# 36


Sheet  Slides


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In the previous lecture we started talking about nucleotides, and in this one we'll talk about how they're synthesized from scratch !

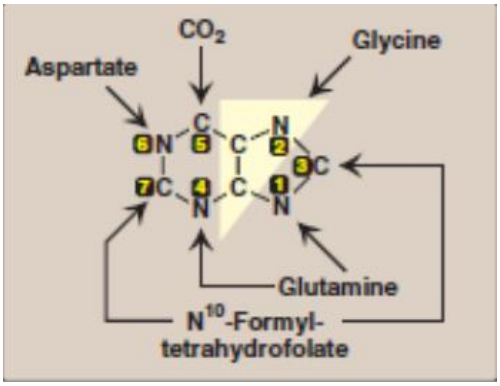
## I. Purine synthesis:

*Purines are bigger, they're made of <u>two connected rings</u>, which indicates that they require a longer pathway to be synthesized than pyrimidines*

- The contributing compounds:

i. **3 kinds of Amino acids** (aspartic acid, glycine and glutamine)
ii. **$CO_2$** (from the surrounding decarboxylation reactions)
iii. **$N^{10}$–formyltetrahydrofolate**

*you're not required to know where each atom came from*




⇒ The purine ring is **constructed primarily in the liver.**

There are actually **two ways** to synthesize a nucleotide,
<u>either</u> **de novo** where it's made from scratch and each atom is added to the ring separately *de novo is the major source of DNA synthesis purines*
<u>or</u> **Salvaged**, where big pieces (like the nitrogenous ring) are priorly synthesized and we just put them together.

- The steps of synthesis (de novo):

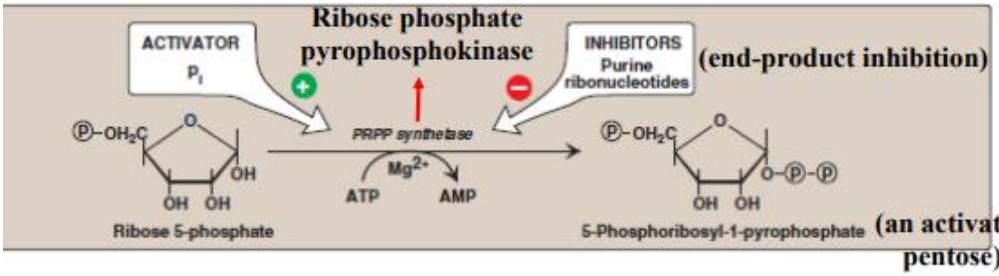
We start with the preformed molecule **Ribose-5-phosphate, and the build up is accomplished by the serial addition of donated nitrogens and carbons.**

⇒ **Ribose 5-phosphate** is synthesized by the **pentose phosphate pathway**

* You're only required to know the names of the main ones like PRPP, IMP. you're not required to know the order of the steps *

Just don't forget that the pentagon ring was formed first.

## Step 1: activation of synthesis by the addition of pyrophosphate to R-5-P




- Activation of of R-5-P occurs by the **addition of a pyrophosphate** from an ATP molecule by the following equation **ATP= $PP_i$ + AMP.**

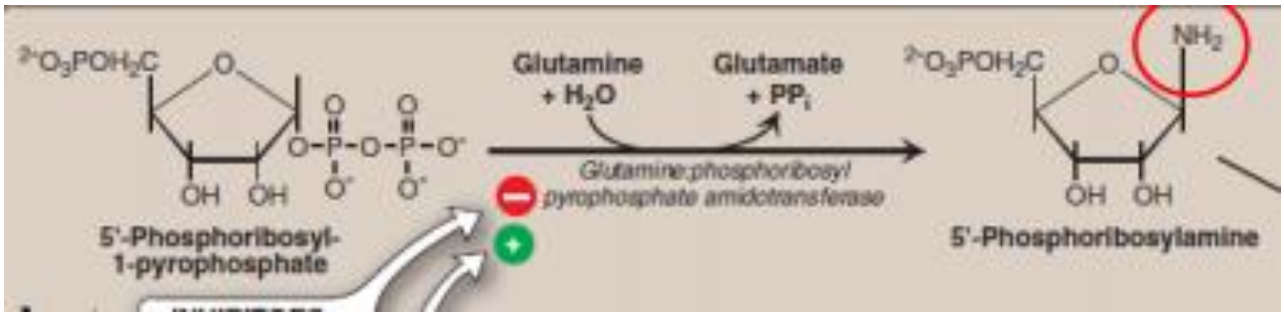
  **Enzyme**: PRPP synthetase (ribose phosphate pyrophosphokinase)
- The **pyrophosphate is added to carbon number one** since it's the eventual attachment site of the nitrogenous base.
  The resulting compound is 5-phosphoribosyl-1-pyrophosphate (PRPP)

