

# Role of extraglandular estrogen in human endocrinology

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### Summary and Conclusions

OBSERVING THAT urine of stallions contained large quantities of estrogen, whereas that of mares had little estrogen, Zondeck (37) in 1934 stated, "I believe that the female hormone which is regularly present in the male organism represents a normal physiological product of the metabolism of the sex hormones, especially since—due to our present chemical knowledge—a conversion of the male hormone into the female one appears to be quite possible." Evidence supporting Zondeck's deduction was reported by Steinach & Kun (33) who in 1937 found that the administration of testosterone propionate increased estrogen in the urine of six men who received this androgen.

Since these early studies, many researchers have reported increased estrogen excretion after the administration of various  $C_{19}$  compounds, principally testosterone, in intact and castrate, male and female human beings and various experimental animals. The first definitive

evidence that the increased estrogen excretion was due to aromatization of the administered steroid was presented in 1956 by West and associates (36). They found estrone and estradiol in the urine of two adrenalectomized, oophorectomized women who had been administered testosterone propionate. Before treatment, estrogen could not be detected in urine from these subjects. This study provided strong support for the thesis that the increased amounts of estrogen found in urine after testosterone treatment arose from the conversion of testosterone to naturally occurring estrogens. Since then, many workers have also shown that the administration of isotopically labeled testosterone to normal men is followed by the appearance of radioactive estrogens in their urine [for review, see (1, 11, 21)].

More recently a number of laboratories have attempted to quantify the extent to which conversion of endogenous androgens contributes to extraglandular estrogen in the human being (3, 9, 15). The demonstration that placental estrogen production in the human being is totally dependent on externally provided  $C_{19}$  steroid precursors (30, 31) focused attention on this new mechanism of hormone production. It has become apparent from these studies that the peripheral conversion of androgens to estrogens in nonpregnant women and men is a physiologically important determinant of the hormonal milieu in both healthy and diseased states. This chapter begins with a description of the experimental approaches the authors have employed in analyzing qualitatively and quantitatively the mechanisms of origin of estrogen in the human being. An understanding of these techniques is essential to a discussion of the physiological studies that have been carried out in these laboratories over the past 10 years.

## EXPERIMENTAL DESIGNS

### Origin of Estrogen in Women

Quantitative studies of steroid dynamics have been made according to the methods reported by Gurgele

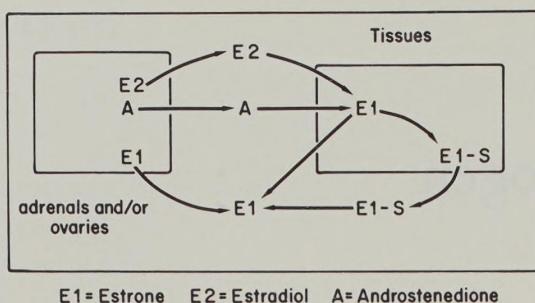


FIG. 1. Possible sources of estrone and estradiol in postmenopausal women. E1-S, estrone sulfate.

and associates (10), Tait & Burstein (34), and MacDonald and colleagues (18-22). Estrogen may arise in women either by glandular (adrenal or ovarian) secretion or by extraglandular aromatization of circulating C<sub>19</sub> precursors, as shown schematically in Figure 1. In order to quantitate estrogen production from circulating precursors, two determinations must be made: 1) the extent of conversion of plasma precursor to the product estrogen hormone, and 2) the amount of endogenous precursor entering the same compartment as the tracer available for this conversion (the plasma production rate of the precursor). It follows that the amount of estrogen produced from a given circulating precursor will equal the product of the amount of the precursor available and the fraction of that precursor converted to the product hormone.

Since little plasma testosterone is available for estrogen production in normal women and, further, since plasma testosterone is inefficiently converted to estradiol in women (0.15%), testosterone does not serve in women as a significant plasma precursor of estrogen (21). Plasma dehydroisoandrosterone and its sulfate ester are also poorly converted to estrogen and do not constitute a significant source of estrogen. However, androstenedione (A) is much more efficiently (1.3% or more) converted to the estrogen product hormone, estrone (E1), and is available normally in sufficient quantity (1.0-3.0 mg/day) to represent a sizable potential source of estrogen production (21). In women, therefore, a study of the origin of estrogen from plasma precursors is confined to the determination of the contribution of androstenedione to estrone production.

The estrone derived from circulating androstenedione may suffer any of three immediate fates: 1) all or part of the estrone thus produced may enter the circulation as free estrone; 2) estrone derived from plasma androstenedione may be released from its site of origin in a form that is eventually convertible to estrone (e.g., estrone sulfate); or 3) the estrone produced from plasma precursors may be in part immediately and irreversibly metabolized before entering the circulation and, accordingly, may not be available to the blood as estrone. Ample evidence for such an occurrence has been presented in the case of testosterone production from circulating androstenedione in the female, in which case testosterone glucuronide formed from androstenedione

in the liver is released into the circulation. In this event the amount of estrone derived from plasma androstenedione that actually enters the circulation as the free hormone would be less than the total amount of estrone produced via this mechanism. The techniques for quantifying each of these alternatives are described below.

**EXTENT OF CONVERSION OF PLASMA ANDROSTENEDIONE TO ESTRONE.** The extent of conversion of circulating androstenedione to estrone is determined as previously described (18, 21). A tracer dose of 6,7-<sup>3</sup>H-estrone and 4-<sup>14</sup>C-androstenedione is administered intravenously by continuous infusion, and the resulting <sup>3</sup>H-to-<sup>14</sup>C ratios of the urinary metabolites estrone, estradiol, and estriol, isolated from a 3-5-day urine collection are determined. The fraction of the administered 4-<sup>14</sup>C-androstenedione converted to <sup>14</sup>C-estrone, or the transfer constant,  $[\rho]_{BU}^{AE1}$ , is calculated as

$$[\rho]_{BU}^{AE1} = R^{I-3H-E1}/R^{I-14C-A} \div ^3H/^{14}C \text{ urinary E1}$$

where  $R^{I-3H-E1}$  is the amount of radioactivity (dpm <sup>3</sup>H) infused as 6,7-<sup>3</sup>H-estrone and  $R^{I-14C-A}$  is the amount of radioactivity (dpm <sup>14</sup>C) infused as 4-<sup>14</sup>C-androstenedione.

This calculation represents the fraction of injected 4-<sup>14</sup>C-androstenedione that was metabolized in the same manner as the injected tritium-labeled estrone. It must be emphasized that this calculation does not represent the fraction of injected 4-<sup>14</sup>C-androstenedione excreted as a <sup>14</sup>C-labeled urinary metabolite of estrone but rather reflects the total amount of circulating androstenedione converted to estrone at the tissue site, or sites, of aromatization.

**PLASMA PRODUCTION RATE OF ANDROSTENEDIONE.** The metabolic clearance rate (MCR) of androstenedione is calculated from the concentration of 4-<sup>14</sup>C-androstenedione per liter of plasma during the continuous intravenous infusion of 4-<sup>14</sup>C-androstenedione. The total dose administered is adjusted to represent the amount that would have been infused during a 24-hr period such that

$$MCR_A (\text{liter}/24 \text{ hr}) = \frac{R^{I-14C-A} \cdot 1,440 \text{ min}/\text{min of infusion}}{\text{dpm } ^{14}C-A/\text{liter plasma}}$$

The plasma production rate of androstenedione,  $PR^P-A$  (expressed as mg/24 hr), equals  $C_A \cdot (MCR_A)$ , where  $C_A$  is the concentration of endogenous circulating androstenedione ( $\mu\text{g}/\text{liter plasma}$ ) and  $MCR_A$  is the metabolic clearance rate of androstenedione (liter/24 hr).

**PRODUCTION RATE OF ESTRONE FROM ANDROSTENEDIONE.** If the amount of circulating androstenedione produced and the fraction converted to estrone are known, the amount of estrone derived by the conversion can be calculated

$$PRE1_A = (PR^P-A) \cdot [\rho]_{BU}^{AE1}$$

**TOTAL ESTRONE PRODUCTION.** The total daily production rate of estrone (PRE1) is determined by the usual isotope

dilution method from the specific activity of urinary estrone glucuronoside

$$\text{PRE1} = \frac{\text{R}^{1-3}\text{H-E1}}{\text{sa-}^3\text{H-E1}} \cdot t$$

where  $\text{R}^{1-3}\text{H-E1}$  is administered dose of radioactivity (dpm 6,  $7-^3\text{H-E1}$ ),  $\text{sa-}^3\text{H-E1}$  is specific activity of urinary estrone referable to tritium (dpm/ $\mu\text{g}$ ), and  $t$  is time in days of urine collection.

