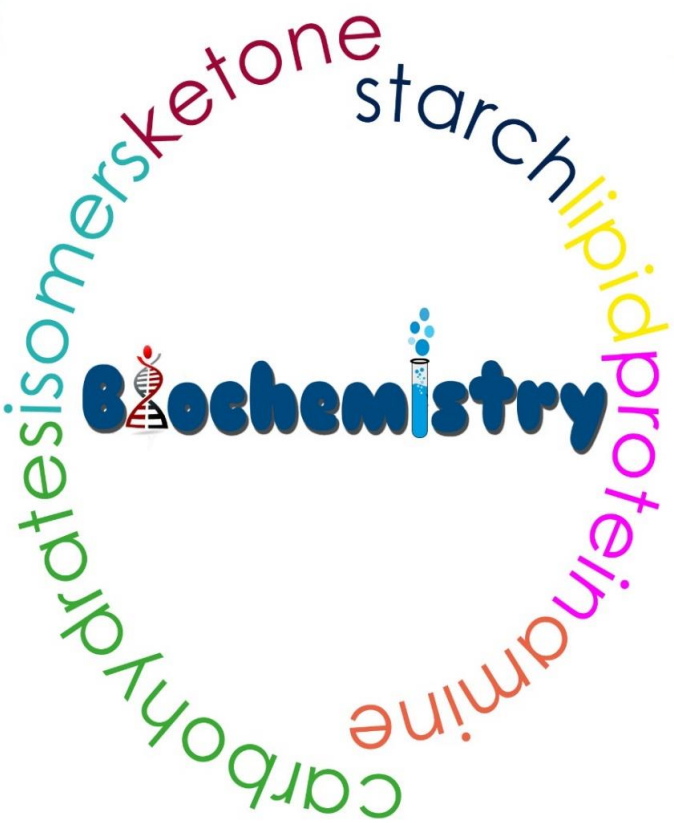


Subject	Metabolism of nucleotides
Done by:	Obada Zalat
Corrected by:	Hamzeh Nakhleh
Number:	34

Metabolism of nucleotides

*There are three sources of nucleotides:

1- **De novo** synthesis

2-**Salvage** synthesis

3-**Diet** (through digestion)

*we will focus on De **novo** synthesis and **Salvage** synthesis.

{Synthesis of purines}

(1) De novo synthesis

-The source of each individual atom in the purine ring in the De novo synthesis:

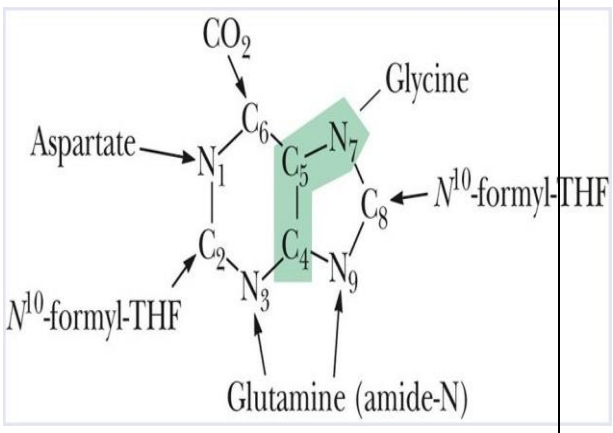
***All the **Glycine** will be utilized in the rung.

***Glutamine** will give nitrogen number 3 and 9

***Formyl** that carried by THF give carbon number 2 and 8

***Aspartate** gives nitrogen number 1

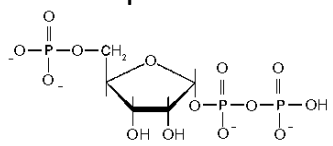
***CO2** gives carbon number 6



-The synthesis started from ribose-5-phosphate, to utilize R-5-P it must be in the active form. The activation is done by the addition of pyrophosphate to R-5-P, and product will be 5-phosphoribosyl-1-pyrophosphate (PRPP). This reaction (activation of the R-5-P) is the start point for the synthesis of purines, pyrimidines and many other reactions like histidine synthesis and salvage pathway.

- **Synthesis of PRPP (5-phosphoribosyl-1-pyrophosphate)**

The synthesis of PRPP from ATP and R-5-p is catalyzed by "ribose phosphate pyrophosphokinase **kinase"** (also it called PRPP synthetase) and the pyrophosphate will be linked at carbon number 1 and the produced AMP is released.



