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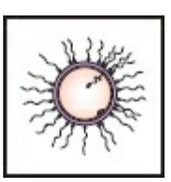

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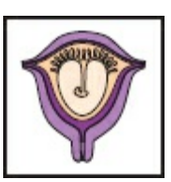

Chapter 7: The Endocrinology of Pregnancy

Hormone Biosynthesis, Metabolism, and Mechanism of Action

1

INTRODUCTION

The classical definition of a hormone is a substance that is produced in a special tissue, from which it is released into the bloodstream and travels to distant responsive cells, in which the hormone exerts its characteristic effects. What was once thought of as a simple voyage is now appreciated as an odyssey that becomes more complex, as new facets of the journey are unraveled in research laboratories throughout the world. Indeed, even the notion that hormones are products only of special tissues has been reconsidered.

Complex hormones and hormone receptors have been discovered in primitive, unicellular organisms, suggesting that endocrine glands are a late development of evolution. Hormones must have appeared even before plants and animals diverged because there are many plant substances similar to hormones and hormone receptors. The widespread capability of cells to make hormones explains the puzzling discoveries of hormones in strange places, such as gastrointestinal hormones in the brain and reproductive hormones in intestinal secretions. Furthermore, because every cell contains the genes necessary for hormonal expression, it is not surprising that dedifferentiated cancer cells can uncover gene expression and, in inappropriate locations and at inappropriate times, make hormones.

Hormones, therefore, are substances that provide a means of communication and should now be viewed broadly as chemical regulatory and signaling agents. The classic endocrine hormones travel through the bloodstream to distant sites, but cellular communication is also necessary at local sites. Paracrine, autocrine, and intracrine depict a more immediate form of communication. As compared to the distant sites of action of endocrine signals, paracrine refers to intercellular communication involving the *local* diffusion of regulating substances from one cell to nearby (contiguous) cells. In further contrast, autocrine and intracrine refer to forms of *intracellular* communication; the former involves secreted substances that act on receptors on the surface of the same cell, while the latter utilizes unsecreted substances to communicate via receptors within the same cell.

Let us follow an estradiol molecule throughout its lifespan and, in so doing, gain an overview of how hormones are formed, how hormones work, and how hormones are metabolized. Estradiol begins its journey with its synthesis in a cell specifically suited for this task. For this biosynthesis to take place, the proper enzyme capability must be present along with the proper precursors. In the adult human female, the principal sources of estradiol are the granulosa cells of the developing follicle and the corpus luteum. These cells possess the ability to turn on steroidogenesis in response to specific stimuli. The stimulating agents are the gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). The initial step in the process that will give rise to estradiol is the transmission of the message from the stimulating agents to the steroid-producing mechanisms within the cells.

Messages that stimulate steroidogenesis must be transmitted through the cell membrane. This is necessary because gonadotropins, being large glycopeptides, do not ordinarily enter cells but must communicate with the cell by joining with specific receptors on the cell membrane. In so doing, they activate a sequence of communication. A considerable amount of investigation has been devoted to determining the methods by which this communication takes place. E. W. Sutherland, Jr, received the Nobel Prize in 1971 for proposing the concept of a second messenger.

Gonadotropin, the first messenger, activates an enzyme in the cell membrane called adenylate cyclase. This enzyme transmits the message by catalyzing the production of a second messenger within the cell, cyclic adenosine 3′,5′-monophosphate (cyclic AMP). The message passes from gonadotropin to cyclic AMP, much like a baton in a relay race.

Cyclic AMP, the second messenger, initiates the process of steroidogenesis, leading to the synthesis and secretion of the hormone estradiol. This notion of message transmission has grown more and more complex with the appreciation of physiologic concepts, such as the heterogeneity of peptide hormones, the up- and downregulation of cell membrane receptors, the regulation of adenylate cyclase activity, and the important roles of autocrine and paracrine regulating factors.

Secretion of estradiol into the bloodstream directly follows its synthesis. Once in the bloodstream, estradiol exists in two forms: bound and free. A majority of the hormone is bound to protein carriers, albumin, and sex steroid hormone–binding globulin. The biologic activity of a hormone is limited by binding in the blood, thereby avoiding extreme or sudden reactions. In addition, binding prevents unduly rapid metabolism, allowing the hormone to exist for the length of time necessary to ensure a biologic effect. This reservoir-like mechanism avoids peaks and valleys in hormone levels and allows a steadier state of hormone action.

The biologic and metabolic effects of a hormone are determined by a cell's ability to receive and retain the hormone. The estradiol that is not bound to a protein but floats freely in the bloodstream readily enters cells by rapid diffusion. For estradiol to produce its effect, however, it must be grasped by a receptor within the cell. The job of the receptor is to aid in the transmission of the hormone's message to nuclear gene transcription. The result is production of messenger RNA, leading to protein synthesis and a cellular response characteristic of the hormone.

Once estradiol has accomplished its mission, it is eventually released back into the bloodstream. It is possible that estradiol can perform its duty several times before being cleared from the circulation by metabolism. On the other hand, many molecules are metabolized without ever having the chance to produce an effect. Unlike estradiol, other hormones, such as testosterone, can either work directly or be metabolized and altered within the cell in which an effect is

produced. In the latter case, a metabolite is released into the bloodstream as an inactive compound. Clearance of steroids from the blood varies according to the structure of the molecules.

Cells that are capable of clearing estradiol from the circulation accomplish this by biochemical means (conversion to estrone and estriol, moderately effective and very weak estrogens, respectively) and conjugation to products that are water soluble and excreted in the urine and bile (sulfo- and glucuroconjugates).

Thus, a steroid hormone has a varied career packed into a short lifetime. In this chapter, we will review the important segments of this lifespan in greater detail, as well as explore the mechanisms by which tropic hormones regulate steroid hormones.

STEROID HORMONE NOMENCLATURE

All steroid hormones are of a basically similar structure, with relatively minor chemical differences leading to striking alterations in biochemical activity. The basic structure is the perhydrocyclopentanophenanthrene molecule. It is composed of three 6-carbon rings and one 5-carbon ring. One 6-carbon ring is benzene, two of the 6-carbon rings are naphthalene, and three 6-carbon rings are phenanthrene; add a cyclopentane (5-carbon ring), and you have the perhydrocyclopentanophenanthrene structure of the steroid nucleus.

The sex steroids are divided into three main groups according to the number of carbon atoms they possess. The 21-carbon series includes the corticoids as well as the progestins, and the basic structure is the **pregnane** nucleus. The 19-carbon series includes all the androgens and is based on the **androstane** nucleus, whereas the estrogens are 18-carbon steroids based on the **estrane** nucleus (**Figure 1.1**).

