ENDOCRINE

Biochemistry

Writer: Mariam Manasrah & Hala Mousa

Corrector: Abdallah Aburoman

Doctor: نافذ

Sheet no.4

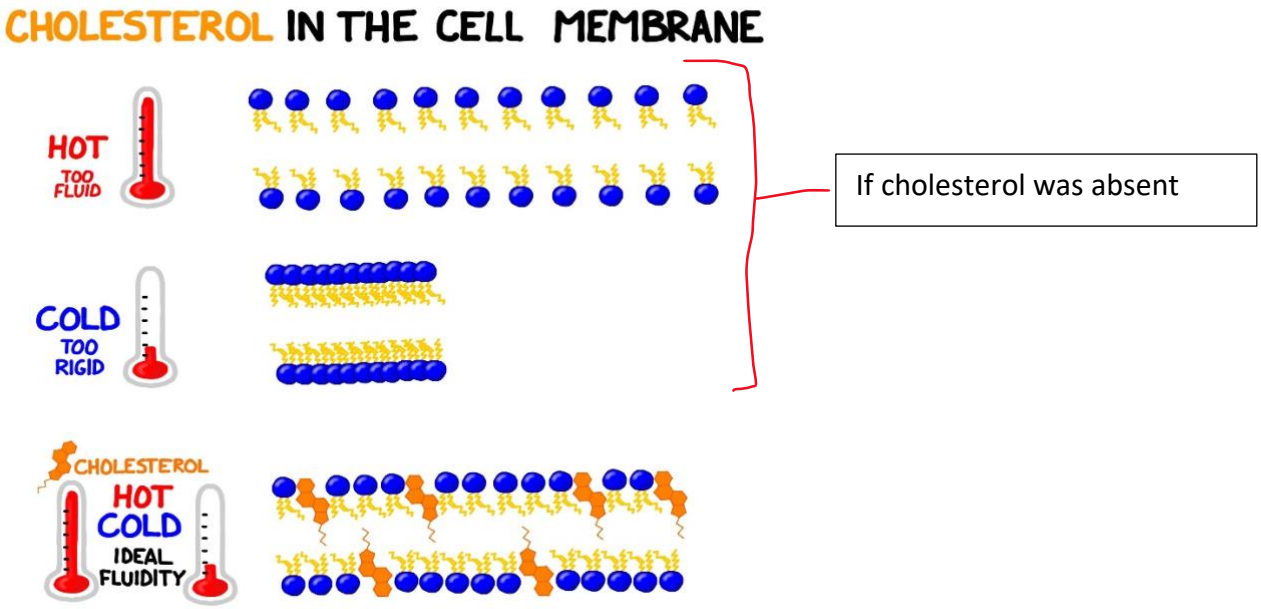
STEROIDOGENESIS

(The pathway by which some cells can manufacture steroids such as aldosterone, estradiol (a form of Estrogen) and testosterone from the parent steroid which is known as cholesterol)

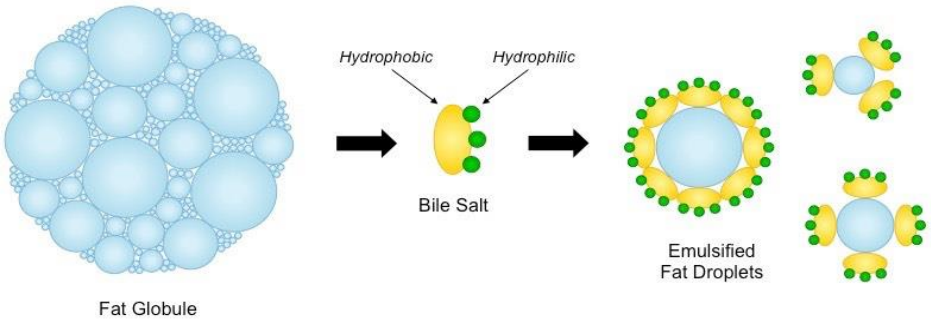
➢ FUNCTIONS OF CHOLESTEROL:

cholesterol has a variety of functions including :

