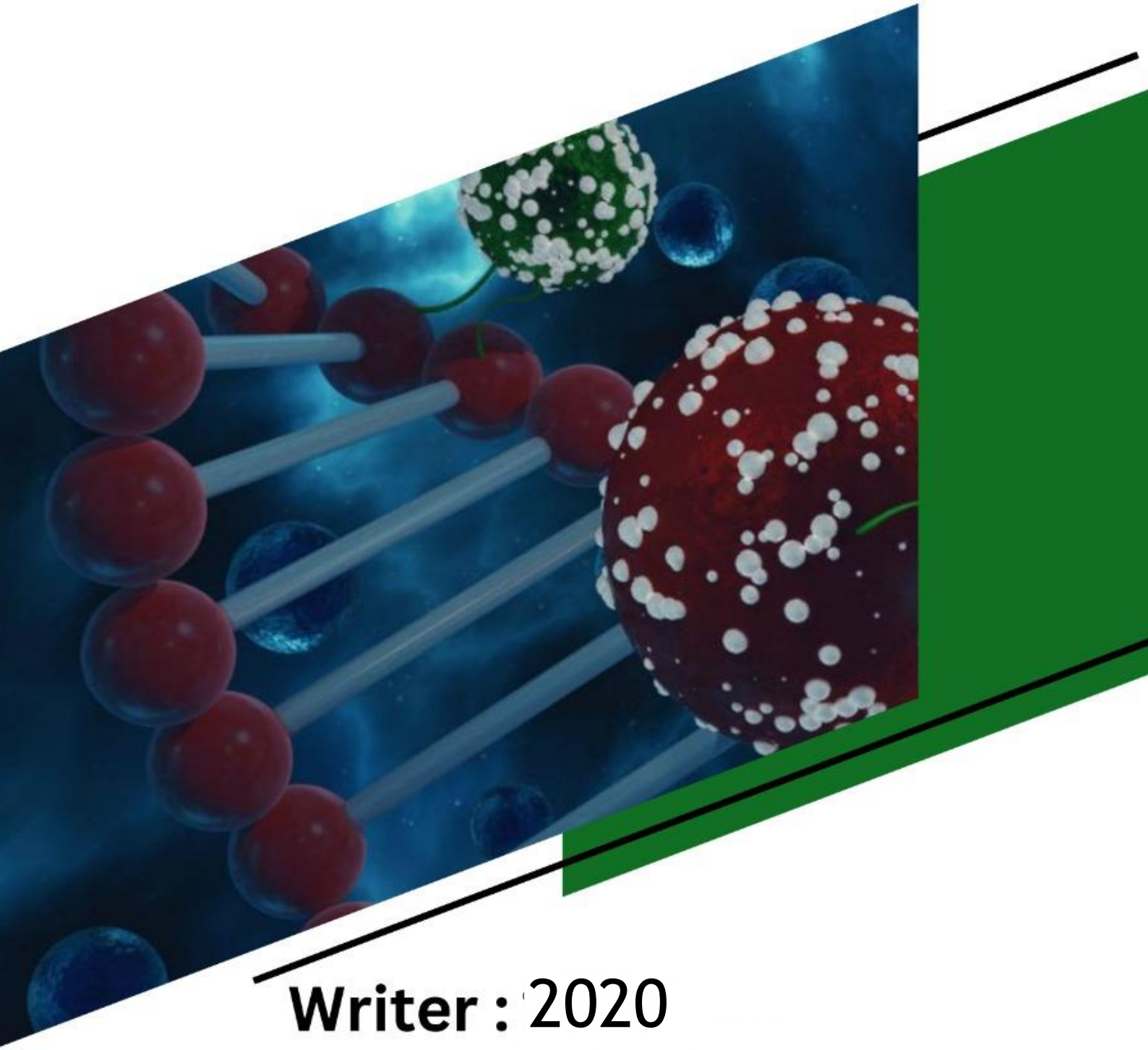
Doctor 021

# METABOLISM

## Sheet no.33



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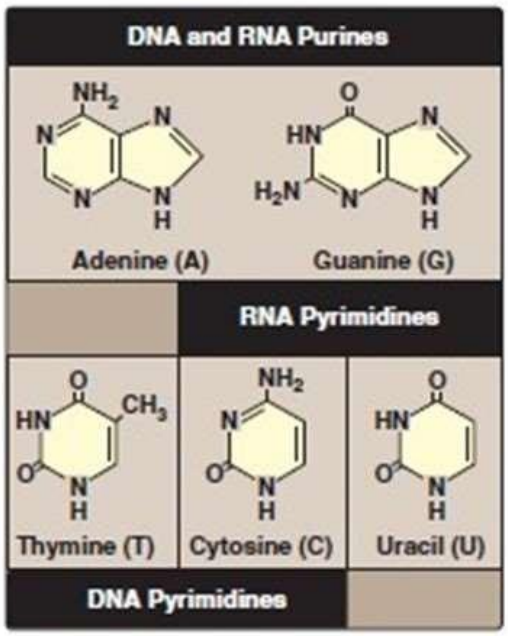
# NUCLEOTIDES METABOLISM




## Purine and pyrimidine structures and functions:

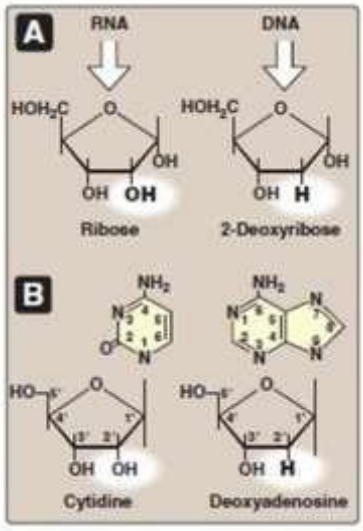
- Pyrimidines (thymine, cytosine, and uracil) composed from one ring made from 6 carbons while purines (adenine and guanine) composed from 2 rings → five membered ring and six membered ring fused to each other.
- Since nucleotides contain purines or pyrimidines (nitrogenous bases) therefore nucleotides considered as a one of the nitrogen containing compounds.
- Purines and pyrimidines are very important molecules that are found in nucleotides and serve different functions:
  1) Essential for RNA and DNA synthesis (deoxyribonucleic acid and ribonucleic acid).
  2) They serve as carriers of activated intermediates in the synthesis of some carbohydrates, lipids, and conjugated proteins, such as, UDP-glucose (glycogen synthesis) and CDP-choline (membrane lipid synthesis).