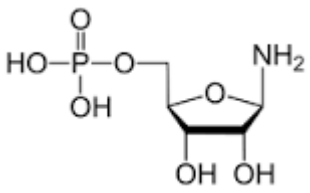
PRPP

This enzyme is under regulation: IMP (inosine monophosphate*), AMP and GMP that will inhabit it; because they are end products of the metabolism pathway as well as when they accumulate, they will inhabit the first enzyme (feedback inhibition). PRPP will activate the enzyme.
-After the synthesis of PRPP, it will enter the purine synthesis pathway.

*Inosine is the nucleoside of the nitrogenous base Hypoxanthine.

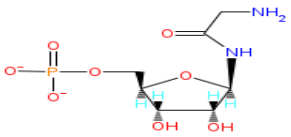
-Purine synthesis pathway steps:

1-**Glatamine** donates the amino group (number 9 in the purine) to PRPP that will bind to carbon number 1 of the ribose, and **PPi** will be released and broken down to make the reaction Irreversible.

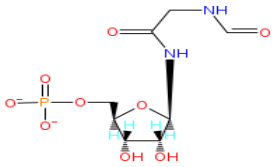


This reaction is the rate limiting step and the committed step in the pathway of purine synthesis, it catalyzed by Glutamine: PRPP amidotransferase or (PRPP glutamyl amidotransferase), this enzyme will be inhabited by all the end product (IMP, GMP and AMP, but when they are **All in Excess**).

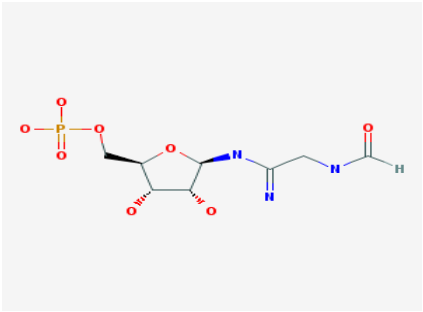
2- **GLysine** is being incorporated **(All the glysine will be linked).**



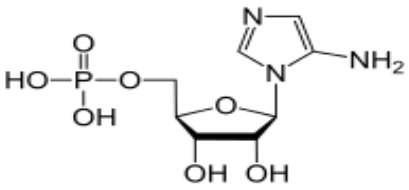
3-**N10-Formyl-THF** will donate **Formyl group** by transfrase enzyme.



4-**Another amino group (from Glutamine)** is being donated.

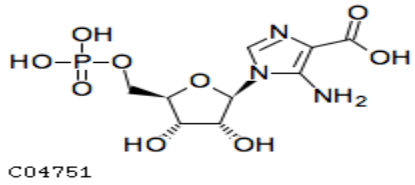


5-**cyclization** happened to form 5 member ring.

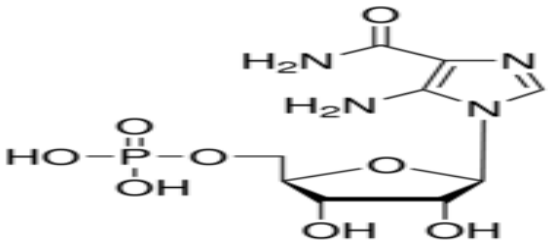


We finished the 5 membered ring. Now we will start the formation of the 6 membered ring:

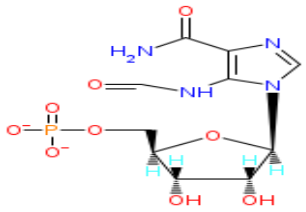
6-**CO2** is being donated via Carboxylase.



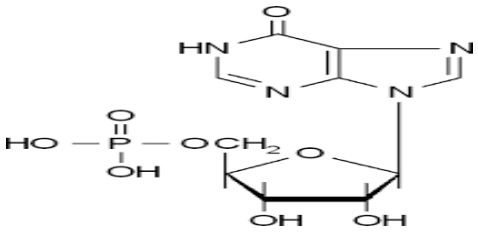
7-**Amino group** from **Aspartate** is being donated, but the mechanism is different: <u>All the aspartate will be linked</u>, then it will be released as fumarate, leaving the amino group linked to the forming ring. This reaction catalyzed by Adenylosuccinate lyase.



8-**Another Formyl group** from **N10-Formyl-THF** is being linked.



9-**The ring will close**.