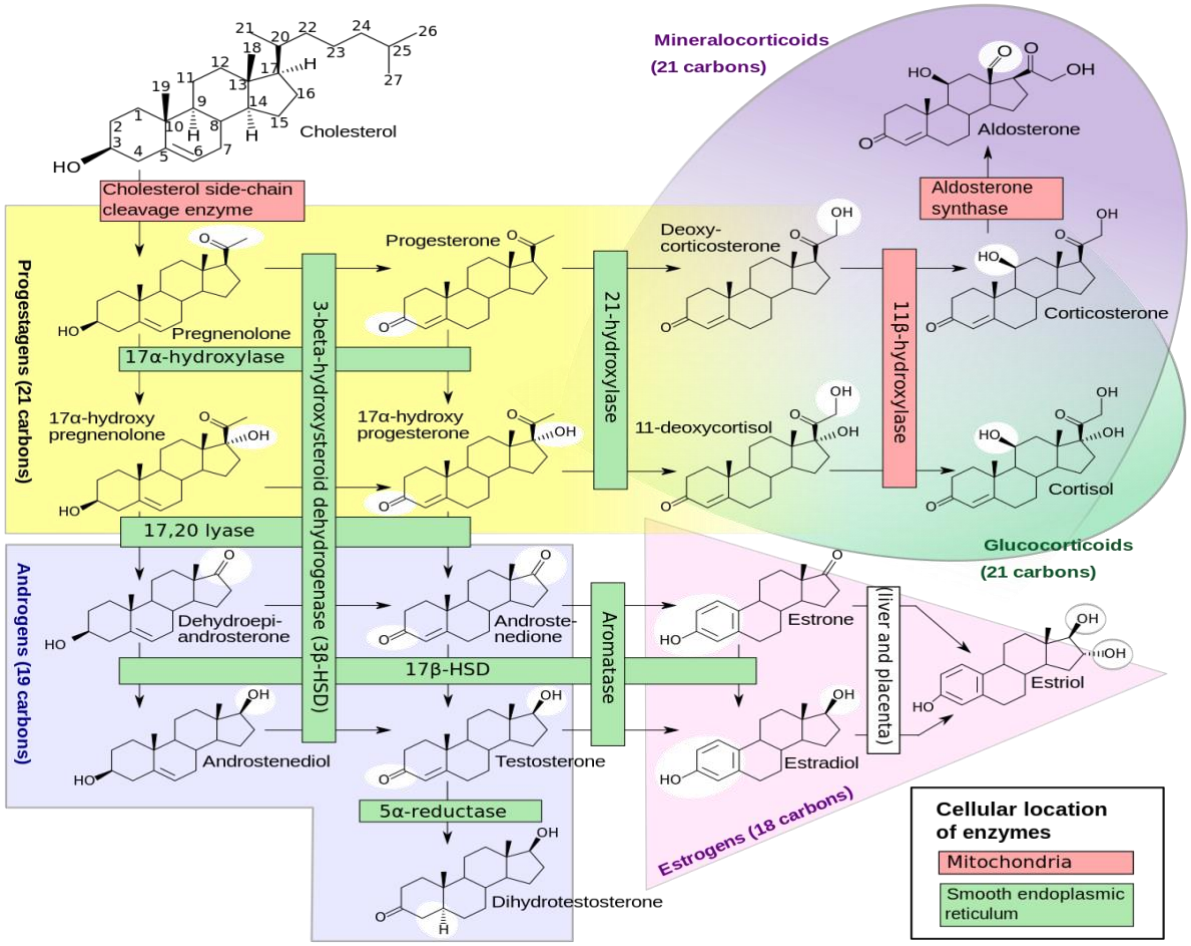
- involvement in stabilizing cell membranes over a wide range of temperatures
  external image:



- used to manufacture bile acids which are used in the emulsification of fat , external image:



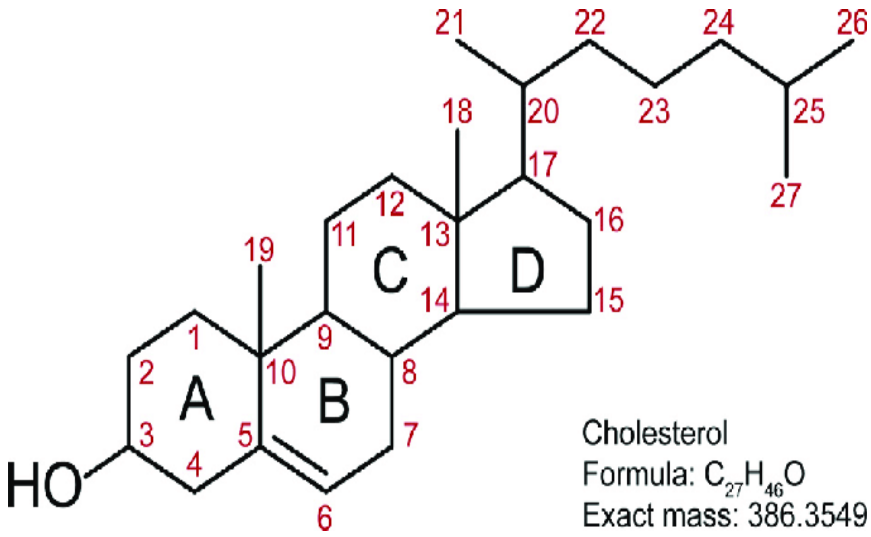
- arguably one of the most important functions of cholesterol is its conversion into steroid hormones.



this diagram is divided into the various types or classes of steroids:

- in yellow we have the progestogens
- down in blue we have the androgens these are the masculinizing hormones (induce male physiological characteristics)
- on the right in pink we have the estrogens or typically feminizing hormones
- in green the glucocorticoids or corticosteroids
- up in purple the one general mineralocorticoid which is aldosterone .

enzymes involved in this process are going to be in different cellular compartments :
the ones in red are going to be mitochondrial enzymes , other enzymes in green are going to be microsomal enzymes meaning they're embedded in the membrane of the smooth endoplasmic reticulum, **smooth not rough**!



pay attention to the numbering system of cholesterol !

some of these enzymes are going to be named based on the number of the carbon where they act

ex : 21 hydroxylase this number in front of this enzyme name (21) gives you an indication of which atom it's acting on

- **CYP11A1** = Cholesterol side-chain cleavage enzyme
- **CYP17A1** = 17α-hydroxylase/17,20-lyase
- **CYP21A2** = 21-hydroxylase
- **CYP11B2** = 11β-hydroxylase
- **CYP18B2/18-HSD** = Aldosterone Synthase
- **CYP19A1** = Aromatase

- Some steroids such as cholesterol, DHEA, and estrone can be sulfated at the 3β by PAPS-dependent **sulfotransferases**.

now some of these enzymes which are p450 enzymes are typically going to be referred to by their gene name.

for example, cholesterol sidechain cleavage enzyme is a p450 enzyme, it's encoded by the gene CYP11A1 and so typically in most textbooks you'll either see this name or the gene name

