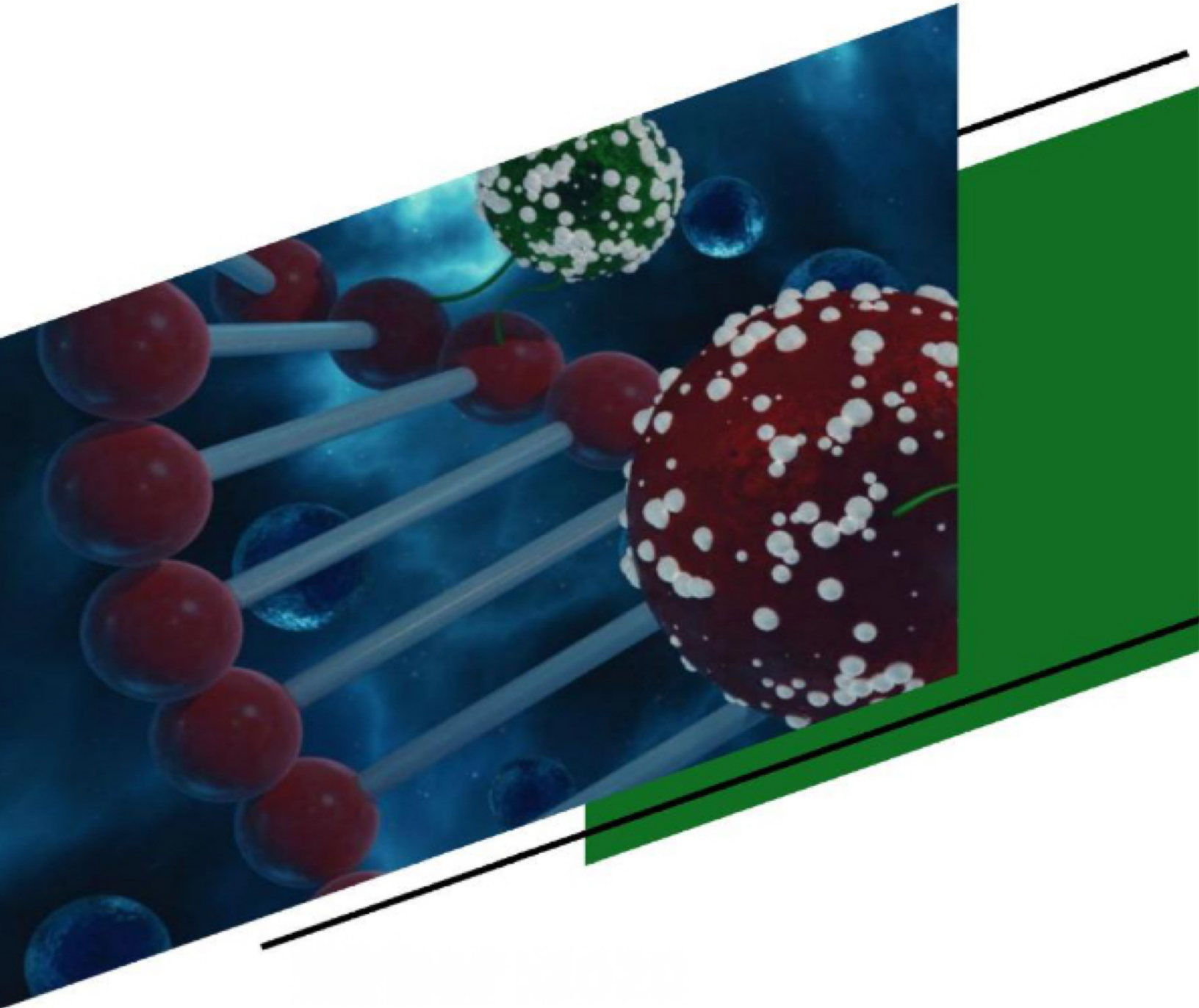


Doctor 021

METABOLISM

Sheet no.34



Doctor : Diala Abu Hasan

Synthesis of Deoxyribonucleotides

U must have noticed that when we synthesised ADP or GDP it was in the ribo form not Deoxyribo that should be in the DNA, so we need to reduce them.

2'-deoxyribonucleotides are produced from ribonucleoside diphosphates by the enzyme **ribonucleotide reductase** during the S-phase of the cell cycle.

