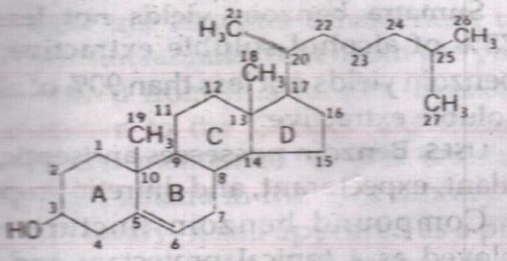
7

Steroids

Steroids constitute a natural product class of compounds that is widely distributed throughout nature. The diversity of biologic activities of steroids includes the development and control of the reproductive tract in humans (estradiol, progesterone, testosterone), the molting of insects (ecdysone), and the induction of sexual reproduction in aquatic fungi (antheridiol). In addition, steroids contribute to a wide range of therapeutic applications, such as cardiotonics (digitoxin), vitamin D precursors (ergosterol), oral contraceptive agents (semisynthetic estrogens and progestins), anti-inflammatory agents (corticosteroids), and anabolic agents (androgens).

NOMENCLATURE

A steroid is any compound that contains a cyclopentanoperhydrophenanthrene nucleus. The chemical nomenclature of steroids is based on this fundamental carbocycle with adjacent side-chain carbon atoms. Each parent tetracyclic hydrocarbon bears a specific stem name, and some of the principal hydrocarbons are shown in Figure 7–1. Steroids are numbered and rings are lettered as indicated in the structural formula for cholesterol. If one or more of the carbon atoms shown in the structure of cholesterol is not present, the numbering of the remainder is undisturbed.



Cholesterol

When the rings of a steroid are denoted as projections onto the plane of the paper, an atom or group attached to a ring is termed α *(alpha)* if it lies below the plane of the paper or β *(beta)* if it lies above the plane of the paper. In formulas, bonds to atoms or groups attached in an α configuration are shown as broken lines, and bonds to atoms or groups attached in a β configuration are shown as solid lines.

The use of a steroid stem name implies that atoms or groups attached at the ring-junction positions 8, 9, 10, 13, and 14 are oriented as shown in Figure 7–2 (8β, 9α, 10β, 13β, 14α), and a carbon chain (R) attached to position 17 is assumed to be β-oriented. The configuration of hydrogen or a substituent at the ring-junction position 5 is always designated by adding α or β after the numeral 5. This numeral and letter are placed immediately before the stem name. The implication of these conventions of nomenclature is that, in most steroids, rings B and C and rings C and D are fused *trans*, whereas rings A and B may be fused either *cis* or *trans*. For example, the bile acid, cholic acid, has a *cis*-fused A/

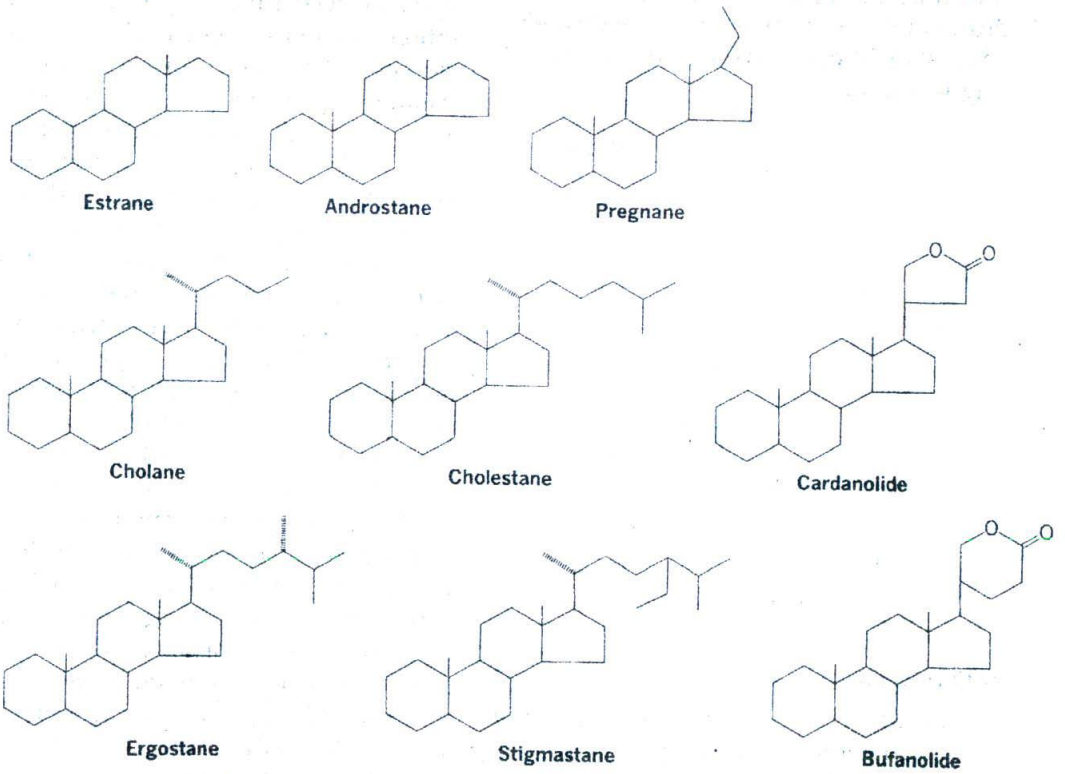


Fig. 7–1. Principal steroid stereoparent hydrocarbons.

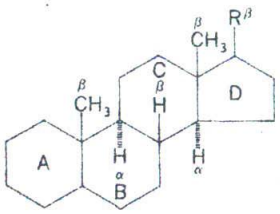
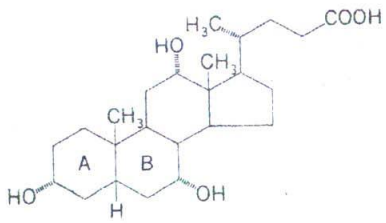
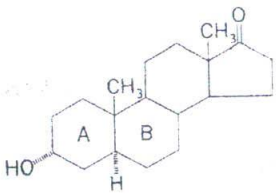


Fig. 7–2. Orientation of steroid substituents.

A/B ring junction. The chemical name of cholic acid is 3α, 7α, 12α-trihydroxy-5β-cholan-24-oic acid. The sex hormone androsterone, chemical name 3α-hydroxy-5α-androstan-17-one, has a *trans*-fused A/B ring junction.



Cholic acid



Androsterone

BIOSYNTHESIS

Steroids are formed biosynthetically from isopentenyl pyrophosphate (active isoprene) and involve the same sequence of reactions as does terpenoid biosynthesis. In fact, the triterpenoid squalene is an intermediate in steroid biosynthesis. Most knowledge of the biosynthesis of steroids has been derived from studies of cholesterol production. Although this compound is not necessarily a direct precursor of all other steroids, its formation may be considered as a general mechanism of steroid biosynthesis. The familiar acetate →→ mevalonate →→ isopentenyl pyrophosphate →→ squalene →→ cholesterol pathway is outlined in Figure 7–3.

The first step in the pathway by which squalene is transformed into sterols is its stereospecific conversion into *S*-squalene 2,3-epoxide by squalene epoxidase. In the next step, the key enzyme involved in the cyclization of squalene 2,3-epoxide to the first cyclic sterol precursor in animals and fungi is 2,3-oxidosqualene:lanosterol cyclase. Lanosterol is replaced in photosynthetic organisms by its isomer cycloartenol, and the enzyme involved is 2,3-oxidosqualene:cycloartenol cyclase. The cyclization reaction has been called one of the most complicated in all of biochemistry. For example, squalene is an acyclic molecule with 6 double bonds, and the lanosterol molecule has 4 rings and 7 asymmetric centers, all properly oriented. As shown in Figure 7–3, a proton initiates the cyclization by attacking the epoxide bond. Each of the rings forms successively, involving attack by a π bond on a specific carbon. The reactions are fast enough and the intermediates rigid enough that stereochemistry is preserved as the rings form. Each π-bond attack leaves behind a carbonium ion, which is the target of the next attack.

After the rings are formed, the resulting carbonium ion intermediate, which has a positive charge at C-20, is stabilized by rearrangements involving 2 hydride shifts (17→20, 13→17) and 2 methyl shifts (14→13, 8→14). These shifts result in the migration of the positive charge to C-8 and, with the loss of a proton from C-9, either the 9,10,19-cyclopropane ring of cycloartenol or the 8,9-double bond of lanosterol may be formed. The conversion of the C_{30} compound, lanosterol, to the C_{27} steroid, cholesterol, involves the loss of 3 methyl groups, the shift of a double bond, and a reduction of a double bond. The sequence in which these reactions take place may vary, depending on the organism. Consequently, numerous intermediates, including zymosterol, have been isolated that represent various stages in this transformation.

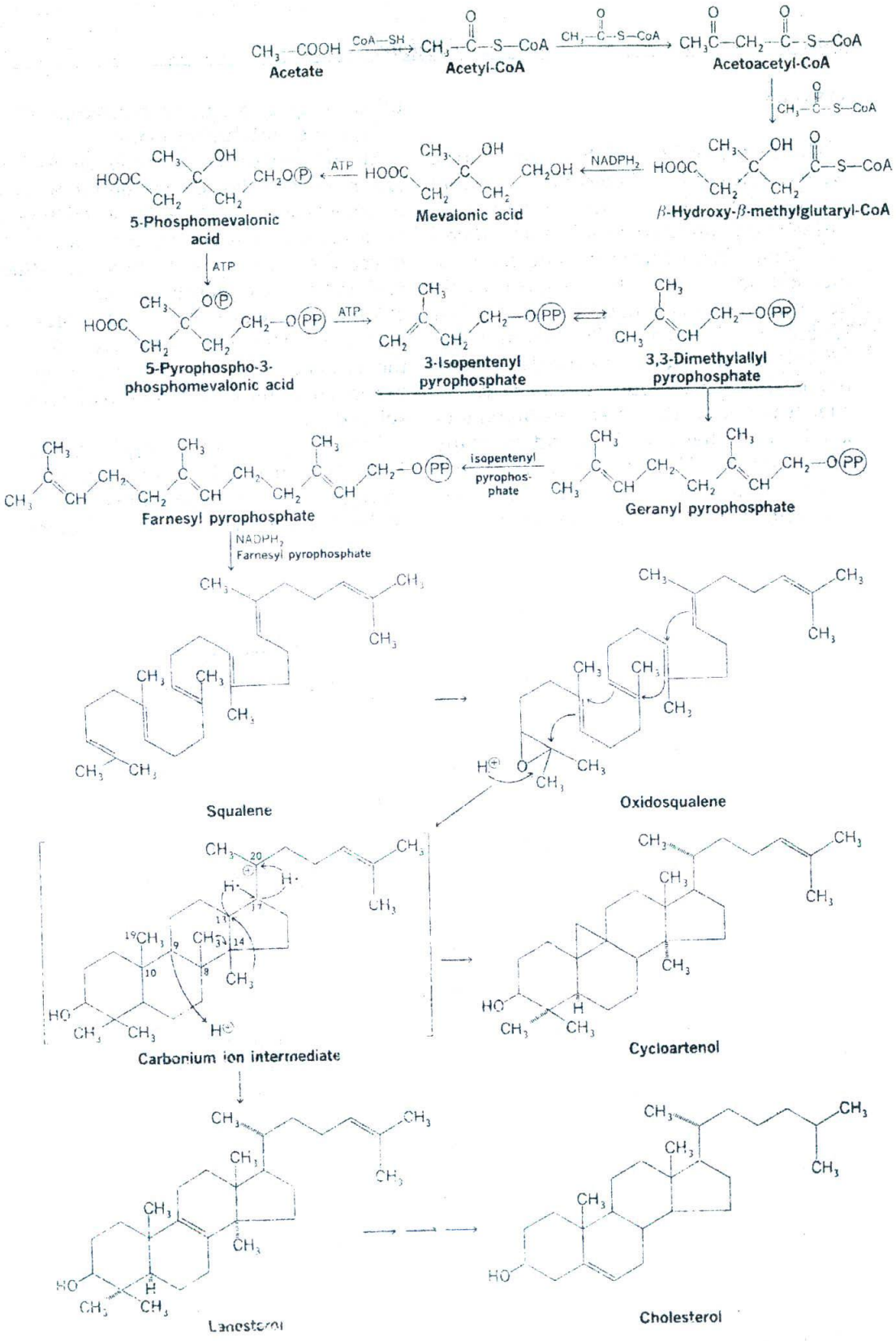


Fig. 7-3. Biosynthesis of cholesterol.

