- **NUCLEOTIDE METABOLISM**

Nucleotide Metabolism

- Chemistry of Nucleotides
- Classfication of Nucleotides
- Function of Nucleotides
- Synthesis,degradation and Associated diseases of purine Nucleotides
- Synthesis,degradation and Associated diseases of pyrimidine Nucleotides

Nucleotides

Nucleotides are composed of a nitrogenous base, a pentose mono saccharides, and one,two or three phosphate groups.

A. Nitrogenous bases: These are either purine or pyrimidines

Purines:

- Adenine → 6 amino purine
- Guanine → 2amino -6- oxy purine

Pyrimidenes:

- Cytosine → 2-oxy-4 amino pyrimidine
- Uracil : → 2.4 Dioxy pyrimidine
- Thymine → 5-methyl pyrimidine

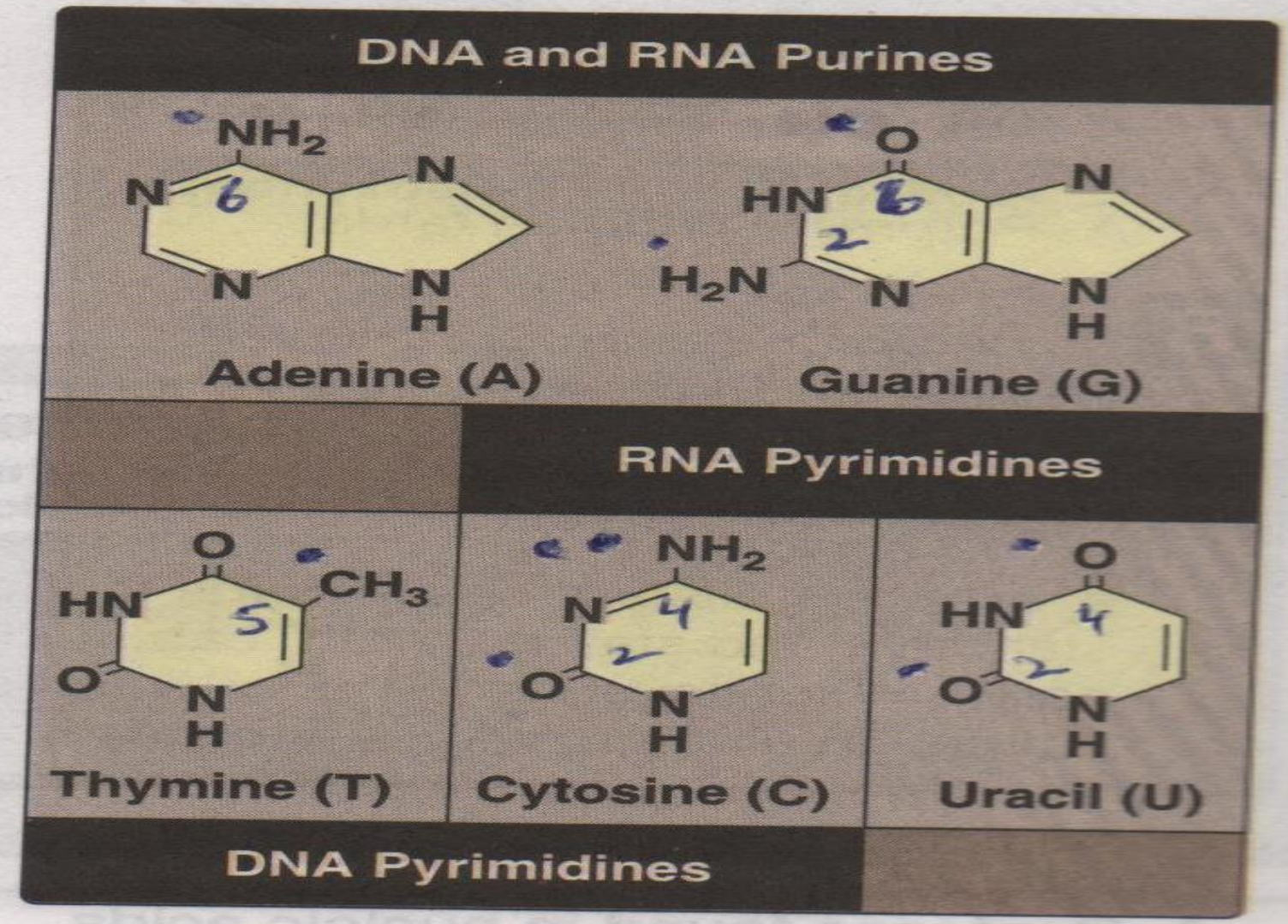


Figure 22.1
Purines and pyrimidines commonly found in DNA and RNA.

B. Sugar: Is either Ribose or Deoxy Ribose

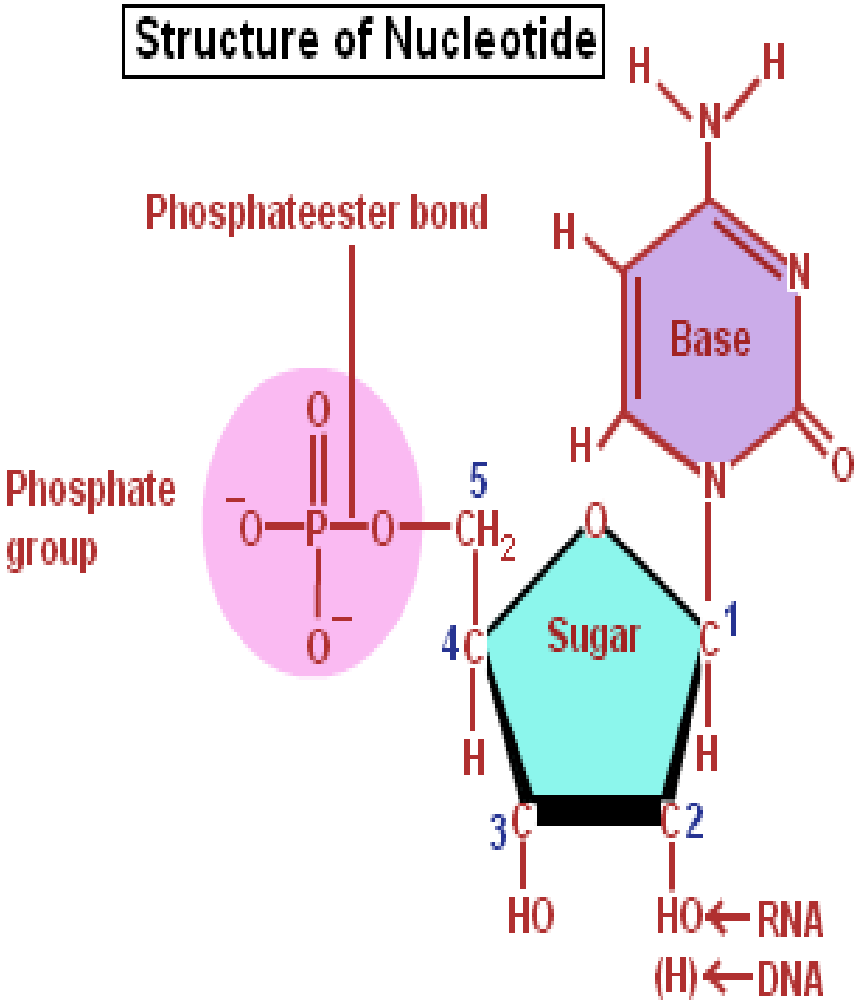
Sugar + Nitrogenous base = Nuleoside

Therefore Nucleosides can be either ribonucleoside or Deoxyribonucleoside e.g

- Adenine + Ribose → Adenosine
- Adenine + Deoxy Ribose → Deoxy Adenosine
- Guanine + Ribose → Guanosine
- Guanine + Deoxy Ribose → Deoxy Guanosine
- Cytosine + Ribose → cytidine
- Cytosine + Deoxy Ribose → Deoxy Cytidine
- Uracil + Ribose → Uridine
- Thymine + Deoxy Ribose → Deoxy Thymidine

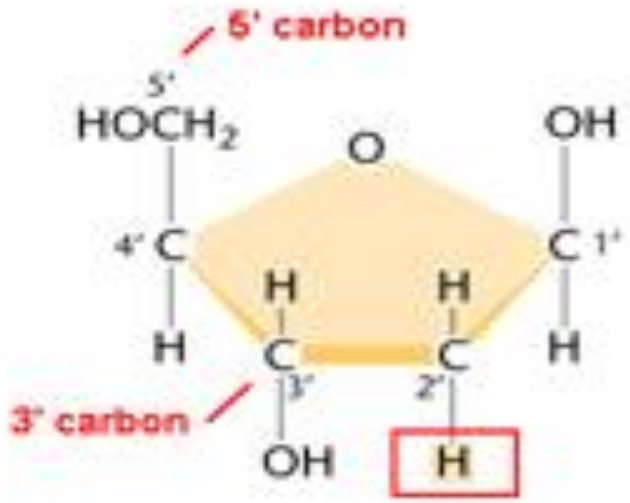
- **PHOSPHORIC ACID:**
- Nucleoside + Phosphoric acid → Nucleotide
- Adenosine + H_3PO_4 → AMP
- Gaunosine + H_3PO_4 → GMP
- Cytidine + H_3PO_4 → CMP
- Uridine + H_3PO_4 → UMP
- In case of Mononucleotides, a phosphoric acid molecule forms an ester linkage with one of the OH groups of sugar of a nucleoside.

Phosphoric acid

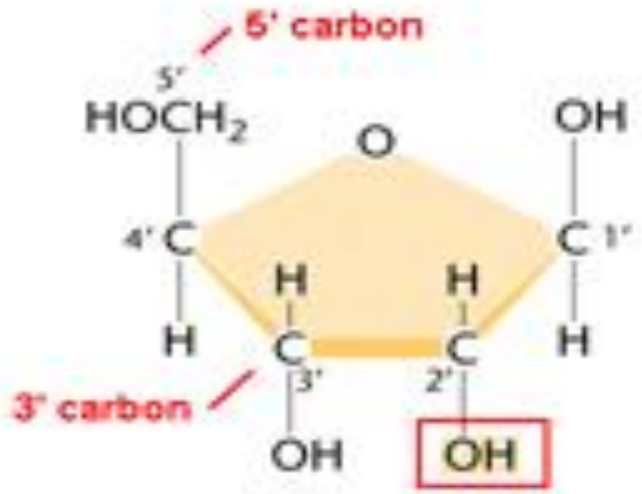


- In case of Ribose, there are three such places (C no. 2,3,5) where this ester linkage can be formed, but in Deoxy ribose ,only two such places i.e.3 & 5 ,because C no.2 of sugar lack an O_2 atom.

RIBOSE & DEOXYRIBOSE



2-Deoxyribose



Ribose

Uncommon or unusual minor bases :-

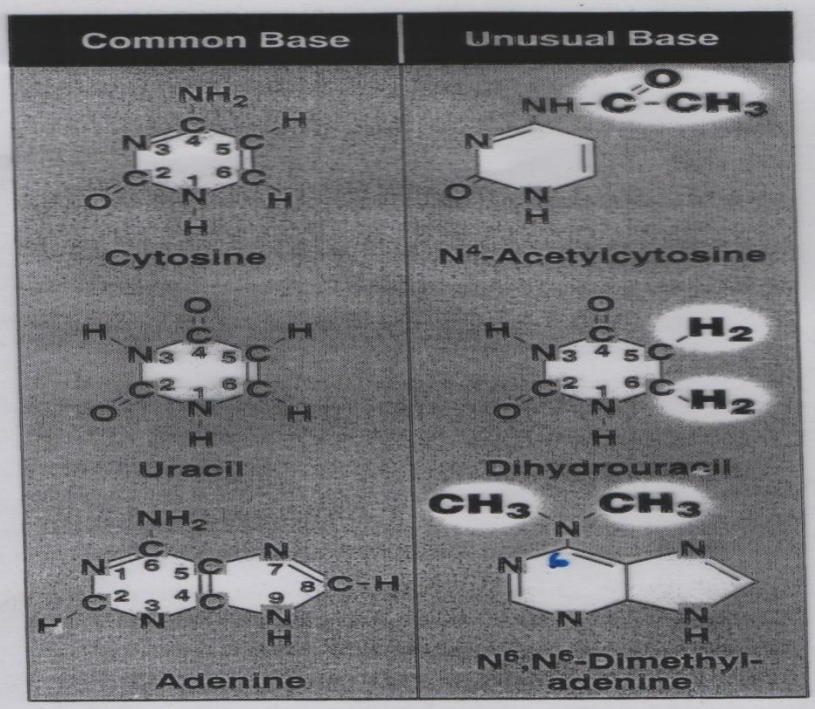


Figure 22.2
Examples of unusual bases.

Plants have a large number of purine bases containing methyl substituents and many of them have definite pharmacological properties :-

1- theophylline :- It is 1,3, dimethyl xanthine — It is found in Tea

2 - Caffein :- It is, 1,3,7 Trimethyl xanthine. It is present in coffee.

CLASSIFICATION

A. *Adenosine nucleotides:* ATP, ADP, AMP and Cyclic AMP.
B. *Guanosine nucleotides:* GTP, GDP, GMP and cyclic GMP.
C. *Uridine nucleotides:* UTP, UDP, UMP, UDP-G.
D. *Cytidine nucleotides:* CTP, CDP, CMP and certain deoxy CDP derivatives of glucose, choline, ethanolamine.
E. *Miscellaneous:* **PAPS ('active' sulphate), "active" methionine (S-adenosyl methiomine),** certain coenzymes like NAD^+ and $NADP^+$, FAD and FMN, Cobamide coenzyme, CoA.

(a) PAPS-Phosphoadenosine phosphosulphate: It is also known as **active sulphate.** It is formed from ATP and SO_4^-.

The compound is formed in liver. For the reaction Vit. is also necessary.

Functions

1. The *sulphatases* are enzymes which catalyse the introduction of a SO_4 group to various biomolecules, e.g.
   - In the biosynthesis of heparin,
   - In the biosynthesis of chondroitin SO_4 A, B, C and D,
   - In Keratosulphate synthesis, and
   - Formation of 'sulpolipids' (sulfatides).
2. It is also required in the conjugation of phenols, indole, skatole to form "ethereal SO_4".

