Metabolism

فريق طوفان الأقصى

Modified N: 14

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Lipid-soluble vitamins

+ *Vitamin B12 and folate*

Prof. Mamoun Ahram

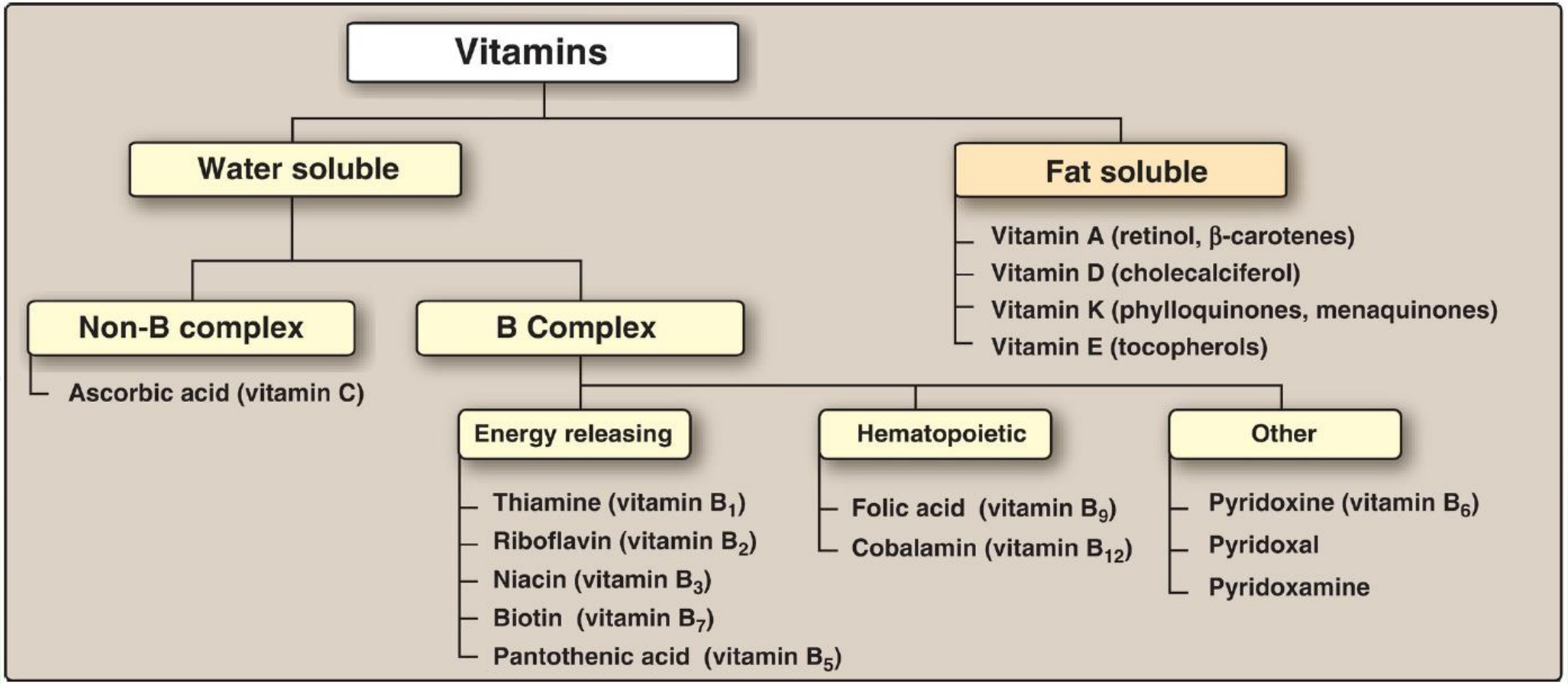
NOTE: Both are water-soluble vitamins

Vitamins

- They are organic compounds an organism requires in low amounts as vital nutrients.
- They cannot be synthesized in sufficient quantities & and must be obtained from diet.
- Thirteen vitamins are universally recognized at present.
- They have diverse biochemical functions:
  - Hormone-like functions (regulators): regulators of mineral metabolism (e.g., vitamin D), or regulators of cell & and tissue growth & and differentiation (e.g., vitamin A)
  - Anti-oxidants (e.g., vitamins E & C)
  - Precursors for enzyme cofactors (vitamin B subclasses)

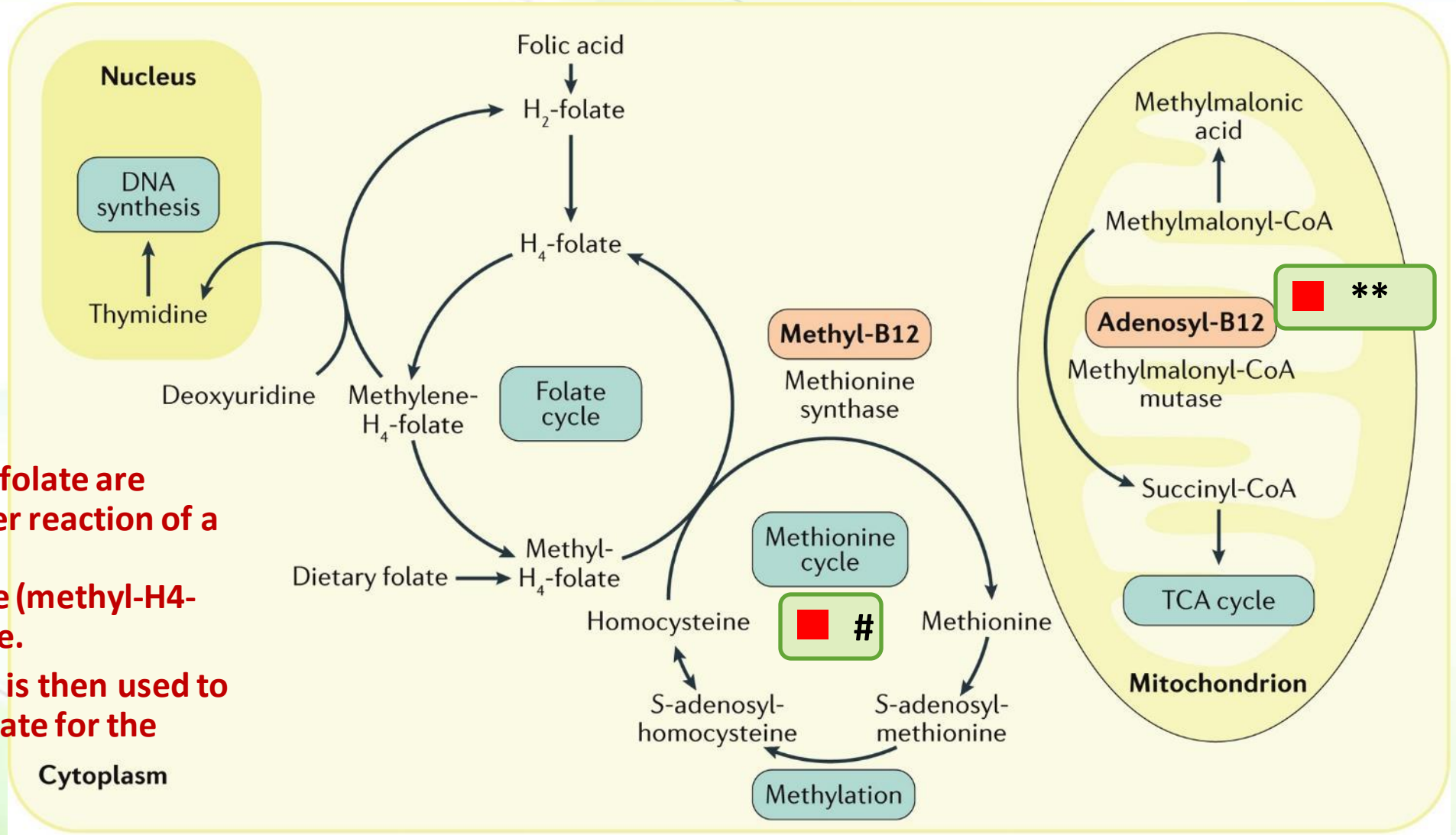
NOTE: By binding to receptor and doing the function

Classification



The connection between folate and vitamin B12

- **Vitamin B12 (B12) and folate are required for the transfer reaction of a methyl group from methyltetrahydrofolate (methyl-H4-folate) to homocysteine.**
- **The resulting H4-folate is then used to make methylene-H4folate for the synthesis of thymidine**



- **The complement in this slide:** So let's talk about Vitamin B12 & Folate, both are interconnected to each other (they regenerate each other) so if one of them is deficient, the other will be deficient as well (one can't function without the other)

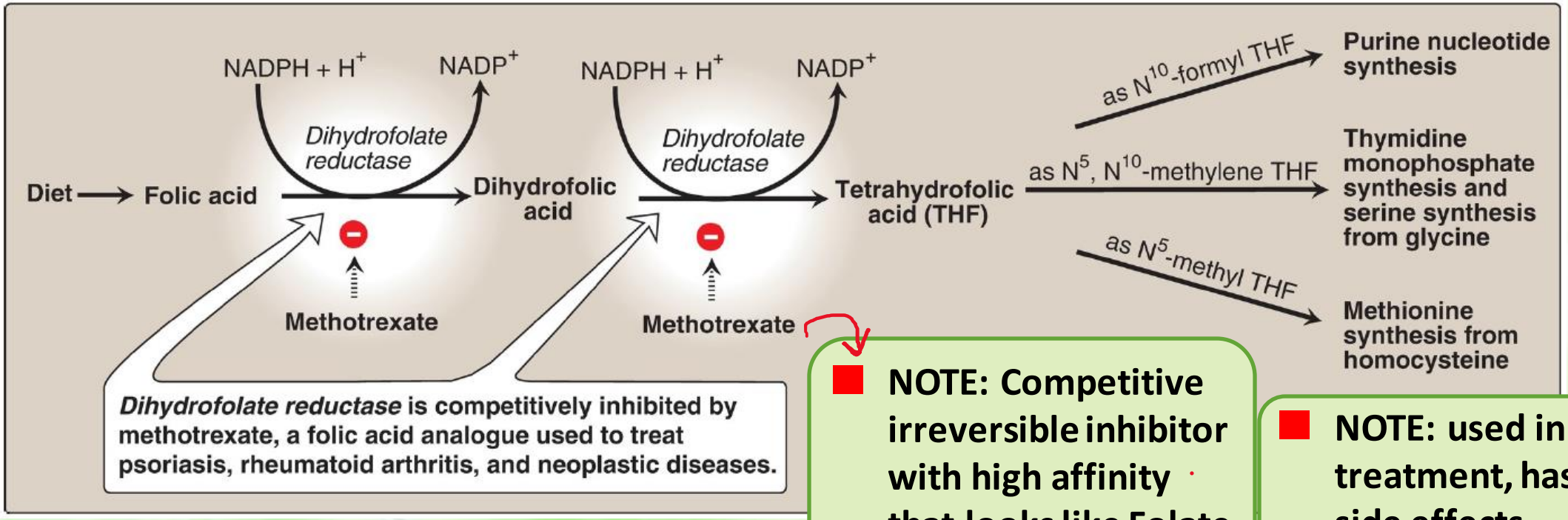
- **: Both are involved in B-oxidation of odd-numbered fatty acids, to refresh your memory, we release one Acetyl CoA at a time, 1, 1, until we reach propionyl CoA, it is carboxylated to methylmalonyl CoA which is isomerised **twice** resulting in succinyl CoA (intermediate in Krebs) and there's loss of energy because you missed some carbons. **What to note is that Vit B12 is important in this reaction**

- #: **Another importance of Vit B12 is in this reaction,** conversion of homocysteine to methionine in Methionine Cycle **(will be discussed in next lectures)** and this step is connected with another reaction, methyl folate —> tetrahydrofolate
- Tetrahydrofolate **is the active form of this vitamin**

- **#:** We obtain Folic acid from diet, it is converted in our body to dihydrofolate, and then tetrahydrofolate, which binds to methylene then methyl, and as we said we'll have regenaration of tetrahydrofolate, **why regenaration is important? Because this H4 folate involved in production of deoxy TMP**, so when we have deficiency in it, we'll have deficiency in DNA synthesis. **That's how Vit.B12 & Folate connected to each other**

Folic acid (folate or vitamin B9)

- Folate mediates one-carbon transfer from donors such as serine, glycine, and histidine to intermediates during the synthesis of amino acids, purine nucleotides, and thymidine monophosphate (TMP).
- The active form of folate is the reduced tetrahydrofolate (THF).

