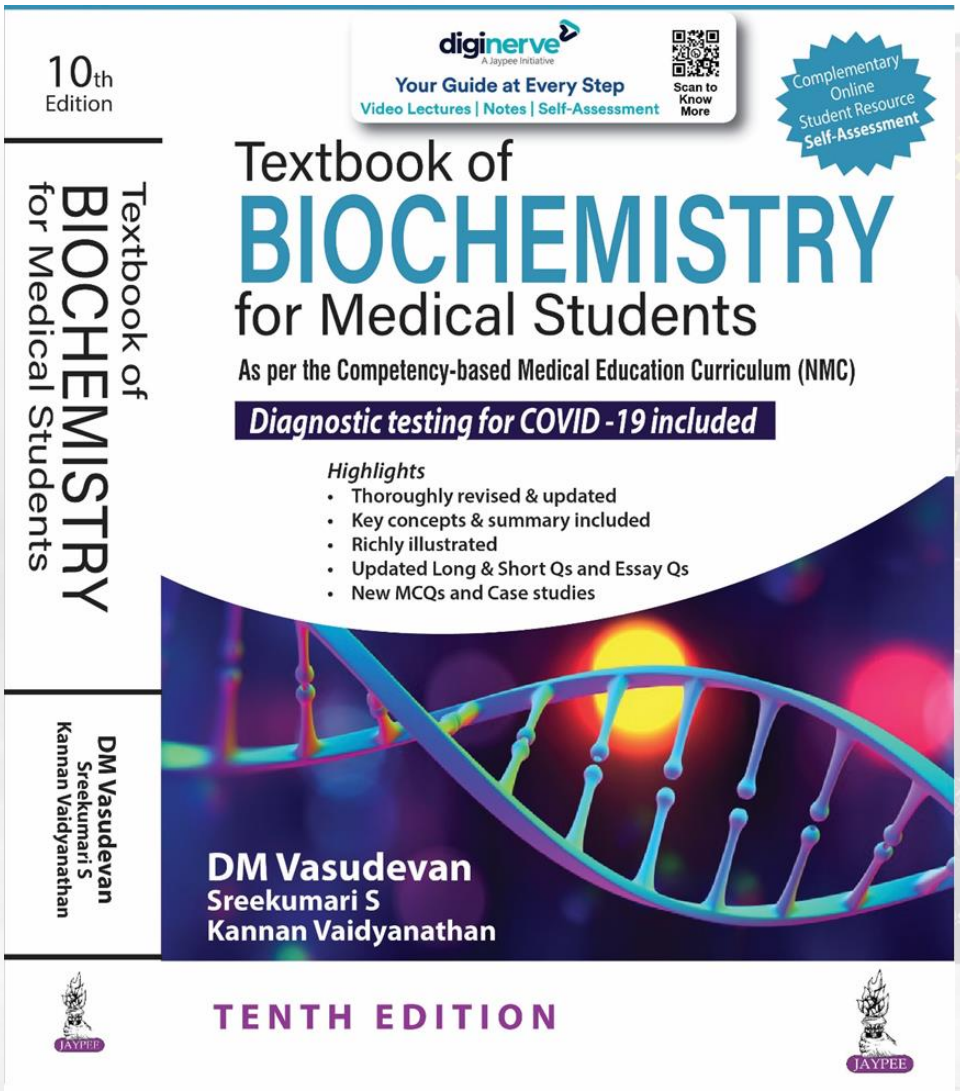


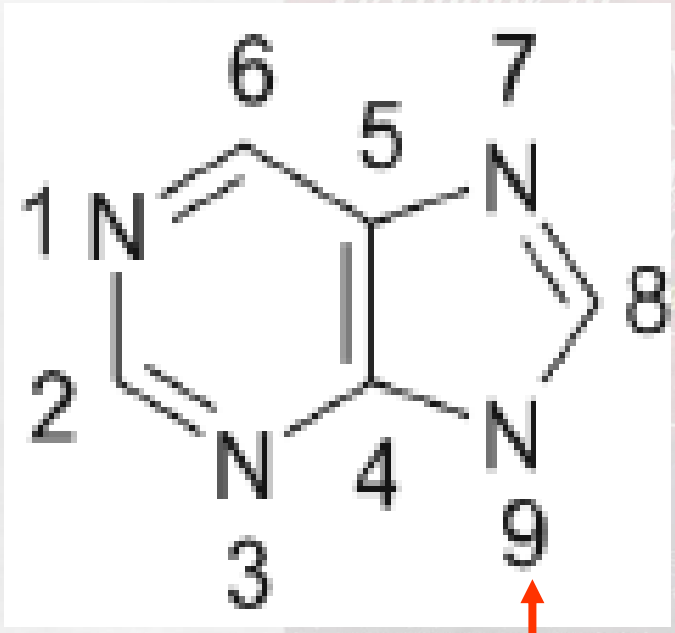
Chapter 33: Nucleotides, Chemistry and Metabolism

Textbook of
BIOCHEMISTRY
for Medical Students

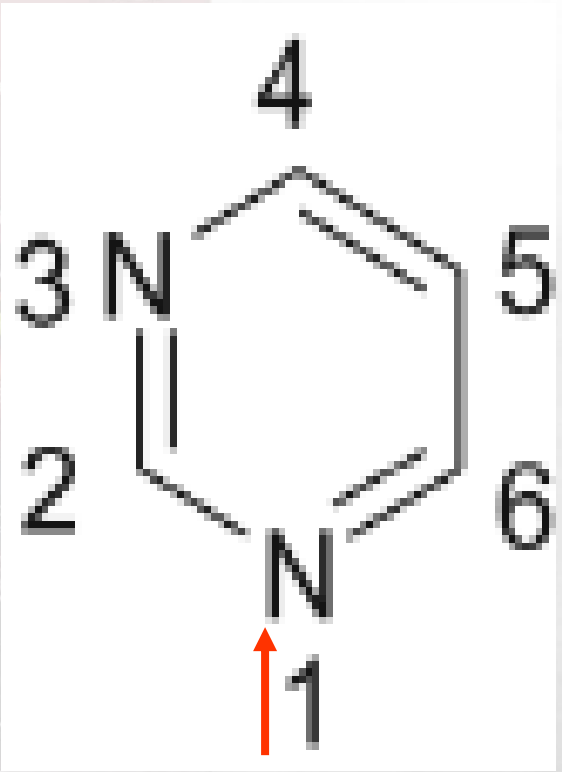
By DM Vasudevan, *et al.*

TENTH EDITION

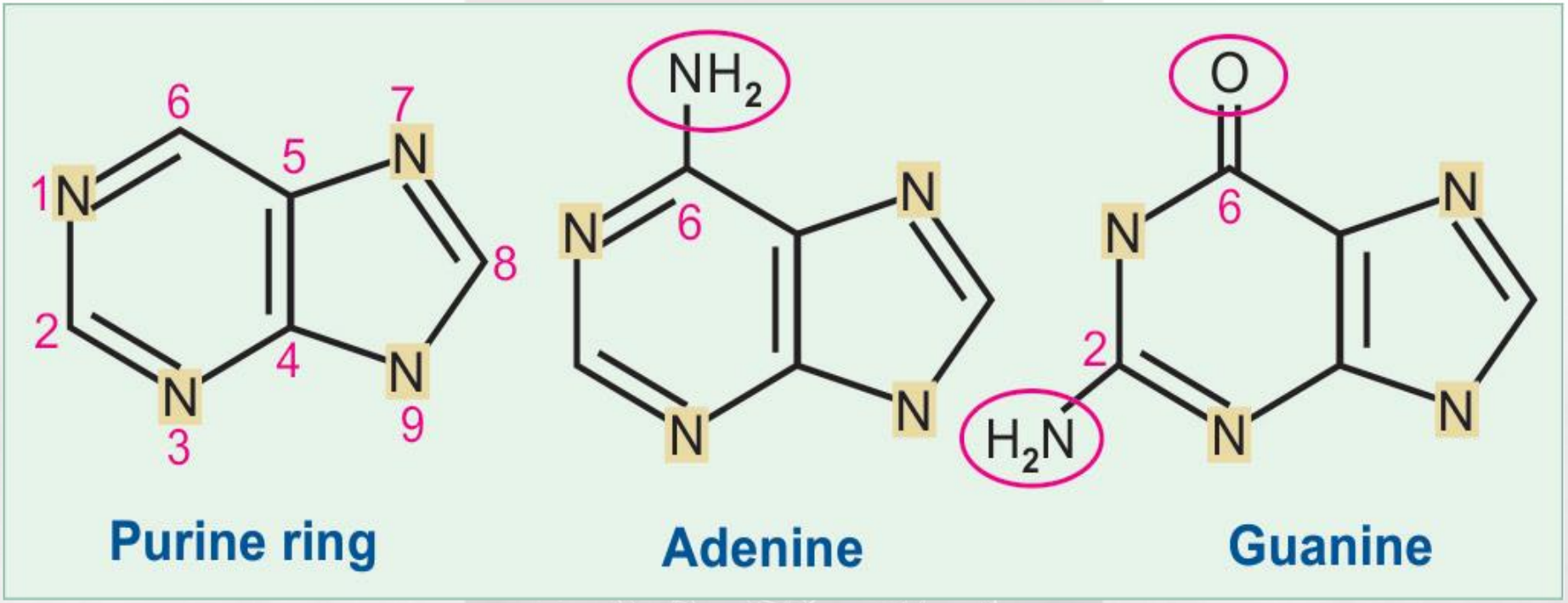
Two types of nitrogenous bases; **purines** and **pyrimidines** are present in nucleic acids.



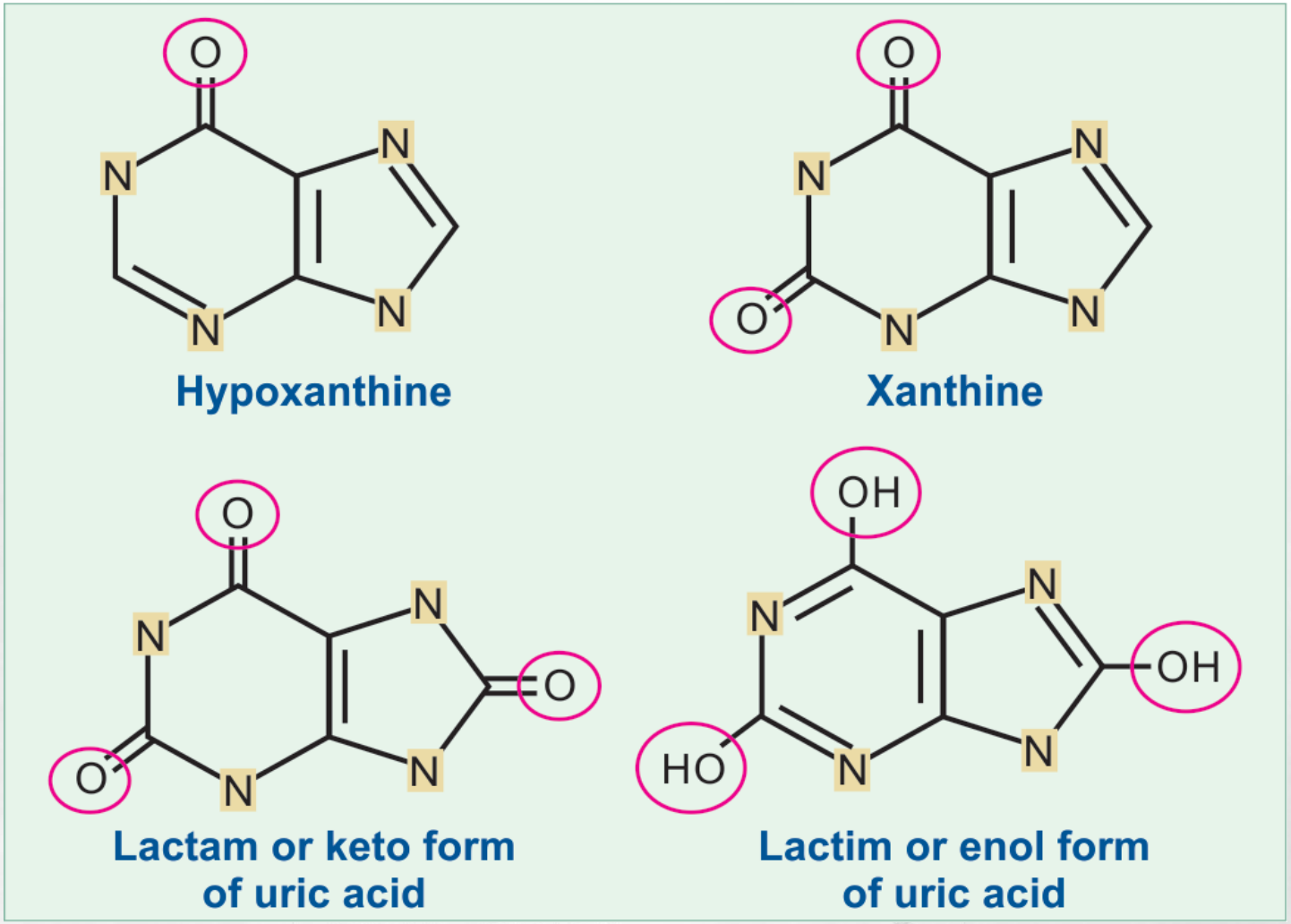
PURINE RING



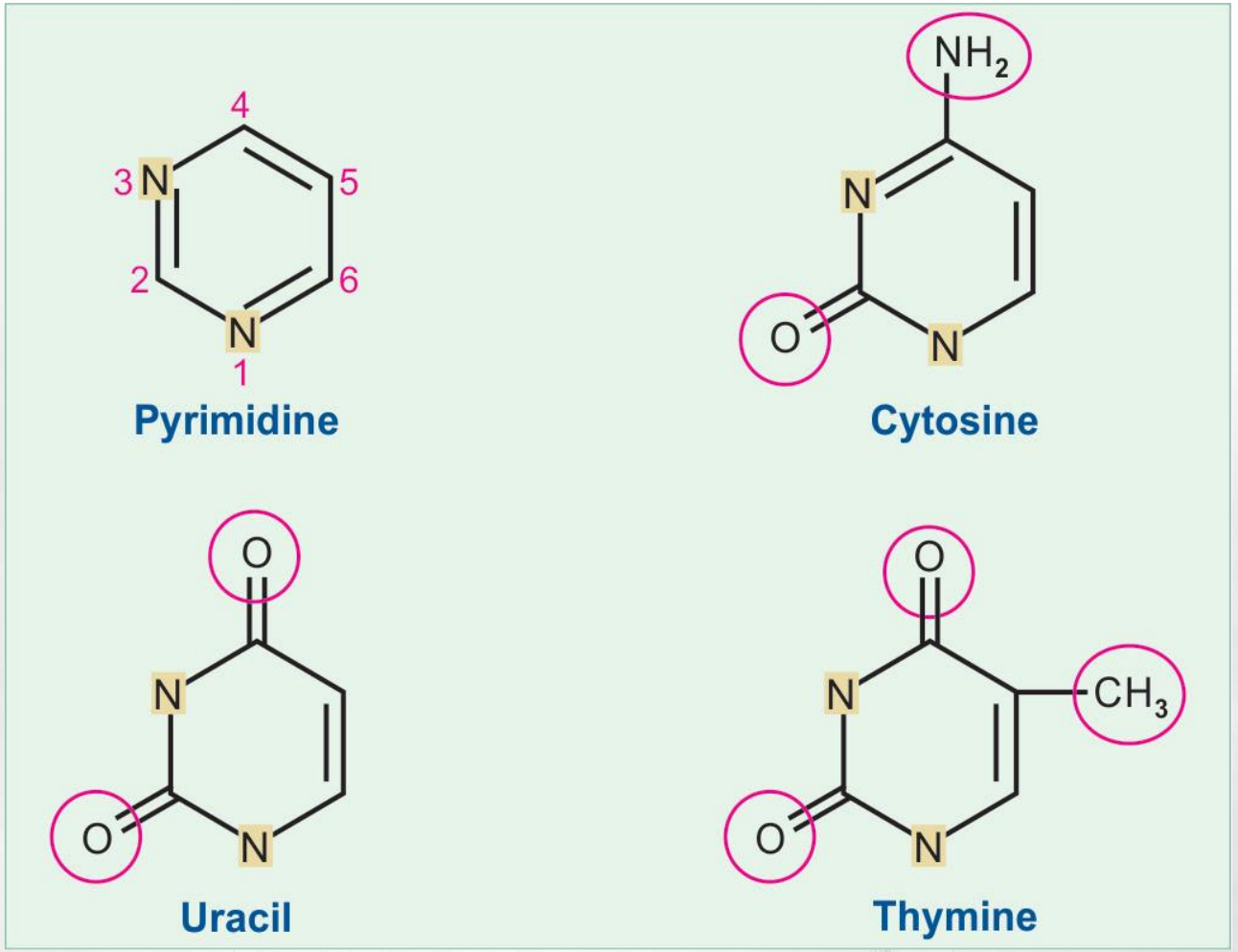
Pyrimidine



Structure of purines.



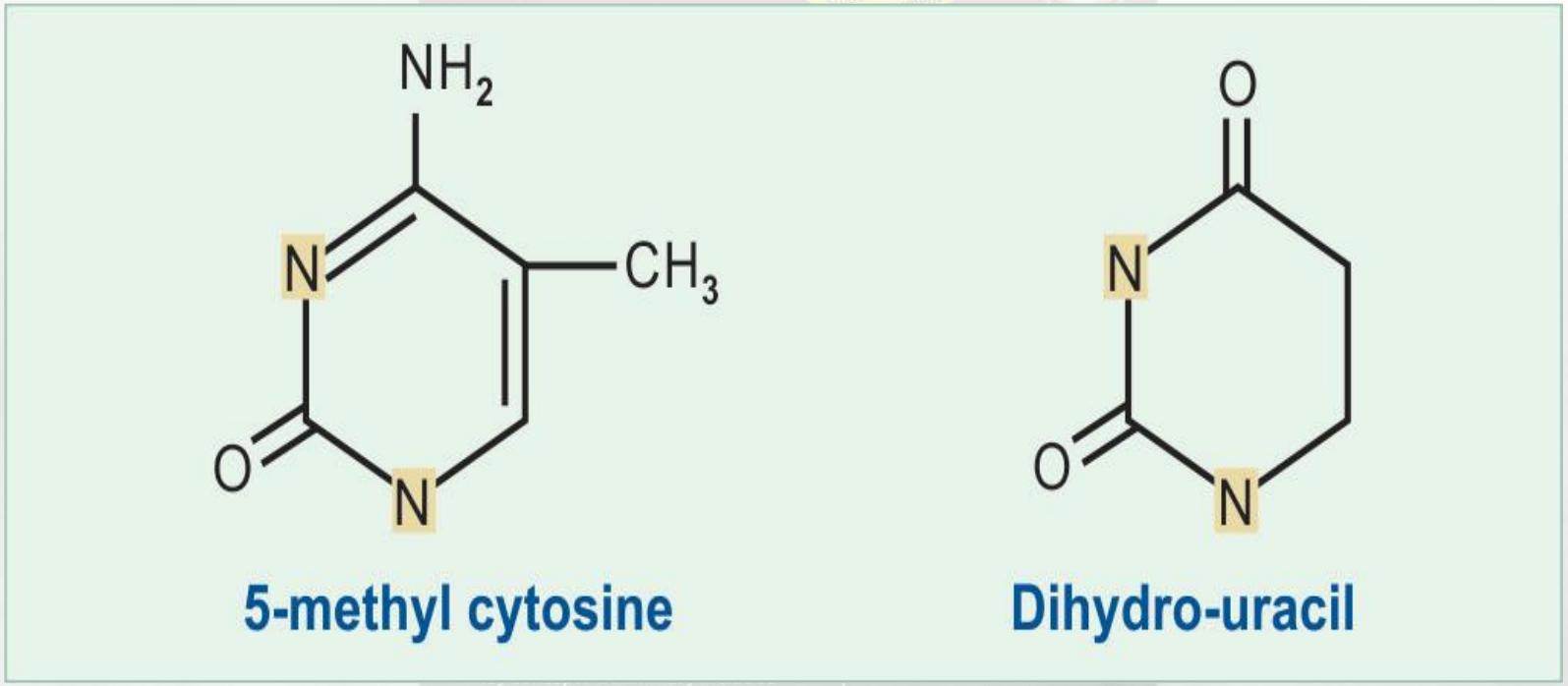
Minor bases seen in nucleic acids



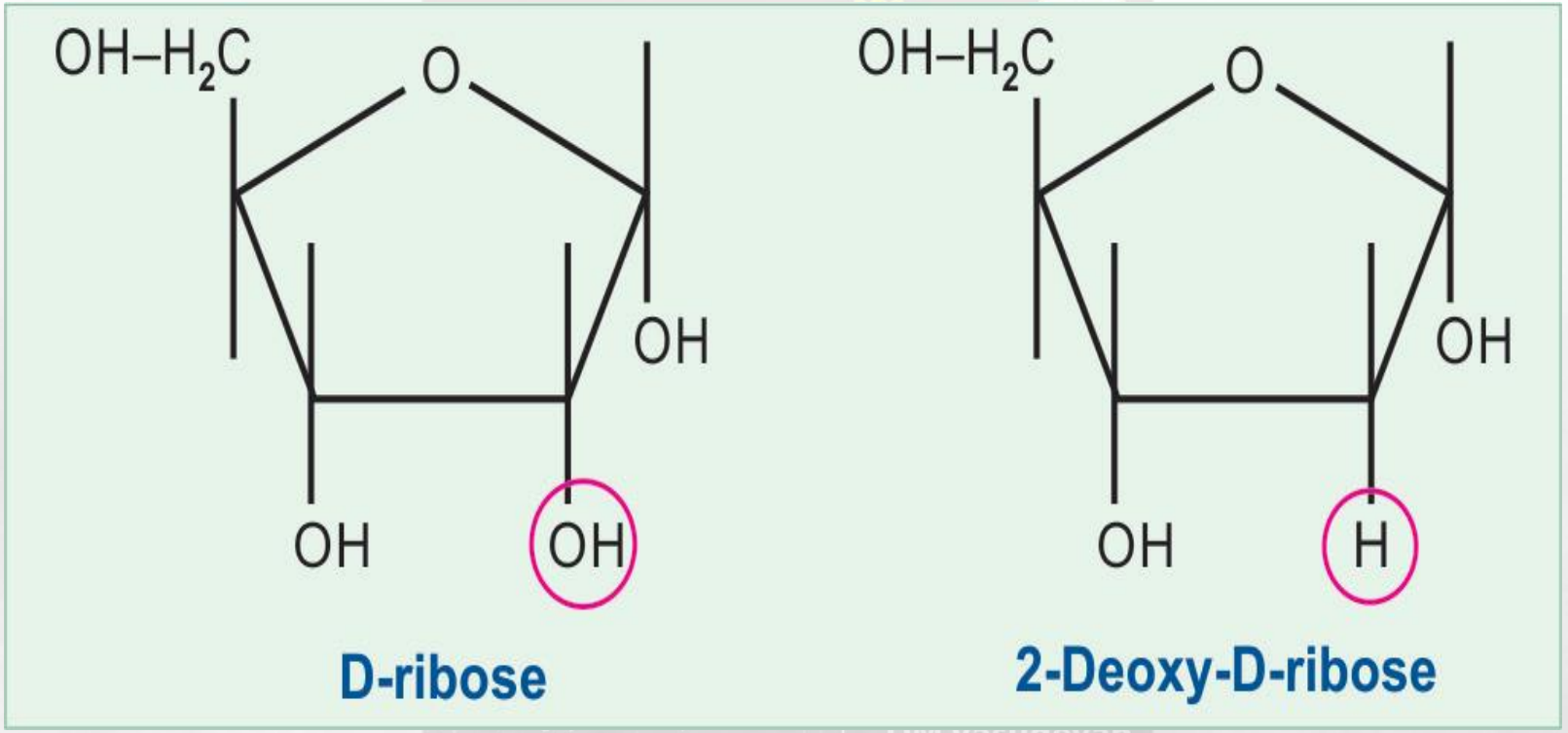
Common pyrimidines.

THYMINE is the base present in DNA

THIAMINE is a member of vitamin B complex



Modified pyrimidine bases.



Sugar groups in nucleic acids.

