SEX HORMONE PRODUCTION AND ACTION

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The mechanisms of the production of estrogens and androgens in women and men are reviewed. Extraglandular estrogen formation from circulating androgens is the principal source of estrogen in men and postmenopausal women. The amount of estrogen formed is dependent upon precursor availability and metabolic factors, such as obesity and hepatic disease. Both androgens and estrogens in the circulation of humans are bound to sex hormone binding globulin which limits their availability to target cell receptors.

The sex steroid hormones have long been suspected of playing a role in autoimmune diseases such as systemic lupus erythematosus (SLE) because of the much higher incidence of these diseases in women than in men. Estrogens are thought to promote the disease process since males with Kleinfelter's syndrome frequently develop gynecomastia and breast cancer and also appear to be more prone to develop SLE (1). The availability of a murine model, the NZB/NZW F_1 mouse, that has many of the characteristics of the human disease provides an opportunity to study the influences of both androgens and estrogens on the disease process. Recent studies have shown that castration or long-term administration of estrogens to males results in a more rapid onset of disease and mortality that is comparable to that found in females (2). Conversely, administration of androgens to castrated females, even late in life, delays the production of autoantibodies and fatal glomerulonephritis (3).

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While these and other studies have clearly demonstrated the important and opposing effects of estrogens and androgens, they have given little insight into the identity, origin, or sites of action of the hormones in intact animals. The simplistic notion that the gonads are the exclusive or even major source of the sex steroids is no longer tenable. It is well established that biologically active androgens and estrogens may be formed in peripheral tissues from inactive precursors arising from the gonads and/or adrenal glands in both men and women (4). Estrogen production by these mechanisms is extremely complex since the amounts formed depend not only upon precursor availability but also on metabolic and other factors. A brief review of the origin, transport, and mechanism of action of the sex steroids and their potential sites of interaction with the immune system is presented in this article.

Androgen and estrogen production in women

Prior to puberty the ovaries secrete small but significant amounts of steroid hormones. Following sexual maturation over a period of 1–2 years, the ovaries commence their dual function of ovum maturation and hormone production that continues until the menopause. The important fluctuations in the serum concentrations of pituitary and ovarian hormones that occur during the normal menstrual cycle are schematically illustrated in Figure 1. A 28-day cycle as shown is generally regarded as the mean length of normal cycles, but the range for different women extends from 25 to 35 days or more. Cycle length may vary considerably in the same woman

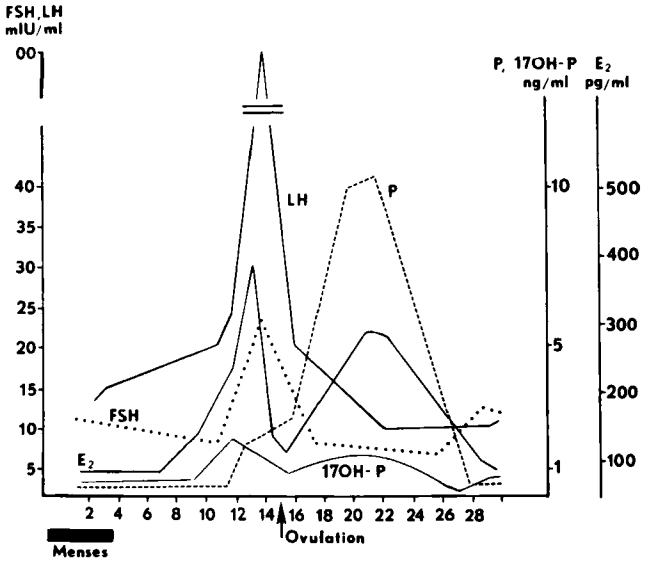

Figure 1. Schematic representation of hormonal changes during the normal menstrual cycle. **LH** = lutenizing hormone; **FSH** = follicle stimulating hormone; E_2 = estradiol; **P** = progesterone; **17OH-P** = 17α-hydroxy progesterone.

particularly at the beginning and end of the fertile years. The follicular phase of the cycle is much more variable than the luteal phase, the latter period being remarkably constant at 13 ± 1 or 2 days. The biologic mechanisms responsible for the constancy of the luteal phase are unknown. However, infertility associated with the so-called "short" luteal phase appears to be due to inadequate gonadotropin stimulation of the developing follicle prior to ovulation.

