Metabolism

فريق طوفان الأقصى

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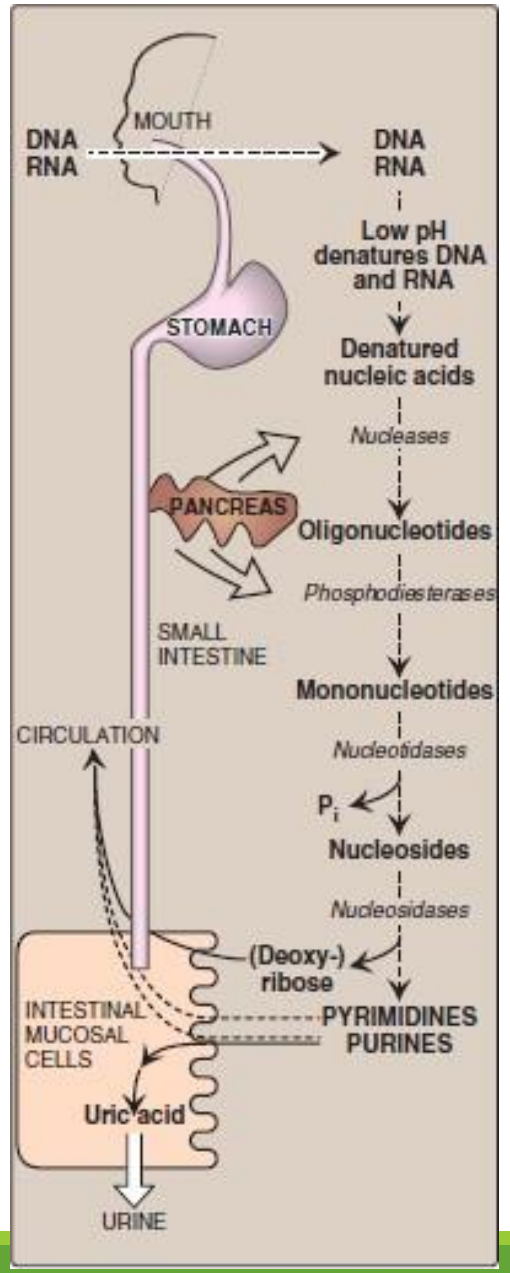
Degradation of Purine Nucleotides

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



- **The complement in this slide:** Nucleotides are degraded either in hepatocytes or intestinal cells or other places. The scheme of degradation is simple. We have nucleotides that contain nitrogen bases, phosphates, sugars → 1st remove phosphate → 2nd remove sugar → 3rd degrade nitrogen bases (purines into uric acids)

Degradation of Purine Nucleotides

A. Degradation of dietary nucleic acids in the small intestine

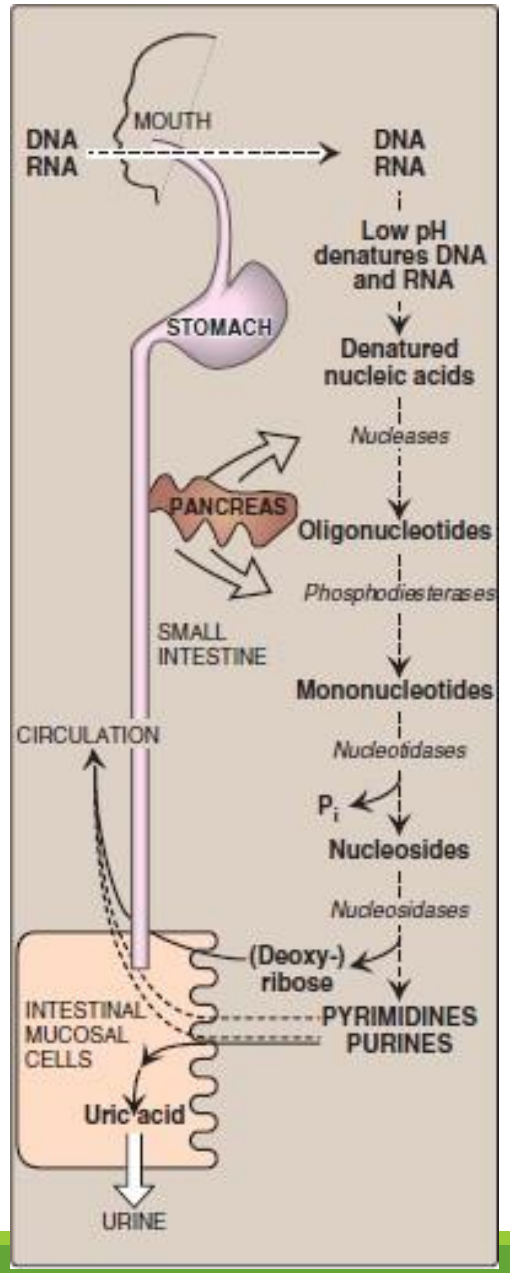
Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA to oligonucleotides.

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.



■ **The complement in this slide:** Degradation of Purine include: 1. digestion from dietary resources 2. degradation as Part of Metabolism (excretion if we have high amounts)

→ If we get these nucleotides from dietary resource (we consume them as nucleic acids i.e. DNA or RNA) --degraded--> Oligonucleotides (10 nucleotides) by **Pancreatic** enzymes called **Nucleases**.

→ Oligonucleotides --Simplified--> Mononucleotides by another Pancreatic enzymes called **phosphodiesterases.**

Inside the Cells:

mononucleotides (from diet, synthesized, or the degradation of mRNA(endogenous source) !!) are degraded by **nucleotidases** to nucleosides and inorganic phosphate.

■ **NOTE:** as we know **mRNA** are used in translation process, to synthesize proteins. As long it is in cytosol, it will be active and produce proteins unless it is deactivated by microRNA; to degrade them into nucleotides.