- 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.

**The pyrophosphate is then removed because the addition of it was just for activation not for building and in its place the building atoms are added starting with $NH_2$**

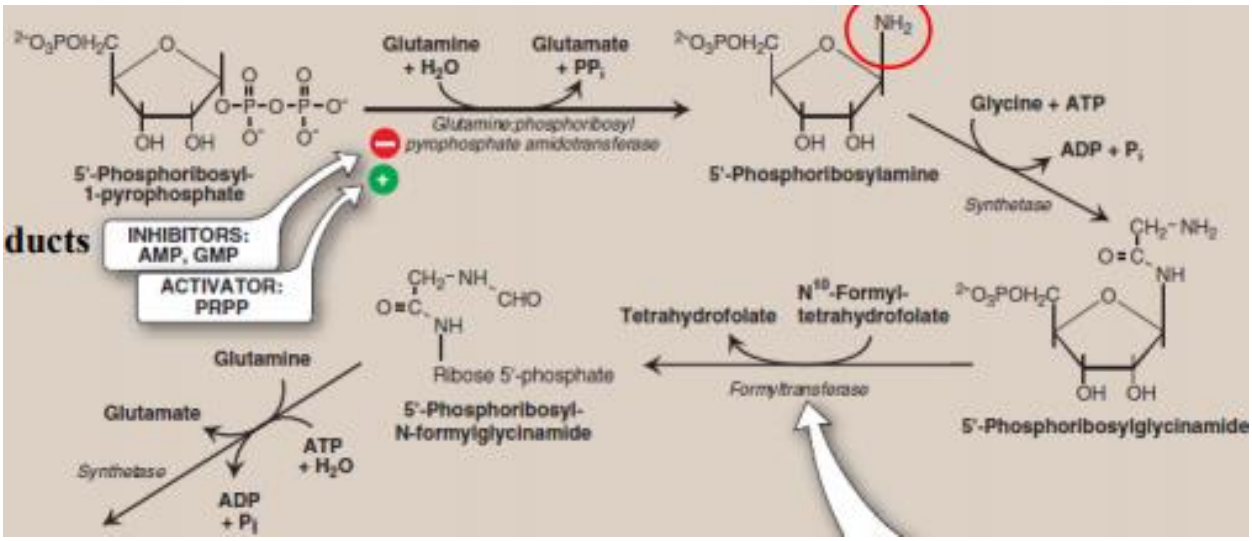
## Step 2: Synthesis of 5'-phosphoribosylamine (first step to IMP)

*the committed step in purine nucleotide biosynthesis*




This step is accomplished by the entry of Glutamine which **gives its NH2 group** and leaves as glutamate. By the enzyme amidotransferase

## Step 3: Synthesis of inosine monophosphate



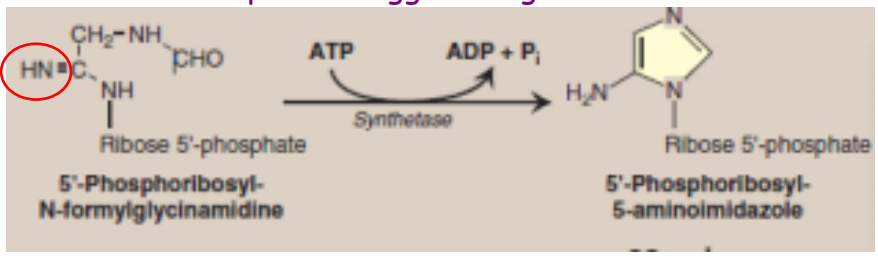

This is accomplished by multistep modification that starts with **addition of glycine (all of it) on the $NH_2$** added previously, with the **use of ATP;** hence the enzyme is called synthetase.