In the early phase of follicular development, serum estradiol levels are very low. Beginning about 8 days before the luteinizing hormone (LH) peak, there is a gradual rise that becomes more accelerated, and estradiol usually reaches maximum levels on the day before the LH peak. Most of the estradiol arises from that follicle which is destined to ovulate, and it is generally believed that this rise in serum estradiol is the primary signal for release of the ovulatory surge of gonadotropins. The serum estradiol concentration drops precipitously as both gonadotropins, LH and follicle stimulating hormone (FSH), are increasing, and it then rises to a peak again around the middle of the luteal phase. By the onset of menses, estradiol levels have fallen to their lowest point seen in the early follicular phase. Serum estrone levels (not shown) are always lower than those of estradiol, but the pattern generally mirrors that of estradiol. As will be shown below, estrone is derived from several sources including small amounts by secretion, larger amounts by peripheral conversion from androstenedione, and by secreted estradiol.

Progesterone is secreted by the ovaries in very small amounts during the follicular phase, and additional small amounts are produced by the adrenals both by secretion and peripheral conversion of secreted pregnenolone and pregnenolone sulfate. Serum progesterone levels increase slightly during the gonadotropin surge and then rise dramatically following ovulation and corpus luteum formation. Maximal progesterone levels of 10–15 ng/ml are reached 5–6 days following ovulation and then fall during the last 2–3 days of the cycle as the corpus luteum regresses. However, if implantation of the fertilized ovum in the uterus occurs, human chorionic gonadotropin (HCG) produced by the trophoblast of the early embryo rescues the corpus luteum, and progesterone is maintained at midluteal phase levels for many weeks.

Other steroids secreted by the ovary include 17α-hydroxyprogesterone and androgens. The pattern of 17α-hydroxyprogesterone secretion is intermediate between that of estradiol and progesterone. A small rise and fall appears to coincide with the estradiol peak, and a second small peak parallels the progesterone pattern during the luteal phase. Both progesterone and 17α-hydroxyprogesterone have been proposed as modulators of the hypothalamic-pituitary events that give rise to the gonadotropin surge. The ovaries normally secrete 1–2 mg/day of androstenedione (A), and the serum level of this androgen reaches its maximum value about the time of the midcycle surge of gonadotropins (5). Serum testosterone levels also follow the same pattern, since 30–40% of the total is derived from peripheral conversion of serum androstenedione. About one-fourth of the circulating testosterone arises from ovarian secretion and the remainder directly or indirectly from the adrenal glands. Small amounts of other androgens including dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), and dihydrotestosterone (DHT) are secreted by the ovary. After menopause the ovaries produce little if any estradiol or progesterone, although testosterone continues to be secreted and may be responsible for the mild hirsutism noted in many older women (6). Not infrequently, however, postmenopausal women are subjected to large amounts of estrogen which earlier were thought to arise by secretion from the adrenal glands.

Extraovarian estrogen production

Evidence for extraglandular estrogen formation was first reported in 1937 by Steinach and Kun (7) who found that administration of testosterone propionate to men increased estrogen excretion in the urine. The first definitive evidence that the increased estrogen excretion was due to peripheral aromatization of the administered steroid was presented in 1956 by West and associates (8). They found estrone and estradiol in the urine of two adrenalectomized, oophorectomized women who had been given testosterone propionate, whereas none could be measured before treatment. Since then many studies have shown that the administration of isotopically labeled testosterone to normal men and women results in the appearance of radioactive estrogens in their urine. Recently, a number of laboratories have examined the extent to which conversion of circulating endogenous androgens contribute to total estrogen production. It has become apparent from these studies that the peripheral conversion of androgens to estrogens in men and nonpregnant women is a physiologically important determinant of the hormonal milieu in both healthy and diseased states (4).

During pregnancy as much as 50% of the DHEAS in the maternal circulation is converted to estradiol in the placenta (9). However, conversion of DHEAS to estrogen does not occur in nonpregnant females (10), and the conversion of free DHEA to estrogen is less than 0.1% (11). On the other hand a significant fraction of circulating androstenedione is converted to estrone in the nonpregnant state. In the early follicular phase of the menstrual cycle, the production rate of estrone is approximately 40 μg/day, most of which arises by peripheral conversion from androstenedione that is derived from both the ovaries and the adrenal glands. About one-half of the total production rate of A (~3 mg/day) is derived from the adrenal glands and the other half from the ovaries. Following the menopause, the ovarian secretion of A falls to negligible amounts while the adrenals continue to secrete approximately 1–1.5 mg of A per day. The conversion of A to E_1 in normal lean premenopausal women is approximately 1–1.5%. The extent of this conversion increases after the menopause to levels of 2–3% so that the production rate of estrone from androstenedione in normal postmenopausal subjects is maintained at about 40 μg/day (12).

