Nucleotide Metabolism

Objectives

- Purine synthesis
- Clinical implications
- Pyrimidine synthesis
- Clinical implications

Clinical case-1

A 46-year-old male presents to the emergency department with severe right toe pain. The patient was in usual state of health until early in the morning when he woke up with severe pain in his right big toe. The patient denies any trauma to the toe and no previous history of such pain in other joints. He did say that he had a "few too many" beers with the guys last night. On examination, he was found to have a temperature of 38.2°C (100.8°F) and in moderate distress secondary to the pain in his right toe. The right big toe was swollen, warm, red, and exquisitely tender. The remainder of the examination was normal. Synovial fluid was obtained and revealed rod- or needle-shaped crystals that were negatively birefringent under polarizing microscopy.

- **What is the likely diagnosis?**
- **How would you make a definite diagnosis?**
- **What is the pathophysiology of this disorder?**

Clinical case 2

In January 2000 a 2 year old boy was referred to a paediatric dental office in Landshut, Germany, because of severe and repeated lip chewing and aggressive tongue biting. A medical history revealed a normal pregnancy with no complications but a diagnosis of muscular hypotonia was made at four months of age. At 18 months a diagnosis was established through biochemical analysis and molecular examinations. The child displayed self-destructive behaviour (biting) since 10 months of his age. Shortly thereafter the patient was supplied with arm cuffs for self-protection which were not tolerated and the self-mutilation continued. Eventually the extraction of all primary teeth was deemed necessary to prevent additional medical problems for this child.

- **FOLLOW-UP:**
- One year after the dental extractions the patient presented with no bite injuries but was now using his fingers to injure himself

