Biosynthesis of steroid hormones
Synthesis and hydroxylation of calciols

Biochemistry II
Lecture 2

2008 (J.S.)

The major sites of steroid hormone biosynthesis

The **adrenal cortex**

- zona fascicularis and reticularis
  - minor mineralocorticoids
  - **glucocorticoids**
  - **adrenal androgens**
- zona glomerulosa
  - **aldosterone**

The **testes**

- the Leydig cells — **testosterone**

The **ovaries**

- the granulosa cells and the developing follicle — **estrogens**
- corpus luteum — **progesterone**

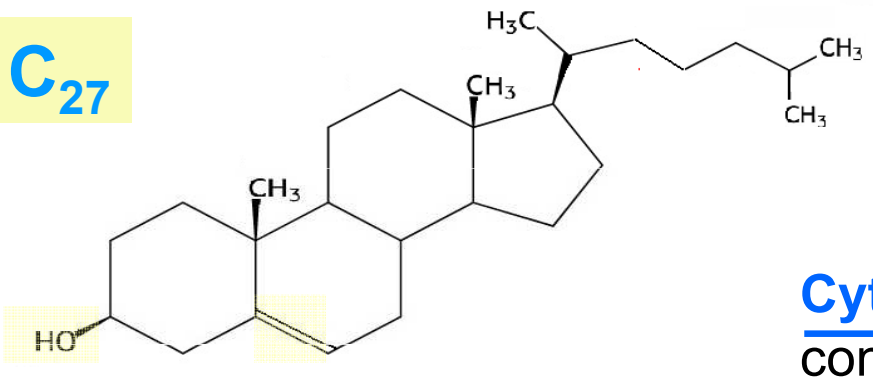
The **placenta**

progesterone (from maternal cholesterol)
estrogens (from both maternal and fetal adrenal androgens)

Cholesterol

(**cholest-5-ene-3β-ol**) is the precursor for all steroid hormone synthesis.

C_{27}



Cytochrome P450 monooxygenases control the steroidogenesis:

The rate limiting step of the biosynthetic pathway that generates C_{21} steroids is catalysed by **P450scc** (side-chain cleaving cholesterol 20,22-desmolase).

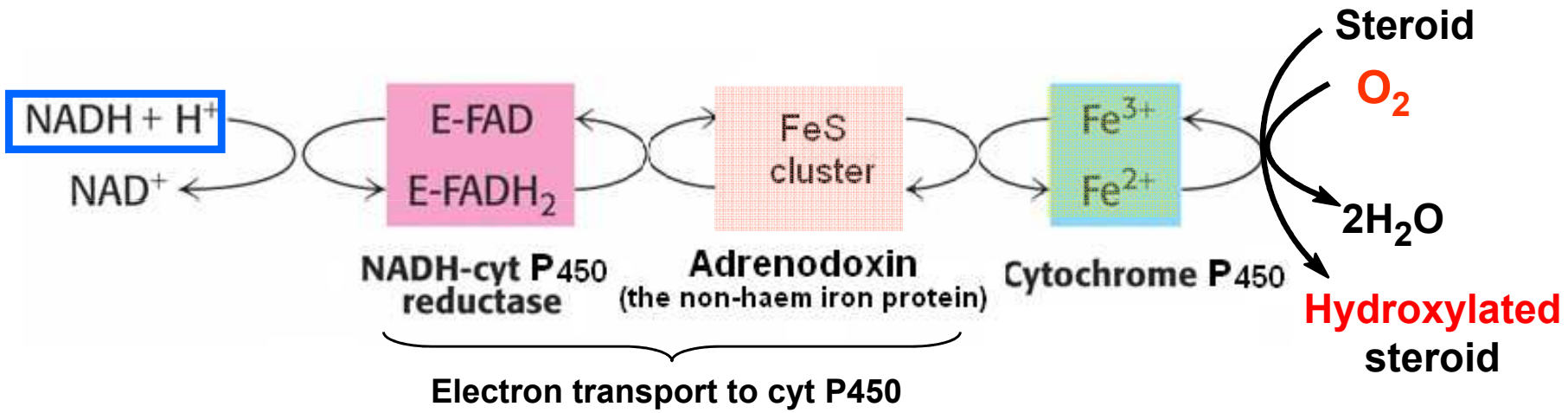
(The dehydrogenation / isomerization is catalysed by **3β-hydroxysteroid dehydrogenase**.)

Three sequential hydroxylations at C-21, C-17α, and C-11β are catalysed by **P450c21, P450c17, P450c11**.

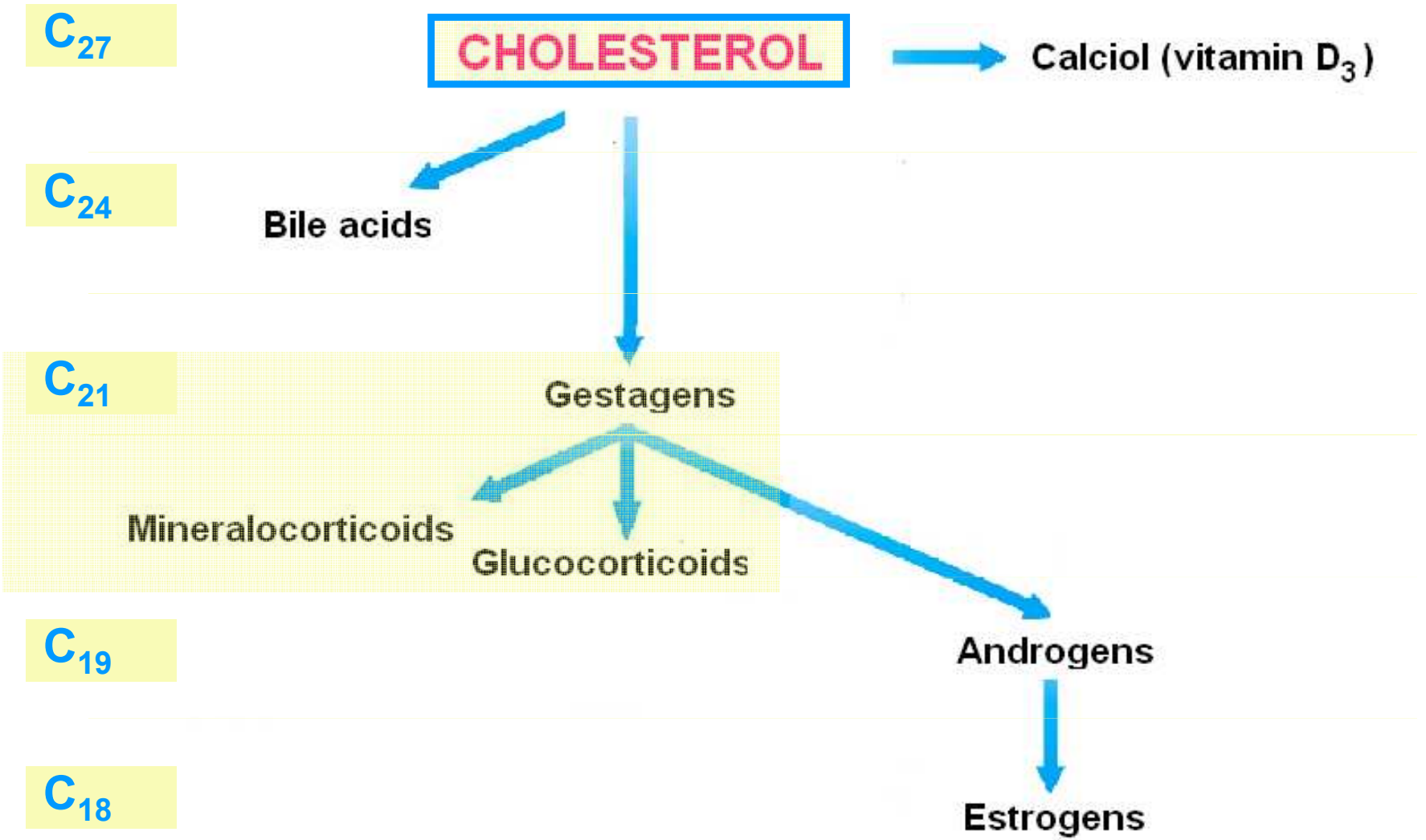
Unique is the hydroxylation / dehydrogenation at C-18catalysed by **P450 aldosterone synthase**.