**PLASMA PRODUCTION OF ESTRONE DERIVED FROM ANDROSTENEDIONE.** The quantity of estrone derived from circulating androstenedione ( $\text{PRE1}_{\text{PA}}$ ) that immediately enters the circulation and likely represents a minimal estimate of the amount of physiologically available estrogen can be calculated by using the value of  $[\rho]_{\text{BB}}^{\text{AE1}}$  as

$$\text{PRE1}_{\text{PA}} = (\text{PR}_{\text{PA}}) \cdot [\rho]_{\text{BB}}^{\text{AE1}}$$

where  $[\rho]_{\text{BB}}^{\text{AE1}}$  is the blood transfer constant calculated from the  $^3\text{H}$ -to- $^{14}\text{C}$  ratio of estrone isolated from plasma obtained during the infusion and the isotope ratio of the infused mixture of tracers as described earlier for  $[\rho]_{\text{BU}}^{\text{AE1}}$ .

**PRODUCTION OF ESTRONE SULFATE FROM PLASMA ANDROSTENEDIONE.** Recent studies have indicated that estrone sulfate (E1S), as well as free estrone, may be derived from circulating androstenedione. Exactly the same experimental design is used to investigate this possibility when  $^3\text{H}$ -estrone sulfate is used in place of  $^3\text{H}$ -estrone. Calculations of  $\text{PRE1S}$ ,  $\text{PRE1S}_{\text{A}}$ , and  $\text{PR}_{\text{PA}}^{\text{E1S}}$  are made by expressions analogous to those described above.

It is evident that estrone may be produced by *a*) glandular biosynthesis and secretion of estrone, *b*) aromatization of circulating androstenedione, *c*) formation of estrone from plasma precursors other than androstenedione, or *d*) formation of estrone via the metabolism of estradiol that is secreted or is produced independently of estrone. It therefore follows, that, if  $\text{PRE1}$  is equal to  $\text{PRE1}_{\text{A}}$ , one must conclude that these other possible mechanisms of estrone production are not operative. Most important, one would conclude that little, if any, estradiol produced independently of estrone was being contributed either by secretion or by formation from other plasma precursors. This would be true since it is known that estradiol is metabolized principally via estrone (7, 10). Estrone-independent estradiol formation either by secretion or formation from another plasma precursor would increase the value of  $\text{PRE1}$  but not the production of estrone from plasma androstenedione,  $\text{PRE1}_{\text{A}}$ . On the other hand, if  $\text{PRE1}$  is greater than  $\text{PRE1}_{\text{A}}$ , one must conclude that a source of estrone or estradiol other than the aromatization of plasma androstenedione exists.

For the purposes of achieving the goals of these studies, it is imperative that the production rate of the estrogen precursor, androstenedione, be determined as its plasma production rate. On the other hand, in order to determine the contribution of plasma androstenedione aromatization to total estrone production (PRE1), it is necessary

to measure both the extent of conversion of plasma androstenedione to estrone,  $[\rho]_{\text{BU}}^{\text{AE1}}$ , and  $\text{PRE1}$  from the isotope ratio and the specific activity with respect to tritium of urinary estrone. Obviously each of these parameters must be measured in the same individual before conclusions concerning the magnitude of glandular secretion can be drawn. Thus the analysis of estrogen production using the plasma production rate of precursor and the urinary producton rate of the product is appropriate and correct. The use of this unique experimental design allows a comparison of the relative quantitative contribution of each mechanism of origin of estrone to total estrone production. Specifically, this experimental design obviates the necessity of measuring either the plasma concentration or metabolic clearance rate of estrone and yet permits the calculation of the plasma production rate of estrone derived from circulating androstenedione (16).

In postmenopausal women (see below)  $\text{PRE1}$  and  $\text{PRE1}_{\text{A}}$  are essentially equal. Accordingly, in these subjects, the measurement of the total estrone production rate represents the measurement of total estrogen production with respect to the estrone-estradiol system. The independent formation of estriol cannot be excluded on the same theoretical grounds but could be detected by a comparison of the specific activities of urinary estrone and estriol.

#### *Origin of Estrogen in Men*

The analysis of estrogen production in the male is more complex because of the much greater availability of testosterone for conversion to estradiol. As noted above, in females, this potential source of estradiol can be ignored except in the presence of a testosterone-secreting tumor.

Of importance in the physiological model utilized for the present experimental design is the fact that estradiol suffers its principal metabolic fate via estrone when estimated by urinary metabolites, that is,  $[\rho]_{\text{BU}}^{\text{E2E1}}$  is usually greater than 90 %, and that a lesser but sizable fraction of estrone is metabolized via estradiol (7, 10). Therefore a consideration of the total daily production rate of estrone as measured by the urinary method takes into account not only estrone produced by secretion or peripheral production but, additionally, that estrone derived from secreted or peripherally formed estradiol. Similarly, estradiol may arise either by direct estradiol production or indirectly from the estrone that is metabolized via estradiol. The experimental model illustrated in Figure 4 was assumed to represent the physiological situation, and the experimental design detailed below was formulated accordingly.

Estrone and estradiol production rates were measured after the simultaneous administration of a tracer dose of  $^3\text{H}$ -labeled estrone and a tracer dose of  $^{14}\text{C}$ -labeled estradiol. The daily production rate of estrone was calculated using the specific activity of urinary estrone (glucuronoside) relative to  $^3\text{H}$ . The daily production

rate of estradiol was calculated from the specific activity of urinary estradiol (glucuronoside) relative to  $^{14}\text{C}$ . Also, from a comparison of the  $^3\text{H}$ -to- $^{14}\text{C}$  ratio of urinary estrone of urinary estradiol with that of the administered tracers, the fraction of estrone suffering its metabolic fate via estradiol,  $[\rho]_{\text{BU}}^{\text{E}1\text{E}2}$ , and that of estradiol metabolized via estrone,  $[\rho]_{\text{BU}}^{\text{E}2\text{E}1}$ , were calculated (20). The total daily production rates of estrone (PRE1) and estradiol (PRE2), as measured by these techniques, were then compared with the amount of estrone calculated to be derived from precursors (androstenedione and estradiol), as well as the amount of estradiol derived from its precursors (testosterone and estrone).

The yield of estrone from circulating androstenedione was determined as described above. The daily production of estradiol derived from plasma testosterone was calculated as follows. In order not to overestimate the contribution of testosterone to estradiol production, the extent of conversion of testosterone to estradiol must be corrected for that derived via the sequence testosterone  $\rightarrow$  androstenedione  $\rightarrow$  estrone  $\rightarrow$  estradiol. Thus

$$d[\rho]_{\text{BU}}^{\text{TE}2} = [\rho]_{\text{BU}}^{\text{TE}2} - [[\rho]_{\text{BB}}^{\text{TA}} \cdot [\rho]_{\text{BU}}^{\text{AE}1} \cdot [\rho]_{\text{BU}}^{\text{E}1\text{E}2}]$$

The  $d[\rho]_{\text{BU}}^{\text{TE}2}$  value multiplied by the daily plasma production rate of testosterone ( $\text{PR}_{\text{P-T}}$ ) equals the production rate of estradiol derived directly from plasma testosterone ( $\text{PRE2}_T$ ).

The amount of estrone production derived from both plasma precursors ( $\text{PRE1}_{\text{A+T}}$ ) then equals the production rate of estrone from circulating androstenedione ( $\text{PRE1}_A$ ) plus the fraction of the production rate of estradiol derived from plasma testosterone that is metabolized via estrone, which is equal to  $(\text{PRE2}_T) \cdot [\rho]_{\text{BU}}^{\text{E}2\text{E}1}$ . Therefore

$$\text{PRE1}_{\text{A+T}} = \text{PRE1}_A + \text{PRE2}_T \cdot [\rho]_{\text{BU}}^{\text{E}2\text{E}1}$$

Similarly the production of estradiol from the two circulating precursors ( $\text{PRE2}_{\text{T+A}}$ ) equals the production rate of estradiol derived from testosterone ( $\text{PRE2}_T$ ) plus the fraction of the production rate of estrone that is ultimately metabolized via estradiol,  $(\text{PRE1}_A) \cdot [\rho]_{\text{BU}}^{\text{E}1\text{E}2}$ . Therefore

$$\text{PRE2}_{\text{T+A}} = \text{PRE2}_T + \text{PRE1}_A \cdot [\rho]_{\text{BU}}^{\text{E}1\text{E}2}$$

The calculated production of estrone and of estradiol from the two circulating precursors can then be compared with the total daily estrone production and total daily estradiol production as independently measured via the urinary isotope dilution techniques previously described.

Metabolic clearance rates of androstenedione and testosterone and transfer constants for their conversion to estrone and estradiol, as measured in plasma and urine, were determined by techniques previously described. Urinary production rates of both estrogens and the extent of their interconversion were calculated according to Gurpide et al. (10).

It should be pointed out that  $[\rho]_{\text{BB}}^{\text{TA}}$  is representative of the conversion of plasma testosterone to plasma androstenedione, whereas the  $[\rho]_{\text{BU}}^{\text{E}1\text{E}2}$  and the  $[\rho]_{\text{BU}}^{\text{E}2\text{E}1}$  values are calculated on the basis of urinary data and do not represent the interconversion of plasma estrone and plasma estradiol. This is, however, appropriate since the precursor contribution is from the plasma product, whereas the total production of estrogen is calculated from urinary data irrespective of the plasma origin of precursor. The contribution of each precursor to total estrogen production is reflected by the  $^3\text{H}$ -to- $^{14}\text{C}$  ratio and specific activity of the urinary metabolite. The experimental design detailed herein obviates the necessity of measuring plasma concentrations or MCR's of estrone and estradiol and yet permits a calculation of the blood production rates of estrone and estradiol derived from their respective circulating precursors.