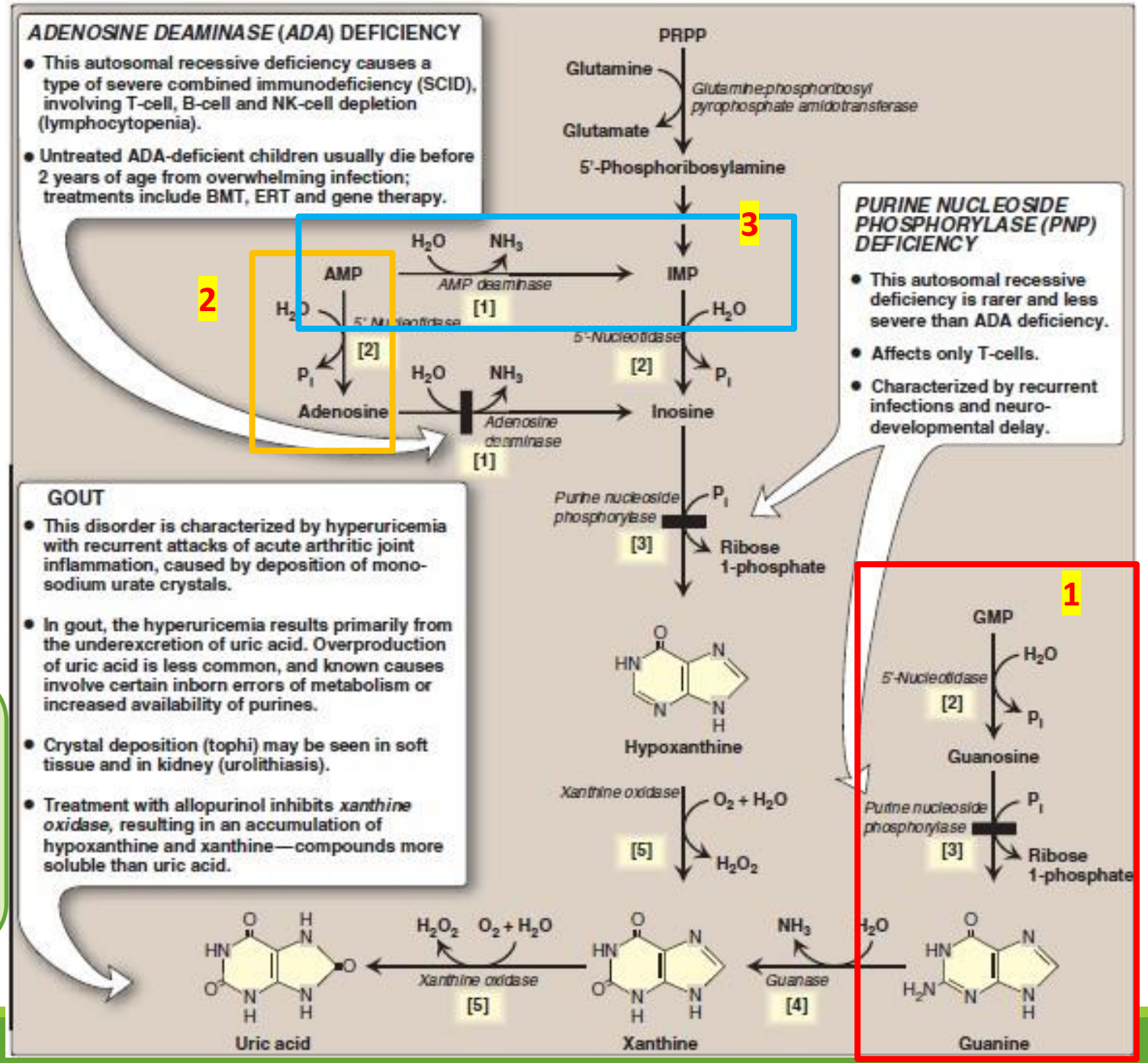
NOTE: Pancreatic enzymes we took so far: Proteases, lipases, amylases, nucleases, phosphodiesterase.

Degradation of Purine Nucleotides

B. Formation of uric acid

NOTE:

- Nucleotidase --> nucleoside+ phosphate
- Nucleosidase --> sugar + nitrogenous base



Degradation of Purine Nucleotides

B. Formation of uric acid

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.

- **The complement in this slide**: Purines are degraded into the same product (Uric Acid), hence the pathways should meet at one point.
- 1. GMP:

GMP → Guanosine by **nucleotidase** (removing phosphate as inorganic phosphate)

Guanosine → Guanine by Purine nucleoside phosphorylase (removing sugar using the free phosphate)

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* Sugar is released as phosphorylated Ribose (Ribose-1-phosphate)
* Nucleotidase & Purine nucleoside phosphorylase are general enzymes (work on GMP & AMP)

Guanine → xanthine releasing free NH3 by Guanase

Xanthine —(Oxidation; O2 → H2O2)—> Uric acid oxidation rxn by xanthine oxidase (**important enzyme**)

* We produced H2O2 (ROS molecule) as a side product.

- **The complement in this slide:**
- **2. AMP:**

AMP → Adenosine by neucleotidases, then we modify nitrogenous bases after that we remove the sugar.

Adenosine → Inosine by **adenosine** deaminase (removing NH3)

Inosine → Hypoxanthine by Purine nucleoside phosphorylase (removing the sugar)

*The sugar is released as Ribose-1-phosphate

The same enzyme (**Xanthine Oxidase**) will work on the 2 coming rxns :

HypoXanthine $\xrightarrow[O2 \rightarrow H2O2]{\text{Oxidation}}$ Xanthine $\xrightarrow[O2 \rightarrow H2O2]{\text{Oxidation}}$ Uric Acid

* 2 H2O2 are produced

3. AMP can be deaminated → IMP by **AMP** deaminase

Then IMP ---> **Inosine** (same intermediate) ----> .. ----> .. ----> Uric acid (same reactions)

Xanthine acts as a connection point between GMP and AMP degradative pathways.

Diseases associated with purine degradation

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

Hyperuricemia due to either the overproduction or underexcretion of uric acid.

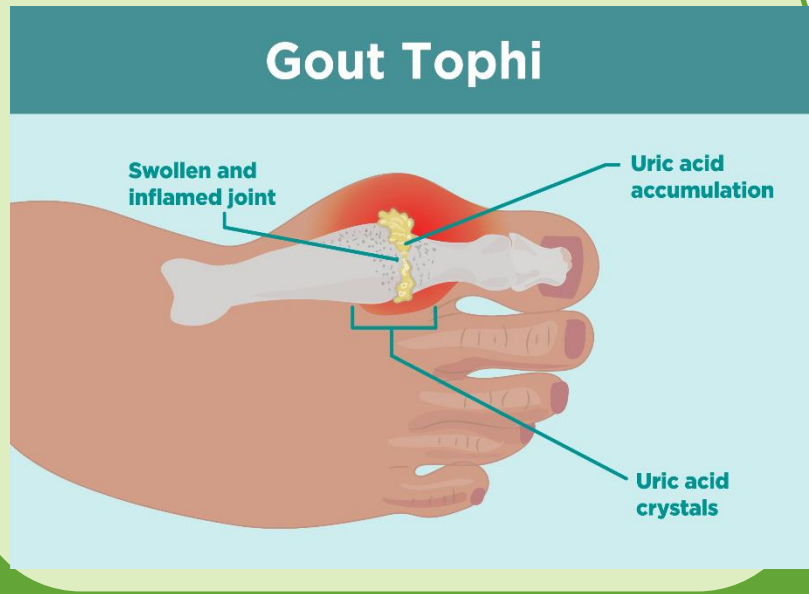
Hyperuricemia lead to the deposition of monosodium urate crystals in the joints, and an inflammatory response to the crystals, causing first acute and then chronic gouty arthritis.

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

In excretion, its concentration in the urine will increase, leading oversaturation
Formation of kidney stones**(Uric acid stones)** in the kidney (urolithiasis).
There are many types of kidney stones, and they differ in their properties
Hyperuricemia is typically asymptomatic and does not lead to gout, but gout is preceded by hyperuricemia.

Figure 22.16
Tophaceous gout.

Attachment:



Diseases associated with purine degradation

1. Gout:

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

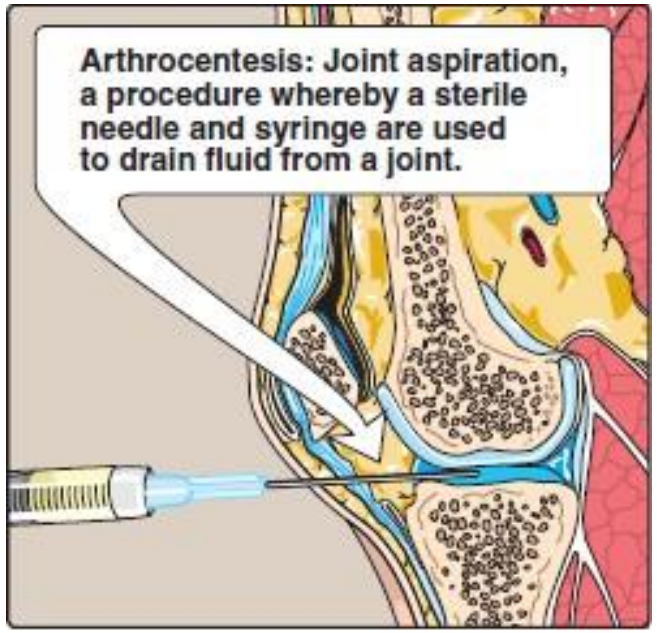


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.

