◉ Sheet ○ Slides

Subject :	Metabolism of A.A
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Number :	35

In the previous lecture we talked about purine synthesis and we said that the first product obtained is IMP and from IMP we get AMP and GMP, we also talked about the salvage pathway. Today we will talk about bases of nucleotides of DNA. DNA accepts deoxynucleotide. So first we will talk about how the reduction of the ribonucleotide to form the deoxynucleotide takes place.

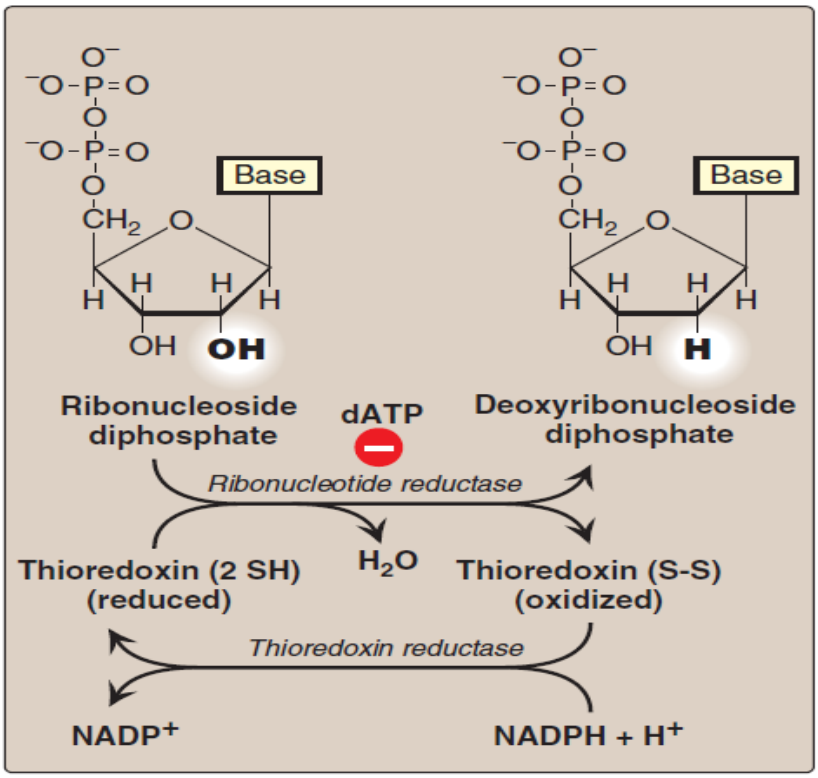
SYNTHESIS OF DEOXYRIBONUCLEOTIDES:

(This is an important reaction and it has a clinical importance since many of the chemotherapeutic drugs work on this pathway. So you should be familiar with it and you should know the biochemical bases for the action of these drugs.)

The nucleotides described thus far all contain ribose (ribonucleotides). The nucleotides required for DNA synthesis, however, are 2'-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle.

[Note: The same enzyme acts on pyrimidine ribonucleotides.]

Note:The Ribonucleotide which is a substrate for this enzyme is a ribonucleoside diphosphate. Therefore, the enzyme Ribonucleotide reductase is also called ribonucleoside diphosphate reductase.

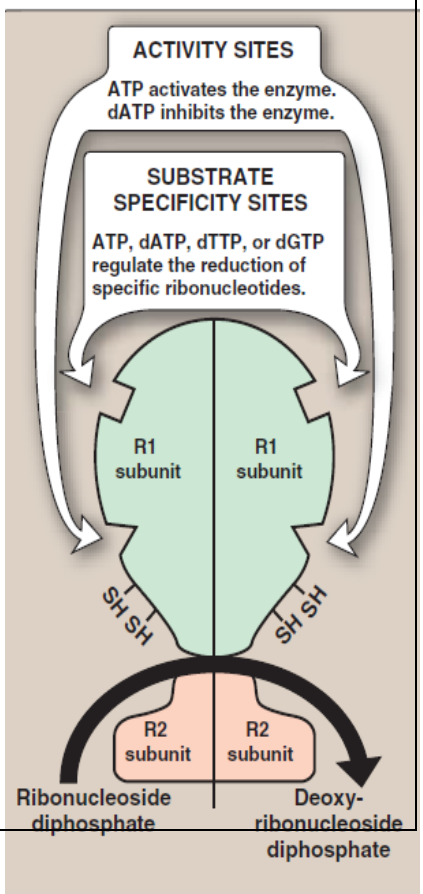


The substrate for getting the deoxyribonucleotide generally is the ribonucleoside diphosphate. And we have one enzyme that works on (CDP,UDP,GDP,ADP), but the regulation of this enzyme is unique and we will talk about it after we see the reactions.