When glycine is added, **4 atoms of the pentagon ring of purine will be present** (three atoms from Gly + N from glutamine), we need to add a fifth one in order to have all the atoms necessary to close the ring.

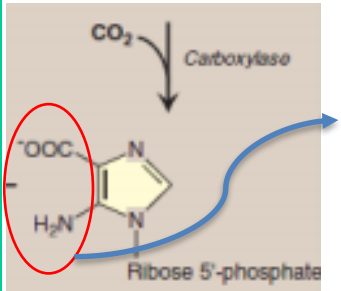
The fifth atom comes from **$N^{10}$- Formyl-tetrahydrofolate** in the form of **-CHO** (H-C=O).

**glutamine** enters and gives **NH which will bind in the form of =NH**, catalyzed by synthetase**.**

*This is the step that triggers ring closure*

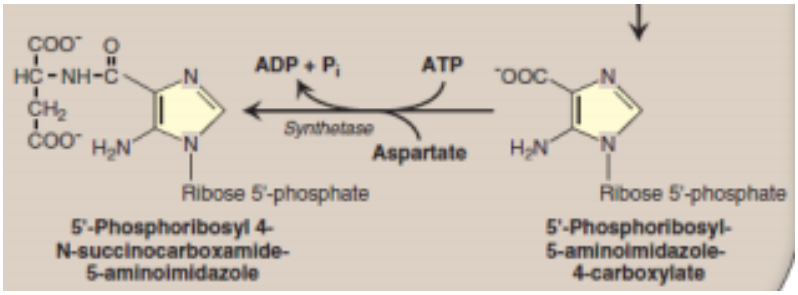


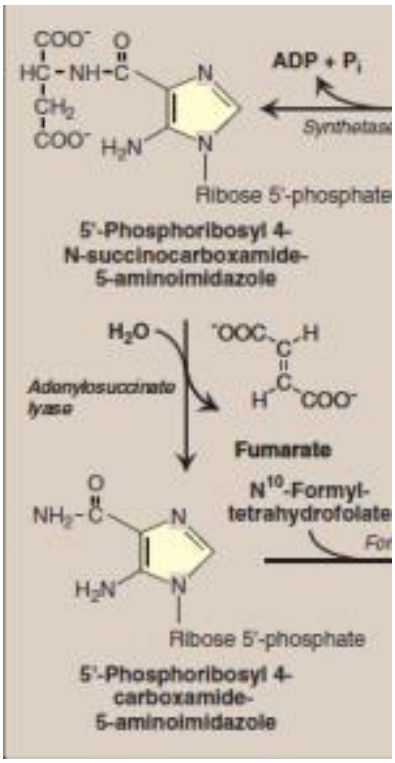

After that a CO2 molecule is added by a carboxylase




**Thus, four atoms of the hexagon ring are present. Two shared with the other ring and two added.**

**Then aspartate is added entirely enters by a synthetase enzyme.**

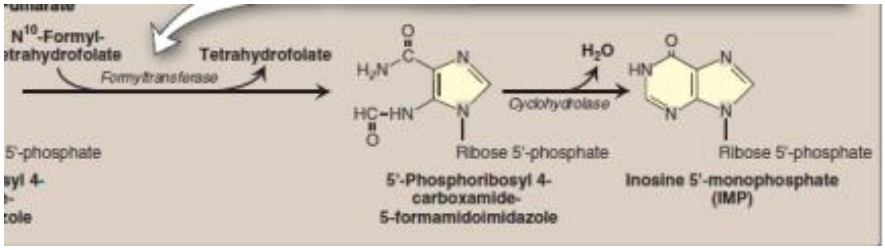



- Then we cut part of this aspartate in the form of **fumarate** and **only NH2 of it remains**.

  * 5 out of 6 ring atoms are present*

- An **$N^{10}$- formyl-tetraydropholate molecule** comes and donates a **carbon atom in the form of -CHO**




**(6 out of 6 atoms are present and the second ring is closed)**

marking the birth of the first nucleotide compound:

Inosine- 5'-monophosphate (IMP) the parent purine nucleotide.