NOTE: monosodium urate crystals

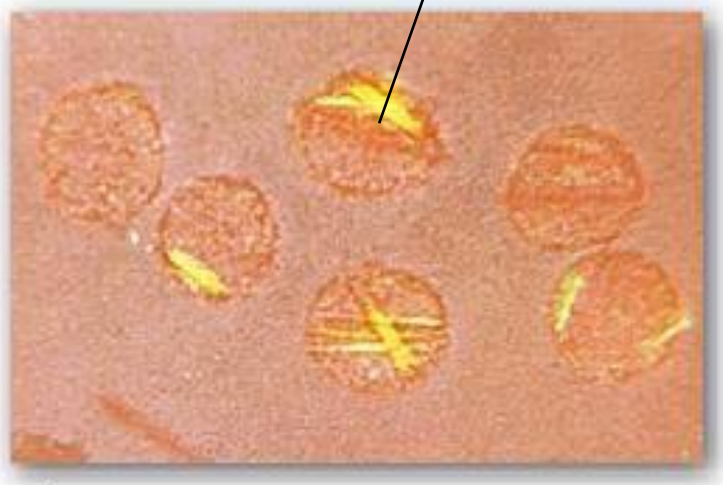


Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within poly-morphonuclear leukocytes.

Causes of hyperuricemia

Underexcretion of uric acid:

Most gout patients In the vast majority of patients,

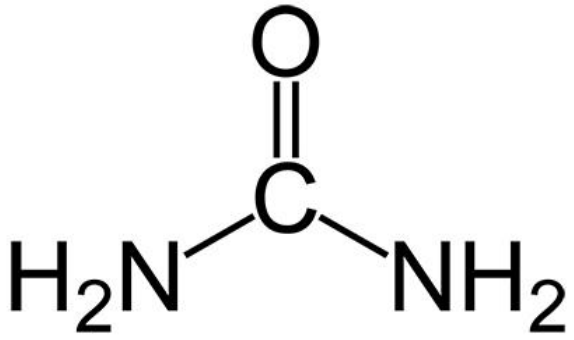
Underexcretion can be primary (due to unidentified inherent excretory defects)

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)

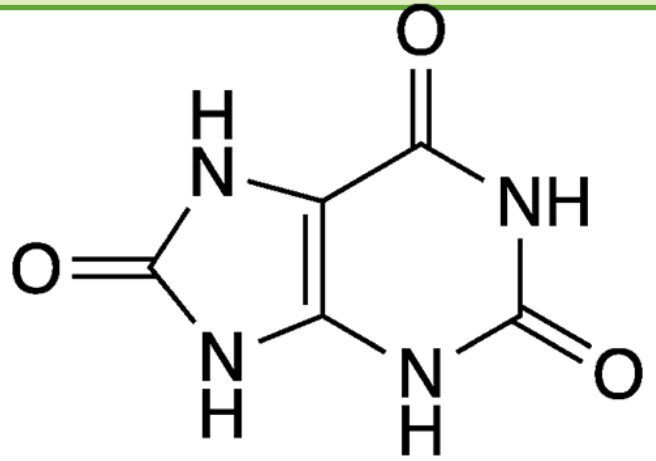
Overproduction of uric acid: less common.
Several identified mutations in the X-linked PRPP synthetase gene that increase PRPP production

- **The complement in this slide:** the treatment depends on the reason whether it is overproduction or under excretion of Uric Acid

- **Overproduction** : inhibit the degradation of Purine especially Xanthine Oxidase (Uric Acid synthesizer)
- **Underexcretion**: Drugs that stimulate more excretion of Uric Acid (thiazide diuretics مدر بول).
- Urea and Uric acid are different structures.



Urea



Uric acid

Pyrimidine Synthesis

The pyrimidine ring is synthesized before being attached to ribose 5-phosphate

Ribose 5-phosphate is donated by PRPP.

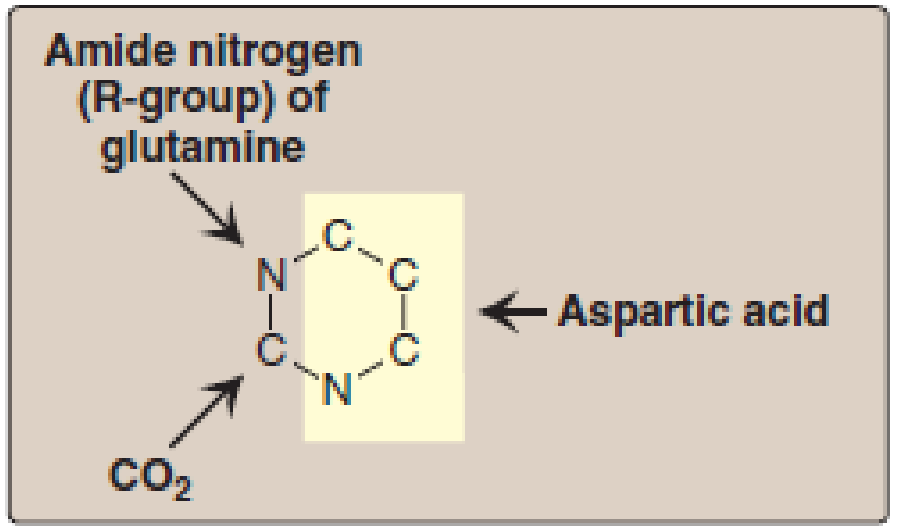


Figure 22.19
Sources of the individual atoms in the pyrimidine ring.

- **NOTE:** de novo pathway of pyrimidine is simpler than purine synthesis.
- Components used : Glutamine, Aspartic Acid, CO_2 .

