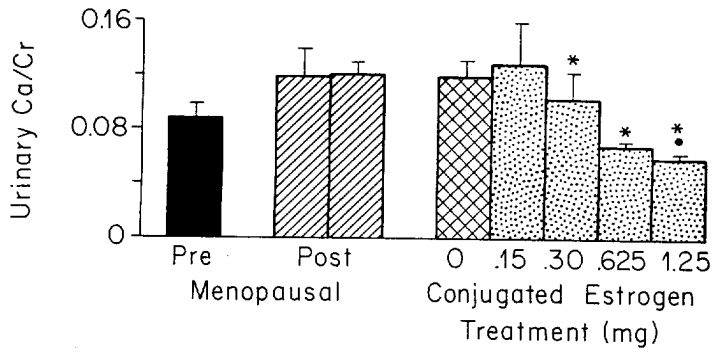

Figure 2. Mean (±S.E.) urinary calcium:creatinine (Ca:Cr) in the same groups of patients. (*From* Geola FL, Fumar AM, Tataryn IV, et al: Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 51:620, 1980; with permission.)

those found in premenopausal women (Fig. 5). In the same study, 5 μg of ethinyl estradiol doubled renin substrate levels and raised the levels of all the carrier proteins (Fig. 6). All other dosages of ethinyl estradiol had even greater actions on these hepatic proteins.

NONORAL ESTROGENS: VAGINAL

The enhanced hepatic effects of estrogen administered orally have been attributed to a so-called "first pass" mechanism. According

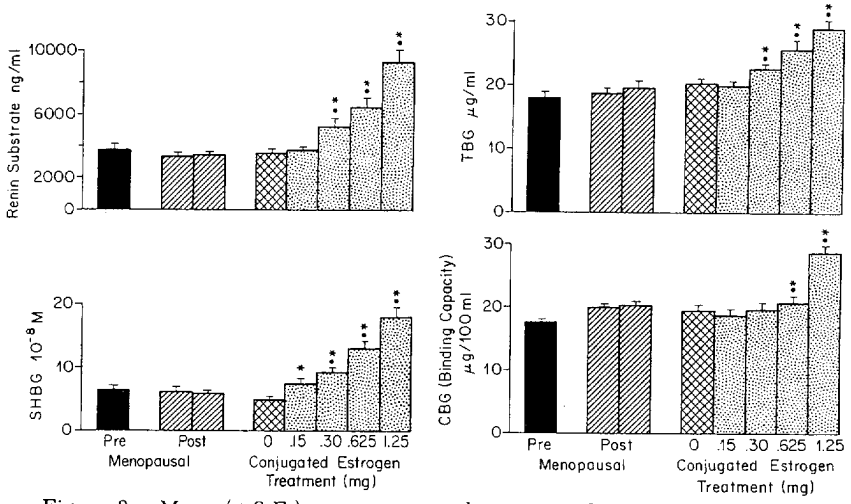

Figure 3. Mean (±S.E.) serum renin substrate, sex hormone–binding globulin (SHBG), and thyroxine-binding globulin (TBG) levels and binding capacity of cortisol-binding globulin (CBG) in the same groups. (*From* Geola FL, Fumar AM, Tataryn IV, et al: Biological effects of various doses of conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 51:620, 1980; with permission.)

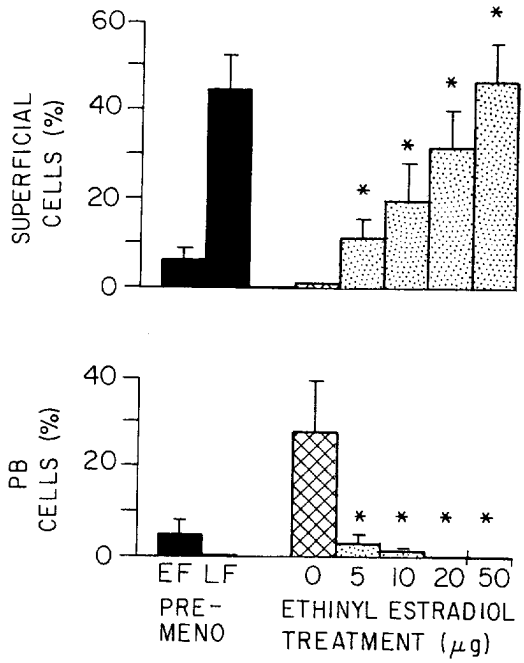

Figure 4. Mean (±S.E.) percentages of vaginal superficial and parabasal cells in postmenopausal women given various doses of ethinyl estradiol for 4 weeks each. Results in premenopausal women are shown for reference. See Fig. 1 legend for symbols. (*From* Mandel FP, Geola FL, Lu JKH, et al: Biologic effects of various doses of ethinyl estradiol in postmenopausal women. Obstet Gynecol 59:673, 1982; with permission.)

to this, estrogens given by mouth are absorbed by the intestines and delivered to the liver before entry into the general circulation. The medication exerts actions on the liver, and then much of it is metabolized to inactive conjugates, with only a fraction of the active hormone entering the general circulation. This might not occur if the medication is given by nonoral routes.

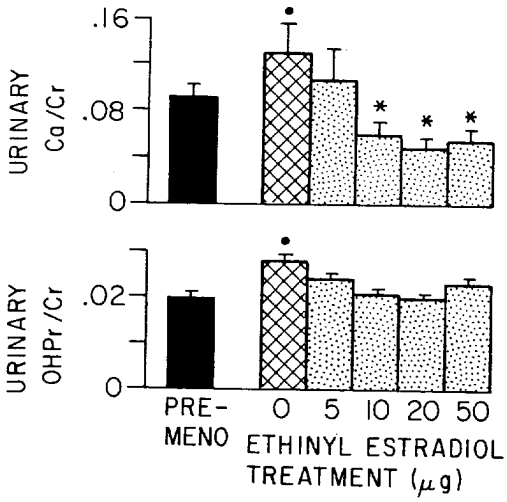

Figure 5. Mean (±S.E.) urinary calcium:creatinine (Ca:Cr) and hydroxyproline:creatinine (OHPr:Cr) in same groups of patients as in Fig. 4. (*From* Mandel FP, Geola FL, Lu JKH, et al: Biologic effects of various doses of ethinyl estradiol in postmenopausal women. Obstet Gynecol 59:673, 1982; with permission.)

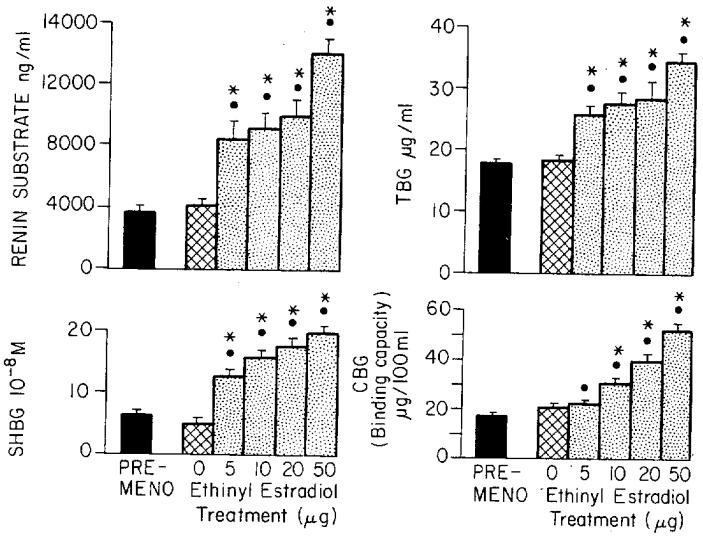

Figure 6. Mean (±S.E.) serum levels of renin substrate, sex hormone–binding globulin (SHBG), and thyroxine-binding globulin (TBG) and serum binding capacity of cortisol-binding globulin (CBG) in same groups. (*From* Mandel FP, Geola FL, Lu JKH, et al: Biologic effects of various doses of ethinyl estradiol in postmenopausal women. Obstet Gynecol 59:673, 1982; with permission.)