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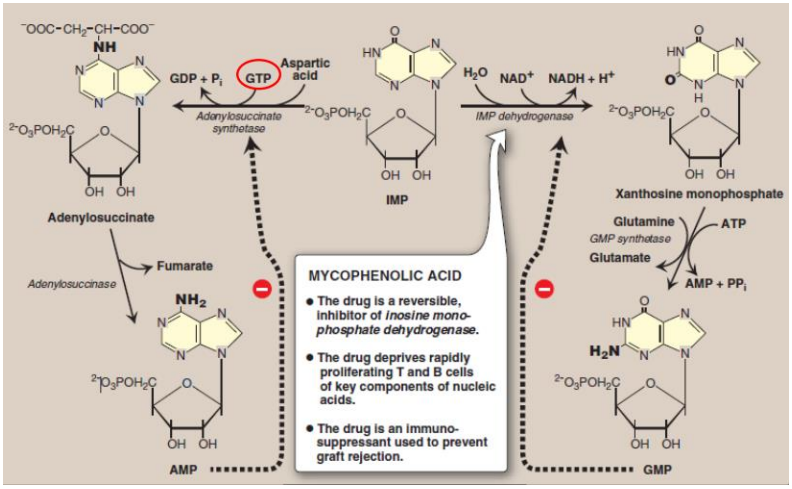
- Inosine monophosphate acts as a branching point:
  GMP and AMP are formed from IMP



### ⇒ AMP production

Adenylosuccinate synthetase adds aspartate (look on the left) by the N atom in place of O on the hexagon ring (GTP used) and **adenylosuccinate** is formed, then most of the structure of succinate is removed in the form of fumarate , only NH2 is left and AMP is produced (adenosine monophosphate)

### ⇒ GMP production

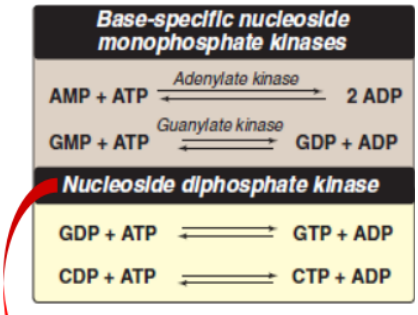
An amine group is added as well but on a different position. First an oxidation-reduction reaction occurs by IMP dehydrogenase (NAD+ reduced to NADH) and we from **xanthosine monophosphate**.

Glutamine donates an amine group, with ATP used as a source of energy, on a different site forming GMP

### How do we regulate this pathway?

- **on the first step**, purines inhibit **PRPP synthetase**, while Pi (high concentration of inorganic phosphate) activates this pathway.
- **First step of IMP production**, final products AMP and GMP if in high concentrations **inhibit the pathway** at this step. The first substrate PRPP activates it.
- **Final products** affect the diversion of the pathway. AMP works on adenylosuccinate synthetase and inhibits it and GMP inhibits IMP dehydrogenase.
- In the final step we have specific inhibition by the needs of the cells.

- Further modification: phosphorylation by kinases to form nucleoside di and tri phosphates




They're phosphorylated in two steps: first, to nucleosides diphosphate by **base specific kinases**:

Adenylate kinase works on AMP and Guanylate kinase works on GMP.

Then, they're phosphorylated to nucleosides triphosphate by a nonbase-specific enzyme:

Nucleoside diphosphate kinase works on both (on ALL actually)

ATP is the general source of the phosphate, since it is present in higher concentrations than the other nucleoside triphosphates.

Adenylate kinase (AK) is particularly active in liver and muscle. AK maintains an equilibrium among AMP, ADP, and ATP (since they use a lot of energy)

⇒ one of the most important functions of nucleotides is **using them in DNA synthesis or DNA replication in cell division**.

We can inhibit purine synthesis and thus stop cell division by some synthetic agents which in turn can be used as treatment for cancer.

Some drugs were produced to affect this pathway like **methotrexate.**

It's a chemotherapeutic agent that **takes the place of tetrahydrofolate** and blocks the procedure of synthesis.

They also used this finding to **inhibit the purine synthesis of microorganisms** like in sulfonamide antibiotics.