  3) They are structural components of several essential coenzymes, such as coenzyme A, FAD, NAD+, and NADP+.
  4) They serve as second messengers in signal transduction pathways, such as cAMP and cGMP.
  5) They are "energy currency" in the cell (ATP).
  6) They act as allosteric regulatory compounds for many metabolic pathways by inhibiting or activating key enzymes (eg: ADP, AMP ….).

## Nucleosides:

- This method is used to name compounds that contain only sugar and a nitrogenous base, to indicate the number of phosphates that the compound contains.

  The main rule → (Nucleoside= Pentose sugar + Base).

  Eg: ATP can be named as adenosine Triphosphate, "sine" suffix is used to indicate the presence of nucleoside (sugar + nitrogenous base) as well as the number of phosphate groups attached to the nucleoside. However, ATP can be named as a "Nucleotide" with no indication for the number of phosphate groups.



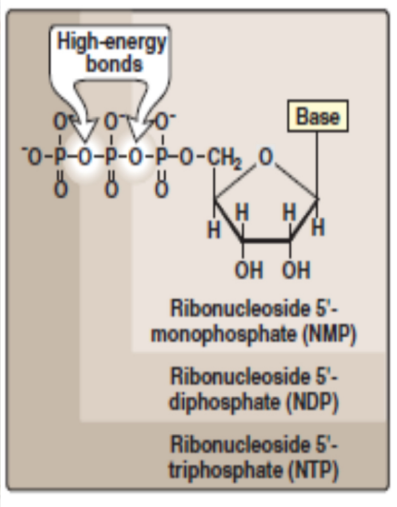

❖ (Ribose + base = Ribonucleoside).
Eg: The ribonucleosides of A, G, C, and U are named **adenosine, guanosine, cytidine, and uridine**, respectively.