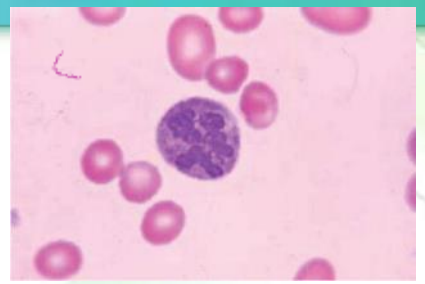


- **NOTE: Competitive irreversible inhibitor with high affinity that looks like Folate**
- **NOTE: used in cancer treatment, has many side effects**

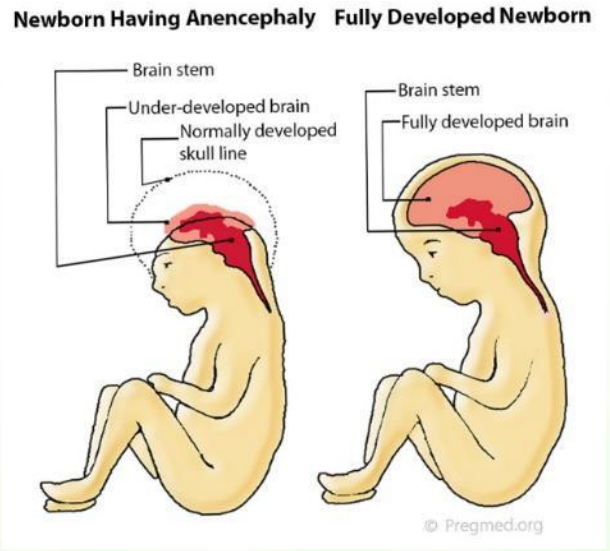
- **The complement in this slide:** As we mentioned the pathway in the previous slide, is is reduced using Dihydrofolate reductase, T4-Folate binds to single carbon group, either formyl or methyl or methylene to get nucleotides, and for that any proliferating cell will be affected if there's deficiency in folate, cancer cells can't proliferate due to DNA synthesis blockage, arthritis (autoimmune cells) blocked also.

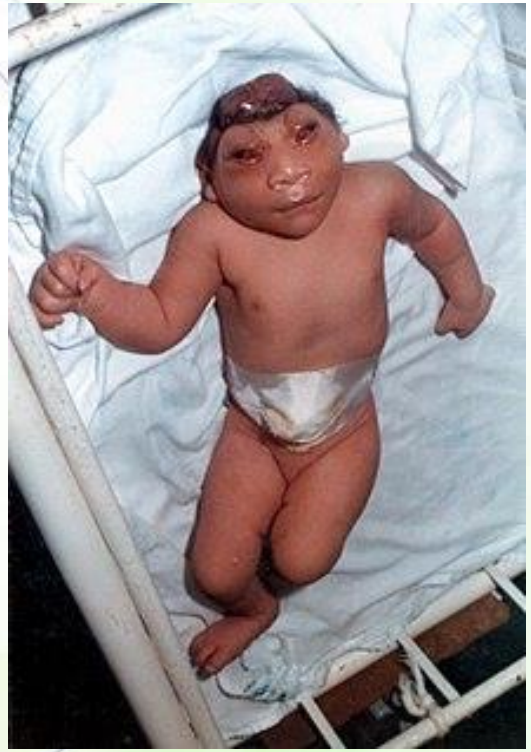
Folate and anemia

NOTE: pregnant women should be given supplement



- Causes of deficiency:
  - Increased demand (pregnancy and lactation)
  - Poor absorption (pathology or alcoholism)
  - Drugs (methotrexate)
  - Folate-free diet (few weeks)
- Deficiency might result in:
  - Megaloblastic anemia: no synthesis of purine nucleotides and TMP → cells cannot make DNA → no cell division.
  - Neural tube defects (NTD): Spina bifida and anencephaly





- **NOTE:** We have good storage of Folate and for that, deficiency takes time
- Megaloblastic anemia is a condition of folate deficiency, enlarging in erythrocytes because they're growing but not dividing
- NTD is another condition, spina bifida and anencephaly (brain look really small) →mental retardation, can be fatal.

Folate supplementation

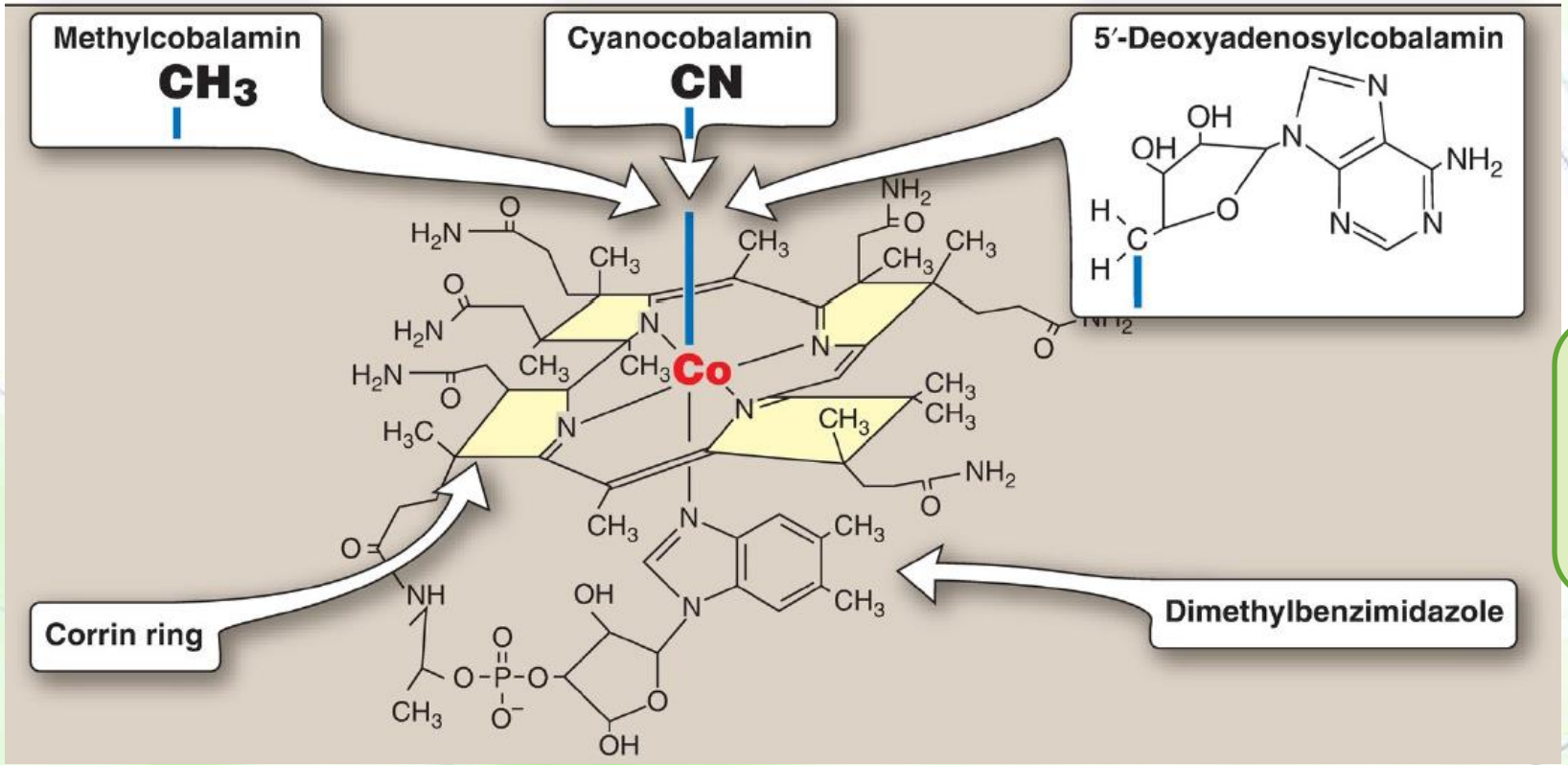
NOTE: For that, pregnant women should be given supplement, because there's many DNA synthesis going on for her and the baby

- Folic acid supplementation before conception and during the first trimester is needed.
- All women of childbearing age need 400 µg/day of folic acid to reduce the risk
  - 10 times if a previous pregnancy was affected.
- In the U.S., folic acid is added to wheat flour and enriched grain products, resulting in a dietary supplementation of ~100 µg/day.