The same enzyme acts on pyrimidine ribonucleotides

1. Ribonucleotide reductase (RR), RR is specific for the reduction of:

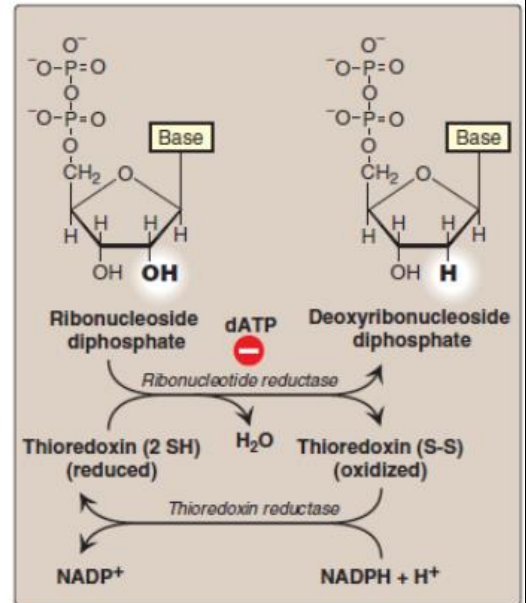
A. Purine nucleoside diphosphates (ADP and GDP) to form their deoxyforms (dADP and dGDP).

B. Pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxyforms (dCDP, and dUDP).

Notice that RR needs **Thioredoxin** to oxidize it and form disulfide bond and Thioredoxin must be reduced again.

2. Regeneration of reduced enzyme: Thioredoxin—a peptide coenzyme of RR

3. Regeneration of reduced thioredoxin: Thioredoxin must be converted back to its reduced form **NADPH + H⁺** are needed. The reaction is catalyzed by **thioredoxin reductase**.



Regulation of deoxyribonucleotide

Ribonucleotide reductase is composed of two non-identical dimeric subunits, R1 and R2

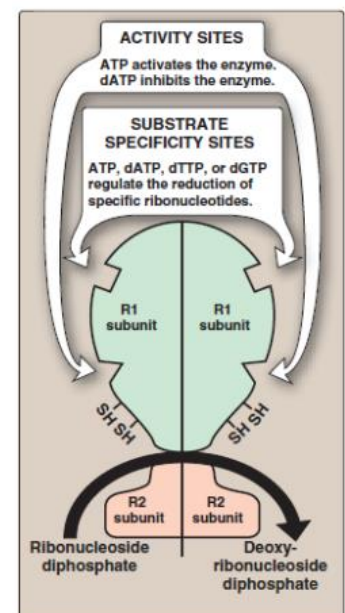
RR is responsible for maintaining a balanced supply of the deoxyribonucleotides required for DNA synthesis.

1. Activity sites (allosteric sites): A- **dATP (product) inhibits the enzyme** and prevents the reduction of **any of the four nucleoside diphosphates** resulting in preventing DNA synthesis.

B- ATP (substrate) activates the enzyme.

2. Substrate specificity sites (allosteric sites): Nucleoside triphosphates regulate substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides. (any ribonucleotides will bind to this site it will activate the reduction of other kinds of ribonucleotides ex:

dTTP binding activates the reduction of GDP to dGDP at the catalytic site



- What's the purpose from this kind of regulation??
- Because we need the 4 deoxyribonucleotides for the DNA so any increase in one of them will result in activation and increase in the other kinds.

Pharmacology application ☹ Hydroxyurea and ribonucleotide reductase

The drug **hydroxyurea** destroys the free radical required for the activity of ribonucleotide reductase

Hydroxyurea inhibits the generation of substrates for DNA synthesis.