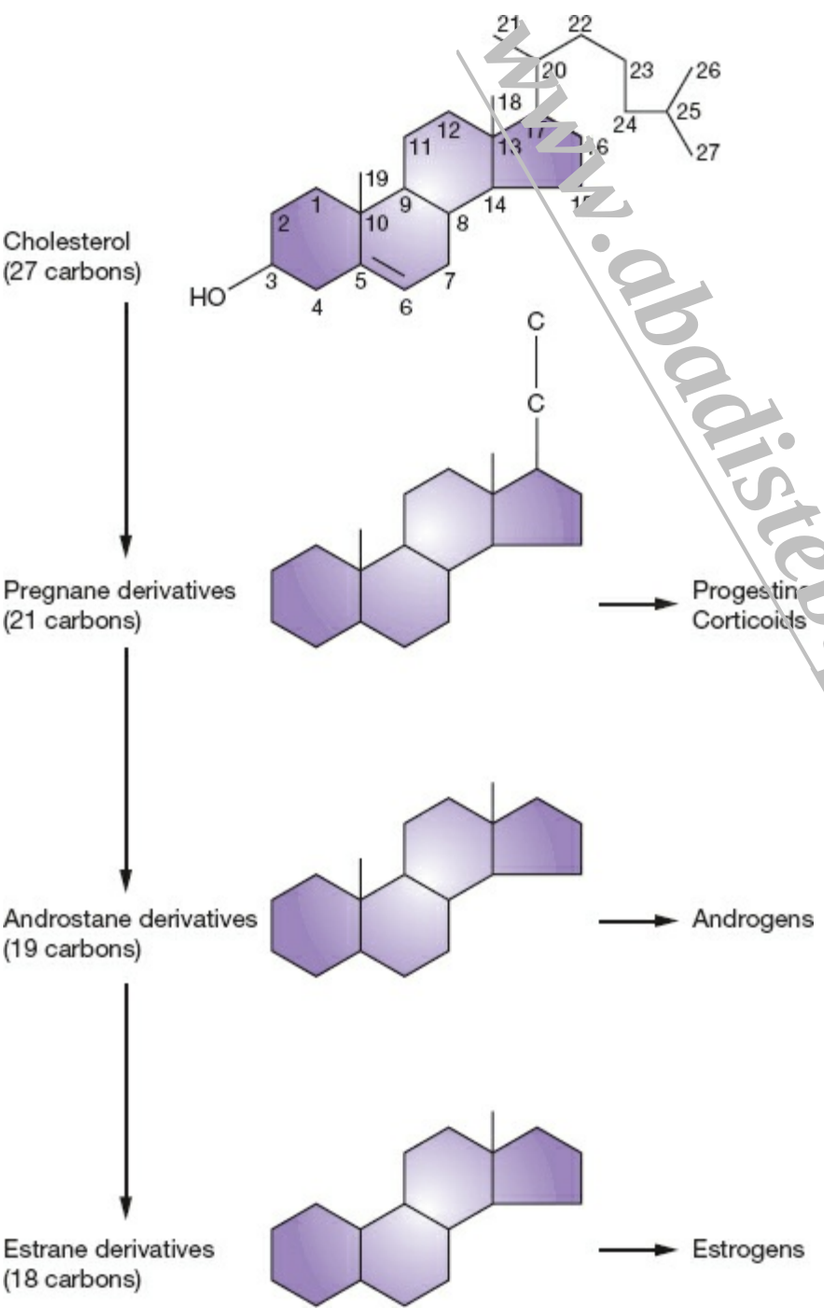

FIGURE 1.1

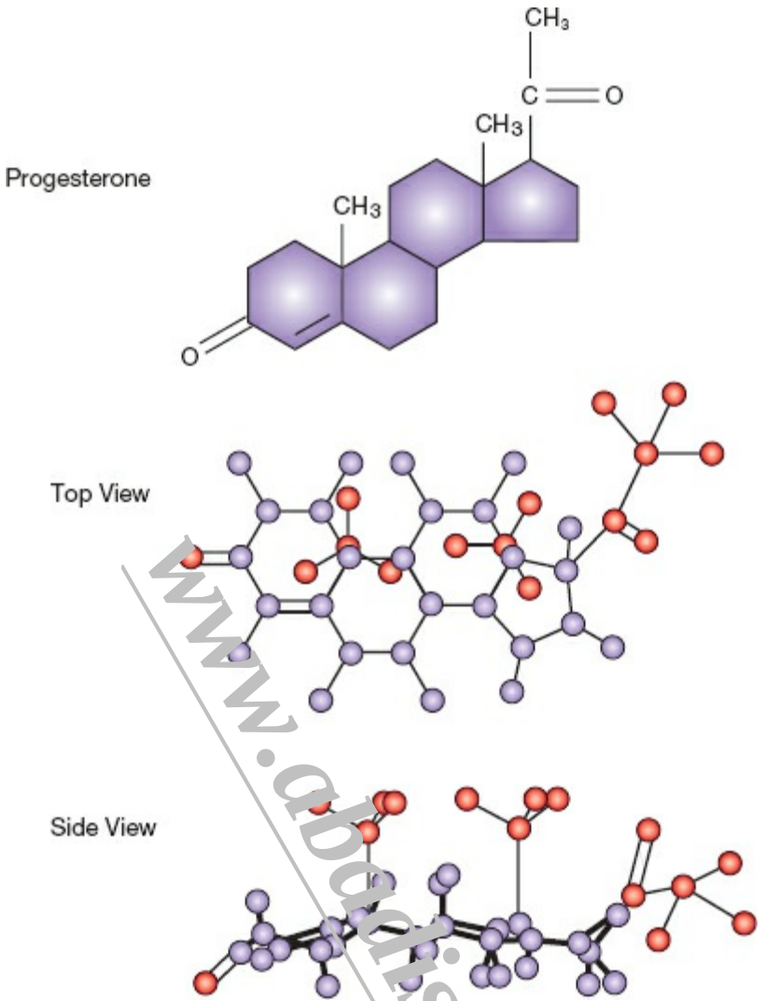

FIGURE 1.2

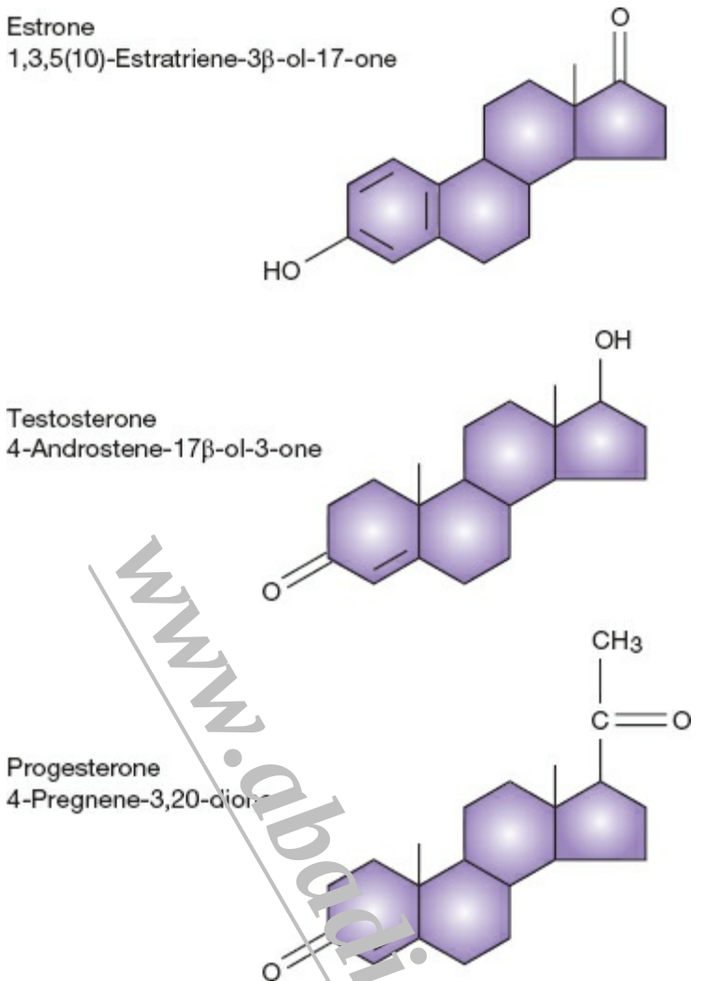

FIGURE 1.3

There are six centers of asymmetry on the basic ring structure, and there are 64 possible isomers. Almost all naturally occurring and active steroids are nearly flat, and substituents below and above the plane of the ring are designated alpha (α) (dotted line) and beta (β) (solid line), respectively. Changes in the position of only one substituent can lead to inactive isomers. For example, 17-epitestosterone is considerably weaker than testosterone, with the only difference being a hydroxyl group in the α-position at C-17 rather than in the β-position (Figure 1.2).

The convention of naming steroids uses the number of carbon atoms to designate the basic name (eg, pregnane, androstane, or estrane). The basic name is preceded by numbers that indicate the position of double bonds, and the name is altered as follows to indicate one, two, or three double bonds: -ene, -diene, and -triene. Following the basic name, hydroxyl groups are indicated by the number of the carbon attachment, and one, two, or three hydroxyl groups are designated -ol, -diol, or -triol. Ketone groups are listed last with numbers of carbon attachments, and one, two, or three groups designated -one, -dione, or -trione. Special designations include deoxy- (elimination of oxygen), nor- (elimination of carbon), and Δ- (location of double bond) (Figure 1.3).

STEROIDOGENESIS

The production of all three major groups of sex steroids is achieved through a common pathway, which begins with the cholesterol molecule. In this section, we will discuss this biosynthesis from the production and uptake of cholesterol through the synthesis of each of the sex steroids.

LIPOPROTEINS AND CHOLESTEROL

Cholesterol is the basic building block in steroidogenesis. All steroid-producing organs except the placenta can synthesize cholesterol from acetate. Therefore, progestins, androgens, and estrogens can be synthesized in situ in the various ovarian tissue compartments from the 2-carbon acetate molecule via cholesterol as the common steroid precursor. However, in situ synthesis cannot meet the demand and, therefore, the major resource is blood cholesterol that enters the ovarian cells and that can be inserted into the biosynthetic pathway or stored in an esterified form for later use. The cellular entry of cholesterol is mediated via a cell membrane receptor for low-density lipoprotein (LDL), the bloodstream carrier for cholesterol.

Lipoproteins are large molecules that facilitate the transport of nonpolar fats in a polar solvent, the blood plasma. There are five major categories of lipoproteins according to their charge and density (flotation during ultracentrifugation). They are derived from each other in the following cascade of decreasing size and increasing density.

Lipoprotein	Diameter (nm)	% Cholesterol	% Triglyceride
Chylomicron	75–1,200	30	4–8

VLDL	30–80	46–50	8–10
IDL	25–50	29	31
LDL	18–28	22	50
HDL	5–15	8	83–84

Chylomicrons

These are large, cholesterol (10%)- and triglyceride (90%)- carrying particles formed in the intestine after a fatty meal.

Very Low-Density Lipoproteins

Also carry cholesterol, these are mostly triglycerides and are denser than chylomicrons.

Intermediate-Density Lipoproteins (IDLs)

These are formed (for a transient existence) by removal of some of the triglycerides from the interior of very low-density lipoprotein (VLDL) particles.

Low-Density Lipoproteins

These are the end products of VLDL catabolism, formed after further removal of triglyceride, leaving approximately 50% cholesterol. They are the major carriers (two-thirds) of cholesterol in the plasma.

High-Density Lipoproteins

The smallest and densest of the lipoproteins with the highest protein and phospholipid content, high-density lipoprotein (HDL) can be further separated into a lighter fraction (HDL^2) and a denser fraction (HDL^3).

The lipoproteins contain four ingredients: (1) cholesterol in two forms, free cholesterol on the surface of the spherical lipoprotein molecule and esterified cholesterol in the molecule's interior; (2) triglycerides in the interior of the sphere; (3) phospholipid; and (4) protein in electrically charged substances on the surface of the sphere and responsible for miscibility with plasma and water. The surface proteins, called **apoproteins**, constitute the sites that bind to the lipoprotein receptor molecules on the cell surfaces. The principal surface protein of LDL is apoprotein B, and apoprotein A-1 is the principal apoprotein of HDL (**Figure 1.4**).

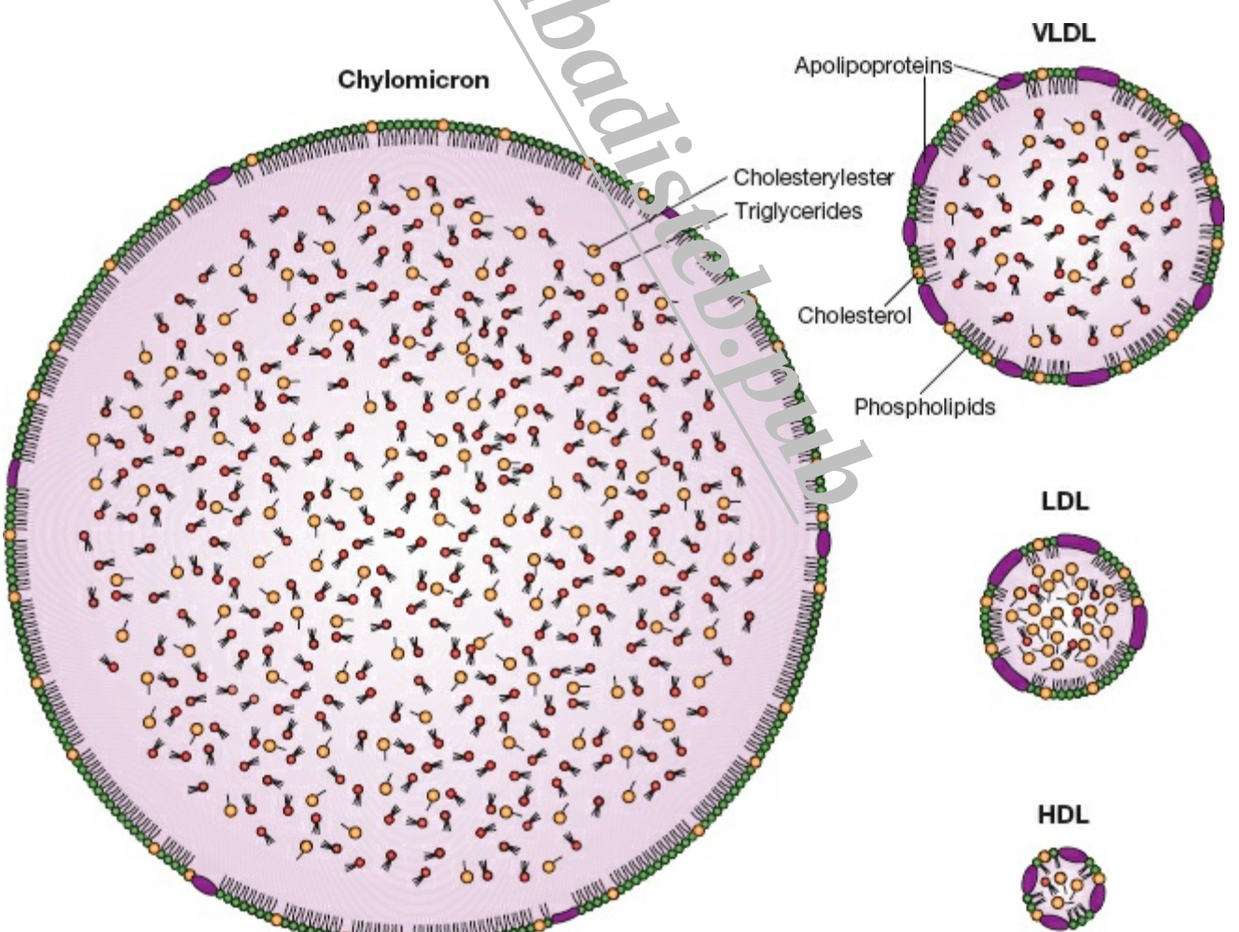

FIGURE 1.4

Lipids for peripheral tissues are provided by the secretion of VLDL by the liver. Triglycerides are liberated from VLDL by lipoprotein lipase located in the

capillary endothelial cells as well as a lipase enzyme located on the endothelial cells in liver sinusoids. In this process, the surface components (free cholesterol, phospholipids, and apoproteins) are transferred to HDL. Finally, the VLDL is converted to LDL, which plays an important role of transporting cholesterol to cells throughout the body. The hepatic lipase enzyme is sensitive to sex steroid changes; it is suppressed by estrogen and stimulated by androgens (**Figure 1.4**).

LDL is removed from the blood by cellular receptors that recognize one of the surface apoproteins. The lipoprotein bound to the cell membrane receptor is internalized and degraded. Intracellular levels of cholesterol are partly regulated by the up- and downregulation of cell membrane LDL receptors. When these LDL receptors are saturated or deficient, LDL is taken up by "scavenger" cells (most likely derived from macrophages) in other tissues, notably the arterial intima. Thus, these cells can become the nidus for atherosclerotic plaques.