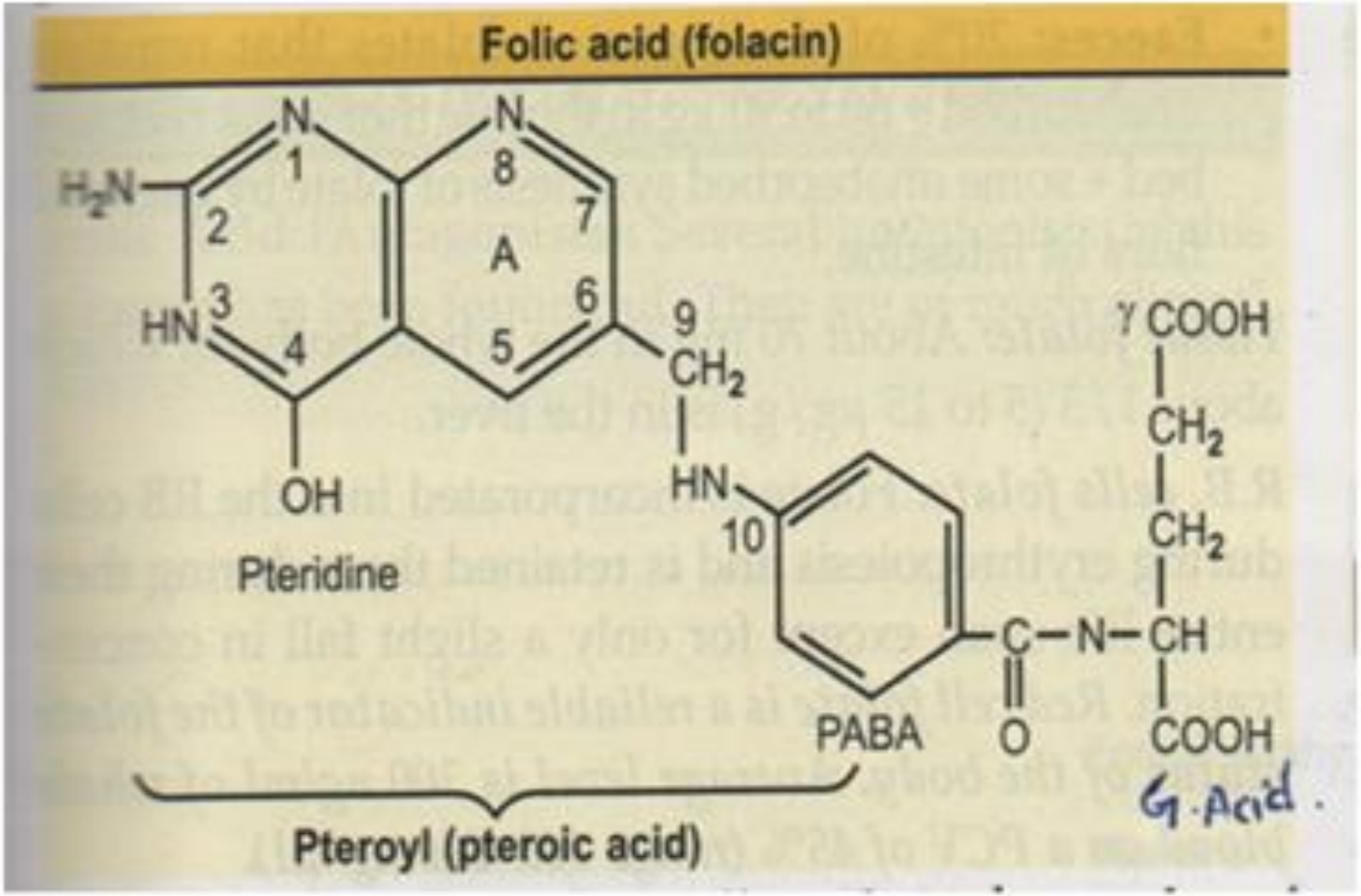
(b) SAM (S-adenosyl Methionine): Also known as "active" methionine (See chapter on "Metabolism of Methionine").

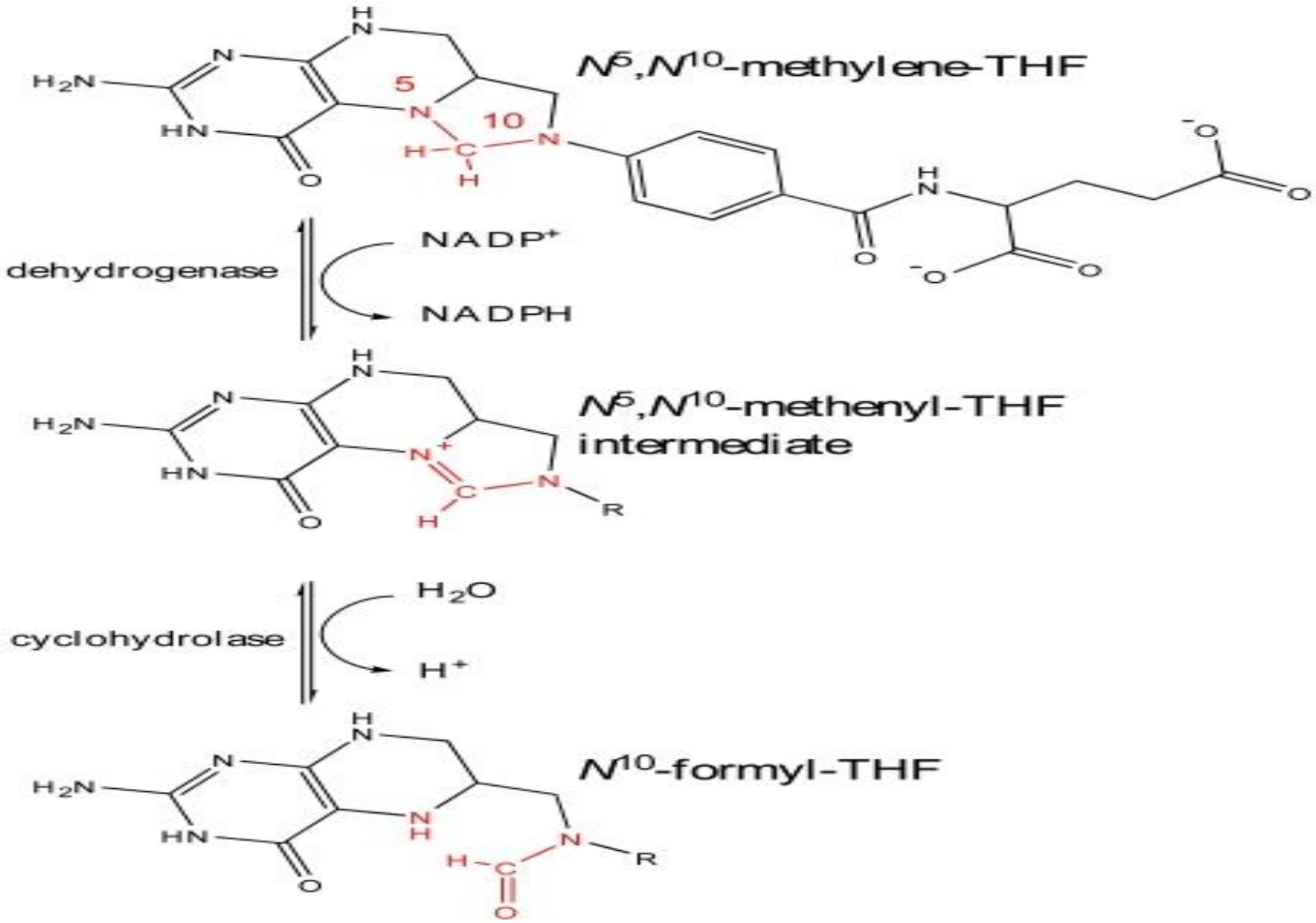
- Functions of Nucleotide
- Formation and Degradation of cyclic AMP
- Function of cyclic AMP
- Function of cyclic GMP

Folic Acids

- Active coenzyme form is called tetra hydro folate (H4 folate)
- Tetra hydrofolate acts as a carrier in transfer of one carbon group
- The one carbon group combines at N^5 ,N^{10} or balanced between nitrogen No 5 and Nitrogen No10 of folic acid
- The one carbon unit may be

1. Formyl group
2. Formamine group
3. Methyl group
4. Methenyl group


Folic acid (folacin)
N
N
1
8
H_2N
2
7
A
3
6
HN
9
4
5
CH_2
OH
HN
10
Pteridine
γ COOH
CH_2
CH_2
C–N–CH
PABA
O
COOH
G. Acid.
Pteroyl (pteroic acid)


N^5,N^{10}-methylene-THF
dehydrogenase
NADP+
NADPH
N^5,N^{10}-methenyl-THF
intermediate
cyclohydrolase
H2O
H+
N^{10}-formyl-THF

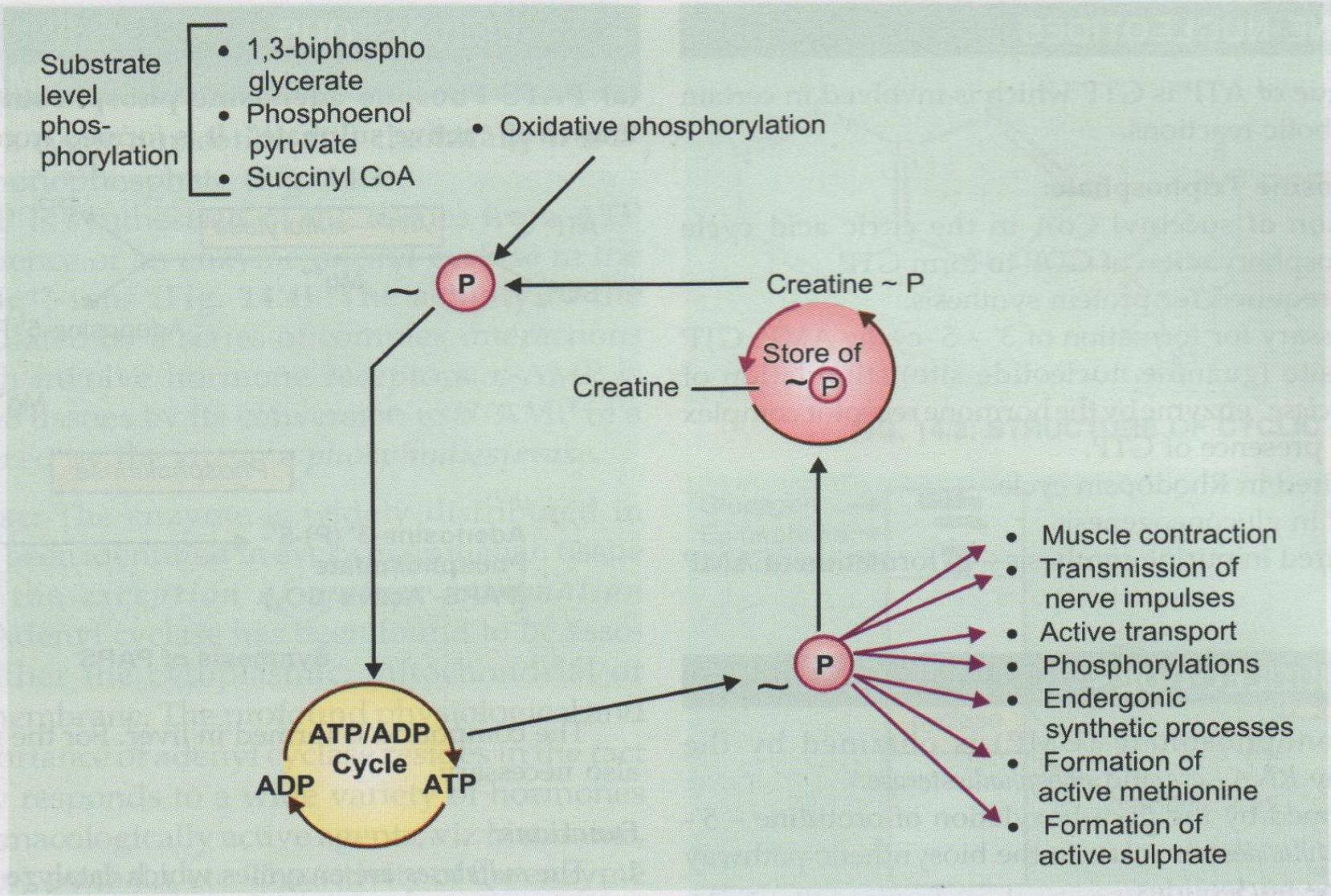
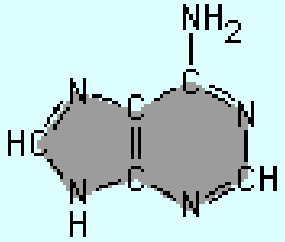


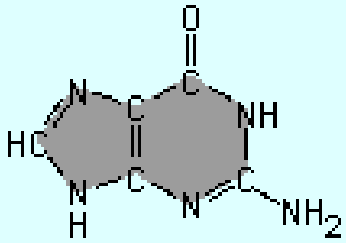
FIG. 14.2: ROLE OF ATP/ADP CYCLE IN TRANSFER OF HIGH ENERGY PHOSPHATE

DNA bases

Purines

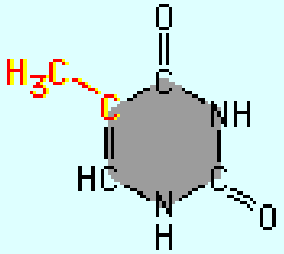


Adenine (A)

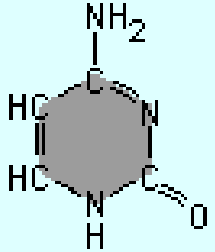


Guanine (G)

Pyrimidines



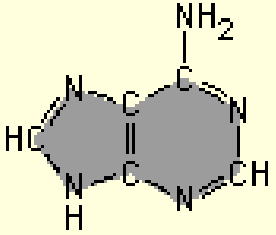
Thymine (T)



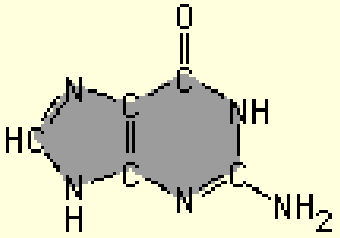
Cytosine (C)

RNA bases

Purines

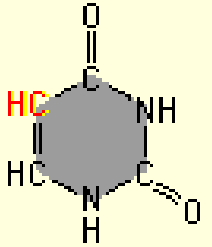


Adenine (A)

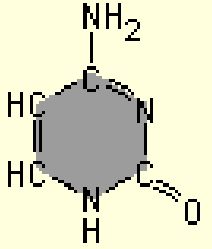


Guanine (G)

Pyrimidines

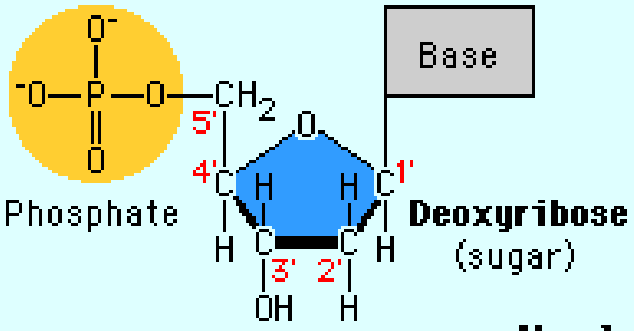


Uracil (U)

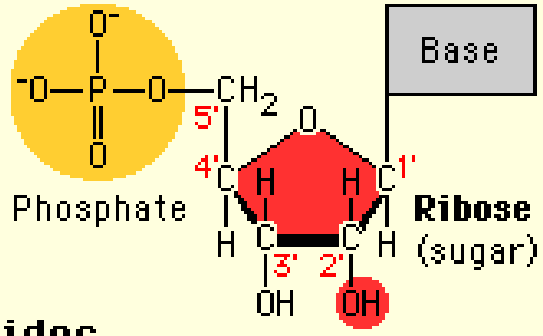


Cytosine (C)

DNA



RNA



Nucleotides

PURINE SYNTHESIS

There are two basic mechanisms to generate purines and pyrimidines

1. *DE NOVO* BIOSYNTHETIC PATHWAYS
(building the bases from simple building blocks)

2. SALVAGE PATHWAYS
(the reutilization of bases from dietary or catabolic sources)

DENOVO –SYNTHESIS OF PURINE NUCLEOTIDES

(i.e SYNTHESIS FROM AMPHIBOLIC INTERMEDIATES or synthesis from different small compounds)

- Purines are built upon a pre-existing ribose -5-phosphate
- Liver is the major site of synthesis
- R.B.C ,W.B.C and brain cannot synthesized purine by Denovo synthesis.

Purines: where do the atoms come from?