Hydroxyurea has been used in the treatment of cancers such as CML (Chronic myeloid leukemia)

Degradation of Purine Nucleotides

The main source of nucleotides is the process of synthesizing them (whether de novo or salvage), also we can obtain them from diet

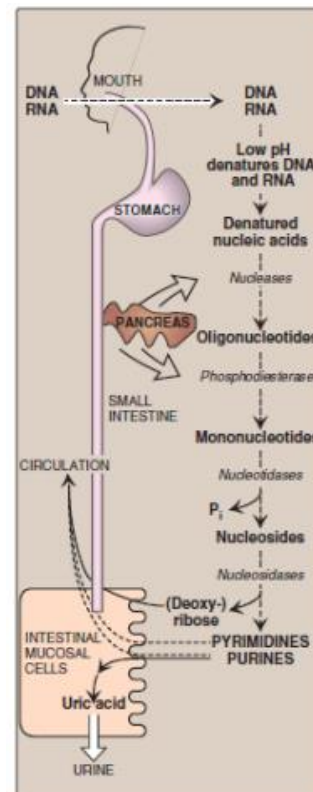
Dietary nucleic acids degradation occurs in the small intestine by a family of pancreatic enzymes (**nucleases and phosphodiesterases**) that hydrolyze the **nucleic acids to nucleotides**.

In the intestinal mucosal cells, purine nucleotides are degraded by **nucleotidases** to **nucleosides** and **free bases**, with **uric acid** as the end product of this pathway.

Purine nucleotides from de novo synthesis are degraded in the liver primarily.

The free bases are sent out from liver and salvaged by peripheral tissues.

Notice no degradation occurs in the mouth or stomach.



Degradation of Purine Nucleotides

1- Degradation of dietary nucleic acids in the small intestine

Ribonucleases and **deoxyribonucleases**, secreted by the pancreas, hydrolyze dietary RNA and DNA to **oligonucleotides**.

Oligonucleotides are further hydrolyzed by pancreatic **phosphodiesterases**, producing a mixture of 3'- and 5'-**mononucleotides**.

In the intestinal mucosal cells, **nucleotidases** remove the phosphate groups hydrolytically, releasing nucleosides that are further degraded to **free bases**.

Dietary purine bases are not an appreciable source for the synthesis of tissue nucleic acids.

Dietary purines are generally converted to uric acid (excreted in urine) in intestinal mucosal cells.

2- Formation of uric acid

General view for GMP first we will convert it to Guanosine via 5'-nucleotidase then we remove the ribose (in the form of ribose 1-phosphate which can be isomerized and used in other pathways) resulting in the Guanine via **Purine nucleoside phosphorylase enzyme** then we will deaminate the guanine through **guanase enzyme** forming xanthine which will be oxidized to uric acid via **xanthine oxidase enzyme**.

For AMP we have 2 pathways that will lead to the same product.

(from doc slides ↓↓) all required

[1] An amino group is removed from AMP to produce IMP by AMP deaminase, or from adenosine to produce inosine (hypoxanthineribose) by adenosine deaminase.

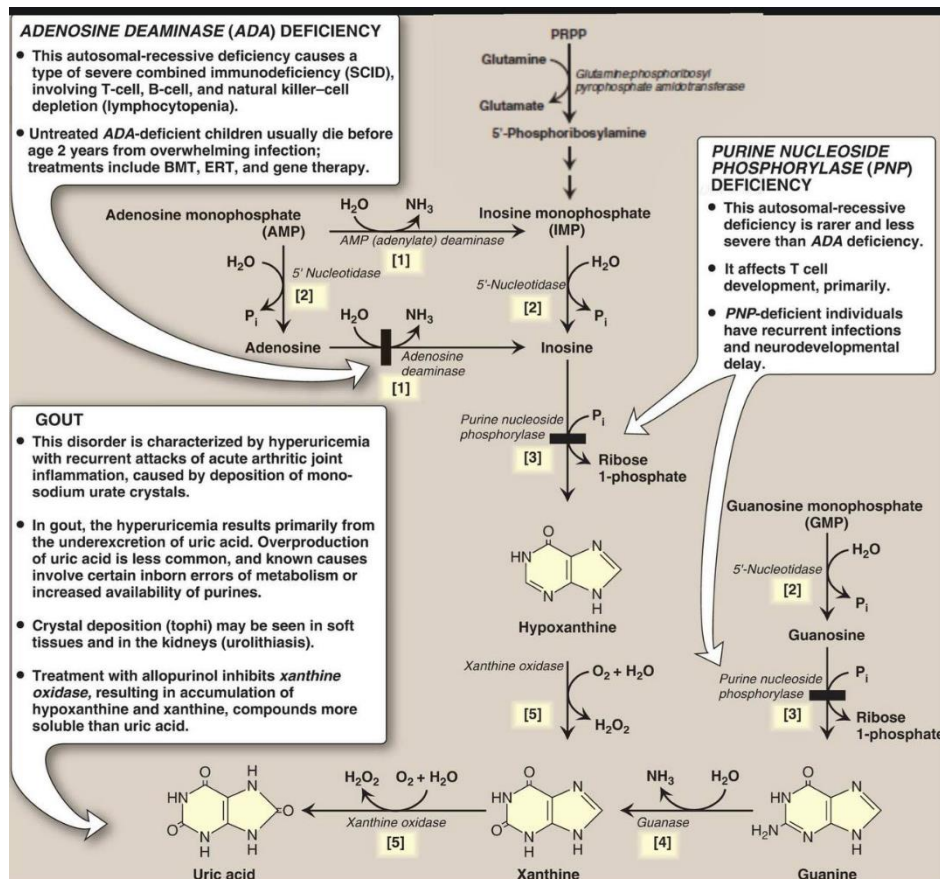
[2] IMP and GMP are converted into their nucleoside forms—inosine and guanosine—by the action of 5'-nucleotidase.-*