سنعود للمحاضرة بعد قليل

External info. about CYP450 , if you aren't interested skip this part please 😊

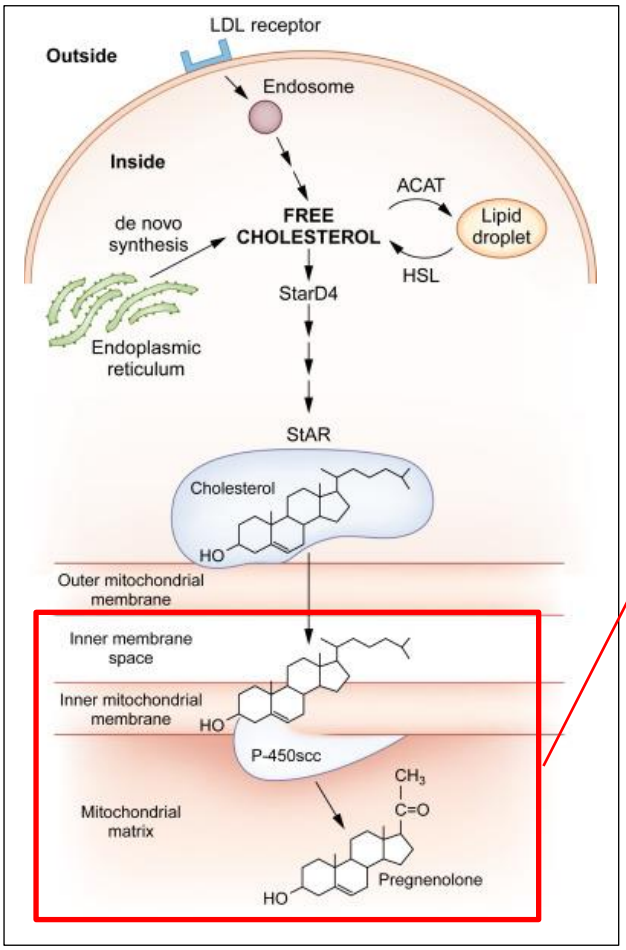
Cytochrome p450 are heme containing proteins , they were named so in 1960s because of their unique feature of appearing chromatic (coloured) pigment in the cell , When the heme iron within the enzyme binds carbon monoxide (CO) and is in its reduced state (Fe^{2+}), the enzyme exhibits a unique absorbance maximum at 450 nanometers (nm) in the visible light spectrum. This is a distinct spectral feature that differentiates them from other heme-containing enzymes.

Among human's 57 functional CYP genes are found , together they form what is called CYP gene superfamily ,superfamilies are defined as a group of proteins or genes of common origin (originate from a single gene that has duplicated) with nonoverlapping functions. If a group of proteins or genes contains a domain of common origin it is a superfamily.

For more info about duplication process :

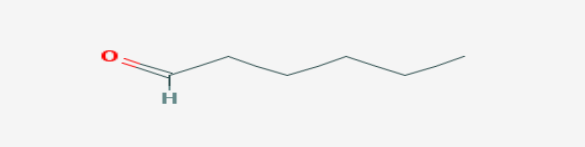
https://www.genome.gov/genetics-glossary/Duplication

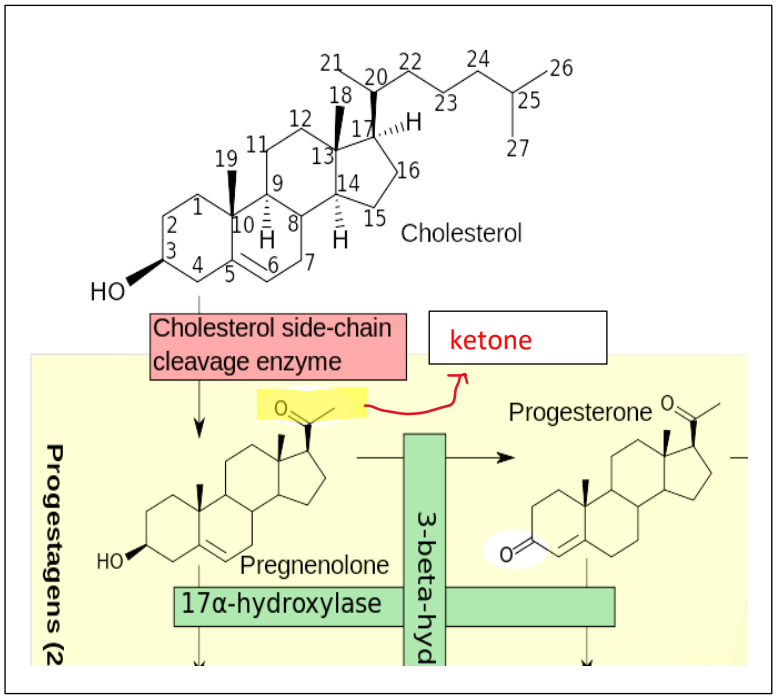
ملاحظة : هذه السبل و العمليات التي تحدث على مستوى الجينات نعلم يقينا أنها بفعل الله الواحد القهار أحاط بها علما و أحصاها عددا (وَكُلُّ شَيْءٍ عِندَهُ بِمِقْدَارٍ)، بل هي من العجائب في هذه الدنيا التي ترشدنا للخالق العزيز الحكيم ، ولا نحيلها كما يفعل الجهلة الى الصدفة و العشوائية بل هذا مشين بحق هذا الجمال و الاتقان في الخلق، فَتَبَارَكَ اللَّهُ أَحْسَنُ الْخَالِقِينَ !



