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Estrogens: Whence, When, Why, How*

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Historical Introduction

Some 1,800 years ago, Galen, omniscient Greco-Roman physician, considered the ovary to be a filter for woman's semen, naming the structure "the female testicle." He was, in a measure, prescient, for the androgen pathway is primary and essential in the synthesis of estrogens by the ovary.

It was not until 1900 that Knauer confirmed the role of the ovary as an endocrine organ by demonstrating the prevention of atrophy of the uterus in ovariectomized rabbits by ovarian transplants. In 1923, Allen and Doisy observed that injections of follicular fluid of hog ovaries induced full estrus in bilaterally oophorectomized mice and rats. A few years later, Aschheim and Zondek (1927) found that pregnancy urine was rich in estrogenic substances, and within the next two years, Doisy in the United States, and Butenandt in Germany, announced the isolation of estrone from urine. Subsequently, estrone and estradiol were isolated from the ovary. At about the same time, Corner and Allen isolated a hormone from sow ovaries which they named progestin.

The isolation of hormones from ovarian tissue and the demonstration of pituitary dependency constitute classic chapters in the literature of reproductive physiology and will not be elaborated upon in this presentation. Attention will be directed toward present-day knowledge of the biosynthesis, physiology, pharmacology and changing views in the clinical use of estrogens.

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Biosynthesis, Physiology, and Metabolism of Estrogens

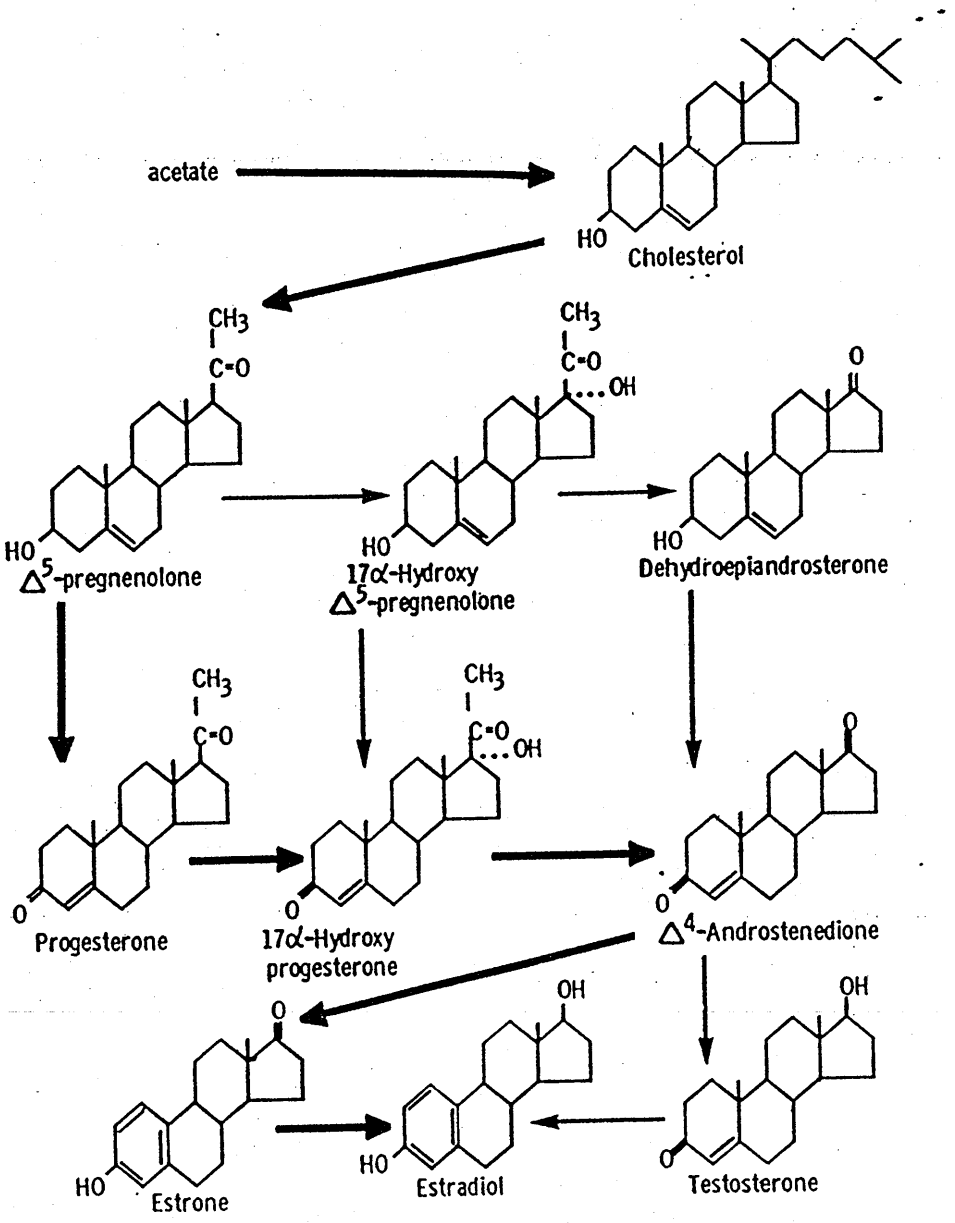
Although the presence of an ovarian hormone that had estrogenic properties was discovered as early as 1923, the precise nature of the secretion of estrogens by the ovary was elucidated much later. In a careful study carried out by Brown in 1957, it was concluded indirectly from experimental evidence that the ovaries secrete estrone (E_1) and estradiol (E_2), but not estriol (E_3).[1]

E_1 and E_2 are synthesized in the ovary from acetate and cholesterol. The synthesis from acetate is believed to be cholesterol-dependent. The various pathways of the synthesis of estrogens in the ovary are shown in Figure 1. Among these, conversion of cholesterol to E_1 and E_2 via Δ^5-pregnenolone to progesterone to 17α-hydroxyprogesterone and Δ^4-androstenedione (Δ^4A) is considered to be a major pathway.