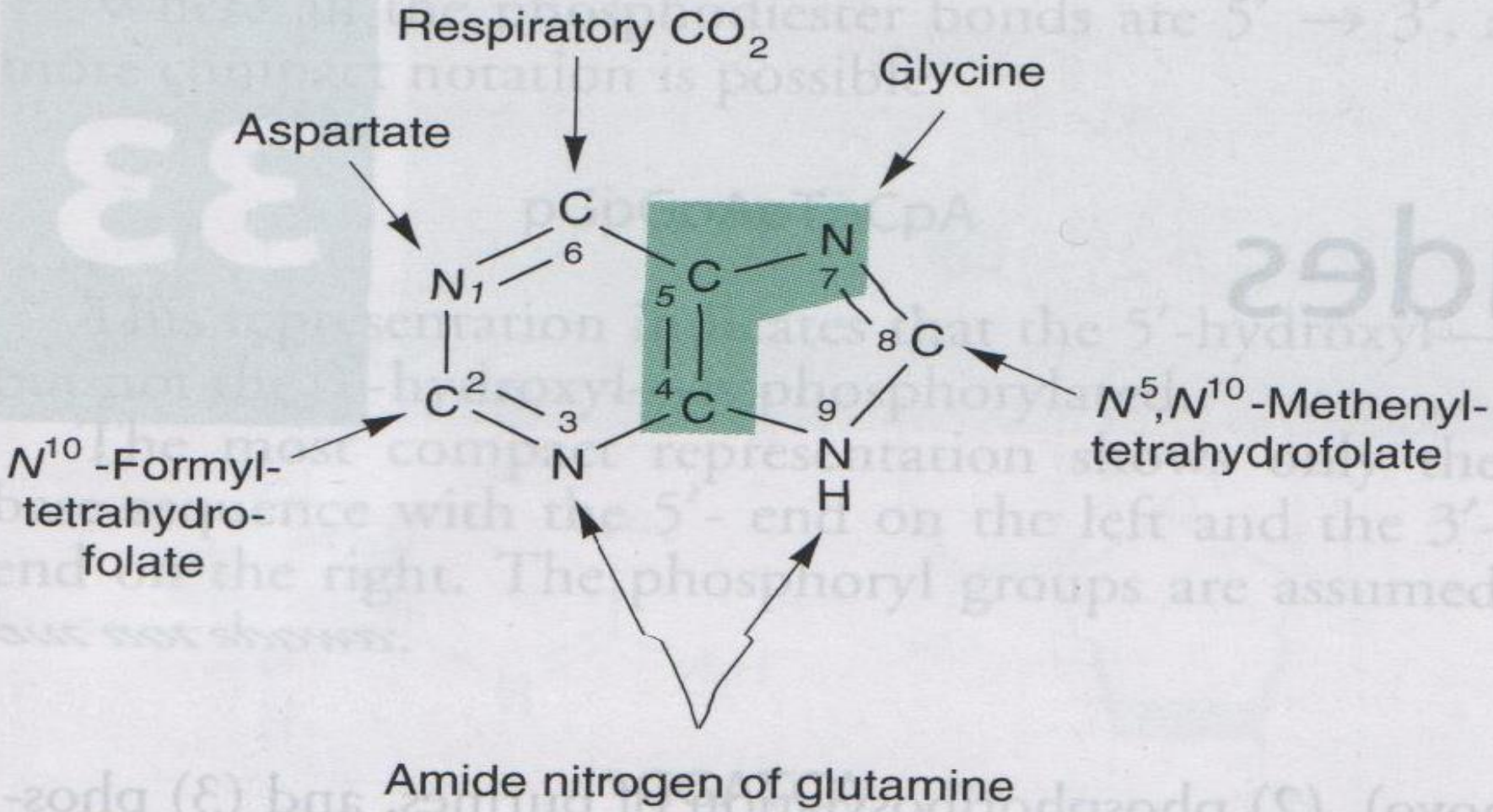


Figure 33–1. Sources of the nitrogen and carbon atoms of the purine ring. Atoms 4, 5, and 7 (shaded) derive from glycine.

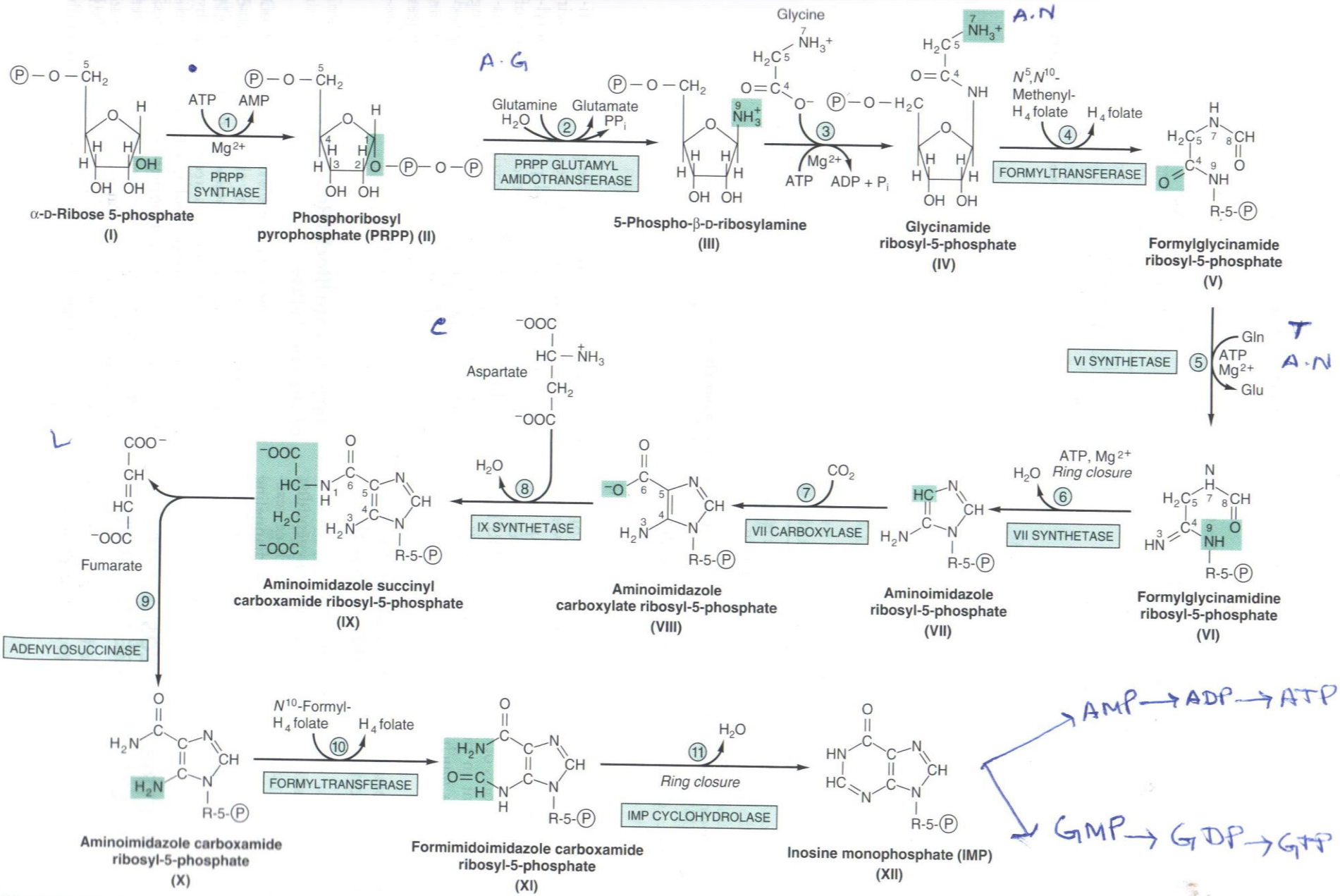
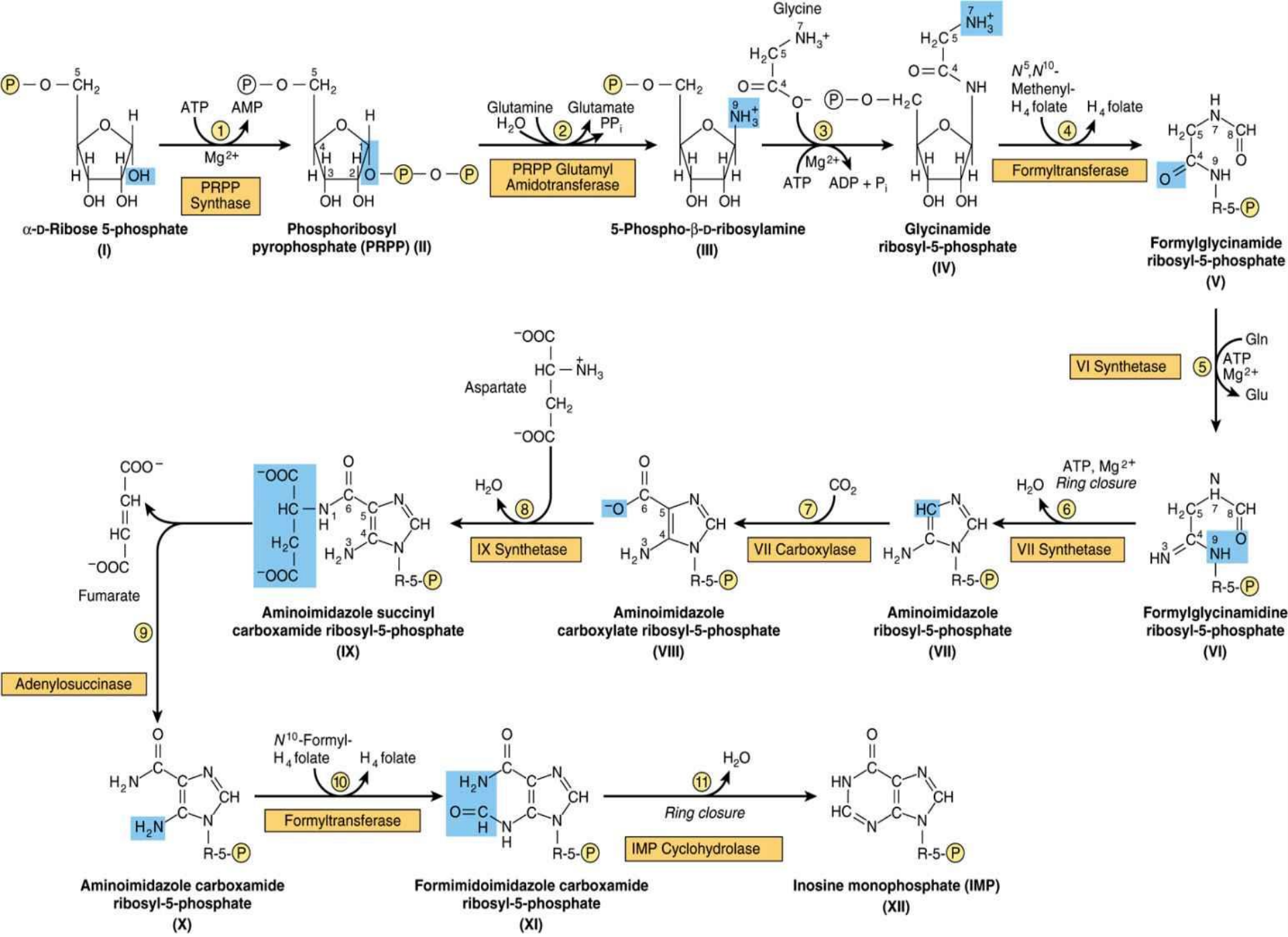


Figure 33–2. Purine biosynthesis from ribose 5-phosphate and ATP. See text for explanations. (Ⓟ, PO_3^{2-} or PO_2^-.)


α-D-Ribose 5-phosphate
(I)
ATP
AMP
Mg2+
1
PRPP Synthase
Phosphoribosyl pyrophosphate (PRPP) (II)
Glutamine
Glutamate
PPi
2
PRPP Glutamyl Amidotransferase
5-Phospho-β-D-ribosylamine
(III)
Glycine
3
Mg2+
ATP
ADP + Pi
Glycinamide ribosyl-5-phosphate
(IV)
N5,N10-Methenyl-H4 folate
H4 folate
4
Formyltransferase
Formylglycinamide ribosyl-5-phosphate
(V)
VI Synthetase
5
Gln
ATP
Mg2+
Glu
Formylglycinamidine ribosyl-5-phosphate
(VI)
ATP, Mg2+
Ring closure
H2O
6
VII Synthetase
Aminoimidazole ribosyl-5-phosphate
(VII)
CO2
7
VII Carboxylase
Aminoimidazole carboxylate ribosyl-5-phosphate
(VIII)
Aspartate
H2O
8
IX Synthetase
Aminoimidazole succinyl carboxamide ribosyl-5-phosphate
(IX)
Fumarate
9
Adenylosuccinase
Aminoimidazole carboxamide ribosyl-5-phosphate
(X)
N10-Formyl-H4 folate
H4 folate
10
Formyltransferase
Formimidoimidazole carboxamide ribosyl-5-phosphate
(XI)
H2O
11
Ring closure
IMP Cyclohydrolase
Inosine monophosphate (IMP)
(XII)

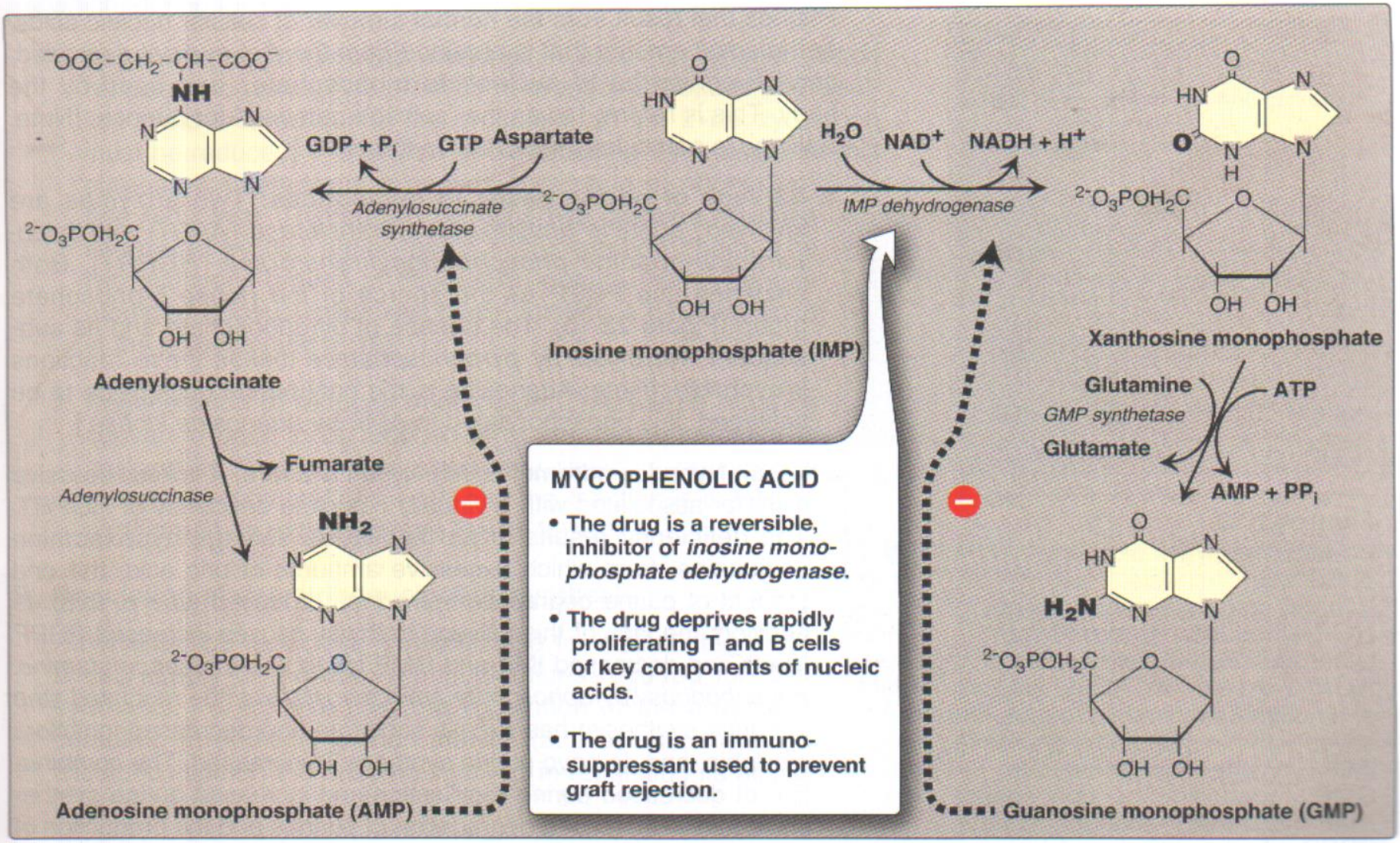
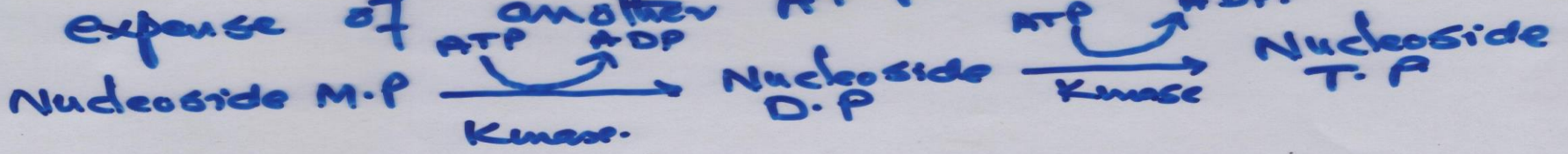


Figure 22.8
Conversion of IMP to AMP and GMP showing feedback inhibition. [Note: AMP is also called adenylate. GMP is also called guanylate.] NAD(H) = nicotinamide adenine dinucleotide; GDP = guanosine diphosphate; GTP = guanosine triphosphate; AMP = adenosine monophosphate; P_i = inorganic phosphate; PP_i = pyrophosphate.