❖ (2-deoxyribose + base = deoxyribonucleoside.)
Eg: The deoxyribonucleosides of A, G, C, and T are named **deoxyadenosine, deoxyguanosine, deoxycytidine, and deoxythymidine**, respectively. (Deoxy sugaís aíe sugaís that havehad a hydíoxyl gíoup íeplaced with a hydíogen atom).

Recall: the carbons in the sugar molecule are numbered with a prime 1', 2' while the carbons of the nitrogenous bases are named without prime (Observe the picture).

-- Numbering is separate and different (prime and on prime)

## Nucleotides:




❖ This method is used to name compounds that contain sugar, nitrogenous base and phosphate without any indication for the number of phosphates that the compound contain.

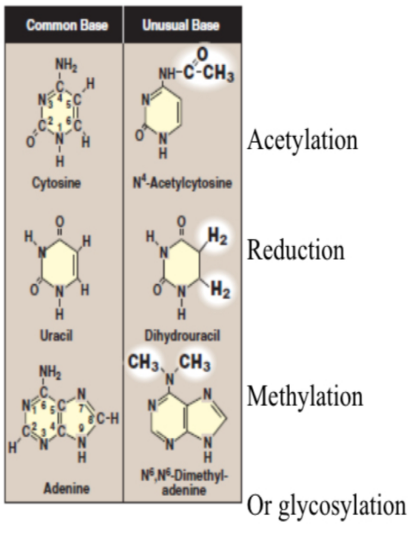
(Nucleoside + one or more phosphate groups= Nucleotide).

❖ The first P group is added by an ester linkage to the 5'-OH of the pentose forming a **nucleoside 5'-phosphate** or a **5'-nucleotide**. The second and third phosphates are each connected to the nucleotide by a "high-energy" bond.
(Nitrogenous base is added to the pentose on the 1' carbon and the phosphate group is added on the 5' carbon).

## The type of pentose is denoted by the prefix in the names "5'-ribonucleotide" and "5'-deoxyribonucleotide."

Recall: The phosphate groups are negatively charged causing DNA and RNA to be nucleic acids.

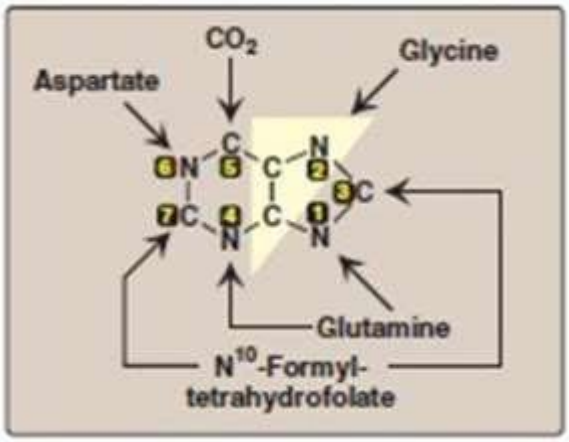
## Base modification:




❖ Nitrogenous bases specifically in the DNA or RNA structures are more susceptible for further covalent epigenetic modifications. Therefore, nucleotide sequences are more able to be recognized by specific enzyme or to be protectable from degradation from certain nucleases.

❖ ***Methylation** for nitrogenous bases is important in gene silencing → preventing transcription factors from binding accordingly no gene expression occur.
***Acetylation** for nitrogenous bases is usually associated with gene activation → allowing for transcription factors to bind accordingly more activation for gene expression. `
***Reduction** is important in URICIL.
***Glycosylation** by adding sugars.

# Nucleotides (IMP) and Purine synthesis:




The purine and pyrimidine bases are synthesized in well fed state (excess of energy) in two ways: 1) **de novo** (From A to Z). 2) **salvage pathways** (reuse of the preformed bases resulting from normal cell turnover).