To test this hypothesis, Mandel and associates[28] studied 20 postmenopausal patients by giving them conjugated estrogen vaginally. The dosages tested were 0.3, 0.625, 1.25, and 2.5 mg, which corresponded to 0.5, 1, 2, and 4 g of cream. The effects of this preparation on vaginal cytology are shown in Fig. 7. The 0.3-mg dosage of vaginally applied conjugated estrogens significantly lowered the percentage of parabasal cells and increased the percentage of superficial cells to values that fell between those seen in premenopausal controls in the early and late follicular phases. Further increases in the percentage of superficial cells were seen with increasing dosages. These data indicated that the 0.3-mg dosage is sufficient to provide physiologic replacement to the vaginal epithelium of the average woman if applied daily. This dosage is one quarter to one eighth the amount currently recommended in the package insert and cannot be administered easily with the currently available applicator.

Figure 8 depicts the effects of vaginally applied conjugated estrogens on the urinary Ca:Cr and hydroxyproline:creatinine ratios (OHPr:Cr). The lowest dosage of vaginally applied conjugated estrogens that significantly reduced the Ca:Cr ratio was 1.25 mg. The effect of 2.5 mg of vaginally applied conjugated estrogens on this ratio fell between those effects exerted by 0.3 and 0.625 mg of orally administered hormone. This suggested that long-term use of 2.5 mg of vaginally applied conjugated estrogens probably would not be protective against osteoporosis.

The effects of vaginally applied conjugated estrogens on hepatic

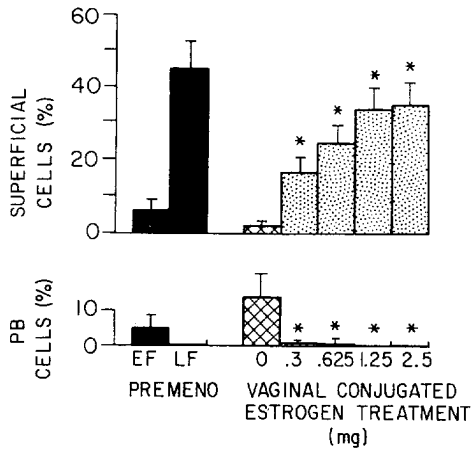

Figure 7. Mean (±S.E.) percentages of vaginal superficial and parabasal cells in premenopausal controls in the early follicular (EF) and late (LF) follicular phases of their cycles compared with values in postmenopausal women before and after vaginal administration of various doses of conjugated estrogens. See Fig. 1 legend for symbols. (*From* Mandel FP, Geola FL, Lu JKH, et al: Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 57:133, 1983; with permission.)

protein synthesis are demonstrated in Figure 9. There were no significant differences in the baseline values observed in the older and younger women. In the postmenopausal subjects, renin substrate levels were significantly increased from baseline by the 2.5-mg dose. SHBG was significantly increased by the 1.25-mg dosage, and the 2.5-mg dose increased the level of TBG. These results indicated that except for the vaginal epithelium, vaginally applied conjugated estrogens continued to elicit pharmacologic actions on the liver at doses that exerted little or no effect on the nonhepatic sites of action.

Goebelsmann and colleagues[19] compared the hepatic impact of oral versus vaginal administration of ethinyl estradiol. Nine healthy postmenopausal women were randomly assigned to receive either a large or a small dose of oral and vaginal ethinyl estradiol, each given over a course of 25 consecutive days. There was a 6-week estrogen-

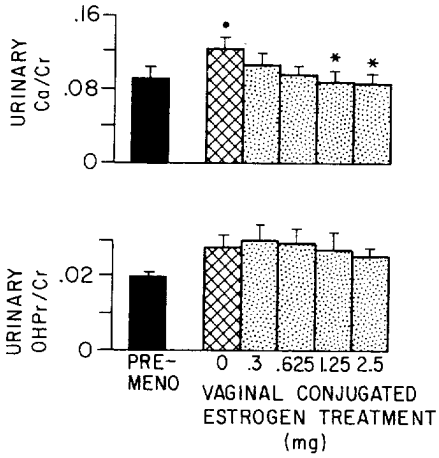

Figure 8. Mean (±S.E.) for the urinary calcium:creatinine (Ca:Cr) and hydroxyproline:creatinine (OHPr:Cr) ratios of the two groups. (*From* Mandel FP, Geola FL, Meldrum DK, et al: Biologic effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 57:133, 1983; with permission.)

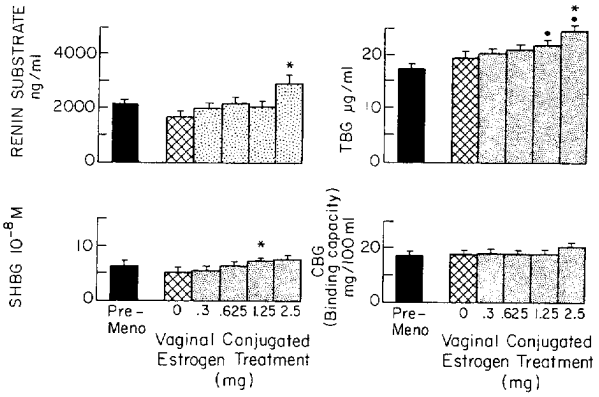

Figure 9. Mean (±S.E.) levels of renin substrate, sex hormone–binding globulin (SHBG), and thyroxine-binding globulin (TBG) and the serum binding capacity of cortisol-binding globulin (CBG) in the two groups studied. (*From* Mandel FP, Geola FL, Meldrum DR, et al: Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab 57:133, 1983; with permission.)

free interval between each subject's course of oral and vaginal ethinyl estradiol, the sequence of which was randomized as well.

The gonadotropin suppressive effects of 5 µg of oral versus 20 µg of vaginal ethinyl estradiol and of 10 µg of oral versus 50 µg of vaginal ethinyl estradiol upon six and three postmenopausal women, respectively, are depicted in Figure 10. The effects were comparable between these orally and vaginally administered dosages, indicating that the potency of orally administered ethinyl estradiol is approximately four to five times greater than vaginally administered hormone on this nonhepatic marker of estrogen action.

Figure 11 shows the effects of these dosages on SHBG levels. This hepatic marker increased significantly in response to both oral dosages as well as to both vaginal dosages. These data indicated that vaginal administration of ethinyl estradiol did not selectively reduce its hepatic effects. The results of this and the previously reported study of vaginally applied conjugated estrogens suggest that nonoral application of these two estrogen preparations does not eliminate their enhanced hepatic actions, and thus the first pass mechanism is not a complete explanation for the greater action of these preparations on the liver.

TRANSDERMAL ESTROGENS

Investigators have shown that the nonoral administration of estradiol appears to reduce if not eliminate the enhanced hepatic actions of the medication while still exerting the desired nonhepatic effects. However, these studies did not use a dose-response study

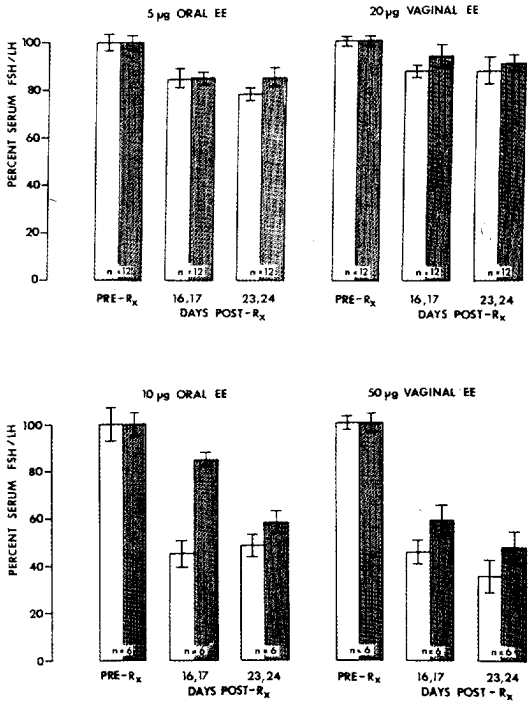

Figure 10. Mean (±S.E.) serum follicle-stimulating hormone (FSH), *open columns*, and luteinizing hormone (LH), *hatched columns*, concentrations in percentage of pretreatment (PRE-R_x) levels prior to and 16 and 17 as well as 23 and 24 days following daily oral or vaginal administration (POST-R_x) of 5 and 20 or 10 and 50 μg of ethinyl estradiol (EE), respectively, in postmenopausal women. (*From* Goebelsmann U, Masbchak CA, Mishell DR: Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 151:868, 1985; with permission.)

design, which made comparison between formulations difficult. To examine this question more closely, Steingold and associates[41] studied the effects of transdermal estradiol administration in a new therapeutic system. Each system consisted of a drug reservoir, an occlusive backing that prevented the outward diffusion of drug, a control membrane that allowed diffusion of medication at a constant, controlled rate into the skin, and an adhesive for attachment to the skin.

