DOCTOR 2020 | JU

METABOLISM

WRITER : ALI ALMAHROOK

CORRECTOR : AHMAD ZAIDAN

DOCTOR: DIALA ABU-HASSAN

المحاضرة دسمه شوي بس انت قدها ⟵ (للتوضيح علي القائل)

لكن بالواقع رح تبلعوا 😀

Last lecture we discussed the De novo pathway of purines and today we will continue the Salvage pathway and pyrimidine synthesis before that we will discuss Synthetic inhibitors of purine synthesis.

Synthetic inhibitors of purine synthesis

Synthetic inhibitors of purine synthesis (the **sulfonamides1**), are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions. (like antibiotics)

Other purine synthesis inhibitors, such as structural **analogs of folic acid** (such as, **methotrexate2**), are used as drugs that control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

Remember that we need N^{10}-Formyl-tetrahydrofolate (folic acid) in 2 reactions in the De novo pathway of purines so if we interfere these reactions, purine synthesis will stop thus inhibit the growth of rapidly dividing microorganisms.

Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, GI tract, immune system, or hair follicles.

Thus, **anticancer drugs result in adverse effects**, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

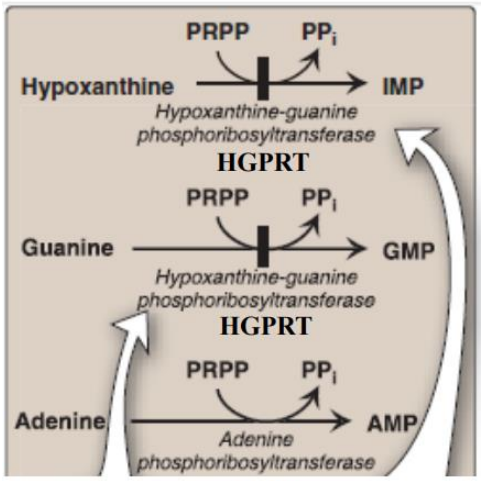
Salvage pathway for purines

Salvage pathway for purines is purine synthesis from:

1. The normal turnover of cellular nucleic acids
2. Diet purines that are not degraded (small amounts)

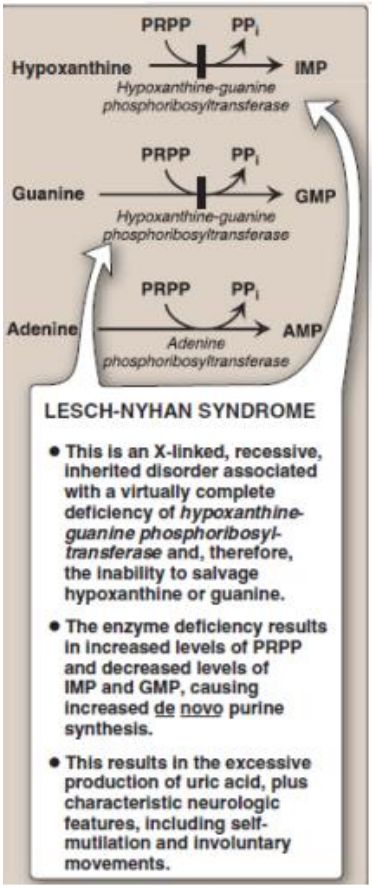
Conversion of purine bases to nucleotides:

Here we have Guanine and by the action of HGPRT enzyme, it takes the ribose 5-phosphate group from PRPP and produce GMP, same thing applies to IMP but it uses Hypoxanthine as well as AMP but the enzyme is APRT as well as the AA is Adenine.



- **Both APRT and HGPRT use PRPP as the source of the ribose 5-phosphate group.**
- **PP is released and hydrolyzed by pyrophosphatase making these reactions irreversible.**
- **Adenosine is the only purine nucleoside to be salvaged. It is phosphorylated to AMP by adenosine kinase.**

Clinical application Salvage pathway for purines-Lesch-Nyhan syndrome



A rare, X-linked, recessive disorder associated with **HGPRT deficiency.**

Notice the AMP salvage pathway isn't affected since it has its own enzyme, so AMP level is higher than GMP which will activate degradation of AMP