Little of the purines and pyrimidines supplied by diet are utilized, and are degraded instead

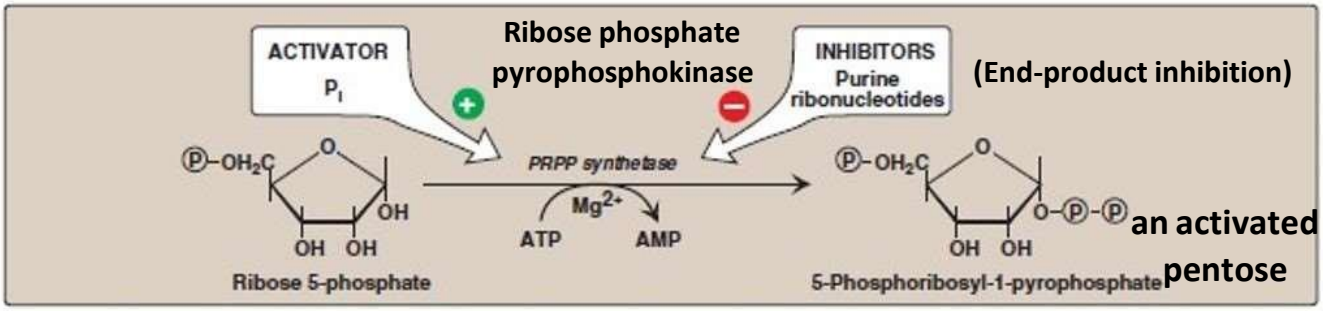
The contributing compounds in purine synthesis are:

1) Amino acids (aspartate, glycine, and glutamine) the major component in purine synthesis used as a source of carbons as well as nitrogen.
2) $CO_2$.
3) N10-formyltetrahydrofolate.

The purine ring is constructed primarily in the liver by a series of reactions that add the donated carbons and nitrogens to a preformed ribose 5-phosphate.

**STEPS OF THE REACRIONS (de novo synthesis of purines):**

## STEP1: Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)




**Ribose-5-phosphate** that comes from pentose phosphate pathway is activated by adding pyrophosphate on the carbon no.1 in a phosphorylation reaction converting ATP to AMP. The product of this activation reaction is **5-phosphoribosyl-1-pyrophosphate (PRPP).** This step is catalyzed by **Ribose phosphate pyrophosphokinase (PRPP synthetase).**

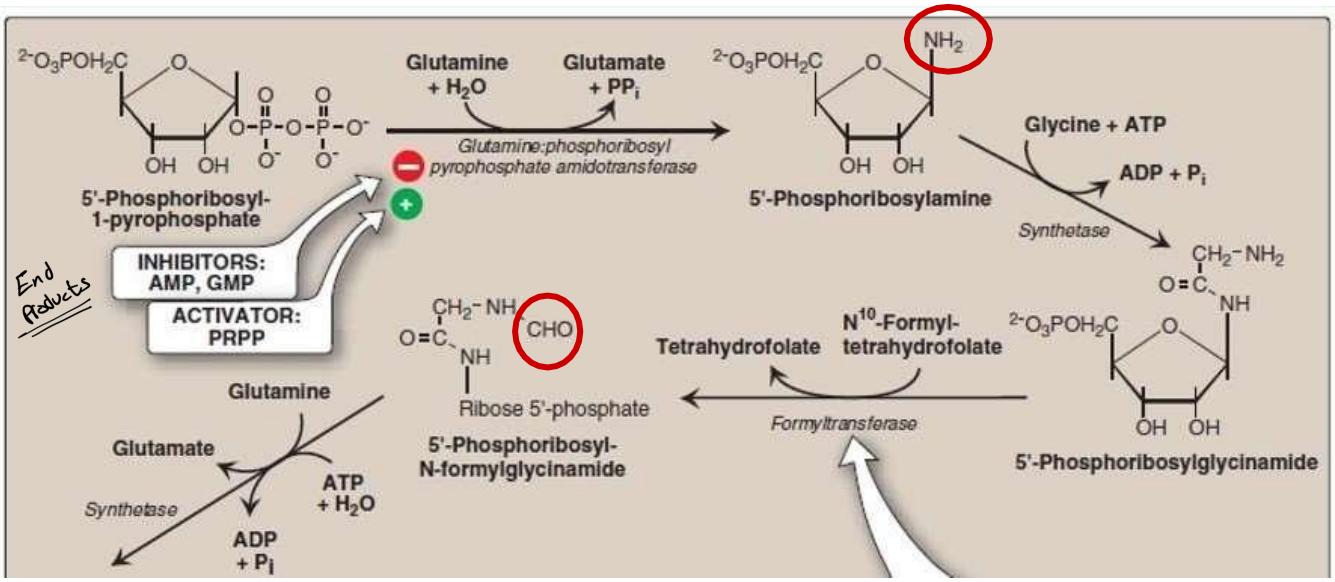
-Notice that pyrophosphate is added on carbon no. 1 where purine synthesis will take place. Therefore, pyrophosphate works as a sign to complete the reaction in nucleotide synthesis-