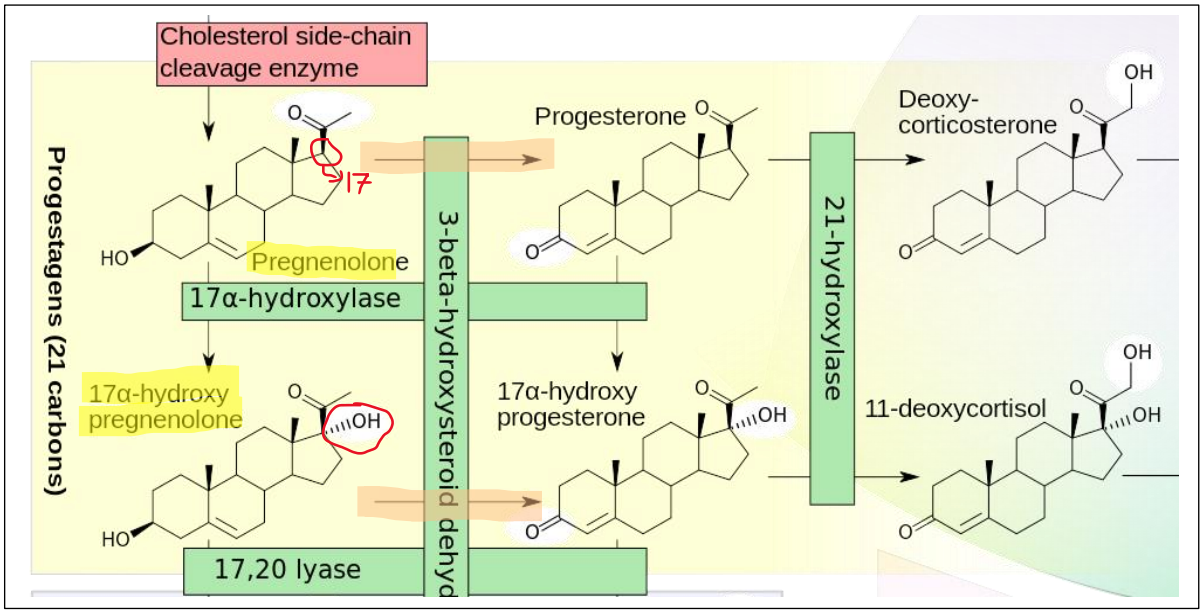
عدنا...

1. the tail of the cholesterol beginning at carbon number 22 and going through 27 must be removed and this is done so by the mitochondrial enzyme :cholesterol sidechain cleavage enzyme CYP11A1 removes this 22 to 27 carbon tail as isocaproaldehyde, which is a 6-carbon aldehyde(C6H12O) → (byproduct)





we get this ketone molecule which is pregnenolone.
in some sources, pregnenolone is considered the parent steroid but that's only because pregnenolone is the first steroid that we see after the tail has been for the most part removed
the doctor in previous lectures clarified that he considers pregnenolone as the parent molecule not cholesterol !

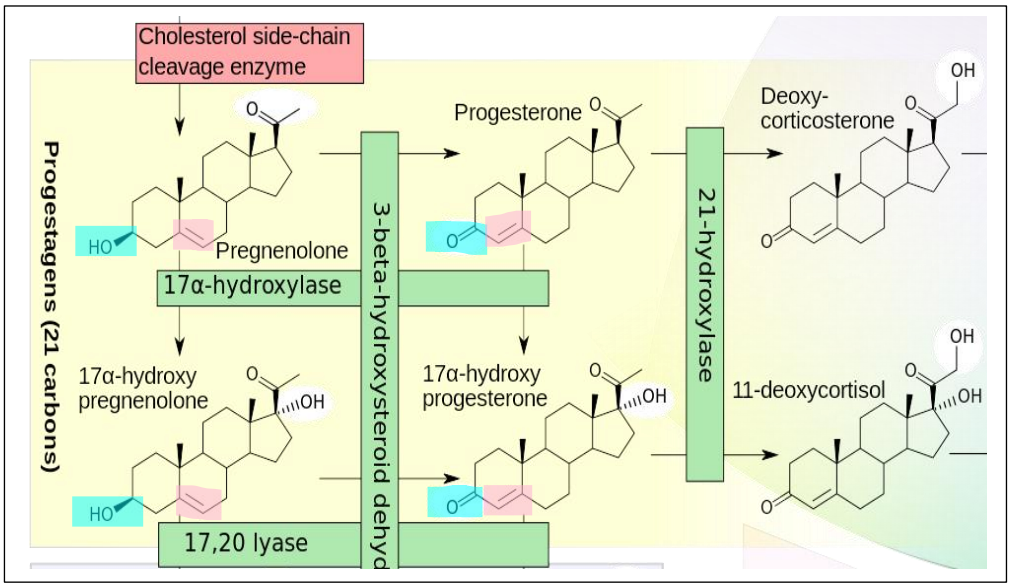


2. okay now pregnenolone can go one of two directions, we're going to go to the right first and see the development of the glucocorticoids and aldosterone

first of all pregnenolone can be hydroxylated into 17 alpha hydroxy pregnenolone, notice at the 17 position which is on the five membered ring components , it's going to get hydroxylated by 17- α- hydroxylase
now both pregnenolone and 17 alpha hydroxy pregnenolone can go to the right and form corticosteroids

pregnenolone will be oxidized into progesterone and progesterone (progestogen or progestin) that we can recognize as a functional mammalian hormone (it regulates menstruation and supports pregnancy in females)

17 alpha-hydroxy pregnenolone is going to be oxidized into 17 alpha hydroxy progesterone



How (pregnenolone → progesterone & 17 alpha-hydroxy pregnenolone →17 alpha hydroxy progesterone) reactions occur ?

