Steroid hormone biosynthesis

The adult adrenal cortex produces three classes of steroid hormones: glucocorticoids, mineralocorticoids, and adrenal androgens. The specialized fetal adrenal, in conjunction with the fetal liver, the placenta, and some maternal organs, produces the steroid hormones of pregnancy. Cortisol, the major glucocorticoid in humans, is rapidly synthesized and secreted in response to ACTH; this is part of a response to stress and increases circulating levels of energy-providing compounds: glucose, free fatty acids and free amino acids. Aldosterone, the major human mineralocorticoid, is synthesized and secreted in response to angiotensin II; it helps prevent ECF depletion by promoting sodium reabsorption and fluid retention. The physiological roles of adrenal androgens are poorly understood. Because the actions of these steroid hormones result from effects on gene expression, the effects generally have a slow onset and are long-lived.

The adrenal is surrounded by the capsule, a tough layer of connective tissue that protects the organ, and helps to maintain its structure and shape. Within the capsule are three layers, or zones, of cells that comprise the steroidogenic structures of the adrenal: the **zona glomerulosa**, the **zona fasciculata** and the **zona reticularis**.

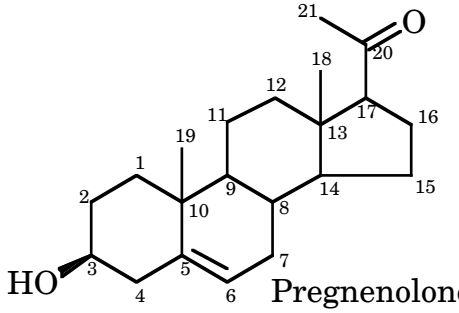
The zona glomerulosa, the outermost zone, is the site of aldosterone production. The zona fasciculata is the largest zone, and is the major site of cortisol synthesis; the zona fasciculata also produces the adrenal androgens DHEA and DHEAS. The innermost steroidogenic zone is the zona reticularis, which also produces cortisol, DHEA, and DHEAS. The steroid profiles produced in the zonæ fasciculata and reticularis are similar. It is not clear why there are two zonæ with the same products, although it has been suggested that the zona reticularis is more involved in adrenal androgen production. This is supported by the fact that the zona reticularis is poorly organized until adrenarche. The cortex surrounds the adrenal medulla, a non-steroid producing, modified neural tissue.

The blood flow in the adrenal is **centripetal** (*i.e.* from the outside toward the center). As a result each zone is exposed to increasing levels of adrenal steroids. This is particularly important in the medulla, which requires high cortisol concentration to induce one of the enzymes necessary for epinephrine biosynthesis.
The **fetal adrenal** consists of two zonæ: the relatively thin **definitive zone** (also called the neocortex) and the **fetal zone**. The definitive zone develops into the cortex after birth. The fetal zone, which is the site of DHEAS synthesis during fetal life, regresses and differentiates into the fasciculata following birth. The adrenal medulla is not present as a discrete structure during fetal life, and only forms during and after regression of the fetal zone. Note that the zona fasciculata is the middle zone of the adult; the reticularis is not fully organized until adrenarche (at age 8-12), when adrenal androgen production increases markedly. Adrenarche is also characterized by the loss of the connective tissue barrier between the cortex and medulla. The fetal adrenal is large compared to the size expected based on the size of the fetus. At term it usually contains about 8 grams of tissue (comparable in size to the adult organ), shrinking to about 5 grams a month later.

The fetal adrenal has low levels of 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase; this results in DHEA and DHEAS being the major products. The other steroidogenic enzymes appear to be present, although probably largely in the definitive zone, and the fetal adrenal does produce some cortisol and aldosterone, either from cholesterol or from maternal progesterone.

The major synthetic pathways for the adrenal steroid hormones are shown on the next page. The chart begins with cholesterol and goes to the adrenal products aldosterone, cortisol, and DHEA(S), and to the non-adrenal steroid hormones progesterone, testosterone, and estradiol. The steroids within the grey box are not produced by the adrenal; these steroids are either produced from cholesterol in gonadal tissues or are produced from circulating DHEA and androstenedione in peripheral tissues. Note that while progesterone is synthesized in the adrenal, adrenal progesterone is generally not released in significant quantities; instead it merely serves as a precursor for some of the adrenal hormones. In normal individuals, hormonally relevant levels of progesterone are produced only by the ovary or by the placenta.