Nucleosides

Base + sugar,

(ribose or deoxy-ribose)

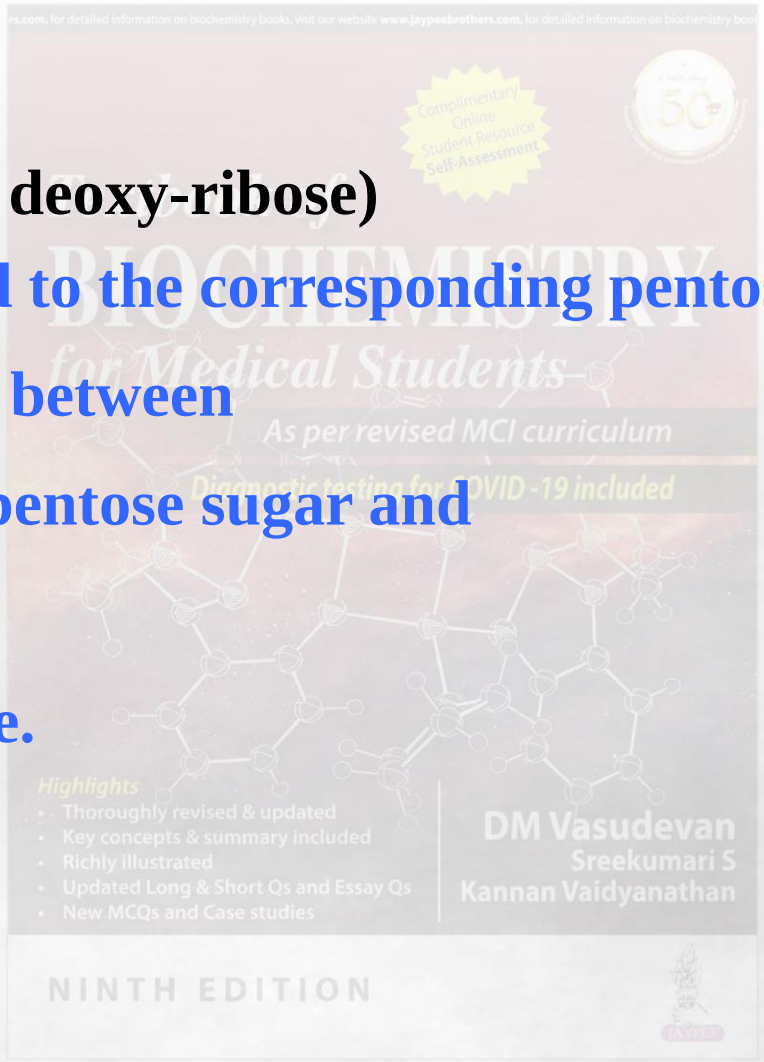
Bases are attached to the corresponding pentose sugar by

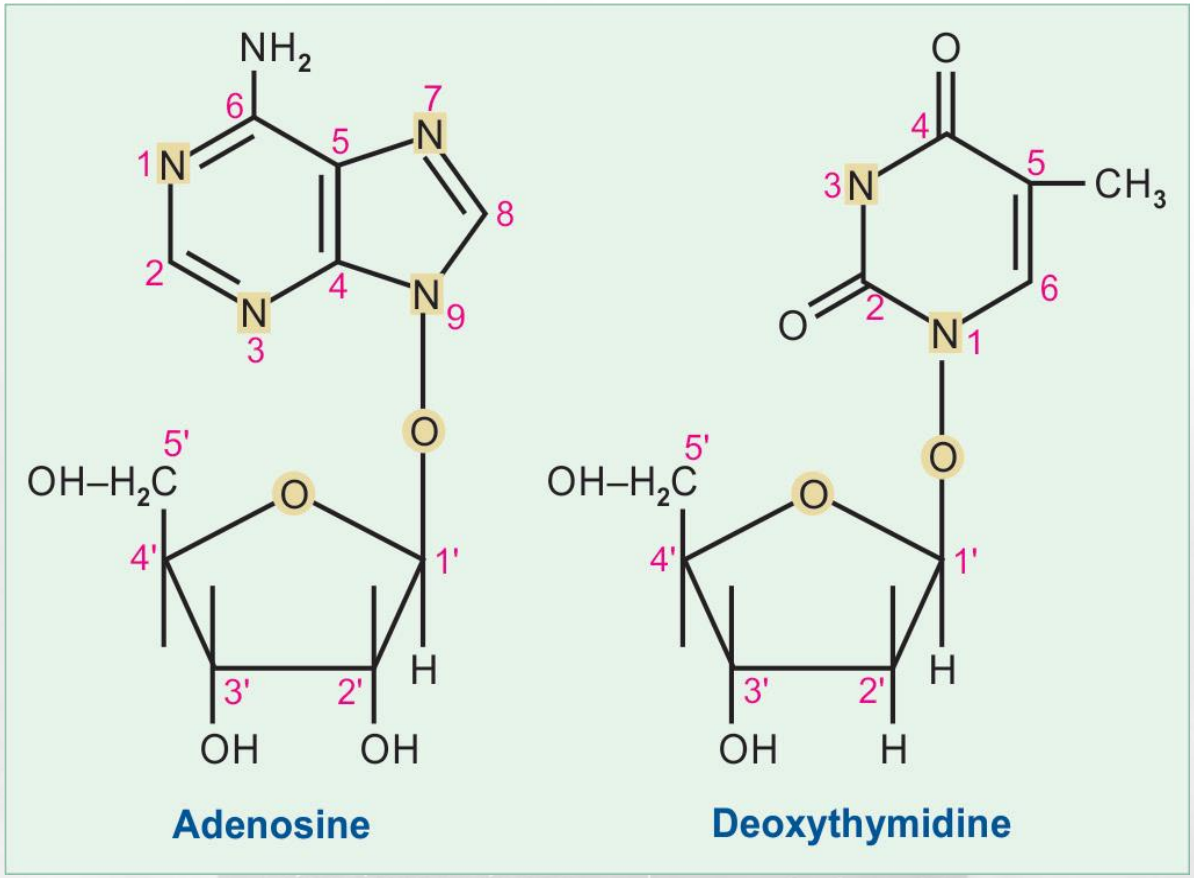
N-glycosidic bond between

1st carbon of the pentose sugar and

N9 of a purine or

N1 of a pyrimidine.





The carbon atoms of the pentose sugar are denoted by using a prime number to avoid confusion with the carbon atoms of the purine or pyrimidine ring

Base + sugar = nucleosides

Ribonucleosides		
Adenine	+ Ribose	→ Adenosine
Guanine	+ Ribose	→ Guanosine
Uracil	+ Ribose	→ Uridine
Cytosine	+ Ribose	→ Cytidine
Hypoxanthine	+ Ribose	→ Inosine
Xanthine	+ Ribose	→ Xanthosine
Deoxyribonucleosides		
Adenine	+ Deoxy ribose	→Deoxy adenosine (d-adenosine)
Guanine	+ Deoxy ribose	→d-guanosine
Cytosine	+ Deoxy ribose	→d-cytidine
Thymine	+ Deoxy ribose	→d-thymidine

Base+sugar+phosphate = nucleotide

Ribonucleotides		
Adenosine	+ Pi	Adenosine monophosphate (AMP) (Adenylic acid)
Guanosine	+ Pi	Guanosine monophosphate (GMP) (Guanylic acid)
Cytidine	+ Pi	Cytidine monophosphate (CMP) (Cytidylic acid)
Uridine	+ Pi	Uridine monophosphate (UMP) (Uridylic acid)
Inosine	+ Pi	Inosine monophosphate (IMP) (Inosinic acid)
Deoxyribonucleotides		
d-adenosine	+ Pi	d-AMP (d-adenylic acid)
d-guanosine	+ Pi	d-GMP (d-guanylic acid)
d-cytidine	+ Pi	d-CMP (d-cytidylic acid)
d-thymidine	+ Pi	d-TMP (d-thymidylic acid)

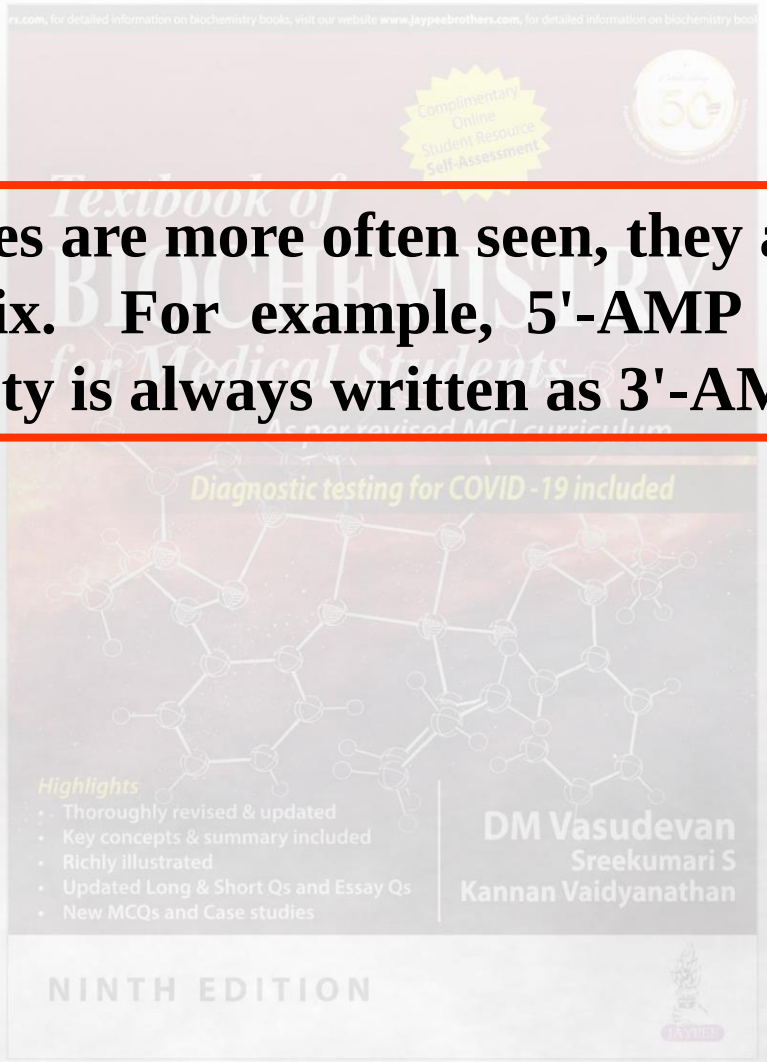
Nucleosides and nucleotides

Base	Sugar	Nucleoside	Phosphoric acid at	Nucleotide
Adenine	ribose	adenosine	5' position	AMP
do	do	do	3' position	3'-AMP
do	deoxyribose	d-adenosine	5' position	d-AMP
do	do	do	3' position	d-3'-AMP
Cytosine	ribose	cytidine	cytidine 5' position	CMP
do	do	do	3' position	3'-CMP
do	deoxyribose	d-cytidine	5' position	d-CMP
do	do	do	3' position	d-3'-CMP

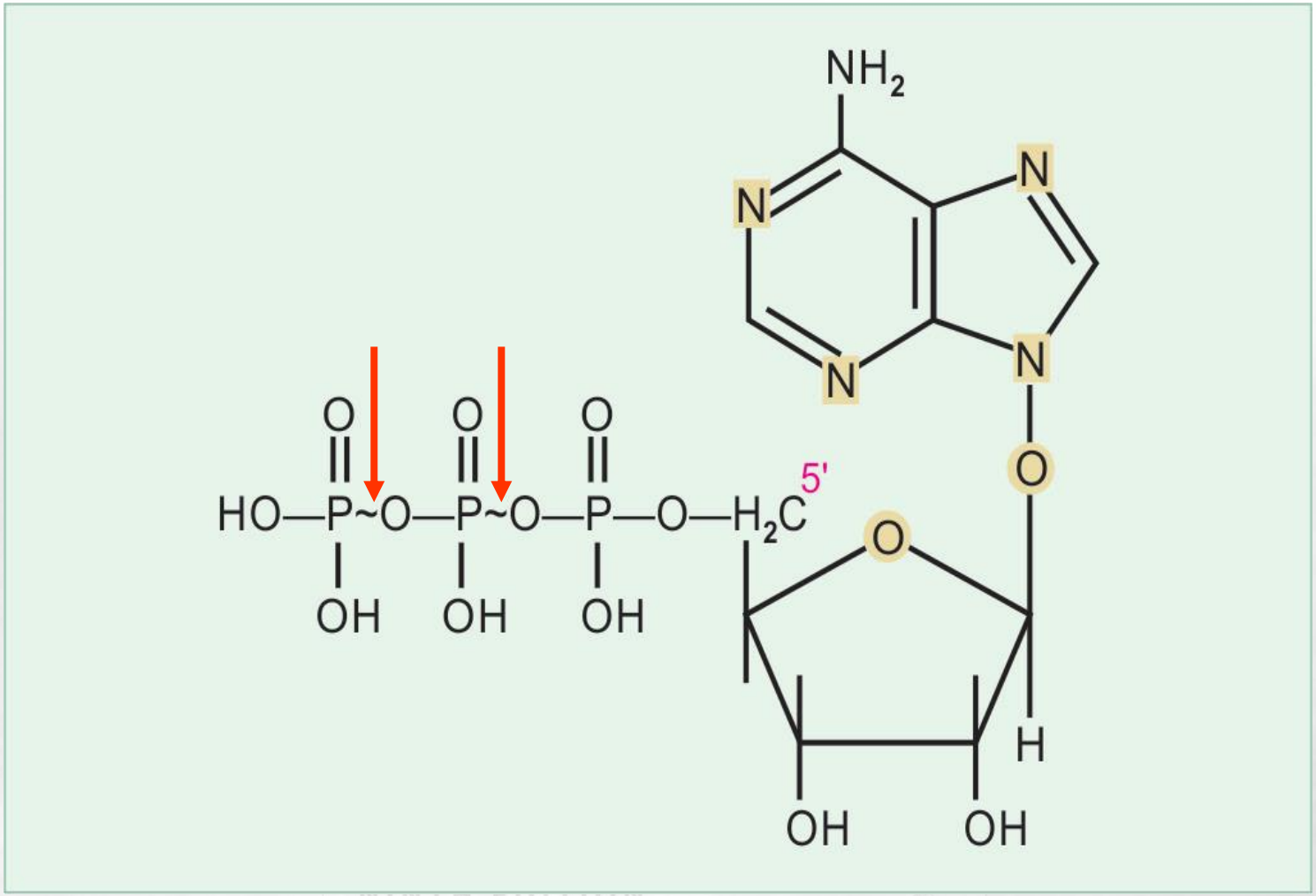
Nucleoside triphosphates

Nucleoside	Nucleoside monophosphate	Nucleoside diphosphate(NDP)	Nucleoside triphosphate (NTP)
Ribonucleoside phosphates			
Adenosine	Adenosine mono-phosphate (AMP)	Adenosine diphosphate (ADP)	**Adenosine triphosphate (ATP)**
Guanosine	GMP	GDP	**GTP**
Inosine	IMP	IDP	**ITP**
Cytidine	CMP	CDP	**CTP**
Uridine	UMP	UDP	**UTP**
Deoxyribonucleoside phosphates			
d-adenosine	d-AMP	d-ADP	**d-ATP**
d-guanosine	d-GMP	d-GDP	**d-GTP**
d-cytidine	d-CMP	d-CDP	**d-CTP**
d-thymidine	**d-TMP**	**d-TDP**	**d-TTP**

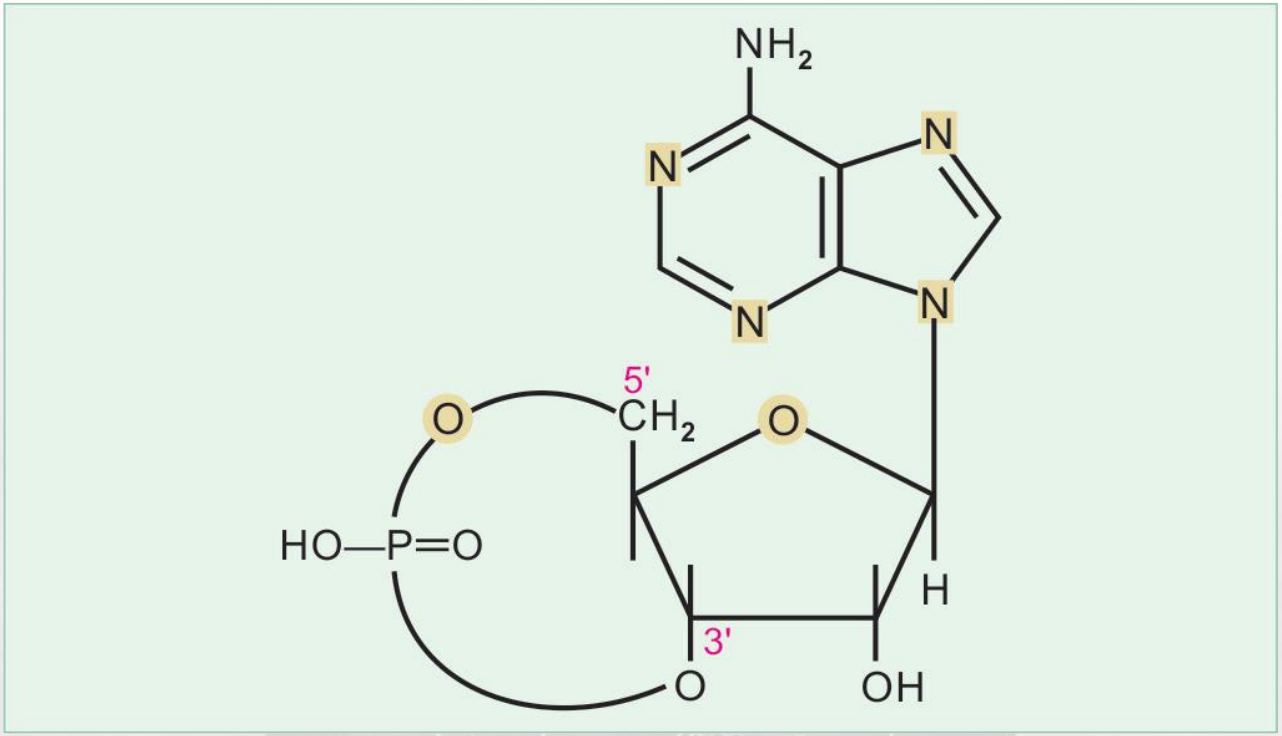
Since 5'-nucleotides are more often seen, they are simply written without any prefix. For example, 5'-AMP is abbreviated as AMP; but 3' variety is always written as 3'-AMP.



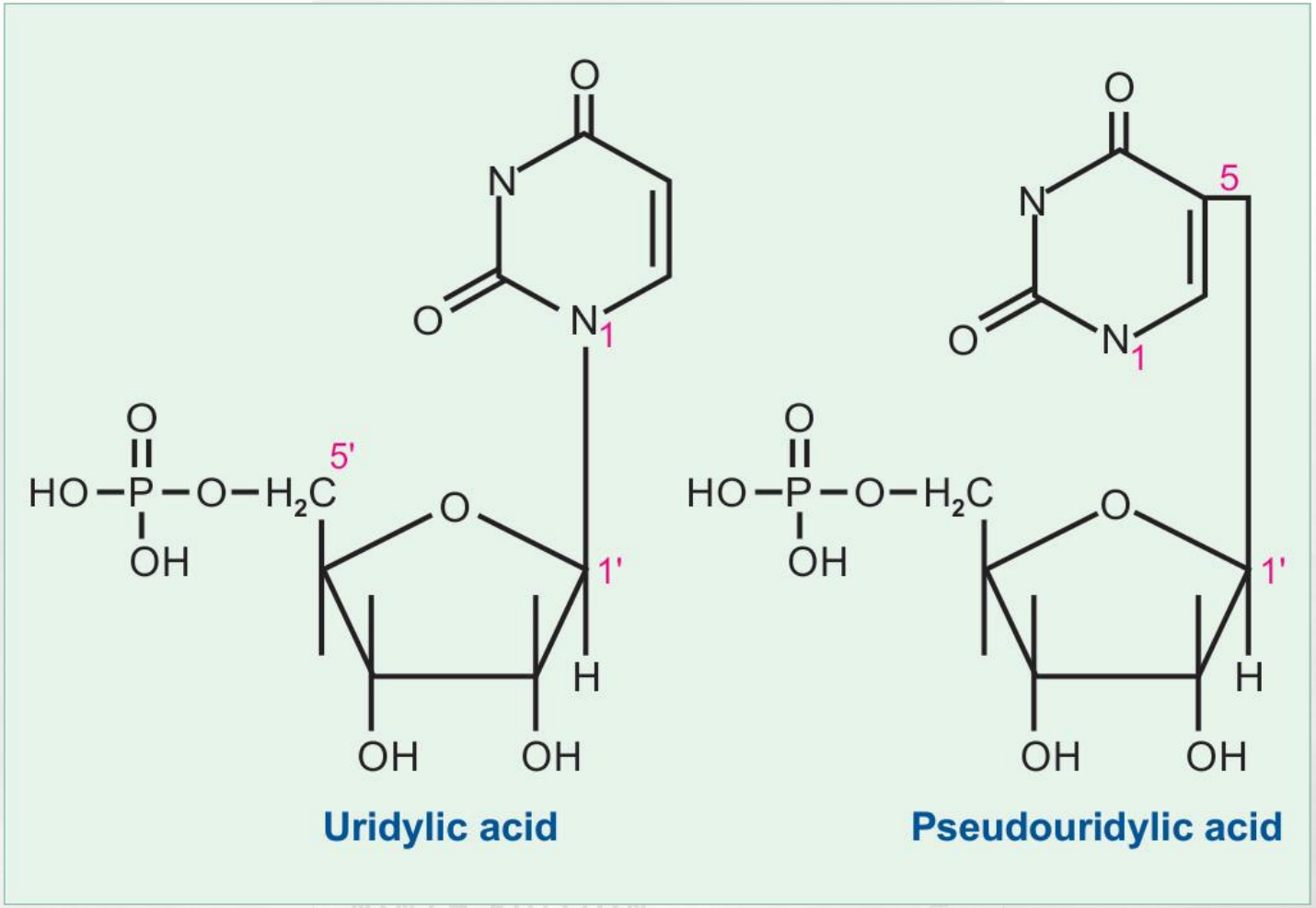
Adenosine triphosphate (ATP)



ATP is the universal energy currency.

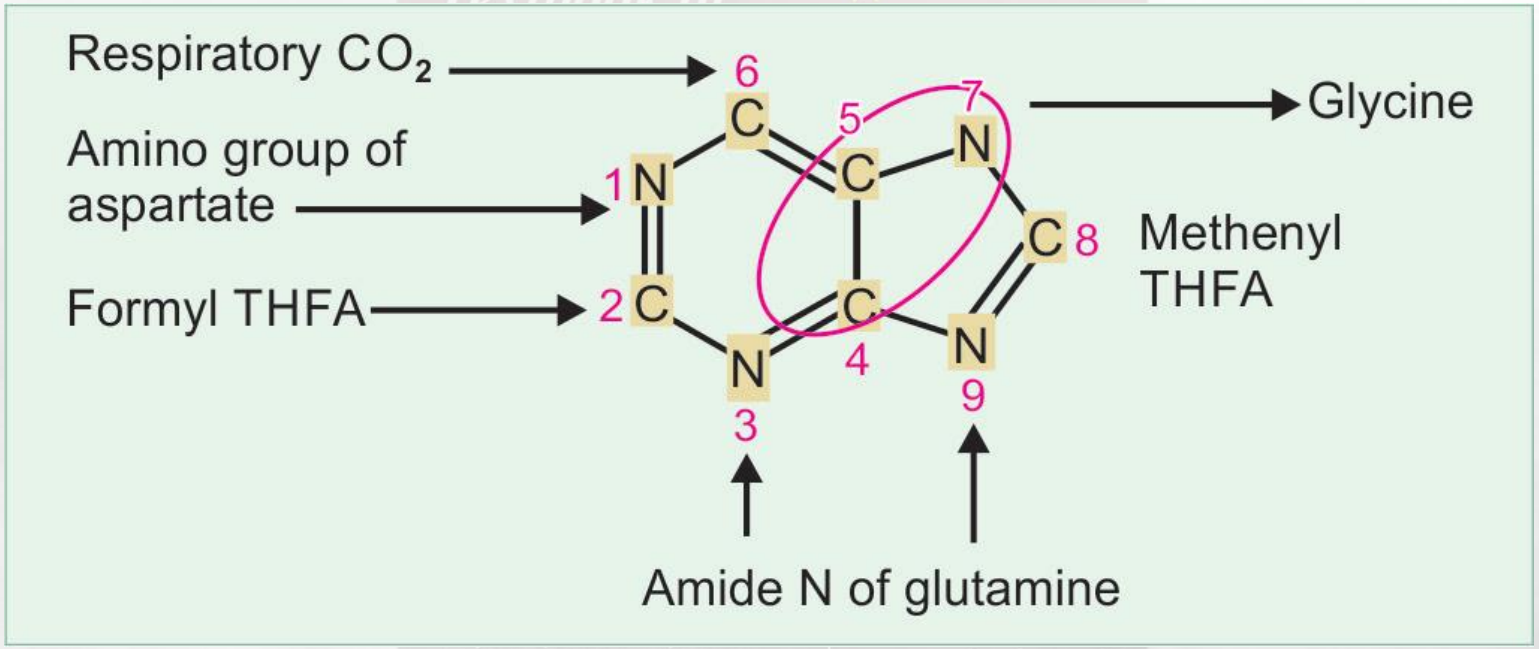


3',5'-cyclic AMP or cAMP. A phosphodiester linkage is formed between the 3' and 5' positions of ribose group. Cyclic AMP and Cyclic GMP is a major metabolic regulators. These are second messengers in mediating the action of several hormones.



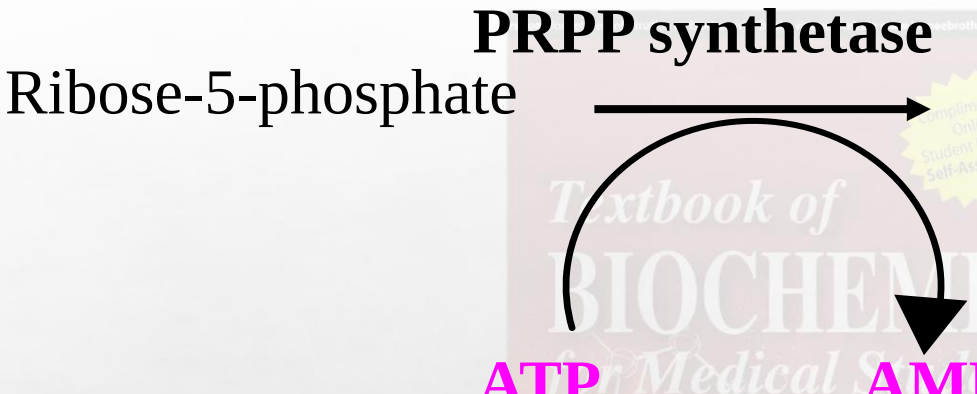
Different attachment of uracil to sugars.