The sugar moiety of PRPP is ribose, therefore, ribonucleotides are the end products of de novo purine synthesis.

When deoxy ribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced

**Regulation:**

Inorganic phosphates are considered as allosteric activators for PRPP synthetase enzyme. While excess of purine ribonucleotides the final product produced considered as an allosteric inhibitor.




**STEP 2**: **Synthesis of 5'-phosphoribosylamine** (the committed step in purine nucleotide biosynthesis).

**Glutamine** donates its own amine group to **PRPP** in the presence of **H2O** producing

**5'-phosphoribosylamine.** Notice that pyrophosphate is removed, and glutamine converted to glutamate.

<u>The first source of nitrogen in purine and pyrimidine synthesis is from glutamine AA</u>

**Regulation:**

AMP and GMP are considered as allosteric inhibitors for this step while PRPP is an allosteric activator

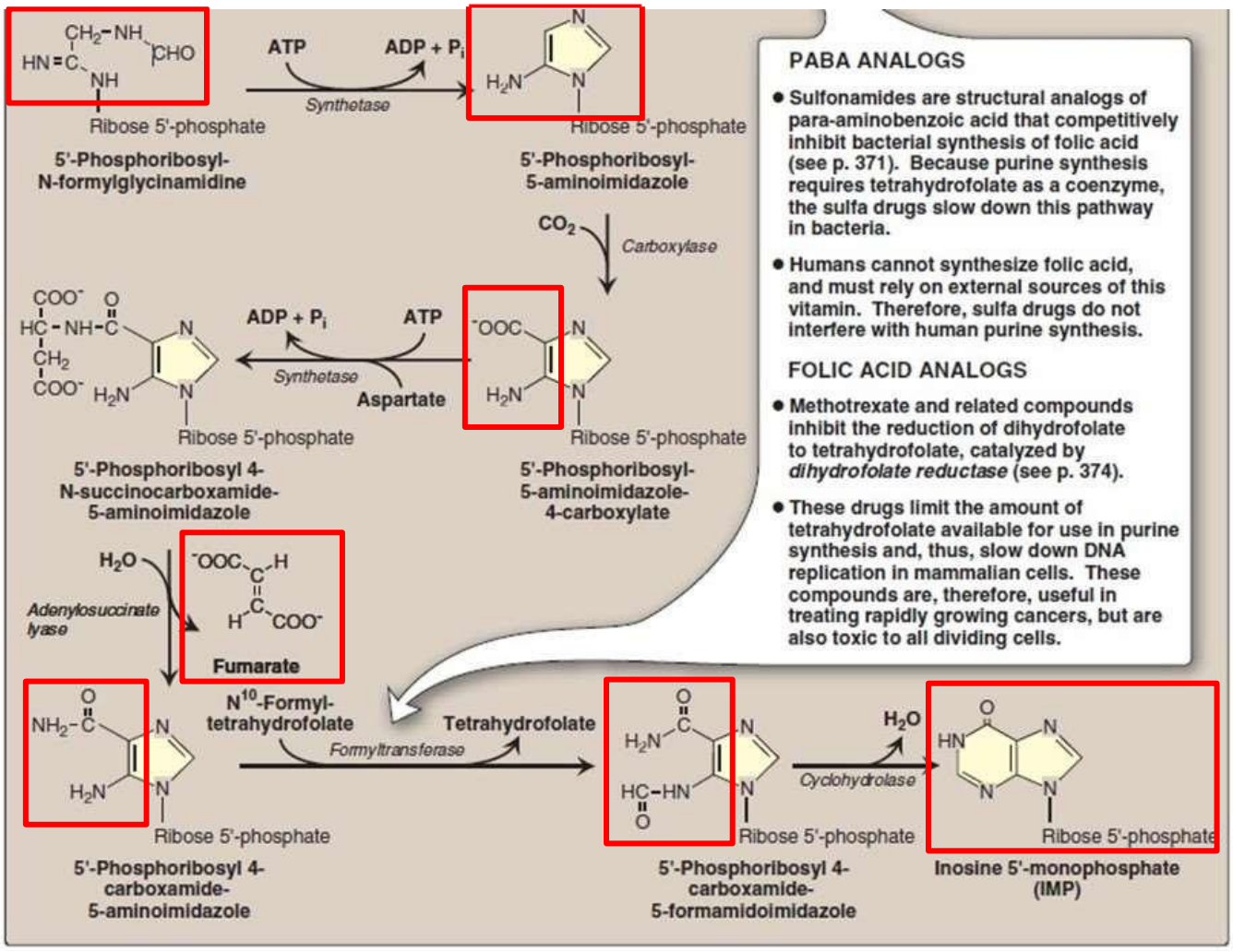
**STEP 3:** **Synthesis of inosine monophosphate, the "parent" purine nucleotide**