- Note: We usually refer to deoxythymidylate(dTMP) as thymidylate , because it always goes without saying that thymidylate is a deoxy, unlike CDP or other nucleotides in which we need to determine the specific form of the compound(either ribo or deoxyribo).

The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond (it give its two hyrogens and form a disulfide bond). the disulfide bond created during the production of the 2'-deoxy carbon must be reduced. The source of the reducing equivalents for this purpose is thioredoxin—a peptide coenzyme of ribonucleotide reductase(it also has two –SH). Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, forming a disulfide bond (it gets oxidized). Now, this thioredoxin must be converted back to its reduced form in order to continue to perform its function, and it is reduced back by NADHP in a reaction catalyzed by another enzyme that is called **Thioredoxin reductase.**

So as you see, always in such reductions the ultimate donor of electrons and protons is NADPH, but it goes sometimes through a carrier (not directly). As we see above that thioredoxin is a carrier to carry this electron and hydrogen atom.



Regulation of deoxyribonucleotide synthesis:

Ribonucleotide reductase is responsible for maintaining a balanced supply of deoxyribonucleotides required for DNA

synthesis. So this enzyme is under complex regulation. Now, before talking about the regulation take a look at this enzyme above, which is composed of two nonidentical dimeric subunits, R1 and R2.
In addition to the catalytic (active) site, there are allosteric sites on the enzyme involved in regulating its activity. Some of these allosteric sites are known as **activity sites**, when ATP is bound to these sites, the enzyme is activated , on the other hand, when dATP is bound to these sites, the catalytic activity of this enzyme is inhibited(thus preventing the conversion of any of the four nucleoside diphosphates(CDP,UDP,ADP,GTP) to their deoxy forms. Other allosteric sites on the enzyme are known as **substrate specificity sites**, and the binding of nucleoside tri - Phosphates to these sites regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis. For example, deoxythymidine triphosphate (dTTP) binding at the specificity sites causes a conformational change that allows reduction of GDP to dGDP at the catalytic site.
To conclude : this enzyme makes sure that there is a balanced supply in deoxy entity. So we have seen that this enzyme has an active site and two regulatory sites(one for the activity and the other for substrate specificity).

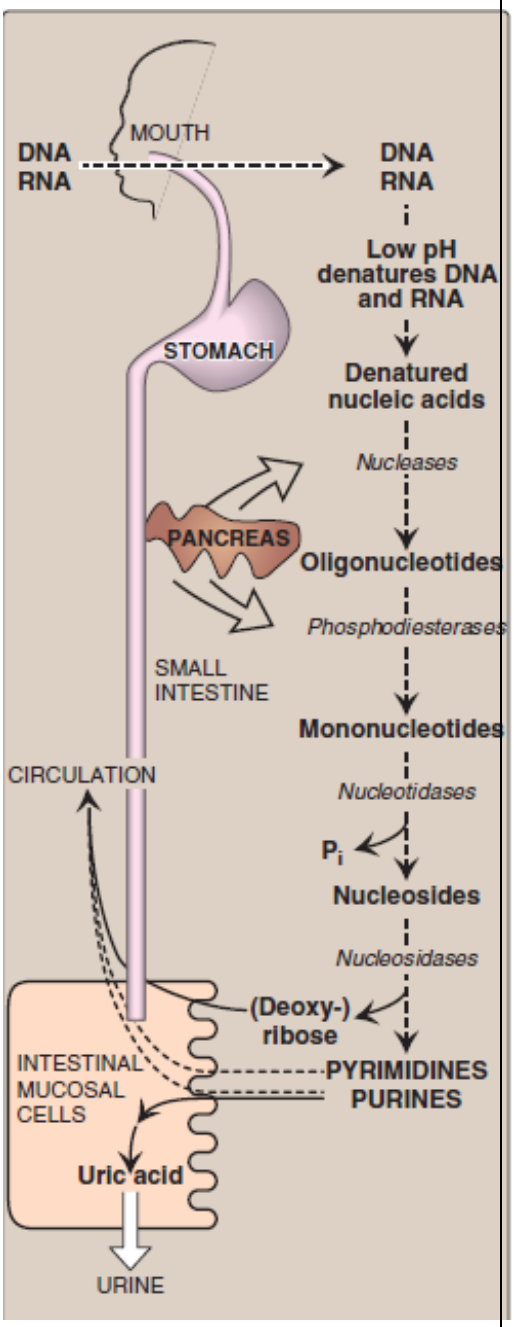
Clinical applications:
-Hydroxyurea : this drug inhibits ribonucleotide redutase, thereby inhibiting the generation of substrates for DNA synthesis, because of this, this drug is an antineoplastic agent that is used in the treatment of some cancers, another use of this drug is in the treatment of sickle cell anemia.
In sickle cell anima, one of the strategies to reduce its bad effects is to increase the level of HbF(fetal hemoglobin) which interrupts the polymerization which occurs in the HbS. Hydroxyurea is known to increase the level of HbF.

