Nucleoprotein Metabolism

Synopsis

- Fates of dietary Nucleoproteins/Nucleic Acids.
- De novo Biosynthesis of Purines and Pyrimidines.
- Salvage of Purines and Pyrimidines
- Catabolism of Purines and Pyrimidines
- Disorders Associated To Nucleic Acid Metabolism.

Fates Of Dietary Nucleoproteins

- Nucleoproteins are conjugated Proteins. containing Nucleic acids as a prosthetic group.

- Nucleoproteins are constituents of each and every living cell.

- **Food substances** of both plant and animal origin **contain Nucleoproteins or Nucleic acids** in them.

- However Nucleoproteins and Nucleic acids are **non essential nutrients.**
- Since biosynthesized in the body.

Digestion and Absorption Of Nucleoproteins

- Dietary Nucleic acids remain unchanged in mouth.

- In Stomach **gastric HCl denatures** Dietary Nucleoproteins.
- Cleaves Hydrogen bonds of Nucleic acids.

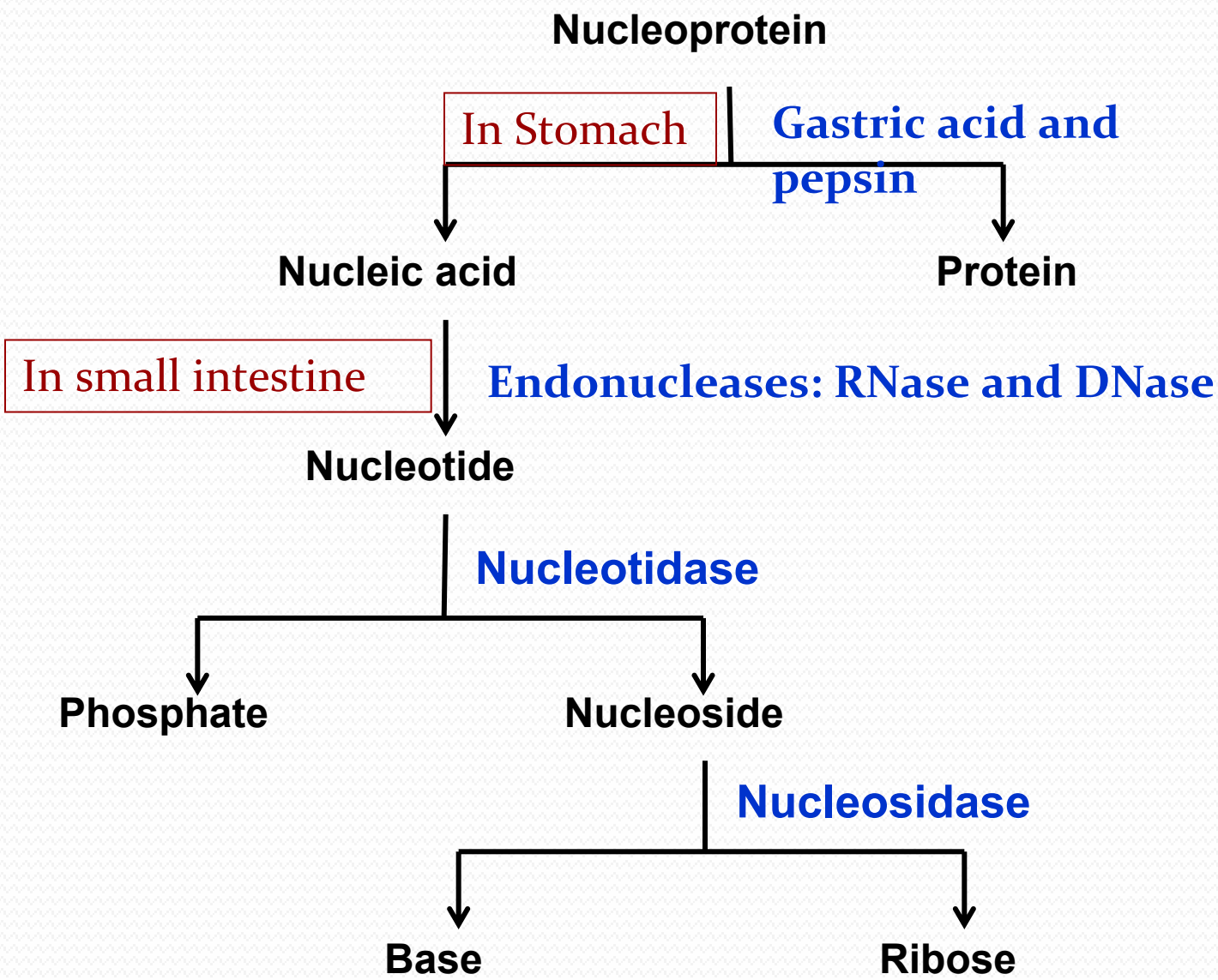
- Predominant and complete digestion of Nucleic acids takes place **in small intestine**

- The **specific Enzymes** required for the **digestion of DNA and RNA** are present in the
- **Pancreatic and Intestinal juice** which specifically act and break the bonds.

- **Nucleic acids** are digested in the small intestine by Deoxyribonuclease / Phosphodiesterase to **generate Nucleotides.**

- **By the catalytic action of Nucleotidase and Nucleosidase.**
- **Nucleotides** and Nucleosides are, **degraded to** three components :
- **Nitrogen Base , Pentose and Phosphate**

Degradation of Nucleoproteins



End Products Of Nucleic Acid Digestion

- **Nitrogen Bases**:
  - Purines and Pyrimidine
- **Sugars:**
  - Ribose and Deoxyribose
- **Phosphoric Acid**

Absorption

- Dietary Purines and Pyrimidines obtained through digestion of Nucleic acids are absorbed through intestinal lumen.
- Some **unabsorbed Purines** are metabolized by intestinal microbial flora and **excreted out through feces**.

- The **absorbed Nitrogen bases are carried to Liver .**

- These are **degraded and excreted out of the body.**

- Thus human body is not dependent upon the dietary Nucleic acids for its use.

- Ribose can be absorbed and catabolized to generate energy.

Nucleotides

- Nucleotides are chemically composed of
  - Nitrogen base: Purines and Pyrimidines
  - Sugar: Ribose / Deoxyribose
  - Phosphate group

Functions of Nucleotides

- **Precursors/Building blocks for DNA and RNA synthesis**
- **Essential carriers of chemical energy, especially ATP (**Energy transformation)
- **Components of the coenzymes NAD$^+$, FAD, and coenzyme A**

- ATP , ADP, and AMP may function as **allosteric regulators** and participate in regulation of many metabolic pathways.
- ATP involved **in covalent modification of enzymes.**

❖cAMP and cGMP, are also cellular **second messengers**.

❖ **Formation of activated intermediates** such as UDP-Glucose and CDP-Diacylglycerol.

Can Cells Biosynthesize Nucleotides?

- Nearly all living organisms biosynthesize Purine and Pyrimidine Nucleotides through "*De novo biosynthesis pathway*"
- Many organisms also "Salvage" Purines and Pyrimidines from diet and degradative pathways.

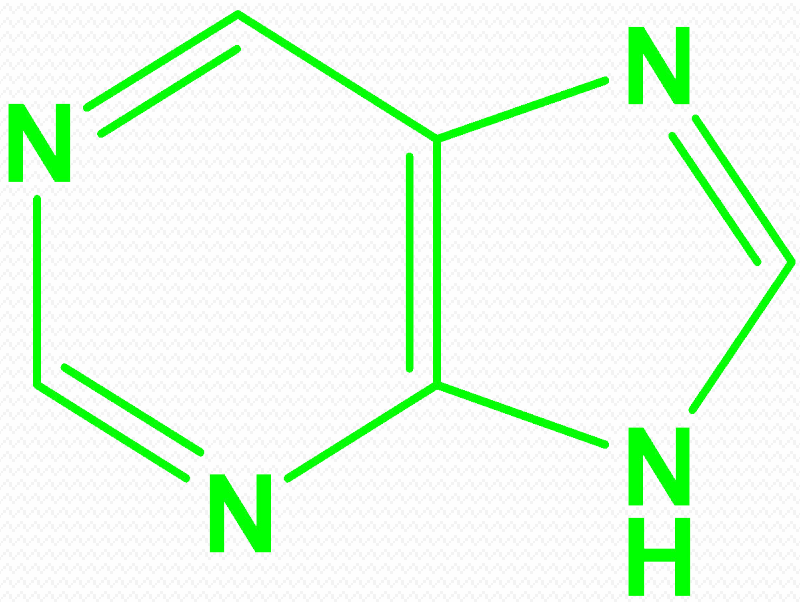
Purine Nucleotide Metabolism

Anabolism

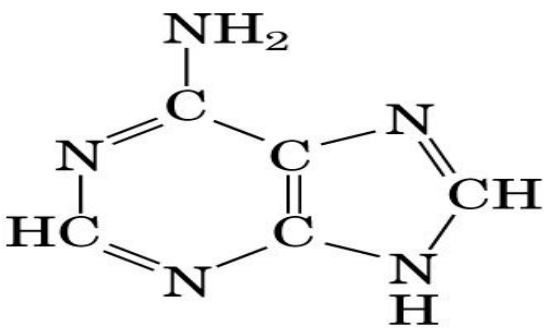
Purine Nucleotide Biosynthesis

De Novo Biosynthesis Of Purine Nucleotides

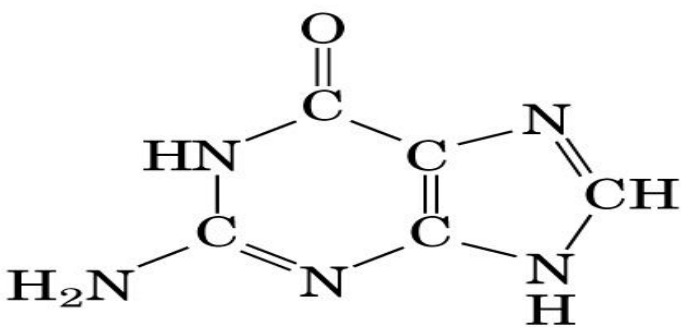
Purine Ring System



Purines And Pyrimidines

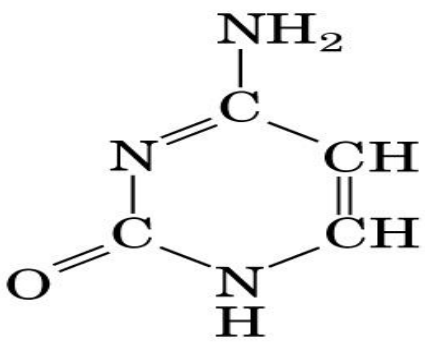


Adenine

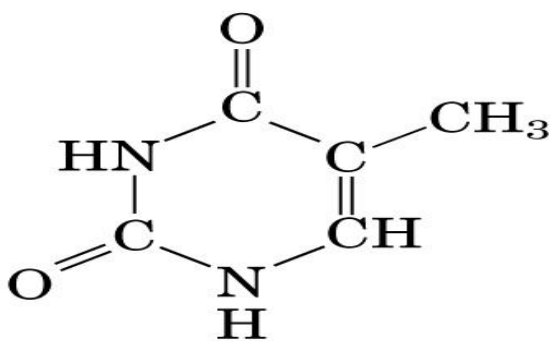


Guanine

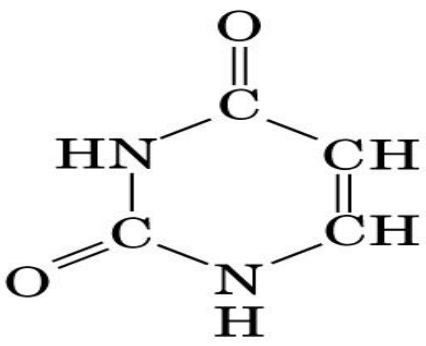
Purines



Cytosine



Thymine
(DNA)



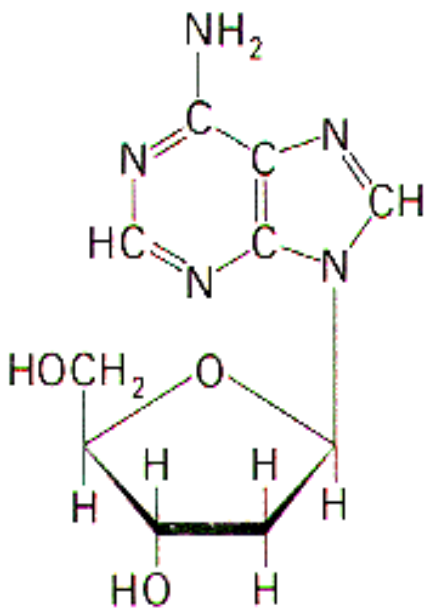
Uracil
(RNA)

Pyrimidines

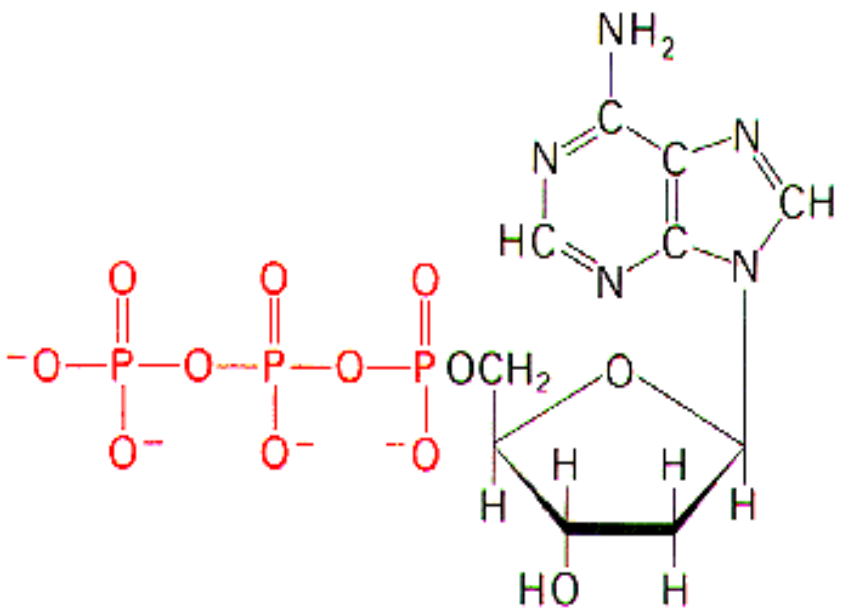
Nucleoside and Nucleotide

Nucleoside = Nitrogenous base — Ribose

Nucleotide = Nitrogenous base — Ribose — Phosphate



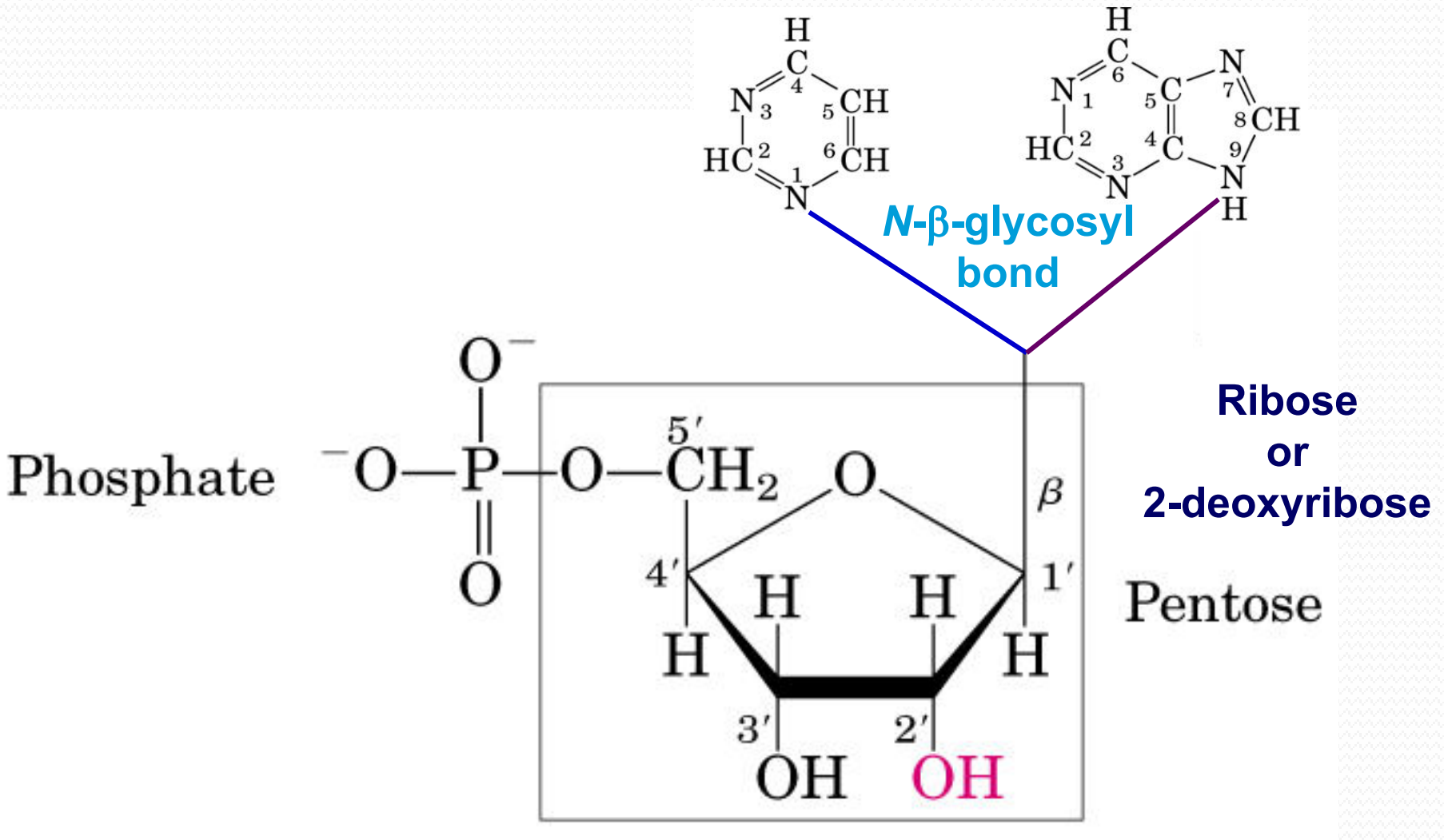
Deoxyadenosine
(A nucleoside)



Deoxyadenosine 5′-triphosphate
(dATP)
(A nucleotide)

Nucleotides are Building blocks of Nucleic acids

Structure of Nucleotides



There are two pathways leading to Biosynthesis of Nucleotides

- **De Novo Biosynthesis:**

- **This is a main synthetic pathway.**
  - **The biosynthesis of nucleotides begins /very new with the use of small metabolic precursors as a raw material:**

- **Amino acids, Ribose-5-phosphate, CO_2, and One-carbon units.**

- **Salvage pathways:**

- **The synthesis of nucleotide by recycle of the free Nitrogen bases or nucleosides released from nucleic acid breakdown.**
- This is important in **Brain and Bone marrow**

De Novo Biosynthesis Of Purine Nucleotides

Site Of Purine Nucleotide Biosynthesis:

- **Predominantly In cytosol of Liver,**
- **To some extent in small intestine and Thymus.**

- In humans, all **necessary enzymes for Purine Nucleotide** biosynthesis are **found in** the **cytoplasm** of the cell.

- Denovo biosynthesis occurs in **most of the cells**' cytosol
- **Except human Brain, Polymorphonuclear leukocytes and Erythrocytes.**

Requirements For De Novo Biosynthesis Of Purine Nucleotides

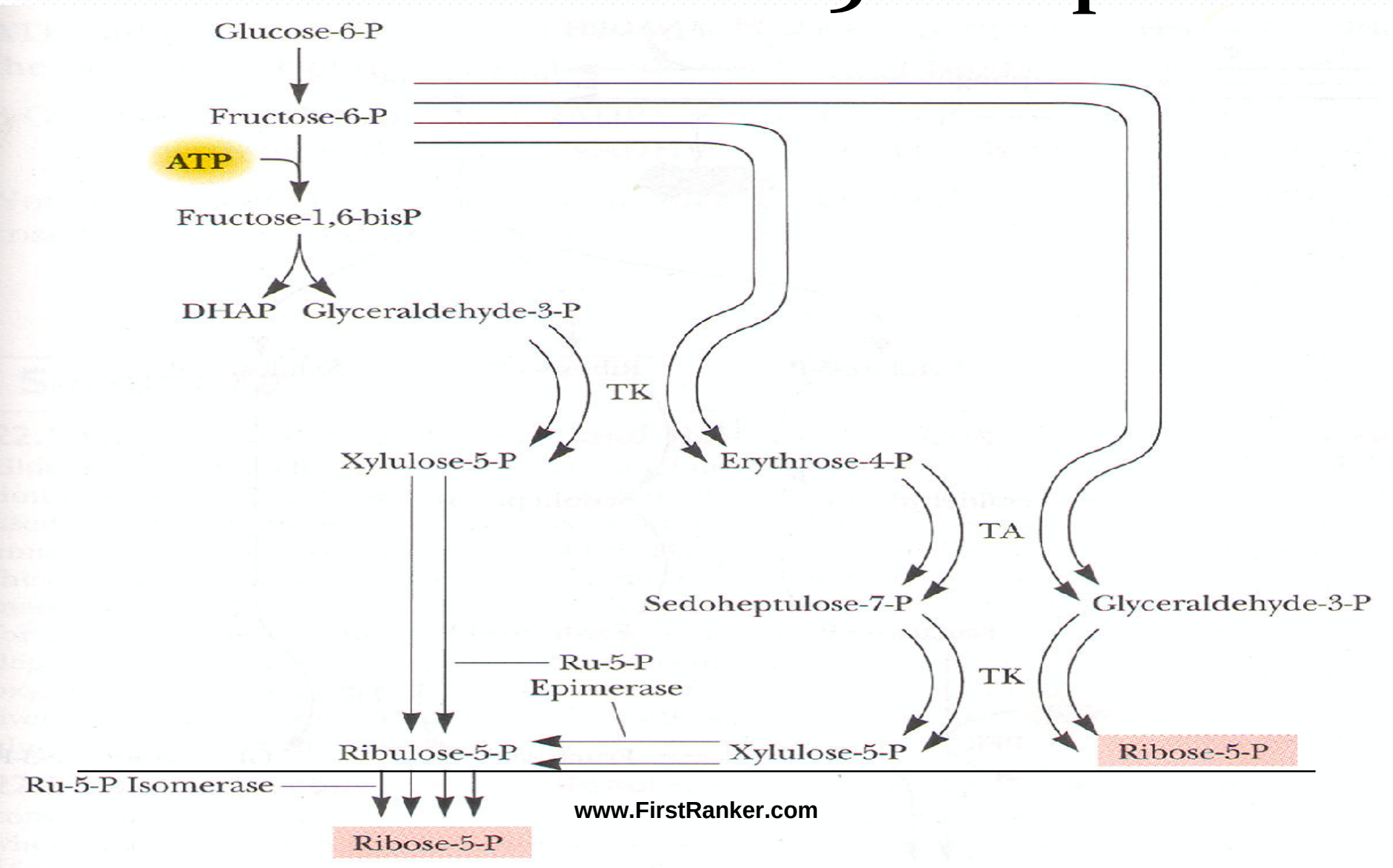
- **Purines are synthesized using 5PhosphoRibose (R-5-P) as the starting material step by step.**

- **PRPP (5-Phosphoribosyl-1-Pyrophosphate) is an active donor of R-5-P.**

- The **Purine ring** is synthesized by a series of biochemical reactions that add the carbon and nitrogen atoms to **a pre-formed Ribose-5-phosphate.**

- The Ribose-5-phosphate is synthesized as part of the **Hexose Mono Phosphate pathway.**

HMP Shunt
Source For Ribose-5-Phosphate

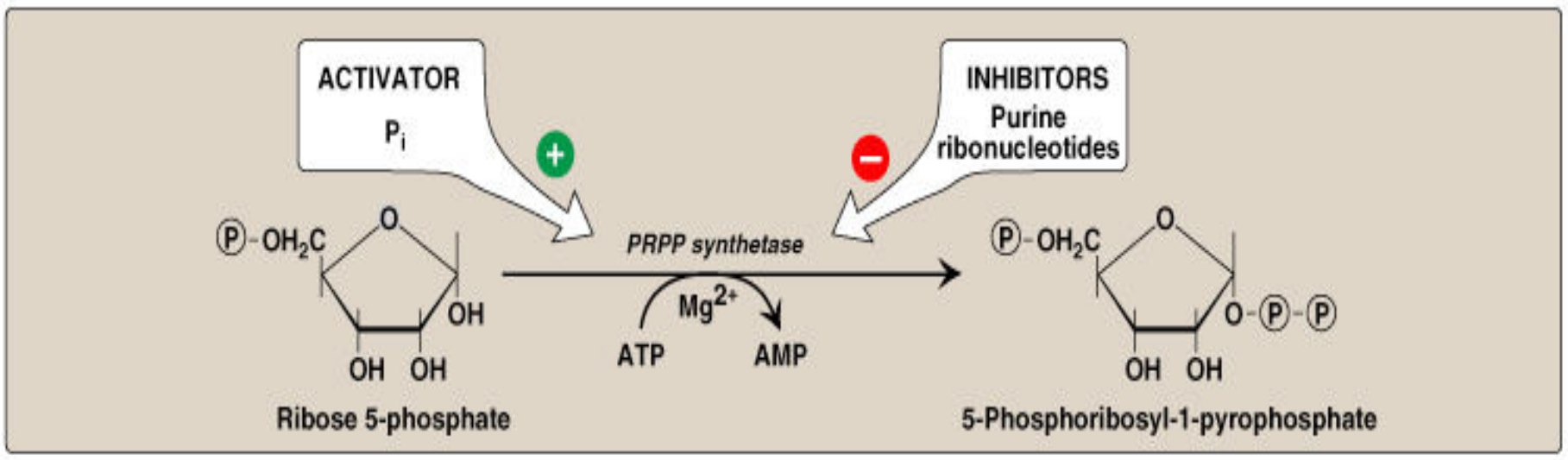


Conversion of Ribose-5-Phosphate to PRPP

- **Phospho Ribosyl Pyro Phosphate (PRPP)** is a starting material for Purine Denovo biosynthesis.
- **PRPP is formed from Ribose-5-Phophate.**

•The Pentose sugar is always a Ribose, which may be reduced to Deoxyribose after nucleotide synthesis is complete.

•**5-Phosphoribosyl-1-pyrophosphate (PRPP)** is also involved in synthesis of Pyrimidine nucleotides, NAD^+, and Histidine biosynthesis.



•The De novo biosynthesis of Purine nucleotide means a very new synthesis using raw materials as

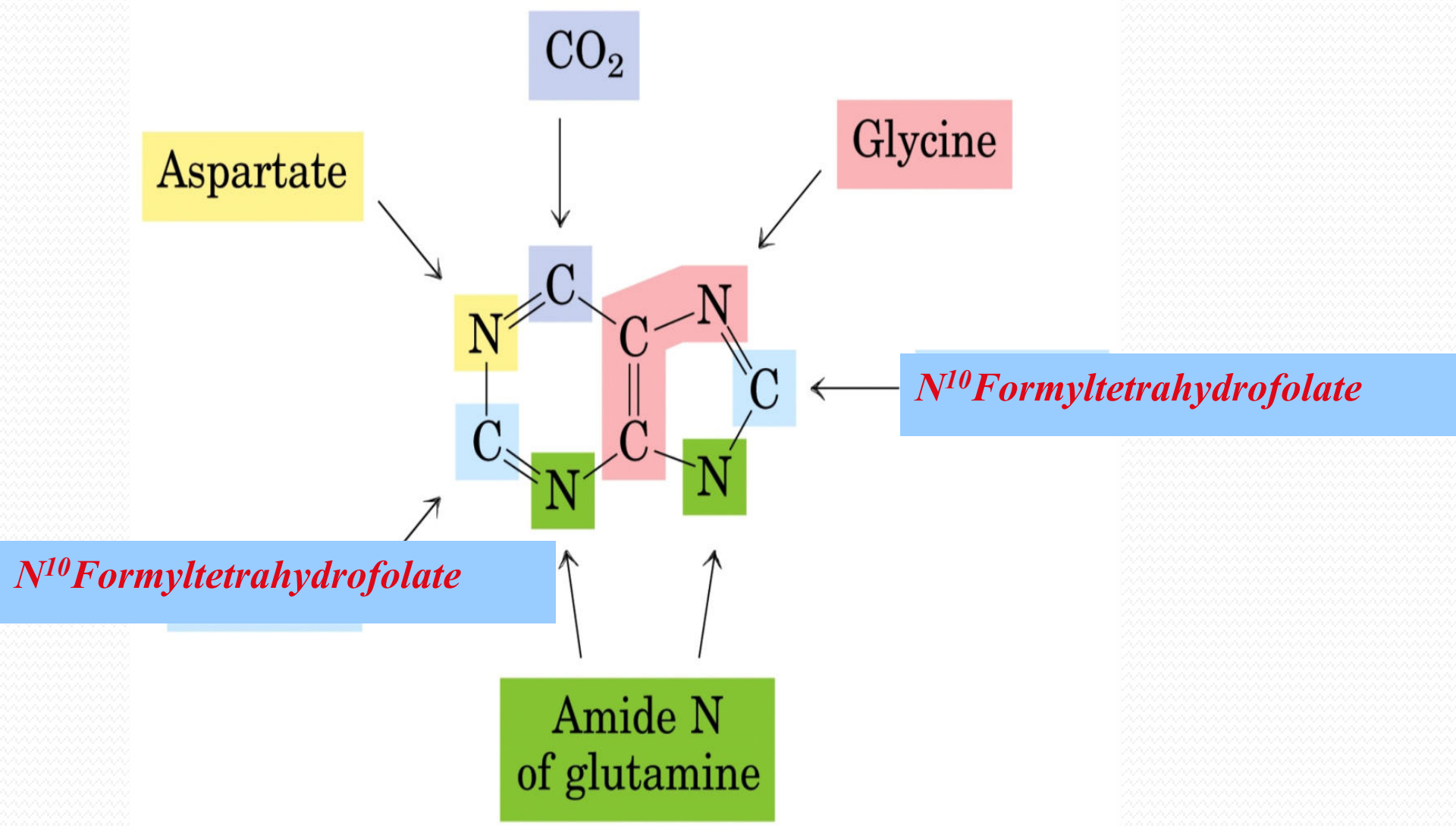
- **Phosphoribose**
- **Amino acids : Gly , Gln and Asp**
- **One carbon units and**
- **CO2**

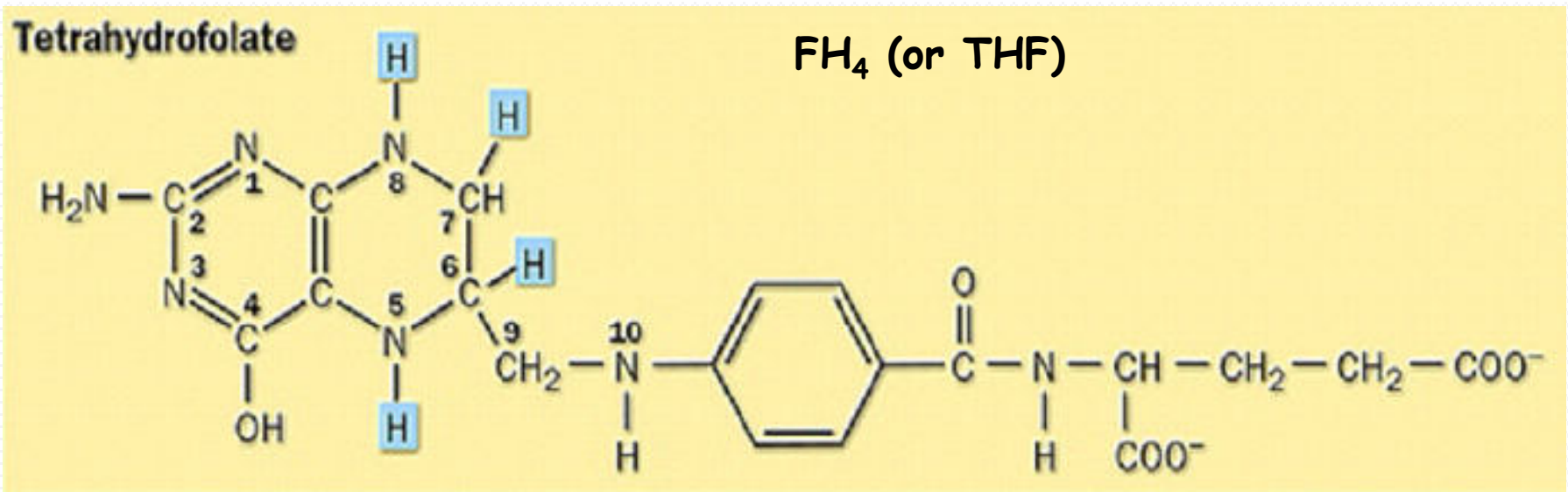
Nitrogen and Carbon Sources Of Purine Ring Biosynthesis

John Buchanan (1948) *"traced" the* ***sources*** *of all* ***nine atoms of Purine ring***

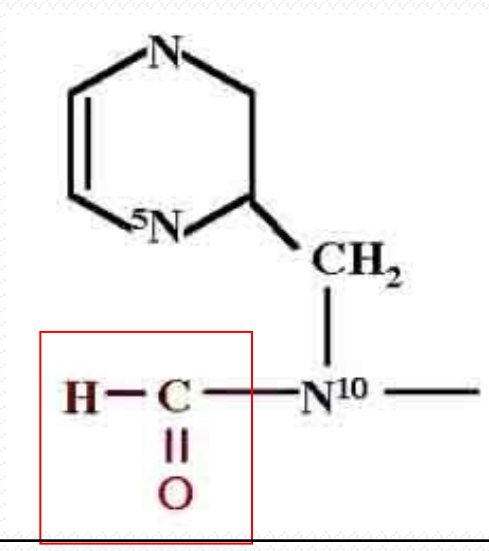
- N-1: Aspartic acid
- N-3, N-9: Glutamine
- C-2, C-8: N^{10}-Formyl-THF- One carbon units
- C-4, C-5, N-7: Glycine
- C-6: CO_2

Element Sources For Purine bases





N^{10}—CHO—FH_4



- **The De Novo synthetic pathway can be divided into two Stages:**

- **Stage one : Formation of Inosine Mono Phosphate (IMP)**

- **Stage two : Conversion of IMP to either AMP or GMP**

- **IMP (Inosine-5'-Monophosphate) is first biosynthesized Purine Nucleotide in this Denovo synthetic pathway.**

- **IMP is a nucleotide with Hypoxanthine as Nitrogen base.**

- **IMP is then converted to AMP and GMP.**

Biosynthesis of Inosine Mono Phosphate (IMP)

- **Basic pathway for De novo biosynthesis of Purine Ribonucleotides**
- Starts from Ribose-5-phosphate(R-5-P)
- Requires 11 steps overall

- Occurs primarily in the **Liver cytosol**.

Steps	Happenings
1	Activation of PRPP
2 and 5	Entry of Glutamine
3	Entry of Glycine
4 and 10	Entry Of N10THF
6	Ring Closure
7	Entry Of CO_2
8	Entry of Aspartate

Steps	Happenings
9	Removal of Fumarate
11	Ring Closure

PRPP Synthetase

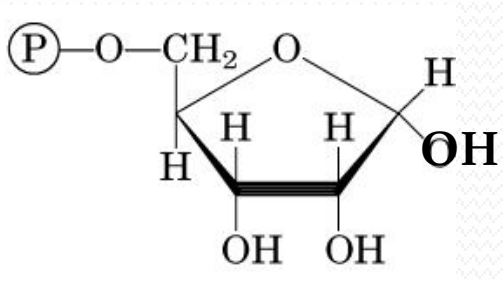
Ribose 5Phosphate + ATP----------→PRPP + AMP

Amidotransferase

PRPP + Glutamine ----------→PRA + Glutamate

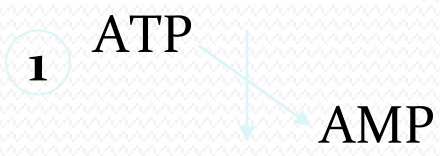
❖Once Phospho Ribosyl Amine (PRA) is formed , the building of the Purine ring structure begins.

❖In nine successive reactions the first Purine nucleotide formed is **IMP** .

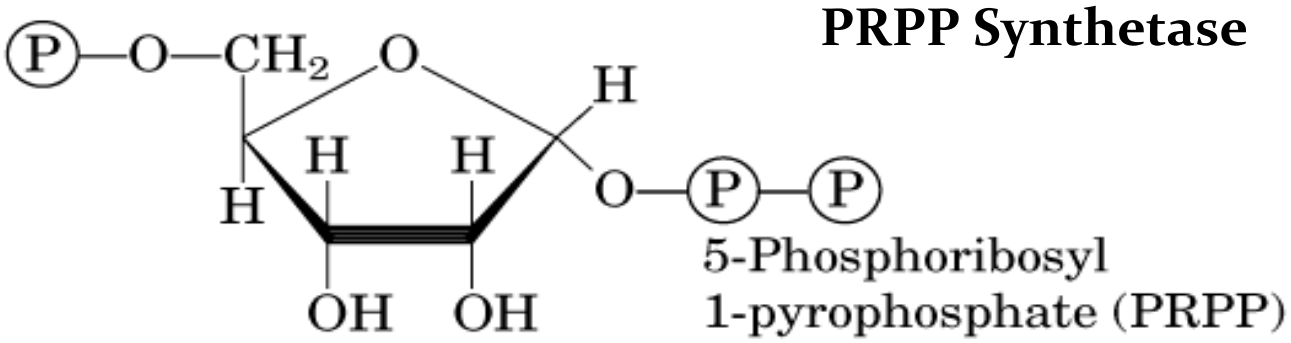


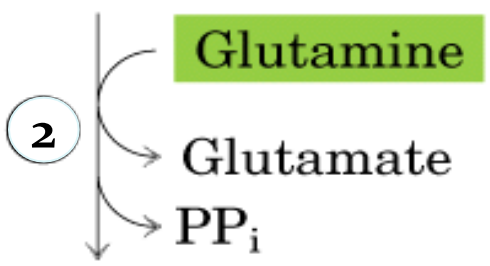
Step 1:Activation of Ribose-5-phosphate

Committed/Regulatory Step



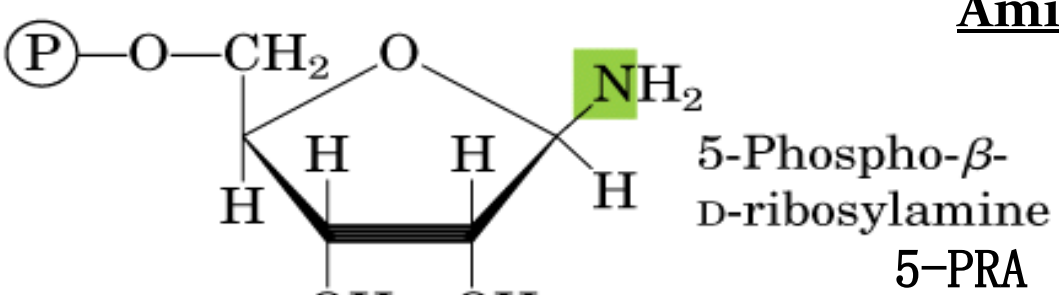
Ribose Phosphate Pyrophosphokinase/ PRPP Synthetase





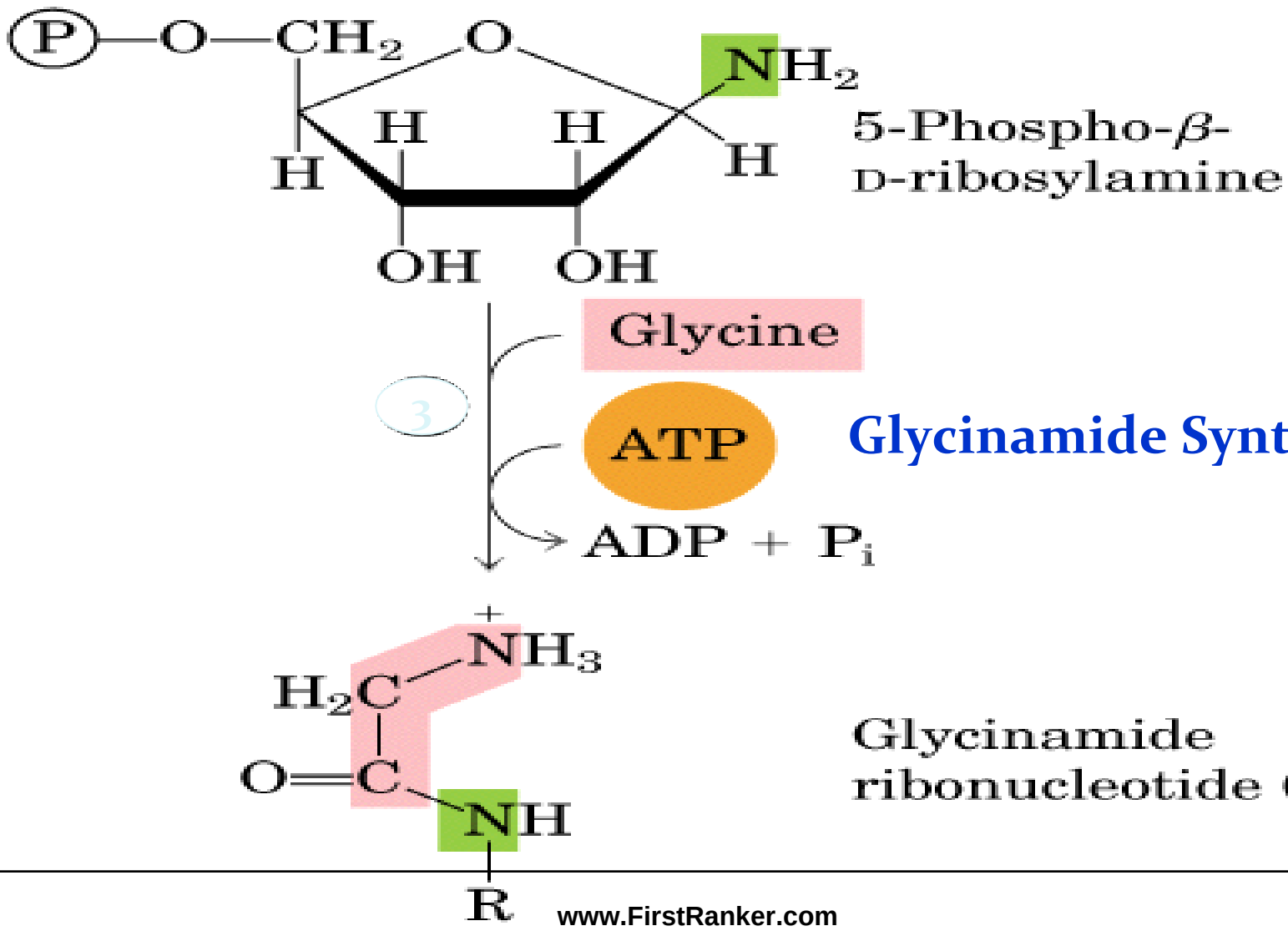
Step 2: Acquisition of Purine atom N9

Gln:PRPP Amidotransferase



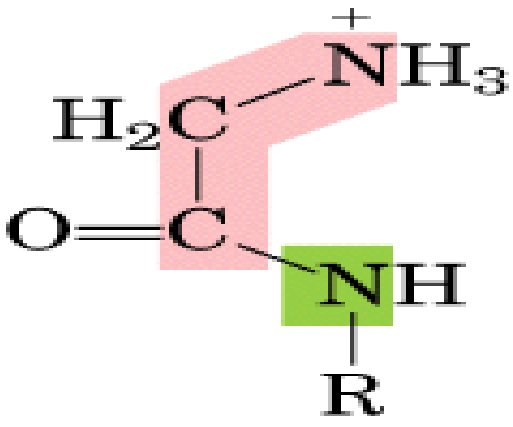
- **Steps 1 and 2 are tightly regulated by feedback inhibition**