Estrogens are present in the blood plasma partly in the free form as E_1 and E_2, and partly conjugated as estrone sulfate, estrone glucuronide and estradiol sulfate. The hormone production at various periods of life ranges from the minimal values found between 5 and 10 years of age, to the maximal values attained during pregnancy. The highest estrogen secretion in the normal non-pregnant adult occurs around the time of ovulation, and is generally referred to as the "ovulation peak." Another, but lesser, peak occurs about the 21st day of the cycle, and is known as the "luteal peak." The estrogens secreted by the ovary undergo extensive metabolism in the body. E_1 and E_2 are freely interconvertible, with the conversion of E_2 to E_1 being more rapid than the reverse.

Although the estrogenic hormone heretofore has been regarded as a female hormone, such a label is misleading, for estrogens are produced by both males and females. Until recent times, the clinical employment of this hormone was gingerly reserved for states of estrogen-deprivation, but new evidence suggests that estrogens are metabolic agents with a wide variety of uses, such as in the treatment of osteoporosis, neuroendocrine disorders (migraine headaches, depression), and even certain instances of breast cancer. This article will try to expose many of the myths and shibboleths about hormone replacement therapy, and point out the source of estrogens, which preparations may be employed in therapy, when and why, how long, by what route, and as well the untoward reactions.

FIGURE 1. Various pathways of the biosynthesis of estrogen in the ovary (dark arrows represent the major pathway).



(Reproduced with permission from Greenblatt RB, et al: Physiologic and clinical aspects of ovarian hormones. *Arch. Derm. 89*:846, 1964.)

Source of Estrogens

Apart from the ovary, estrogens are produced by the testes, placenta and the adrenals. After bilateral oophorectomy, significant estrogen levels are found in the blood and urine, the source presumed to be the adrenals. It has been suggested that the adrenals compensate for the loss of estrogens after menopause by acting as an auxiliary gonad.

Present-day studies indicate that the postmenopausal ovary does not produce estrogens *de novo*. This may also be true for the normal adrenal, although adrenal tumors may possess this capacity. Androgens, whether produced by the menopausal ovary, the adrenals, or exogenously administered, are converted peripherally to estrogens in varying degrees. The aromatization of androgens by the kidney, liver, brain, muscle and adipose tissue is an important source of estrogen in man[2,3] and in the postmenopausal woman.[4,5] Bulbrook and Greenwood found estrogens in the urine of women with breast carcinoma after ovariectomy, adrenalectomy and hypophysectomy.[6] Unexplained are the plasma estrogen levels after both bilateral ovariectomy and adrenalectomy (Table 1).

Extra-Ovarian Sources of Estrogen

What is the source of estrogen in the postmenopausal woman who presents with an estrogenized vaginal smear and a proliferative endometrium? Feinberg and Cohen demonstrated by histochemical methods that the aging ovary with hyperthecosis was apparently steroidogenic.[7] Hammerstein *et al.* were able to show that Δ^4A, dehydroepiandrosterone (DHEA), and testosterone (T) were the

TABLE 1. Plasma levels (pg/ml) of estrone and estradiol in 3 women who had undergone bilateral oophorectomy and adrenalectomy for breast cancer.

Patient	Estrone	Estradiol
A.C.	26.14	15.22
E.K.	29.42	34.66
L. McK.	53.07	40.76
Normal Values	60-120	18-120

(Reproduced with permission from Greenblatt RB and Vasquez JM: Extragonadal sources of oestrogens *Pharmatherapeutica* 2(Suppl 2):76, 1980).

major radioactively-labeled steroids produced *in vitro* by the ovarian stromal compartment, and this was later confirmed by Mattingly and Huang.[8,9]