#### NORMAL VALUES

##### *Premenopausal Women*

The extent of conversion of circulating androstenedione to estrone in nonpregnant subjects of the premenopausal age group ranges from 0.6 to 1.9% as indicated in Table 1. The average conversion in six normal ovulating women was 1.3%. The same conversion of plasma androstenedione to estrone was also observed in castrate and adrenalectomized women, and therefore estrogen produced by this mechanism is considered to be "extra-glandular." The average conversion of androstenedione to estrone in all premenopausal patients, including normal, castrate, and adrenalectomized patients, those with increased levels of androstenedione (e.g., patients with arrhenoblastoma and polycystic ovarian disease), and those with decreased levels of circulating androstenedione, was not significantly different from that in normal subjects, which averaged 1.2%.

Several groups of investigators have reported the concentration of androstenedione to be in the range of 0.1–0.15  $\mu\text{g}/100\text{ ml}$  of plasma with no major changes occurring during the menstrual cycle. The mean value recorded in this laboratory is 0.14  $\mu\text{g}/100\text{ ml}$ , which together with a MCR of approximately 2,000 liter/day yields a plasma production rate of androstenedione of

TABLE 1. Extent of Conversion of  $^{14}\text{C}-\Delta^4$ -Androstenedione to  $^{14}\text{C}$ -Estrone

Subject	Age	Number	Extent of Conversion, %	
			Range	Average
Normal	21–37	6	1.0–1.7	1.3
Castrate	29;32	2	1.1;1.3	1.2
Adrenalectomized	25;37	2	1.1;1.7	1.4
All premenopausal patients*	17–39	28	0.6–1.9	1.2

\* Premenopausal subjects include normal, castrate, and adrenalectomized individuals, as well as patients with arrhenoblastoma, polycystic ovarian disease, and gonadal dysgenesis.

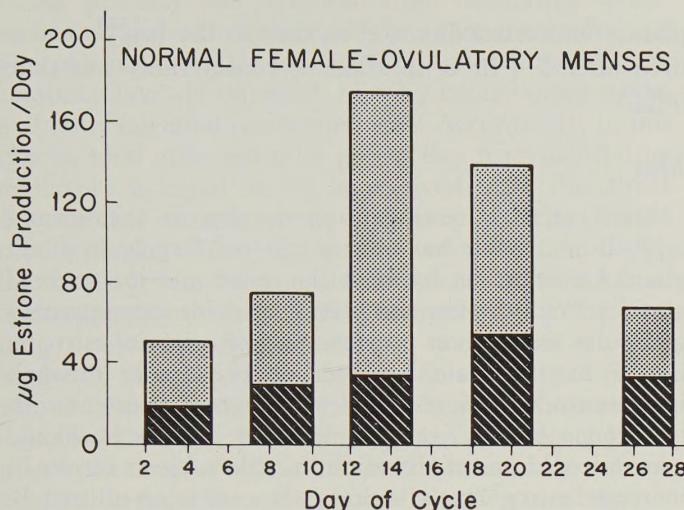


FIG. 2. Sources of estrogen production during a normal ovulatory menstrual cycle. Total height of bars represents sum of estrone and estradiol production; the hatched portion, that amount derived from plasma androstenedione.

about 3 mg/day. On the basis of analyses of both adrenal and ovarian vein plasma it has been estimated that the adrenals contribute about two-thirds and the ovaries one-third of the total androstenedione secretion (see chapter by Tagatz and Gurpide in this volume of the *Handbook*). Thus the conversion of androstenedione to estrone normally contributes about 40  $\mu\text{g}/\text{day}$  to total estrogen production in premenopausal women.

The contribution to total estrone production made by the conversion of circulating androstenedione to estrone in nonpregnant, premenopausal subjects is diagrammatically illustrated in Figure 2. In this diagram the total daily estrone production found in a normal female during various phases of an ovulatory cycle is represented by the total height of each bar. The portion of total estrone-estradiol production contributed by the utilization of circulating androstenedione is indicated by the hatched part of each bar. It is evident that from 10 to 50% of the total estrone production arose from the utilization of plasma androstenedione in this subject. The remainder of estrone production is derived principally from ovarian secretion of estradiol, which is peripherally metabolized via estrone and thereby contributes to its production rate. Thus it can be seen that the extraglandular formation of estrone from androstenedione results in a relatively constant basal level of estrogen upon which is superimposed the fluctuating secretion of estradiol by the developing follicles or corpus luteum, or both.

#### Postmenopausal Women

Until recently it was generally held that the human adrenal glands secrete significant amounts of estrogens, even though little direct evidence for this belief had been provided. Numerous studies in postmenopausal and oophorectomized women demonstrated that such individuals continue to excrete measurable amounts of urinary estrogens. The adrenal glands were implicated

as the source of these estrogens since the administration of ACTH caused an increase, whereas adrenalectomy led to a decrease, in the levels of urinary estrogens. For example, Brown et al. (6) found that total urinary estrogen excretion by an oophorectomized woman exceeded that observed for ovulatory women after 2 days of treatment with ACTH. Also, some direct evidence for adrenal secretion of estrogens was recently reported by Baird et al. (4) who demonstrated higher estrone levels in adrenal venous blood than in peripheral blood after ACTH administration in two of the three subjects. However, since no differences were noted under basal conditions the significance of these findings is presently unclear.

Several observations suggested an alternative mechanism of estrogen production in the postmenopausal woman. As indicated earlier, the conversion of parenterally administered radioactive androgens to urinary estrogen in nonpregnant women had been demonstrated by several workers. These qualitative findings, together with the demonstration of the quantitative importance of adrenal "androgen" precursors for placental estrogen production during pregnancy (30, 31), led to the hypothesis that extraglandular estrogen production in the human being may arise from C<sub>19</sub> steroid precursors that are normally present in the peripheral circulation (21).

In Figure 3, data obtained in six endocrinologically normal women are summarized. The blood production rates of androstenedione (mean = 1.75 mg/24 hr) in postmenopausal women are about one-half those reported for premenopausal women in the follicular phase of the menstrual cycle (3, 9). The average extent of conversion of plasma androstenedione to estrone in normal postmenopausal women is about 2.7% as compared to the average value of 1.3% in premenopausal women. An increase in conversion of circulating precursors to estrogen (androstenedione  $\rightarrow$  estrone and testosterone  $\rightarrow$

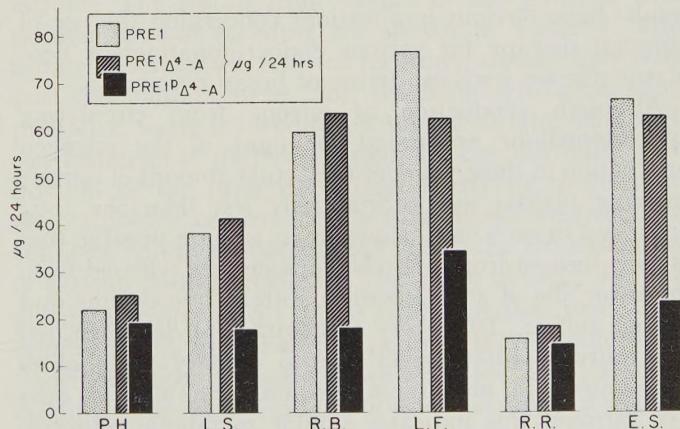


FIG. 3. Estrogen production from circulating androstenedione in normal postmenopausal women. PRE1, total estrone production; PRE1 $\Delta^4$ -A, production rate of estrone from plasma androstenedione; and PRE1 $P\Delta^4$ -A, amount of estrone derived from androstenedione that enters the plasma as free estrone. As indicated in text, formation and release of estrone sulfate likely accounts for difference between PRE1 $\Delta^4$ -A and PRE1 $P\Delta^4$ -A.

estradiol) with advancing age has been observed in male subjects, as well as in certain postmenopausal women who have evidence of excessive estrogen production (see below). In this connection it is of interest that other workers have reported a steady rise in urinary estrogen excretion after the menopause (25, 26). An increase in the value of  $[\rho]_{\text{BU}}^{\text{AEI}}$  with age could provide a plausible explanation for this paradoxical phenomenon. The average production rate of estrone arising from this conversion is 45  $\mu\text{g}/\text{day}$ , and, as is expected, this value is considerably lower than the values found at midcycle in premenopausal women by Goering & Herrmann (8). Also, as one would expect, this value is considerably lower than the urinary estradiol production rates measured in postmenopausal women treated with ACTH, as reported by Barlow et al. (5). However, the recent report by Longcope (13) in which the blood production rate of estrone in postmenopausal women estimated from mean values of MCR and plasma estrone levels was calculated to be about 40  $\mu\text{g}/\text{day}$  is in agreement with our findings. Earlier estimates of blood production rates of estrone in both men and women were much greater, due presumably to overestimation of both endogenous levels and the metabolic clearance rate (3, 15).

