Nucleotide Metabolism

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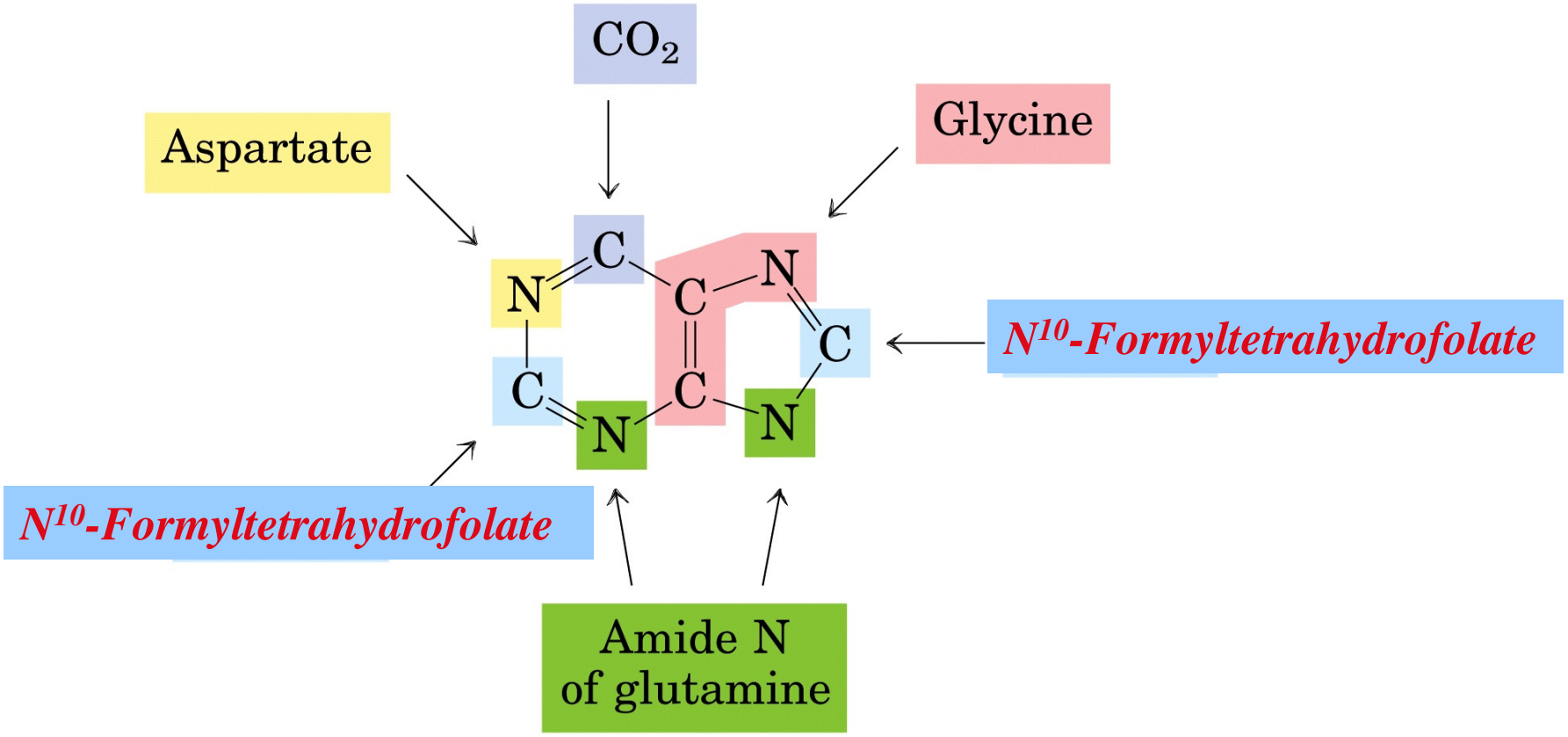
Nucleiotide Needs

- DNA synthesis
- RNA synthesis
- Energy Currency like
  - **ATP**
- Secondary messanger
  - **C-AMP, C-GMP**
- Intermediate in metabolisms = CO-ENZYME
  - **NAD, NADP, FAD, AMP, ATP**

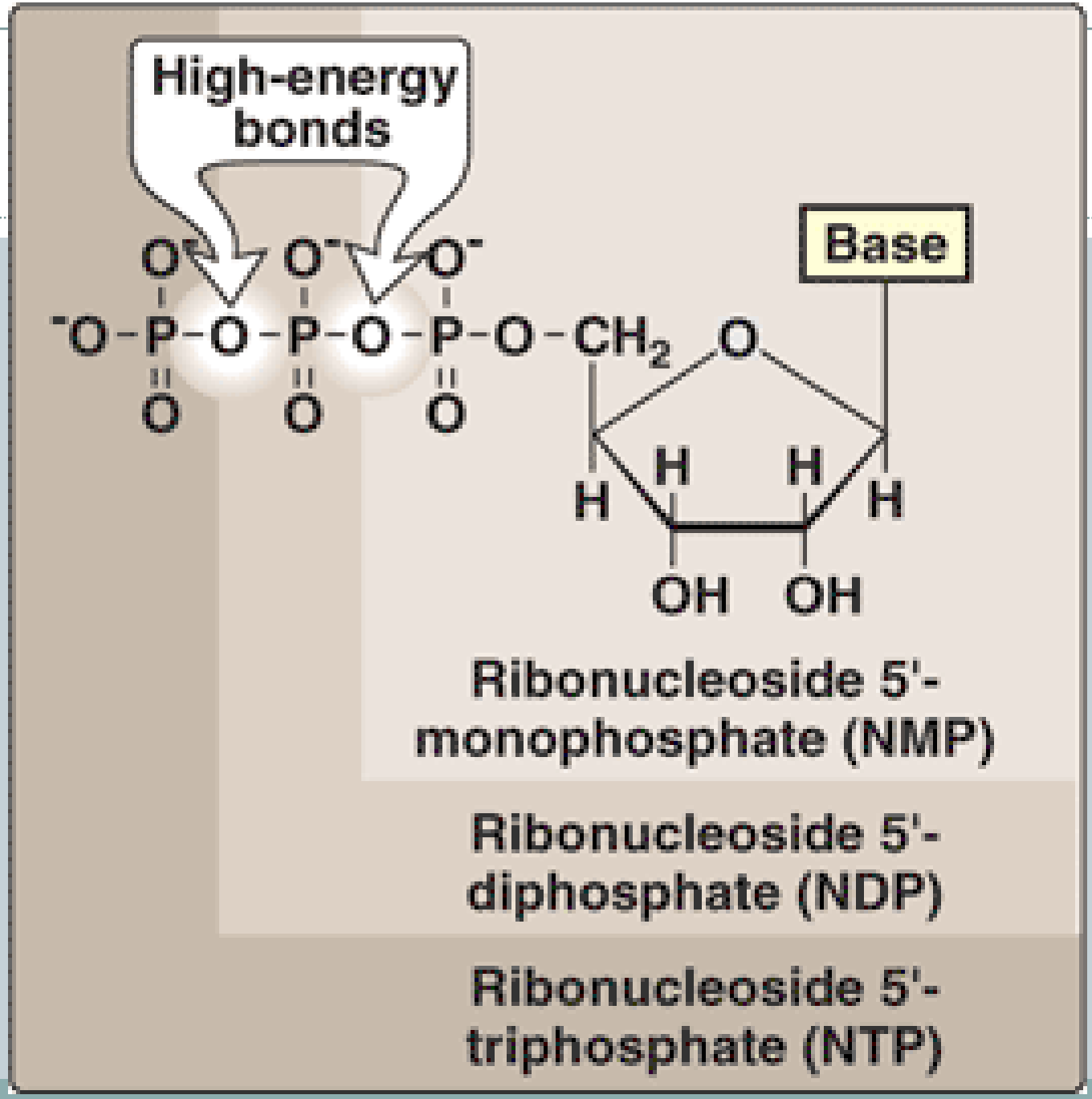
Source of Nucleotide

- Denovo Synthesis of Nucleiotide
- Salvage Pathway (Normal cell turnover)
- Diet

Element sources of purine bases

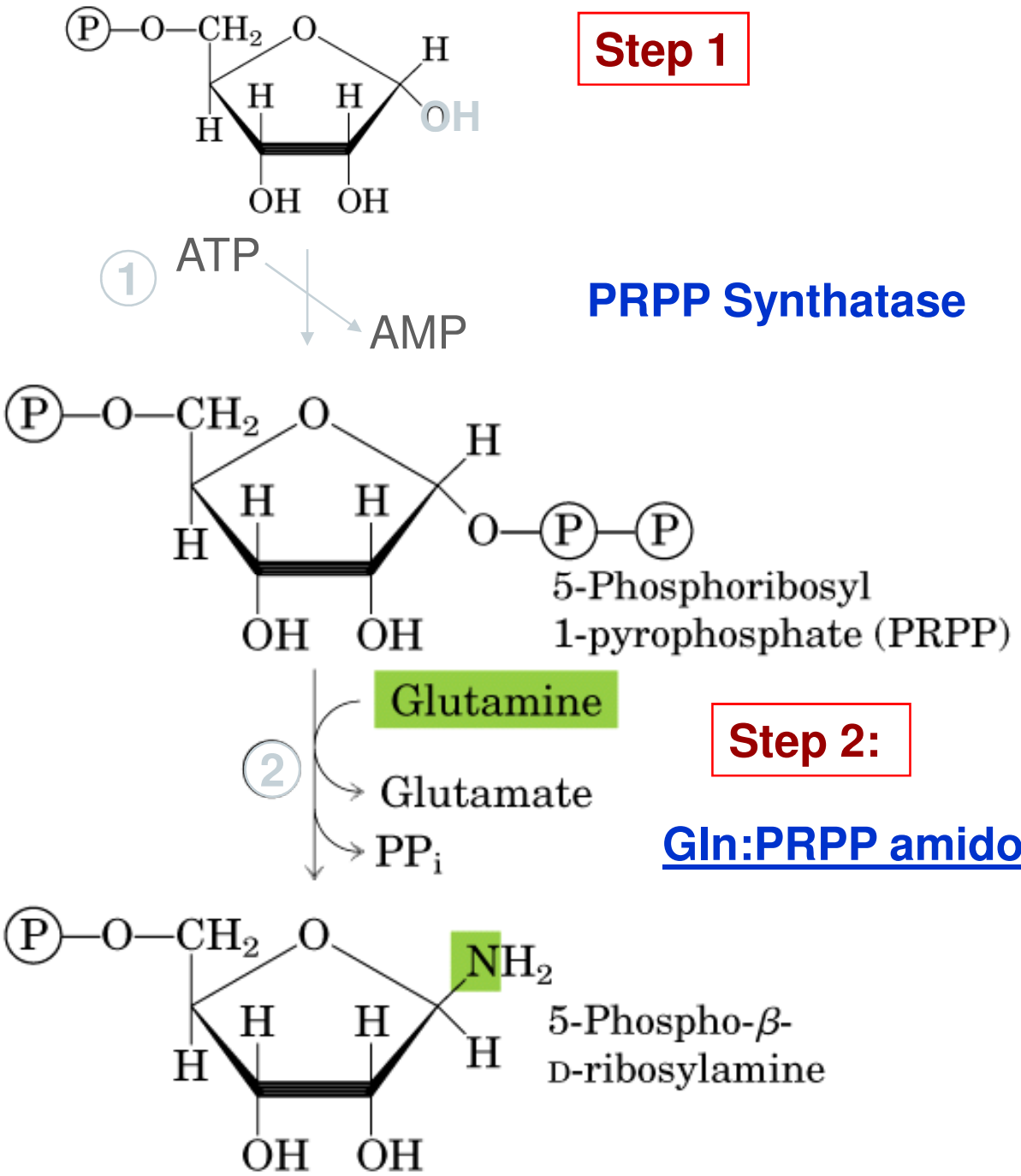


First, synthesis Inosine-5'-Monophosphate, IMP

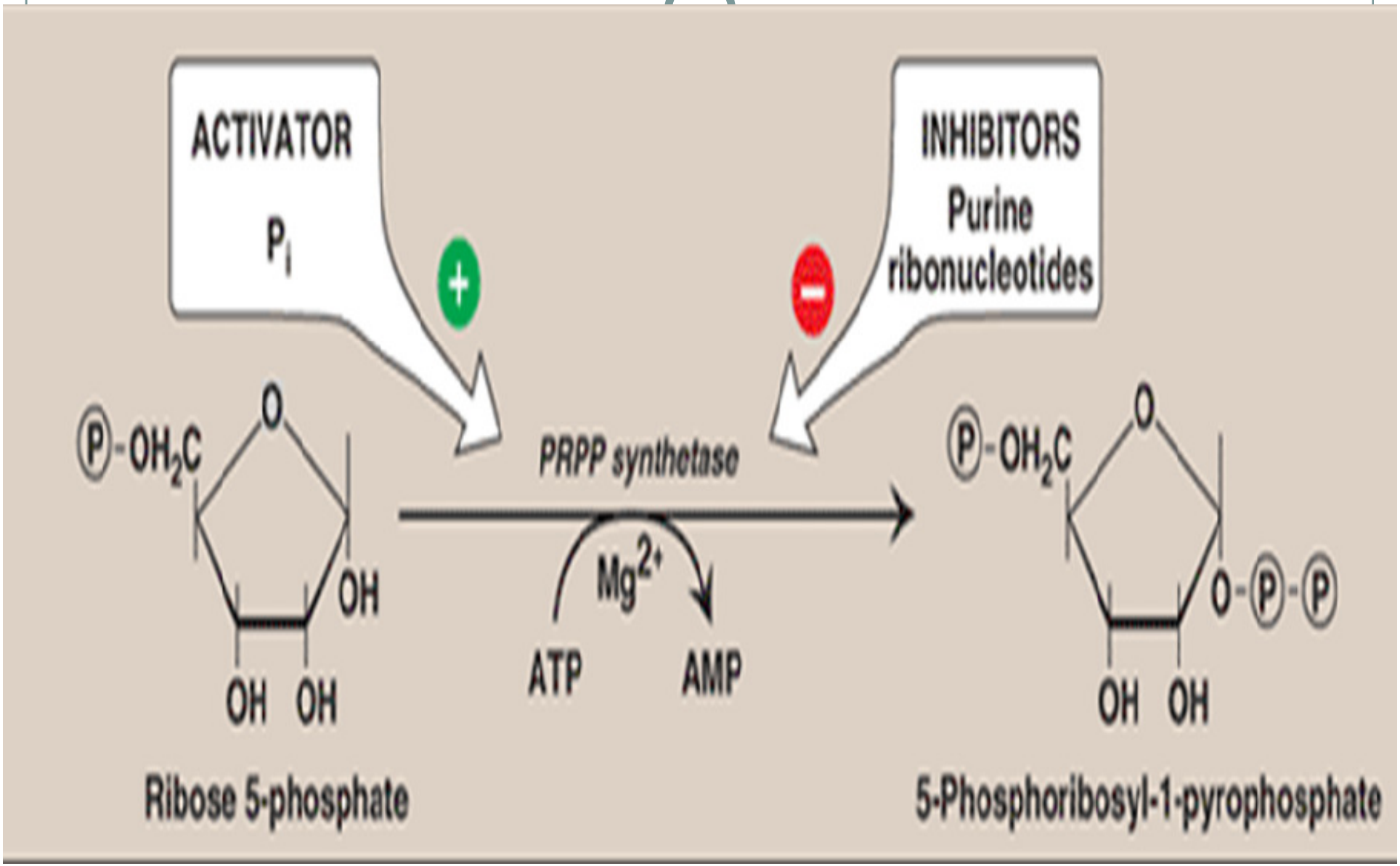

High-energy bonds
Base
Ribonucleoside 5'-monophosphate (NMP)
Ribonucleoside 5'-diphosphate (NDP)
Ribonucleoside 5'-triphosphate (NTP)

Synthesis of Inosine Monophosphate (IMP)

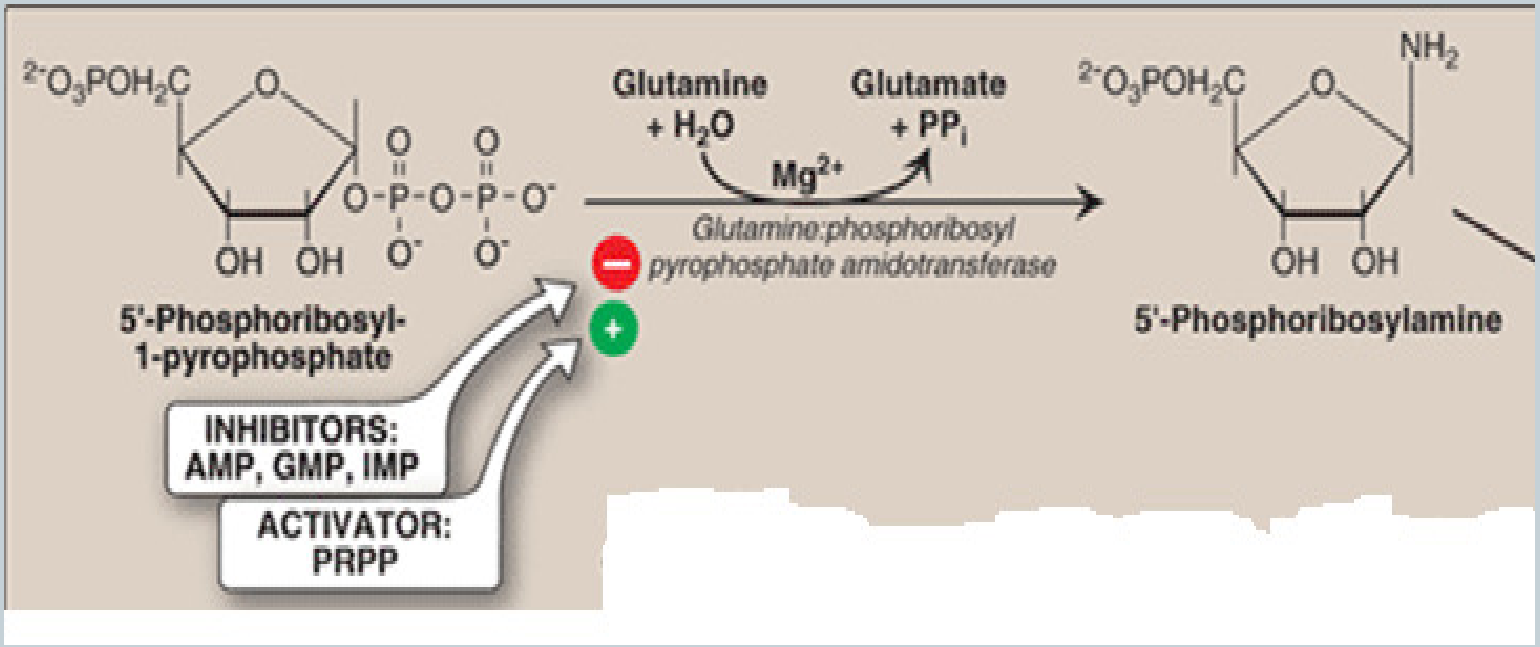
- Basic pathway for biosynthesis of purine ribonucleotides
- Starts from ribose-5-phosphate(R-5-P)
- Requires 11 steps overall
- occurs primarily in the liver


Step 1
ATP
AMP
PRPP Synthatase
5-Phosphoribosyl
1-pyrophosphate (PRPP)
Glutamine
Glutamate
PPi
Step 2:
Gln:PRPP amidotransferase
5-Phospho-β-
D-ribosylamine

PRPP synthetase
(ribose phosphate pyrophosphokinase



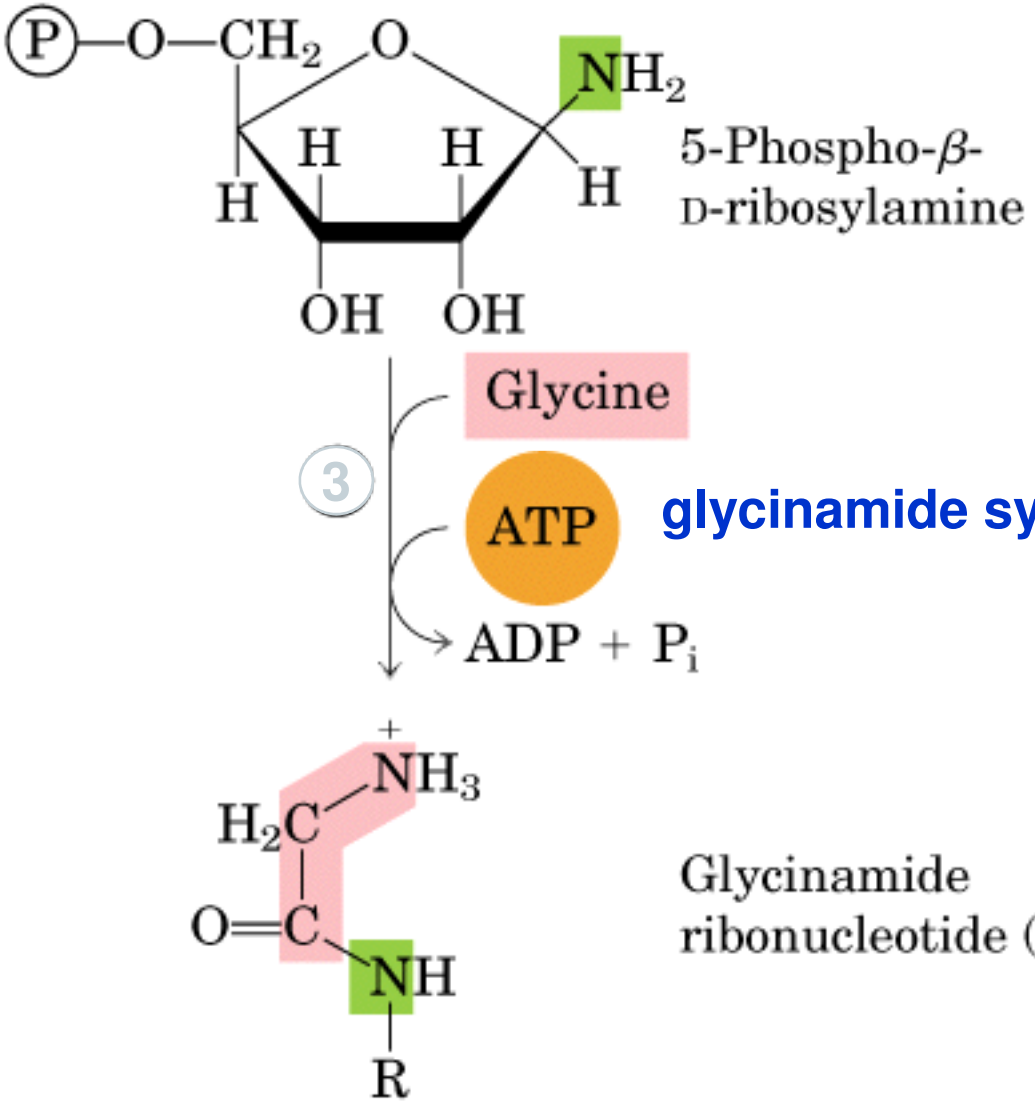
Glutamine :Phosphoribosyl pyrophosphate amidotransferase