Step 3: Acquisition of Purine atoms C4, C5, and N7



Glycinamide Synthetase

•Step 4: Acquisition of Purine atom C8



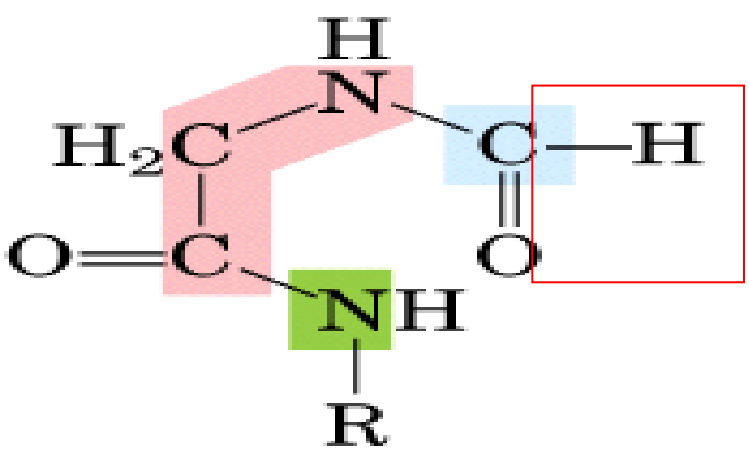
Glycinamide ribonucleotide (GAR)

4

N^{10}-Formyl H_4 folate

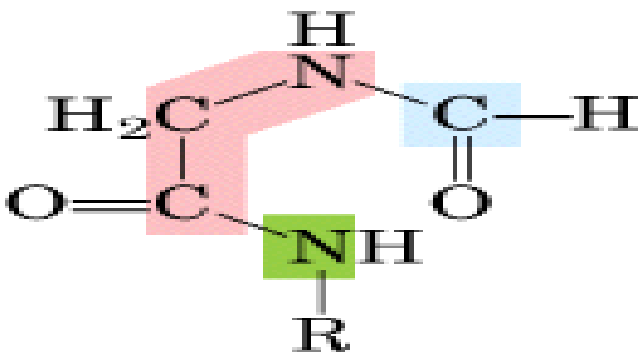
H_4 folate

GAR transformylase



Formylglycinamide ribonucleotide (FGAR)

Step 5: Acquisition of Purine atom N3



Formylglycinamide ribonucleotide (FGAR)

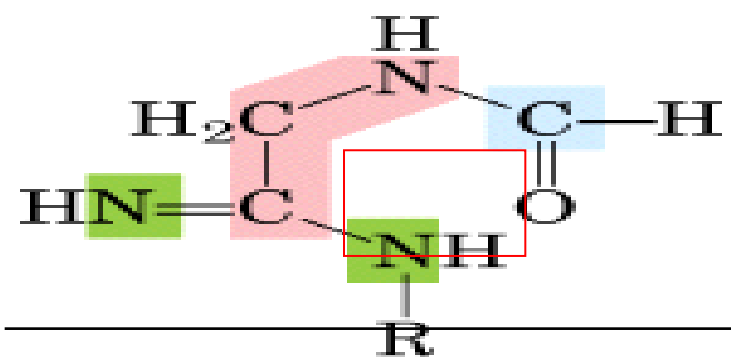
Glutamine

Glutamate

5

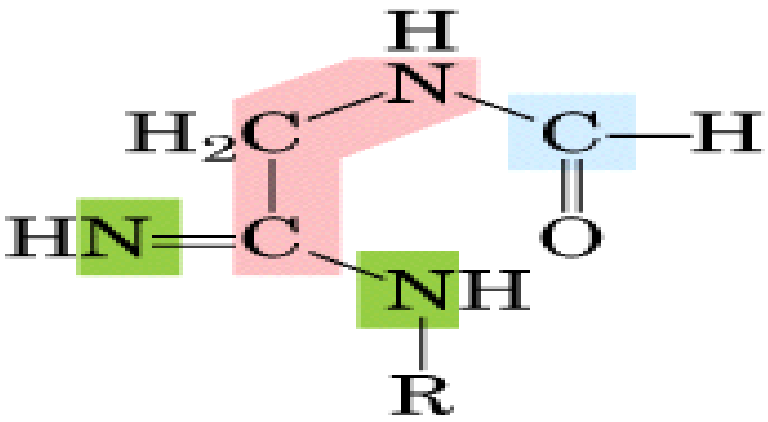
ATP

ADP + P_i

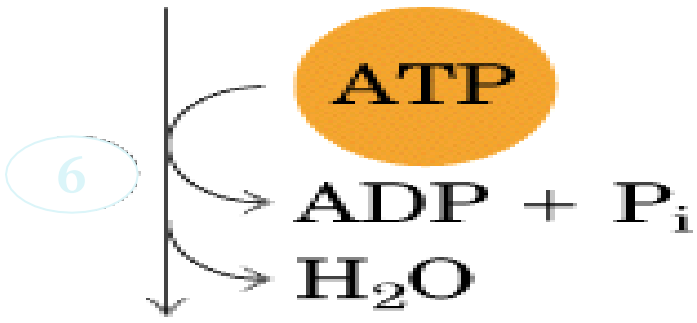


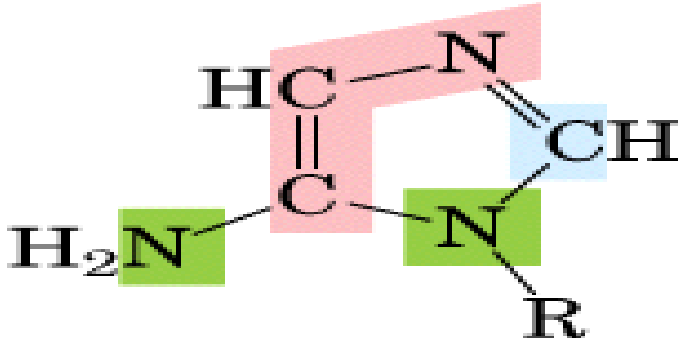
Formylglycinamidine ribonucleotide (FGAM)

•Step 6: Closing of the Imidazole ring



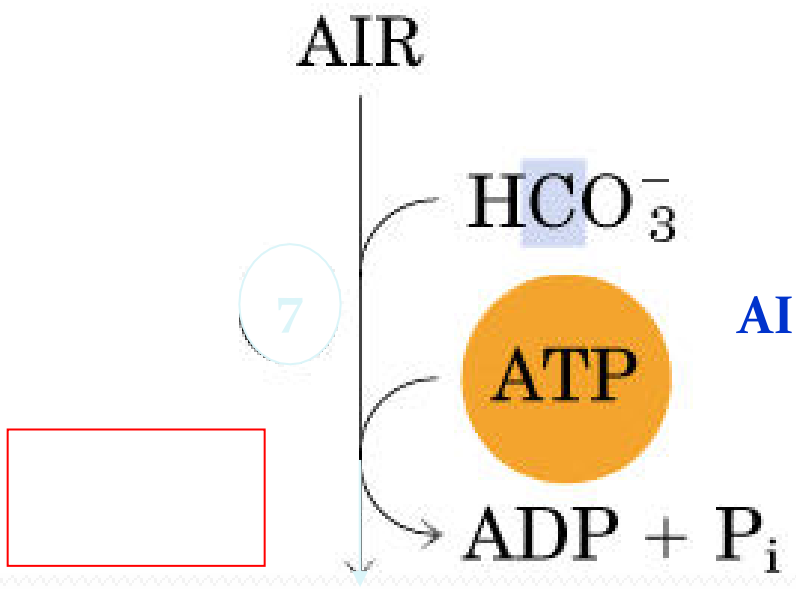
Formylglycinamidine ribonucleotide (FGAM)



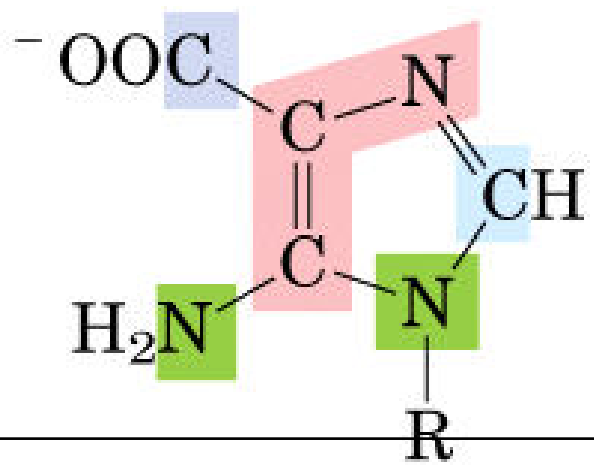


5-Aminoimidazole ribonucleotide (AIR)

Step 7: Acquisition of C6

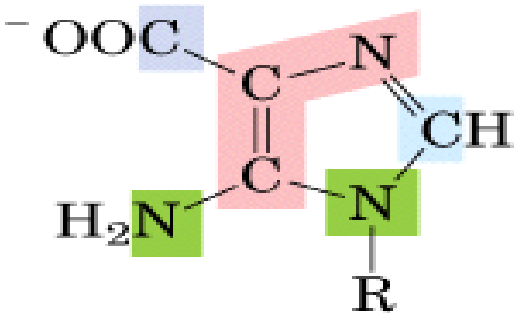


AIR carboxylase

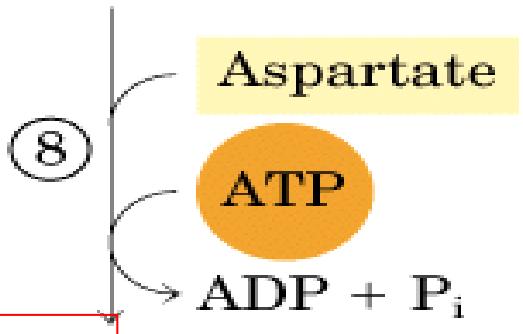


Carboxyaminoimidazole ribonucleotide (CAIR)

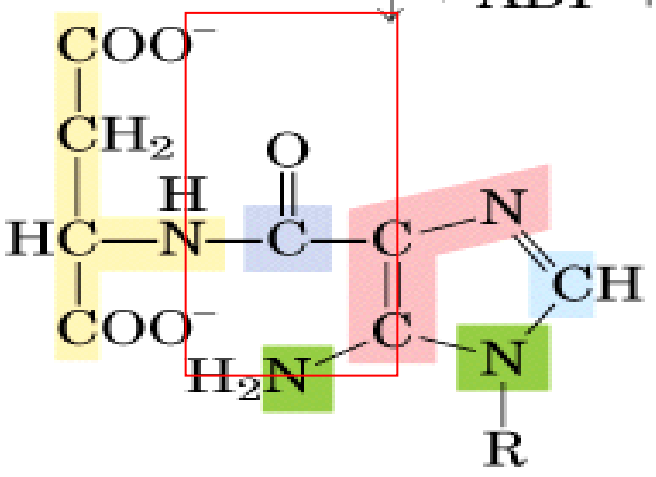
Step 8: Acquisition of N1



Carboxyaminoimidazole ribonucleotide (CAIR)

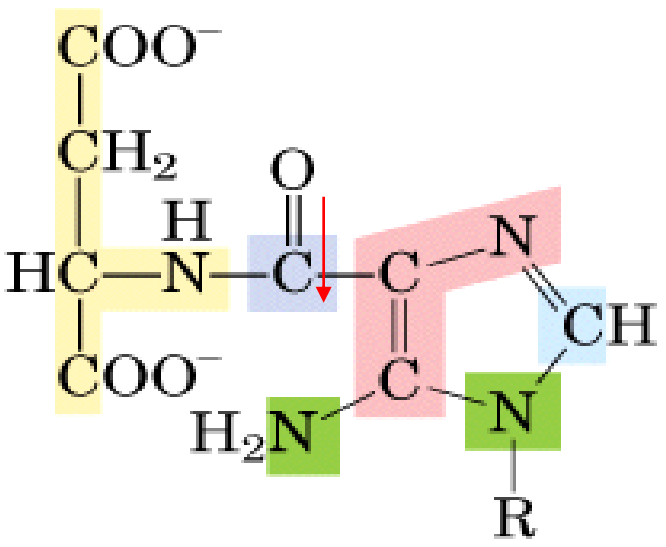


SAICAR synthetase

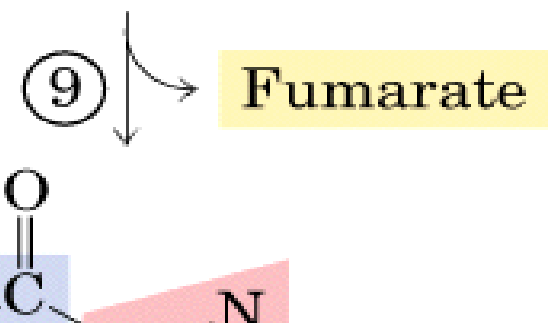


N-Succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)

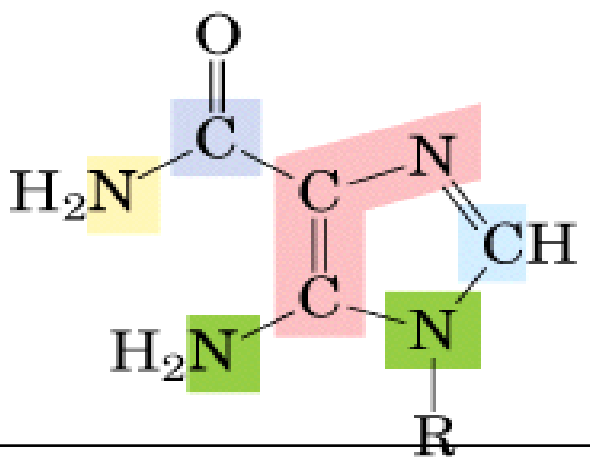
Step 9: Elimination of Fumarate



N-Succinylo-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)

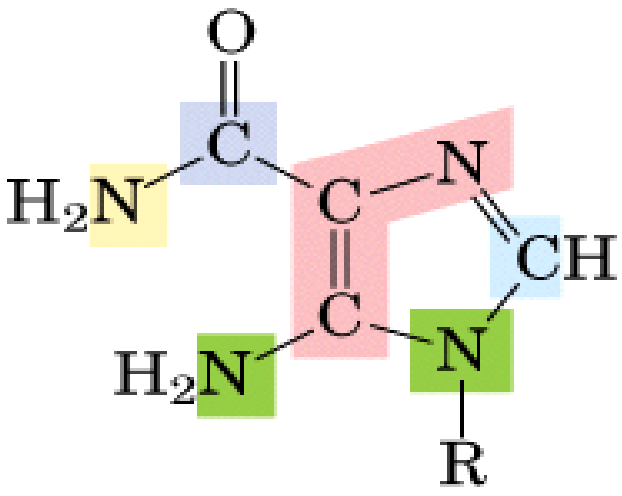


Adenylosuccinate Lyase

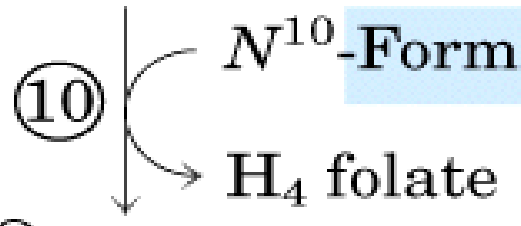


5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)

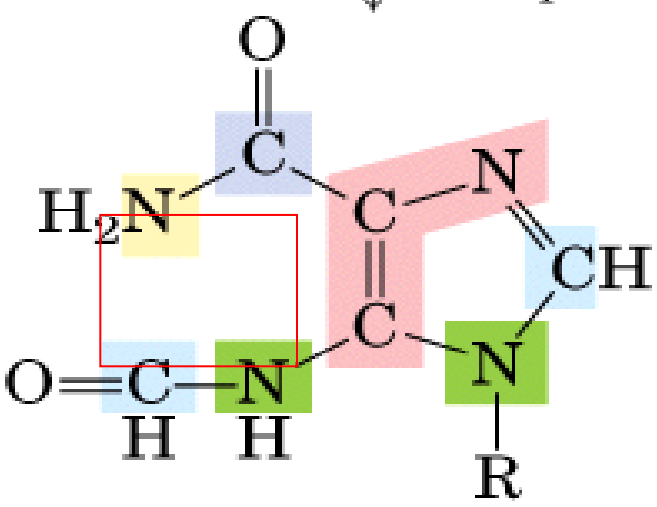
Step 10: Acquisition of C2



5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)

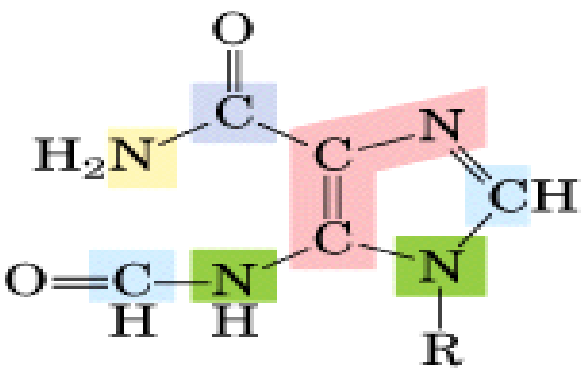


AICAR Transformylase

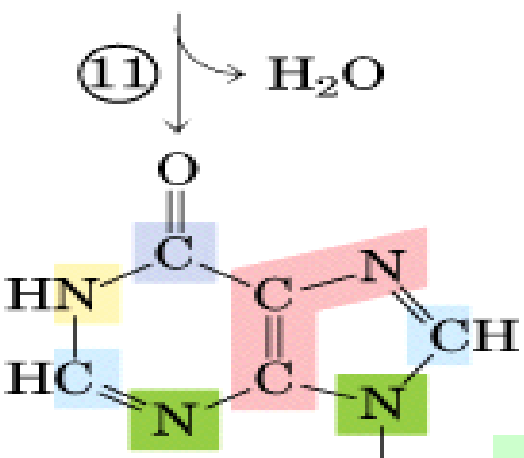


N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)

Step 11: Ring Closure to form IMP



N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)



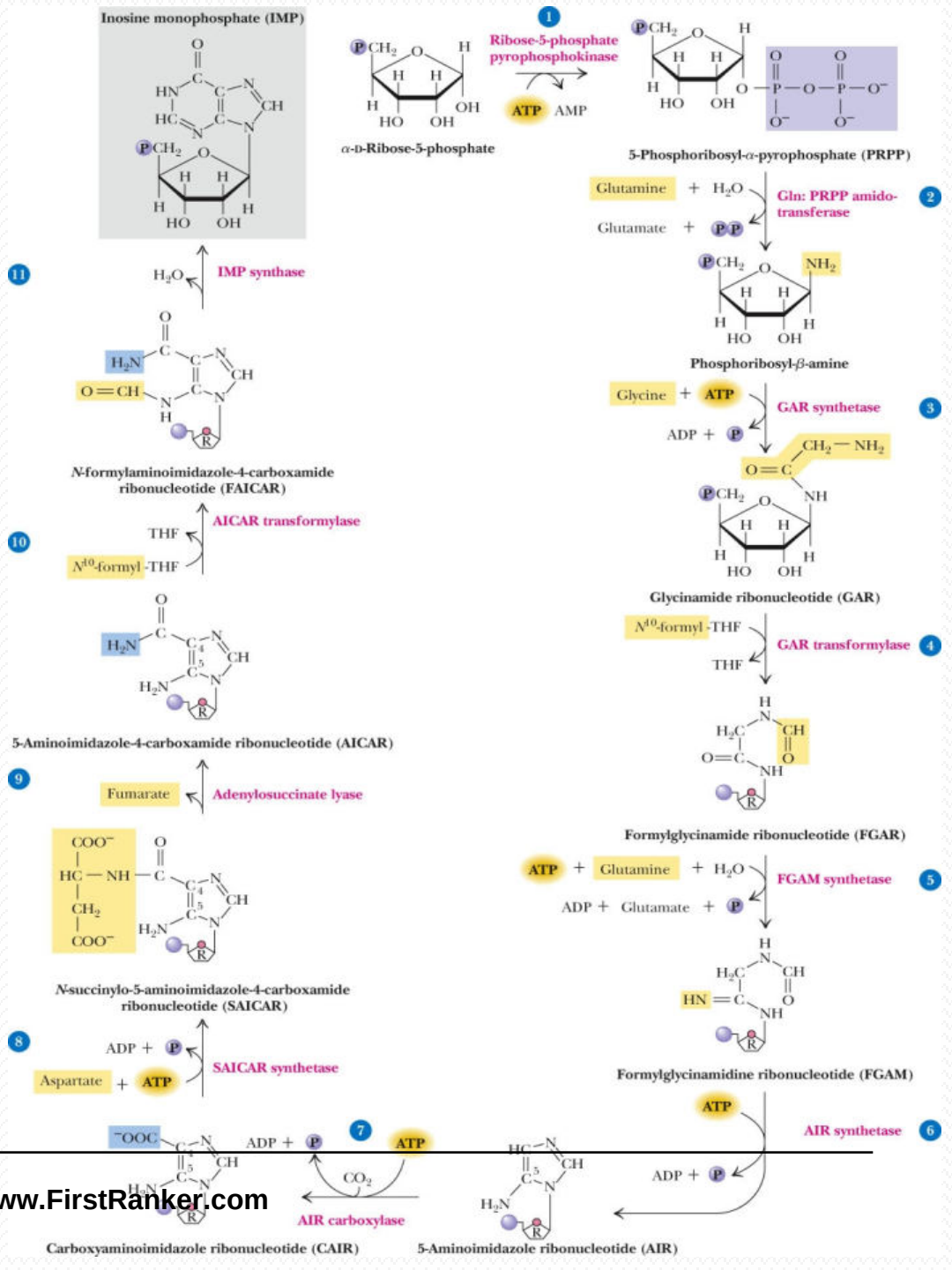
Inosinate (IMP)

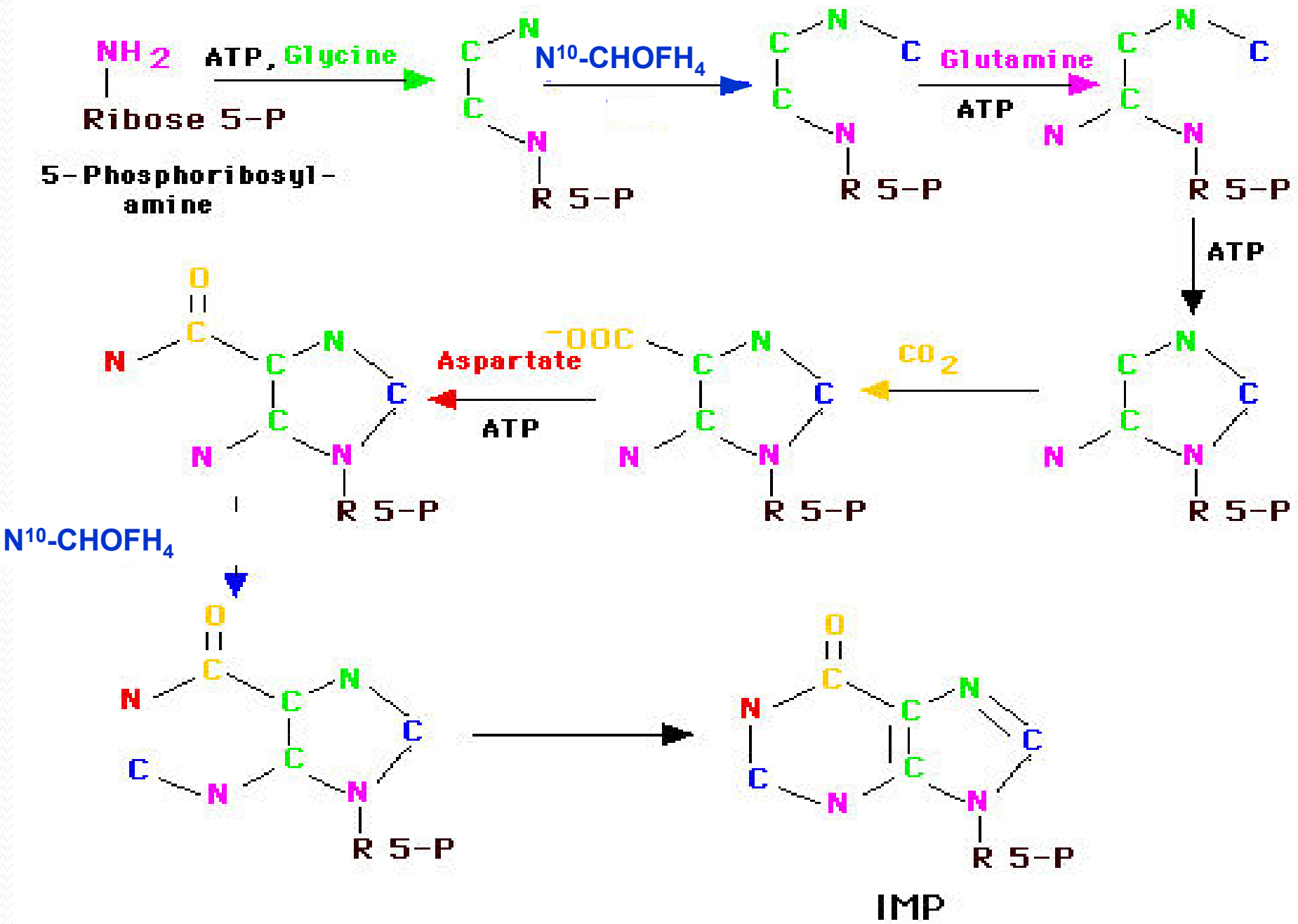
- **Once formed, IMP is rapidly converted to AMP and GMP (it does not accumulate in cells).**

- IMP is a nucleotide of Nitrogen base Hypoxanthine(6 OxyPurine).
- IMP is the first Purine Nucleotide synthesized in Denovo Synthesis mechanism.

The *De Novo* pathway for Purine biosynthesis.

Step 1: Ribose-5-phosphate pyrophosphokinase.
Step 2: Glutamine phosphoribosyl pyrophosphate amidotransferase.
Step 3: Glycinamide ribonucleotide (GAR) synthetase.
Step 4: GAR transformylase.
Step 5: FGAM synthetase (FGAR amidotransferase).
Step 6: FGAM cyclase (AIR synthetase).
Step 7: AIR carboxylase.
Step 8: SAICAR synthetase.
Step 9: adenylosuccinase.
Step 10: AICAR transformylase.
Step 11: IMP synthase.



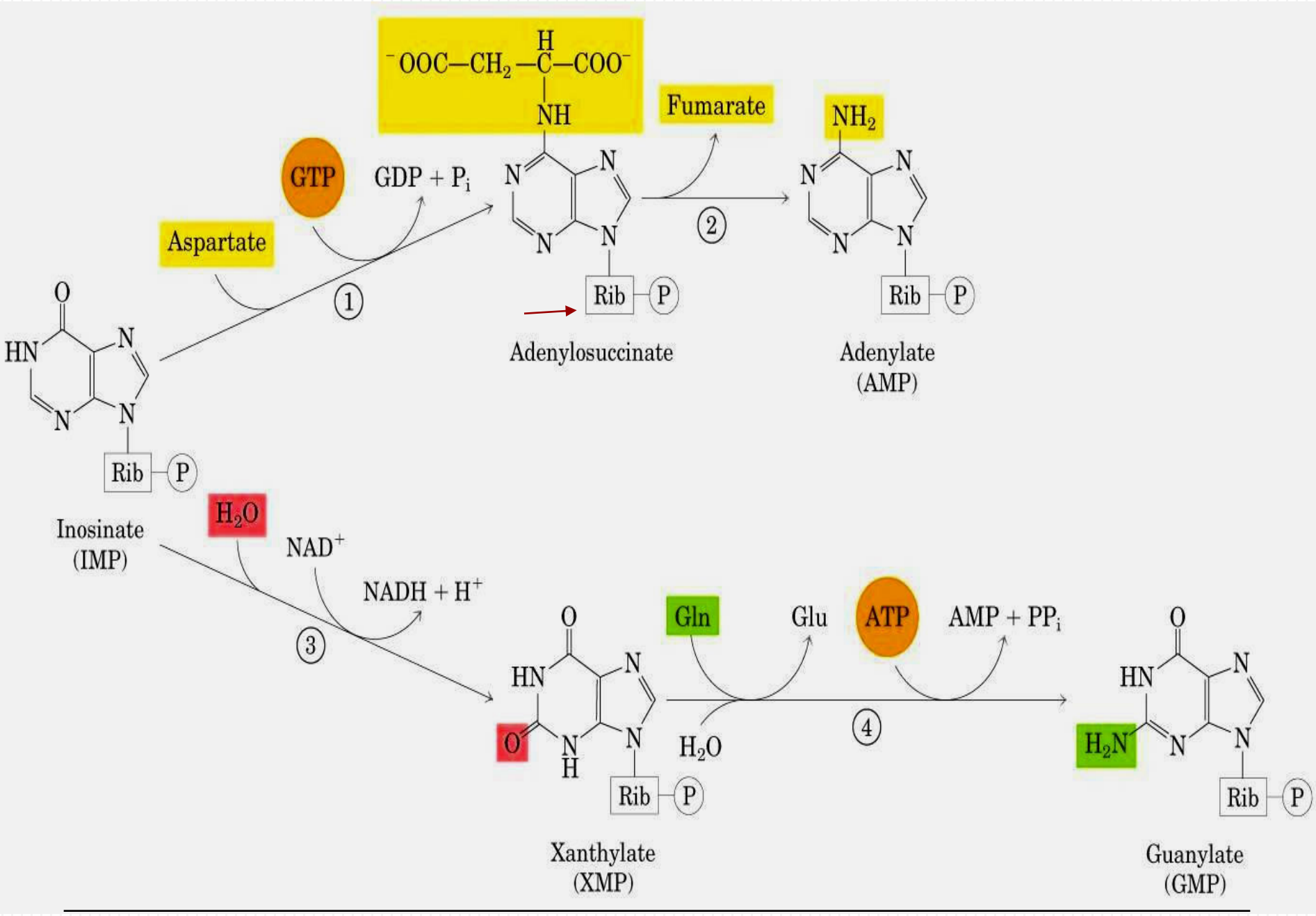


- **6 ATPs** are **required** in the **Purine biosynthesis** from Ribose-5-phosphate to IMP.
- Since in one step ATP is converted to AMP.
- Hence this is really **7 ATP equivalents.**

Conversion of IMP to AMP and GMP

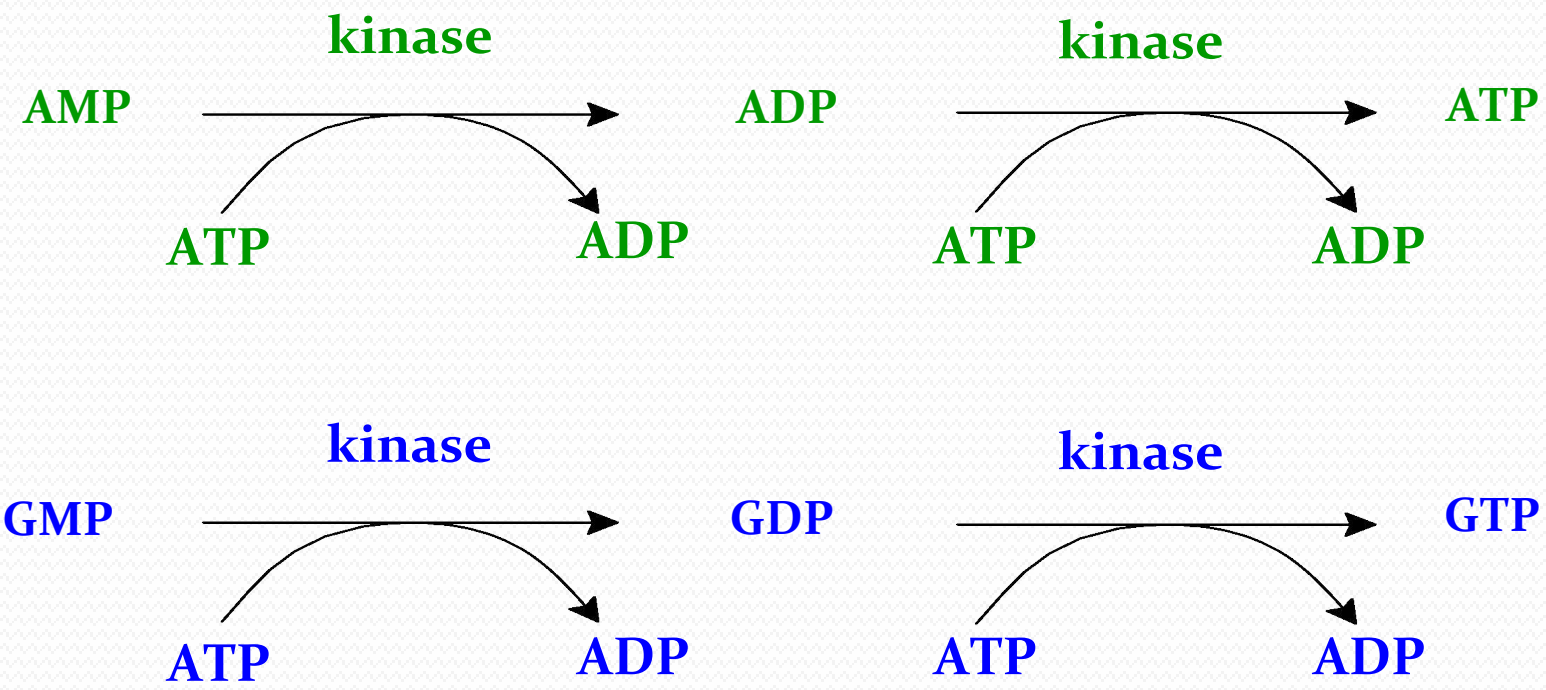
Aspartate and GTP is used for AMP synthesis.

Glutamine and ATP is used for GMP synthesis.



IMP is the precursor for both AMP and GMP.

ADP, ATP, GDP and GTP Biosynthesis



Regulation of Purine Nucleotide Biosynthesis

- Purine Nucleotide biosynthesis is well regulated to meet the cellular demand.

- **Two enzymes** are the **key regulatory enzymes** for the Purine Nucleotide De novo biosynthesis.

- **PRPP Synthase** synthesizing PRPP (Phosphoribosyl Phosphate).
  - **PRPP** is "**Feed-forward" activator**
- **PRPP Glutamyl Amidotransferas**

- The intracellular concentration of PRPP regulates the Purine biosynthesis to large extent.

- More **availability of PRPP increases** more synthesis of Purine nucleotides if the enzyme PRPP Synthetase is not inhibited by feed back control.

- **IMP, AMP and GMP** availability to sufficient concentration inhibits the regulatory enzymes by. **feed back mechanism**.

- PRPP activates **PRPP Glutamyl Amidotransferase**
- **IMP , AMP and GMP** inhibit PRPP synthetase.

- **Sufficient AMP:**
- Inhibits conversion of IMP to AMP

- **Sufficient GMP :**
- Inhibits conversion of IMP to GMP.

- **Regulation of AMP synthesis:**
  - Adenylosuccinate synthetase is feedback-inhibited by AMP

- **Regulation of GMP synthesis:**
  - IMP Dehydrogenase is feedback-inhibited by GMP

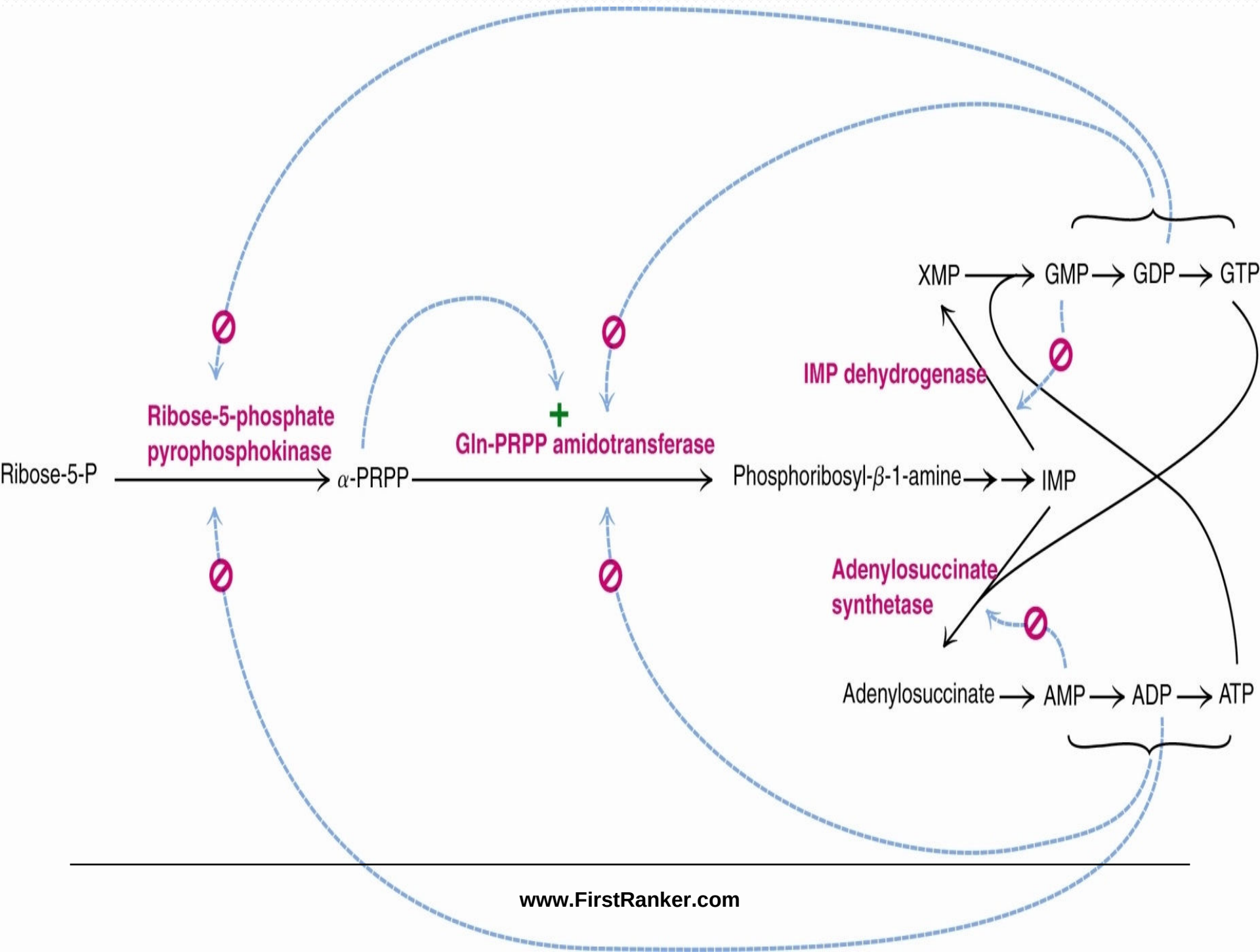
- ATP stimulates conversion of IMP to GMP
- GTP stimulates conversion of IMP to AMP.

- That ensures a balanced synthesis of both families of Purine nucleotides.

Significance of Regulation Of Denovo Synthesis:

- **Meet the sufficient need of the nucleotides to body function, without wasting.**
- **AMP and GMP control their respective synthesis from IMP by a feedback mechanism, [GTP]=[ATP]**

Purine Nucleotide biosynthesis is Regulated by Feedback inhibition



Antimetabolites /Inhibitors of Purine Nucleotides

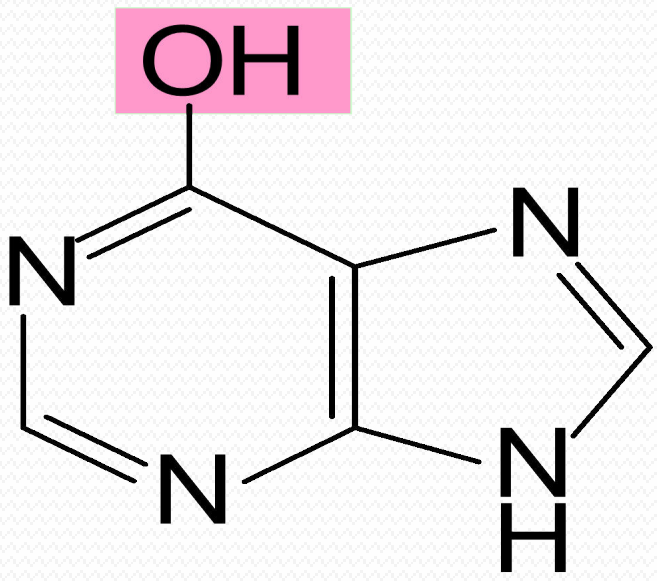
- Nucleotide biosynthesis pathways are good targets fo **anticancer/antibacterial strategies**.