-In adenosine deaminase deficiency (ADA deficiency), dATP levels increase and as a result of this increase, the enzyme ribonucleotide reductase will be inhibited. Therefore, the supply of the substrates for DNA(deoxyribonucleotide) will be suppressed, as a result the immune cells which multiply and require

deoxyribonucleotides will be low, and as a result of that, we see that the patient suffers from deficiency in his immunity (severe combined immunodeficiency, immunodeficiency syndrome).

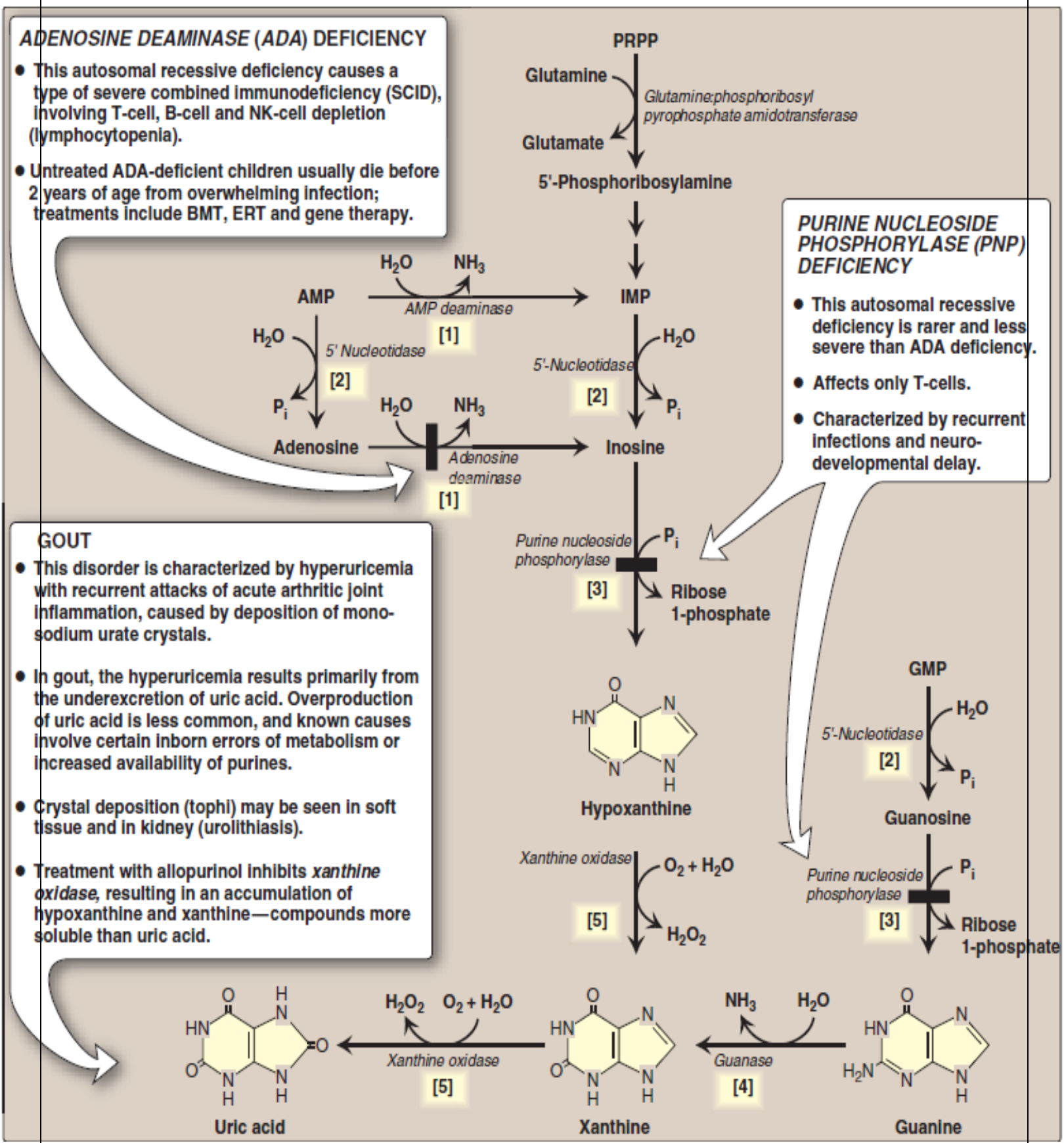
DEGRADATION OF PURINE NUCLEOTIDES:

In the degradation of purine nucleotides, we said before that the dietary sources of the purine bases is poor, we ingest some DNA and RNA in the food which undergo denaturation in the stomach, due to its acidity, and then they will be subjected to pancreatic enzymes such as Nucleases which break down DNA and RNA to small pieces called Oligonucleotides, then these Oligonucleotides are further hydrolyzed by pancreatic phosphodiesterases, producing a mixture of 3'- and 5'-mononucleotides. In the intestinal mucosal cells, a family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that are further degraded by nucleosidases(nucleoside phosphorylases) to free bases(pyrimidines and purines) plus (deoxy) ribose 1-phosphate. Ribose will be absorbed and will go to the circulation and we still have pyrimidines and purines. The majority of purine undergoes degradation to uric acid as we will see just in a moment, and small portion can be absorbed. So in patients with high uric acid levels, one of the advices that are given to them is to reduce their intake of food rich in nucleotides, such organ meats as liver and kidney etc…,and a kind of fish called anchovies, sardines. So these forms of food must be reduced since they give more uric acid.



Pyrimidines are usually metabolized to small soluble compounds without problems, but purines are degraded to uric acid, so most dietary purines in the intestinal mucosal cells are converted to uric acid, so we do not have these purines in the blood to reutilize them.
Note: 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.

How are purine nucleotides degraded ?
We have GMP and AMP and some IMP .
[1] AMP has two modes of degradation :
An amino group is removed from AMP to produce IMP by AMP Deaminase, then a Nucleotidase enzyme removes the phosphate group from this IMP to get inosine // or a Nucleotidase removes a phosphate group from AMP to get adenosine, then Adenosine deaminase removes an amino group from this adenosine to get inosine(hypoxanthineribose).
So both pathways lead to inosine.
[2] GMP is converted into its nucleoside form (guanosine) by the action of Nucleotidase .
[3] Purine nucleoside phosphorylase converts inosine and guanosine into their respective purine bases, hypoxanthine and guanine.
[4] Guanine is deaminated to form xanthine by the enzyme Guanase.
[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. Uric acid is excreted primarily in the urine.
Note: IMP can participate in both synthetic (to form GMP,AMP) and degradative (to form urate) pathways, and the main factor that determines whether IMP molecules will mainly go through one of the pathways or the other is the need of the cell.