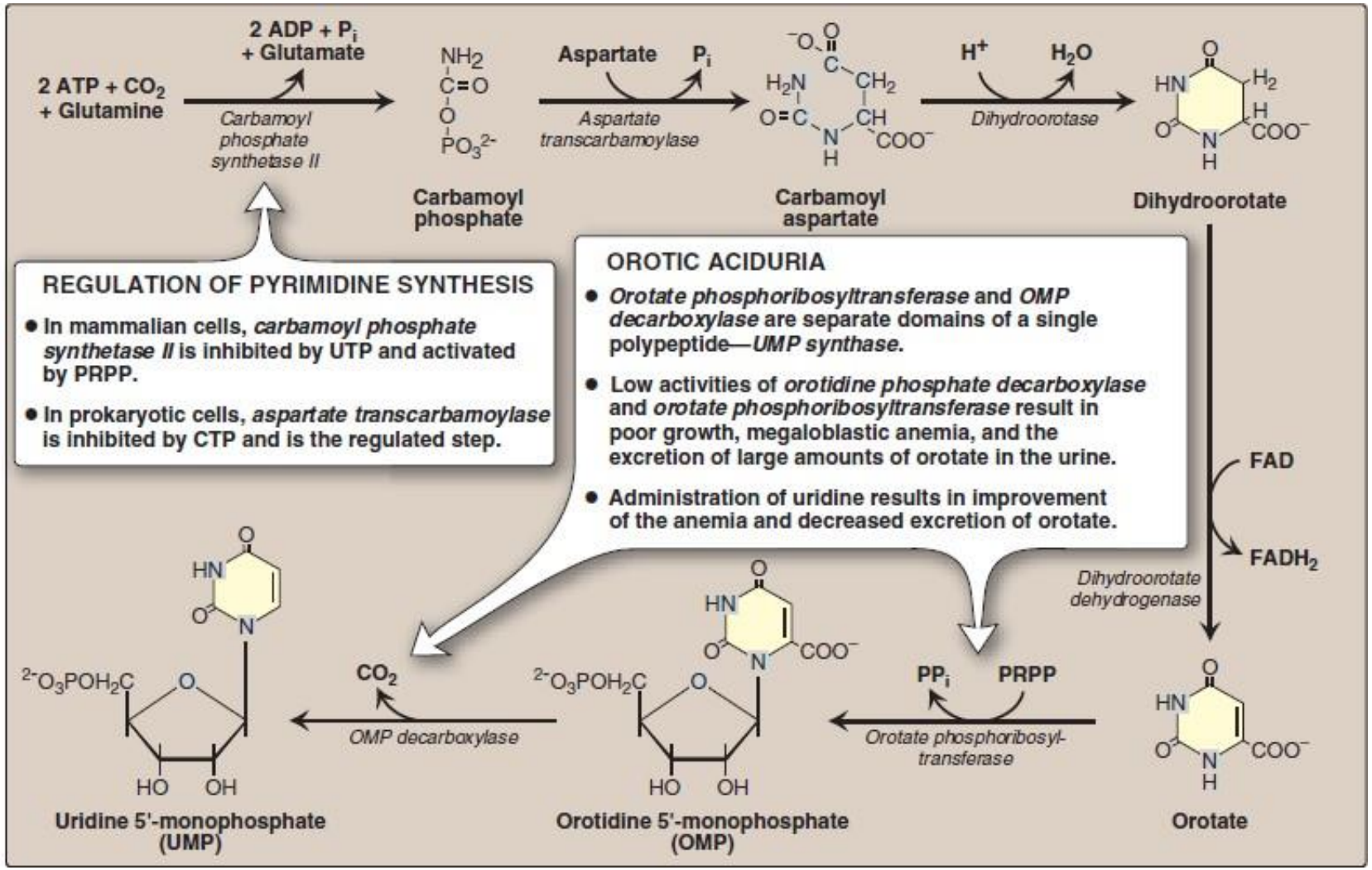
*we used also Glycine & formyltetrahydrofolate in purine synthesis 😄

Pyrimidine Synthesis

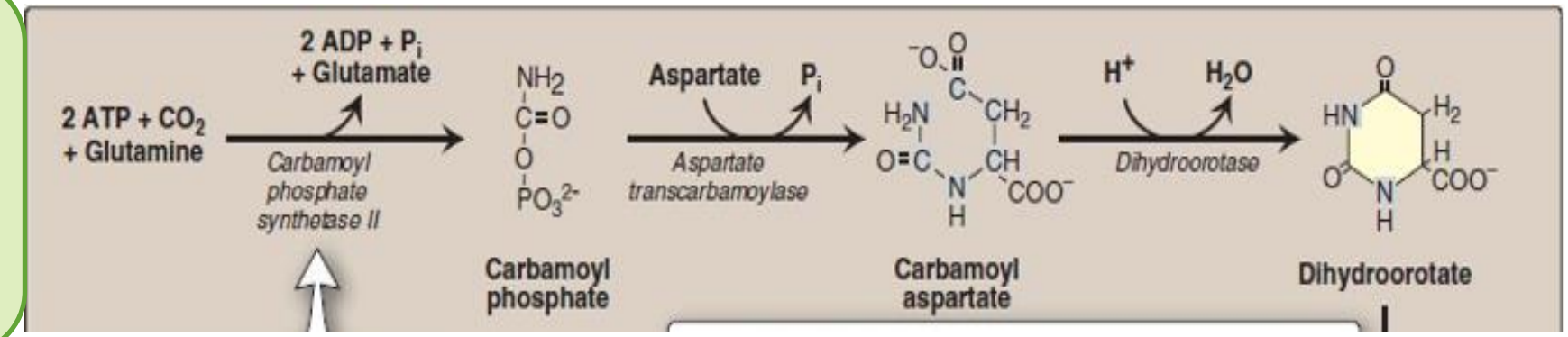
A. Synthesis of carbamoyl phosphate

The regulated step of this pathway in mammalian cells is the synthesis of carbamoyl phosphate from glutamine and CO2