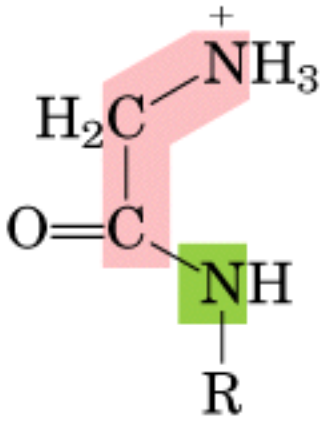
Synthesis of IMP ("Parent" purine nucleotide)

- **Next nine steps** in purine nucleotide biosynthesis leading to the synthesis of **IMP** (whose base is hypoxanthine).
- Requires
  - **four ATP.**
  - **N^{10}-formyltetrahydrofolate.**
  - **Aspartate.**
  - **Glycine**

Step 3: acquisition of purine atoms C4, C5, and N7



•**Step 4: acquisition of purine atom C8**



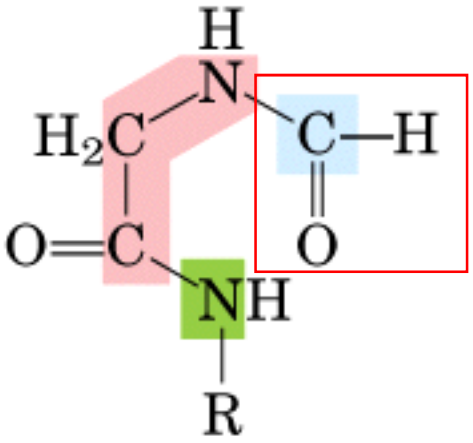
Glycinamide ribonucleotide (GAR)

4

N^{10}-Formyl H_4 folate

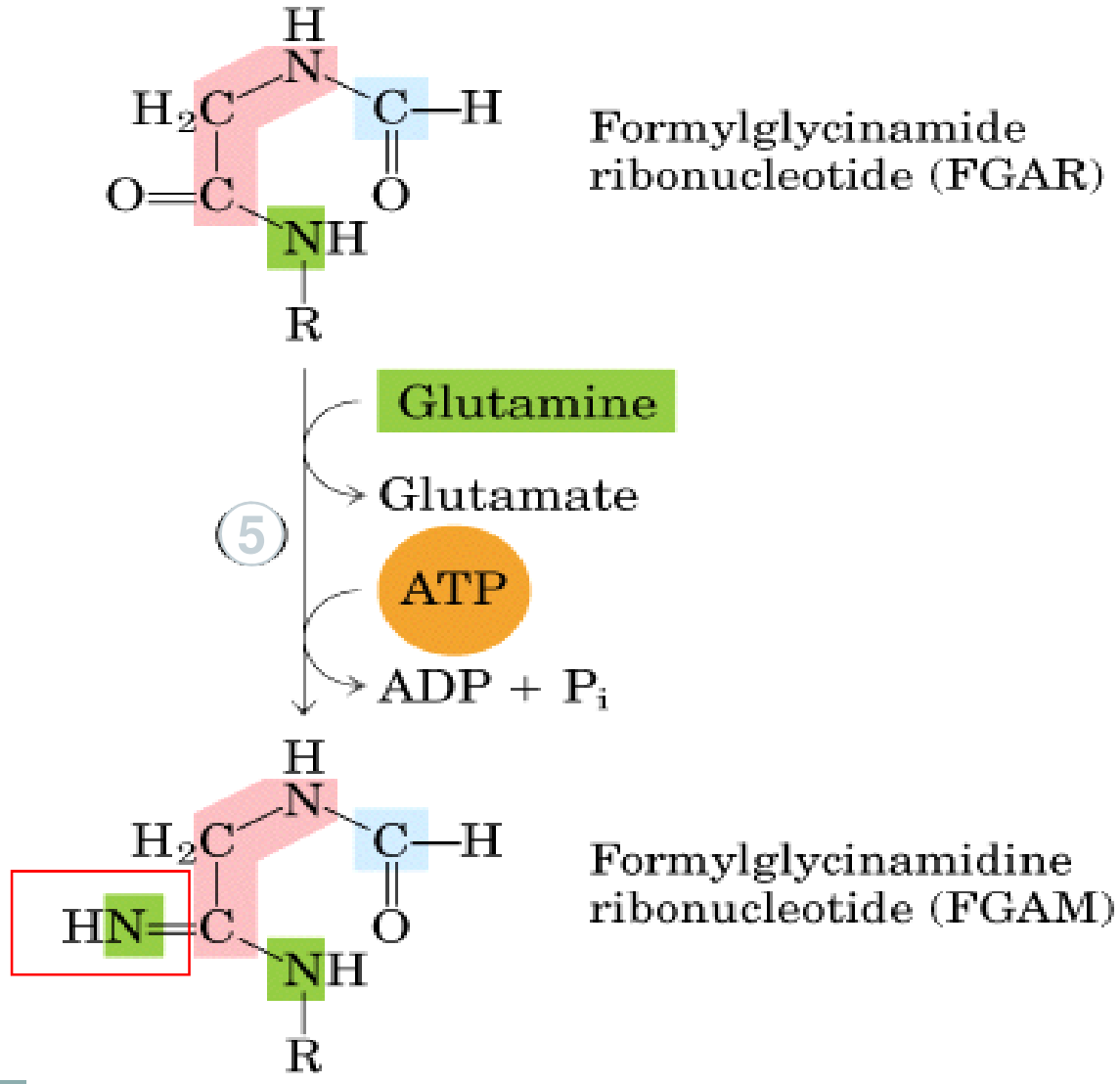
H_4 folate

GAR transformylase

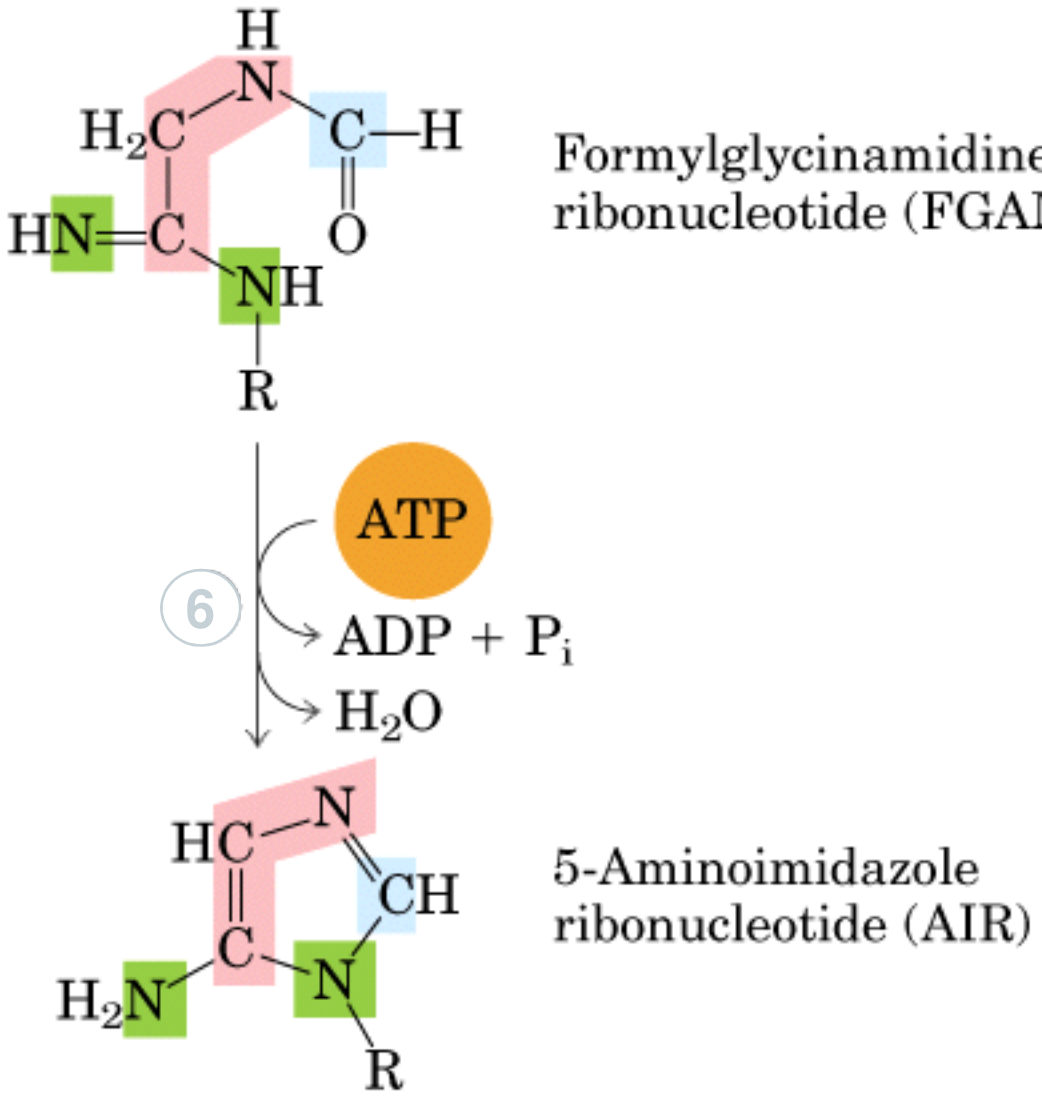


Formylglycinamide ribonucleotide (FGAR)

Step 5: acquisition of purine atom N3



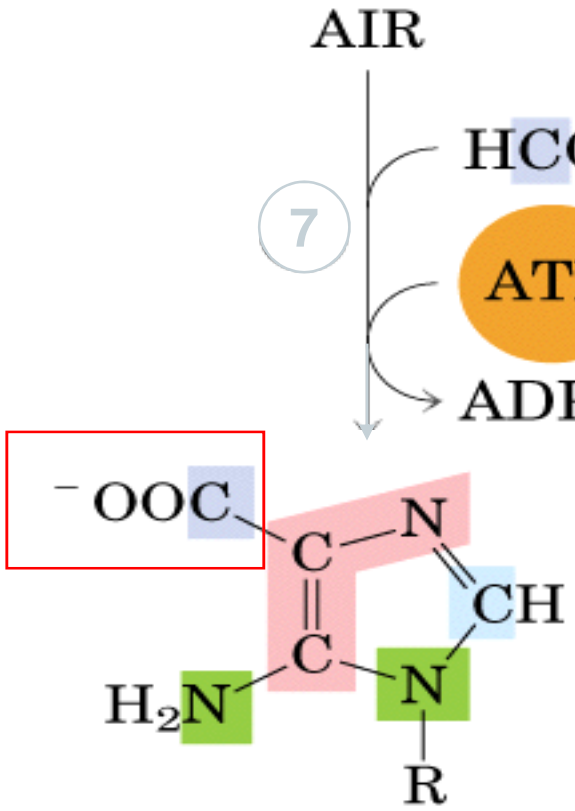
•**Step 6: closing of the imidazole ring**



Formylglycinamidine ribonucleotide (FGAM)

5-Aminoimidazole ribonucleotide (AIR)

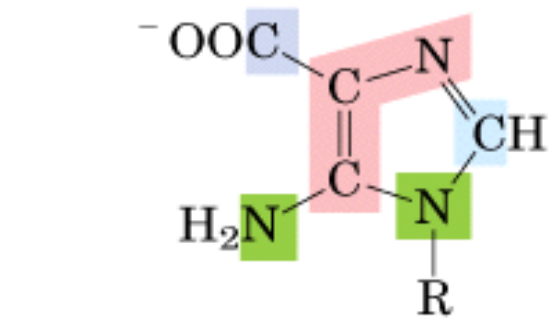
Step 7: acquisition of C6



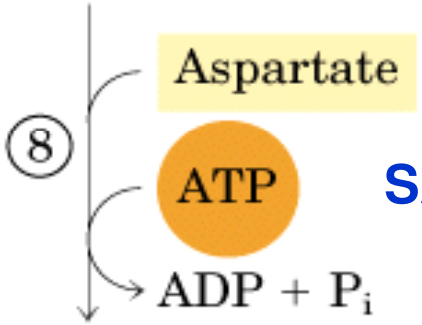
AIR carboxylase

Carboxyaminoimidazole ribonucleotide (CAIR)

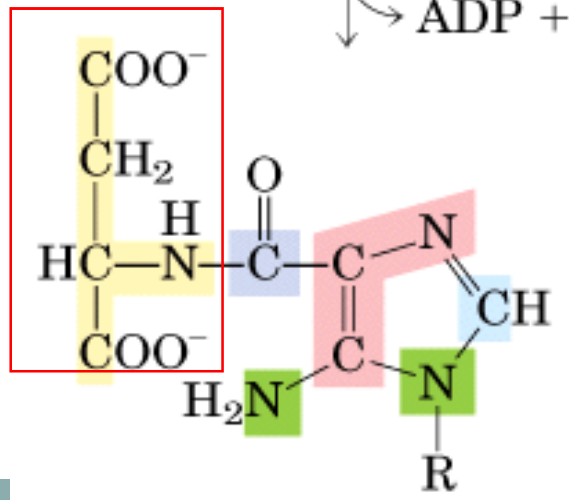
Step 8: acquisition of N1



Carboxyaminoimidazole ribonucleotide (CAIR)

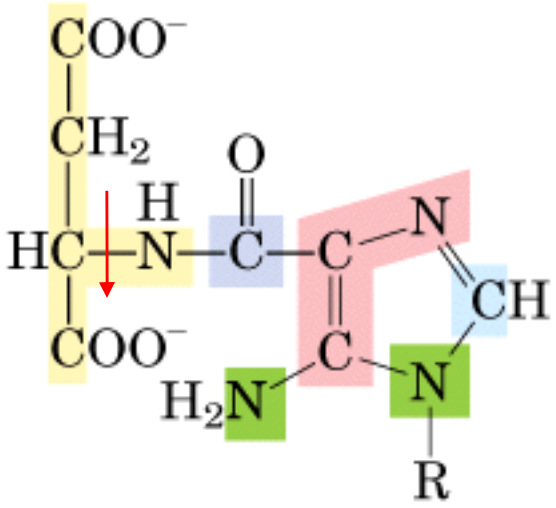


SAICAR synthetase



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

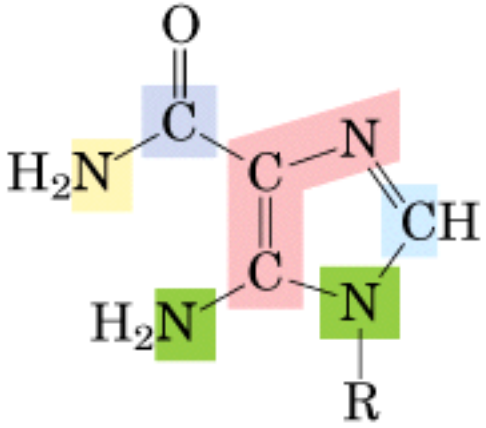
Step 9: elimination of fumarate



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

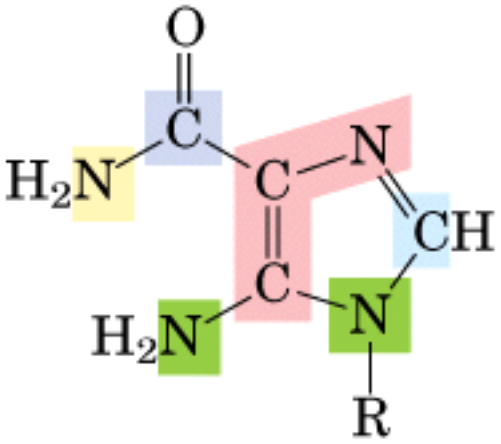
9 → Fumarate

adenylosuccinate lyase



5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)

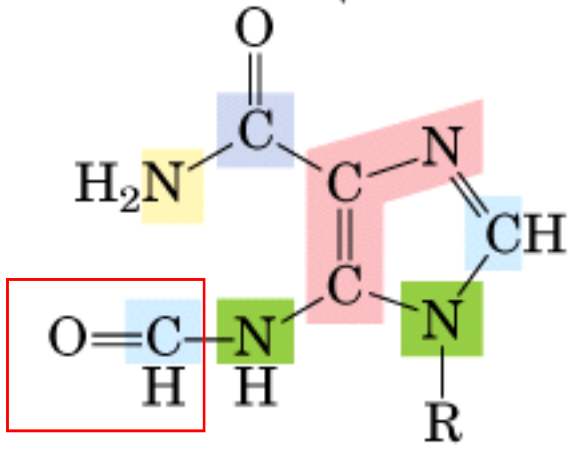
Step 10: acquisition of C2



5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)

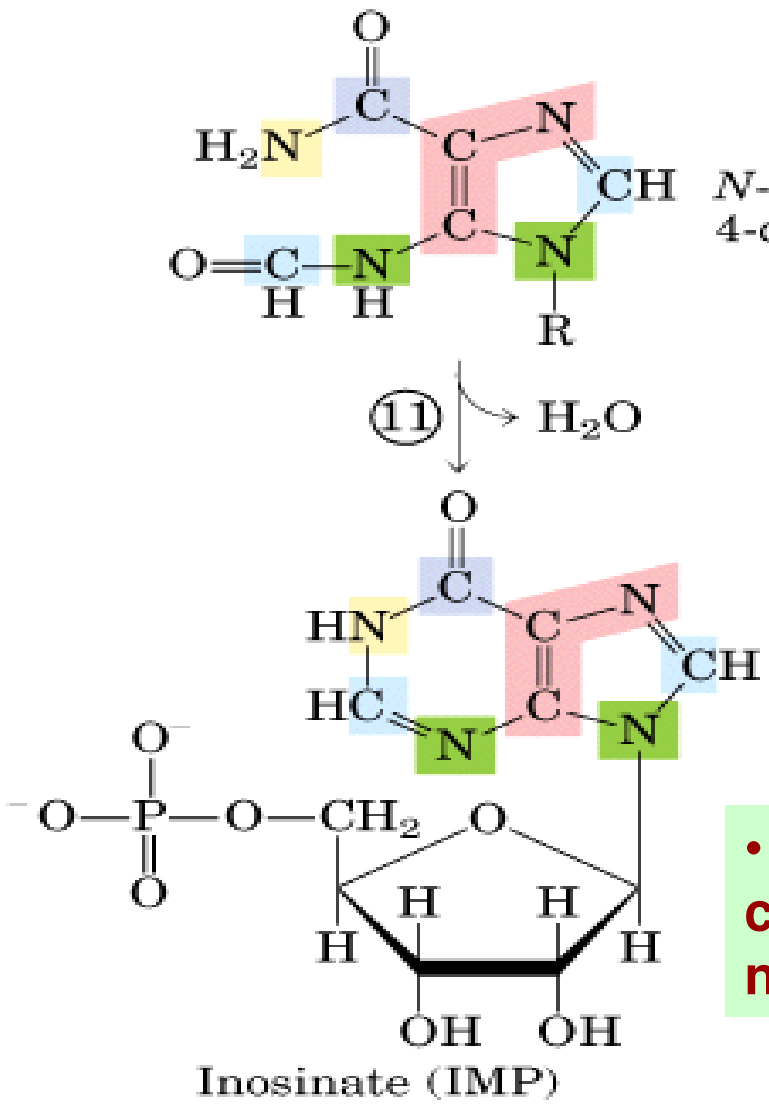
10 N^{10}-Formyl H_4 folate → H_4 folate

AICAR transformylase



N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)