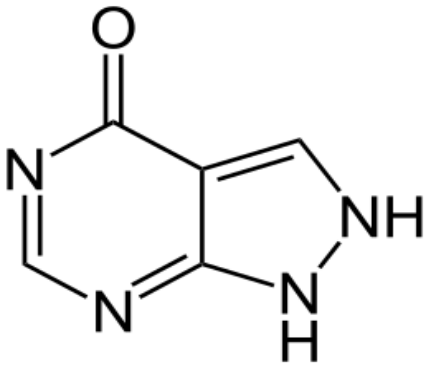

ADENOSINE DEAMINASE (ADA) DEFICIENCY
This autosomal recessive deficiency causes a type of severe combined immunodeficiency (SCID), involving T-cell, B-cell and NK-cell depletion (lymphocytopenia).
Untreated ADA-deficient children usually die before 2 years of age from overwhelming infection; treatments include BMT, ERT and gene therapy.
PRPP
Glutamine
Glutamate
Glutamine:phosphoribosyl pyrophosphate amidotransferase
5'-Phosphoribosylamine
AMP
H_2O
NH_3
AMP deaminase
[1]
IMP
H_2O
5' Nucleotidase
[2]
P_i
5'-Nucleotidase
[2]
H_2O
P_i
Adenosine
H_2O
NH_3
Adenosine deaminase
[1]
Inosine
PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFICIENCY
This autosomal recessive deficiency is rarer and less severe than ADA deficiency.
Affects only T-cells.
Characterized by recurrent infections and neuro-developmental delay.
Purine nucleoside phosphorylase
[3]
P_i
Ribose 1-phosphate
GOUT
This disorder is characterized by hyperuricemia with recurrent attacks of acute arthritic joint inflammation, caused by deposition of mono-sodium urate crystals.
In gout, the hyperuricemia results primarily from the underexcretion of uric acid. Overproduction of uric acid is less common, and known causes involve certain inborn errors of metabolism or increased availability of purines.
Crystal deposition (tophi) may be seen in soft tissue and in kidney (urolithiasis).
Treatment with allopurinol inhibits xanthine oxidase, resulting in an accumulation of hypoxanthine and xanthine—compounds more soluble than uric acid.
Hypoxanthine
GMP
H_2O
5'-Nucleotidase
[2]
P_i
Guanosine
Xanthine oxidase
$O_2 + H_2O$
[5]
H_2O_2
Purine nucleoside phosphorylase
P_i
[3]
Ribose 1-phosphate
Uric acid
H_2O_2
$O_2 + H_2O$
Xanthine oxidase
[5]
Xanthine
NH_3
H_2O
Guanase
[4]
Guanine

Xanthine Oxidase requires Molybdenum(Mo).
Uric acid is antioxidant compound. However, many problems are associated with it.
Problems with uric acid:

When the concentration of uric acid reaches (6.5-7 mg/dl) and above, its solubility will be low and it precipitates as crystals which induce inflammation in the joints which is painful (Gout disease), also, formation of uric acid stones in the kidney may be seen.

One of the therapeutic strategies for gout disease is to inhibit the enzyme xanthine oxidase, thereby reducing the amount of uric acid in the body and resulting in the accumulation of xanthine or hypoxanthine. Xanthine and hypoxanthine are more soluble than uric acid and in patients with normal levels of HGPRT the hypoxanthine can be salvaged , reducing the levels of PRPP and therefore de novo purine synthesis. Allopurinol is a powerful drug for this problem.

Allopurinol is a widely used drug for gout treatment. It looks like hypoxanthine with small difference in the location of N and C atoms of the 5-membred ring. This Allopurinol inhibits xanthine Oxidase. So the end products will be xanthine and hypoxanthine. The mechanism of action of this drug is that the enzyme xanthine Oxidase 'thinks' that allopurinol is hypoxanthine and works on it as a substrate and binds to it and then the active site will be locked, so we call this committing suicide(suicide inhibition). Allopurinol also reacts with PRPP and forms allopurinolribonucleotides, reducing PRPP pool and purine synthesis.



Treatment with allopurinol inhibits xanthine oxidase, resulting in an accumulation of hypoxanthine and xanthine—compounds more soluble than uric acid.

Adenosine deaminase (ADA) deficiency:

ADA is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme. A deficiency of ADA results in an accumulation of adenosine, which is converted to its ribo -nucleotide or deoxyribonucleotide forms by cellular kinases. As dATP levels rise, ribonucleotide reductase is inhibited, thus preventing the production of all deoxyribose-containing nucleotides. Consequently, cells cannot make DNA and divide. [Note: The dATP and adenosine that accumulate in ADA deficiency lead to developmental arrest and apoptosis of lymphocytes.]
In its most severe form, this autosomal recessive disorder causes a type of severe combined immunodeficiency disease (SCID), involving a decrease in T cells, B cells, and natural killer (NK) cells. It is estimated that in the United States, ADA deficiency accounts for approximately 14% of all cases of SCID.

Now back to problems with regard to uric acid:
The problems of uric acid is either by its overproduction or under excretion, the overproduction is less common than under excretion.

Overproduction of uric acid:

A less common cause of gout is hyperuricemia from the overproduction of uric acid. Primary hyperuricemia is, for the most part, idiopathic (having no known cause). However, several identified mutations in the gene for X-linked PRPP synthetase result in the enzyme having an increased Vmax for the production of PRPP, a lower Km for ribose 5-phosphate, or a decreased sensitivity to purine nucleotides—its allosteric inhibitors. In each case, increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid. Lesch-Nyhan syndrome also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and guanine, and the subsequent increased availability of PRPP. Secondary hyperuricemia is typically the consequence of increased availability of purines, for example, in patients with myeloproliferative disorders or who are undergoing chemotherapy and so have a high

rate of cell turnover. Hyperuricemia leading to gout can also be the result of seemingly unrelated metabolic diseases,such as von Gierke disease or fructoseintolerance.