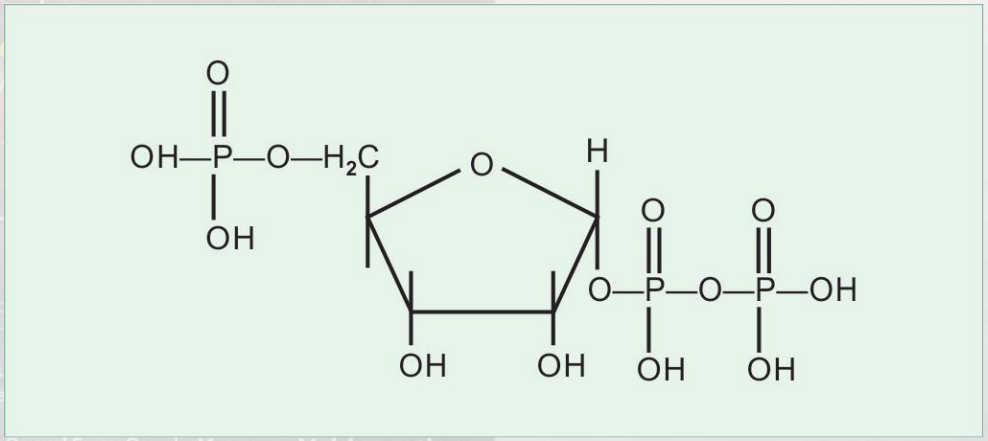
The major pathway is denoted as de novo synthesis, because the purine ring is synthesised from different small components.



The assembly of purine ring is from various sources.
THFA (FH4) = tetrahydrofolic acid.

Step 0 (Preparatory Step), PRPP Synthesis





The purine ring is later on assembled on the ribose-5-phosphate.

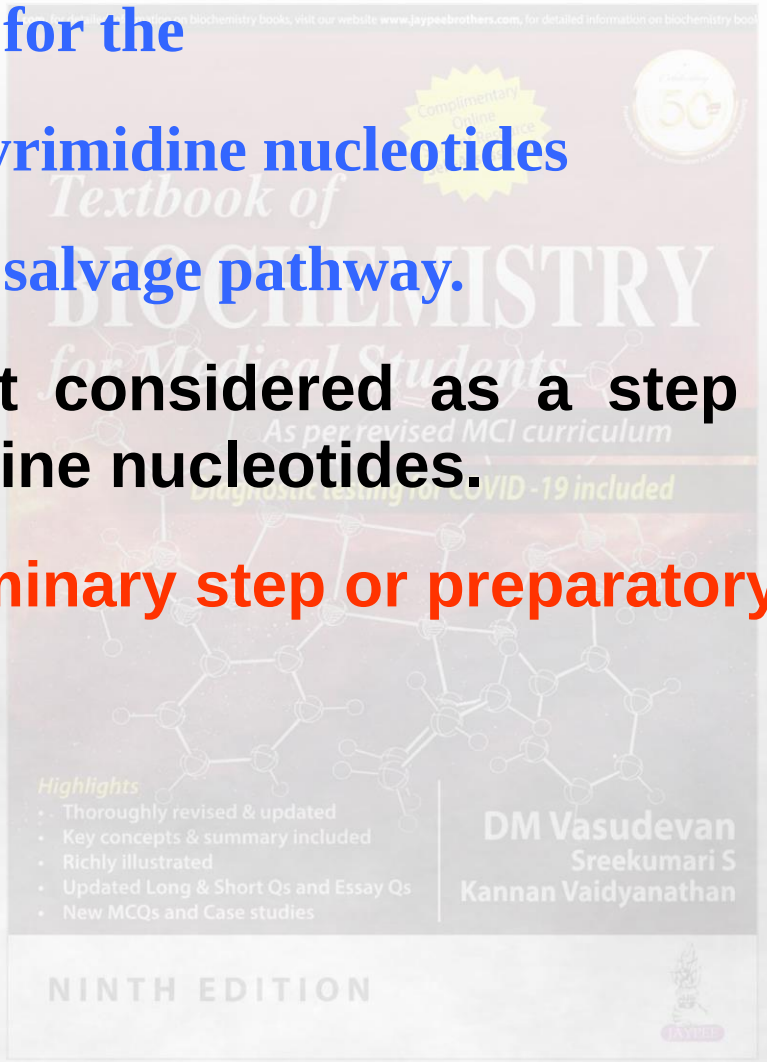
PRPP is also used for the

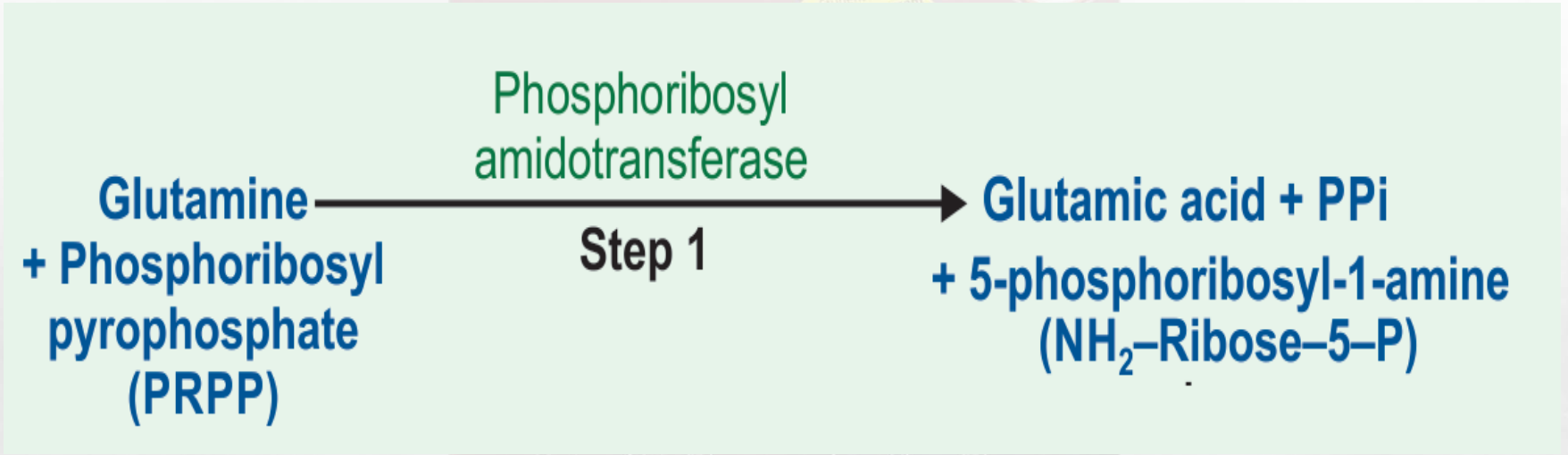
i) synthesis of pyrimidine nucleotides

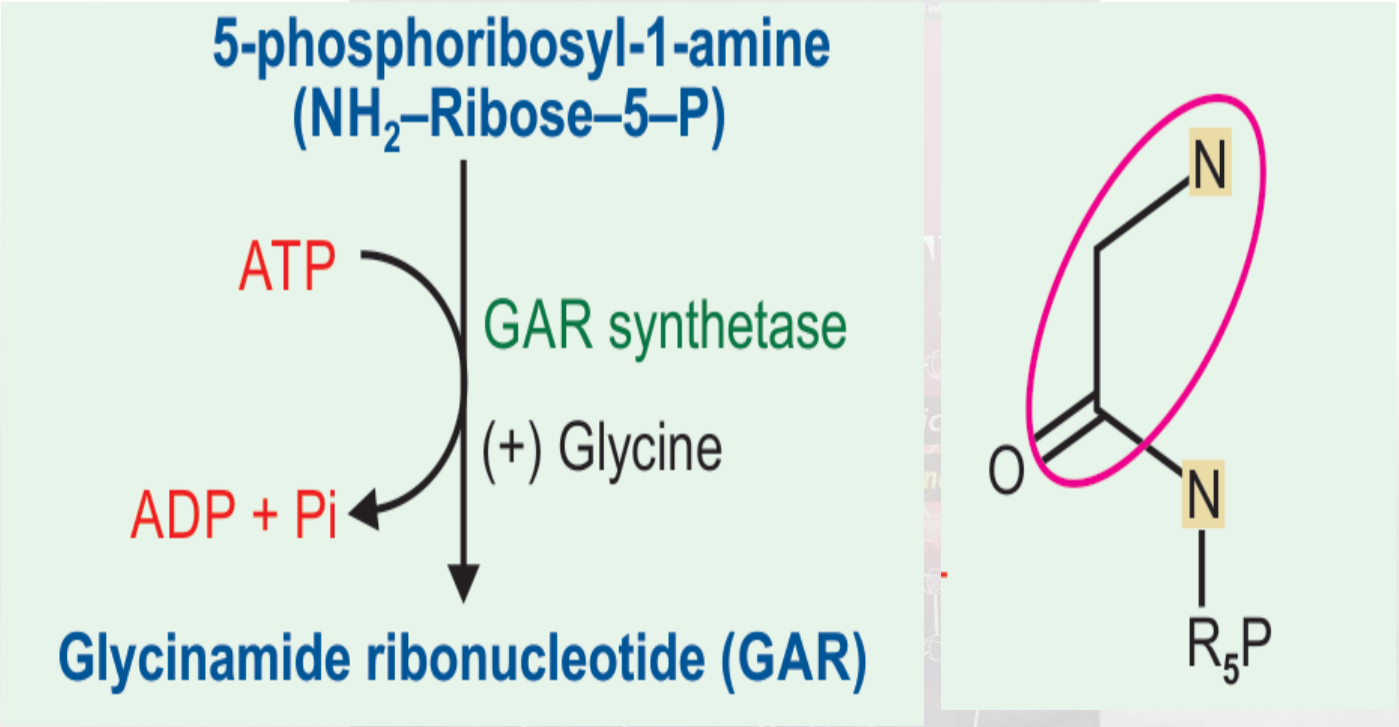
ii) ii) and for the salvage pathway.

So PRPP is not considered as a step in the de novo synthesis of purine nucleotides.

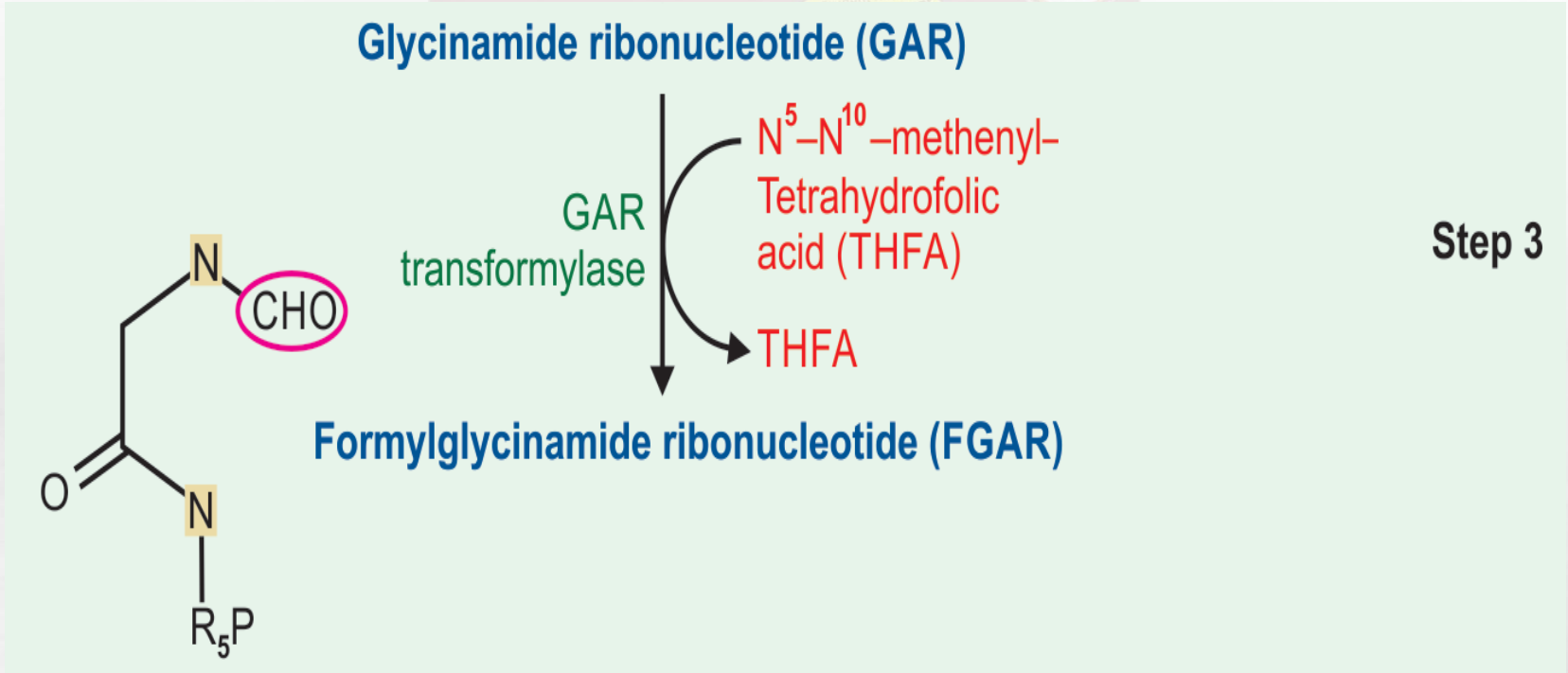
It is called preliminary step or preparatory step or Step 0



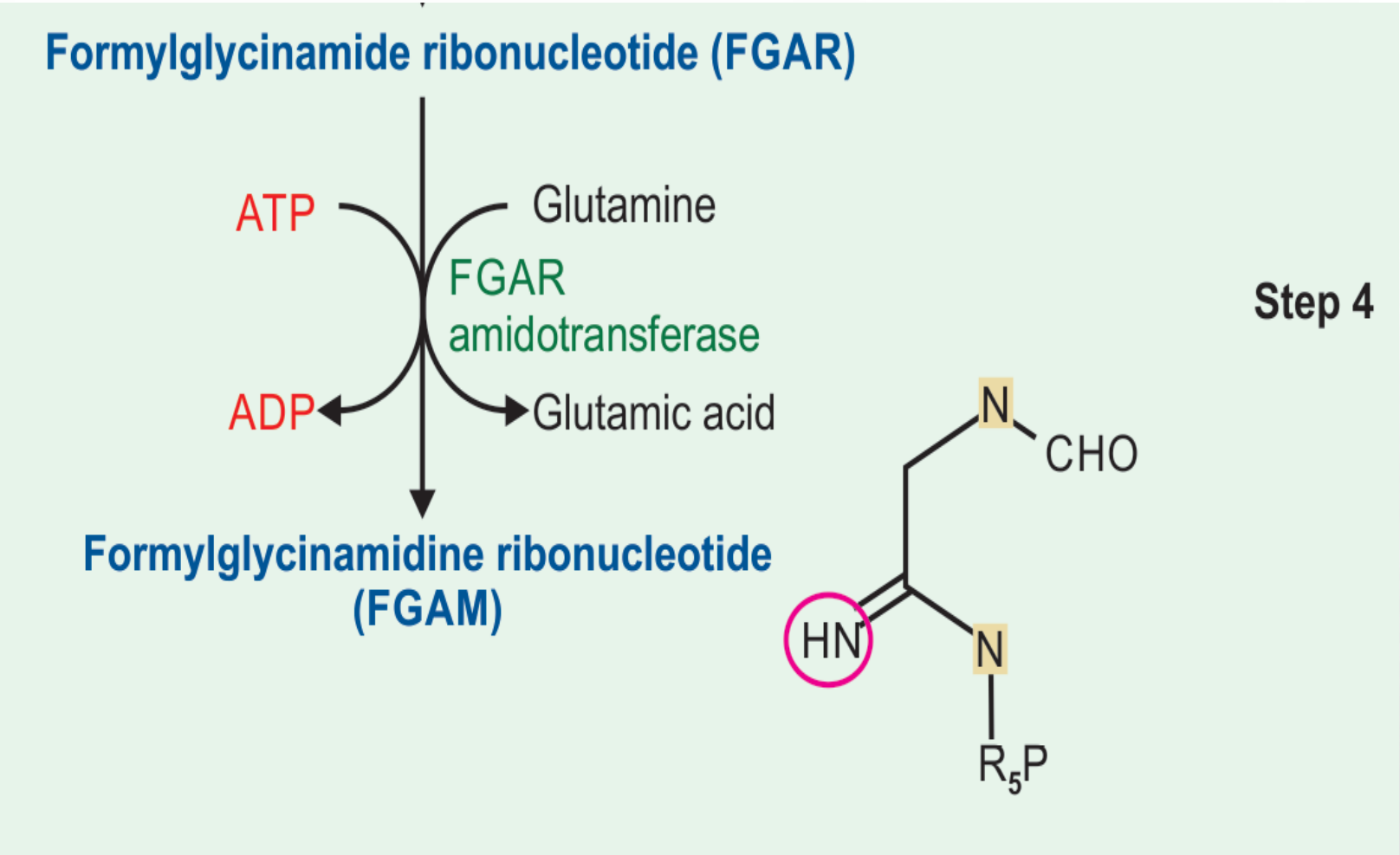

Glutamine
+ Phosphoribosyl
pyrophosphate
(PRPP)
Phosphoribosyl
amidotransferase
Step 1
Glutamic acid + PPi
+ 5-phosphoribosyl-1-amine
(NH_2–Ribose–5–P)