Cobalamin (vitamin B12)

Structure and coenzyme forms

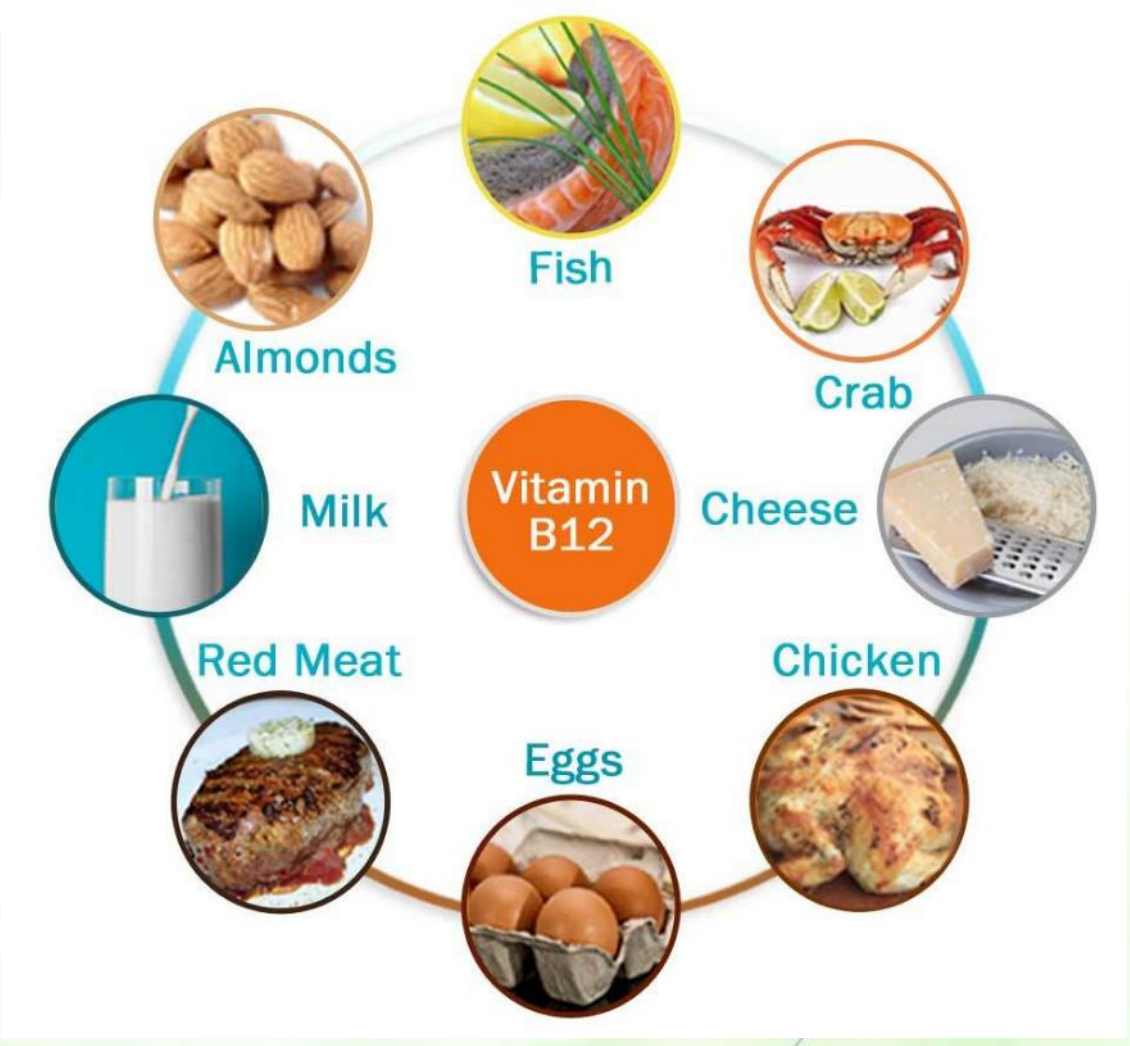
- It contains a “corrin” ring that looks like a pyrrole ring. *Not important*
- The physiologic coenzymes are: *5’-deoxyadenosylcobalamin and methylcobalamin*
- The commercial form has a cyanide is added (cyanocobalamin).



- **Notice the cobalt Co in the middle**

Sources of cobalamin

- Vitamin B12 is synthesized only by intestinal microbiota or by eating foods derived from other animals, but not from plants.
- Cobalamin is present in good amounts in liver, red meat, fish, eggs, dairy products, and fortified cereals.

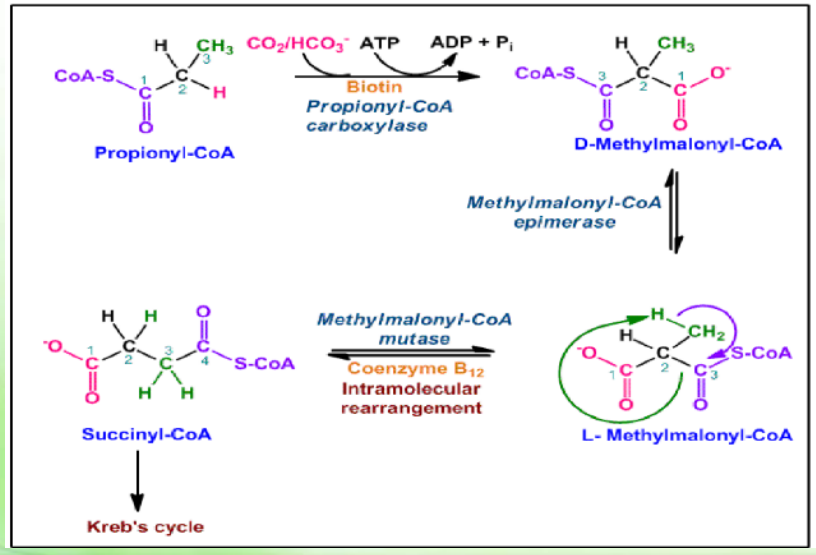


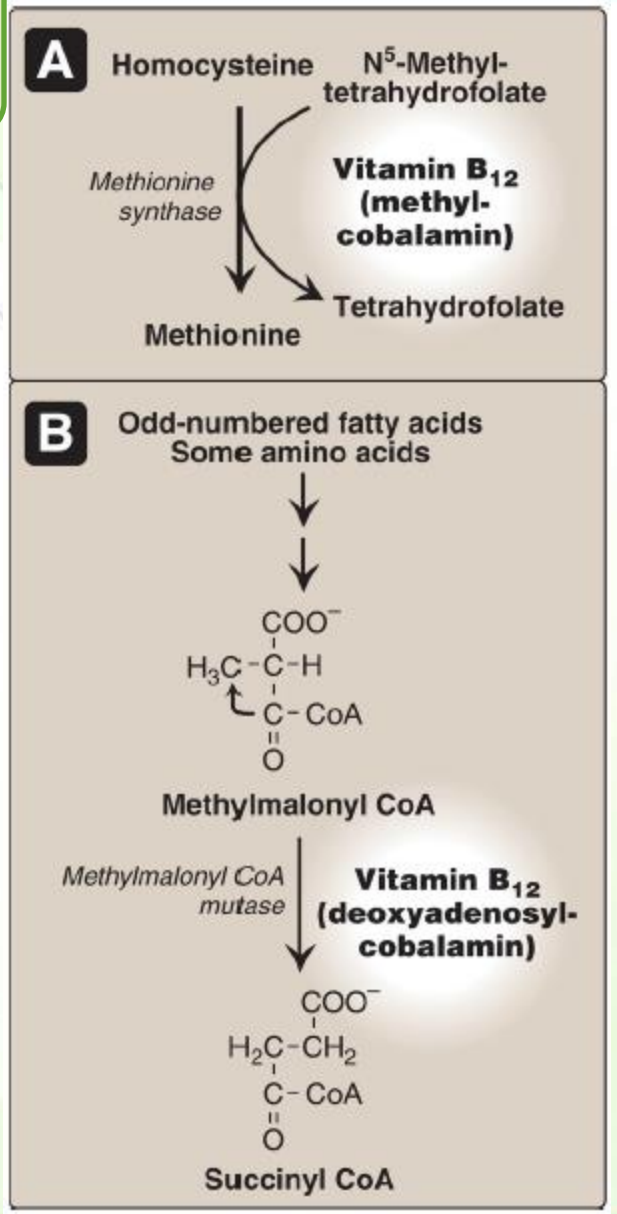
Biochemical need of cobalamin (vitamin B12)

- **NOTE: Here the importance of Vit.B12 is mentioned (we talked about them in previous slides)**
- **B12 required in metabolism of odd FAs and branched amino acids**

- It is required for two essential enzymatic reactions:
  - Re-methylation of homocysteine (Hcy) to methionine
  - Isomerization of methylmalonyl coenzyme A (CoA) during:
    - Degradation of some amino acids (methionine and the branched isoleucine, valine, and threonine)
    - Degradation of fatty acids (FA) with odd numbers of carbon atoms.

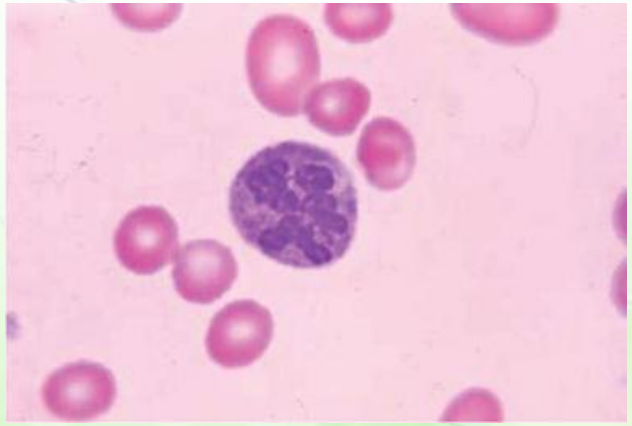
- When deficient,
  - Branched amino acids accumulate resulting in neurologic manifestations of vitamin B12 deficiency.
  - Homocysteine is not re-methylated resulting in its accumulation.



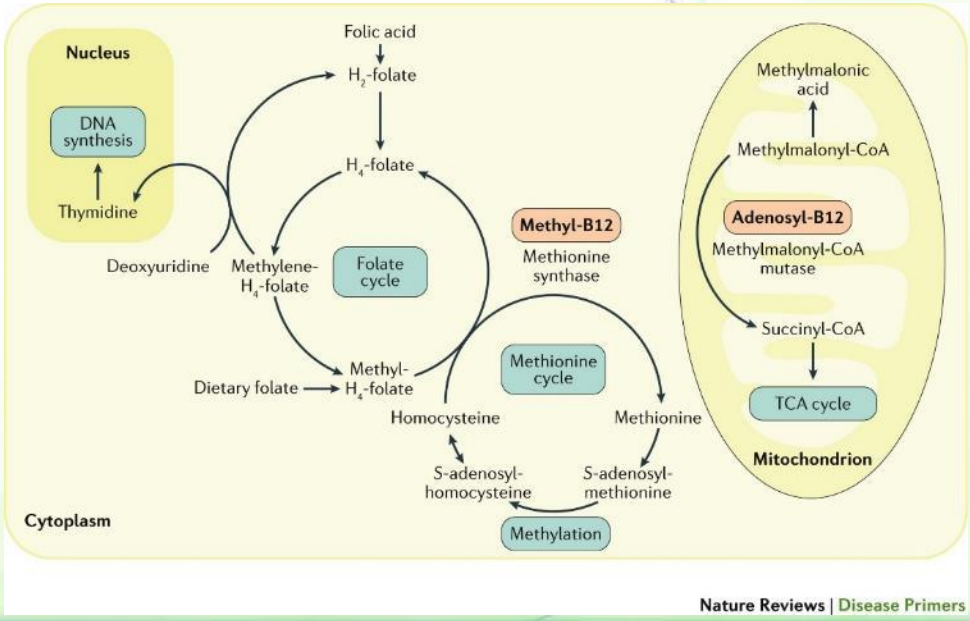


Folate trap hypothesis

- Cobalamin deficiency affects rapidly dividing cells, such as the erythropoietic tissue of bone marrow and the mucosal cells of the intestine since they need both the N5,N10-methylene and N10-formyl forms of THF for the synthesis of nucleotides.
- In cobalamin deficiency, the utilization of the N5-methyl form of THF is impaired and, hence, it accumulates.
- Deficiency of THF forms results in megaloblastic anemia.