STEROLS

The first steroids isolated from nature were a series of C_{27}–C_{29} alcohols that were found in the lipid fractions of many tissues. These compounds were solids and therefore named **sterols** from the Greek *stereos*, meaning solid (Fig. 7–4). The most widely occurring sterol is cholesterol. It was first isolated from human gallstones and, because it is a constituent of animal cell membranes, it has been found in all animal tissue. It is one of the chief constituents of lanolin and therefore is found in many drug products. Until recently, cholesterol was thought to be restricted to the animal kingdom; however, it has now been identified in algae, fungi, actinomycetes, bacteria, ferns, and higher plants.

Much has been written about cholesterol and human health. Cholesterol is present in atherosclerotic plaques, and feeding of cholesterol to susceptible animals has induced atherosclerosis. In humans, atherosclerosis is frequently associated with conditions in which the blood cholesterol is elevated. However, at the present time, the evidence of a causal relationship between cholesterol and atherosclerosis is still indirect.

The principal sterol in fungi is ergosterol. This C_{28} sterol arises biosynthetically through a transmethylation reaction of the cholestane side chain involving *S*-adenosyl

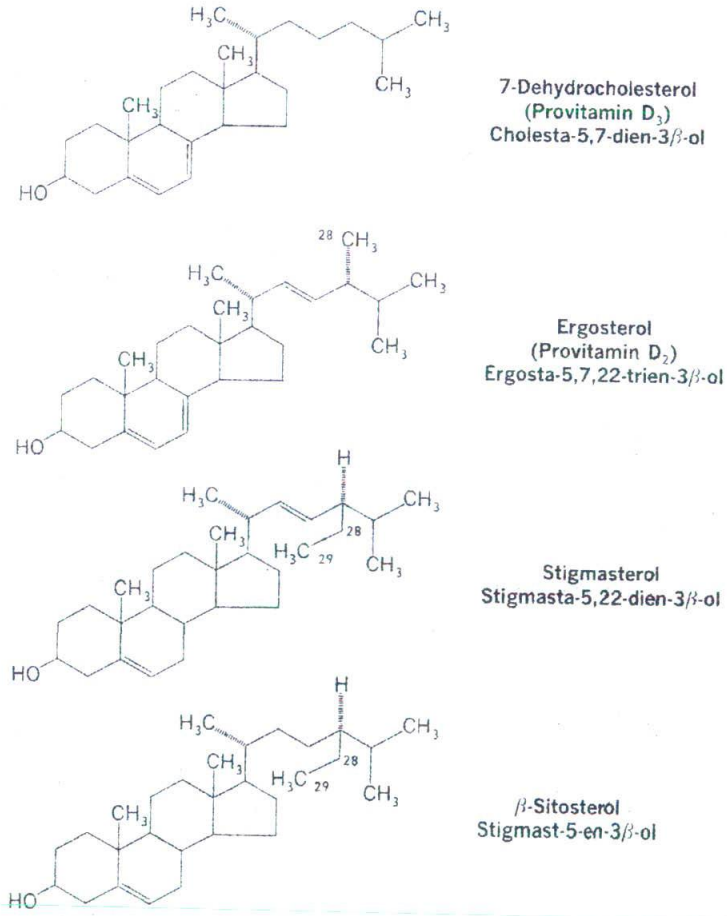


Fig. 7–4. Sterols.

methionine. Ergosterol is also known as provitamin D_2 because, upon ultraviolet irradiation, a series of isomerizations with the subsequent opening of ring B results in the formation of vitamin D_2. Vitamin D_3 is formed in the same manner from 7-dehydrocholesterol. This compound occurs in small amounts with cholesterol in animal tissue, including human skin, where irradiation from the sun catalyzes the formation of vitamin D_3. The vitamin D compounds are discussed in the chapter on vitamins.

The most common sterol in plants is β-sitosterol (stigmast-5-en-3β-ol), a C_{29} compound. It has been shown that a second transmethylation from methionine accounts for the C-29 atom. In general, sitosterols are widely distributed throughout the plant kingdom and may be obtained from wheat germ oil, rye germ oil, corn oil, cottonseed oil, and other seed oils.

Closely related to β-sitosterol is the sterol, stigmasterol, which was first isolated from calabar beans but is also found in soybean oil. The double bond at position 22 of stigmasterol allows it to be more readily converted into the pregnane-type steroid hormones than β-sitosterol; consequently, the extraction of stigmasterol from soybean oil is an important commercial process.

BILE ACIDS

In the liver of humans and other animals, the side chain of cholesterol is degraded to C_{24} steroids, which possess a C-24 carboxyl. These steroids are collected in the bile; therefore, they are referred to as the bile acids (Fig. 7–5). The primary bile acids formed in the human liver are cholic acid and chenodesoxycholic acid. Desoxycholic acid and lithocholic acid are also found in substantial amounts in mammalian bile; however, they are not formed in the liver. They are produced in the intestinal tract by the action of microorganisms on cholic acid to form desoxycholic acid and on chenodesoxycholic acid to form lithocholic acid. Their presence in the bile is attributed to enterohepatic circulation. Generally, the bile acids do not exist in the free state but are conjugated through a peptide bond to either glycine or taurine (Fig. 7–6). The conjugated bile acids are discharged into the duodenum where they act as emulsifying agents to aid in the intestinal absorption of fat. Bile salts are the sodium salts of the conjugated acids and are the principal constituents of ox bile extract. It is usually given in a dose of 300 to 600 mg daily. After absorption, the bile acids exhibit choleretic action by increasing bile flow. They also have a mild laxative effect, and since there is no evidence of efficacy in replacement therapy for bile deficiencies, the principal use of ox bile extract is as a laxative.

Ox bile extract is prepared by partial evaporation of fresh ox bile, precipitation of the mucus and albuminous matter with alcohol, filtering, washing, and evaporating the combined filtrates to dryness at a temperature not exceeding 80° C. It contains an amount of the sodium salts of glycocholic acid and taurocholic acid equivalent to not less than 45% of cholic acid.

NONPRESCRIPTION PRODUCT. Bilron Pulvules®.

Chenodiol (chenodesoxycholic acid) suppresses hepatic synthesis of both cholesterol and cholic acid, gradually replacing the cholic acid and its metabolite, desoxycholic acid, in an expanded bile acid pool. This contributes to biliary cholesterol desaturation and gradual dissolution of radiolucent cholesterol gallstones. Chenodiol has no effect on radiopaque (calcified) gallstones or on bile pigment gallstones.

The recommended dosage of chenodiol is 13 to 16 mg/kg/day, divided into 2 doses. The duration of treatment may be 2 years or more. In addition, stones may recur when therapy is discontinued. Because there is a high incidence of adverse effects, such as elevated liver enzyme levels and a dose-related diarrhea, this agent is rec-

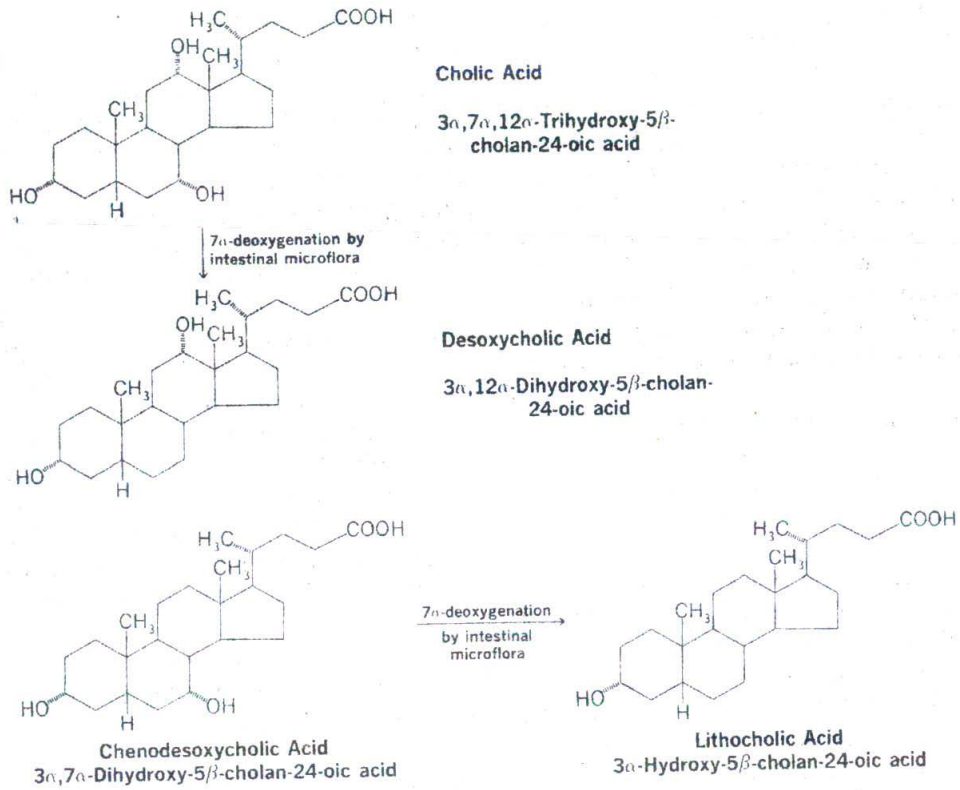


Fig. 7–5. Bile acids.

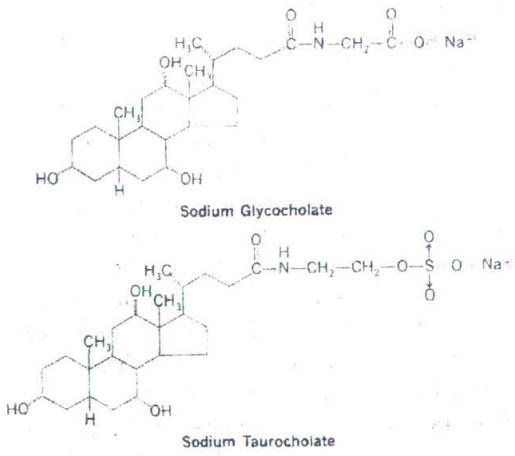
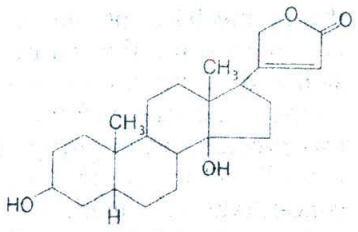


Fig. 7–6. Conjugated bile acids.

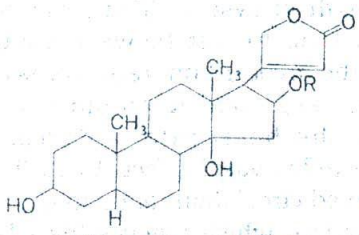
ommended only in patients who are poor surgical risks for a cholecystectomy.

CARDIAC GLYCOSIDES

Some steroids present in nature are characterized by the highly specific and powerful action that they exert on the cardiac muscle. These steroids occur as glycosides with sugars attached at the 3-position of the steroid nucleus. Because of their action on the heart muscle, they are named cardiac glycosides (Fig. 7–7). The steroid aglycones or genins are of 2 types: a cardenolide or a bufadienolide. The more prevalent in nature are the cardenolides, which are C_{23} steroids that have as a 17β side chain an α,β-unsaturated 5-membered lactone ring. The bufadienolides are C_{24} homologs of the cardenolides and carry a doubly unsaturated 6-membered lactone

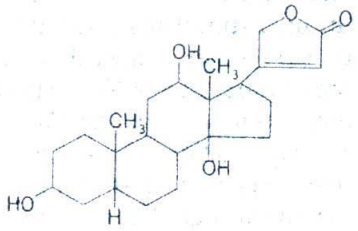


Digitoxigenin
3β,14-dihydroxy-5β,14β-card-20(22)-enolide

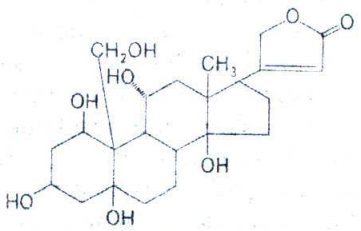


Gitoxigenin R = H
3β,14,16β-trihydroxy-5β,14β-card-20(22)-enolide

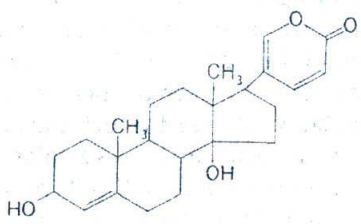
Gitaloxigenin R = CHO
16β-formyloxy-3β,14-dihydroxy-5β,14β-card-20(22)-enolide



Digoxigenin
3β,12β,14-trihydroxy-5β,14β-card-20(22)-enolide



Ouabagenin
1β,3β,5,11α,14,19-hexahydroxy-5β,14β-card-20(22)-enolide



Scillarenin A
3β,14-dihydroxy-14β-bufa-4,20,22-trienolide

Fig. 7–7. Structural formulas of several aglycones of cardiac glycosides.

ring at the 17-position. The bufadienolides derive their name from the generic name for the toad, *Bufo* (the prototype compound bufalin was isolated from the skin of toads). An unusual aspect of the chemistry of both cardenolides and bufadienolides is that the C/D ring junction has the *cis*-configuration. To obtain optimum cardiac activity, the aglycone should possess an α,β unsaturated lactone ring that is attached β at the 17-position of the steroid nucleus and the A/B and C/D ring junctions should have the *cis*-configuration. Metabolic reduction of the double bond in the lactone ring of digoxin to form dihydrodigoxin may explain why certain individuals are refractory to digoxin therapy. If the glycoside is cleaved, the aglycone retains cardiac activity; however, the sugar portion of the glycoside confers on the molecule solubility properties important in its absorption and distribution in the body. Oxygen substitution on the steroid nucleus also influences the distribution and metabolism of glycosides. In general, the more hydroxy groups on the molecule, the more rapid the onset of action and the subsequent dissipation from the body.