The next nine steps lead to the synthesis of IMP, whose base is hypoxanthine
This pathway requires ATP as an energy source.

- **Glycine AA** is attached to the amine group (observe the picture above) forming the first 4 atoms of purine's first ring. -ATP dependent step-
- <u>Carbonyl group</u> is added from **N10-formyl-tetrahydrofolate** forming 5 atoms of purine's ring.
- <u>Amine group</u> is added from **glutamine** and **cyclization reaction** occur producing the first ring of purine structure and the <u>first nitrogen</u> atom in the second ring. -ATP dependent step-
- Adding **$CO_2$** molecule forming the first 4 atoms of the second ring. (2 atoms from the first ring in addition to the <u>amine group and $CO_2$</u>).
- **Aspartate AA** gives the <u>$5^{th}$ atom (nitrogen)</u> of the second ring and leaves the reaction as fumarate.
- <u>A carbonyl group</u> is added from **N10-formyltetrahydrofolat** ($6^{th}$ atom of the second ring).

- **Cyclization reaction** occur and finally the two rings of purine are ready. This step produces **inosine 5'-monophosphate IMP** (Inosine-5'-monophosphate= purine ring + sugar + phosphate).

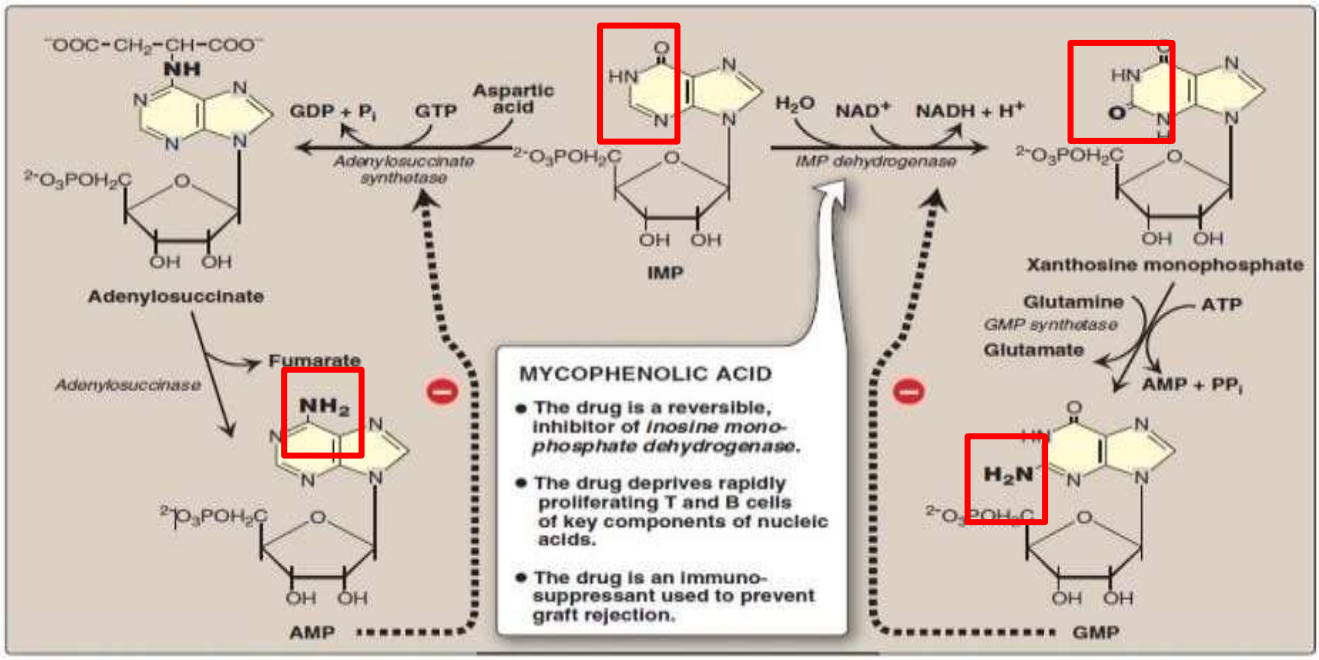
تبعوا مع الصور كل خطوة بخطوتها عشان تفهموا , التفاصيل المطلوب حفظها مكتوبة بالخطوات فوق و حاولوا احضرو مع الدكتورة لانها كانت تختصر الخطوات , فاحضروا المحاضرةعشان تفهموا مزبوط وين تركزوا.




## Conversion of IMP to AMP and GMP: (observe the picture below)

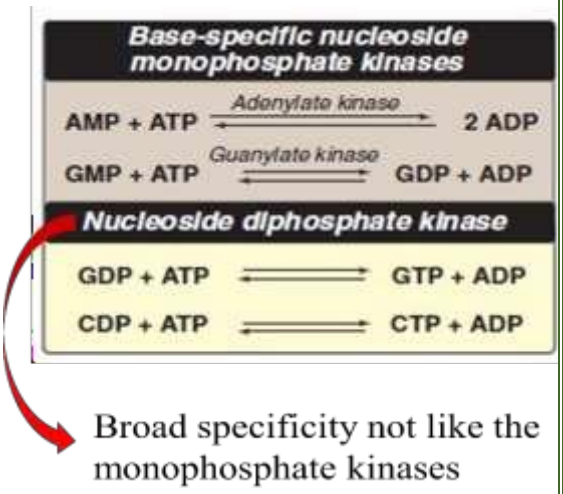
Recall that there are two types of purines; adenine and guanine → the difference between them is only in the position of amine group. In the previous pathway IMP is synthesized and ready to be modified into AMP or GMP.

- AMP:
  *__Aspartate__ is added to IMP as a source of nitrogen and **GTP** as a source of energy producing **adenylosuccinate**. This step is catalyzed by **adenylosuccinate synthetase**.
  *Fumarate is released producing AMP.
  **Regulation:** AMP is an allosteric inhibitor (feedback inhibition).
- GMP:
  *IMP got oxidized by **IMP dehydrogenase** producing **Xanthosine monophosphate** and NADH.
  *__Glutamine__ is added as a source of nitrogen and **ATP** as a source of energy producing GMP.
  **Regulation**: GMP is an allosteric inhibitor (feedback inhibition).