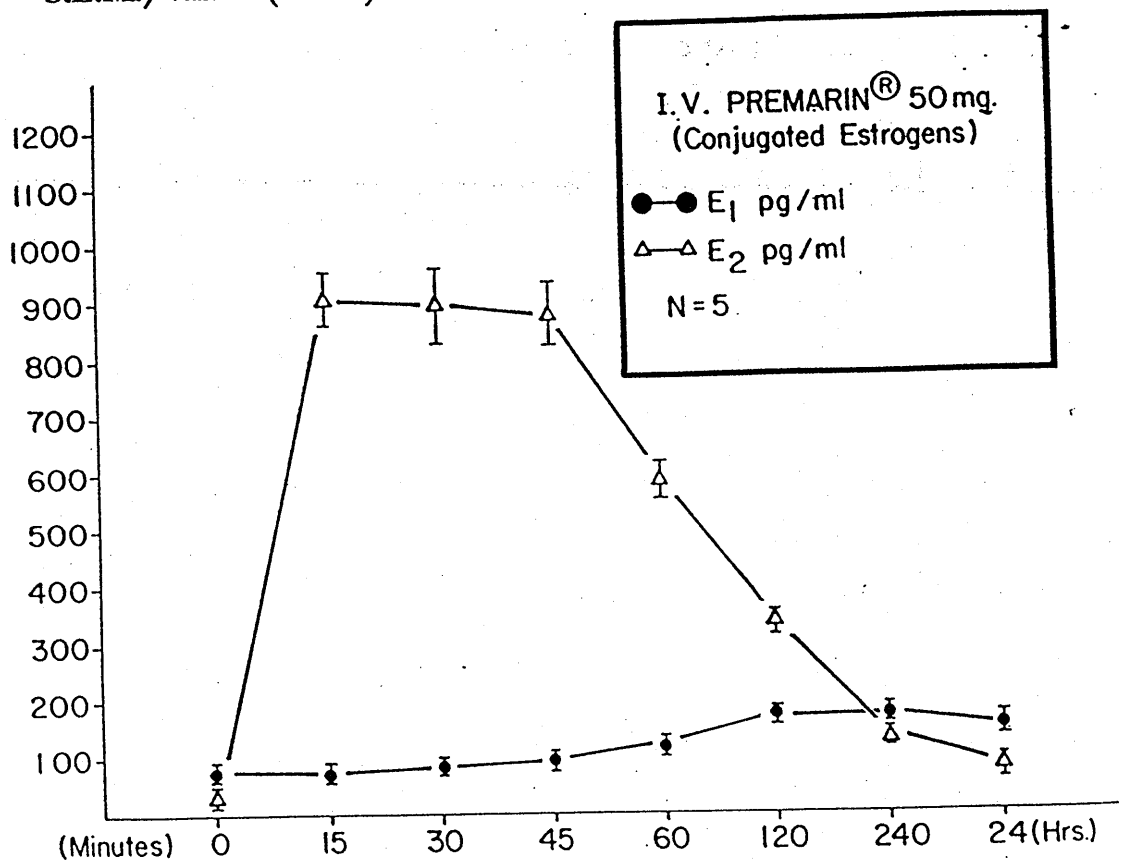
It is only in the past decade that confirmation for steroid production by the postmenopausal ovary has come to the fore. Poortman *et al.* stated, "It is likely that after the menopause, all estrogens are derived from peripheral conversion of androgens without secretion by the ovaries."[10] That E_2 was mostly converted to E_1 was shown by Yen *et al.* when they studied the effect of orally administered micronized E_2 (Estrace®) on the serum levels of 9 menopausal women.[11] However, Greenblatt and his associates demonstrated that E_2 levels remained high when a bolus of 50 mg of conjugated estrogens (Premarin IV) was administered intravenously or pellets of crystalline estradiol were implanted subcutaneously (Figures 2 and 3).[12] However, when a large bolus of E_1 or E_2 was administered orally, they, just as Whitehead *et al.*, found that E_1 levels rose considerably while E_2 levels were relatively low (Figures 4 and 5).[13]

Judd *et al.* found that the mean ovarian vein levels of E_1 and E_2 were significantly higher than the mean peripheral vein levels.[14] Greenblatt *et al.* obtained ovarian, adrenal, and peripheral vein blood by catheterization, and samples were taken at various intervals before and after intravenous injection of 5,000 units of human chorionic gonadotropin (hCG). Ovarian vein E_2 levels in menopausal women were 2-3 times lower than those found in women during the follicular phase. Furthermore, hCG did not evoke an increase of E_2 production in the menopausal ovary but did so in the ovary of the normal female (Figure 6); hCG induced a rise in Δ^4A and T in the postmenopausal ovary.[15]

Adrenal Estrogens

Are estrogens produced *de novo* by the adrenal cortex or are they systemic estrogens filtrating (coursing) through the adrenal? Our studies revealed that adrenocorticotropin (ACTH) does not stimulate E_2 production in either the ovaries or the adrenals. The concept that the adrenal takes over the estrogen production after the menopause is more conjectural than factual. The levels of E_2 found in ovarian and adrenal vein blood of postmenopausal women, in all probability, are circulating systemic estrogens and not necessarily evidence of inherent or *de novo* production, since ovarian and ad-

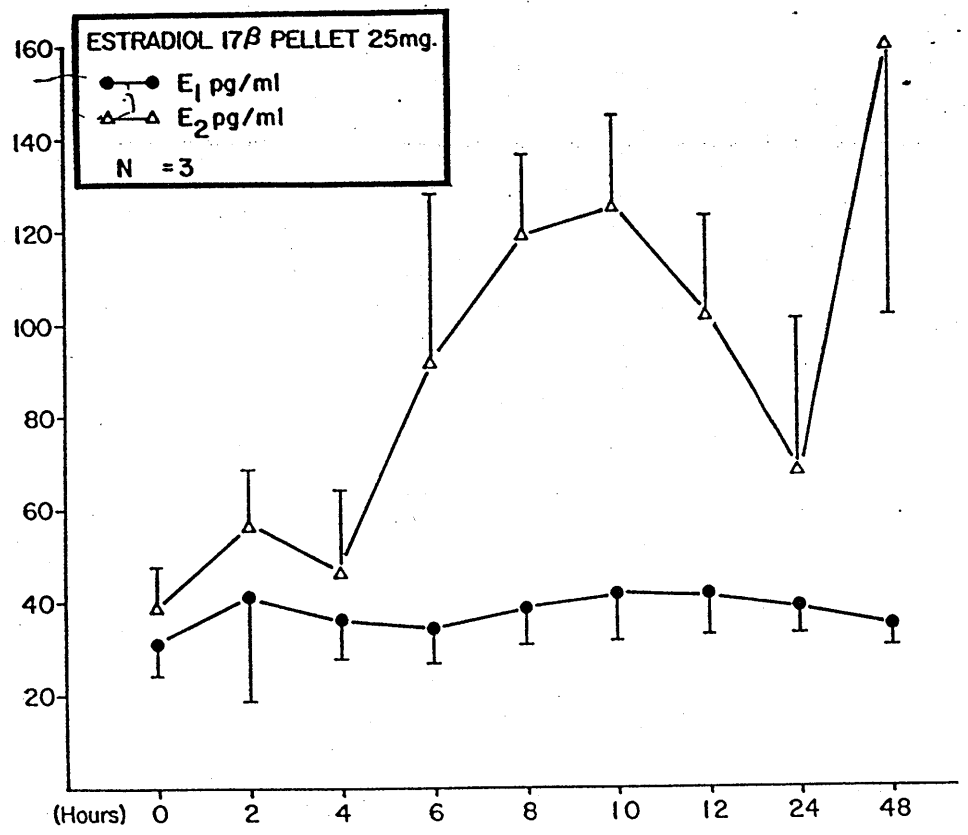
FIGURE 2. Rapid conversion to estradiol of a bolus of 50 mg conjugated estrogens (mostly estrone sulfate) given intravenously: mean (± S.E.M.) values (n = 5).[12]



renal estrogen levels remain more or less parallel. We were unable to obtain any elevation of E_2 in adrenal vein blood when intravenous ACTH was administered to normal adult women, hirsute women with polycystic ovaries, or postmenopausal women. ACTH, however, stimulated Δ^4A, DHEA, and T output (Figures 7 and 8).