- Antimetabolites of Purine nucleotides are **structural analogs of**
- **Purine,**
- **Amino acids and**
- **Folic acid.**

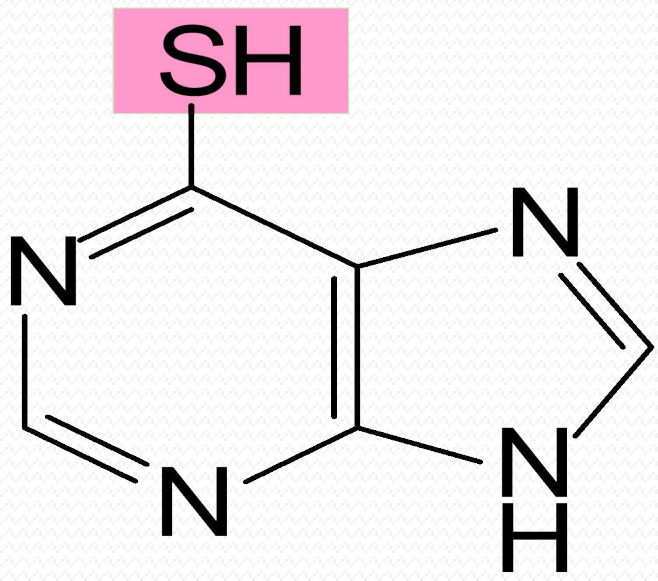
- They can interfere, inhibit or block biosynthesis pathway of Purine nucleotides and further block synthesis of DNA, RNA, and proteins.
- Widely used to **control cancer(Chemotherapeutic Agent).**

Purine Analogs

- **6-Mercaptopurine (6-MP) is a analog of Hypoxanthine.**



hypoxanthine

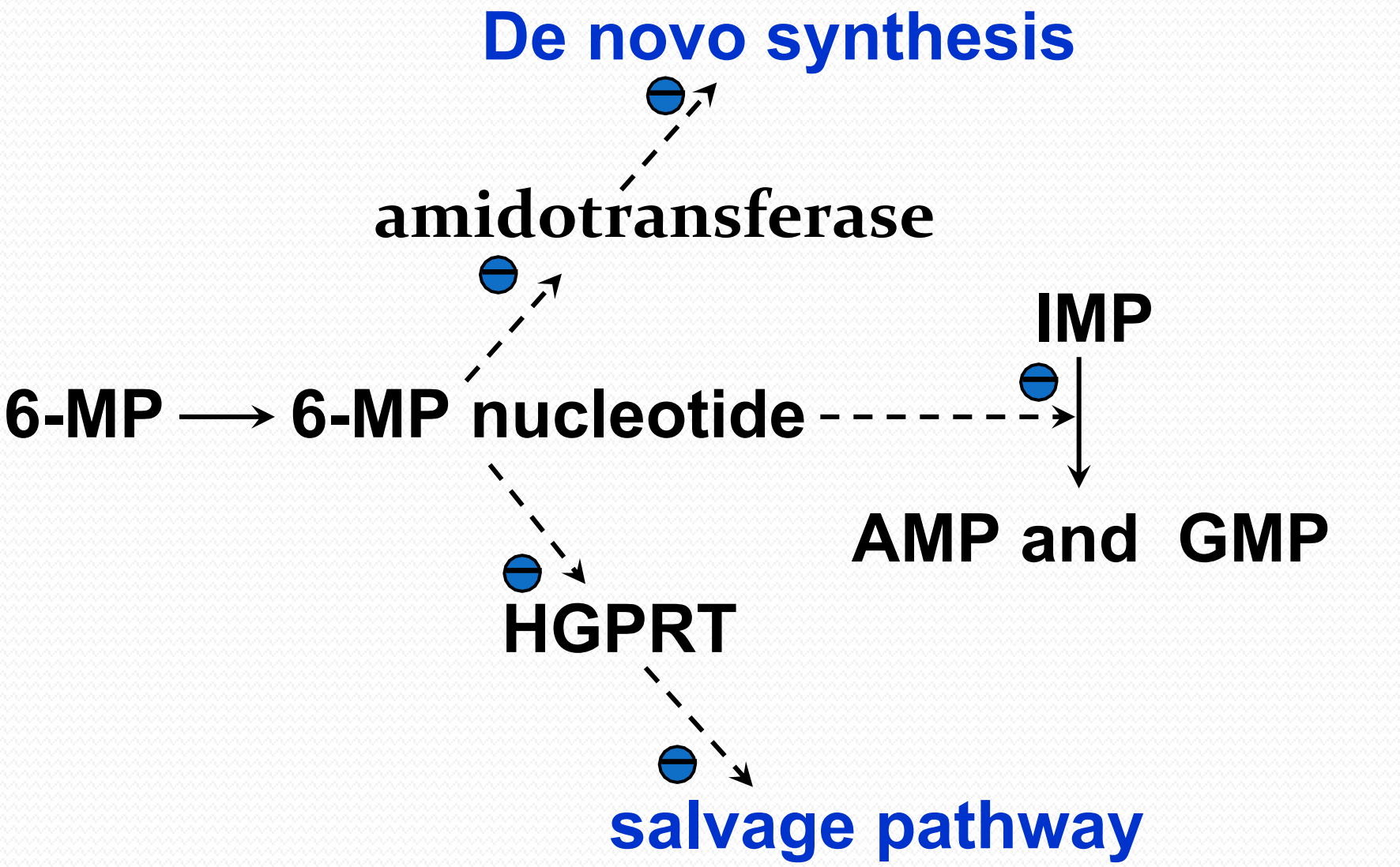


6-MP

6 Mercapta Purine

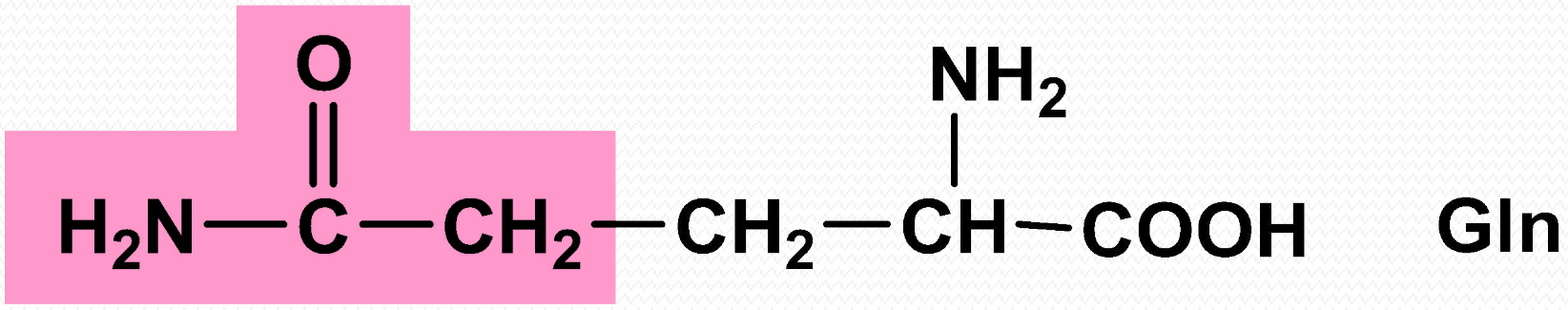
- 6 Mercapta Purine is an inhibitor of Enzymes:
  - **Adenyl Succinase**
  - **IMP Dehydrgenase**
- Decreases levels of AMP and GMP

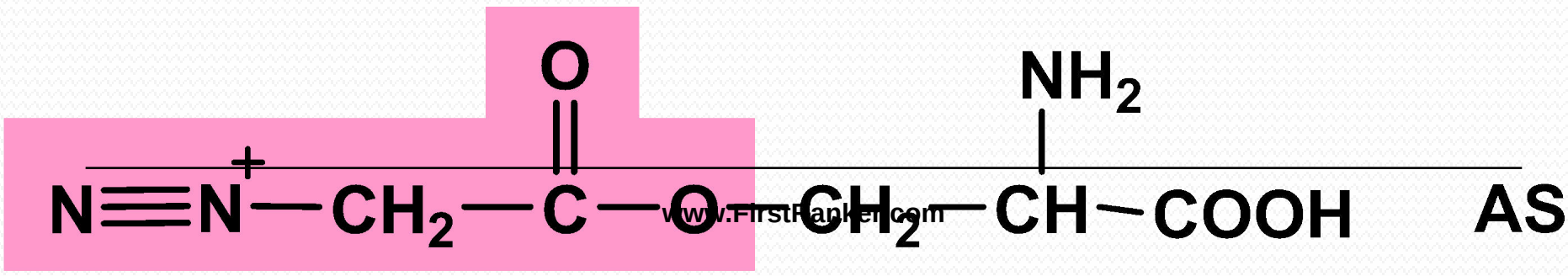
- **6-MP nucleotide is a analog of IMP**



Amino acid Analogs

- **Azaserine (AS) is a analog of Gutamine.**
- **It inhibits 5th step of Purine biosynthesis.**





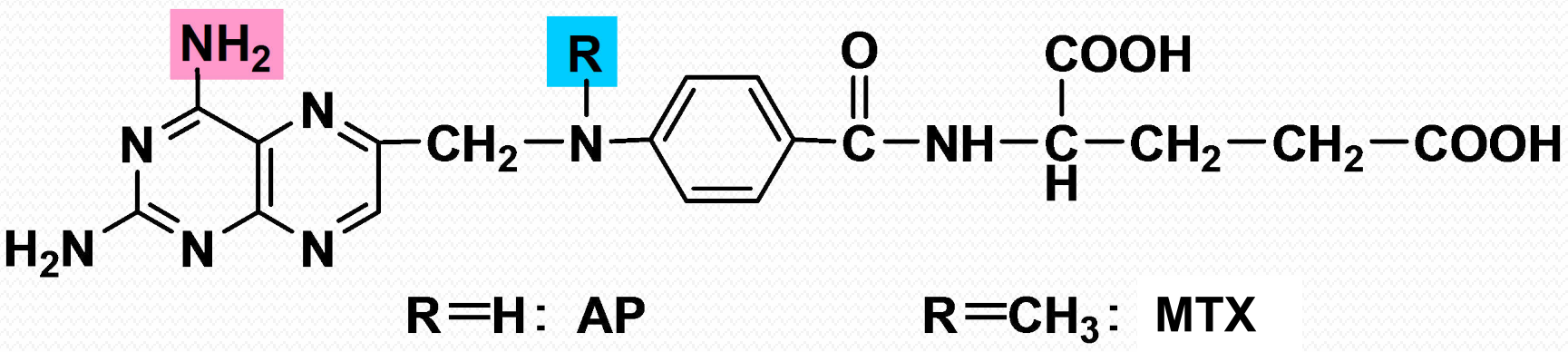
Folate Analogs

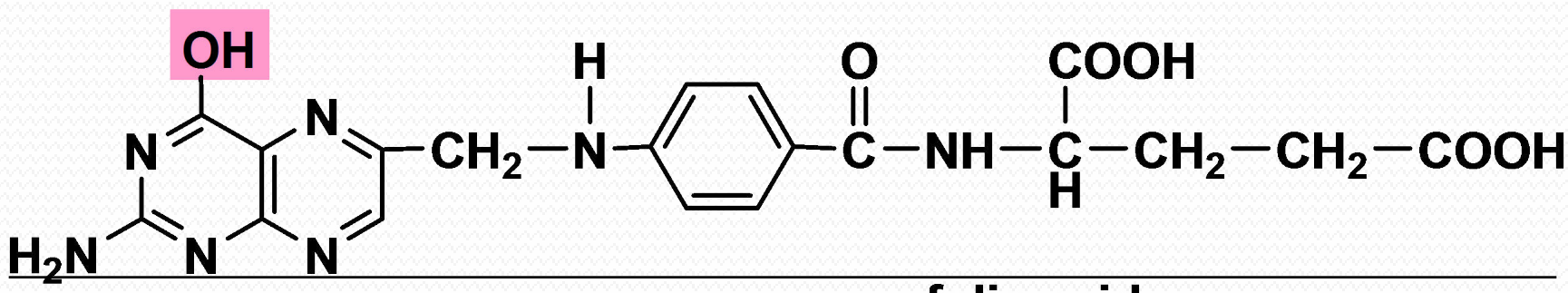
- Folate analogs Methotrexate and Sulfonamides block Purine biosynthesis

- **Sulfonamides structural analogs of PABA** inhibits **Folate Synthesis in microbes.**
- It indirectly inhibit Purine biosynthesis
- Since THFA is a carrier of one carbon moiety N10FormylTHF.

Folic acid Analogs

- **Aminopterin (AP) and Methotrexate (MTX)**

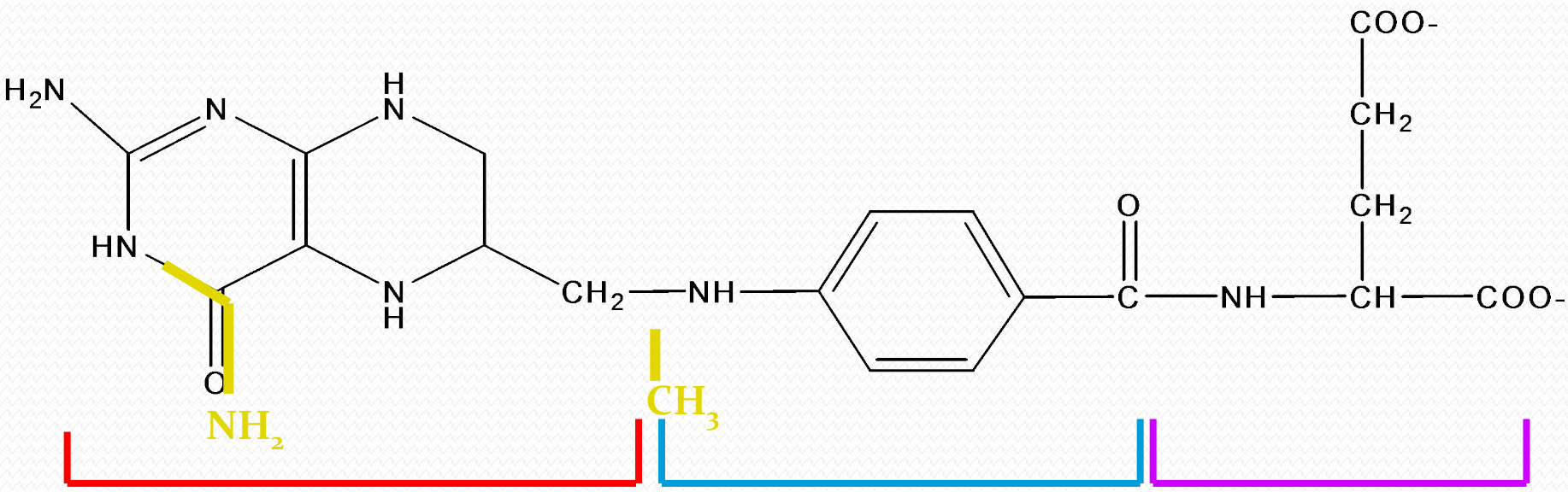




folic acid

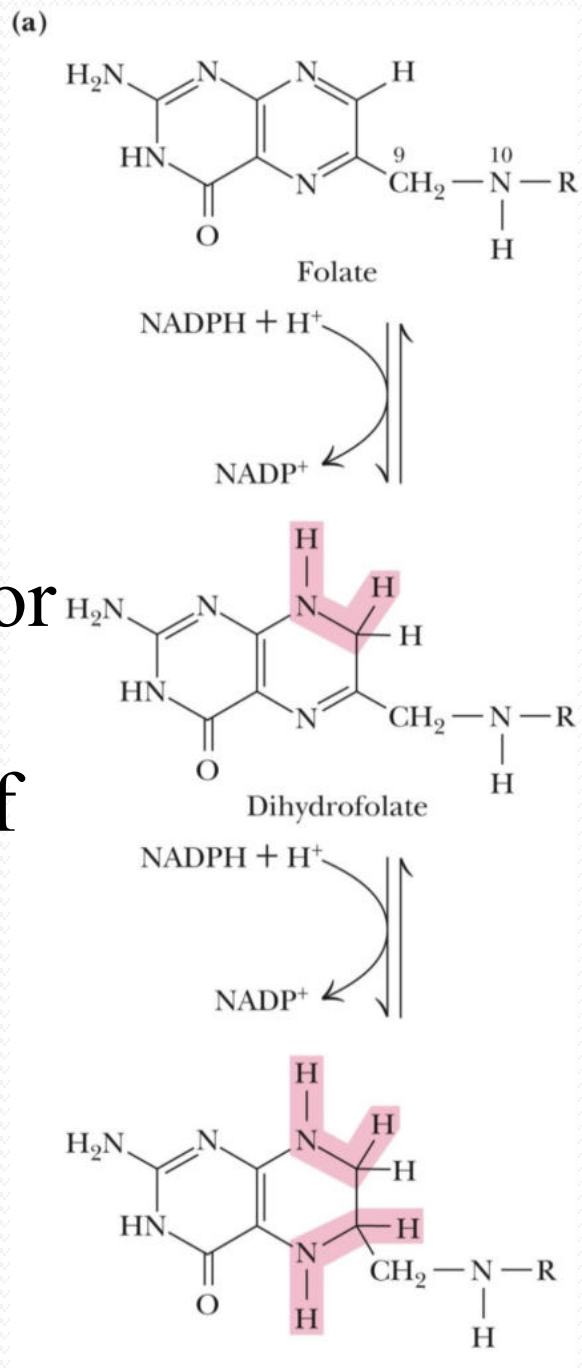
- **Methotrexate and Aminopterin Folate analogs** are inhibitors of **Folate Reductase** which form THFA.
- Presence of these inhibitors **affect the reduction of Folate to THFA.**
- **THFA is not available** for **1 Carbon moiety transfer** in Purine biosynthesis.

Methotrexate



6 methyl pterin ***p*-amino benzoic acid** **glutamate**

Tetrahydrofolate and One-Carbon Units



- Folic acid, a B vitamin found in green plants, fresh fruits, yeast, and liver, is named from *folium*, Latin for "leaf".
- Folates are acceptors and donors of one-carbon units for all oxidation levels of carbon except CO_2 (for which biotin is the relevant carrier).
- The active/coenzyme form is Tetrahydrofolate.

Tetrahydrofolate and One-Carbon Units

Oxidation States of Carbon in One-Carbon Units Carried by Tetrahydrofolate

Oxidation Number*	Oxidation Level	One-Carbon Form†	Tetrahydrofolate Form
−2	Methanol (most reduced)	—CH_3	N^5-Methyl-THF
0	Formaldehyde	—CH_2—	N^5,N^{10}-Methylene-THF
2	Formate (most oxidized)	—CH=O	N^5-Formyl-THF
		—CH=O	N^{10}-Formyl-THF
		—CH=NH	N^5-Formimino-THF
		—CH=	N^5,N^{10}-Methenyl-THF

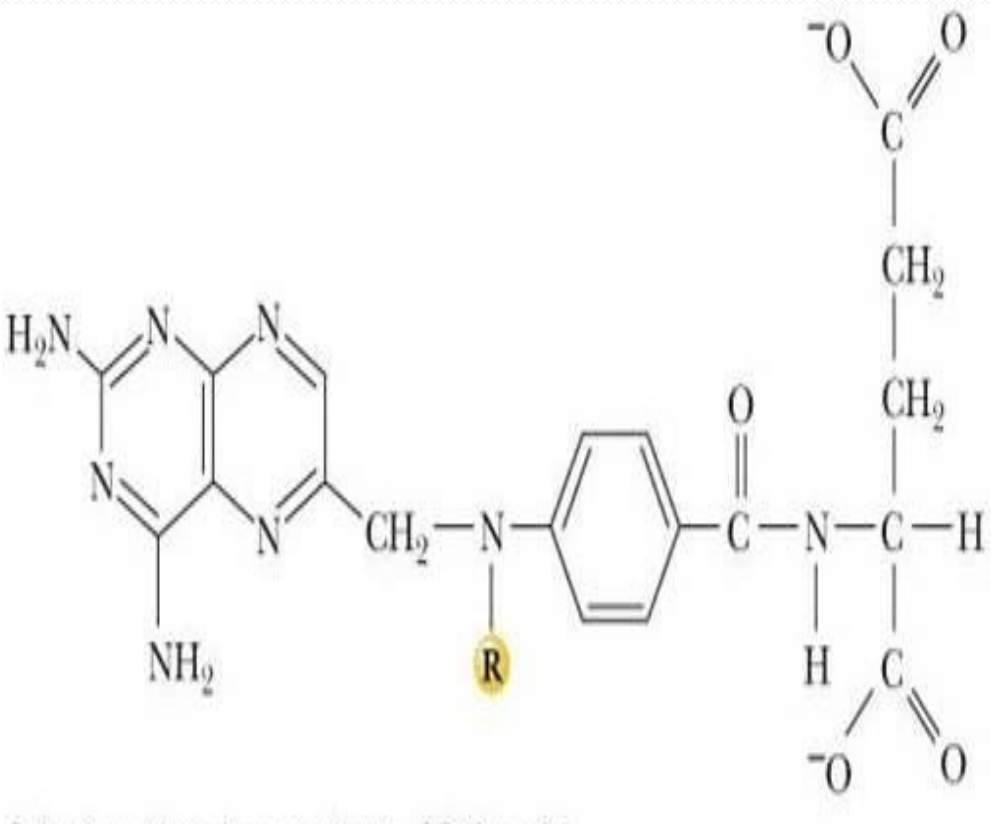
Folates are acceptors and donors of one-carbon units for all oxidation levels of carbon except CO_2 (for which biotin is the relevant carrier).

Folate Analogs as Antimicrobial and Anticancer Agents

De novo Purine biosynthesis depends on folic acid compounds at steps 4 and 10

- For this reason, antagonists of folic acid metabolism indirectly inhibit Purine formation and, in turn, nucleic acid synthesis, cell growth, and cell development
- Rapidly growing cells, such as infective bacteria and fast-growing tumors, are more susceptible to such agents

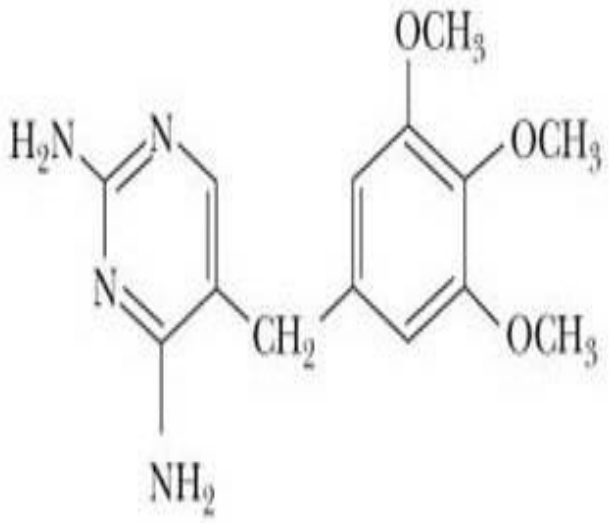
- Sulfonamides are **effective anti-bacterial agents**
- Methotrexate and Aminopterin are folic acid analogs that have been used in **cancer chemotherapy**



2-Amino, 4-amino analogs of folic acid

R = H Aminopterin

R = CH_3 Amethopterin (methotrexate)



Trimethoprim

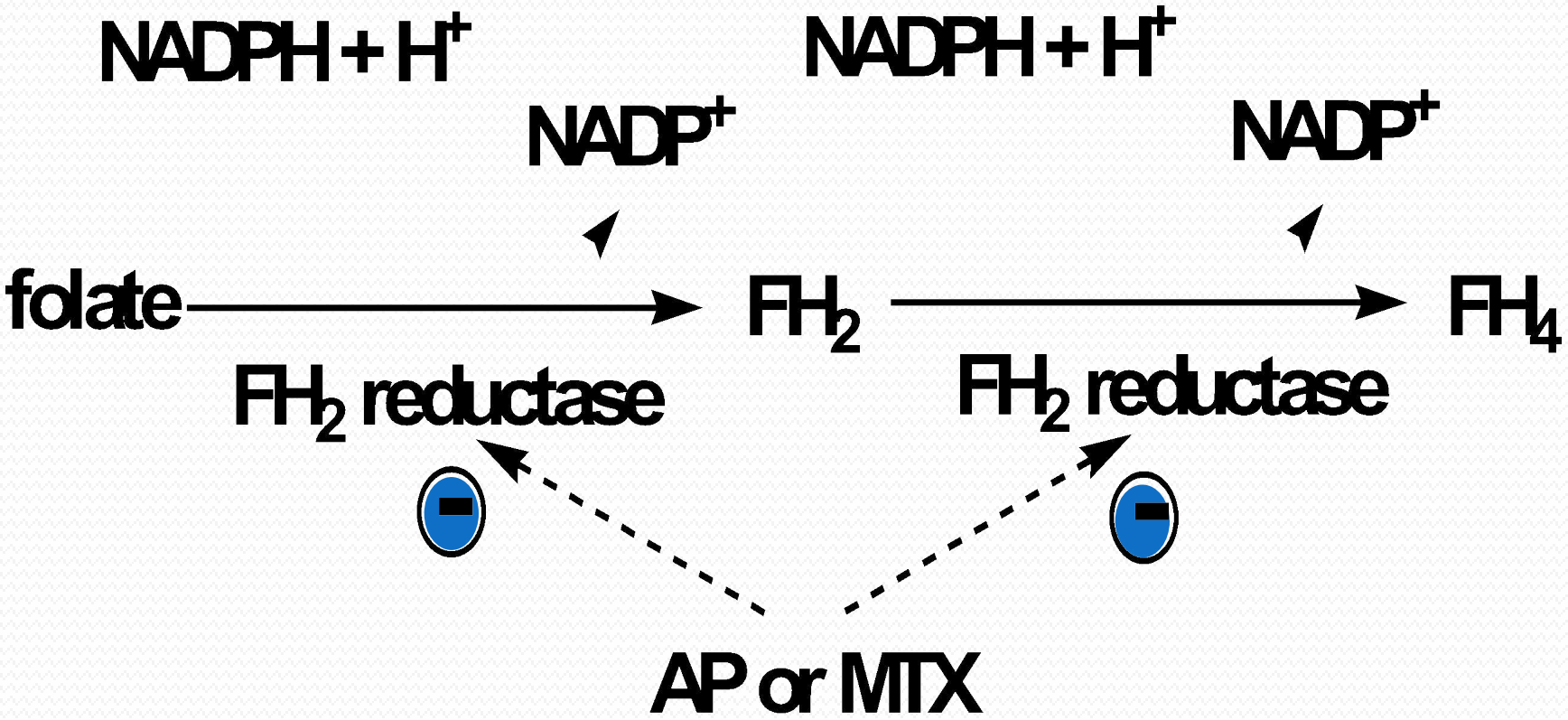
- Precursors and analogs of Folic acid employed as antimetabolites: sulfonamides , as well as methotrexate, aminopterin, and trimethoprim,
- These compounds shown here bind to dihydrofolate reductase (DHFR) with about 1000-fold greater affinity than DHF and thus act as virtually **irreversible inhibitors.**

Anti Cancer Drugs: Methotrexate

- Methotrexate, one of the earliest anti-cancer drugs, inhibits folate metabolism
- Folate provides methyl groups for biosynthetic reactions
  - It is essential for the conversion of dUMP to TMP
  - It provides carbon for the purine ring.

Methotrexate and Cancer

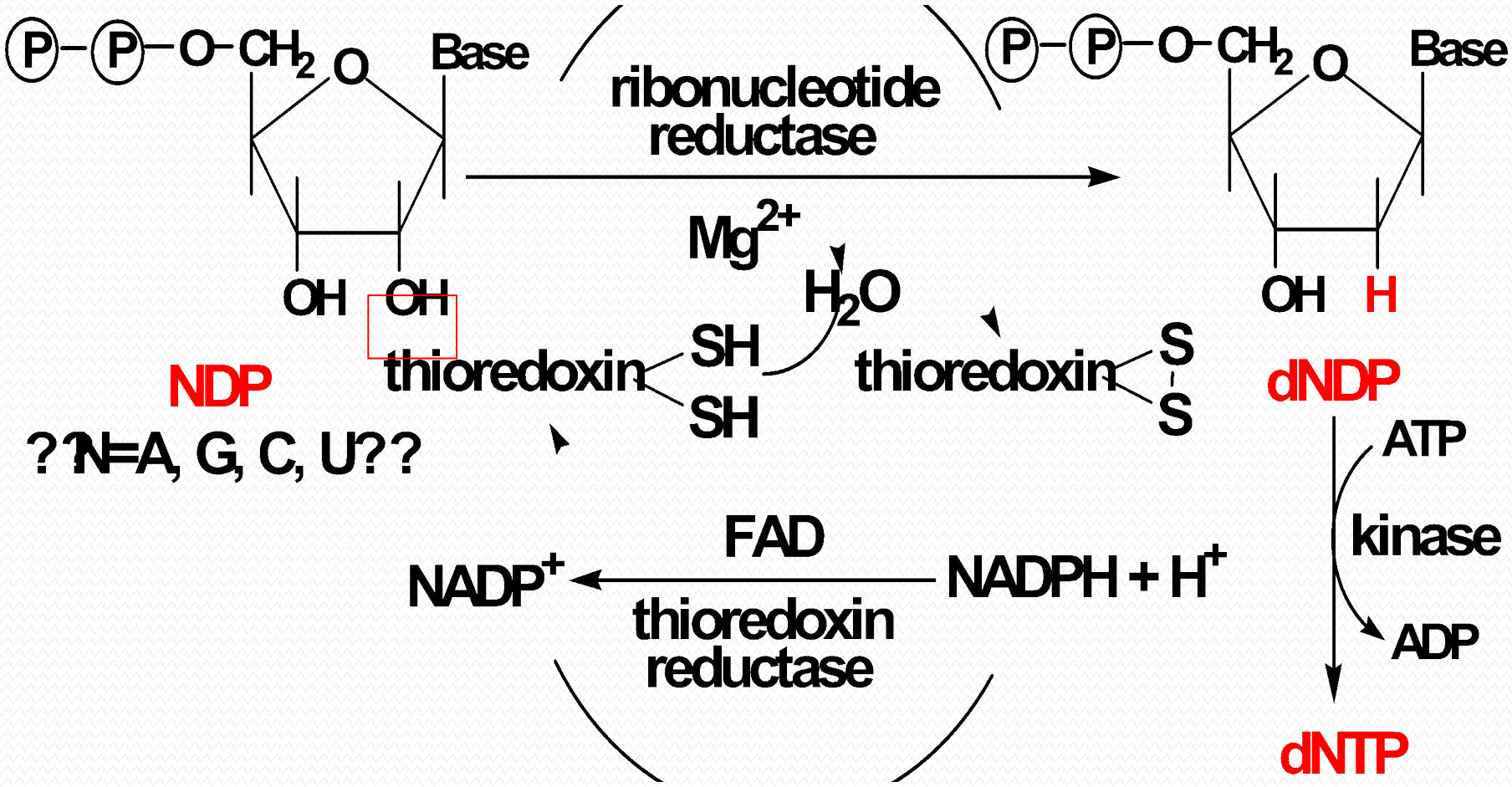
- Affects rapidly growing cells
- Adverse events include anemia, scaly skin, GI tract disturbances (diarrhea), and baldness
- Resistance to MTX is caused by amplification of dihydrofolate reductase gene



- The structural analogs of folic acid(e.g. MTX) are widely used to **control cancer** (e.g. Leukemia).
- Notice: These inhibitors also affect the proliferation of normally growing cells. This causes many **side-effects** including anemia, baldness, scaly skin etc.

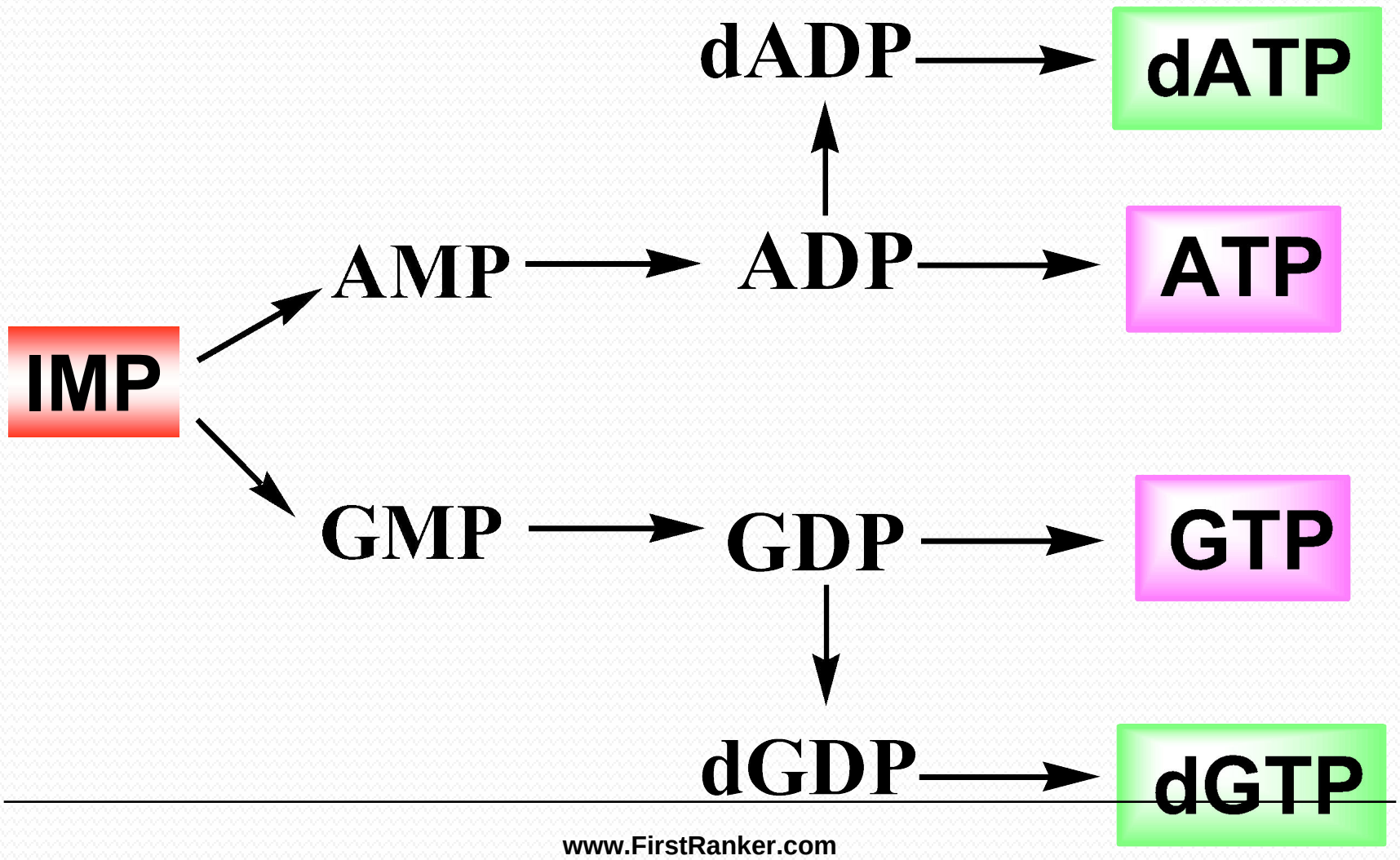
Formation of Deoxyribonucleotide

- **Formation of Deoxyribonucleotide** involves the **reduction of the sugar** moiety of Ribonucleoside Diphosphates (ADP, GDP, CDP or UDP).
- Deoxyribonucleotide synthesis occurs at the nucleoside diphosphate(NDP) level.



Deoxyribonucleotide synthesis at the NDP level

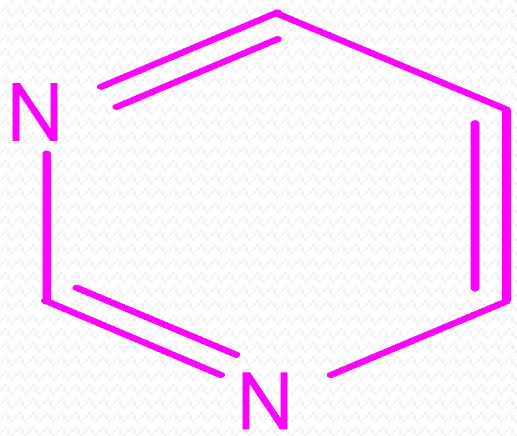
Summary of Purine biosynthesis



Biosynthesis Of Pyrimidines Nucleotides

Biosynthesis of Pyrimidine Nucleotides

Pyrimidine Ring System



Pyrimidine Nucleotide Metabolism

- There are also two synthesis pathways of Pyrimidine nucleotides:
- Denovo Synthesis and Salvage pathway.

De Novo Synthesis Pathway

- In De novo pathway the Pyrimidine ring is assembled first and then linked to Ribose phosphate.

- The carbon and nitrogen atoms in the Pyrimidine ring are derived from:
  - **Bicarbonate**
  - **Aspartate**
  - **Glutamine**

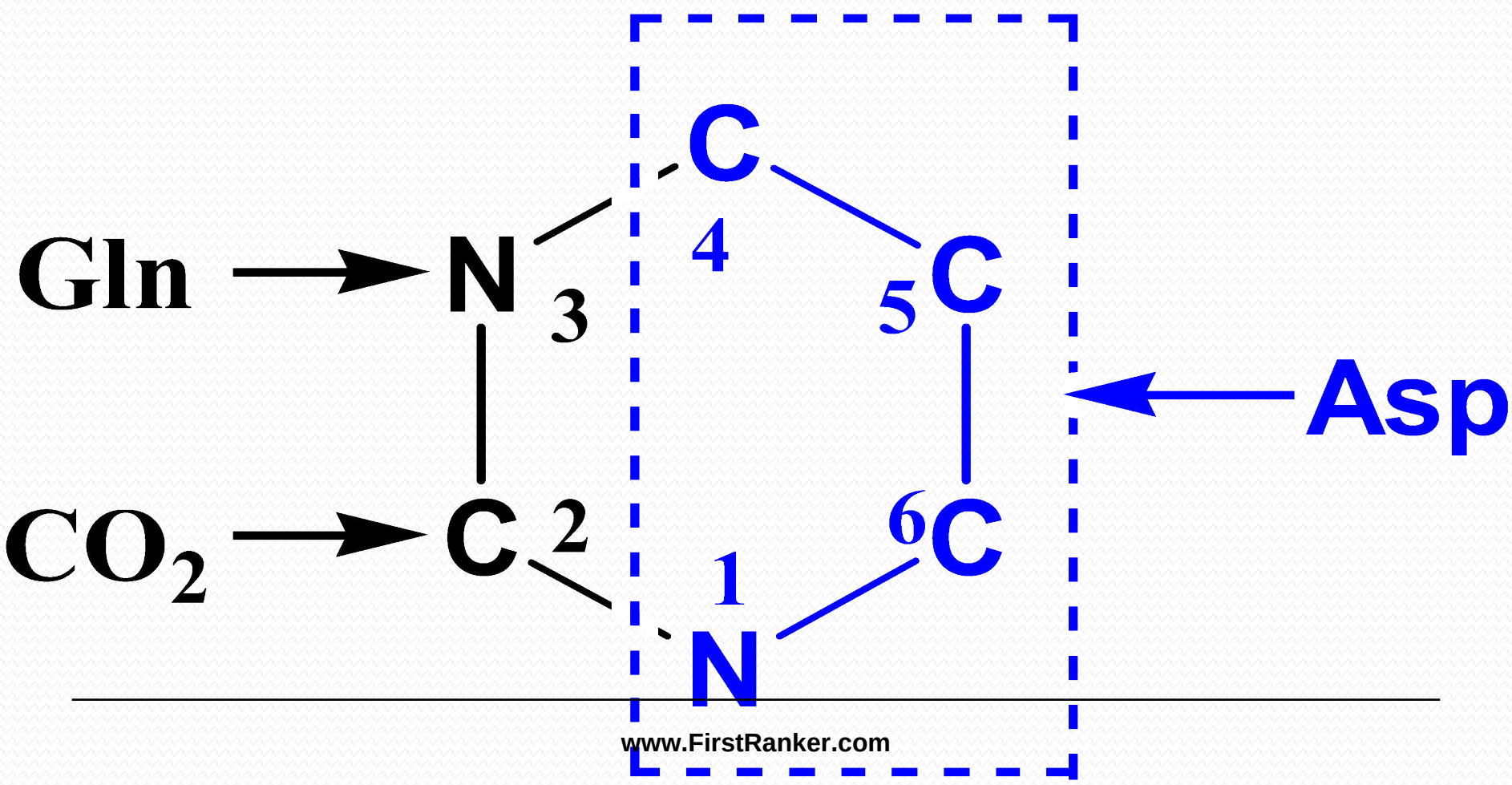
- Shorter pathway than for Purine Synthesis
- Pyrimidine ring is made first, then attached to ribose-P (unlike Purine biosynthesis)

- Pyrimidine Denovo synthesis requires 6 steps

  (instead of 11 steps for Purine)
- The product is UMP (Uridine Monophosphate)

- Only 3 precursors are used for Pyrimidine Denovo synthesis.
- These contribute to the 6-membered ring
  - **Aspartate**
  - **Glutamine**
  - **HCO_3^-**