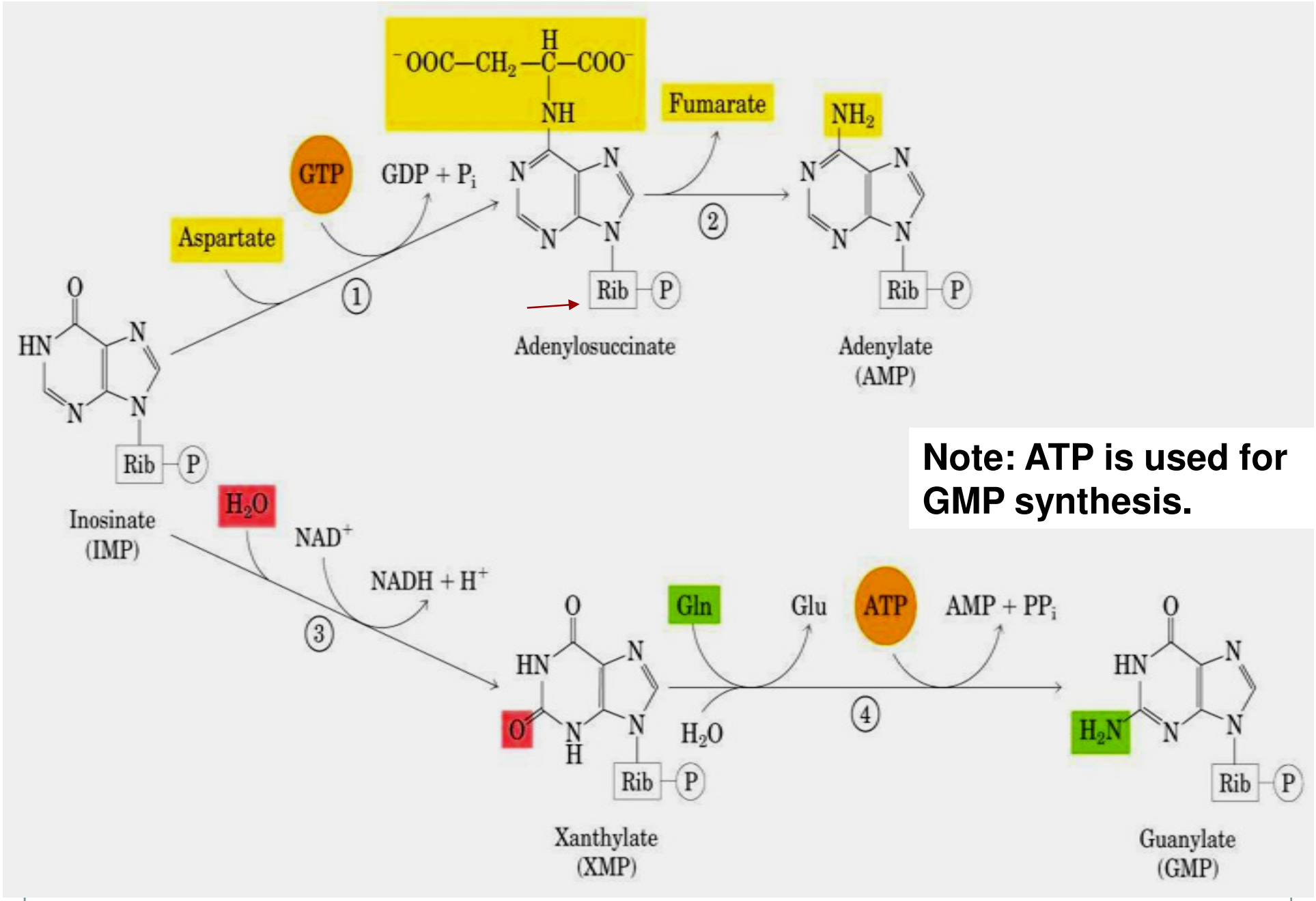
Step 11: ring closure to form IMP



N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)

Inosinate (IMP)

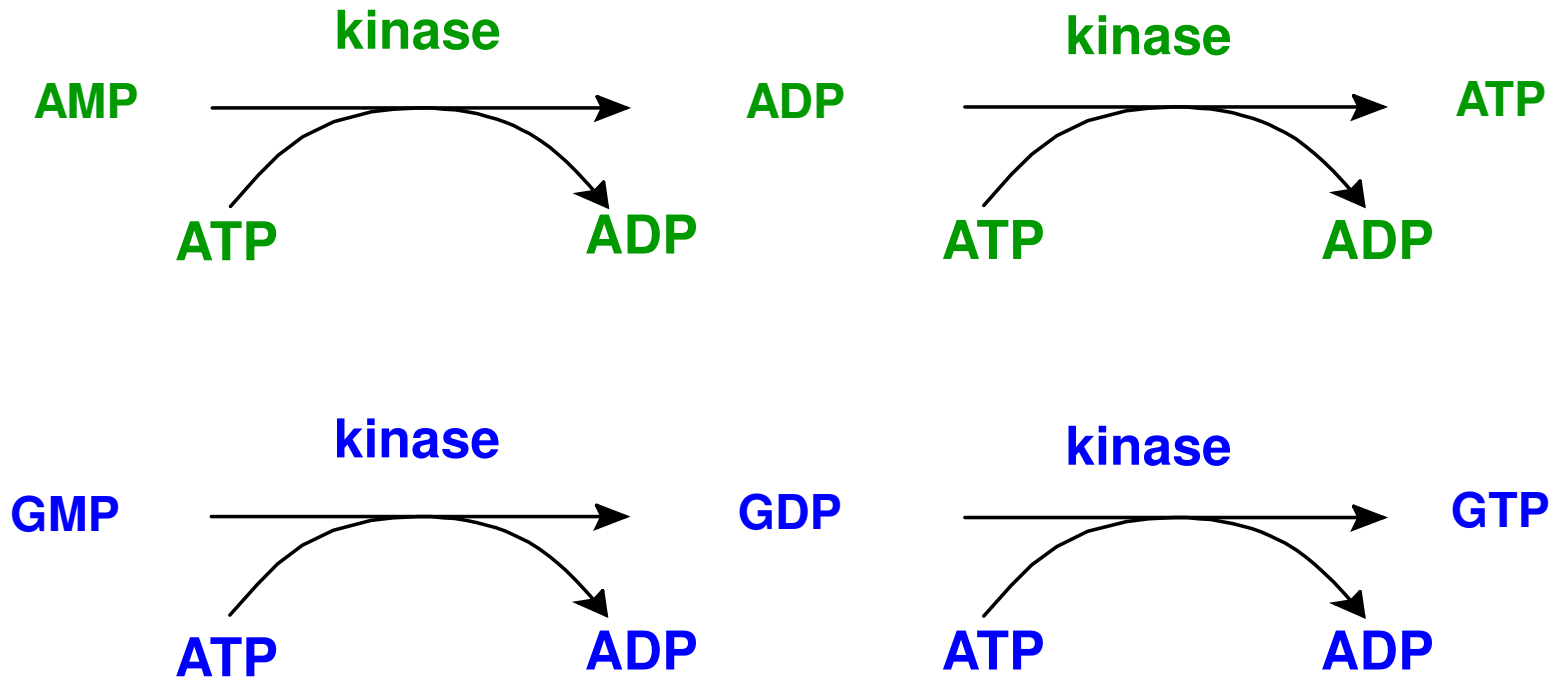
- **Once formed, IMP is rapidly converted to AMP and GMP (it does not accumulate in cells).**

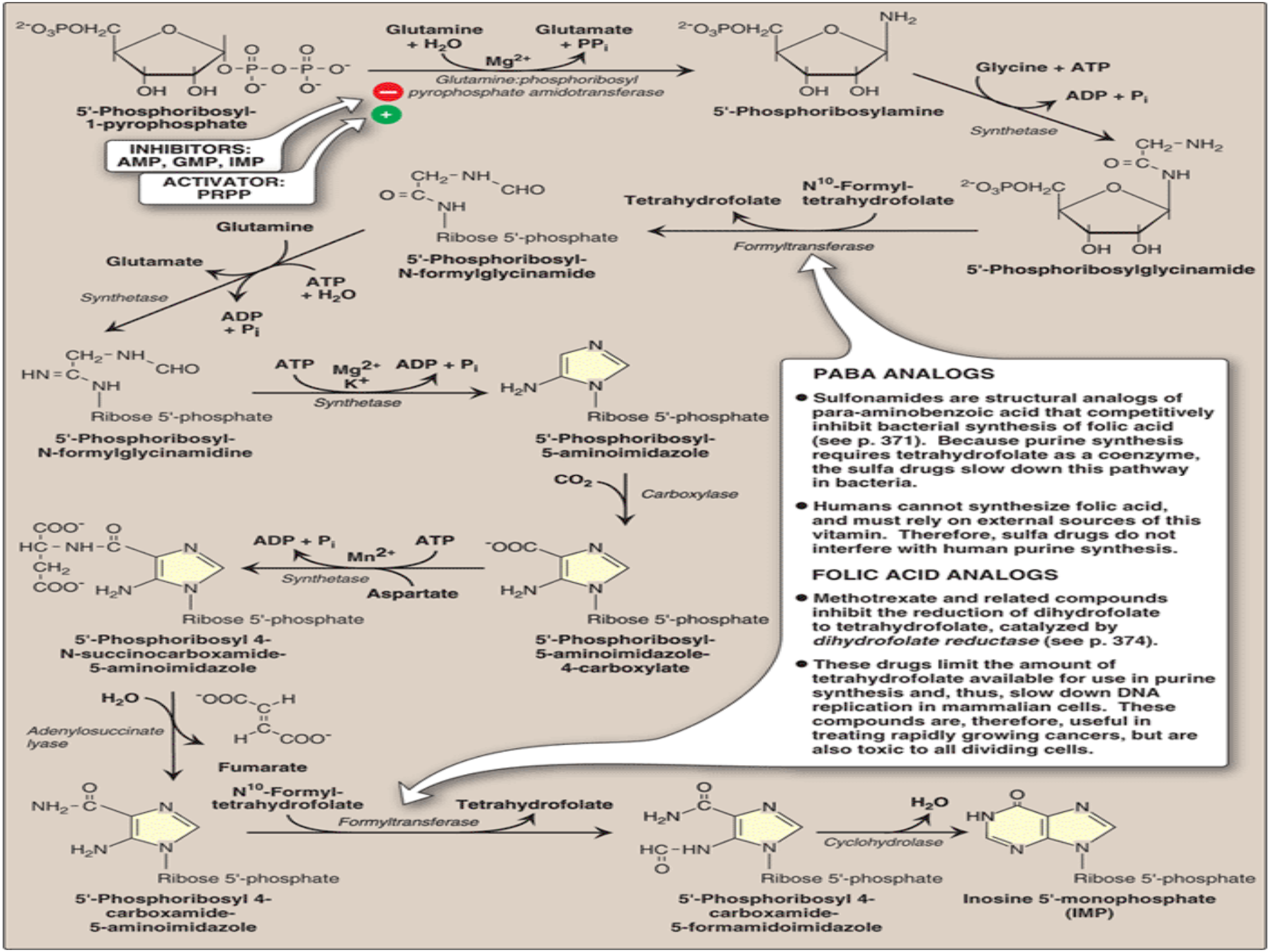


Note: ATP is used for GMP synthesis.

IMP is the precursor for both AMP and GMP.

4. ADP, ATP, GDP and GTP biosynthesis





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.

FOLIC ACID ANALOGS

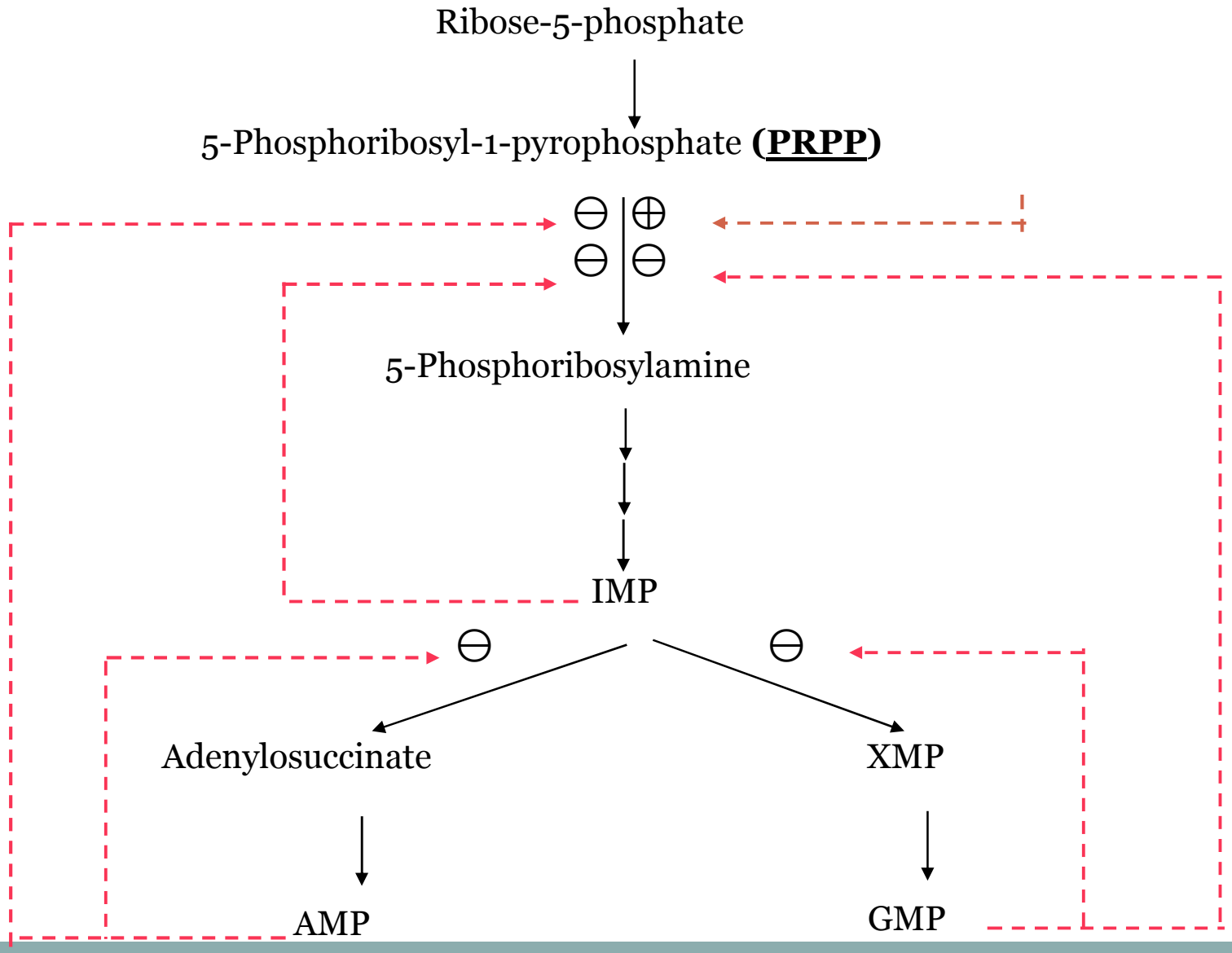
- Methotrexate and related compounds inhibit the reduction of dihydrofolate to tetrahydrofolate, catalyzed by *dihydrofolate reductase* (see p. 374).
- These drugs limit the amount of tetrahydrofolate available for use in purine synthesis and, thus, slow down DNA replication in mammalian cells. These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

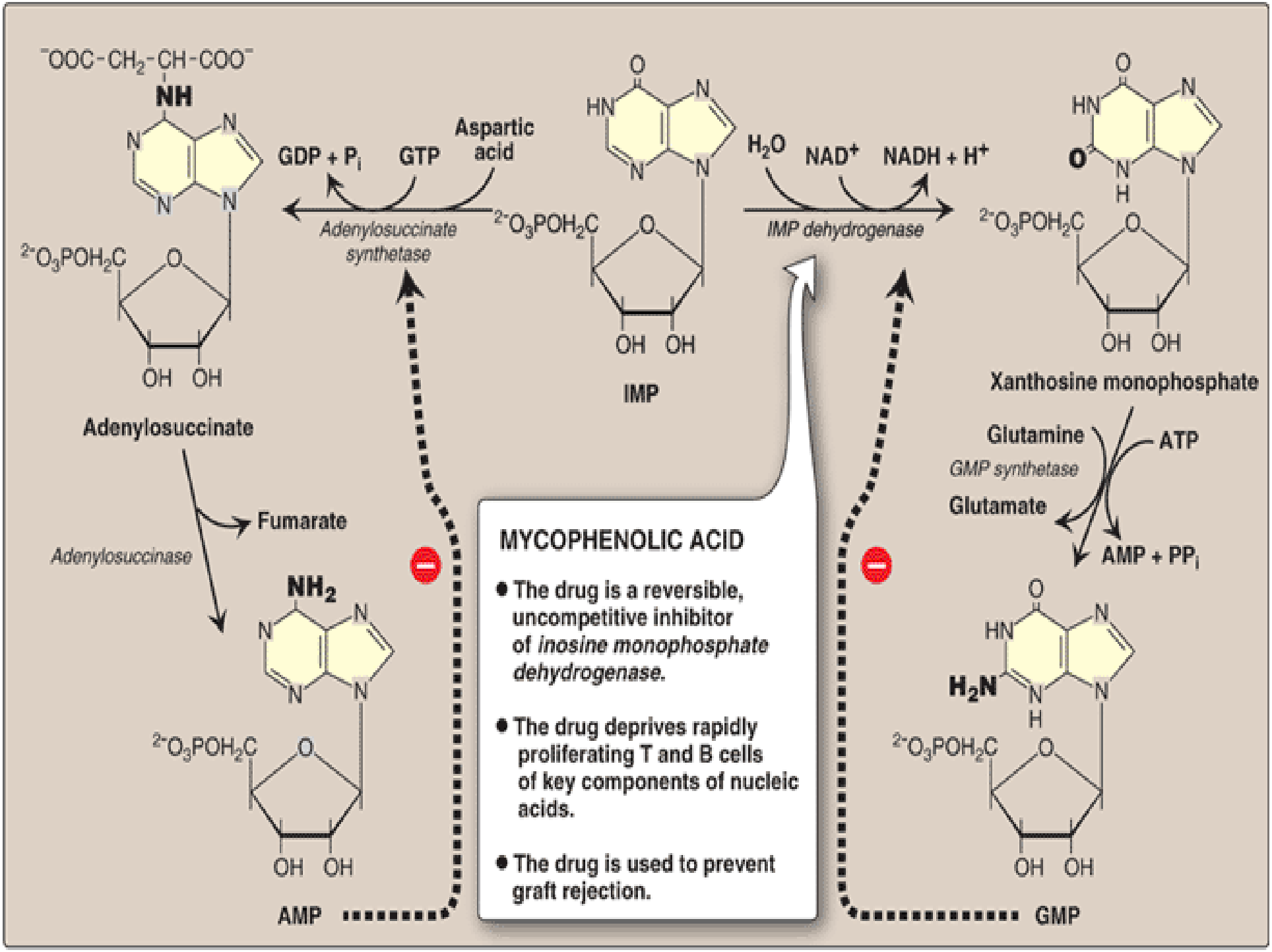
Inhibitors of Purine Synthesis

- **Sulfonamides** (antibiotic) = PABA analogs)
- **Trimethoprim**
- = Folate analogs

  = Selective inhibition of bacterial dihydrofolate reductase.
- **Methotrexate (chemotherapy)** = Folic acid analogs
  - Inhibitors of human purine synthesis
  - Inhibit Rapidly replication cell.
  - **Bone marrow supression.**
  - **Nause – Vomiting – Gastritis – Ulcer**
  - **Hair loss.**

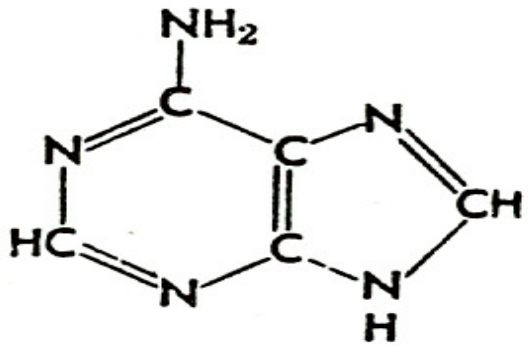
Summary of Regulation




Aspartic acid
GDP + P_i
GTP
Adenylosuccinate synthetase
H_2O
NAD^+
$NADH + H^+$
IMP dehydrogenase
Adenylosuccinate
IMP
Xanthosine monophosphate
Glutamine
ATP
GMP synthetase
Glutamate
$AMP + PP_i$
Fumarate
Adenylosuccinase
MYCOPHENOLIC ACID
• The drug is a reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase.
• The drug deprives rapidly proliferating T and B cells of key components of nucleic acids.
• The drug is used to prevent graft rejection.
AMP
GMP

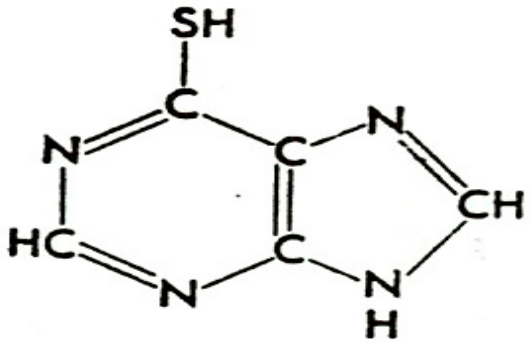
Inhibition of Purine Biosynthesis by the Antitumor Agent