Of more importance, however, is the striking agreement between the production rate of estrone derived from androstenedione and the total estrone production rate (45.4 vs. 46.3  $\mu\text{g}/24 \text{ hr}$ , respectively). Thus, within the limits of technical error, which can be estimated to be about 15% in each experiment, it must be concluded that the major source of estrogen in the postmenopausal female is peripheral formation of estrone from plasma androstenedione and not ovarian or adrenal secretion. That the results obtained in the absence of ovaries (subjects L.F. and R.R.) were essentially the same as those obtained in the other four subjects strengthens this conclusion. Longcope (13) recently has reached a similar conclusion in apparently normal postmenopausal women by using a quite different experimental approach. These results have obvious implications concerning the use of ablation therapy for various endocrinopathies or neoplastic disease, such as uterine or breast carcinoma.

Although production of estrone from circulating androstenedione accounted for most of the estrogen production in these women, the actual amount of estrone entering plasma was substantially less than the total produced in each case. However, it is quite possible that estrone formed from androstenedione was released from the tissue site of aromatization both as free estrone and estrone sulfate. Twombly & Levitz (35) demonstrated that estrone sulfate gives rise to urinary metabolites conjugated with glucuronic acid, much the same as does free estrone; this indicates similar metabolic patterns. More recently it has been shown that, although estrone sulfate gives rise to free estrone in blood, it has a much lower metabolic clearance rate in both males and females than does estrone (14, 27). Taken together these observations would explain the finding that total estrone production can be accounted for by the conversion of

plasma androstenedione, even though the  $[\rho]_{\text{BB}}^{\text{AEI}}$ , measured after 3–4 hr of infusion, is considerably less than  $[\rho]_{\text{BU}}^{\text{AEI}}$ .

### Men

Until recently, estrogen production in the normal adult human male has been attributed largely to direct glandular secretion by both the testes and the adrenal cortices. With but few exceptions the evidence suggesting glandular secretion as a mechanism of origin of estrogen in the human male has accrued principally through indirect studies, the results of which were consistent with this concept. For example, whereas human chorionic gonadotrophin administration to male subjects results in increased estrogen production rates, castration ultimately leads to a reduction in urinary estrogen excretion. The results of similar physiological studies in which ACTH has been administered have suggested that the adrenal cortex may also be a significant source of estrogen in the normal adult male. Isolated reports of the *in vitro* conversion of isotopically labeled androgens to estrogen in human testicular and neoplastic adrenal tissue have lent support to the concept of a glandular source of estrogen in normal males. Although direct experimental evidence for estrogen secretion by the human testis recently has been obtained by estrogen measurements in spermatic vein blood in several laboratories (11, 17), the quantitative importance of such secretion remains controversial.

An analysis of estrogen production in a normal male, as carried out by the authors, is shown in Figure 4. The daily production rate of androstenedione was 2.5 mg/day, of which 1.7% was converted to the product estrogenic hormone, estrone, yielding 42  $\mu\text{g}/\text{day}$ . In the same subject, 7.0 mg testosterone was produced per day, 0.35% of which was converted directly to the product hormone, estradiol. This yielded 20  $\mu\text{g}$  estradiol from plasma testosterone. In turn, 98% (18  $\mu\text{g}$ ) of the estradiol so produced suffered its ultimate metabolic fate via estrone and resulted in new entry of steroid into the estrone compartment. Accordingly, in this subject, 60  $\mu\text{g}$

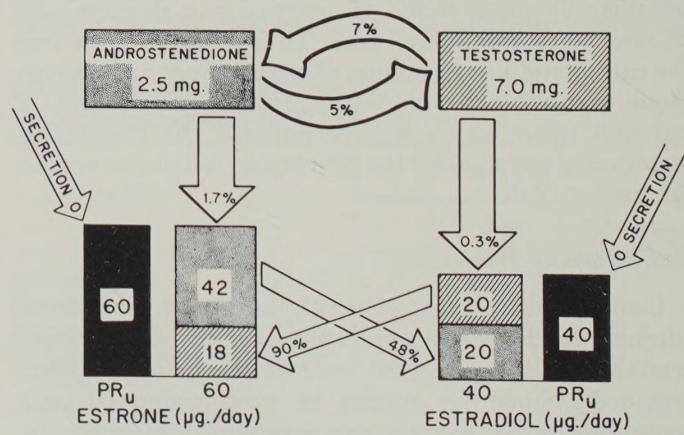


FIG. 4. Analysis of androgen and estrogen production in normal male. PR<sub>u</sub> = the total production rate measured from the specific activity of a urinary metabolite. [From MacDonald et al. (19).]

estrone per day was produced from circulating testosterone and androstenedione. Of the 42 µg estrone derived from plasma androstenedione, 48% suffered its ultimate metabolic fate via estradiol, thereby contributing to the total daily estradiol production rate. Accordingly, in this subject, total daily estradiol production from circulating precursors is equal to 20 µg derived from the direct conversion of testosterone to estradiol plus 20 µg derived from androstenedione via estrone, or a total of 40 µg/day. If the production rates of estrone and estradiol from circulating precursors are then compared with the total daily production rate of estrone and estradiol independently measured from the specific activity of urinary estrone and estradiol, it can be seen that these are virtually identical. Accordingly, in this subject, 100% of estrone and of estradiol production could be accounted for from the utilization of plasma precursors.

Very similar results have been obtained in similar studies of four normal men. Specifically, in these normal men, 98–105% of total daily estrone production is accounted for by the utilization of plasma androstenedione and testosterone, whereas 80–100% of estradiol production is similarly explained. Thus it must be concluded that in the absence of chromosomal abnormalities (see below) the testes of the normal male secrete only small amounts of estradiol. Earlier indirect estimates of secretion by Longcope et al. (15) indicated that as much as 50–70% of total estrone and estradiol production results from secretion. However, these workers used mean values for production rates of precursors and transfer constants, which likely accounts for the discrepancy. More recently, Kelch et al. (11) by direct measurement have estimated that testicular estradiol secretion amounts to about 10 µg/day or approximately one-fourth of the total production rate. Analysis of similar data published by Longcope et al. (17) suggests that testicular estradiol secretion amounts to 40 µg/day, which would account for virtually total estradiol production. However, the mean values reported for spermatic vein plasma concentrations of both testosterone and estradiol differ greatly in these studies. Kelch et al. (11) reported mean values of 1,049 pg/ml and 712 ng/ml, whereas Longcope reported values of 341 pg/ml and 58 ng/ml for estradiol and testosterone, respectively. Although it is not known whether these differences are due to methodological difficulties, it is apparent that the exact extent of testicular estradiol secretion in the male remains uncertain.

#### PHYSIOLOGICAL MANIFESTATIONS OF EXTRAGLANDULAR ESTROGENS

##### *Postmenopausal Bleeding*

The gynecologist commonly observes women who have clinical evidence of increased estrogen production after the menopause. This may be evident by lack of postmenopausal symptomatology combined with evidence of vaginal epithelial growth, cornification of exfoliated cells of the vaginal epithelium, production of clear

TABLE 2. *Estrogen Production in Postmenopausal Patients with Uterine Bleeding*

Patient	Age	Weight, lb	MCRA <sub>A</sub> , <sup>*</sup> liter/day	C <sub>A</sub> , <sup>†</sup> µg/ liter	PRA <sub>A</sub> , <sup>‡</sup> mg/day	[ρ] <sub>BU</sub> <sup>AE1§</sup> %	PRE <sub>E</sub> , <sup>  </sup> µg/day	PRE <sub>A</sub> , <sup>#</sup> µg/day
D.H.	73	195	1,992	0.57	1.14	4.4	57	47
B.B.	57	363	2,656	1.04	2.76	9.2	230	240
A.W.	69	200	1,924	0.98	1.89	5.7	96	101
A.O.	63	255	2,928	0.85	2.49	4.3	86	101
E.S.	63	185	2,548	1.04	2.65	2.0	49	50
<i>Mean</i>								
	65	240	2,410	0.90	2.19	5.1	104	108

\* Metabolic clearance rate of androstenedione. † Concentration of endogenous circulating androstenedione. ‡ Total daily production rate of androstenedione. § Extent of conversion of androstenedione to estrone. || Total daily production rate of estrone. # Quantity of estrone derived from circulating androstenedione.

cervical mucus in abundant quantities, growth of uterine myomata, and of course proliferation of the endometrium to the extent that vaginal bleeding occurs. Since estrogen in postmenopausal women is derived exclusively via the extraglandular aromatization of plasma androstenedione, it is apparent that alterations in estrogen production may arise via at least two mechanisms involving this system. First, there may be increases in the extent of conversion of androstenedione to estrone or estrone sulfate, or both. Alternatively, there may be increased production of plasma androstenedione for conversion to estrogen. In either of these circumstances, if the estrone produced is ultimately available to the endometrium in increased quantities, endometrial proliferation or hyperplasia, or both, may ultimately give rise to postmenopausal bleeding. Many such patients have been observed and studied in the authors' laboratories. Most commonly, increased endogenous estrogen production of the postmenopause is the result of increased extraglandular conversion of androstenedione to estrone. The results from studies of five patients with postmenopausal bleeding are shown in Table 2. This group of women was selected for study because they were postmenopausal, were not receiving estrogens, and presented vaginal bleeding of uterine origin. Although the histological patterns varied, as did the daily production of the principal estrogen, estrone, a constant endocrinologic occurrence was that the estrone produced could be completely accounted for by the aromatization of plasma androstenedione. The value of [ρ]<sub>BU</sub><sup>AE1</sup> for this group of women (5.1%) is approximately twice that obtained from similar studies performed on nonbleeding, "normal" postmenopausal women (see Fig. 3). Accordingly the estrone production in this group is slightly more than double that of the asymptomatic postmenopausal women. This higher value of [ρ]<sub>BU</sub><sup>AE1</sup> appears to be due to the fact that this group has a mean weight 100 lb heavier than the normal postmenopausal group. As is pointed out below, an increase in conversion of plasma

androstenedione to estrone has also been observed in patients with liver disease and diabetes.