NOTE: No regenaration of H4 folate, resulting in accumulation of methylene H4 folate.



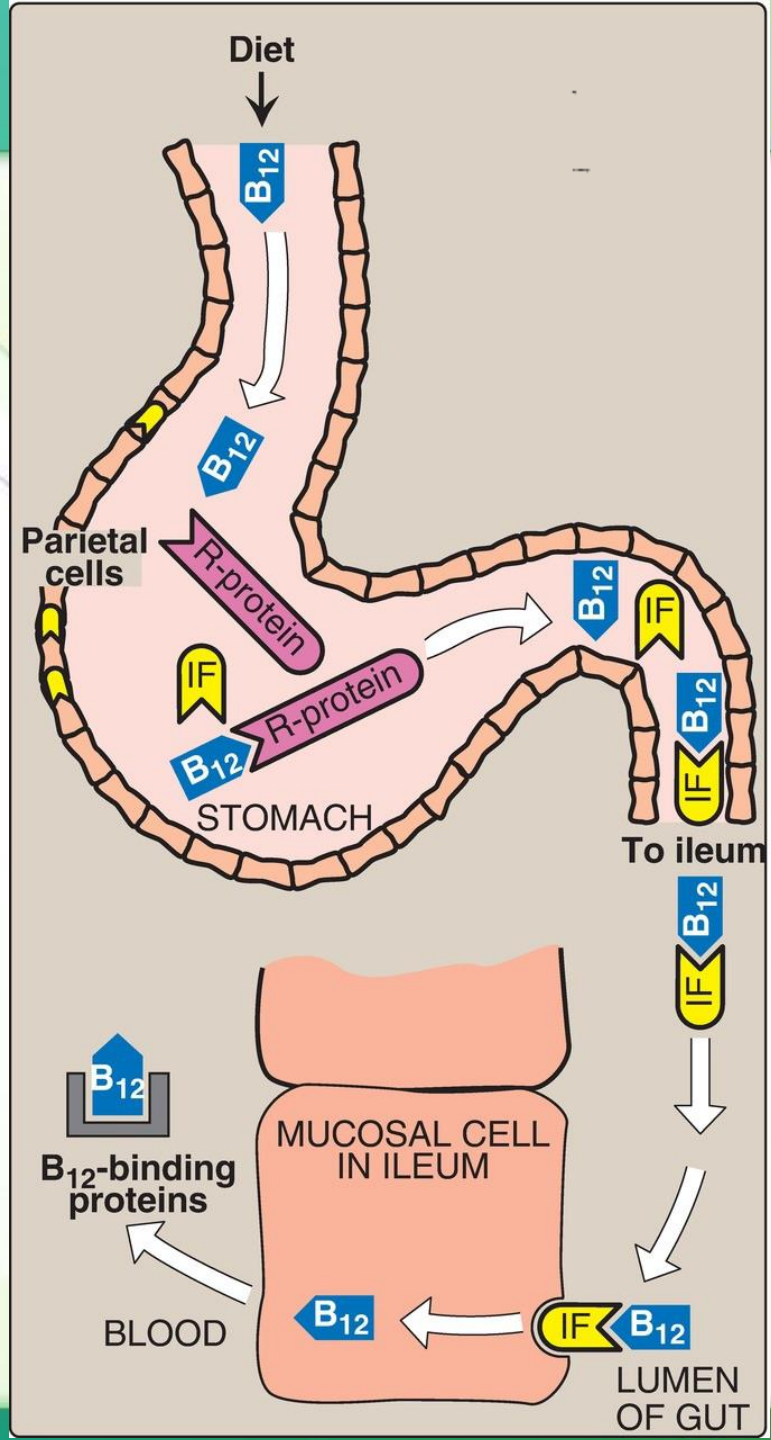
Clinical indications for cobalamin

- In contrast to other water-soluble vitamins, significant amounts (2–5 mg) of vitamin B12 are stored in the body.
- Clinical symptoms may develop after several years of deficiency.
- Deficiency happens much more quickly (in months) if absorption is impaired.

Absorption of vitamin B12 and Pernicious anemia

- Severe malabsorption of vitamin B12 leads to pernicious anemia.
  - *Pernicious: harmful gradually and subtly.*
- It is, most commonly, a result of an autoimmune destruction of the gastric parietal cells that are responsible for the synthesis of intrinsic factor (IF), which is a protein that helps in its absorption in the intestines via binding to a receptor called cubilin.
- Malabsorption in the elderly causes achlorhydria (no HCl).
- Individuals with cobalamin deficiency are usually anemic (folate recycling is impaired).

Ahram side advice : It's nice to learn something new all the time



- **The complement in this slide:** How can the deficiency in B12 occur? If there's impaired absorption of it.
- How the absorption occur? B12 binds to a protein called **intrinsic factor (a glycoprotein released from parietal stomach cells)** in the duodenum, and the complex binds to **cubilin receptor (receptor in mucosal cells of ileum and intestines)** and is absorbed. Note that this occur when pH goes up
- People can have defect in either IF or in the cubilin receptor due to mutations and for that, **they are given injections of B12 because they can't absorb it.**
- What is trend nowadays is taking B12 in any weak situation you are passing through, Is that high level of B12 toxic? **It can't be toxic because it is water soluble..** Normal Range 100-900 microg/dL, can be 2000? Yes, Toxic? No. Preferred to be more than 300.

- **Ahram side advice : It's nice to learn something new all the time**

- **NOTE: Student Q: Does B12 enhance memory? Dr.Ahram:** B12 importance is not in enhancing memory, as you can see it helps in **proliferation** of cells.

- **NOTE: Student Q: Should we take B9 before B12? Dr Ahram:** recall that **B9 is the folic acid** and we saw the interconnection between them. It is recommended to take them together

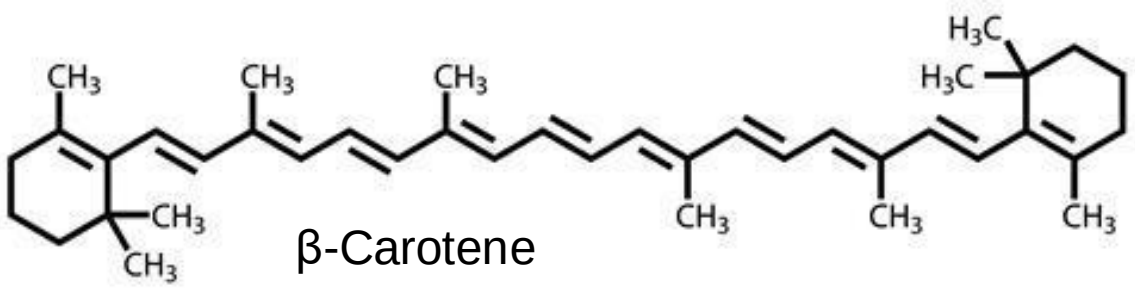
Lipid-soluble vitamins

Vitamin	Main function	Deficiency
A	Roles in vision, growth, reproduction	Night blindness, cornea damage
D	Regulation of Ca^{+2} & phosphate metabolism	Rickets (children), Osteomalacia (adults)
E	Antioxidant	RBCs fragility
K	Blood coagulation	Subdermal hemorrhaging

All fat-soluble vitamins are carried in chylomicrons.

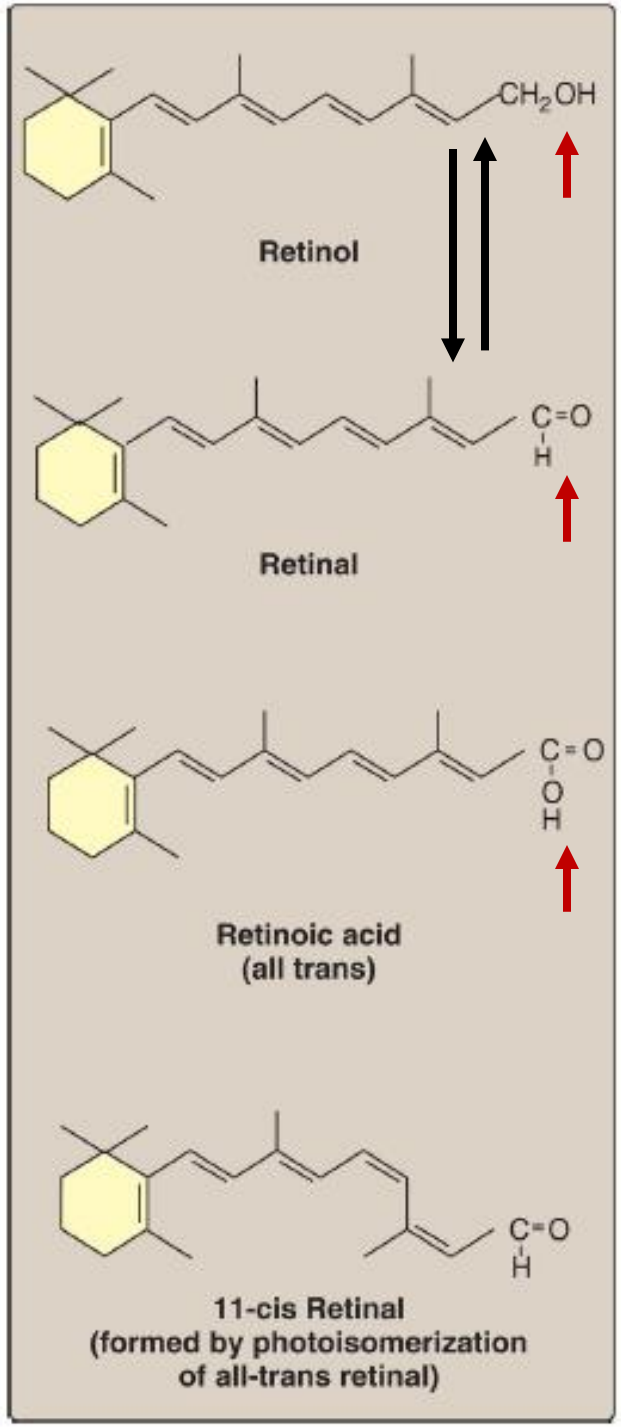
- **NOTE ABOUT TOXICITY:** Water-soluble vitamins can’t be toxic because they can be eliminated easily
- Lipid-soluble vitamins could be toxic since they are hardly eliminated.