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

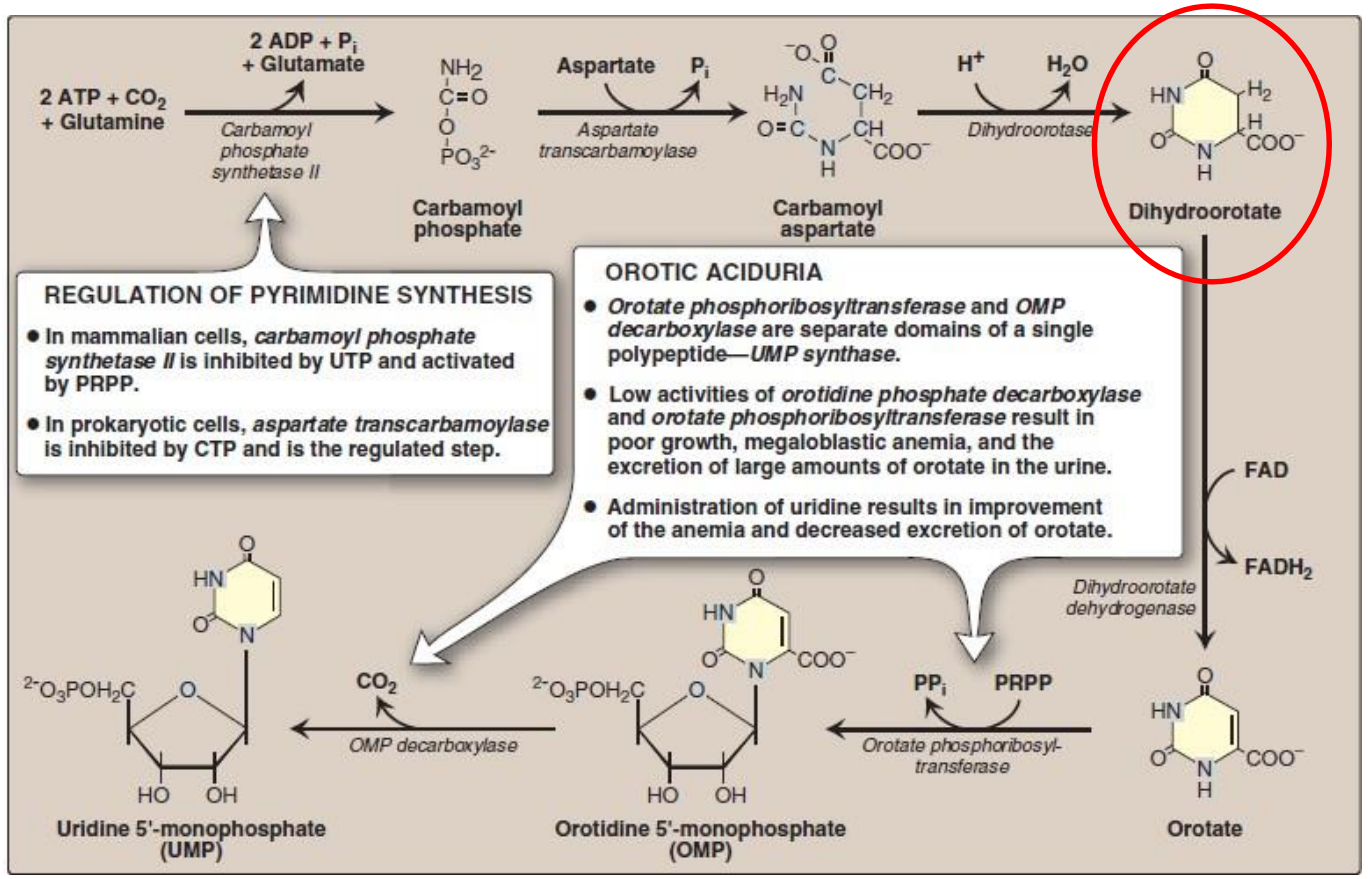


We start with 2ATP, CO2 and Glutamine



- **The complement in this slide**: Carbamoyl phosphate is synthesized from Phosphate + Glutamine + CO_2 by CPS II.
- *Remember, we took CPS I in Urea Cycle, CPS I work on free NH_3 while CPS II take NH_3 from Glutamine.
- Carbamoyl Phosphate → Carbamoyl aspartate, by reacting with Aspartate & releasing Phosphate. Enzyme used Aspartate transcarbamoylase
- * Now we have 6 atoms for the pyrimidine cycle, then enzyme dihydroorotase will induce dehydration rxn يلف بخليه and close the ring ---> Dihydroorotate.

Pyrimidine Synthesis



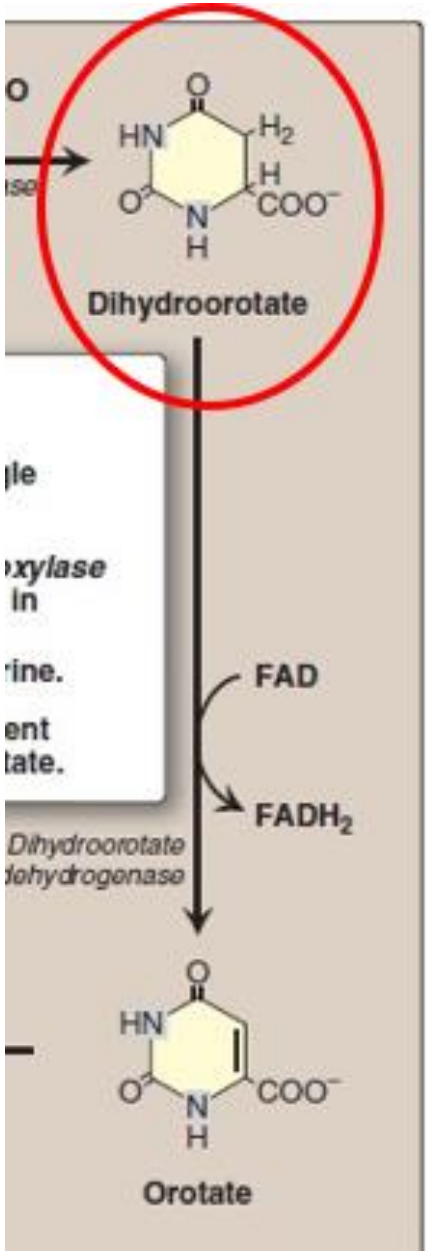
B. Synthesis of orotic acid

The enzyme that produces orotate, dihydroorotate dehydrogenase, is associated with the **inner mitochondrial membrane**.

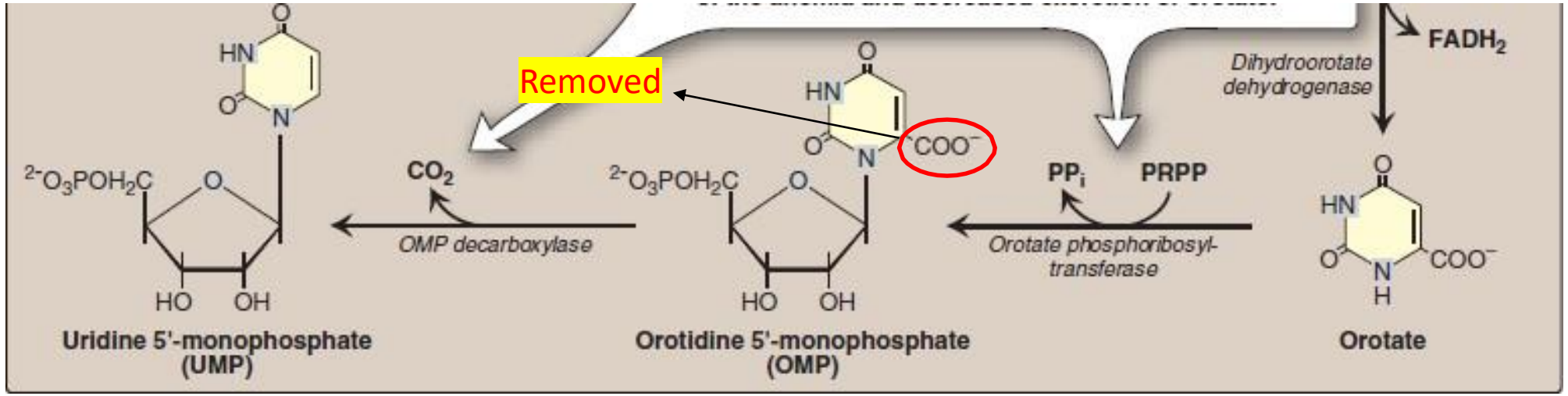
All other enzymes in pyrimidine biosynthesis are **cytosolic**.

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)

- **The complement in this slide:** Now Dihydroorate is the first cycle compound, we need to modify it for the formation of pyramidines (Uracil, Thymine, Cytosine).
- Dihydroorotate ---Oxidation---> Orotate } Enzyme Dihydroorotate Dehydrogenase
  FAD ---Reduction---> FADH2



Pyrimidine Synthesis



C. Formation of a pyrimidine nucleotide

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.

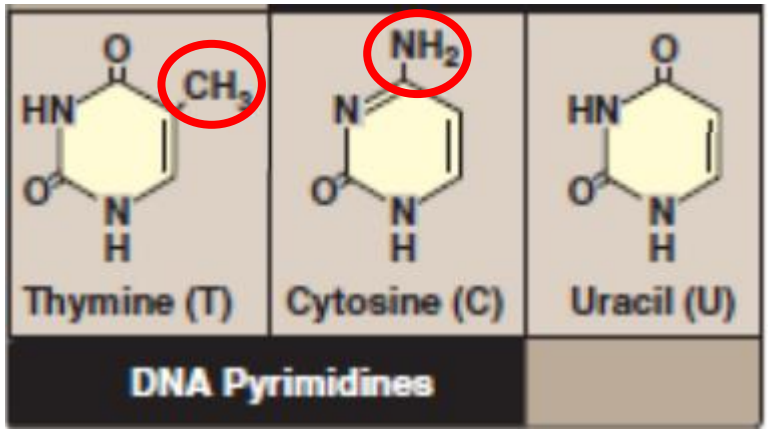
Orotate phosphoribosyl transferase and orotidylate decarboxylase are catalytic domains of a single polypeptide chain called UMP synthase.