[3] Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine.

Note: A mutase interconverts ribose 1- and ribose 5-phosphate.

[4] Guanine is deaminated to form xanthine.

[5] Hypoxanthine is oxidized by xanthine oxidase to xanthine, which is further oxidized by xanthine oxidase to uric acid, the final product of human purine degradation.



Disease associated with purine degradation

Gout: high levels of uric acid in blood (hyperuricemia)

Hyperuricemia due to either the **overproduction (minor cause)** or **under excretion of uric acid (major cause)**.

Hyperuricemia lead to the deposition of monosodium urate crystals in the joints, and an inflammatory response to the crystals, causing first acute and then chronic gouty arthritis.

Nodular masses of monosodium urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout.



Formation of uric acid stones in the kidney (**urolithiasis**)

Hyperuricemia is typically asymptomatic and does not lead to gout, but gout is preceded by hyperuricemia.

Diagnosis of gout requires aspiration and examination of synovial fluid from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped monosodium urate crystals.

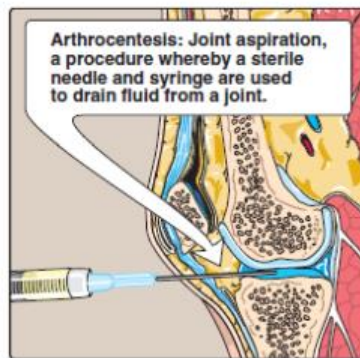


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.



Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.

Causes of hyperuricemia

As a physician I must know what's the cause of hyperuricemia to choose the appropriate treatment so if the patient cause is under-excretion I must give him drug that enhance the kidney functions without affecting the purine synthesis, but if the cause was overproduction I should give him a drug that interferes with the purine synthesis.

Underexcretion of uric acid:

Most gout patients in the vast majority of patients,

Underexcretion can be primary (due to unidentified inherent excretory defects) Or secondary to:

1. A known disease that affects the kidney function in handling urate, such as lactic acidosis (lactate and urate compete for the same renal transporter)