This process applies for both reactions ! (**Oxidation** and **isomerization**)

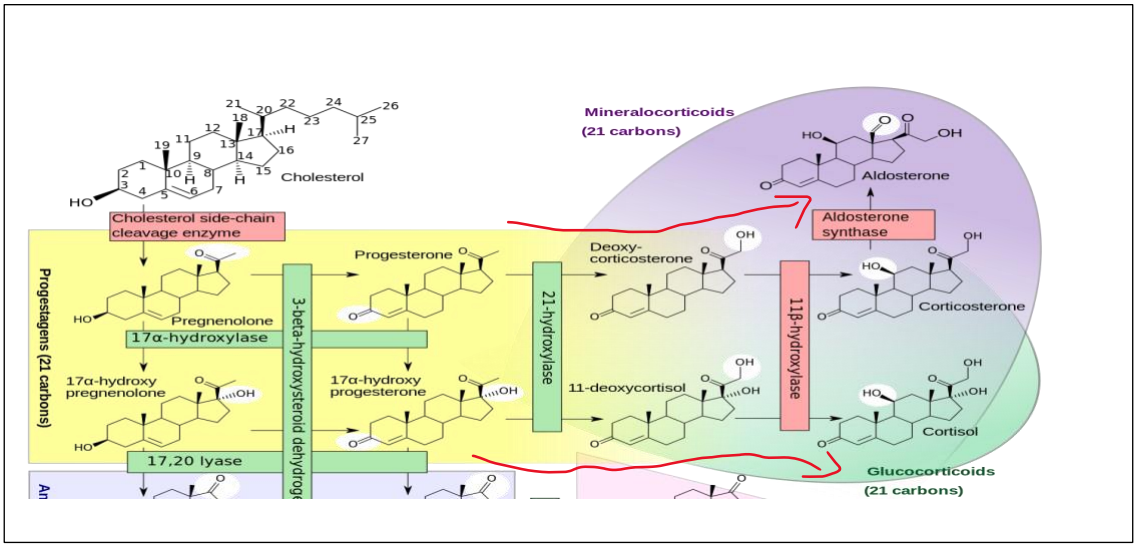
The enzyme 3-beta-hydroxysteroid dehydrogenase is going to

- oxidize the hydroxyl group at 3 position (thus the name 3 beta) , so this hydroxyl group is going to become a ketone
- other thing is isomerization of the double bond between positions 5 & 6 which eventually going to be between positions 4 & 5 .

---

progesterone right here can act independently to produce its functions **or** progesterone can be processed into aldosterone.

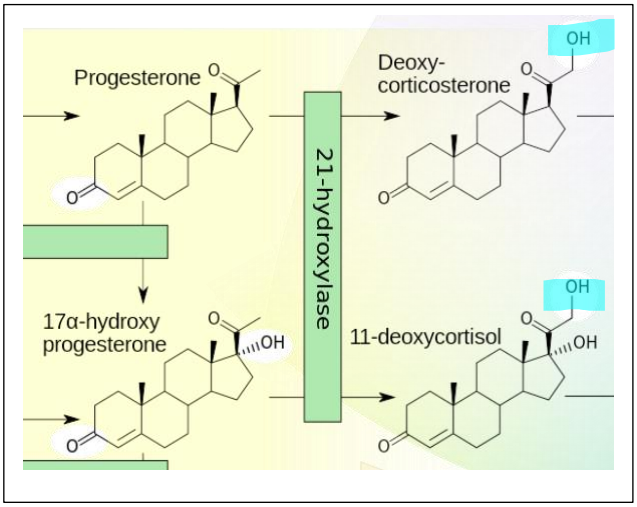
17-alpha-hydroxy progesterone is going to be processed into cortisol.



3. next enzyme in this pathway(regardless of which pathway you're taking whether it's progesterone or the hydroxylated version) , is going to be 21 hydroxylase so this carbon number 21 is going to be hydroxylated .

- 21 hydroxylase is another p450 enzyme which is actually encoded by CYP21A2

there's another version of this enzyme (just know that)



progesterone will be hydroxylated into deoxy-corticosterone

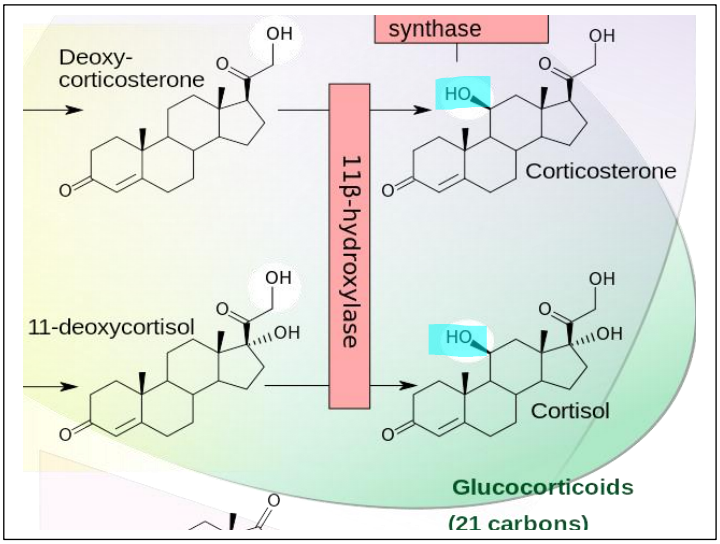
17 alpha hydroxy progesterone will be hydroxylated into 11-deoxycortisol

now both enzymes (21 hydroxylase and the 3 beta hydroxy steroid dehydrogenase) are microsomal enzymes (SER Enzyme)

---

4. the next enzyme is a mitochondrial enzyme!
so Deoxy-corticosterone & 11-deoxycortisol must be transported into the mitochondria , they can just move mostly by simple diffusion into the mitochondria because they're hydrophobic molecules.

11-beta-hydroxylase is going to hydroxylate the 11 position clearly



#11-deoxycortisol will be hydroxylated into cortisol
cortisol as we know is so forth the primary glucocorticoid or corticosteroid that's present in humans, it is the chronic stress hormone that we see in in many mammals such as humans. Cortisol can act independently (doesn't require further processing) !