However, virilizing adrenal cortical tumors in the female and feminizing adrenal tumors in the male are believed to elaborate estrogens.[16,17] Furthermore, elevated levels of estrogens, aside from androgens and pregnanetriol, are found in congenital adrenal hyperplasia.[18]. In adrenal hyperplasia due to Cushing's disease, a 3- to 5-fold increase in estrogen secretion has been demonstrated after intravenous ACTH.[19] Baird *et al.* found increased E_1 levels, but not E_2, in adrenal venous plasma after ACTH administration.[20] It appears that with abnormal conditions (tumor, congenital adrenal hyperplasia, Cushing's disease), the adrenal may produce estrogens,

FIGURE 3. Estrone and estradiol levels after subcutaneous implantation of 25-mg pellet of crystalline estradiol: mean (± S.E.M.) values (n = 3).[12]

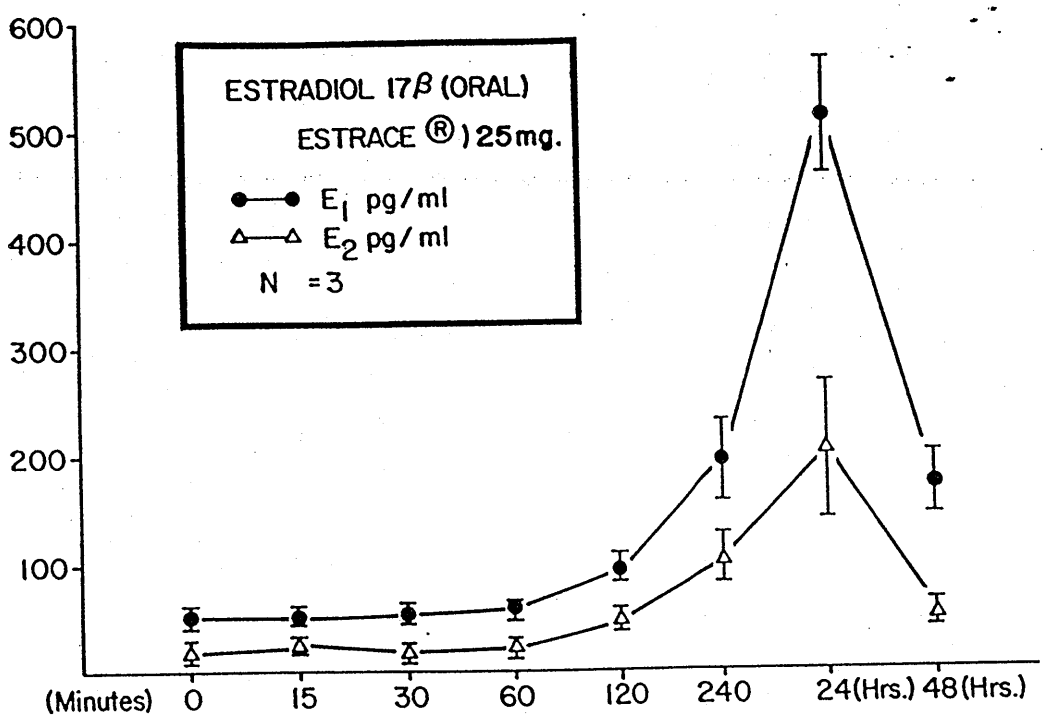


but no clear-cut evidence has been presented that the normal adrenal has that capacity.

Testicular Estrogens

The human testicle produces estrogens; the removal of testes in adult males is usually followed by severe vasomotor symptoms. Ruder *et al.* found that the administration of hCG will induce a significant and rapid estrogen and androgen response.[21] We were able to corroborate these findings (Figure 9).[22] Furthermore, after injection of testosterone propionate, Steinach and Kun noted the excretion of estrogenic material in urine of men.[23] Our own studies revealed that serum E_1 and E_2 levels usually rose after parenteral injection of testosterone compounds to ovariectomized women (Table 2, p. 62).

FIGURE 4. Estrone and estradiol levels after oral ingestion of 25 mg oral estradiol (Estrace®): mean (± S.E.M.) values (n = 3).[12]