Metabolite

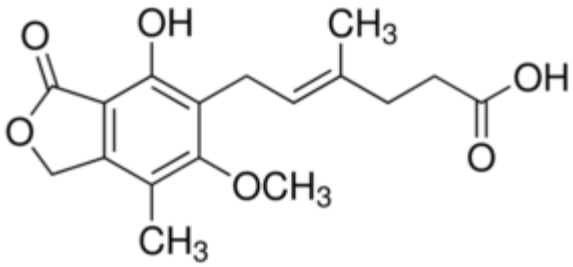


Adenine

Antimetabolite



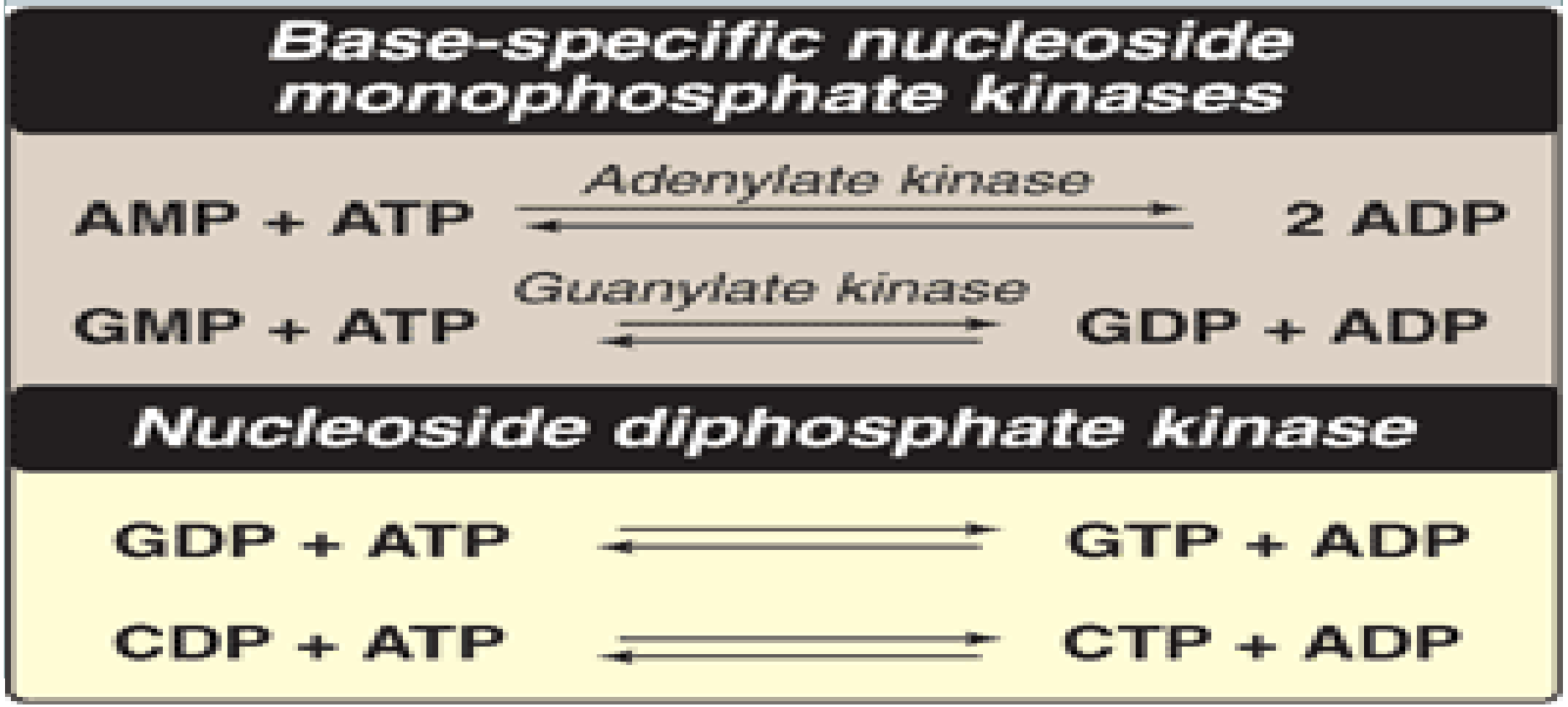
6-Mercaptopurine



Mycophenolic acid

Conversion of ?MP = ?DP and ?TP

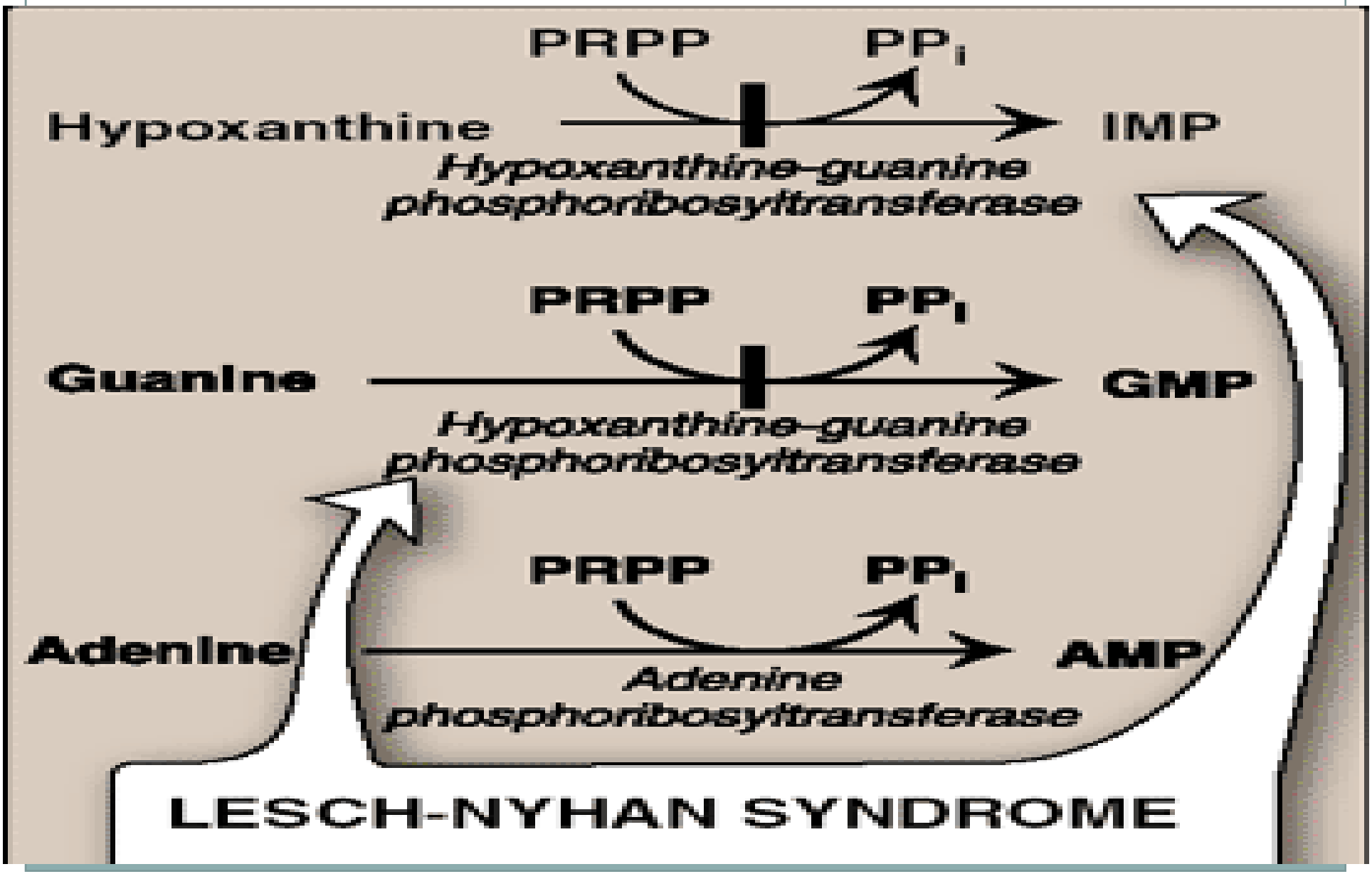
- Adenylate kinase = active in liver and muscle.
- Nucleoside diphosphate kinase = broad specificity.



Salvage Pathway

- Sources of NTP
  - from denovo synthesis
  - from the **diet.**
  - from normal **cellular turnover.**
- ***HGPRT (Hypoxanthine Guanine Phosphoribosyl Transferase)***

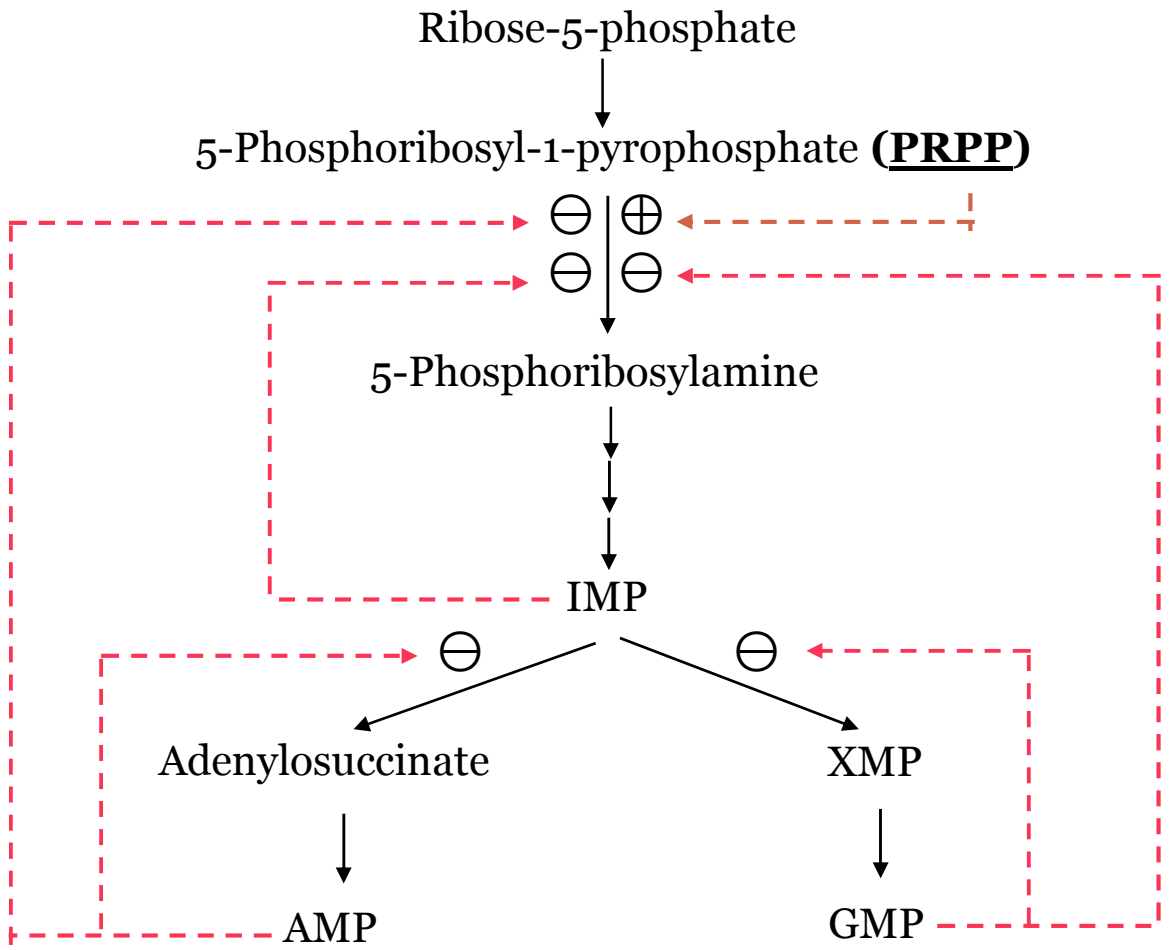
Salvage Pathway



Enzyme: Hypoxanthine-guanine phosphoribosyltransferase (HGPRTase)

hypoxanthine + PRPP ⟶ IMP + PP_i

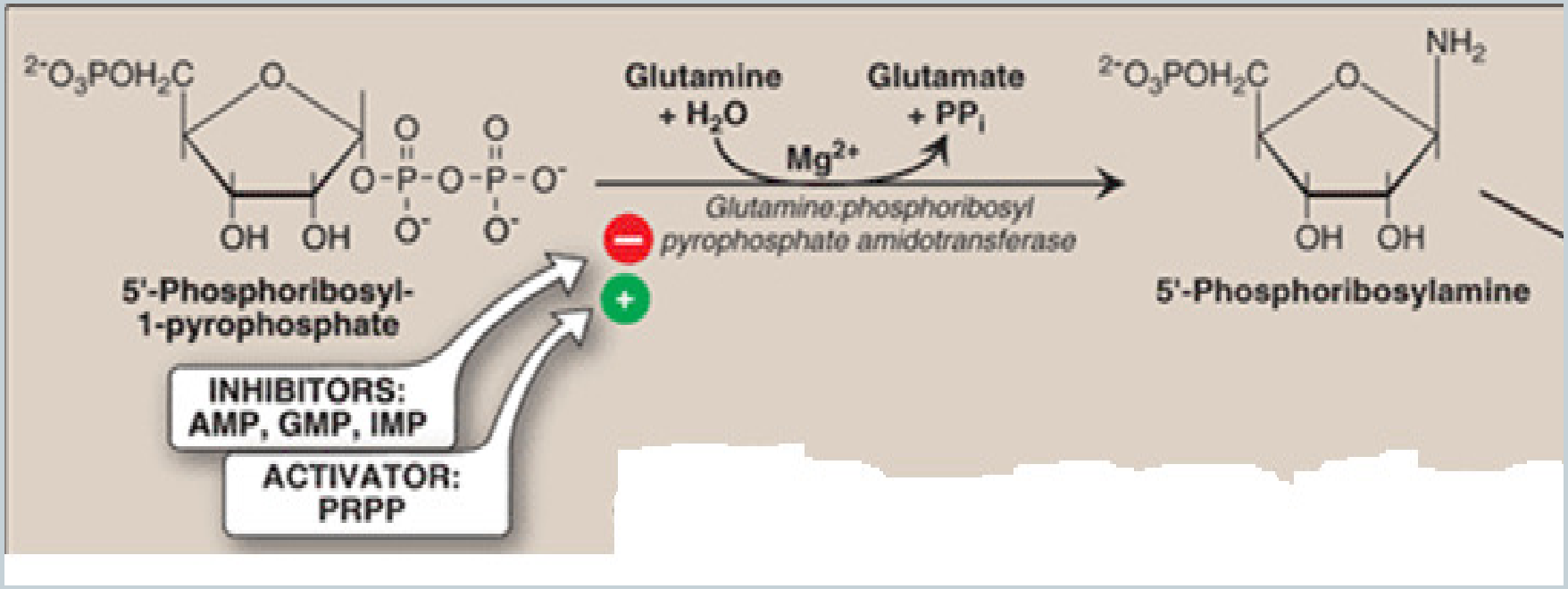
guanine + PRPP ⟶ GMP + PP_i



Lesch-Nyhan syndrome

- **Complete deficiency of HGPRT**.
- Inability to salvage hypoxanthine or guanine
- **Increased PRPP.**
- **Decreased IMP and GMP.**

- **What will be effect on following reaction of Denovo synthesis of Purine due to**
  - **Increased PRPP &**
  - **Decreased IMP and GMP ?**

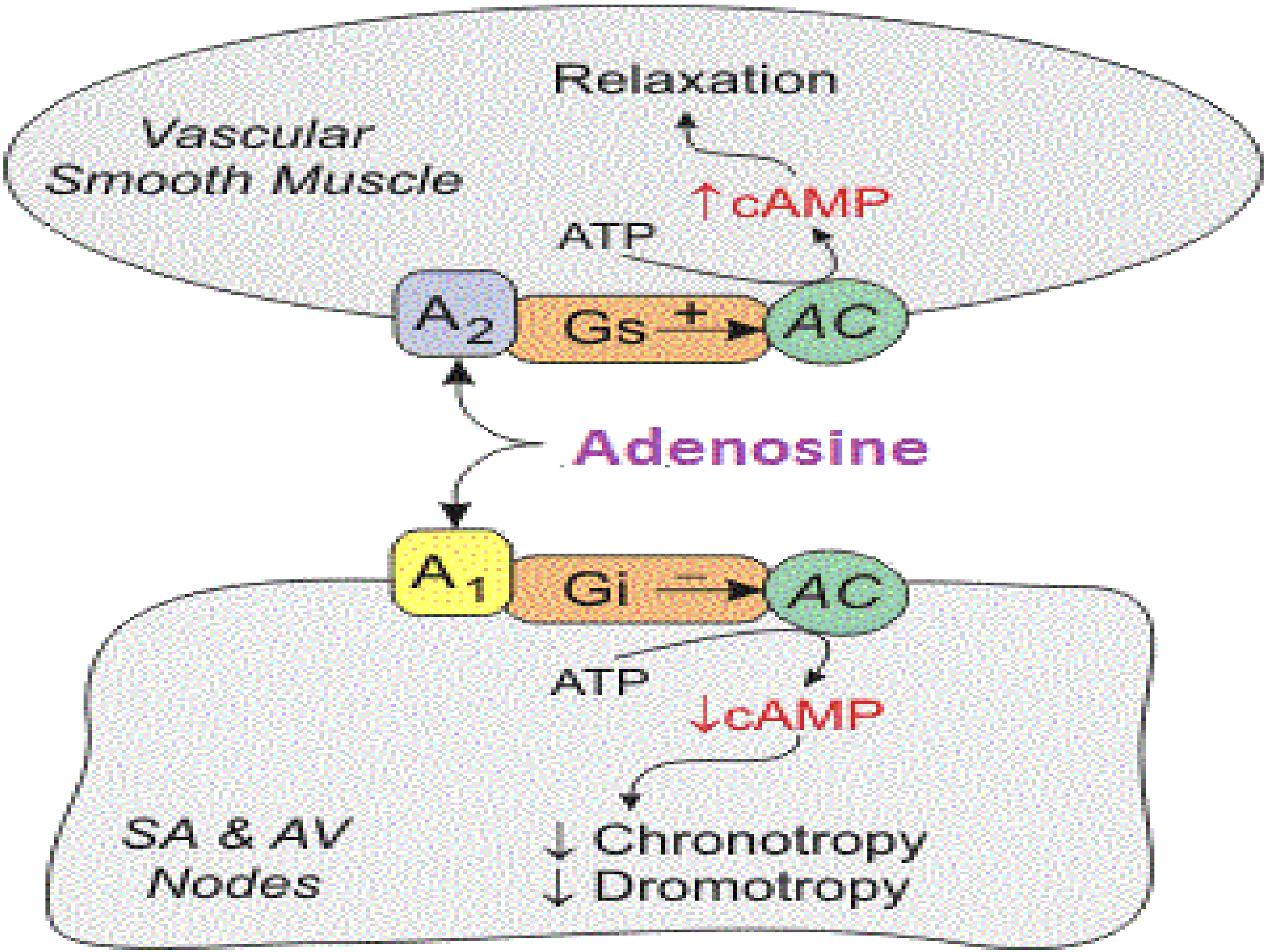


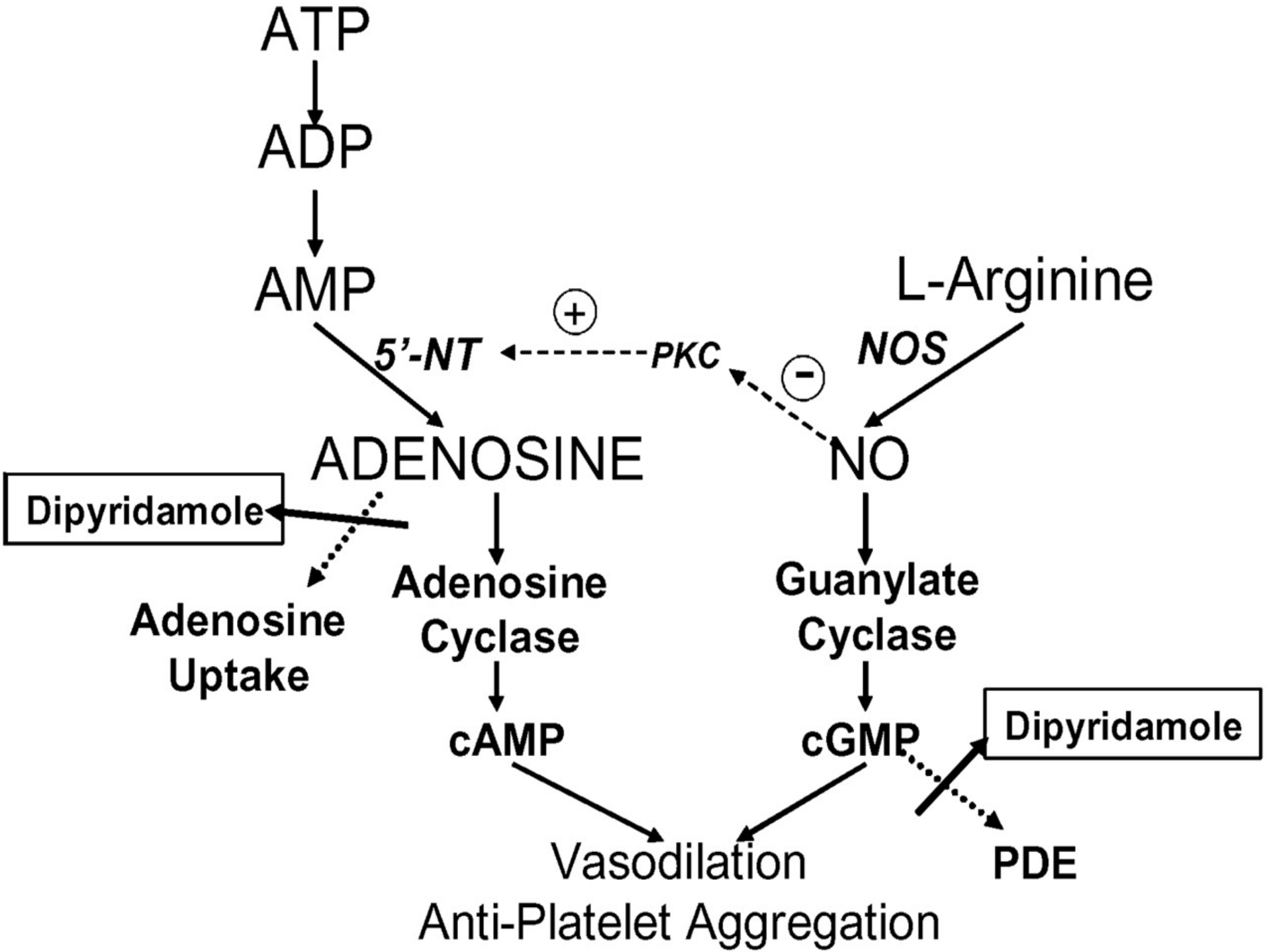
Lesch-Nyhan syndrome

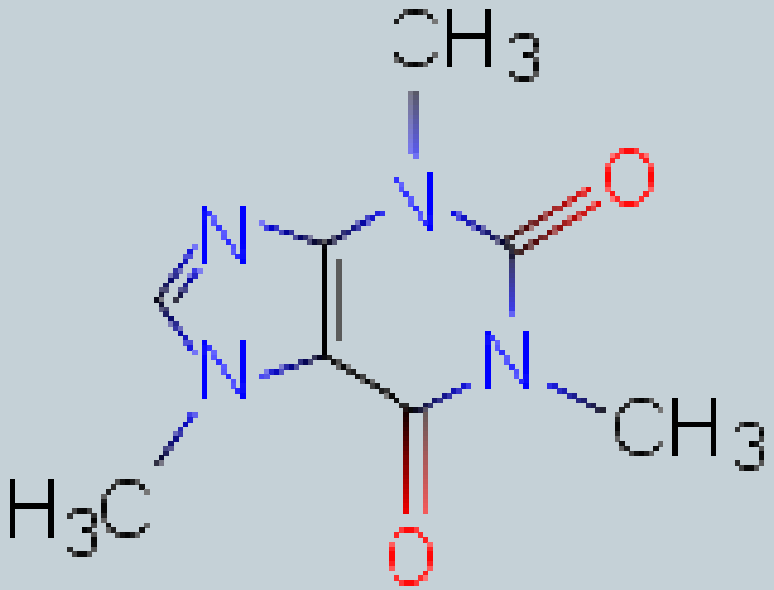
- For Glutamine:phosphoribosylpyrophosphate amidotransferase
  - excess substrate (PRPP)
  - decreased product (IMP)
- **Purine denovo synthesis is increased.**
- **Decreased purine reutilization.**
- Increased degradation of purines
- Production of **large amounts of uric acid (Hyperuricemia)**