HDL is secreted by the liver and intestine or is a product of the degradation of VLDL. Cholesteryl ester molecules move to form a core in a small spherical particle, the HDL^3 particle. These particles accept additional free cholesterol, perhaps mediated by receptors that recognize apoprotein A-1. With the uptake of cholesterol, the particle size increases to form HDL^2, the fraction that reflects changes in diet and hormones. HDL^3 levels remain relatively stable.

The protein moieties of the lipoprotein particles are strongly related to the risk of cardiovascular disease, and genetic abnormalities in their synthesis or structure can result in atherogenic conditions. The lipoproteins are a major reason for the disparity in atherosclerosis risk between men and women. Throughout adulthood, the blood HDL cholesterol level is about 10 mg/dL higher in women, and this difference continues through the postmenopausal years. Total and LDL cholesterol levels are lower in premenopausal women than in men, but after menopause, they rise rapidly.

The protective nature of HDL is due to its ability to pick up free cholesterol from cells or other circulating lipoproteins. This lipid-rich HDL is known as HDL^3, which is then converted to the larger, less-dense particle, HDL^2. Thus, HDL converts lipid-rich scavenger cells (macrophages residing in arterial walls) back to their low-lipid state and carries the excess cholesterol to sites (mainly the liver) where it can be metabolized. HDL can also remove free cholesterol via its uptake from cell membranes, and once esterified, the free cholesterol is moved to the core of the HDL particle, where it can be delivered to sites for utilization (steroid-producing cells) or metabolism and excretion (liver).

Low blood concentration of cholesterol is associated with good cardiovascular health. Tight regulation of the blood concentration of cholesterol is, therefore, critical. Cholesterol is transported by esterifying the cholesterol and packaging the ester within the cores of plasma lipoproteins. Cholesterol is then delivered into cells via lipoprotein receptors. After binding the lipoprotein with its package of esterified cholesterol, the complex is delivered into the cell by receptor-mediated endocytosis (discussed later in this chapter), in which the lysosomes liberate cholesterol for use by the cell.

Major protection against atherosclerosis depends on the high affinity of the receptor for LDL and the ability of the receptor to recycle multiple times, allowing large amounts of cholesterol to be delivered while maintaining a healthy low blood level of LDL. Cells can control their uptake of cholesterol by increasing or decreasing the number of LDL receptors according to the intracellular cholesterol levels. Thus, a high-cholesterol diet influences the liver to reduce the number of LDL receptors on its cells, causing an elevated blood level of LDL. Statins protect against atherosclerosis by reducing cholesterol biosynthesis, increasing LDL receptors in the liver, and lowering circulating levels of LDL cholesterol.

STEROID BIOSYNTHETIC PATHWAY

The overall steroid biosynthesis pathway, shown in **Figure 1.5,** is based primarily on the pioneering work of Kenneth J. Ryan and his coworkers.[1,2] This pathway follows a fundamental pattern displayed by all steroid-producing endocrine organs. Hence, the normal human ovary produces all three classes of sex steroids: estrogens, progestins, and androgens. The importance of ovarian androgens is appreciated, not only as obligate precursors to estrogens but also as clinically important secretory products. The ovary differs from the testis in its fundamental complement of critical enzymes and, hence, its distribution of secretory products. The ovary is distinguished from the adrenal gland by its deficiency in 21-hydroxylase and 11β-hydroxylase reactions. Glucocorticoids and mineralocorticoids, therefore, are not produced in normal ovarian tissue.

During steroidogenesis, the number of carbon atoms in cholesterol or other steroid molecule can be reduced but never increased. The following reactions can take place:

1. **Cleavage of a side chain** (desmolase reaction)
2. **Conversion of hydroxy groups into ketones or ketones into hydroxy groups** (dehydrogenase reactions)
3. **Addition of hydroxy group** (hydroxylation reaction)
4. **Creation of double bonds** (removal of hydrogen)
5. **Addition of hydrogen to reduce double bonds** (saturation)

The traditional view of steroidogenesis was that each step was mediated by many enzymes, specific to tissue type. A fundamental simplicity to the system emerged when the responsible complementary DNAs and genes were cloned.[3–5]

Steroidogenic enzymes are either dehydrogenases or members of the cytochrome P450 group of oxidases. Cytochrome P450 (CYP) is a generic term for a family of oxidative enzymes, termed 450 because of a pigment (450) absorbance shift when reduced. P450 enzymes can metabolize many substrates; for example, in the liver, P450 enzymes (CYPs) metabolize toxins and environmental pollutants. The human genome contains genes for 57 cytochrome P450 enzymes; 7 of these enzymes localize to the mitochondria and 50 to the endoplasmic reticulum (the major site for metabolic clearance). The following distinct P450 enzymes are involved in steroidogenesis: P450scc is the cholesterol side-chain cleavage enzyme; P450c11 mediates 11β-hydroxylase, 18-hydroxylase, and 19-methyloxidase; P450c17 mediates 17α-hydroxylase and 17,20-lyase; P450c21 mediates 21-hydroxylase; and P450arom mediates aromatization of androgens to estrogens (**Table 1.1**). Marked differences in the exon–intron organization of the *P450* genes are compatible with an ancient origin; the superfamily of *P450* genes diverged more than 1.5 billion years ago.

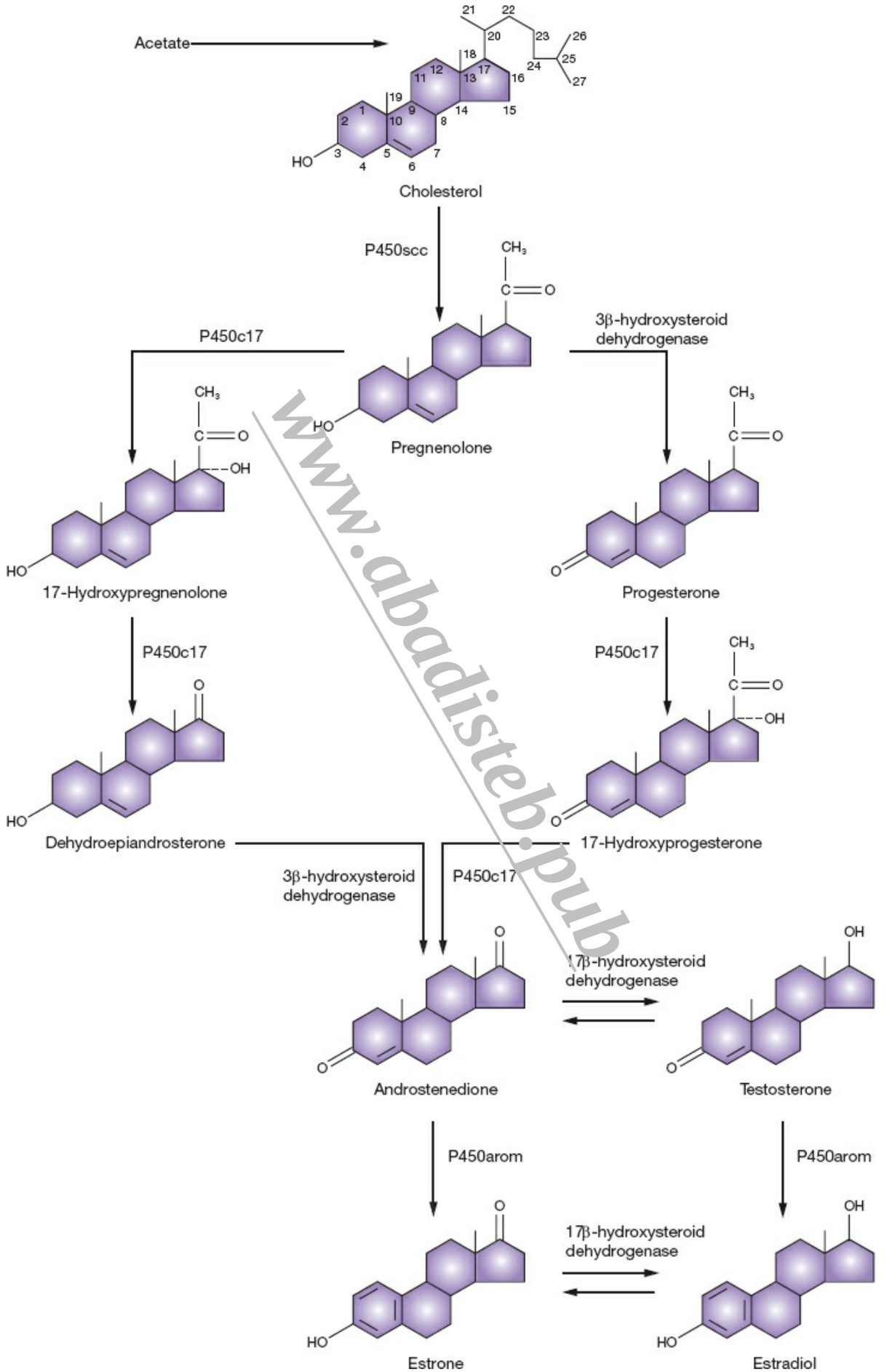

FIGURE 1.5

The structural knowledge of the P450 enzymes that has been derived from amino acid and nucleotide sequencing studies demonstrated that all the steps between cholesterol and pregnenolone were mediated by a single protein, P450scc, bound to the inner mitochondrial membrane. Cloning data revealed that this protein is encoded by the *CYP11A1* gene on chromosome 15. These experiments indicated that multiple steps did *not* require multiple enzymes. Varying activity in different tissues may reflect posttranslational modifications. In addition, P450-encoding genes contain tissue-specific promoter sequences, allowing for distinct regulatory mechanisms in different tissues (eg, placenta and ovary). *CYP11A1* mutations are very rare, producing impaired steroidogenesis in both the adrenal glands and the gonads and causing abnormal sexual development and adrenal failure.[6]

Conversion of cholesterol to pregnenolone involves hydroxylation at the carbon 20 and 22 positions, with subsequent cleavage of the side chain. Conversion of cholesterol to pregnenolone by P450scc takes place within the mitochondria. It is one of the principal effects of tropic hormone stimulation, which also causes the uptake of the cholesterol substrate for this step in the ovary. The tropic hormones from the anterior pituitary bind to the cell surface receptor of the G protein system, activate adenylate cyclase, and increase intracellular cyclic AMP. Cyclic AMP activity leads to gene transcription, which encodes the steroidogenic enzymes and accessory proteins. In a process that is faster than gene transcription, cyclic AMP stimulates the hydrolysis of cholesteryl esters and the transport of free cholesterol to the mitochondria.

TABLE 1.1 Cytochrome P450 Enzymes

Enzyme	Cellular Location	Reactions
P450scc	Mitochondria	20α-Hydroxylase 22-Hydroxylase Cholesterol side-chain cleavage
P450c11	Mitochondria	11β-Hydroxylase 18-Hydroxylase 19-Methyloxidase
P450c17	Endoplasmic reticulum	17 α-Hydroxylase 17, 20-Lyase
P450c21	Endoplasmic reticulum	21-Hydroxylase
P450arom	Endoplasmic reticulum	Aromatase

As mentioned, the cholesterol used for steroid synthesis is derived from circulating LDLs, followed by the mobilization and transport of intracellular stores.[5,7,8] LDL cholesterol esters are incorporated into the cell by tropic hormone stimulation of endocytosis via clathrin-coated pits (a mechanism discussed later in this chapter). Cholesterol is stored in the cell in the ester form or as free cholesterol. Indeed, the rate-limiting step in steroidogenesis is the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where fully active P450scc waits for substrate. The rate-limiting transfer of hydrophobic cholesterol through the aqueous space between the outer and the inner mitochondrial membranes is mediated by protein activation stimulated by the tropic hormone. Long-term, chronic steroidogenesis requires gene transcription and protein synthesis, but short-term, acute responses occur independently of new RNA synthesis (although protein synthesis is still necessary—specifically the proteins that regulate cholesterol transfer across the mitochondrial membrane).