The use of the cardiac glycosides in therapeutics stems from the ability of these compounds to increase the force of systolic contraction. An increase in contractility in the failing heart results in a more complete emptying of the ventricle and a shortening

in the length of systole. Thus, the heart has more time to rest between contractions. As the myocardium recovers as a result of increased cardiac output and circulation, the heart rate is decreased through a reflex vagal effect. In addition, the improved circulation tends to improve renal secretion, which relieves the edema often associated with heart failure.

In the use of cardiac glycosides to treat congestive heart failure, the patient is given an initial loading dose of the drug in order to bring the heart under the influence of the drug. Because the amount required varies with the patient and the drug used, the preparation is given in divided doses while titrating the dose against signs of improvement. The patient is usually maintained indefinitely after the loading dose by administering a daily maintenance dose that replaces the amount of drug that is metabolized and excreted. In toxic concentrations, the glycosides may increase cardiac automaticity and lead to ectopic tachyarrhythmia. Ventricular extrasystoles are the most frequent effect. With all the glycosides, the therapeutic level appears to be approximately 50 to 60% of the toxic dose. This finding explains why dosage must be carefully determined experimentally for each patient.

Despite numerous experimental investigations, the mechanism of action of the cardiac glycosides is still not completely known; however, observations have implicated Na^+, K^+-ATPase as the receptor enzyme. This enzyme catalyzes the active transport of Na^+ out of the cell and the subsequent transport of K^+ into the cell.

Na^+, K^+-ATPase operates in all cell membranes to maintain the unequal distribution of Na^+ and K^+ ions across the membrane. However, in the myocardium the ion exchange is rapid because it is required after each heart beat; therefore, an inhibition of Na^+, K^+-ATPase has a greater effect on heart tissuethan on other cells of the body. When the heart beats, a wave of depolarization passes through it, changing the permeability of the cell membranes. Na^+ moves into the cell by passive diffusion and K^+ moves out. Na^+, K^+-ATPase supplies the energy from ATP to reverse this process and to pump the Na^+ out of the cell and the K^+ into the cell against a concentration gradient.

Inhibition of Na^+, K^+-ATPase by the cardiac glycoside results in an increase in Na^+ and a decrease in K^+ within the cell which, in turn, stimulates a secondary Na^+ Ca^{++} exchange mechanism that functions to remove intracellular Na^+ with a subsequent increase in intracellular Ca^{++}. The positive inotropic action or muscle contraction enhancement of cardiac glycosides is mediated through the increase in Ca^{++}. Ca^{++} interacts with troponin which then, through its action on tropomyosin, unmasks the binding sites on actin that bind myosin, allowing for the formation of the contractile protein actomyosin (Fig. 7–8).

Drug Interactions. This postulated mechanism implicating intracellular cation levels explains the development of toxicity symptoms in patients with certain plasma-electrolyte imbalances who receive cardiac glycoside therapy. Potassium depletion increases the susceptibility to cardiac glycoside toxicity; therefore, patients on concomitant therapy with such potassium-depleting drugs as thiazide diuretics and corticosteroids with mineralocorticoid activity may require potassium supplementation or a reduced dosage of cardiac glycosides. Conversely, patients treated with cardiac glycosides should not commence the excessive ingestion of any product containing absorbable calcium, e.g., milk, calcium gluconate, and dibasic and tribasic calcium phosphate. Also, such patients should not be given parenteral calcium because hypercalcemia can potentiate the cardiac effect.

Digitalis

Digitalis or foxglove is the dried leaf of *Digitalis purpurea* Linné (Fam. Scrophulariaceae) (Fig. 7–9). Its potency is such that,

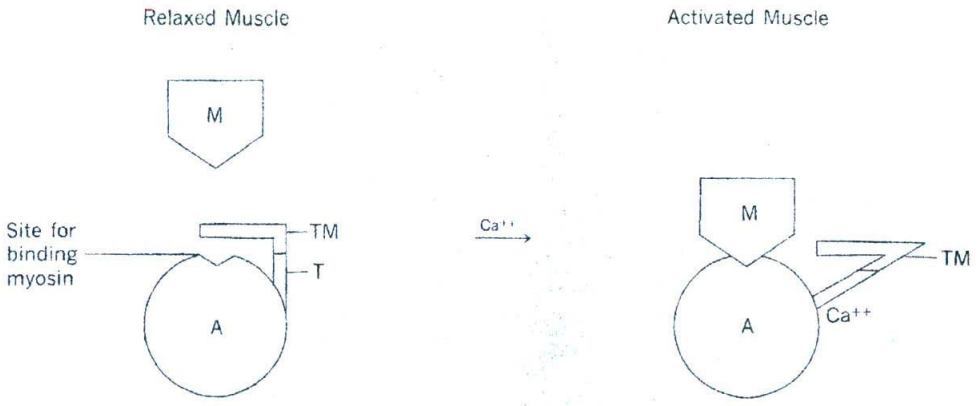


Fig. 7–8. Schematic diagram showing the interaction of contractile protein during muscle contraction. A = actin, M = myosin, TM = tropomyosin, T = troponin. In the relaxed muscle, tropomyosin masks the sites on actin to which myosin binds through steric blockage. In the activated muscle, Ca^{++} interacts with troponin, which brings about a conformational change in tropomyosin, unmasking the actin-to-myosin binding sites and allowing for the formation of actomyosin.

when assayed as directed, 100 mg are equivalent to not less than 1 USP digitalis unit (100 mg of the USP Digitalis Reference Standard). *When digitalis is prescribed, powdered digitalis is to be dispensed.*

Powdered digitalis is digitalis dried at a temperature not exceeding 60° C, reduced to a fine or a very fine powder, and adjusted, if necessary, to conform to the official potency by admixture with sufficient lactose, starch, exhausted marc of digitalis, or with a powdered digitalis that has either a lower or a higher potency.

Digitalis is from the Latin *digitus*, meaning finger, and refers to the finger-shaped corolla, so named by Tragus in 1539; *purpurea* is Latin and refers to the purple color of the flower. The plant is a biennial herb, probably indigenous to central and southern Europe and naturalized in various parts of Europe and in the northern and western United States and Canada.

Digitalis seems to have been used externally by the Welsh. Parkinson recommended it in 1640, but its internal use was not in vogue until its recommendation by Withering in 1776. It is an important drug and has been official in most pharmacopeias of the world since the 18th century.

The leaves of other *Digitalis* species, *D. dubia, D. ferruginea, D. grandiflora, D. lanata, D. lutea, D. mertonensis, D. nervosa, D. subalpina*, and *D. thapsi*, also show the presence of cardiac glycosides.

CULTIVATION OF DIGITALIS. Until recently, digitalis was cultivated in Pennsylvania by the S. B. Penick Company. At present, however, digitalis and the digitalis glycosides used in the U.S. are obtained principally from England and Germany. In Germany, *D. purpurea* seeds, which have been developed through strain selection to yield plants with maximum drug potency and with resistance to plant diseases, are sown in greenhouses in March. From the middle of May until the beginning of June, the young plants are planted outside in relatively small plots (1 to 10 acres). The areas of cultivation are centered around a commercial drying unit for medicinal plants at a distance of not more than 20 km. To ensure potency, the leaves must be rapidly and gently dried at 50 to 60° C as soon as the plants are harvested. This procedure must be followed because the leaf contains hydrolytic enzymes which, if not rapidly inactivated,

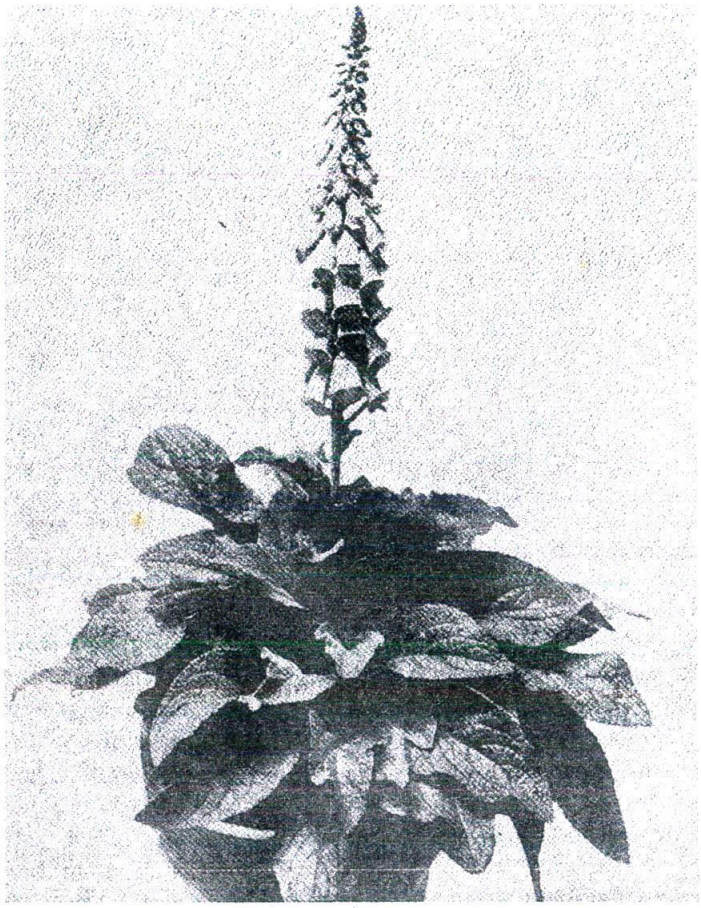


Fig. 7–9. Specimen plant of *Digitalis purpurea*.

cleave the glycosidic linkages, thereby giving rise to the less active genins. Also excess heat may split off water from the tertiary hydroxy group at position 14 of the steroid nucleus, thereby forming the inactive anhydro compound.

The annual crop is harvested from the middle of September to the end of October. The weight of a fresh plant ranges from 200 to 500 g. The yield per acre, depending on the quality of the soil and the effort and skill of the farmer, may vary from 2.5 to 5.5 tons fresh weight/acre, which corresponds to approximately 0.6 to 1.4 tons dry weight/acre.

The harvested crop utilizes only the first year's leaves (Fig. 7–10), which develop as a dense rosette. Some of the plants remain undisturbed to permit development of the flowering stalk during the second season. These flowering stems are the source of seeds for future use. With the exception of the plants used for seed production, all other plants are harvested; consequently, fresh cultivation of young plants is begun each year.

CONSTITUENTS. The drug contains a large number of glycosides, of which the most important from a medicinal viewpoint are digitoxin, gitoxin, and gitaloxin. The total concentration of these 3 glycosides varies appreciably with the plant source and the conditions of growth. Also, because all are secondary glycosides derived by hydroly-

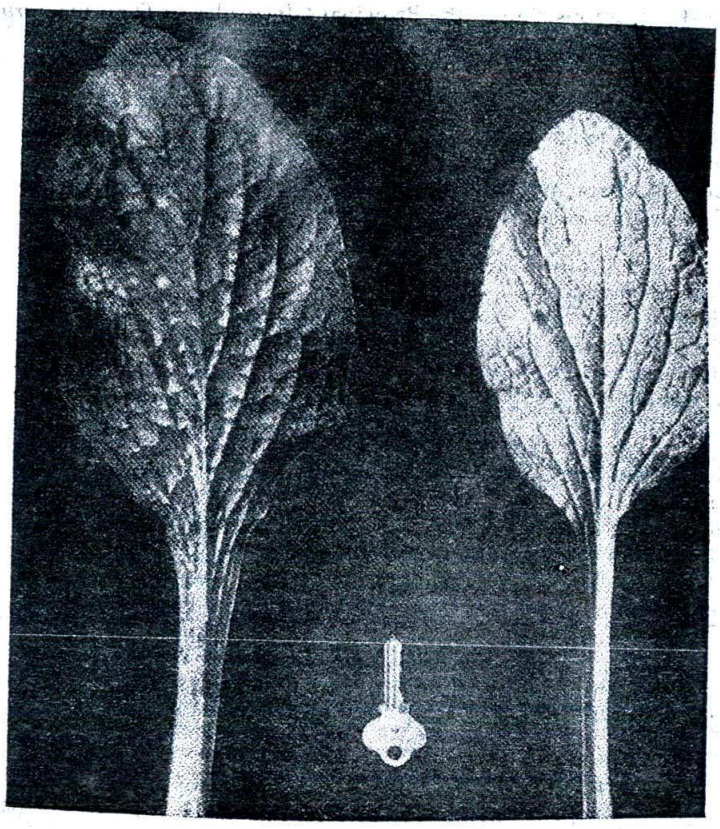


Fig. 7–10. Mature digitalis leaves showing the prominent veins of the dorsal and ventral surfaces. Note the winged petiole.

sis of some of the sugars from the primary or parent glycosides occurring in the leaf, their concentration depends on the manner of treatment of the plant material following harvesting. Careful experiments have revealed that the secondary glycoside content in the leaf is about 10 to 20% of the primary glycoside concentration. Reported total concentrations of digitoxin, gitoxin, and gitaloxin range from 0.09% in a poor-quality Spanish sample to 0.225% in a superior Japanese leaf; the average concentration approximates 0.16%.