Vitamin A



β-Carotene

- The retinoids are different forms of vitamin A.
- They are derived from β-Carotene, which is oxidatively cleaved in the intestines to yield 2 molecules of retinal.
- Retinal and retinol are inter-convertible.
- Retinoic acid mediates most of the actions of the retinoids, except for vision and spermatogenesis.

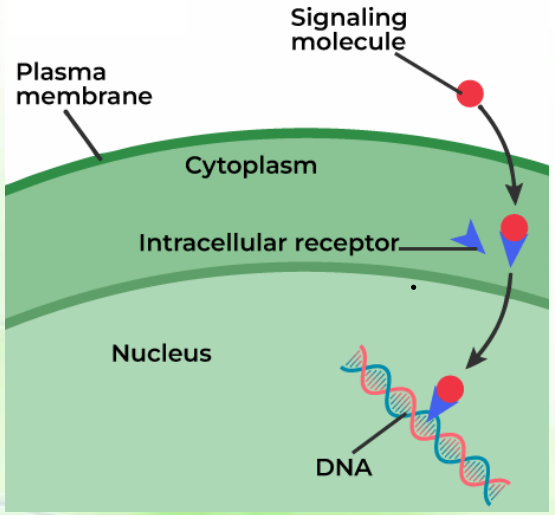


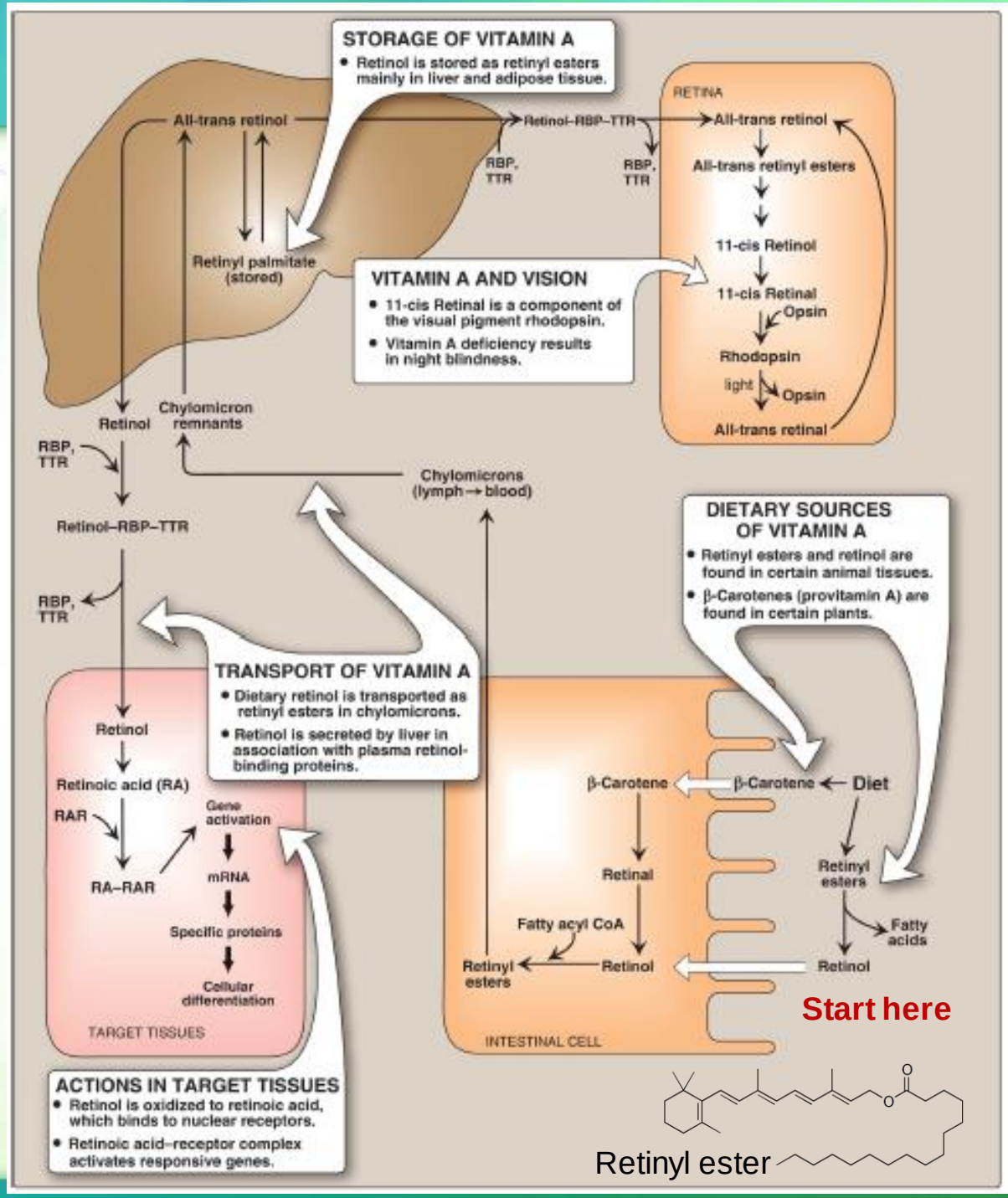
- **The complement in this slide:** Starting with Vitamin A, it is obtained from **B-carotene** (in diet) (notice its structure), which is cleaved from the middle to give 2 Retinal molecules.
- Vitamin A have 3 forms: **Retinol** & **Retinal** (which are inter-convertible), & **Retinoic Acid**
- Retinol can change to Retinal (oxidation) and Retinal can change to Retinol (reduction), and you can convert Retinal to Retinoic acid (By oxidation) **but you can't convert back Retinoic acid to Retinal or Retinol**
- That doesn't change the fact that this 3 forms are important

- **Retinol<—>Retinal → Retinoic Acid**

Absorption & transport

- Intestinal cells absorb β-Carotene and retinol (from retinyl esters), then re-esterified.
- Retinal esters are transported to the liver via chylomicrons for storage.
- Retinol binds the plasma retinol-binding protein (RBP) complexed with transthyretin for transport and entry into target cells.
- In the retina, retinol is oxidized to retinal, which is involved in vision.
- In other target cells, retinol is oxidized to retinoic acid, which binds to intracellular receptors and the complex binds to DNA elements regulating transcription.
- Example: the keratin genes in most epithelial tissues

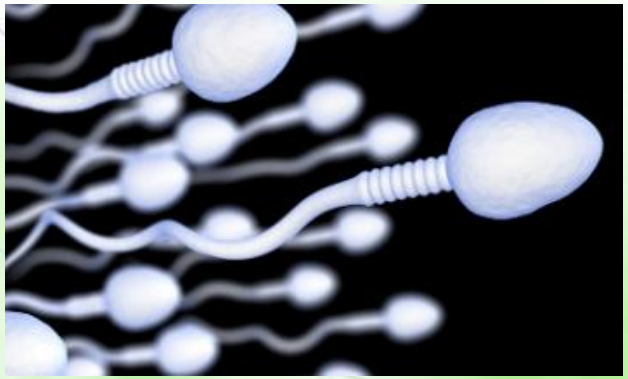




- The complement in this slide: FOLLOW THE PICTURE!
- Let's Start with the absorption of Vitamin A, it is absorbed by intestinal cells, Retinyl ester (which is a Retinol with fatty acid, like palmitate, obtained from diet) should be cleaved, removing FA to have Retinol that could be absorbed by intestinal cells. And when it is absorbed, it is re-esterfied and then packaged in chylomicrons, chylomicrons are released and get into the liver where Retinyl ester is released (and that's how liver store Retinyl ester)
- Liver is the delivery site of Vitamin A in our body, Vitamin A binds to RBP (Retinol Binding Protein)(synthesized in liver) and this RBP is complexed with TTR (Transthyretin) TTR—RBP—VitA.
- In target cells, Retinol is released from the complex and taken up by them
- Retina gets the Retinol, Retinol which is all-trans (all double bonds are in trans configuration.) And then it's converted to 11-cis Retinol then 11-cis Retinal (and this is how we SEE)(will be discussed later in 3rd year)
- In other target cells, Retinol is taken up, converted to Retinoic acid, which diffuses and binds to intracellular receptor, and this compound goes to the nucleus and binds to DNA elements to regulate certain genes like keratin genes (which is important for tissue toughness)

Functions of vitamin A

- **Reproduction**: Retinol and retinal (not retinoic acid) are essential for spermatogenesis in the male and preventing fetal resorption in the female
- **Growth** (retinoic acid): Vitamin A deficiency results in a decreased (growth rate and bone development) in children.
- **Maintenance of epithelial cells** (retinoic acid): Vitamin A is essential for normal differentiation of epithelial tissues & and mucus secretion.
- Animals given vitamin A only as retinoic acid from birth are blind and sterile.

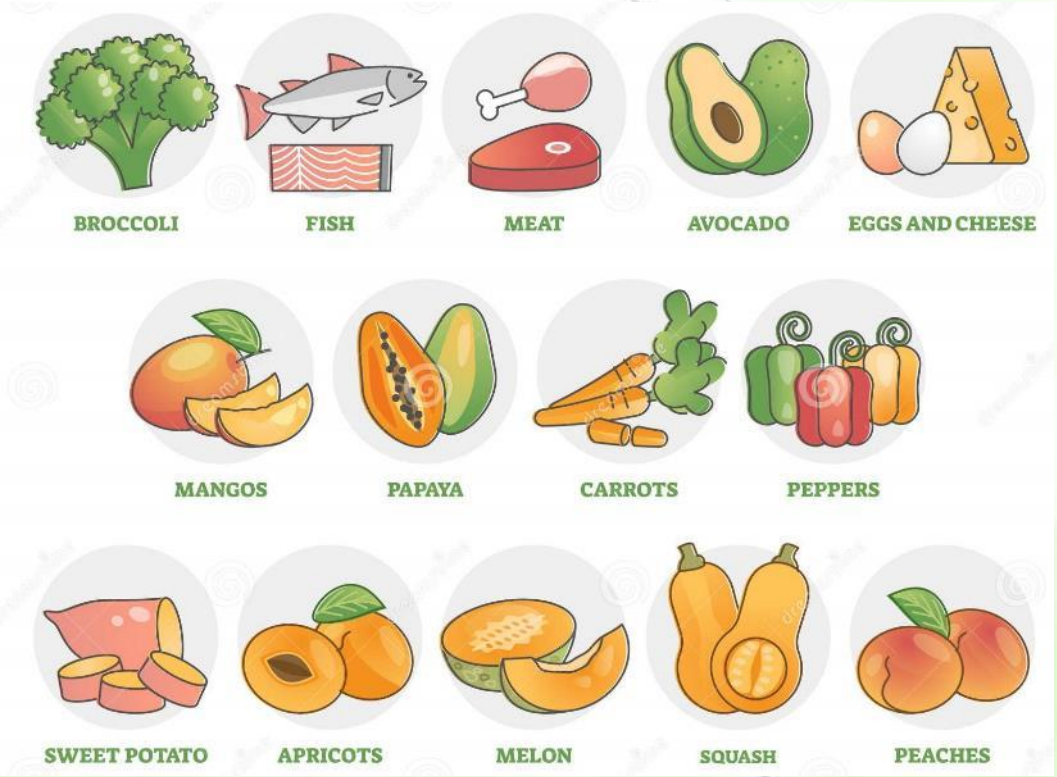


That's why this mouse is given عصاي ونظارة

Distribution & Requirements

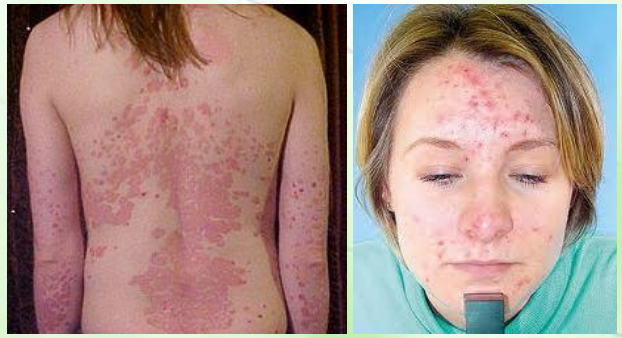
- Liver, kidney, cream, butter, and egg yolk are good sources of preformed vitamin A.
- Yellow, orange, and dark-green vegetables and fruits are good sources of the β-carotene (provitamin A).