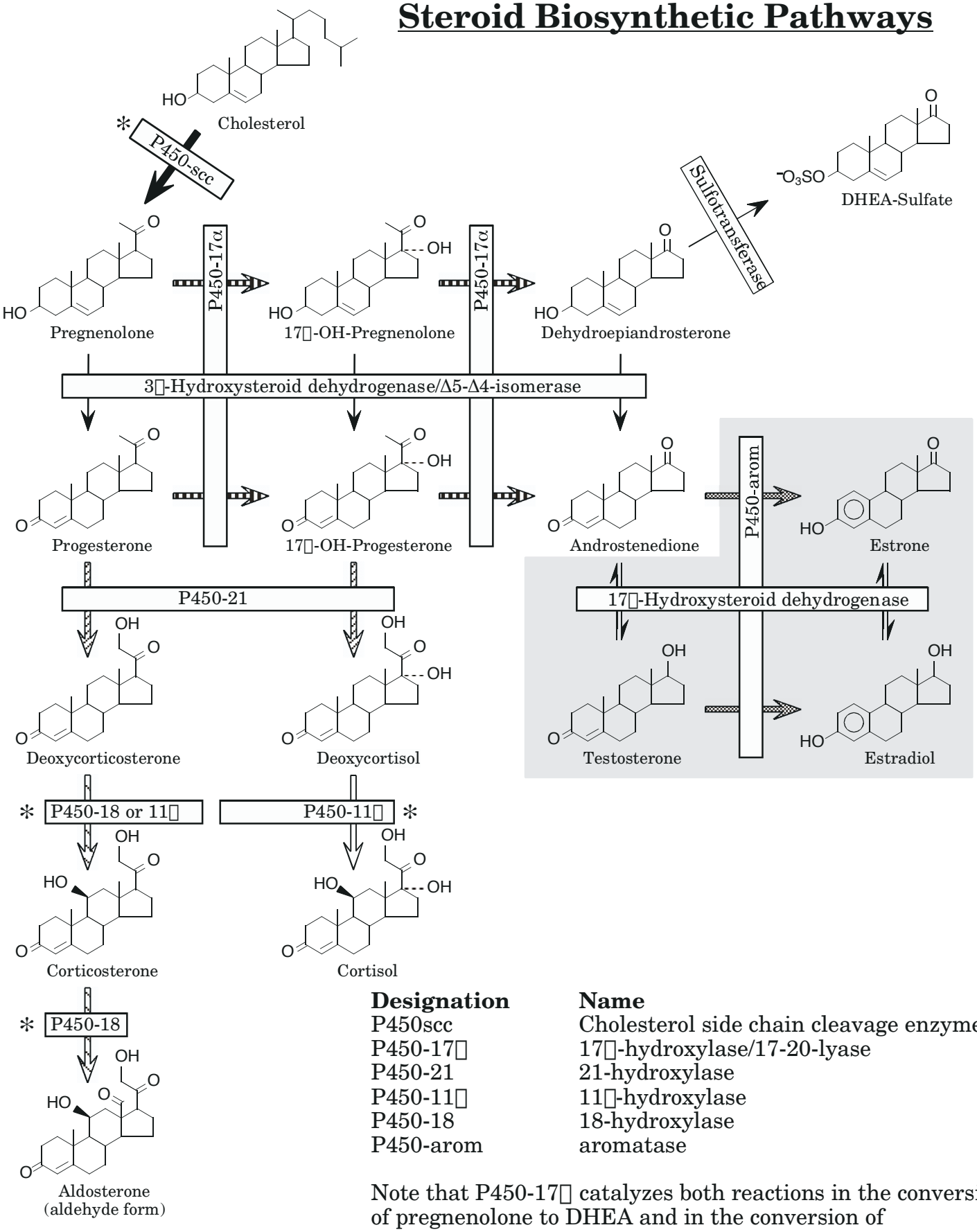
The chart appears complex but is logically organized. In each case the difference between one row and the next lower row is the reaction catalyzed by a single enzyme. Each of the reactions linked by a box is catalyzed by the same enzyme. Thus, the **first row** (with the steroids pregnenolone, 17β-hydroxy-pregnenolone, and dehydroepiandrosterone (DHEA) contains **3β-hydroxy-Δ5 steroids**, where Δ5 refers to the presence of a double bond between the 5 and 6 postions. The **second row** (progesterone, 17β-hydroxy-progesterone, and androstenedione) contains **3-keto-Δ4 steroids**. All are produced from first row steroids by the enzyme 3β-hydroxysteroid dehydrogenase/Δ5-Δ4-isomerase. The **third row** contains **21-hydroxysteroids** (deoxycorticosterone and deoxycortisol). These are produced from the corresponding second row steroids by the enzyme 21-hydroxylase. The **fourth row** contains **11β-hydroxysteroids** (corticosterone and cortisol), products of the enzyme 11β-hydroxylase (cortisol, and some corticosterone), or 18-hydroxylase (corticosterone); in the case of corticosterone production, the same reaction can be catalyzed by two different enzymes. The **fifth row** contains a single steroid, aldosterone, the final product of the 18-hydroxylase.