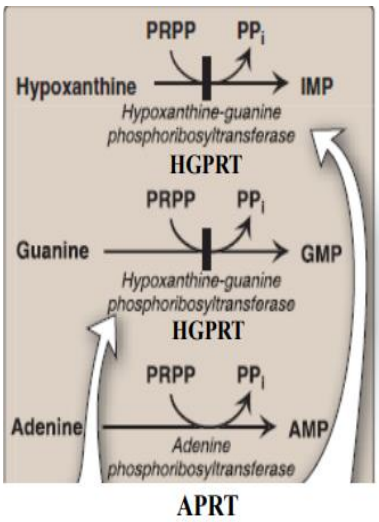
**FOLIC ACID ANALOGS**

- **Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by *dihydrofolate reductase* (see p. 374).**
- **These drugs limit the amount of tetrahydrofolate available for use in purine synthesis and, thus, slow down DNA replication in mammalian cells. These compounds are, therefore, useful in treating rapidly growing cancers, but are also toxic to all dividing cells.**

**PABA ANALOGS**

- **Sulfonamides are structural analogs of para-aminobenzoic acid that competitively inhibit bacterial synthesis of folic acid (see p. 371). Because purine synthesis requires tetrahydrofolate as a coenzyme, the sulfa drugs slow down this pathway in bacteria.**
- **Humans cannot synthesize folic acid, and must rely on external sources of this vitamin. Therefore, sulfa drugs do not interfere with human purine synthesis.**

- Salvage pathway for purines (recycling pathway)



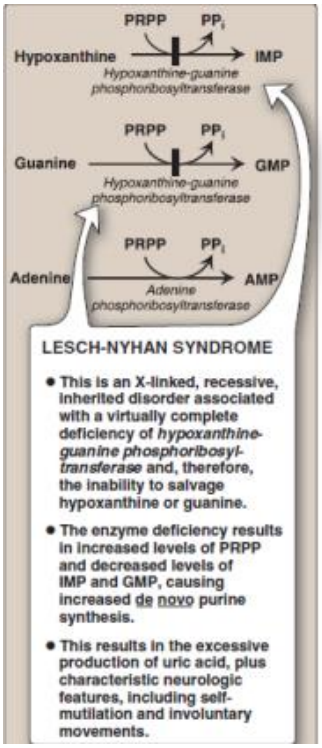

We get the nitrogenous base from wherever and just add PRPP to it, then pyrophosphate is removed, and N-base is placed instead of it and nucleotides are produced.

**Adenine** by the enzyme APRT is recycled to→ AMP
**Guanine** by the enzyme HGPRT is recycled to→ GMP
**HGPRT** works on two substrates, it can add PRPP to guanine and produce GMP or add PRPP to hypoxanthine and produce IMP which can proceed to form AMP and GMP.

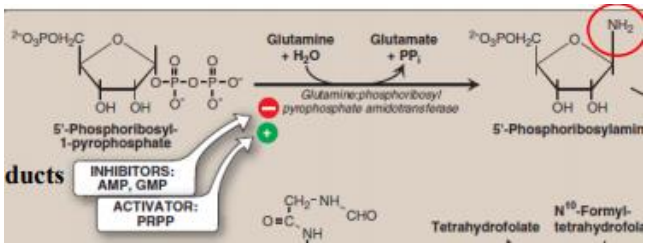
⇒ Adenosine is the only **nucleoside** we can salvage.

## Salvage pathway for purines-Lesch-Nyhan syndrome

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



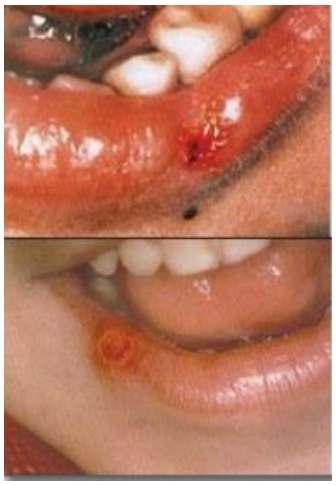

- **Inability to salvage hypoxanthine** or **guanine** resulting in **high amounts of uric acid** (the end product of purine degradation)




- **Because of 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 to make up for it.