The splitting at C-17 that generates C_{19} steroids is catalysed by **P450c17 17-20 lyase / desmolase**.

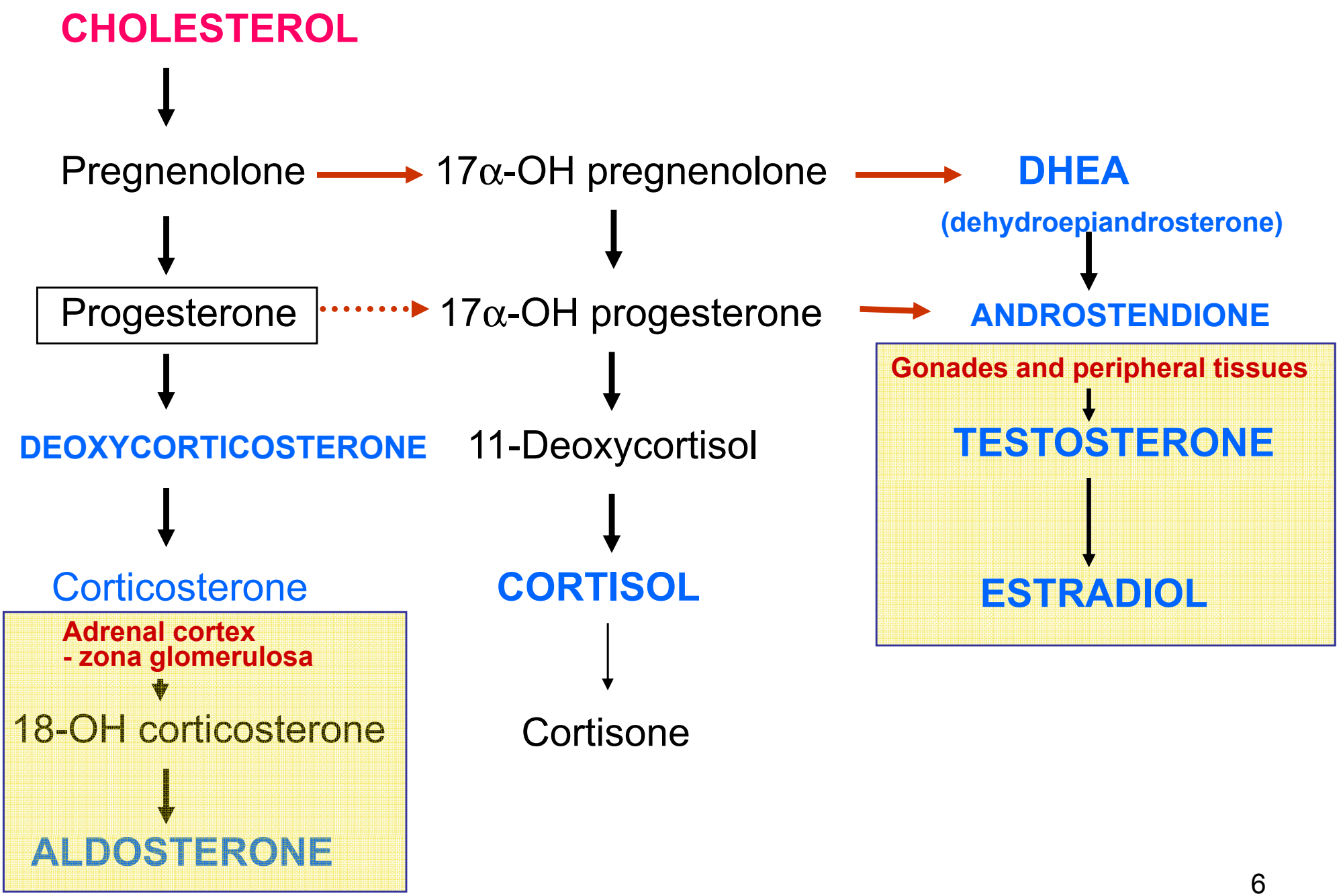
Steroid hormones are hydroxylated by the cytochrome P450 systems located partly in membranes of endoplasmic reticulum (P450c17, P450c21, P450aro), partly **in mitochondria** (P450scc, P450c11β, P450AS). These systems differs from the hydroxylating cyt P450 systems in membranes of endoplasmic reticulum in the liver – the reduction of mitochondrial cyt P450 involves an additional component, the iron-sulphur protein **adrenodoxin** between the reductase and the cytochrome.



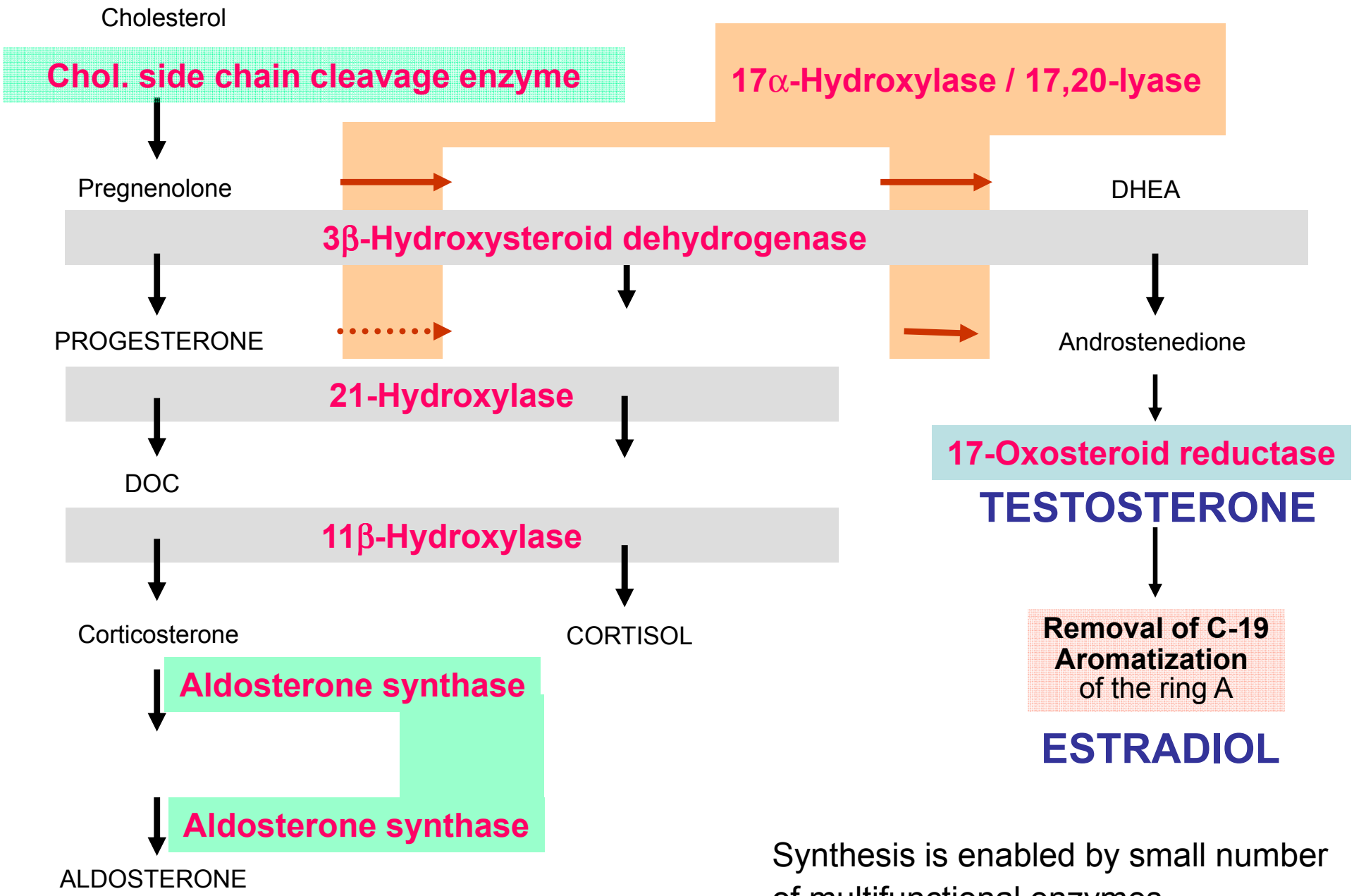
Cholesterol is the precursor for other important steroids:



Steroidogenesis



Enzymes catalysing steroid synthesis:



Synthesis is enabled by small number of multifunctional enzymes.