- **The complement in this slide:**
- a Phosphorylated Sugar from PRPP will be added to Orotate ----forming----> OMP(the first nucleotide that is formed) by Orotate phosphoribosyl transferase.

*PRPP & PRPP synthetase enzyme are not used as an **initial step** in pyrimidine synthesis, unlike Purine synthesis. In Purine synthesis, we synthesized PRPP then I start the building of nitrogenous base on it but in pyrimidine, we formed nitrogenous base (not in the final form(not A,C,U)) then we added PRPP.

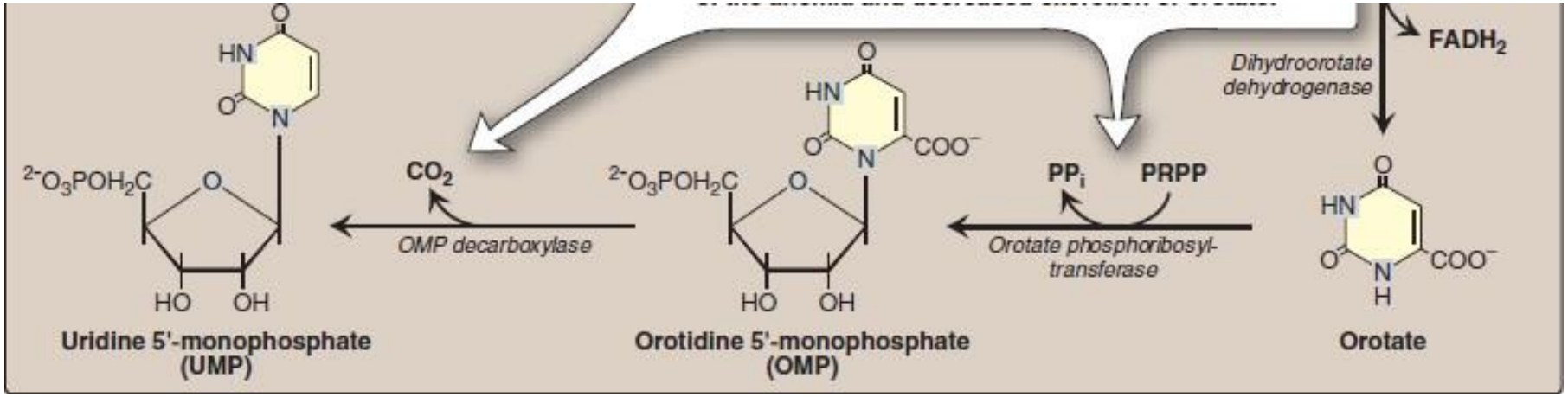
- ❑ OMP is decarboxylated by OMP decarboxylase ----forming---> UMP (Finally !)
- **UMP** is a **branching point** in the pathway, like IMP in purines

- Thymine can be produced by methylation (adding methyl)
- Cytosine can be produced by removing O and adding NH2 group



Pyrimidine Synthesis and Metabolism

One phosphate
and
Ribose sugar
Nitrogenous base



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.

Pyrimidine Synthesis

D. Synthesis of UTP and cytidine triphosphate (CTP)

Some CTP is dephosphorylated to CDP (a substrate for ribonucleotide reductase)

dCDP can be phosphorylated to dCTP for DNA synthesis.

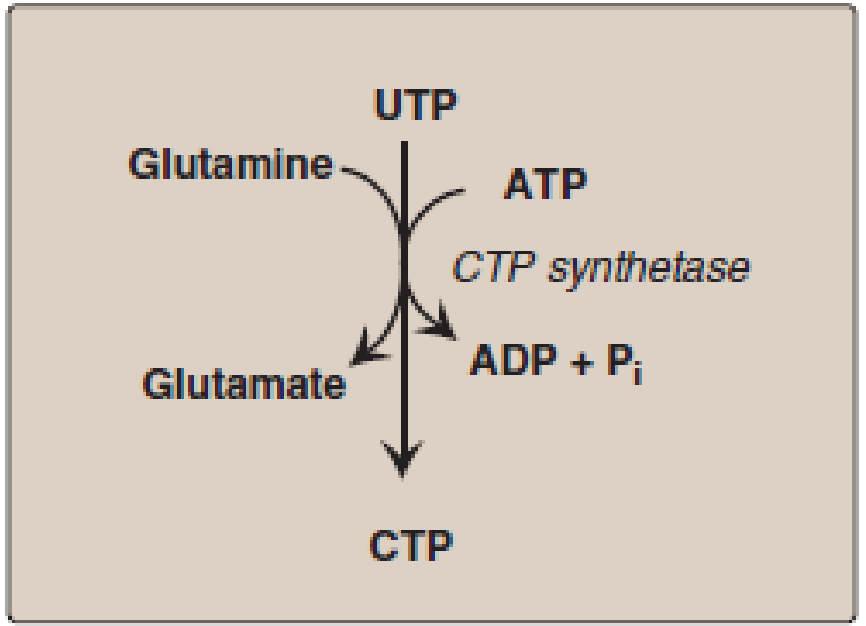


Figure 22.22
Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.]

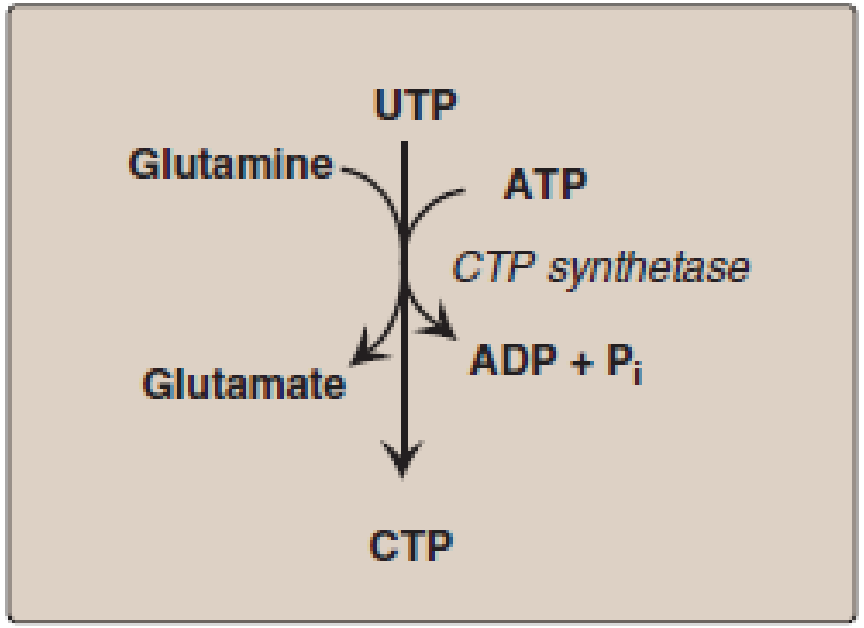


Figure 22.22
Synthesis of CTP from UTP. [Note: CTP, required for RNA synthesis, is converted to dCTP for DNA synthesis.]