A number of conditions are known to increase the production of estrone from circulating androstenedione. These include hyperthyroidism (13), aging, hepatic disease, and obesity (4). The conversion of A to E_1 may be elevated as much as 4 to 5-fold in obese postmenopausal women or women with cirrhosis, and estrone production rates are correspondingly elevated to 100–200 μg/day. Elevated serum levels of both E_1 and E_2 have been measured in obese postmenopausal women (14), and uterine bleeding is common in postmenopausal women who produce more than 80 μg/day by this process. On the other hand, estrone production may be increased without an increase in the conversion of A to E_1 if the production rate of androstenedione is elevated. This has been observed in postmenopausal women with nonendocrine tumors of the ovary with associated stromal hyperplasia (15). In younger women with polycystic ovarian disease (PCOD), the production rate of A may be 10 mg/day or even greater. PCOD (Stein-Levinthal syndrome) is the most common abnormality associated with chronic anovulation and is characterized by elevated serum LH, hirsutism of varying degree, and obesity (16). Both the ovaries and adrenal glands have been implicated as the source of excessive androgen production. In most cases however, it would appear that the ovaries produce large amounts of A. Testosterone and DHT are derived peripherally from circulating androstenedione and smaller amounts are secreted. On the other hand, elevated A production leads to chronically elevated estrone production in peripheral tissues. Thus PCOD is a unique situation in which hyperandrogenic and hyperestrogenic states may coexist due to the conversion of a biologically inactive precursor (A) to both classes of sex hormone. This syndrome also illustrates the importance of serum sex hormone binding globulin (SHBG) in hormone dynamics since an elevation of both the percentage free estradiol and testosterone is usually found (see below).

Despite extensive study, the principal site of peripheral aromatization and the mechanism by which this process becomes more active under some circumstances are still not certain. A number of studies have suggested that most extraglandular estrogen formation occurs in adipose tissue. Low levels of the aromatase enzyme have been demonstrated in adipose tissue in vitro by several investigators (17–19). Also, the extent of conversion of plasma androstenedione to estrone is highly significantly correlated with excessive body weight in postmenopausal women (20) as well as in young ovulatory and anovulatory women (21). When ^{14}C-androstenedione is infused intravenously, the ^{14}C-estrone formed in extraglandular sites enters the blood much

more slowly in obese subjects than in nonobese subjects (22). This slow release of estrone formed from infused androstenedione is also reflected in the rate of excretion of its urinary metabolites in obese women as compared to nonobese subjects. These observations have been interpreted to indicate that the extent of A to E_1 conversion in adipocytes is proportional to their lipid content. This could be due to the lipophilic nature of A and its sequestration in fat cells. High lipid content would favor the accumulation of A from the circulation and also retard release of E_1 in the reverse direction. However, markedly increased conversion of androstenedione to estrone also occurs in subjects with hepatic disease in the absence of obesity (4). This increased conversion of A to E_1 may be explained by reduced hepatic clearance of A and consequent greater availability to adipose tissue and the aromatase enzyme. However, in a study of the effects of acute weight loss on the conversion of A to E_1 in obese women, no change or even an increase in conversion was observed following an average weight loss of approximately 100 pounds (23). These unexpected results suggested that obesity may influence the conversion of A to E_1 indirectly and that aromatization of circulating A may occur in other tissues including the liver. For example, the hepatic fatty metamorphosis that occurs in obese subjects may reduce the normal hepatic metabolism of A to 17-ketosteroids and favor estrogen formation within the liver. It is of interest in this regard that hepatic 5α-reductase activity is markedly reduced during starvation (24).

Several early reports demonstrated that human fetal liver could transform androgens into estrogens (25–26). Our more recent studies have shown that the concentrations of the aromatase enzyme in fetal liver is at least 50–60% that of the corresponding placenta (27). In marked contrast, the C_{19} 5α- and 5β-reductases which catalyze the first step in the formation of 17-ketosteroids and androstanediols in adult liver were virtually undetectable in fetal liver. These findings raise the possibility that the decline in hepatic aromatase activity following birth in the human may not be due to genetic or other restriction of aromatase enzyme synthesis. The appearance of the much more active C_{19} 5α- and 5β-reductase enzymes following birth may drastically limit aromatization by competing for Δ^4-3-ketosteroid substrates (A) that enter the liver. A recent report (28) described a young boy with florid gynecomastia in whom the conversion of androstenedione to estrone was 50 times higher than normal. However, the biochemical basis for this inborn error of steroid metabolism was not ascertained.