This system was chosen for study because of several unique features. First, it delivers the primary ovarian estrogen, estradiol, into the systemic circulation. Second, this delivery is constant and controlled, resulting in steady serum estradiol levels and steady rates of excretion of estradiol conjugates, both throughout the wearing of a single system and during a 3-week period of repetitive, consecutive wearings of these systems. Third, it delivers sufficient estradiol into the circulation to raise estradiol levels to concentrations similar to those in women in the early to mid-follicular phases of their men-

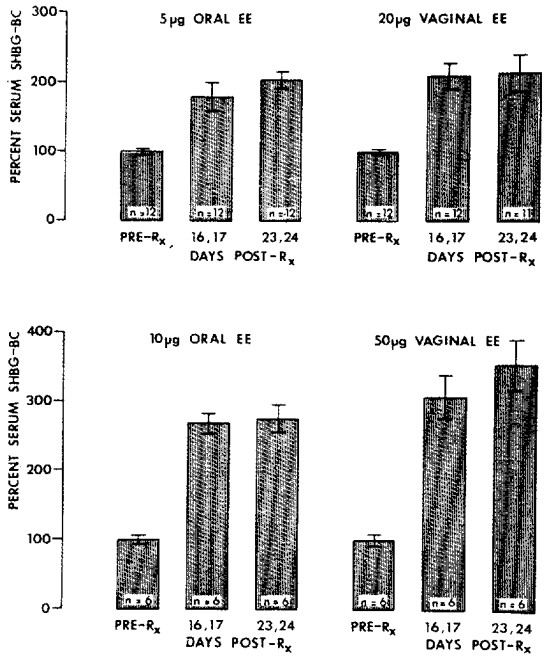

Figure 11. Mean (± S.E.) serum binding capacity of sex hormone–binding globulin (SHBG-BC) in percentage of pretreatment (PRE-R_x) levels prior to and 16 and 17 as well as 23 and 24 days following daily oral or vaginal administration (POST-R_x) of the same dosage of ethinyl estradiol (EE) in postmenopausal women. (*From* Goebelsmann U, Masbchak CA, Mishell DR: Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 151:868, 1985; with permission.)

strual cycles.[24] Fourth, the system can be applied or removed with ease. Thus, its use allowed systematic assessment of the effects of hormone administration on the relief of hot flashes in terms of circulating estradiol levels.

To prove the efficacy of these systems, 50 postmenopausal women with frequent hot flashes (more than 10 per day) were studied in a double-blind, prospective fashion.[41] Each patient was assigned prospectively to receive one of five dosages of medication, including placebo, on a blinded basis. The systems were applied to the anterior abdominal wall and were changed twice weekly. The patients returned for re-study on the 20th day with the last system in place. The systems delivered 25, 50, 100, and 200 μg per 24 hours of estradiol.

Figure 12 shows the mean levels of serum estradiol before and during cutaneous application of the systems in the five groups of patients in comparison to the mean value in premenopausal women in the early follicular phase of their cycles. Baseline levels were similar between the postmenopausal groups. With the exception of placebo, all estradiol dosages significantly raised the serum level in a dose-dependent manner to means of 18, 38, 72, and 100 pg per ml for the 25,

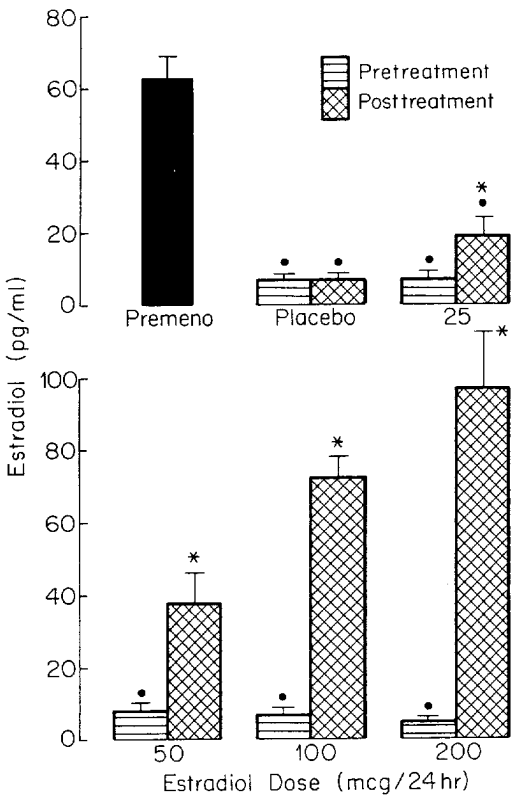

Figure 12. Mean (±S.E.) serum estradiol levels before and during transdermal administration of this estrogen in five study groups. The mean value in premenopausal women during the early follicular phase of their cycle is shown for comparison. See Fig. 1 legend for symbols. (*From* Steingold KA, Laufer L, Chetkowski RJ, et al: Treatment of hot flashes with transdermal estradiol administration. J Clin Endocrinol Metab 61:627, 1985; with permission.)

50, 100, and 200 μg per 24 hour dosages, respectively. At the dosage of 50 μg per 24 hr or greater, the mean values were not significantly different from the levels in the premenopausal women.

Figure 13 shows the effects of the systems on objectively measured hot flashes. Baseline rates of hot flashes varied between 0.64 and 0.83 hot flashes per hour in the five groups of symptomatic women. Administration of placebo did not influence the occurrence of these symptoms. The lack of effect with placebo also indicated that this method of assessing flashes is reproducible, an observation we have made earlier.[9,23] Application of systems containing estradiol resulted in a linear reduction of flashes with increasing dosages of estradiol. The 50 μg per 24 hours or higher dosages significantly reduced the number of flashes. At the 200 μg per 24-hour dosage, a 91 per cent reduction occurred.

Figure 14 shows the linear regression of hot flashes per hour versus treatment estradiol levels. A significant negative correlation was found. Based on this regression, a 100 per cent reduction in flashes should occur at the X-intercept or 122 pg per ml, whereas a 50 per cent reduction should occur at an estradiol level of 61 pg per ml. There was a range of responses of flashes to estradiol levels, with

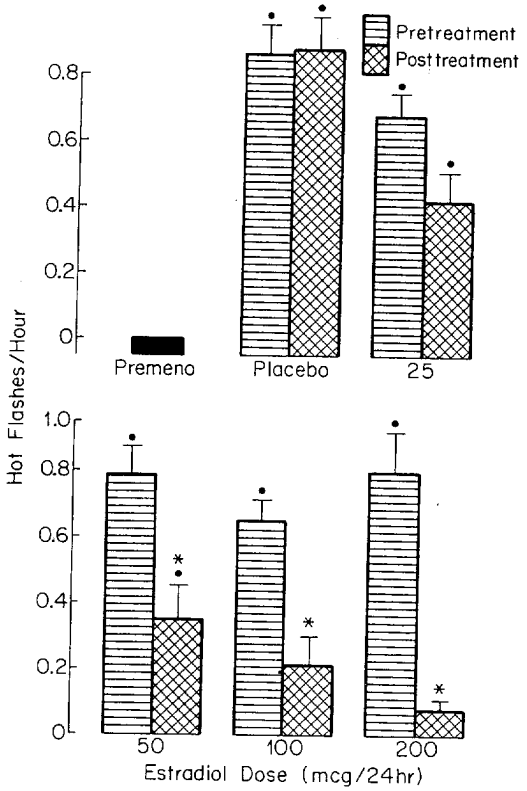

Figure 13. Mean (± S.E.) rate of occurrence of hot flashes in the study groups and premenopausal women before and during transdermal estradiol administration. (*From* Steingold KA, Laufer L, Chetkowski RJ, et al: Treatment of hot flashes with transdermal estradiol administration. J Clin Endocrinol Metab 61:627, 1985; with permission.)

complete elimination of symptoms in two patients with estradiol levels of 32 and 34 pg per ml; one patient had 0.28 hot flashes per hour with an estradiol level of 99 pg per ml. The 95 per cent confidence limits for 50 per cent reduction of hot flashes were 24 and 85 pg per ml. For 100 per cent reduction, they were 97 and 173 pg per