Several proteins have been characterized and proposed as regulators of acute intracellular cholesterol transfer. These proteins include sterol carrier protein 2 (SCP2), which is able to bind and transfer cholesterol between compartments within a cell, steroidogenesis activator polypeptide (SAP), which is a small molecule, and translocator protein (TSPO; previously called peripheral benzodiazepine receptor [PBR]), which affects cholesterol flux through a pore structure. However, the most studied and favored protein as a regulator of acute cholesterol transfer is **steroidogenic acute regulatory (StAR) protein**.[9–13] StAR messenger RNA and proteins are induced concomitantly with acute steroidogenesis in response to cyclic AMP stimulation. StAR protein increases steroid production and is imported and localized in the mitochondria. Congenital lipoid adrenal hyperplasia, an autosomal recessive disorder, is a failure in adrenal and gonadal steroidogenesis due to a variety of mutations in the *StAR* gene.[14,15] With several of these mutations, a low level of steroidogenesis is possible, even permitting feminization at puberty, but continuing tropic hormonal stimulation results in an accumulation of intracellular lipid deposits that destroy steroidogenic capability.[16] Mutations of the *StAR* gene are the only inherited disorder of steroidogenesis not caused by a defect in one of the steroidogenic enzymes.

StAR is required for adrenal and gonadal steroidogenesis (for which it mediates the transport of cholesterol into mitochondria) and, therefore, is necessary for normal male sexual differentiation. StAR moves cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, where it can enter the steroidogenic pathway by being converted to pregnenolone. A group of proteins structurally related to StAR have been identified, designated StARD4, StARD5, and StARD6. StARD4 is responsible for binding free cholesterol, as it is produced in the cytoplasm and transporting it to the outer mitochondrial membrane.[12] Because steroid-producing cells do not store large amounts of hormones, acute increases in secretion depend on this system to produce rapid synthesis.

Once pregnenolone is formed, further steroid synthesis in the ovary can proceed by one of two pathways: via Δ^5-3β-hydroxysteroids or via the Δ^4-3-ketone pathway. The first (the Δ^5 pathway) proceeds by way of pregnenolone and dehydroepiandrosterone (DHEA), and the second (the Δ^4 pathway) via progesterone and 17α-hydroxyprogesterone.

The conversion of pregnenolone to progesterone involves two steps: the 3β-hydroxysteroid dehydrogenase and Δ^{4-5} isomerase reactions that convert the 3-hydroxyl group to a ketone and transfer the double bond from the 5 to 6 position to the 4 to 5 position. The 3β-hydroxysteroid dehydrogenase enzyme catalyzes both the dehydrogenation and isomerization reactions and exists in two forms (type I and type II), encoded by two separate genes on chromosome 1. The type I gene is expressed in the placenta, breast, and other nonglandular tissues, while the type II gene is expressed in the gonads and the adrenal glands. Once the Δ^{4-5} ketone is formed, progesterone is hydroxylated at the 17 position to form 17α-hydroxyprogesterone. 17α-Hydroxyprogesterone is the immediate precursor of the C-19 (19 carbons) series of androgens in this pathway. By peroxide formation at C-20, followed by epoxidation of the C-17, C-20 carbons, the side chain is split off, forming androstenedione. The 17-ketone may be reduced to a 17β-hydroxy to form testosterone by the 17β-hydroxysteroid dehydrogenase reaction. Both C-19 steroids (androstenedione and testosterone) can be converted to corresponding C-18 phenolic steroid estrogens (estrone and estradiol) by microsomal reactions in a process referred to as aromatization. This process includes hydroxylation of the angular 19-methyl group, followed by oxidation, loss of the 19-carbon as formaldehyde, and ring A aromatization (dehydrogenation). As an alternative, pregnenolone can be directly converted to

the Δ^5-3β-hydroxy C-19 steroid, DHEA, by 17α-hydroxylation, followed by cleavage of the side chain. With the formation of the Δ^4-3-ketone, DHEA is converted into androstenedione.

The four reactions involved in converting pregnenolone and progesterone to their 17-hydroxylated products are mediated by a single enzyme, P450c17, bound to smooth endoplasmic reticulum and encoded by the *CYP17A1* gene on chromosome 10q24.32. 17-Hydroxylase and 17,20-lyase were historically thought to be separate enzymes; however, these two different functions of the single P450c17 enzyme are not genetic or structural but represent the effect of posttranslational influencing factors.[17] In the adrenal gland pathway to cortisol formation, very little 17,20-lyase activity is expressed. In the ovarian theca cells, the testicular Leydig cells, and the adrenal reticularis, both 17-hydroxylase and 17,20-lyase activities are expressed, directing the steroidogenic pathway via DHEA. In the corpus luteum, the principal pathway is via progesterone.

Hydroxylation of progesterone and 17α-hydroxyprogesterone is mediated by the P450c21 protein, also known as 21-hydroxylase. Characterization of this protein and gene cloning indicate that its encoding gene, *CYP21*, is located on chromosome 6p21.3. An inactive pseudogene, *CYP21P*, is nearby. Many of the mutations that affect *CYP21* and cause congenital adrenal hyperplasia are gene conversions involving recombinations between *CYP21* and inactivating mutations in *CYP21P*.

Aromatization is mediated by P450arom found in the endoplasmic reticulum.[18,19] Aromatase cytochrome P450 is encoded by the *CYP19A1* (cytochrome P450; family 19—denoting oxidation of the C-19 methyl group; subfamily A; polypeptide 1) gene on chromosome 15q21.1. Aromatization in different tissues with different substrates is the result of the single P450arom enzyme encoded by this single gene. Aromatase deficiency because of an inactivating mutation of *CYP19A1* is very rare; only a handful of cases have been reported.[20] Affected females present at birth with virilization because the placenta cannot convert fetal adrenal androgens to estrogens; thus, maternal virilization during the pregnancy is usually also present.

Aromatase transcription is regulated by several promoter sites that respond to cytokines, cyclic nucleotides, gonadotropins, glucocorticoids, and growth factors.[21] Tissue-specific expression is regulated by tissue-specific promoters that allow the extremes of highly regulated expression in the ovary in response to cyclic AMP and gonadotropins, expression in adipose tissue stimulated by prostaglandin E^2, and nonregulated expression in the placenta and adipose. Very specific inhibitors of P450arom have been developed, called "aromatase inhibitors," which allow intense blockage of estrogen production, with clinical applications that include the treatment of breast cancer (eg, anastrozole and letrozole) and ovulation induction. The aromatase complex also includes NADPH-cytochrome P450 reductase, a ubiquitous flavoprotein involved in reduction reactions.

The 17β-hydroxysteroid dehydrogenase and 5α-reductase reactions are mediated by non-P450 enzymes. The 17β-hydroxysteroid dehydrogenase is bound to the endoplasmic reticulum and the 5α-reductase to the nuclear membrane. The 17β-hydroxysteroid dehydrogenase enzymes convert estrone to estradiol, androstenedione to testosterone, and DHEA to androstenediol, and vice versa. Fourteen different isozymes have been cloned and characterized in mammals.[22] Each has a unique gene, subcellular, and tissue distribution as well as preferred catalyst, substrate, and cofactor. The cell-specific production of each of these isoforms is a method for regulating the local concentration of estrogens and androgens.

ESTROGENS

Androgens are the precursors of estrogens. 17β-Hydroxysteroid dehydrogenase activity converts androstenedione to testosterone, which is not a major secretory product of the normal ovary. It is rapidly demethylated at the C-19 position and aromatized to estradiol, the major estrogen secreted by the human ovary. This process involves the well-characterized "two-cell system," described at length in Chapter 5 (**Figure 1.6**). Estradiol also arises to a major degree from androstenedione via estrone, and estrone itself is secreted in significant daily amounts. Estriol is the peripheral metabolite of estrone and estradiol and not a secretory product of the ovary. The formation of estriol is typical of general metabolic "detoxification," (ie, the conversion of biologically active material to less active forms) (**Figure 1.7**).

The conversion of steroids in peripheral tissues is not always a form of inactivation. Free androgens are peripherally converted to free estrogens, for example, in the skin and adipose cells. The location of the adipose cells influences their activity. Women with central obesity produce more androgens.[23] The work of Siiteri and MacDonald[24] demonstrated that enough estrogen can be derived from circulating androgens to produce bleeding in the postmenopausal woman. In the female, the adrenal gland remains the major source of circulating androgens, particularly androstenedione (**Figure 1.8**). In the male, almost all of the circulating estrogens are derived from peripheral conversion of androgens. The precursor androgens consist principally of androstenedione, DHEA, and dehydroepiandrosterone sulfate.

It can be seen, therefore, that the pattern of circulating steroids in the female is influenced by the activity of various processes outside the ovary. Because of the peripheral contribution to steroid levels, the term *secretion rate* **is reserved for direct organ secretion, whereas** *production rate* **includes organ secretion plus peripheral contribution via conversion of precursors. The** *metabolic clearance rate (MCR)* **equals the volume of blood that is cleared of the hormone per unit of time. The** *blood production rate (PR)* **then equals the MCR multiplied by the concentration of the hormone in the blood**.