2. Environmental factors such as drugs (thiazide diuretics)

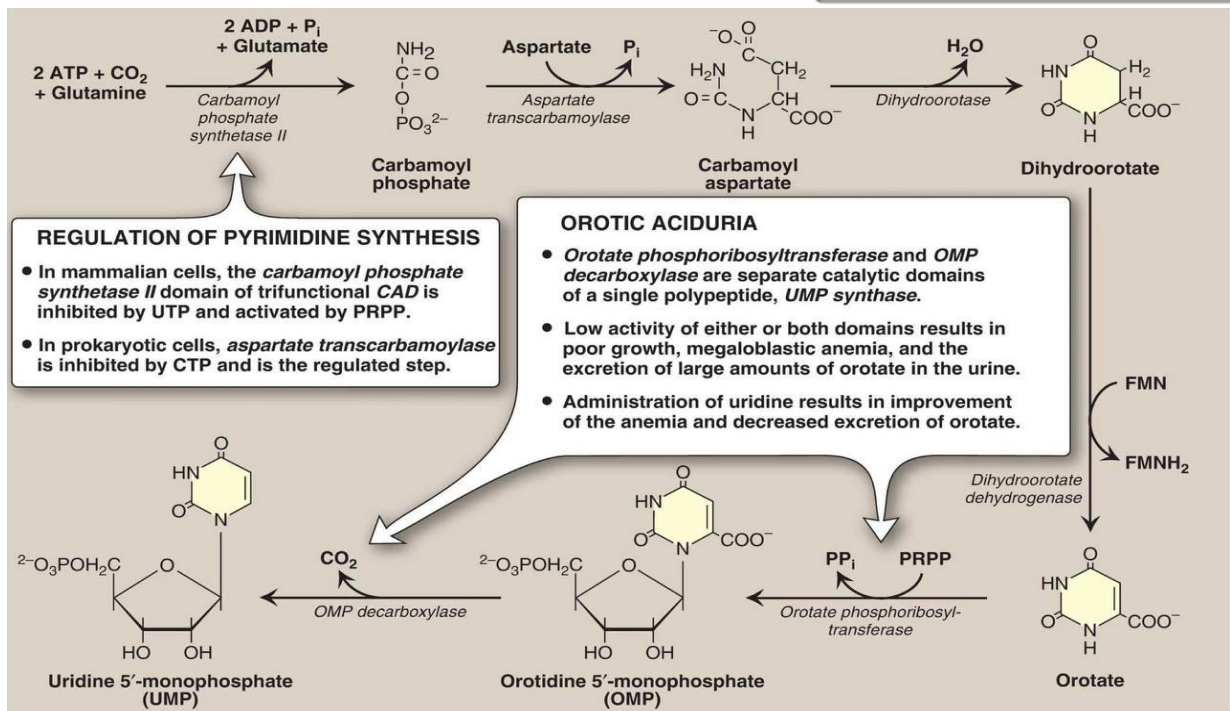
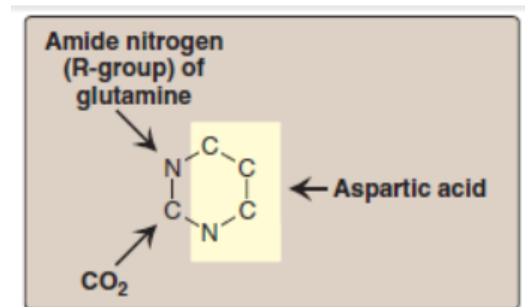
3. Exposure to lead (saturnine gout)

Overproduction of uric acid: less common.

Several identified mutations in the X-linked PRPP synthetase gene that increase PRPP production

Pyrimidine Synthesis

- ❖ The pyrimidine ring is synthesized before being attached to ribose 5-phosphate.
- ❖ Ribose 5-phosphate is donated by PRPP.
- ❖ Sources of the 6 atoms ring: Aspartic acid (gives 4), glutamine (1) and CO₂ (1)



1.Synthesis of carbamoyl phosphate

The regulated step of this pathway (pyrimidine synthesis) in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO₂ and 2 ATP via Carbamoyl phosphate synthetase II (CPS II).

CPS II is inhibited by UTP (the end product of this pathway) CPS II is activated by PRPP.

- ❖ Remember (CPS I) catalyze the same reaction in Urea cycle but the question is how the cell decide if the Carbamoyl phosphate that result from this reaction will continue into urea cycle or pyrimidine synthesis?? by the special regulation meaning that every enzyme is located in different places in the cell CPS I in the mitochondria in the hepatocytes while CPS II in the cytosol in many cells.

2.Synthesis of orotic acid

- ❖ After we form carbamoyl phosphate, aspartate AA will enter and form a **carbamoyl aspartate** via aspartate **transcarbamoylase** releasing phosphate, carbamoyl aspartate will be reduced through a **reductase** and form **dihydroorotate** which will be oxidized via **dihydroorotate dehydrogenase** and form **Orotate**.
- ❖ The enzyme that produces orotate, dihydroorotate dehydrogenase, is associated with the inner mitochondrial membrane.
- ❖ All other enzymes in pyrimidine biosynthesis are cytosolic.
- ❖ The first three enzymic activities in this pathway (CPS II, aspartate transcarbamoylase, and dihydroorotase) are three different catalytic domains of a single polypeptide chain (CAD)