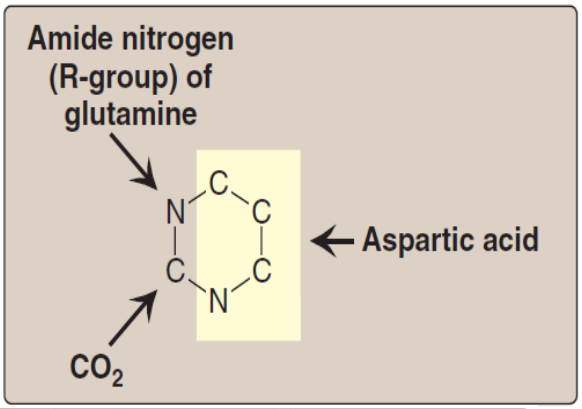
Underexcretion of uric acid:

In the vast majority of patients, the hyperuricemia leading to gout is caused by underexcretion of uric acid. Underexcretion can be primary, due to as-yet-unidentified inherent excretory defects, or secondary to known disease processes that affect how the kidney handles urate, for example lactic acidosis (lactate and urate compete for the same renal transporter), and to environmental factors such as the use of drugs, for example, thiazide diuretics, or exposure to lead (saturnine gout).

»thus we finished talking about degradation of purine nucleotides but still we haven't talk about pyrimidines

PYRIMIDINE SYNTHESIS AND DEGRADATION:

Unlike the synthesis of the purine ring, which is constructed on a preexisting ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP. The sources of the atoms in the pyrimidine ring are glutamine, CO2, and aspartic acid. [Note: Glutamine and aspartic acid are thus required for both purine and pyrimidine synthesis.]



steps:

A. Synthesis of carbamoyl phosphate:

Carbamoyl phosphate synthetase-1(CPS-1) is an enzyme that is involved in the formation of carbamoyl phosphate in the mitochondria(which we talked about in sheet 30) in urea biosynthesis. We have an

	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

enzyme that is similar, but not identical, to CPS-1 (made by a different gene), which is called CPS-2 (CPS-ll). This enzyme does not take a free ammonia as CPS-1 does (which is in the mitochondria), rather than that, CPS-2 (which is in the cytosol) takes the ammonia from glutamine with bicarbonate and 2ATP to make carbamoyl phosphate.

This enzyme (CPS-2) is the rate limiting enzyme in mammalian cells in Pyrimidine synthesis pathway, and it is inhibited by the end product which is pyrimidine nucleotide (UTP). In the bacterial system it is different. This carbamoyl phosphate now donates the carbamoyl group to aspartate to get carbamoyl aspartate in a reaction catalyzed by the enzyme aspartate transcarbamoylase. In the bacterial system(prokaryotic) the enzyme aspartate transcarbamoylase(ATC) is a regulatory enzyme and allosteric enzyme and consists of multisubunits and it is inhibited by CTP and activated by ATP.