Any vegetable that has a color is considered a source of vitamin A



Dietary deficiency of vitamin A

- Mild (night blindness, nyctalopia)
  - *Night=nyx, blind=alaos, eye=ōps*
- Prolonged (irreversible loss for some visual cells)
- Severe (xerophthalmia)
  - Xerophthalmia: ulceration & dryness of conjunctiva & cornea, followed by scar & blindness (affecting over 500,000 children worldwide every year)
  - Acne and psoriasis: effectively treated with retinoic acid



Toxicity - Hypervitaminosis A

- Excess vitamin A causes hypervitaminosis A.
- The upper limit is 3 mg of preformed vitamin A/day
- Amounts exceeding 7.5 mg/day of retinol should be avoided.
- Pregnant women are at risk of teratogenesis
  - Congenital malformations are produced in an embryo or fetus.
- Prolonged treatment with isotretinoin (an isomer of retinoic acid) can increase TAG and cholesterol, providing some concern for an increased risk of CVD.

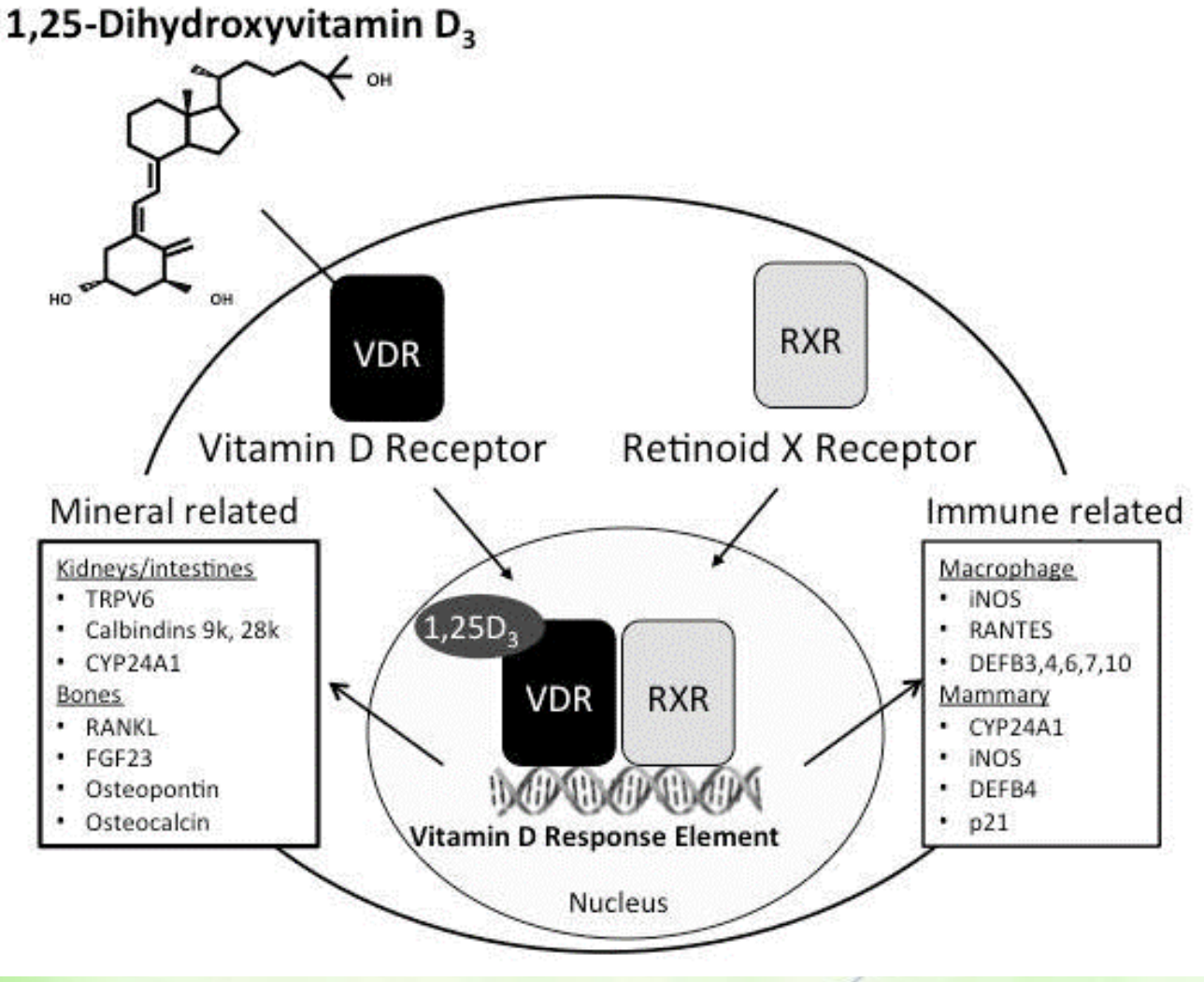
Vitamin D

Derived From cholesterol

- It is a sterol with hormone-like functions.
- The active molecule: 1,25-dihydroxycholecalciferol (or calcitriol) binds to intracellular receptor proteins.

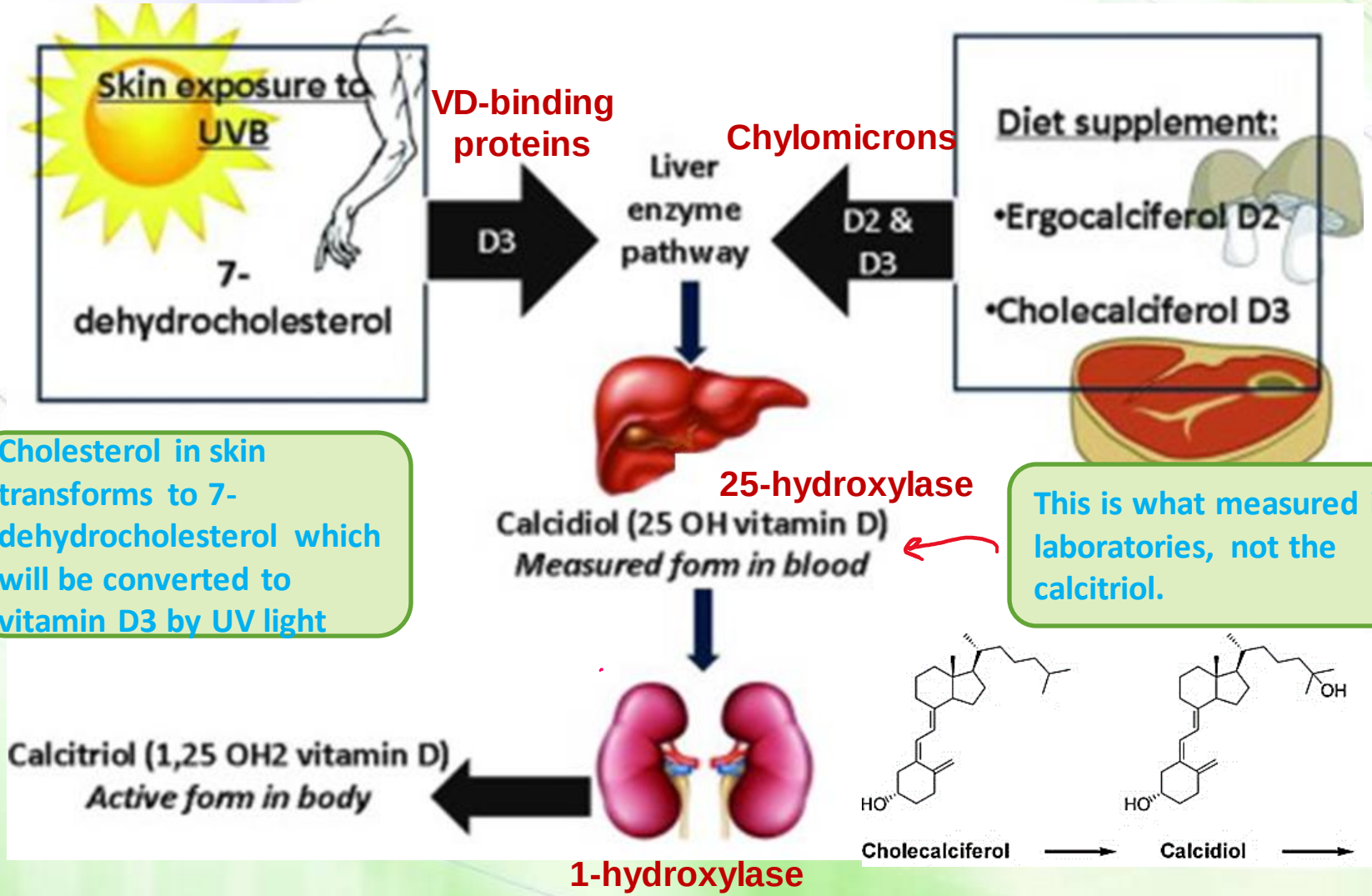
And then the vitamin D and its receptor will heterodimerise with another receptor and make a bigger complex.

- The complex interacts with response elements on the DNA of target cells and selectively regulates gene transcription.