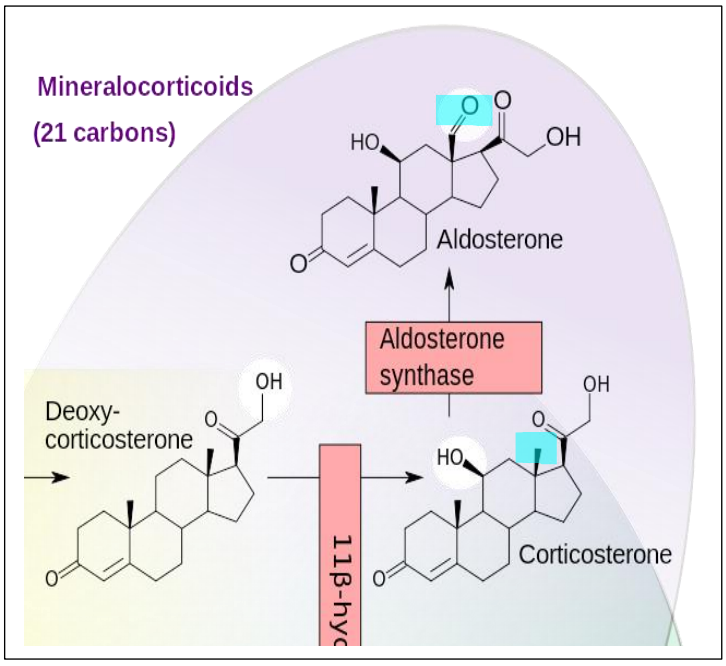
#11-beta-hydroxylase is going to hydroxylate Deoxycorticosterone into corticosterone which is present in humans and possess some of the glucocorticoid activity, but corticosterone have a minor effect in humans (the major is cortisol), however in some organisms such as mice and rats corticosterone becomes a primary glucocorticoid and cortisol more or less becomes a minor one but in general these are both glucocorticoids

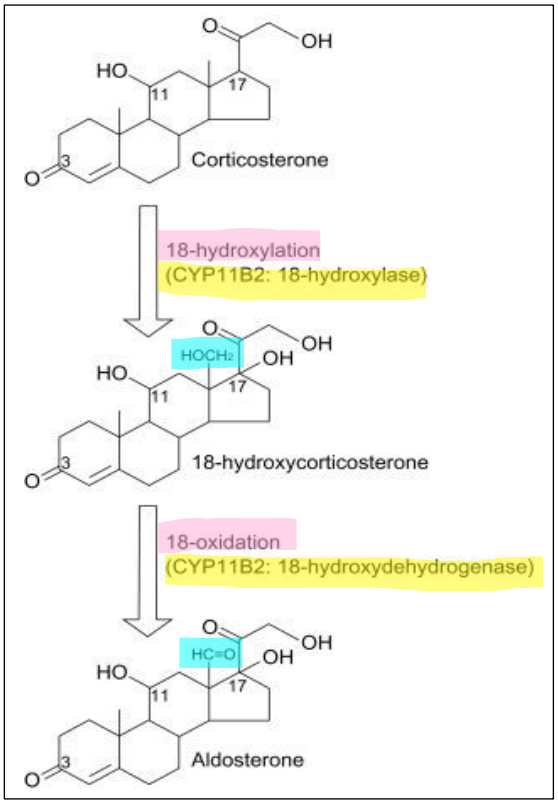
5. corticosterone can be further processed into the one general mineralocorticoid known as aldosterone which is involved in salt and water retention at the kidneys

aldosterone synthase ,another mitochondrial enzyme which mainly do two things:

1-first , it hydroxylates position number 18 (so sometimes this enzyme is actually called CYP18B2)
2-then it will oxidize the hydroxyl group into an aldehyde (the reason aldosterone gets its name is because it's an aldehyde)

aldosterone synthase generally have these two activities a hydroxylase activity which is at the site 18 and then an 18 hydroxy steroid dehydrogenase convert that hydroxyl into the aldehyde



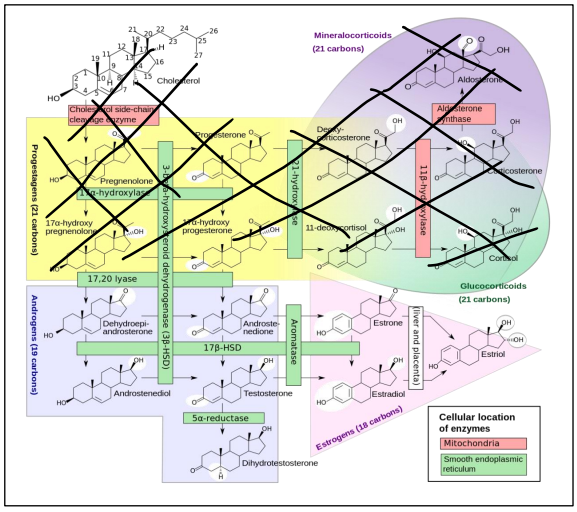


---

aldosterone and cortisol are going to be sort of dead ends of this part of steroidogenesis because these two hormones are gonna have one of two things:

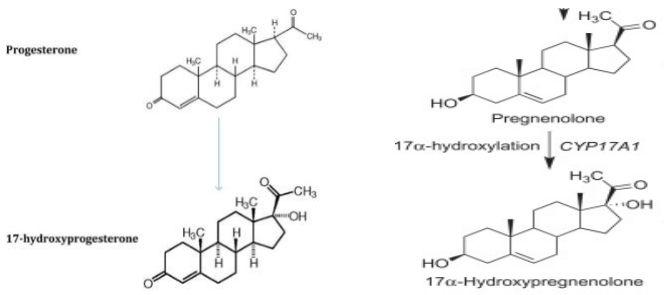
they're either going to act independently at their corresponding receptors throughout the body
or
they're just going to be metabolized in the liver

so we're done with this part of the pathway!