Elevated estrone production resulting in postmenopausal bleeding due to increased availability of precursor androstenedione for extraglandular conversion to estrone has also been observed as a clinical entity. This particular clinical problem arises in women with polycystic ovarian disease (see below) and in instances of cortical stromal hyperplasia of the ovary associated with nonsecretory ovarian tumors, such as the pseudomucinous cystadenoma (Fig. 5), or secretory tumors, such as the lipoïd cell tumor (Fig. 6). In these and similar cases the increased extraglandular estrogen resulted from a marked increase in the secretion of androstenedione by the stromal cells of the ovary contiguous to the nonsecretory ovarian tumor and not from the tumor itself. Thus, with increased availability of substrate without necessarily an increase in extent of conversion, markedly increased quantities of extraglandular estrogen may give rise to clinical problems associated with hyperestrogenism.

The possible role of extraglandular estrogen in the regulation of metabolic functions, such as calcium metabolism, in postmenopausal women is unknown. At this time one can only phrase questions and give speculative answers. Estrogen may play an important role in decreasing calcium resorption from bone by its ability to block parathyroid hormone action (2). Is the osteoporosis of the menopausal woman due to deficient extraglandular estrone formation? An increase in the extent of conversion of androstenedione to estrone is frequently, but not always, observed after the menopause. Is it possible that unknown compensatory mechanisms increase the extraglandular mechanisms of aromatization after cessation of ovarian estradiol secretion? A strong positive correlation between  $[P]_{BU}^{AE1}$  and body weight has been observed in

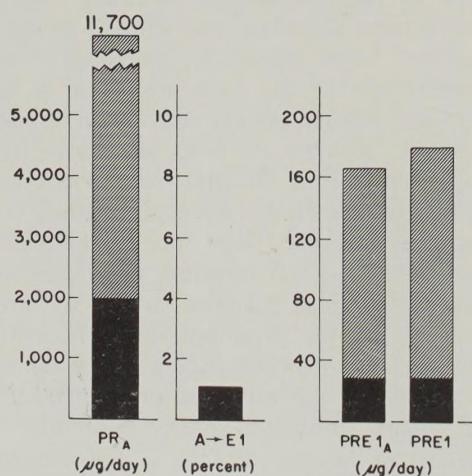


FIG. 5. Analysis of estrogen production in a 57-year-old female, 18 years postmenopausal, with ovarian mucinous cystadenocarcinoma and concomitant luteinization of adjacent uninvolved stroma. The latter resulted in the almost sixfold increase in the daily production of androstenedione ( $PR_A$ ), the aromatization of which totally accounted for the fourfold increase of daily estrone production ( $PRE1$ ). Solid portions of each bar represent normal values.  $PRE1_A$ , production rate of estrone derived from circulating androstenedione; A, androstenedione; E1, estrone.

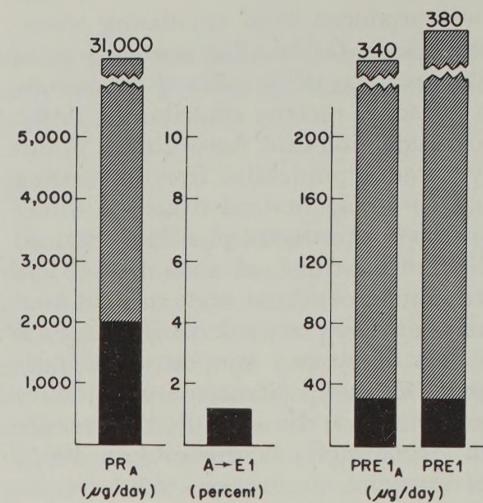


FIG. 6. Estrogen production in an anovulatory 36-year-old female with a virilizing lipoïd cell tumor of the ovary and ovarian stromal hyperplasia. Estrogen production was totally accounted for by the aromatization at a normal rate of the massively increased daily production of androstenedione. Solid portion of each bar represents normal value.  $PR_A$ , daily production of androstenedione; A, androstenedione; E1, estrone;  $PRE1_A$ , estrone derived from circulating androstenedione;  $PRE1$ , daily estrone production.

studies of over 100 women of all ages carried out by the authors. Together with evidence suggesting that adipose tissue possesses the aromatase enzyme, albeit in low concentration, these findings suggest that gradual deposition of body fat in old age may serve to maintain a favorable estrogen environment by increasing the efficiency of extraglandular estrogen formation. This effect would ultimately be offset by declining adrenal production of steroids, in particular precursor androstenedione. As shown above, in males, both adrenal androstenedione and testicular testosterone production that continues relatively unabated throughout life contributes to estrogen production. Therefore, during old age, extraglandular production of estrogen may provide a more favorable estrogen level in males than in females or may provide males with the more active hormone, estradiol, and thereby the less frequent occurrence of osteoporosis and bone breakage in males can be explained.

#### Polycystic Ovarian Disease

In premenopausal women with chronic anovulation but continued gonadotrophin production, a state analogous to chronic estrus may obtain. However, in many of these cases the source of estrogen that produces the chronic estrous state is principally extraovarian. The clinical evidence of estrogen production is apparent by organ growth or development maintained by estrogen, or both, plus the ready withdrawal bleeding that occurs after a provocative test dose of progesterone. In many of these subjects with failure of follicular maturation and theca and stromal hyperplasia, increased secretion of androstenedione by the ovaries is observed. Thus, as in postmenopausal women with increased availability of precursor, increased extraglandular estrone production

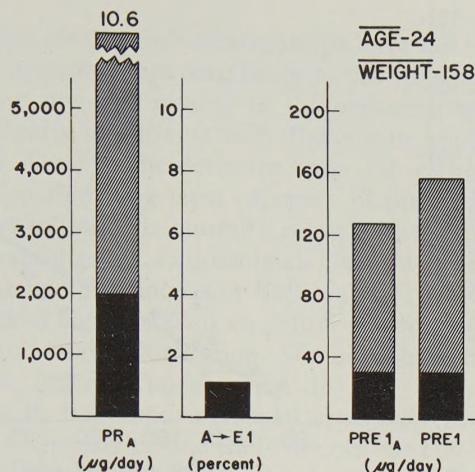


FIG. 7. Estrogen production in a group of anovulatory women (mean age, 24 years; mean wt, 158 lb) with polycystic ovarian disease. Estrone derived primarily by peripheral aromatization of androstenedione, the daily production of which was 5 times elevated. Solid portion of each bar represents normal value.

results. As indicated in Figure 7, in many such patients the majority of total estrogen production is in fact estrone that was derived from circulating androstenedione. It is tempting to speculate that this large source of constant extraglandular estrogen is etiologically related to the acyclicity of gonadotrophin release and ultimately to the failure of adequate follicular maturation occurring in response to gonadotrophin stimulation. This mechanism would readily explain the therapeutic effects of ovarian wedge resection and adrenal suppression in some patients since both maneuvers would decrease androstenedione and, in turn, estrone production. It is also important to note that the hormonal milieu of chronic estrus or polycystic ovarian disease in these premenopausal women is the extraglandular production of estrone rather than follicular estradiol-17 $\beta$ . As is pointed out below, this is the same hormonal milieu of endometrial neoplasia, and fully 80% of women under age 40 who develop endometrial carcinoma have coexisting polycystic ovarian disease.

#### *Endometrial Hyperplasia and Carcinoma*

For over 50 years controversy has surrounded the hypothesis that endogenously produced or exogenously administered estrogen is causally related to the development of endometrial carcinoma. The proponents of this theory cite the concomitant finding, or even the antecedence, of endometrial hyperplasia and endometrial carcinoma; the high incidence in patients with estrogen-secreting tumors; the occurrence of endometrial carcinoma in subjects taking estrogen; the higher incidence of corticostromal hyperplasia of the ovary in endometrial carcinoma patients; the report of high levels of vaginal cornification or even "estrogenic hormone" in the urine of these subjects; the apparent rarity of the disease in those women with senile vaginitis or vasomotor symptoms; the dependency on estrogen of endometrial carcinoma produced in rabbits; the near uniqueness of the

simultaneous existence of polycystic ovaries and anovulation or estrogen-secreting tumors with endometrial carcinoma in premenopausal women.