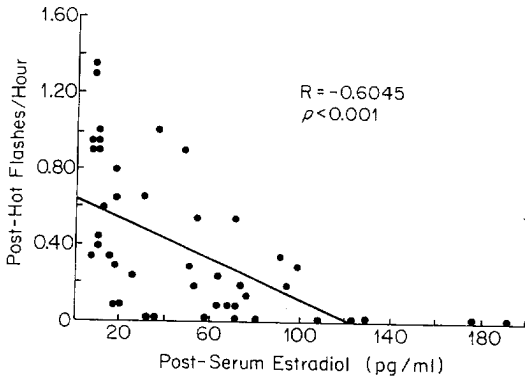

Figure 14. The linear regression of the number of hot flashes per hour versus estradiol levels during transdermal administration of estradiol. (*From* Steingold KA, Laufer L, Chetkowski RJ, et al: Treatment of hot flashes with transdermal estradiol administration. J Clin Endocrinol Metab 61:627, 1985; with permission.)

ml. These data provide guidelines as to what serum level of estradiol is needed to sustain relief of these symptoms and indicate that this can be expected with concentrations in the range found in women in the early to mid-follicular portion of their cycles.

To examine a more prolonged effect of the systems on hot flashes and other symptoms of the menopause, Padwick and co-workers[30] conducted an open trial at Kings College Hospital, London. Twelve symptomatic volunteers attending the menopause clinic were recruited for the study. Two pretreatment visits occurred 2 weeks apart, and therapy was started immediately after visit 2. The transdermal estradiol systems were prescribed for 3 weeks, followed by 1 treatment-free week; this sequence was repeated three times. Visits during the treatment phase occurred at the end of the third treatment week in each sequence. Fifty micrograms per 24-hour systems were used.

The changes in the average daily number of hot flashes experienced during the previous week are illustrated in Figure 15. The results are expressed as a percentage of the mean of the two pretreatment values. With each successive treatment cycle, there was a trend toward a further reduction in the number of flashes. At the end of the study, the average number of flashes per day had declined from pretreatment levels by 91 per cent.

Shown in Figure 16 are the mean Graphic Rating Scale scores for menopausal symptoms from pretreatment to treatment. Transdermal estradiol exerted a significant beneficial effect on hot flashes, sleep disturbance, irritability, and poor concentration. The relief of vaginal dryness and anxiety just failed to reach significance.

With these studies showing the efficacy of the transdermal systems to relieve menopausal symptoms, Chetkowski and col-

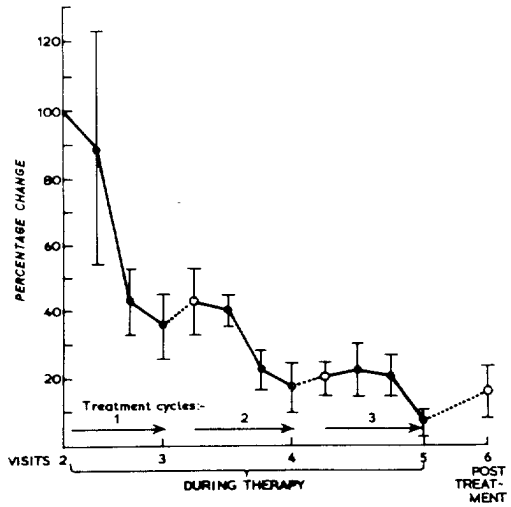

Figure 15. Percentage change in mean (±S.E.) daily number of hot flashes. ●——● = during treatment; ○——○ = off treatment. (*From* Padwick M, Endacott J, Whitehead MI: Efficacy, acceptability, and metabolic effects of transdermal estradiol in the management of postmenopausal women. Am J Obstet Gynecol, 1985; with permission.)

leagues[7] embarked on a study to compare the effects of the system on nonhepatic and hepatic markers of estrogen action in comparison to the two most commonly used dosages of conjugated estrogen.

Twenty-three postmenopausal women were given transdermal estradiol in four increasing dosages (25, 50, 100, and 200 μg per 24 hours), followed by the daily oral administration of conjugated estrogen in two dosages (0.625 and 1.25 mg per day). All dosages were administered for 28 days. The medication-free interval between each dose was 7 days, and the "washout" period between administrations of the two types of medication was 28 days. All the subjects were studied before the start of therapy and on the last day of each dosage.

The mean levels of estradiol and estrone are shown in Figure 17. For comparison, the values observed in 15 premenopausal women during the early and late follicular phases of their menstrual cycles are also shown. As expected, the basal estrogen levels were lower in the postmenopausal than in the premenopausal women. With the transdermal systems, estradiol levels rose in a dose-response fashion. Conjugated estrogen also raised estradiol levels above baseline, but the concentrations remained lower with both doses than in the premenopausal subjects. Circulating estrone levels also rose after the four dosages of transdermal estradiol. As expected, conjugated estrogen increased estrone concentrations substantially.

Mean gonadotropin levels in the same subjects are shown in Figure 18. In the postmenopausal women, the high baseline levels showed stepwise decreases with increasing dosages of both estrogenic preparations, but even the largest dosages of these medications did not lower the gonadotropin levels to the levels observed in the premenopausal subjects. The decreases elicited with 50 and 100 μg per 24 hours of transdermal estradiol corresponded to those seen with 0.625 and 1.25 mg of conjugated estrogen, respectively.

The results of vaginal cytologic studies are shown in Figure 19. In the postmenopausal women, all dosages of both preparations re-

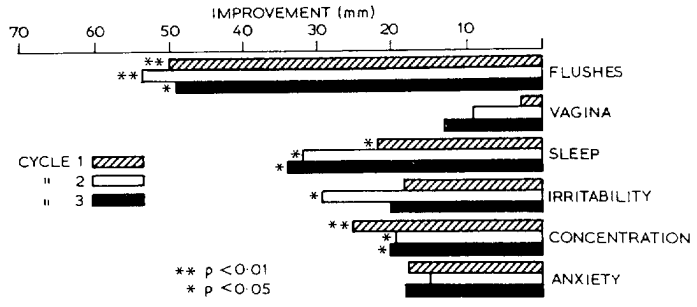

Figure 16. Mean changes in Graphic Rating Scale score (mm) in the last week of therapy in treatment cycles 1, 2, and 3, compared with pretreatment. Significance of difference compared with pretreatment values. (*From* Padwick M, Endacott J, Whitehead MI: Efficacy, acceptability, and metabolic effects of transdermal estradiol in the management of postmenopausal women. Am J Obstet Gynecol 152:1085, 1985; with permission.)

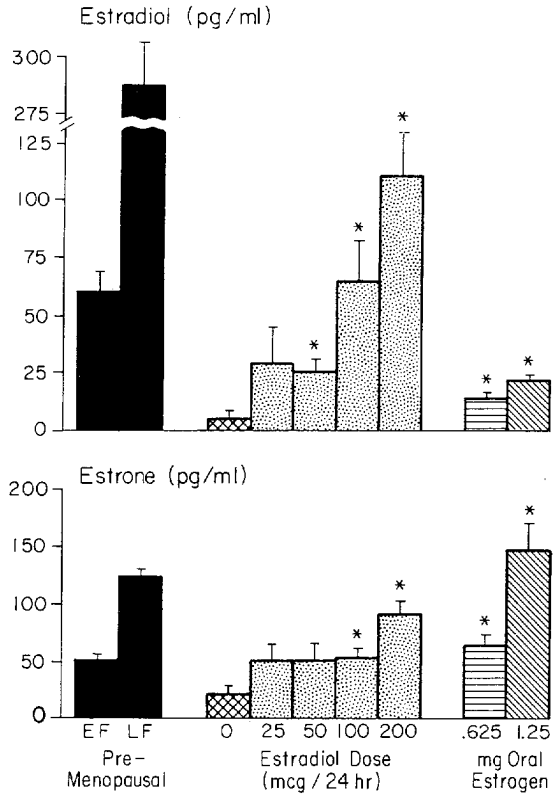

Figure 17. Mean (±S.E.) estradiol and estrone levels in premenopausal women during the early follicular (EF) and late follicular (LF) phases of their menstrual cycles and postmenopausal women before and during transdermal administration of estradiol and oral administration of conjugated estrogens. See Fig. 1 legend for symbols. (*From* Chetkowski RJ, Meldrum DR, Steingold KA, et al: Biologic effects of transdermal estradiol. N Engl J Med 314:1615, 1986; with permission.)

duced the mean percentage of parabasal cells significantly, whereas the lowest dosages that significantly increased the percentage of superficial cells were 100 μg per 24 hours and 1.25 mg of transdermal estradiol and conjugated estrogen, respectively. Again, the dosages of 50 and 100 μg per 24 hours of transdermal estradiol elicited effects similar to the 0.625-mg and 1.25-mg dosages of conjugated estrogen, respectively.