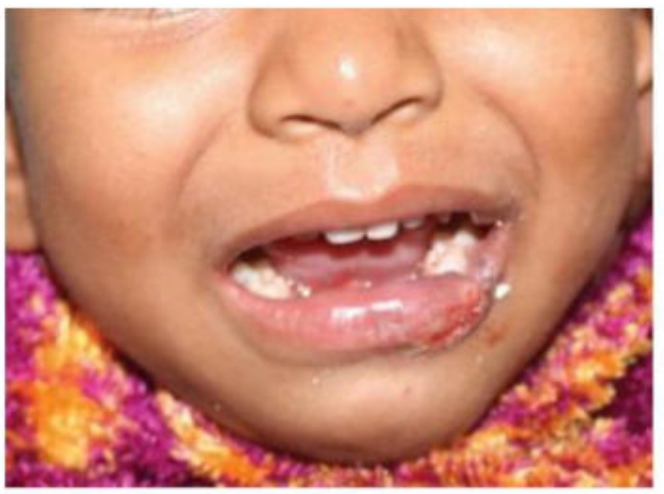


Fig. 1: Patient's extraoral appearance

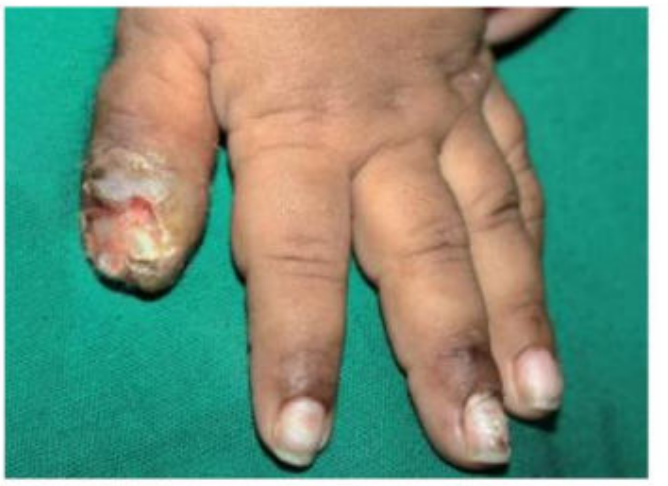


Fig. 2: Mutilated thumb of left hand

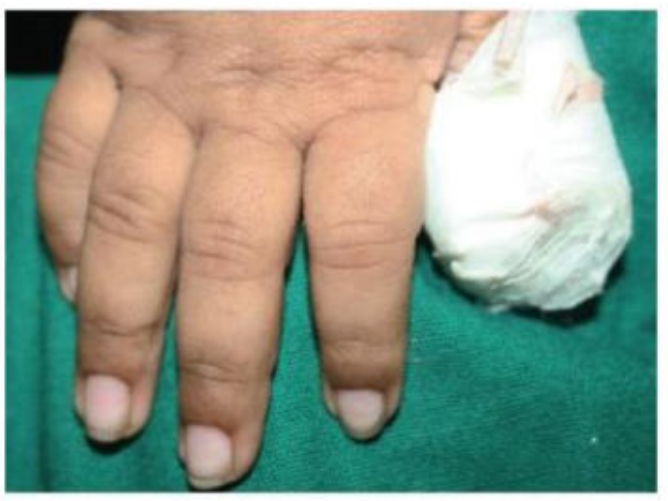


Fig. 3: Bandaged thumb on right hand

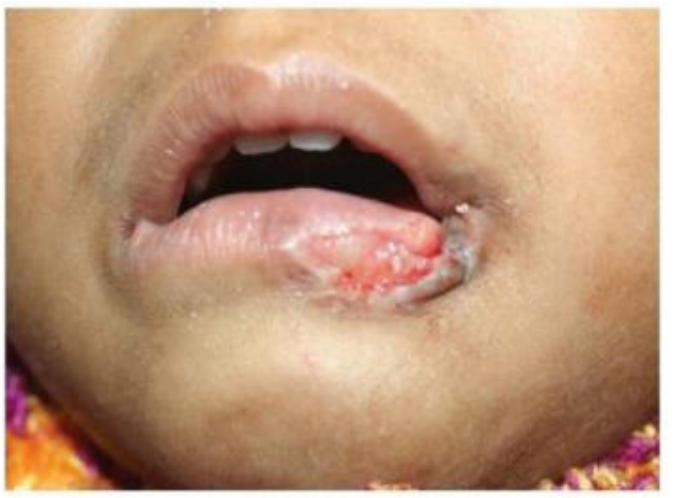


Fig. 4: Severely injured lower lip

- What is the likely diagnosis?
- How would you make a definite diagnosis?
- What is the pathophysiology of this disorder?

Clinical Case 3

A 4 year old girl presented to the clinic with Megaloblastic anaemia, growth failure and mental retardation. History revealed that the child was born normally. The RBC count was 2.55 million /cmm and Hb was 6 g/dl, She was given antibiotics and transfusion. Despite that the anaemia worsened. There was no response following treatment with B12, Folic acid and , or Pyridoxine.

A prominent feature of the child's urine was a crystalline sediment, which was found to be orotic acid. Orotic acid in amounts as high as 1500 mg (9.6 mmol) was excreted daily (Normal 1.4mg/day, 9µmol).

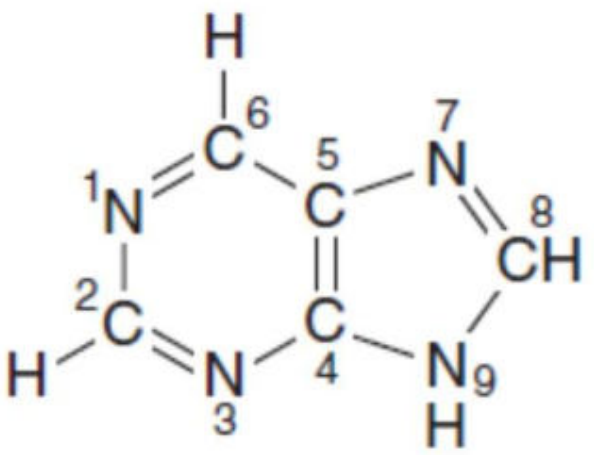
- What is the likely diagnosis?
- How would you make a definite diagnosis?
- What is the pathophysiology of this disorder?
- What could be the treatment?

Purine nucleotide biosynthesis

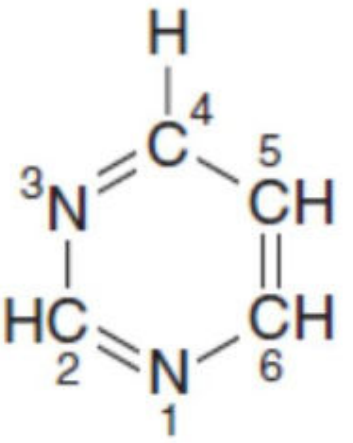
- De novo synthesis
- Phosphoribosylation of purines
- Phosphorylation of purine nucleoside

- **De novo synthesis of Purines**

Purine and pyrimidine. The atoms are numbered according to the international system.