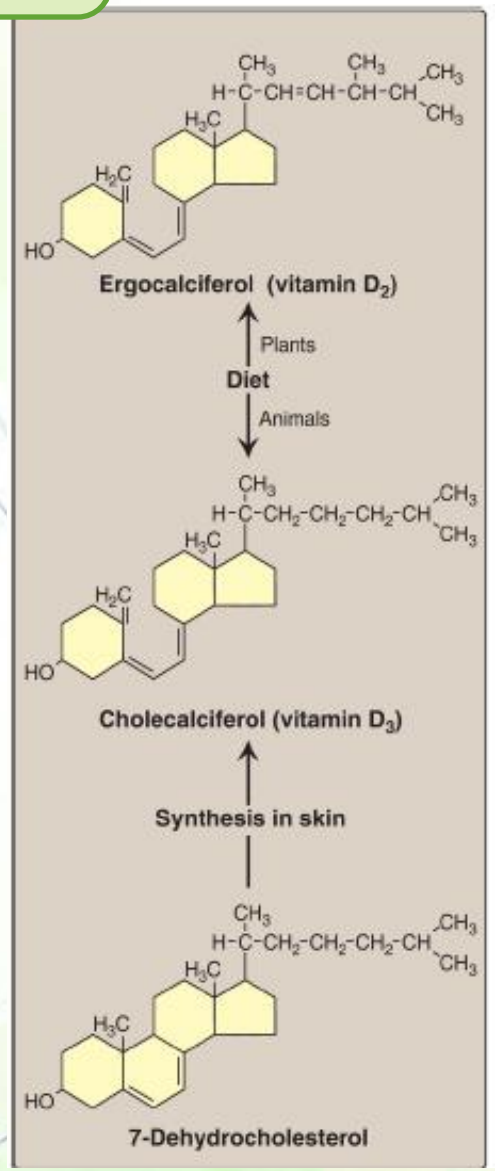
Synthesis of vitamin D

Patients with MS (Multiple Sclerosis) are recommended to take Vit.D



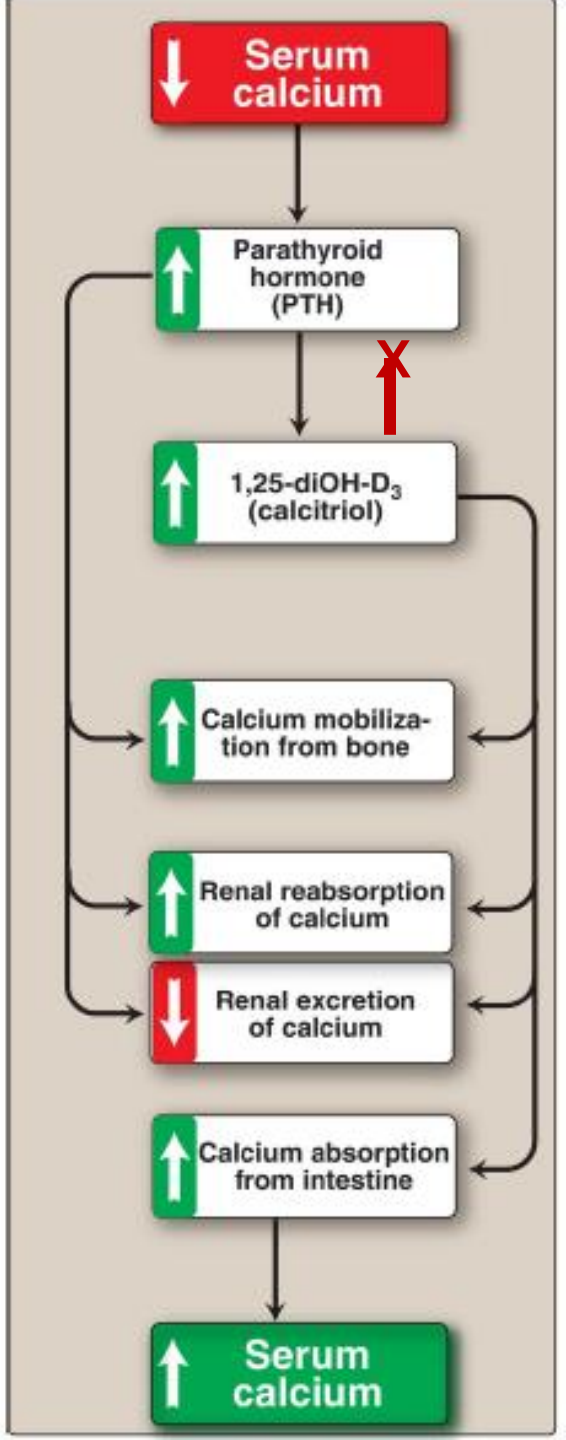
Cholesterol in skin transforms to 7-dehydrocholesterol which will be converted to vitamin D3 by UV light

This is what measured in laboratories, not the calcitriol.



Functions and regulation of vitamin D

- Functions: **regulating the serum levels of calcium and phosphorus.**
- Formation of calcitriol is regulated by the level of serum phosphate (PO_4^{3-}) and calcium ions (Ca^{2+}).
- 25-Hydroxycholecalciferol 1-hydroxylase activity is increased:
  - Directly by low serum PO_4^{3-}
  - Indirectly by low serum Ca^{2+} (through parathyroid hormone)
- Hypocalcemia caused by insufficient dietary Ca^{2+} results in elevated levels of serum 1,25-diOH-D3.(look to >>>)
- 1,25-diOH-D3 inhibits the expression of parathyroid hormone, forming a negative feedback loop.
  - It also inhibits the activity of the 1-hydroxylase.



Distribution and requirement

- The RDA for individuals ages 1–70 years is 15 µg/day and 20 µg/day if over age 70 years
  - 1 µg vitamin D = 40 international units (IU)
- Naturally in fatty fish, liver, and egg yolk
- Milk, **unless it is artificially fortified,** is not a good source
- Because breast milk is a poor source of vitamin D, supplementation is recommended for breastfed babies.

EXTRA FROM GOOGLE
RDA= recommended dietary allowance
Average daily dietary intake level that is sufficient to meet nutrient requirement of nearly all health people.

Toxicity

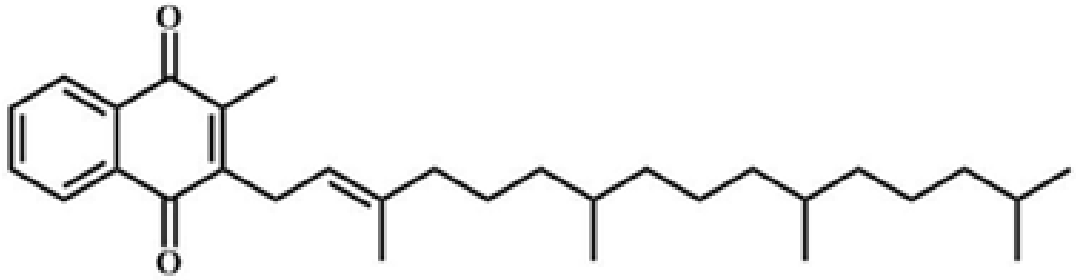
- High doses (100,000 IU for weeks or months) can cause loss of appetite, nausea, thirst, and weakness.
  - The upper limit is 100 µg/day (4,000 IU/day) for individuals ages 9 years or older, with a lower level for those under age 9 years.
- Enhanced Ca^{2+} absorption and bone resorption results in hypercalcemia, which can lead to the deposition of calcium salts in soft tissue (metastatic calcification). **Vitamin D toxicity can lead to hyperkalemia too.**
- Toxicity is only seen with the use of supplements.

Forms of vitamin K

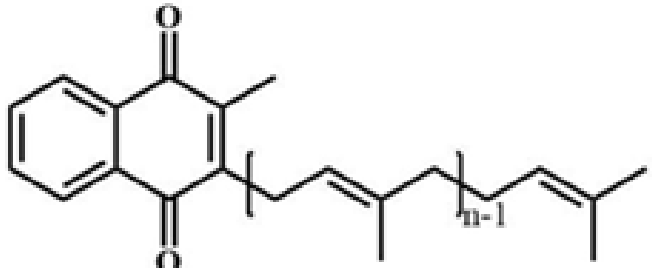
K from coagulation but in latin

- Vitamin K exists in several active forms:
  - As phylloquinone (or vitamin K1) in plants
  - As menaquinone (or vitamin K2) in intestinal bacteria
  - A synthetic form of vitamin K, menadione, can be converted to K2.

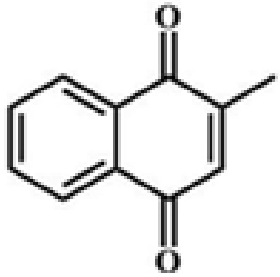
So it is difficult to have vitamin k deficiency because bacteria take care of it.



Vitamin K_1
(phylloquinone)



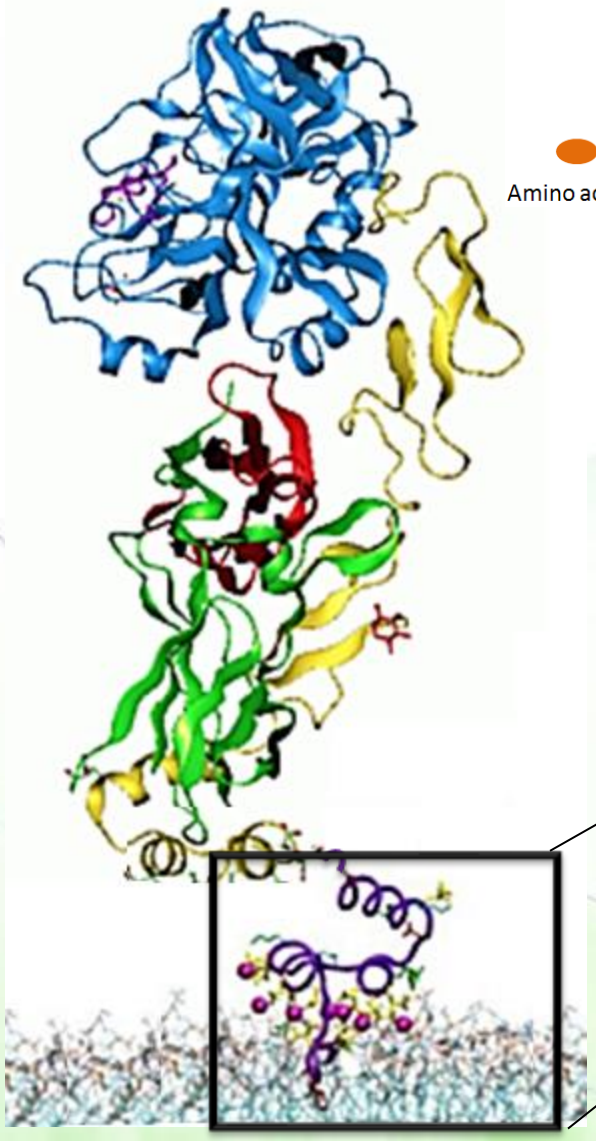
Vitamin K_2
(menaquinone)