Nearly 30 other glycosides have been identified in the drug. The major glycosides, in terms of concentration, include purpurea glycoside A, purpurea glycoside B, glucogitaloxin, glucodigitoxigenin-bis-digitoxiside, glucogitaloxigenin-bis-digitoxiside, glucoevatromonoside, glucogitoroside, glucolanadoxin, digitalinum verum, glucoverodoxin, stropeside, and verodoxin (see Table 7–1).

ASSAY. Digitalis and its preparations must be assayed biologically to ensure their potency; however, because the crystalline glycosides are definite chemical entities, they can be assayed chemically. A number of test animals have been used in the past: guinea pigs, frogs, and cats. The animal now employed in the assay procedure is the pigeon.

Standardization is determined by comparison of the effect of a known dilution of the drug with that of a similar dilution of the USP Digitalis Reference Standard.

Table 7–1. Composition of the Principal Glycosides of *Digitalis purpurea*

Glycoside	Sugars
Derivatives of Digitoxigenin	
Purpurea glycoside A	3 digitoxose, 1 glucose
Digitoxin	3 digitoxose
Gluco-digitoxigenin-bis-digitoxoside	2 digitoxose, 1 glucose
Gluco-evatromonoside	1 digitoxose, 1 glucose
Derivatives of Gitoxigenin	
Purpurea glycoside B	3 digitoxose, 1 glucose
Gitoxin	3 digitoxose
Gluco-gitoroside	1 digitoxose, 1 glucose
Digitalinum verum	1 digitalose, 1 glucose
Stropeside	1 digitalose
Derivatives of Gitaloxigenin	
Gluco-gitaloxin	3 digitoxose, 1 glucose
Gitaloxin	3 digitoxose
Gluco-gitaloxigenin-bis-digitoxoside	2 digitoxose, 1 glucose
Gluco-lanadoxin	1 digitoxose, 1 glucose
Gluco-verodoxin	1 digitalose, 1 glucose
Verodoxin	1 digitalose

Adult pigeons are anesthetized lightly with ether, immobilized, and their alar vein is exposed and cannulated. Definite volumes of the diluted preparation are introduced at 5-minute intervals until the pigeon dies from cardiac arrest.

The bioassay of digitalis leaf can be criticized because of the inability of the method to predict oral potency of the drug. For example, gitoxin in the leaf would contribute to the intravenous assay potency, but because it is poorly absorbed from the gastrointestinal tract, it would not contribute significantly to the cardiac effect. This observation assumes additional significance when one considers that the amount of gitoxin present in the leaf may vary greatly, depending on the genetics of the plant or the manner in which the drug is harvested and prepared for market. As a precautionary measure, care should be taken to maintain patients on one brand of digitalis tablets. This precaution decreases the chances of dispensing a preparation with an oral potency that is either reduced or greater than that obtained by the patient from a prior prescription.

In monitoring patient therapy with digitoxin and digoxin, radioimmune assay techniques have been developed that allow for the measurement of nanogram quantities of these glycosides in the blood serum. The underlying principle is that nonradioactive glycoside (in known standard solution or in patients' sera) will compete with radioactively labeled glycoside for combining sites on antidigitalis antibody. If one mixes varying quantities of unlabeled glycoside with a standard amount of radiolabeled glycoside, the amount of radioactivity bound by a standard amount of antibody will decrease as increasing amounts of unlabeled glycoside are added. A standard curve can then be constructed from which the concentration of glycoside in a patient's blood serum can be determined on the basis of the decrease it causes in the binding of radioactive glycoside by specific antibody. Radiolabeled glycosides and antisera are commercially available. If stored properly, antibodies are stable for many years, and 1 ml of antiserum may be employed in more than 100,000 determinations.

USES AND DOSE. Digitalis is used in the form of tablets or capsules to treat conges-

tive heart failure, paroxysmal atrial tachycardia, atrial flutter, and atrial fibrillation. See Table 7–2 for the usual dose of digitalis. Dose must be reduced by 25 to 50% for the elderly, for patients with lean body mass, and for patients with metabolic or electrolyte disorders. The onset of action is 2 to 4 hours, and maximal effect occurs in 12 to 14 hours. Complete dissipation of the drug from the body takes 2 to 3 weeks.

PRESCRIPTION PRODUCT. Digiglusin® contains the products from the specially prepared leaf of *D. purpurea*.

Digitoxin

Digitoxin is a cardiotonic glycoside obtained from *D. purpurea*, *D. lanata*, and other suitable species of *Digitalis*. On hydrolysis, digitoxin yields 1 molecule of digitoxigenin and 3 of digitoxose. It is a highly potent drug and should be handled with exceptional care. Digitoxin occurs as a white or pale buff, odorless, microcrystalline powder. It is a bitter substance that is practically insoluble in water and slightly soluble in alcohol. It is the most lipid-soluble of the cardiac glycosides used in therapeutics.

The major pharmacokinetic parameters for digitoxin include complete oral absorption, which distinguishes it from other cardiac glycosides. Upon oral administration, the onset of action is 1 to 4 hours with a

Table 7–2. Dosage Schedules of Various Forms of Digitalis and Cardiac Glycosides

Drug	Dosage Form	Route of Administration	Usual Initial Loading Dose	Usual Maintenance Dose
Digitalis	tablets or capsules	oral	1.2 g divided in equal doses administered every 6 hours	100 to 200 mg daily
Digitoxin	tablets	oral	rapid: 600 μg followed by 400 μg, then 200 μg at 4- to 6-hour intervals	50 to 300 μg daily
			slow: 200 μg twice daily for 4 days	
	injection	IV	same as rapid oral loading dose	100 to 200 μg daily
Digoxin	tablets	oral	rapid: 0.75 to 1.25 mg divided into 2 or more doses, each administered every 6 to 8 hours	125 to 500 μg daily
			slow: 125 to 500 μg daily for 7 days	
	capsules	oral	rapid: 400 to 600 μg initially, then 100 to 300 μg every 6 to 8 hours until desired effect is clinically evident	50 to 350 μg as 1 or 2 doses daily
			slow: 50 to 350 μg daily divided into 2 doses and repeated for 7 to 22 days as needed to reach steady-state serum concentrations	
	injection	IV	400 to 600 μg with additional doses of 100 to 300 μg every 4 to 8 hours	125 to 500 μg daily in single or divided doses
Deslanoside	injection	IV	1.6 mg as a single dose or 800 μg initially and repeated every 4 hours	—
		IM	800 μg given at each of 2 separate injection sites	—

peak at 8 to 14 hours. Approximately 50 to 70% of the glycoside is converted by the liver to inactive genins, which are excreted in the kidneys. Because of a long plasma half-life (168 to 192 hours), it may take from 3 to 5 weeks for complete dissipation of the drug from the body following discontinuation of therapy. It is estimated that a drug-serum level of 14 to 26 ng/ml is required for full therapeutic effect, and levels exceeding 35 ng/ml may produce symptoms of toxicity.

It has the same uses and precautions as digitalis. The dose is given in Table 7–2.

PRESCRIPTION PRODUCT. Digitoxin is represented by Crystodigin®.

Digitalis Lanata

Digitalis lanata or Grecian foxglove is the dried leaves of *Digitalis lanata* Ehrhart, a plant indigenous to southern and central Europe. It is the source of digoxin and desacetyllanatoside C; however, nearly 70 different glycosides have been detected in the leaves of *D. lanata*. The composition of 19 of the most important of these is listed in Table 7–3. All are derivatives of 5 different aglycones, 3 of which (digitoxigenin, gitoxigenin, and gitaloxigenin) also occur in *D. purpurea*. The other 2 types of glycosides derived from digoxigenin and diginatigenin occur in *D. lanata* but not in *D. purpurea*. As noted in the table, the 5 types of primary glycosides are designated lanatosides A through E, according to the identity of the aglycone. The lanatosides are sometimes referred to as digilanids, especially in the older literature.

None of the primary glycosides of *D. lanata* is identical to those found in *D. purpurea*. Even those that have the same aglycone differ by the presence of an acetyl group attached to the third digitoxose residue. Removal of the acetyl group and sugar residues by selective hydrolysis results in secondary glycosides, some of which, e.g., digitoxin, occur in both species. NOTE: Glycosides derived from aglycones of the C and D series may be obtained only from *D. lanata*.

Digoxin

Digoxin is the most widely used of the cardiotonic glycosides, and it is obtained from the leaves of *D. lanata*. On hydrolysis digoxin yields 1 molecule of digoxigenin and 3 of digitoxose. It is a highly potent drug and should be handled with exceptional care. Digoxin occurs as a white, crystalline powder.

Digoxin tablets are 60 to 80% absorbed, and variable bioequivalence among different brands of digoxin tablets has been demonstrated. Because of the low therapeutic index of the drug, it is recommeneded that, in the absence of good comparative bioavailability data, a patient should not be changed from one brand of tablet to another after a reasonable therapeutic effect has been achieved with one preparation. Otherwise, either a toxic or nontherapeutic effect may result owing to a change in the bioavailability of the drug.

USE AND DOSE. A solution-filled capsule is available that the manufacturer claims provides a 100% bioavailability.

Upon oral administration, the onset of action is 30 minutes to 2 hours, with a peak at 2 to 6 hours. Digoxin is also administered parenterally for a more rapid effect. The major route of elimination is the kidneys, and with a plasma half-life of 30 to 40 hours, complete dissipation of effects following discontinuation of therapy takes from 6 to 8 days. It is estimated that a drug-serum level of 0.5 to 2 ng/ml is required for full therapeutic effect, and levels exceeding 2.5 ng/ml may produce symptoms of toxicity.

Digoxin has the same uses and precautions as digitalis and is indicated when the risk of digitalis intoxication is great, since it is relatively short-acting and rapidly eliminated when compared with digitoxin. However, digitoxin may be indicated in patients with impaired renal function.

The usual dosage schedule is given in Table 7–2.

PRESCRIPTION PRODUCTS. Lanoxin®, Lanoxicaps®.

Table 7–3. Composition of the Principal Glycosides of *Digitalis lanata*

Glycoside	Sugars
Derivatives of Digitoxigenin	
Lanatoside A	3 digitoxose, 1 acetyl group, 1 glucose
Acetyldigitoxin (α and β forms)	3 digitoxose, 1 acetyl group
Digitoxin	3 digitoxose
Gluco-evatromonoside	1 digitoxose, 1 glucose
Gluco-digitoxigenin-glucomethyloside	1 glucomethylose, 1 glucose
Gluco-digifucoside	1 fucose, 1 glucose
Neo-gluco-digifucoside	1 fucose, 1 glucose
Derivatives of Gitoxigenin	
Lanatoside B	3 digitoxose, 1 acetyl group, 1 glucose
Gluco-gitoroside	1 digitoxose, 1 glucose
Digitalinum verum	1 digitalose, 1 glucose
Derivatives of Gitaloxigenin	
Lanatoside E	3 digitoxose, 1 acetyl group, 1 glucose
Gluco-lanadoxin	1 digitoxose, 1 glucose
Gluco-verodoxin	1 digitalose, 1 glucose
Derivatives of Digoxigenin	
Lanatoside C	3 digitoxose, 1 acetyl group, 1 glucose
Desacetyllanatoside C	3 digitoxose, 1 glucose
Acetyldigoxin (α, β, and γ forms)	3 digitoxose, 1 acetyl group
Digoxin	3 digitoxose
Gluco-digoxigenin-bis-digitoxoside	2 digitoxose, 1 glucose
Derivatives of Diginatigenin	
Lanatoside D	3 digitoxose, 1 acetyl group, 1 glucose

Deslanoside

Deslanoside is desacetyllanatoside C, which on hydrolysis yields 1 molecule of digoxigenin, 3 of digitoxose, and 1 of glucose. Deslanoside occurs as a white, crystalline powder. It is hygroscopic, absorbing about 7% of moisture when exposed to air, and is highly potent.