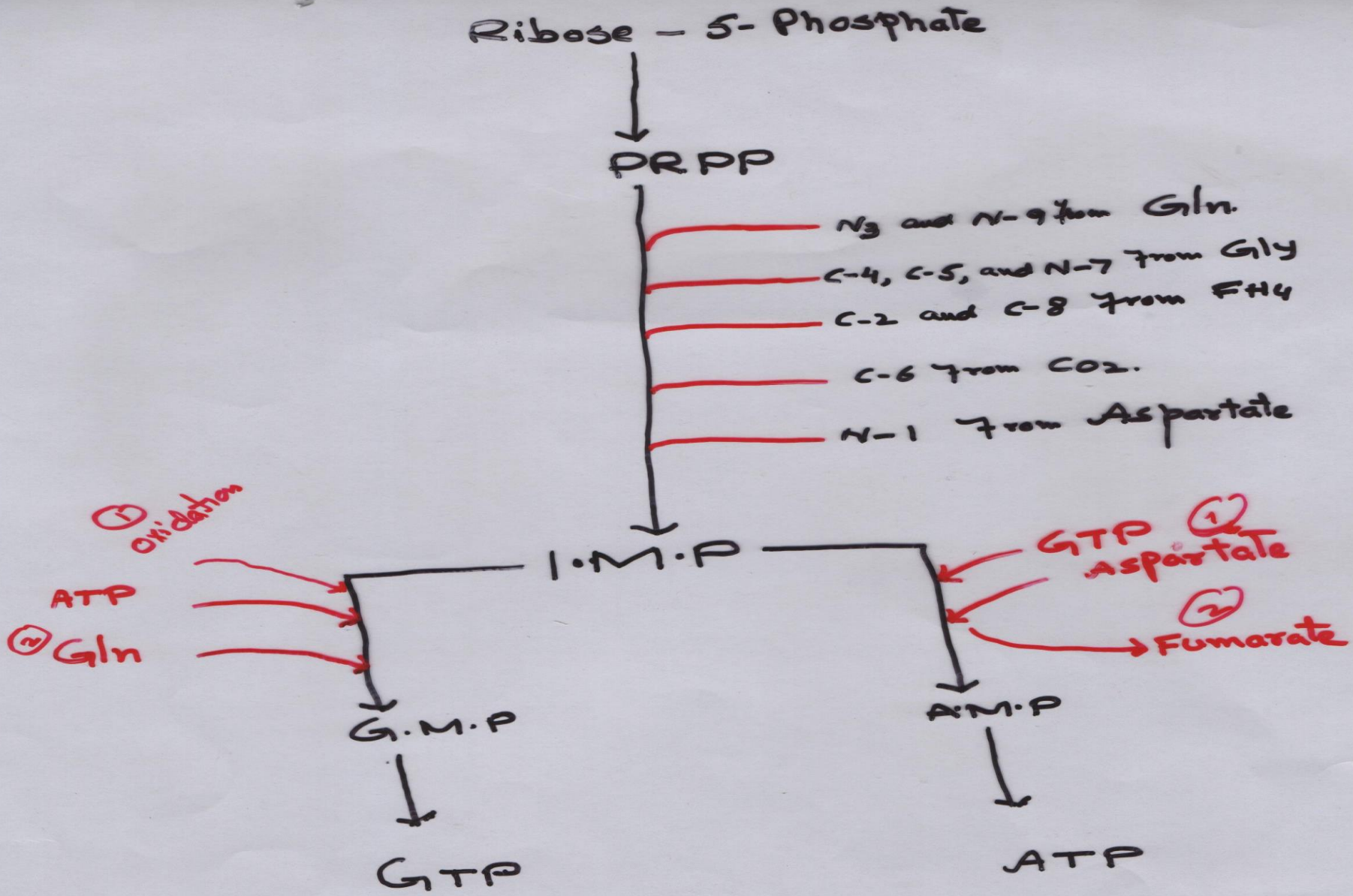
Phosphoryl Transfer from ATP Converts Mono Nucleotides to Nucleosides Di- AND Triphosphates :—

the mono nucleotides AMP and GMP are Converted in to ADP and GDP via Phosphoryl Transfer from ATP Catalyzed by Nucleoside monophosphate Kinase

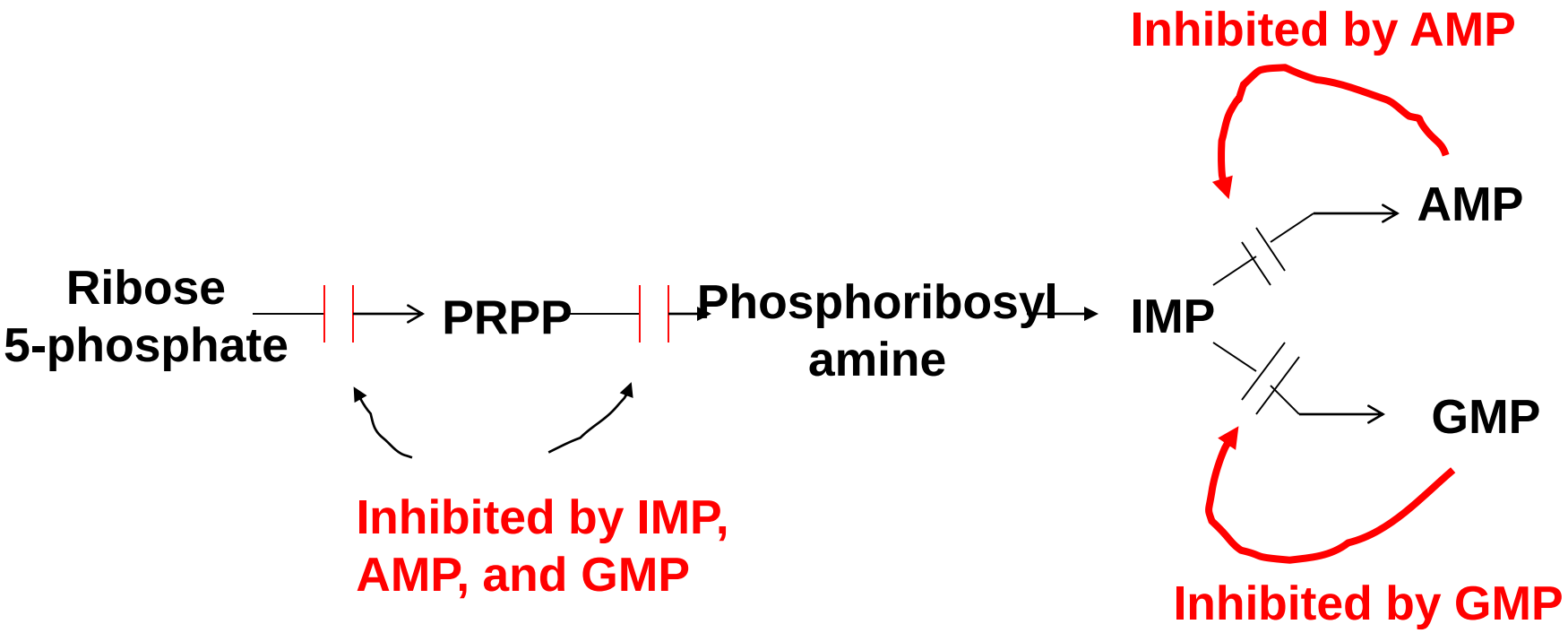
GDP is then Converted to GTP by nucleoside diphosphate Kinase at the expense of another ATP.

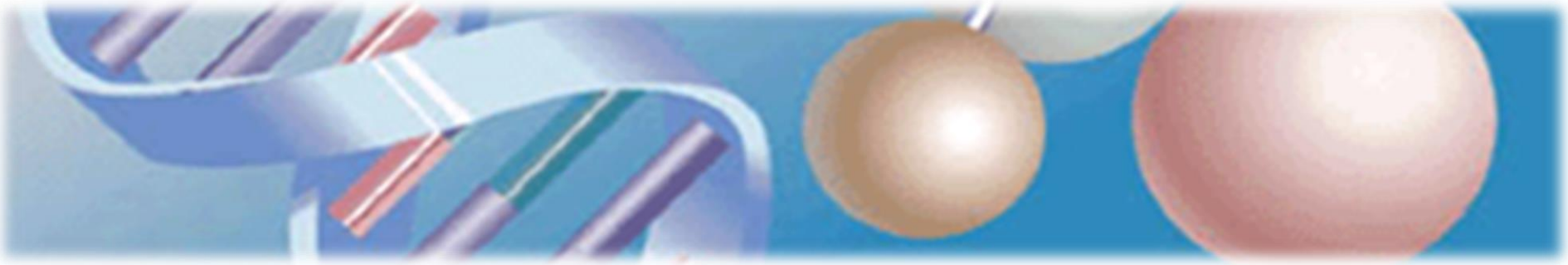


Conversion of ADP to ATP is achieved primarily by oxidative phosphorylation and secondarily by reactions of glycolysis and citric acid cycle


Ribose – 5- Phosphate
PRPP
N3 and N-9 from Gln.
C-4, C-5, and N-7 from Gly
C-2 and C-8 from FH4
C-6 from CO2.
N-1 from Aspartate
1. oxidation
ATP
2. Gln
I.M.P
GTP
1. Aspartate
2. Fumarate
G.M.P
A.M.P
GTP
ATP

The regulation of purine biosynthesis is a classic example of negative feedback





- Antifolate Drugs and Glutamine Analogs Block Purine Nucleotide Biosynthesis

- Six ATP utilized in de novo synthesis so energetically expensive process.
- Amino Acid required are Glycine, Aspartic Acid and glutamine.
- Co enzymes and Co factors are, formylated FH4, Mg++.
- Purines & Pyrimidines are dietarily nonessential
- Purines are synth mainly in the cytosole of liver cell.

- Nucleotides do not enter cell directly but are converted to nucleosides by the cell membrane nucleosidases
- In the cell nucleoside is either converted to the Nucleotide again by a kinase enzyme or degraded to corresponding base by the enzyme nucleoside Phosphorylase .

SALVAGE PATHWAY FOR PURINES

- Purines that result from the normal turnover of cellular nucleic acids or that are obtained from the diet and not degraded can be reconverted into nucleoside triphosphates and used by the body.

Significances of salvage pathway

- 1. Denovo synth is expensive in terms of use of high energy phosphate bonds.
- 2. Enzyme PRPP Glutamyl amido Transferase is not present in certain tissues e.g Neutrophil , R.B.C and Brain cells.
- So denosynthesis cannot take place in these tissues.

- Two pathways are available

1. One step synthesis
2. Two step synthesis

ONE STEP SYNTHESIS

Two Enzymes are involved

- i) APRT
- ii) HGPRT
- Both enzymes utilize PRPP as a source of the R-5-Phosphate group.

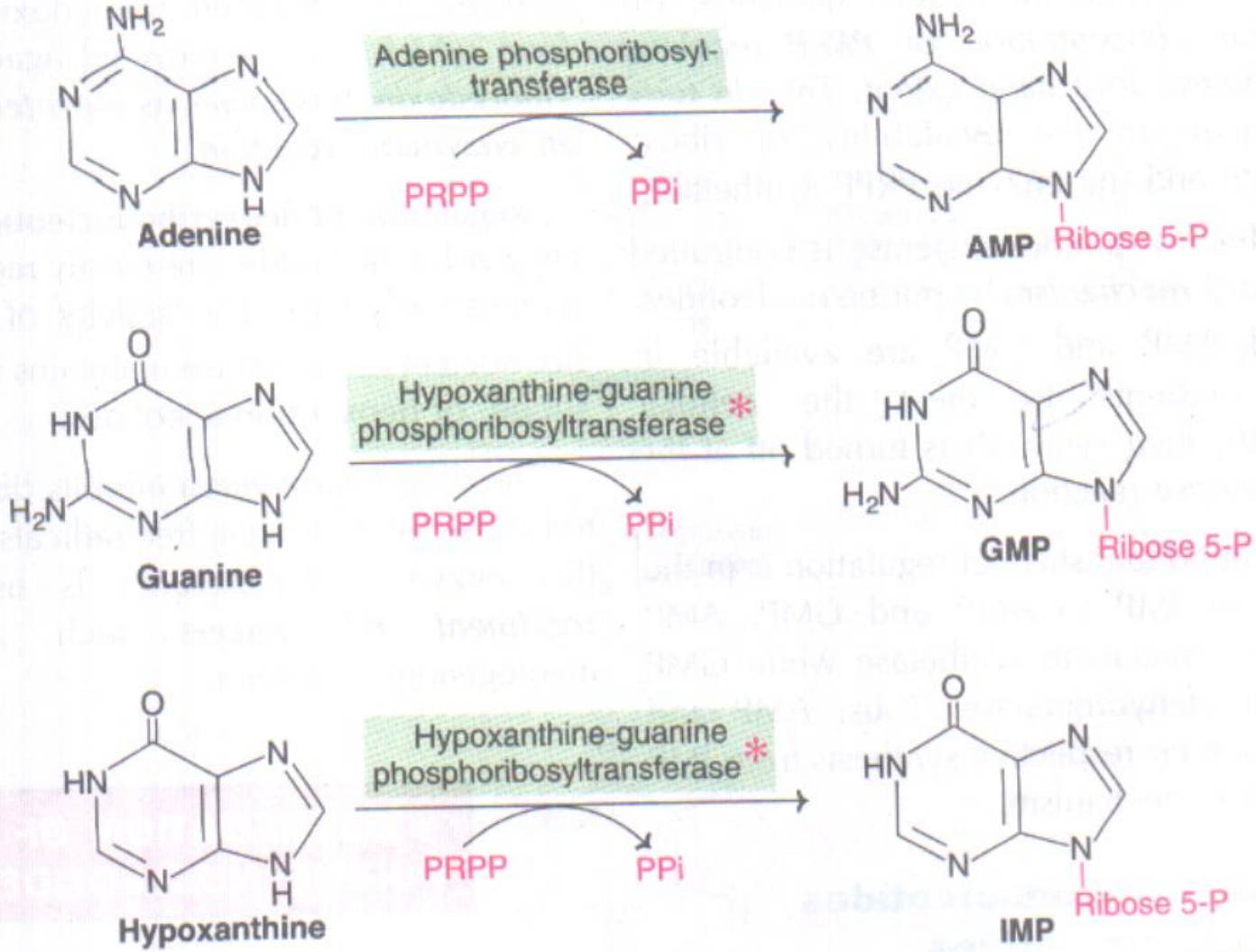


Fig. 17.5 : Salvage pathways of purine nucleotide synthesis (*PRPP–Phosphoribosyl pyrophosphate;* PPi–Inorganic pyrophosphate; AMP–Adenosine monophosphate; GMP–Guanosine monophosphate; *IMP–Inosine monophosphate;* * *Deficiency of HGPRT causes Lesch-Nyhan syndrome).*