- **The complement in this slide:**
- In order to continue toward the synthesis of cytosine we have to use **UTP** not UMP, so I have to phosphorylate it.
- The first phosphate is added by specific enzyme (if u remember purines, we also added the first phosphate by specific enzyme)
- The second one is added by non-specific enzyme.
- As we said we have to add amino group to produce CTP.
- The source of amino group is **glutamine** which will be converted into glutamate by an enzyme called **CTP synthetase**. (anabolic pathway so we **used energy** as well)

Pyrimidine Synthesis

E. Synthesis of thymidine monophosphate (TMP) from dUMP

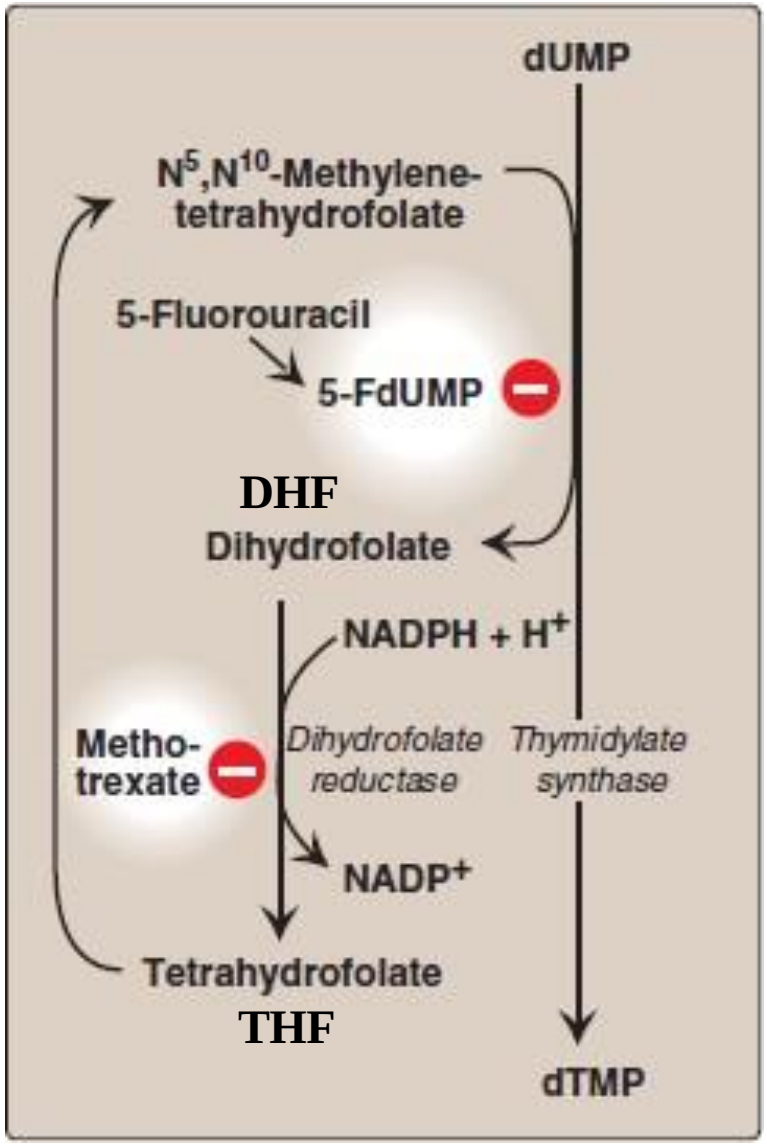
Thymidylate synthase inhibitors include thymine analogs such as 5-fluorouracil (antitumor agents).

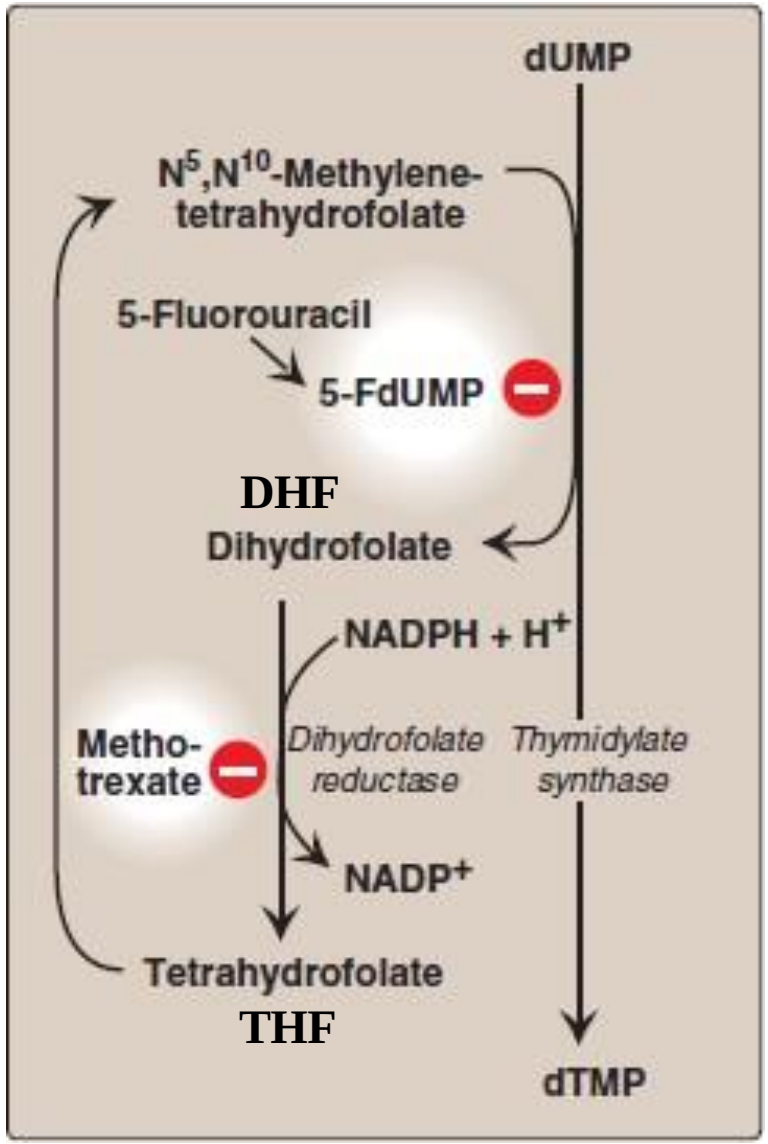
5-Fluorouracil (**suicide inhibitor**) is converted to 5-FdUMP that **permanently** binds to the inactivated thymidylate synthase

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**





- **The complement in this slide:**
- In thymine synthesis we can't use UMP or UTP so we use **dUMP** (remember Ribonucleotide reductase **(RR)** can work on all nucleotides but it can only identify **diphosphates**)
- So, UMP ---(Base-specific enzyme)---> UDP ---(RR reduce it)---> dUDP ---(Removing phosphate)---> dUMP
- dUMP ----------> dTMP, this methylation reaction is catalyzed by **Thymidylate synthase**.
- The source of methyl is tetrahydrofolate that carries it in the form of methylene-tetrahydrofolate.
- If we look at the structure of methylene-tetrahydrofolate we will notice that the carbon is attached to N^5 and N^{10} (less number of hydrogen on it), so if I want to take CH3 I will have to take H from tetrahydrofolate **releasing** it as Dihydrofolate.
- I need to recycle it to tetrahydrofolate so I can use it again, so I reduce it by **Dihydrofolate reductase**, oxidizing **NADPH**
- We used NADPH in purines as well.