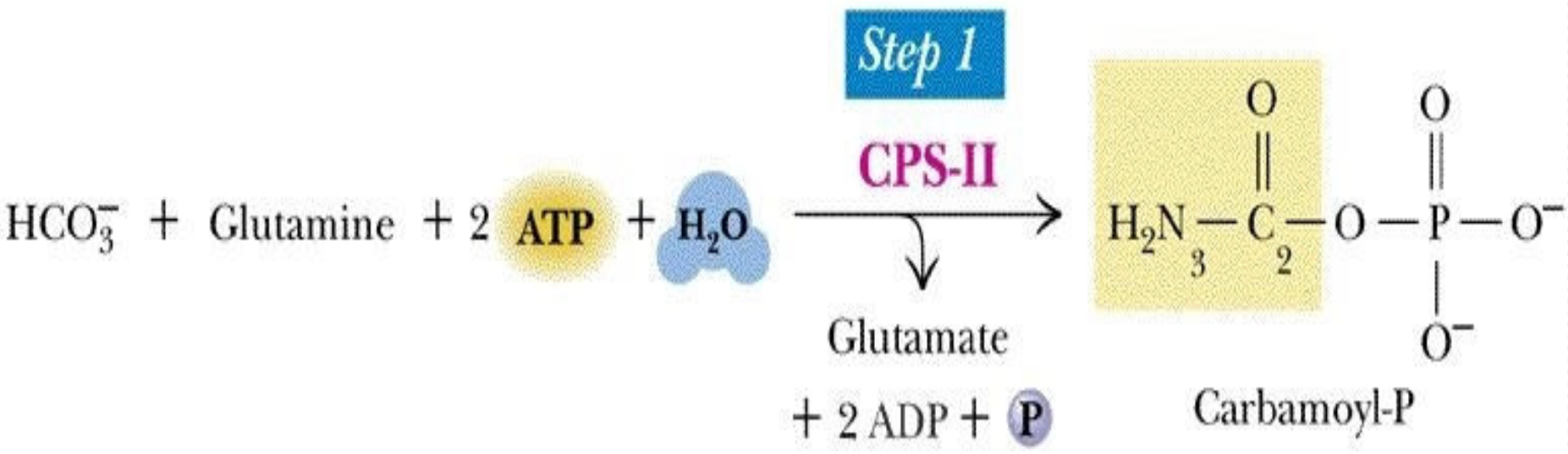
Element Sources of Pyrimidine base



- Pyrimidine Biosynthesis involves 2 ATPs

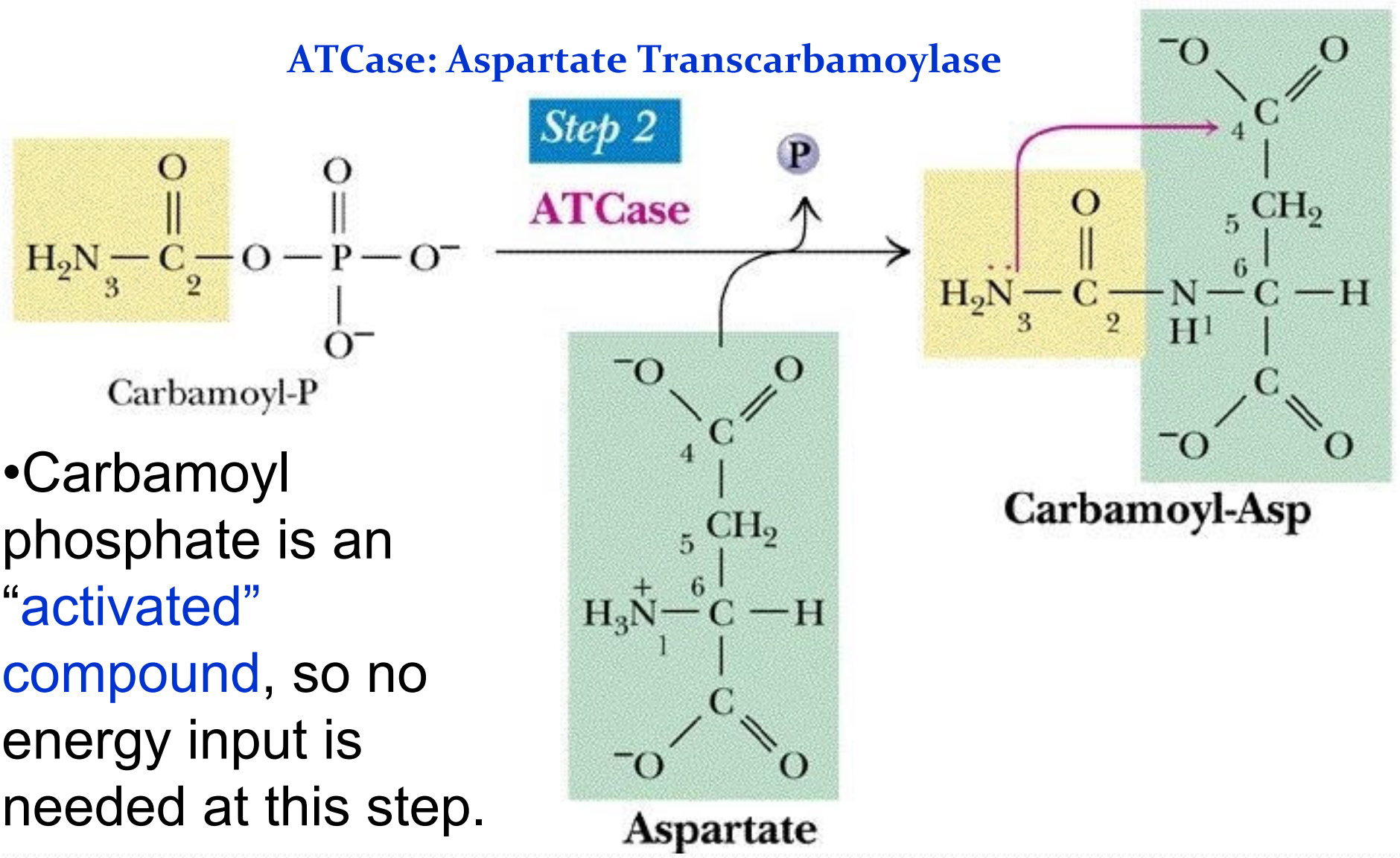
Steps	Happenings
1	Entry of CO_2 and Glutamine
2	Entry of Aspartate
3	Ring Closure with Dehydration
4	Oxidation of Di Hydro Orotate
5	Entry of PRPP
6	Decarboxylation To form UMP

Step 1:
Synthesis of Carbamoyl Phosphate



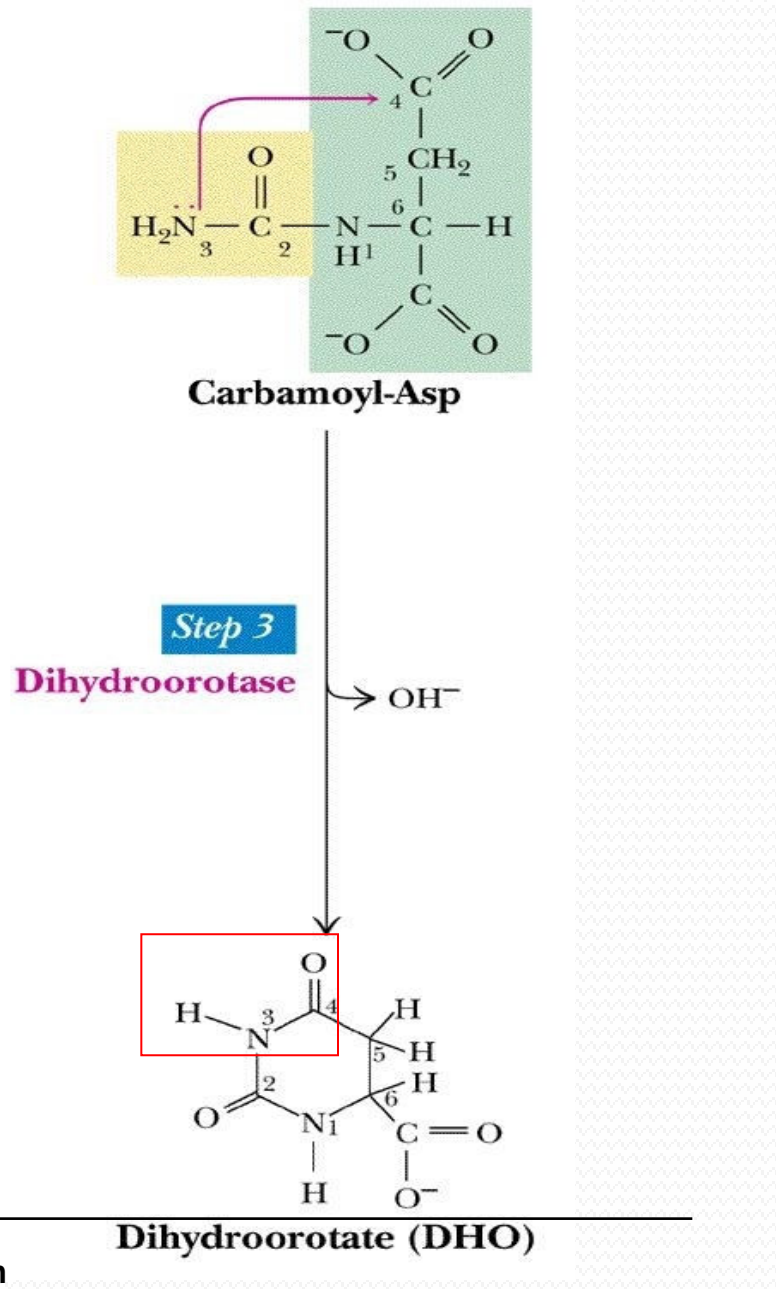
- Carbamoyl phosphate synthetase(CPS) exists in 2 types:
  - **CPS-I**, a mitochondrial enzyme, is dedicated to the urea cycle and arginine biosynthesis.
  - **CPS-II**, a Cytosolic enzyme, used here. It is the committed step in animals.

Step 2:
Synthesis of Carbamoyl Aspartate

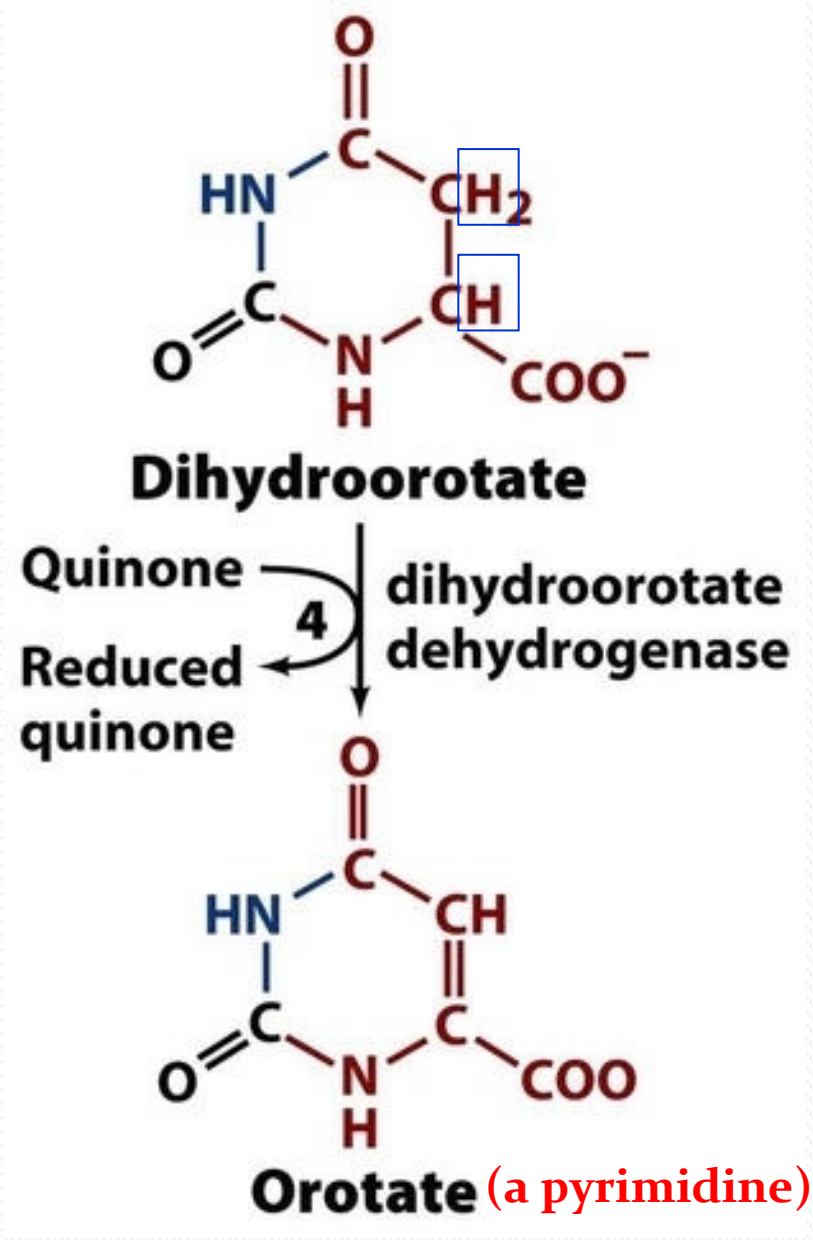


•Carbamoyl phosphate is an "activated" compound, so no energy input is needed at this step.

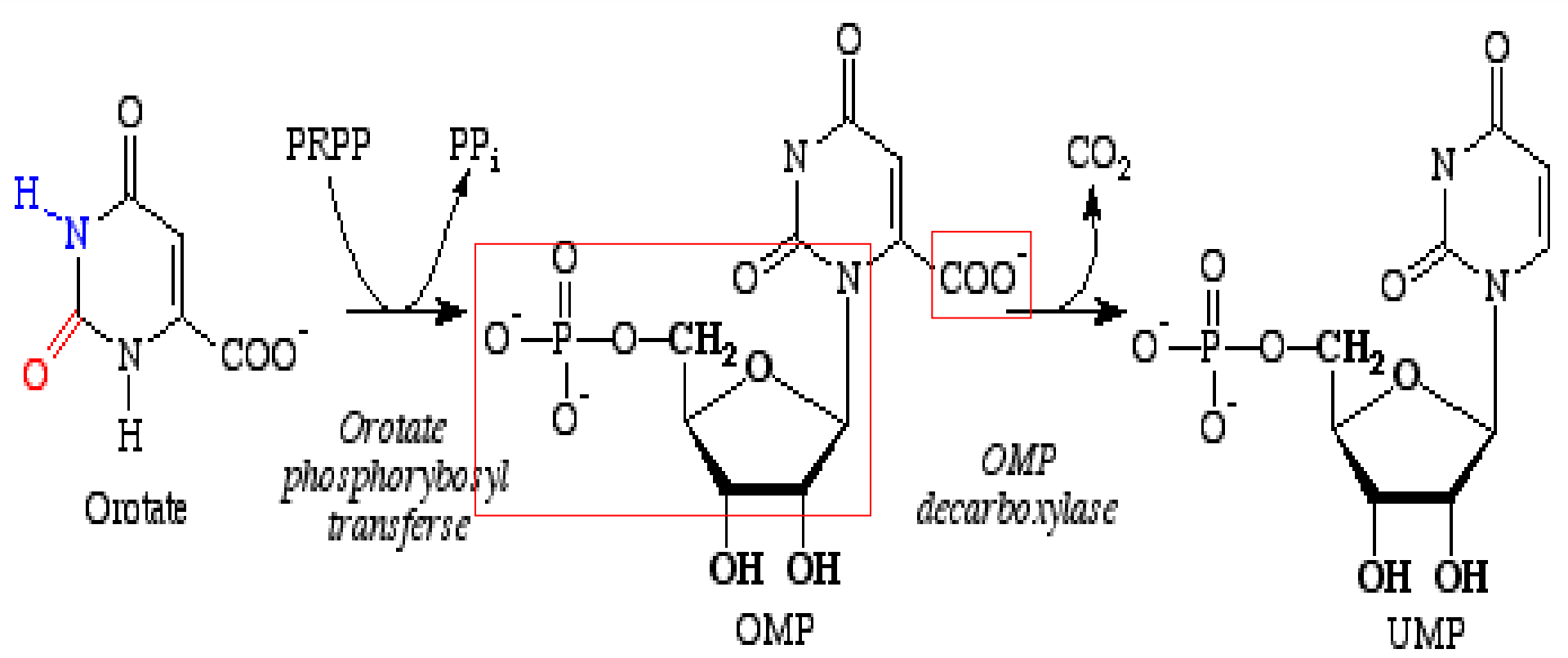
Step 3:
Ring closure to form DihydroOrotate



Step 4: Oxidation of DihydroOrotate To Orotate

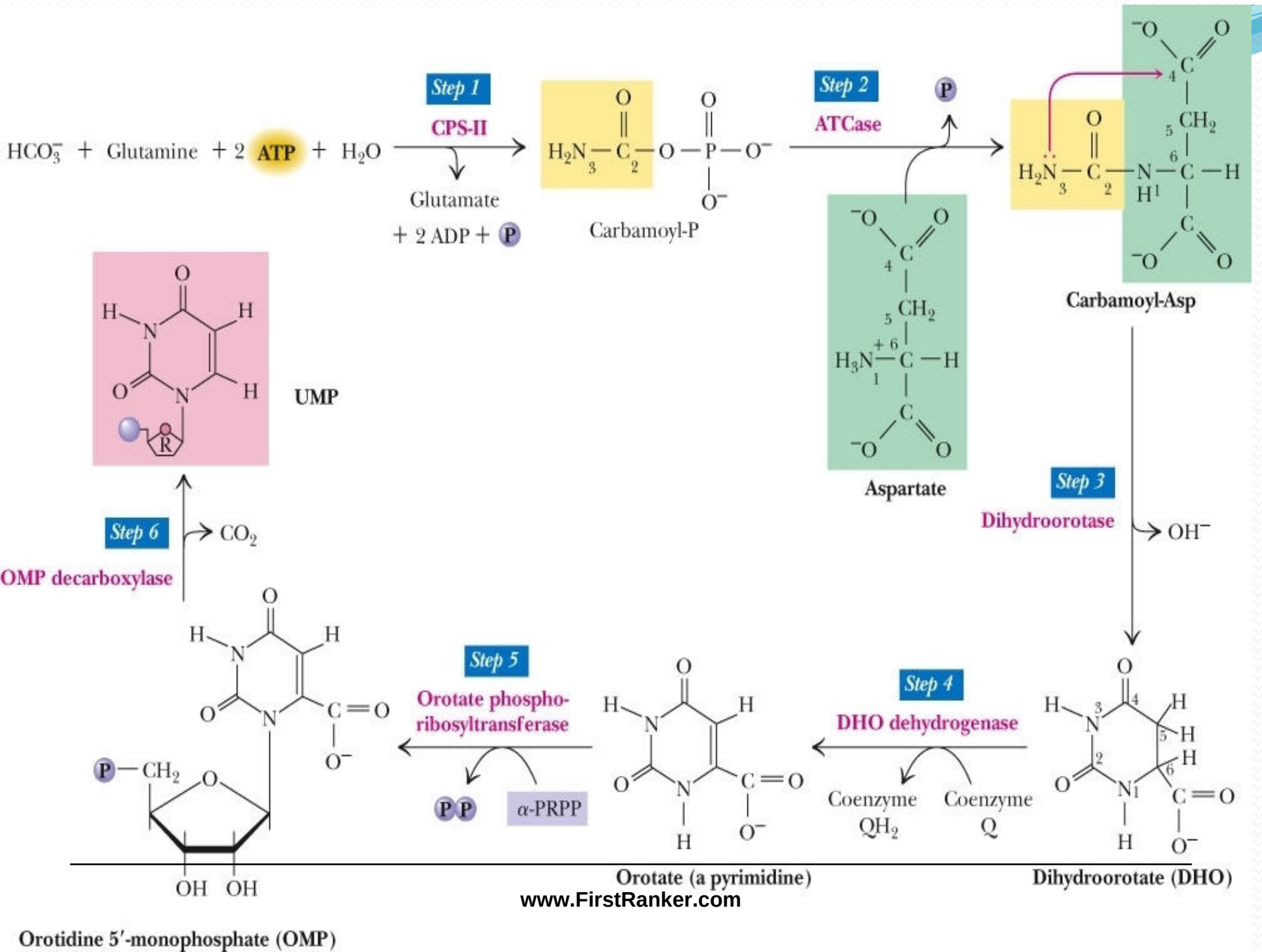


Step 5: Acquisition of Ribose Phosphate moiety

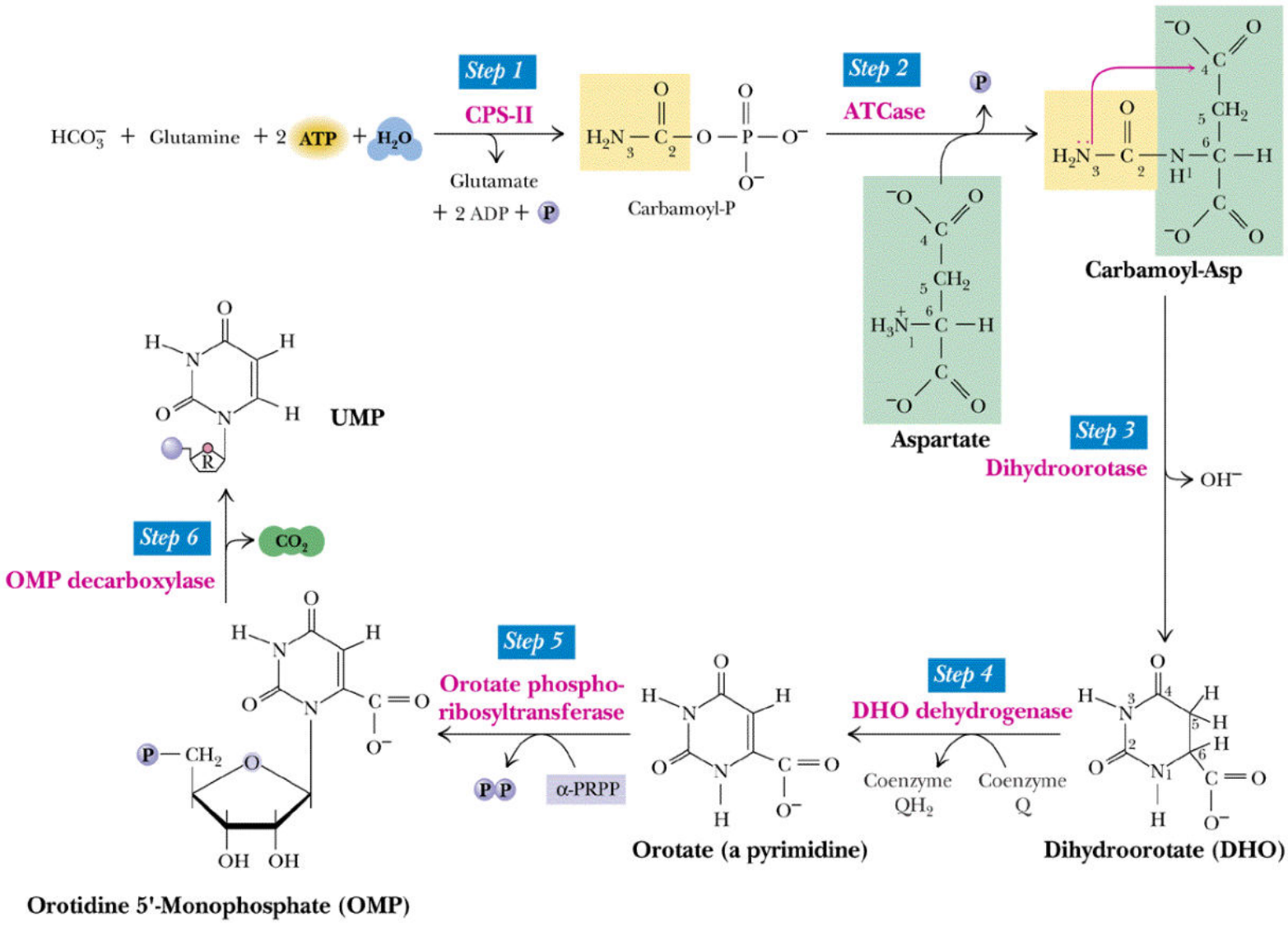


Step 6:
Decarboxylation of OMP

- OMP is decarboxylated to UMP



Garrett/Grisham, Biochemistry with a Human Focus
Figure 21.36



UMP Is Converted To CMP and TMP

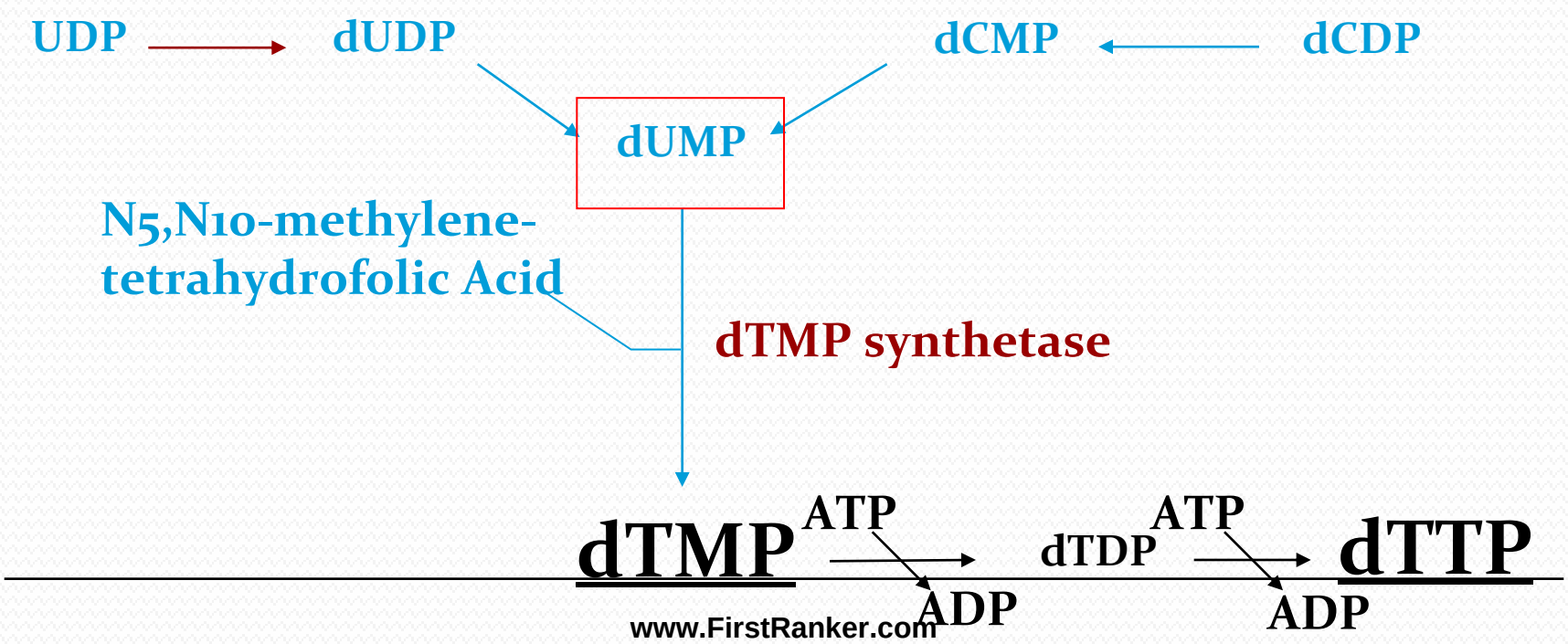
Conversion Of UMP to CMP

- UMP is converted to CMP in presence of Glutamine and ATP

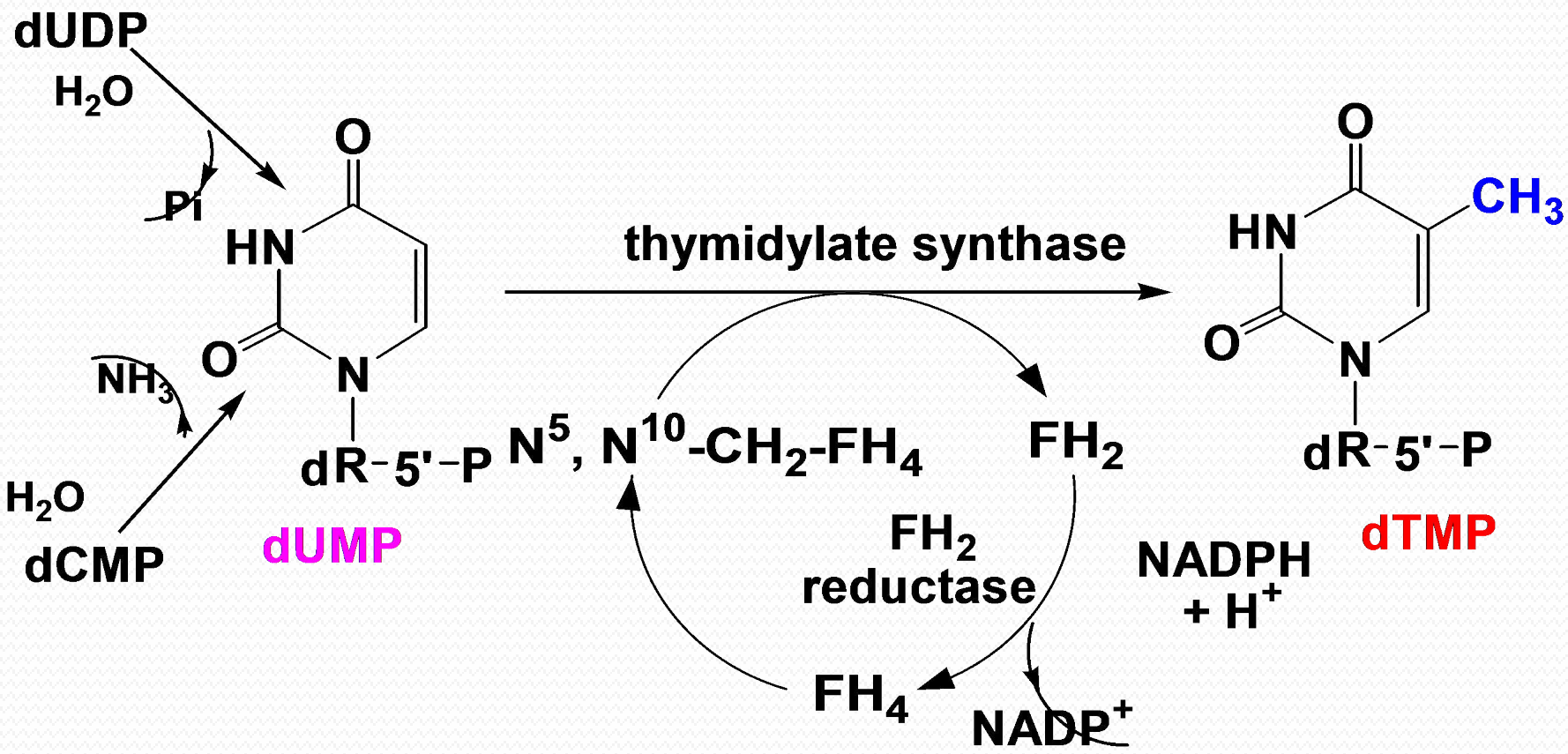
Formation of dTMP

The immediate precursor of thymidylate (dTMP) is dUMP.

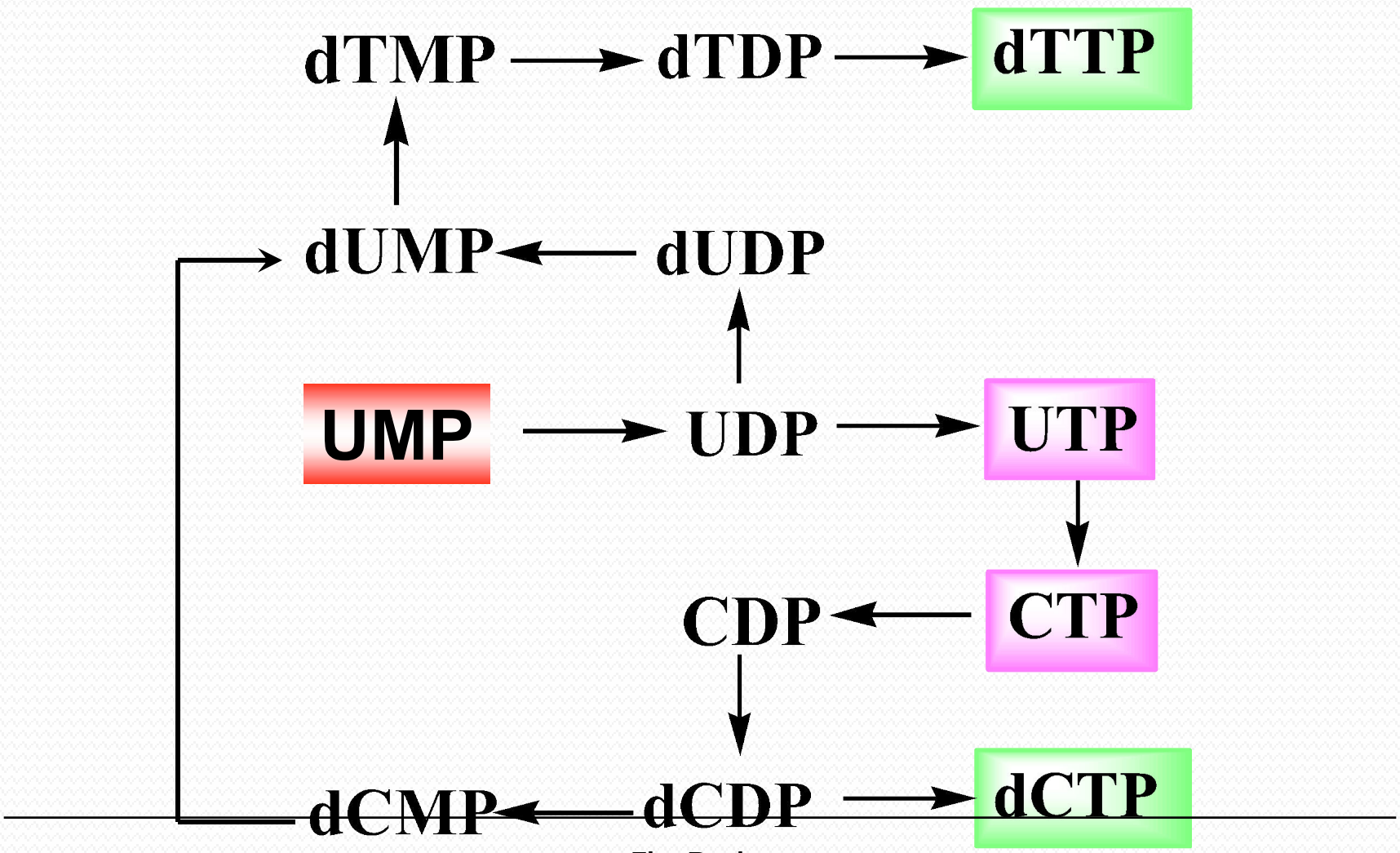
The formation of dUMP either by deamination of dCMP or by hydrolyzation of dUDP. The former is the main route.



dTMP synthesis at the Nucleoside Monophosphate level.



Summary of pyrimidine biosynthesis

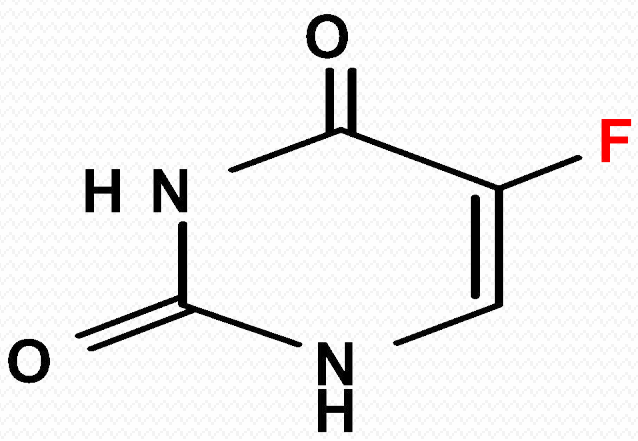


Antimetabolites of Pyrimidine Nucleotides

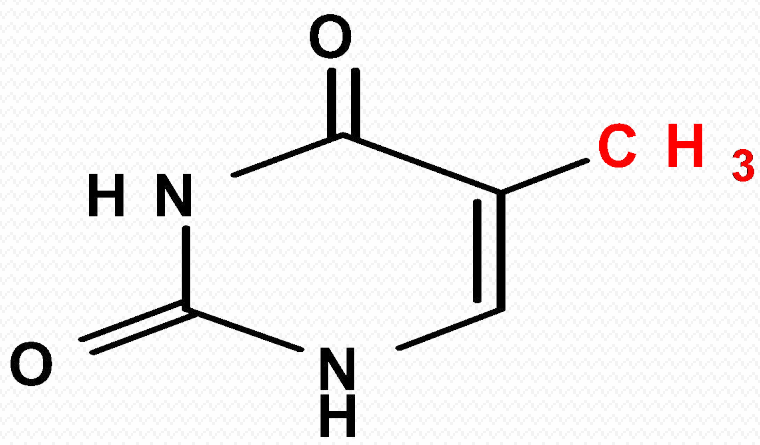
- **Antimetabolites of Pyrimidine nucleotides are similar with them of Purine nucleotides.**

Pyrimidine Analogs

- **5-fluorouracil (5-FU) is a analog of Thymine.**



5-FU

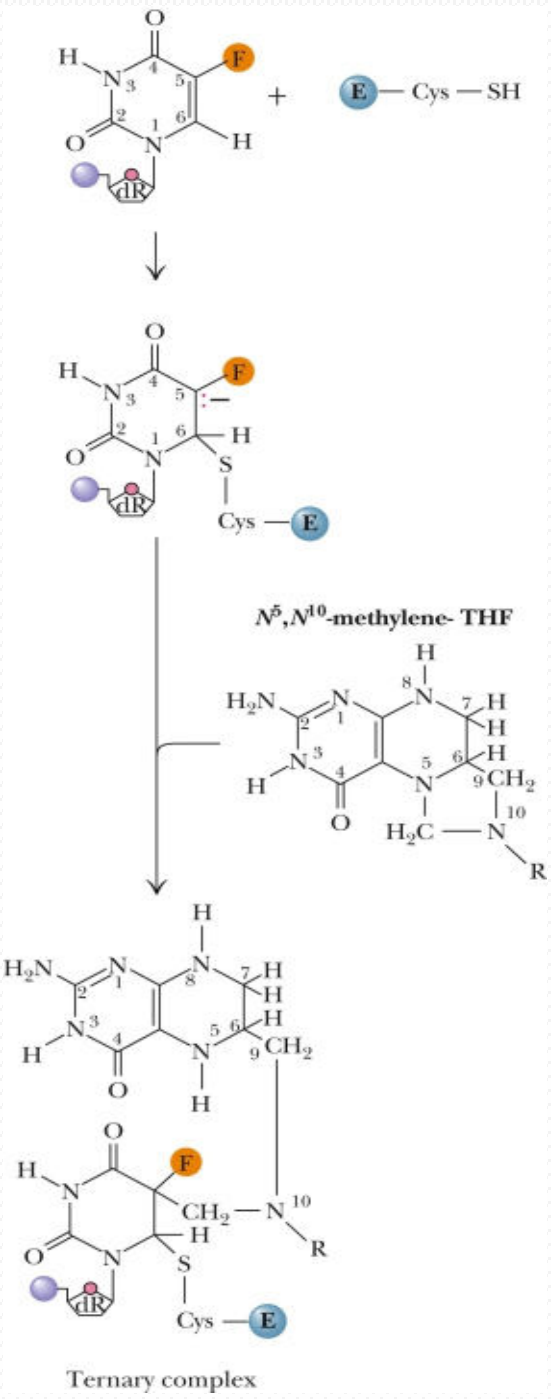


thymine

- Synthesis of dTMP from dUMP is catalyzed by Thymidylate Synthase
  - This enzyme methylates dUMP at the 5-position to create dTMP
  - The methyl donor is the one-carbon folic acid derivative N^5, N^{10}-Methylene-THF

- The reaction is a reductive methylation; the one-carbon unit is transferred at the methylene level of reduction and then reduced to the methyl level
  - The THF cofactor is oxidized to yield DHF
- DHFR reduces DHF back to THF for serving again
  - dTMP synthesis has become a preferred target for inhibitors designed to disrupt DNA synthesis

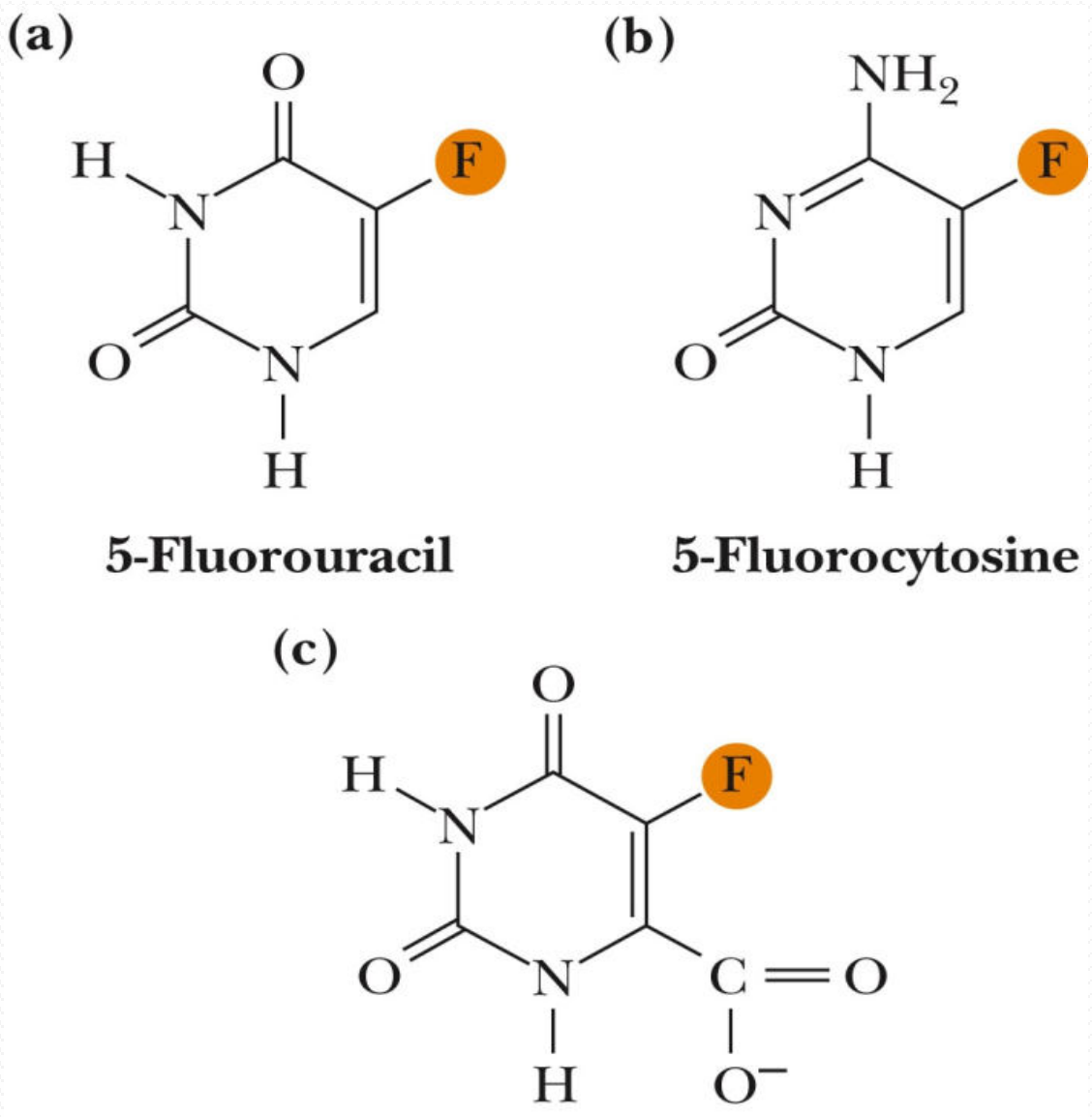
- Fluoro-substituted analogs as therapeutic agents



5-fluorouracil (5-FU) is used as a chemotherapeutic agent in the **treatment of cancers**

5-fluorocytosine is used as an **antifungal drug**

5-fluoroorotate is an ~~effective~~ **antimalarial drug**



- The **5-Fluoro** substitution inhibits on the mechanism of action of Thymidylate Synthase.
- Which in turn affects DNA synthesis.