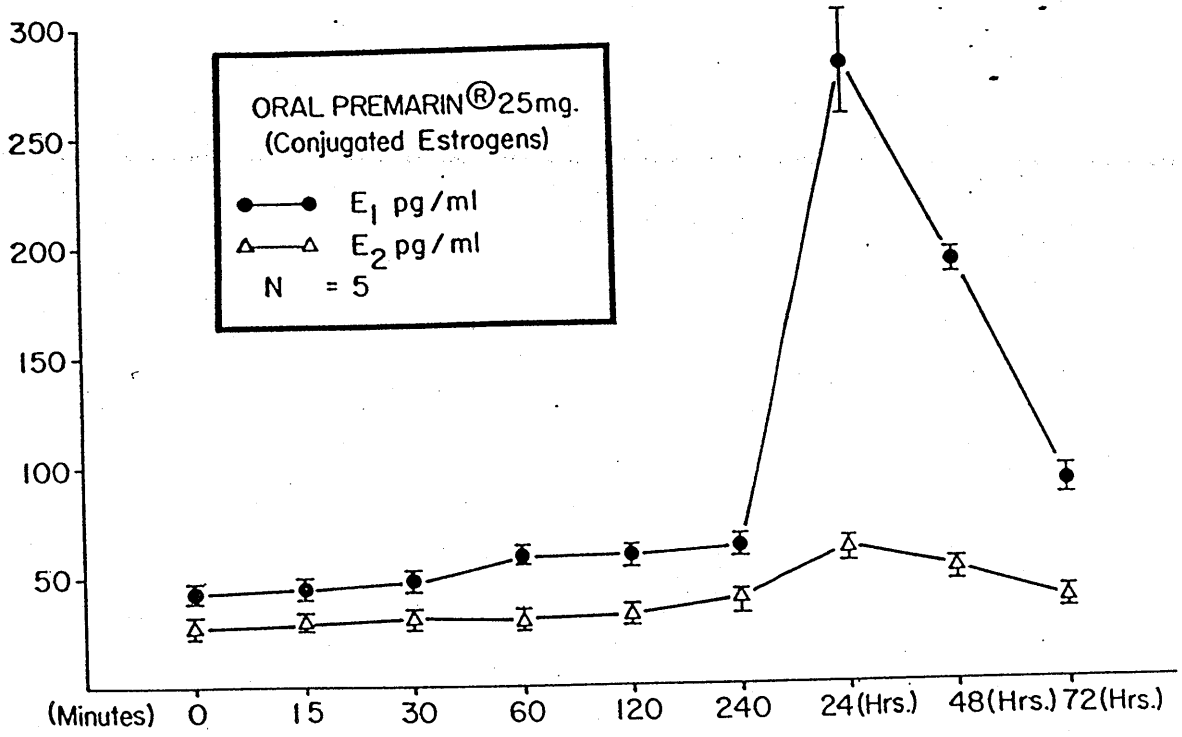
Placental Estrogens

The idea that both the fetus and the placenta are involved in the biosynthesis of estrogens was originally advanced by Diczfalusy, who developed the concept of the feto-placental unit as an integrated steroid-producing system.[24] During pregnancy, E_1 and E_2 excretion is increased about 100 times over nonpregnant levels, while maternal E_3 excretion is about 1,000-fold. For the production of estrogens, the placenta derives its precursors from outside sources. Approximately 90% of estriol can be accounted for by DHEA production by the fetal adrenal.

Foodstuff Estrogens

Estrogens derived from ingested foodstuffs, plants and grasses, are thought to be responsible for estrogen levels found in gonadectomized, adrenalectomized, and hypophysectomized men and

FIGURE 5. Estrone and estradiol levels after oral ingestion of 25 mg of conjugated estrogens (Premarin®).[22]



women. Estrogens have been found in the sex organs of plants, for example in the catkins of willow trees and palm kernels. Alfalfa is said to contain estrogenic substances.

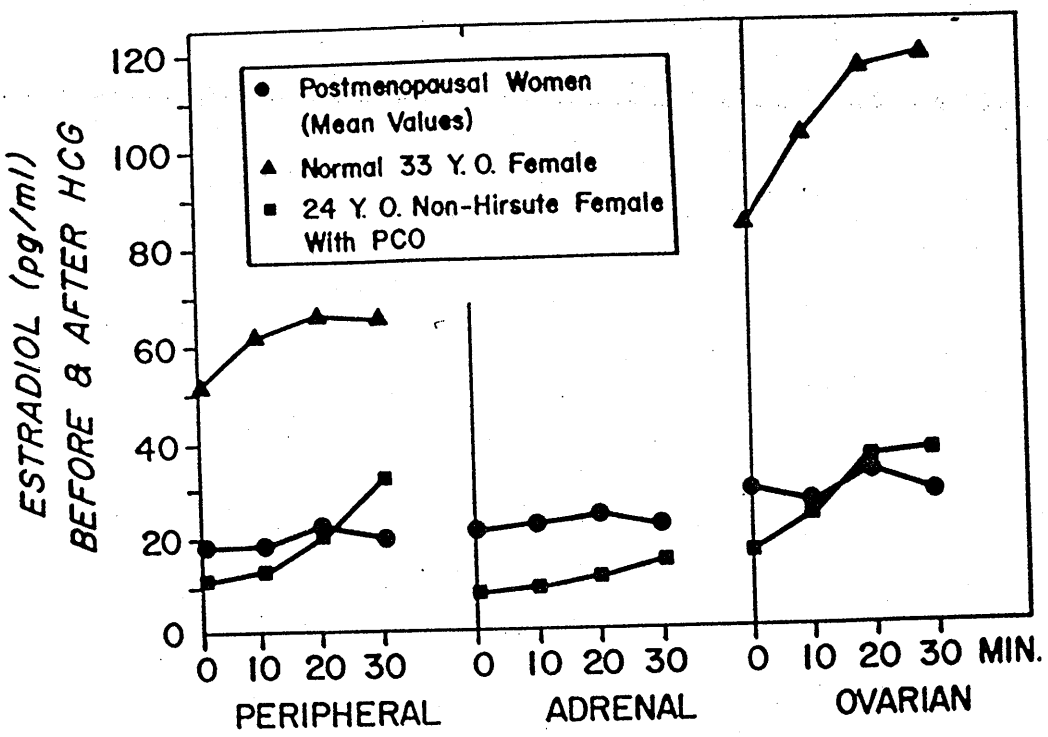
Common Estrogen Preparations Available for Clinical Use

Biologic or Natural Estrogens:

a. Conjugated equine estrogens (Premarin® orally, Premarin cream® for vaginal use, IV Premarin®).
b. Estrone sulfate (Evex®, Estratab®).
c. Estradiol—micronized (Estrace®)
—pellet implants (Estrapel®, Progynon®).*
d. Percutaneous estradiol (now under investigation in the United States).

*Available to physicians with an IND which may be obtained by applying to FDA.