Shown in Figure 20 are the mean urinary calcium levels and the ratios of calcium and hydroxyproline to creatinine in the same subjects. In the baseline samples, the postmenopausal women had higher values of all these markers of bone metabolism than those of the premenopausal women. These presumably reflected greater bone resorption in the older women. All dosages of both estrogenic preparations significantly lowered the urinary calcium levels and the ratio of Ca:Cr. Again, responses induced with 50 and 100 μg per 24

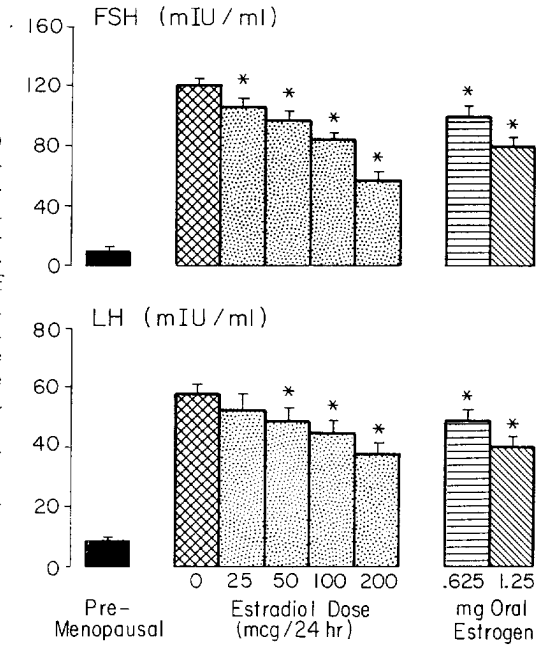

Figure 18. Mean (±S.E.) levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in postmenopausal women before and during transdermal administration of estradiol and oral administration of conjugated estrogens. The mean values in premenopausal women during the early follicular phase of their menstrual cycle are shown for comparison. (*From* Chetkowski RJ, Meldrum DR, Steingold KA, et al: Biologic effects of transdermal estradiol. N Engl J Med 314:1615, 1986; with permission.)

hours of transdermal estradiol were similar to those seen with 0.625 and 1.25 mg of conjugated estrogen, respectively, except for the urinary hydroxyproline:creatinine ratio after the 1.25-mg dosage. Since 0.625 mg of conjugated estrogen has been shown to inhibit bone loss,[26] it is possible that the transdermal estradiol dosages of 50 μg per 24 hours or greater will also. Randomized, long-term studies using bone-density measurements and assessments of fracture rates are under way to investigate this important issue.

The mean levels of renin substrate and binding globulins are shown in Figure 21. With the administration of all dosages of transdermal estradiol, no significant change was noted, whereas both dosages of conjugated estrogen elicited significant elevations in the levels of these hepatic proteins. Of particular importance was the elevation of renin substrate levels with oral conjugated estrogen, in view of the association of hypertension with the administration of these medications.[8] The marked increase in the level of this protein accentuates or initiates the development of high blood pressure in susceptible women with other predisposing factors.[22] The ability of transdermal estradiol to provide adequate amounts of estrogen to suppress hot flashes, correct vaginal atrophy (see Fig. 19), and reduce urinary calcium excretion (see Fig. 20) without increasing the synthesis of renin substrate represents an advantage of this nonoral form of estrogen replacement.[41]

The effects of these estrogenic preparations on circulating clot-

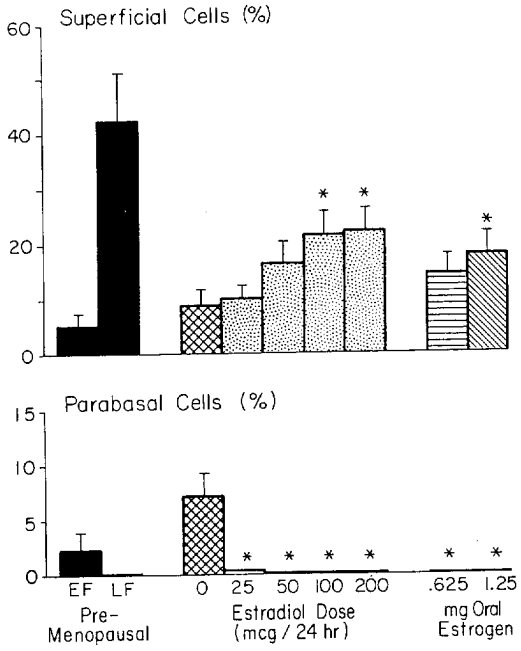

Figure 19. Mean (±S.E.) percentages of vaginal superficial and parabasal cells in premenopausal women during the early follicular (EF) and late follicular (LF) phases and in postmenopausal women before and during the administration of transdermal estradiol or oral conjugated estrogens. (*From* Chetkowski RJ, Meldrum DR, Steingold KA, et al: Biologic effects of transdermal estradiol. N Engl J Med 314:1615, 1986; with permission.)

ting factors are shown in Figure 22. The baseline levels of these clotting factors were similar in postmenopausal and premenopausal women and did not change significantly with any dosage of either preparation. The four clotting parameters studied were specifically chosen because they have been shown to be altered by oral contraceptives.[1,14,29] The absence of a discernible effect on the concentration of antithrombin III or fibrinopeptide A, a measure of thrombin action in fibrinogen, with either preparation suggests that the dosages studied do not promote intravascular clotting.

A major difficulty in the investigation of the laboratory and clinical aspects of hormone replacement and thromboembolism is that clinical expression of the disorder occurs infrequently.[1] In addition, it has long been appreciated that deep-vein thrombosis is frequently a clinically silent condition and spontaneous resolution is the rule.

The measurement of plasma fibrinogen levels by chromatography offers an alternative approach to this problem. This method quantifies the respective proportions and absolute amounts of high molecular weight fibrinogen complexes, monomeric fibrinogen, and derivatives of fibrinogen smaller than the parent molecule in plasma.[1,16] Plasma fibrinogen chromatography quantifies disorders of fibrinogen metabolism after intravascular coagulation or the presence of a thrombus and is a sensitive method for detecting the presence of even small clinically silent thrombi.

The presence of high molecular weight fibrinogen complexes in

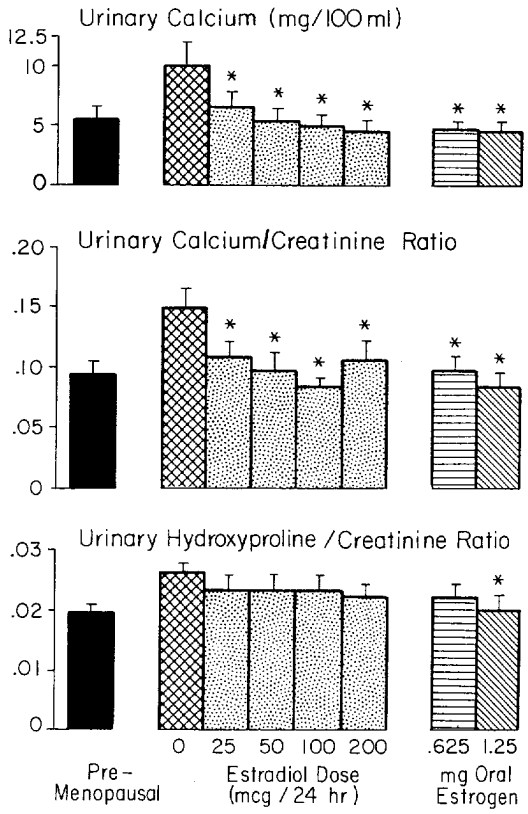

Figure 20. Mean (±S.E.) levels of urinary calcium, the urinary calcium:creatinine ratio, and the urinary hydroxyproline: creatinine ratio in the same study subjects. (*From* Chetkowski RJ, Meldrum DR, Steingold KA, et al: Biologic effects of transdermal estradiol. N Engl J Med 314:1615, 1986; with permission.)

excess of two standard deviations of the mean normal value for either plasma proportions or absolute plasma concentrations has been shown in postoperative patients to be highly correlated ($P < 0.001$) with the presence of clinically silent thrombi detected by isotope scanning using I^{125}-labeled fibrinogen.[1] High levels have also been observed in 27 per cent of women within 1 month of commencing oral contraceptive usage with preparations containing 50 or 100 μg of ethinyl estradiol.[16] Thus, this assay represents a sensitive, indirect measurement of the presence of abnormal amounts of intravascular coagulation. The absence of elevated levels of this fraction of fibrinogen in the patients given transdermal estradiol and conjugated estrogen also supports the concept that the doses tested of both preparations do not enhance intravascular coagulation.