Pregnenolone

The columns are similarly organized; the **first column contains 21-carbon steroids**, **the second column contains 21-carbon, 17β-hydroxysteroids**, and the **third column contains 19-carbon steroids**. Note that P450-17β catalyzes two separate reactions: 17β-hydroxylation of pregnenolone and progesterone, and the cleavage of the short side chain to produce the 19-carbon steroids. The enzyme can release the 21-carbon 17β-hydroxysteroids, allowing the production of (eventually) cortisol, or can catalyze the second step, and release the 19-carbon final product.

Steroid Biosynthetic Pathways



Designation	Name
P450scc	Cholesterol side chain cleavage enzyme
P450-17α	17α-hydroxylase/17-20-lyase
P450-21	21-hydroxylase
P450-11β	11β-hydroxylase
P450-18	18-hydroxylase
P450-arom	aromatase

Note that P450-17α catalyzes both reactions in the conversion of pregnenolone to DHEA and in the conversion of progesterone to androstenedione.

* -- Denotes mitochondrial enzyme

The thick arrows in the steroid biosynthesis pathway chart are cytochrome P450 enzymes. (Note that these all have two names, which are given in the table on the chart.) These enzymes are members of a large superfamily of heme-containing proteins that catalyze mixed function oxidation reactions, using molecular oxygen and electrons from NADPH to modify the substrate. The mechanisms of these enzymes are very complex, but allow an attack on an unsubstituted carbon atom, a process that is chemically very difficult. Most cytochrome P450 enzymes catalyze the insertion of an oxygen atom into a carbon-hydrogen bond, to yield a hydroxyl group, or the addition of an oxygen across a carbon-carbon double bond, to yield an epoxide.

$$\text{R-H} + O_2 + \text{NADPH} \xrightarrow{\text{cytochrome P450}} \text{R-OH} + H_2O + \text{NADP}^+$$

In some cases the process ends there, while for other enzymes the reaction process continues with further steps, such as the cleavage of carbon-carbon bonds.

Three of the enzymes, all cytochromes P450 (P450scc, P450-11β, P450-18) are located within the mitochondria; all of the other steroidogenic enzymes are found in the smooth endoplasmic reticulum.

The first enzyme in the pathway is cytochrome P450scc, also known as cholesterol side chain cleavage enzyme. **P450scc catalyzes the first, slowest, and therefore, rate limiting step for hormone synthesis**. As you might expect from the observation that P450scc catalyzes the rate-limiting step, P450scc is also the major site of physiological regulation. In each steroid synthetic tissue, the control hormone for that endocrine gland increases the activity of P450scc.

Pregnenolone, the product of the P450scc reaction, is converted to the final hormone product by sequential steps along the pathway depending on the enzymes that are present in that tissue. Thus, the zona glomerulosa makes aldosterone, because it contains 3β-HSD, P450-21, and P450-18, and lacks P450-17α. Gonadal tissues lack P450-21 and therefore can only make the sex steroids progesterone, testosterone and estradiol.

P450-18 is closely related (about 95% sequence identity) to P450-11β, and has both 11β-hydroxylase activity and the ability to catalyze the additional reactions that produce aldosterone. The majority of human corticosterone is produced by the action of 11β-hydroxylase on deoxycorticosterone present in the zona fasciculata; normally, 18-hydroxylase carries out all of the reactions required to convert deoxycorticosterone to aldosterone without releasing the intermediates.

This chart is important in understanding the normal biosynthetic processes involved in steroid production. In cases of genetic enzyme deficiencies, however, the patient will make products determined by enzymes that are still present. For example, a patient lacking P450-21 can only make androgens. A patient lacking P450-17α has an entire adrenal that acts like the zona glomerulosa, producing large amounts of deoxycorticosterone, corticosterone, and aldosterone, but nothing else.