  The **increased purine synthesis** due to de novo activation and **decreased purine reutilization** results in **increased degradation of purines** and the production of large amounts of uric acid **(hyperuricemia)**

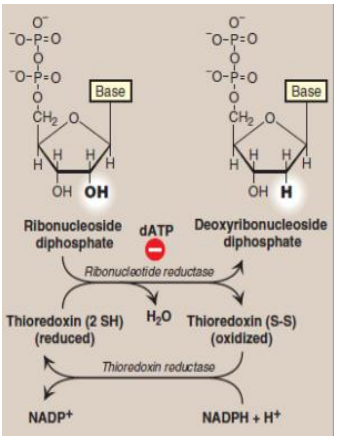


  Increased concentration of uric acid leads to:

  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)



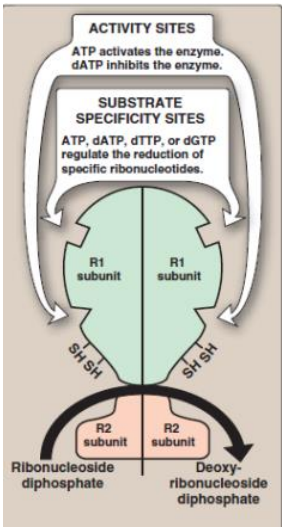

## Synthesis of Deoxyribonucleotides: (to be used in DNA)

The enzyme doing the work is Ribonucleotide reductase (RR)

It replaces the oxygen with a hydrogen by a reduction reaction. It works on **nucleotide diphosphates (C, G, A and T)**

**Thioredoxin**— is the peptide coenzyme of RR. It gets oxidized and forms a **disulfide bridge**.

**Thioredoxin** gets reduced back (recycled) by the **oxidation of NADPH** to **NADP+**.
Through the enzyme thioredoxin reductase.




This enzyme doesn't distinguish between purines and pyrimidines or any of their subtypes and it needs to be **highly regulated**. It has four subunits (R1 R1, R2 R2).

It has different allosteric (regulatory) sites:

- **activity sites**, where activators and inhibitors bind and determine the activity of the enzyme
    - Example:
      dATP → decreases activity
      ATP→ increases activity of the enzyme on all types.
- **substrate specificity sites**, differs in that the binding of a certain nucleotide activates or inhibits the production of a certain nucleotide with a different nitrogenous base.
    - Example:
      dTTP binds the allosteric site and activates conversion of GDP to dGDP.

Hydroxyurea and ribonucleotide reductase:
This enzyme was targeted to specifically block the **synthesis of DNA**.
A drug made for such purpose is Hydroxyurea, which was used as an anti-cancerous agent for the treatment of cancers like **Chronic Myelogenous Leukemia (CML)**
The drug hydroxyurea destroys the free radical required for the activity of ribonucleotide reductase.

## II. purine degradation

First, Digestion: Dietary nucleic acids degradation occurs in the small intestine by a family of pancreatic enzymes:

nucleases break DNA into fragments.
phosphodiesterases break fragments into free nucleotides.

Then, **In the intestinal mucosal cells**, purines are degraded as such:

Phosphates are removed from nucleotides to make them nucleosides by Nucleotidases
Sugars are separated from bases by Nucleosidases. Dietary purines are generally degraded in the intestinal mucosal cells.

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

* degradation of the base that occurs in all types of cells producing end products common between all purines as we'll see next*

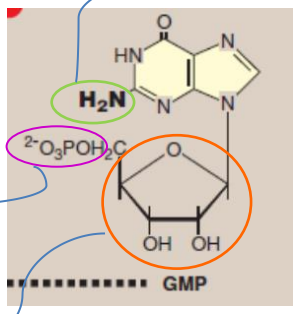
Main principle:

Phosphates are removed from nucleotides to make them nucleosides by Nucleotidases
Sugars are separated from bases by phosphorylase (phosphorylated sugar)

Then specific Nitrogenous base degradation follows:

## GMP degradation:

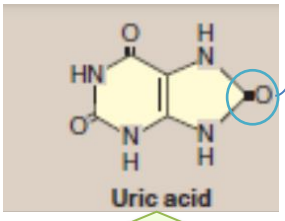
**1**-First phosphate is removed by nucleotidase, and guanosine is formed.