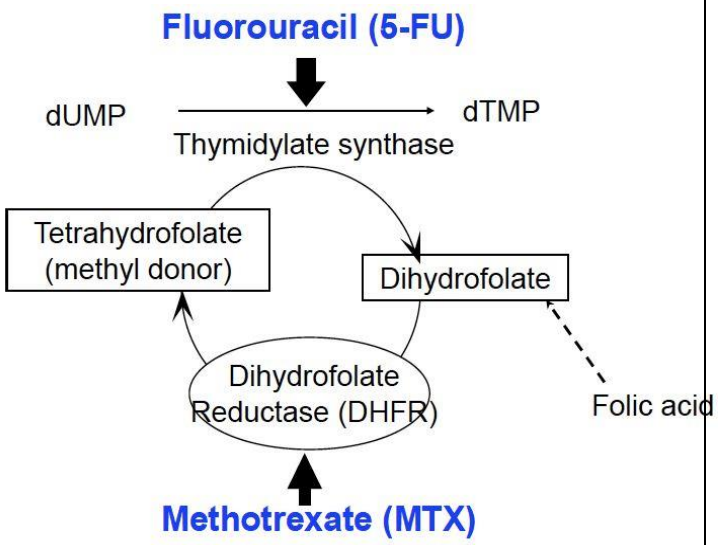
See **Figure 22.7. It is easier to study the pathway from it.**

-The first purine ring made is the <u>Hypoxanthine</u>, and because it linked to R-5-P it called **IMP (inosine monophosphate)** or **Inosinate.**

-we have Two N10-Formyl-tetrahydrofolate molecules in the pathway, so any drug that subjected towared **THF will be inhibitors for the pathway.**

-The first drug is **para-aminobenzoic acid (PABA) analogs that** are portion of THF. **Sulfonamide drugs** one of these analogs to PABA, this drug affecting bacteria, because the only pathway for bacteria to get the folic acid is the synthesis pathway, and these drugs inhabit this process. (Has no effects on humans since we don't produce folic acid)

-we will see in the pyrimidines synthesis that THF after donation the Formyl group will converted to dihydrofolate , and to active again it must converted to THF via **dihydrofolate reductase**. This reductase is inhabited by a **Folate analog,** the most important analog is **Methotrexate** that used widely as **anti-cancer drug,** it inhabits the formation of THF , this mean the supply line of THF(In the nucleotide synthesis pathway) has been interfered with, and this explain the mechanism of action of these drugs. But these drugs have side effect to normal cells (toxic effect).



-Since methotrexate inhibits the production of of THF, this drug affects purine and pyrimidine synthesis pathways (slows DNA replication).

-The regulation mechanisms in purine synthesis pathway

-We will take about the **Rate limiting enzyme** that link the nitrogen number 9 to carbon number 1 (First enzyme in the pathway), accumulation of AMP alone or GMP alone or IMP alone didn't inhabit the enzyme, but when they accumulate at the same time, the effect will be **synergistic,** they inhabit the enzyme by making it in the **dimmer form**

(inactive state). On the other hand **PRPP** is activator (by forming the **monomer form** (which is the **active state**). The effect of these activators and inhibitors illustrated in the kinetic profile of the pathway in the slides (Glutamate is a substrate and the curve is hyperbolic as well as PRPP is a substrate but the curve is sigmoidal).

-In this pathway we see how to form monophosphate nucleotide, but most of nucleotides in the cell present in the (Di and Tri)-phosphate, so there is enzyme called **nucleoside monophosphate kinase** that convert monophosphate into Di-phosphate (Mainly, the phosphate group came from ATP as it might come from GTP, CTP..), this enzyme is specific for each base (for example, A has its own one , G has its own one and so on….) but not specific for the sugar(it doesn't discriminate between ribose and deoxyribose) , the most important and predominant (From the monophosphate kinase enzymes) is the one that work on AMP that called **Adenylate kinase** (AMP + ATP ⇨2ADP). However, **nucleoside diphosphate kinase** is 10-100 fold times more than monophosphate kinase, because the Triphosphate nucleosides are more than Diphosphate and the (Di and Tri) much more than monophosphate nucleosides, nucleosides and bases

* **nucleoside diphosphate kinase** (coverts Di to Tri by using any triphsphate nucleoside like ATP).

*the nucleoside alone can undergo salvage pathway (phosphorylation), but this reaction is actually present only for Adenosine, that convert Adenosine to adenosine monophosphate by Adenosine kinase enzyme.

- The purine synthesis pathway is end with IMP, but how we can get GMP and AMP?