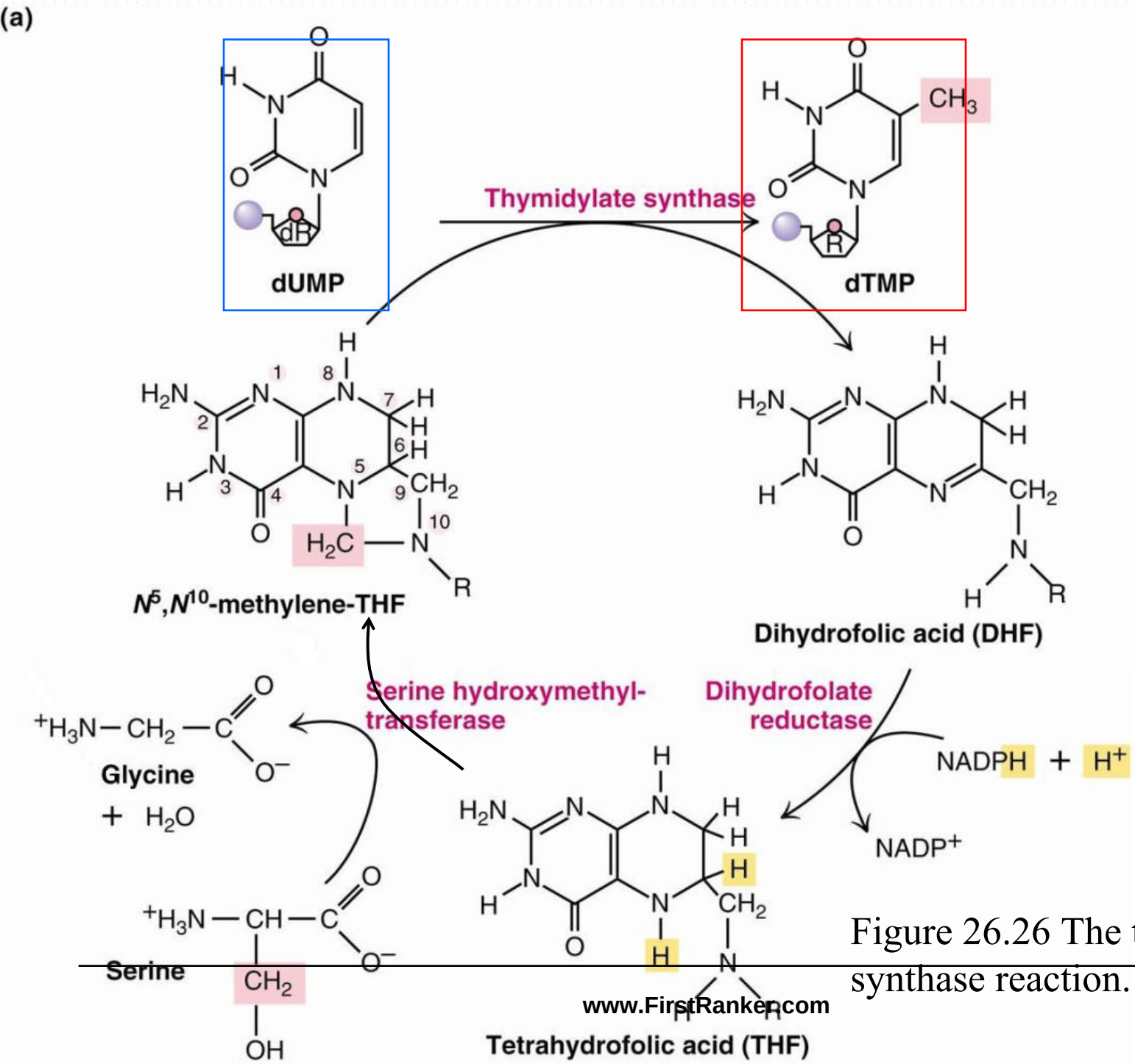


Figure 26.26 The thymidylate synthase reaction.

Amino acid analogs

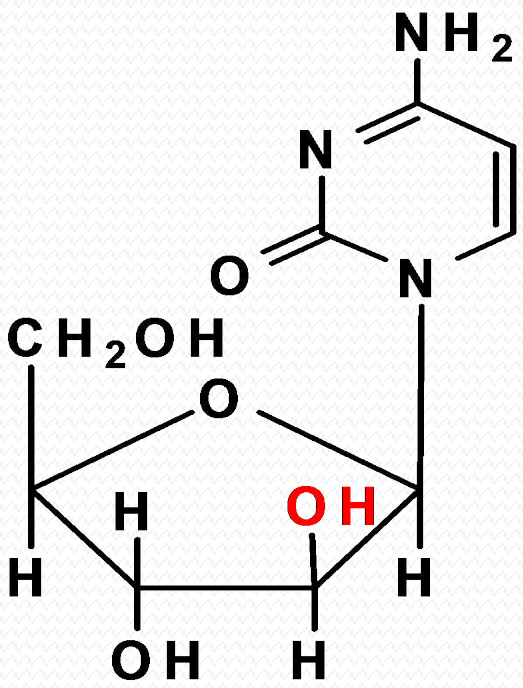
- **Azaserine (AS) inhibits the synthesis of CTP.**

Folic acid Analogs

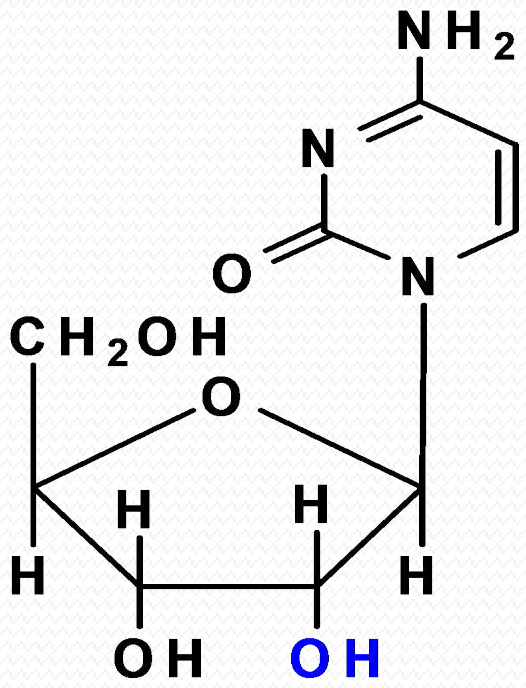
- **Methotrexate (MTX) inhibits the synthesis of dTMP.**

Nucleoside Analogs

- **Arabinosyl cytosine (Ara-c) <u>inhibits the synthesis of dCDP</u>.**



ara-c



cytosine

Salvage Pathway

- **Salvage Pathway** is important in **Brain and Bone marrow**
- Where Denovo synthesis of Purine and Pyrimidine nucleotide do not occur.

Salvage Pathway of Purine Nucleotides

- Salvage pathway have mechanisms to retrieve Purine bases and Purine nucleosides. They are used to synthesize Purine nucleotides.

- **Purine bases created by degradation of RNA or DNA and intermediate of purine synthesis can be directly converted to the corresponding nucleotides.**
- **The significance of salvage pathway :**
  - **Save the fuel.**
  - **Some tissues and organs such as brain and bone marrow are only capable of synthesizing nucleotides by salvage pathway.**

- **Two Phosphoribosyl transferases are involved:**
  - **APRTase**

    (Adenine phosphoribosyl transferase) for Adenine.
  - **HGPRTase**

    (Hypoxanthine guanine phosphoribosyl transferase) for guanine or Hypoxanthine.

From Nitrogen Base to Nucleotides

APRTase

Adenine + PRPP----------→AMP + ppi

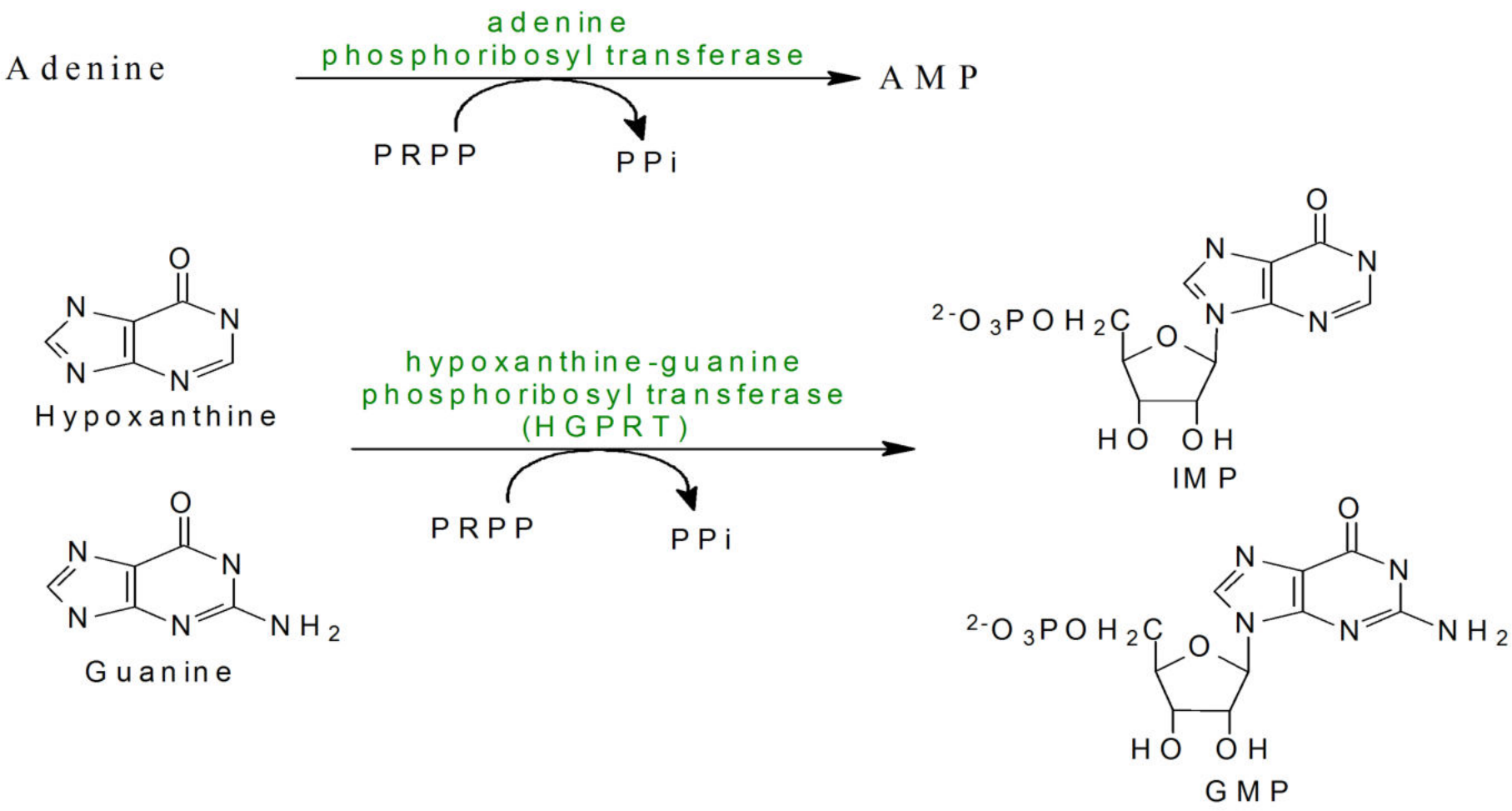
HGPRTase

Hypoxanthine + PRPP----------→ IMP + ppi

HGPRTase

Guanine + PRPP----------→GMP + ppi

Purine Salvage Pathway



Absence of activity of HGPRTase leads to Lesch-Nyhan Syndrome.

From Nucleoside to Nucleotide

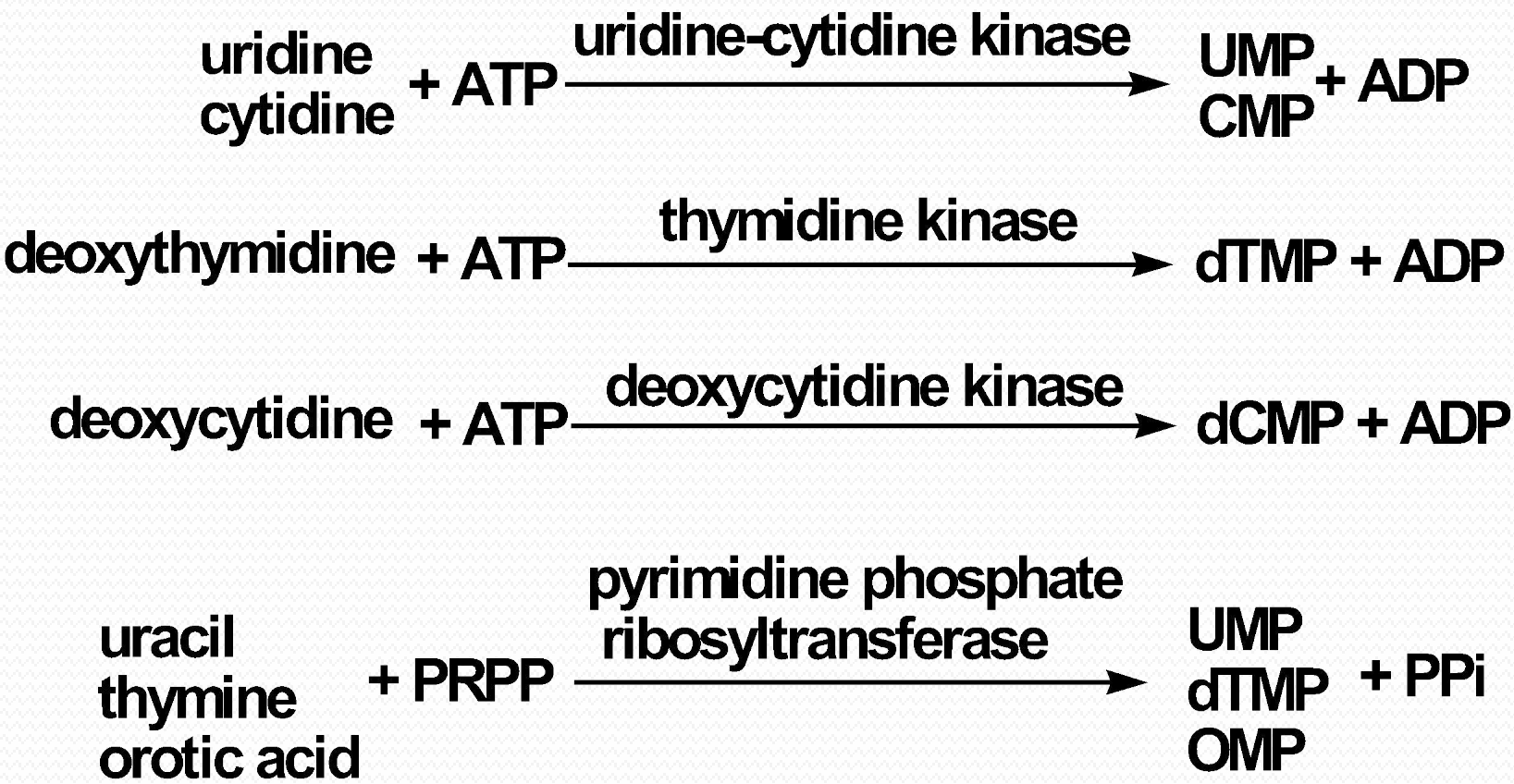
AR kinase

AdenineRibose + ATP----------→AMP + ADP

In comparison to De novo pathway, salvage pathway is energy-saving.

In brain and bone marrow tissues salvage pathway is the only pathway of nucleotide synthesis.

Pyrimidine Salvage pathway



Salvage Pathway

Pyrimidine Phosphoribosyl Transferase (**PPRTase**) catalyzes the following Salvage reaction.

Uracil + PRPP- ---→ UMP + ppi

- In some organisms, free Pyrimidines are salvaged and recycled to form Pyrimidine nucleotides
  - In humans, Pyrimidines are recycled from Nucleosides, but free Pyrimidine bases are not salvaged

- Uridine Kinase catalyzes the formation of UMP from Uridine and ATP.

UR + ATP-------→ UMP + ADP

Formation of Deoxynucleotides

- Deoxynucleotides are formed by **reducing Ribonucleotide Diphosphates**.

Ribonucleotide Reductase

NDP + NADPH + H+----------→dNDP + H2O + NADP+

- In the reaction of Ribonucleotide Reductase Hydrogen atoms are not directly donated by NADPH.

- **Coenzyme Thioredoxin,** a Protein with **two sulfhydryl groups** mediates the **transfer of hydrogen atoms** from **NADPH to Ribonucleotide Reductase.**

- Then the enzyme catalyzes the reduction of NDP, to form dNDP.

NDP reductase

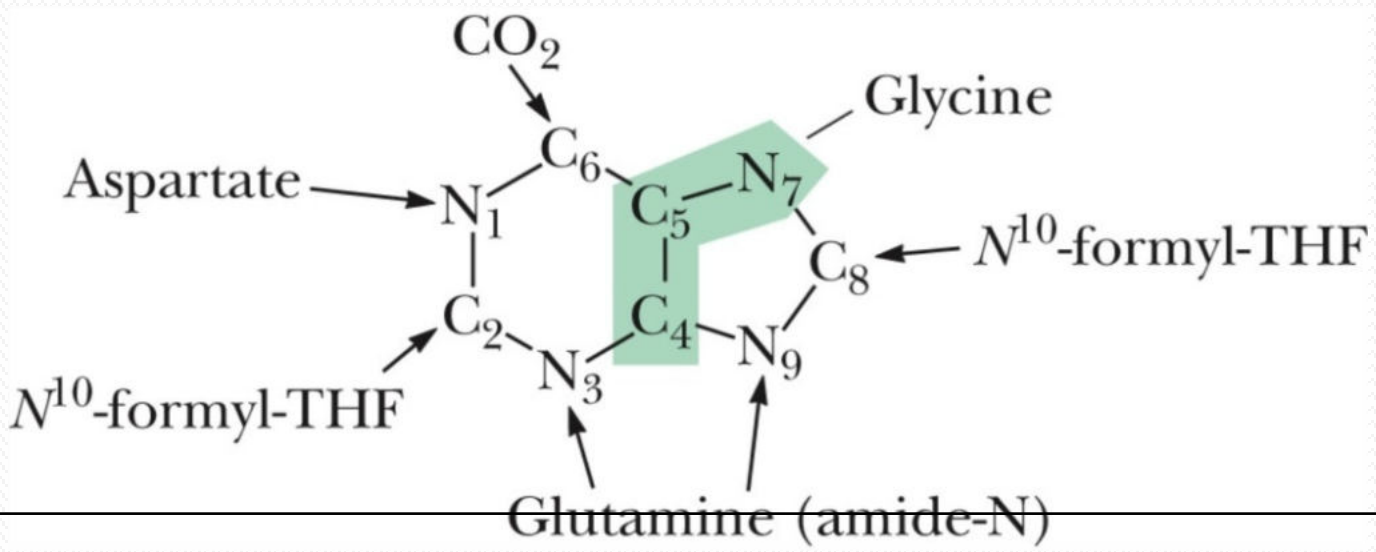
- NDP + Thioredoxin (SH)2 --------→ dNDP + Thioredoxin (-S-S-)

- The regeneration of **reduced Thioredoxin** is catalyzed by Thioredoxin reductase.
- Thioredoxin Reductase converts Oxidized Thioredoxin to functional Reduced Thioredoxin.
- Thioredoxin is NADPH+ H^+ requiring enzyme

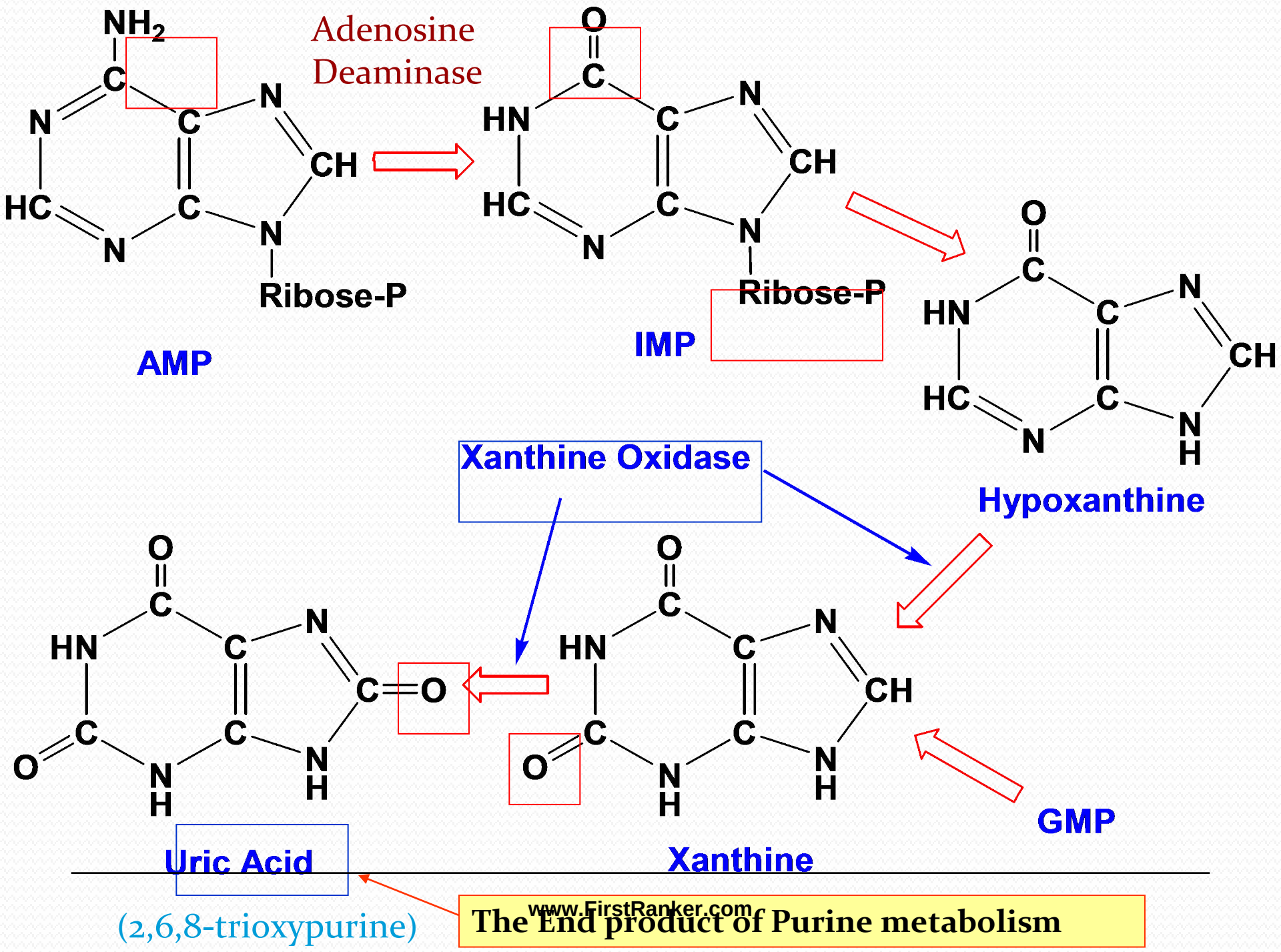
- Thioredoxin (-S-S-) +NADPH +H+→ Thioredoxin (SH)2+NADPH

- **NDP Reductase** is an allosteric enzyme, Its activity is controlled by various **NTPs and dNTPs.**

Catabolism Of Purine Nucleotides



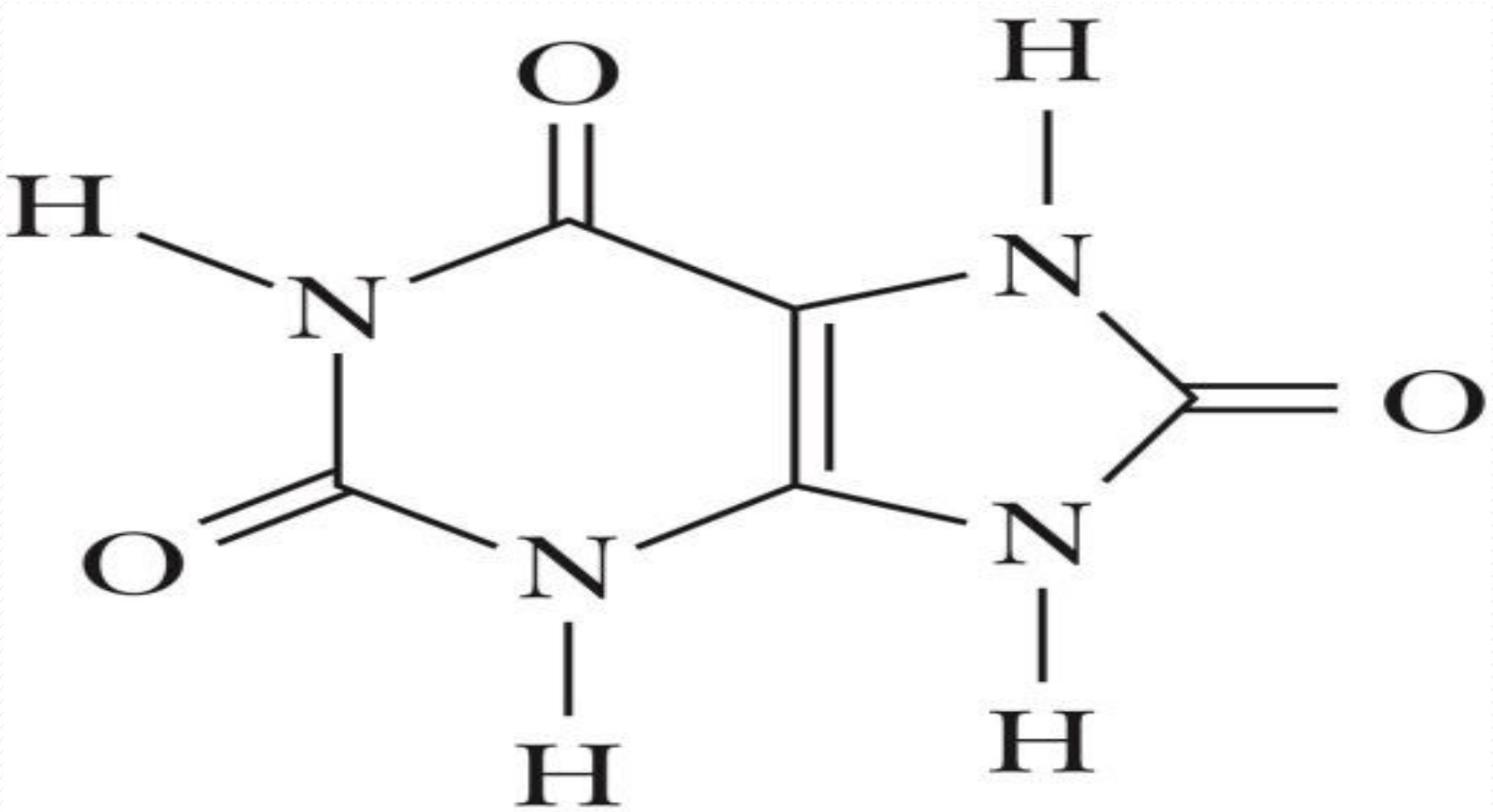
Degradation of Purine Nucleotides



Uric acid

- Uric acid is a NPN, waste excreted end product of Purine catabolism.
- The rate of uric acid excretion by the normal adult human is about 0.6 g/24 h in urine, arising in part from ingested purines and in part from the turnover of the purine nucleotides of nucleic acids.
- The normal concentration of uric acid in the serum of adults is in the range of 3-7 mg/dl.

2, 6,8 Tri Oxy Purine



Uric acid

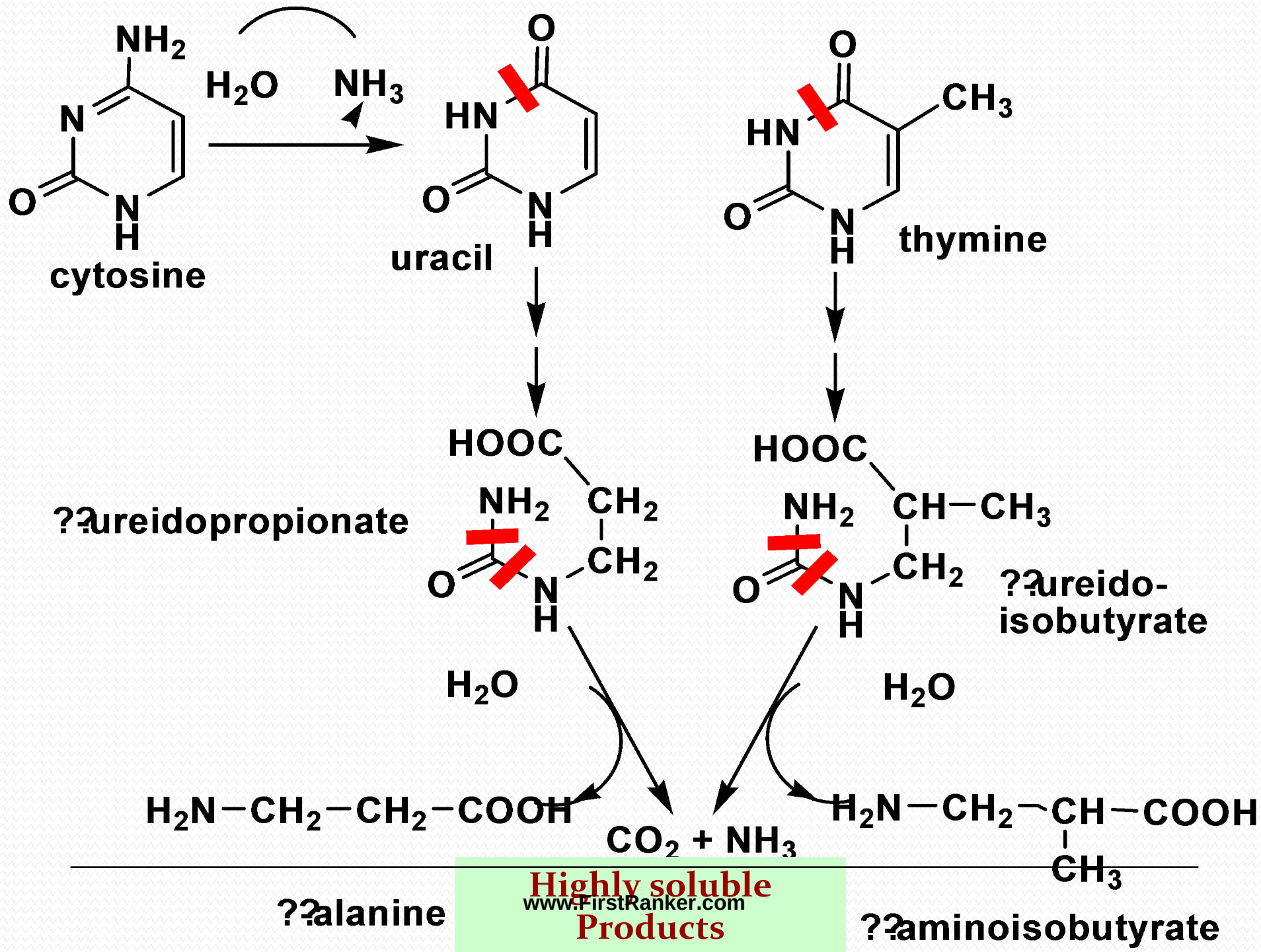
Catabolism Of Pyrimidines

Degradation of Pyrimidine Nucleotides

How Are Pyrimidines Degraded?

- Catabolism of Pyrimidine Nitrogen Bases **Cytosine** and **Uracil** yields :
  - β-Alanine,
  - Ammonium ions
  - CO_2
- β-Alanine can be recycled into the synthesis of coenzyme A

- Catabolism of **Thymine** yields:
  - β-Aminoisobutyric acid
  - Ammonium ions
  - CO_2



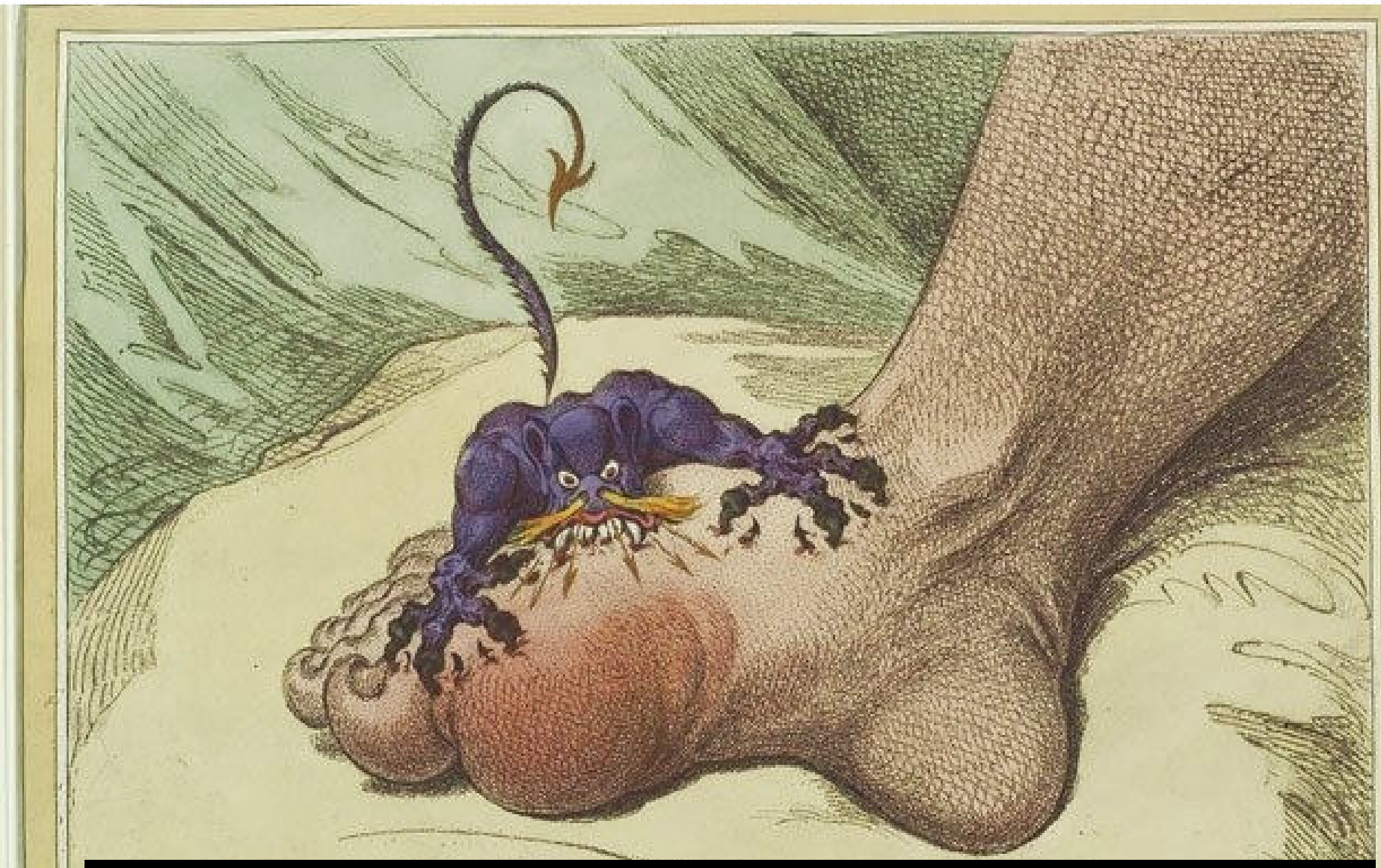
Principal differences between metabolism of Purines and Pyrimidines

Character	Purines De Novo Synthesis	Pyrimidines De Novo Synthesis
Number Of Steps Involved	11 Steps	6 Steps
Precursors Of Ring	Amino acids :Asp Gly and Gln N10FormylTHF CO2	Amino acids :Asp and Gln CO2
Major Portion Of Ring provided by	Glycine	Aspartate

Character	Purines De Novo Synthesis	Pyrimidines De Novo Synthesis
Acquisition of Ribose-Phosphate	In Starting Steps	In End Steps
Formation of N-Glycosidic bond	In 1st step of their biosynthesis (PRPP is the 1st Substrate)	a heterocyclic ring is formed first, then it reacts with PRPP
products of degradation	**Uric acid** (poor solubility in H_2O) NH_3	CO_2, NH_3, β-Amino Isobutyrate and βAla (soluble in H_2O)

Character	Purines De Novo Synthesis	Pyrimidines De Novo Synthesis
Number Of ATPs Involved	6 ATPs	2ATPs
Nucleotide Produced in End	IMP	UMP
Ring Closure At	6 and 11 steps	3rd Step

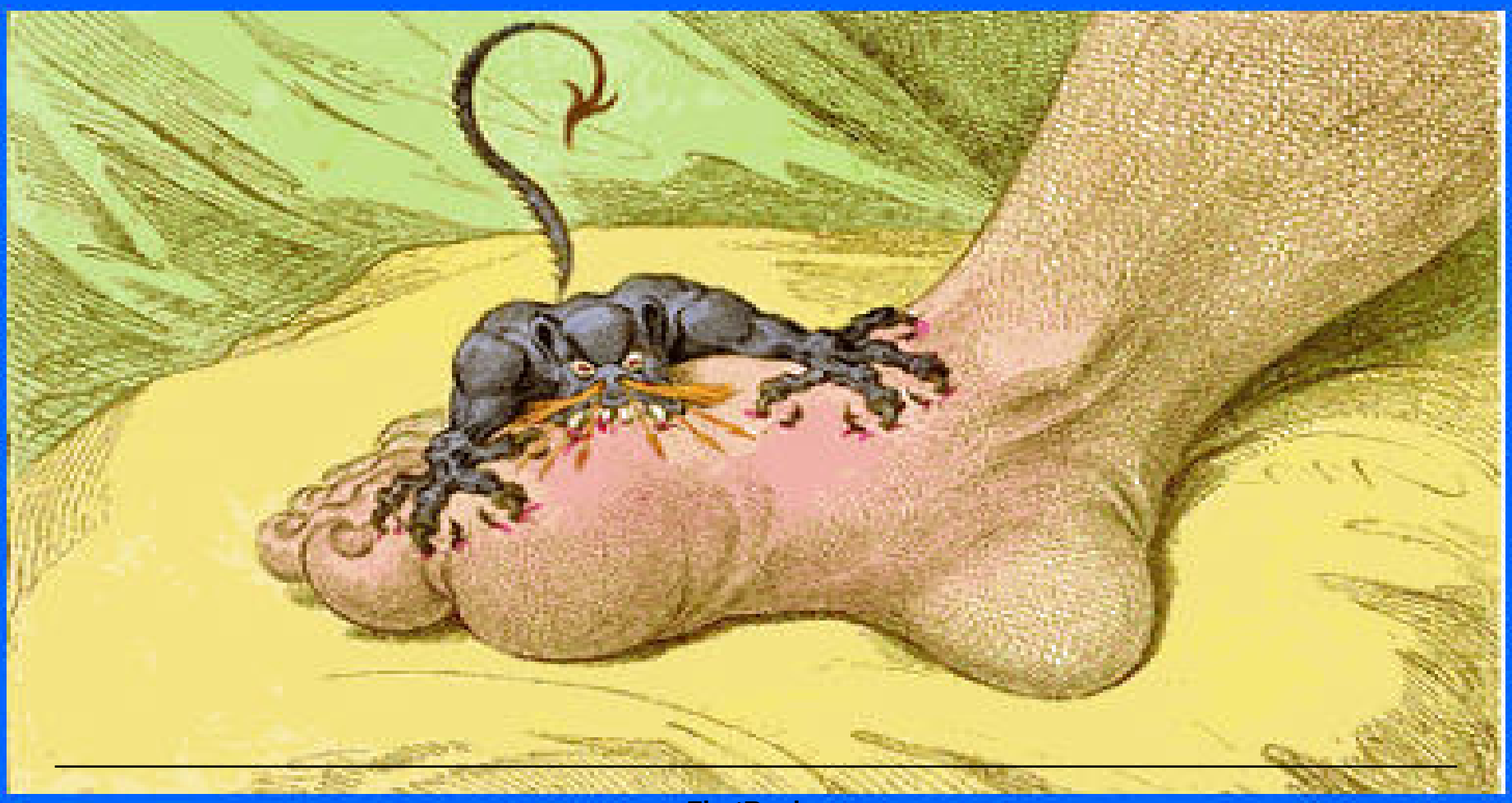
Disorders Of Nucleic Acid Metabolism



Disorders of Purine Nucleotides Metabolism

Gout

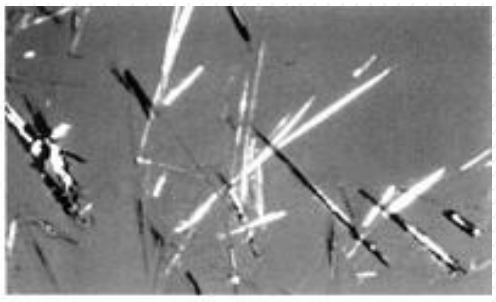
Gouty Arthritis



- Gout derived from Latin Word: **GUTTA**
- Meaning '**A drop of liquid**'

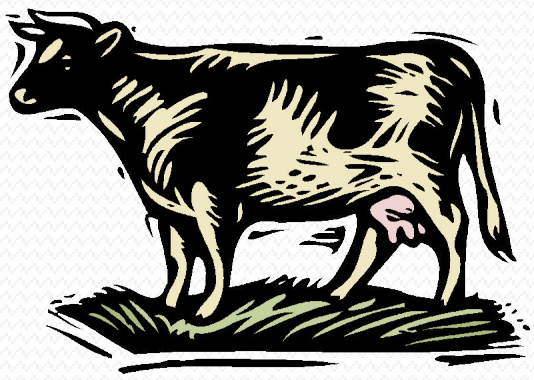
- Gout is a common **metabolic disorder of Purine metabolism characterized by :**
  - **Persistent Hyperuricemia**
  - **Hyperuricaciduria and**
  - **Joint pain**

GOUT


Sodium Urate Crystals

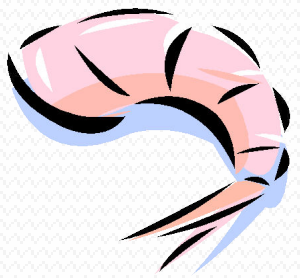
- **Gout**, is a disease of the joints, usually in males, caused by an **elevated concentration of uric acid** in the blood and tissues.
- The **joints** **become inflamed, painful, and arthritic**, owing to the **abnormal deposition of crystals of sodium urate**.
- The **kidneys** are also affected, because excess uric acid is deposited in the kidney tubules.

Gout:"Disease of Kings"



- Rich foods have a higher concentration of Nucleoproteins.
- This could cause major problems for a person afflicted with Gout.

- **ORGAN MEATS**
- **WILD GAME**
- **SEAFOOD**
- **LENTILS**
- **PEAS**
- **ASPARAGUS**
- **YEAST**
- **BEER**



Types and Causes Of Gout

Types Of Gout

- **Primary Gout** (Genetic Cause)
- **Secondary Gout**

Basic Cause Of Gout

- **Hyperuricemia**

- Over Production Of Uric acid
- Under Excretion Of Uric acid

Primary Gout

- Primary Gout is an inherited sex linked recessive disorder.
- Affecting more Males.

Causes Of Primary Gout

- Basic cause of **primary Gout is genetic cause.**
- **It** has **Enzyme** defects concerned with:
  - **Over Production** Of Purine Nucleotides **than the functional use**.
  - **Over catabolism** of Purine Nucleotides
  - **Results in Hyperuricemia**

5 Enzyme Defects Causing Primary Gout

1. PRPP Synthetase

(Increased Activity))

2. PRPP Glutamyl Amido Transferase

(Increased Activity)

3. HGPRTase

(Decreased Activity)

4. Glucose 6 Phosphatase

(Decreased Activity)

5. Glutathione Reductase

(Decreased Activity)

- The defect of above 5 Enzymes in primary Gout
- Directly or indirectly increases the Denovo Biosynthesis of Purine nucleotides.