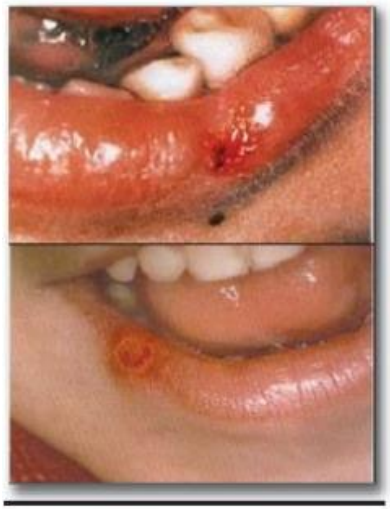
Inability to salvage hypoxanthine or guanine resulting in high amounts of uric acid (the end product of purine degradation)

Increased PRPP levels and decreased IMP and GMP levels.

The committed step in purine synthesis has excess substrate and decreased inhibitors available, and **de novo purine synthesis is increased.**

When **de novo purine synthesis increase this we result in both increasing AMP and GMP. The decreased purine reutilization and increased purine synthesis results in increased degradation of purines and the production of large amounts of uric acid (hyperuricemia)**

Lesch-Nyhan syndrome



Hyperuricemia results in:

1. Uric acid stones in the kidneys (urolithiasis)

2. The deposition of urate crystals in the joints (gouty arthritis) and soft tissues.

The syndrome is characterized by:

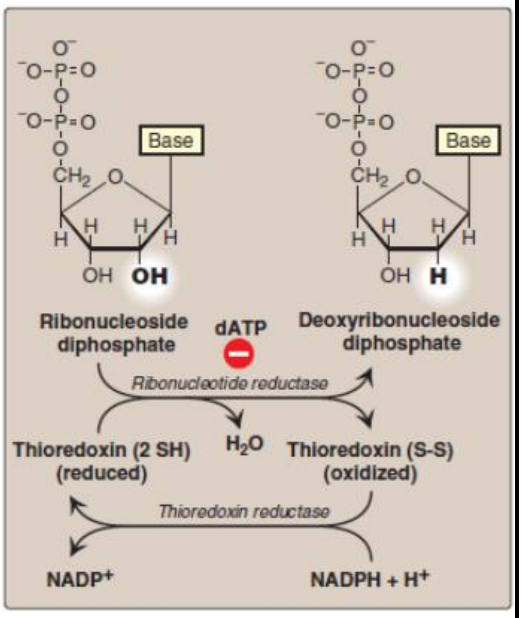
Motor dysfunction

Cognitive deficits

Behavioral disturbances that include self-mutilation (biting of lips and fingers).

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 dislufide bond and Thioredoxin must be reduced again.

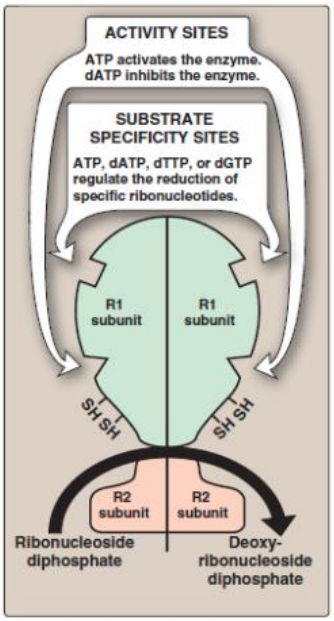
2. Regeneration of reduced enzyme: Thioredoxin—a peptide coenzyme of RR

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 active 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.**

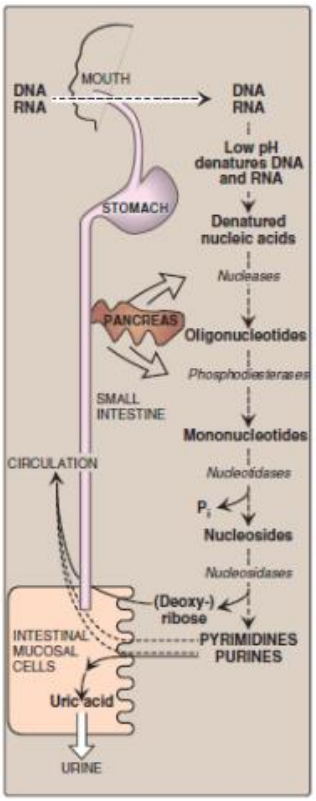
Pharmacology application ☹ Hydroxyurea and ribonucleotide reductase

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 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.