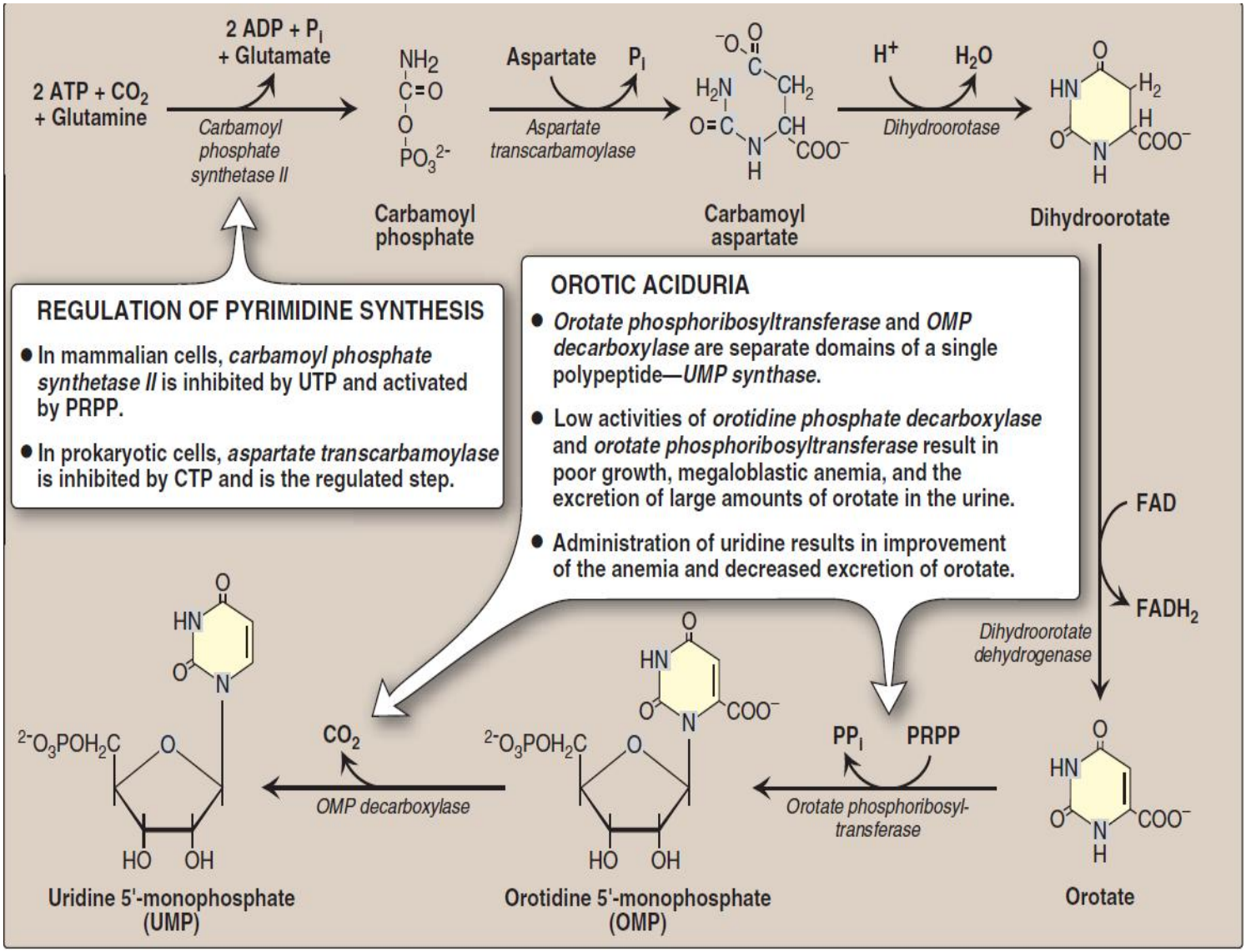
The third enzyme in the pathway is dihydroorotase, it will convert carbamoyl aspartate to dihydroorotate and the ring is closed.

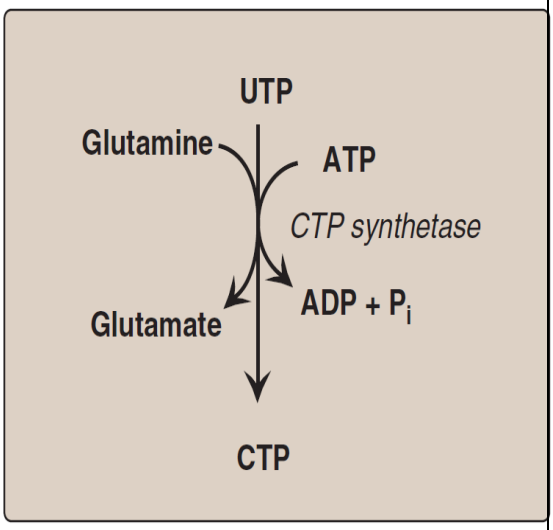
- The first three enzymic activities in this pathway (CPS II, aspartate transcarbamoylase, and dihydroorotase) are actually three different catalytic domains of a single polypeptide chain known as CAD from the first letter in the name of each domain. This is an example of a multifunctional or multicatalytic polypeptide that facilitates the ordered synthesis of an important compound.
- These three reactions occur in the cytosol.

Now**,** dihydroorotate will enter the mitochondria and undergoes oxidation to produce **Orotate** (Orotic acid) by the enzyme *dihydroorotate dehydrogenase*. This is the only reaction that takes place in the mitochondria. However, all other enzymes in pyrimidine biosynthesis are cytosolic. Dihydroorotate dehydrogenase is a flavoprotein associated with the inner mitochondrial membrane. If you remember, we saw that in purine synthesis the ribose 5-phosphate is present from the beginning of the synthesis and the synthesis is done based on it atom by atom, but here in pyrimidine synthesis

the pyrimidine base is made first, then ribose 5-phosphate will come. Ribose 5-phosphate will be donated by PRPP to Orotate to get OMP(Orotidine Monophosphate) also known as orotidylate in a reaction catalyzed by the enzyme orotate phosphoribosyltransferase. Finally OMP, the parent pyrimidine mononucleotide, is converted to uridine monophosphate (UMP, aka uridylate) by orotidylate decarboxylase, which removes the acidic carboxyl group.