Congenital Adrenal Hyperplasia (CAH) results from an inherited partial or total deficiency in any one of five enzymes (P450scc, P450-21, P450-11β, P450-17β, or 3β-HSD). The deficiency causes a decreased ability to synthesize cortisol, and leads (as a result of continual stimulation by ACTH) to hyperplasia of the adrenal and (usually) to elevated levels of other adrenal steroid products. (One additional (rare) cause of CAH, usually termed Congenital Adrenal Lipoid Hyperplasia, is due to a defect in the mitochondrial cholesterol transport pathway.)

The most common form of CAH is due to defects in the gene for P450-21 (more than 90% of cases). P450-21 has a functional gene and a 98% identical pseudogene, and both are located in the HLA locus on chromosome 6. Splicing errors, gene deletions, gene conversions, and unequal crossing-over between the gene and the pseudogene account for many new cases of 21-hydroxylase deficiency. Inactive P450-21 results in lack of aldosterone as well as cortisol deficiency; partial loss of activity (about 100-times more common) usually yields low to normal cortisol but *elevated* aldosterone levels (as little as 1% of normal levels of P450-21 is enough to produce sufficient aldosterone); both forms present with elevated androgen levels.

Due to the fact that many of the possible mutations result in reduced rather than abolished 21-hydroxylase activity, and to the fact that there is significant variation between individuals in the ability to 21-hydroxylate adrenal steroids in peripheral tissues, the severity of 21-hydroxylase deficiency is quite variable. Patients may present with mineralocorticoid levels ranging from low to high, and cortisol levels ranging from zero to within the normal range. The most common form of the disorder is “non-classical” 21-hydroxylase deficiency, in which symptoms may not be apparent until early puberty or even adulthood. In general, these patients have sufficient 21-hydroxylase activity to produce both cortisol and aldosterone early in life; however, alterations in steroid synthesis patterns during early puberty often result in insufficient capacity to produce cortisol, and therefore in excessive androgen production. Individuals with non-classical 21-hydroxylase deficiency may have transient (life-threatening) salt-wasting episodes. Non-classical deficiency in 21-hydroxylase is one of the most common genetic disorders, with an incidence of ~1% in the general population. Approximately 15-20% of the general population (with higher incidence in some ethnic groups, such as Askenazi Jews) are carriers of at least mild defects in 21-hydroxylase activity.

P450-11β deficiency (~4% of total cases of CAH) generally presents with hypertension (either due to elevated DOC levels or to elevated levels of other steroids with mineralocorticoid activity). Since a separate enzyme (P450-18) catalyzes the 11β-hydroxylation of DOC, most cases of P450-11β deficiency only require glucocorticoid supplementation. However, the zona glomerulosa may not recover immediately from suppression, and therefore patients must be monitored to prevent salt-wasting until the zona glomerulosa function is restored.

In complete 3β-HSD deficiency, only some pathway intermediates and (large amounts of) DHEA are produced by the adrenal. Most of these patients are able to make at least some sex steroids due to the activity of the Type I gene in peripheral tissues. While in males 3β-HBD deficiency often results in ambiguous genitalia at birth due to marked decrease in testicular androgen synthesis, in females the

peripheral production of androgens may be sufficient to cause androgenital syndrome.

All forms of CAH are life-threatening disorders, and must be recognized and treated. Untreated complete 21-hydroxylase or 3□-HSD deficiencies are rapidly fatal within two weeks after birth due to lack of mineralocorticoids. While 21-hydroxylase deficiency in females is generally diagnosed (due to the associated androgenital syndrome), in males it may be missed or misdiagnosed (since the elevated androgens have little additional phenotypic effect in male infants and the hypoaldosteronism does not result in symptoms until several days postpartum), often with lethal consequences.

P450-21 and P450-11□ (and many cases of 3□-HSD) deficiencies usually lead to **androgenital syndrome**. Androgenital syndrome is a consequence of the extremely high levels of adrenal androgens produced in response to overstimulation of the adrenal by ACTH; in males it leads to early puberty, and in females to ambiguous genitalia at birth, and to virilization and hirsuitism later in life. There have been some (somewhat controversial) attempts to begin treatment *in utero* (beginning in first trimester) to prevent the formation of ambiguous genitalia in female infants, especially in cases of P450-21 deficiency.