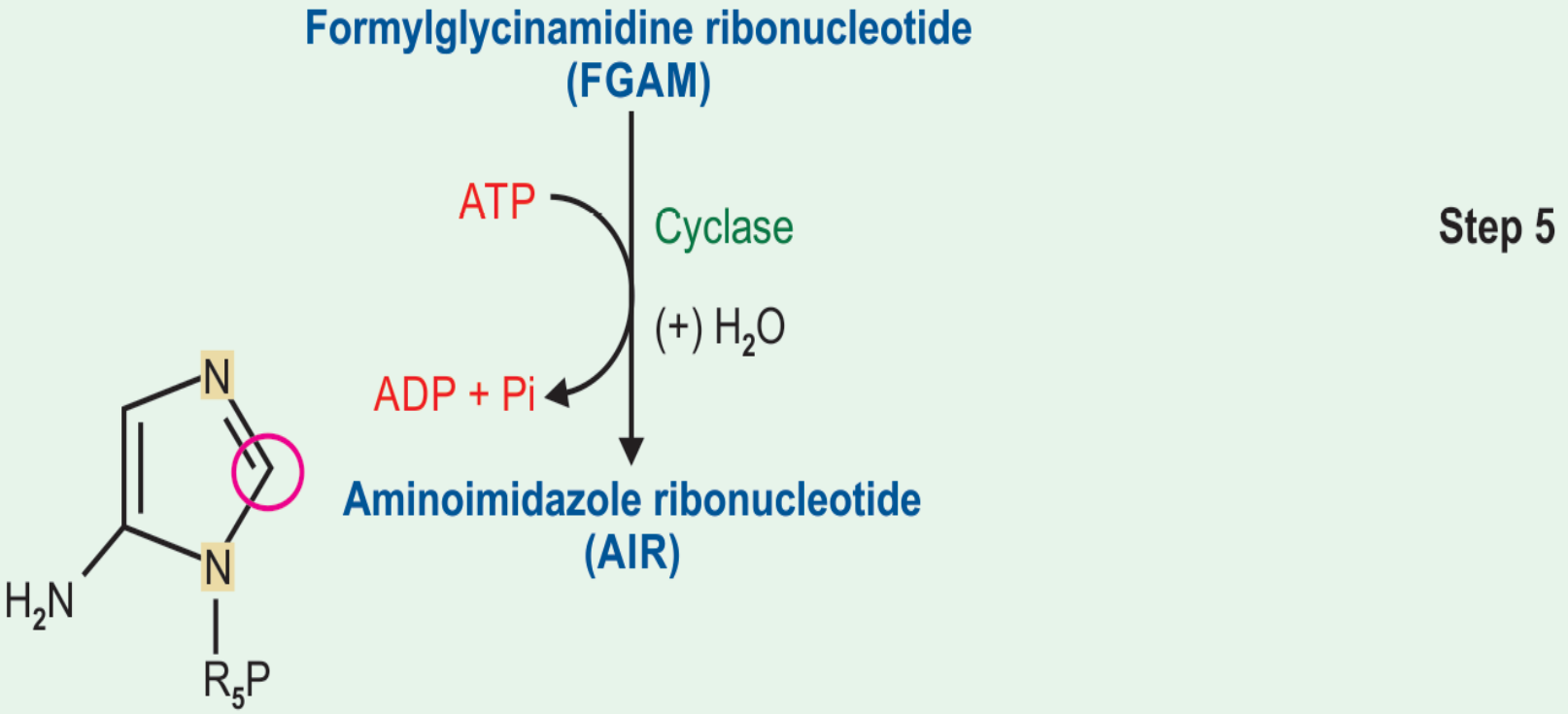
Step 2 of Purine synthesis GAR



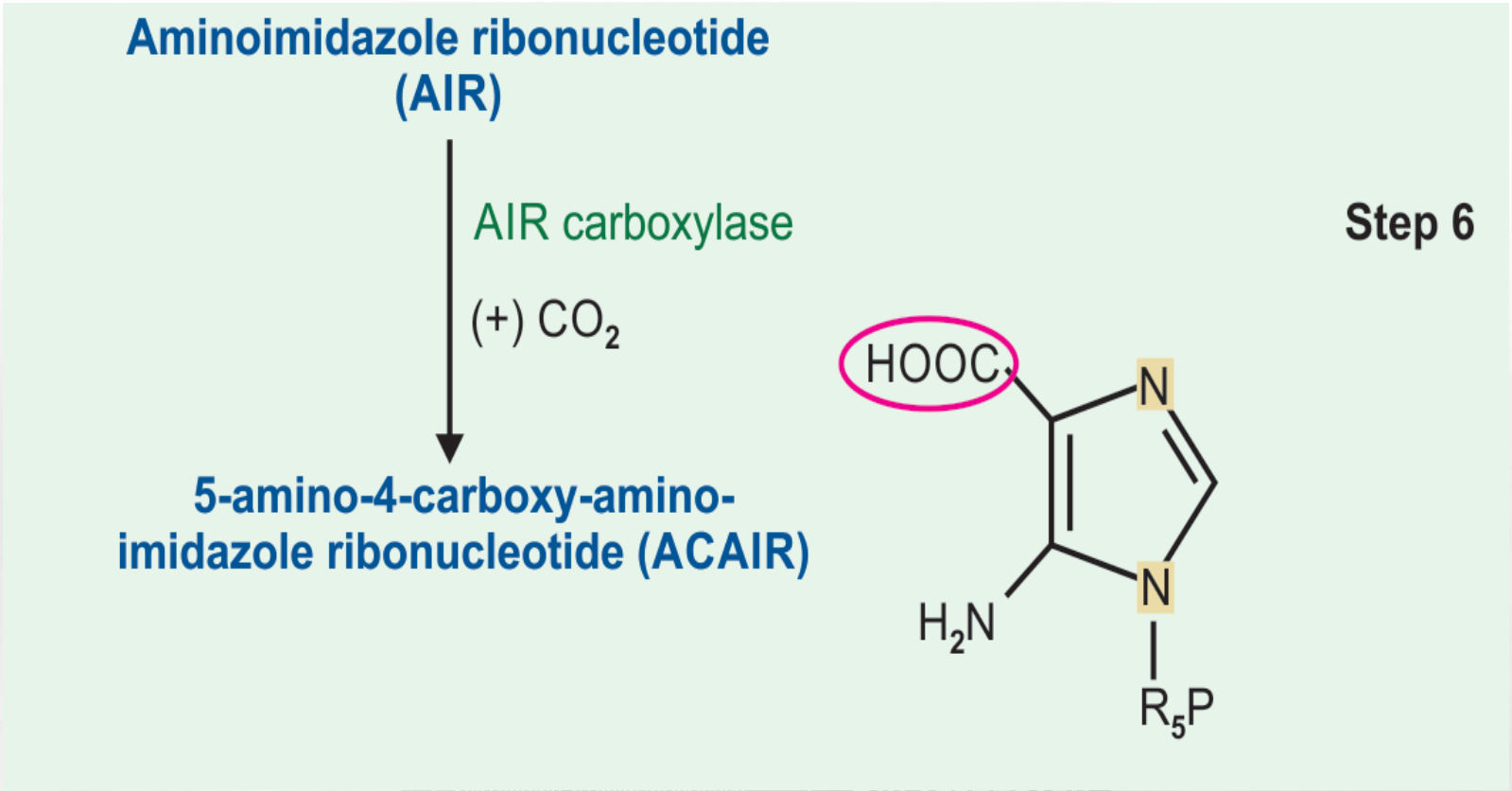
Step 3 of Purine synthesis



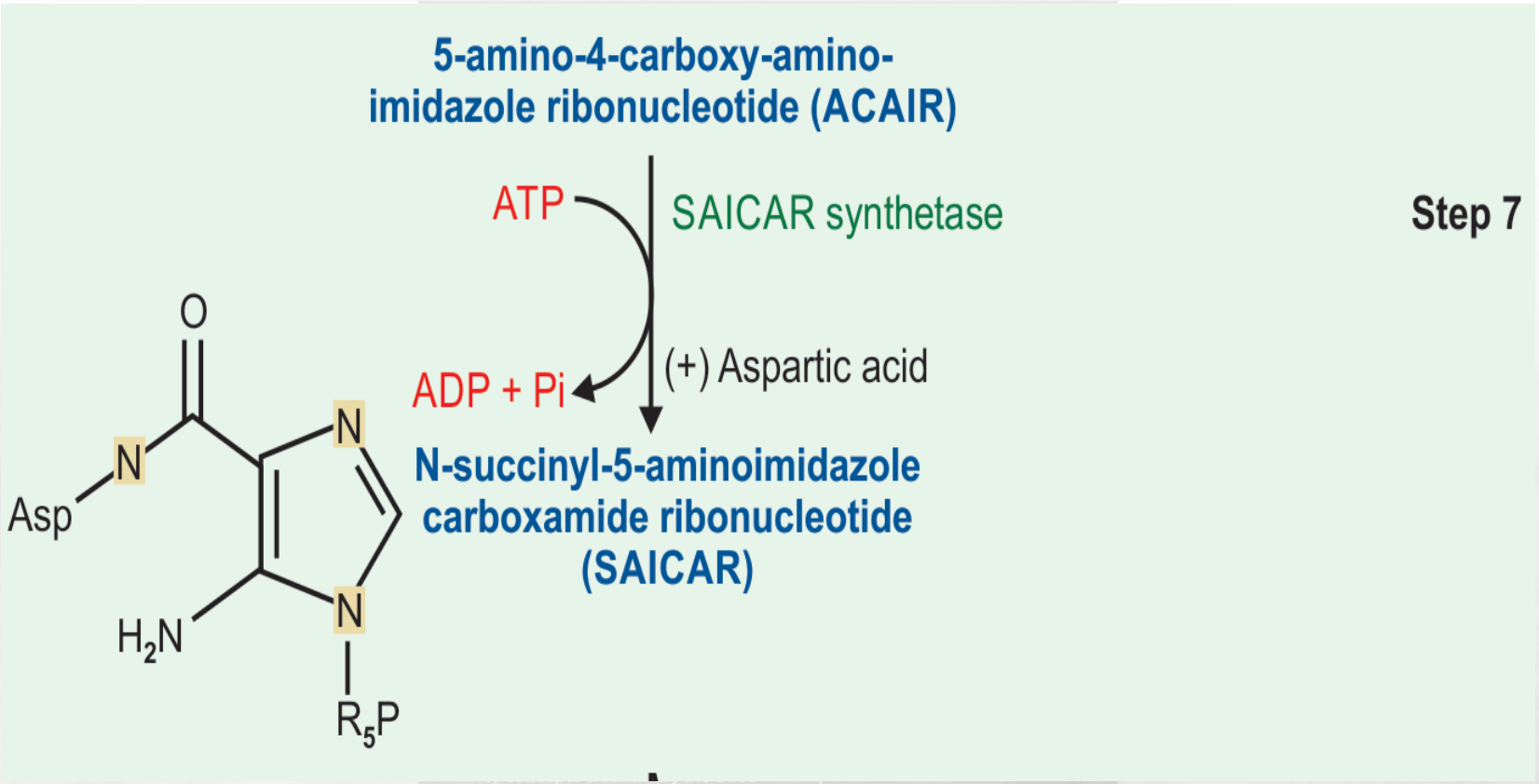
Step 4 of Purine synthesis



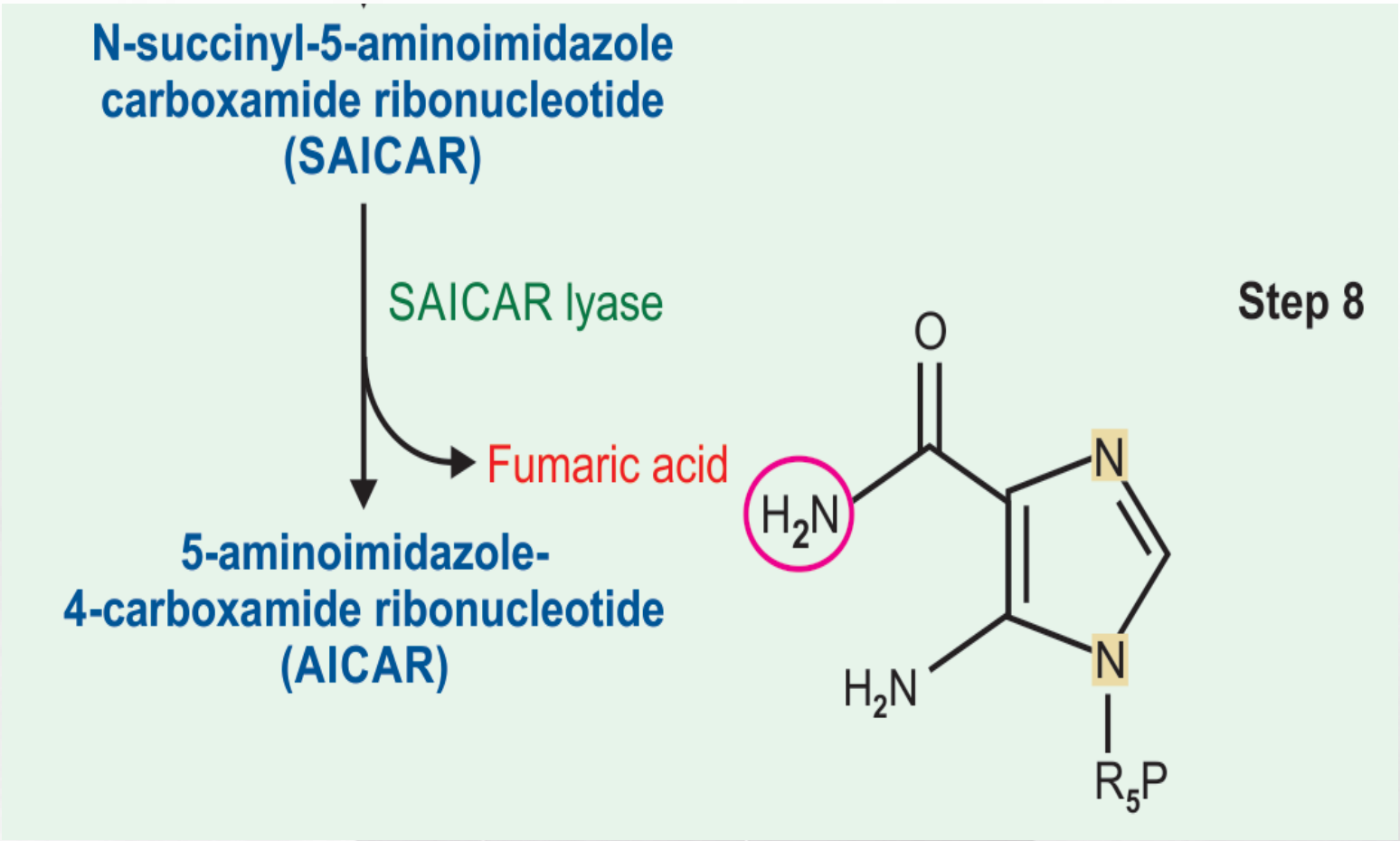
Step 5 of Purine synthesis



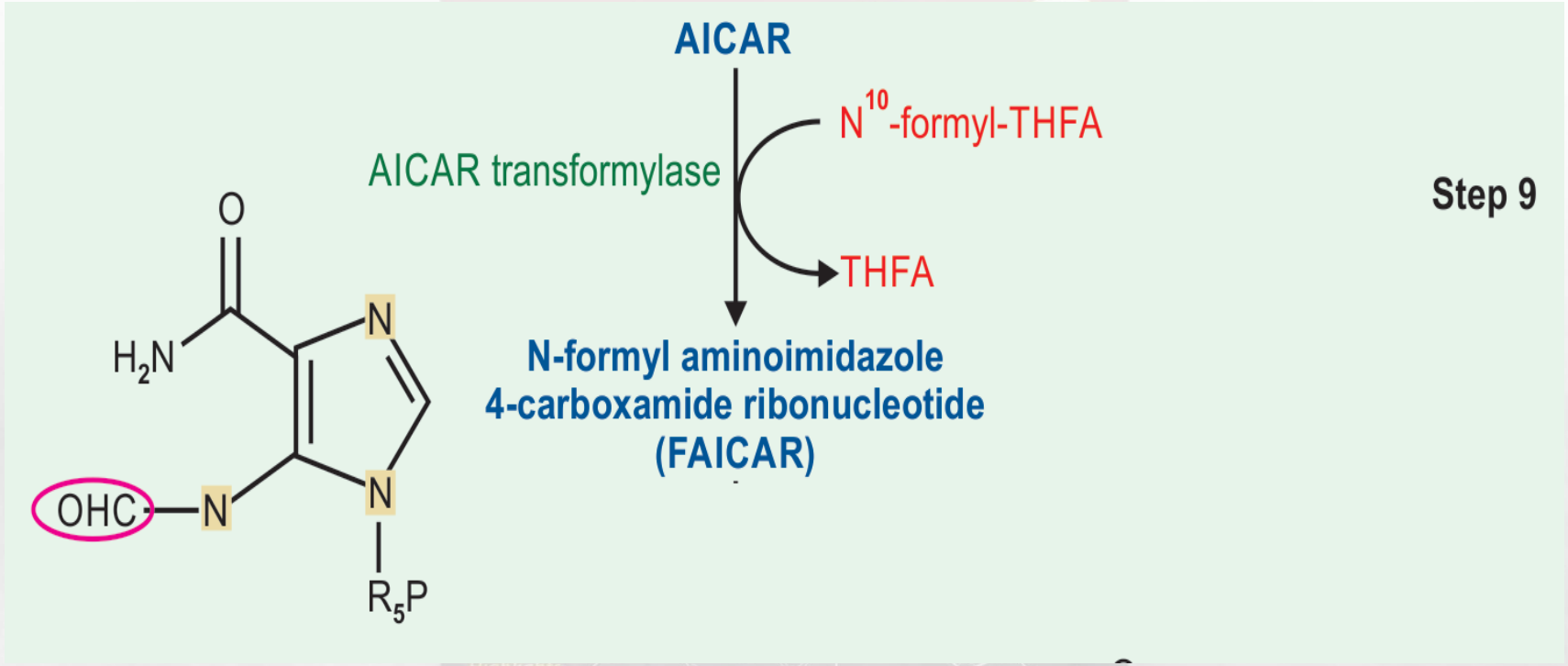
Step 6 of Purine synthesis



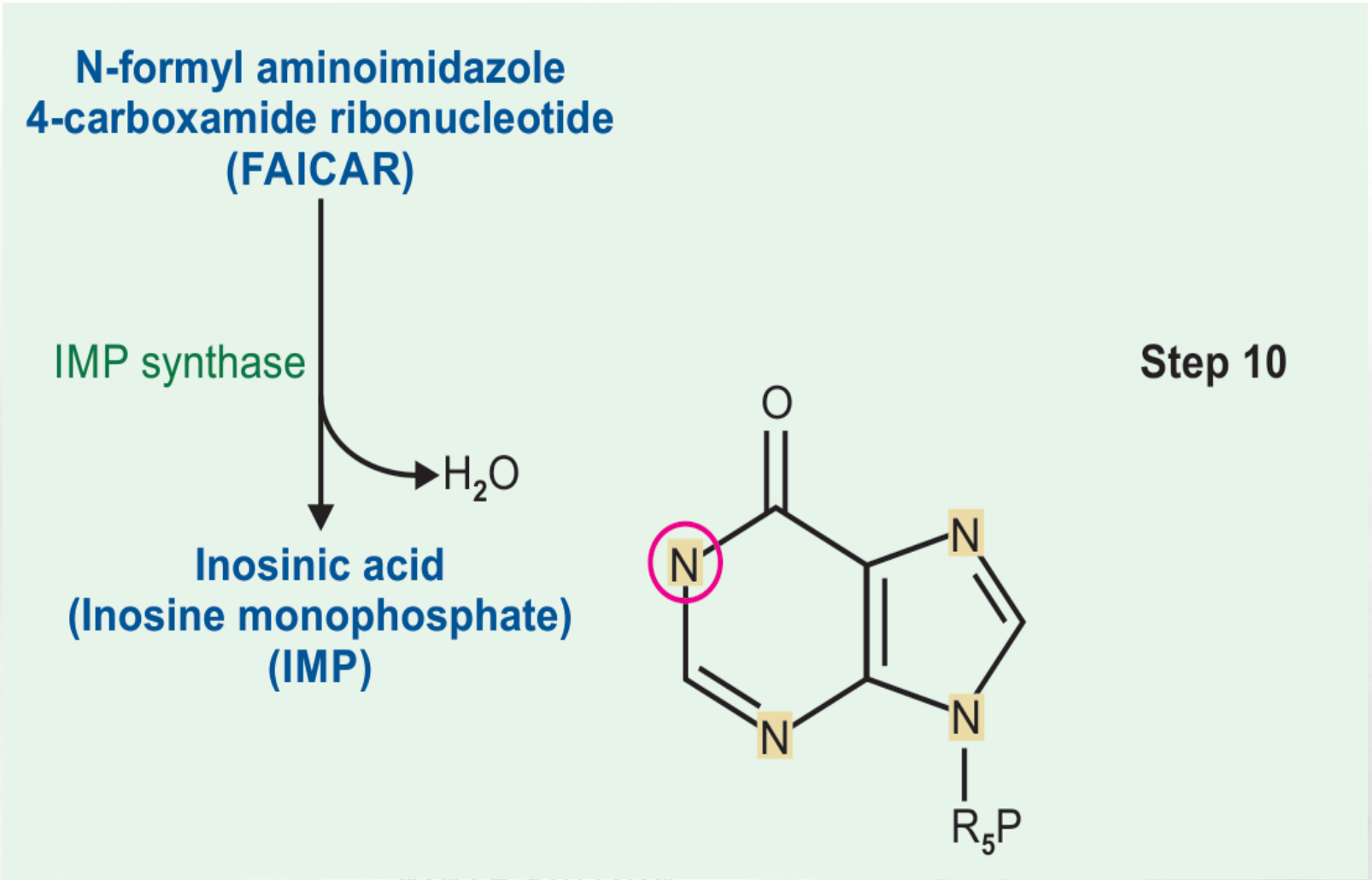
Step 7 of Purine synthesis



Step 8 of Purine synthesis



Step 9 of Purine synthesis

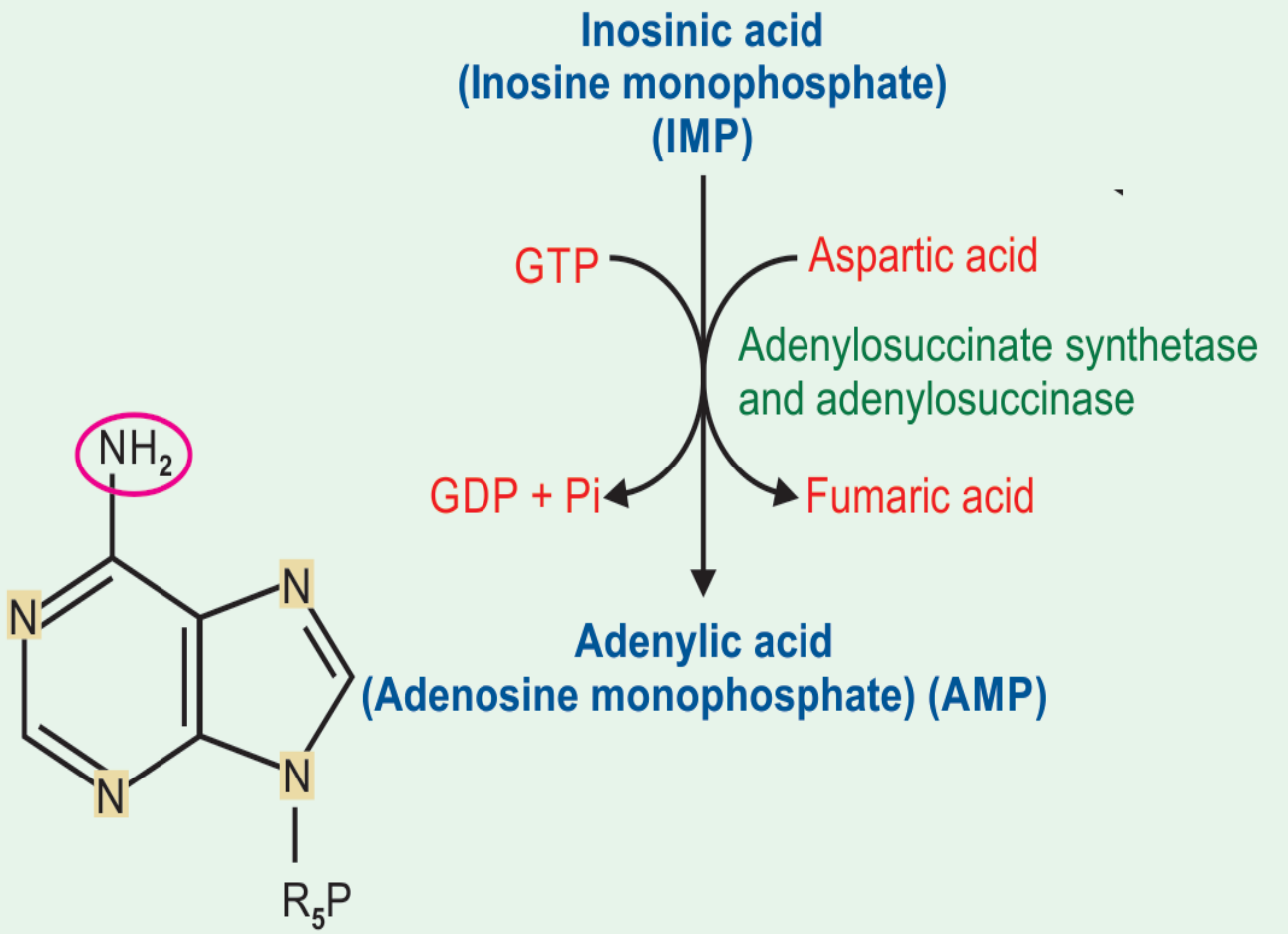


Step 10 of Purine synthesis

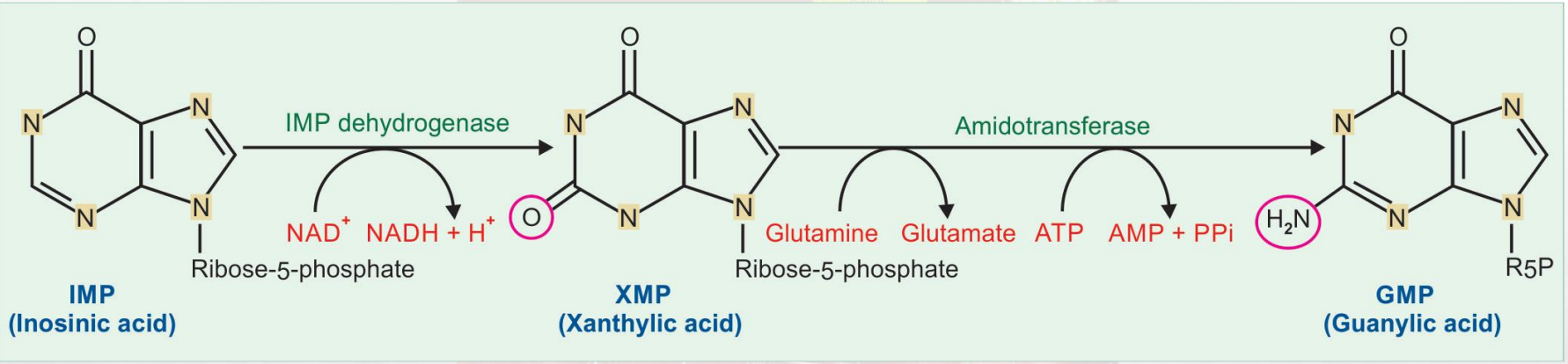
Summary of steps of purine synthesis

Step	Donor	Added atom	Product
1	Glutamine	N9 (Rate limiting)	PRA
2	Glycine (ATP required)	C4, 5, N7	GAR
3	Methenyl-THFA	C8	FGAR
4	Glutamine	N3 (ATP required)	FGAM
5	–	Ring closure (ATP)	AIR
6	Carbon dioxide	C6	ACAIR
7	Aspartic acid	N1 (ATP required)	SAICAR
8	–	Fumarate removed	AICAR
9	Formyl-THFA	C2	FAICAR
10	–	Ring closure	IMP

PRA = phosphoribosyl amine. GAR = glycinamide ribonucleotide. FGAR = formyl glycinamide ribonucleotide. FGAM = formyl glycinamidine ribonucleotide. AIR = amino imidazole ribonucleotide. ACAIR = amino carboxy amino imidazole ribonucleotide. SAICAR = succinyl amino imidazole carboxamide ribonucleotide. AICAR = amino imidazole carboxamide ribonucleotide. FAICAR = formyl amino imidazole .



Step 11 of Purine synthesis



Conversion of inosine monophosphate (IMP) to guanosine monophosphate (GMP) (R5P: ribose-5-phosphate).

Multifunctional Catalysts of Purine Nucleotide Synthesis

Prokaryotes : Each reaction catalysed by a different polypeptide

Eukaryotes : Gene fusion → single polypeptide with multiple catalytic function.

Multifunctional catalysts function.

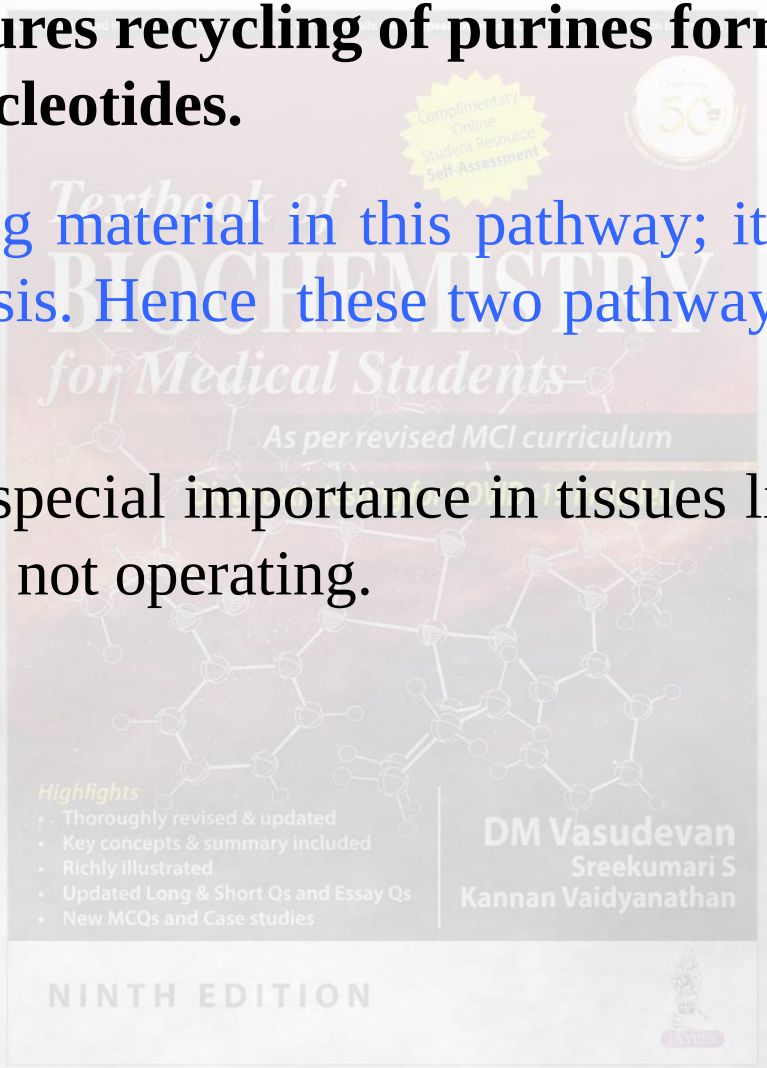
- Catalyse reaction 3,4 & 6 - phophoribosyl glycinamide synthase,formyl transferase,aminoimidazole ribosyl 5 phosphate synthase
- Catalyse reaction 7,8 - aminoimidazole ribosyl 5 phosphate carboxylase,aminoimidazole succinyl carboxamide ribosyl 5 phosphate synthase
- Catalyse reaction 10 & 11 – formyl tranferase, IMP cyclohydrolase

Salvage Pathway

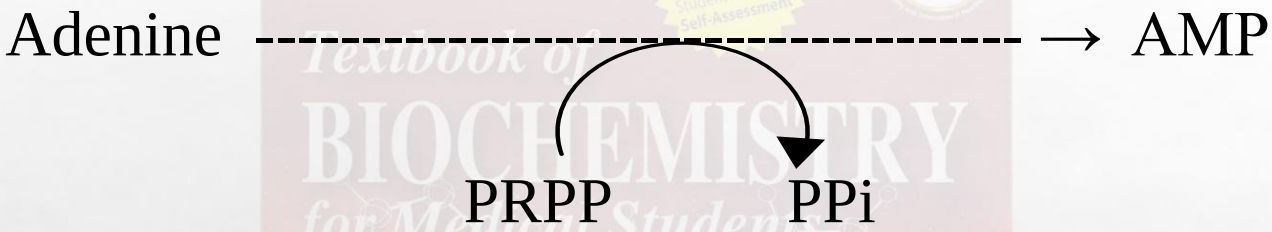
This pathway ensures recycling of purines formed by degradation of nucleotides.

PRPP is the starting material in this pathway; it is also a substrate for *de novo* synthesis. Hence these two pathways are closely inter-related.

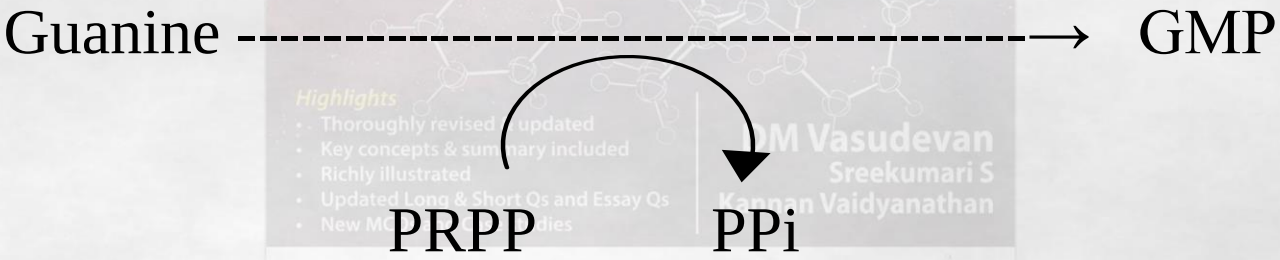
The pathway is of special importance in tissues like brain where the de novo pathway is not operating.