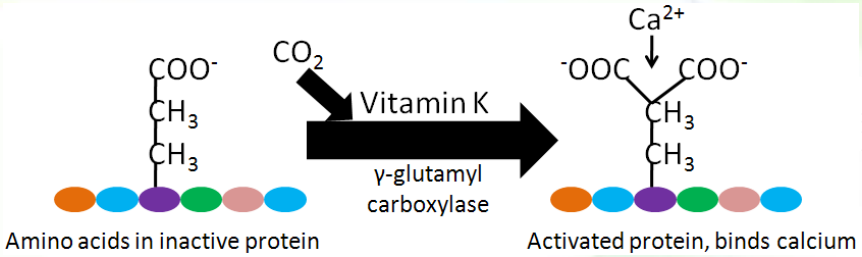


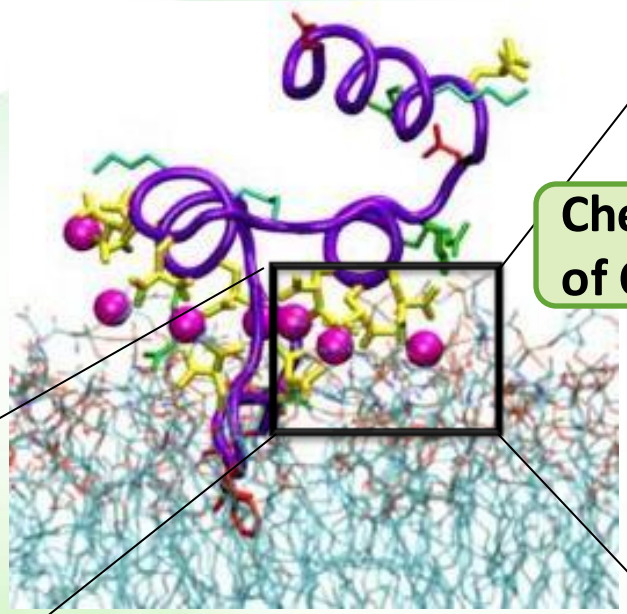
Vitamin K_3
(menadione)

- It is found in cabbage, spinach, egg yolk, and liver.
- There is also synthesis of the vitamin by the gut microbiota.

Zoom into the zoom



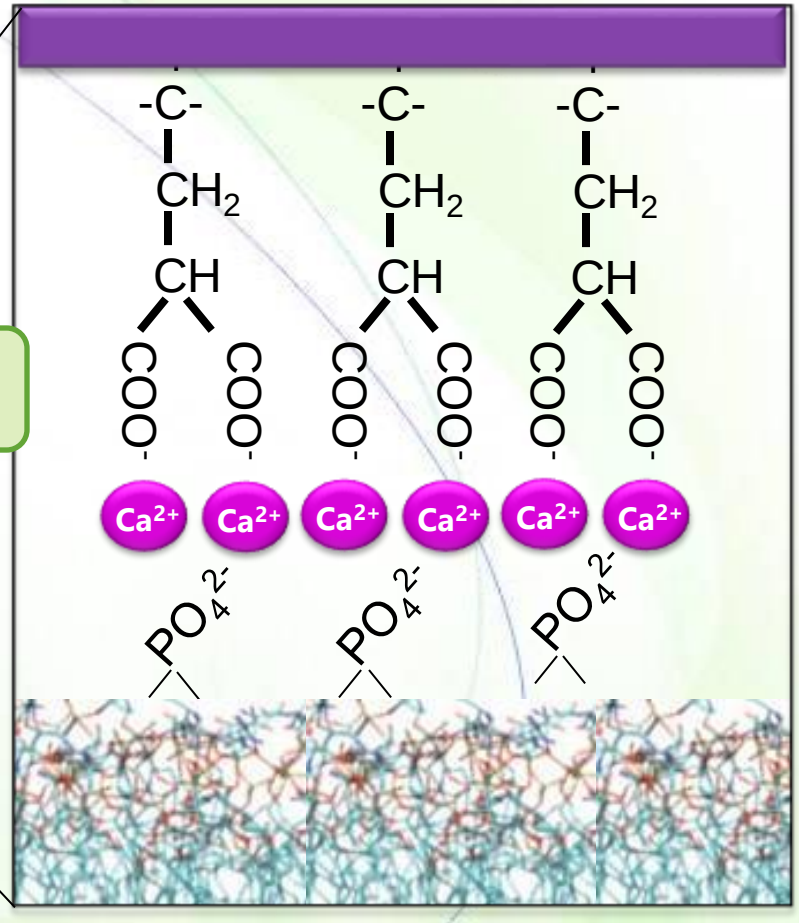




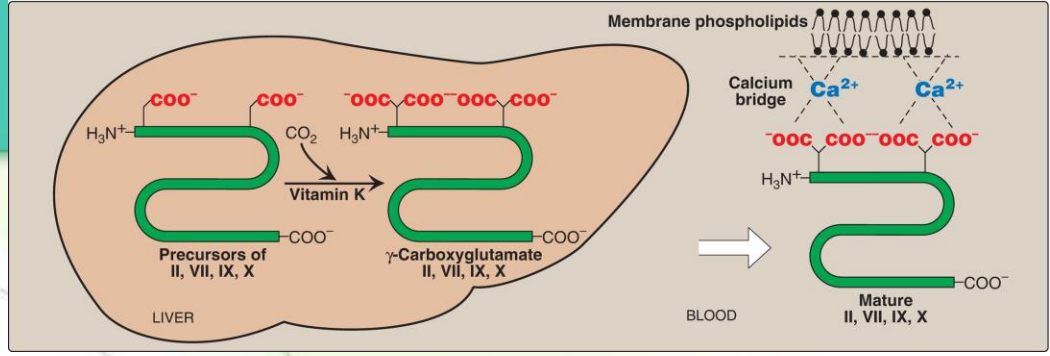
A carboxyglutamate (γ) domain

FLγγVRKGNLγRγCVγγTCSYγγAFγALγSSTATDVFWAKYTA

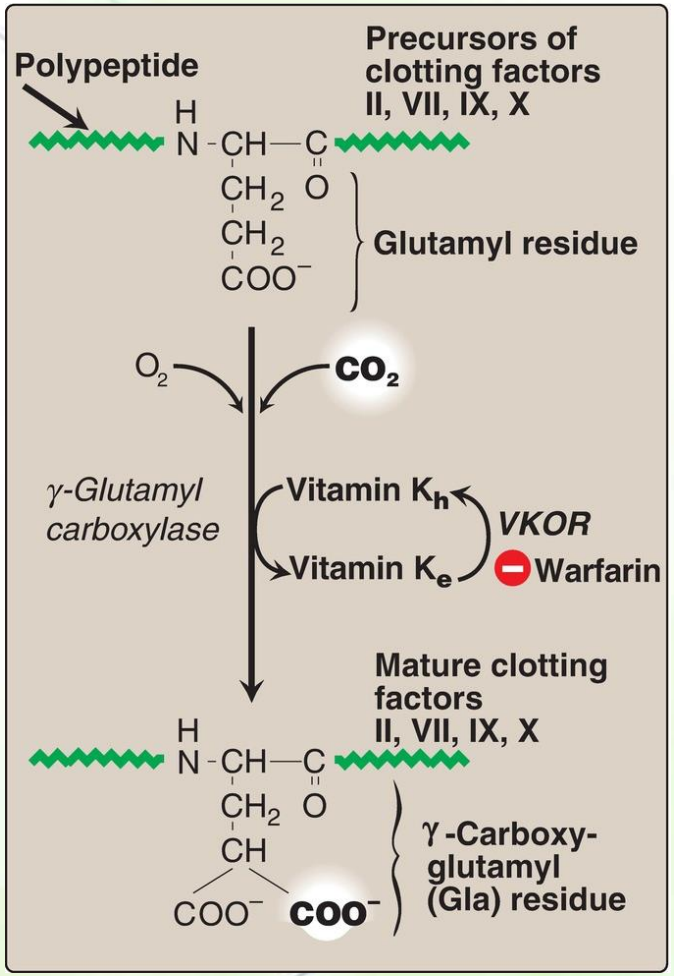
Chelation of Ca^{2+}



Function of vitamin K



- It serves as a coenzyme in the carboxylation of certain glutamate residues (γ-Carboxyglutamate) of proteins involved in blood clotting.
- γ-Carboxyglutamate allows the attachment of these proteins on the surface of platelets via calcium chelation facilitating their activation and promotion of blood clotting.
- Formation of Gla (γ-Carboxyglutamate) residues is sensitive to inhibition by warfarin, a synthetic analog of vitamin K that inhibits vitamin K epoxide reductase, the enzyme required to regenerate the functional hydroquinone form of vitamin K.



Clinical indications for vitamin K

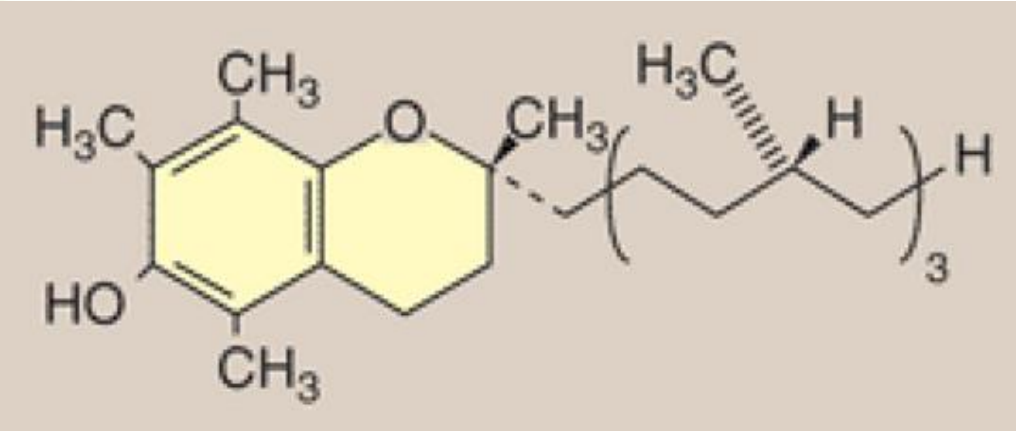
- Vitamin K deficiency in adults is unusual but can occur after long antibiotic treatment.
  - Certain cephalosporin antibiotics (for example, cefamandole) cause hypoprothrombinemia, apparently by a warfarin-like mechanism that inhibits vitamin K epoxide reductase.
    - Treatment is usually supplemented with vitamin K.
- Deficiency is mainly restricted in newborns due to:
  - Sterile intestines.
  - Human milk is a poor source of vitamin K.
    - it is recommended that all newborns receive a single intramuscular dose of vitamin K as prophylaxis against hemorrhagic disease of the newborn.

Toxicity

- Prolonged administration of large doses of menadione can produce hemolytic anemia and jaundice in the infant, because of toxic effects on the RBC membrane.
- Therefore, it is no longer used to treat vitamin K deficiency.
- No upper limit for the natural form has been set.

Vitamin E

- There are different forms of vitamin E, but α-tocopherol is the most active form.
- The primary function is as an antioxidant.
  - It has a long hydrocarbon chain that is inserted in plasma membrane so it protect membrane fatty acids from ROS



- V2:Retinoic acid → Retinal.
- Slide 24

- V3–> Slide 25
- **(which is important for tissue toughness) "it was somehow written in wrong place along with the right place"**
- Slide 31–> Additional things