Shown in Figure 23 are the lipid levels measured in the study.[7] All the baseline levels were similar or higher in the postmenopausal women. This presumably reflected the differences in age and body weight of the two groups. Again, the administration of transdermal estradiol had no discernible effect on the levels of any of these circulating lipids, whereas the 1.25-mg dosage of conjugated estrogen re-

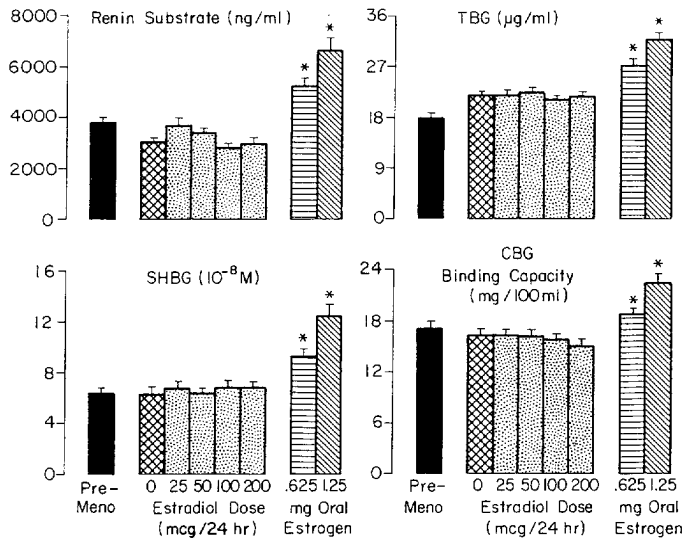

Figure 21. Mean (±S.E.) levels of renin substrate, sex hormone–binding globulin (SHBG), cortisol-binding (CBG) activity, and thyroxine-binding globulin (TBG) in the same study subjects. (*From* Chetkowski RJ, Meldrum DR, Steingold KA, et al: Biologic effects of transdermal estradiol. N Engl J Med 314:1615, 1986; with permission.)

sulted in small but significant reductions in total cholesterol and LDL cholesterol and elevations in HDL cholesterol and in the ratio of HDL/LDL.

The clinical implications of estrogen actions on hepatic lipid metabolism are mixed, as mentioned previously. An increased occurrence of gallbladder disease has been reported with oral contraceptive usage and estrogen replacement therapy.[5] This increase is

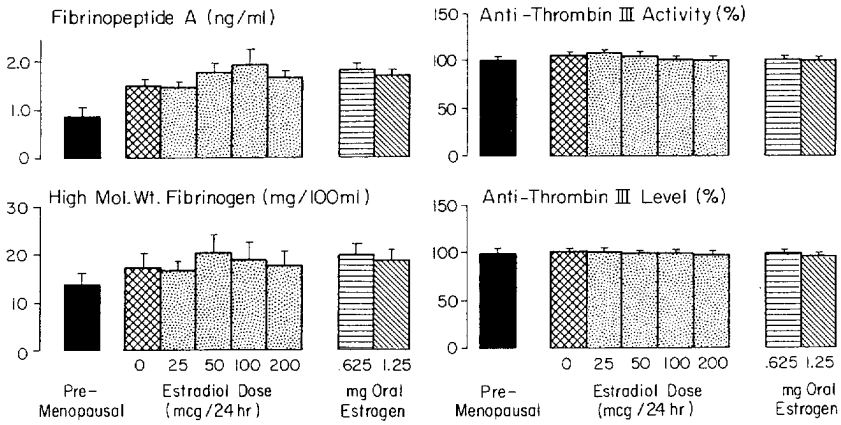

Figure 22. Mean (±S.E.) levels of fibrinopeptide A, high-molecular-weight fibrinogen, antithrombin III activity, and antithrombin III level in the same subjects.

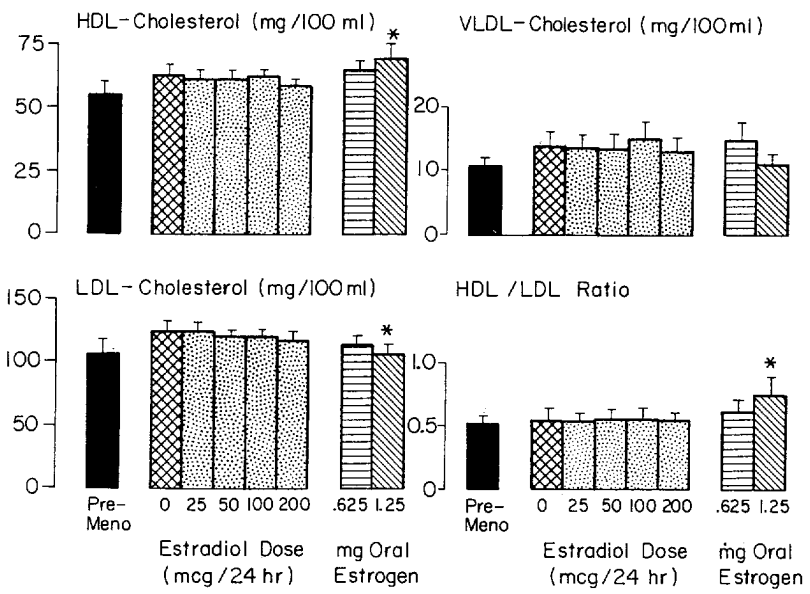

Figure 23. Mean (±S.E.) levels of high-density lipoprotein (HDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and HDL/LDL ratios in the same study subjects.

thought to relate to the effects of estrogens on the saturation of cholesterol in bile. The absence of any discernible effect of transdermal estradiol on circulating lipids suggests that this method of administration may not alter hepatic lipid synthesis and thus may not enhance gallstone formation. Further studies are needed to confirm this possibility.

The effects of the 1.25-mg dosage of conjugated estrogen on lipoprotein levels have potentially beneficial implications for heart disease. As mentioned previously, a growing body of information suggests that loss of ovarian function is associated with an increased occurrence of heart disease,[2,20] while estrogen replacement therapy is accompanied by a decreased incidence.[36] If future studies support these observations, then the prevention of heart disease should become by far the most important indication for estrogen replacement.

The mechanisms by which cessation of ovarian function and estrogen replacement affect the incidence of heart disease are not known. It is thought that the effects exogenously administered estrogens have on circulating lipoprotein levels may provide the explanation, but careful review of the literature makes this conclusion tenuous. Only a few studies have assessed the impact of natural or surgical menopause on circulating lipid levels. Most of these studies have been group comparisons and have revealed higher cholesterol levels in women with oophorectomies than women without, particularly before the age of 50. The failure to control for other known

determinants of cholesterol levels such as degree of obesity, alcohol consumption, physical activity, and cigarette smoking could have influenced these results.

Investigators who have studied premenopausal women before and after bilateral oophorectomy have made less conclusive findings. Blumenfeld and colleagues[3] showed an increase in total cholesterol levels that was attributable to a rise in VLDL cholesterol levels. Removing the ovaries had little impact on total HDL cholesterol levels. The latter observation confirmed previous findings by other investigators. These studies were criticized because only a small number of patients were studied and the impact of oophorectomy was assessed just 1 month after surgery. Obviously, the impact of cessation of ovarian function on lipoprotein levels needs to be examined critically.