Adenine phospho ribosyl transferase (APRTase)



Hypoxanthine guanine phosphoribosyl transferase (HGPRTase)



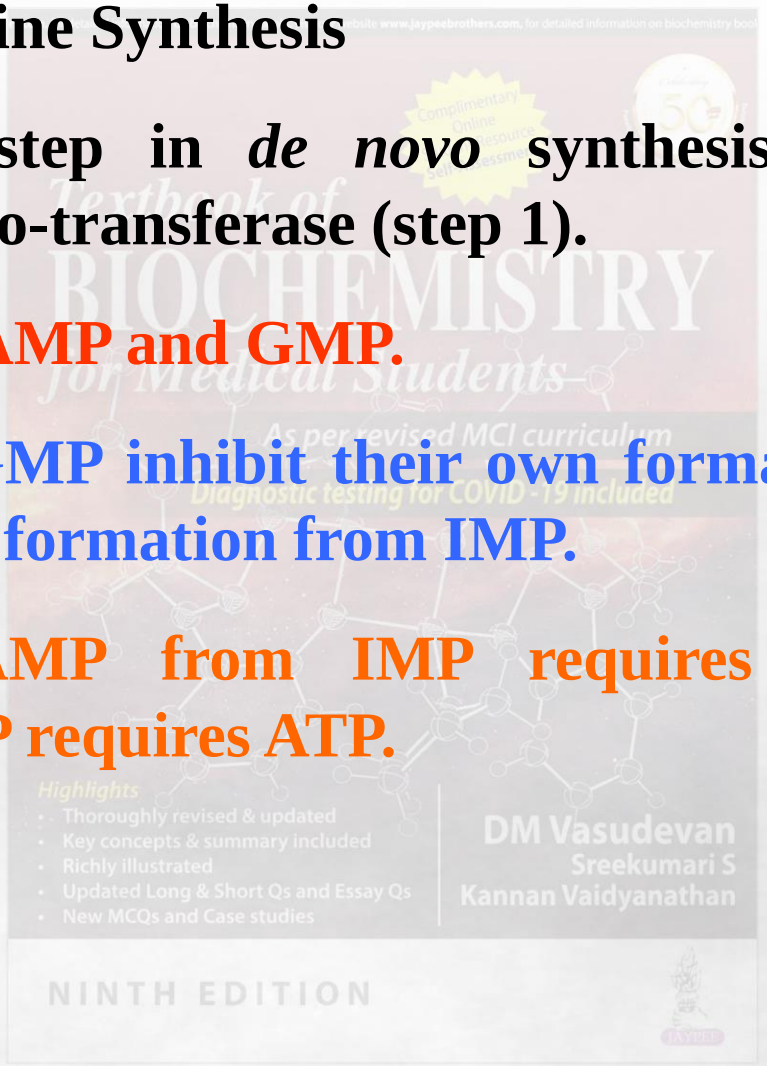
Regulation of Purine Synthesis

The committed step in *de novo* synthesis is the reaction catalysed by amido-transferase (step 1).

It is inhibited by AMP and GMP.

Both AMP and GMP inhibit their own formation by feedback inhibition of their formation from IMP.

Formation of AMP from IMP requires GTP; similarly formation of GMP requires ATP.



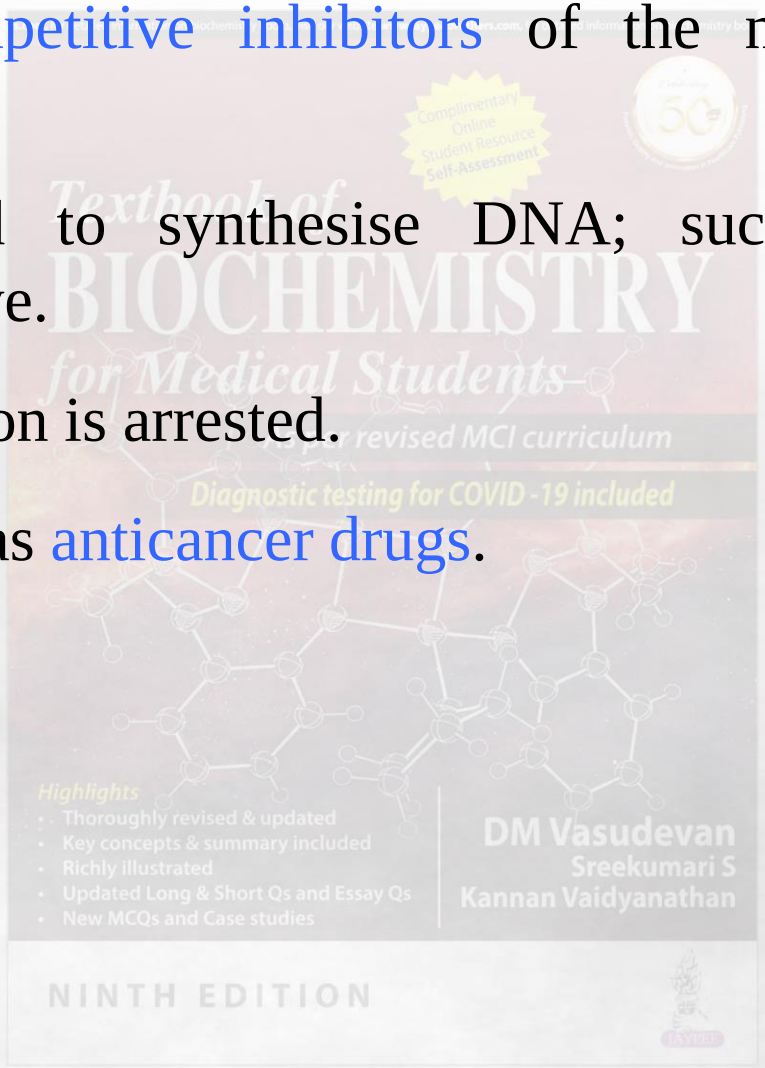
Analogues as Purine Synthesis Inhibitors

They act as competitive inhibitors of the naturally occurring nucleotides.

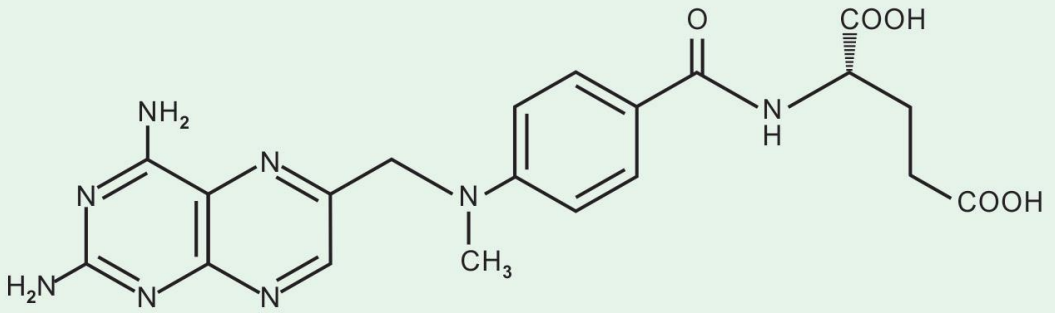
They are utilised to synthesise DNA; such DNA becomes functionally inactive.

Thereby cell division is arrested.

So they are useful as anticancer drugs.



6-Mercaptopurine



Methotrexate

6-Thioguanine

8- Azaguanine

Purine synthesis inhibitors.

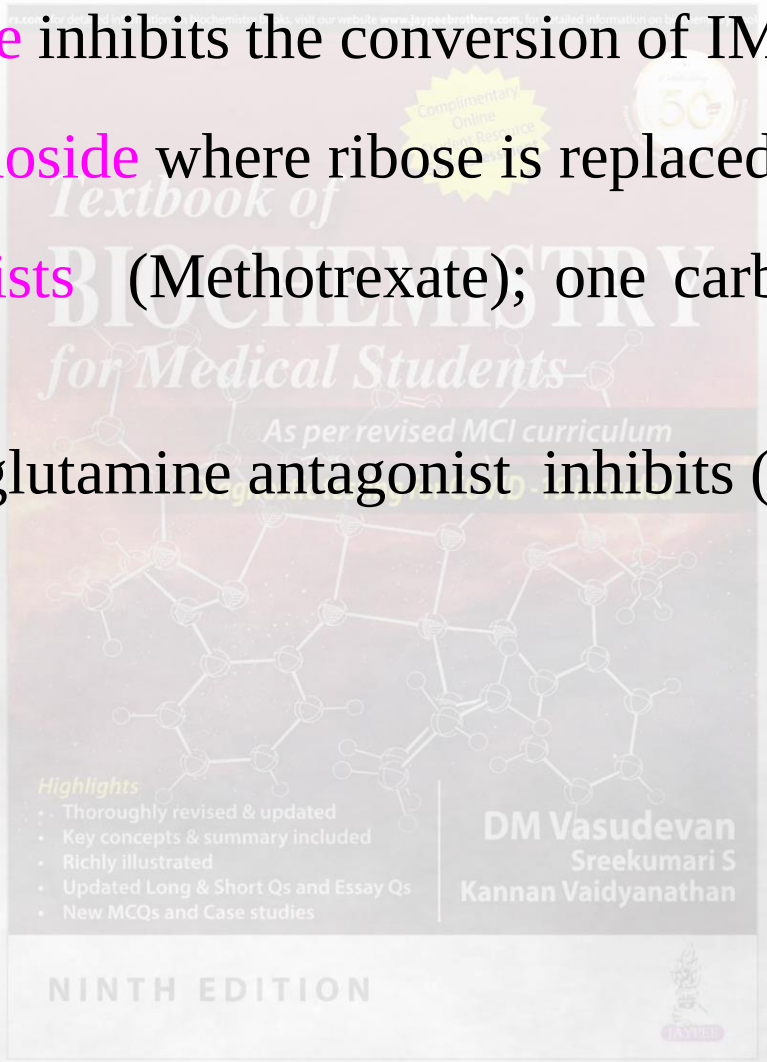
Analogues as Purine Synthesis Inhibitors

a) Mercaptopurine inhibits the conversion of IMP to GMP & AMP

b) Cytosine arabinoside where ribose is replaced by arabinose.

c) Folate antagonists (Methotrexate); one carbon groups are not available.

d) Azaserine is a glutamine antagonist inhibits (steps 1& 4).



2. **Glutamine analogues**

Azaserine : inhibits reactions involving Gln –

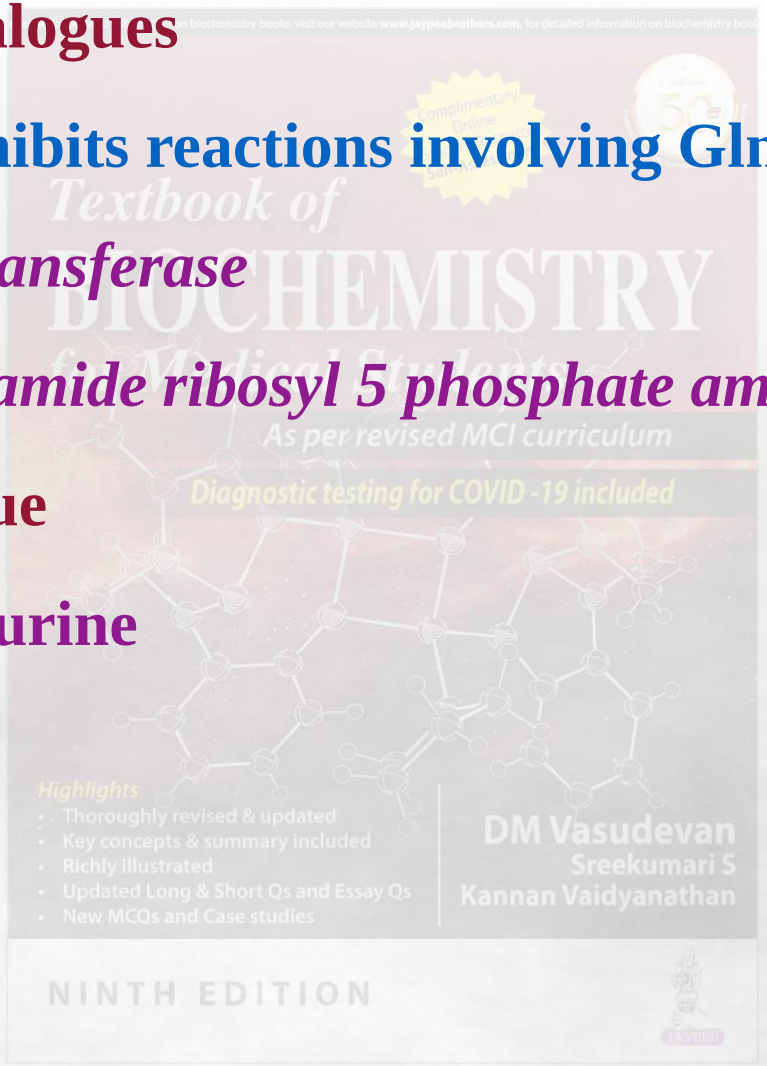
PRPP amidotransferase

Formyl glycinamide ribosyl 5 phosphate amidotransferase

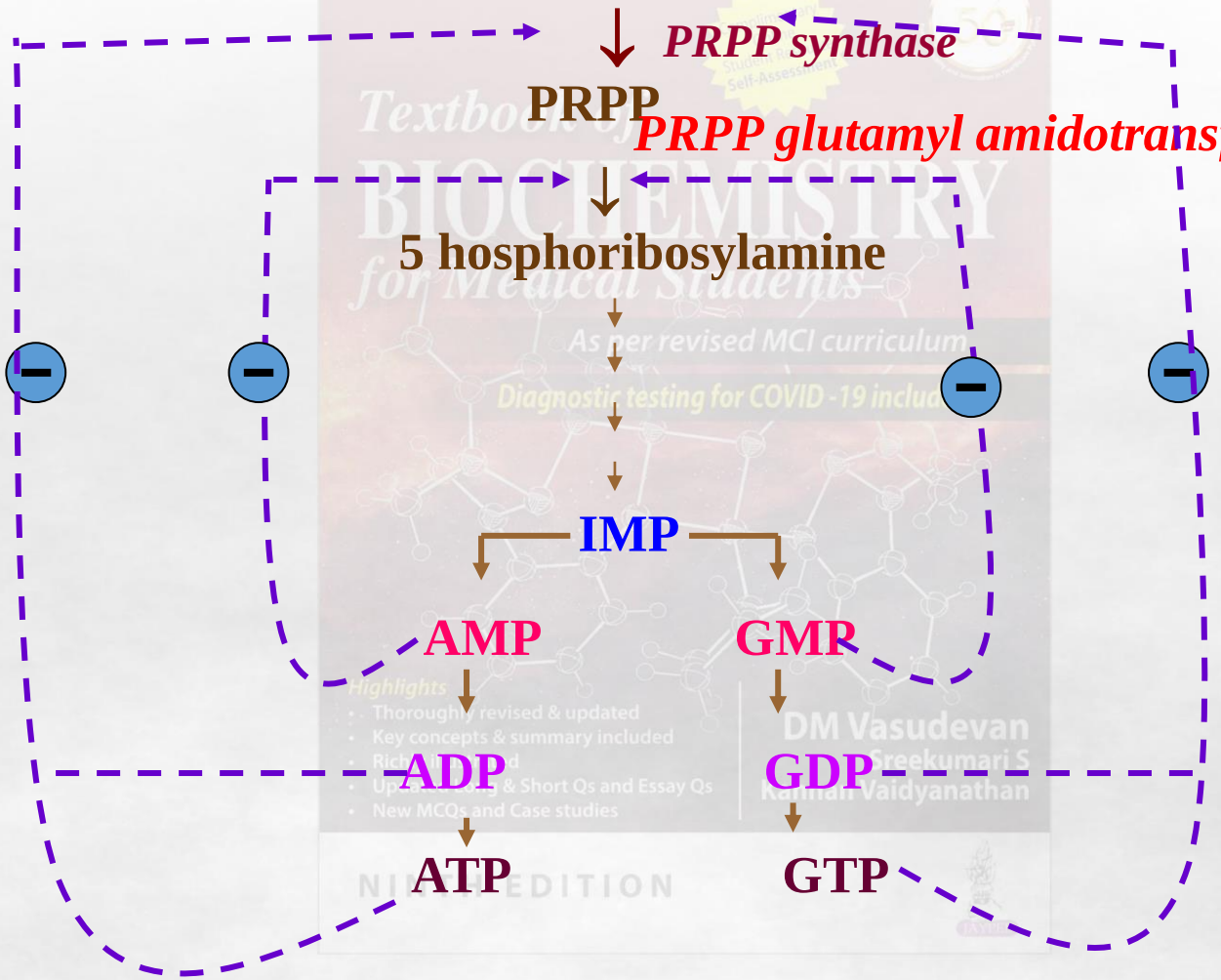
3. **Purine analogue**

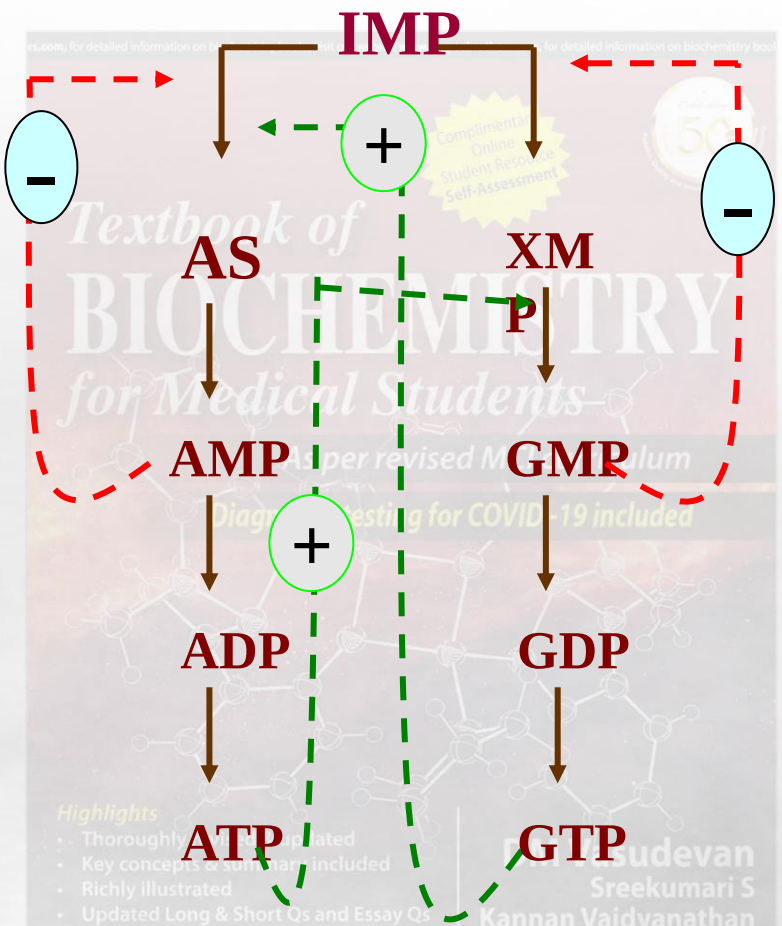
6 –mercaptopurine

Thioguanine

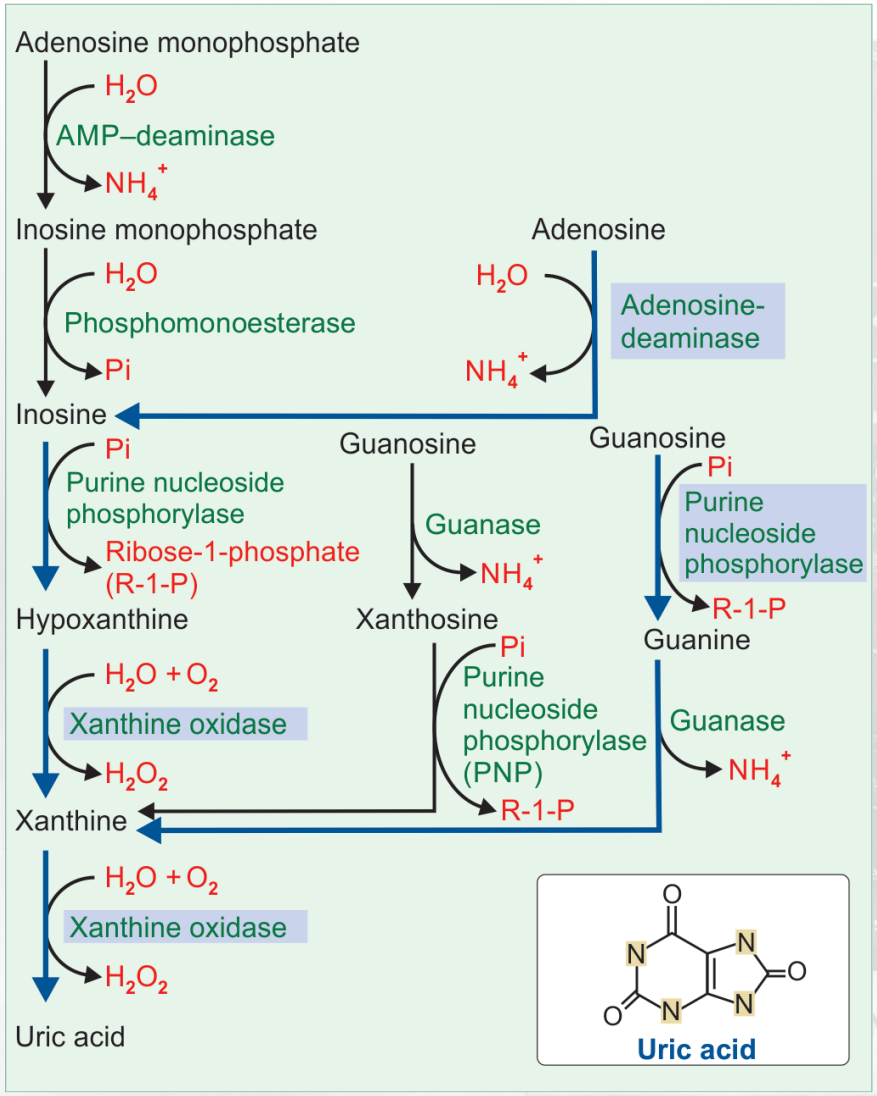


RIBOSE 5 PHOSPHATE + ATP





AMP & GMP feedback regulate their formation from IMP and also inhibit HGPRT

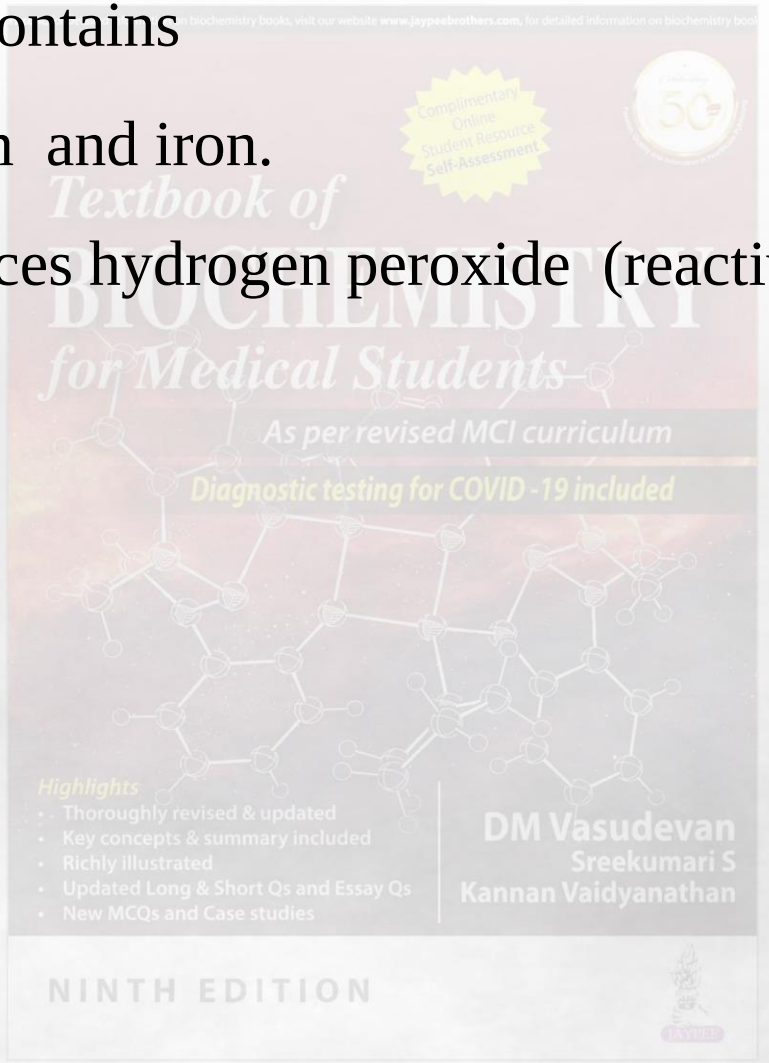


Degradation of purine nucleotides. Main pathway is shown in blue arrows.

Xanthine oxidase contains

FAD, molybdenum and iron.

The reaction produces hydrogen peroxide (reactive oxygen species).



Disorders of purine metabolism

Disorder	Defect	Nature of defect
Gout	PRPP synthetase, HGPRT, glucose-6-phosphatase	Hyperuricemia
Lesch Nyhan syndrome	HGPRT	Lack of the enzyme
Immunodeficiency	Purine nucleotide phosphorylase	Lack of the enzyme
Xanthinuria	Xanthine oxidase	Hypouricemia, xanthine renal lithiasis

Uric Acid

The normal blood level of uric acid

2-5 mg/dl in females;

3-7 mg/dl in males.

Nucleic acid content is more in non-vegetarian diet.

Uric acid is sparingly soluble in water.

Hyperuricemia;

serum uric acid concentration exceeding 6 mg/dl in female and 7 mg/dl in male

Uricosuria;

increased excretion of uric acid in urine.

Gout

It is due to accumulation of urate crystals in the synovial fluid resulting in inflammation leading to acute arthritis.