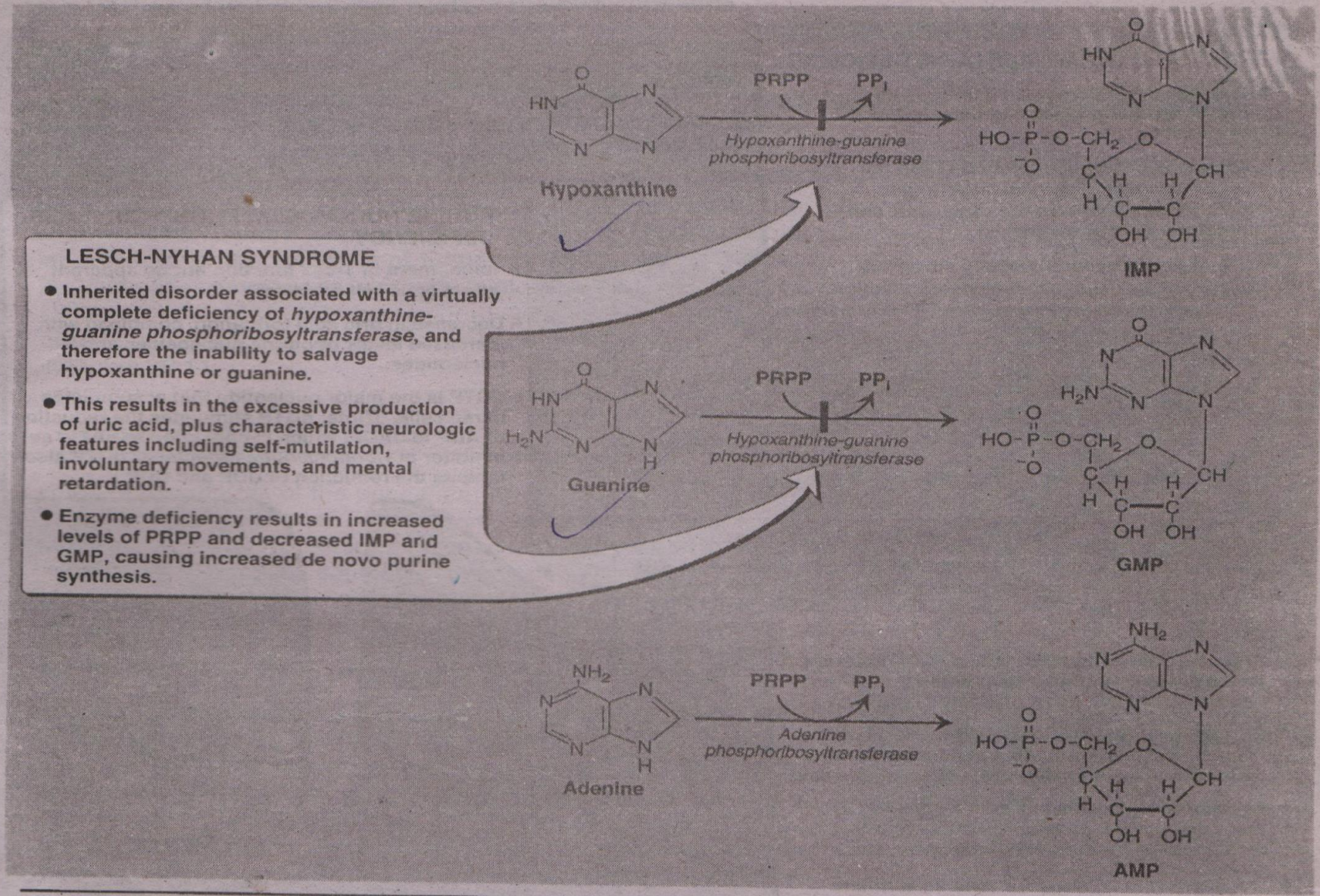
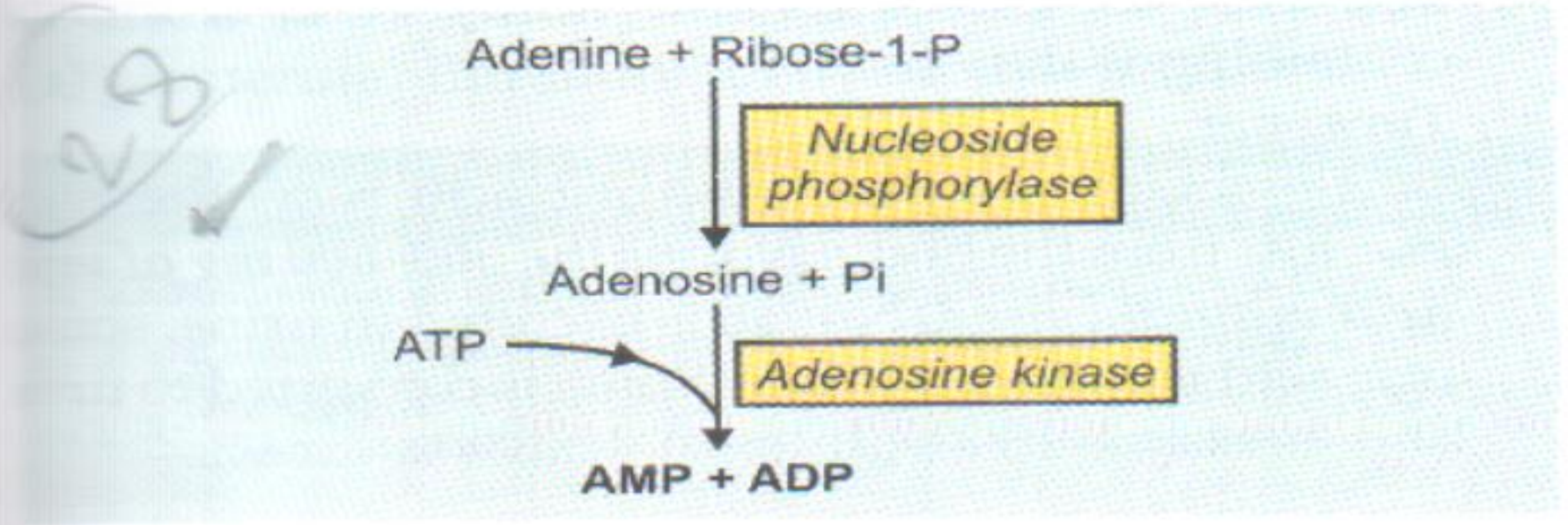


Figure 29.9
Salvage pathways of purine nucleotide synthesis.

TWO STEP PATHWAY (Nucleoside phosphorylase-Nucleosside kinase pathway)

- *Formation of AMP*



- Neither guanosine nor inosine kinase have been detected
- So adenine is the only purine that may be salvaged by the two steps pathway.

Regulation of purine synthesis

- PRPP synthetase :

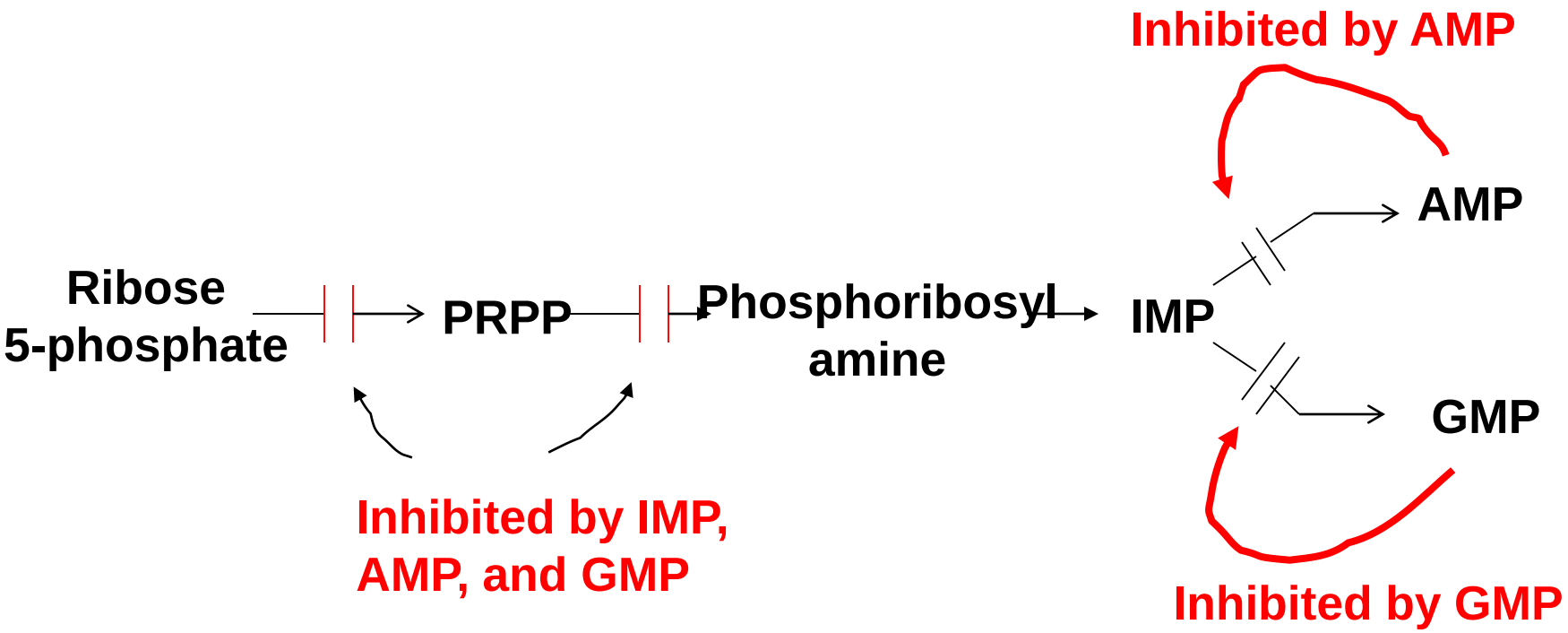
Allosterically inhibited by PRPP and AMP,GMP ,ADP,GDP,NAD ,FAD.

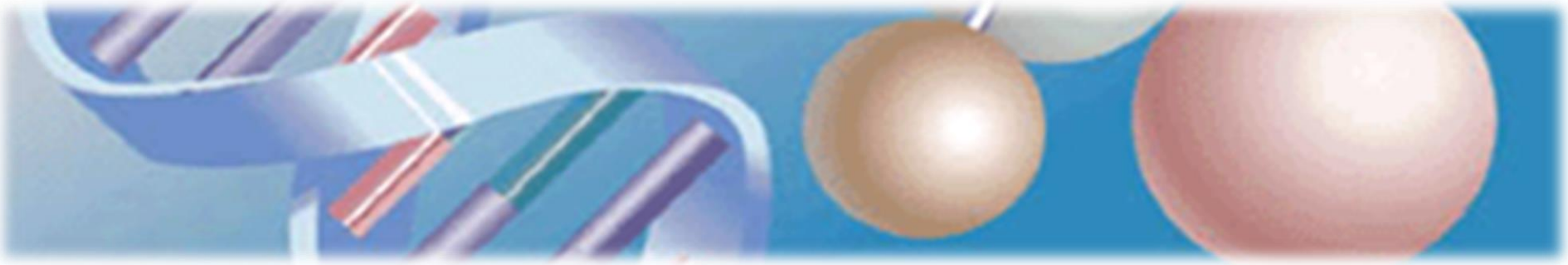
- Glutamin PRPP amidotransferase :

Feedback inhibition by AMP and GMP

- A proper balance between the adenine and guanine conc maintain by adenylosuccinate synthetase and IMP dehydrogenase

The regulation of purine biosynthesis is a classic example of negative feedback





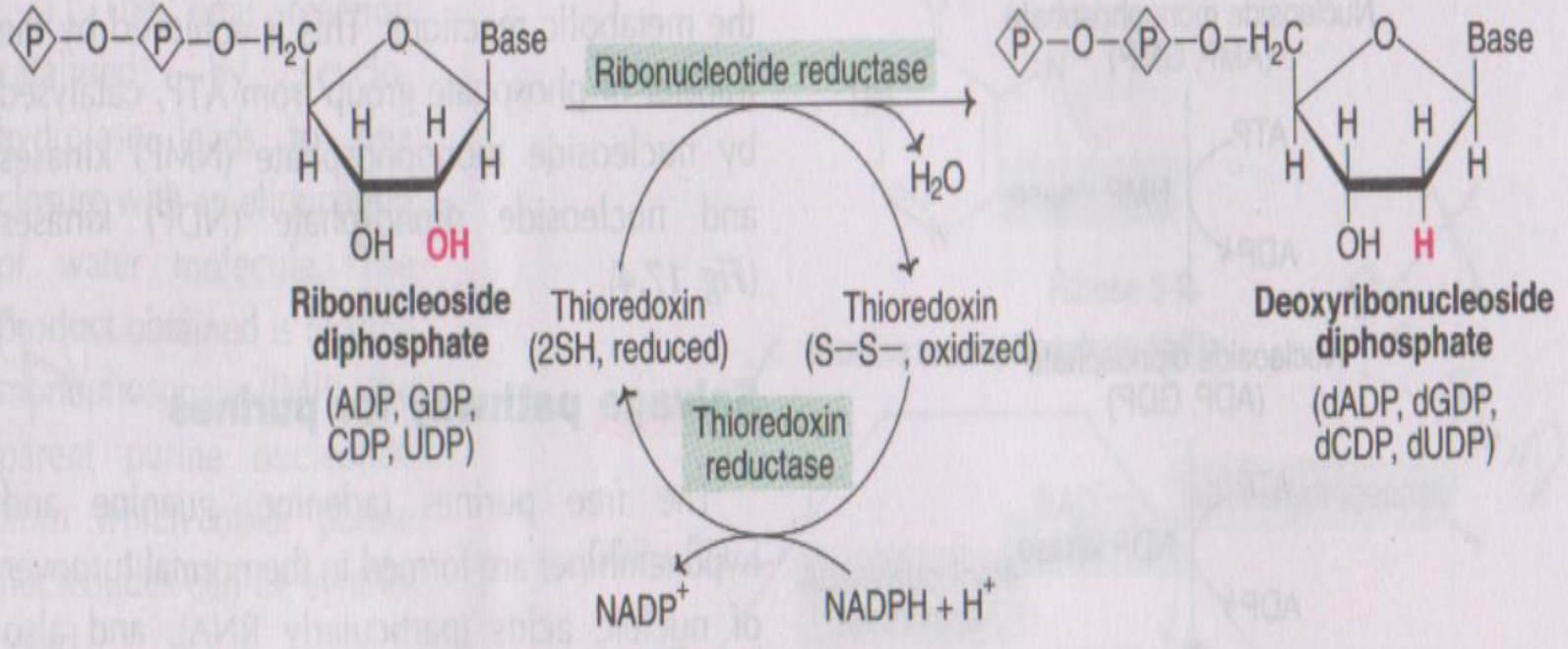


Fig. 17.6 : Formation of deoxyribonucleotides from ribonucleotides.

Ribonucleotide reductase provides the hydrogen atoms needed for reduction from its sulfhdryl groups.