Deslanoside is frequently used to attain rapid initial loading by parenteral administration. Onset of action is 10 to 30 minutes; maximal effects occur in 2 to 3 hours, with dissipation in 3 to 6 days. The usual dosage schedule, intramuscularly or intravenously for digitalization, is given in Table 7–2. The same precautions of use that apply to digitalis also apply to deslanoside.

PRESCRIPTION PRODUCT. Cedilanid D®.

Other Cardioactive Drugs

A number of plants contain cardioactive glycosides, and some of them have been employed for many years as cardiac stimulants and diuretics. Several are more potent than digitalis, but they are less reliable because their dosage cannot be controlled properly. Although most of these drugs were recognized officially for years and were considered efficacious, they have been superseded by digitalis and its derivatives. A few are currently under reinvestigation.

Convallaria or lily-of-the-valley root is the dried rhizome and roots of *Convallaria majalis* Linné (Fam. Liliaceae). More than 20 cardioactive glycosides have been isolated from this drug. Principal among these is convallatoxin, a monoglycoside composed of the genin of K-strophanthin (strophanthidin) and the sugar of G-strophanthin (rhamnose). Other minor glycosides include convallatoxol and convalloside.

Apocynum, black Indian hemp, dog bane, or Canadian hemp consists of the

dried rhizome and roots of *Apocynum cannabinum* Linné or *A. androsaemifolium* Linné (Fam. Apocynaceae). The chief constituent is cymarin, although apocannoside and cyanocannoside have also been isolated from *A. cannabinum*.

Adonis or pheasant's eye is the dried overground portion of *Adonis vernalis* Linné (Fam. Ranunculaceae). Cardioactive glycosides identified in the drug include adonitoxin, cymarin, and K-strophanthin.

Cactus grandiflorus or night-blooming cereus consists of the fresh, succulent stem of wild-growing *Selenicereus grandiflorus* (Linné) Britton et Rose (Fam. Cactaceae).

Black hellebore or Christmas rose is the dried rhizome and roots of *Helleborus niger* Linné (Fam. Ranunculaceae). The chief constituent is hellebrin. Black hellebore possesses cardiac stimulant properties in contrast to green hellebore (see veratrum viride), which is a cardiac depressant.

Another plant that contains a cardiac glycoside is *Nerium oleander* Linné (Fam. Apocynaceae). The leaves have been used to treat cardiac insufficiency. The chief constituent is oleandrin, a 3-glycosido-16-acetyl derivative of gitoxigenin.

Strophanthus is the dried, ripe seed of *Strophanthus kombe* Oliver, or of *S. hispidus* DeCandolle (Fam. Apocynaceae), that is deprived of the awns. Strophanthus seeds have long been used by native Africans in the preparation of arrow poisons. These poisons were first observed in western Africa by Hendelot and in East Africa by Livingstone. Early specimens sent to Europe established the powerful cardiac properties of the seeds.

K-strophanthoside, also known as strophoside, is the principal primary glycoside in both *S. kombe* and *S. hispidus*. It is composed of the genin, strophanthidin, coupled to a trisaccharide consisting of cymarose, β-glucose, and α-glucose. α-Glucosidase removes the terminal α-glucose to yield K-strophanthin-β, and the enzyme, strophanthobiase, contained in the seed converts this to cymarin plus glucose. A mixture of these glycosides, existing in the seed in concentrations of up to 5%, was formerly designated strophanthin or K-strophanthin. Recent studies have revealed additional glycosides as minor constituents.

Ouabain is a glycoside of ouabagenin and rhamnose. It may be obtained from the seeds of *Strophanthus gratus* (Wall et Hook.) Baillon or from the wood of *Acokanthera schimperi* (A. DC.) Schwf. (Fam. Apocynaceae). It is extremely poisonous. Ouabain is also known as G-strophanthin.

Squill or squill bulb consists of the cut and dried, fleshy, inner scales of the bulb of the white variety of *Urginea maritima* (Linné) Baker, known in commerce as white or Mediterranean squill; or of *U. indica* Kunth, known in commerce as Indian squill (Fam. Liliaceae). The central portion of the bulb is excluded during its processing.

Squill contains about a dozen cardioactive glycosides. The principal one, scillaren A, comprises about two thirds of the total glycoside fraction. On hydrolysis, it yields the aglycone scillarenin, a bufadienolide, plus rhamnose and glucose. Other minor glycosides include glucoscillaren A (scillarenin + rhamnose + glucose + glucose) and proscillaridin A (scillarenin + rhamnose).

Squill is an expectorant, but it also possesses emetic, cardiotonic, and diuretic properties.

Red squill consists of the bulb or bulb scales of the red variety of *U. maritima*, which is imported for use as a rat poison. It should not be present in the medicinal squill and may be detected by the presence of red, pink, or purple epidermal or parenchymal tissues.

Most of the squill imported into the United States is of the red variety. Each year, a considerable tonnage is used as a rodenticide. Rodents lack the vomiting reflex, which makes red squill particularly lethal to these animals. The inadvertent

ingestion by human beings of plant materials that contain cardiac glycosides induces the vomiting reflex and reduces the life-threatening aspects of the toxic manifestations.

STEROID HORMONES

The steroid hormones can be divided into 2 classes, the **sex hormones** and the **adrenocortical hormones.** The former are produced primarily in the gonads and mediate the growth, development, maintenance, and function of the reproductive tract and the accessory sex organs. These hormones fall into 3 chemically and physiologically distinct categories: the **estrogens** and **progestins,** which regulate various functions of the female reproductive tract, and the **androgens**, which stimulate the development of the male reproductive organs. The adrenocortical hormones are produced by the outer cortical portion of the adrenal glands, and they are divided into 2 classes, depending on their biologic activity. The hormones that principally affect the excretion of fluid and electrolytes, with a subsequent sodium retention, are called mineralocorticoids; those that affect intermediary metabolism are termed glucocorticoids.

The production of steroid hormones in the body is initiated by the releasing factors of the hypothalamus, which travel to the anterior lobe of the pituitary gland where they induce the release of tropic hormones into the blood. When stimulated by the appropriate tropic hormone, steroids are synthesized at the target site, either the adrenal cortex or the gonads. Steroid level in the blood is held in balance by a mechanism of feedback regulation that is mediated through the hypothalamus. When excess active steroid is in the blood that reaches the hypothalamus, the production of the hypothalamic releasing factors is stopped (Fig. 7–11).

This phenomenon of feedback regulation can cause problems in drug therapy

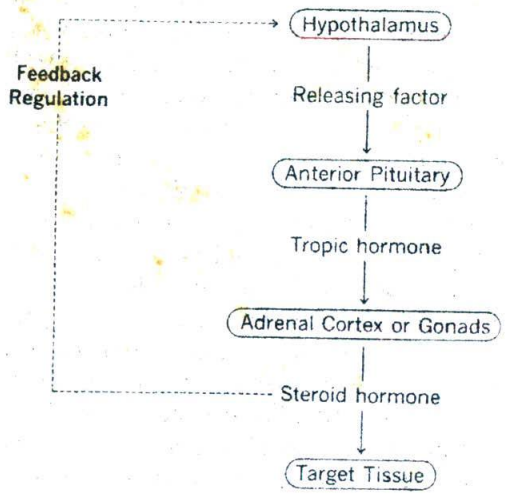


Fig. 7–11. Regulation of steroid hormone production.

with steroid hormones. For example, prolonged therapy with corticosteroids may cause irreversible atrophy of the adrenal cortex. A high corticosteroid level in the body suppresses the hypothalamus from secreting the corticotropin-releasing factor which, in turn, suppresses release of corticotropin. The lack of stimulatory impact of this anterior pituitary hormone results in atrophy of the adrenal cortex.

Biosynthesis of Steroid Hormones. Biosynthesis of the numerous steroid hormones of the adrenal cortex, gonads, and placenta is an extremely complex specialty field. Only the briefest essentials can be presented here. When one realizes that more than 70 different steroids have been isolated from the adrenal gland alone, one can easily understand why the biosynthetic relationships are complex.

Like other steroids of biologic origin, these hormones are derived from the well-known acetate-mevalonic acid pathway which, in this case, leads first to cholesterol (see Fig. 7–3 for details). Partial side-chain degradation of cholesterol leads to pregnenolone and then to progesterone, both of which serve as precursors of the other steroid hormones.

The conversion of cholesterol to preg-

nenolone is catalyzed by a mixed-function oxidase enzyme complex that involves a desmolase and requires O_2 and NADPH. This conversion appears to be the rate-limiting step in steroid hormone biosynthesis and is under the influence of the tropic hormones of the anterior pituitary. In the case of ACTH stimulation of steroidogenesis, ACTH activates adrenal cortical adenyl cyclase, which causes a rise in cyclic AMP and a subsequent activation of glycogen phosphorylase. This enzyme breaks down glycogen to produce glucose-6-phosphate, which then is oxidized via the hexose monophosphate shunt pathway, yielding NADPH. An increase in the availability of this coenzyme increases the activity of the desmolase and the hydroxylase reactions.

Enzymes in the adrenals and the gonads remove the side chain and hydroxylate the steroid nucleus in the 17 α-position to form the androgens. After loss of the angular 19-methyl group, androgens are aromatized to estrogens. The adrenals also hydroxylate progesterone in positions 21, 11, and/or 17 to produce the classic adrenocortical hormones. Production of aldosterone involves 18-hydroxylation and dehydrogenation reactions.

Some of the principal conversions are illustrated in the simplified scheme shown in Figure 7–12.

The steroid hormones are bound to proteins, primarily albumins, for transport in the blood. These steroid-protein complexes per se are physiologically inert and protect the steroid from metabolic inactivation. The strength of binding varies and can be generalized by classification as follows: the corticosteroids tend to be weakly bound, the estrogens are more strongly bound, and progesterone and testosterone are intermediate between the 2 extremes.

Reductive processes are normally involved in the metabolism of steroid hormones. The di-, tetra-, and hexahydric metabolites may be formed and usually entail progressive reduction of the 4-ene, 3-keto, and 20-keto functions. The reduced forms are usually excreted as the more soluble uronides or sulfate esters involving the 3-oxygen function. In the case of the metabolism of estradiol and testosterone, the initial metabolic reaction is oxidative, involving the 17-hydroxyl function, but subsequent metabolic steps are reductive, with eventual conjugation.

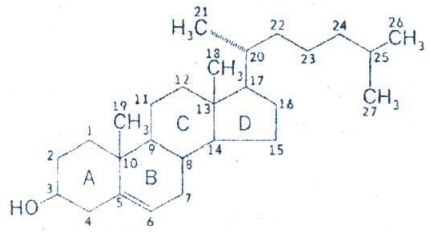
Mechanism of Action. The steroid hormones have diverse actions, and several specific receptor proteins, varying with the particular target tissue, have been isolated for each action. Structural changes of the hormone may affect the affinity or activity on one receptor and have little or no effect on other receptors. For example, changes in the chemical structure of testosterone allow for the separation of the androgenic activity from the anabolic activity of this hormone. The same applies when separating the glucocorticoid/mineralocorticoid activity of corticosteroids. Also, steroid hormones do not act through an increase in cyclic AMP but rather through a stimulation of protein synthesis. A possible explanation of this mechanism is that the steroid binds with the specific receptor protein in the cytoplasm of the target cell. This complex enters the nucleus, where it is bound to the chromosome through a specific acceptor protein associated with chromatin. The interaction of steroid, of cytoplasm receptor protein, and of the chromosomal receptor protein may lead to a derepression of a segment of chromosome, which would result in the increased production of a particular enzyme protein. For example, mineralocorticoids produce an increase in the synthesis of enzymes that are necessary for active transport of Na^+, which leads directly to increased Na^+ reabsorption in the renal tubules.

Commercial Production of Steroids. The steroid hormones and their semisynthetic analogs represent a multimillion-dollar annual business for the American drug industry. When one considers the social, political, and economic implications asso-

7

Steroids

Steroids constitute a natural product class of compounds that is widely distributed throughout nature. The diversity of biologic activities of steroids includes the development and control of the reproductive tract in humans (estradiol, progesterone, testosterone), the molting of insects (ecdysone), and the induction of sexual reproduction in aquatic fungi (antheridiol). In addition, steroids contribute to a wide range of therapeutic applications, such as cardiotonics (digitoxin), vitamin D precursors (ergosterol), oral contraceptive agents (semisynthetic estrogens and progestins), anti-inflammatory agents (corticosteroids), and anabolic agents (androgens).