Corticoids

are steroid hormones secreted from the **adrenal cortex**. The principal types of adrenal steroid hormones are the glucocorticoids and mineralocorticoids.

Glucocorticoids (such as **cortisol**)

- promote gluconeogenesis and the formation of glycogen,
- enhance the degradation of proteins and fat, and
- inhibit the inflammatory response.

They enable animals to respond to stress.

Mineralocorticoids (primarily **aldosterone**)

- act on the kidney to increase the reabsorption of Na^+ and the excretion of K^+, which leads to an increase in blood volume and blood pressure.

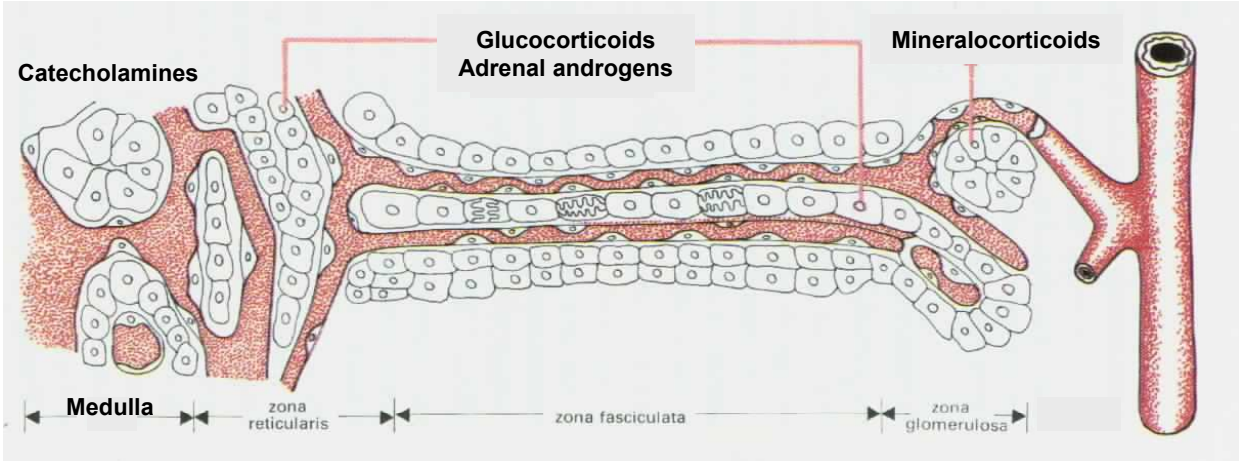
They are effective in keeping the water and mineral balance.

Adrenal androgens - **dehydroepiandrosterone** and **androstenedione** - are secreted from adrenal cortex, too.

In adrenal cortex, there are three distinct cellular zones differing in the enzymatic equipment. The three zones form **two functional units** that are controlled independently.

The cells of the outer layer – **zona glomerulosa** – do not express 17α-hydroxylase so that they do not produce the precursors of glucocorticoids and adrenal androgens. On the other hand, the gene for aldosterone synthase is expressed only in that zone – the unique **site of aldosterone production**. The synthesis and secretion of aldosterone is **controlled by renin-angiotensin** systém; the influence of adrenocorticotropin (ACTH) is very weak and transient.

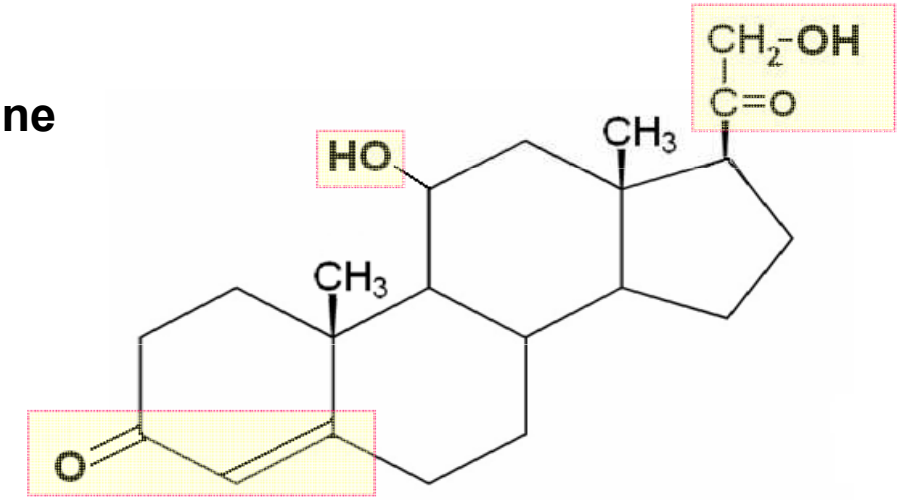
Both inner zones of adrenal cortex – **zona fasciculata and reticularis** – produce **glucocorticoids and androgens**, the production of less effective mineralocorticoids deoxycorticosterone (DOC) and corticosterone is not very important. The synthesis and secretion is **controlled by ACTH**.



Mineralocorticoids

Corticosterone

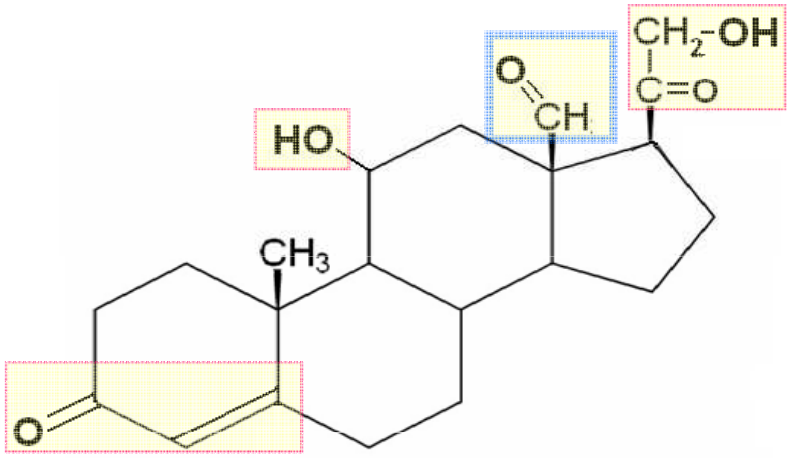
11β,21-Dihydroxypregn-4-ene-3,20-dione

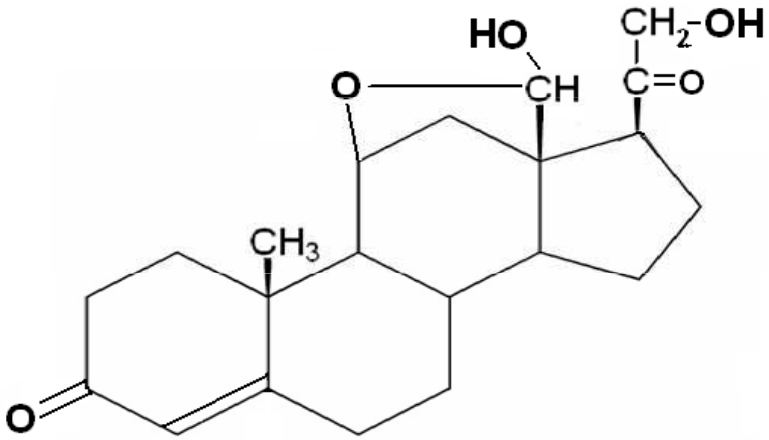


is produced also in the zona fasciculata and reticularis, but its biological effects are **much weaker** than the effect of aldosterone.