DEGRADATION OF PURINE NUCLEOTIDES
(Formation of uric acid)

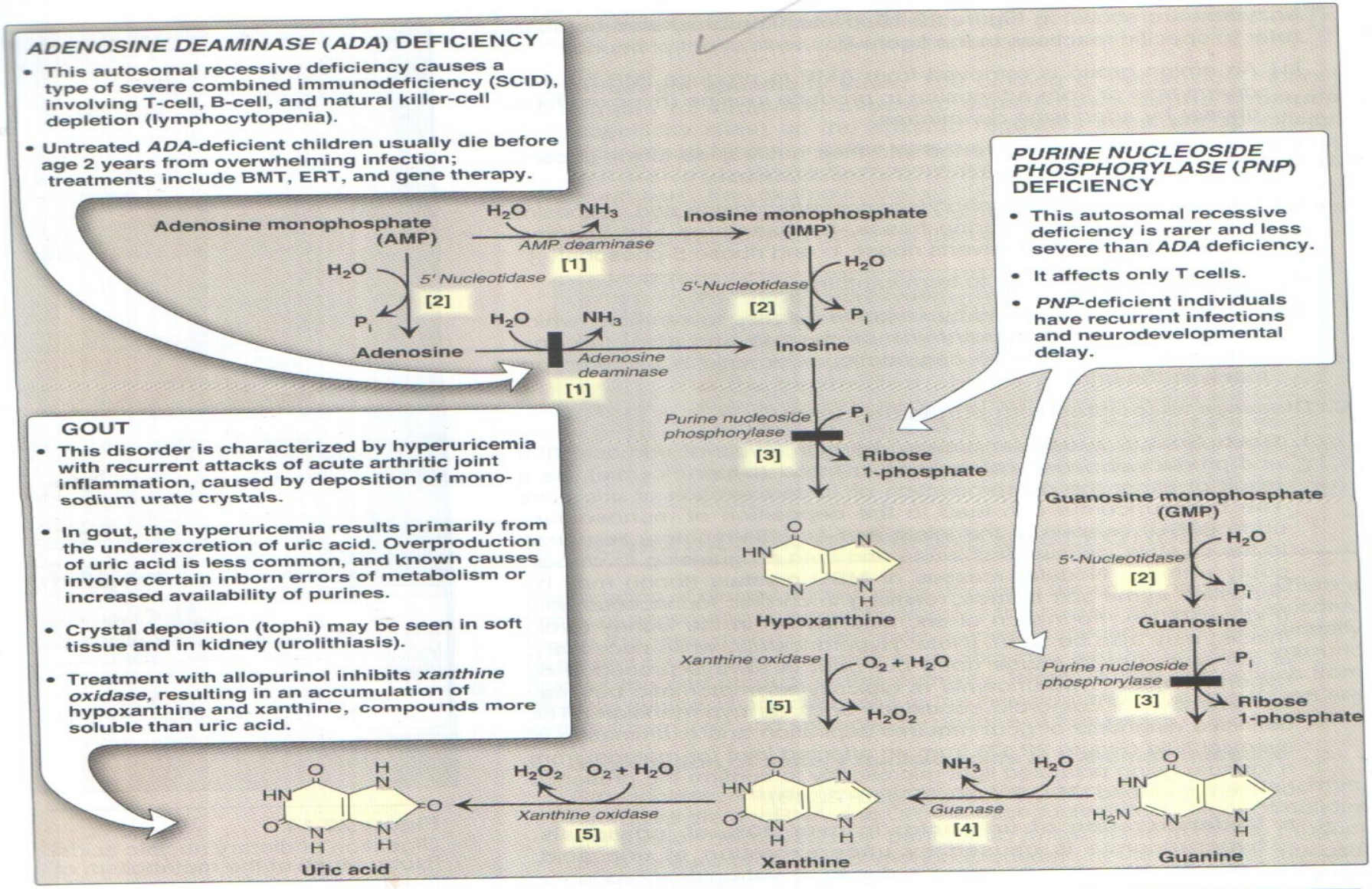


Figure 22.15
The degradation of purine nucleotides to uric acid, illustrating some of the genetic diseases associated with this pathway. [Note: The numbers in brackets refer to the corresponding numbered citations in the text.] BMT = bone marrow transplantation; ERT = enzyme replacement therapy; P_i = inorganic phosphate.

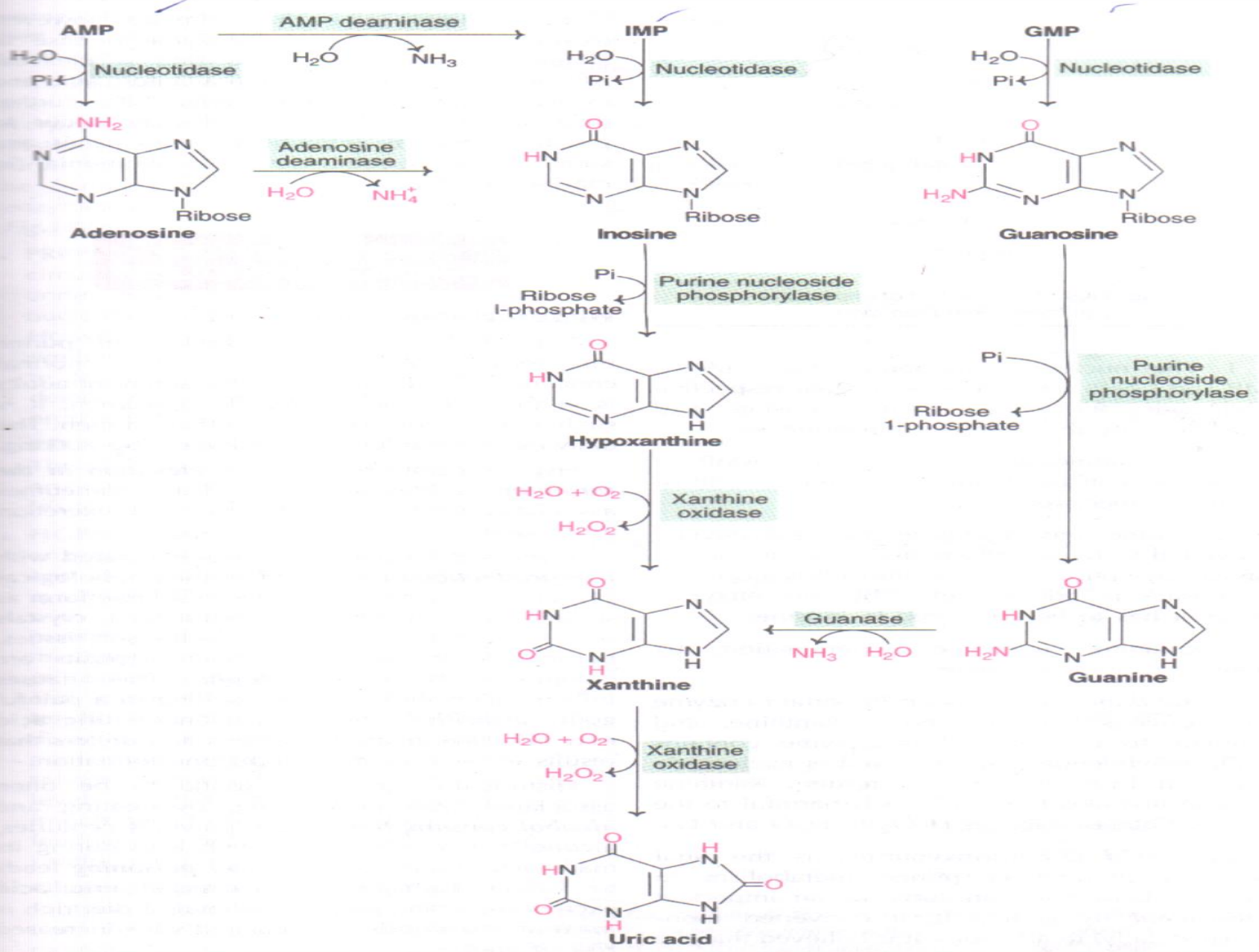


Fig. 17.7 : Degradation of purine nucleotides to uric acid (AMP–Adenosine monophosphate; IMP–Inosine monophosphate; GMP–Guanosine monophosphate).

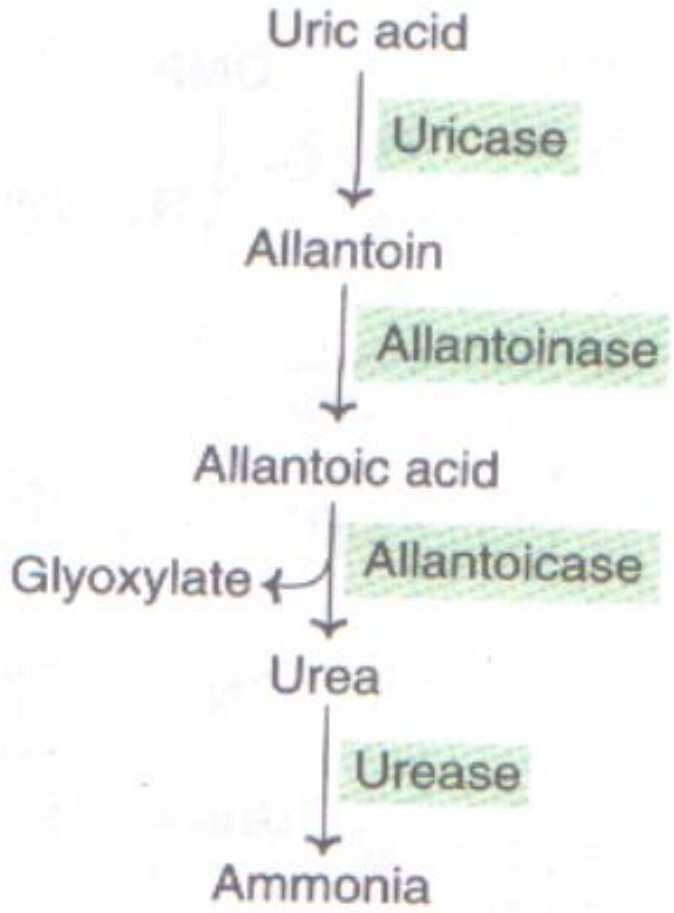


Fig. 17.8 : *Degradation of uric acid in animals other than man.*

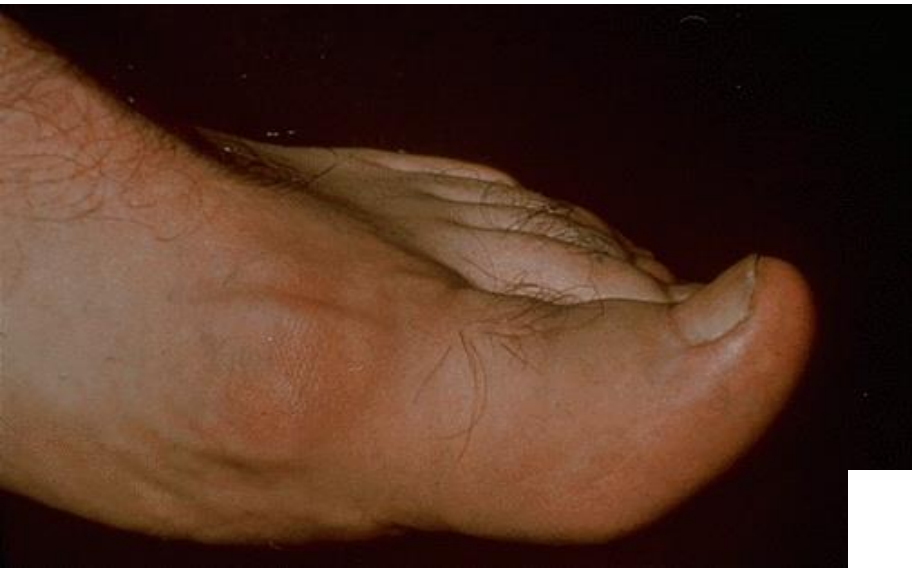
- Xanthine oxidase contain FAD ,molybdenum and iron and is exclusively found in liver and small intestine.
- Molecular oxygen is reduced at each stage to super oxide (O^-_2) which is converted to

  H_2O_2 by super oxide dismutase.
- Catalase cleves H_2O_2 to H_2O and O_2

- Allopurinol is competitive inhibitor of Xanthine oxidase.
- Allopurinol is a best example of suiccidal inhibition.
- Allopurinol $\xrightarrow{\text{xanthine oxidase}}$ Alloxanthine which is a stronger inhibitor of xanthine oxidase.

DISORDERS OF PURINE METABOLISM

Gout



Sodium Urate Crystals

GOUT

- Gout is not a single disease.
- The term is used to describe a number of disorders in which crystals of Mono sodium urate monohydrate (uric acid) derived from Hyperuricemic body fluid give rise to
- Inflammatory arthritis
- Urolithiasis
- Renal disease

3.4-------7mg/dl in males

2.4-------5.8mg /dl in Females

- Uric acid is formed by oxidation of purine bases , which may be exogenous or endogenous in origin.
- It is formed in the liver and excreted largely through kidneys (2/3),some is excreted in bile,some is converted to urea and amonia by the intestinal bacteria.
- U.A is completely filtered at Glomerulus, reabsorbed at prox conv Tubules and secreted further along the Tubules.

Hyperuricemia occurs as a result of

- A. Over production of U.A (Metabolic)
- B. Under excretion of U.A (Renal)

A.Over production

1.Genetic disorders

PRPP synthetase :

Variant forms of PRPP synthetase ,may be

i. Superactive
ii. Resistant to feed back inhibition
iii. Low Km for ribose-5-Phosphate

PRPP glutamylamidotransferase:

The lack of feedback control

HGPRT deficiency :

Von –Gierke`s disease: (G6-Phosphatase deficiency)

Fructose intolerance

2. High purine and Fructose intake:
3. Increase tissue breakdown (Treatment of malagnancies)
4. Alcohol consumption (lactic acidosis)

B. Under Excretion

Renal diseases:

Hypertension : (Essential)

Enhances prox Tub reabsorbtion and

Depresses renal Tub secr of U.A.

Diuretic Therapy: (Similar Mechanism)

Diabetes Mellitus:(Increase Insulin. similar mechanism)

Clinical feature of Gout

1.Metatarso Phalangeal Joint of a great Toe (70%) (Podagra).

- Other Joints may be affected .
- The effected joint is Hot,Red and swollen with shiny overlying skin and dilated veins.
- It is severely painful and Tender.

2. Deposition of urate crystals in soft tissues (Tophaceous gout).

3. Renal Complication

Such as pain in the lumber region due to stone formation and the stones may cause renal failure.

Von Gierke,s Diseas

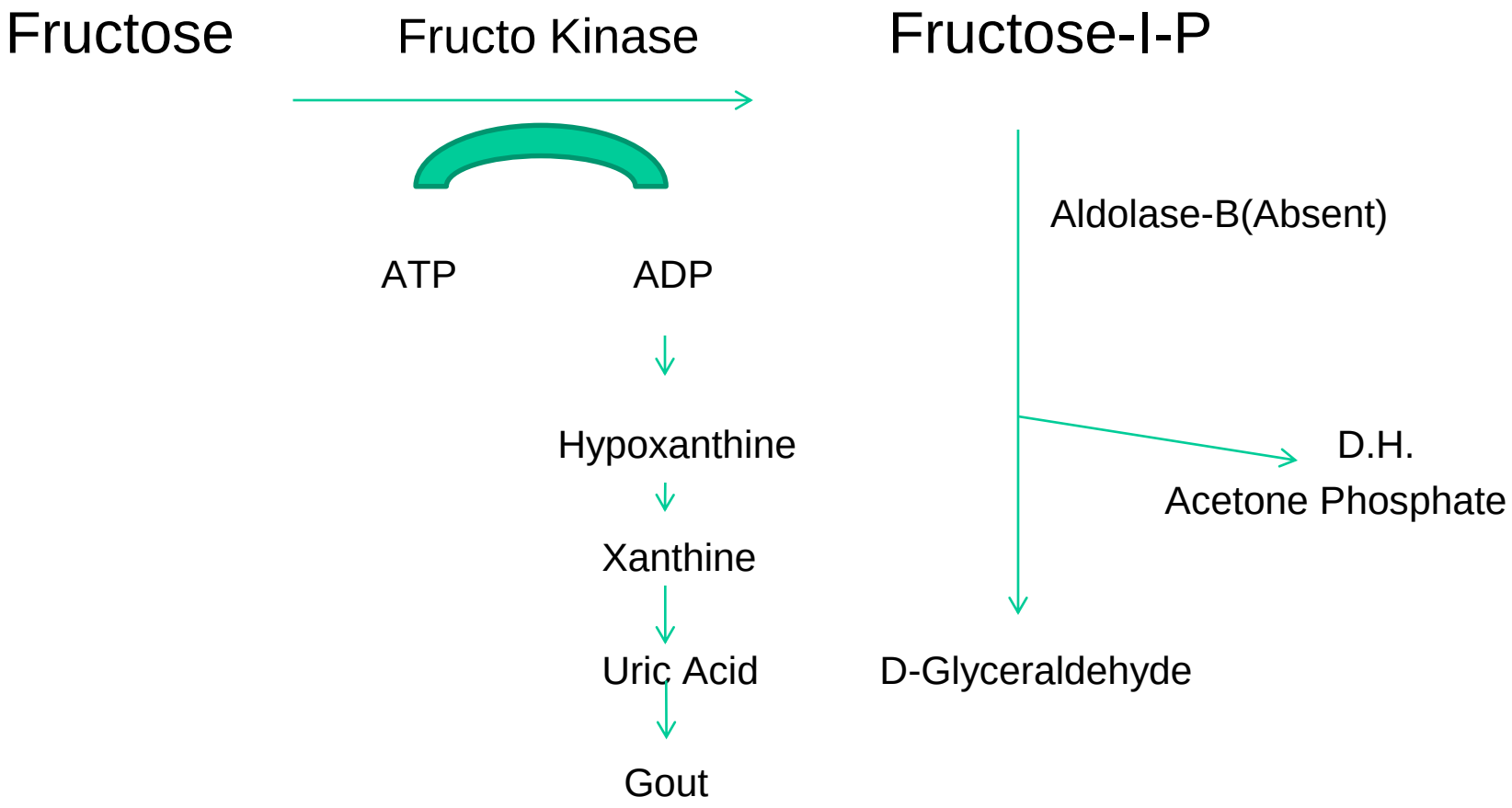
- Hyper uricemia occurs due to glucose-6-phosphatase-deficiency
- Glucose
- 
- G-6-P
- So more G-6-P will be shunted into HMP Shunt, Resulting in more formation of

R-5-P, PRPP and Purine over production .