- There is overproduction of Purine Nucleotides more than their functional use
- Which further catabolizes them to produce increased Uric acid levels (Hyperuricemia)

Secondary Gout

- It is an **acquired cause**:
- In some pathological states where there is **abnormal and excessive breakdown of cells** releases Nucleic acids and Nucleotides.
- Whose catabolism produces increased Uric acid levels (Hyperuricemia)

Conditions Of Secondary Gout

- Leukemia
- Lymphomas
- Polycythemia
- Treatment Of Large Tumors
- Traumatic Conditions
- Radiation Injury

Renal Gout

- Type of Gout caused due to **insufficiency of Renal System**.
- Where there is reduced excretion of Uric acid through Urine.
- Retention of the Uric acid in blood leading to Hyperuricemia.

Conditions Of Renal Gout

- Renal Failure
- Use of Thiazide diuretics
- Metabolic Acidosis
  - **Ketoacidosis and Lacticacidosis affects the excretion of Uric acid through Urine.**

Incidence Of Gout

- Primary Gout accounts for 90% of cases
- Affects primarily middle aged men

Risk Factors of Gout

- Obesity (High BMI)
- Hypertension (HTN)
- Use of Thiazide diuretics
- Diet high in meat & seafood
- Excess Alcohol use
  - Highest with Beer

- **Diet high in Purines may trigger an attack in a susceptible persons.**

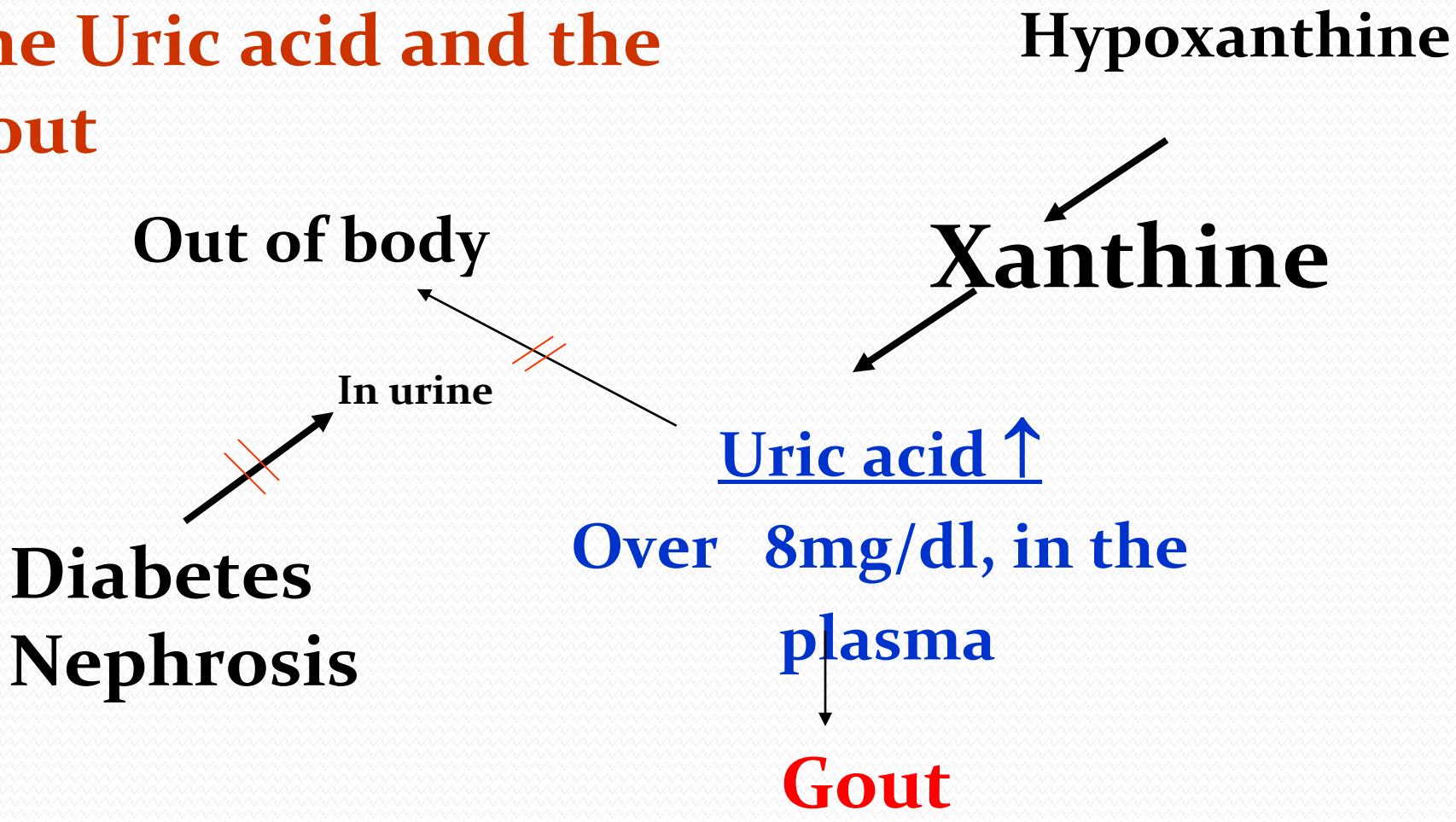
RISK FACTORS OF GOUT

- Male Gender
- Postmenopausal female
- Older Persons
- Pharmaceuticals: Cyclosporine

Pathophysiology Of Gout

- **Uric acid** is **NPN compound**
- **Waste end product** of Purine metabolism
- **Excreted** by the kidneys through **urine**.

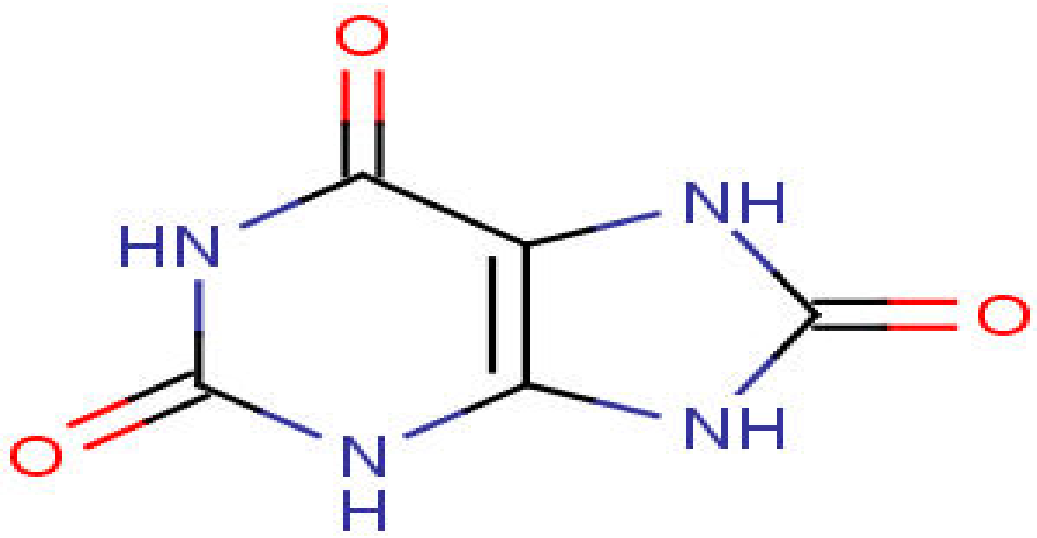
The Uric acid and the Gout



Urate crystallization in joints, soft tissue, cartilage and kidney

- The normal serum Uric acid level in adults is 2-7 mg%
- 0.5-1 g of uric acid is formed daily in the organism.
- In Gout the serum Uric acid levels rises above 8 mg%.
- Uric acid in miscible pool of Gout patients is increased up to 2000-4000 mg% (normally 1200mg%).

- Uric acid is poorly soluble in water.



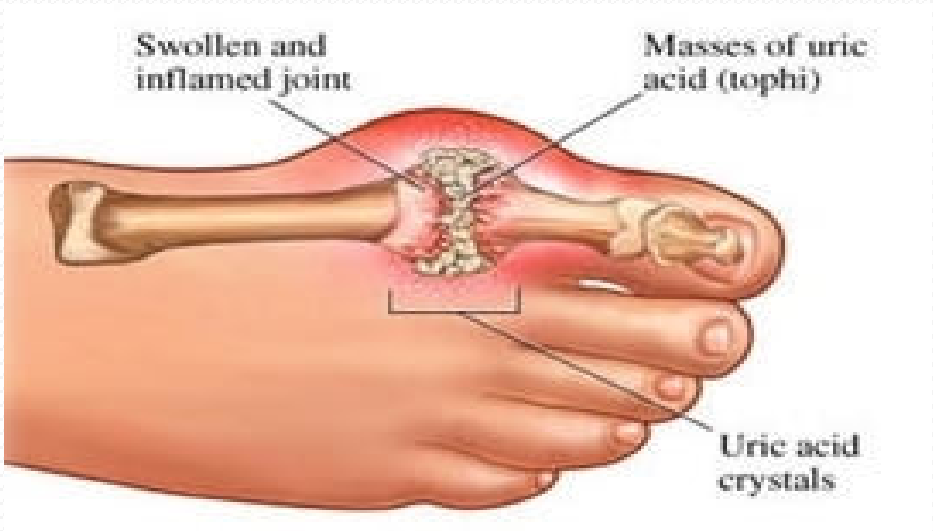
- The **increased Uric acid** levels
- **Decreases the solubility** of Uric acid and
- Get **crystallized** to form Mono Sodium Urate Crystals.

- The **Mono Sodium Urate Crystals** get **deposited** in the **synovial spaces of joints**

- In periarticular ,articular and extra articular tissues to **form Tophi (Hard Mass/ Swelling)**

- Deposition of Urate crystals in synovial spaces **affects the movements of joints.**
- Leads to **pain , inflammation, stiffness and redness of joints** known as **Gouty Arthritis.**

- Deposits of sodium urate crystals in articular, periarticular, and subcutaneous tissues in Gout



HYPERURICEMIA & GOUT

- Hyperuricemia caused by
  - Overproduction of Urate
  - Under excretion of Urate
- No Gout w/o crystal deposition

THE GOUT CASCADE

Urate

Over production **Under excretion**

Hyperuricemia

- **Silent Tissue Deposition** — **Gout**
- **Renal Manifestations**
- **Associated CV events & mortality**

Clinical Manifestations Of Gouty Arthritis

- Onset of Gout is usually nocturnal, with sudden swelling and excruciating pain
- May have low grade fever

- Usually subsides within 2-10 days
- Joints are normal, with no symptoms between attacks

- **Gouty arthritis** in one or more joints (but less than four)
- **Great /big toe joint (Metatarsophalangeal)** most common first manifestation **(Monoarticular)**

- Other joints may be the foot, ankle, knee, or wrist (Polyarticular)

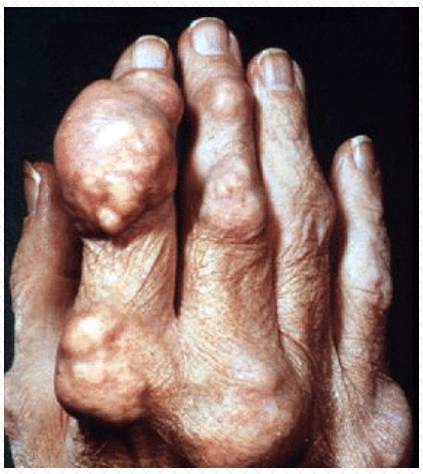
- Joints become tender /stiff & cyanotic
- Recurrent attacks of pain and swelling of the joints.

- Constant recurring vermicular movements of hands and feet.
- Involuntary and Jerky movements
- Spasticity
- Mental Retardation

- Urate crystals trigger a local immune-mediated inflammatory reaction.
- With one of the key proteins in the inflammatory cascade being interleukin 1β.
- Causing inflammation of the area.

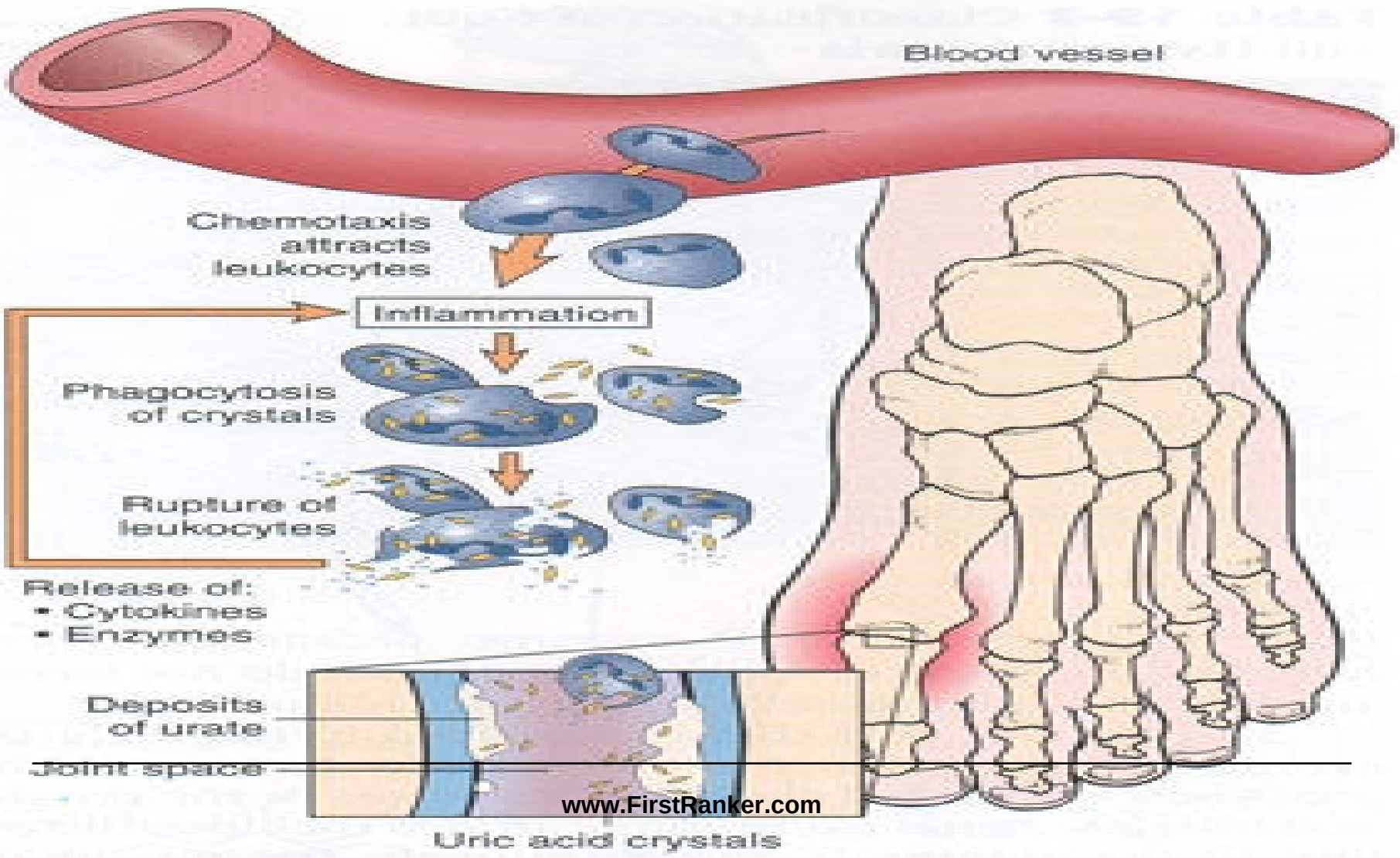
Gouty Arthritis
Main Symptoms

- **Joint Pain**
  - Affects one or more joints : hip, knee, ankle, foot, shoulder, elbow,wrist, hand, or other joints
  - Great toe, ankle and knee are most common
- **Swelling of Joint**
  - Stiffness
  - Warm and red
  - Possible fever
- **Tophi/Skin Lump**
  - which may drain chalky material



- **Gouty Arthritis may be precipitated by :**
  - Trauma
  - Surgery
  - Alcohol ingestion
  - Infection

Gouty Arthritis



Stages of Gout

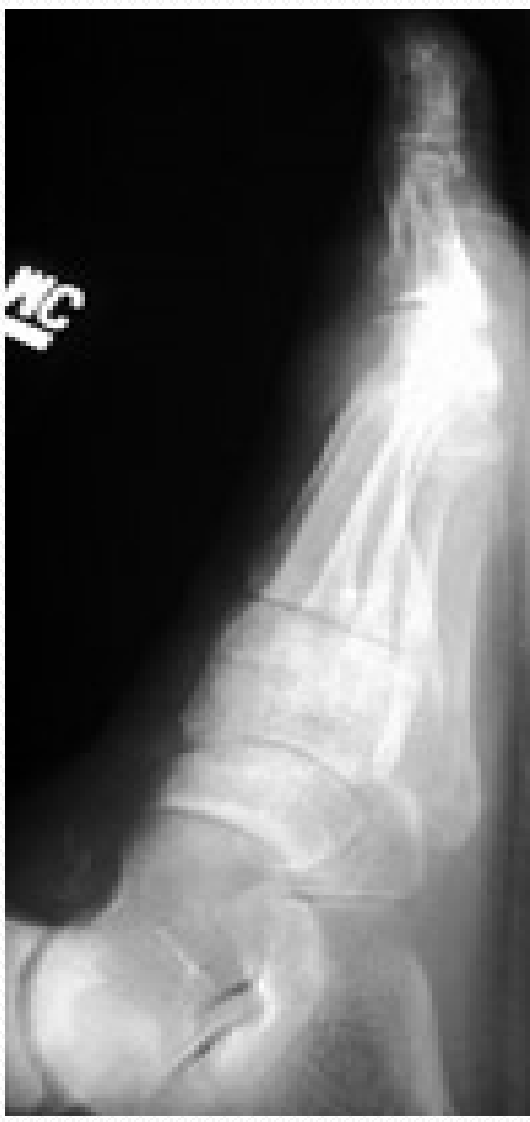
- Asymptomatic Hyperuricemia
- Acute Flares of Crystallization
- Intervals between flares/Intercritical Stage
- Advanced/Chronic Gout
- Complications of Gout

Stage 1
Asymptomatic Hyperuricemia.

- Very initial stage of Gout
- When serum Urate concentration is greater than 8 mg/dL,
- Urate crystals may start to deposit in the joints.
- No evidence that treatment is required.

ASYMPTOMATIC

- A meaning without indicates that there are **no symptoms associated**
- Patient will be unaware of what is happening
- Gout can only be determined with the help of a physician



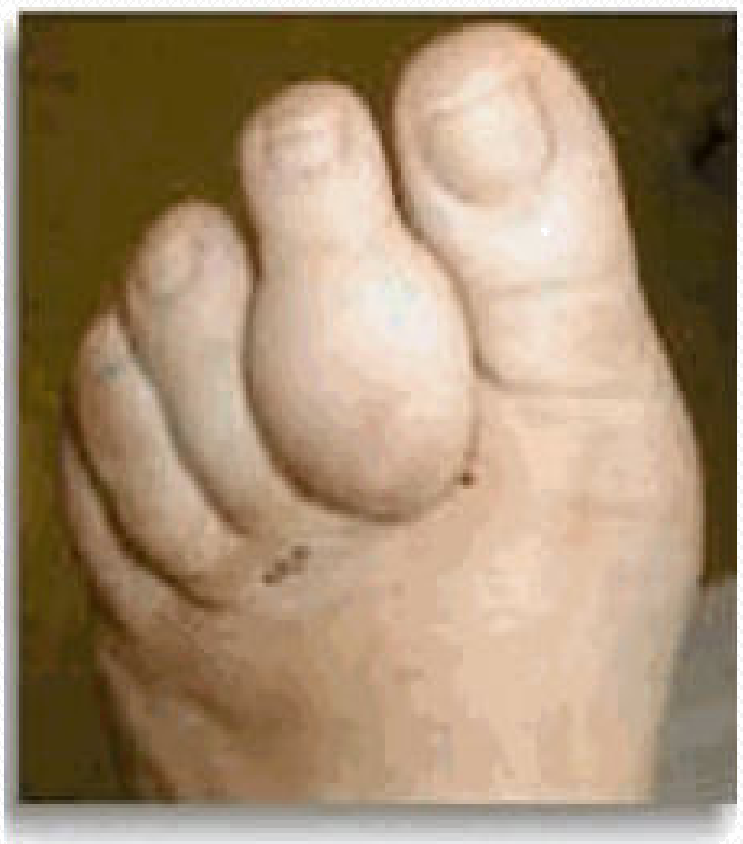
Stage 2
Acute Gout

- If **sufficient urate deposits around joints**, and if the local environment or some trauma triggers
- The release of crystals into the joint space, **an inflammatory response occurs.**
- These **flares** can be self resolving but are **likely to recur**.

ACUTE GOUTY FLARES

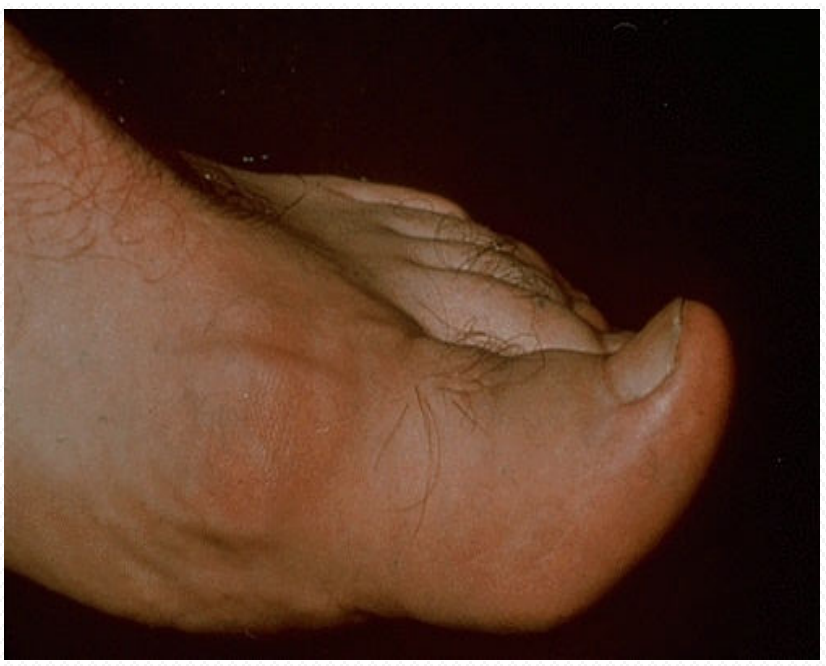
- **Abrupt onset of severe joint inflammation, often nocturnal**
- **Warmth, swelling, erythema, & pain; Possibly fever**
- If untreated get **resolves in 3-10 days**
- 90% 1st attacks are **monoarticular**
- 50% are **podagra** (Gout of big Toe)

ACUTE GOUT



SITES OF ACUTE FLARES

- 90% of gout patients eventually **have podagra : 1st MTP joint**

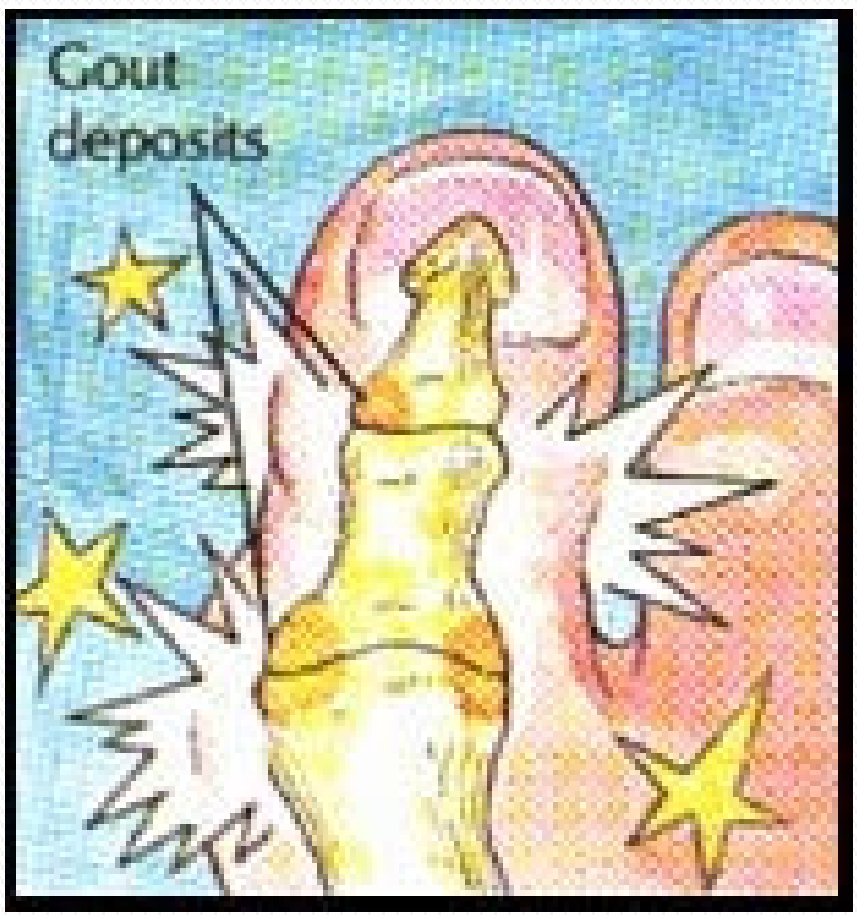


Stage 3
Intercritical periods

- These are the **intervals between attacks.**
- During these periods, **crystals may still be present at a low level in the synovial tissue and fluid, resulting in future attacks.**

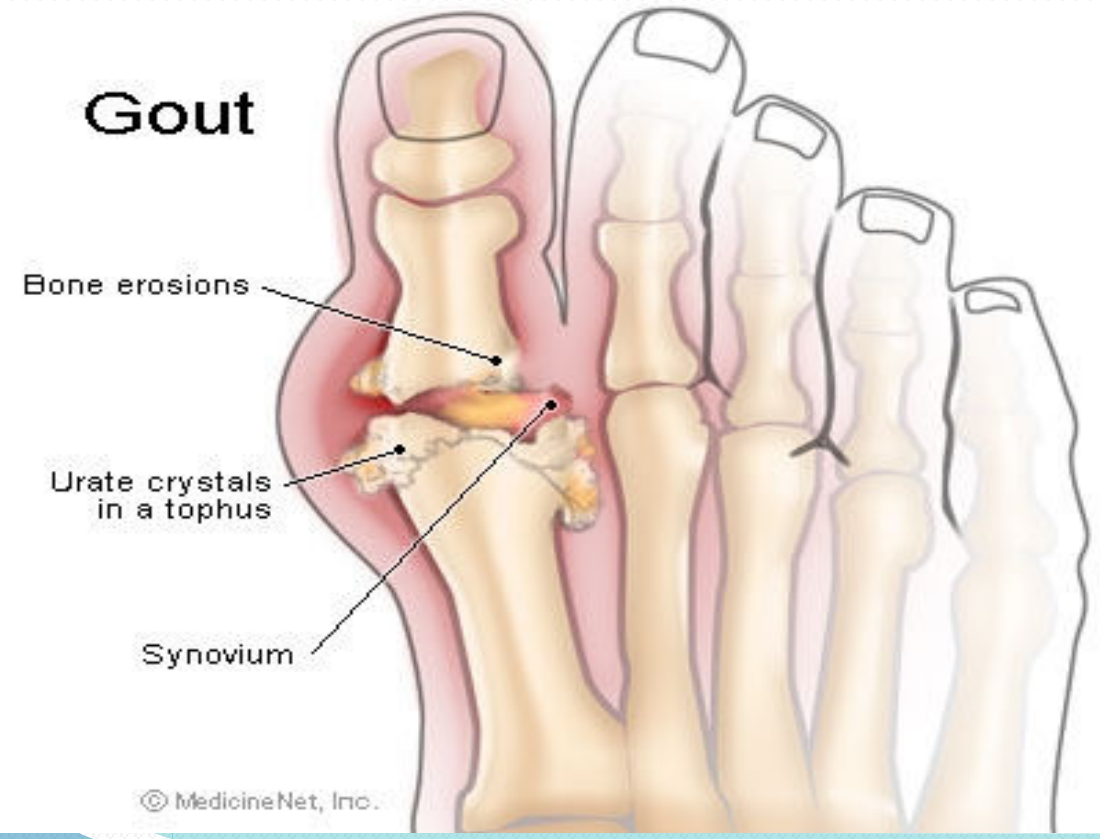
INTERCRITICAL

- More concentration of uric acid crystals
- Typically no need for drug intervention at the time.



FLARE INTERVALS

- Silent tissue deposition & Hidden Damage

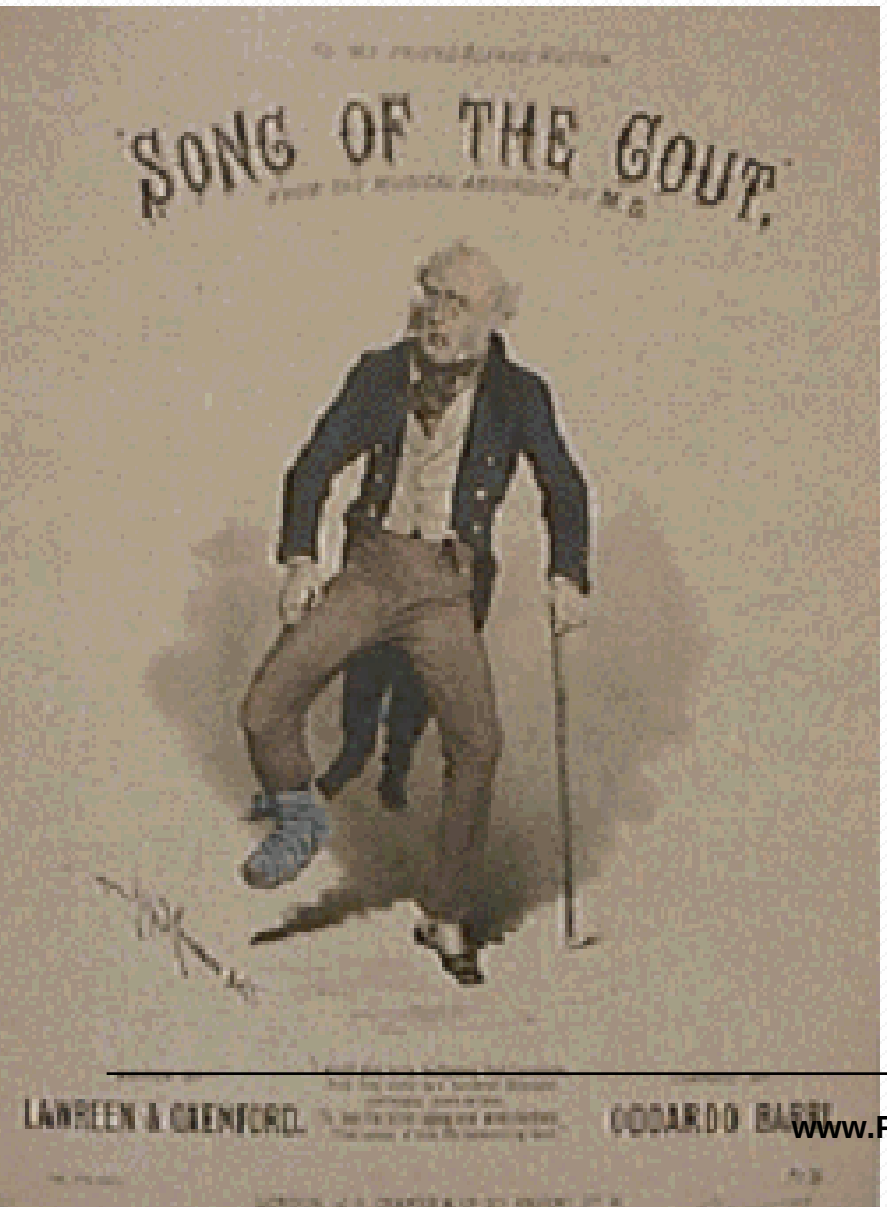


Stage 4 Advanced /Chronic Gout.

- If crystal deposits continue to accumulate, patients may develop **chronically stiff, swollen joints and tophi.**

- This **advanced stage of Gout** is relatively uncommon generally avoidable with therapy.

CHRONIC GOUT



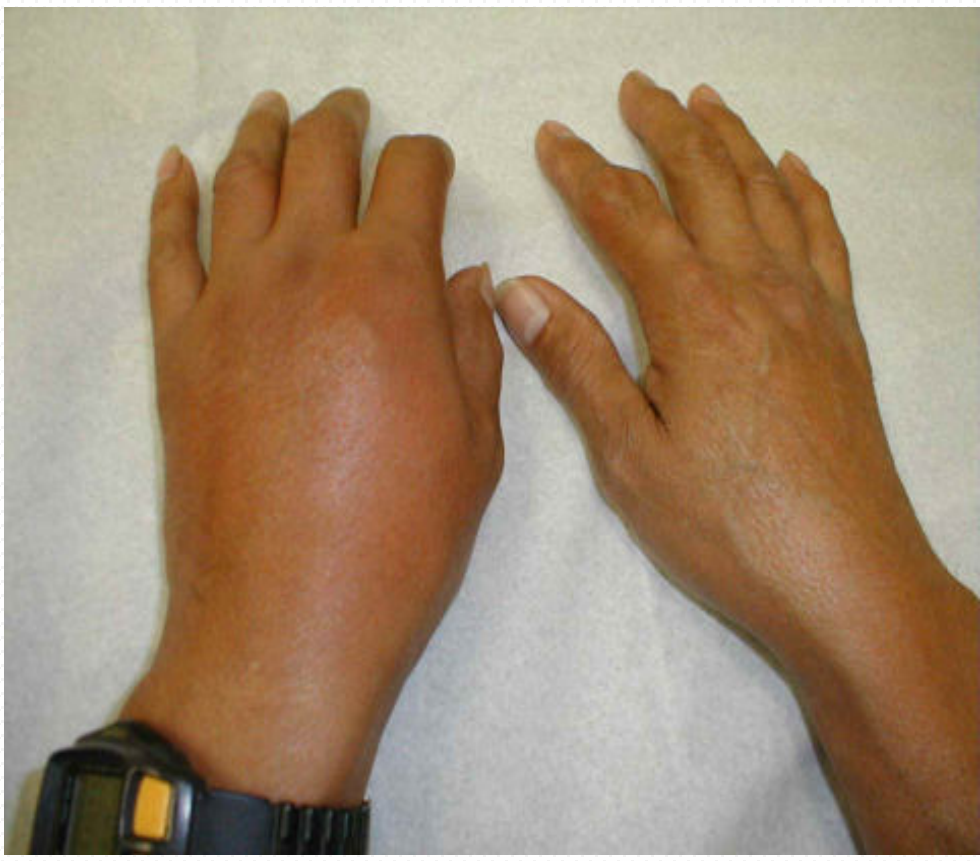
- Continuous or persistent over a long period of time
- Treatment required
- Not easily or quickly resolved

IN ADVANCED GOUT

- Chronic Arthritis
- X-ray Changes noted
- Tophi Developed
- Acute Flares continues

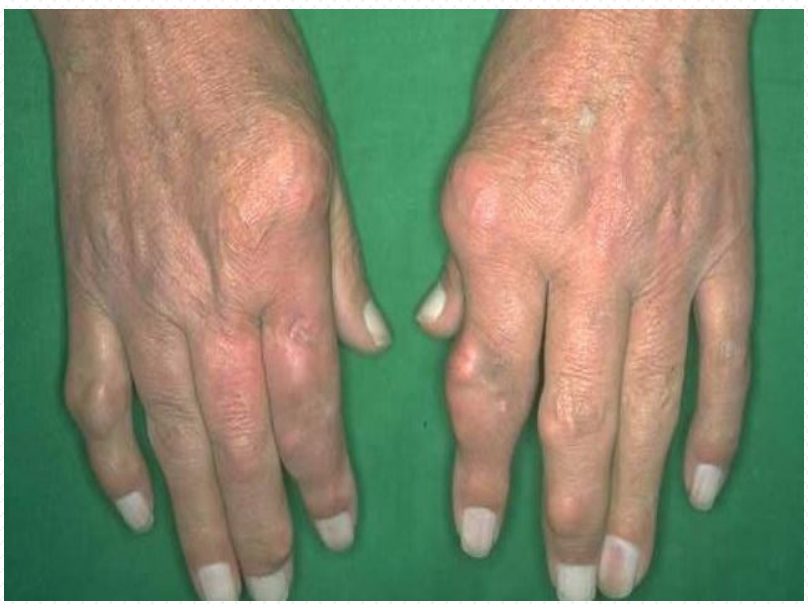
ADVANCED GOUT

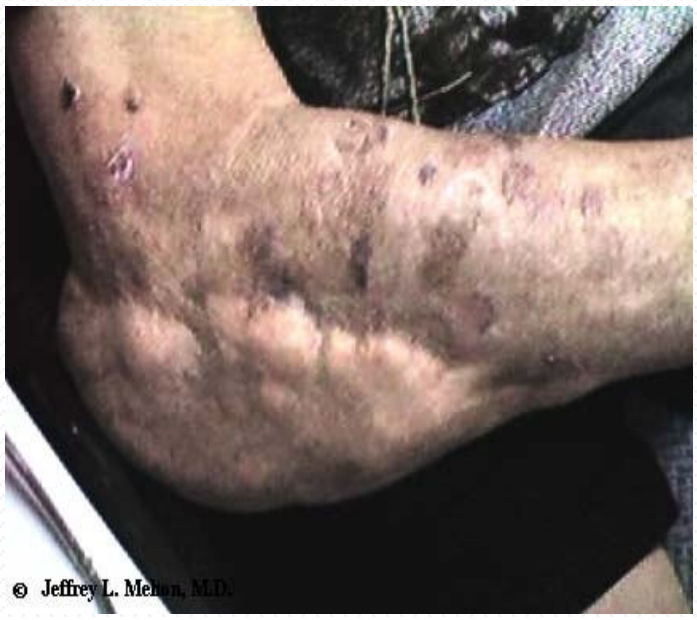
- Chronic Arthritis
- Polyarticular acute flares with upper extremities more involved



Sites

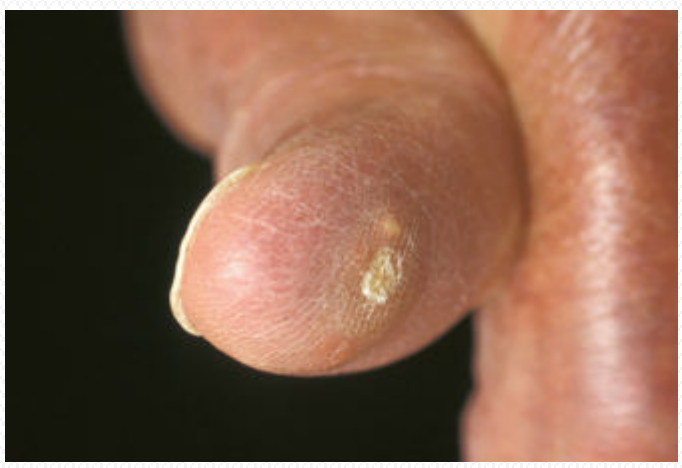
- Can occur in other joints, bursa & tendons