The recent observations of Longcope and associates suggest that adipose tissue is not the only site of peripheral aromatization. They observed that both muscle and adipose tissue of the human forearm can convert androstenedione to estrone, but that total aromatization by these two tissues could account for only about 30–40% of total aromatization (29). While many assumptions were necessary to reach this conclusion, these results suggest that many tissues may be involved in this process. Indeed, it has recently been reported that adult human liver contains demonstrable levels of the aromatase enzyme (30). Progress in understanding the sites of synthesis and the regulation and function of peripheral estrogen synthesis has been severely hampered by the lack of suitable animal models for study.

The known factors that determine the amount of peripheral estrogen produced and available for interaction with receptors in target cells are summarized in Figure 2. It is of interest that many of the conditions that increase the peripheral formation of estrone, either by elevated androstenedione production or an increase in the efficiency of its aromatization, are also associated with an increased risk for development of endometrial and/or breast cancer (31). For example, obesity is the most common clinical abnormality found in endometrial cancer patients, and, although the incidence rises dramatically following the menopause, younger patients may frequently have polycystic ovarian disease. It thus appears that prolonged exposure of target tissues to estrogen as compared with cyclical interactions with estrogen and progesterone favors cancer development. The many reports that have appeared recently indicating that consumption of exogenous estrogens by postmenopausal women is associated with increased risk for endometrial cancer are in accord with this view

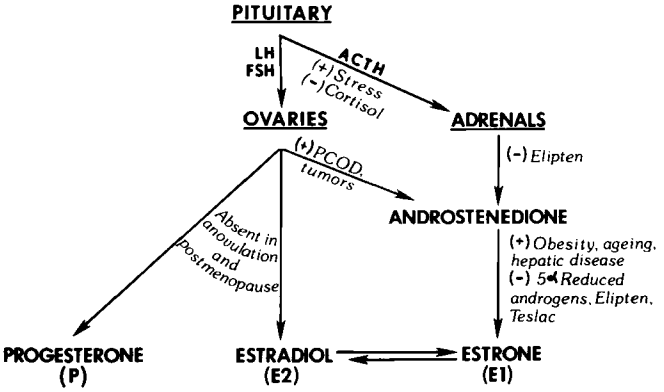

Figure 2. Factors that modulate the production of estrogens from adrenal androstenedione in anovulatory women.

(32–36). As is evident in Figure 2, peripheral estrone production is reduced or eliminated by many of the therapeutic measures used in breast and endometrial cancer patients. Ablation of either the pituitary or adrenal glands reduces the production rate of androstenedione to very low levels in postmenopausal women.

Treatment with cortisol or synthetic glucocorticoids has the same effect by suppressing pituitary ACTH secretion which reduces adrenal androgen secretion to very low levels. It is of interest in this regard that uterine bleeding may cease during glucocorticoid treatment in postmenopausal women who have higher than normal conversion of androstenedione to estrone. Treatment with the antibreast cancer drug Teslac (testolactone; Squibb and Sons) an extremely weak androgen that inhibits the aromatase enzyme, reduces the peripheral conversion of androstenedione to estrone in vivo (37). The drug Elipten (aminoglutethimide; Ciba Pharmaceutical Co.) is even more potent in this regard and therefore is highly effective in reducing estrone production since it also is a well known inhibitor of adrenal steroidogenesis (38). Also shown to be inhibitory are 5α-reduced androgens. Compounds such as 5α-androstenedione and dihydrotestosterone have been shown to be the most potent naturally occurring aromatase inhibitors (37). Whether they function to regulate aromatase activity in vivo, however, is not known.