## Conversion of AMP and GMP to ADP/ATP and GDP/GTP respectively:

Phosphate groups are added separately so that one phosphate group is added each time by different enzyme. Accordingly, the enzymes that is responsible for adding the first phosphate group are specific enzymes **"Base specific nucleoside monophosphate kinases"** for each nucleotide. While the enzymes that is responsible for adding the second phosphate group are not specific **"nucleoside diphosphate kinase".**




- AMP is phosphorylated to ADP by **adenylate kinase (AK)** and GMP is phosphorylated to GDP by **guanylate kinase**.
  Both AMP and GDP hydrolyze ATP as a source of phosphate since it is present in higher concentrations than the other nucleoside triphosphates.
- GDP and ADP are phosphorylated to GTP and ADP respectively by **nucleoside diphosphate kinase**. Both AMP and GDP hydrolyze ATP as a source of phosphate (the enzyme that catalyzes this step can phosphorylate any type of nucleotide, whether these nucleotides contain purines or pyrimidines).

  **NOTE:** Base-specific nucleoside monophosphate kinases do not discriminate between ribose or deoxyribose in the substrate and Adenylate kinase (AK) is particularly active in liver and muscle also AK maintains an equilibrium among AMP, ADP, and ATP

## 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.

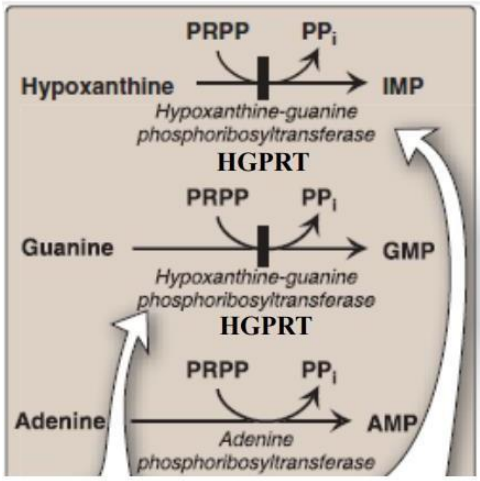
## Salvage pathway for purines

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)

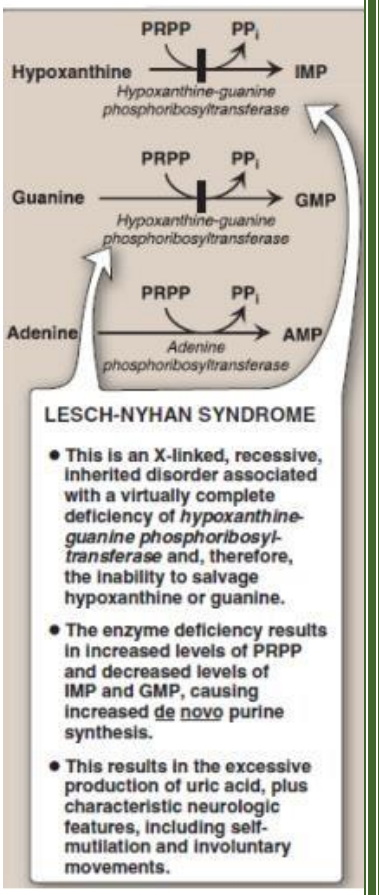
Conversion of purine bases to nucleotides:

**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**

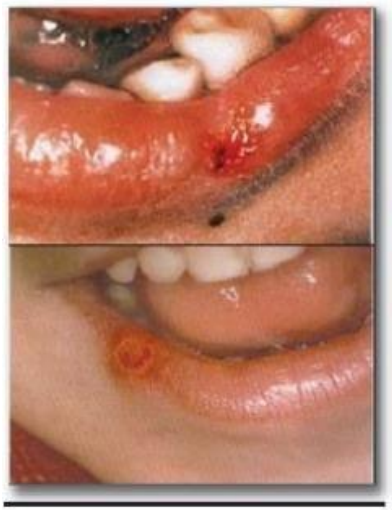
Inability to salvage hypoxanthine or guanine resulting in high amounts of uric acid (the end product of purine degradation)

**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).**

# v1