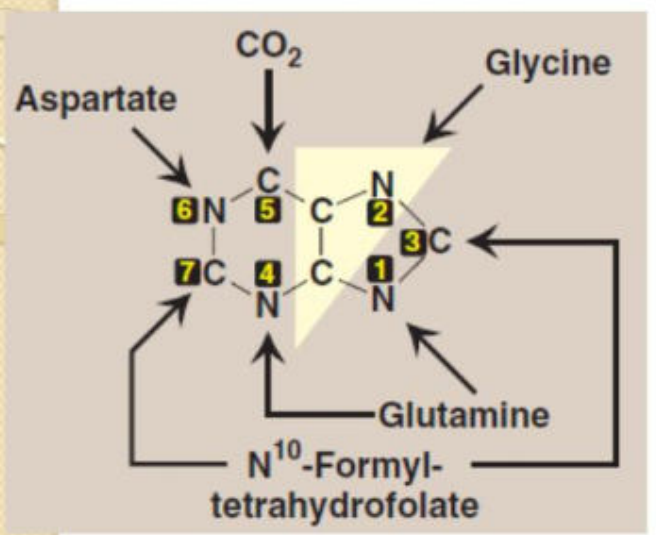


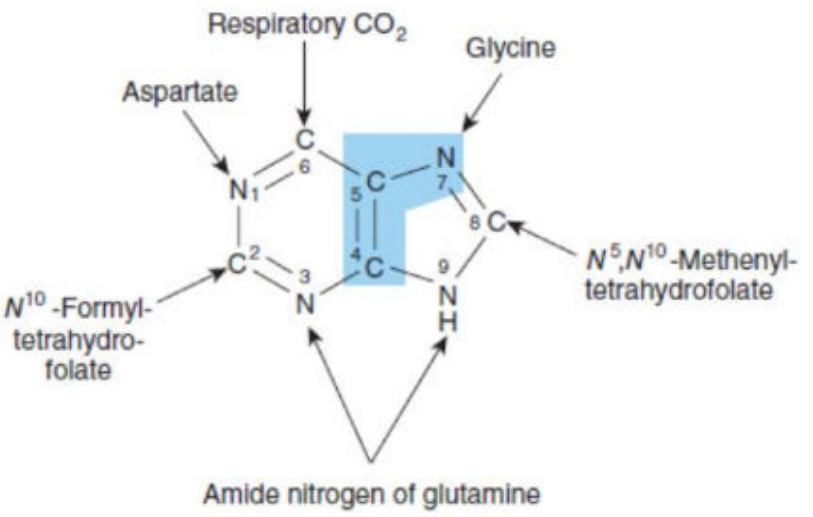
Purine

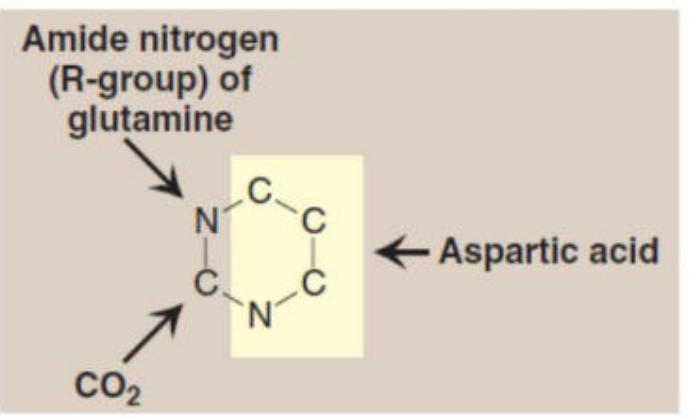