Androgen and estrogen production in men

The interstitial cells of Leydig located in the connective tissue stroma of the testis between the seminiferous tubules are the source of testosterone (T) secretion. These cells are activated around the seventh week of fetal life when androgen-dependent sexual differentiation begins. Both testosterone and its metabolic product dihydrotestosterone (DHT) are required for complete development of the male genital organs (see article in this issue by J. D. Wilson, page 1275). The activation of testosterone synthesis is likely, although not proven, to be due to placental HCG secretion which is maximal during this stage of pregnancy. The Leydig cells become quiescent following birth until puberty when pituitary LH is secreted in sufficient quantities to stimulate adult level Leydig cell testosterone secretion. The normal production rate of testosterone is approximately 7 mg/day, and normal blood levels range from 500 to 1,000 ng/dl. The testes also secrete small amounts of DHT, A, and DHEA. The Sertoli cells and perhaps the interstitial cells of the testis secrete small amounts of estradiol, but this accounts for only 10–20% of total estrogen production. The remainder is derived from peripheral conversion of A to E_1 and T to E_2 as shown in Figure 3 (39).

The importance of peripheral estrogen production in males is demonstrated by the fact that gynecomastia is commonly observed in the absence of normal

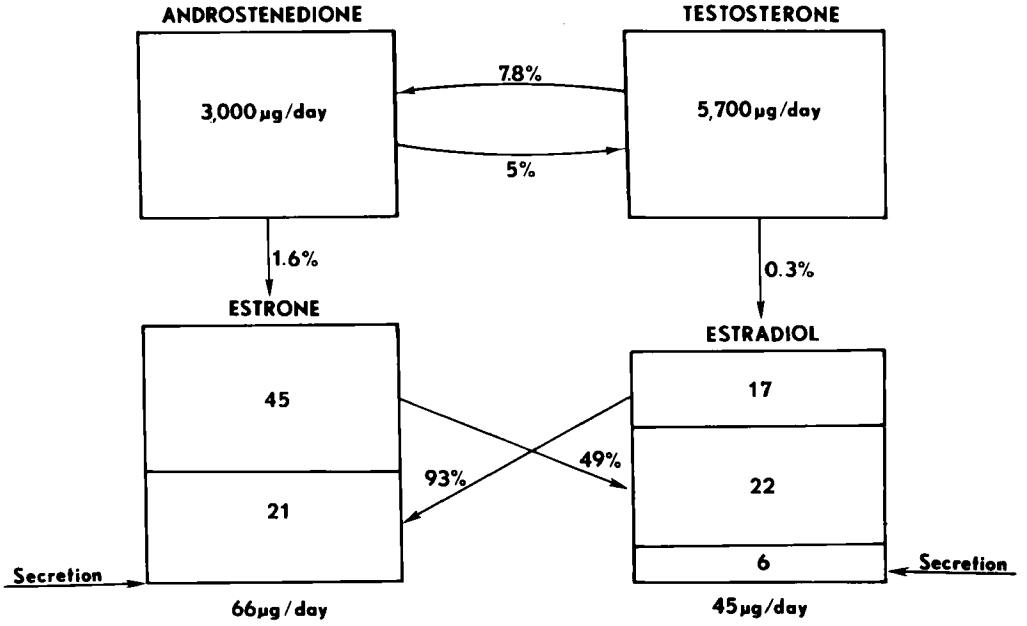

Figure 3. Origin of estrogen in normal men. Numbers inside boxes indicate appropriate blood production rates.

testicular testosterone secretion (4) as occurs in anorchia or after testicular damage. Peripheral estrone formed from adrenal androstenedione is apparently sufficient to feminize the male breast. Kleinfelter's syndrome also is frequently associated with gynecomastia (4) and breast cancer. Increased secretion of estradiol by the testes has been demonstrated and is sufficient to feminize the male breast despite fairly normal testosterone production. Therefore the male breast appears to be under dual control in which only slight changes in the normal ratio of androgen to estrogen production can lead to proliferation of estrogen-sensitive cells. However, the mechanism(s) by which androgens and estrogens have opposing effects on target cells are not yet known. Synergistic effects of these two classes of hormone also may occur, as recently demonstrated in androgen-induced benign prostatic hypertrophy in the dog (40). Simultaneous administration of estradiol with androstenediol accelerated prostatic growth, and later studies showed that estrogen administration increases the tissue level of androgen receptors (41).