- **The complement in this slide:**
- Methotrexate as we said before it works in purine synthesis on the steps that have N5,N10-tetraydrofolate stopping the formation of A and G
- But here it will stop the coenzyme regeneration (inhibit **dihydrofolate reductase**) **not** thymidylate synthase.
- So, it is going to inhibit dihydrofolate reductase resulting in higher concentration of dihydrofolate that can't be loaded with methylene, affecting the thymine synthesis.
- So, methotrexate act on the synthesis of **A, G and T.**

- Another anti cancerous agent called **Fluorouracil** that inhibit **thymidylate synthase**, so it interferes with the formation of thymine.
- Now, why uracil is not found in DNA since we have the deoxy form of it?

Because there is an enzyme called **dUTPase** that degrades dUTP, preventing it incorporation with DNA structure.

- **NOTE:** let's assume that we are going to synthesize nucleotides in hepatocytes to make a comparison .

- **NOTE: how the cell can know that if the carbamoyl phosphate is for urea cycle or pyrimidine synthesis?**
- By something called Spatial regulationالتنظيم المكاني .
- CPS I is found in the mitochondria while CPS II in the cytosol.
- CPS I take ammonia from free ammonia while in CPS II the source of ammonia is derived from the amide group of glutamine.

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 Versus CPS II

	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

UTP as inhibitor because it is the first type of pyrimidine nucleotides that appear during this synthetic pathway

Pyrimidine Salvage and Degradation

Salvage:

Few pyrimidine bases are salvaged in human cells.

Mechanism:

Pyrimidine nucleosides —(Nucleoside kinases; ATP)→ Nucleotides

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.

- **The complement in this slide:**
- In purines we do salvage for nitrogenous base, but in pyrimidines we do salvage for nucleo**sides.**
- In pyrimidine salvage we add phosphate group by kinase enzyme, phosphate from ATP.

Adenosine is the only purine nucleoside to be salvaged.
It is phosphorylated to AMP by adenosine kinase.

Slide 14

- Adenosine(**the only purine**) can be salvaged by 2 ways either by PRPP add to the nitrogenous base or by phosphorylated (nucleo**side** --> nucleo**tide**).

- **Degradation:**
- Pyrimidine degradation is similar to purine degradation (I have to get rid of phosphate group by nucleo**tidase** then remove of the sugar by nucleo**sidase**(nucleoside phosphorylase)) by the way the released Ribose-1-phosphate is isomerized to Ribose-5-phosphate by **mutase** enzyme.
- Then I have the ring structure, so the first step is the open of the ring then cleavage.
- Production of free NH3 and CO2 as a side products.
- The main products are **β-alanine** and **β-aminoisobutyrate.**
- **All produce the same products(T,C,U) because they are similar to each other structurally.**

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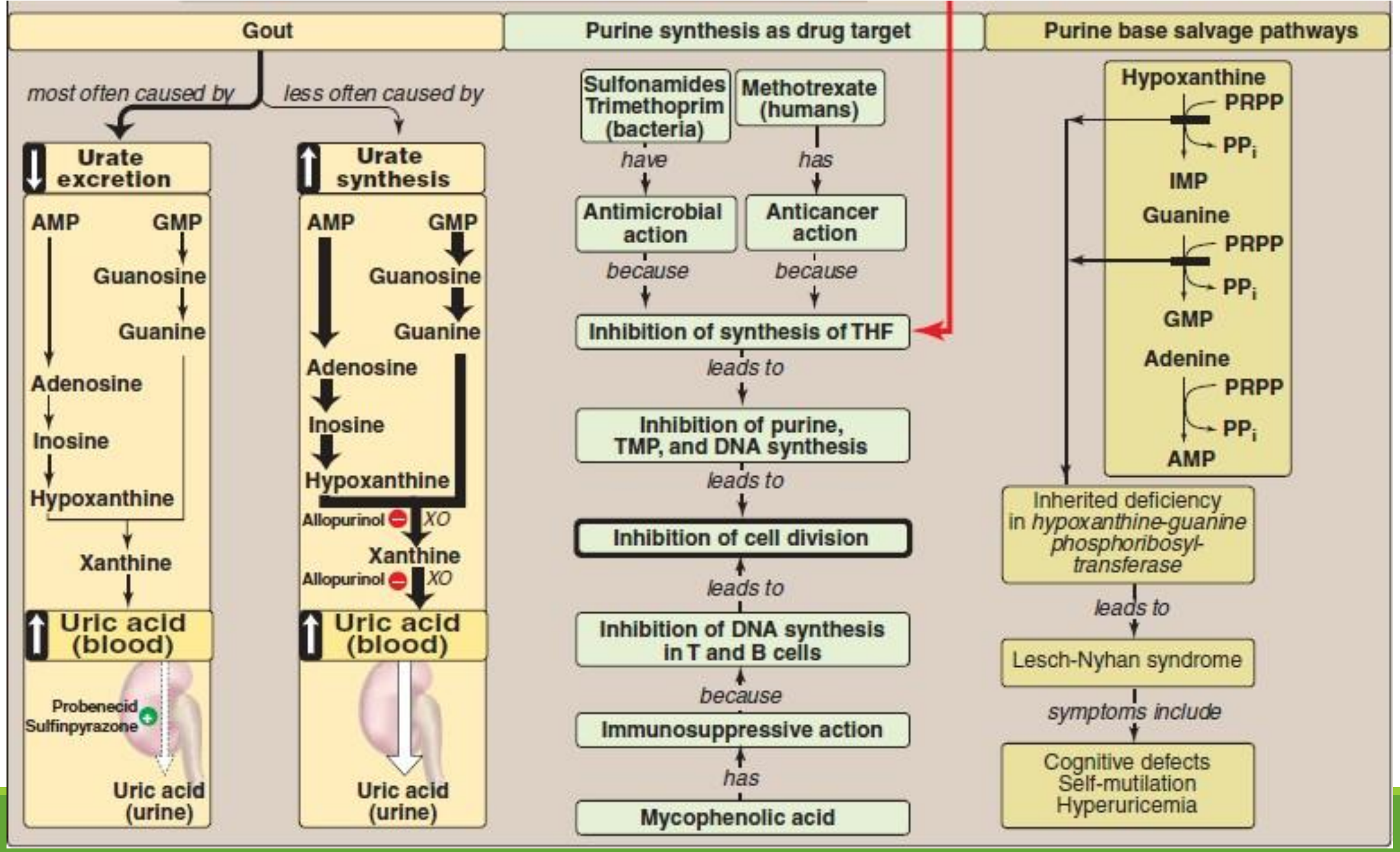
Gout
most often caused by
less often caused by
Urate excretion
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Xanthine
Uric acid (blood)
Probenecid
Sulfinpyrazone
Uric acid (urine)
Urate synthesis
AMP
GMP
Guanosine
Guanine
Adenosine
Inosine
Hypoxanthine
Allopurinol
XO
Xanthine
Allopurinol
XO
Uric acid (blood)
Uric acid (urine)
Purine synthesis as drug target
Sulfonamides Trimethoprim (bacteria)
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have
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because
because
Inhibition of synthesis of THF
leads to
Inhibition of purine, TMP, and DNA synthesis
leads to
Inhibition of cell division
leads to
Inhibition of DNA synthesis in T and B cells
because
Immunosuppressive action
has
Mycophenolic acid
Purine base salvage pathways
Hypoxanthine
PRPP
PPi
IMP
Guanine
PRPP
PPi
GMP
Adenine
PRPP
PPi
AMP
Inherited deficiency in hypoxanthine-guanine phosphoribosyl-transferase
leads to
Lesch-Nyhan syndrome
symptoms include
Cognitive defects
Self-mutilation
Hyperuricemia