3.Formation of a pyrimidine nucleotide

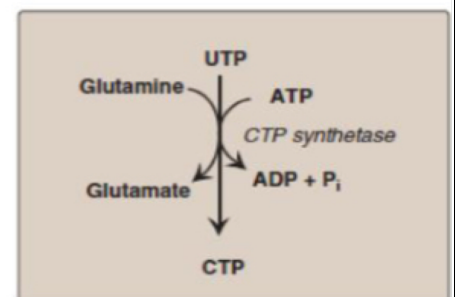
- ❖ After we form an orotate a ribose sugar with phosphate will be transferred from **PRPP** to orotate and forms orotidine 5-monophosphate (OMP) via **Orotate phosphoribosyl transferase** enzyme then OMP will get decarboxylated via **OMP decarboxylase** and forms UMP.
- ❖ The completed pyrimidine ring is converted to the nucleotide orotidine 5' monophosphate (OMP), or the parent pyrimidine mononucleotide.
- ❖ **The reaction releases pyrophosphate, thus, it is irreversible.**
- ❖ Both purine and pyrimidine synthesis require Gln, Asp, and PRPP as essential precursors.
- ❖ Orotate phosphoribosyl transferase and orotidylate decarboxylase are catalytic domains of a single polypeptide chain called UMP synthase.

لا تنسى بعد كل خطوة ترجع للصورة فوق...

- Orotic aciduria, a rare genetic defect, caused by a deficiency of one or both activities of the bifunctional UMP synthase resulting in orotic acid in the urine
- UMP is phosphorylated to UDP and then UTP.
- The UDP is a substrate for ribonucleotide reductase, which generates dUDP.
- The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase (dUTPase).
- dUTPase reduces the available dUTP for DNA synthesis, thus preventing incorporation of uracil into DNA.

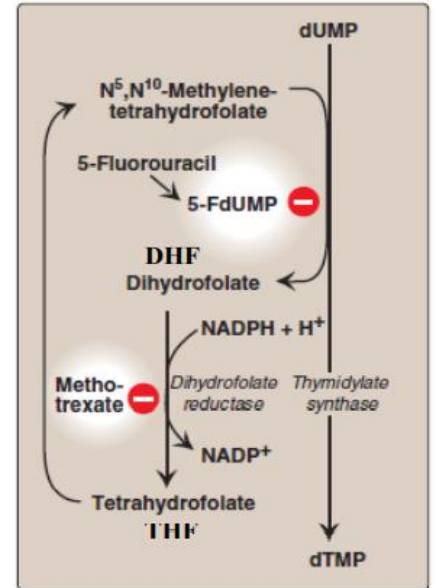
4.Synthesis of UTP and cytidine triphosphate (CTP)

- ❖ After we form UMP it will get phosphorylated to UTP after that a **CTP synthetase** enzyme will take an amino group from glutamine and form a CTP.
- ❖ Some CTP is dephosphorylated to CDP (a substrate for ribonucleotide reductase)
- ❖ Remember ribonucleotide reductase works on nucleoside diphosphate not triphosphate.
- ❖ dCDP can be phosphorylated to dCTP for DNA synthesis.



5.Synthesis of thymidine monophosphate (TMP) from dUMP

- ❖ To form TMP we need a dUMP so the UMP that results from the pyrimidine synthesis must be reduced to form dUMP, then dUMP will get a methyl group from N5, N10 methylene tetrahydrofolate (folic acid) via **Thymidylate synthase** and forms dTMP.
- ❖ N5, N10 methylene tetrahydrofolate after it donates the methyl group, it become Dihydrofolate(DHF) and should be reduced again via Dihydrofolate reductase to form the tetrahydrofolate (THF).
- ❖ Thymidylate synthase inhibitors include thymine analogs such as 5-fluorouracil (antitumor agents).
- ❖ 5-Fluorouracil (**suicide inhibitor**) is converted to 5-FdUMP that **permanently** binds to the inactivated thymidylate synthase
- ❖ Methotrexate inhibits dihydrofolate reductase
- ❖ Methotrexate reduces THF, inhibits purine synthesis and prevents methylation of dUMP to dTMP, resulting in **DNA synthesis inhibition and cell growth slow down** .
- ❖ 5-Fluorouracil and Methotrexate are **anti cancerous agents**.



CPS I Versus CPS II

- ❖ Carbamoyl phosphate, which is synthesized by CPS I, is a precursor of urea.
- ❖ Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate.