The opponents of the hypothesis cite, on the other hand, a) the evidence that occurrence of endometrial carcinoma is principally a disease of the postmenopause, when estrogen production has declined; b) the rarity of its development in women treated with estrogen for even decades; c) the failure of many investigators to find significantly increased amounts of estrogen metabolites in the urine of endometrial carcinoma victims; d) the refractoriness of experimental animals (including the monkey) in development of uterine carcinoma with estrogen treatment; e) the development of endometrial carcinoma in women many years after castration and even in subjects with gonadal dysgenesis.

To date, however, most authors have considered the problem to be whether or not hyperestrogenism or unopposed estrogen may be etiologically related to the development of endometrial carcinoma. Either view will not satisfactorily explain the diverse findings in this common neoplasia, nor will either concept adequately explain the common association of the constitutional stigmata known to favor the development of endometrial neoplasia (aging, obesity, diabetes, liver disease, non-endocrine tumors of the ovary, polycystic ovarian disease, and ovarian corticostromal hyperplasia).

Studies, such as those described above, have shed light on the endocrine status of individuals who have or who by their constitution are highly susceptible to endometrial neoplasia. These data offer a working hypothesis that may resolve in large measure the apparent conflicts between those who favor a causal relationship between estrogen production and the development of endometrial disease and those who oppose this view.

The results of these studies clearly demonstrate that the common endocrinologic feature of these diverse groups of individuals who develop endometrial neoplasia is the exclusive production of estrone via the aromatization of androstenedione at some extraglandular site, or sites (18, 29). Those subjects with the constitutional stigmata that predispose to its development may produce more extraglandular estrone by one of several metabolic alterations. The hypothesis emerging from these studies is that the exclusive production of estrone (rather than the more potent follicular hormone, estradiol-17 $\beta$ ) is the requisite hormonal event that interacts on a predetermined genetic predilection for the development of endometrial anaplasia. With a great genetic predilection, a normal quantity of postmenopausal estrone is a sufficient provocateur, whereas even with a lesser genetic risk the increased quantity of estrone produced in those individuals with certain constitutional stigmata may bring about neoplasia.

This hypothesis therefore does not necessarily embrace or contradict the hyperestrogenic postulate; rather, it amends the proposition to consider the hormonal milieu of these subjects as a "dysestrogenic" state of varying magnitude.

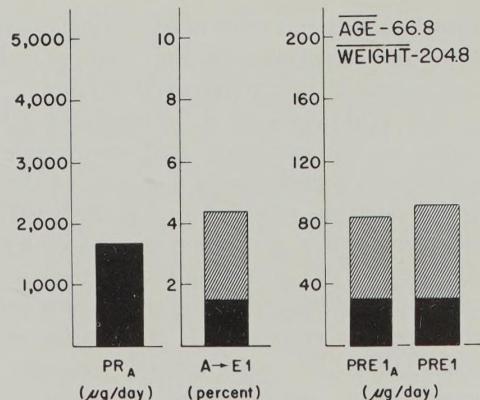


FIG. 8. Estrone production in women ( $n = 8$ ), more than 10 years after menopause, all but one of whom had symptoms of estrogen excess. Estrone production was about twice normal as a result of increased values of  $[P]_{BU}^{AE1}$ . Solid portion of each bar represents normal value.

In Figure 8 the results obtained in a group of eight women with excessive estrogen production, who were studied more than 10 years after the menopause, are presented. The mean age for this group was 66.8 years, and their mean weight was 205 lb. The production rate of androstenedione was similar to the normal younger group, 1,700  $\mu\text{g}/24\text{ hr}$ , but there was a considerably higher extent of its conversion to estrone, 4.4 %. Thus the conversion of androstenedione resulted in the formation of 86  $\mu\text{g}/24\text{ hr}$  of estrone, as compared with a total estrone production rate of 92  $\mu\text{g}/24\text{ hr}$ . These values are not significantly different, so again it may be concluded that total estrogen production in this group of women was derived from circulating androstenedione. Of the eight women in this group, all but one presented with uterine bleeding due either to adenomatous and/or atypical hyperplasia or endometrial carcinoma. This is a selected group of patients in that they sought medical attention and cannot be considered representative of women in this age category. Nevertheless, it is of interest to ask why the extent of conversion of androstenedione to estrone was elevated in these subjects.

As already indicated, a strong correlation (correlation coefficient = 0.74) exists between the extent of conversion of androstenedione to estrone and total body weight (Fig. 9). A similar analysis of age and extent of conversion showed no significant correlation, but there appears to be a rather abrupt increase in conversion after the menopause. Thus these data suggest that several factors, including excessive weight and unknown changes occurring at menopause, may lead to an elevated conversion of androstenedione to estrone.

Figure 10 illustrates the results obtained in a 55-year-old subject who weighed 130 lb and was hospitalized with severe cirrhosis of the liver and uterine bleeding. The androstenedione production rate was found to be at a premenopausal level of 2,900  $\mu\text{g}/24\text{ hr}$ , but the extent of conversion of androstenedione to estrone was 5.7 %. This conversion resulted in the production of 141  $\mu\text{g}/24\text{ hr}$  of estrone, which again was about equal to the total estrogen production rate. Similar results have been ob-

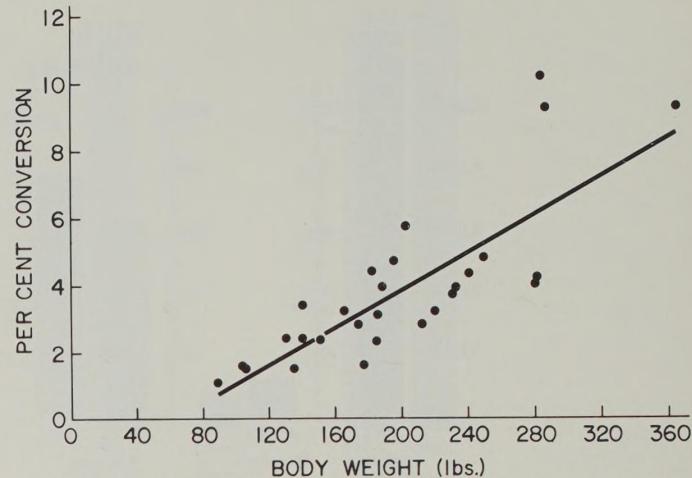


FIG. 9. Correlation of extent of conversion of androstenedione to estrone,  $(P)_{BU}^{AE1}$ , with body weight in postmenopausal women. Correlation coefficient = 0.74.

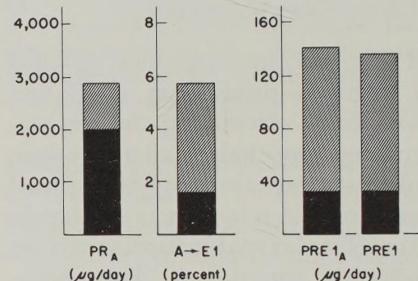


FIG. 10. Estrone production in 55-year-old postmenopausal woman (wt, 130 lb) with cirrhosis and uterine bleeding. Solid portion of each bar represents normal value. PR<sub>A</sub>, daily production of androstenedione; A, androstenedione; E1, estrone; PRE<sub>1A</sub>, estrone derived from circulating androstenedione; PRE<sub>1</sub>, daily estrone production.

tained in a group of six women with various degrees of liver disease without associated obesity, all of whom showed an elevated conversion of androstenedione to estrone. It seems likely that the increased conversion of circulating androstenedione to estrone in patients with liver disease results from reduced irreversible liver catabolism of androstenedione to 17-ketosteroids, which results in more androstenedione being available to the tissue site, or sites, of estrone production.

The alternative means by which elevated estrone production occurs via this same mechanism arises when abnormally large amounts of precursor androstenedione are produced, as discussed above for women with polycystic ovarian disease. The results of these studies demonstrate that production of excessive amounts of estrone via the aromatization of circulating androstenedione is a frequent occurrence in all those situations commonly associated with an increased incidence of endometrial carcinoma. Thus this hitherto unrecognized mechanism of estrogen production provides a common endocrine denominator in women who are highly susceptible to endometrial cancer. It should be pointed out that a high incidence of hypertension and diabetes in women with endometrial carcinoma has also been suggested by a number of authors. Although numbers of patients suf-

ficient to establish clearly such relationships have not yet been studied, it appears more than likely at this time that these associations are best related to the commonplace occurrence of obesity in patients with diabetes or hypertension, or both. These studies form the basis of a working hypothesis stating that long-term exposure of the endometrium to estrone may be causally related to the development of premalignant, endometrial, atypical hyperplasia or endometrial carcinoma or both.