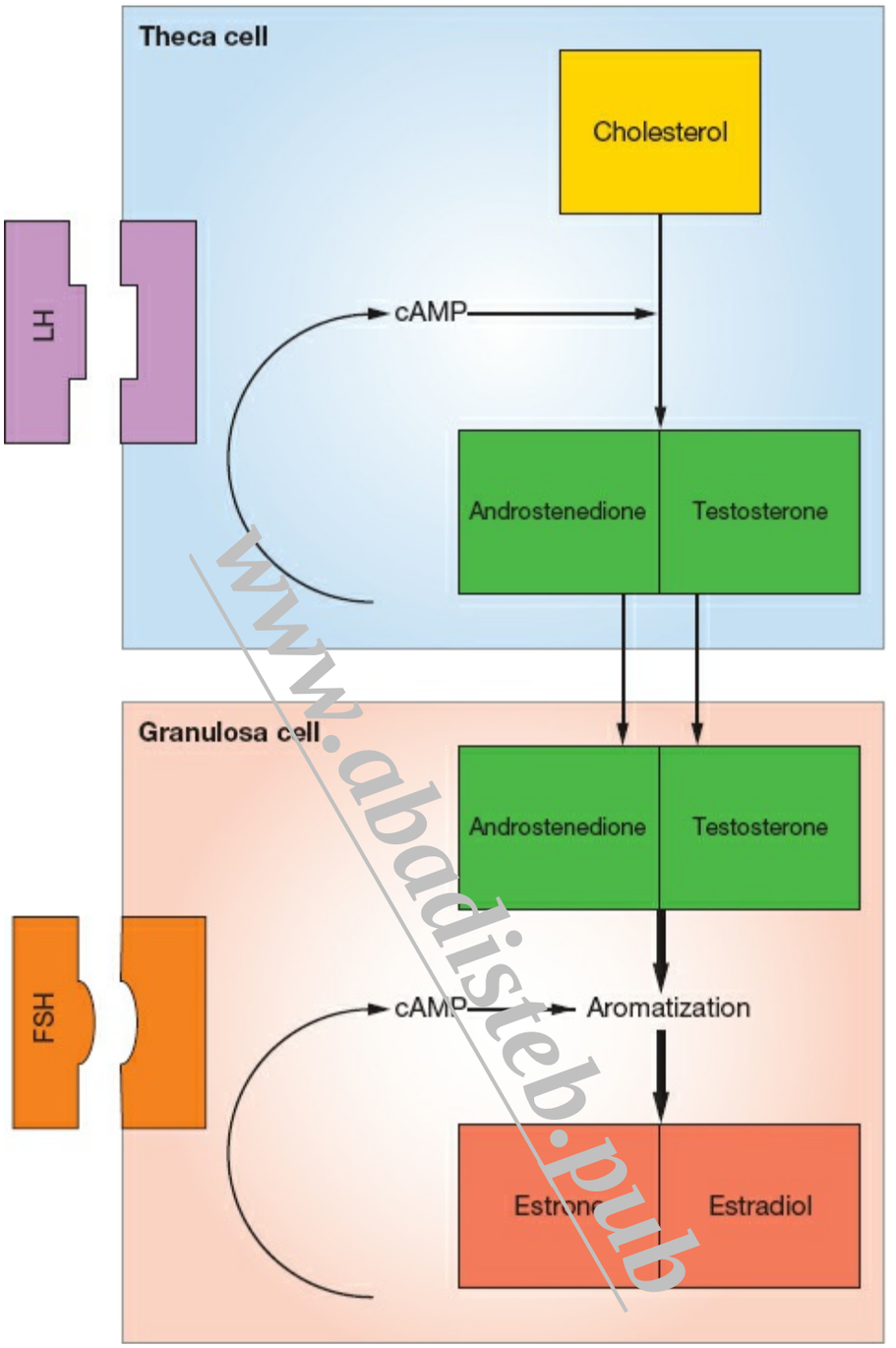

FIGURE 1.6

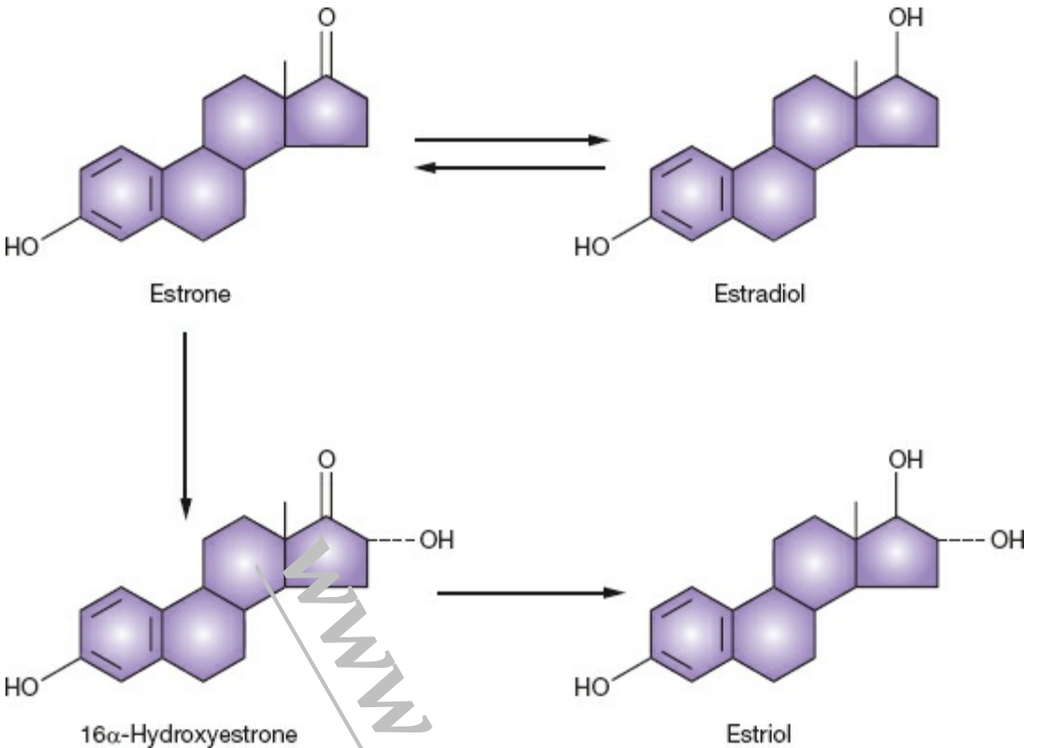

FIGURE 1.7

MCR = Liters/Day
PR = MCR × Concentration (Liters/Day × Amount/Liter = Amount/Day)

In a typical nonpregnant female, estradiol is produced at the rate of 100 to 500 μg/d, but it circulates and functions within cells in concentrations of pg/mL. The production of androstenedione is about 3 mg/d, and the peripheral conversion (~1%) of androstenedione to estrone accounts for about 20% to 30% of the estrone produced per day. Because androstenedione is secreted in milligrams, even a small percentage of conversion to estrogen results in a significant contribution to estrogens, which exist and function in the circulation in picogram amounts. Thus, the circulating estrogens in the female are the sum of direct ovarian secretion of estradiol and estrone, plus peripheral conversion of C-19 precursors (**Figure 1.9**).

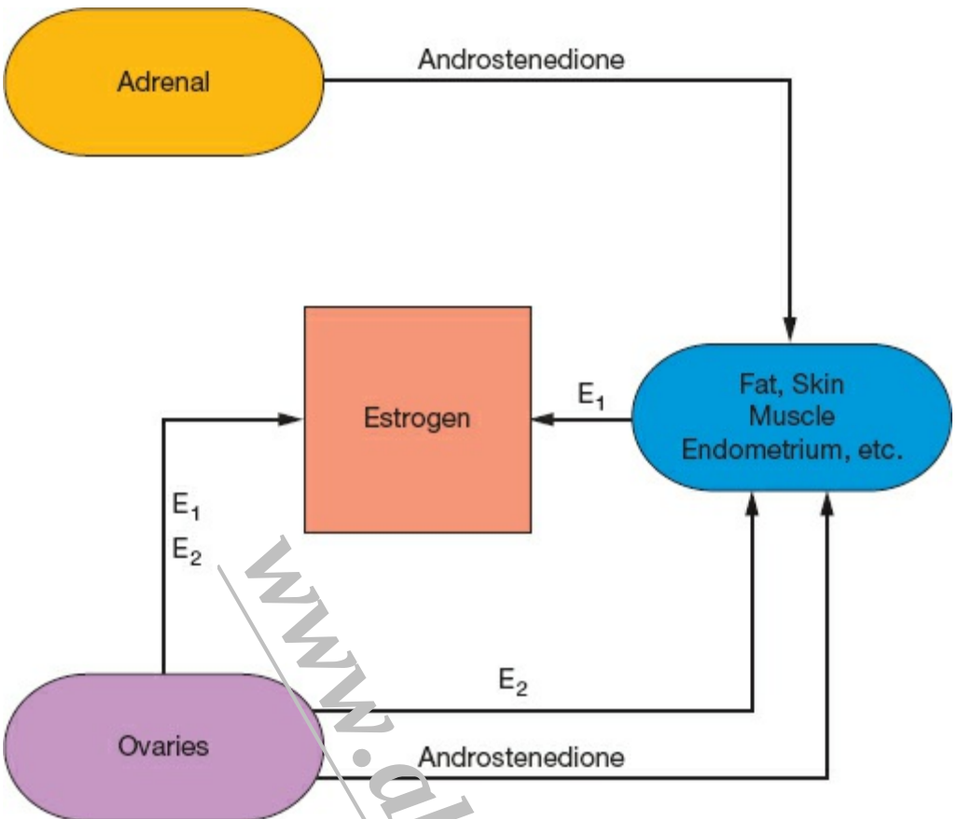

FIGURE 1.8

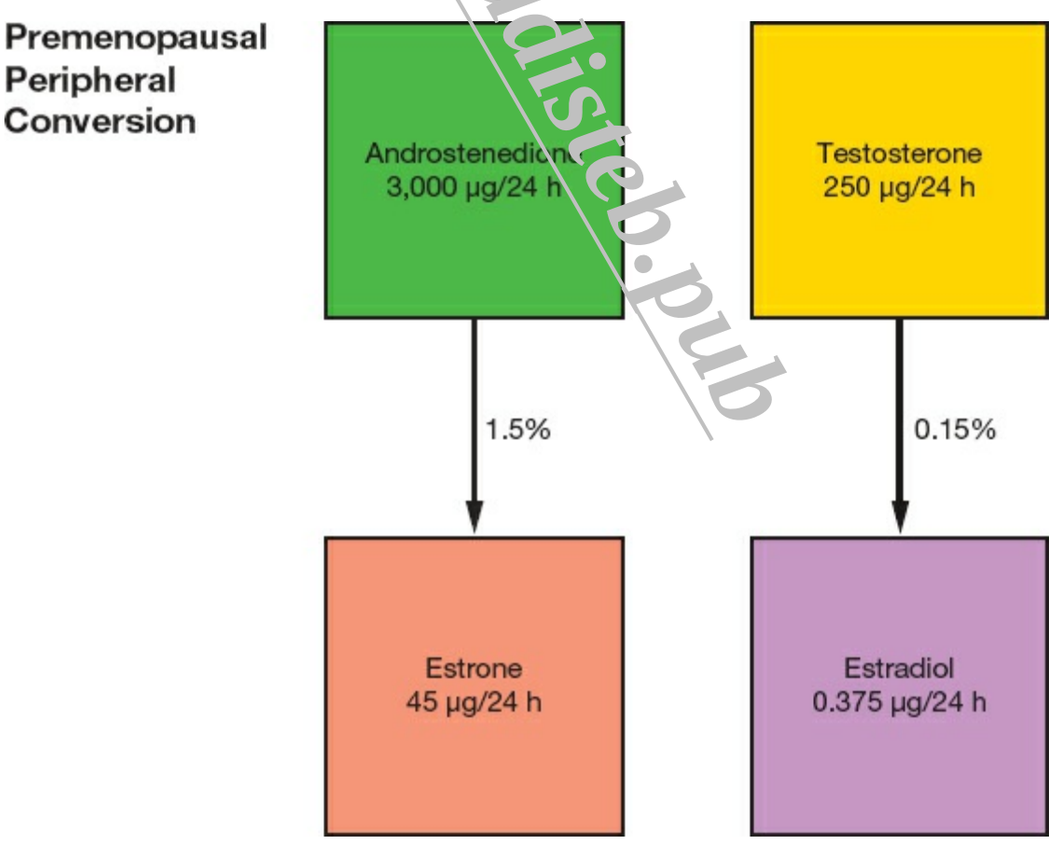

FIGURE 1.9

PROGESTERONE

Peripheral conversion of steroids to progesterone is not seen in the nonpregnant female; rather, the progesterone PR is a combination of secretion from the adrenal and the ovaries. Including the small contribution from the adrenal, the blood PR of progesterone in the preovulatory phase is less than 1 mg/d. During the luteal phase, production increases to 20 to 30 mg/d.

In the preovulatory phase in adult females, in all prepubertal females, and in the normal male, the blood levels of progesterone are at the lower limits of immunoassay sensitivity: less than 1 ng/mL. After ovulation, that is, during the luteal phase, progesterone ranges from 3 to 15 ng/mL. In congenital adrenal hyperplasia, progesterone blood levels can be as high as 50 times above normal. Pregnanediol and pregnanetriol are metabolites of progesterone that are excreted in the urine (**Figure 1.10**).

ANDROGENS

The major androgen products of the ovary are DHEA and androstenedione (and minimal testosterone), which are secreted mainly by stromal tissue derived from theca cells. With excessive accumulation of stromal tissue or in the presence of an androgen-producing tumor, testosterone becomes a significant secretory product. Occasionally, a nonfunctioning tumor can induce stromal proliferation and increased androgen production. The normal accumulation of stromal tissue at midcycle results in a rise in circulating levels of androstenedione and testosterone at the time of ovulation.

The adrenal cortex produces three groups of steroid hormones: the glucocorticoids, the mineralocorticoids, and the sex steroids. The adrenal sex steroids represent intermediate by-products in the synthesis of glucocorticoids and mineralocorticoids, and excessive secretion of the sex steroids occurs only with neoplastic cells or in association with enzyme deficiencies. Under normal circumstances, adrenal gland production of the sex steroids is less significant than gonadal production of androgens and estrogens. About one-half of the daily production of DHEA and androstenedione comes from the adrenal gland. The other half of androstenedione is secreted by the ovary, while the other half of DHEA is secreted almost equally from the ovary and peripheral tissues. The PR of testosterone in the normal female is 0.2 to 0.3 mg/d and approximately 50% arises from peripheral conversion of androstenedione (and a small amount from DHEA) to testosterone, whereas 25% is secreted by the ovary and 25% by the adrenal.