Lesch-Nyhan syndrome

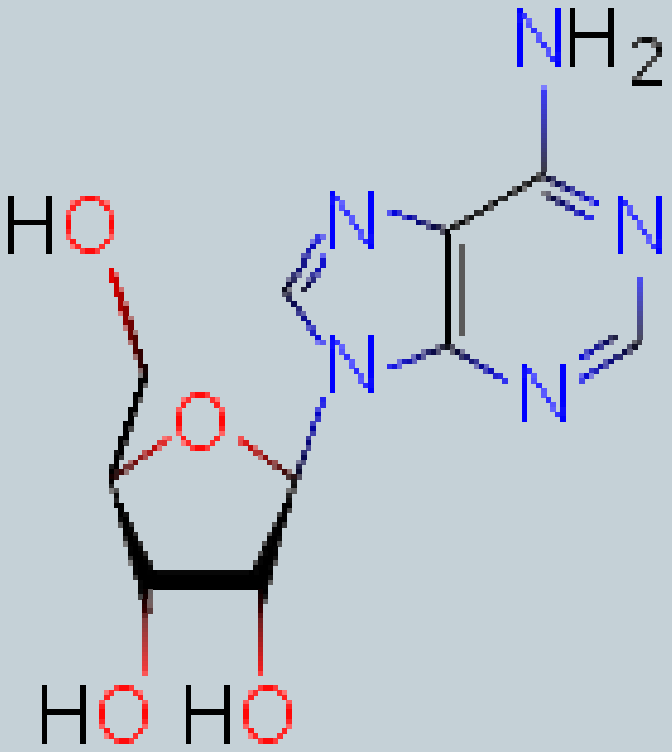
- Uric acid stones
- Gouty arthritis.
- Motor dysfunction, cognitive deficits
- *Self-mutilation (biting of lips and fingers).*


Relaxation
Vascular
Smooth Muscle
↑cAMP
ATP
A2
Gs
+
AC
Adenosine
A1
Gi
AC
ATP
↓cAMP
SA & AV
Nodes
↓ Chronotropy
↓ Dromotropy

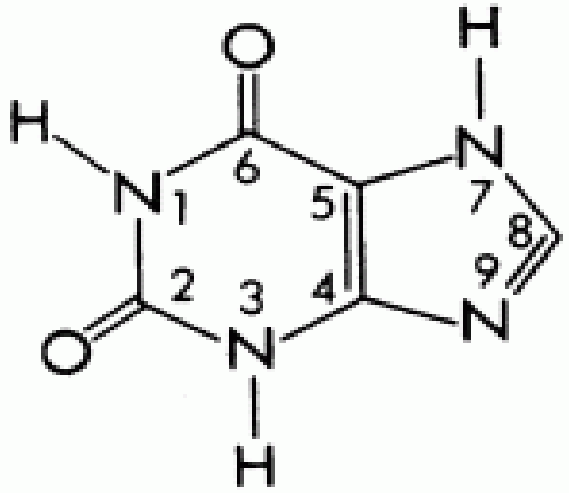

ATP
ADP
AMP
L-Arginine
+
5'-NT
PKC
NOS
−
ADENOSINE
NO
Dipyridamole
Adenosine Uptake
Adenosine Cyclase
Guanylate Cyclase
cAMP
cGMP
Dipyridamole
PDE
Vasodilation
Anti-Platelet Aggregation



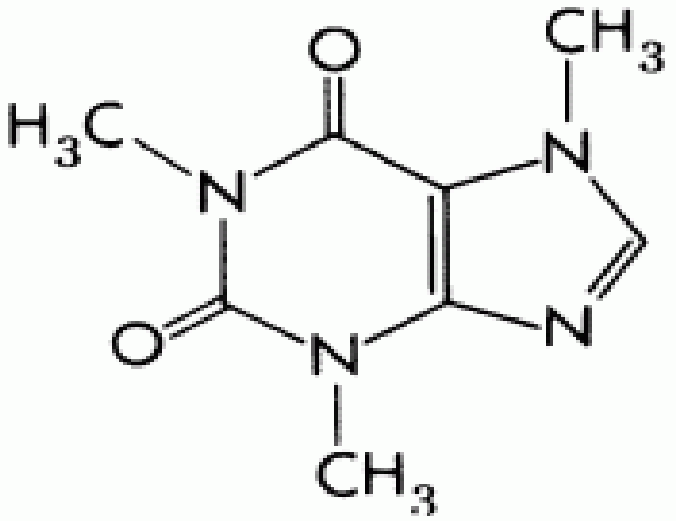
Caffeine



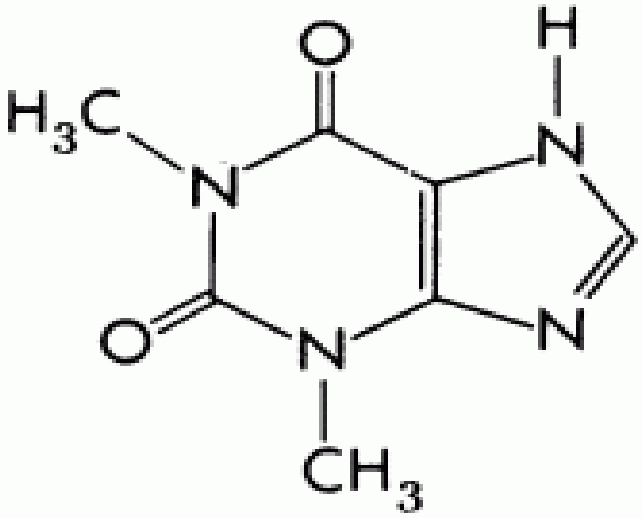
Adenosine



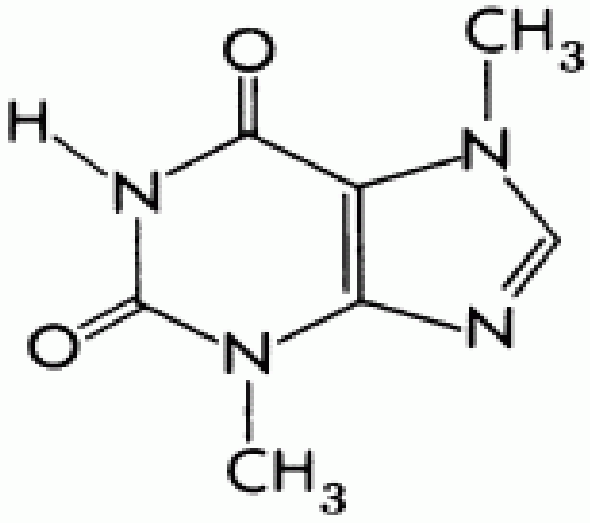
XANTHINE



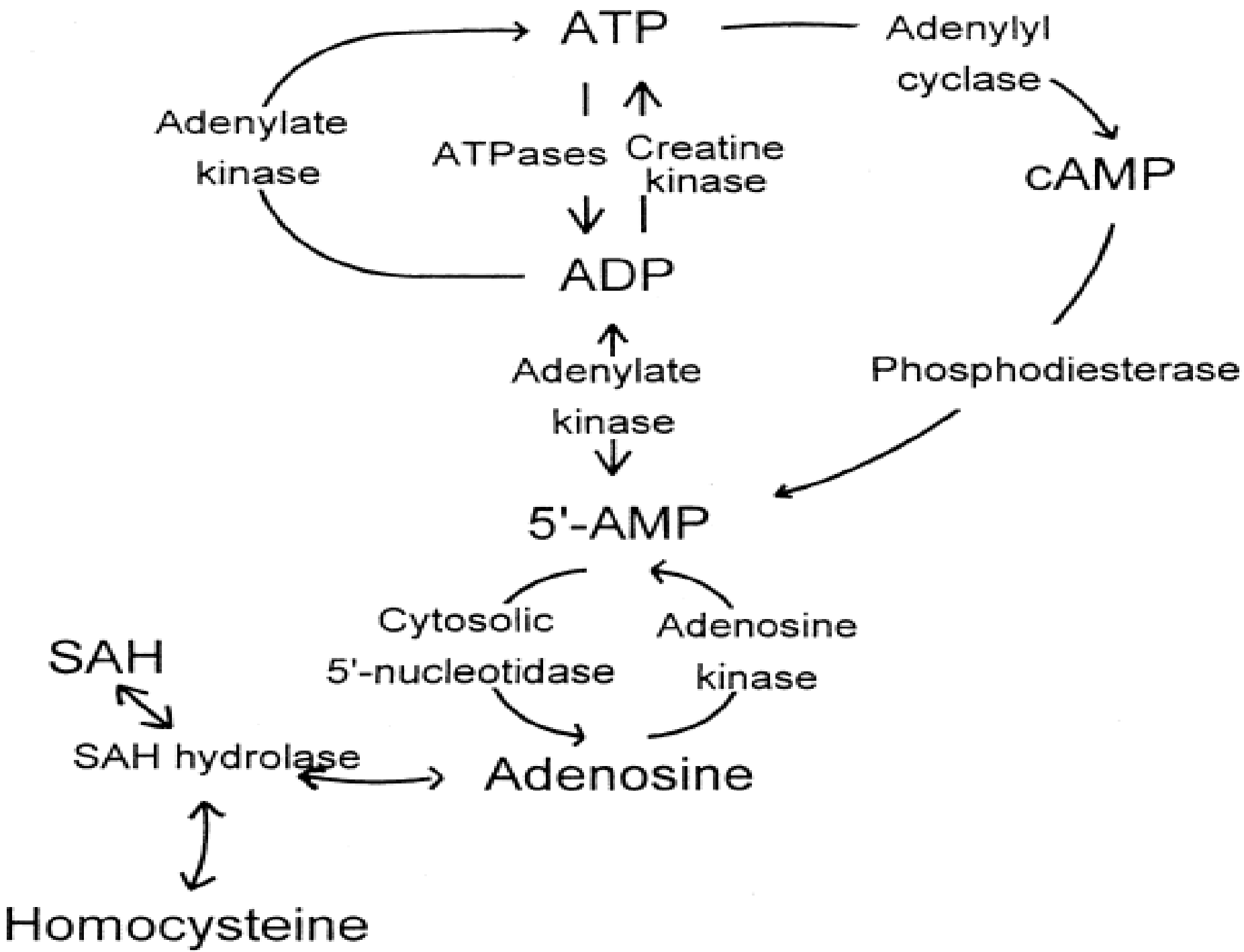
CAFFEINE



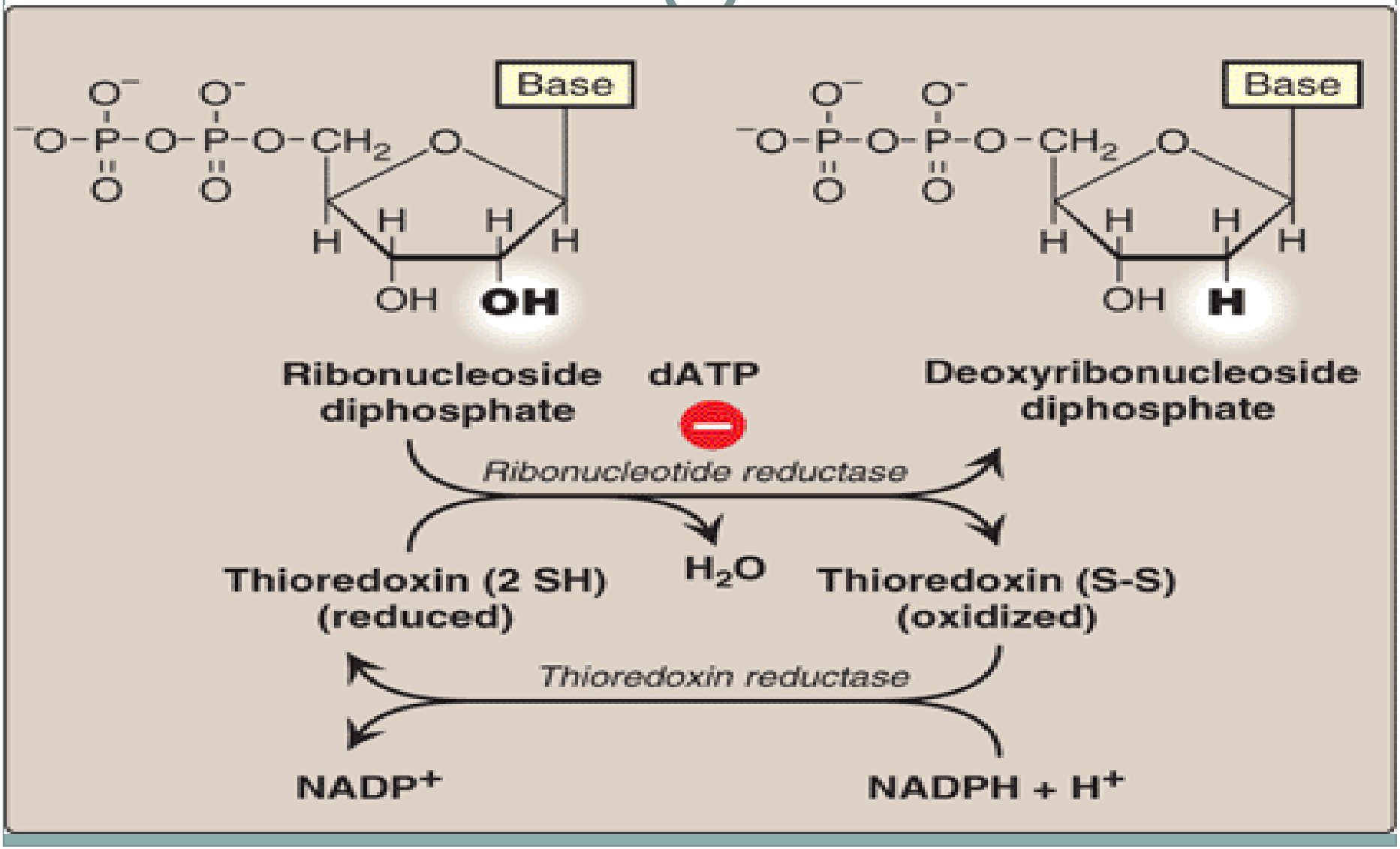
THEOPHYLLINE



THEOBROMINE


ATP
Adenylyl cyclase
Adenylate kinase
ATPases
Creatine kinase
cAMP
ADP
Adenylate kinase
Phosphodiesterase
5'-AMP
Cytosolic 5'-nucleotidase
Adenosine kinase
SAH
SAH hydrolase
Adenosine
Homocysteine

Synthesis of Deoxyribonucleotides



Regulation of Deoxyribonucleotides

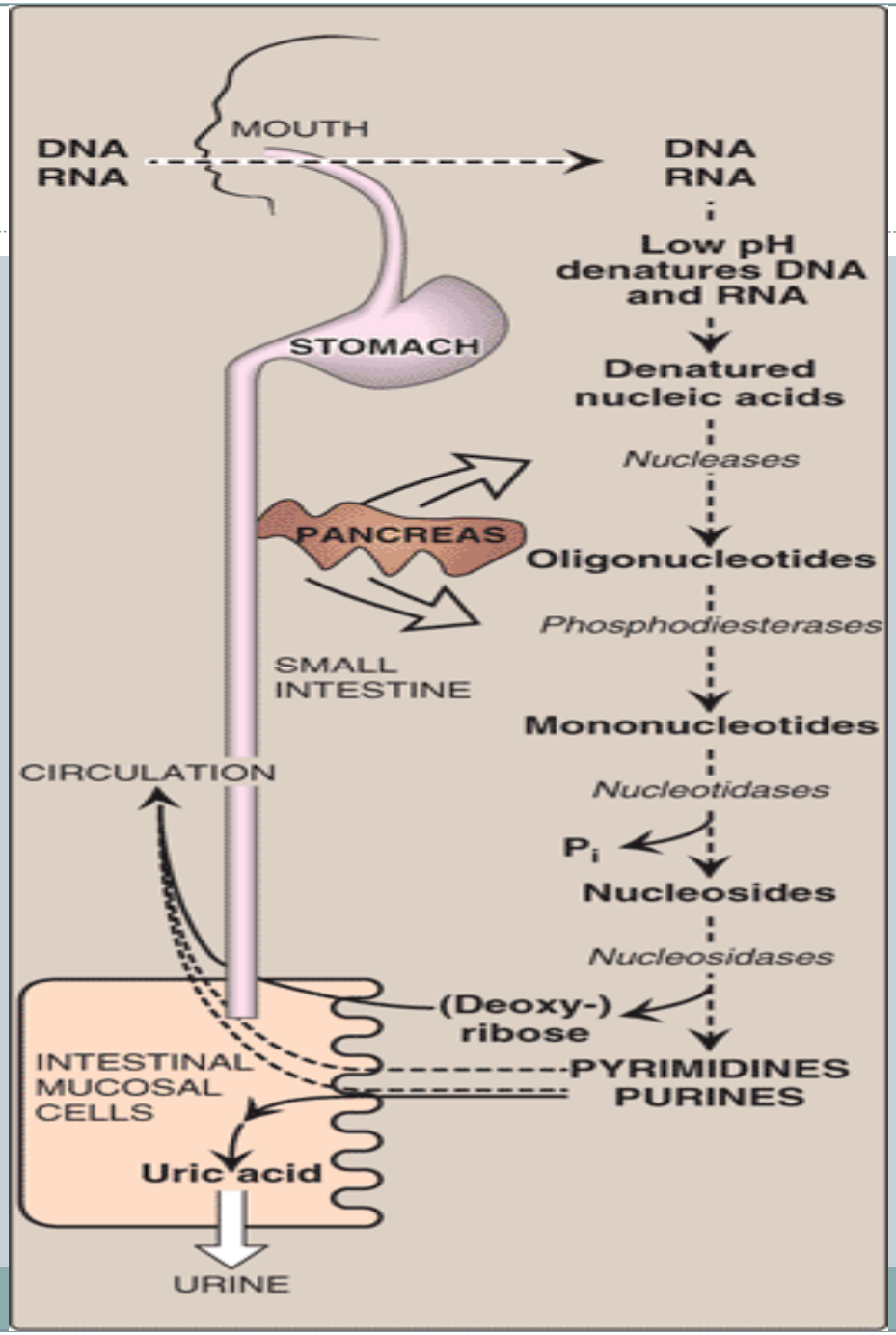
- Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain
- **dATP**
  - **Allosteric inhibit enzyme**
  - **Inhibit reduction of all four nucleoside diphosphates.**
- **dATP increase = in Adenosine deaminase deficiency**
- This effectively prevents DNA synthesis
- **Hydroxyurea** destroys the free radical required for enzyme activity of ribonucleotide reductase
- Used in Cancer treatment.
- E.g. Chronic Myelogenous Leukemia.

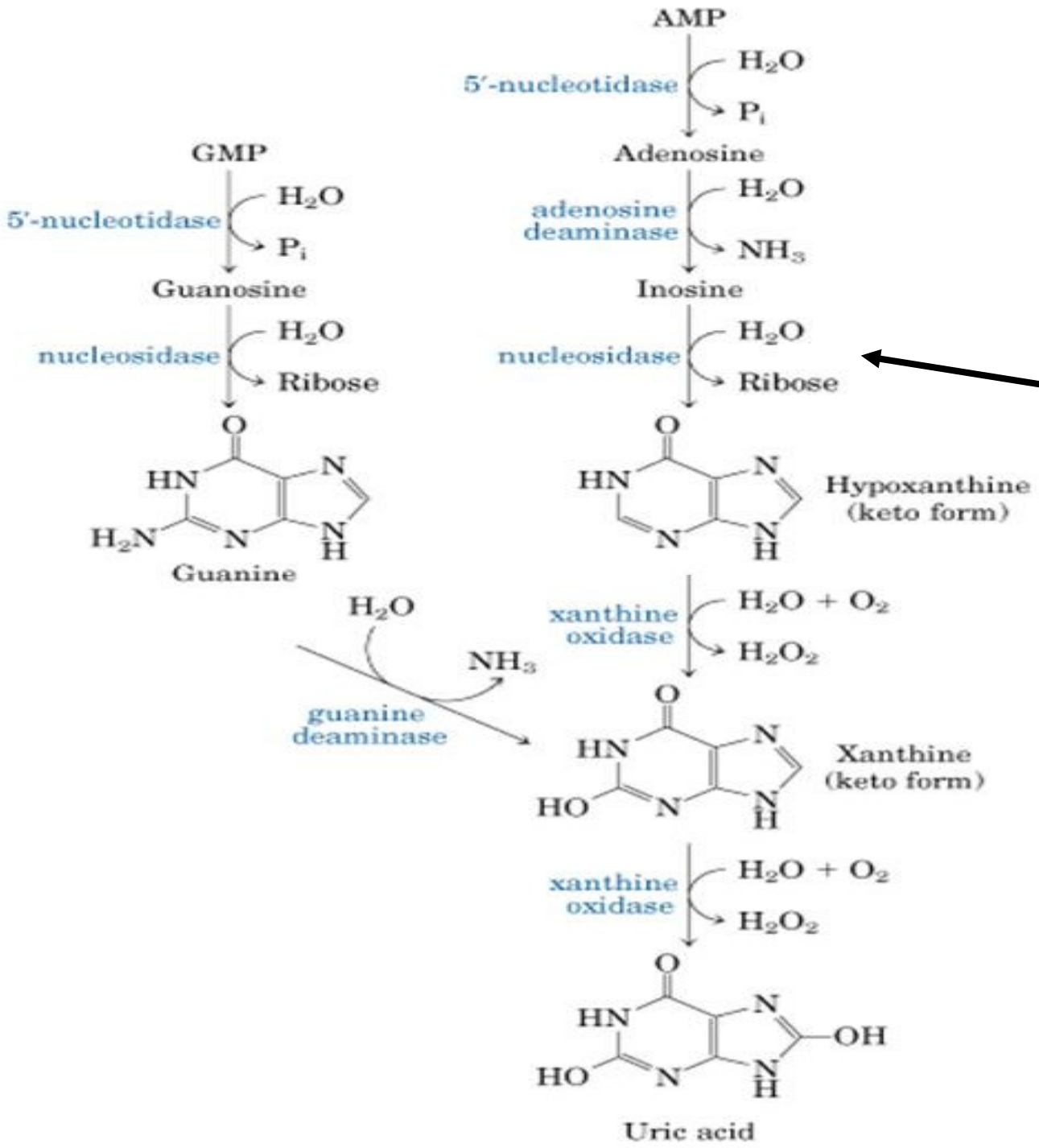
Purine Degradation

- Occurs in the small intestine
- Pancreatic enzymes hydrolyzes the nucleotides to nucleosides and free bases.
- Inside cells, **Purine nucleotides = Uric acid**.
- In Mammals & Some organism
  - **Uric acid = Allantoin.**
  - **Uric acid = Urea / Ammonia.**
- Humans excrete about 0.6 g uric acid every 24 hours.