The biochemical basis for such an action of estrone on the endometrium awaits elucidation. We have reported that estrone is indeed concentrated in the human endometrium with little conversion to estradiol either within or without the uterus, and therefore estrone can be classified as a primary hormone on this basis (28). Furthermore, in studies carried out to investigate the interaction of estrone and estradiol with receptors of human endometrium, it has been found that both hormones can bind to the cytoplasmic 8S receptor, and subsequently both estrone and estradiol receptor complexes are transferred to the nucleus. The only difference between the two hormones thus far detected is the lower affinity of estrone for the cytoplasmic receptor, a relation that has been shown in several other species. Nevertheless, if the plasma estrone concentration is sufficiently high, it would appear that estrone can effect the same early biochemical functions in the target cell as does estradiol, particularly in the relative absence of the latter. Therefore the early hypothesis of unopposed estrogen may be valid since prolonged exposure to either estradiol or estrone may ultimately lead to abnormal proliferation and neoplasia by the same mechanism, or mechanisms. Alternatively, qualitative differences in the biochemical events that follow the interaction of the estrone receptor complex with the nucleus of the target cell and that lead to neoplasia may exist.

#### Gynecomastia

Gynecomastia has long been a perplexing clinical and laboratory problem. The most puzzling aspect of this expression of apparent estrogen excess has been the diversity of conditions with which gynecomastia is commonly associated. Perhaps best understood is gynecomastia due to deficient or absent testosterone production resulting from a variety of causes or due to excessive estrogen production by testicular or adrenal tumors. These observations indicate that growth of the male breast may be subject to a balanced control involving both androgen and estrogen, as first suggested by Lewin (12). However, a unified explanation has not been put forth for the physiological gynecomastia of adolescence and of aging or of pathological forms associated with diseases, such as cirrhosis, bronchogenic carcinoma, or Graves disease. Equally obscure have been the physiological mechanisms underlying drug-induced gynecomastia brought about by the administration of dissimilar nonestrogenic drugs, such as digitalis and spironolactone. However, new insight into these problems has been gained by the development of an experimental model

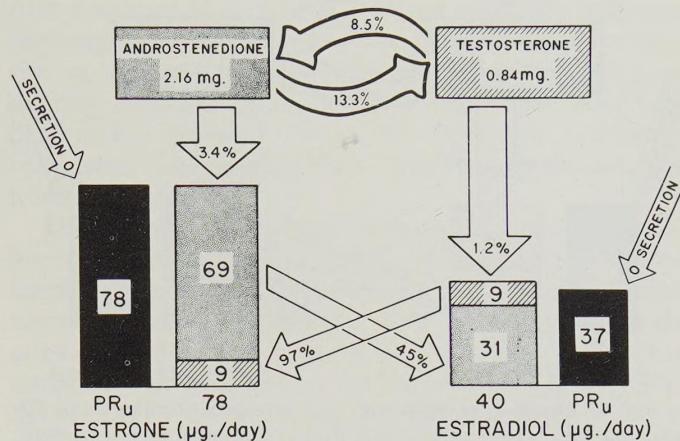


FIG. 11. Analysis of androgen and estrogen production in 68-year-old male with gynecomastia. Markedly reduced testosterone production as a result of mumps orchitis and relatively normal estrogen production are evident (cf., Fig. 4).

that adequately represents the physiological relation between androgen and estrogen production in man. This physiological model is represented in Figure 4.

As indicated, one cannot consider only excessive estrogen production either by secretion or peripheral conversion as a provocateur of gynecomastia in the male. The quantities of estrogen and of androgen (probably testosterone exclusively) that are produced and the relation between the production of these two hormones must be examined. This has become abundantly evident from studies carried out in over 30 males with gynecomastia of greatly differing etiology. Of all males studied (with the exception of those who have drug-induced prolactin secretion) the only common hormonal denominator has been a decreased ratio of testosterone to estrone plus estradiol (T/E) production.

**ABNORMAL TESTOSTERONE PRODUCTION.** A marked reduction in testosterone production, such as occurs with testicular atrophy, castration, anorchia, or drug administration (e.g., spironolactone) but with continued aromatization of plasma estrogen precursors, specifically androstenedione, results in a dramatically lowered T/E production ratio. Even though no increase in the absolute amount of estrogen produced is observed (in fact it may be less than normal, as has been observed frequently by urinary excretion studies), the relative absence of testosterone results in gynecomastia. Figure 11 illustrates the results of a study in a 69-year-old male who developed gynecomastia at age 45 after bilateral mumps orchitis at age 18. At the time of study he had enormous pendulous breasts, no libido, and little facial hair growth. It can be seen that the testosterone production rate was markedly diminished (0.84 mg/day). It should be noted that the conversion of both androstenedione and testosterone to estrogen was higher (two and four times, respectively) than normal. This resulted in slightly greater than normal estrone and a normal estradiol production rate. No evidence for glandular secretion of either estrogen was obtained. Nevertheless, the T/E ratio was markedly lower (ca. 7) than normal (ca. 100), which suggested

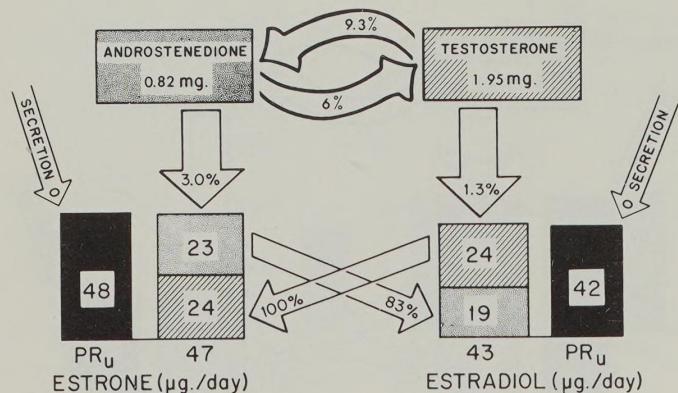


FIG. 12. Analysis of androgen and estrogen production in 60-year-old male with gynecomastia due to spironolactone treatment. Production rates of both androstenedione and testosterone were reduced to one-third of normal, but estrogen production was normal.

that the patient's breasts responded to normal estrogen production in the absence of adequate testosterone.

Figure 12 illustrates the results of a study in a patient who developed gynecomastia while being treated for primary aldosteronism with large doses of spironolactone. It can be seen that the production rates of both androstenedione and testosterone were depressed to about 30% of normal. Again the efficiency of conversion of both androgens to estrogen was higher than normal (consistent with aging) so that the total estrogen production rates were relatively normal. Before surgery for removal of bilateral adrenal adenomas, medication was stopped and the patient's plasma levels of androgens, including dehydroisoandrosterone, promptly returned to normal and the gynecomastia improved. The results of this study suggested that spironolactone decreases testosterone levels by interfering with testicular and adrenal androgen biosynthesis ( $T/E = 20$ ); thus relatively normal amounts of estrogen were allowed to stimulate the breasts. Indeed, it has been demonstrated that spironolactone effectively inhibits pregnenolone or progesterone conversion to testosterone in slices of mouse or human testes. Inhibition was shown to occur at the 17,20-lyase enzymatic step of the conversion by the marked accumulation of  $17\alpha$ -hydroxylated intermediates (P. K. Siiteri and P. C. MacDonald, unpublished observations).

**INCREASED EXTRAGLANDULAR ESTROGEN.** In men, as in women, both androstenedione and testosterone are converted to their product hormones, estrone and estradiol, respectively, in amounts that increase with advancing age, as indicated in Figure 13. Thus, with a slowly declining testosterone production in old age, the development of gynecomastia is more likely since there is an increased conversion of the remaining plasma precursors to their respective product estrogenic hormones. On the other hand, it has been suggested that the efficiency of extraglandular aromatization is high in the newborn and gradually declines during childhood to adult levels by age 10–12 (D. Hemsell, J. Madden, P. K. Siiteri, and P. C. MacDonald, unpublished results). Since the rising production of adrenal steroids (androstenedione) at

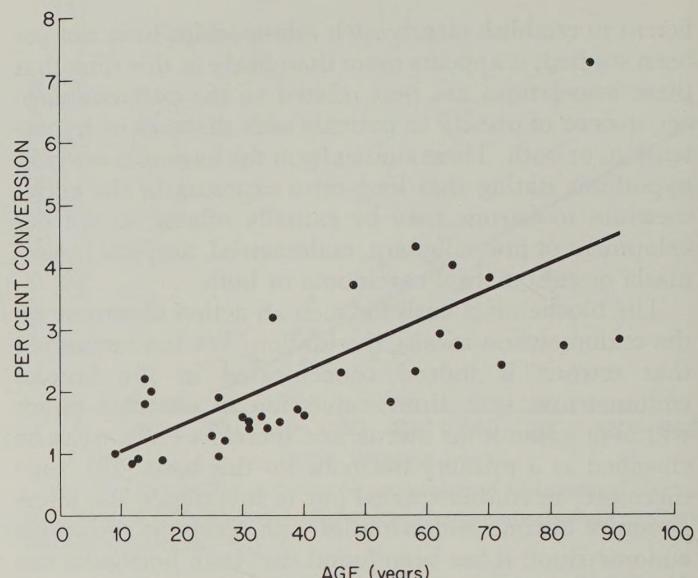


FIG. 13. Correlation of extent of conversion of androstenedione to estrone with age in males. Correlation coefficient = 0.62.

adrenarche occurs at about the same age, the transient gynecomastia of adolescence may represent excessive extraglandular estrogen produced during a period when these phenomena overlap, before pubertal testosterone secretion has begun. Alternatively the efficiency of testosterone aromatization may remain at levels sufficient to produce feminizing amounts of estradiol for a short time after the onset of testicular testosterone secretion. This estrogen would also be overridden by maturation of testicular function and increasing testosterone secretion after puberty.