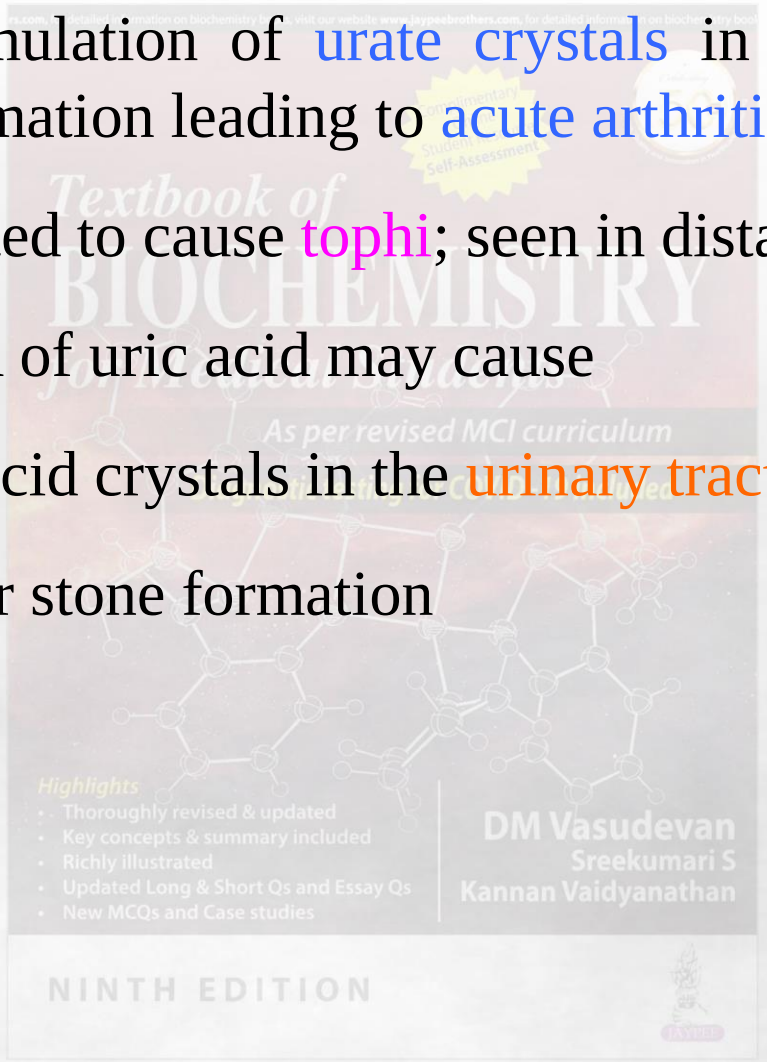
Uric acid is deposited to cause tophi; seen in distal joints of foot.

Increased excretion of uric acid may cause

deposition of uric acid crystals in the urinary tract;

leading to calculi or stone formation

with renal damage.



Primary Hyperuricemia

1. Abnormal 5-phosphoribosyl amido transferase

It is not sensitive to feedback regulation; leading to over -production of purine nucleotides.

Glucose-6-phosphatase Deficiency

von Gierke's disease (glycogen storage disease, type I).

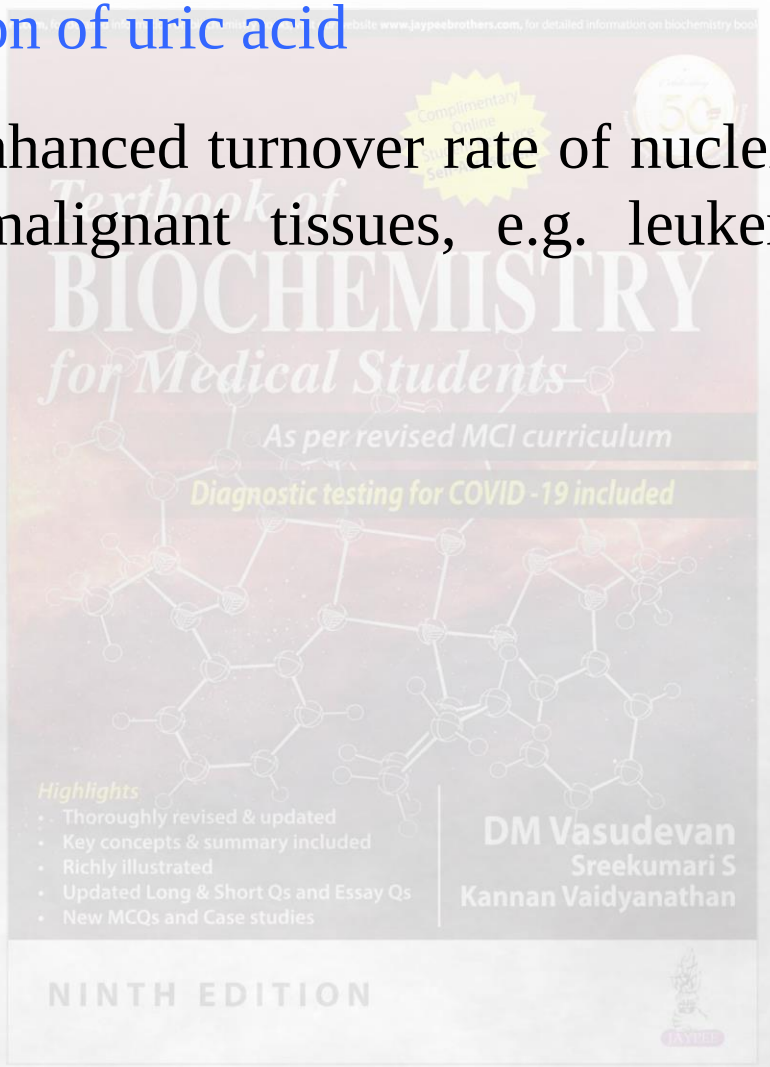
Glucose-6-phosphate cannot be converted to glucose.

So more glucose is channelled into the pentose-phosphate shunt pathway, resulting in increased availability of ribose-5-phosphate. This would lead to increased formation of PRPP.

Secondary Hyperuricemia

Increased production of uric acid

It may be due to enhanced turnover rate of nucleic acids as seen in rapidly growing malignant tissues, e.g. leukemias, lymphomas, polycythemia

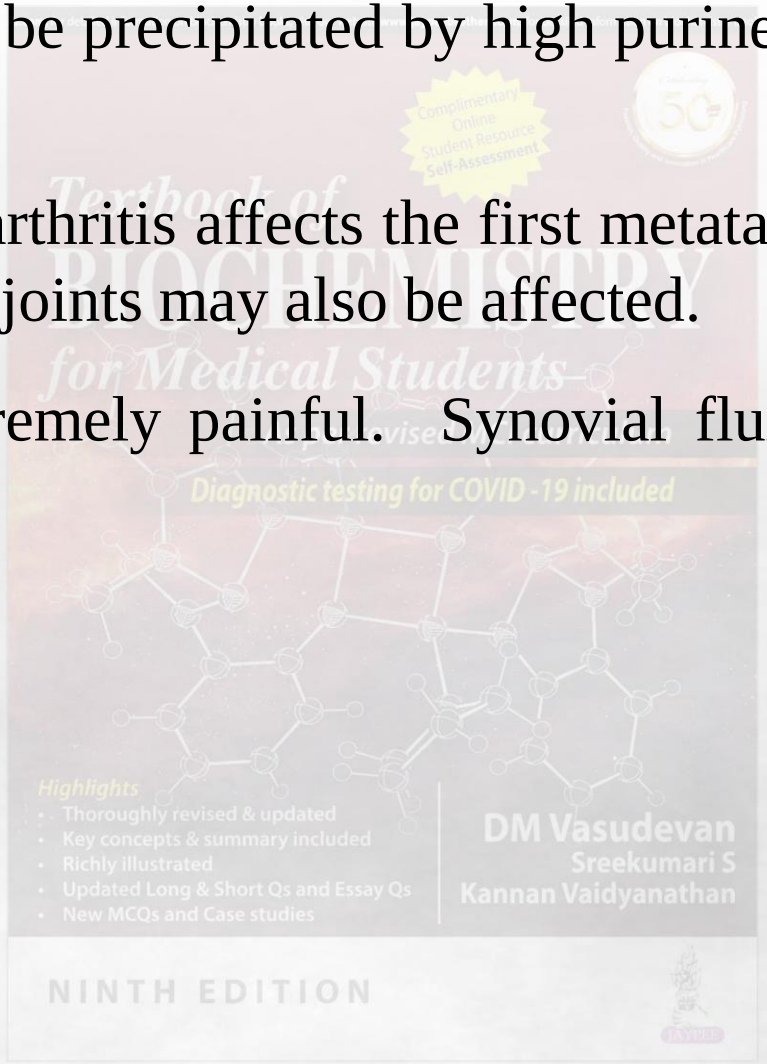


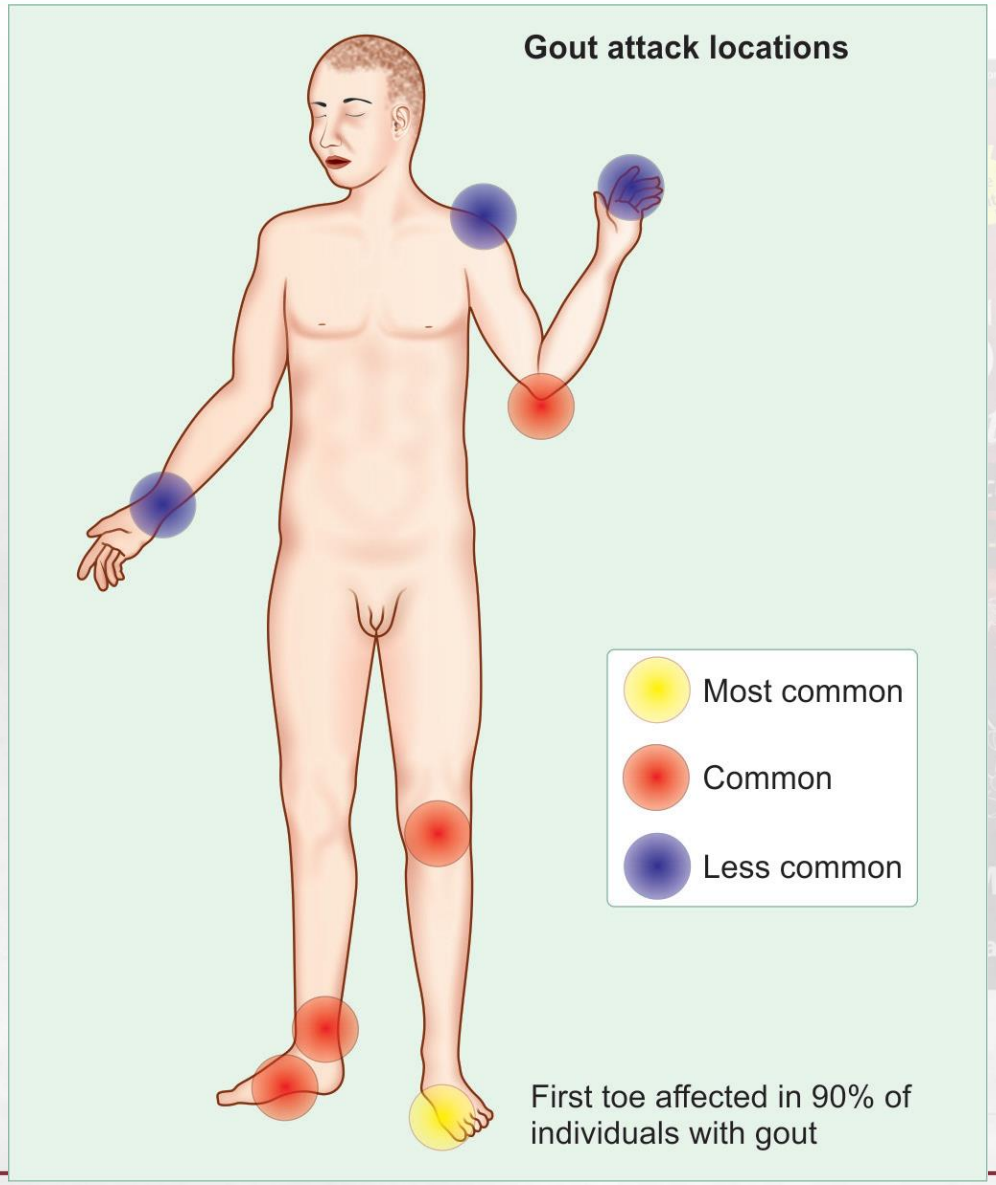
Clinical Findings of Gout

Gouty attacks may be precipitated by high purine diet and increased intake of alcohol.

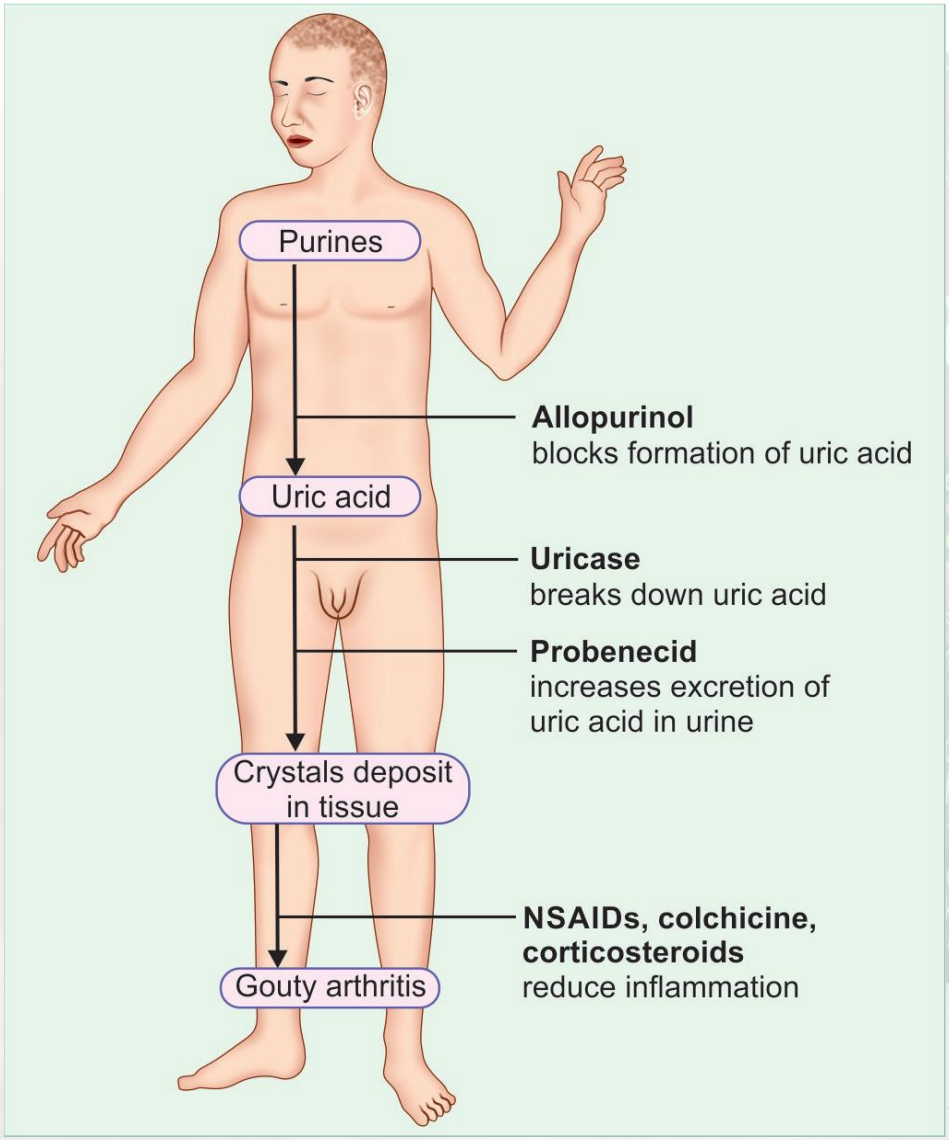
The typical gouty arthritis affects the first metatarsophalangeal joint (big toe), but other joints may also be affected.

The joints are extremely painful. Synovial fluid will show urate crystals.





Location of gout attack.



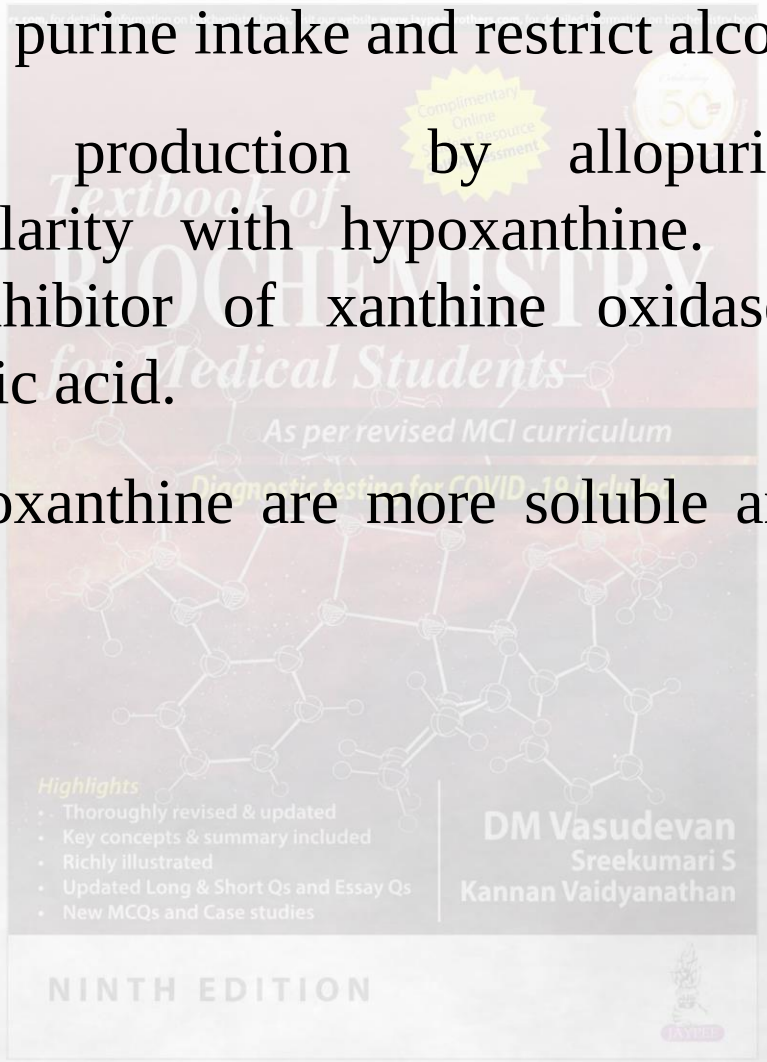
Action of medicines in gout.

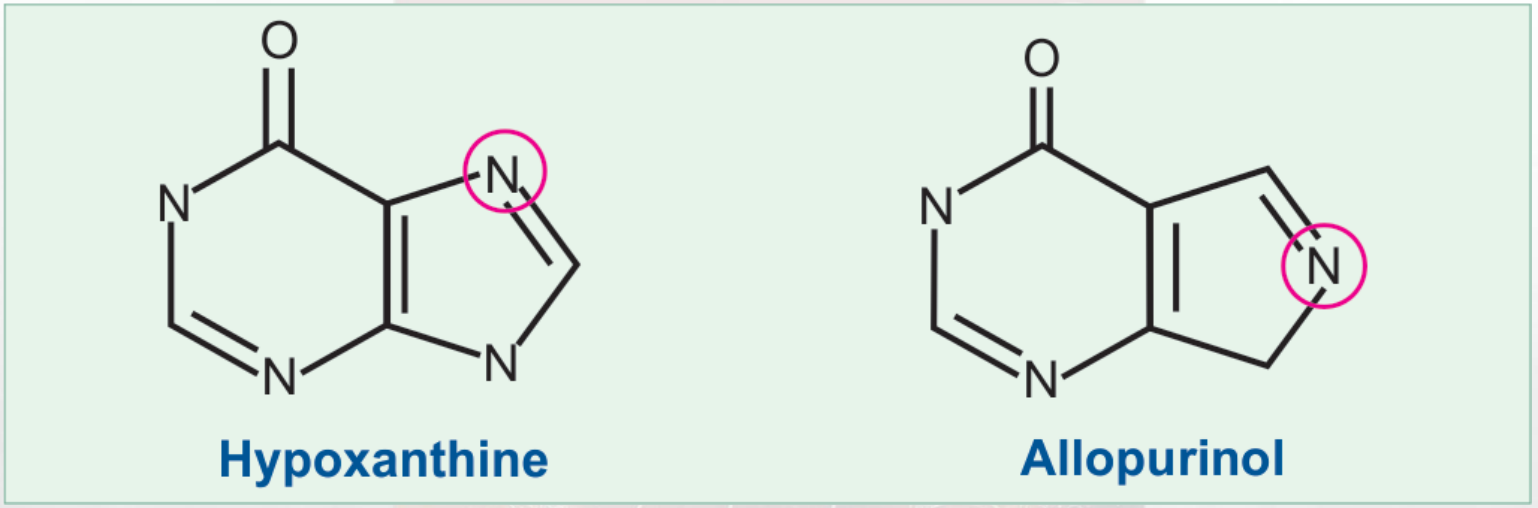
NSAIDs: nonsteroidal anti-inflammatory drugs

Treatment Policies in Gout

1. Reduce dietary purine intake and restrict alcohol.
2. Reduce urate production by allopurinol, which has structural similarity with hypoxanthine. Allopurinol is a competitive inhibitor of xanthine oxidase, decreasing the formation of uric acid.

Xanthine and hypoxanthine are more soluble and so are excreted more easily.



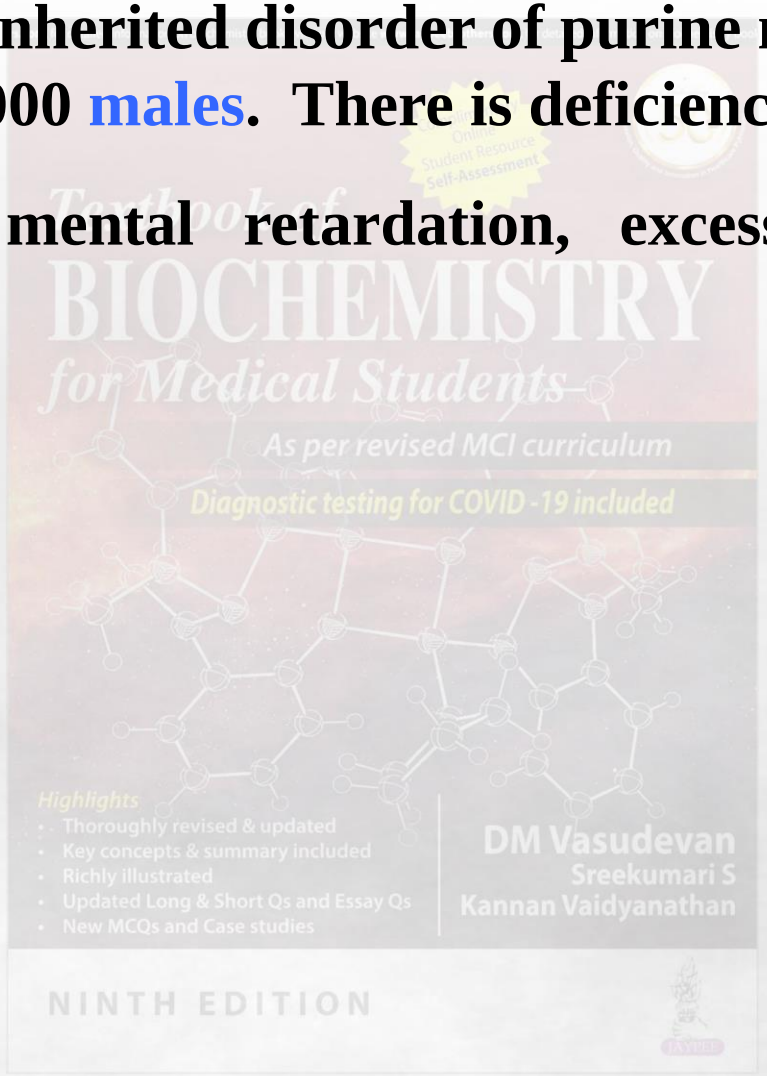


Allopurinol inhibits xanthine oxidase; an example of competitive inhibition.

Lesch-Nyhan Syndrome

It is an X-linked inherited disorder of purine metabolism. Incidence is 1:10,000 males. There is deficiency of HGPRTase.

Self mutilation, mental retardation, excess uric acid and nephrolithiasis.



Hypouricemia.

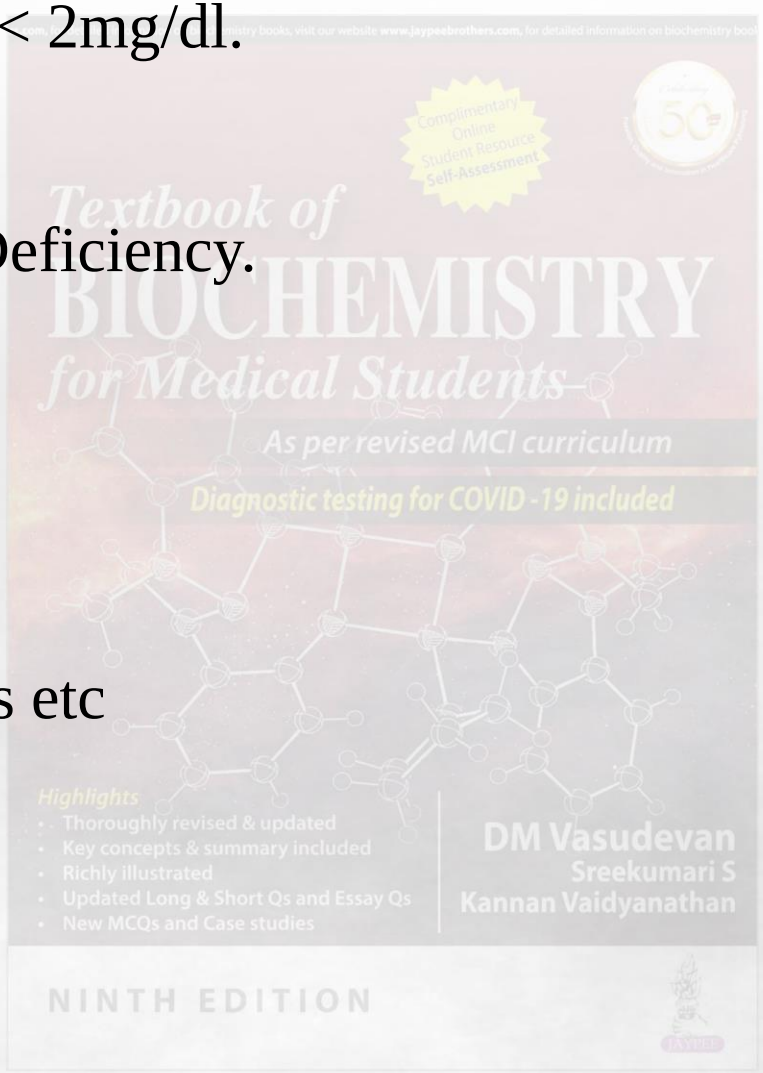
S.Uric Acid level < 2mg/dl.