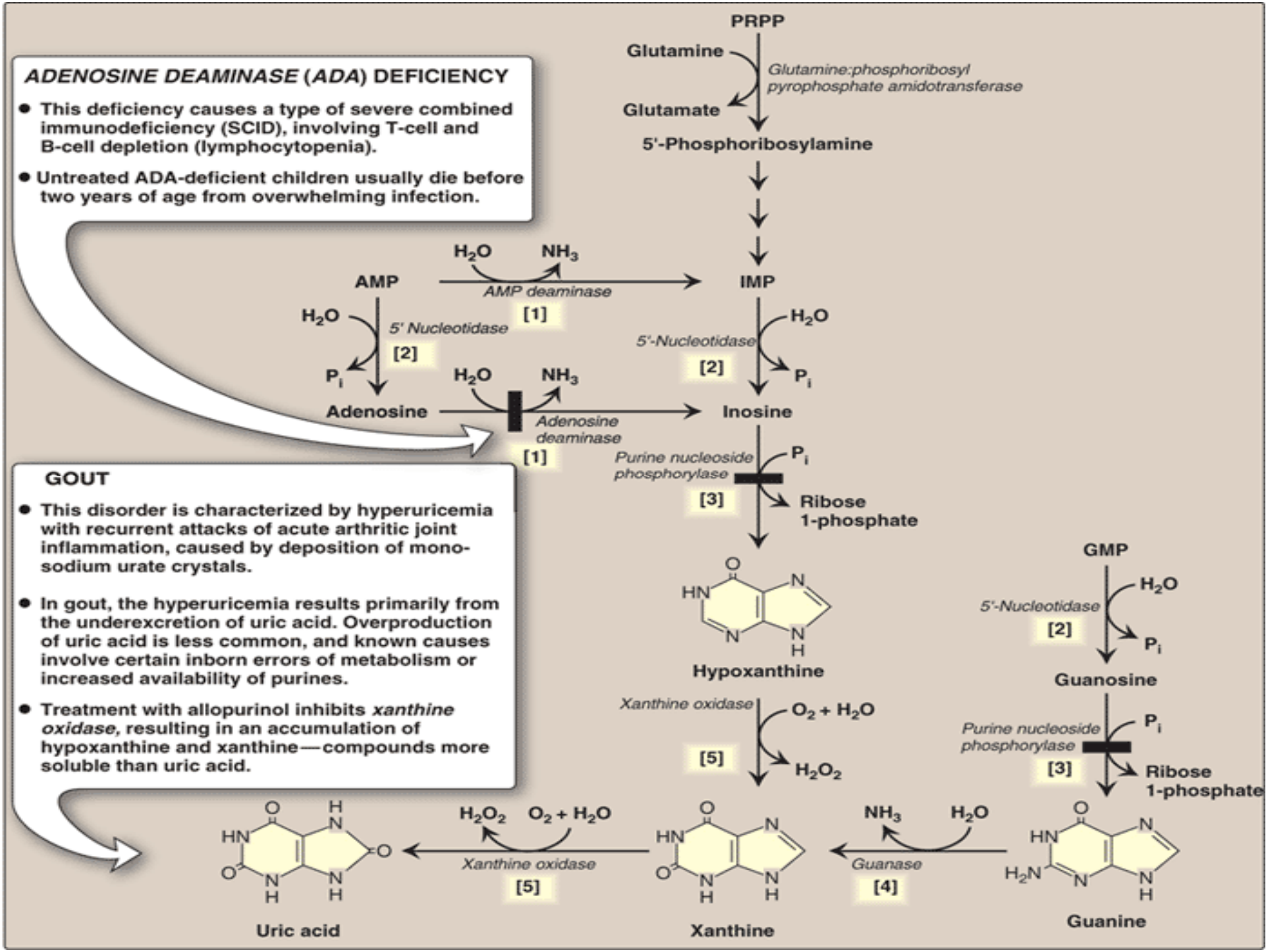
Degradation of Dietary Nucleotide

- Pancrease Release
  - Ribonucleases and Deoxyribonucleases
    - hydrolyze RNA and DNA to Oligonucleotides.
  - Pancreatic phosphodiesterases
    - Oligonucleotides to 3′- and 5′-mononucleotides.
  - Nucleotidases
    - Removes phosphate = Nucleosides
    - Nucleosides = Free bases.
- Purines and pyrimidines are not used for synthesis of tissue nucleic acids.
- Dietary purines = Uric acid


DNA
RNA
MOUTH
DNA
RNA
Low pH
denatures DNA
and RNA
STOMACH
Denatured
nucleic acids
Nucleases
PANCREAS
Oligonucleotides
Phosphodiesterases
SMALL
INTESTINE
Mononucleotides
CIRCULATION
Nucleotidases
P_i
Nucleosides
Nucleosidases
(Deoxy-)
ribose
INTESTINAL
MUCOSAL
CELLS
PYRIMIDINES
PURINES
Uric acid
URINE



The enzyme "nucleotidase" is also known as purine nucleotide phosphorylase (PNP)


ADENOSINE DEAMINASE (ADA) DEFICIENCY
This deficiency causes a type of severe combined immunodeficiency (SCID), involving T-cell and B-cell depletion (lymphocytopenia).
Untreated ADA-deficient children usually die before two years of age from overwhelming infection.
GOUT
This disorder is characterized by hyperuricemia with recurrent attacks of acute arthritic joint inflammation, caused by deposition of mono-sodium urate crystals.
In gout, the hyperuricemia results primarily from the underexcretion of uric acid. Overproduction of uric acid is less common, and known causes involve certain inborn errors of metabolism or increased availability of purines.
Treatment with allopurinol inhibits xanthine oxidase, resulting in an accumulation of hypoxanthine and xanthine—compounds more soluble than uric acid.
PRPP
Glutamine
Glutamate
Glutamine:phosphoribosyl pyrophosphate amidotransferase
5'-Phosphoribosylamine
AMP
IMP
H2O
NH3
AMP deaminase
[1]
5' Nucleotidase
[2]
5'-Nucleotidase
Pi
Adenosine
Inosine
Adenosine deaminase
Purine nucleoside phosphorylase
[3]
Ribose 1-phosphate
Hypoxanthine
GMP
Guanosine
Guanine
Xanthine oxidase
O2 + H2O
H2O2
[5]
Guanase
[4]
Xanthine
Uric acid

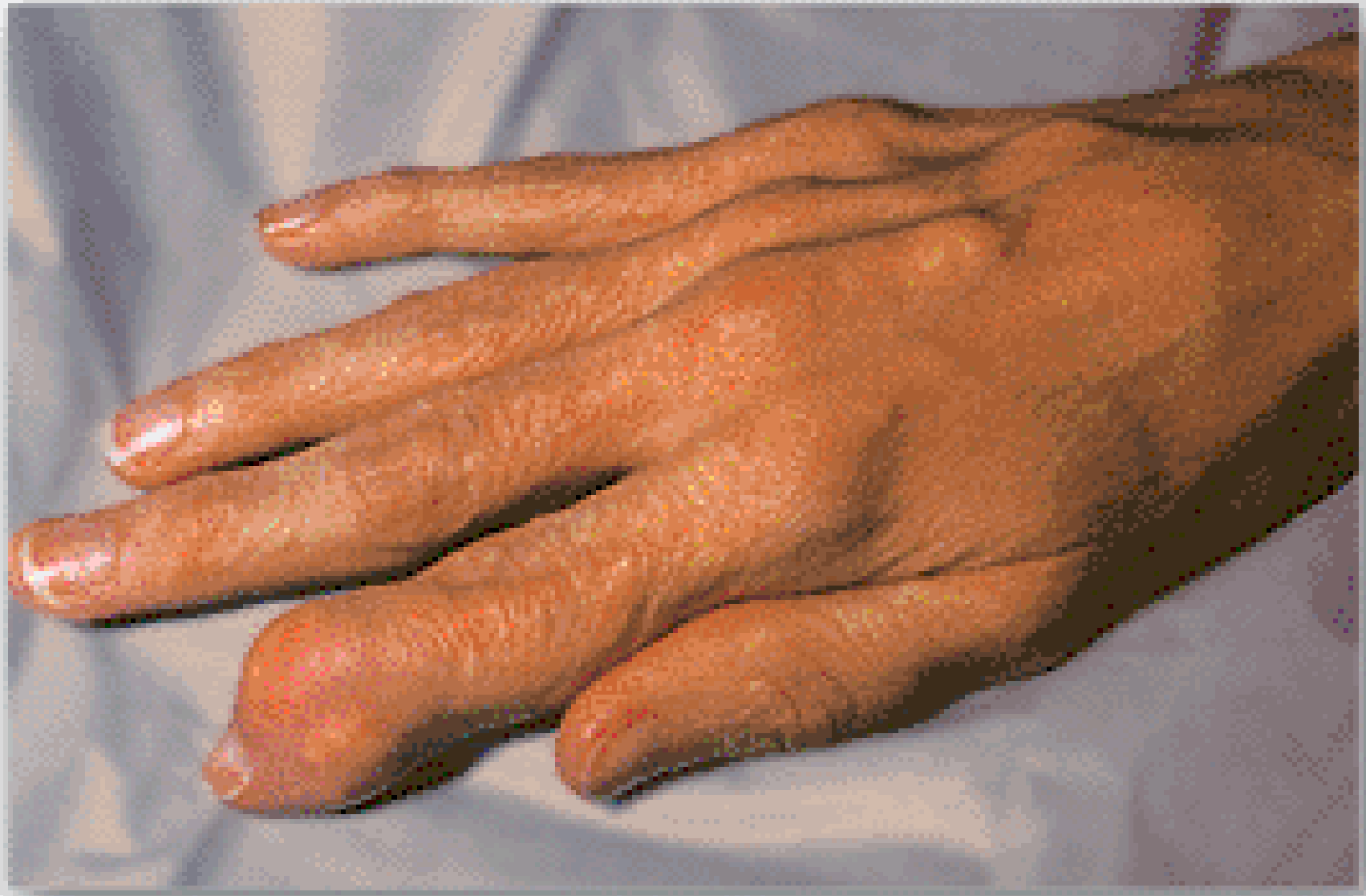
GOUT

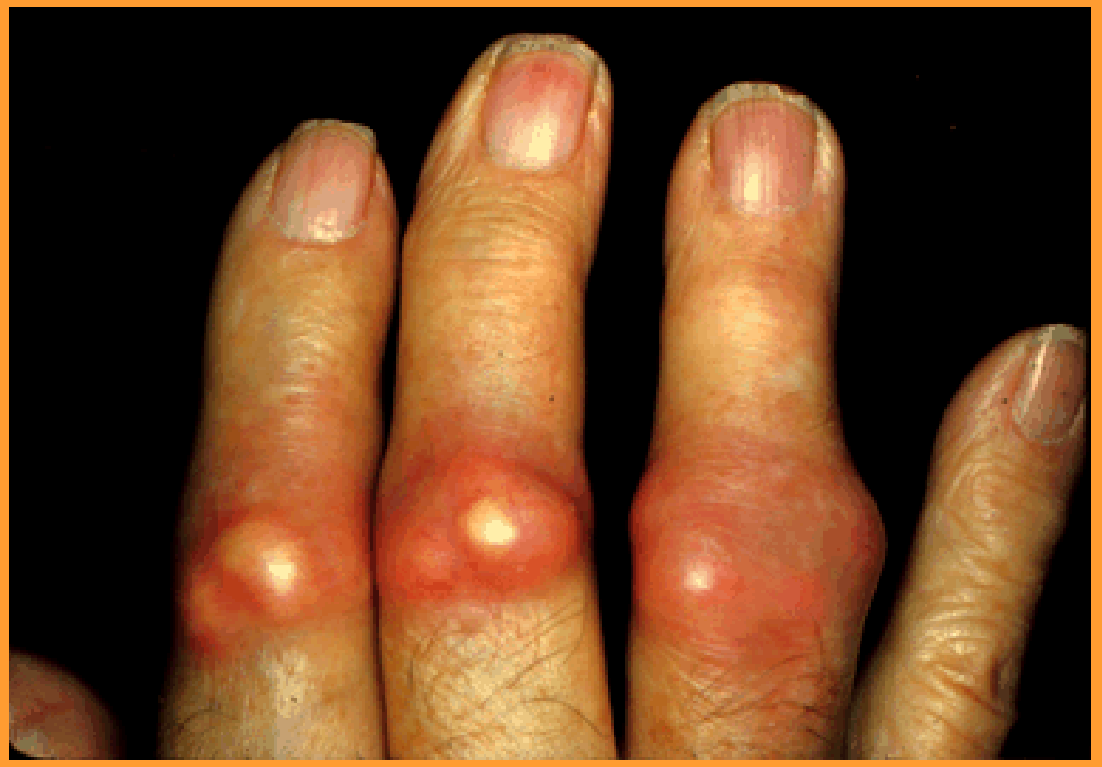
- Accumulation of **Monosodium Urate crystals** in synovial fluid
- Inflammation in surrounding area = **Acute Arthiritis**.
- At 30 °C & in acidic pH solubility is lower.
- Deposited in cooler areas of body.
- **Tophi = M**ass of monosodium urate crystals
- Deposited in the soft tissues
- Deposition of uric acid crystals in the urinary tract.
- Stone damage to kidney

URIC ACID

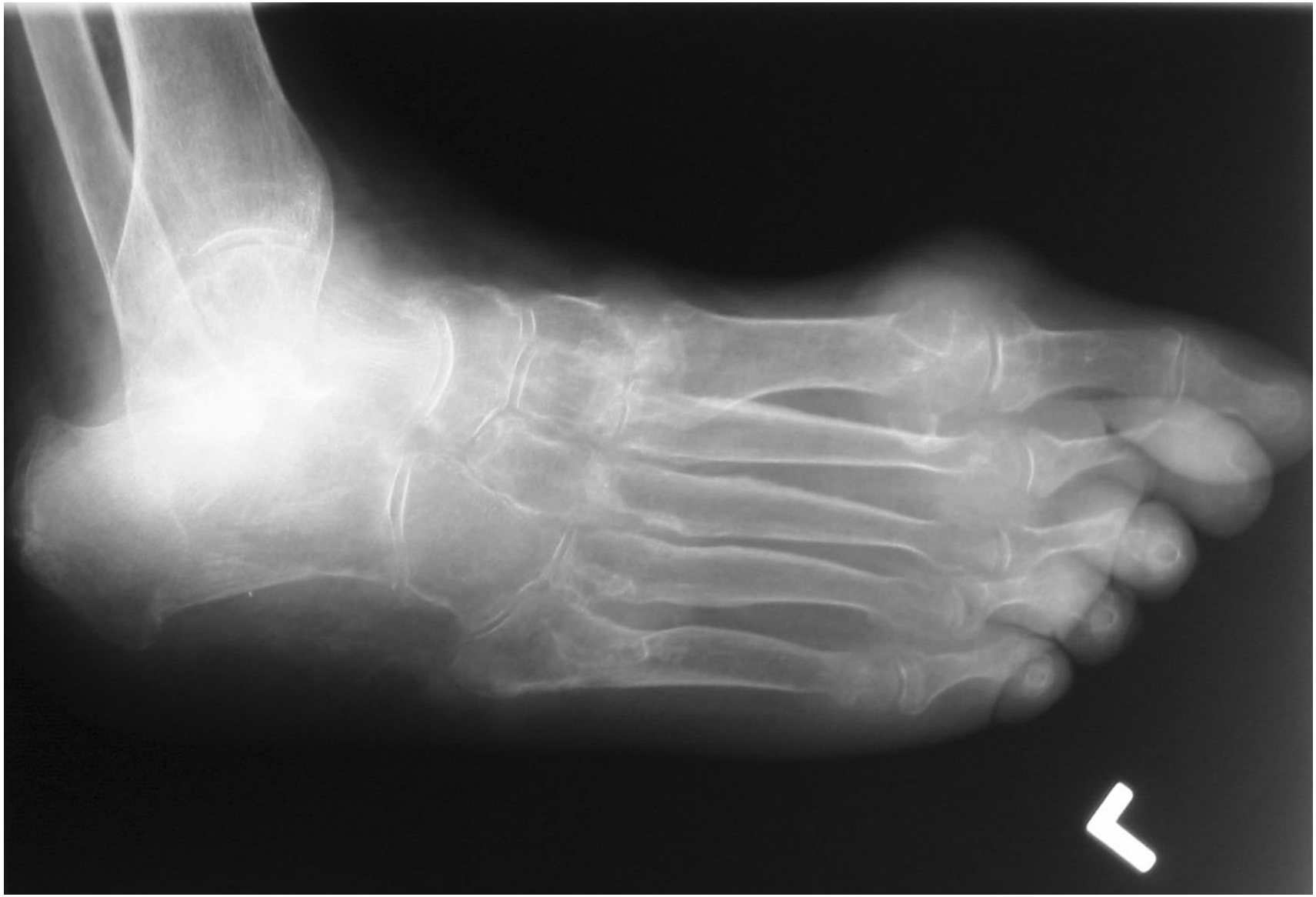
- Serum Uric acid = 2-5 mg/dl in females
  = 3-7 mg/dl in males.
- Elevated uric acid = hyperuricaemia (> 7mg/dl)
- The manifestation are due to low solubility of uric acid in water.

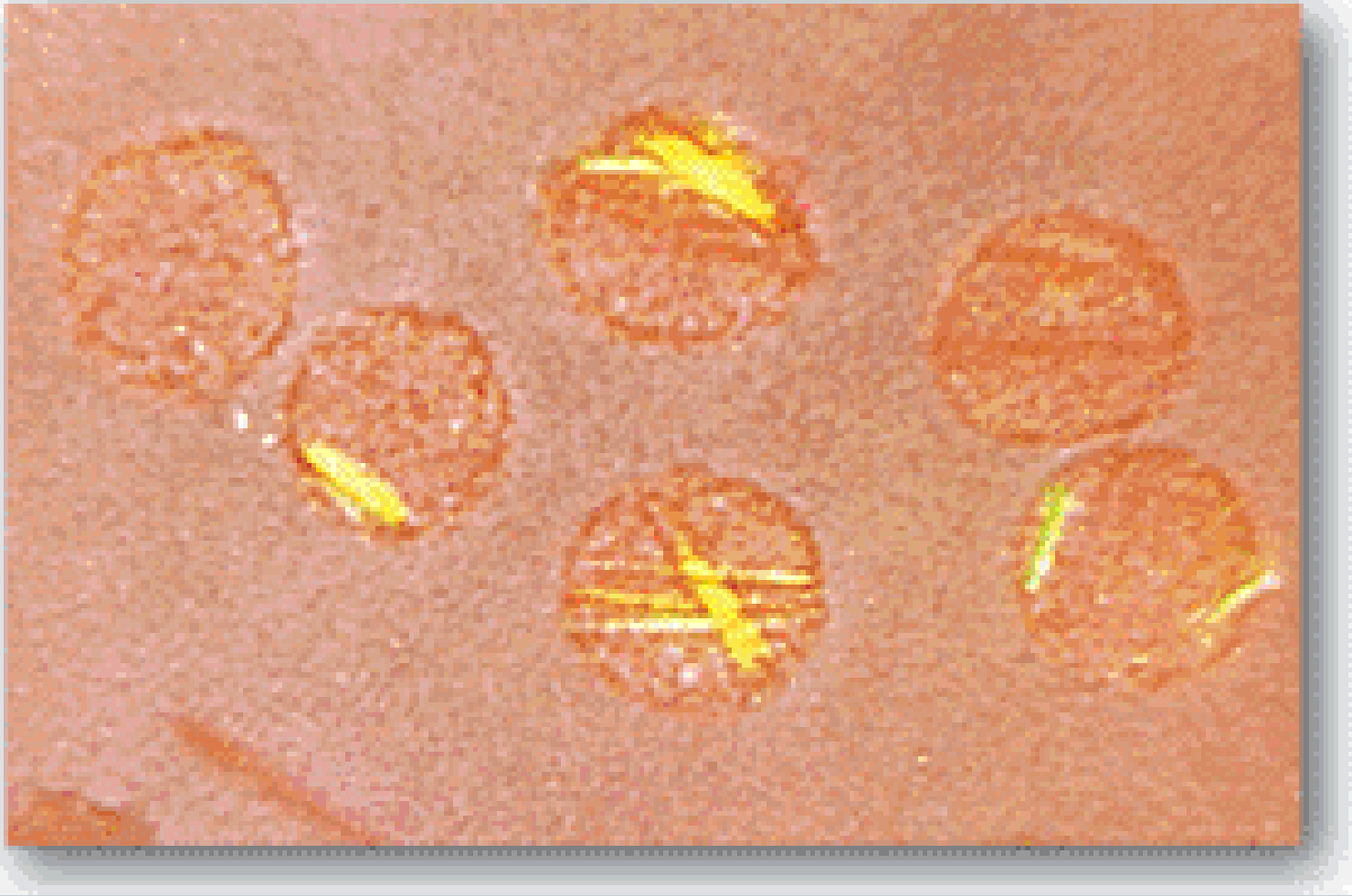
Tophi





Inflamed tophaceous gout Three inflamed tophi over the proximal interphalangeal joints in a patient with chronic tophaceous gout. Several of the lesions ruptured spontaneously over the next three days, exuding a pasty material composed of urate crystals and inflammatory cells but no organisms. The inflammation largely subsided over one week after the administration of a nonsteroidal antiinflammatory drug. Courtesy of Michael A Becker, MD.