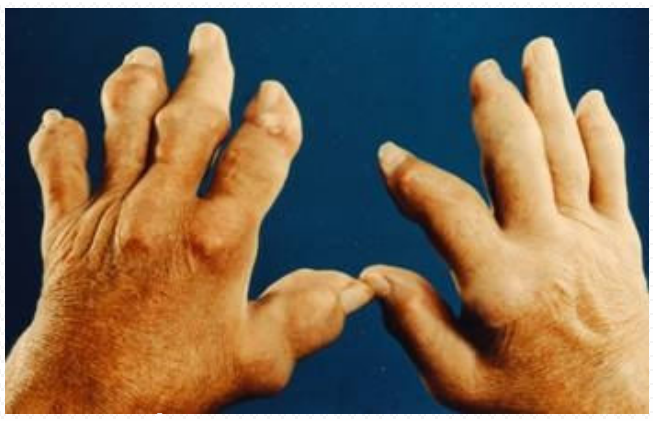


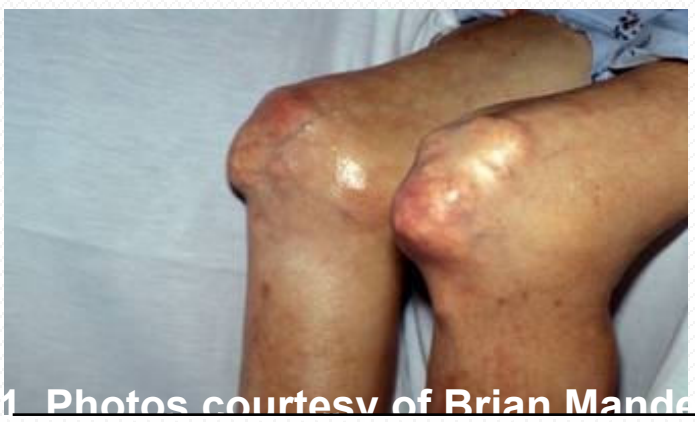


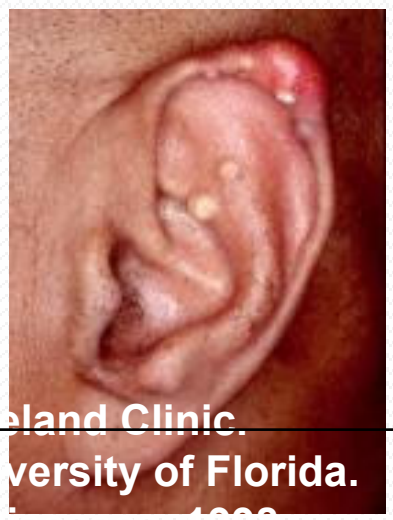
Advanced Gout

Clinically Apparent Tophi

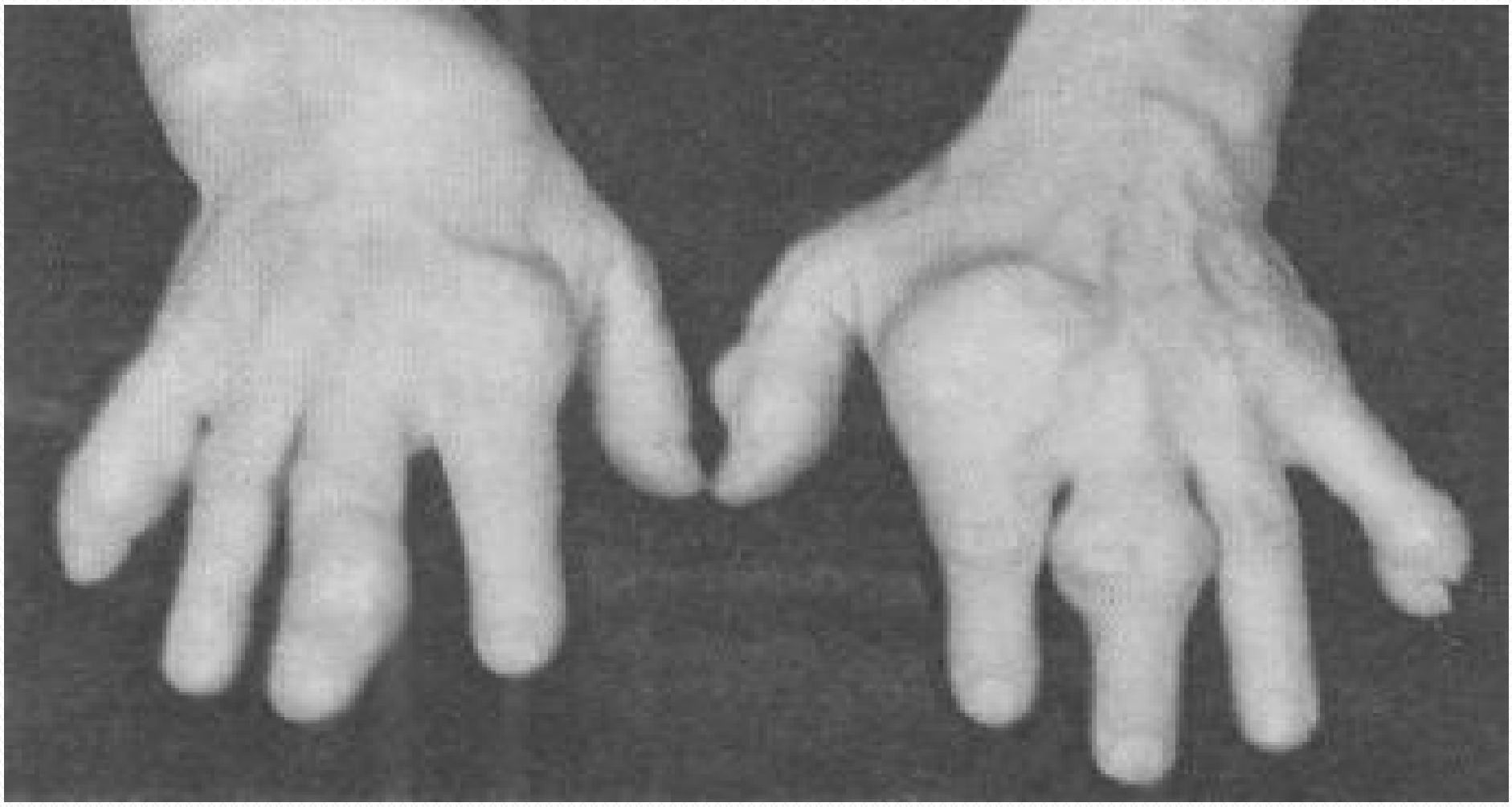


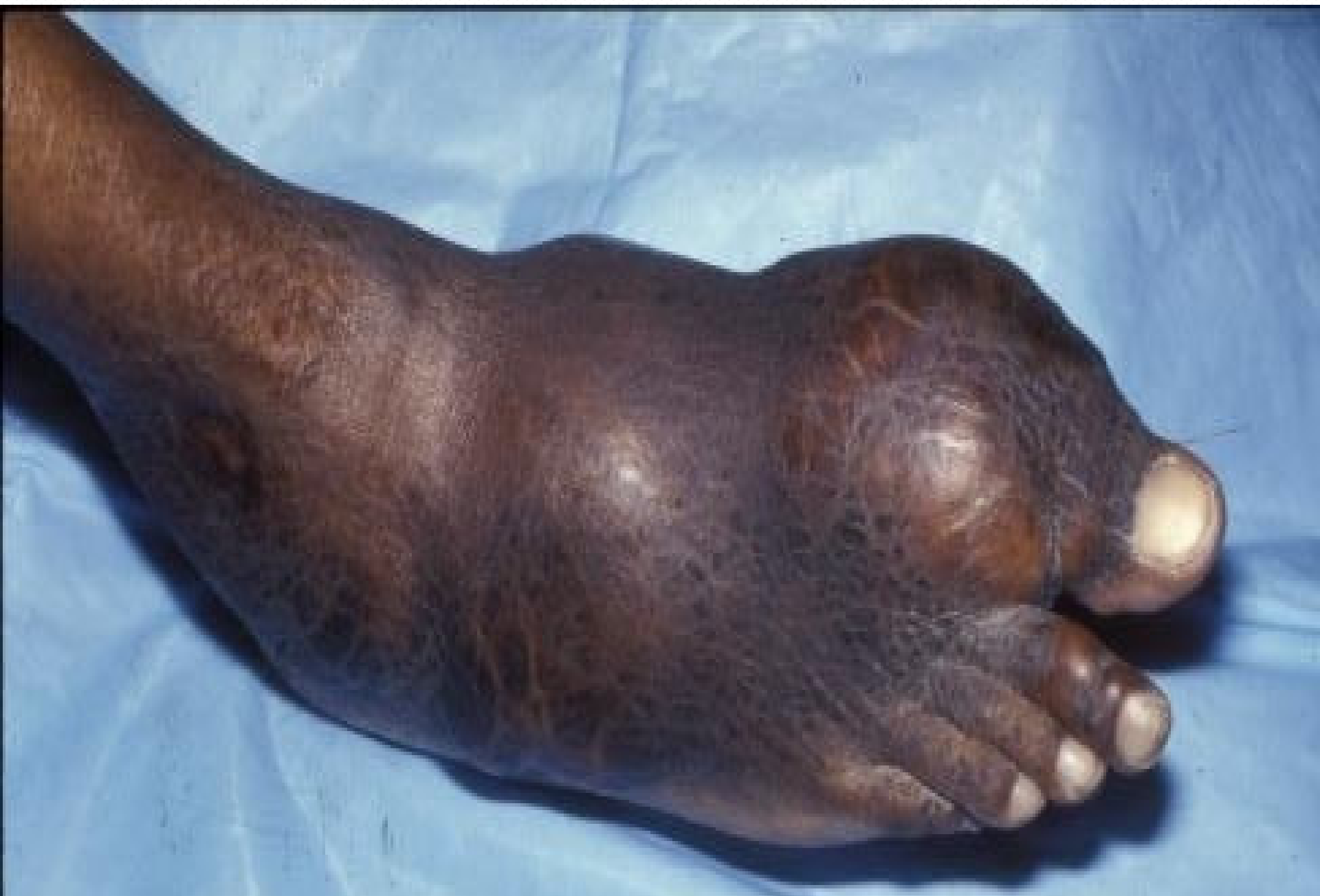






1. Photos courtesy of Brian Mande[illegible]eland Clinic.
2. Photos courtesy of [illegible]versity of Florida.
3. ACR Clinical Slide Collection [illegible]



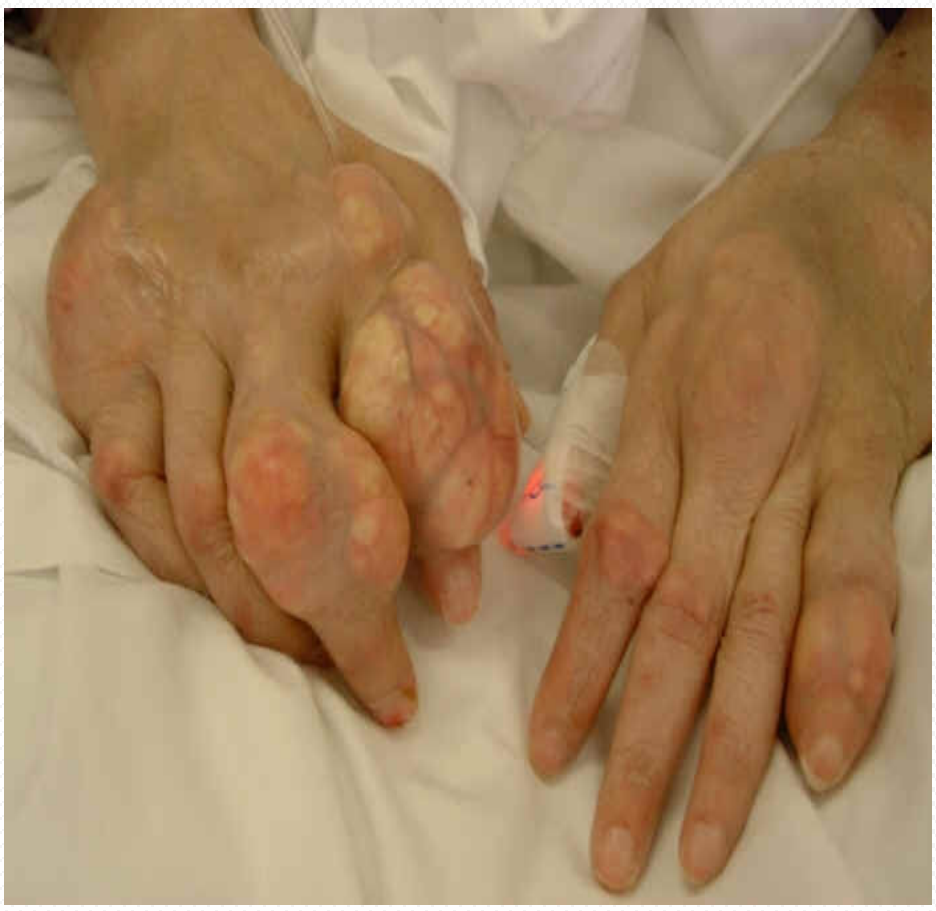


Acute Intermittent Gout

- Initial episode usually follows decades of asymptomatic hyperuricemia
- Characterized by intense pain and inflammation (warmth, swelling, erythema)
- Usually begins as monoarticular involvement with first MTP joint

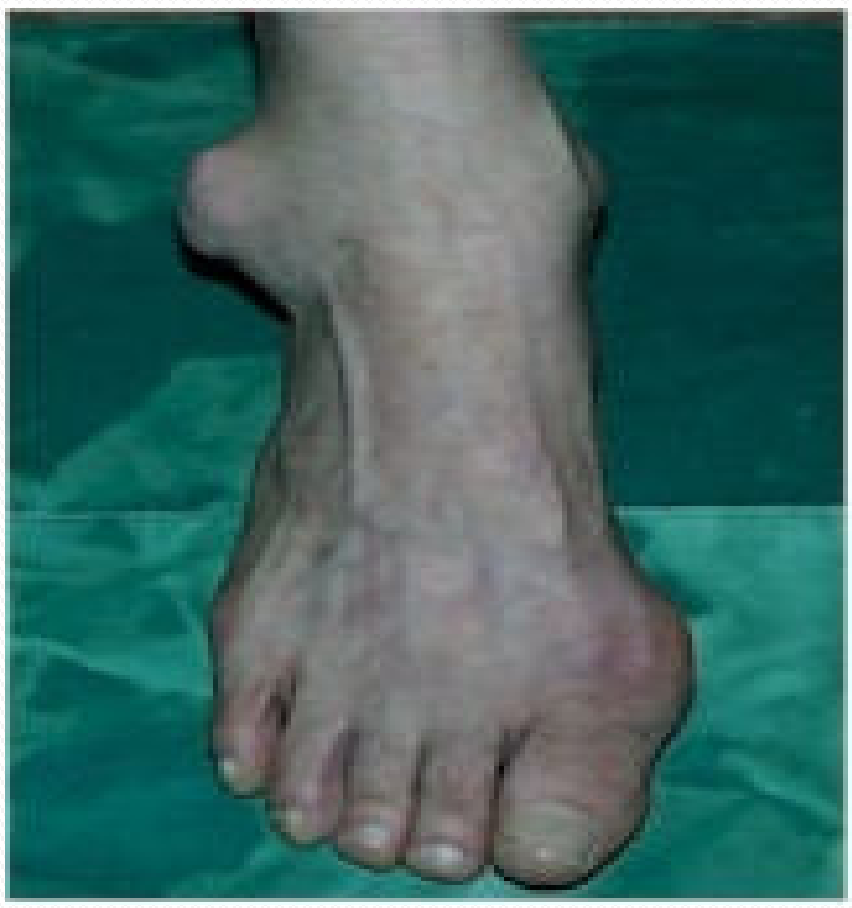
TOPHI

- Solid urate deposits in tissues



TOPHI

- Irregular & destructive



Complications Of Gout

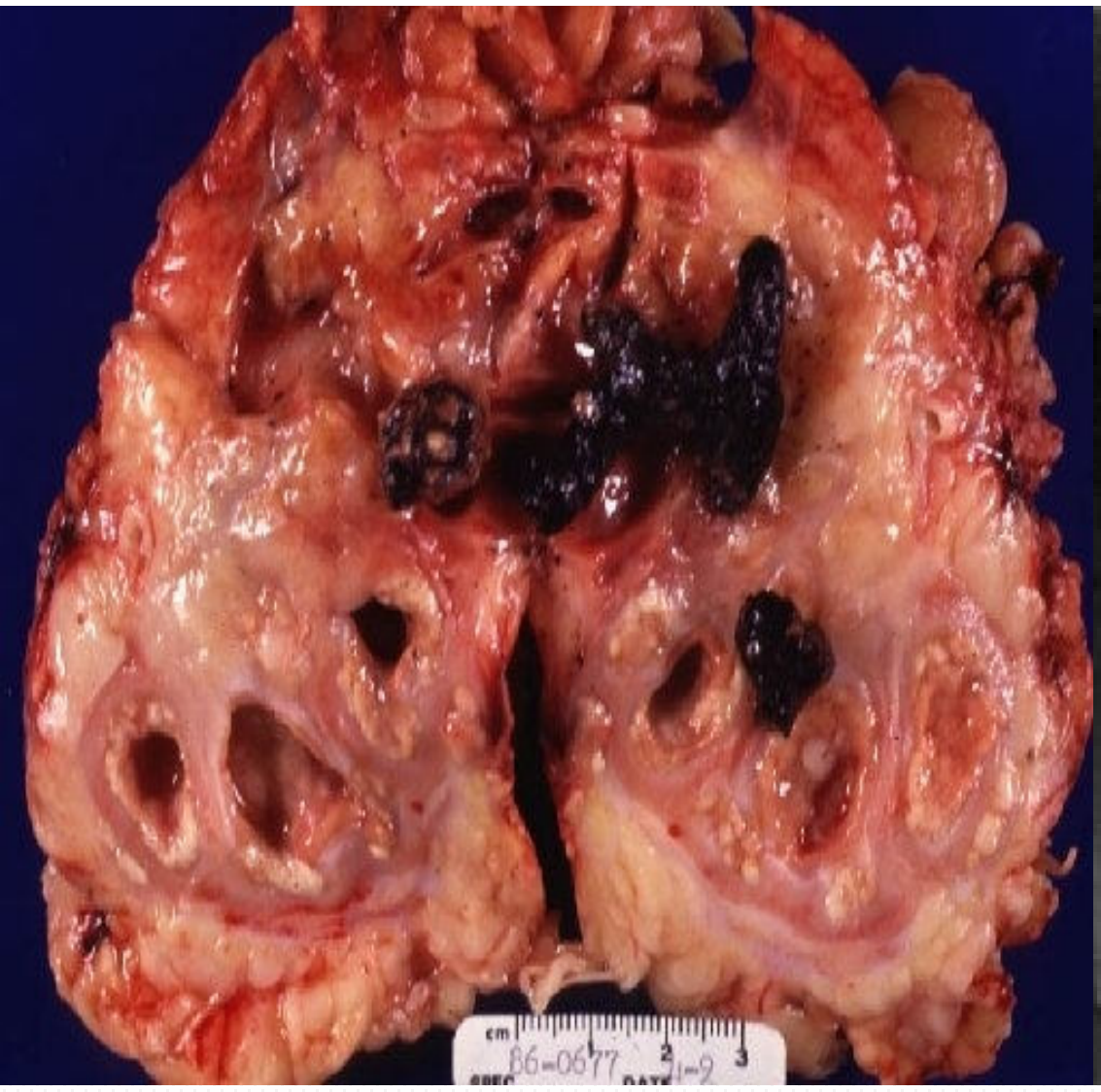
- Joint deformity
- Osteoarthritis

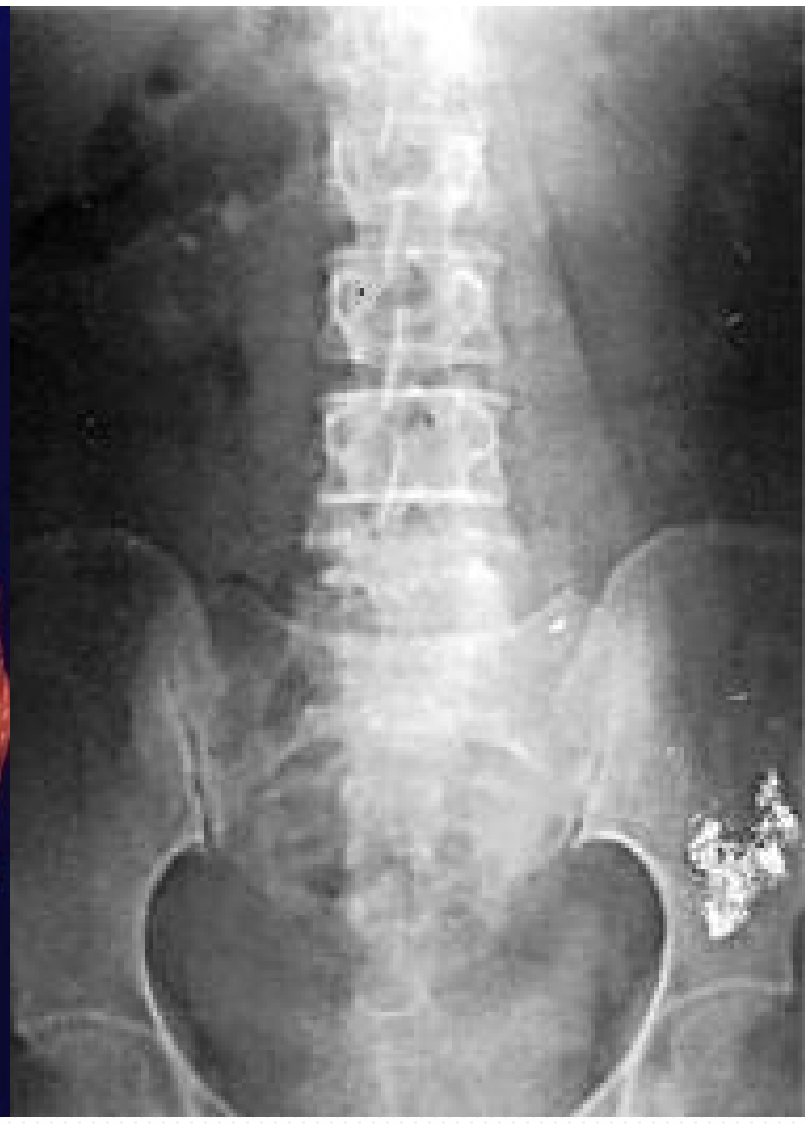
- Tophi may produce draining sinuses that may become infected.

- Renal stones, pyelonephritis, obstructive renal disease.

Assessment for Gout Complications

- Formation of kidney stones
- Hypertriglyceridemia
- Hypertension





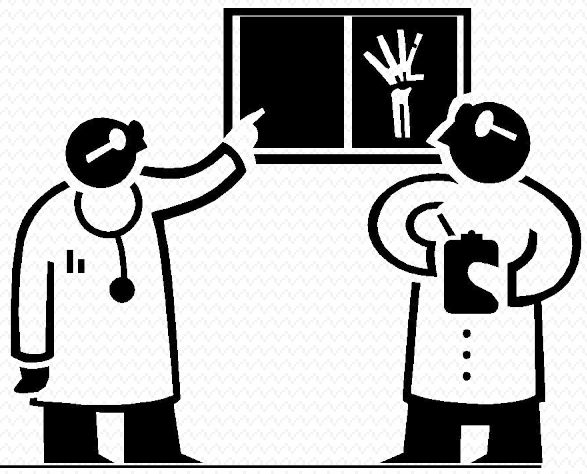
Gout: Kidney Stones

Diagnosis Of Gout

- **History taking & physical examination**
- Family history of Gout
- Clinical symptoms alone are sufficient to make accurate diagnosis in most cases
- **Performing Diagnostic studies** may help in knowing **the stage and progression of Gout.**

Gout Diagnosing Studies

- Examination of joint fluid (**Arthrocentesis** extraction of joint fluid).
- X-rays of joint
- Blood Examination



Diagnostic Profile

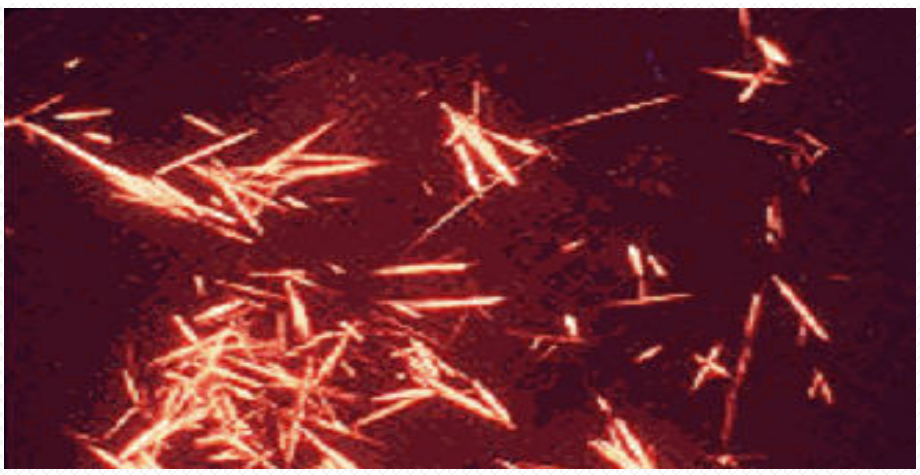
- Serum Uric acid levels usually elevated.
- 24 hour urine Uric acid levels increased.
- WBC Count elevated during acute attacks.
- ESR (elevated)
- Synovial fluid aspiration contains Urate crystals
- X-rays appear normal in early stages; Tophi appear as eroded areas of bone

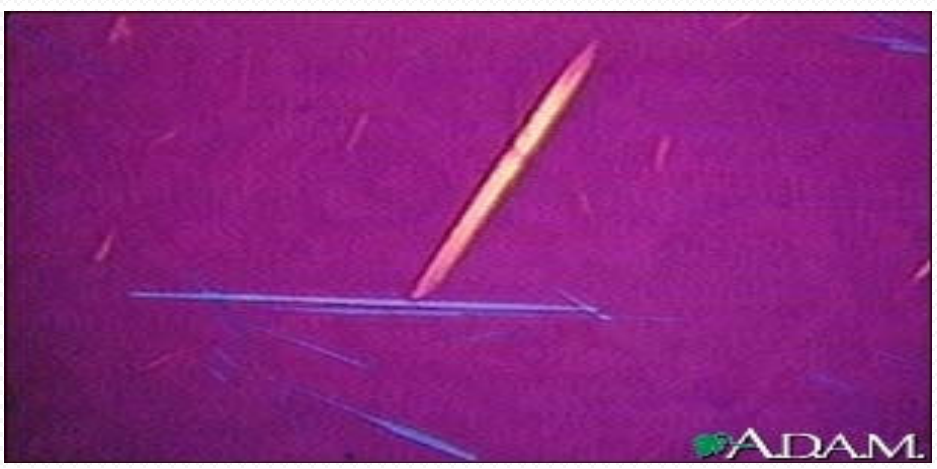
SYNOVIAL FLUID ANALYSIS (Polarized Light Microscopy)

- **Considered as the Gold standard**
- Urate Crystals are intracellular during attacks
- Needle & rod shaped Urate crystals
- With strong negative birefringence

SYNOVIAL FLUID

Microcopy Of Urate Crystals





Treatment Of Gout

Palliative Treatment

- Bed rest : No much movements of joints.
- Bed rest : With a position for comfort

Treatment and Nursing Care

- Joint immobilization and protect joint from pressure
- Local application of heat or cold around the joint area.

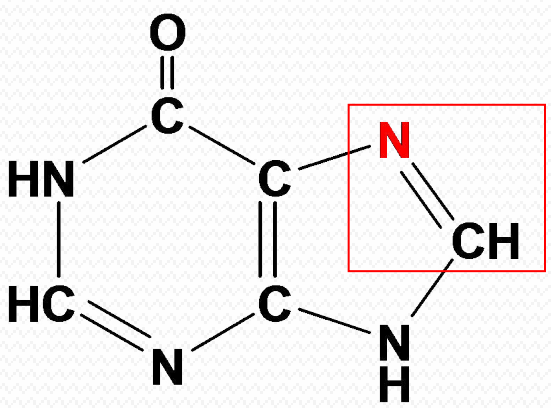
- Restrict intake of diet rich in Purine content.
- Restrict Alcohol consumption
- Avoid dehydration
- Drink lots of Water

Specific Treatment

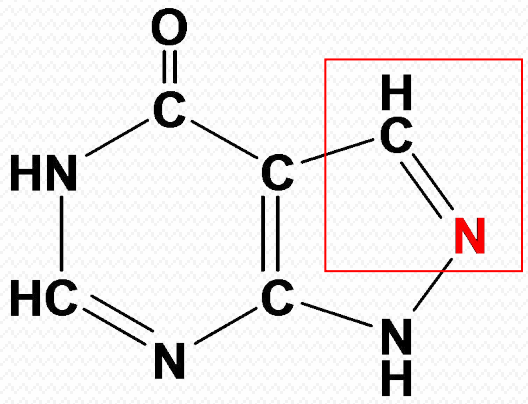
- **Allopurinol (Zyloprim) is a drug of choice for Treatment of Gouty arthritis.**
- Allopurinol is a structural analog of Hypoxanthine.

- **Allopurinol** is a Competitive inhibitor of Enzyme **Xanthine Oxidase.**
- Prevents conversion of Hypoxanthine and Xanthine to Uric acid.
- **Prevents accumulation of Uric acid** and its crystallization and deposition.
- **Hypoxanthine and Xanthine** are **more water soluble form** and readily excreted out.
- **Allopurinol is transformed to Alloxanthine** and excreted out.

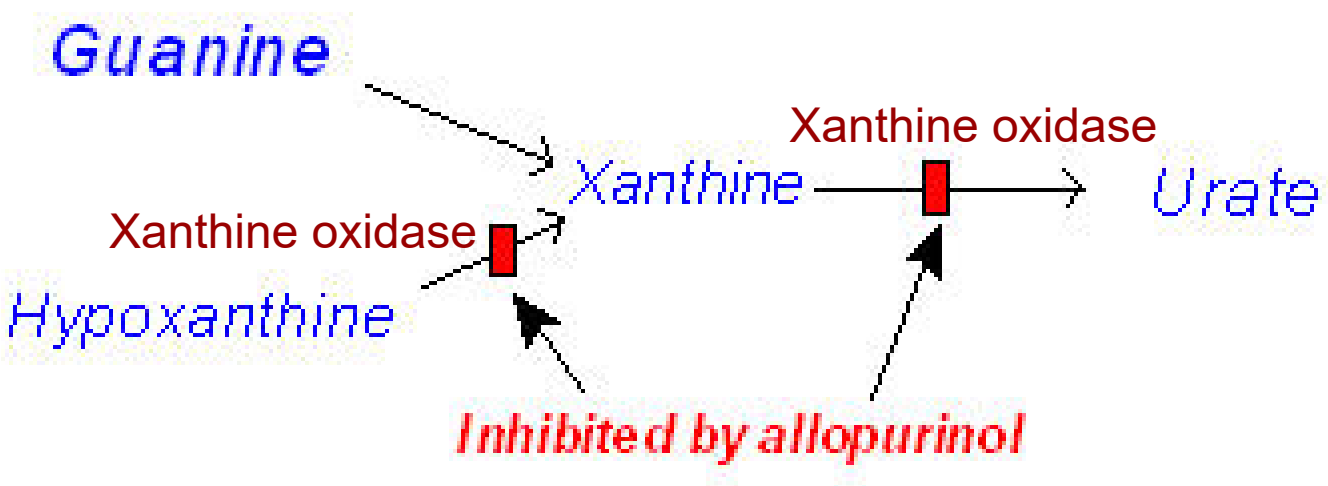
Allopurinol – a Suicide inhibitor used to treat Gout



Hypoxanthine



Allopurinol



- **Allopurinol Dosage:**
- **Initial Stages**
- 100-200 mg/day
- **For Maintenance**
- 200-600 mg/day

- **Administration of Uricosuric drugs :**
- Which **decreases renal reabsorption of Uric acid from renal tubules**
- Thereby **increasing Uric acid excretion.**
- Example : **Probenecid Salicylates**.

- **Using Anti inflammatory agents** to arrest pain and inflammation in Gouty arthritis:
  - Colchicine
  - NSAIDS : Diclofenac
  - Ibufren
  - Proxivan

TREATMENT WITH

- **Colchicine**- reduces pain, swelling, and inflammation; of Gouty arthritis.
- Pain subsides within 12 hrs and relief occurs after 48 hrs.

Collaborative Care

Prevention of Acute Attacks

- **Colchicine combined with:**
  - **Allopurinol** (Zyloprim, Alloprim) – blocks production of uric acid
  - **Probenecid** (Benemid), sulfinpyrazone (Anturane) – inhibit tubular reabsorption of uric acid
  - **Febuxostat** (Uloric) – inhibits xanthine oxidase, recently shown to reduce serum uric acid levels

Collaborative Care

- Dietary measures
  - Weight reduction
  - Avoidance of Alcohol

- **Avoidance of Foods high in Purines**
  - **High Risk:** Yeast , Sardines, Calms Anchovies, Herring, Mussels, liver, kidney, goose, venison, meat soups, sweetbreads, beer & wine
  - **Moderate Risk:** Chicken, Salmon, Crab, Veal, Lobster , mutton, bacon, Pork, Turkey , beef, Ham

Collaborative Care

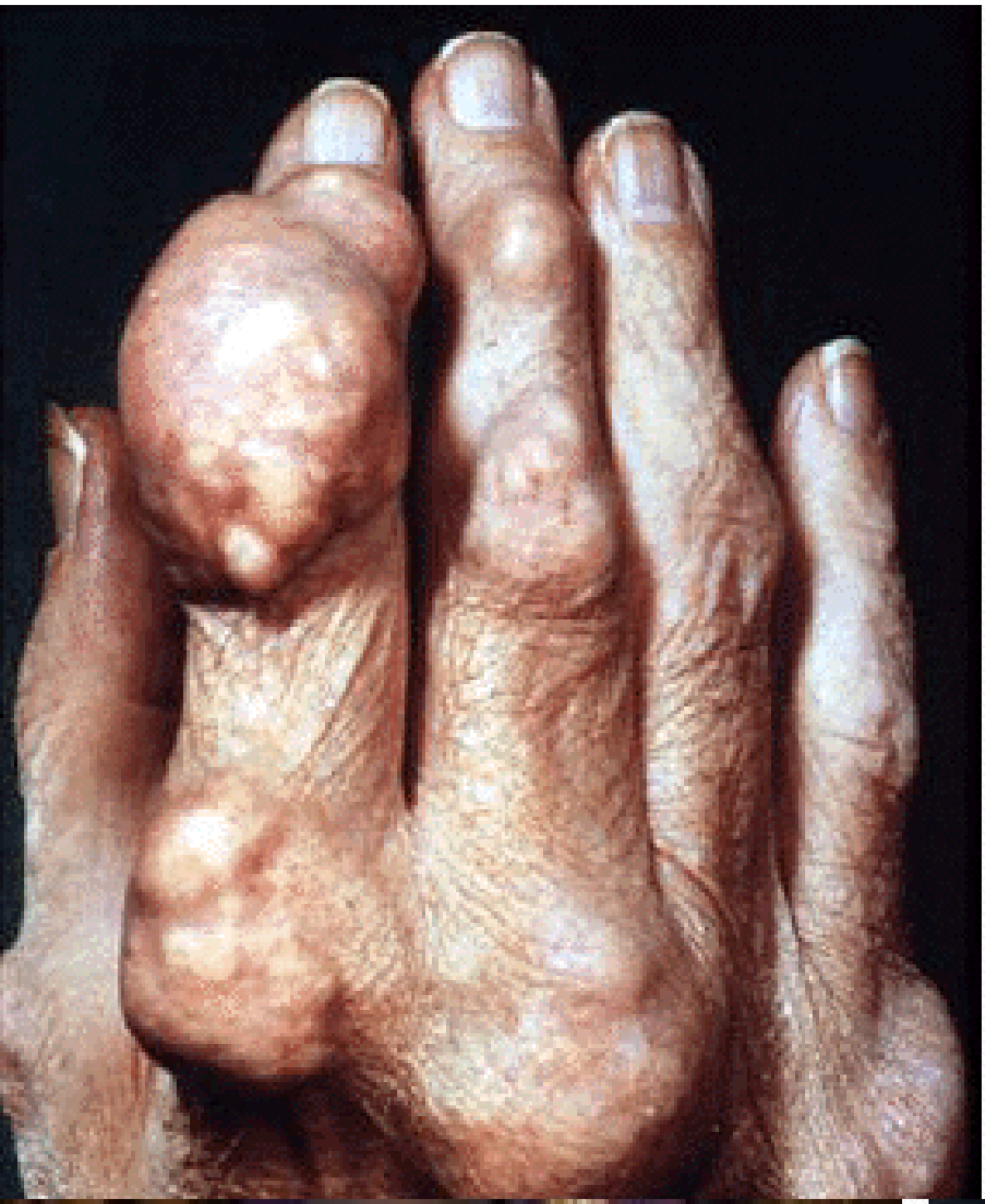
- **Prevention of Renal stones**
  - Increase fluid intake to maintain adequate urine output
  - Allopurinol
  - ACE inhibitor Losartin (Cozaar) – promotes urate Diuresis

Prevent Drugs That Promote Gout

Diuretics	**Leads to increased uric acid reabsorption**
Low-dose aspirin	**Over 6% increase in mean serum urate and 23% decrease in uric acid clearance**
Pyrazinamide **Ethambutol** **Niacin**	**Gout observed at higher incidence**

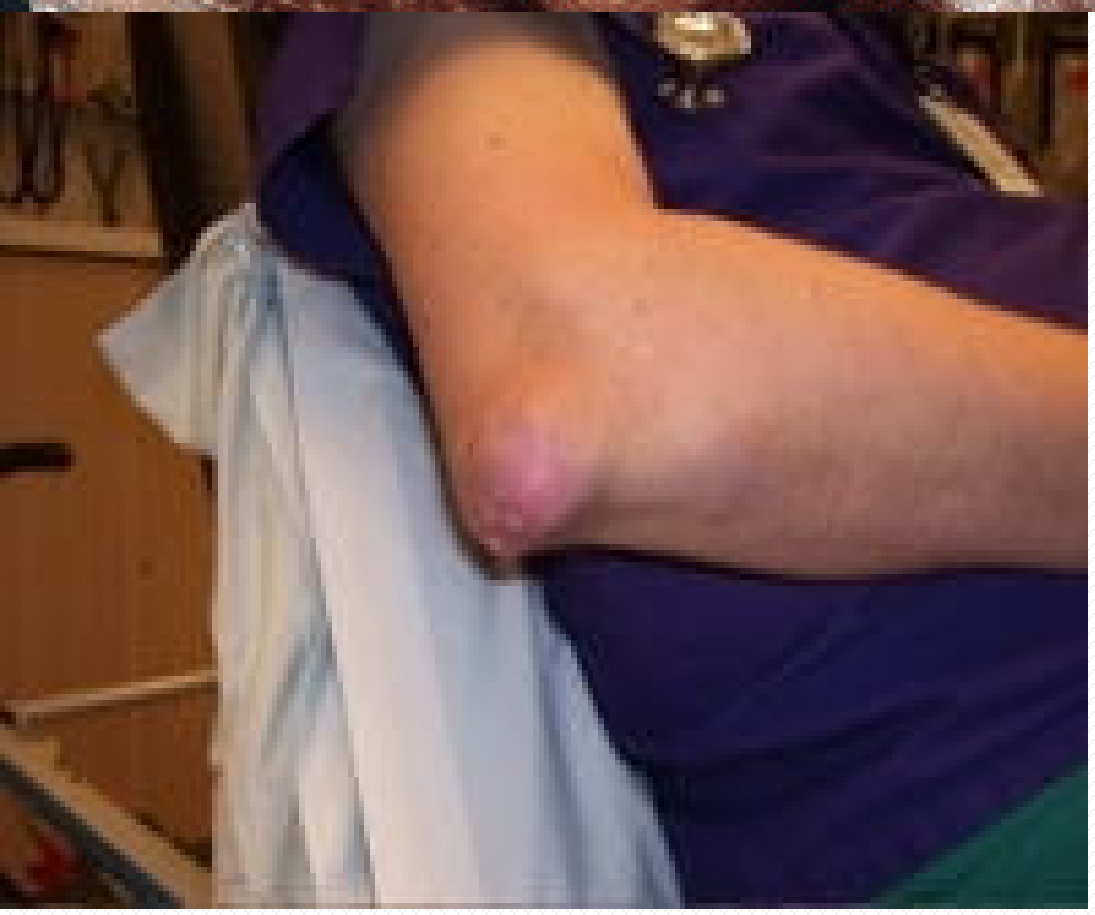
Factors Triggering Gouty Arthritis

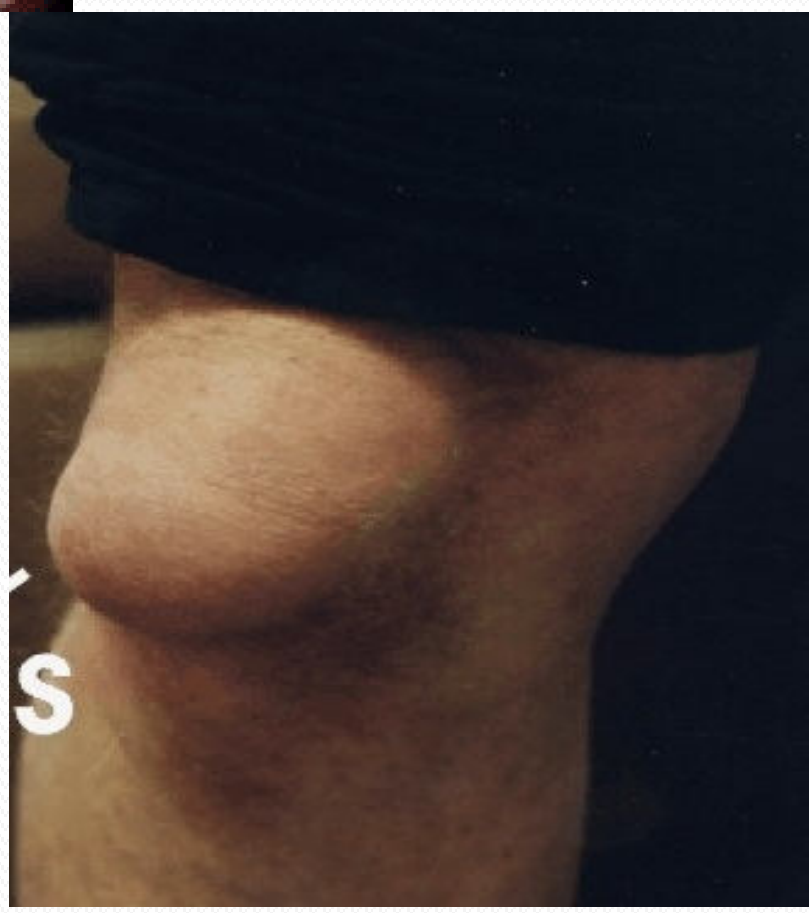
- Cool temperatures
- Rapid changes in uric acid level,
- Acidosis
- Articular hydration, and
- Extracellular Matrix Proteins, such as Proteoglycans, Collagens, and Condroitin Sulfate

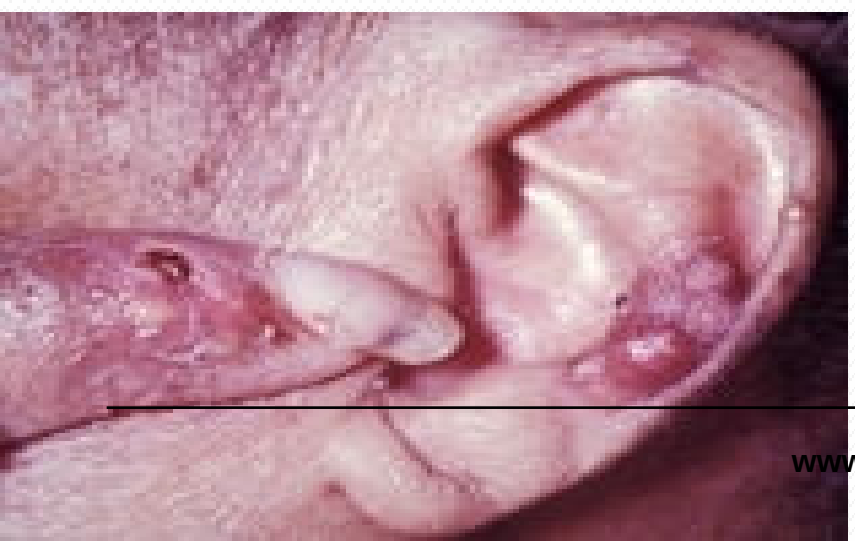


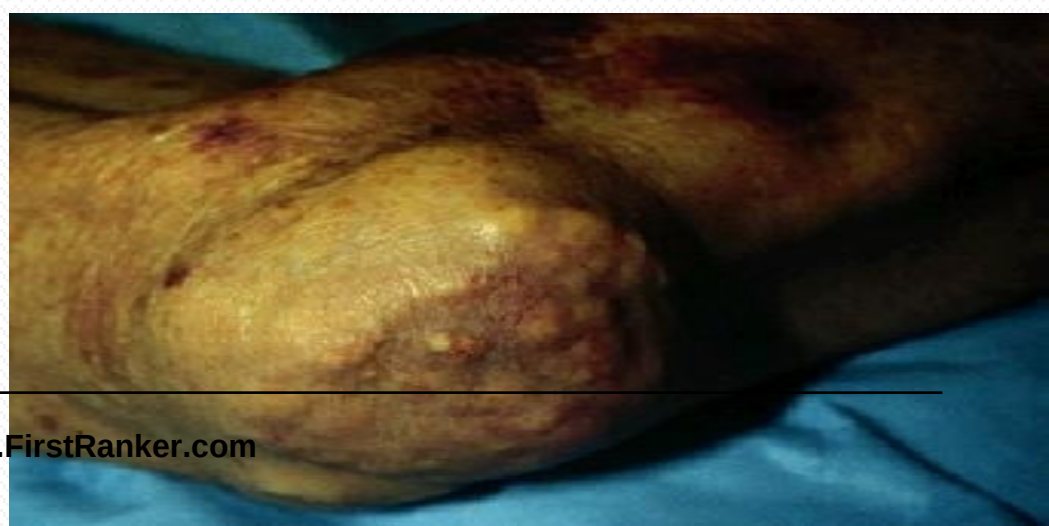
Gout: accumulation of Uric acid salts in joints

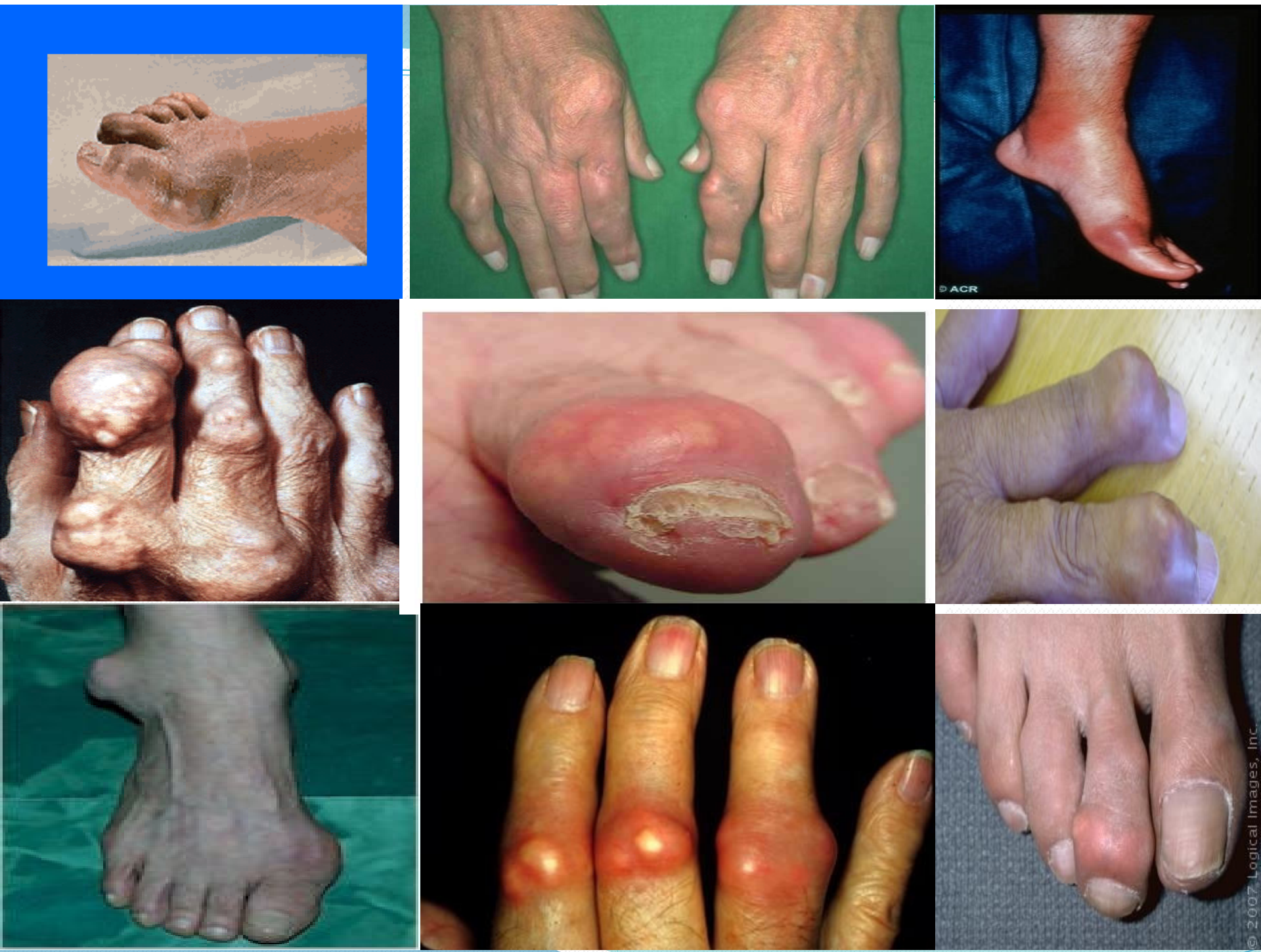
Gout: *Tophuses* – accumulation of uric acid salts in cartilages, under skin.