GMP and AMP are formed from IMP. **IMP dehydrogenase** will introduce another keto group on IMP producing **Xanthosine monophosphate**, then Glutamine donate an amino group producing **GMP** by **GMP synthatase**, we can notice that to synthesis GMP we need ATP (as

source of energy), once the product (GMP) accumulates it will feedback inhabit IMP dehydrogenase (inhbited only by GMP). For the synthesis of AMP, all what we need is an amino group, that provided by aspartate and this reaction require GTP, as we know when aspartate donate an amino group, all the aspartate molecule will linked to the acceptor (in this case IMP), then it will be released as fumarate leaving the amino group and we will have AMP, and when AMP accumulate it will inhabit the first enzyme in its branch. *See the slides.

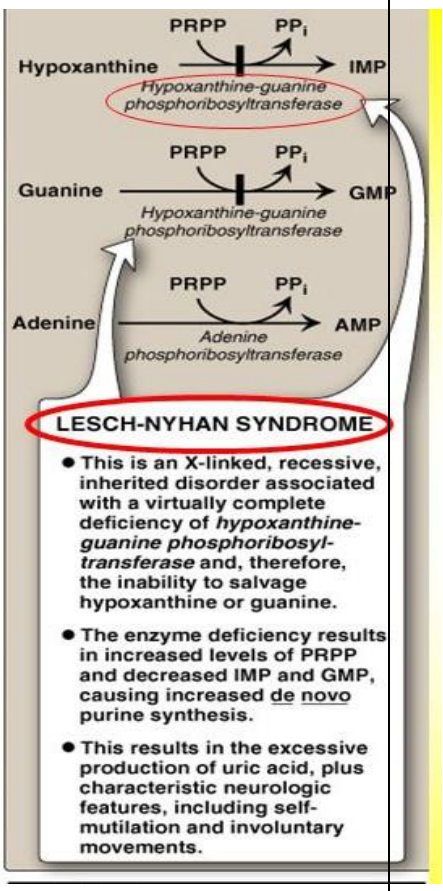
-**Mycophenolic acid** is a drug that used to prevent the rejection of organ graft, rejection of graft is involve immune cells (T and B cells), therefore to prevent that we must suppress these fast proliferating cells by inhabiting **IMP dehydrogenase**, thus this cells are no longer able to synthesis GMP (which ultimately making GTP) and this GTP is required for synthesis of nucleotide which is important for T and B cells replication.

(2) salvage pathway

-Nucleotides can be degraded to bases. Then they go to the blood to all tissues. We studied how the bases are synthesized in a long pathway, as well as the salvage pathway is very important in the brain (the De novo is not fully active in the brain), so the body doesn't throw or degraded these bases, instead the body use them in salvage pathway to get the nucleotide back.

-There is salvage enzyme that require PRPP an activated donor of the ribose phosphate which linked with the base and we will have new nucleotide (One single reaction), for purine there are two enzymes, **Hypoxanthine-guanine phosphoribosyltransferase (HGPRT)** that act on (Hypoxanthine and Guanine), and **Adenine phosphoribosyltransferase (APRT)** that work on adenine. Salvage enzyme act in many tissues, but it is very important **in the brain**.

-**Lesch-Nyhan syndrome** (Rare): In this syndrome HGPRT is defective, from the consequences of the syndrome; we will appreciate the value of the salvage pathway. This enzyme is X-linked, recessive disorder associated with a virtually complete deficiency of HGPRT. The deficiency leads to degrade Hypoxanthine and Guanine to form **Uric acid** (excess amount will be present in the body). In addition, the lack of this salvage pathway causes decreased level of the ribonucleotides (IMP and GMP) and increased level of PRPP; these 2 factors will lead to increase De novo purine synthesis* as well as there will increase in nucleotides that will be degraded to bases, and these bases are not able to be used another time (by salvage pathway), so they will undergo degradation and produce uric acid, that cause **hyperuricemia.** The brain need salvage pathway, so when it's defect, the brain will be affected, and this effect is characterized by motor dysfunction, cognitive deficits and behavior disturbances that include self-mutilation(for example, biting of lips and fingers). Remember that the solubility of uric acid is very low, so once it increases it will precipitate as urate crystals, especially in the joint (gouty arthritis), soft tissues that induce inflammation and this state is called **gout**.



*Remember: IMP and GMP feedback inhibit De novo purine synthesis. PRPP is an activator for De novo purine synthesis.

#To sum up:

*In normal situation nucleotides give us bases, and some of these bases will be converted to nucleotide in salvage pathway. But salvage pathway is defective in Lesch-Nyhan syndrome, so we will have excess in PRPP and low level of IMP and GMP, this will increase the synthesis (by De novo pathway). At the end, this will lead to increase in degradation and producing of Uric acid (hyperuricemia).