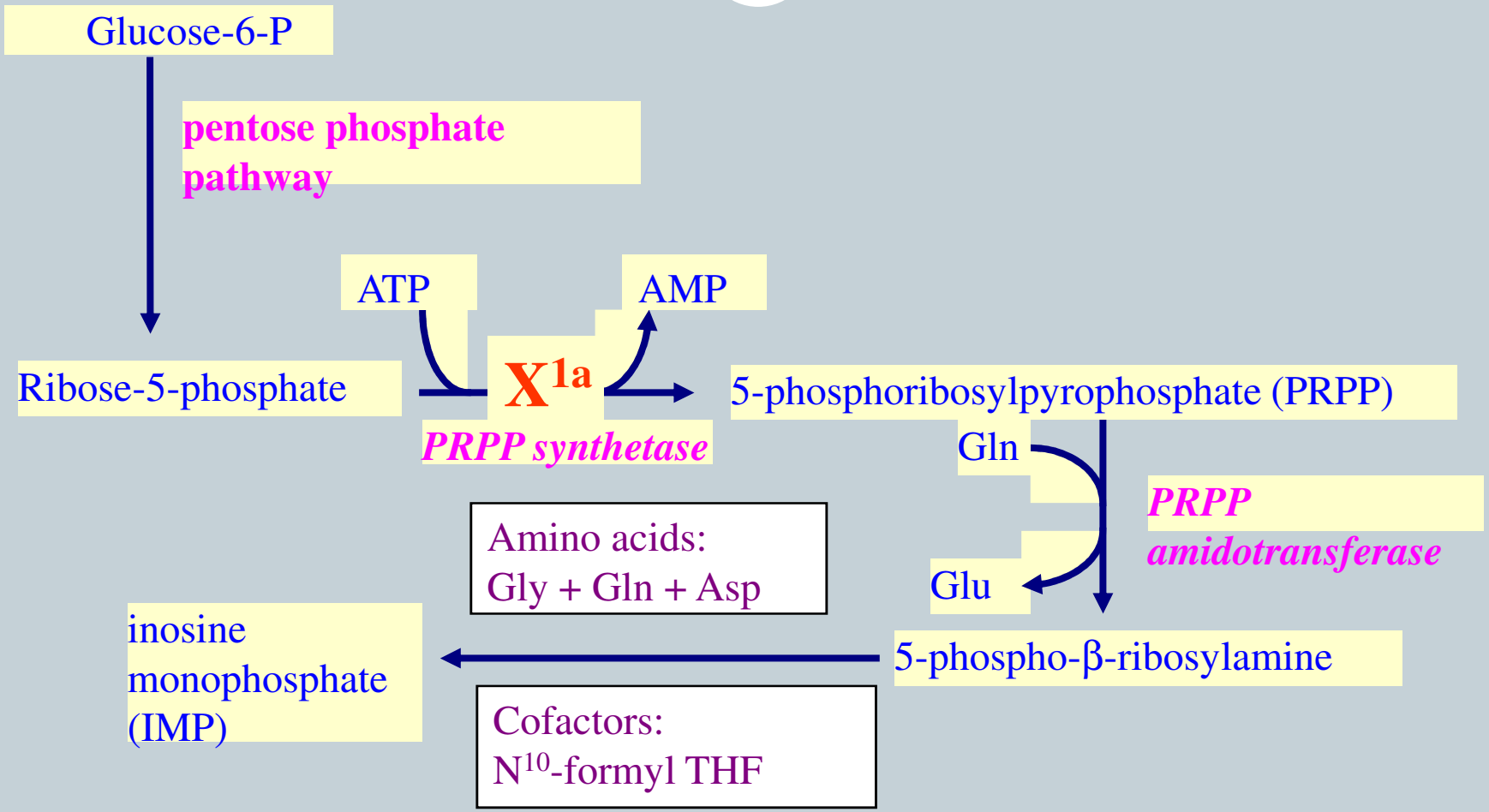


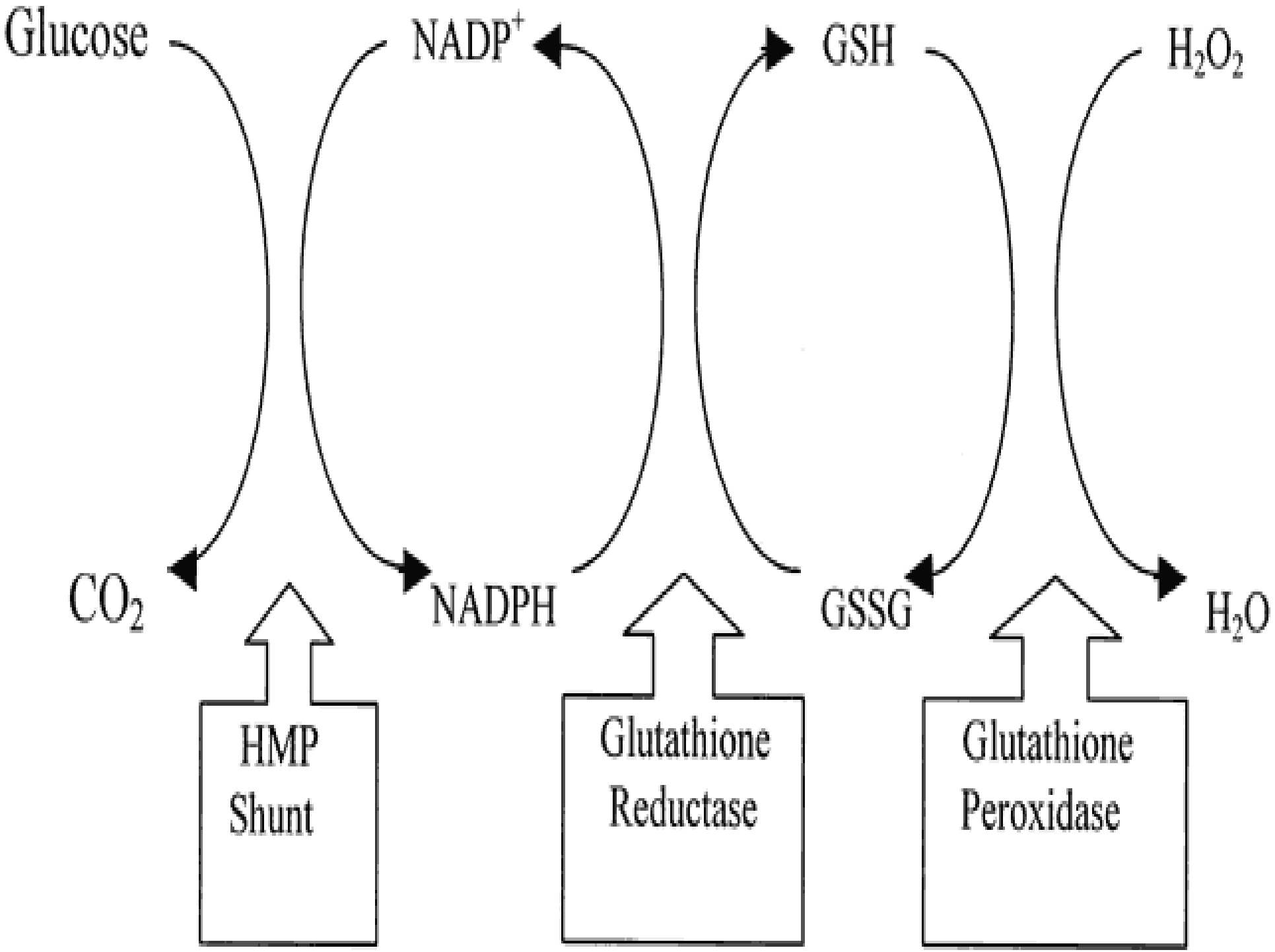


- Synovial Fluid Examination in microscope
- Light microscopy = Presence of needle-shaped monosodium urate crystals

Primary hyperuricaemia

- **Over activity of 5-phosphoribosyl amido transferase**
- **Over activityof PRPP synthase activity**
  - Increased V_{max} & lower K_m for ribose 5-phosphate
- **Deficiency of enzymes of salvage pathway**
  - Lesch-Nyhan syndrome
  - increased availability of PRPP.
- **Glucose-6-phosphatase deficiency**
  - Von Gierke disease
- **Glutathione reductase variant**


Glucose-6-P
pentose phosphate pathway
ATP
AMP
Ribose-5-phosphate
X1a
PRPP synthetase
5-phosphoribosylpyrophosphate (PRPP)
Gln
PRPP amidotransferase
Glu
Amino acids:
Gly + Gln + Asp
inosine monophosphate (IMP)
5-phospho-β-ribosylamine
Cofactors:
N10-formyl THF


Glucose
NADP+
GSH
H2O2
CO2
NADPH
GSSG
H2O
HMP Shunt
Glutathione Reductase
Glutathione Peroxidase

Secondary Hyperuricaemia

Increased production of uric acid

- leukemias,lymphomas,polycythemias
- Radiotherapy
- Chemotherapy
- Raised rate of catabolism in starvation

Reduced excretion of uric acid

- Renal failure
- Lactic acidosis
- Ketoacidosis
- Thiazide diuretics (inhibit secretion of uric acid)

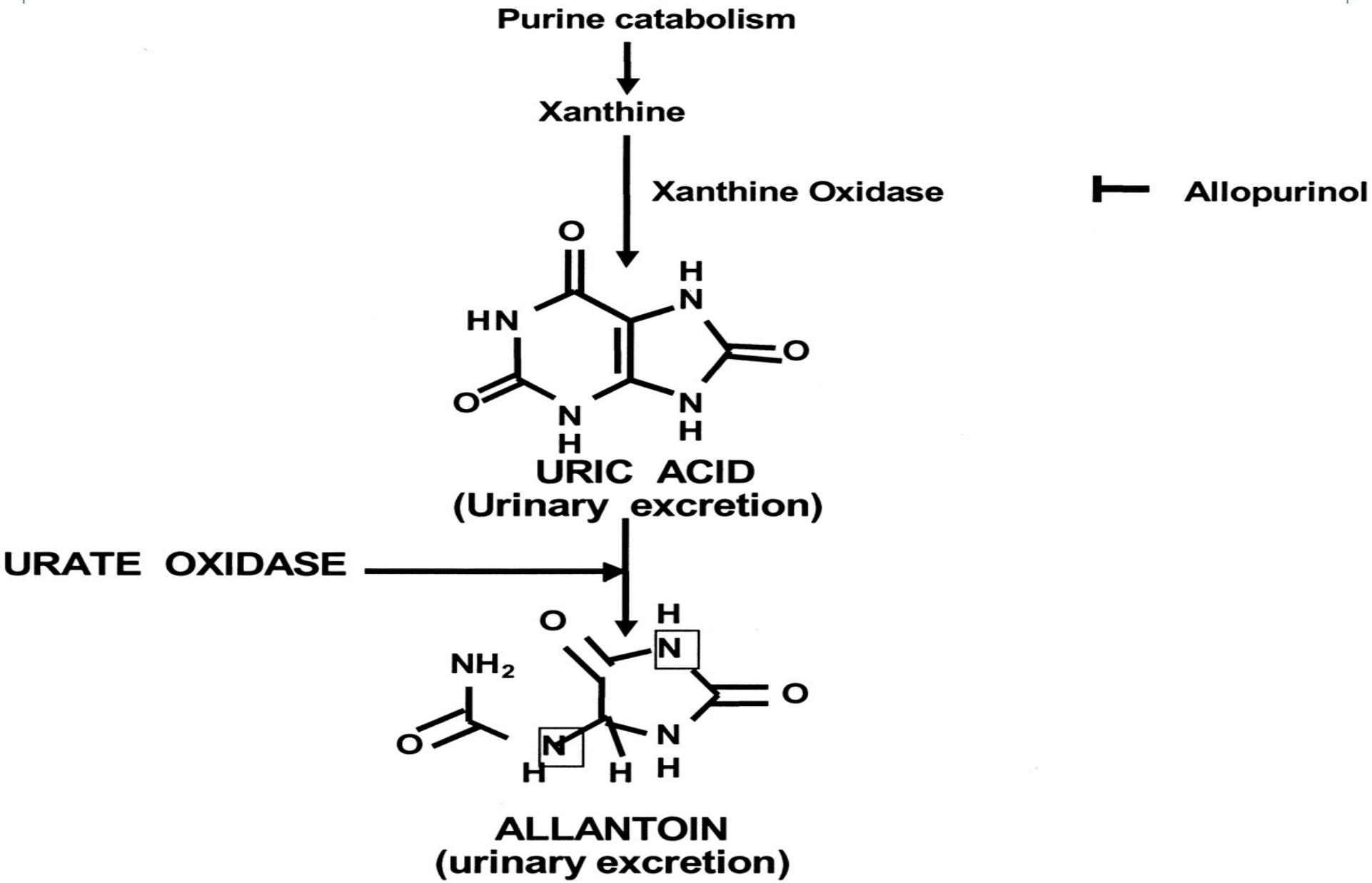
Clinical features

- Gouty attacks may be precipitated by high purine and high intake of alcohol.
- Alcohol leads to accumulation of lactic acid.
- Metatarsophalangeal joints.
- Extremely painful.
- Synovial fluid will bifringent urate crystals.
- (**Tophi**) = Chronic cases uric acid deposited in joints.
- Deposition of urate crystal in renal = urolithiasis & renal damage.

Treatment

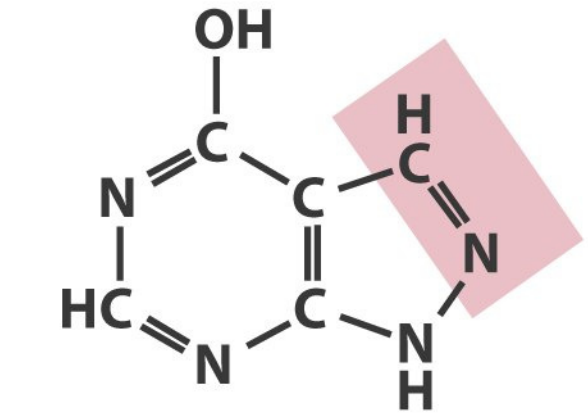
- **In Acute attack**
  - **Colchicine**
  - **Prednisone**
  - **Indomethacin.**
- **Uricosuric agents = Probinoside**
- **Reduce urate production**
- **Allopurinol & Febuxostat,**
- Allopurinol is analogue of hypoxanthine.
- Xanthine and hypoxanthine are more soluble & so are excreted more easily.

Recombinant Urate Oxidase
Resburicase (Fasturtec)

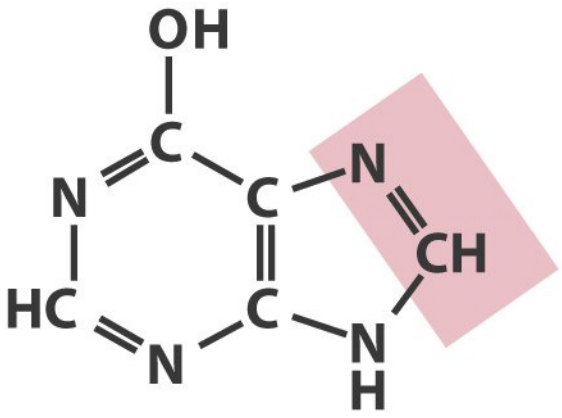


Precipitating Factor

- Excessive consumption of ethanol.
- Organ meats, anchovies, sardines, and legumes by diet.

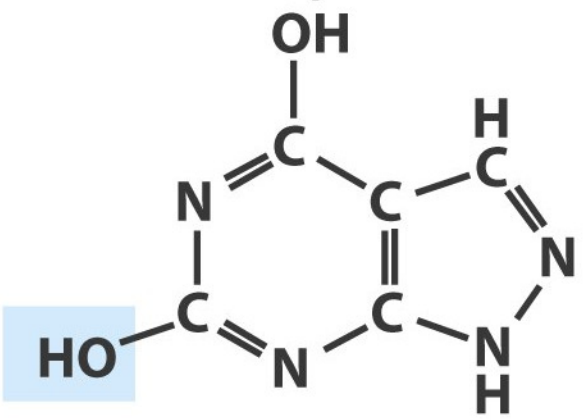


Allopurinol



Hypoxanthine
(enol form)

xanthine
oxidase



Oxypurinol

Excess uric acid causes gout, treated with allopurinol, inhibitor of xanthine oxidase

Adenosine deaminase (ADA) deficiency

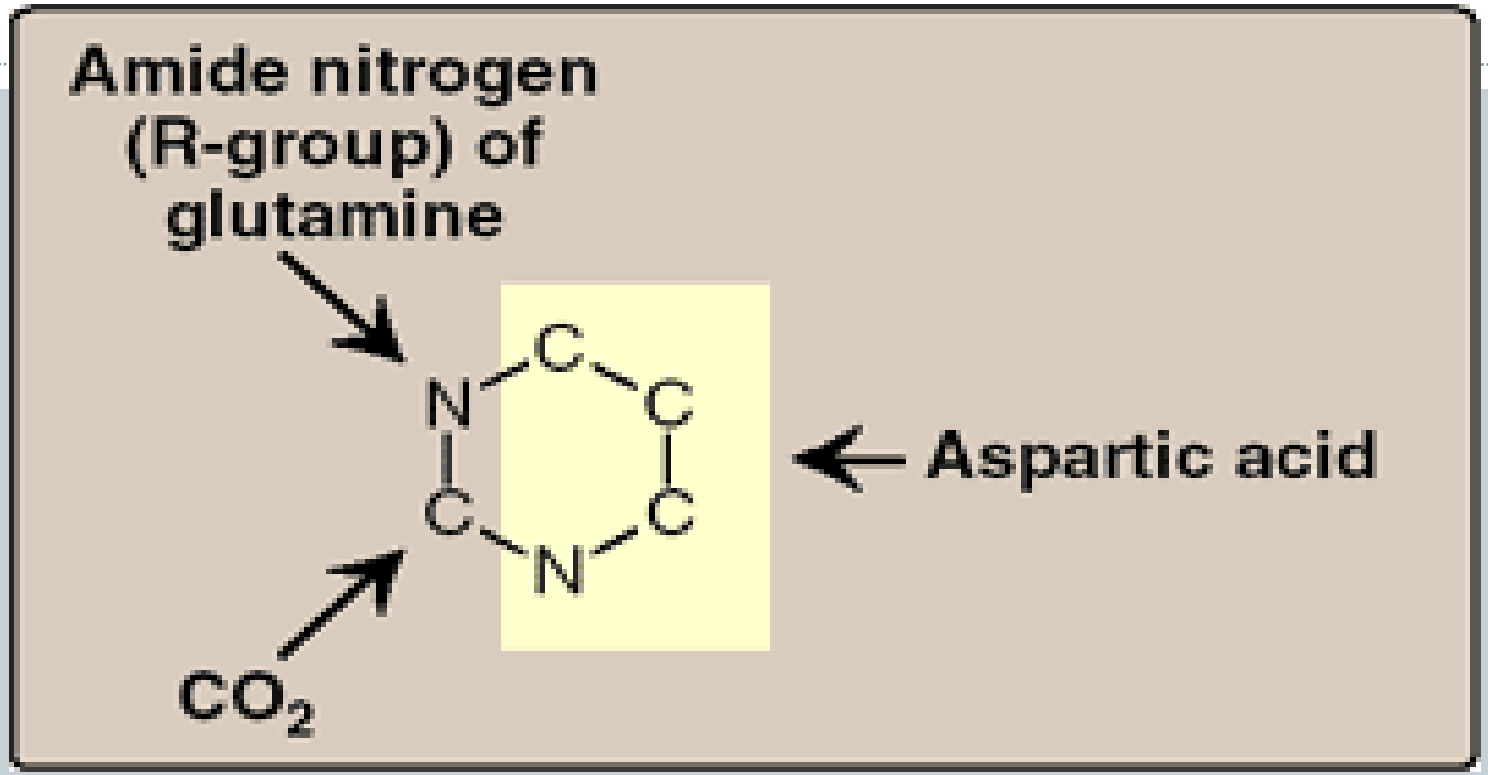
- ADA is expressed in the cytosol of all cells
- Lymphocytes have the highest activity of this enzyme.
- Accumulation of adenosine
- Increrase Adenosine =

  = Increse Ribonucleotide or Deoxyribonucleotide

  = Increase dATP levels.
- Ribonucleotide reductase is inhibited
- Inhibit production of all deoxyribose-containing nucleotides.
- Decrease dGTP, dCTP, dTTP production

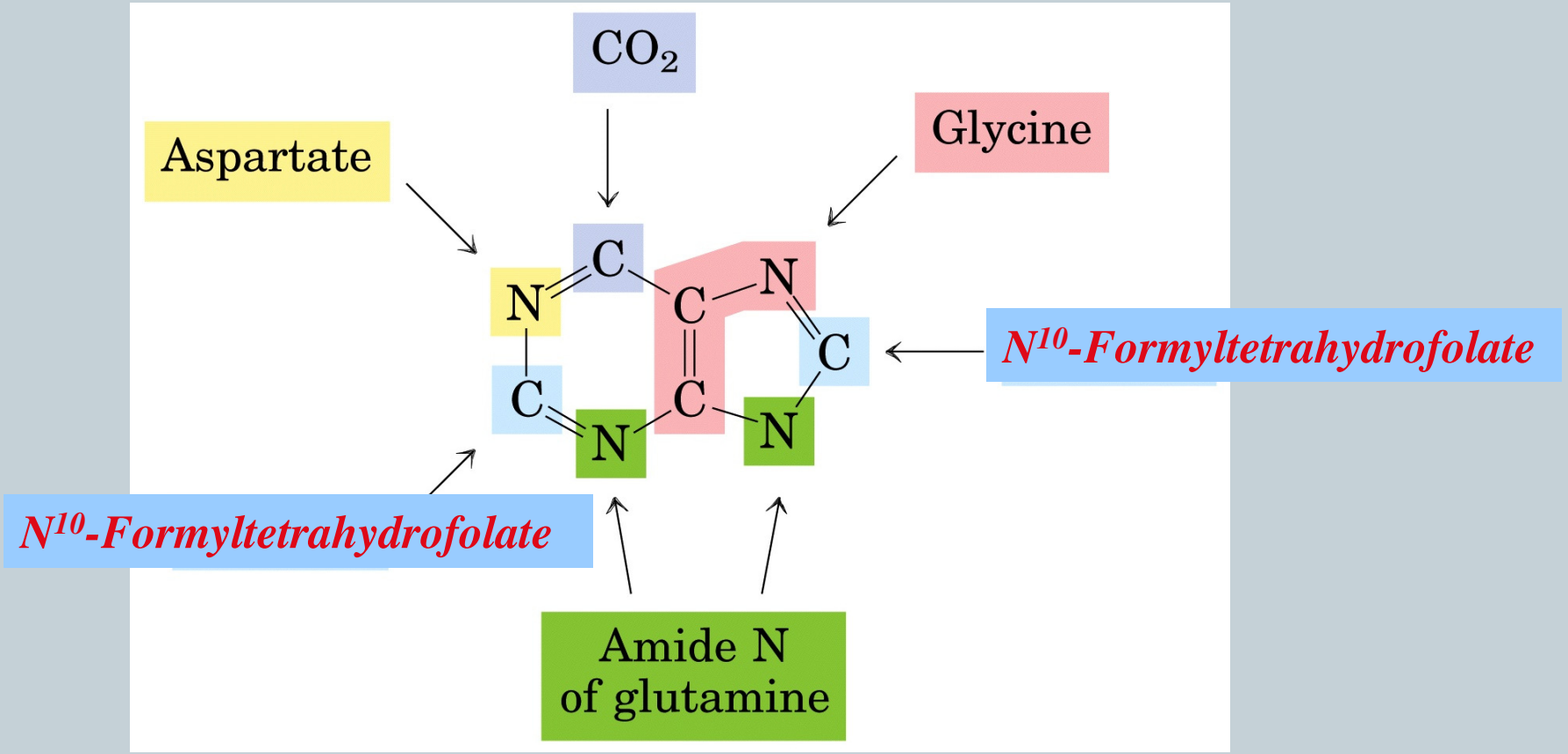
- DNA formation during cell division inhibited.
- Severe combined immunodeficiency disease
- Involving a decrease in both T cells and B cells.
- Treatment
  - Bone marrow replacement
  - Enzyme replacement therapy
  - Gene Therapy.
- Without treatment, children die by the age of two years.