Reduction of the Δ^4 unsaturation (an irreversible pathway) in testosterone is very significant, producing derivatives very different in their spatial configuration and activity. The 5β-derivatives are not androgenic, and this is not a clinically important pathway; however, the 5α-derivative (a very active pathway) is extremely potent. Indeed, dihydrotestosterone (DHT), the 5α-derivative, is the principal androgenic hormone in a variety of target tissues and is formed within the target tissue itself.

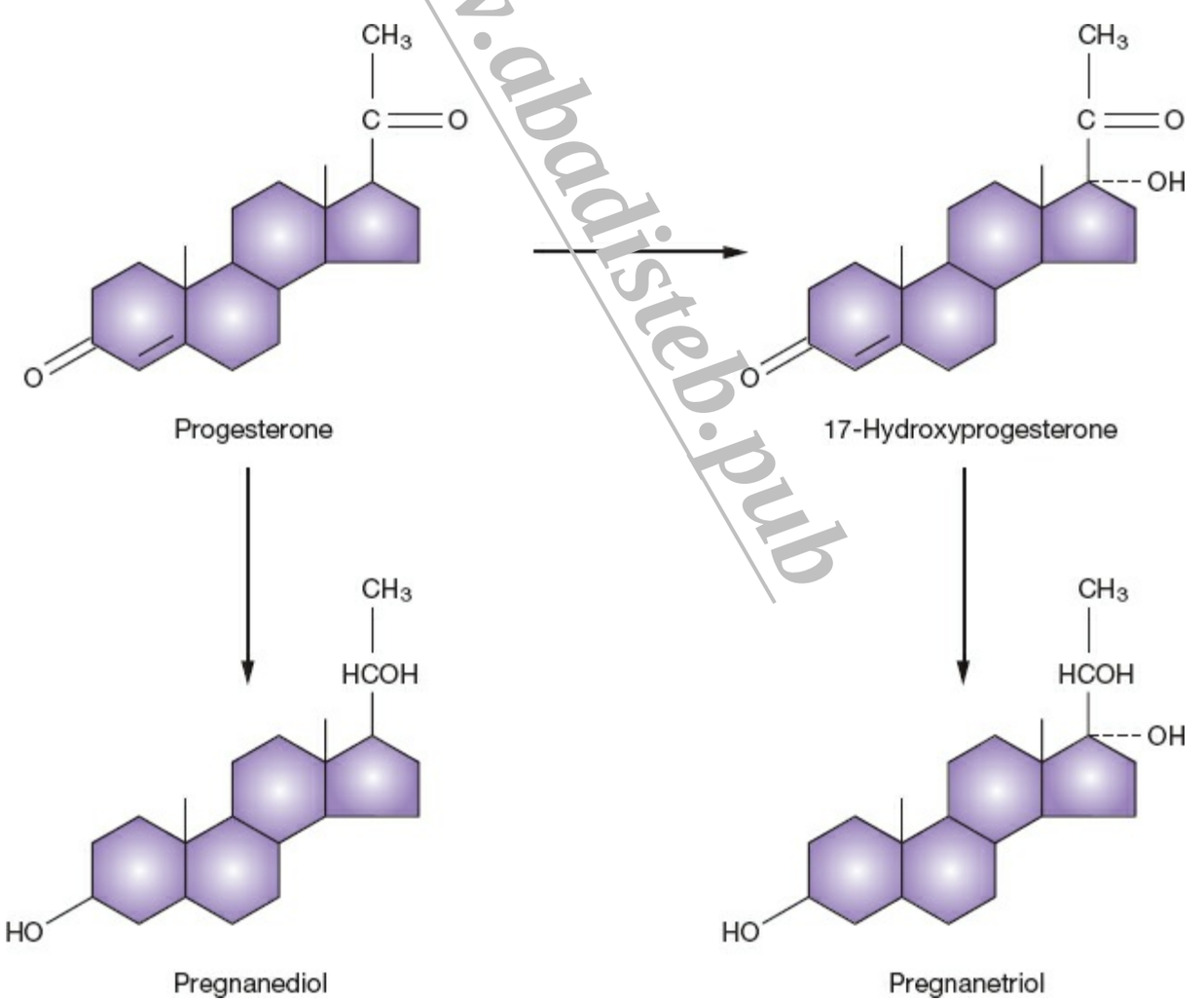

FIGURE 1.10

In men, the majority of DHT is derived from testosterone that enters a target cell and is converted by means of 5α-reductase. In women, because the PR of androstenedione is greater than testosterone, DHT is derived primarily from androstenedione and partly from DHEA.[25] Thus, in women, the skin production of

DHT is predominantly influenced by androstenedione. DHT is, by definition, an intracrine hormone that is formed in and acting within target tissues.[26] The 5α-reductase enzyme exists in two forms, types I and II, each encoded by a separate gene, with the type I enzyme found in the skin and the type II reductase predominantly expressed in reproductive tissues.[27]

DHT is largely metabolized intracellularly; hence, the blood DHT is only about one-tenth of the level of circulating testosterone. In tissues sensitive to DHT (which includes hair follicles), only DHT enters the nucleus to provide the androgen message. DHT can also perform androgenic actions within cells that do not possess the ability to convert testosterone to DHT. DHT is further reduced by a 3α-keto-reductase to androstanediol, which is relatively inactive. The metabolite of androstanediol, 3α-androstanediol glucuronide, is the major metabolite of DHT and can be measured in the plasma, indicating the amount of target tissue conversion of testosterone to DHT (**Figure 1.11**).

Not all androgen-sensitive tissues require the prior conversion of testosterone to DHT. In the process of masculine differentiation, the development of the Wolffian duct structures (epididymis, the vas deferens, and the seminal vesicle) is dependent on testosterone as the intracellular mediator, whereas the development of the urogenital sinus and urogenital tubercle into the male external genitalia, urethra, and prostate requires conversion of testosterone to DHT.[28] Muscle development is under the direct control of testosterone. Testosterone is also aromatized to a significant extent in the liver and breast, and in some circumstances, including in the brain, androgenic messages can be transmitted via estrogen.

BLOOD TRANSPORT OF STEROIDS

While circulating in the blood, most of the principal sex steroids (ie, estradiol and testosterone) are bound to a protein carrier known as sex hormone–binding globulin (SHBG), which is produced mainly in the liver. Additionally, 30% is loosely bound to albumin, and a very small percentage binds to corticosteroid-binding globulin, leaving only about 1% unbound and free. Hyperthyroidism, pregnancy, and estrogen administration all increase SHBG levels, whereas corticoids, androgens, progestins, growth hormone, insulin, and insulin-like growth factor I (IGF-I) decrease SHBG.

The circulating level of SHBG is inversely related to body weight and, thus, significant weight gain can decrease SHBG and produce important changes in the unbound levels of the sex steroids. Additionally, circulating SHBG levels are reduced in the setting of insulin resistance and hyperinsulinemia[29,30]; this, in fact, may be the major mechanism that mediates the impact of increased body weight on SHBG. The relationship between levels of insulin and SHBG is so strong that SHBG concentrations are a marker for hyperinsulinemic insulin resistance, and a low level of SHBG is a predictor for the development of type 2 diabetes mellitus.[31] Similarly, SHBG is a biomarker and therapeutic target for patients with polycystic ovary syndrome (PCOS)[32] (Chapter 11).

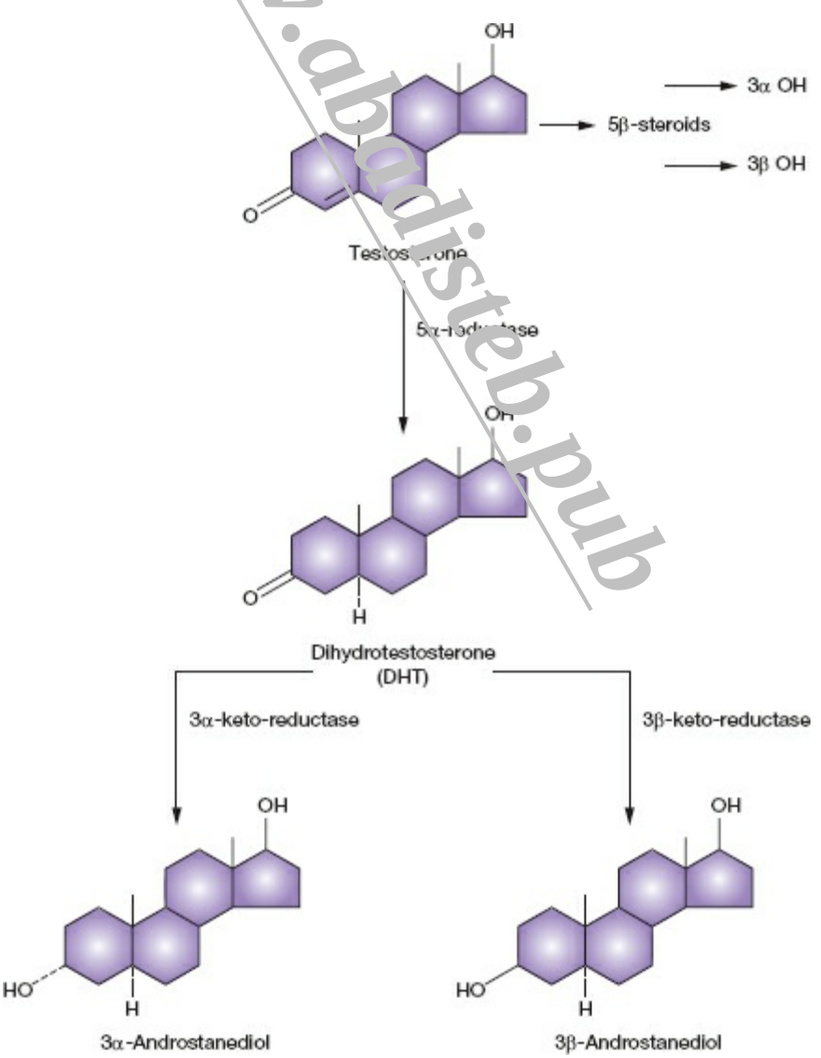

FIGURE 1.11

The distribution of body fat also has a strong influence on SHBG levels. Android or central fat is located in the abdominal wall and visceral-mesenteric locations. This fat distribution is associated with hyperinsulinemia, hyperandrogenism, and decreased levels of SHBG.[33] The common mechanism for these changes is probably the hyperinsulinemia.

While SHBG is a homodimeric glycoprotein composed of two monomers, it contains a single binding site for androgens and estrogens. Its encoding gene has been localized to the short arm of chromosome 17.[34] Genetic studies have revealed that the *SHBG* gene also encodes the androgen-binding protein present in the seminiferous tubules, synthesized by the Sertoli cells.[35,36] Dimerization is believed to be necessary to form the single steroid-binding site. Specific genetic abnormalities with decreased or abnormal SHBG have not been reported. SHBG gene expression has now been identified in other tissues (brain, placenta, and endometrium), with the biologic significance actively being explored. For example, SHBG expression in the hypothalamus has been found to correlate to pregnancy, labor, and breastfeeding.[37]

As previously mentioned, SHBG is decreased by androgens; hence, the binding capacity in men is lower than in women. The binding globulin level in women with increased androgen production is also depressed, such as in PCOS. Androgenic effects are dependent on the unbound fraction that can move freely from the vascular compartment into the target cells. Routine assays determine the total hormone concentration, bound plus free. Thus, a total testosterone concentration can be in the normal range in a woman who is hirsute, but because the binding globulin level is depressed by the androgen effects, the percent-free and active testosterone is elevated.

Transcortin, also called corticosteroid-binding globulin, is a plasma glycoprotein that binds cortisol, progesterone, deoxycorticosterone, corticosterone, and some of the other minor corticoid compounds. Normally, about 75% of circulating cortisol is bound to transcortin, 15% is loosely bound to albumin, and 10% is unbound or free. Progesterone circulates in the following percentages: less than 2% unbound, 80% bound to albumin, 18% bound to transcortin, and less than 1% bound to SHBG. Binding in the circulation follows the law of mass action: the amount of the free, unbound hormone is in equilibrium with the bound hormone. Thus, the total binding capacity of a binding globulin will influence the amount that is free and unbound.