An increase in estrogen production giving rise ultimately to a decrease in the T/E production ratio has been observed in a number of the clinical entities associated with gynecomastia (32). This may occur as the consequence of the increased conversion of the plasma C<sub>19</sub> precursors to estrogens observed in liver disease (cirrhosis) or marked obesity, as already described above in women. In cirrhosis of the liver there is an increased conversion of testosterone to androstenedione due to reduced clearance of testosterone by the liver. In this circumstance there is increased estrogen production for two reasons: 1) since plasma androstenedione is a much more efficient precursor of extraglandular estrogen production than is testosterone, increases in the conversion of testosterone to androstenedione will increase the efficiency of conversion of testosterone to estrogen; and 2) decreased hepatic extraction of the C<sub>19</sub> precursors makes more substrate available to the extrahepatic aromatizing sites, and thus the conversion of both precursors to estrogen is increased.

In addition to those causes of increased extraglandular conversion of androgen to estrogen that have been cited above, increased conversion of yet another precursor to estrogen may be observed in patients with choriocarcinoma (22). In this circumstance dehydroisoandrosterone sulfate is converted in the trophoblastic tissues of

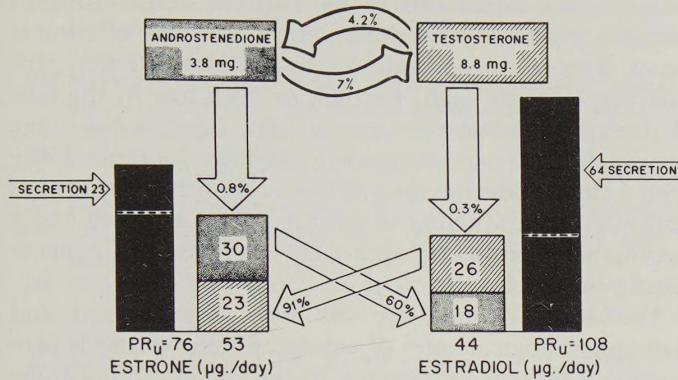


FIG. 14. Analysis of androgen and estrogen production in 18-year-old male with gynecomastia and mosaic Klinefelter's syndrome. Androgen production was normal, but excessive estradiol production arose presumably by testicular secretion.

the choriocarcinoma to estradiol. Thus, again, a decrease in the testosterone-to-estrogen production ratio may occur via the utilization of a plasma precursor for estrogen production.

**ESTROGEN SECRETION BY THE TESTIS.** As indicated earlier in this chapter, estrogen production in the male derives primarily from the peripheral conversion of androstenedione to estrone and of testosterone to estradiol. Although in the normal male there is probably some small secretion of estradiol by the testis, this likely accounts for only 10–20% of the total estradiol production, and there is very little contribution by the testis to estrone production. In a number of males that we have studied, however, there has been direct secretion of estradiol or estrone, or both, probably by the testes. This has occurred almost exclusively in males with chromosomal abnormalities or in males with increased gonadotrophin production. These chromosomal abnormalities have been consistent with those giving rise to Klinefelter's syndrome (Fig. 14). Thus males with chromosomal constitutions of XXY, XY/XXY, XO/XY, and XY (testicular feminization) have been found to have decreased testosterone-to-estrogen production ratios as a result of increased estrogen derived from direct secretion by the testis.

#### SUMMARY AND CONCLUSIONS

The studies cited and presented above clearly show that the peripheral utilization of plasma C<sub>19</sub> steroids for estrogen production at extraglandular sites occurs throughout life in the human being. The physiological potential of this hitherto neglected hormonal mechanism is enormous since it is evident in the fetus in utero and is a major determinant of estrogen production in the elderly. The mechanism, or mechanisms, of estrogen production independent of glandular secretion have been clearly established. The output of this mechanism of estrogen production is not static but may vary markedly depending on the transfer constants of conversion of androstenedione to estrone or testosterone to estradiol.

Also a variety of metabolic patterns markedly influence the magnitude of these transfer constants and thus give rise to a system in which extraglandular estrogen production displays dynamic alterations that are fundamentally different from the processes ordinarily considered in hypothalamic-hypophysial-gonadal sex hormone production.

These studies have shown that the product estrogenic hormones derived from plasma precursors are clearly defined and qualitatively quite different under specified circumstances. Specifically, plasma androstenedione gives rise exclusively to estrone and/or estrone sulfate, the estrogens that predominate in the postmenopausal state. Paradoxically, in males, plasma testosterone gives rise to estradiol, the ovarian secretory product, and also to estrone via the sequence plasma testosterone → plasma androstenedione → estrone. The relative quantitative importance of extraglandular estrogen production from circulating precursors depends on the physiological or pathophysiological state being considered. In normal premenopausal women the extraglandular production of estrone from circulating androstenedione at the extremes of the menstrual cycle may account for 50% or more of total estrogen production, whereas at midcycle, during the follicular surge in estradiol secretion by the ovary, extraglandular estrone may account for only 10% of total estrogen production. On the other hand, in premenopausal women subjected to acyclic gonadotrophin stimulation resulting in polycystic ovarian disease, extraglandular estrone production constitutes, quantitatively, the most important source of estrogen. Increased androstenedione secretion by the ovaries (and/or adrenals) of these subjects, despite failure of follicular maturation and a normal conversion to estrone, results in extraglandular estrone (sulfate) production that may approximate in amount normal ovarian estradiol secretion. Thus there exists in such subjects a situation in which an endogenous, but extraovarian, contraceptive dose of estrogen is being produced.

In postmenopausal women estrogen production is principally, if not exclusively, the result of peripheral aromatization of plasma androstenedione. However, the postmenopause cannot be considered as a time of quiescence with respect to estrogen production. Although it is well recognized that the dynamic processes of hypothalamic-pituitary-ovarian estrogen production have ceased, the amount of estrone derived from plasma androstenedione of adrenal origin is subject to wide variation. A markedly increased conversion of androstenedione to estrone has been observed in a variety of metabolic aberrations, including obesity, liver disease, aging, and perhaps other factors such as diabetes and certain drug treatments. In addition, increased availability of precursor androstenedione, as occurs with adrenal tumors, with nonsecretory ovarian tumors, or with corticostromal hyperplasia of the ovary, provides the setting for increased estrogen production as the result of the increased availability of plasma substrate. Therefore the extent of extraglandular estrogen production

following the menopause is subject to many possible factors involving total body metabolism. Of significant interest in this regard is the fact that many of these metabolic aberrations are exactly those that have been considered constitutional stigmata of endometrial neoplasia. Therefore it seems clear that the hormonal milieu of atypical endometrial hyperplasia and carcinoma is the exclusive production of estrone or its sulfate ester, or both. This strong clinical and laboratory correlation between endometrial neoplasia and the exclusive production of the weaker estrogen, estrone, is a most provocative finding meriting further study to ascertain if a causal relation exists between the two.

Equally intriguing is the complex relation between androgen production and estrogen origin in normal males and in males with certain endocrinopathies that suggest estrogen dominance. These studies show clearly that estrogen production in the male is principally the consequence of extraglandular formation from plasma precursors. Specifically estrone is derived almost exclusively from the extraglandular utilization of plasma androstenedione and testosterone. Additionally 75–100% of estradiol production in males is similarly derived from the utilization of testosterone and androstenedione. Therefore only a small fraction of estradiol and little, if any, estrone is directly secreted by the testes or adrenal cortex. If one considers this complexity of origin of androgen and its ultimate utilization for estrogen production, it is no

wonder that alterations in the ratio of testosterone to estrogen production are observed in a variety of clinical states. This gives rise to situations in which estrogen production, whether high, normal, or even low in the face of decreased testosterone production, may set the stage for the development of breast growth in the male. Only with a clear understanding of these relative contributions and rates of production of androgen and estrogen can a reasonable therapeutic approach to males with gynecomastia be developed.

Yet to be defined are the true physiological and pathophysiological roles of extraglandular estrogen production. Of great importance to the elucidation of these roles is the identification of the tissues involved in the aromatization of circulating androgens. What effects on the hypothalamic and/or hypophysial control systems are initiated by the local aromatization in the brain of estrogen precursors (23, 24)? The relative importance of estrogen derived from circulating precursors in a given aromatizing site and that obtained by secretion will certainly be the subject of great interest in the future. Future studies of the role of extraglandular estrogen will no doubt concentrate on the importance of extraglandular estrogen, and specifically of estrone (sulfate) to neoplasia, the mechanism by which fetal aromatization is ultimately repressed, and the possibilities of stimulation or inhibition of extraglandular aromatization in various metabolic and disease states.

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