Aldosterone

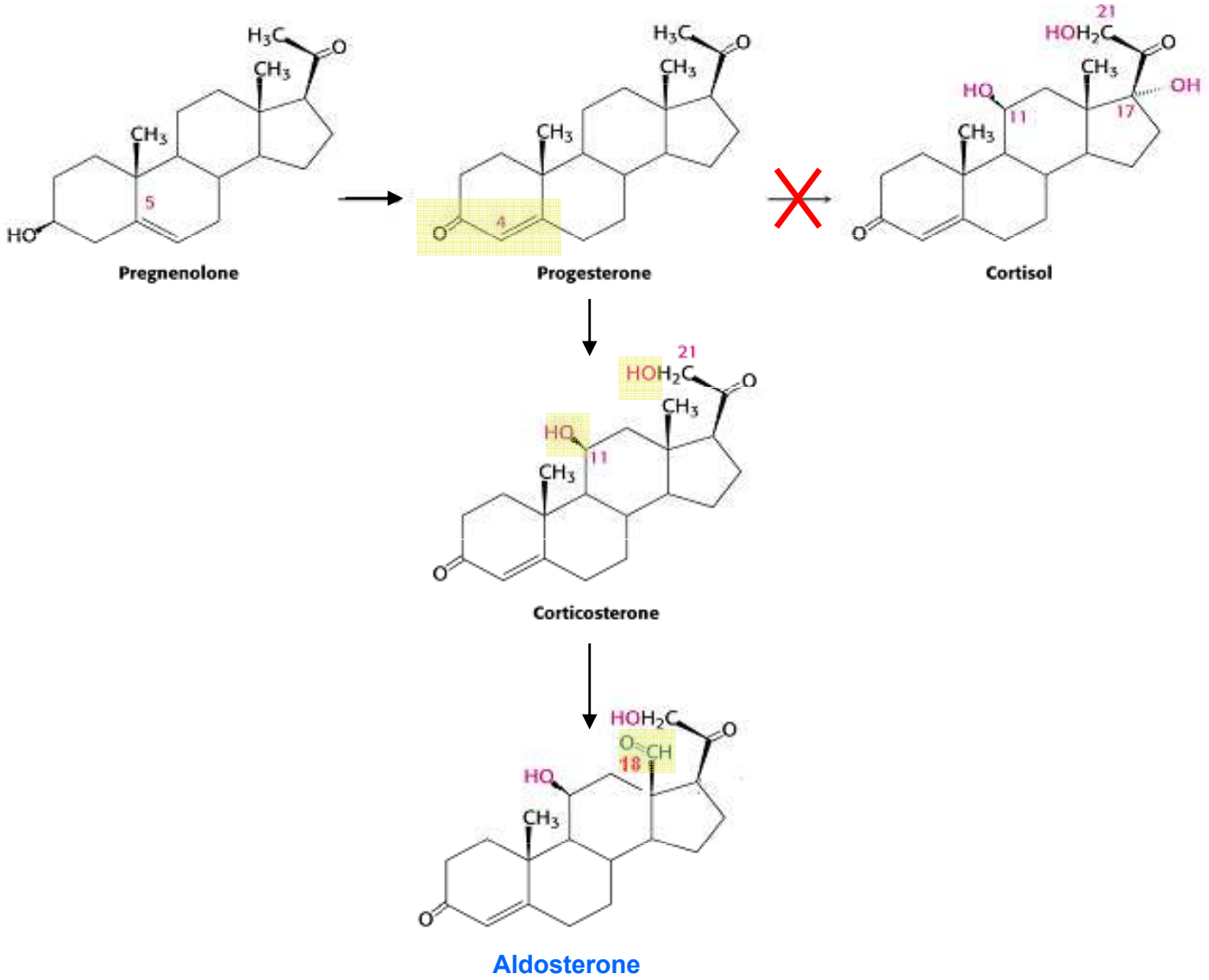
is synthesized <u>only in the zona glomerulosa</u> of adrenal cortex.





(hemiacetal form)

Synthesis of aldosterone in zona glomerulosa



Synthesis of aldosterone in zona glomerulosa of adrenal cortex

CHOLESTEROL

Chol. side chain cleavage enzyme

↓

Pregnenolone

3β-Hydroxysteroid dehydrogenase

↓

Progesterone

21-Hydroxylase

↓

DOC

11β-Hydroxylase

↓

Corticosterone

Aldosterone synthase

↓

18-OH corticosterone

Aldosterone synthase

↓

ALDOSTERONE → **REABSORPTION of Na^+, excretion of K^+**

ANGIOTENSIN II

Carboxypeptidase **ACE**

ANGIOTENSIN I

RENIN (proteinase)

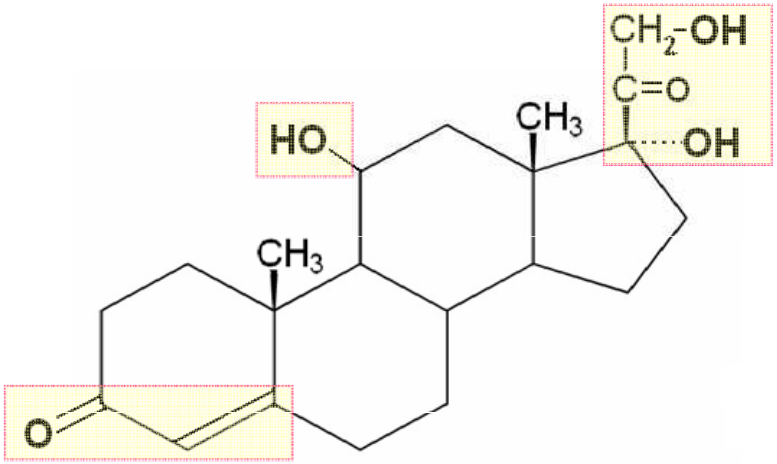
ANGIOTENSINOGEN (plasma α_2-globulin)

Secretion of the juxtaglomerular cells is stimulated by
- pressure decrease in arteriolae afferentes
- circulating catecholamines
- the decrease of $[Na^+] + [Cl^-]$ in the tubular liquid (detected by cells of macula densa)

Glucocorticoids

Cortisol (hydrocortisone)