Lesch-Nyhan Syndrome

(LNS)

Lesch-Nyhan Syndrome(LNS)

- First described in 1964 by Michael **Lesch** and William L. **Nyhan**.

- LNS is a **genetic disorder**
- Affects **Salvage pathway** of Purine Metabolism.

- Caused due to **defect or lack in** the **HGPRTase an enzyme of Purine Salvage.**

- Severely **affects the Brain growth and development.**

- LNS is a **Sex-linked genetic recessive** disease that is **linked to the X chromosome.**

- Affects only Males

Biochemical Defect

HGPRTase role in the body

- Hypoxanthine-Guanine Phosphoribosyl Transferase is a **Purine Salvage enzyme** that
- Plays a key role in the recycling of the Purine bases, Hypoxanthine, and Guanine into Purine nucleotide pools through **Salvage pathway.**

Purine Bases are Catabolized To Uric Acid In LNS

- In **HGPRTase** deficiency the **free Purine** bases are **not recycled** through Salvage pathway
- Instead Purines are broken down and **excreted as Uric acid.**

- The rate of Purine synthesis is increased about 200-fold in LNS

- Lack of HGPRTase activity in Lesch-Nyhan Syndrome causes a buildup of PRPP.
- This PRPP activates the De novo biosynthesis of Purine nucleotides.

Loss of HGPRTase leads to

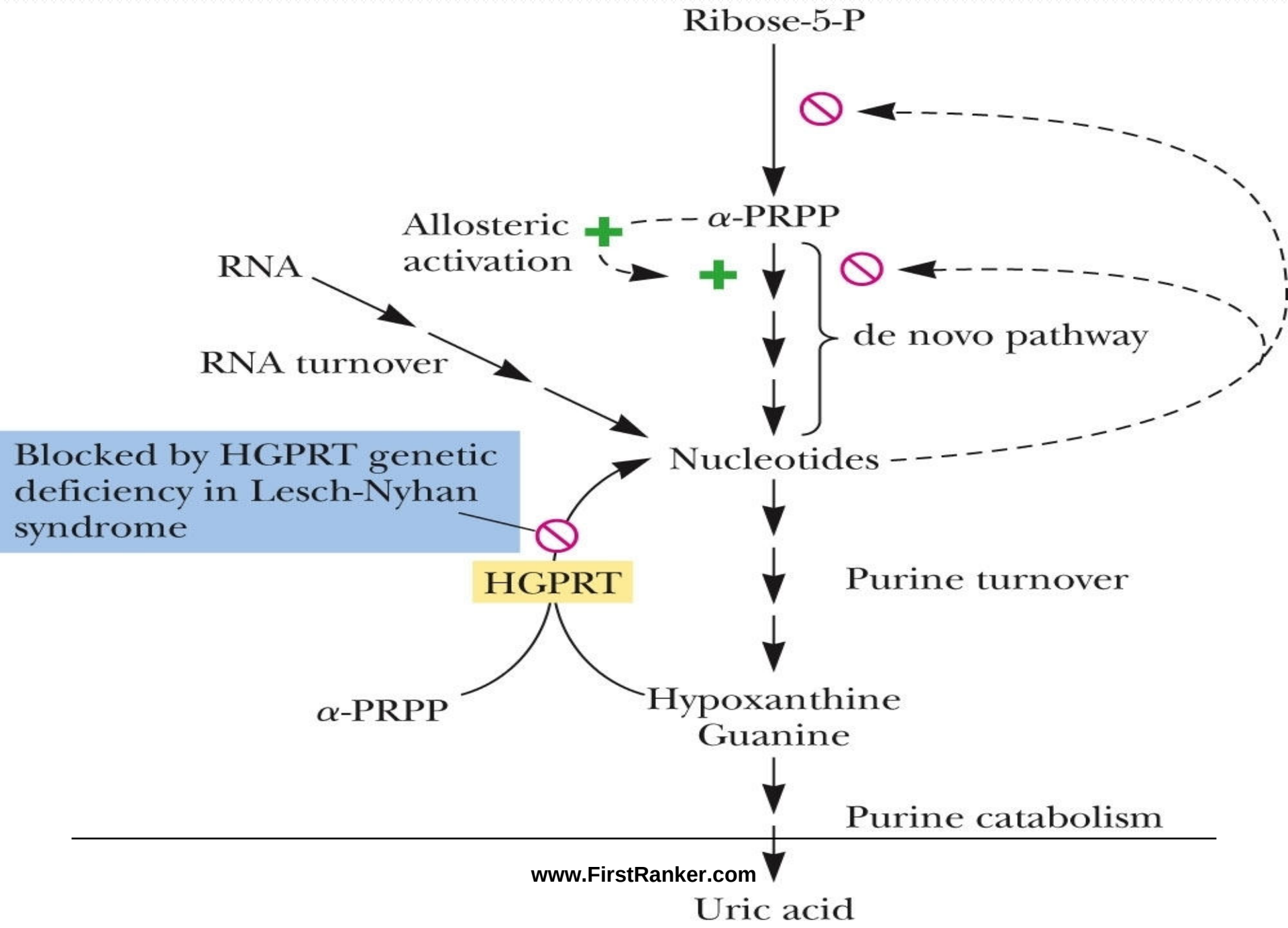
- **No use of PRPP** in the Salvage step
  - More availability of **unused PRPP**
  - **PRPP** allosterically stimulates **PRPP Synthetase** of De novo Purine synthesis.

- Purines synthesis is more than its functional use.
- Later these Purines are catabolized to end high Uric acid levels in blood and body.

hypoxan hine-guanine
phosphoribosyl transferase

Guanine + PRPP → Guanylate + PPi

Hypoxanthine + PRPP → Inosinate + PPi



LNS Is A Cause For Primary Gout

- LNS is characterized with **hyperuricemia** (Uric acid level rises) and suffers from Gout.
- In addition there are mental aberrations.
- LNS patients will self-mutilate **(self harming)** by biting lips and fingers off.

Hyperuricemia In LNS

- LNS is characterized with Hyperuricemia (high concentration of uric acid in the blood).
- A high concentration of uric acid, solidifies and deposits in the tissues **forming Gouty Tophi.**
- The deposits in the joints causes **inflammation and Gouty arthritis.**
- The kidneys excrete the extra uric acid, which increases the risk of **forming Urate stones.**

- The urate stones may pass as a sandy sludge or may **obstruct urine flow.**
- This **increases** the **risk for hematuria and urinary tract infections.**

Symptoms of LNS

*All of the following symptoms of **LNS** are a result of an **overproduction of Uric Acid***

- Swelling of the joints
- Urate crystal formations, which look like orange sand, are deposited in diapers of the babies
- Kidney stones
- Blood in the urine

- **Basis of neurological aberrations in LNS**
- **May be due to defect in Brain Salvage pathway.**

- As in LNS there is defect in Salvage Pathway primarily carried out in Brain.
- This might **affects the Brain growth and development**.
- There by leading to **Nervous dysfunction and related manifestations.**

- Athetosis (uncontrolled spastic muscle movements of the arms and legs)
- Involuntary joint movements
- Chorea (purposeless repetitive movements)
- Moderate mental retardation
- Irritability
- GIT disturbances are also noted

- **LNS Behavioral Elements**

- Cognitive dysfunction and aggressive and impulsive behaviors

-Severe self injurious behavior is common

LNS and Cerebral Palsy

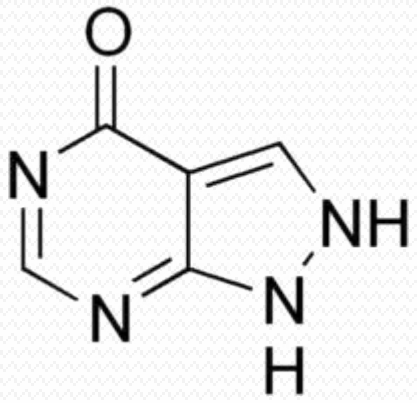
- "***Cerebral palsy*** is a group of **movement disorders** that result from damage to the brain, either before, during or shortly after birth."
- Thus, **LNS** is often a cause for the **damage to the brain that triggers cerebral palsy.**

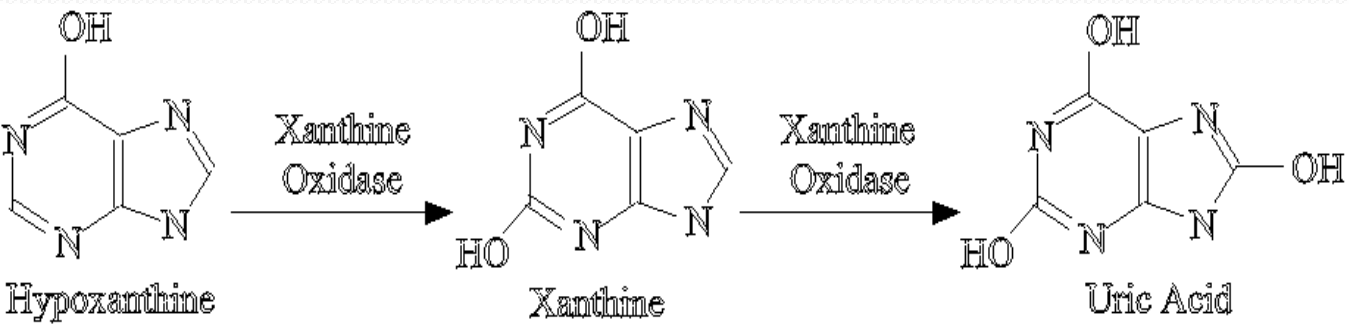
LNS Treatment and Prognosis

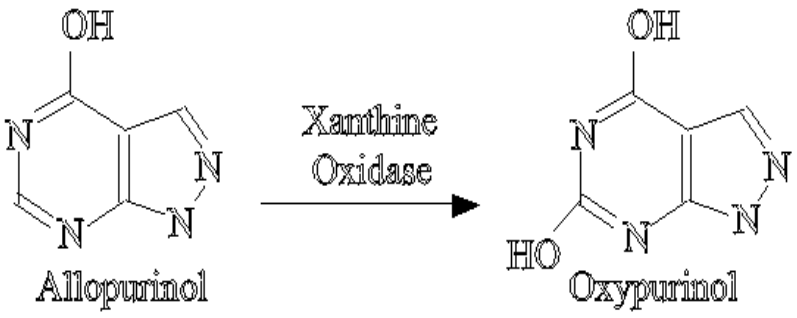
- **Treatment:**

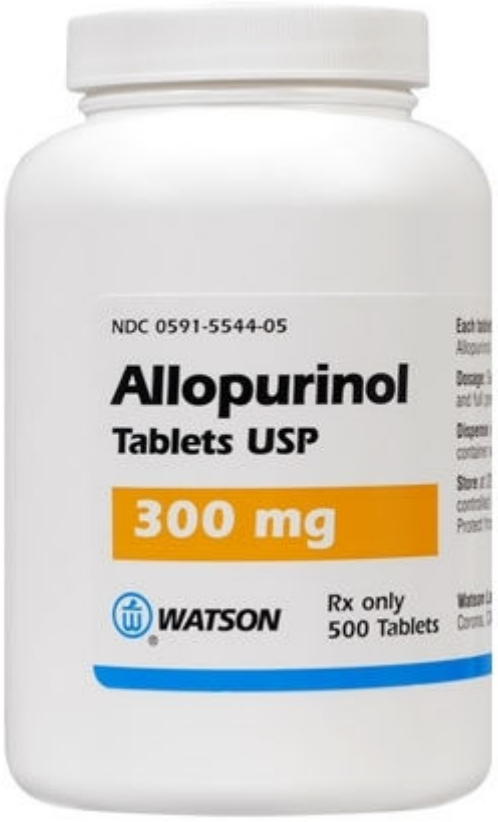
- Enzyme defect in LNS cannot be treated.
- Only the symptoms of LNS can be treated.
- The **drug Allopurinol** may be used to control excessive amounts of uric acid.

Treatment: Allopurinol – Competitive Inhibitor of Xanthine Oxidase





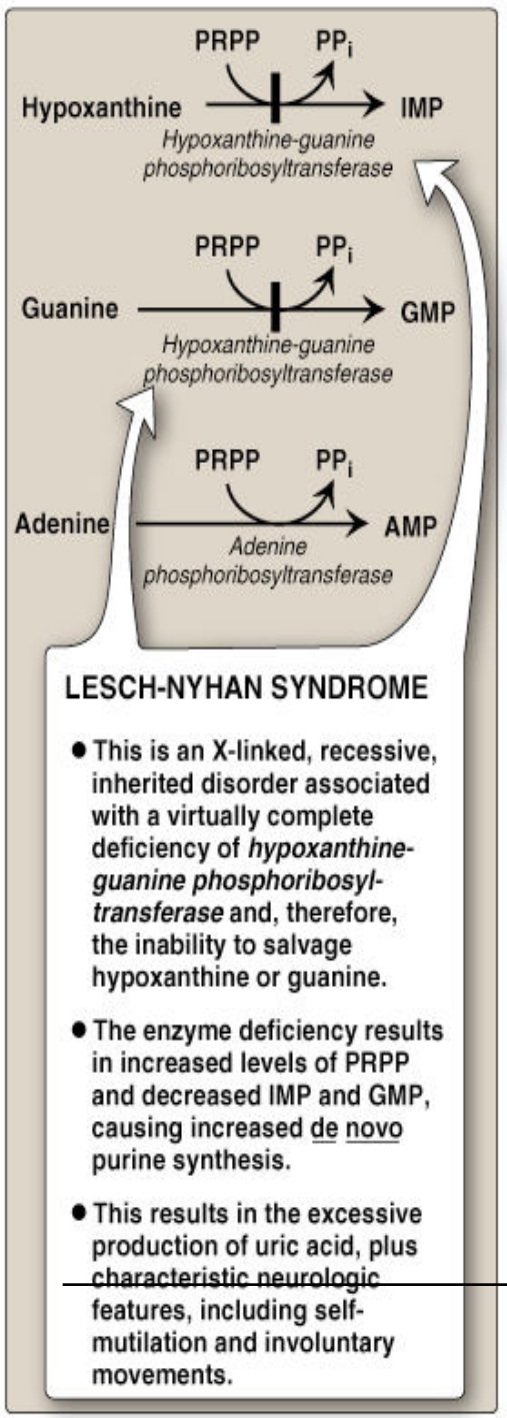




- Kidney stones can be treated with **lithotripsy**
- There are unfortunately **no treatments for the behavioral and neurological effects of LNS**

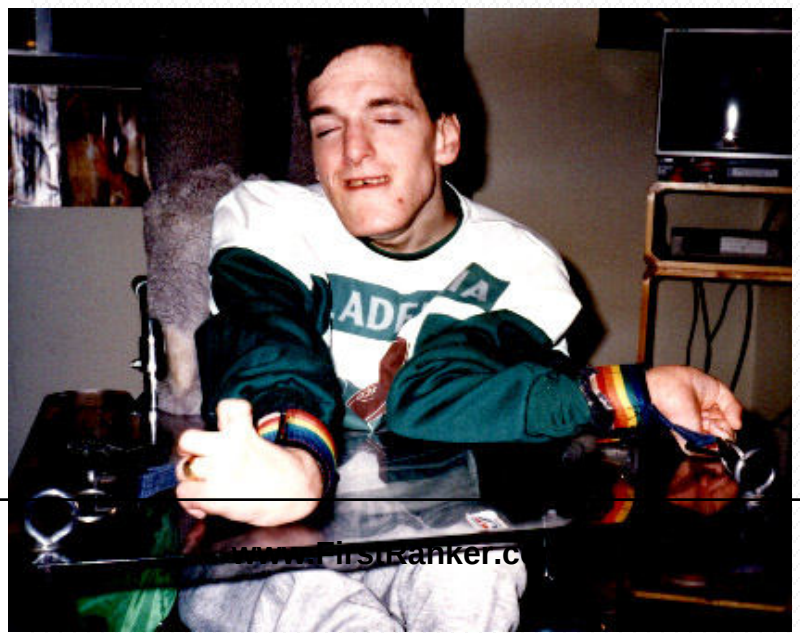
- **Prognosis:**

- The **prognosis for LNS is poor**
- Because there are **no treatments** for the neurological effects of the syndrome as **self-mutilation and may result in severe retardation and death**.
- The **build-up of excessive uric acid** in the body **causes painful episodes** of joints.

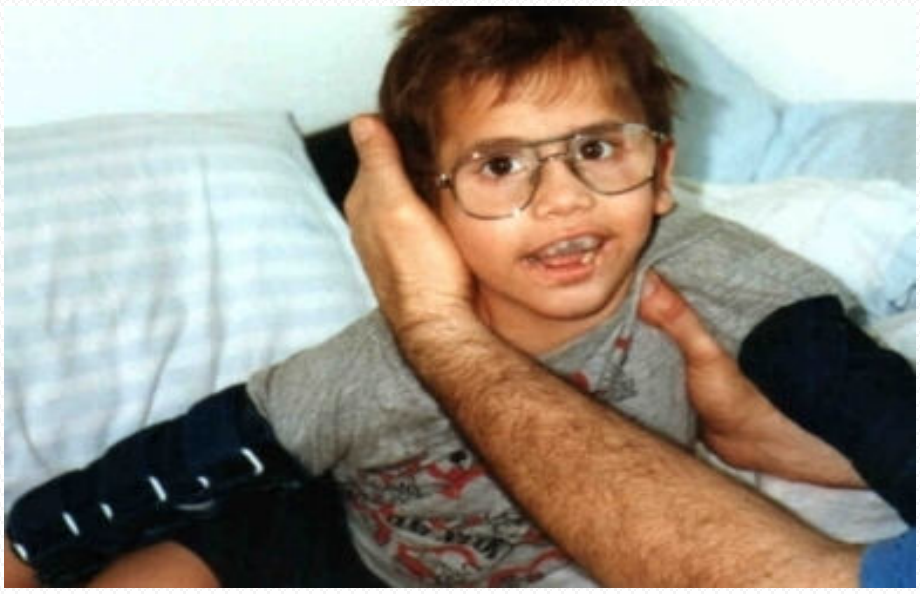


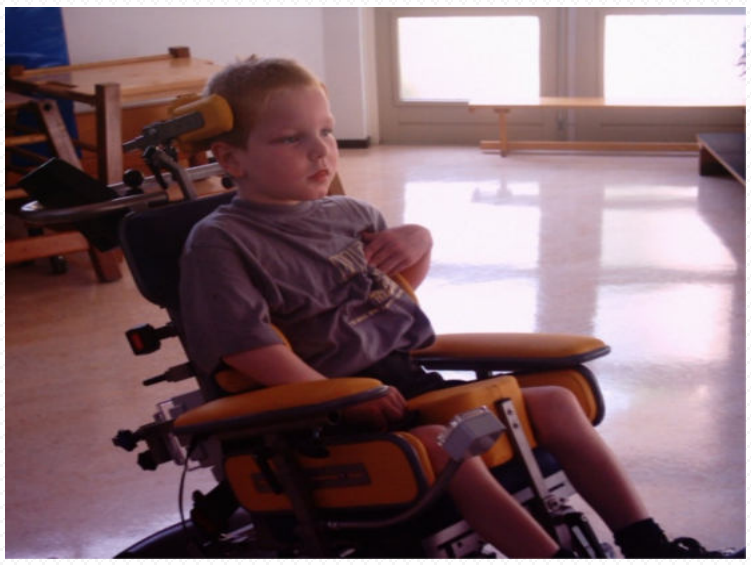
Lesch-Nyhan Syndrome

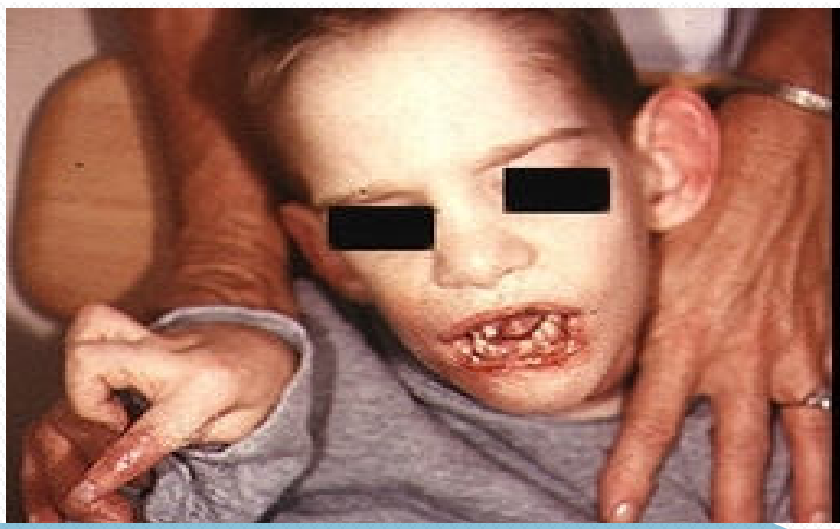
- Build up of Hypoxanthine and Guanine
- Degradation of hypoxanthine and guanine results in increased **uric acid**
- Excess uric acid in urine often results in orange crystals in the diaper of affected children
- Severe mental retardation
- Self-mutilation
- Involuntary movements
- Gout

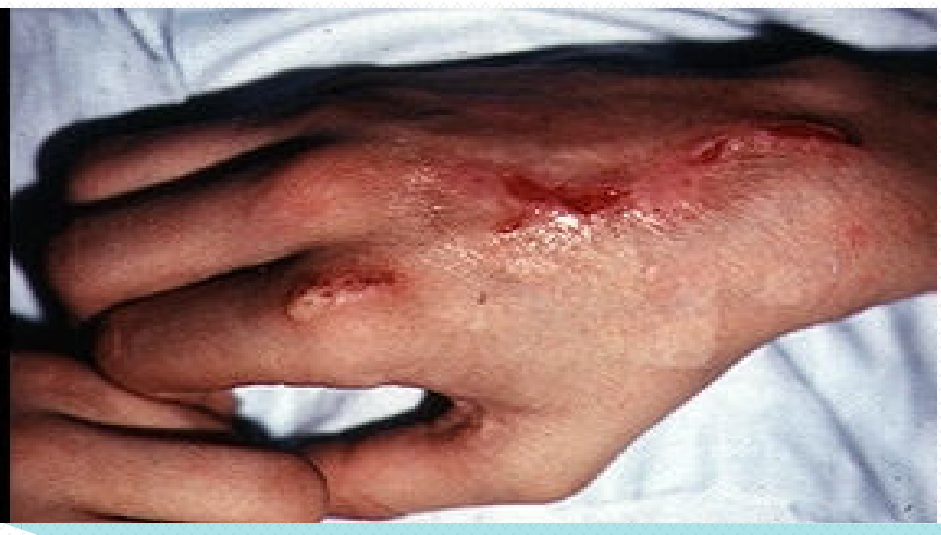


Lesch-Nyhan Syndrome









Orotic Aciduria

- **Oroticaciduria** is a **rare inherited** disorder of **Pyrimidine synthesis**.
- Caused by a deficiency of the enzyme
  - ***Orotate Phospho Ribosyl Transferase*** *(OPRTase)*
  - ***OMP Decarboxylase.***

Type I Oroticaciduria

- Both **OPRTase** and **OMP Decarboxylase** Enzyme deficient.
- Bifunctional deficiency.

Type II Oroticaciduria

- Only **OMP Decarboxylase deficient.**

- Enzyme defects **accumulates Oroticacid** in **blood**
- Increased excretion of **Orotic acid in urine**

(Oroticaciduria : 1.0-1.5 g)

Symptoms

- Mental and Physical retarded growth
- Severe Megaloblastic Anemia

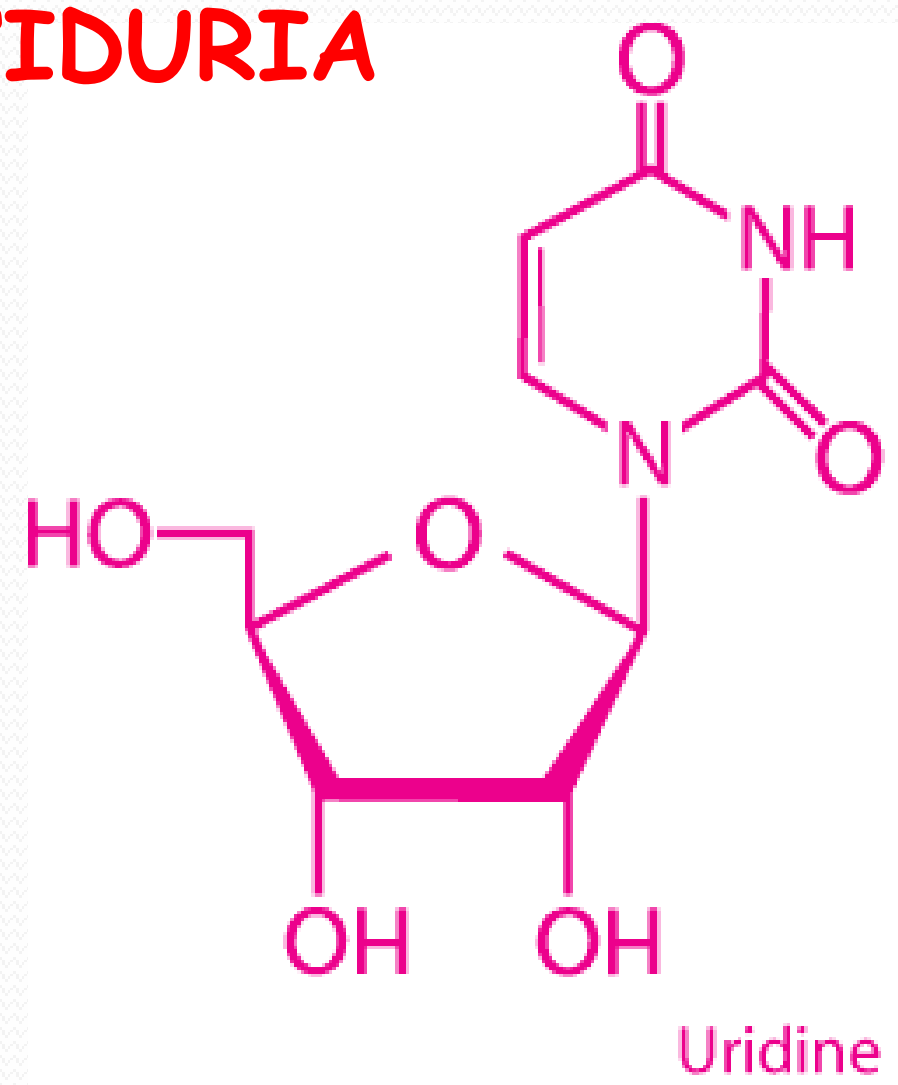
Treatment

- Treat with feeding diet rich in Uridine /Cytidine
- This provide Pyrimidine nucleotides through Salvage Pathway.
- Promotes DNA and RNA synthesis.

- Also the introduced Pyrimidine bases inhibits CPS II enzyme by **feed back mechanism and block synthesis of Oroticaciduria.**

TREATMENT OF OROTACIDURIA

Taking of Cytidine and **Uridine** during the whole life



Uridine

Adenosine Deaminase (ADA) defects
OR
Severe Combined Immuno Deficiency (SCID)

SCID Induced by Adenosine Deaminase Defects

- **Adenosine Deaminase** (ADA) is an Enzyme involved in **Purine catabolism**

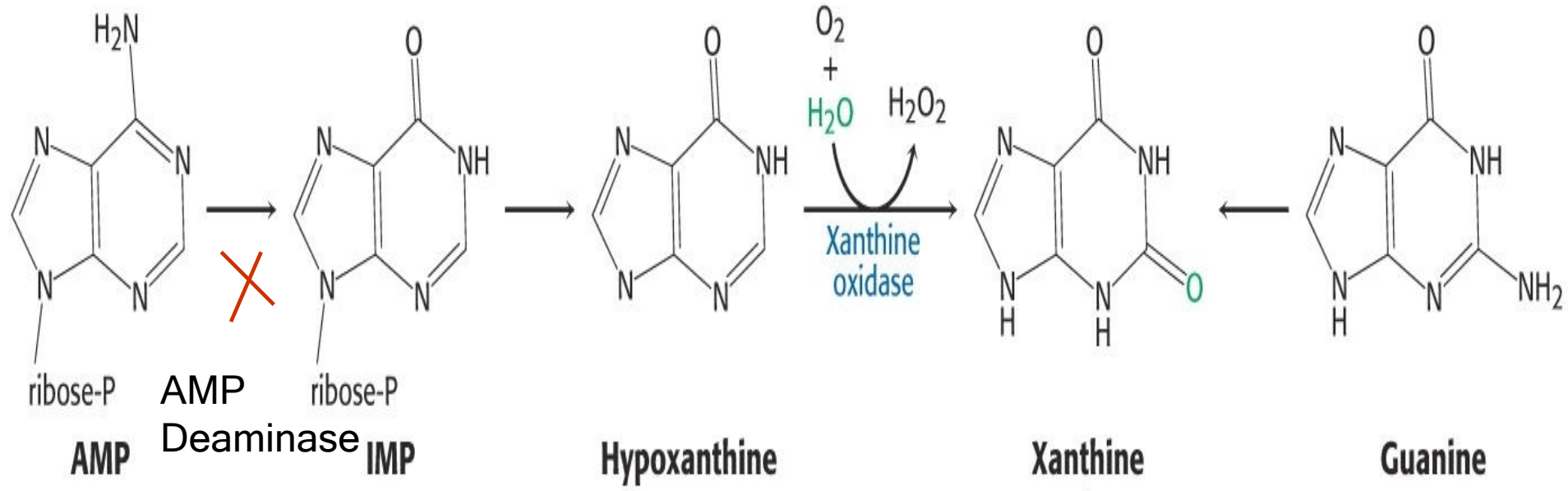
- **Deficiency of ADA enzyme leads to Immunological disorder –Severe Combined Immuno Deficiency (SCID)**

- The enzyme Adenosine Deaminase is encoded by a **gene on chromosome 20.**
- ADA deficiency is inherited in an **Autosomal recessive manner**.

Biochemical Defect

ADENOSINE DEAMINASE DEFICIENCY

- IN PURINE DEGRADATION, ENZYME **Adenosine Deaminase catalyzes** the conversion of:
- **ADENOSINE/AMP → INOSINE/IMP**



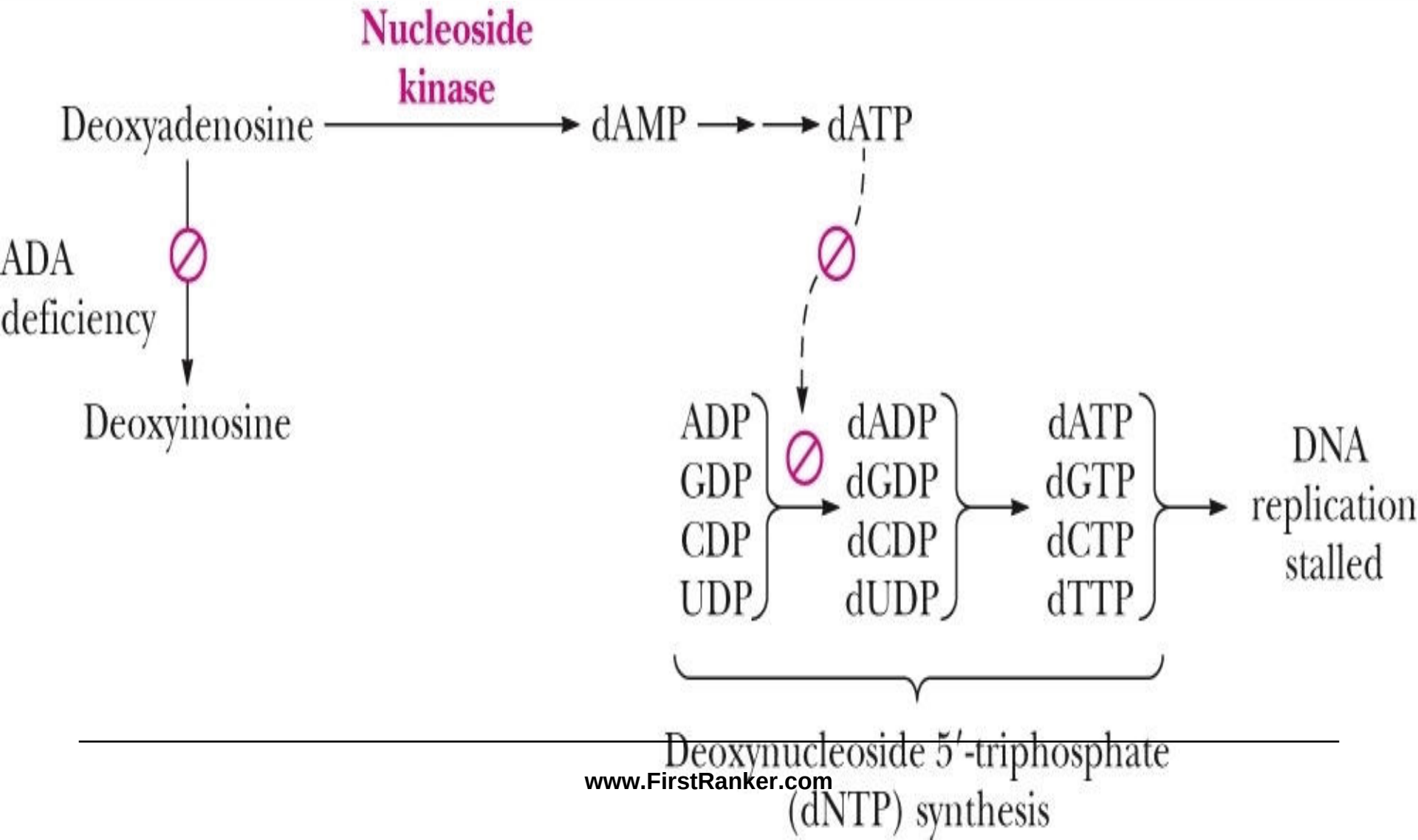
ADA Deficiency Affects DNA Synthesis

- ADA deficiency accumulates Adenosine/AMP later **transformed to** dAMP and **dATP** by enzyme **Nucleoside Kinases**.
- The formed dATP is an inhibitor of enzyme **Ribonucleotide Reductase.**

- **Ribonucleotide reductase** is an enzyme which catalyzes conversion of dNDPs to dNTPs.

- Inhibited Ribonucleotide Reductase thus **unable to produce dNTPs to support DNA biosynthesis.**

Cause Of
Severe Combined Immunodeficiency Syndrome (SCID)



- Thus Deficiency of ADA results in accumulation of AMP and dATP formed through Kinases.
- dATP is an inhibitor of **Ribonucleotide reductase** and inhibit the biosynthesis of other Deoxynucleotides like dCTP

ADA Deficiency Affects The Growth and Multiplication Of Rapidly Dividing Cells

- Low availability of dNTPs affect the DNA biosynthesis.
- This affects the rapidly dividing cells of the body.

- **The low levels of dCTP affects DN replication.**
- Which further affects the growth of rapidly dividing **immune cells T and B lymphocytes and other cells**
- leading **to IMMUNO DEFICIENCY.**

ADA Deficiency Leads To Immuno Deficiency

- Defects in AMP Deaminase **prevent biodegradation of AMP**
- AMP is converted into dATP by Kinases
- dATP inhibits the synthesis of other Deoxyribonucleotide by Ribonucleotide reductase,
- Causing problems with the Immune System (death of lymphocytes, immunodeficiency disease)

- **Decreased dATP, dGTP levels inhibit DNA replication**

- Function of Immune System depends upon Lymphocyte Proliferation.
- ADA deficiency inhibits Ribonucleotide Reductase and has Low dNTPs.

- This inhibits DNA Synthesis of Lymphocytes and its proliferation.
- Immune System is compromized due to non functional T and B cells.

SCID

- SCID is also known as
  - Alymphocytosis
  - Glanzmann-Riniker Syndrome
  - Sever Mixed Immunodeficiency Syndrome
  - Thymic Alymphoplasia

Incidence Of SCID

- 1 in 100 , 000 births.
- Some predict 1 in 50 ,000 live births

SCID

- **SELECTIVELY KILLS LYMPHOCYTES**
- **Absence of Functional**
- BOTH B- and T-CELLS
- Natural Killer Cells (NK)

- SCID exhibits **defective antibody response.**
- SCID sufferers are **extremely susceptible to infectious diseases**(Bacterial , Viral ,Fungal).

SCID Treatment

- Bone Marrow transplant
- Gene therapy
- Enzyme Replacement Therapy - PEG-ADA

ADA DEFICIENCY

- ONE OF FIRST DISEASES TO BE TREATED WITH GENE THERAPY
- ADA GENE INSERTED INTO LYMPHOCYTES; THEN LYMPHOCYTES RETURNED TO PATIENT
- PEG-ADA TREATMENTS
- ACTIVITY LASTS 1-2 WEEKS

- On **September 14, 1990**, the first gene therapy to combat this disease was performed by Dr. William French Anderson
- On a **four year old girl**, **Ashanti DeSilva**, at the **National Institutes of Health, Bethesda, Maryland, U.S.A.**

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

- If **ADA is deficient** or absent, Deoxyadenosine is not converted into Deoxyinosine as normal.
- This **elevates the levels of Deoxyadenosine** of Purine metabolism.
- Deoxyadenosine is **salvaged by** a **Nucleoside Kinase,** which converts it to dAMP, leading to accumulation of **dATP** and

- **Inhibition of Deoxynucleotides synthesis** through **Ribonucleotide reductase**.
- Thus, **DNA replication is ceased.**
- **This affects the rapidly growing cells.**

Points To Remember

- **Synthesis of Purine Nucleotides**
  - **De novo synthesis:** Site, Characteristics, Element sources of Purine bases
  - **Salvage pathway:** definition, significance, enzyme, **Lesch-Nyhan Syndrome**
  - Formation of Deoxyribonucleotide: **NDP level**

- **Degradation of Purine Nucleotides**
  - **Uric acid, Gout**
- **Synthesis of Pyrimidine Nucleotides**
  - **De novo synthesis:** Characteristics, Element sources of Pyrimidine bases
  - Salvage pathway
  - Antimetabolites of Pyrimidine nucleotides
- **Catabolism of Pyrimidine Nucleotides**
- **Related Disorders.**

- **Antimetabolites of Purine and Pyrimidine Bases and Nucleotides:**
  - **Uses of Purine, Amino acid, and Folic acid analogs.**

QUESTIONS

- **Long Essays.**

- 1) Draw the Purine ring; write the sources of carbon and Nitrogen atoms of the ring.
  - OR
- Give the outline of Purine biosynthetic pathway and a note on regulation and inhibition of Purine nucleotide biosynthesis.

- 2) Describe metabolism of Pyrimidine metabolism / synthesis and Degradation Pyrimidine nucleotides.
- 3) Catabolism of Purine nucleotides / formation of uric acid. Add a note on Inborn Errors of Nucleotide metabolism.

- **Short Notes:**

- 1) Gout
- 2) Inter conversion of IMP to AMP & GMP
- 3) Salvage pathway.
- 4) Lesch Nyhan syndrome
- 5) PRPP

- 6) Digestion of Nucleic acids/ Fate of Dietary Nucleic acid
- 7) Allopurinol /Treatment of Gout
- 8) Adenosine Deaminase Deficiency/SCID
- 9) Orotic aciduria.

THE END

THANK YOU