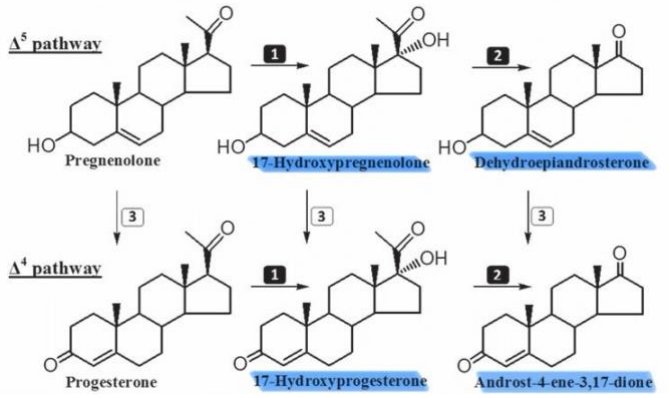


Now let's perceive how androgens and estrogens are formed.

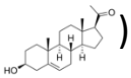
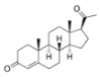
In order to make **androgens**, **2 progestogens** have to be formed, these 2 are the **hydroxylated forms of pregnenolone** and **progesterone** which are **17α-hydroxypregnenolone and 17α-hydroxyprogesterone** respectively.



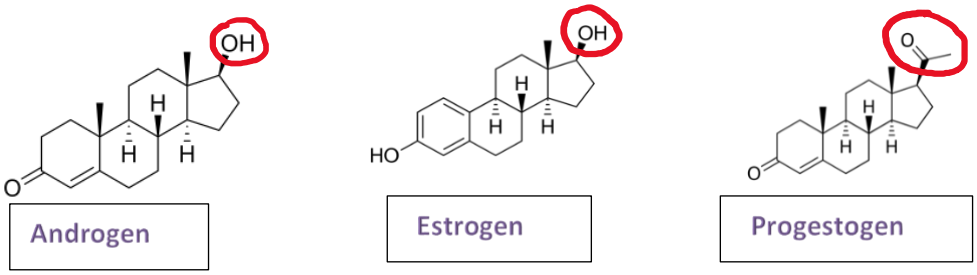
Now we have another enzyme that is called **17,20 lyase** which is the same enzyme as **17α -hydroxylase** but with an extra action. Its secondary activity is the conversion of the previously produced **17α-hydroxypregnenolone** and **17α-hydroxyprogesterone** into **DHEA** and **Androstenedione** respectively.



Enzyme no.2 is 17,20 lyase

Sometimes, steroids are released from the enzyme prematurely and that's okay because they gives us **17α-hydroxypregnenolone and 17α-hydroxyprogesterone** which are going to be processed to **aldosterone and cortisol** respectively. But if **17,20 lyase** performs its secondary activity, it will remove the hydroxyl group that it has just added (remember that it's primary activity is **17α-hyroxylase**) to induce the removal of the ketone group on **pregnenolone** () and **progesterone** () to produce **DHEA** and **Androstenedione**. These are the two first recognizable androgens and notice the difference between **androgens** and **progestogens** the figures below👇. The main difference is that the tails in **progestogens** have been completely removed in **androgens**. Additionally, tails have

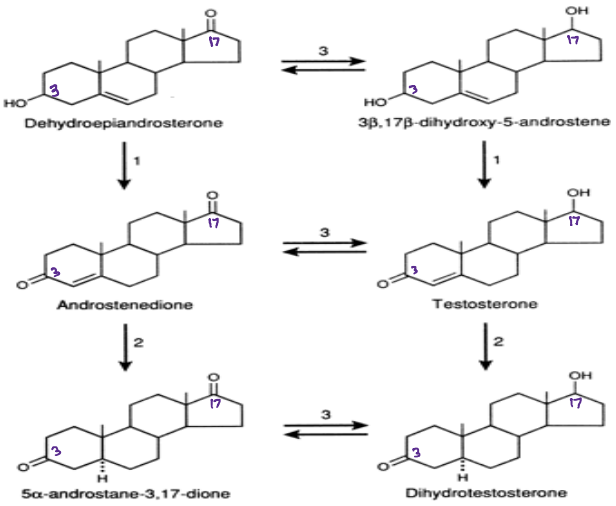
been removed in **estrogens** and that's a characteristic of both sex hormones (**estrogens and androgens**).



Now there's another enzyme at play here and it's the **3β-Hydroxysteroid dehydrogenase** which we've seen before. It will oxidize the **3-beta hydroxyl group** in **DHEA** into a **ketone** in **Androstenedione**, also we have a net ismoeration of the double bond between **C5** and **C6** into a double bond between **C4** and **C5**. Afterwards, **17β HSD** will reduce the ketone group in **Androstenedione** into a hydroxyl group forming **Testosterone**.

Testosterone can also be produced via a longer pathway which is through the reduction of **DHEA** (Hydroxyl group on **C17**) into **Androstenediol** (Ketone group on **C17**), then an oxidation takes place to convert the hydroxyl (on **C3**) back to a ketone (on **C3**) with an additional isomeration of the double bond **(C5,6 👉 C4,5).**

Testosterone is the 2nd most potent androgen, hence having 2 pathways to be generated through. However, the most potent androgen is **Dihydrotestosterone** which is formed from **Testosterone** by the enzyme **5α -reductase** along the cofactor **NADPH**. Using the reducing power of NADPH, double bond between **C4 and C5** will be removed, this reduction process increases the androgenic power of **dihydrotestosterone** up to 20-100 folds compared with testosterone.