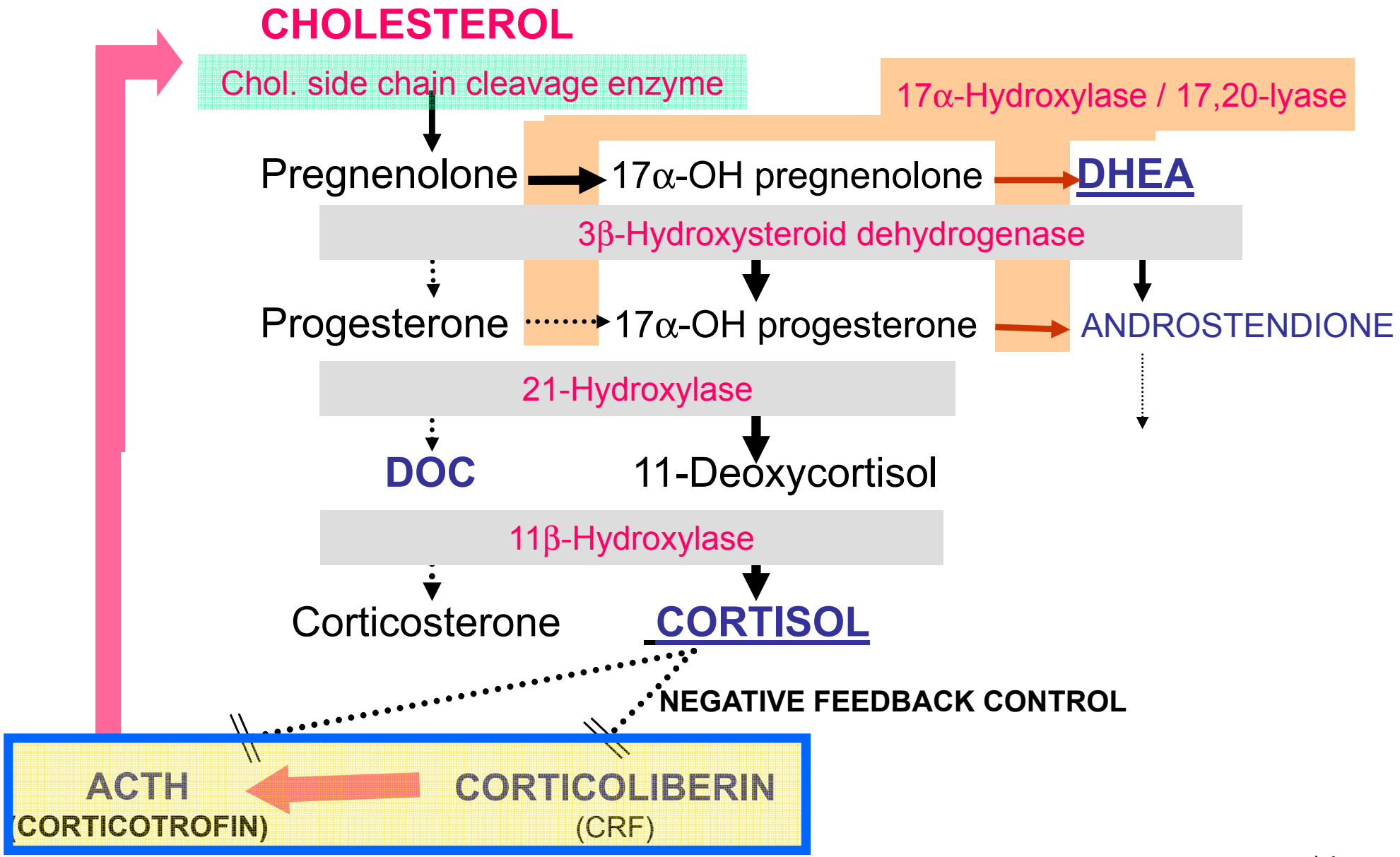
11β,17α,21-Trihydroxypregn-4-ene-3,20-dione



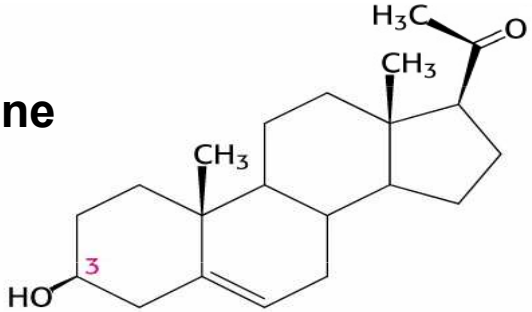
Cortisol, the major glucocorticoid, is synthesized from progesterone by hydroxylations at C-17, C-21, and C-11.

Cortisol secretion under basal (i.e., non-stressed) conditions ranges from 22 µmol/d to 70 µmol/d (8 – 25 mg/d).

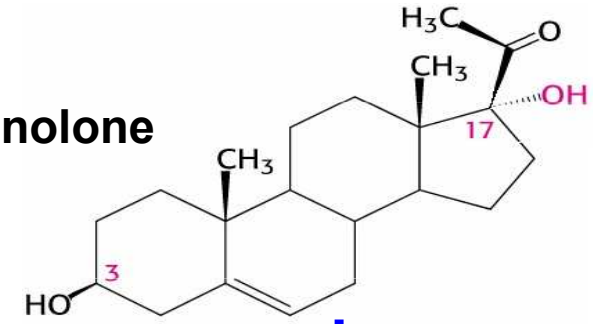
Synthesis of steroids in zona fasciculata and zona reticularis:



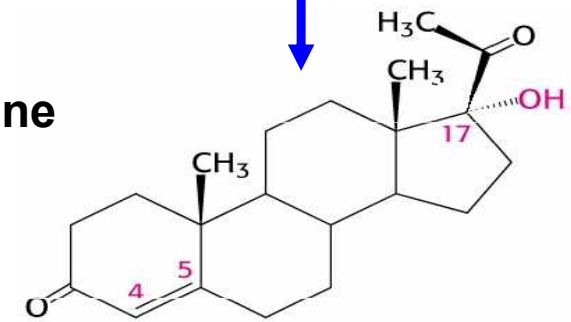
Pregnenolone



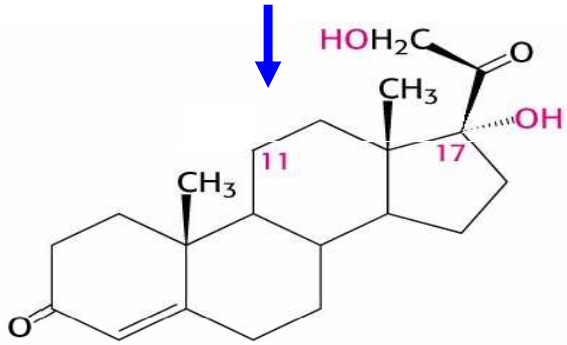
17α-Pregnenolone



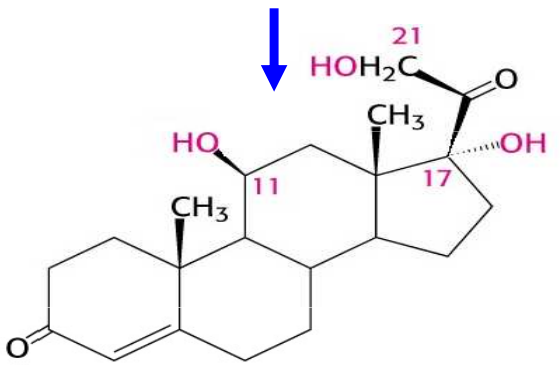
17α-Progesterone



11-Deoxycortisol

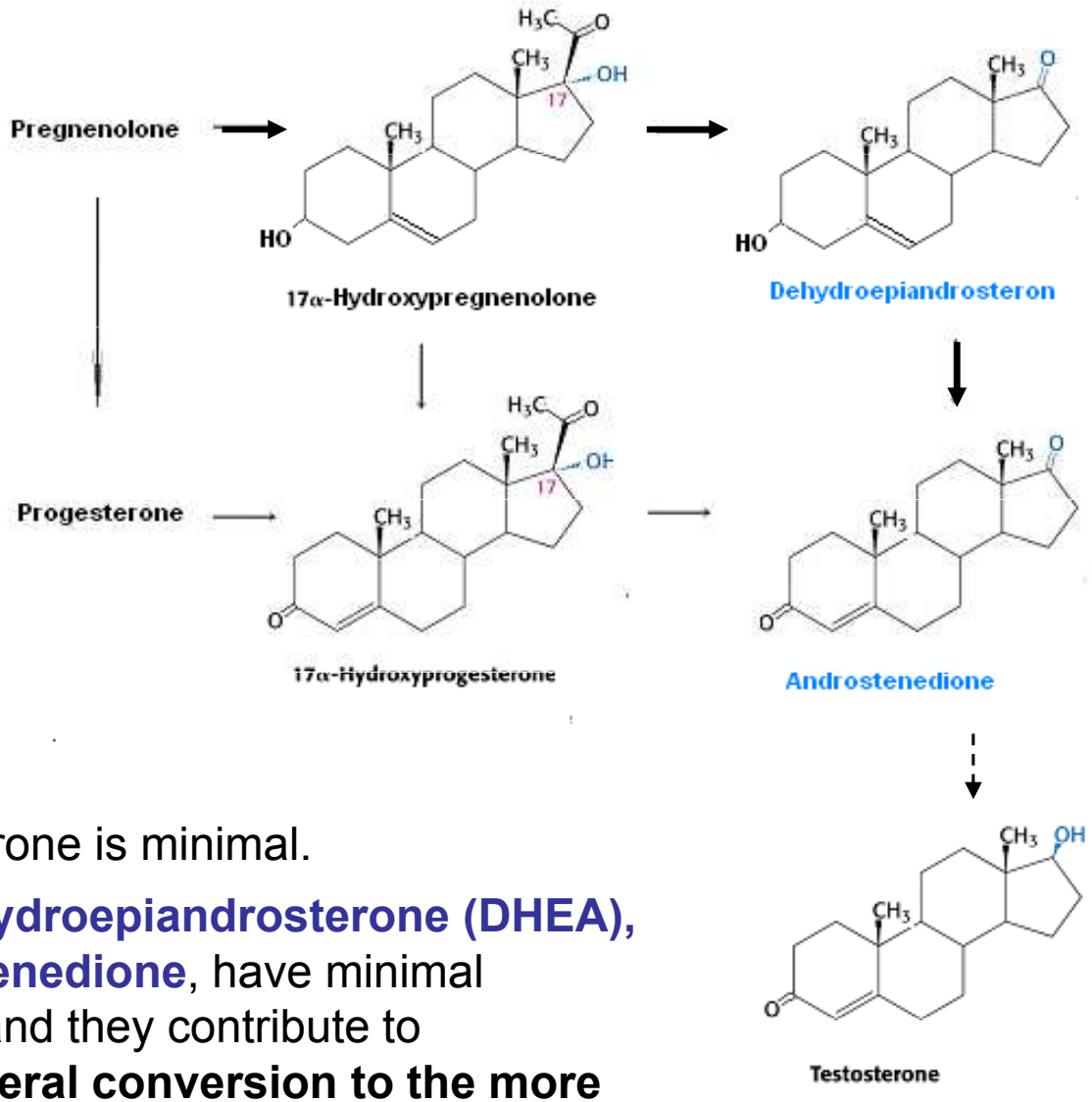


Cortisol
(Hydrocortisone)



Adrenal androgens

The production of adrenal androgens requires prior 17α-hydroxylation and thus does not occur in the zona glomerulosa.



Adrenal secretion of testosterone is minimal.

The adrenal androgens, **dehydroepiandrosterone (DHEA), DHEA sulfate, and androstenedione**, have minimal intrinsic androgenic activity, and they contribute to androgenicity by their **peripheral conversion to the more potent androgens testosterone and dihydrotestosterone**.

Gonadal steroids

In **male** reproductive function, the three steroids of primary importance are testosterone, dihydrotestosterone and estradiol.

In the **female** reproductive system, the mature ovary synthesizes and secretes estrogens, progesterone, androgens, and their precursors.

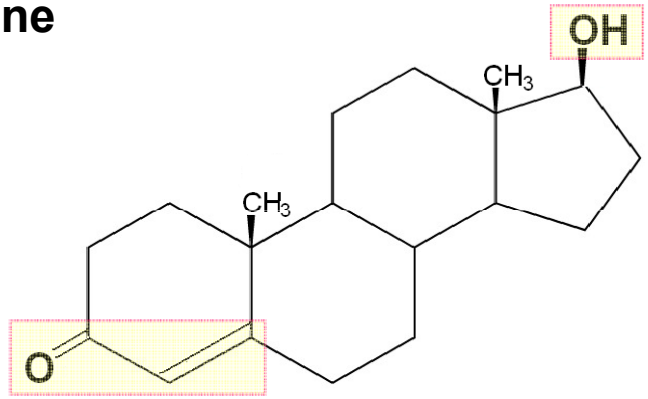
Testicular androgens

C_{19}

are responsible for the development of male secondary sex characteristics. From a quantitative standpoint, the most important androgen is testosterone secreted by the testicular Leydig cells.

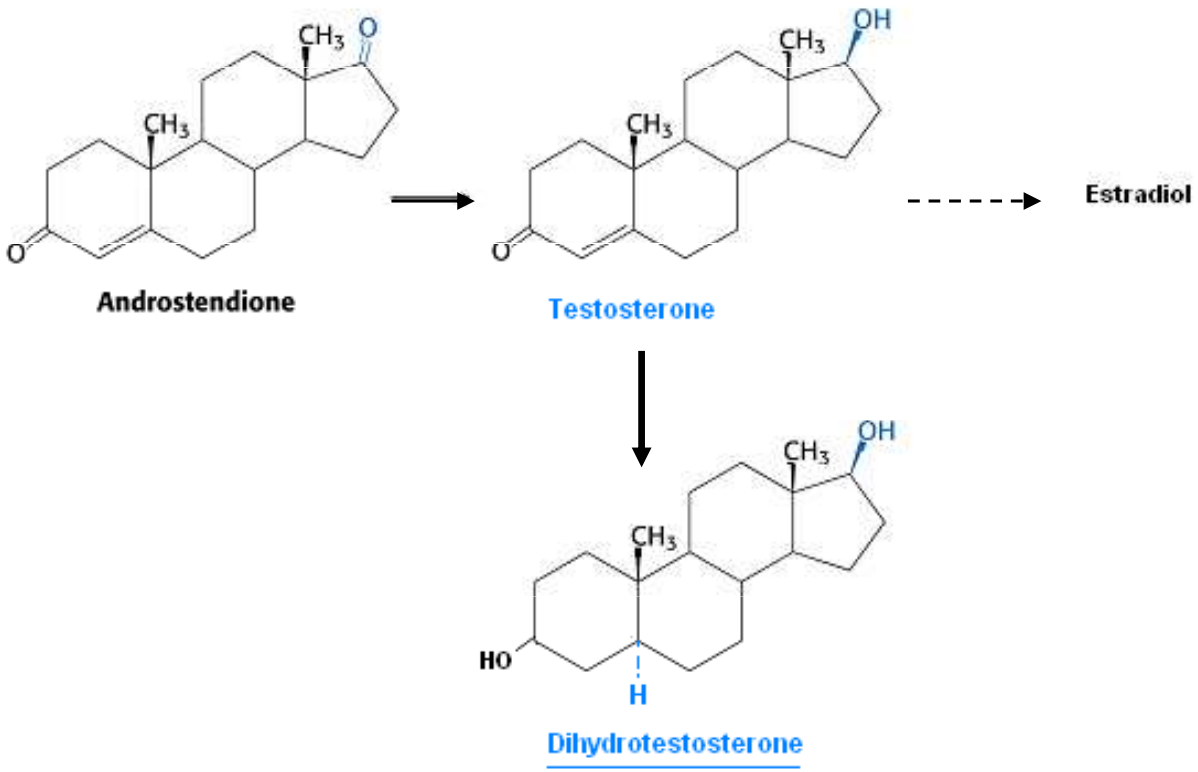
Testosterone

17β-Hydroxyandrost-4-ene-3-one



In addition to testosterone, the testes secrete small amounts of the potent androgen dihydrotestosterone, the weak androgens DHEA and androstenedione, and estradiol, progesterone, etc.

About 80 % of the circulating concentrations of dihydrotestosterone and estradiol is derived by **conversion of testosterone and its precursors in peripheral tissues** by the microsomal reductase.



Steroid hormones of the ovary

Estrogens

C_{18}

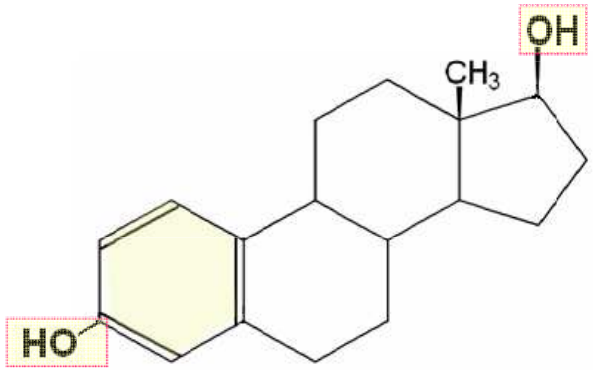
are required for the development of female secondary sex characteristics and, along with progesterone, also participate in the ovarian cyclus.

The ovary is normally the major source of estrogens, although the conversion of androgen precursors in other tissues is clinically important after the menopause (and in some women with disorders of ovarian function).