FIGURE 6. Effect of 5,000 units of hCG intravenously on estradiol production by the normal female ovary. (▲--▲ = normal 33-year-old female; ●--● = postmenopausal woman (mean values); ■--■ = 24-year-old non-hirsute female with polycystic ovaries.[22])

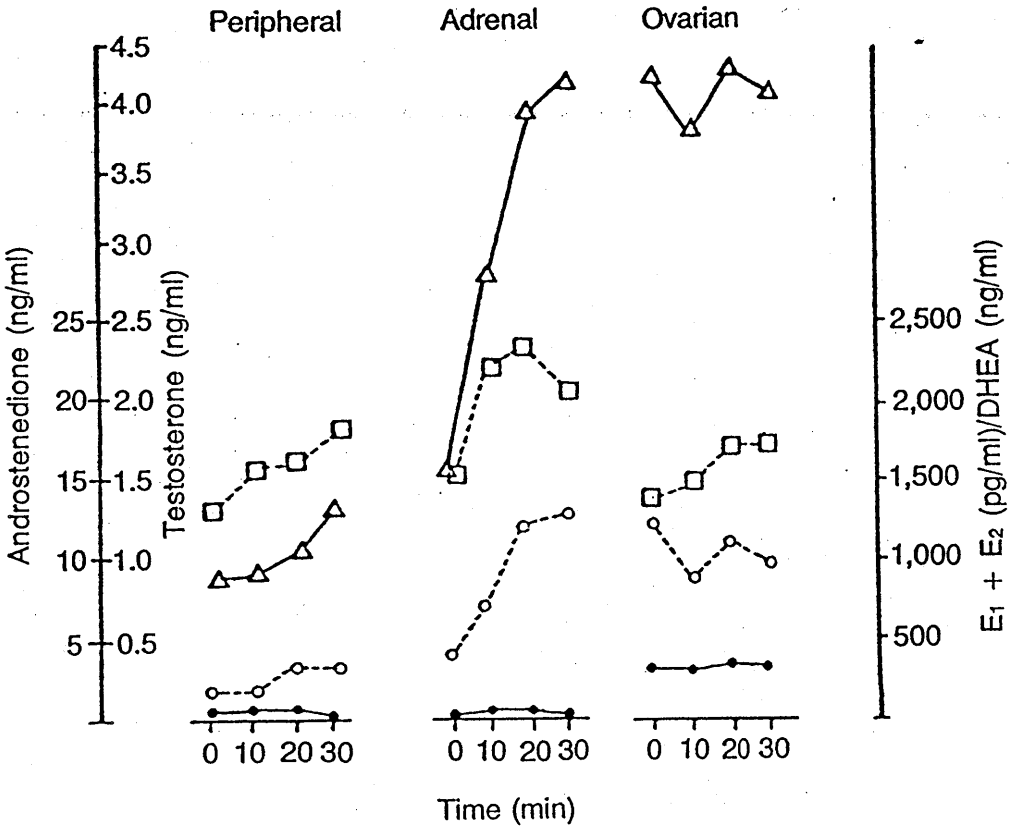


Synthetic Estrogens (do not occur naturally)

a. Diethylstilbestrol
b. Chlorotrianisene (Tace®)
c. Ethinyl estradiol (Estinyl®)
d. Quinestrol (Estrovis®)
e. Piperazine estrone sulfate (Ogen®)
f. Injectable estradiol compound (Delestrogen®, Depo-Estradiol®)
g. Creams (dinestrol)

The human female is programmed to utilize endogenous estrogens. Exogenously administered oral estrogens affect hepatic metabolism differently than do parenteral estrogens, because the latter avoid the first-pass effect within the liver. Synthetic oral es-

FIGURE 7. Effects of 40 units ACTH intravenously on levels of estradiol, testosterone, Δ^4-androstenedione and dehydroepiandrosterone in a young hirsute female with suspected polycystic ovaries.[12]