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.

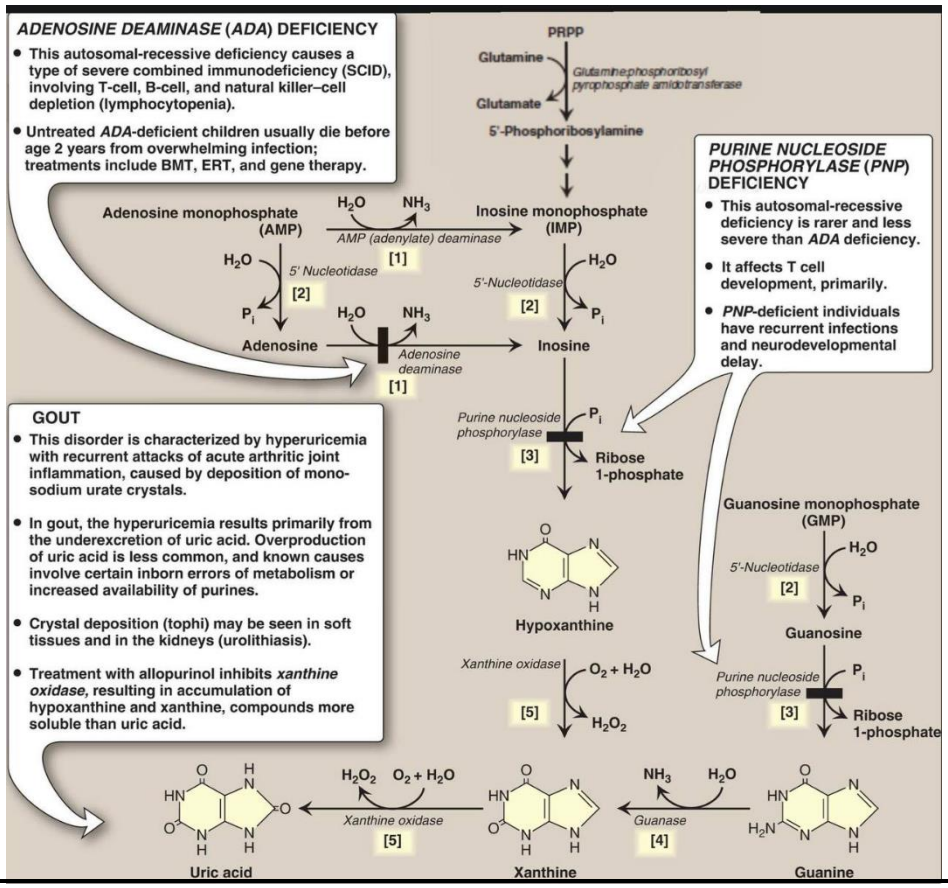
[2] IMP and GMP are converted into their nucleoside forms—inosine and guanosine—by the action of 5'-nucleotidase.-*

[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.

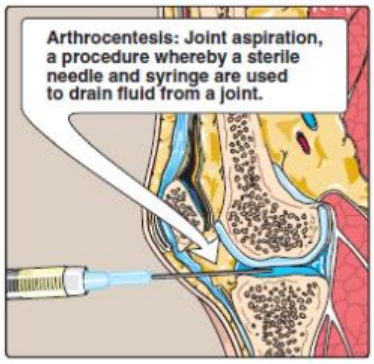


Figure 22.17
Analysis of joint fluid can help to define causes of joint swelling or arthritis, such as infection, gout, and rheumatoid disease.

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)

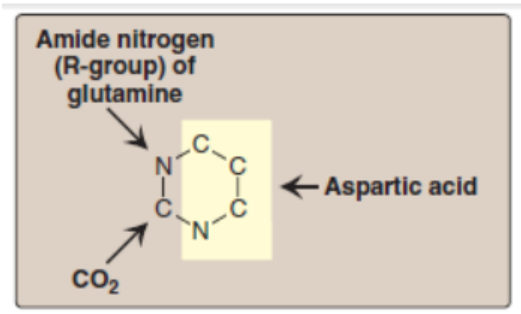
2. Environmental factors such as drugs (thiazide diuretics)