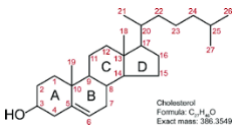
1= 3ß - hydroxysteroid isomerase

2 = 5 reductase

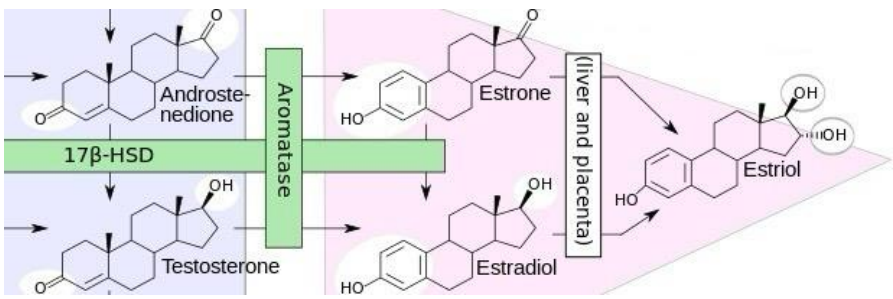
3 = 17ß-hydroxysteroid dehydrogenase

Now we're going to trace the synthetic machinery of estrogens. The 2 formerly synthesized androgens, **Androstenedione** and **Testosterone** are converted directly into estrogens. Bear in mind that in order to make **androgens** which are converted to **estrogens**, **progestogens** have to be formed, [**Progestogens** 👉 **Androgens** 👉 **Estrogens**]. The enzyme involved in the conversion is an aromatase and we're going to follow its activity on Androstenedione and Testosterone respectively.

1) It reduces the ketone group (on **C3**) in **Androstenedione**
2) The **A ring** becomes completely aromatic with **3 conjugated double bonds.**



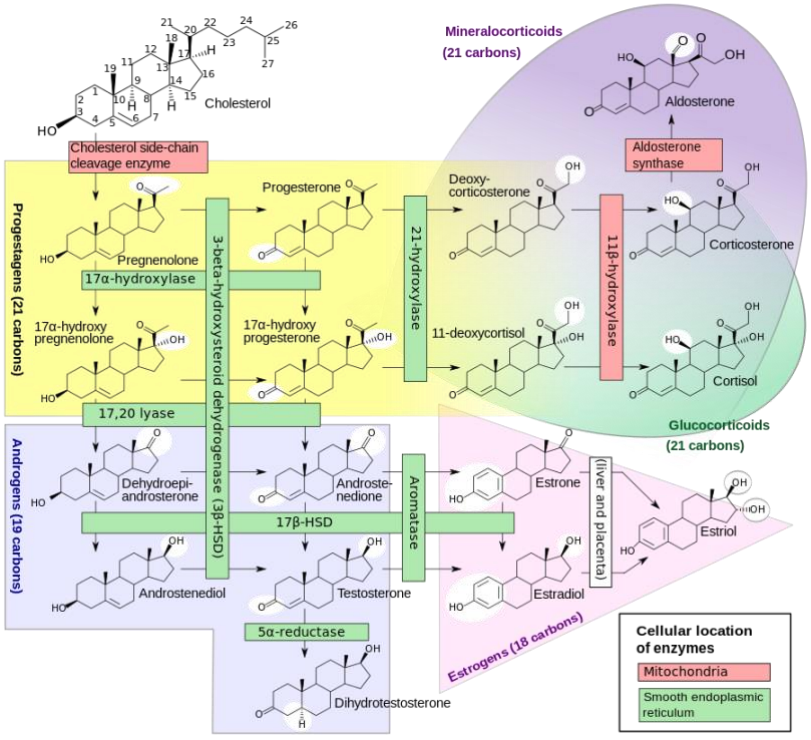
Consequently, **Androstenedione** is converted into **Estrone** which is the <u>least active estrogen</u>. Similarly, **aromatase** <u>reduces and then aromatizes</u> **Testosterone** into **Estradiol** which is the <u>primary estrogen in all mammalian</u> species. <u>Spot that all **estrogens** have an aromatic ring 'A ring'</u> which is produced by the **aromatase enzyme encoded by CYP19A1** and hence the



name *'aromatase'*. Both of **Estrone** and **Estradiol** can be <u>metabolized in the liver and placenta</u> to produce **Estriol** which is the 3rd estrogen. Estradiol's 5 membered ring **'D ring'** is hydroxylated (C16) to produce **Estriol**. While **Estrone's ketone group on C17** is first reduced using **17β HSD** and then **C16** gets **hydroxylated**. So, the hyroxylation process is **common** between <u>[Estrone 👉 Estriol] and [Estradiol 👉 Estriol]</u>, but the **reduction** process is **exclusive** for <u>[Estrone 👉 Estriol].</u>

Referring to steroidogenic enzymes, **3** of them are located in the **mitochondria** which are **<u>Cholesterol side chain cleavage enzyme</u>**, **<u>Aldosterone synthase</u>** and **<u>11-β hydroxylase enzymes</u>**, with the rest being **embedded** in the **membrane of the smooth endoplasmic reticulum** (**<u>microsomal</u>**).

Another thing to mention is that while <u>cholesterol is known to be the parent steroid molecule</u>, **Progestogens** in general can also be considered <u>parent molecules</u>. For an elaboration, in order to synthesize **mineralcorticoids**, **glucocorticoids** which come from **progestogens** are needed. On top of that, in order to make **estrogens**, **androgens** which also come from **progestogens** are required.



Done !! Congratulations 😊

سبحانك اللهم وبحمدك أشهد أن لا إله إلا أنت أستغفرك و أتوب إليك

قال أبىّ : قلت : يا رسول الله، إنى أكثر الصلاة عليك فكم أجعل لك من صلاتى ؟ قال : " ما شئت " قلت : الربع ؟ قال : ما شئت، وإن زدت فهو خير لك. قلت : النصف ؟ قال : ما شئت، وإن زدت فهو خير لك. قلت : الثلثين ؟ قال : ما شئت، وإن زدت فهو خير لك. قلت : أجعل لك صلاتى كلها ؟ قال : إذًا يكفيك الله ما أهمك من أمر دنياك وآخرتك. وفى لفظ : إذا تكفى همك، ويغفر ذنبك.

-أجعل لك من صلاتى ؟ يعنى من دعائى؛ فإن الصلاة فى اللغة هى الدعاء

أكثروا من الصلاة على النبي !

صلى الله عليه و على آله و صحبه و سلم