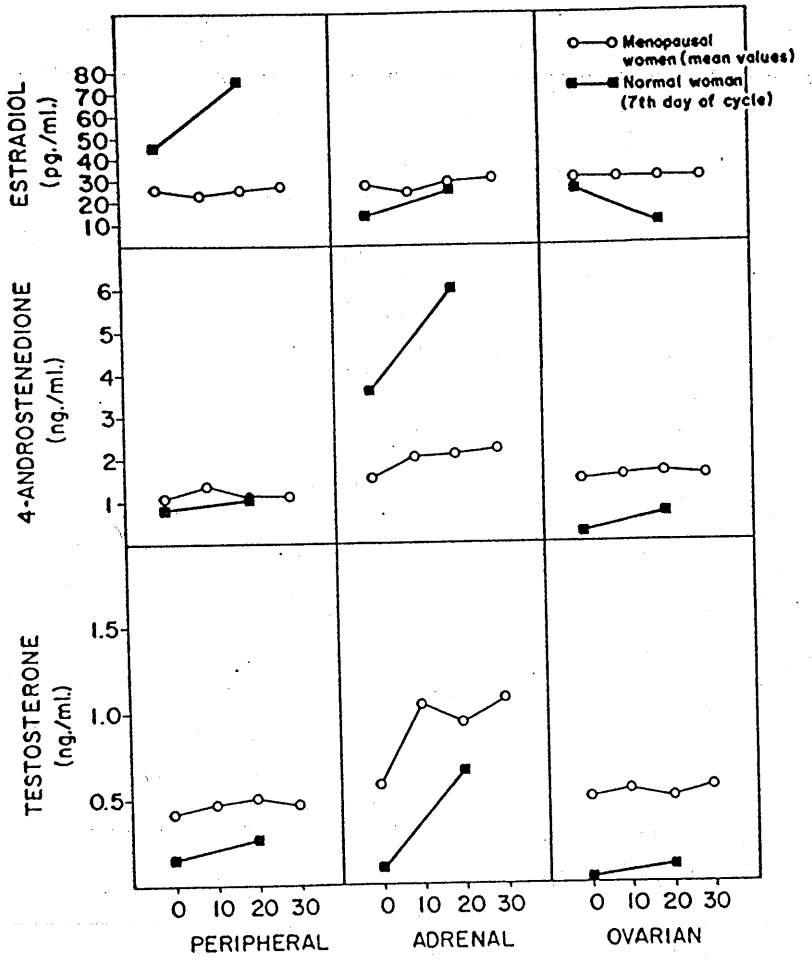


trogens place more of an added strain on liver function than their natural counterparts.[25] Estrogens increase sex-hormone-binding globulin, cortisol-binding globulin, and renin substrate in a dose-dependent fashion. Synthetic estrogens like stilbestrol and ethinyl estradiol provoke even greater responses.[26] Antithrombin III activity was more readily depressed by oral synthetic estrogens than by natural estrogens,[27] while no significant change in activity followed percutaneous administration of estrogens.[28] The major differences between the two routes is how they affect liver function, especially in the synthesis of plasma proteins, globulins, clotting factors, and lipoprotein moieties.

Indications for Estrogen Administration[29]

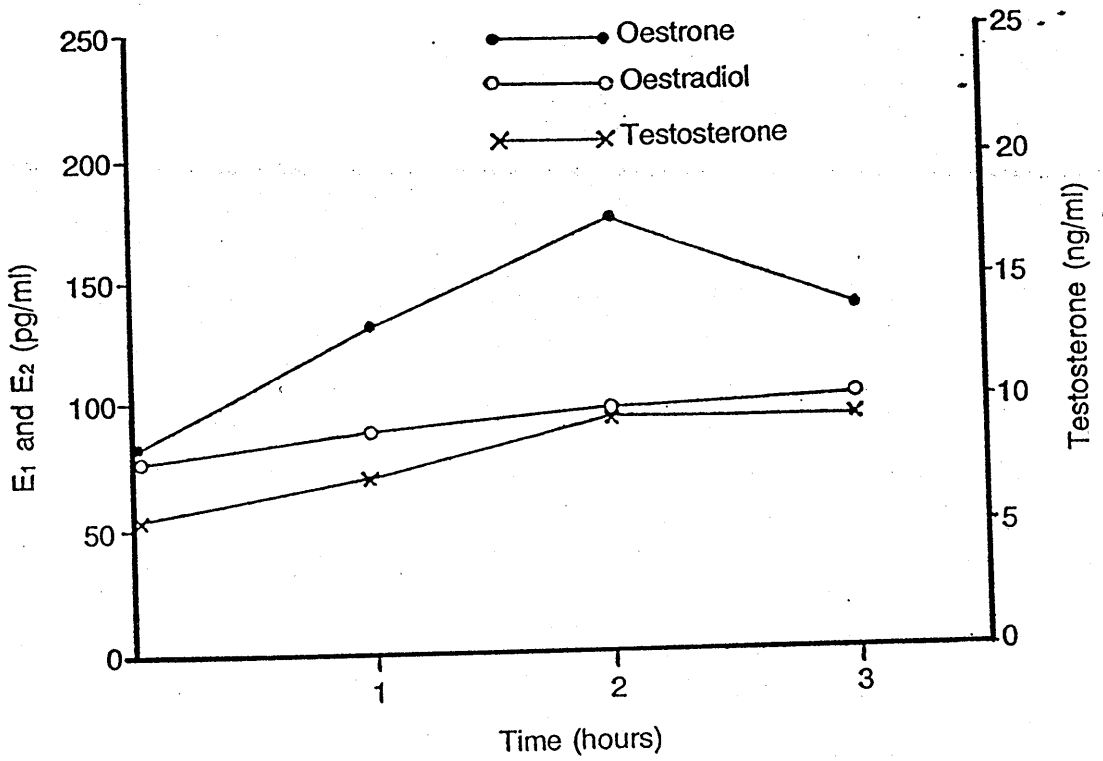
A. Estrogen deficiency states

FIGURE 8. Effect of 40 units ACTH intravenously on levels of estradiol, testosterone and Δ^4-androstenedione in normal women and menopausal women. (■--■ = normal women; o--o = menopausal women, mean values).[22]



1. a. Gonadal dysgenesis
   b. Hypothalamic and/or hypopituitary amenorrhea
   c. Premature ovarian failure
   d. Gonadotropin-resistant ovary syndrome
2. Menopausal syndrome
3. Estrogen deficiency osteopenia (osteoporosis)

FIGURE 9. Effect of 10,000 units of hCG intramuscularly on estrone, estradiol, and testosterone levels: mean values in 3 adult male volunteers.[22]



B. Pharmacologic use of estrogens in non-deficiency states
   1. To inhibit ovulation
      a. Severe dysmenorrhea (primary)
      b. Severe premenstrual tension states
      c. Mittelschmerz (middle-of-the-month pain)
      d. Contraception
   2. Acne and/or idiopathic hirsutism
   3. Arrest of excessive growth—"Tall Girl Syndrome"
   4. Dyspareunia due to atrophic vaginitis
   5. Urinary incontinence (non-bacterial; non-anatomic)
   6. Metastatic breast cancer and prostatic cancer
   7. Cardiovascular disease
   8. Arthritides
   9. Neuroendocrine disturbances—perimenopausal depression or emotional instability; migrainoid headaches; insomnia

TABLE 2. Estrone and estradiol levels (pg/ml) after injection of 100 mg testosterone cypionate to 2 ovariectomized women.

Treatment	Patient M.S.		Patient F.F.	
	Estrone	Estradiol	Estrone	Estradiol
Before	74.00	23.80	38.70	31.62
After 2 days	104.30	55.31	45.52	22.24
After 4 days	94.36	61.35	51.87	41.36
After 6 days	100.54	58.53	—	—

(Reproduced with permission from Greenblatt RB and Vasquez JM: Extragonadal sources of oestrogens. *Pharmatherapeutica* 2(Suppl 2):76, 1980.)