**3**-$H_2O$ is added to guanine by guanase, removing $NH_2$ and making it **xanthine** (=O instead of $NH_2$)

**2**-Sugar is removed from guanosine in the form ribose-1-phosphate by the enzyme purine nucleoside phosphorylase turning it into guanine.

**4**-$H_2O$ and $O_2$ are then added by xanthine oxidase adding an oxygen in this position making it uric acid (the final product)



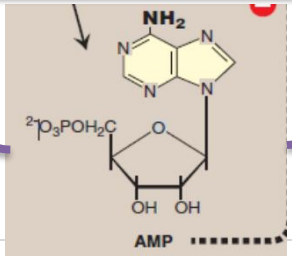

## AMP degradation:

there are two possible ways:

**2**-Then, the sugar is **phosphorylytically removed** by phosphorylase. After that **hypoxanthine is formed**, an oxygen is then double bonded on a certain site making it **xanthine**. Finally, $O_2$ and $H_2O$ enter by xanthine oxidase adding an oxygen and yielding **uric acid**

**1A**-phosphate is removed by nucleotidase, making it adenosine, then the amino group is removed by adenosine deaminase converting it into inosine

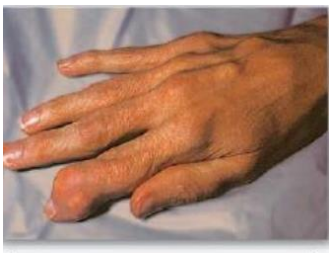
**1B**-The other choice is to do deamination first (by AMP deaminase) to IMP then remove the phosphate by nucleotidase forming inosine



⇒Intermediates of degradation have some resemblance to intermediates of synthesis, like IMP for example. So, we might actually start synthesizing a molecule then find that there's too much of it and decide to degrade it from that point on.

## III. Diseases associated with purine degradation

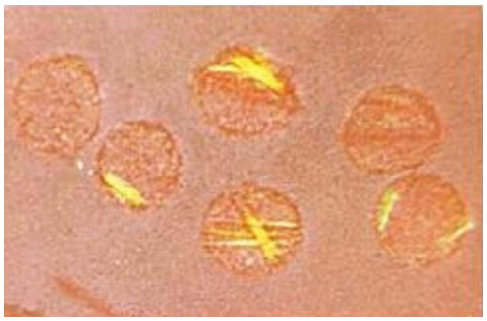
### Gout



accumulation of uric acid due to over production or under excretion of uric acid.

Uric acid's concentration increases,

Leading to:



1. Formation of **monosodium urate crystals** that **deposit in the synovial fluid** of joints. These crystals will be recognized by the immune system inducing an **inflammatory reaction** causing acute then chronic gouty arthritis.
2. Nodular masses of monosodium urate crystals (tophi) may be deposited in the soft tissues, resulting in chronic tophaceous gout.
3. Formation of uric acid stones in the kidney (urolithiasis)

**Underexcretion of uric acid:** *Most gout patients have under excretion*

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

   This disease called kings' disease because it's related to over ingestion of meat which increases the amount of uric acid.

**Treatment** depends on the cause, under excretion we give things to enhance excretion, over production we fix that.

## Adenosine deaminase (ADA) deficiency: (autosomal recessive)

It's involved in **AMP degradation**.

ADA is expressed in many tissues, but lymphocytes have the highest activity in humans. A **deficiency of ADA** results in an **accumulation of adenosine**, which is **converted to its ribonucleotide or deoxyribonucleotide** forms by cellular kinases.

- **dATP levels rise** and ribonucleotide reductase is inhibited, **thus preventing deoxynucleotides production**, and **DNA synthesis for cell division stops.**
- The dATP and adenosine that accumulate lead to **developmental arrest** and **apoptosis of lymphocytes** (arrest of growth)

**Treatment** requires either **bone marrow transplantation** (BMT) or **enzyme replacement therapy** (ERT).

*Without appropriate treatment, children usually die by the age of two*

"Molecules, when broken, yield as much energy as was invested in them when they were made.. Everything in life works as such, you must invest today as much as you want to yield tomorrow."