Causes.

Cong. X.Oxidase Deficiency.

Clinical features.

- Xanthinuria.
- Hypouricemia.
- Xanthine Stones etc



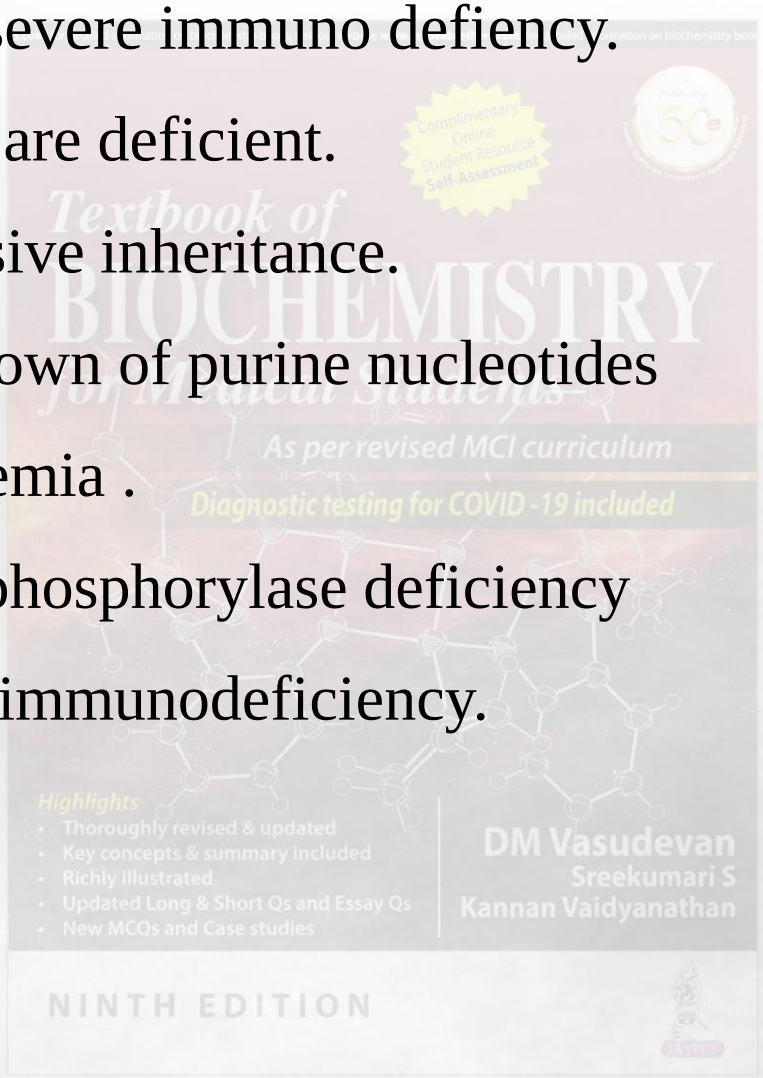
Adenosine Deaminase Deficiency

- Associated with severe immuno defiency.
- Both T & B cells are deficient.
- Autosomal recessive inheritance.
- Defective breakdown of purine nucleotides

Leads to Hypouricemia .

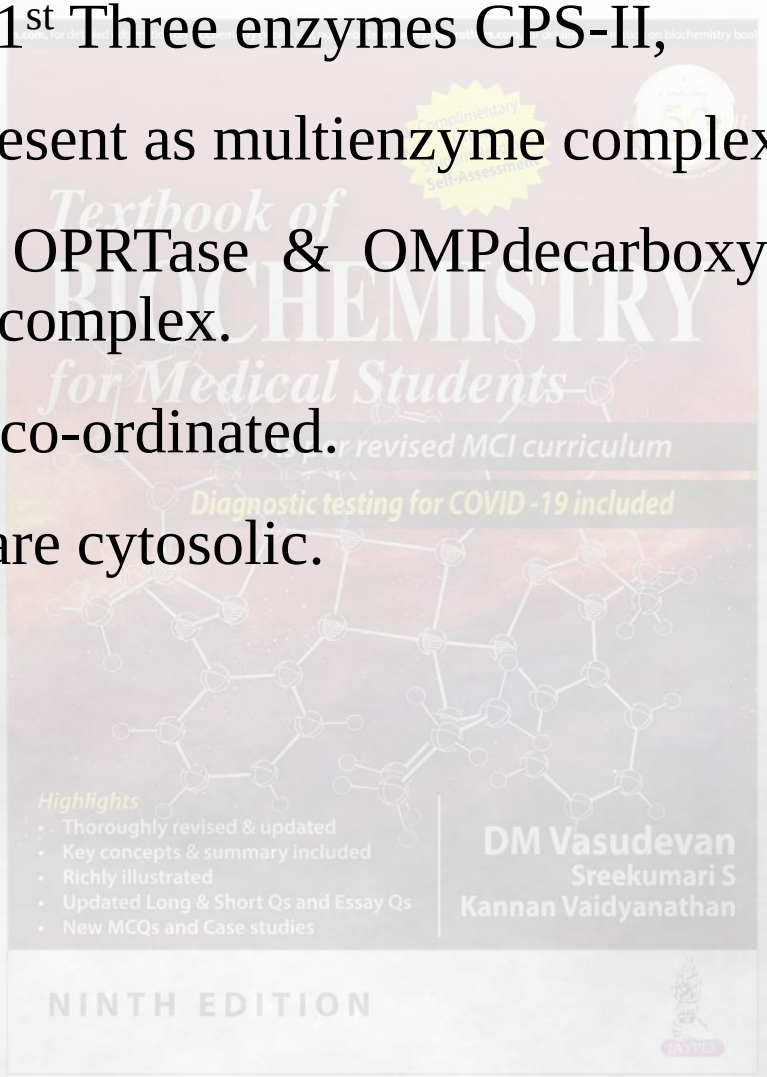
Purine nucleoside phosphorylase deficiency

Manifest as severe immunodeficiency.



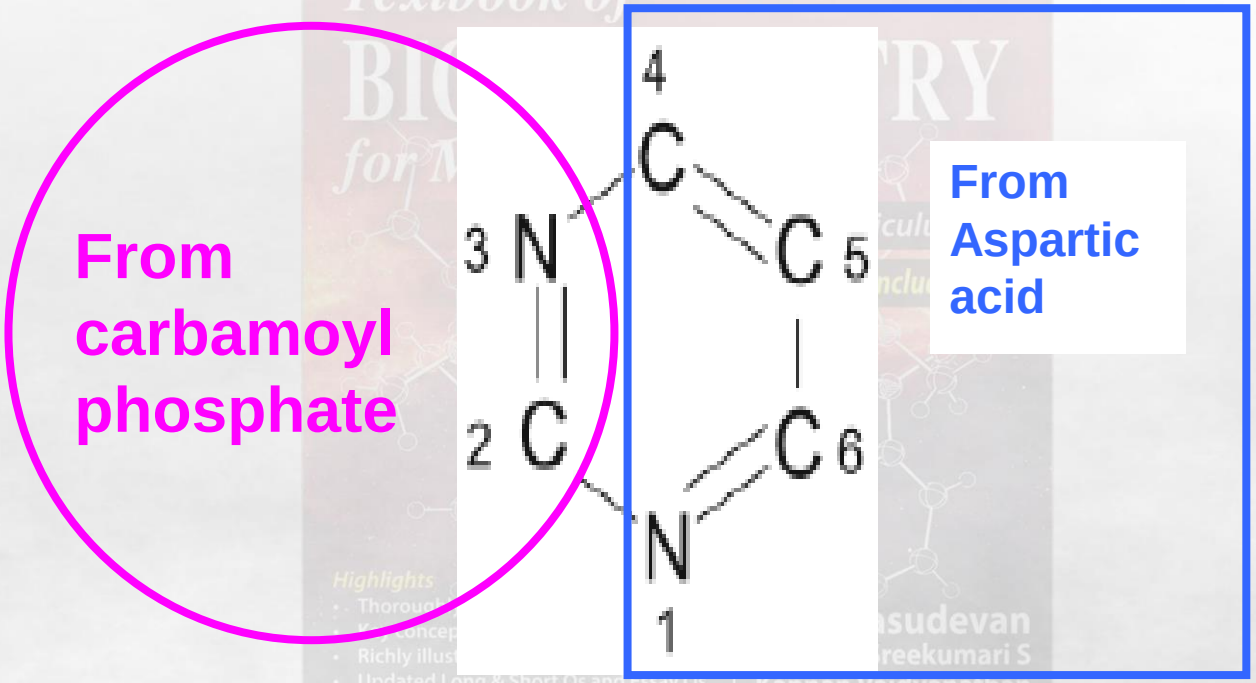
Regulation

- In eukaryotes the1st Three enzymes CPS-II,
- ATC,DHO ase present as multienzyme complex referred as CAD.
- Last 2 enzymes OPRTase & OMPdecarboxylase– present as a single functional complex.
- Synthesis is well co-ordinated.
- Both complexes are cytosolic.

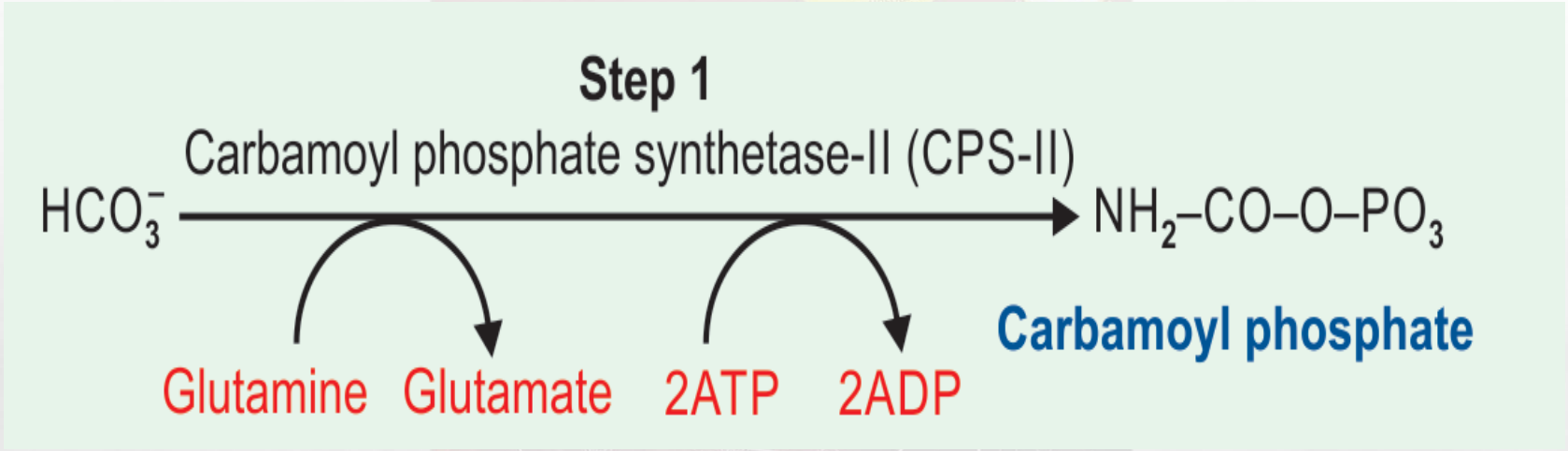


De Novo Synthesis of Pyrimidine

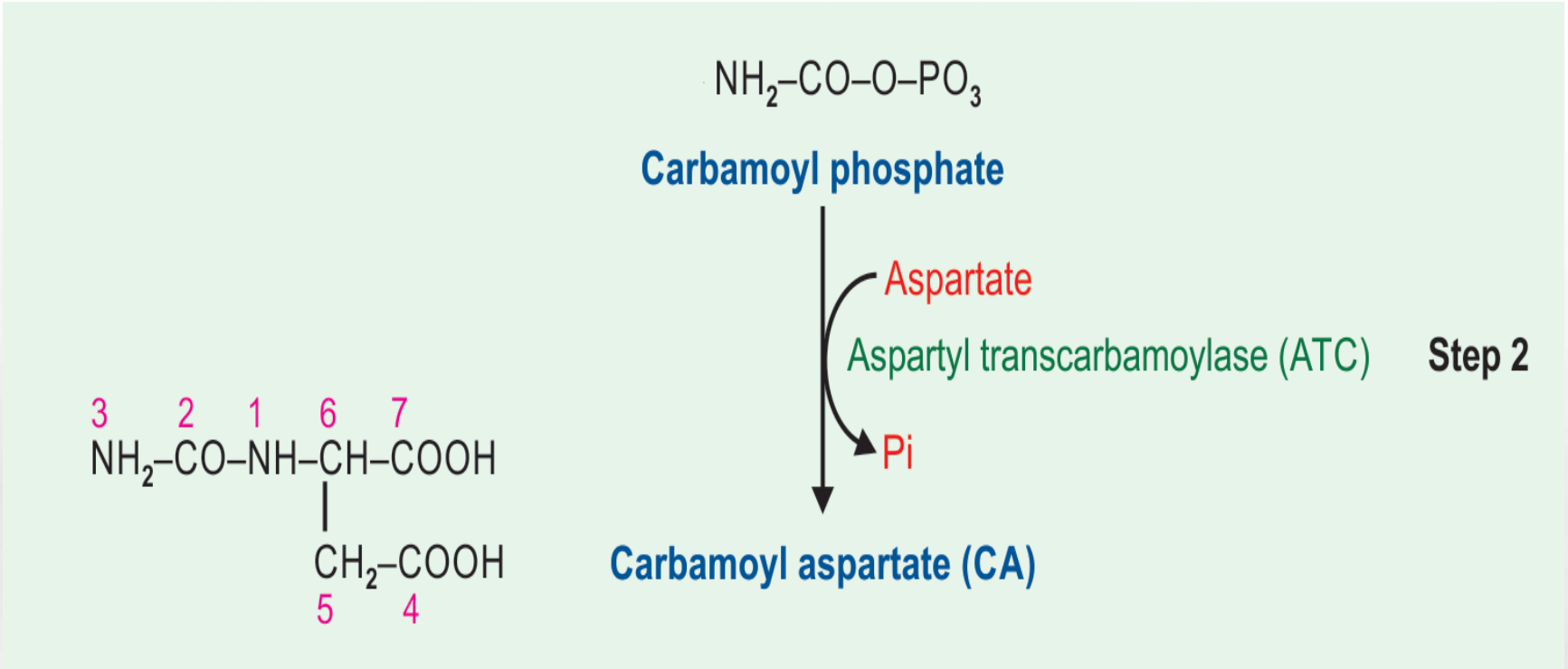
The pyrimidine ring (unlike the purine) is synthesised as free pyrimidine and then it is incorporated into the nucleotide.



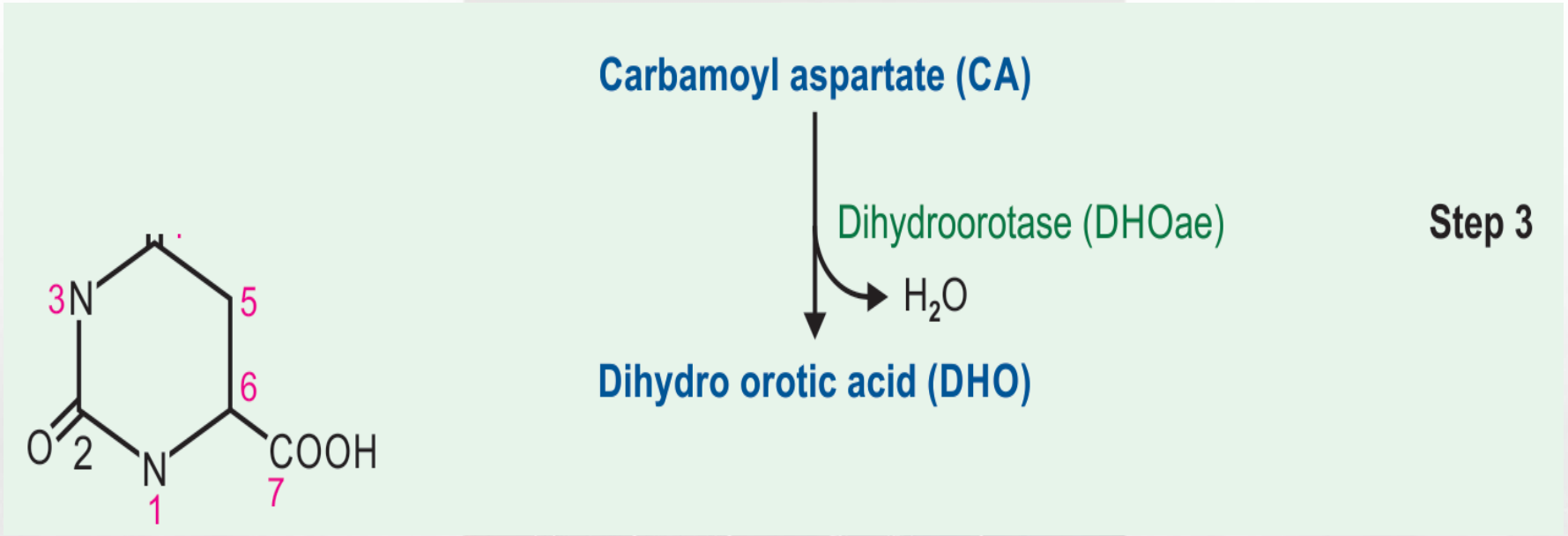
Sources of C and N atoms of pyrimidine



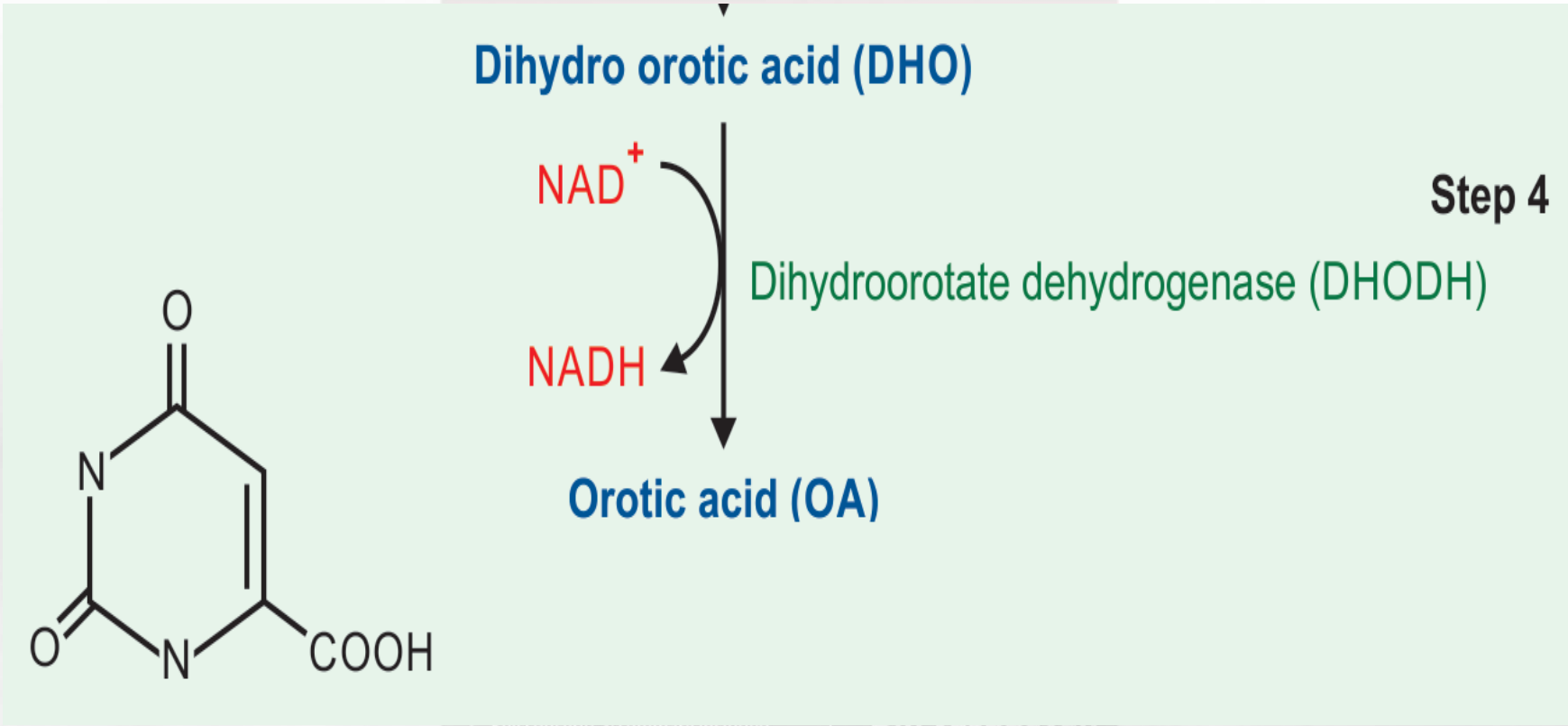
Step 1, de novo synthesis of pyrimidine,



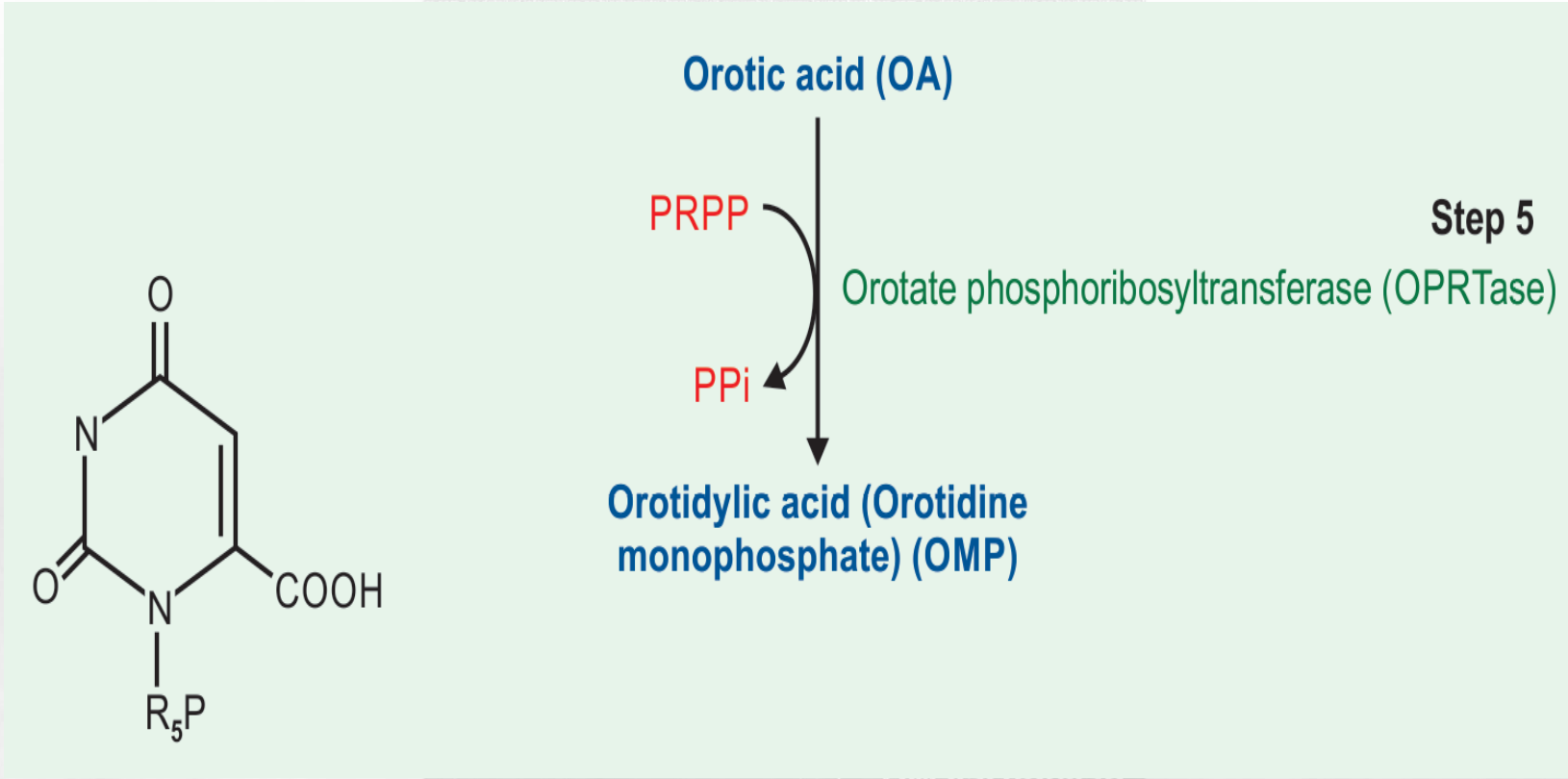
Step 2, de novo synthesis of pyrimidine,