Cholesterol

NOMENCLATURE

A steroid is any compound that contains a cyclopentanoperhydrophenanthrene nucleus. The chemical nomenclature of steroids is based on this fundamental carbocycle with adjacent side-chain carbon atoms. Each parent tetracyclic hydrocarbon bears a specific stem name, and some of the principal hydrocarbons are shown in Figure 7–1. Steroids are numbered and rings are lettered as indicated in the structural formula for cholesterol. If one or more of the carbon atoms shown in the structure of cholesterol is not present, the numbering of the remainder is undisturbed.

When the rings of a steroid are denoted as projections onto the plane of the paper, an atom or group attached to a ring is termed α *(alpha)* if it lies below the plane of the paper or β *(beta)* if it lies above the plane of the paper. In formulas, bonds to atoms or groups attached in an α configuration are shown as broken lines, and bonds to atoms or groups attached in a β configuration are shown as solid lines.

The use of a steroid stem name implies that atoms or groups attached at the ring-junction positions 8, 9, 10, 13, and 14 are oriented as shown in Figure 7–2 (8β, 9α, 10β, 13β, 14α), and a carbon chain (R) attached to position 17 is assumed to be β-oriented. The configuration of hydrogen or a substituent at the ring-junction position 5 is always designated by adding α or β after the numeral 5. This numeral and letter are placed immediately before the stem name. The implication of these conventions of nomenclature is that, in most steroids, rings B and C and rings C and D are fused *trans*, whereas rings A and B may be fused either *cis* or *trans*. For example, the bile acid, cholic acid, has a *cis*-fused A/

coniferyl benzoate (60 to 70%), plus smaller amounts of free benzoic acid (10%), the triterpene, siaresinol, (6%), and a trace of vanillin.

Sumatra benzoin contains free balsamic acids, chiefly cinnamic (10%) and benzoic (6%), as well as esters derived from them. Triterpene acids, especially 19-hydroxyoleanolic and 6-hydroxyoleanolic, and traces of vanillin, phenylpropyl cinnamate, cinnamyl cinnamate, and phenylethylene are also present.

Sumatra benzoin yields not less than 75% of alcohol-soluble extractive; Siam benzoin yields not less than 90% of alcohol-soluble extractive.

USES. Benzoin possesses antiseptic, stimulant, expectorant, and diuretic properties.

Compound benzoin tincture is employed as a topical protectant and is applied as required. It contains benzoin, aloe, storax, and Tolu balsam and is valuable as an expectorant when vaporized.

PROPRIETARY PRODUCT. VapoSteam®.

Benzoic acid is now a synthetic product but was first obtained by sublimation from Sumatra benzoin.

It occurs as white crystals, usually in the form of scales or needles. It has a slight odor of benzoin and is volatile at moderate temperatures, freely so in steam.

Benzoic acid and its sodium salt are extensively used as preservatives of foods, drinks, fats, pharmaceutic preparations, and other substances. Medicinally, benzoic acid is used primarily as an antifungal agent. It is an ingredient in benzoic and salicylic acids ointment (Whitfield's ointment), which is effective in the treatment of athlete's foot and, to a lesser extent, ringworm.

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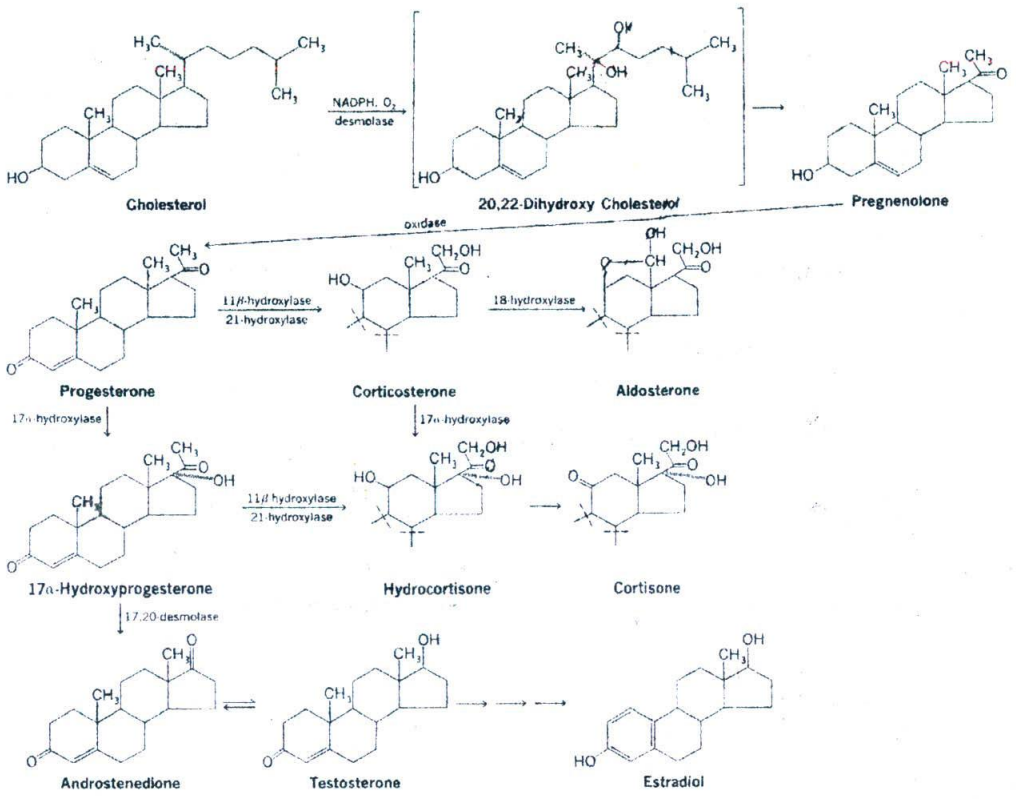


Fig. 7–12. Biosynthesis and bioconversion of steroid hormones.

ciated with the use of oral contraceptive drugs, the importance of steroids to mankind cannot be questioned. At the present time, the principal source of the steroid chemical nucleus used in the drug industry is the plant kingdom; however, in the not too distant past, the source of steroid hormones was from the gonads and adrenal glands of animals that were used as food by humans. The amount of hormone present in these glands was extremely small, and large quantities of glands were required to isolate milligram quantities of hormone; consequently, it was not practical to use the pure hormone in therapy. For example, in 1934, Schering Laboratories, Berlin, needed 625 kg of ovaries from 50,000 sows in order to obtain 20 mg of pure crystalline progesterone.

Today, the steroid industry represents the culmination of efforts by many scientists; however, a few can be singled out for their pioneering work in steroid chemistry. One of these men is Russell E. Marker. Marker is responsible for the discovery of a commercially feasible conversion of steroidal sapogenins to progesterone. His early work involved the search for plant species that were rich in steroidal sapogenins. When he found that Mexican yams, various species of *Dioscorea*, were rich in these compounds, he moved to Mexico City in 1943, where he isolated diosgenin from *D. macrostachya* (*D. mexicana*), known in Mexico as *cabeza de negro*. From diosgenin, employing the chemical degradation illustrated in Figure 7–13, he managed to prepare more than 3 kg of progesterone (at the time valued at $8 a gram). This hormone and the process used to prepare it were the foundation stones for the Syntex Company.

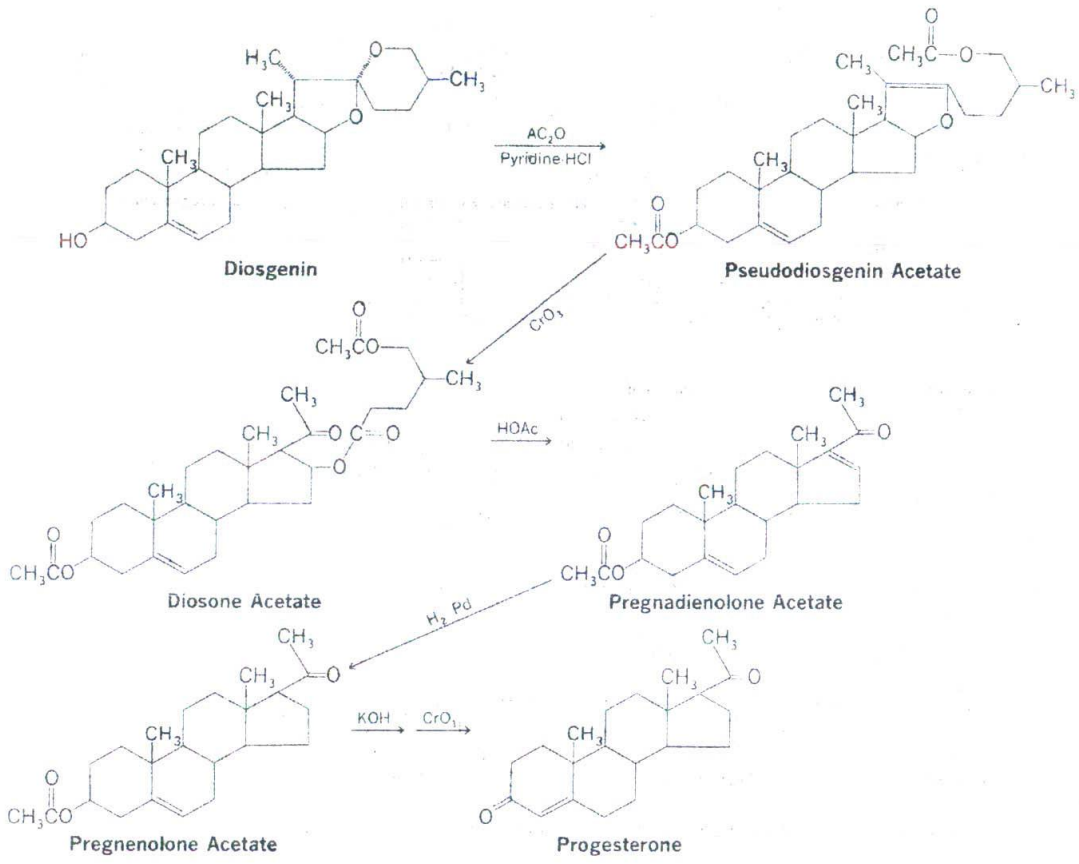
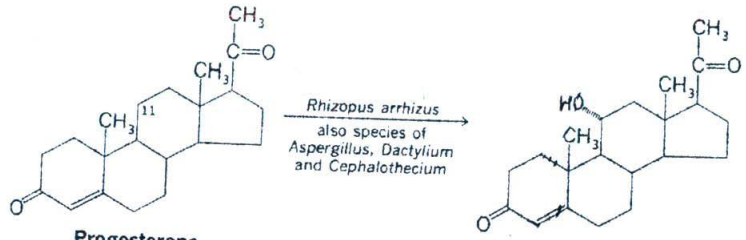


Fig. 7–13. The Marker degradation.

During the 1930s, several scientists, including E. C. Kendall, a chemist at the Mayo Clinic, and T. Reichstein, a chemist at the Federal Institute of Technology, Zurich, Switzerland, almost simultaneously and independently isolated steroids from the adrenal cortex of cattle. Stimulated by the potential therapeutic importance of these compounds, the Merck Company in 1944 successfully produced 15 mg of cortisone from 1 kg of desoxycholic acid utilizing 36 separate chemical steps. However, in 1949, when P.S. Hench of the Mayo Clinic announced cortisone's dramatic effectiveness in treating rheumatoid arthritis, the increased demand for cortisone required a more readily available and inexpensive source. The problem was solved in 1952 when scientists at the Upjohn Company found a microorganism, *Rhizopus arrhizus*, that could convert progesterone, a readily available starting material because of the Marker degradation, to 11α-hydroxyprogesterone in an 80 to 90% yield. The extremely difficult problem of introducing an oxygen function in the 11-position of the steroid nucleus by using chemical methods was therefore solved (Fig. 7–14).

A vast amount of research resulted in extension and improvement of this basic procedure with other precursors and numerous microorganisms. Relatively inexpensive starting materials, such as stigmasterol from soybeans, hecogenin from the sisal industry, or diosgenin from *Dioscorea* species, are now employed.

Stigmasterol may be converted chemi-



Progesterone

11α-Hydroxyprogesterone

Fig. 7–14. Introduction of oxygen function into the 11-position of the steroid nucleus.

cally to progesterone, which is, in turn, incubated in large fermentors with suitable microorganisms under specified conditions to yield 11α-hydroxyprogesterone, which may then be converted chemically to cortisone. Similarly, cortexolone (Reichstein's substance S) is prepared chemically from diosgenin and is then converted by *Streptomyces fradiae* or *Cunninghamella blakesleeana* to cortisol (hydrocortisone).

Cortisone or cortisol is dehydrogenated in the Δ^1-position by *Corynebacterium simplex* or by *Fusarium* species to yield prednisone or prednisolone, respectively.

Certain microorganisms also can hydroxylate synthetically prepared fluorosteroids in the 16α-position to produce triamcinolone (Fig. 7–15).