Transport and mechanism of action of sex steroids

Steroid hormones in the blood of humans are reversibly bound to albumin and other proteins including corticosteroid binding globulin (CBG) and sex hormone binding globulin (SHBG). Binding to albumin is weak ($K_D \approx 10^{-5} M$) and nonspecific, whereas binding to CBG and SHBG is of much higher affinity (10^{-8} to $10^{-9} M$ K_D) and demonstrates steroid specificity. CBG binds both cortisol and progesterone, whereas SHBG, a glycoprotein of 94,000 molecular weight, binds both androgens and estrogens. Dihydrotestosterone is most avidly bound by SHBG ($K_D = 10^{-9} M$), whereas testosterone and estradiol are about 3 and 10 times less tightly bound, respectively. Other important serum androgens such as androstanediol also bind with intermediate affinity, whereas those having a 17-keto function, such as androstenedione, have very low affinity. In normal women the SHBG binding capacity (~60 nM) is about twice that found in men. The difference is presumably due to the fact that estrogens stimulate whereas androgens suppress SHBG synthesis (42). The highest levels of SHBG (approximately 200 nM) are found during late pregnancy and in thyrotoxic states, and lower than normal levels are found in obese subjects (42). The administration of either estrogens or thyroid hormones to men and women increases the concentrations of serum SHBG. It is generally assumed, but not proven, that SHBG is synthesized in the liver.

As a consequence of SHBG and albumin binding of testosterone, only a small fraction (2–3%) of the total steroid in serum is free, i.e., not protein bound. Whether it is only this small amount that is biologically active is not clear, although it is likely that SHBG bound steroid is not available to cells (43). Support for this contention comes from studies of the metabolic clearance rate (MCR) of various steroids. The values of MCR, defined as the volume of blood that is irreversibly cleared of a substance per unit time, for the sex steroids are inversely proportional to their binding affinity to SHBG (Table 1). Other studies have shown that the clinical degree of androgen excess is more accurately reflected by an increase in the apparent free testosterone level than by the total serum testosterone concentration in hirsute women (42). The apparent free testosterone level is estimated by measuring the percentage of steroid that is free after the addition of a radioactive tracer by using ultrafiltration or equilibrium dialysis and multiplying by the total serum concentration of testosterone. The values obtained by this procedure re-

Table 1. Metabolic clearance rates and approximate affinities of steroid for serum proteins and tissue receptors

	Metabolic clearance rates,* liter/day	Dissociation constant K_D $M \times 10^9$		
		Serum binder		Target tissue receptor†
		SHBG	CBG	
Estradiol	1,300	5	>10	0.1 (E)
Estrone	2,200	>10	>100	0.3 (E)
Androstenedione	2,000	—	—	—
Testosterone	600	2	>100	1 (A)
Dihydrotestosterone	400	1	>100	1 (A)
Progesterone	2,200	>100	2	1 (P)

* Normal female values.
† E = estrogen receptor; A = androgen receptor; P = progesterone receptor.

flect the minimum fraction of hormone in serum that is available to target cells in vivo. It is likely that the albumin-bound fraction which has been estimated to be 30–50% of the total is also available because of its ease of dissociation (44). Nevertheless, reduced SHBG levels due to elevated androgen production and/or obesity uniformly increase the apparent free concentration of T and other active androgens in women with PCOD who are virilized even if the total concentration of androgens is normal.

Relatively little attention has been given to the possibility that similar relationships may exist for estradiol binding to SHBG. Mikhail and colleagues measured the levels of SHBG and the percentage of free estradiol throughout the menstrual cycle and found no significant changes (45). Kirschner et al found no abnormality in free estradiol as measured by equilibrium dialysis in breast cancer patients (46). On the other hand, recent work has indicated that the free estradiol fraction may be elevated in grossly obese men due to depressed SHBG levels (47). In preliminary studies we have found that SHBG levels are markedly depressed and the percentage of free estradiol is increased in obese postmenopausal women despite the fact that peripheral estrogen production is markedly increased (48). This unexpected finding suggests that reduced SHBG and the resulting increase in free estradiol may amplify the effect of increased estrogen production associated with obesity. In other studies using a new method of centrifugal ultrafiltration/dialysis we have observed that the percentage of free estradiol is increased in serum from breast cancer patients with normal SHBG levels (49). Preliminary results suggest that the binding characteristics of SHBG from cancer patients are normal and therefore it is possible that competing androgens or other substances may be responsible for the elevation in free estradiol. It is not known at present why SHBG levels are decreased in obese subjects. It may be speculated that SHBG synthesis is depressed due to a subclinical state of hypothyroidism. Reduced hepatic conversion of thyroxine to triiodothyronine or hepatic thyroid hormone receptor defects may be reflected by depressed SHBG synthesis. Considered in this light the measurement of SHBG may serve as an exquisitely sensitive indicator of functional thyroid hormone action. In any event a relationship between thyroid hormones, SHBG, and increased free estrogens may provide a rational explanation for much evidence suggesting a link between hypothyroidism and increased risk for breast and endometrial cancer.