The effects of estrogen replacement on circulating lipid levels are better established. Administration of high dosages of estrogens clearly elevates HDL levels and its subfraction HDL_2 and lowers LDL cholesterol concentrations.[15] The impact of estrogen administration with dosages employed for hormone replacement is less clear. Most earlier studies have examined the effect of conjugated estrogen on total HDL or HDL_2 levels by using study designs that compared the levels of lipoproteins in groups of women who were or were not receiving the hormone.[45] The failure to control for other known determinants of HDL likely influenced the results.

The Chetkowski study indicated that the impact of conjugated estrogen on lipoprotein levels is small, particularly for the 0.625-mg dosage.[7] Similar findings have been made with conjugated estrogen and medroxyprogesterone acetate usage for 18 months. Since the 0.625 mg-dosage has been shown to provide protection against heart disease equal to the 1.25-mg dosage,[36] questions must be raised whether all of the beneficial effects of estrogen replacement on heart disease can be explained through the lipoprotein mechanism. These questions are supported by the observations of the Lipid Research Laboratory Study, which found the protective effect of estrogen on all-cause mortality of their patients could not be explained solely on the differences of lipid levels measured in their subjects. Thus, mechanisms other than the effects of estrogens on lipoproteins may be responsible for some or all of the protective actions estrogens appear to have on heart disease. For example, estradiol infusions increase the coronary blood flow in sheep.[35] Estrogen also alters vascular connective tissue and prostaglandin metabolism. Properly designed studies are badly needed to critically determine the possible role of estrogens in heart diseases and the mechanisms by which this steroid exerts these actions.

ORGAN-SPECIFIC EXTRACTION OF CIRCULATING ESTROGENS

Thus, it is apparent that the transdermal administration of estradiol exerts none of the enhanced actions of estrogens on hepatic

function. This also is seen with the use of subcutaneous pellets and skin creams containing estradiol. We now believe there are at least two mechanisms responsible for the enhanced hepatic actions of estrogens, and depending on the preparation administered, one or the other mechanism predominates. The first is the first pass mechanism described earlier. The second is the increased entry of circulating estrogens into the liver, as compared with other organs. During passage through the vascular system, the fraction of circulating estrogens that exit the vascular system, enter the interstitial space, and are available to cells for action is much greater in the liver than in other organs.[31] Several factors influence the transport of steroid hormones from the circulation to the cells of an organ. These include capillary membrane permeability barriers, plasma protein binding of steroids, and the anatomy of the microvasculature of an organ. Each of these will be discussed briefly.

For a steroid hormone to leave the vascular system, it must exit the capillary by passing between or through the capillary endothelial cells. The plasma membrane of these cells consists of a lipid-protein matrix. Studies indicate that steroids have different permeabilities to capillary endothelial cells.

Two factors that influence the capillary membrane characteristics of steroids are the lipid solubility and the polarity of the hormone. The latter is a function of the hydrogen bonds forming functional groups and the charged functional groups. In general, the polarity of a hormone appears to predict membrane permeability much better than lipid solubility. In blood, there are proteins that bind to steroids. Albumin is known to bind with a low affinity to most steroids. There are also globulins in the circulation that bind with high affinity to specific steroids. For example, SHBG binds to estradiol but not estrone.

Last, the anatomy of the microvasculature of organs is different. For example, it is well recognized that the microvasculature of the brain is different from other organ systems because there are tight junctions between the capillary endothelial cells. Thus, proteins cannot cross the blood-brain barrier unless there is a specific transport mechanism. Each of these factors comes into play when considering the exit of estrogens used for replacement therapy into specific organs.

Using double isotope, single injection tissue-sampling methods, Steingold and co-workers[42] have assessed the in-vivo delivery of estrogens into the brain, uterus, and liver during a single passage through these organs' capillary beds in female Sprague-Dawley rats anesthetized with pentobarbital. In studies involving the brain, after anesthesia, the rat was placed in the supine position, and the right common carotid artery was isolated. The test solution, in a volume of 250 μl, was rapidly injected as a bolus via the carotid artery through a sharp 27-gauge needle. The bolus contained a tritium-labeled estrogen and ^{14}C-labeled butanol (a freely diffusible reference) with human postmenopausal serum as the injection vehicle. Fifteen seconds after injection, the animal was decapitated; the cerebral

hemisphere ipsilateral to the injection was removed from the cranium, and the tissue processed. For studies involving the uterus, rats were ovariectomized and then studied 7 to 14 days later. The lower abdominal aorta and the right common iliac artery were surgically exposed. The latter was clamped as was the left femoral artery. The test solution was administered into the lower abdominal aorta. Fifteen seconds after the injection, the left uterine horn was resected. Influx into the liver was determined after injection into the portal vein immediately after ligation of the hepatic artery. Eighteen seconds after the injection, the right major lobe was removed.

The brain, uterine, and liver tissues were processed, and the brain influx index, uterine influx index, and the liver influx index were calculated as previously described.[25,32,43] The brain influx index (BII), uterine influx index (UII), and liver influx index (LII) were computed as follows:

$$\text{BII, UII or LII} = \frac{(^3\text{H}: ^{14}\text{C})\text{ dpm ratio in tissue}}{(^3\text{H}: ^{14}\text{C})\text{ dpm ratio in injectate}} \times 100$$

The brain influx index, uterine influx index, or liver influx index is equal to the ratio of the extraction of the estrogen divided by the extraction of the butanol reference at 15, 15, and 18 seconds, respectively, after the injection. The extraction for butanol was measured previously, equaling 90 per cent for the brain,[32] 77 per cent for the uterus,[25] and 84 per cent for the liver.[43] Therefore, the brain extraction = (BII) (0.90), the uterus extraction = (UII) (0.77), and the liver extraction = (LII) (0.84).

The tissue extractions (percentages) by the brain, uterus, and liver of ^{3}H labeled estradiol, estrone, estrone sulfate, ethinyl estradiol, and diethylstilbestrol from postmenopausal serum relative to that of ^{14}C butanol are shown in Figure 24. Data are mean ± S.E. (n = three to six animals per point). The background values of dextran for the brain and uterus and inulin for the liver correspond to the amounts of test substances that have been demonstrated to remain in the brain and uterine vasculature at 15 sec and hepatic vasculature at 18 sec.

With regard to the brain, the extractions of different estrogens varied markedly, being highest for estrone and barely above background dextran for estrone sulfate. The extractions of estrogens by the uterus resembled those seen in the brain, with the exception of diethylstilbestrol in which the extraction by the uterus was greater. By contrast, the extractions of the different estrogens by the liver did not vary appreciably, and the values were much higher than those seen in the brain and uterus.

The major permeability barrier for transport into the brain and uterus is the plasma membrane of the capillary endothelial cells. For the liver, its wide sinusoidal pores allow for instantaneous equilibration of plasma proteins and steroids between intravascular and inter-

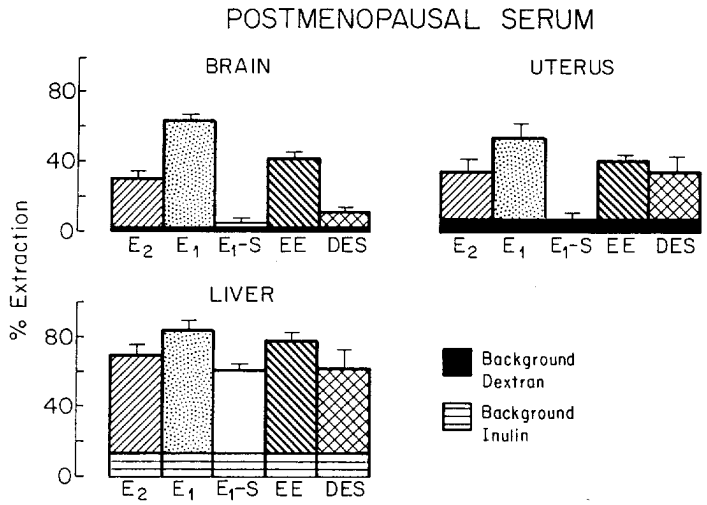

Figure 24. Mean tissue extractions (percentage) by the brain, uterus, and liver of ^{3}H-labeled estrone (E_1), estradiol (E_2), estrone sulfate (E_1-S), ethinyl estradiol (EE), and diethylstilbestrol (DES) from postmenopausal serum relative to that of (^{14}C) butanol are shown. Data are the mean ± S.E. (3–6 animals/point). The background values of dextran for the brain and uterus and inulin for the liver correspond to nonspecific isotope remaining 15 seconds after injection in the brain and uterus and 18 seconds after injection in liver capillaries, respectively. (*From* Steingold KA, Cefalu W, Pardridge W, et al: Enhanced hepatic extraction of estrogens used for replacement therapy. J Clin Endocrinol Metab 62:761, 1986; with permission.)

stitial spaces. This organ's microvascular permeability barrier is the hepatocyte plasma membrane. Influx of steroids is related exponentially to the surface area of the organ's microcirculation. The large hepatocyte surface area relative to the capillary endothelia of the brain and uterus presumably contributes to the greater influx of steroids into the liver.