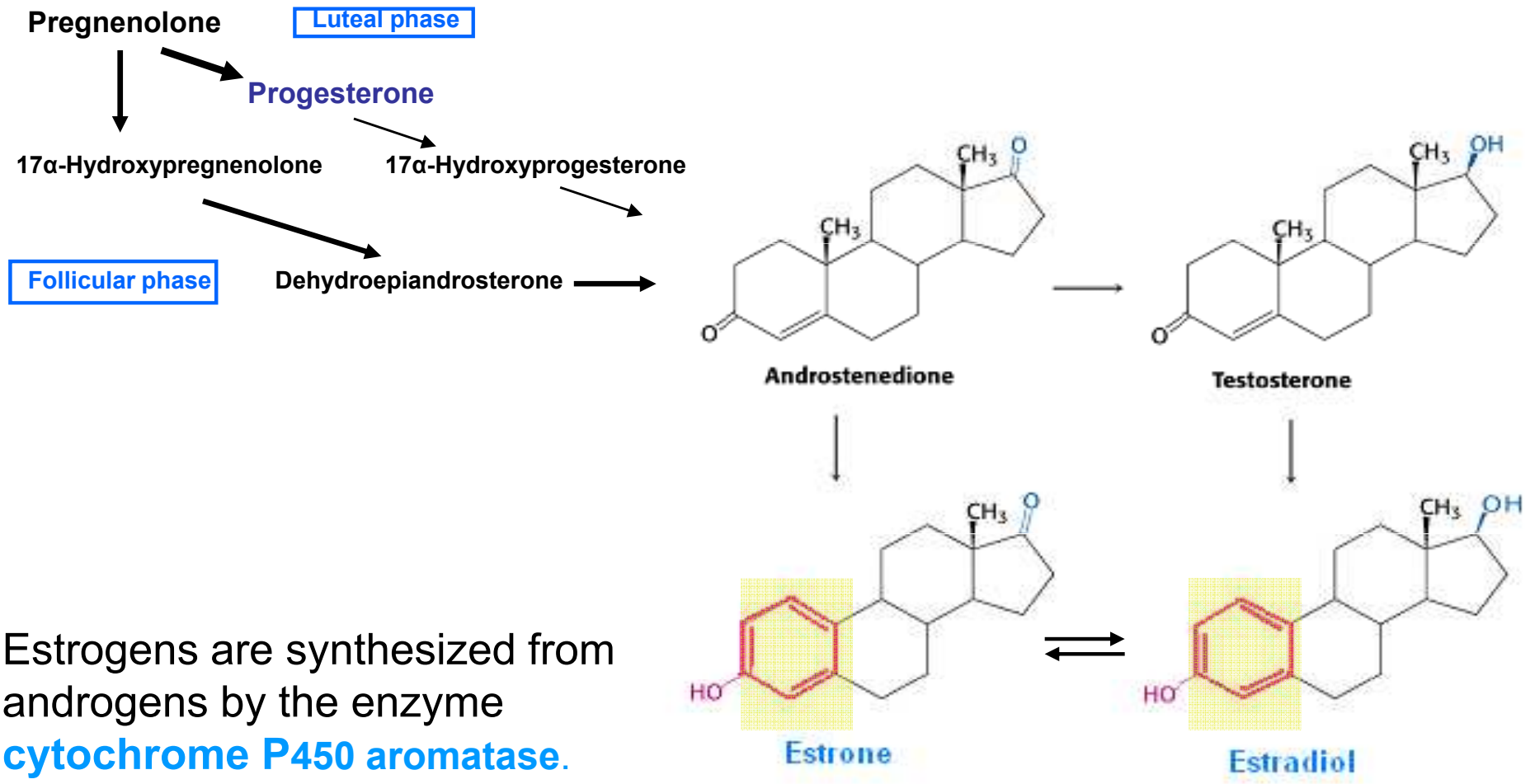
The ovary is also the source of small amounts of **testosterone and other androgens** that serve not only as precursors to estrogen synthesis but also are released into the circulation to act on peripheral tissues.

Estradiol

Estra-1,3,5(10)-triene-3,17β-diol



Synthesis of estrogens in the ovary



Estrogens are synthesized from androgens by the enzyme **cytochrome P450 aromatase**.

The process involves three steps:

- two successive hydroxylations of the methyl group at C-19 *(*the *gem*-diol then spontaneously loses water to give aldehyde), and
- a third hydroxylation at C-2 which results in a product that rapidly and nonenzymatically loses formic acid and forms an aromatic ring A.

Gestagens

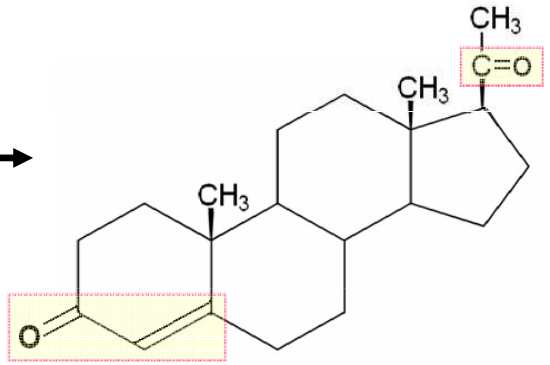
C_{21}

Progesterone (pregn-4-ene3,20-dione)

prepares the lining of the uterus for implantation of an ovum and is also essential for the maintenance of pregnancy.
The ovary produces and secretes large amounts of progesterone during the **luteal phase** of the cycle.

However, it also serves as a minor precursor for androgens and estrogens.

Cholesterol → **Pregnenolone** →



Progesterone

--→ Androgens

⇣

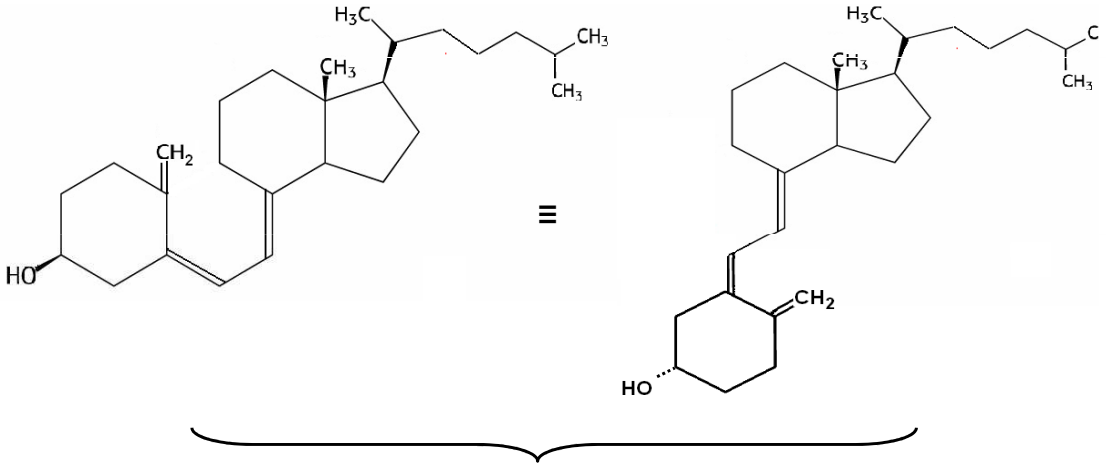
Estrogens

Progesterone is rapidly cleared from the circulation converted (by reduction) to **pregnanediol** and **conjugated** to glucuronate in the liver.

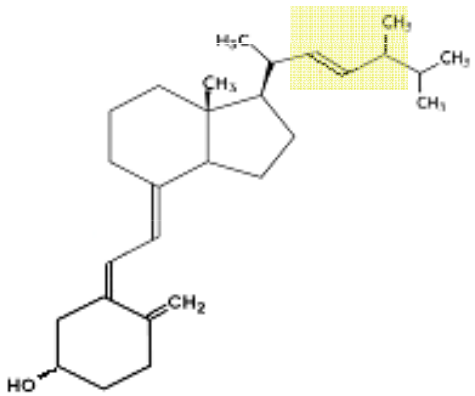
Synthesis and hydroxylation of calciols

The calciols (formerly calciferols, several forms of vitamin D) are a family of sterols that affect calcium homeostasis.