Despite the evidence cited above the true physiologic function of serum steroid binding proteins remains in question. Some experimental evidence suggests that their function may be quite the opposite in that they may promote the entry of steroid hormones into target tissues (50). On the other hand, wide species differences in occurrence and binding specificities suggest that they are not essential to hormone action. For example, rodents do not have SHBG-like proteins, whereas in species such as the rabbit, serum binding proteins occur that bind androgens but not estrogens (51). A seemingly trivial but nonetheless important function of serum binding proteins may be to retain steroid hormones in the vascular tree so that they may be delivered to appropriate target tissues rather than being partitioned into lipid-rich adipose or other nontarget cells.

Steroid hormone receptors

Steroid hormones leave the blood and accumulate in target tissues because they bind with high affinity to specific intracellular proteins called receptors. This was first demonstrated in studies in which it was found that following administration of radiolabeled estrogens to rats, target tissues, such as breast and uterus, achieved higher levels and retained the hormone for longer periods of time than nontarget tissues. Extensive study in many laboratories (for recent reviews see 52–54) has led to the general scheme shown in Figure 4. The free steroid hormone appears to enter cells by passive diffusion and avidly binds to a specific receptor

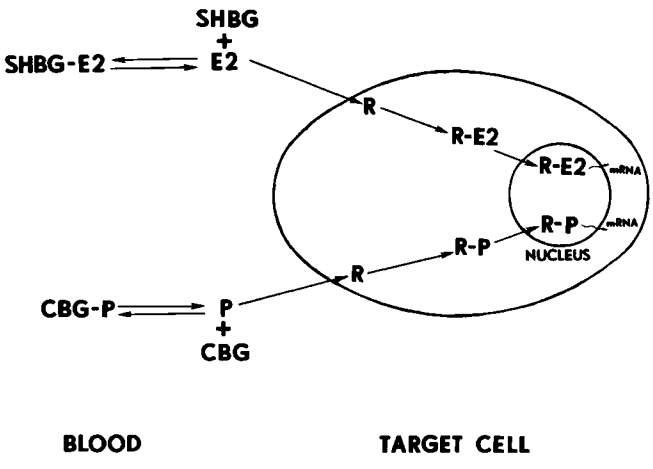

Figure 4. Schematic representation of the interaction of estradiol (E_2) and progesterone (P) with serum sex hormone binding globulin (SHBG) and corticosteroid binding globulin (CBG) and their tissue receptors (R).

present only in the cytoplasm of target cells. The receptor-steroid complex then appears to undergo a temperature and hormone dependent transformation or activation that results in its accumulation in the cell nucleus where it is bound to both specific and nonspecific sites on chromatin called acceptor sites. The interaction of the complex with specific acceptor sites apparently leads to the synthesis of greater quantities of specific messenger RNA (mRNA) by mechanisms not yet clarified. Depending upon the hormone and tissue in question, this may involve one or many mRNA species that direct the synthesis of new or existing proteins. The appearance of the receptor complex in the nucleus is a specific process for each hormone that can be demonstrated under physiologic circumstances. In the case of estrogen-stimulated uterine growth, it has been shown that nuclear occupancy of the receptor-steroid complex must be sustained for 6–8 hours in order to obtain a full response. For estrogens there exists a reasonably good correlation between biologic activity of various compounds and their binding affinity for the estrogen receptor. Thus it would appear that nuclear retention of the complex is dependent upon steroid binding affinity to the receptor. On the other hand, antiestrogens such as nafoxidine bind with relatively low affinity to receptors and have very weak activity and yet may be found in the nucleus for prolonged periods of time (weeks) following their administration (55). Thus it appears that recycling of the receptor between nucleus and cytoplasm is also necessary for sustained action.