SUMMARY

Thus, the effects that a specific estrogen has on the liver is the summation of several mechanisms, including the first pass mechanism and the enhanced delivery of circulating estrogens to this organ. For example, the major estrogen in conjugated estrogen is estrone sulfate. Based on the present data, very little circulating estrone sulfate is available to the brain or uterus (Fig. 25). Since most of the hypothalamus is behind the blood-brain barrier, it is unlikely estrone sulfate exerts a direct action on gonadotropin-releasing hormone (GnRH) neurons to reduce its secretion and, subsequently, gonadotropin levels. Estrone sulfate also does not interact with the estrogen receptor. It is possible the small amount of estrone sulfate that crosses the blood-brain barrier is converted to unconjugated es-

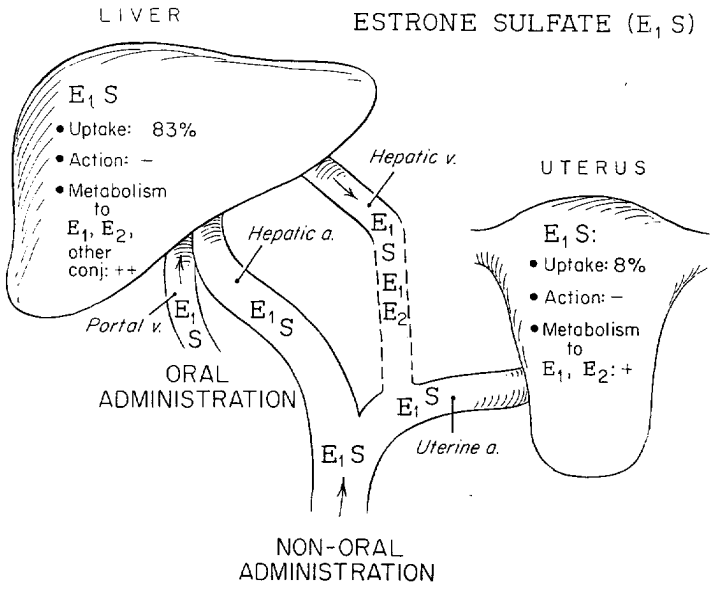

Figure 25. Comparison of oral versus nonoral administration of estrone sulfate (E_1-S). Note the enhanced hepatic uptake regardless of the route of administration and the required hepatic metabolism to estrone (E_1) and estradiol (E_2) for biological action.

trogens locally[33] and these could have function. More likely, the major mechanism by which estrone sulfate suppresses GnRH release is through conversion to unconjugated estrogens, principally estrone, in the liver.[38] The large extraction of estrone sulfate by the liver allows accessibility of the hepatocyte for this conversion. Based on this concept, conjugated estrogen must enter the liver to be converted to its active forms. The route of administration then should have little impact on changing the relative potency of this preparation on hepatic and nonhepatic markers of estrogen action.[28]

For ethinyl estradiol, the preparation is orally active because it is rapidly and almost completely absorbed from the stomach and undergoes limited hepatic metabolism before entry into the general circulation (Fig. 26).[21] This limited hepatic metabolism reduces the impact of the first pass mechanism on ethinyl estradiol. Thus, the enhanced hepatic action of ethinyl estradiol is principally related to the greater entry of this estrogen into the liver than other organs (Fig. 27). Consequently, the route of administration should have little impact on the exaggerated hepatic actions of this estrogen.[19]

Orally administered estradiol undergoes substantial hepatic metabolism to less active forms, principally estrogen conjugates.[13] The amount of estradiol leaving the liver following oral administration is substantially less than that which enters it through the portal vein. The systemic administration of estradiol avoids this initial hepatic metabolism. Furthermore, only 25 per cent of nonorally administered estrogen will go to the liver at each pass, and nonhepatic

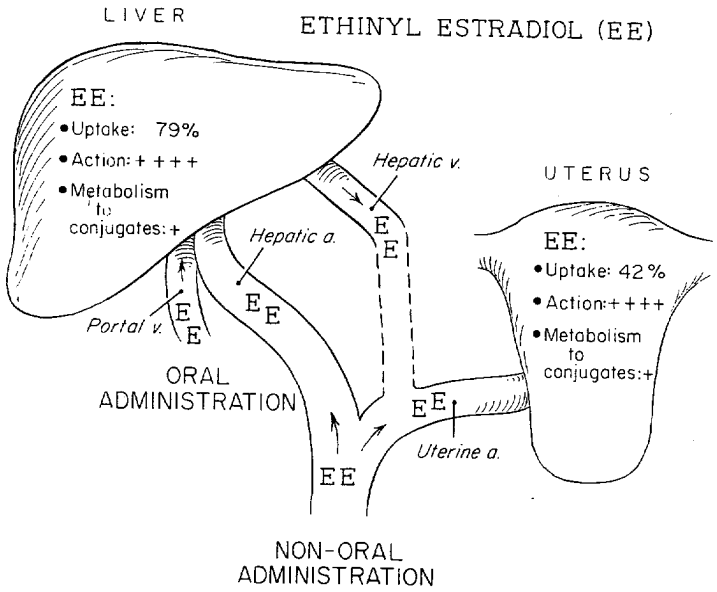

Figure 26. Comparison of oral versus nonoral administration of ethinyl estradiol (EE). Note there is minimal liver metabolism of EE, but liver uptake predominates regardless of the route of administration.

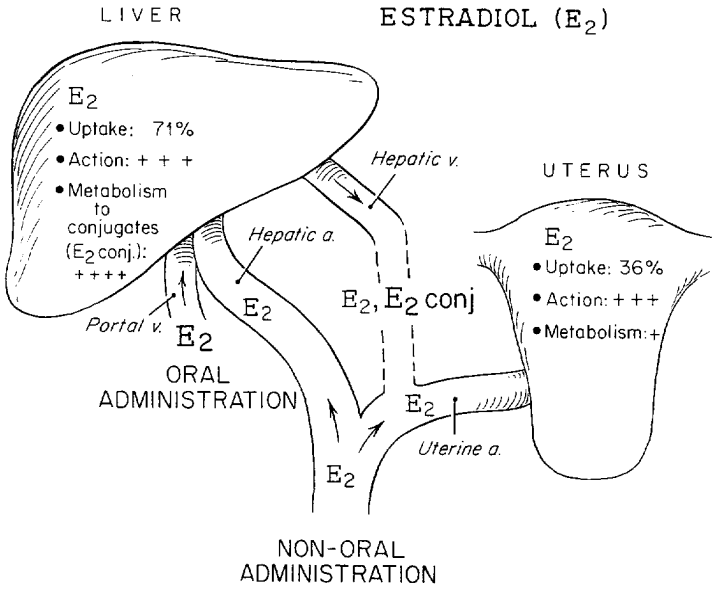

Figure 27. Comparison of oral versus nonoral administration of estradiol (E_2). Note orally administered E_2 is largely converted to conjugates in the liver. Nonoral administration provides estradiol to the general circulation while bypassing the increased hepatic metabolism and action.

tissues would be exposed to a greater extent than after oral administration. Thus, peripheral administration of estradiol reduces the exaggerated hepatic responses in comparison to nonhepatic actions.[15,24]

In summary, we have presented several arguments that suggest exaggerated actions of estrogens on the liver for the most part should be avoided. The nonoral administration of estradiol, but not conjugated estrogen or ethinyl estradiol, does this. Since it is anticipated that postmenopausal women may be using hormone replacement for extended periods of time to prevent symptoms, osteoporosis, and possibly heart disease, it seems reasonable to provide therapy that reproduces ovarian function as closely as possible. The nonoral administration of estradiol represents one of these approaches.

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