3. Exposure to lead (saturnine gout)

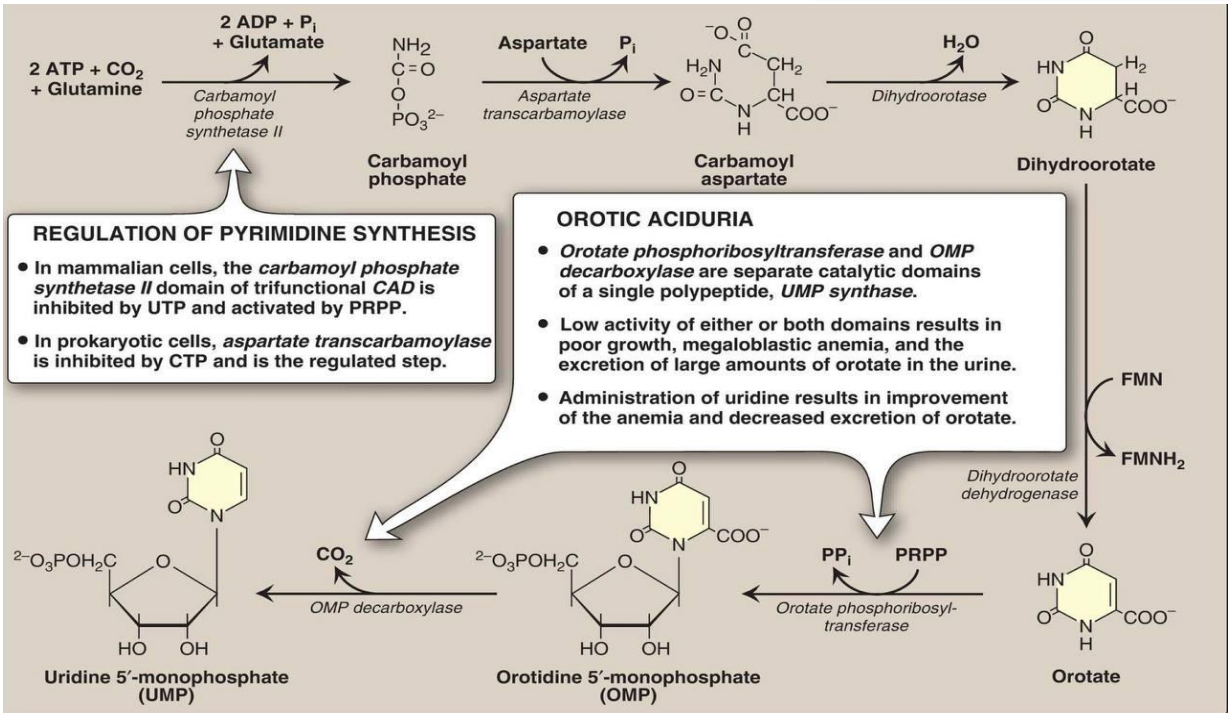
Overproduction of uric acid: less common.

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) catalaze 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-monophospahe (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.**

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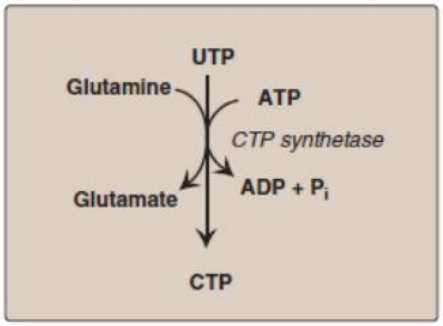
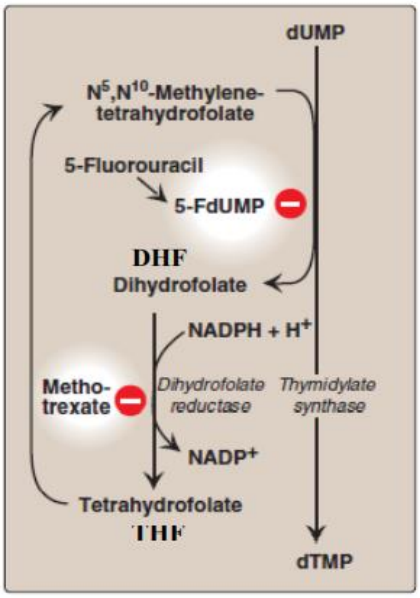
4.Synthesis of UTP and cytidine triphosphate (CTP)

- **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 from 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

صلّ على النبي

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 NH3 and CO2.

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.