Adrenal Cortex

The adrenal cortex is essential to life. Removal of about 85% of cortical tissue is lethal in a few days. In animals so treated, life may be maintained by the administration of extracts of hormones of the adrenal cortex.

Cortical deficiency in animals is marked by a loss of appetite and weight, vomiting and diarrhea, weakness, and a fall in temperature, metabolism, and blood pressure. There is a loss of blood fluid, with resulting concentration of blood, and a fall in serum sodium, with a rise in serum glucose and potassium. Kidney damage is frequently present. These developments can be prevented or restored to normal by the administration of cortical extracts and frequently by the simple use of a high sodium, low potassium intake.

The human counterpart of this deficiency picture is seen in the clinical development of Addison's disease (chronic adrenocortical insufficiency), usually owing to tuberculosis or tumor of the adrenal cortex. Associated with this disease are degeneration of the gonads, a marked increase in capillary permeability, and an increased sensitivity to insulin. Sodium loss with potassium retention may be the outstanding condition of the disease. If untreated, Addison's disease terminates fatally in 1 to 3 years, usually owing to hypoglycemia, dehydration, nutritional disturbances, or secondary infection.

Excessive adrenal cortical activity, as in tumors or because of the presence of accessory cortical tissue, results in profound growth abnormalities, especially seen in the external genitalia and in the secondary sex characteristics. In young children, there is precocious sexual development and desire and obesity or unusual muscular development. In adult females, virilism usually develops, associated with a masculine appearance, often with homosexuality. The bearded lady of the circus frequently falls into this category. Treatment of cortical hyperactivity is principally surgical.

Some 70 or more steroids have been isolated from cortical extracts. These exhibit in some degree the action of adrenal cortex. Some, in addition, manifest estrogenic, androgenic, and progesteronelike activity,

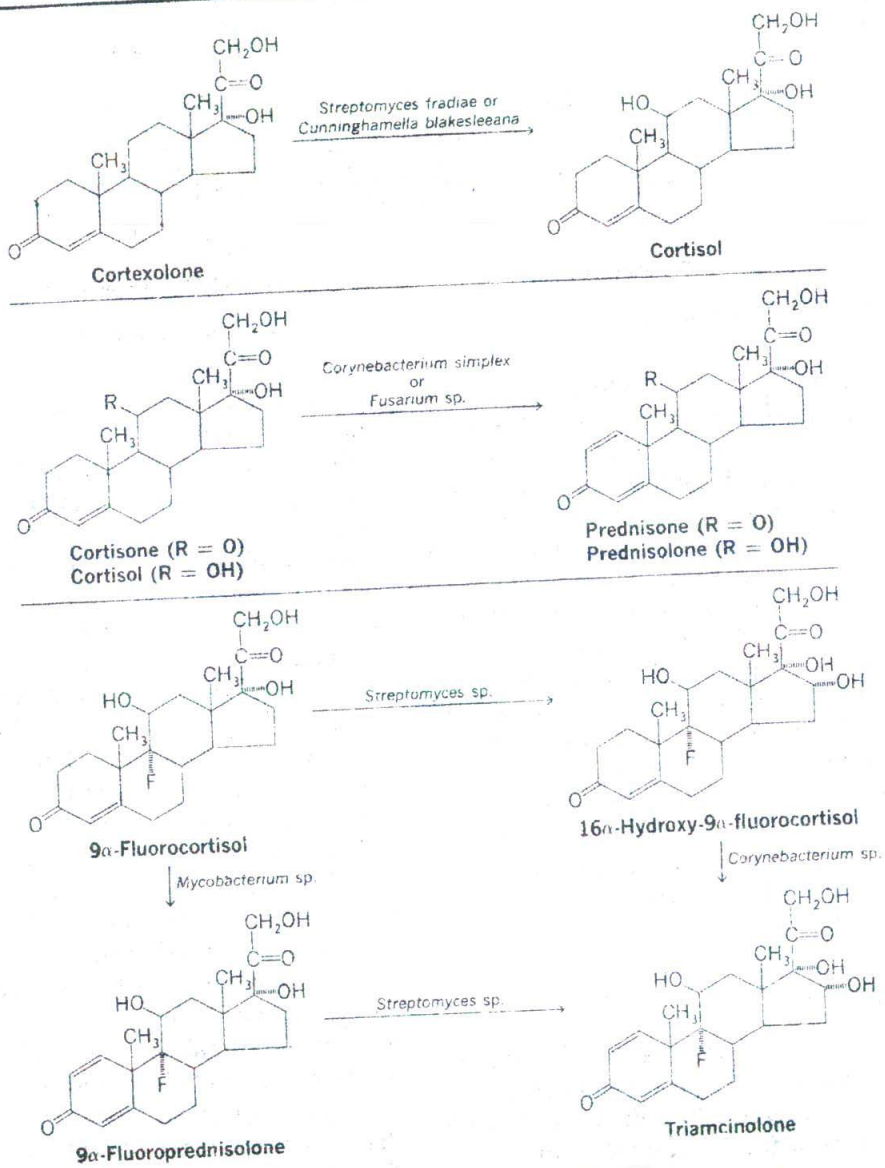


Fig. 7–15. Microbiologic transformation in production of glucocorticoids.

further indicating the close relationship between the adrenal cortex and the gonads.

Adrenocortical steroids include cortisone, hydrocortisone, desoxycorticosterone, and aldosterone. Cortisone and hydrocortisone constitute the majority of the hormones that regulate protein and carbohydrate metabolism. They have been referred to as the glucocorticoids. Aldosterone and desoxycorticosterone have been referred to as mineralocorticoids. Aldosterone is the principal adrenal steroid that regulates sodium, potassium, and water balance in the organism; however, it is not available for therapeutic use. Also, many agents that are considered primarily glucocorticoids possess variable mineralocorticoid activity as well.

Preparations of the adrenal cortex are used most effectively in replacement therapy for such conditions as Addison's disease or surgically caused adrenal cortex de-

ficiency. An injection containing a mixture of hormonal substances from the adrenal cortex has been used in replacement therapy. Presumably, use of such a crude mixture offers the advantage of administering all of the active glandular hormones rather than only individual hormones that have been recognized and are available in pure form. However, controlling responses with undefined preparations is difficult, and the subtle responses potentially elicited by the mixed extracts are not easily recognized; thus, the trend in replacement therapy favors the use of pure hormonal substances.

The glucocorticoids are also used for their anti-inflammatory activity; therapy based on this pharmacologic response is an effective palliative approach in rheumatoid arthritis and a number of other conditions involving the inflammatory response. However, caution must be used in balancing the advantages and disadvantages of prolonged administration of corticosteroid therapy, such as may be involved in arthritic conditions. Exogenous sources of corticosteroids may cause a disruption in the physiologic balance among the biosynthetically related steroid hormones; toxic manifestations in such situations often involve changes that are normally considered to be dominated by gonadal hormones. As was discussed earlier, another potential problem of serious consequence is irreversible atrophy of the adrenal cortex.

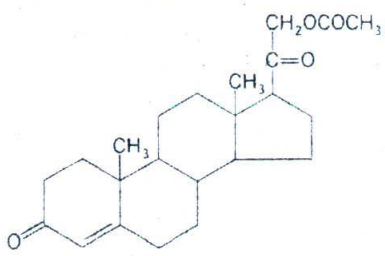
Glucocorticoid therapy provides palliative treatment of symptoms in many allergic disorders, such as bronchial asthma, and is lifesaving for patients in anaphylactic shock. These compounds are used as immunosuppressive agents in organ transplants and autoimmune disorders and as antitumor agents in the treatment of malignancies, especially in certain leukemias and lymphomas.

Drug Interactions. Barbiturates and phenytoin can induce the hepatic drug-metabolizing enzymes, such as hydrocortisone hydroxylase, which results in an increased degradation of corticosteroids. Therefore, concomitant therapy with one of these drugs may require an increase in the dose of the corticosteroid.

Because of an increase in hepatic gluconeogenesis during glucocorticoid therapy, the dose of hypoglycemic agents may have to be adjusted upward in diabetic patients receiving corticosteroids.

Desoxycorticosterone or desoxycortone is 21-hydroxypregn-4-ene-3,20-dione, a steroid hormone that was identified by Reichstein and his associates in 1938. Later, it was synthesized from stigmasterol. Present drug supplies are obtained by synthetic means.

This hormone is classified as a mineralocorticoid. Desoxycorticosterone functions primarily to restore a balance of sodium and potassium in body fluids and to restore kidney function in cortical deficiency. Death from hypoglycemia may occur when Addison's disease is treated with desoxycorticosterone alone; such cases also require the use of a glucocorticoid.



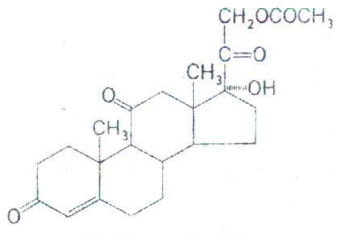
Desoxycorticosterone Acetate

The hydroxyl function at C-21 of desoxycorticosterone is esterified, normally with acetic acid, in pharmaceutic formulations. It is effective when administered buccally, but better and more uniform results follow intramuscular injection. Pellets can be successfully implanted in the subcutaneous tissues for even more prolonged action. The usual dose of desoxycorticosterone acetate, intramuscularly or subcutaneously, is 1 to 5 mg daily.

PRESCRIPTION PRODUCTS. Doca Acetate®, Percorten Acetate®, Percorten Pivalate® (the trimethylacetate ester).

Cortisone or 17,21-dihydroxypregn-4-ene-3,11,20-trione is one of the glucocorticoid substances of the adrenal cortex. The acetate ester of this hormone is used intramuscularly, orally, and topically to treat a wide variety of situations, such as rheumatoid arthritis, other collagen diseases, Addison's disease, and certain allergic and asthmatic conditions. An appreciable sodium-retaining property can be a major problem with the systemic use of cortisone. The usual dose, orally, is 25 to 300 mg a day; intramuscularly, 20 to 300 mg a day.

PRESCRIPTION PRODUCT. Cortone® Acetate.



Cortisone Acetate

Cortisol or hydrocortisone (Kendall's compound F) is 11β,17,21-trihydroxypregn-4-ene-3,20-dione. It is considered the principal glucocorticoid substance of the adrenal cortex. This hormone and its acetate ester are used intramuscularly, orally, and topically for the same purposes as cortisone acetate. Intra-articular administration of cortisol always involves the acetate ester. Hydrocortisone sodium phosphate and hydrocortisone sodium succinate are water-soluble and are used in parenteral formulations when intravenous administration is indicated.

There are indications that cortisol is slightly more potent in some patients than cortisone and gives slightly better overall effects. However, it may exhibit the same disadvantages of sodium retention that were noted with cortisone.

The usual oral dose of hydrocortisone is 20 to 240 mg daily as a single dose or in divided doses. Topically, it is applied as a 0.5 to 2.5% cream or ointment. Hydrocortisone acetate is usually administered intra-articularly, intralesionally, or by soft-tissue injection, 5 to 75 mg at each site, repeated at 2- to 3-week intervals. Both hydrocortisone sodium phosphate and hydrocortisone sodium succinate are employed intravenously or intramuscularly in usual doses equivalent to 100 to 500 mg of hydrocortisone, repeated at 2- to 6-hour intervals, depending upon patient response.

PRESCRIPTION PRODUCTS. Cortef®, Cortef® Acetate, Solu-Cortef®, Hydrocortone®, Hydrocortone® Phosphate.

The potential therapeutic utility of the glucocorticoids has promoted intensive efforts to discover modifications of the naturally occurring hormones that will be more potent and more specific in their activity. The best success has been achieved with desired increases in potency. Prednisone (Deltasone®, Meticorten®) and prednisolone (Delta-Cortef®, Sterane®) represent early achievements in these efforts. Elimination of any mineralocorticoid activity has been a major objective; a degree of success has been attained with such compounds as betamethasone (Celestrone®), dexamethasone (Decadron®, Dexone®, Hexadrol®), methylprednisolone (Medrol®), paramethasone (Haldrone®), and triamcinolone (Aristocort®, Kenacort®), but the ideal of total separation of mineralocorticoid activity from glucocorticoid substances has not yet been achieved. It is interesting to note that successful modifications in the basic steroid molecule fall into 4 categories:

1. Δ^1-dehydrogenation
2. 16α-hydroxylation
3. 6α- or 9α-fluorination
4. 6α-, 16α-, or 16β-methylation.

Gonads

The ovaries and testes are exocrine (ova, sperm) as well as endocrine (hormonal) in function. They develop under the influence of anterior pituitary hormones, particularly:

1. The follicle-stimulating hormone (FSH) leads to the development of the

ovarian follicles, to their formation of ova and of estrogen, and to the development of the testes and the maturation of the spermatozoa.