Lactic Acidosis

- Increased lactic acid competes with uric acid for excretion, resulting in retention of uric acid .

Fructose Intolerance and Gout



Disorders of Purine Catabolism

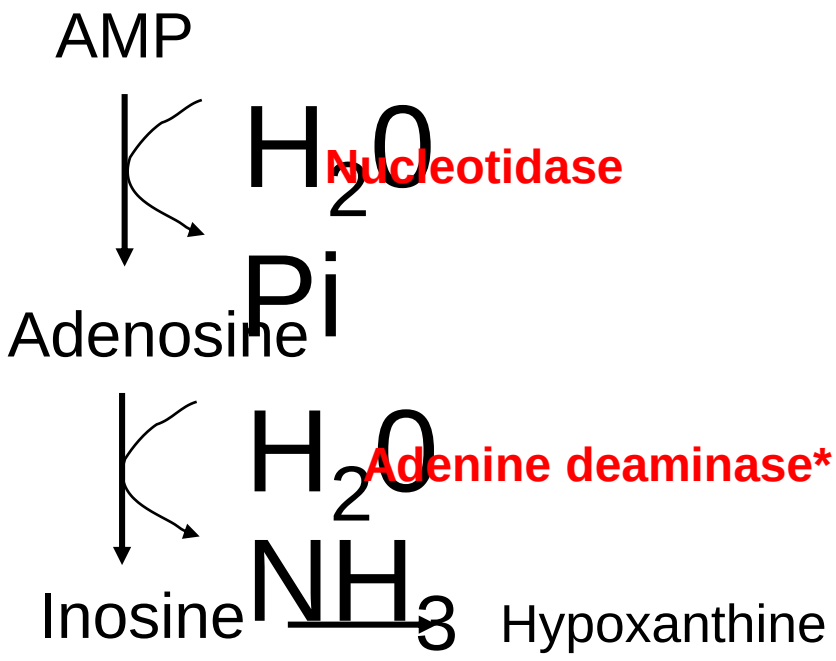
- 1. Gout
- 2.Lesch-Nyhan Syndrome
- 3.Von Gierke Disease
- 4. Hypouricemia
- 5.Adenosine Deaminase Deficiency
- 6. Purine Nucleoside Phosphorlylase Deficiency.

Table 36–1. Inherited disorders of purine metabolism and their associated enzyme abnormalities.

Clinical Disorder	Defective Enzyme	Nature of the Defect	Characteristics of Clinical Disorder	Inheritance Pattern
Gout	PRPP synthetase	Superactive (increased V_{max})	Purine overproduction and overexcretion	X-linked recessive
Gout	PRPP synthetase	Resistance to feedback inhibition	Purine overproduction and overexcretion	X-linked recessive
Gout	PRPP synthetase	Low K_m for ribose 5-phosphate	Purine overproduction and overexcretion	Probably x-linked recessive
Gout	HGPRTase[1]	Partial deficiency	Purine overproduction and overexcretion	X-linked recessive
Lesch-Nyhan syndrome	HGPRTase[1]	Complete deficiency	Purine overproduction and overexcretion; self-mutilation	X-linked recessive
Immunodeficiency	Adenosine deaminase	Severe deficiency	Combined (T cell and B cell) immunodeficiency, deoxyadenosinuria	Autosomal recessive
Immunodeficiency	Purine nucleoside phosphorylase	Severe deficiency	T cell deficiency, inosinuria, deoxyinosinuria, guanosinuria, deoxyguanosinuria, hypouricemia	Autosomal recessive
Renal lithiasis	Adenine phosphoribosyltransferase	Complete deficiency	2,8-Dihydroxyadenine renal lithiasis	Autosomal recessive
Xanthinuria	Xanthine oxidase	Complete deficiency	Xanthine renal lithiasis, hypouricemia	Autosomal recessive

[1]HGPRTase = hypoxanthine-guanine phosphoribosyltransferase (Figure 36–6).

SCID-Severe Combined Immunodeficiency Syndrome



Autosomal recessive disorder Mutations in ADA

ADA deficiency

- Lymphocytes have highest concentration of ADA.
- Deficiency of Enzyme results in accumulation of Adenosine, which is converted in the ribonucleotide & deoxy ribonucleotide form.
- As dATP level rises, it inhibits ribonucleotide reductase thus preventing the production of all deoxyribonucleotide containing nucleotides so cell cannot make DNA & divide.
- Since T-cells & B-cells are mitotically most active cells

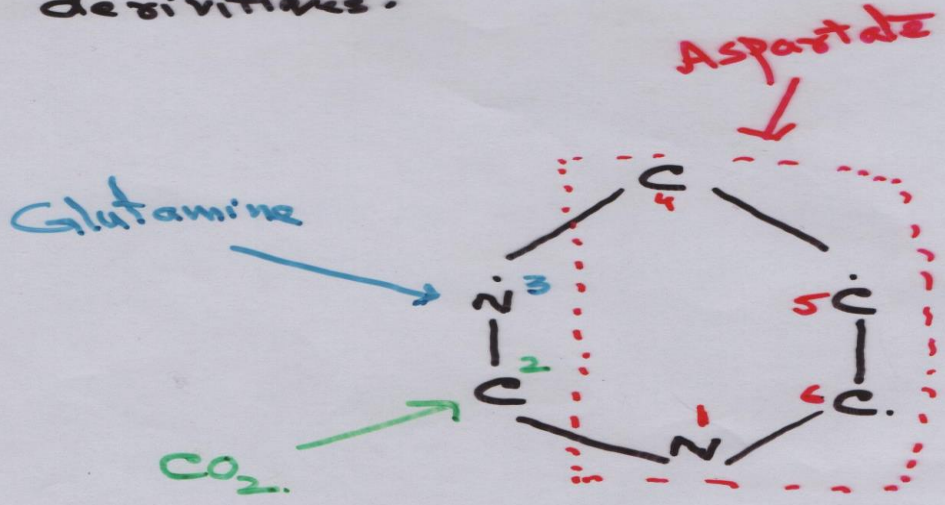
  so there is decrease in T-cells,B-cells
- Children usually die by the age of 2yrs by infections.

- PYRIMIDINE SYNTHESIS

Pyrimidine Synthesis :.

- Unlike the Synth of Purine ring, where the ring is constructed on a pre-existing ribose – 5 – phosphate, the Pyrimidine ring is Synth before being attached to ribose-5-phosp, which is denoted by PRPP..

- Purimidine and Purine nucleoside biosynth share several common precursors i.e PRPP, glutamine, CO_2, aspartate and Tetrahydro folate derivitives.



Sources of individual atoms in Pyrimidine ring.

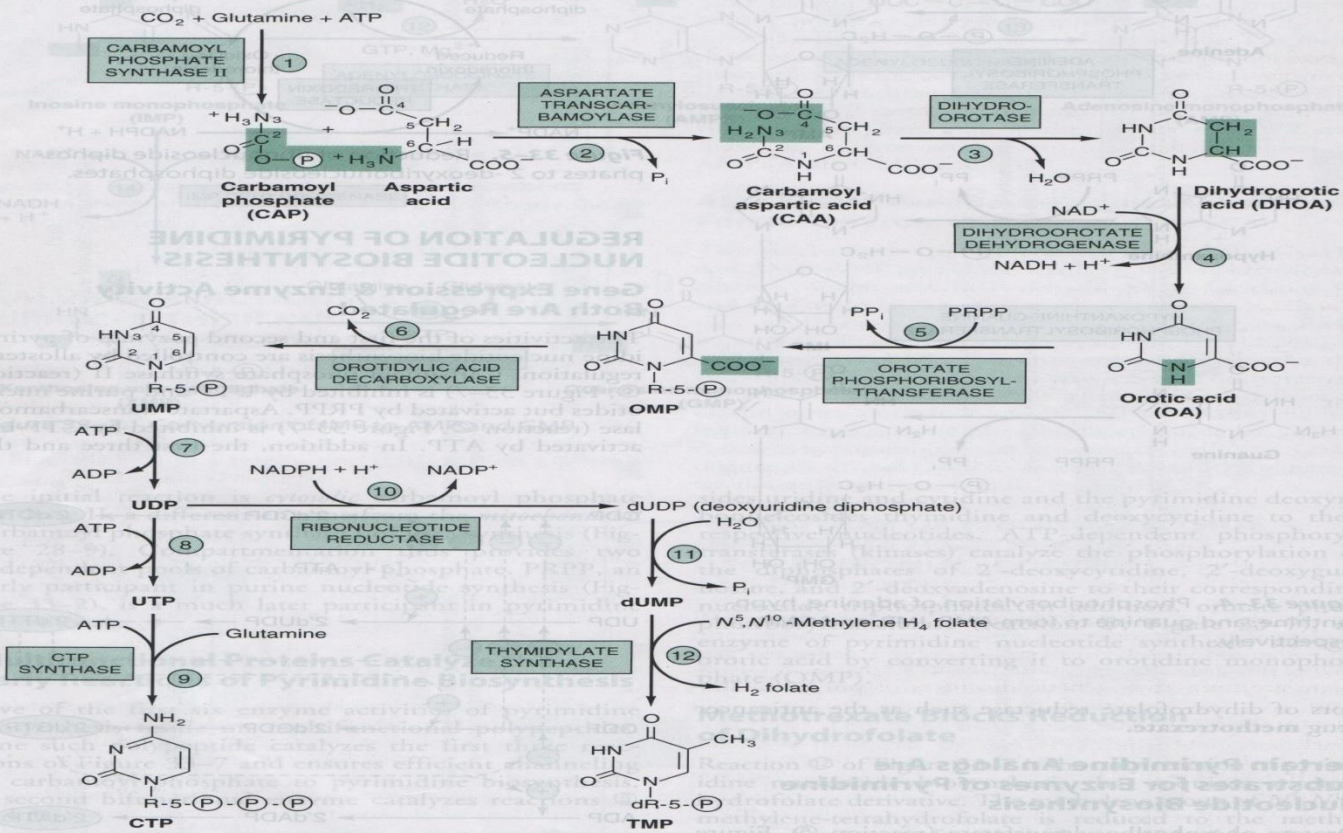


Figure 33–7. The biosynthetic pathway for pyrimidine nucleotides.

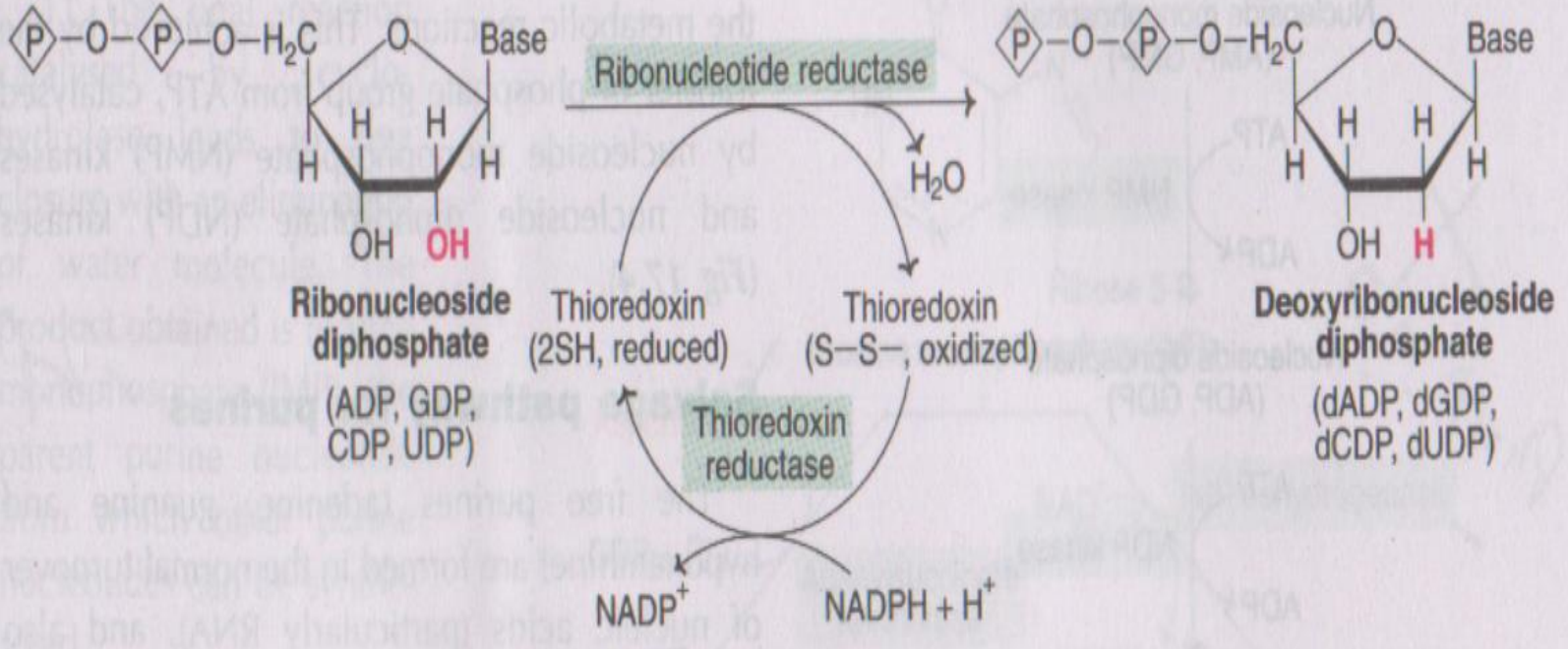


Fig. 17.6 : Formation of deoxyribonucleotides from ribonucleotides.

Ribonucleotide reductase provides the hydrogen atoms needed for reduction from its sulfhdryl groups.