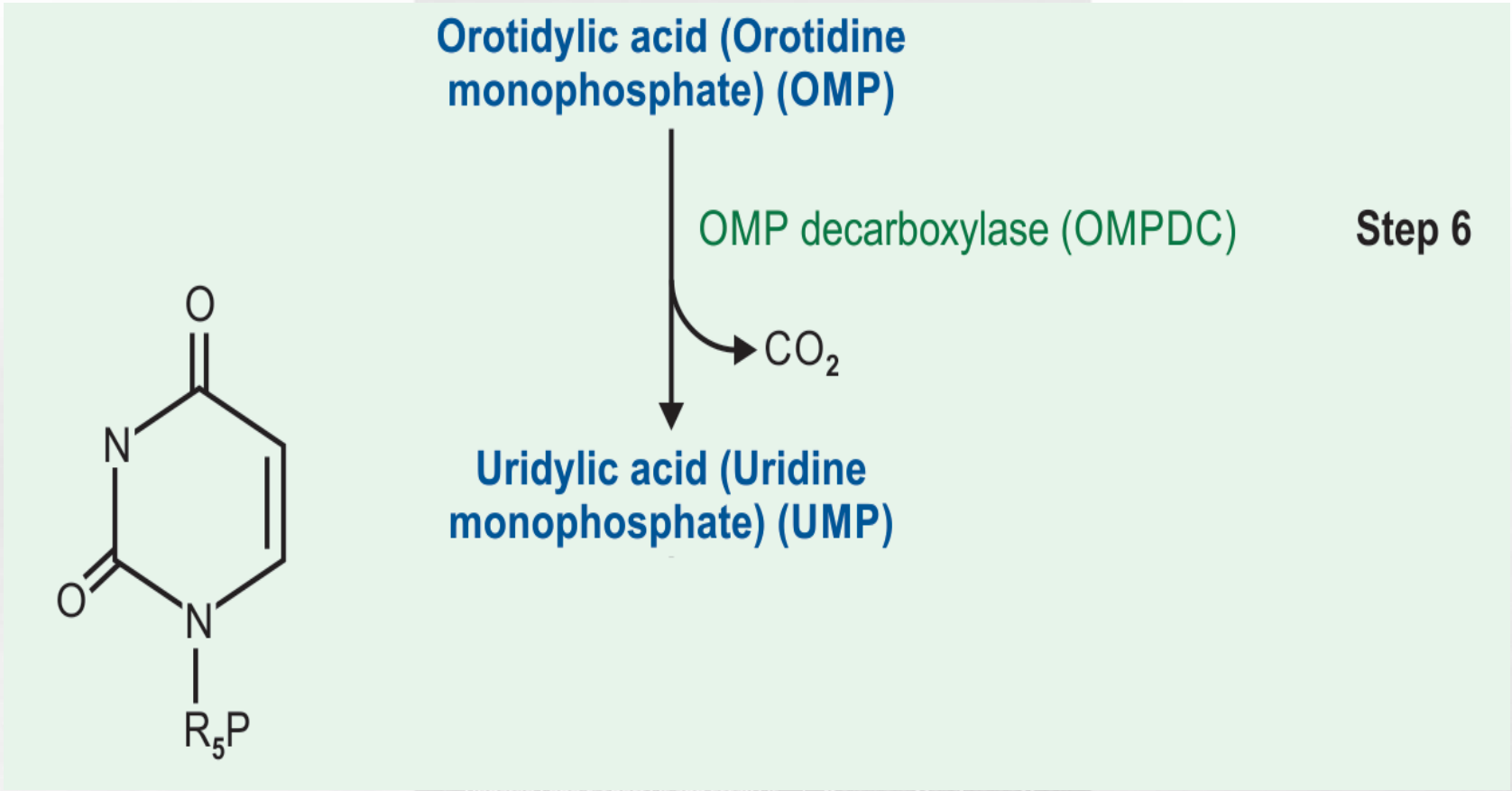
Step 3, de novo synthesis of pyrimidine



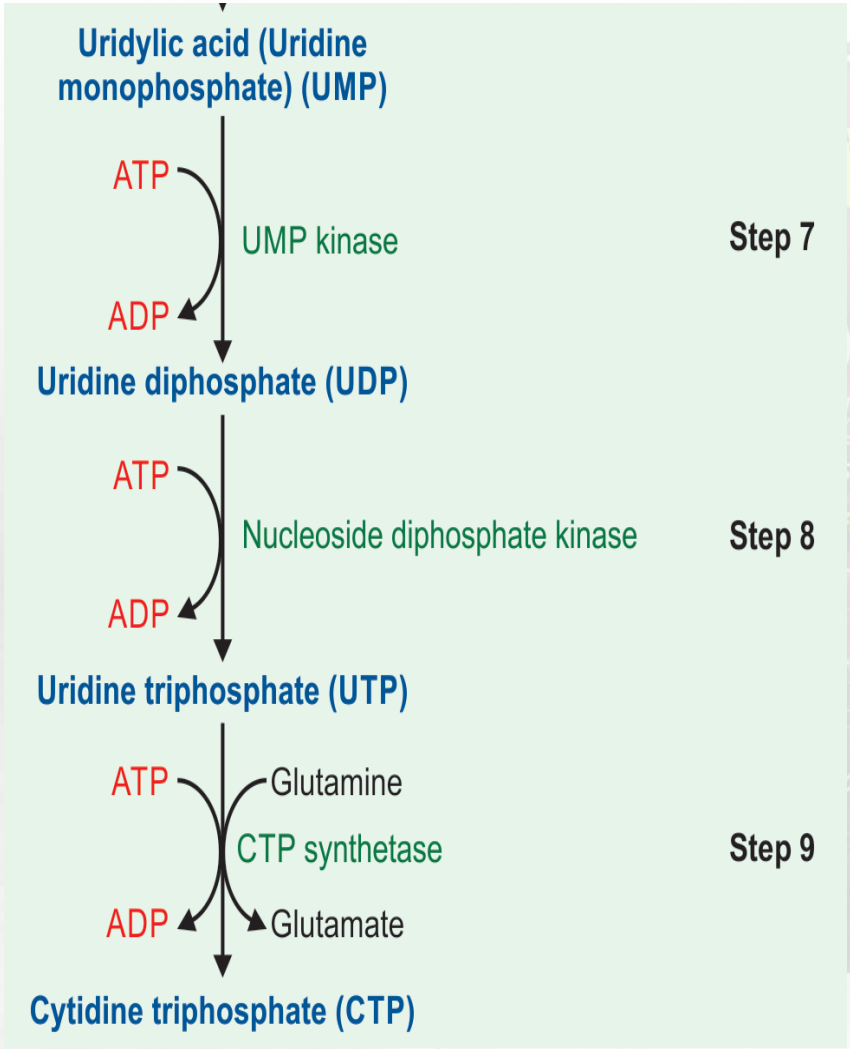
Step 4, de novo synthesis of pyrimidine.

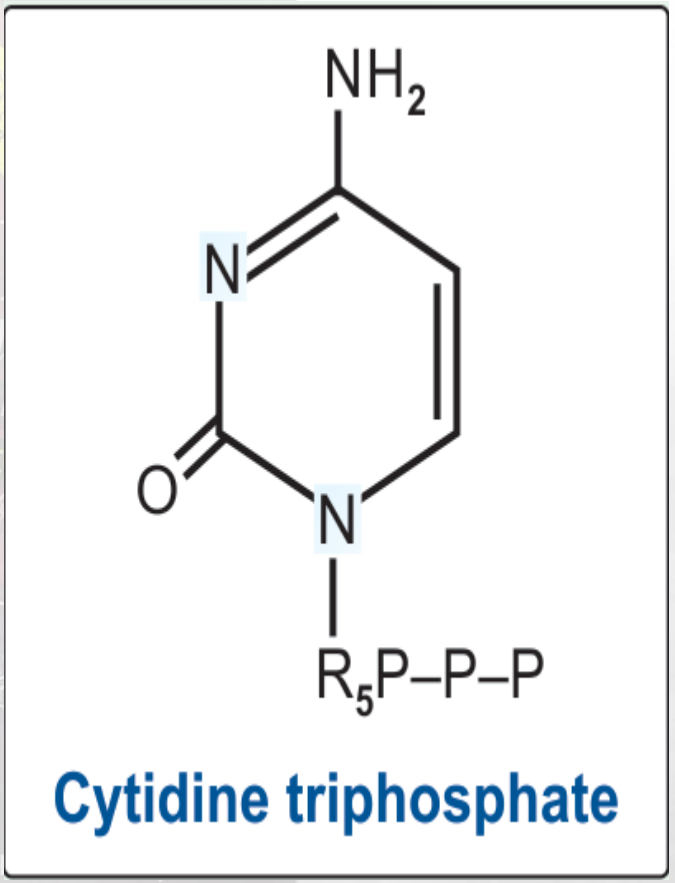


Step 5, de novo synthesis of pyrimidine.



Step 6, de novo synthesis of pyrimidine.





Steps 7,8,9, de novo synthesis of pyrimidine.

Disorders of Pyrimidine Metabolism

Disorder	Defective Enzyme
Orotic aciduria Type I	OPRTase, OMP decarboxylase
Orotic aciduria Type II	OMP decarboxylase
Orotic aciduria	Ornithine Transcarbamoylase
Drug induced Orotic aciduria	OMP decarboxylase
Beta-amino isobutyric aciduria	Transaminase, affects urea cycle function, deamination of alpha-amino acids to alpha-keto acids

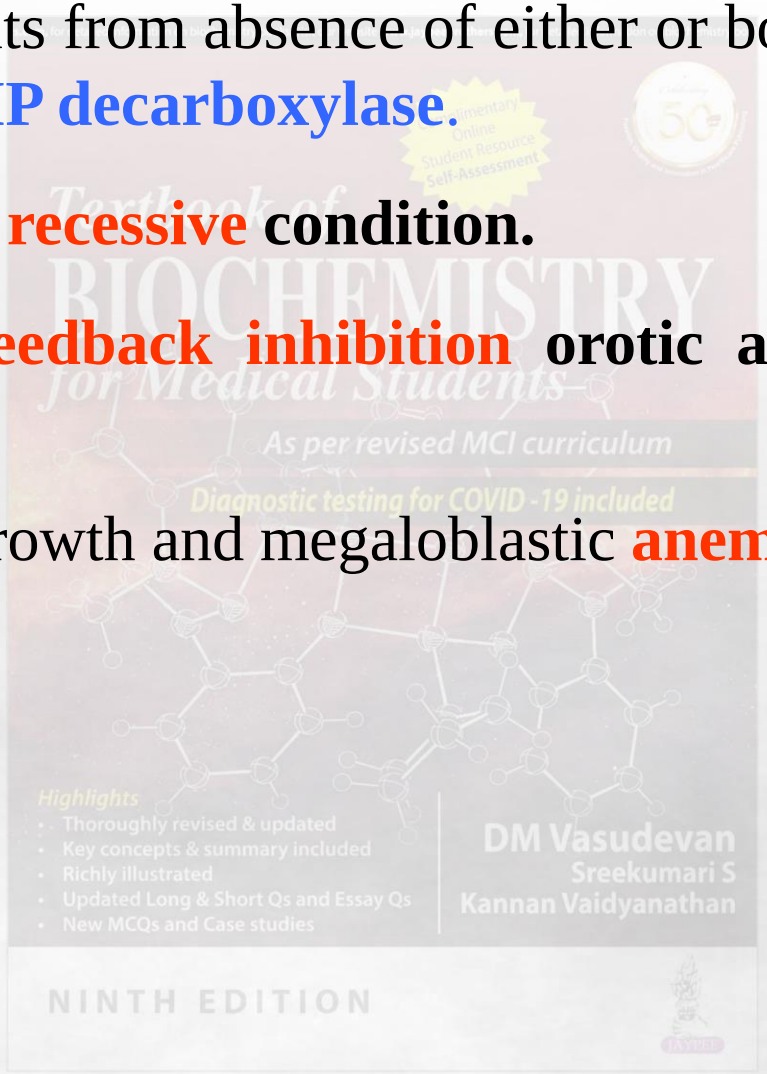
Orotic Aciduria

The condition results from absence of either or both of the enzymes, **OPRTase and OMP decarboxylase.**

It is an autosomal recessive condition.

Due to lack of feedback inhibition orotic acid production is excessive.

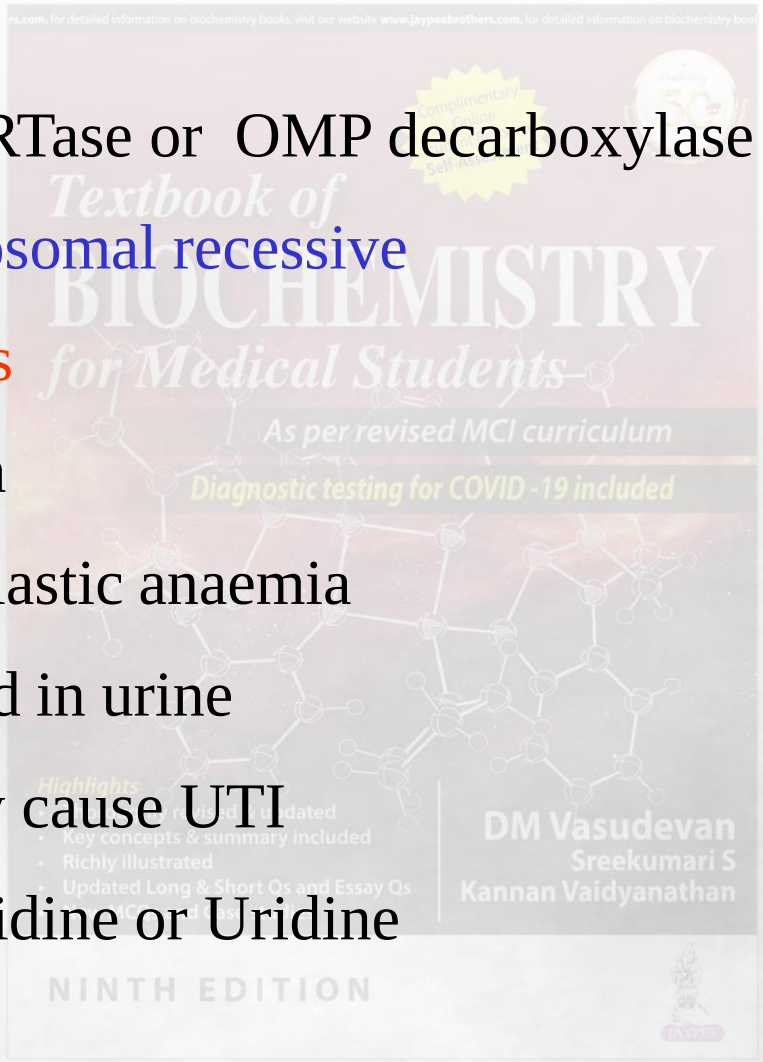
There is retarded growth and megaloblastic **anemia.**

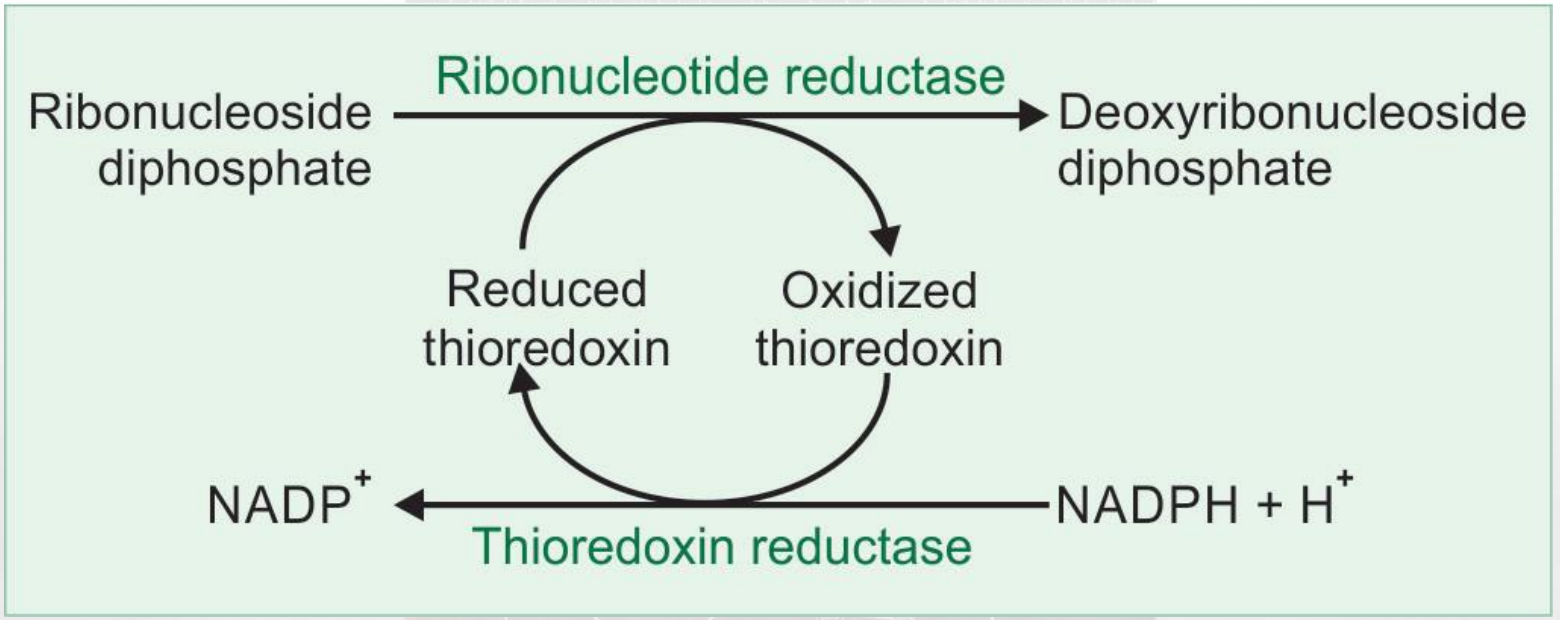


Orotic Aciduria

Causes

- Absence of OPRTase or OMP decarboxylase
- Inherited as autosomal recessive
- Clinical Features
- Retarded growth
- Severe megaloblastic anaemia
- Crystals excreted in urine
- Cryslalluria may cause UTI
- R_x – Feeding cytidine or Uridine

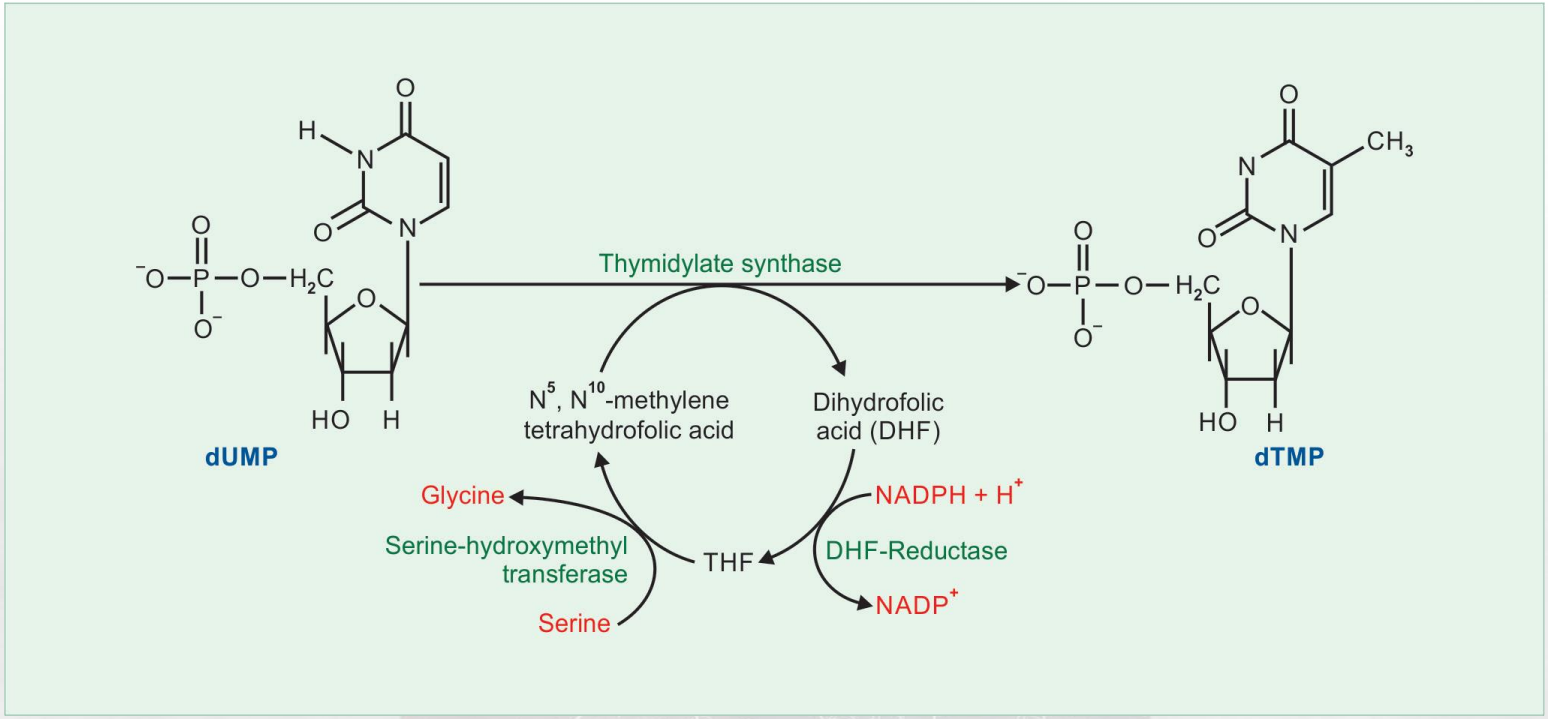




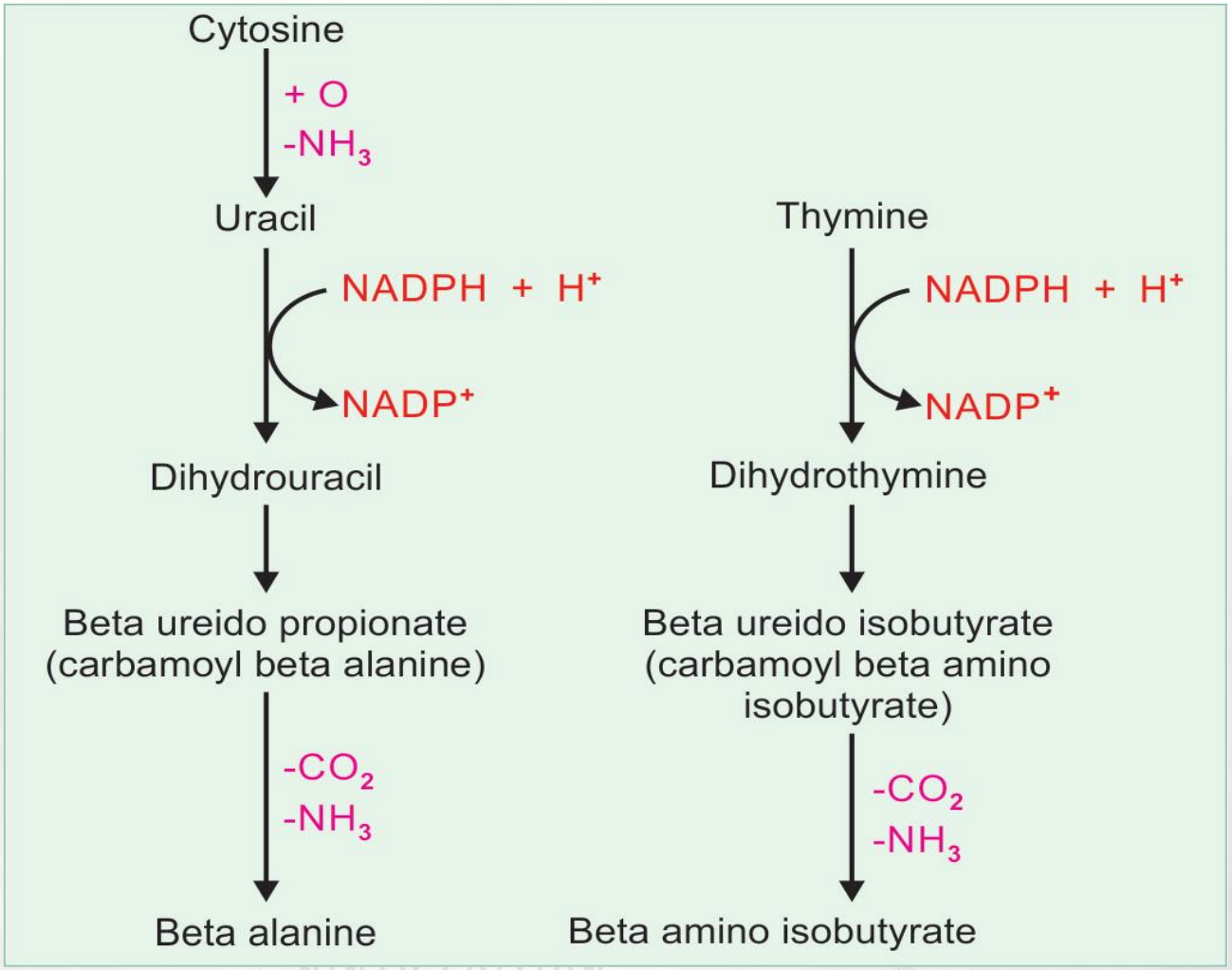
Formation of 2'-deoxyribonucleoside diphosphates.

Regulation of deoxyribonucleotide formation

Reaction	+ve regulator	Inhibitor
CDP → d-CDP	ATP	dATP, dGTP, dTTP
UDP → d-UDP	ATP	dATP, dGTP
ADP → d-ADP	dGTP	dATP, ATP
GDP → d-GDP	dTTP	dATP



Production of dTMP from dUMP, by the enzyme thymidylate synthase. The reaction needs one carbon units, and folic acid. Methotrexate inhibits the enzyme DHF-reductase. So dTMP synthesis is inhibited, in turn DNA synthesis is inhibited. (DHF: dihydrofolic acid; THF: tetrahydrofolic acid; TMP: thymidine monophosphate; UMP: uridine monophosphate).



Catabolism of pyrimidines.