The daily requirement for calciols is 5 - 20 μg (200 – 800 IU).
The **D-provitamins**, ergosterol and 7-dehydrocholesterol, are widely distributed in the animals and plants.
Most natural foods have a low content of **vitamin D_3**. It is present in egg yolk, butter, cow's milk, beef and pork liver, animal fat and pork skin.
The most important vitamin D (D_2) source is fish oil, primarily liver oil.



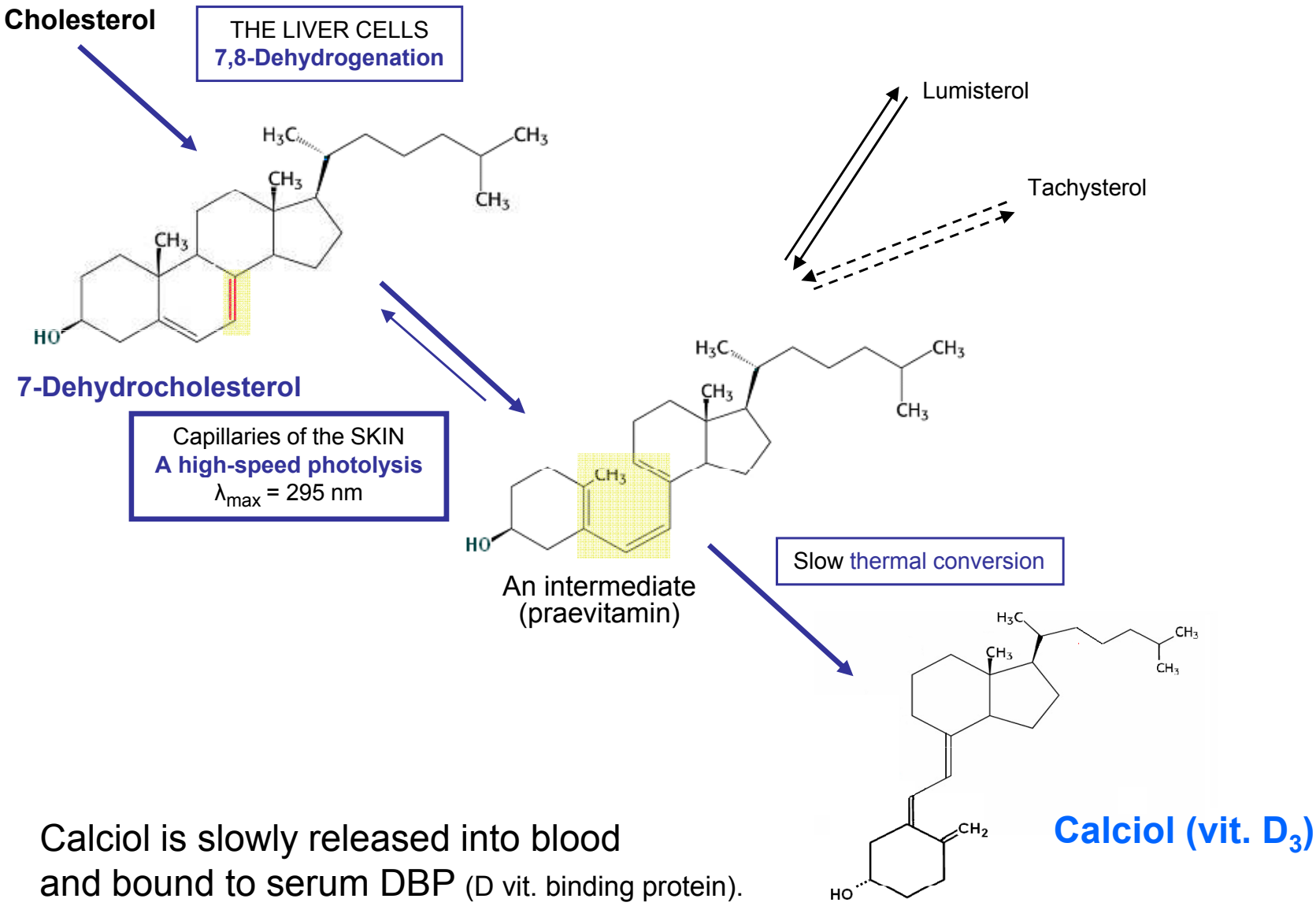
Calciol (cholecalciferol, vitamin **D_3**)



Ercalciol (ergocalciferol, vitamin **D_2**)

The calciols are 9,10-*seko*steroids, in which the ring B is opened.

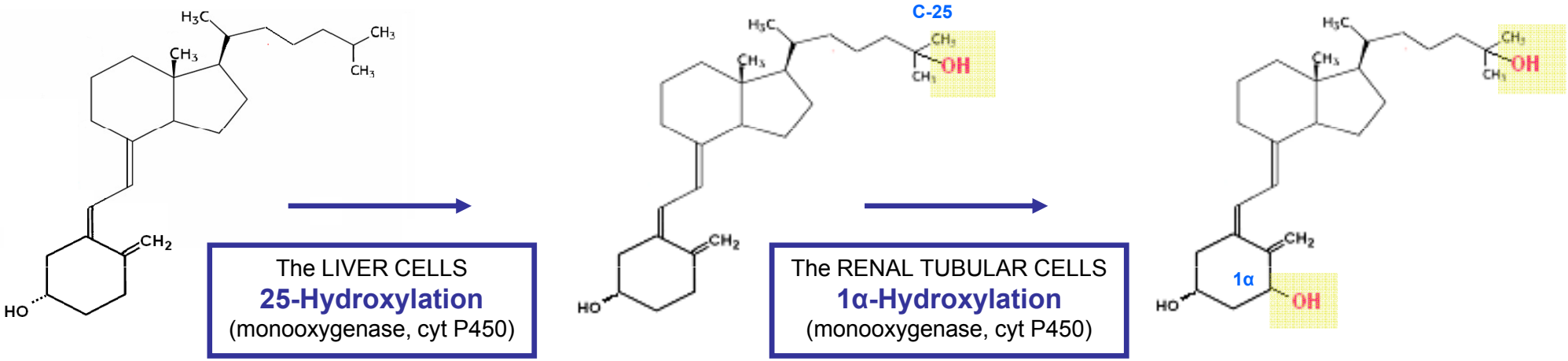
Calciol (vit. D_3) is synthesized from 7-dehydrocholesterol by photolysis that is a cause of opening of the ring B:



Calciol is slowly released into blood and bound to serum DBP (D vit. binding protein).

Calciol is an inactive precursor of **calcitriol**, the most potent biologically active form of vitamin D.

The hydroxylation of calciols



Calciol
(Cholecalciferol)

Calcidiol
(25-Hydroxycholecalciferol)

Calcitriol
(1α,25-Dihydroxycholecalciferol)
A CALCIOTROPIC STEROID HORMONE

Calcidiol is the major circulating metabolite of calciol. Its biological half-life is rather long, approx. 20 - 30 days. The concentration of calcidiol in blood plasma informs of the body calciol saturation. Seasonal variations are observed.

25-Hydroxylation of calcidiol is inhibited by the high concentrations of calcidiol and calcitriol (feedback control), calcitonin, and the high intake of calcium in the diet.

Calcitriol has a short biological half-life. 1α-Hydroxylation is stimulated by parathyrin (PTH), inhibited by calcitonin and high concentrations of calcitriol.

Biological action of calcitriol ($(1,25\text{-}(OH)_2D_3)$)

In the intestine – increased absorption of Ca^{2+} by enterocytes:

- Induction of a change in conformation of the calmodulin in cytosol – transfer of Ca^{2+} across the cytoplasmatic membrane is supported;
- Induction of the **calbindin synthesis** in cytosol (CaBP, calcium-binding protein), CaBP enables the transport of Ca^{2+} within the cell;
- Induction of the **Ca^{2+}-ATPase synthesis**, that enables the export of Ca^{2+} into the extracellular fluid.

In the bone – it **regulates both the resorption and regeneration** of bone tissue

- Induction of the **osteocalcin synthesis** (bone Gla protein, BGlaP), but the control mechanismus is not yet fully explained.