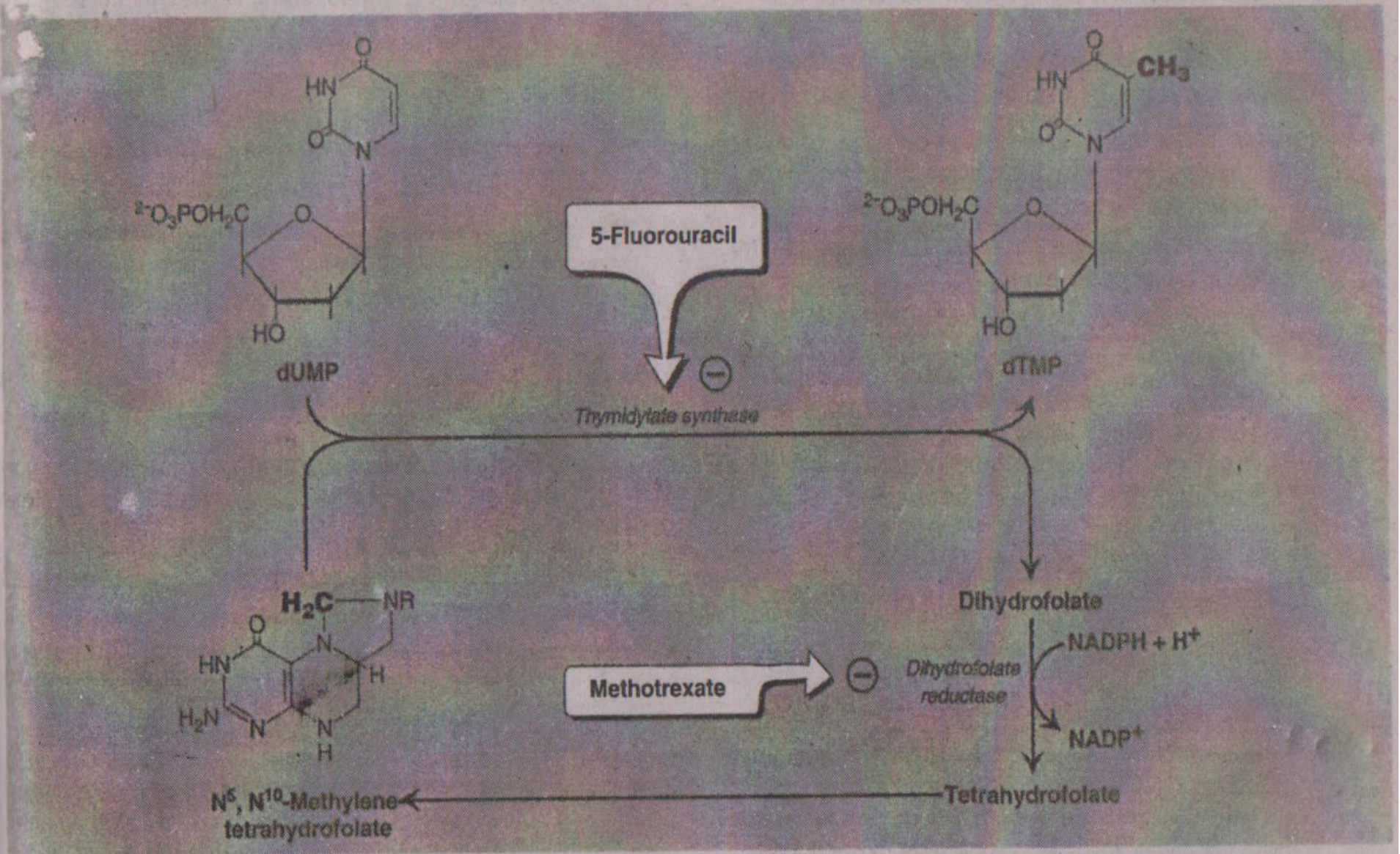


Figure 29.17
Synthesis of dTMP from dUMP illustrating sites of action of antineoplastic drugs.

1. The first three enzymes of the cycle are domains of single polypeptide chain.
2. Orotate phosphoribosyl Transferase and OMP decarboxylase are domains of single Polypeptide chain.

Dihydro orotate Dehydrogenase

- Is the only mitochondiral enzyme of the cycle .
- Contain FMN, FAD, Fe, S as Prosthetic group and require NAD as Coenzyme

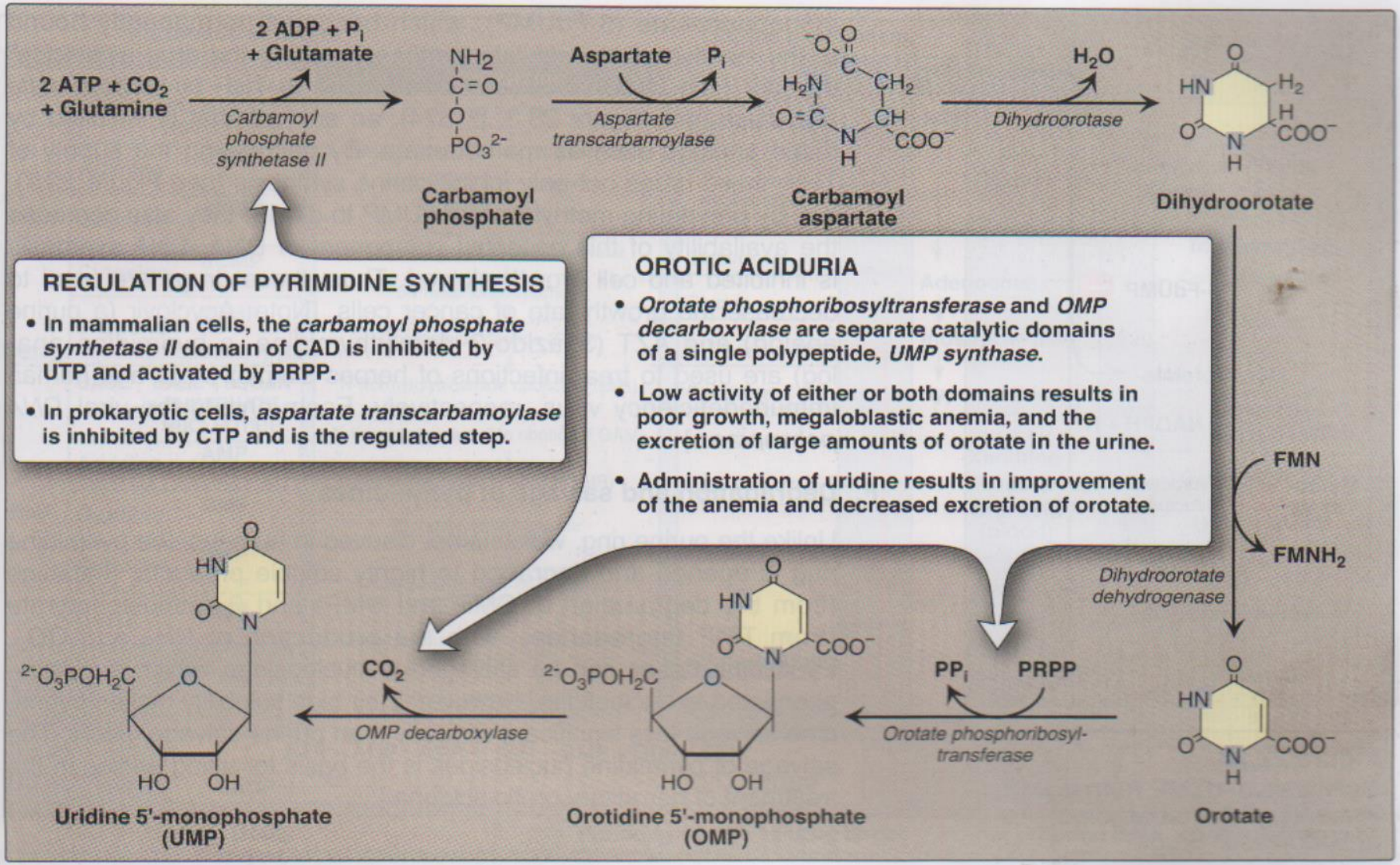


Figure 22.21

De novo pyrimidine synthesis. ADP = adenosine diphosphate; P_i = inorganic phosphate; FMN(H_2) = flavin mononucleotide; CTP = cytidine triphosphate; PRPP = 5-phosphoribosyl-1-pyrophosphate; PP_i = pyrophosphate.

Orotic Aciduria

Mainly of two types

Type-1. Orotate phosphoribosyl transferase and OMP decarboxylase(Both) are deficient.

- Results in accumulation of orotate in blood causing growth retardation .Megaloblastic Anaemia.

Type-2: Deficiency of OMP decarboxylase

- Megaloblastic Aneamia .

Other causes of orotic Aciduria

1. Reye syndrome------
   Damaged mitochondria
2. Deficiency of urea cycle enzyme i.e
   Ornithine transcarbamyolase.
3. Drugs, e.g Allopurinol and 6-
   Azauridine.

$CO_2 + NH_4^+ + 2ATP \longrightarrow$ Carbamoyl Phosphate

(3) Ornithine Transcarbamylase

+ ornithine

↓ = citrulline

If ornithine Transcarbamylase is deficient or results in increased excretion of orotic acid and uridine.

Carbamoyl phosphate exits in the cytosol, where it stimulates pyrimidine biosynthesis, the substrate undergoes utilized

Result in mild Orotic Aciduria.

Orotic Aciduria = Results from the absence of either one or both of the enzymes (1) OPRTase and (2) OMP decarboxylase

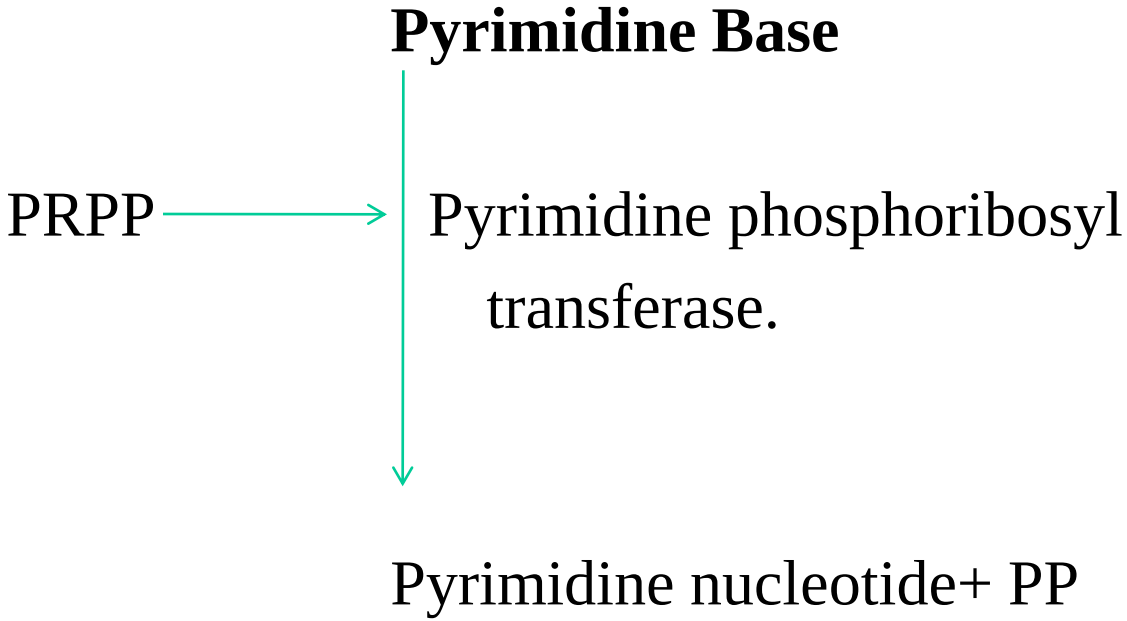
- Due to feed back inhibition lack, orotic acid production is excessive. The condition can be treated by feeding cytidine or uridine, which are converted to UTP, which acts as feed back inhibitor

- OMP and OPRTase are domains of single polypeptide chain.

Orotic Aciduria Causes U.T.Obst → Megaloblastic Anemia. Crystals

PYRIMIDINE BASE SALVAGE

- The enzyme pyrimidine phosphoribosyl transferase catalyzes the formation of pyrimidine nucleotide, using PRPP as the donor of ribosyl moiety.



Synthetases:

ATP dependent enzymes catalyzing biosynthetic reactions and belong to Ligases

e.g - Carbamoyl-PO4 synthetase

- Arginine Succinate synthetase

- Glutamine synthetase

Synthases:

Enzymes catalyzing biosynthetic reactions but do not require ATP. they belong to classes other than Ligases. e.g

Glycogen synthase,

ALA synthase

IMP synthase

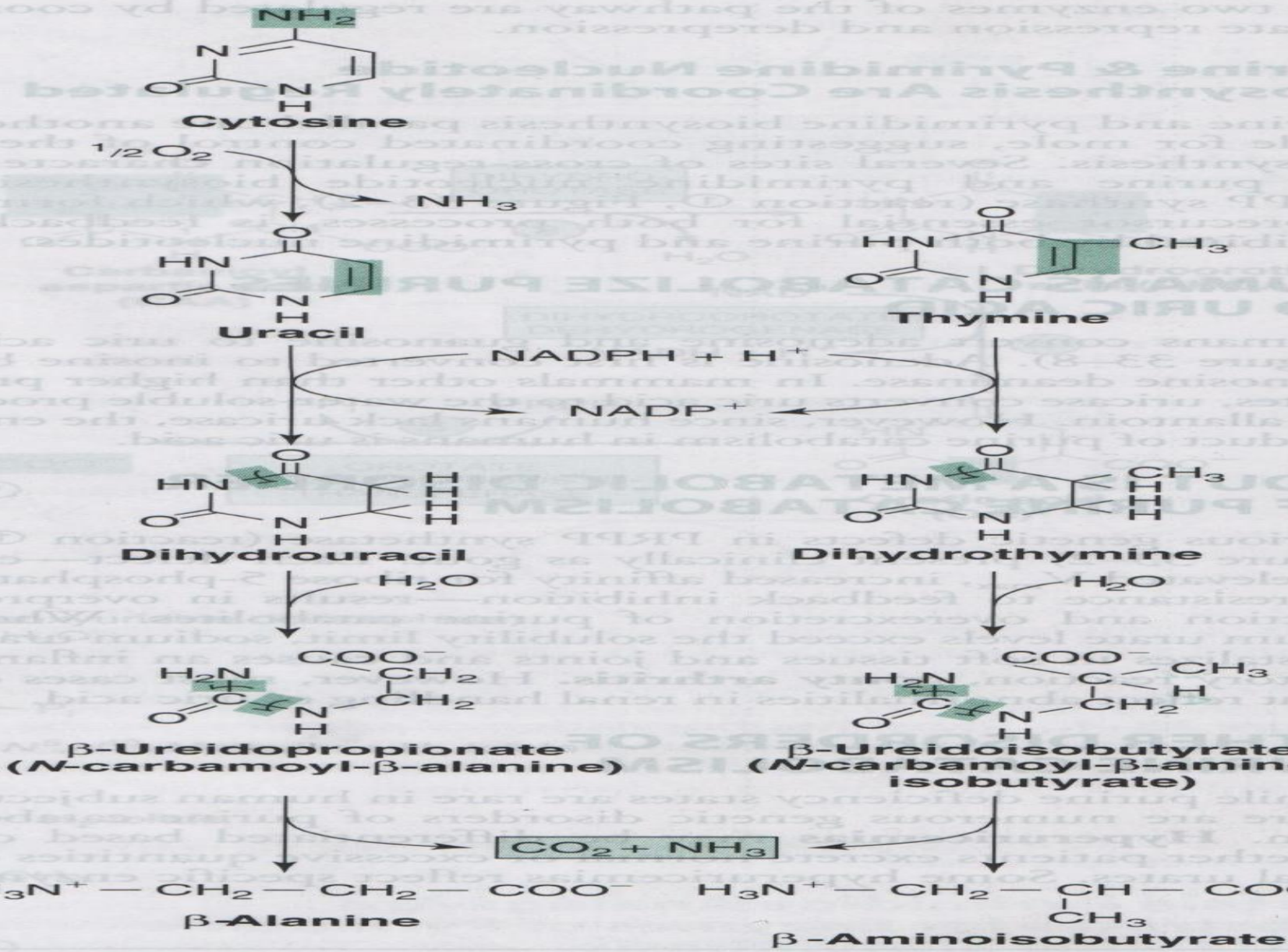


Figure 33–9. Catabolism of pyrimidines.

Chinese or Japanese ancestry routinely excrete β-amino-isobutyrate. Humans probably transaminate β-amino-isobutyrate to methylmalonate semialdehyde, which then forms succinyl-CoA (see Figure 20–2).