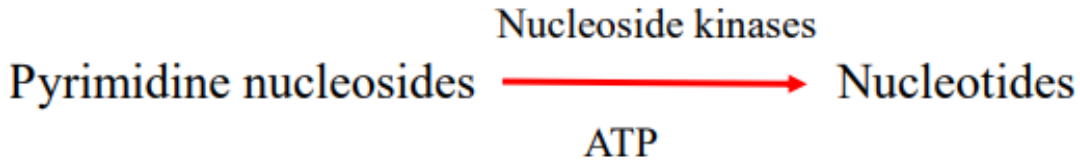
	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ -Amide group of glutamine
Regulators	Activator: N-acetyl-glutamate	Activator: PRPP Inhibitor: UTP



Pyrimidine Salvage and Degradation Salvage

Few pyrimidine bases are salvaged in human cells

Mechanism:



Degradation:

The pyrimidine ring is opened and degraded to highly soluble products (β -alanine and β -aminoisobutyrate) with the production of NH_3 and CO_2 .

Remember the degradation of purines produce Uric acid as we mentioned above.

Important points

Orotic aciduria, a rare genetic defect, caused by a deficiency of one or both activities of the bifunctional UMP synthase resulting in orotic acid in the urine.

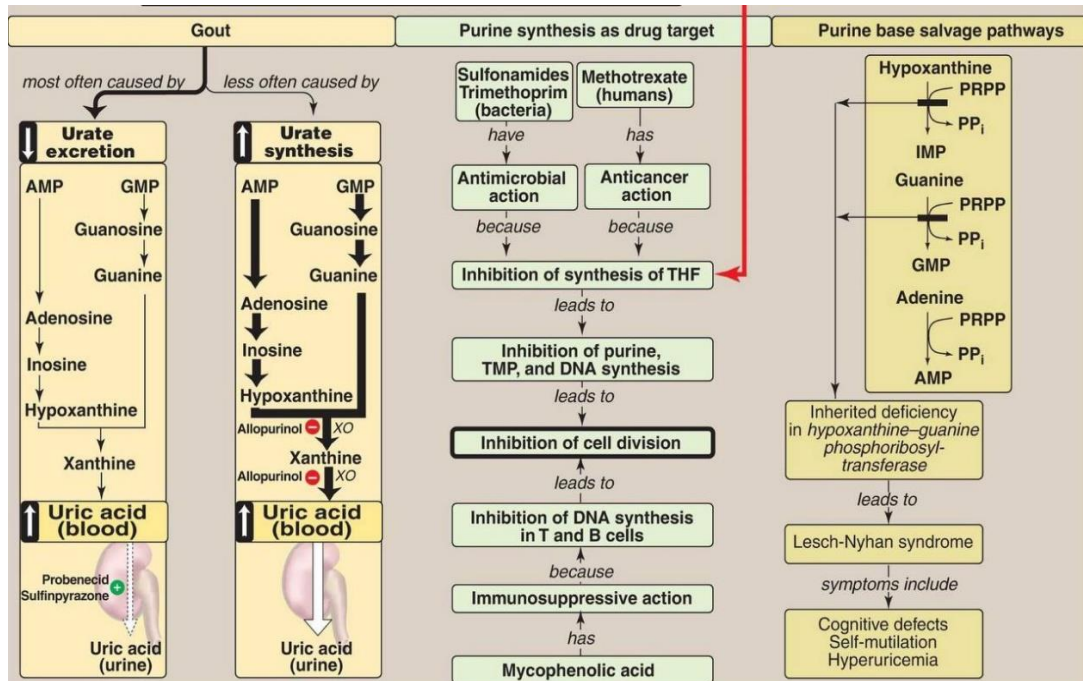
UMP is phosphorylated to UDP and then UTP.

The UDP is a substrate for ribonucleotide reductase, which generates dUDP.

Remember Uracil can't be in the DNA so What prevents the dUTP from being used in DNA synthesis?? **It's the dUTPase**

The dUDP is phosphorylated to dUTP, which is rapidly hydrolyzed to dUMP by **UTP diphosphatase (dUTPase)**.

dUTPase reduces the available dUTP for DNA synthesis, thus preventing incorporation of uracil into DNA.



V1