References

1. Brown JB: Relationship between urinary oestrogens and oestrogens produced in the body. *J. Endocrinol. 16*:202, 1957.
2. Longcope C, Kato T and Horton R: Conversion of blood androgens to estrogens in normal adult men and women. *J. Clin. Invest. 48*:2191, 1969.
3. Siiteri PK and MacDonald PC: Role of extraglandular estrogen in human endocrinology. In: Handbook of Physiology, Section 7, Vol. II, Part I, p. 615. RO Greep, EB Astwood, eds., American Physiological Society, Washington, DC, 1973.
4. Frieden EH, Patkin JK and Mills M: Effect of follicle stimulating hormone (FSH) upon steroid aromatization in vitro. *Proc. Soc. Exper. Biol. Med. 129*:606, 1968.
5. Longcope C: Steroid production in pre- and post-menopausal women. In: The Menopausal Syndrome. RB Greenblatt, VB Mahesh, PG McDonough, eds., Medcom Press, New York, 1974, p. 6.
6. Bulbrook RD and Greenwood FC: Persistence of urinary oestrogen excretion after oophorectomy and adrenalectomy. *Br. Med. J. 2*:662, 1957.
7. Feinberg R and Cohen RB: A comparative histochemical study of the ovarian stromal lipid band, stromal theca cell and ovarian follicular apparatus. *Am. J. Obstet. Gynecol. 92*:958, 1965.
8. Hammerstein J, Rice BG and Savard K: Steroid hormone formation in the human ovary. I. Identification of steroids formed in vitro from acetate 1-14C in the corpus luteum. *J. Clin. Endocrinol. Metab. 24*:597, 1964.
9. Mattingly RF and Huang WY: Steroidogenesis of the menopausal and postmenopausal ovary. *Am. J. Obstet. Gynecol. 103*:679, 1969.
10. Poortman J, Thijssen JHH and Schwarz F: Androgen production and conversion to estrogens in normal postmenopausal women and in selected breast cancer patients. *J. Clin. Endocrinol. Metab. 37*:101, 1974.
11. Yen SSC, Martin PL, Burnier AM, et al.: Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17-estradiol in postmenopausal women. *J. Clin. Endocrinol. Metab. 40*:518, 1975.
12. Greenblatt RB, Colle ML and Mahesh VB: Ovarian and adrenal steroid production in the postmenopausal woman. *Obstet. Gynecol. 47*:383, 1976.
13. Greenblatt RB, Natrajan PK, Aksu MF, et al.: The fate of a large bolus of exogenous estrogen administered to menopausal women. *Maturitas 2*:29, 1979.
14. Judd HL, Judd GE, Lucas WE, et al.: Endocrine function of the postmenopausal ovary: Concentration of androgens and estrogens in ovarian and peripheral vein blood. *J. Clin. Endocrinol. Metab. 39*:1020, 1974.
15. Greenblatt RB, Colle ML and Mahesh VB: Ovarian and adrenal steroid production in the postmenopausal woman. *Obstet. Gynecol. 47*:383, 1976.
16. Frank RT: A suggested test for functional cortical adrenal tumor. *Proc. Soc. Exper. Biol. Med. 31*:1204, 1933.
17. Gabrilove JL, Sharma DC, Wotz HH, et al.: Feminizing adrenocortical tumors in the male. A review of 52 cases, including a case report. *Medicine 44*:37, 1965.

18. Bongiovanni AM, Eberlein WR and Cara J: Studies on the metabolism of adrenal steroids in the andrenogenital syndrome. *J. Clin. Endocrinol. Metab. 14*:409, 1954.
19. Labhart A: Clinical Endocrinology. Springer-Verlag, New York, Heidelberg, Berlin, 1974.
20. Baird DT and Guevara A: Concentration of unconjugated estrone and estradiol in peripheral plasma in nonpregnant women throughout the menstrual cycle, castrated and postmenopausal women and in men. *J. Clin. Endocrinol. Metab. 29*:879, 1969.
21. Ruder HJ, Loriaux DL, Sherins RS, et al.: Leydig cell function in men with disorders of spermatogenesis. *J. Clin. Endocrinol. Metab. 38*:244, 1974.
22. Greenblatt RB and Vasquez JM: Extragonadal sources of oestrogens. *Pharmatherapeutica 2*:76, 1980.
23. Steinach S and Kun H: Transformation of male sex hormones into a substance with the action of a female hormone. *Lancet 2*:845, 1937.
24. Diczfalusy E: Endocrine functions of the human fetoplacental unit. *Fed. Proc. 23*:791, 1964.
25. Maschak CA, Lobo RA, Dozono-Takano R, et al.: Comparison of pharmacodynamic properties of various estrogen formulations. *Am. J. Obstet. Gynecol. 144*:511, 1982.
26. Geola Fl, Frumar AM, Tataryn IV, et al.: Biological effects of various doses of conjugated equine estrogens in postmenopausal women. *J. Clin. Endocrinol. Metab. 51*:620, 1980.
27. Notelovitz M: Clotting Factors and Oestrogen Replacement Therapy. In: Female and Male Climacteric. PA van Keep, DM Serr and RB Greenblatt, eds., MTP Press, Lancaster, England, 1979, p. 103.
28. Elkik F, Compel A and Mauvais-Jarvis P: In: The Controversial Climacteric. PA van Keep *et al.*, eds., MTP Press, Lancaster, England, 1982, p. 103.
29. Gambrell RD, Jr. and Greenblatt RB: The menopause: Indications for estrogen therapy. In: Current Problems in Obstetrics and Gynecology, Volume II, Number 5. Year Book Medical Publishers, Inc., Chicago, London, 1979.