- These last two enzymes Orotate phosphoribosyltransferase and orotidylate decarboxylase are also catalytic domains of a single polypeptide chain called UMP synthase . Orotic aciduria—a rare genetic defect—may be caused by a deficiency of one or both activities of this bifunctional enzyme,resulting in orotic acid in the urine.





As you see, the first pyrimidine we get is UMP.
Now this UMP is sequentially phosphorylated to UDP and UTP. When UDP is formed, an amino group can be added from glutamine by CTP synthetase to get CTP. CTP is an inhibitor for the enzyme CTP synthetase(end product inhibition) and GTP is an activator for it.
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), so this enzyme reduces the availability of dUTP for DNA synthesis, which prevents erroneous incorporation of uracil into DNA.

Synthesis of deoxythymidine monophosphate (dTMP) from dUMP:

dUMP is converted to dTMP by thymidylate synthase, which uses N5,N10-methylene tetrahydrofolate as the source of the methyl group.
This is an unusual reaction in that tetrahydrofolate (THF) contributes not only a one carbon unit but also two hydrogen atoms from the pteridine ring, resulting in the oxidation of THF to dihydrofolate (DHF). Inhibitors of thymidylate synthase include thymine analogs such as 5-fluorouracil, which serve as successful antitumor agents. 5-Fluorouracil is metabolically converted to 5-FdUMP, which becomes permanently bound to the inactivated thymidylate synthase; for this reason, the drug is called a “suicide” inhibitor. DHF can be reduced to THF by dihydrofolate reductase, an enzyme that is inhibited by drugs such as methotrexate.
By decreasing the supply of THF, these folate analogs not only inhibit purine synthesis, but, by preventing methylation of dUMP to dTMP, they also lower the cellular concentration of this essential component of DNA. DNA synthesis is inhibited and cell growth slowed. Drugs such as those described above, therefore, are used to decrease the growth rate of cancer cells. [Note: Trimethoprim, a folate analog, has potent antibacterial activity because of its selective inhibition of bacterial dihydrofolate reductase.]

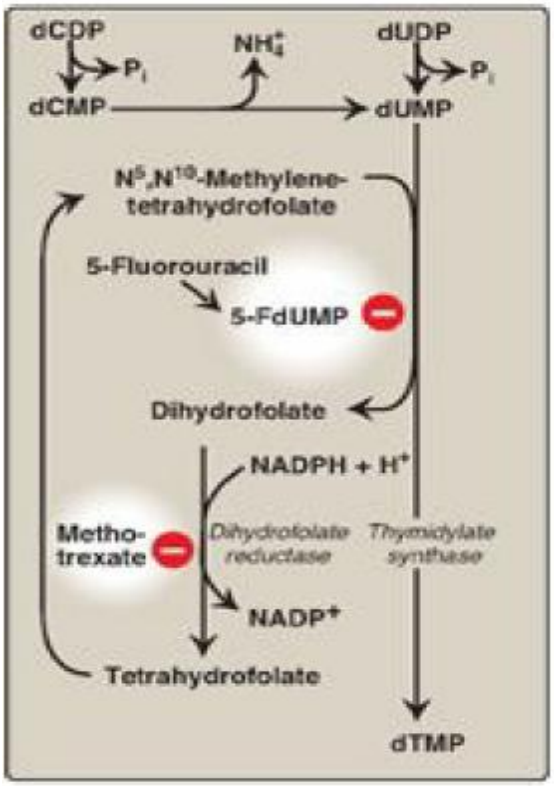
Salvage of pyrimidines:

Few pyrimidine bases are salvaged in human cells. Pyrimidine bases can be salvaged to nucleosides, which can be converted to nucleotides by nucleoside kinases that utilize ATP in the phosphorylation of the nucleosides to nucleotides.

[Note: The salvage of pyrimidine nucleosides is the basis for using uridine in the treatment of hereditary orotic aciduria.]

Degradation of pyrimidine nucleotides:

Unlike the purine ring, which is not cleaved human cells, the pyrimidine ring is opened and degraded to highly soluble products, β-alanine and β-aminoisobutyrate, with the production of NH3 and CO2.



The End