2. The luteinizing hormone (LH) is necessary to the development of the corpora lutea in the ovarian follicles after ovulation, to the formation of progesterone by the corpora lutea, and to the production of androgen in the matured testis.

Androgens (male hormones) and estrogens (female follicular hormones) act to:

1. Develop and maintain the secondary characters of sex.
2. Depress anterior pituitary function, leading in turn to the depression of the testis or the ovary.

Progesterone (corpus luteum hormone) similarly depresses anterior pituitary function and presents a mixed antagonism-synergism with estrogenic activity, as will be indicated later.

Gonadal hyperactivity or excessive therapy may thus result in a picture of precocious or excessive sexual development, with the generalized effects of anterior pituitary depression. Gonadal hypoactivity, as occurs in the natural menopause or following surgical removal of the gonads, results in a mixed picture of sexual regression and enhanced anterior pituitary activity, with psychic disturbance and the involvement of other endocrine glands, particularly the thyroid.

Testes

Following castration in the male, the sex organs atrophy, and sexual desire and activity are diminished. These functions are restored by the administration of testis hormone. Hypogonadism (eunuchoidism) is inadequate development of the testes owing to pituitary disorder, infection, or other disease. Therapy of this condition is still in the experimental stages.

Hypergonadism is most frequently seen in young males, owing to testis tumors; this results in precocious development of sex organs and male characteristics. Therapy is usually surgical.

Testosterone is believed to be the true testis hormone, although it has been identified only in the bull's testis. It was synthesized by Ruzicka from cholesterol in 1936. Androsterone and dehydroandrosterone are urinary excretion products, relatively inactive in man.

Testis hormone preparations have been valuable in the replacement therapy of male castrates and eunuchoid states and in the treatment of certain female ovarian dysfunctions. Much of this therapy is still in the experimental stages. Testosterone is not an aphrodisiac, and its use may produce the general effects of anterior pituitary depression. It may produce virilism in the female, and skin reactions similar to acne vulgaris may frequently develop.

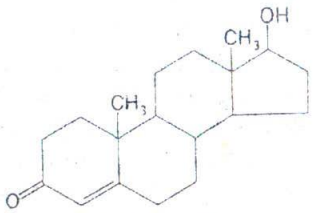
Anabolic effects, especially with regard to protein synthesis and nitrogen retention in the body, have been noted with androgens. This action is potentially useful as supportive therapy in a number of debilitating conditions. Attempts have been made to prepare steroid compounds that separate anabolic effects from other androgenic activities, and the ultimate limitations on this therapeutic approach are keyed to the success of these efforts. The ideal separation has not been achieved with such compounds as methylandrostenediol, methandrostenolone, and other anabolic substances that are currently available.

Testosterone or 17β-hydroxyandrost-4-en-3-one is the active male hormone. The quantities used for drug purposes are prepared synthetically. The 17-hydroxyl function of testosterone is readily oxidized and metabolized to the much less physiologically active keto compound. Thus, testosterone is not administered orally. The hormone may be used buccally, implanted subcutaneously, or injected intramuscularly. However, many formulations for these purposes utilize derivatives of the

hormone, such as the cypionate, ethanthate, and propionate esters of the 17-hydroxyl group, which are characterized by delayed absorption and destruction. The usual dose of testosterone, intramuscularly, is 25 mg as needed; implantation, 150 to 400 mg every 3 to 6 months.

PRESCRIPTION PRODUCTS. Delatestryl®, Depo-testosterone®.

The introduction of a methyl substituent at C-17 is another manipulation that has been used to circumvent the chemical and metabolic instability of testosterone. Preparations of methyltestosterone (Android®, Metandren®, Oreton® Methyl) are used buccally and orally for androgenic purposes.



Testosterone

Ovary

The human ovaries are paired organs. One is situated on each lateral pelvic wall in the posterior layer of the broad ligament, behind and below the lateral extremity of each fallopian tube (oviduct). Each is about the size and shape of an unshelled almond and weighs about 4 to 8 g.

Ova develop within primitive ovarian follicles (graafian follicles) under the influence of the follicle-stimulating hormone of anterior pituitary. Ovulation with the extrusion of one ovum from a ripened follicle normally occurs each month during the childbearing period. The ruptured follicle undergoes cellular change to become the corpus luteum under the influence of the luteinizing hormone of the anterior pituitary. The ovary elaborates 2 types of hormones: the estrogens, elaborated in the developing graafian follicle and probably also in the placenta during pregnancy; and the progestins, normally elaborated by the corpus luteum and, in the later half of pregnancy, by the placenta.

Estrogens. Deficiency in estrogenic activity is most frequently experienced in the normal menopause or following surgical removal of the ovaries. Local changes in the tissues of the vagina and vulva may result from estrogenic deficiency of any cause. The estrogens are necessary to:

1. Develop and maintain secondary female sex characteristics.
2. Develop and maintain the uterus and the vagina.
3. Aid in the presecretory development of the mammary glands.
4. Act as a growth hormone for uterine smooth muscle cells during pregnancy.

Estrogens act further to excite or sensitize the uterine muscle and to depress the anterior pituitary function. Preparations of estrogenic substances are employed in the management of:

1. Symptoms of the natural or surgical menopause.
2. Local atrophic and degenerative changes in the adult vagina and vulva, resulting from estrogen deficiency.
3. Gonorrheal vaginitis in the young female child, by inducing an adult type of vaginal epithelium resistant to the gonococcus.
4. Suppression of lactation in engorged, painful mammary glands, presumably by a direct action in the breast.
5. Prostatic cancer in the male, presumably by balancing an excessive persistence of androgen—the principle of "biochemical castration."

The natural ovarian hormones are steroids. The 3 major estrogenic hormones are estradiol and its oxidation products, estriol, and estrone. These hormones can be isolated from urine during pregnancy and can be prepared synthetically. Other estrogenic substances occur naturally, and amorphous mixtures of some of these steroids obtained from a pregnant mare's

urine are used in therapy under the designations of conjugated and esterified estrogens.

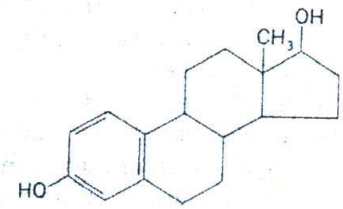
Estrogens may be administered orally, parenterally, by implantation, or by inunction for systemic activity. Orally administered natural estrogens are destroyed in greater part. Estriol is the best of the pure, naturally occurring estrogens for oral use; oral efficiency of estriol is about one-fifth that achieved by parenteral administration. Conjugated and esterified estrogens are also used orally, and the introduction of an ethinyl substituent at C-17 of estradiol gives a potent, orally effective compound; the usual dose of ethinyl estradiol (Estinyl®, Feminone®) is 50 μg, 1 to 3 times a day.

As much as 90% of parenterally administered natural estrogens may be destroyed. This factor, in addition to rapid absorption, tends to diminish their efficiency and the effective period of therapy. Pharmaceutic manipulations, which have proved useful in achieving a prolonged action, include the use of esters, such as cypionate or valerate, and of formulations involving sterile vegetable oils. These manipulations slow absorption and destruction of the hormones; they also lessen the side effects of nausea and vomiting.

Implantation of the estrogens or their esters provides an even longer duration of action than do preparations administered intramuscularly. Suppositories containing estrogenic substances provide local treatment of changes in the vagina or vulva, or treatment of gonorrheal vaginitis in female children, with a minimum of systemic effect.

The natural estrogens exhibit carcinogenic properties upon prolonged administration to animal strains having hereditary susceptibility to mammary cancer. On this basis, some feel that use of estrogens should be contraindicated in women who have a personal or family history of mammary or genital cancer.

Estradiol. Estradiol or estra-1,3,5(10)-triene-3,17β-diol is used orally, injected intramuscularly, and implanted subcutaneously. The usual dose, orally, is 1 to 2 mg daily; implantation, 25 mg, as necessary.



Estradiol

The usual intramuscular maintenance doses of the estradiol esters are 1 to 5 mg every 1.5 to 2 months for the cypionate (Depo-Estradiol®) and 10 to 20 mg every 4 weeks for the valerate (Delestrogen®).

Estrone. Estrone or estra-1,3,5(10)-trien-3-ol-17-one is used intramuscularly. The usual dose is 100 to 500 μg 2 to 3 times a week for menopausal symptoms.

PRESCRIPTION PRODUCT. Theelin®.

The designation **conjugated estrogens** refers to a mixture of the sodium salts of the sulfate esters of the estrogenic substances that are of the type excreted by pregnant mares. This mixture of estrogenic substances must contain not less than 50% and not more than 65% of sodium estrone sulfate and not less than 20% and not more than 35% of sodium equilin sulfate. Equilin is estra-1,3,5(10),7-tetraen-3-ol-17-one and is one of the estrogens that appears in pregnant mare's urine in increasing quantities as the stage of pregnancy advances; equilin is only slightly less potent than estradiol. Conjugated estrogens may be administered orally or parenterally. The usual dose for menopausal symptoms, orally, is 625 μg to 1.25 mg, daily, cyclically, and a progestin may be added concurrently or sequentially.

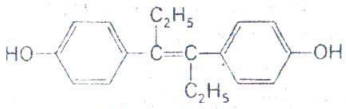
PRESCRIPTION PRODUCT. Premarin®.

The designation **esterified estrogens** also refers to a mixture of the sodium salts of the sulfate esters of the estrogenic substances that are of the type excreted by pregnant mares. This mixture differs from

conjugated estrogens because it has more estrone and less equilin metabolites. It must contain not less than 75% and not more than 85% of sodium estrone sulfate and not less than 6.5% and not more than 15% of sodium equilin sulfate. It is used orally for the same purposes and in the same dosage range as are preparations of conjugated estrogens.

PRESCRIPTION PRODUCT. Menest®.

A number of stilbene derivatives, as well as various other compounds, have estrogenic activity. These synthetic substances are active orally and have been used in some instances as therapeutic substitutes for the estrogenic steroids. These stilbene derivatives are absorbed rapidly, destroyed slowly, and active for a prolonged period. However, the side effects of nausea and vomiting also tend to be enhanced. Diethylstilbestrol is probably the best known of these substances, but other useful derivatives include chlorotrianisene (Tace®) and dienestrol.



Diethylstilbestrol

Corpus Luteum—Progestin. The corpus luteum is essential to the maintenance of human pregnancy during the first half of the term. Its principal hormonal functions are:

1. Preparation of the uterine mucosa to receive the fertilized ovum.
2. Development of the maternal placenta.
3. Continuation of the development of the mammary glands in preparation for lactogenic action of anterior pituitary.
4. Suppression of ovulation for the duration of pregnancy.
5. Antagonism of the stimulating effect of estrogens on the uterine muscle to produce a relaxation of the uterus.

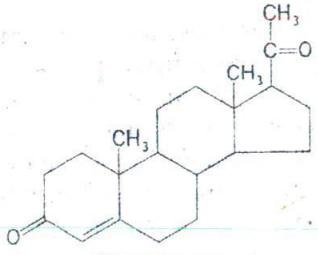
The active hormone of the corpus luteum is progesterone. It can be prepared synthetically from a number of steroidal substances. Progesterone is relatively inactive on oral administration, and it is given buccally or parenterally. This hormone is used in the treatment of amenorrhea, dysmenorrhea, endometriosis, functional uterine bleeding, premenstrual tension, and threatened or habitual abortion.

Progesterone. Progesterone is pregn-4-ene-3,20-dione. The usual dose, intramuscularly, is 50 to 100 mg for one dose only, or 5 to 10 mg a day for 6 days for functional uterine bleeding.

PRESCRIPTION PRODUCTS. Femotrone in Oil® and Progestaject®.

A number of synthetic progestins have been developed that have such advantages over progesterone as fewer side effects when administered over prolonged periods, oral efficacy, and greater potency. Such compounds as hydroxyprogesterone caproate in oil (Delalutin®), methoxyprogesterone acetate (Provera®), and norethindrone (Norlutin®) may be used as therapeutic substitutes for the natural hormone.

One of the normal physiologic functions of progesterone is to suppress ovulation during pregnancy. This hormone is not formed during the first half of a normal menstrual cycle, but administration of it or of some other progestational agent during this part of the menstrual period offers an effective means of birth control. When progestins are used as oral contraceptives, some estrogenic substance is frequently added, either by combined formulation or sequential administration, to the therapeutic approach to reduce side effects.



Progesterone

Progesterone is also available in an intrauterine device (IUD). The hormone is dissolved in silicone oil, and the flexible polymer of the IUD acts as a membrane to allow for the slow release of progesterone (65 μg daily) into the uterine cavity. The IUD contains enough progesterone to last 1 year, and the failure rate is about 2%. The failure rate of the same device without progesterone is approximately 18%. The product is called Progestasert®.

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