The biologic effects of the major sex steroids are determined largely by the unbound portion, known as the free hormone. In other words, the active hormone is unbound and free, whereas the bound hormone is relatively inactive. This concept is not without controversy. The hormone–protein complex may be involved in an active uptake process at the target cell plasma membrane.[38–40] The albumin-bound fraction of steroids may also be available for cellular action because this binding has low affinity. Because the concentration of albumin in plasma is much greater than that of SHBG, the contribution of the albumin-bound fraction can be significant. Routine assays determine the total hormone concentration, bound plus free, and special steps are required to measure the active free level of testosterone, estradiol, and cortisol.

THE IMPORTANCE OF LOCAL SEX HORMONE PRODUCTION

While blood transport of the sex steroids is a key mediator of their biologic effects, it is important to note that the circulating levels of sex hormones do not always reflect concentrations in target cells. In premenopausal women, target tissues synthesize and metabolize most of the testosterone produced. Thus, in women, testosterone functions as a paracrine and intracrine hormone. In men, abundant secretion of testosterone creates circulating levels that are sufficient to allow testosterone to function as a classic hormone. In women, the same description applies to estradiol. Estradiol functions as a classical circulating hormone until menopause, after which both estradiol and testosterone activities are due to local target tissue synthesis, using precursors derived from the circulation. Clinical interventions after menopause, therefore, are directed to local hormone production—for example, the use of aromatase inhibitors to treat breast cancer.

EXCRETION OF STEROIDS

Active steroids and metabolites are excreted as sulfo- and glucuroconjugates. Conjugation of a steroid converts a hydrophobic compound into a hydrophilic one and generally reduces or eliminates the activity of a steroid. This is not completely true, however, because hydrolysis of the ester linkage can occur in target tissues and restore the active form. Furthermore, estrogen conjugates can have biologic activity, and it is known that sulfated conjugates are actively secreted and may serve as precursors, present in the circulation in relatively high concentrations because of binding to serum proteins. Ordinarily, however, conjugation by the liver and intestinal mucosa is a step in deactivation preliminary to, and essential for, excretion into urine and bile (**Figure 1.12**).

For example, the metabolic fate of progesterone, as expressed by its many excretion products, is relatively complex. About 10% to 20% of progesterone is excreted as pregnanediol. Pregnanediol glucuronide is present in the urine in concentrations of less than 1 mg/d until ovulation. Postovulation pregnanediol excretion reaches a peak of 3 to 6 mg/d, which is maintained until 2 days prior to menses. The assay of pregnanediol in the urine now has little clinical use. Pregnanetriol is the chief urinary metabolite of 17α-hydroxyprogesterone and has clinical significance in congenital adrenal hyperplasia, in which an enzymatic defect (most frequently 21-hydroxylase) results in accumulation of 17α-hydroxyprogesterone and increased excretion of pregnanetriol (**Figure 1.10**). However, the plasma or serum assay of 17α-hydroxyprogesterone is a more sensitive and accurate index of this enzyme deficiency than measurement of pregnanetriol.

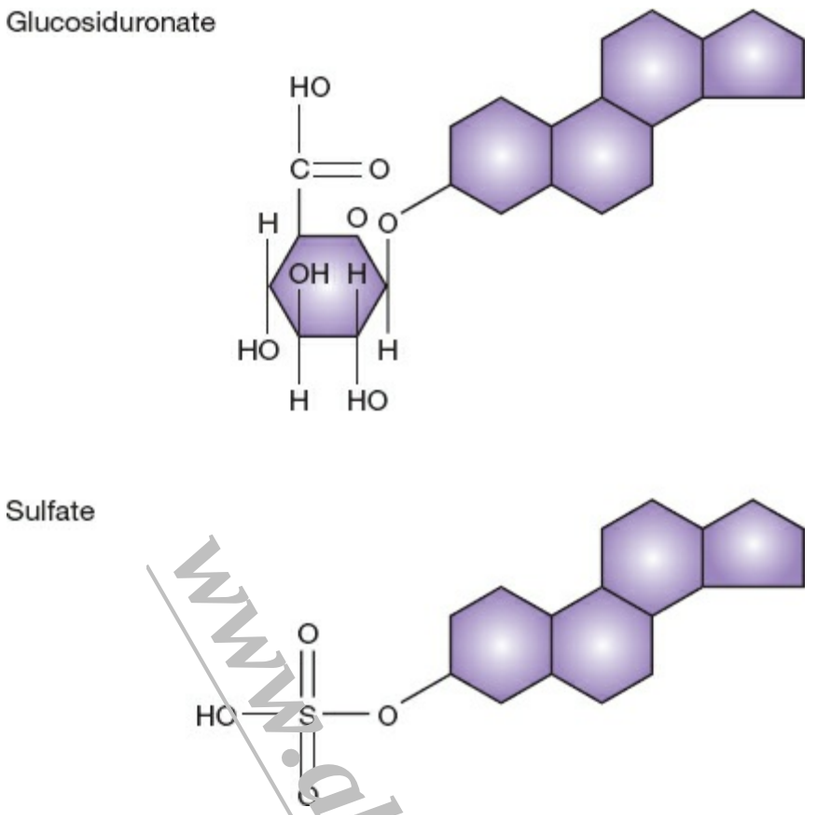

FIGURE 1.12

MECHANISM OF ACTION FOR STEROID HORMONES

The specificity of the reaction of tissues to sex steroid hormones is due to the presence of intracellular receptor proteins. Different types of tissues, such as liver, kidney, and uterus, respond in a similar manner. The mechanism includes (1) steroid hormone diffusion across the cell membrane, (2) steroid hormone binding to a receptor protein, (3) interaction of a hormone–receptor complex with nuclear DNA, (4) synthesis of messenger RNA (mRNA), (5) transport of the mRNA to the ribosomes, and, finally, (6) protein synthesis in the cytoplasm that results in specific cellular activity (**Figure 1.13**). The steroid hormone receptors primarily affect gene transcription but also regulate posttranscriptional events and nongenomic events. Steroid receptors regulate gene transcription through multiple mechanisms, not all of which require direct interactions with DNA.

Each of the major classes of the sex steroid hormones, including estrogens, progestins, and androgens, acts according to this general mechanism. Glucocorticoid and mineralocorticoid receptors, when in the unbound state, reside in the cytoplasm and move into the nucleus after hormone–receptor binding. Estrogens, progestins, and androgens are transferred across the nuclear membrane and bind to their receptors within the nucleus.

Steroid hormones are rapidly transported across the cell membrane by simple diffusion. The factors responsible for this transfer are unknown, but the concentration of free (unbound) hormone in the bloodstream seems to be an important and influential determinant of cellular function. One study in Drosophilia has contested this by identifying a necessary membrane transporter, but this alternative mechanism to simple diffusion has not yet been confirmed in mammalian studies.[41] Once in the cell, the sex steroid hormones bind to their individual receptors. During this process, **transformation** or **activation** of the receptor occurs. Transformation refers to a conformational change of the hormone–receptor complex revealing or producing a binding site that is necessary in order for the complex to bind to the chromatin. In the unbound state, the receptor is associated with heat shock proteins that stabilize and protect the receptor and maintain a conformational shape that keeps the DNA-binding region in an inactive state. Activation of the receptor is driven by hormone binding that causes a dissociation of the receptor–heat shock protein complex.

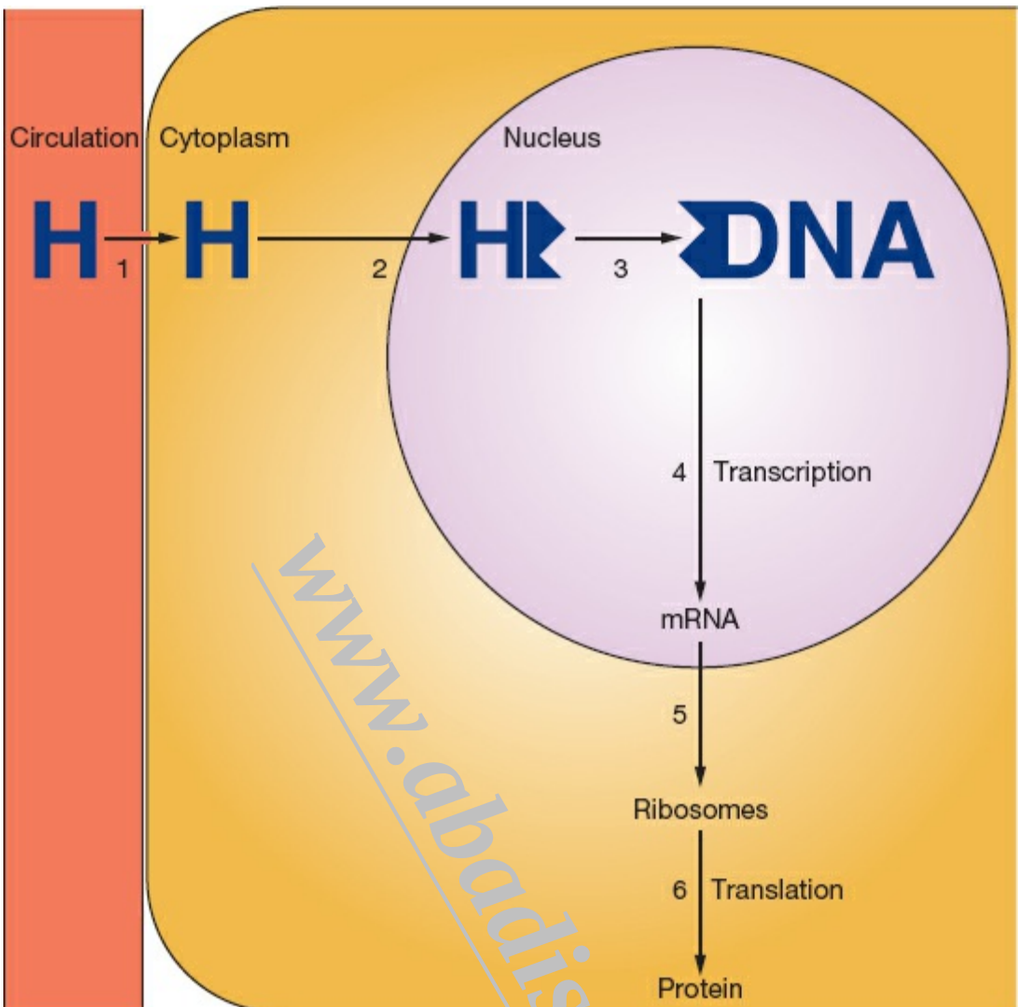

FIGURE 1.13

The hormone–receptor complex binds to specific DNA sites (**hormone-responsive elements**) that are located upstream of the gene. The specific binding of the hormone–receptor complex with DNA results in RNA polymerase initiation of transcription. Transcription is followed by translation and mRNA-mediated protein synthesis on the ribosomes. The principal action of steroid hormones is the regulation of intracellular protein synthesis by means of the receptor mechanism.

Biologic activity is maintained only while the nuclear site is occupied with the hormone–receptor complex. The dissociation rate of the hormone and its receptor and the half-life of the nuclear chromatin–bound complex are factors in the biologic response because the hormone response elements are abundant and, under normal conditions, are occupied only to a small extent.[42] Thus, an important clinical principle is the following: **duration of exposure to a hormone is as important as dose**. One reason only small amounts of estrogen need be present in the circulation is the long half-life of the estrogen hormone–receptor complex. Indeed, a major factor in the potency differences among the various estrogens (estradiol, estrone, estriol) is the length of time the estrogen–receptor complex occupies the nucleus. The higher rate of dissociation with the weak estrogen (estriol) can be compensated for by continuous application to allow prolonged nuclear binding and activity. Cortisol and progesterone must circulate in large concentrations because their receptor complexes have short half-lives in the nucleus.