Pyrimidine Synthesis



- Purine synthesis = Constructed on a pre-existing ribose 5-phosphate.
- Pyrimidine Synthesis = Before attached to ribose 5-phosphate.

Element sources of purine bases



First, synthesis Inosine-5'-Monophosphate, IMP

Two Main Domain of Pyrimidine Synthesis

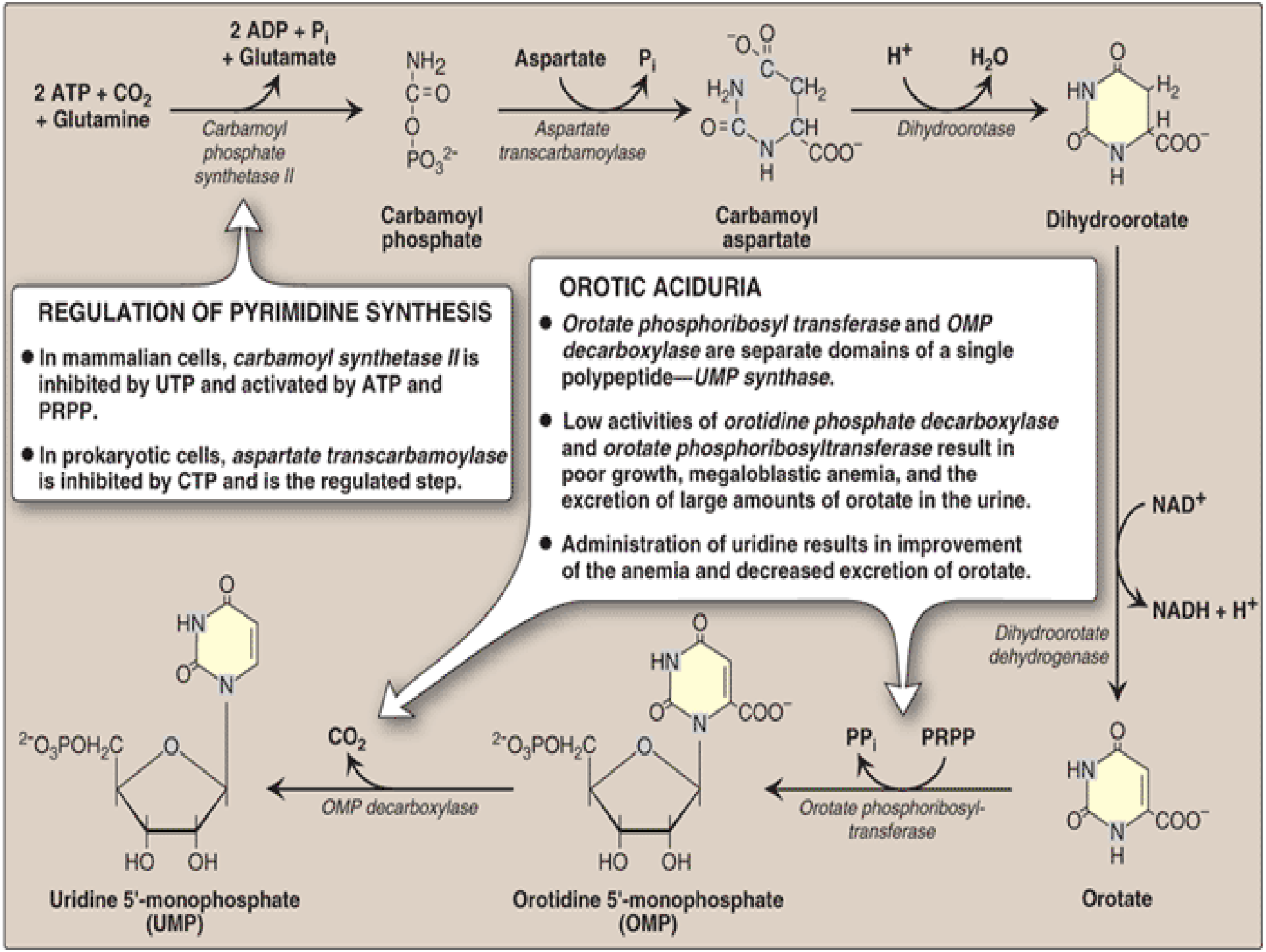
1. Carbamoyl Phosphate Synthetase (CPS) II
   a. CPS II
   b. Aspartate transcarbamoylase
   c. Dihydroorotase
2. UMA Synthase
   a. Orotidylate decarboxylase
   b. Orotate phosphoribosyltransferase

Synthesis of Carbamoyl phosphate

- Carbamoyl phosphate synthetase (CPS) II.
  - Multifunctional polypeptide
  - Three different catalytic domains of a single polypeptide chain.
  - CPS II, Aspartate transcarbamoylase & Dihydroorotase
- Glutamine and CO_2, catalyzed by
- CPS II
  - Inhibited by UTP (the end product)
  - Activated by ATP and PRPP.

Carbamoyl Phosphate

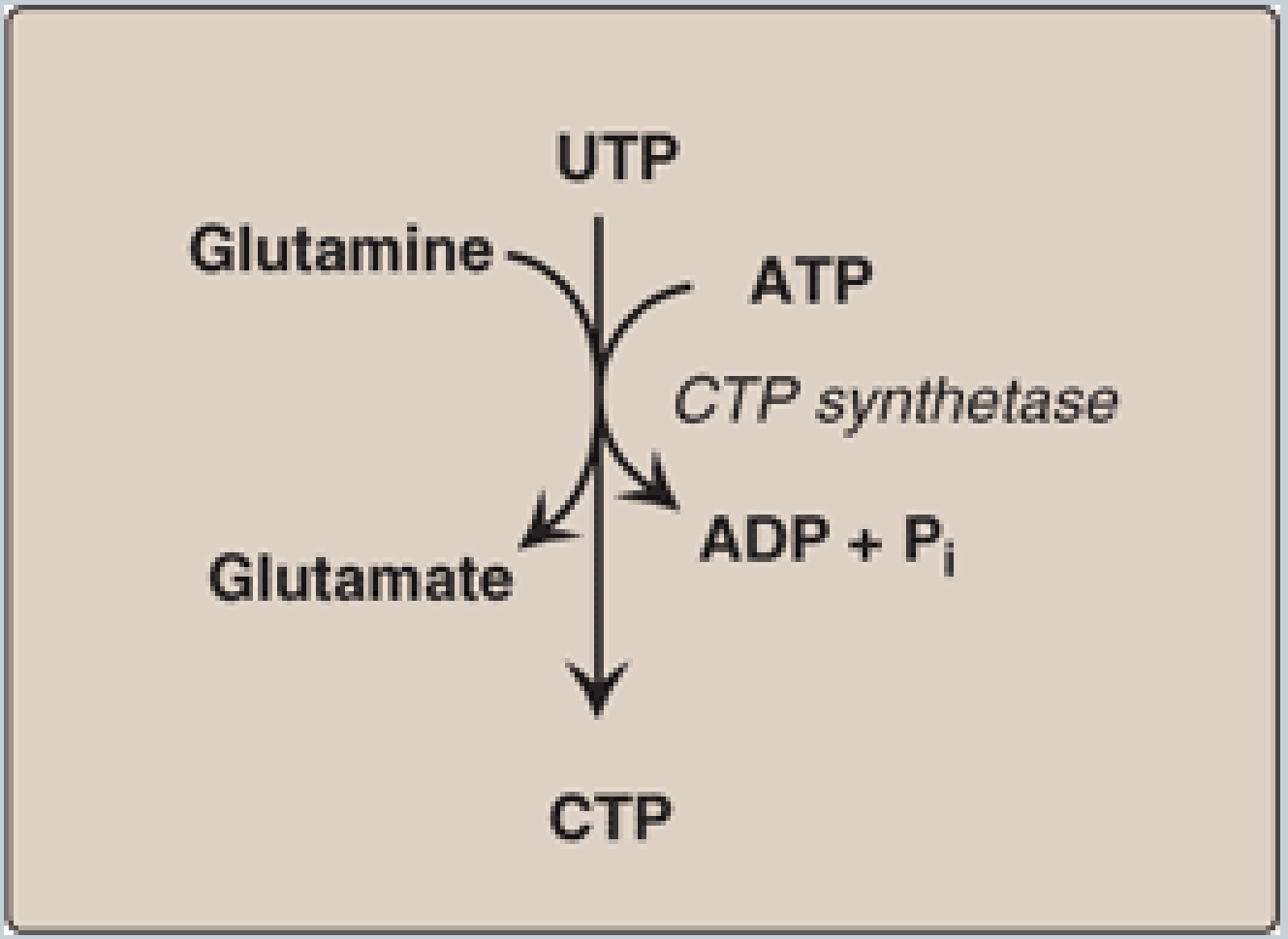
	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N—acetyl-glutamate	Inhibitor: UTP Activator: ATP


2 ATP + CO_2 + Glutamine
2 ADP + P_i + Glutamate
Carbamoyl phosphate synthetase II
Carbamoyl phosphate
Aspartate
P_i
Aspartate transcarbamoylase
Carbamoyl aspartate
H^+
H_2O
Dihydroorotase
Dihydroorotate
REGULATION OF PYRIMIDINE SYNTHESIS
In mammalian cells, carbamoyl synthetase II is inhibited by UTP and activated by ATP and PRPP.
In prokaryotic cells, aspartate transcarbamoylase is inhibited by CTP and is the regulated step.
OROTIC ACIDURIA
Orotate phosphoribosyl transferase and OMP decarboxylase are separate domains of a single polypeptide—UMP synthase.
Low activities of orotidine phosphate decarboxylase and orotate phosphoribosyltransferase result in poor growth, megaloblastic anemia, and the excretion of large amounts of orotate in the urine.
Administration of uridine results in improvement of the anemia and decreased excretion of orotate.
NAD^+
$NADH + H^+$
Dihydroorotate dehydrogenase
PP_i
PRPP
Orotate phosphoribosyl-transferase
Orotate
CO_2
OMP decarboxylase
Uridine 5'-monophosphate (UMP)
Orotidine 5'-monophosphate (OMP)

Formation of a Pyrimidine nucleotide

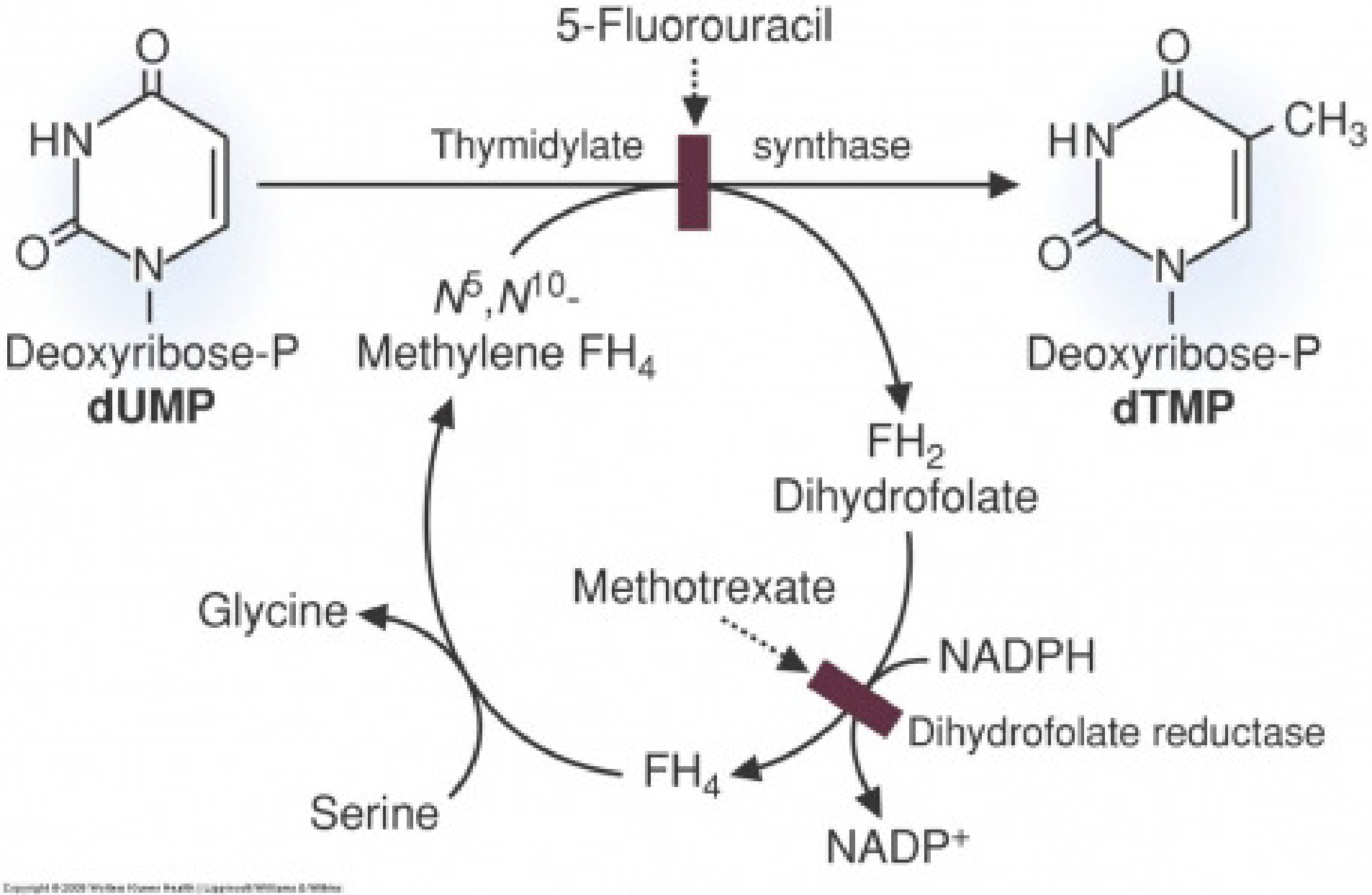
- PRPP is again the ribose 5-phosphate donor.
- Both purine and pyrimidine synthesis thus require glutamine, aspartic acid, and PRPP as essential precursors.
- **UMP synthase = domains of a single polypeptide chain**
  - **Orotidylate decarboxylase**
  - **Orotate phosphoribosyltransferase**

Synthesis of CTP from UTP



Orotic aciduria

- **Deficiency of**
  - **UMA Synthase**
    - **Orotidylate decarboxylase**
    - **Orotate phosphoribosyltransferase**
- Orotic acid in the urine.
- Megaloblastic Anaemia.
- Rx
- Uridine ???????.


5-Fluorouracil
Thymidylate synthase
HN
O
O
N
Deoxyribose-P
dUMP
N^5,N^{10}-
Methylene FH_4
HN
CH_3
O
O
N
Deoxyribose-P
dTMP
FH_2
Dihydrofolate
Methotrexate
NADPH
Dihydrofolate reductase
Glycine
Serine
FH_4
$NADP^+$

Salvage of Pyrimidines

- Nucleoside kinases that utilize ATP
- Through phosphorylation of the nucleosides to nucleotides.
- The salvage of pyrimidine nucleotides is the basis for using uridine in the treatment of hereditary orotic aciduria.

26.3 Pyrimidine Salvage Pathways

PYRIMIDINES:

uracil

Uracil + PRPP ----------> UMP + PPi

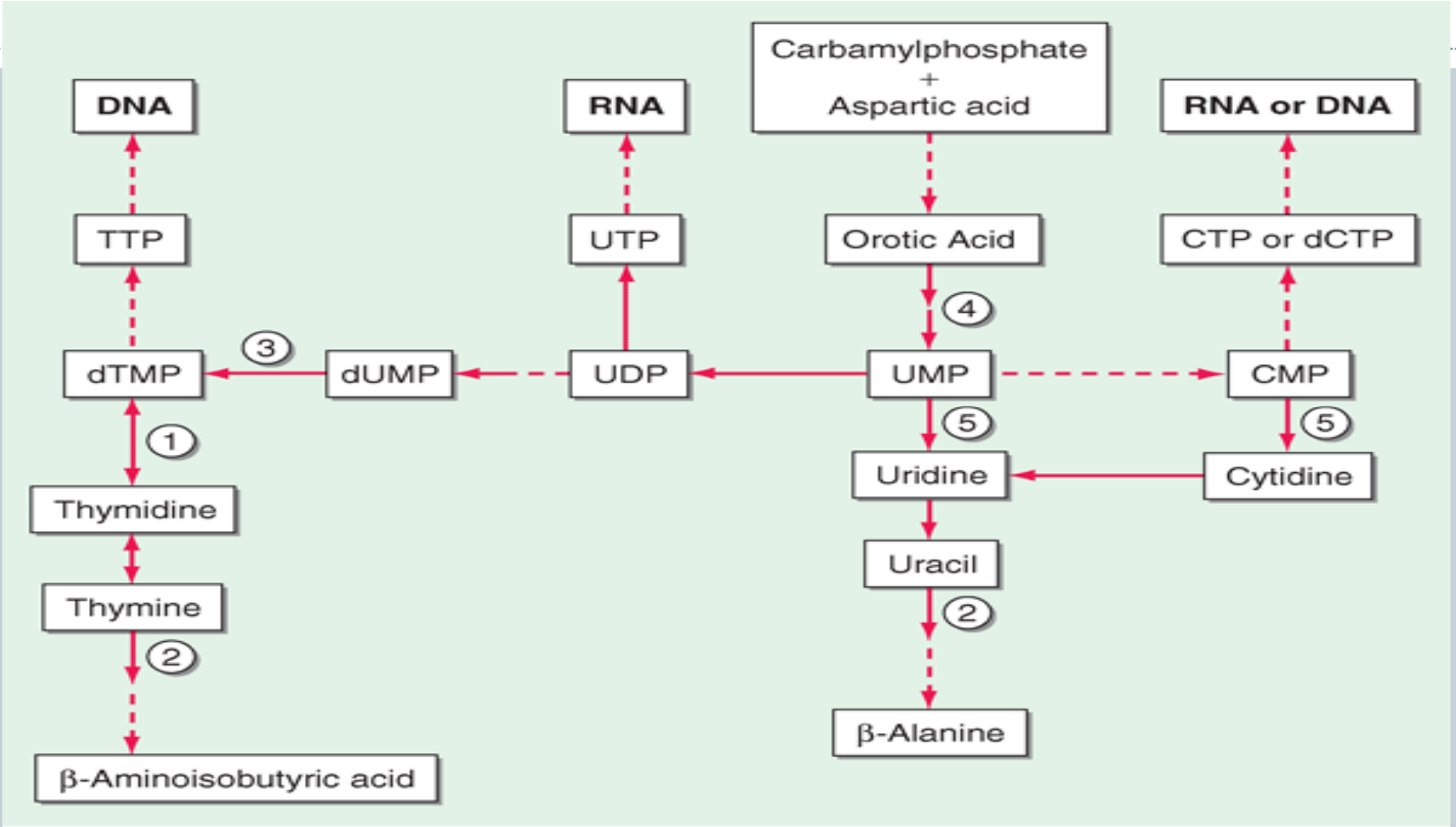
phosphoribosyl transferase

Orotate orotate

Uracil + PRPP ----------> PyMP + PPi

Cytosine phosphoribosyl transferase

Pyrimidine Degradation



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine, 18th Edition*: www.accessmedicine.com

Degradation of pyrimidine nucleotides

- Pyrimidine ring is opened
- Degraded to highly soluble products,
  - **β-alanine**
  - **β-aminoisobutyrate,**
  - **with the production of NH_3 and CO_2.**

Bedaquiline