While there is a considerable amount of data that support the scheme shown in Figure 4, Gorski and Gannon have recently pointed out several problems that are usually ignored (56). While there is no question about nuclear accumulation of steroid-receptor complexes, the precise intracellular location of the receptor prior to interaction with steroid is not certain. Virtually all studies of the estrogen receptor (ER) in uterus have utilized low ionic strength buffers during tissue disruption. The use of these highly unphysiologic conditions that are known to disrupt membranes and subcellular organelles, including the nucleus, casts doubt upon the "cytoplasmic" location of the receptor. Indeed, evidence has recently been presented for the presence of estrogen receptors on the surface membrane of endometrial cells (57). Furthermore, careful kinetic analysis of hormone uptake in vivo strongly suggested that the transformation of the receptor complex actually occurs in the nucleus of cells in the rat uterus (58). These findings suggest that the scheme shown in Figure 4 may be based to some extent on experimental artifacts and should be viewed with caution. Nonetheless, the general characteristics of steroid receptors are important since their presence usually identifies a target tissue and their measurement has proved to be of clinical value in identifying estrogen-dependent breast tumors and in understanding the androgen resistance of testicular feminization. On the other hand, the relative ease of demonstrating a "receptor-like" binding in tissues has resulted in a flood of recent reports suggesting new target tissues for steroid hormones without any physiologic basis for such claims. This seems to be the case for several reports describing progesterone receptors in the prostate.

Of interest are recent studies concerning the regulation of tissue receptor levels. Tissue receptor concentrations vary widely depending on hormonal, developmental, and genetic circumstances. Positive and negative control of receptor levels by hormones is best understood for estrogen and progesterone. Estrogen target tissues of the neonatal rat have very low estrogen receptor levels. Receptors are detectable at about 10 days of life after which time they can be increased greatly by the administration of estrogen. Many studies have shown that estrogen not only stimulates synthesis of its own receptor but also that of progesterone receptors. Conversely, progesterone has a negative effect on both classes of receptor. This "inactivation" of both receptors by progesterone is not fully understood but could be exerted at the level of synthesis, recycling, or decay. Nevertheless, receptor measurements in both animals and humans are consistent with the well known priming effect of estrogen required for progesterone action in the uterus and the antiestrogenic properties of progesterone. Following the pioneering work of Jensen and his coworkers (59), many investigators have demonstrated that the presence of a minimal threshold of estrogen receptors in breast tumors increases the probability that they will respond to endocrine therapy. It has been suggested by some authors that the simultaneous presence of both estrogen and progesterone receptors signifies that the estrogen receptor is functional and therefore further improves the chances of response (60). While the total absence of androgen receptor has been observed in the complete testicular feminization syndrome, intermediate levels have been found in patients with less severe defects, indicating a gene dosage effect on receptor synthesis. However, the finding of a normal complement of cytoplasmic androgen receptor in some androgen-resistant states also indicates that abnormal receptors may be synthesized and that subsequent steps such as chromatin binding may be defective (61).

Interaction of the endocrine and immune systems

While extensive study of glucocorticoid effects on the thymus gland and circulating lymphoid cells has been carried out, relatively little attention has been given to the influence of sex steroids on thymic function even though a relationship between the thymus and the gonads has been reported in 1898 (62). It was early recognized that the thymus gland undergoes involution in both sexes after puberty and the onset of adult gonadal function. Furthermore, it was apparent that reversible involution of the thymus occurs during pregnancy and lactation (63). When the steroid hormones became available, a number of studies demonstrated that large doses of estrogens or androgens caused thymic involution. Studies of the effect of thymectomy during the neonatal period have shown that there is a reciprocal interaction between thymic hormones and the pituitary-ovarian axis in females. In 1969 Nishizuka and Sakakura reported that neonatal thymectomy in the mouse between 2 to 4 days of age results in a high frequency of ovarian dysgenesis which is characterized by a rapid loss of oocytes and subsequently a decrease in the number of follicles and corpora lutea in the mouse (64). Depending upon the strain, various degrees of disruption of normal ovarian morphology have been reported, and indeed the development of ovarian tumors in a high percentage of certain strains of mice following neonatal thymectomy has been reported. These changes in ovarian function can be prevented by grafts or cell suspensions of the thymus indicating the production of substance(s) necessary for normal ovarian function. However, despite a number of reports, the nature of the interaction between the thymus, the pituitary, and the ovary has not been delineated. Very recently it has been reported that the thymus gland of the rat and the cow contains estradiol binding proteins which have the characteristics of steroid receptor proteins (65–66). However, these studies have only demonstrated binding in cytosol from these tissues and have not demonstrated nuclear uptake. Indeed, it is not clear whether the estrogen binding was due to lymphocytes or epithelial cells.

Thus, our present understanding of the sites of interaction of androgens and estrogens with the immune system is extremely limited. Obviously there are many other potential sites at which the sex steroids can act on both the humoral and cellular immune system. Hopefully, increased interest in this area will lead to new knowledge in these neglected areas.

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