An important action of estrogen is the modification of its own and other steroid hormones' activities by affecting receptor concentrations. Estrogen increases target tissue responsiveness to itself and to progestins and androgens by increasing the concentration of its own receptor and that of the intracellular progestin and androgen receptors. Progesterone and clomiphene, on the other hand, limit tissue response to estrogen by blocking this mechanism, thus decreasing over time the concentration of estrogen receptors.

The synthesis of the sex steroid receptors obviously takes place in the cytoplasm, but for estrogen and progestin receptors, synthesis must be quickly followed by transportation into the nucleus. There is an amazingly extensive nuclear traffic.[43] The nuclear membrane contains 3,000 to 4,000 pores. A cell synthesizing DNA imports about 1 million histone molecules from the cytoplasm every 3 minutes. If the cell is growing rapidly, about three newly assembled ribosomes will be transported every minute in the other direction. The typical cell can synthesize 10,000 to 20,000 different proteins. How do they know where to go? The answer is that these proteins have localization signals. In the case of steroid hormone receptor proteins, the localization signal sequences are in the hinge region.

Estrogen and progestin receptors exit continuously from the nucleus to the cytoplasm and are actively transported back to the nucleus. This is a constant shuttle; diffusion into the cytoplasm is balanced by the active transport into the nucleus. This raises the possibility that some diseases are due to poor traffic control. This can be true of some acquired diseases as well (eg, Reye syndrome, an acquired disorder of mitochondrial enzyme function).

The fate of the hormone–receptor complex after gene activation is referred to as hormone–receptor **processing** (Figure 1.14). In the case of estrogen receptors, processing involves the rapid degradation of receptors unbound with estrogen and a much slower degradation of bound receptors after gene transcription. The rapid turnover of estrogen receptors has clinical significance. The continuous presence of estrogen is an important factor for continuing response.

The best example of the importance of these factors is the difference between estradiol and estriol. Estriol has only 20% to 30% affinity for the estrogen receptor compared with estradiol; therefore, it is rapidly cleared from a cell. However, if the effective concentration is kept equivalent to that of estradiol, it can produce a similar biologic response.[44] In pregnancy, where the concentration of estriol is very high, it can be an important hormone, not just a metabolite.

The depletion of estrogen receptors in the endometrium by progestational agents is the fundamental reason for adding progestins to estrogen treatment programs. The progestins accelerate the turnover of preexisting receptors, and this is followed by inhibition of estrogen-induced receptor synthesis. Using monoclonal antibody immunocytochemistry, this action has been pinpointed to the interruption of transcription in estrogen-regulated genes. The mechanism is different for androgen antiestrogen effects. Androgens also decrease estrogen receptors within target tissues, especially in the uterus.[45,46]

The Nuclear Receptor Superfamily

Recombinant DNA techniques have permitted the study of the gene sequences that code for the synthesis of nuclear receptors. Steroid hormone receptors share a common structure with the receptors for thyroid hormone, 1,25-dihydroxyvitamin D3, and retinoic acid; thus, these receptors are called a superfamily (**Figure 1.15**).[47,48] Each receptor contains characteristic domains that are similar and interchangeable. Therefore, it is not surprising that the specific hormones can interact with more than one receptor in this family. Analysis of these receptors suggests a complex evolutionary history, during which gene duplication and swapping between domains of different origins occurred. This family now includes hundreds of proteins, present in practically all species, from worms to insects to humans. Some are called **orphan receptors** because specific ligands for these proteins have not been identified, but the number of orphan receptors is gradually diminishing ("deorphanization"). It has been convincingly argued that the six steroid receptors originated in a common ancestral receptor gene.[49] The identification of steroid receptors in the sea lamprey dates the origin to over 450 million years ago, and the characterization of a receptor that functions like an estrogen receptor in the mollusk suggests that the ancient and initial sex steroid receptor was an estrogen receptor.[50] Knowledge of the complete human genome has confirmed that there are 48 nuclear receptors in this superfamily.[48]

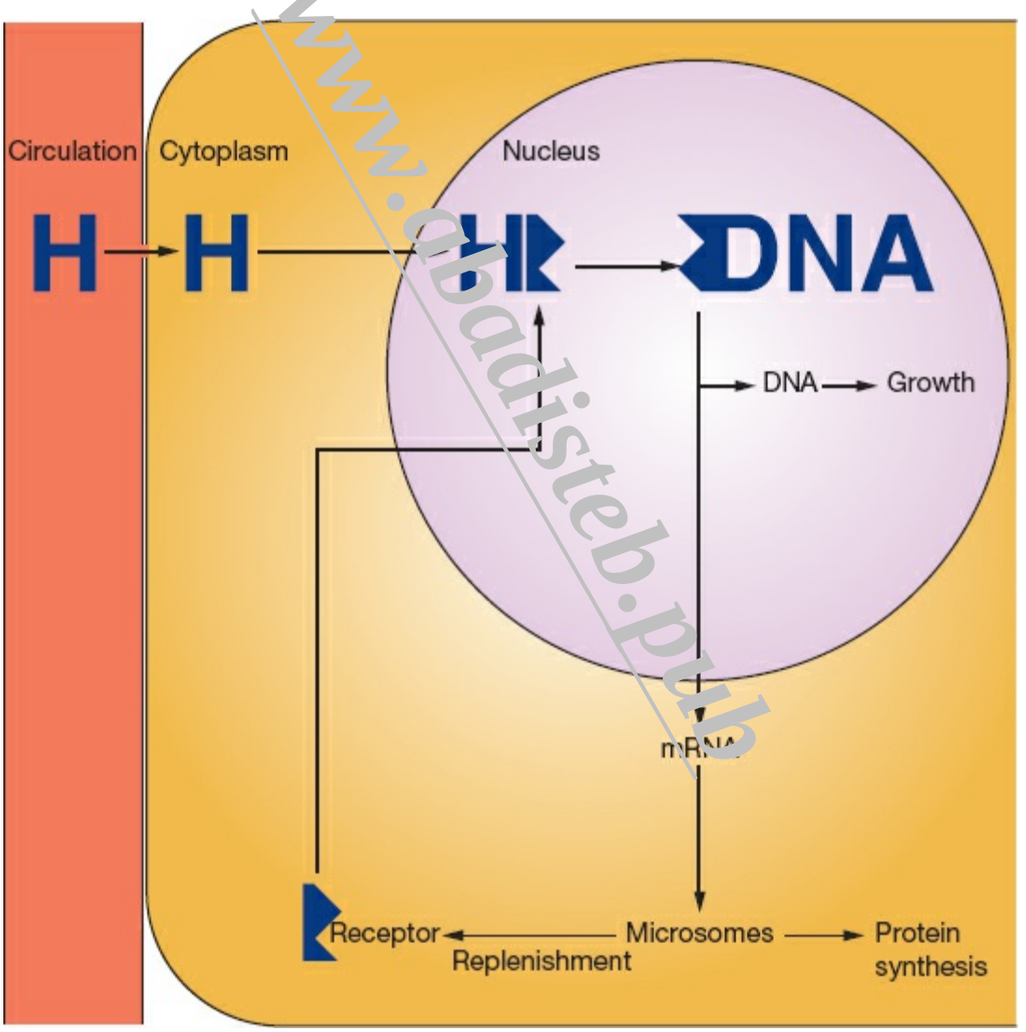

FIGURE 1.14

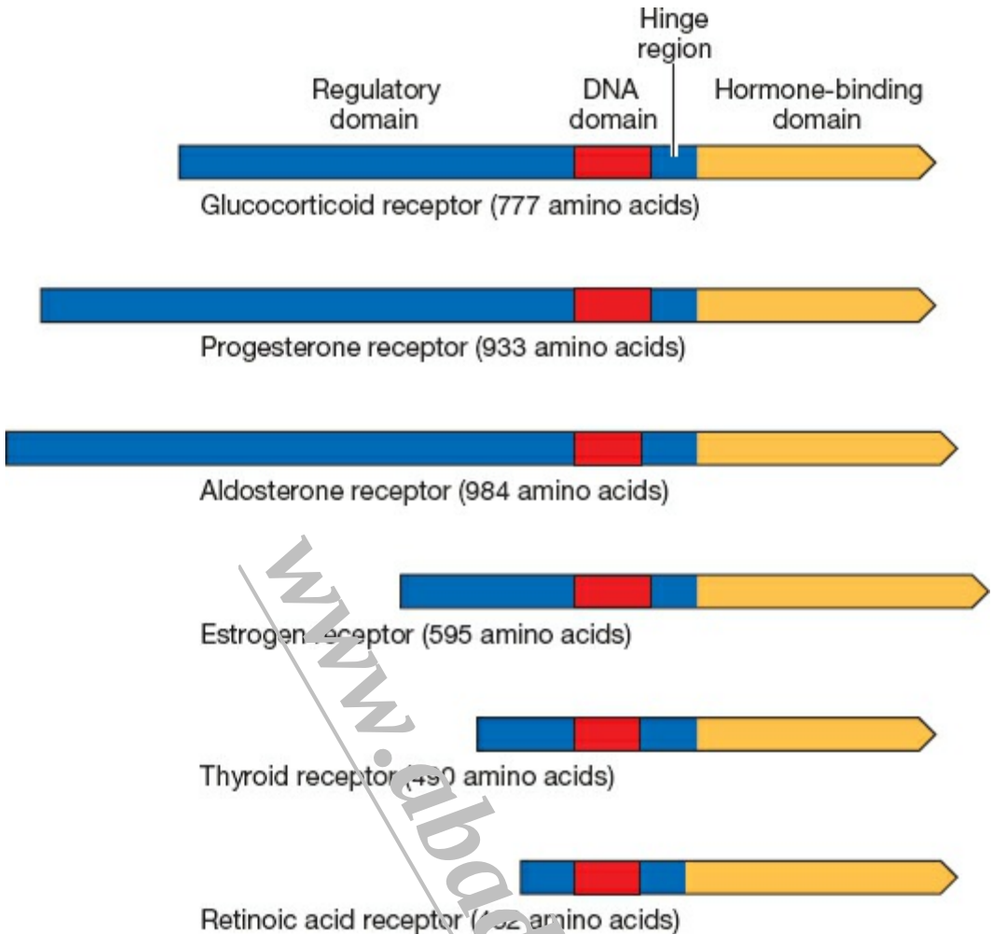

FIGURE 1.15

The Estrogen Receptors

Two estrogen receptors have been identified, designated as estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β) (**Figure 1.16**).[51,52] The estrogen receptor-α was discovered about 1960, and the amino acid sequence was reported in 19[illegible].[55] The estrogen receptor-α is translated from a 6.8-kilobase mRNA derived from a gene that contains eight exons on the long arm of chromosome 6. It has a molecular weight of approximately 66 kDa with 595 amino acids. The receptor-α half-life is approximately 4 to 7 hours; thus, the estrogen receptor-α is a protein with a rapid turnover. The more recently discovered estrogen receptor-β, a protein with 530 amino acids, is encoded by a gene localized to chromosome 14q23.2, in close proximity to genes related to Alzheimer disease.[57,58] Multiple isoforms of ER-β exist, including five full-length forms.

Orphan receptors have been identified that are related to the estrogen receptors and have been named estrogen-related receptor (ERRα, ERRβ, and ERRγ). ERRα may be regulated by coactivator proteins and interacts with typical steroid signaling pathways.[59,60] These orphan receptors are expressed in most tissues and may be involved in typical estrogen activities, such as the proliferation and differentiation of target cells in the bone and in the breast. Nevertheless, they do not bind estrogens, and no endogenous ligand has yet been identified.[61]

The story is further complicated with the recognition that members of the nuclear receptor superfamily are each associated with multiple isoforms.[62] This increases the number of possible signaling pathways in physiology and disease. In this discussion, we will mention only the most biologically important isoforms.

The estrogen receptors are divided into six regions in five domains, labeled A to F. The ER-β is 96% homologous in amino acid sequence, with the α estrogen receptor in the DNA-binding domain and 60% homologous in the hormone-binding domain. The full comparison is shown in **Table 1.2**.[57,63,64]