Pyrimidine

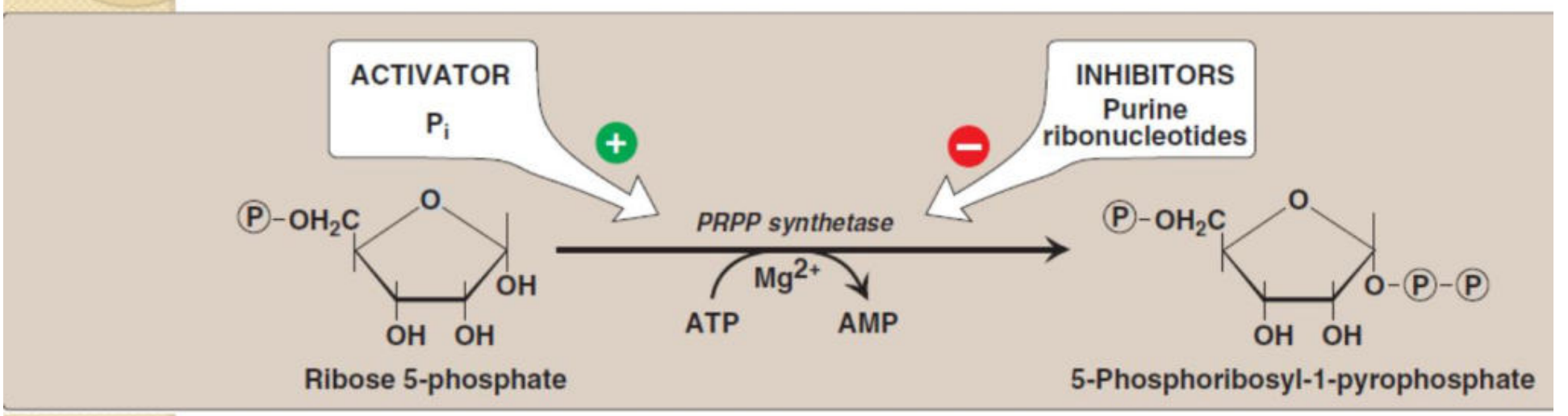
Sources of individual atoms in the purine ring



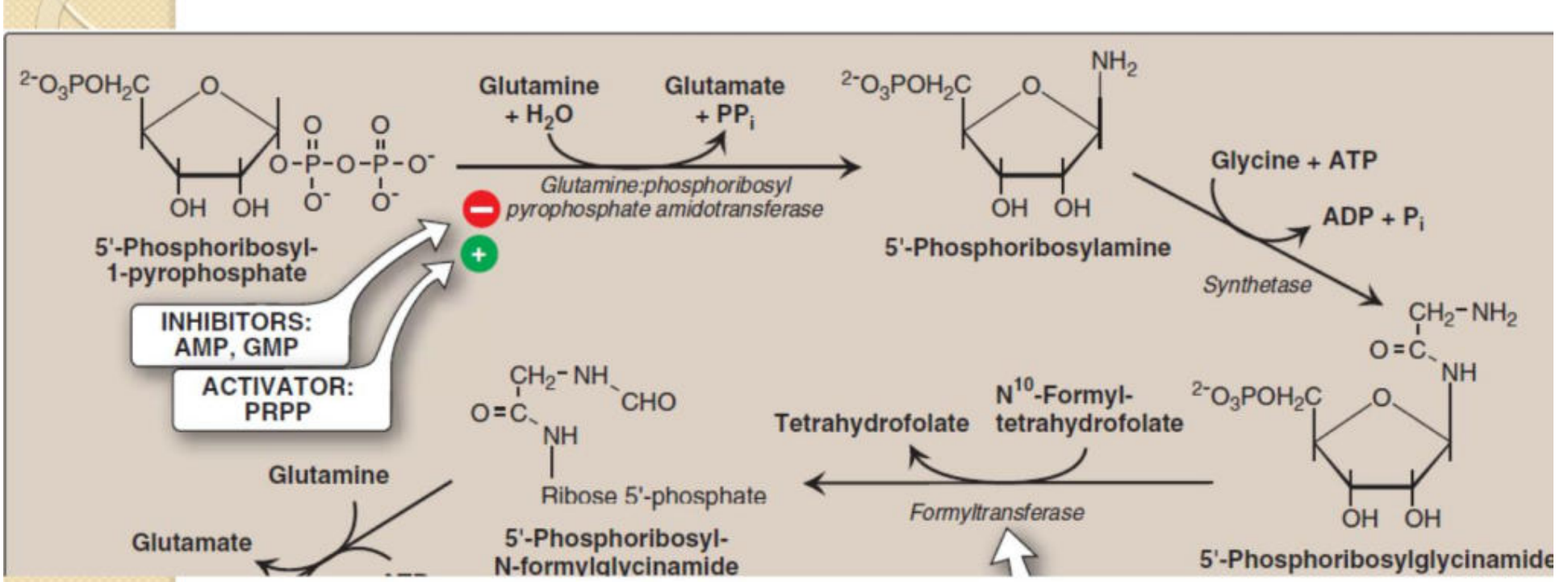


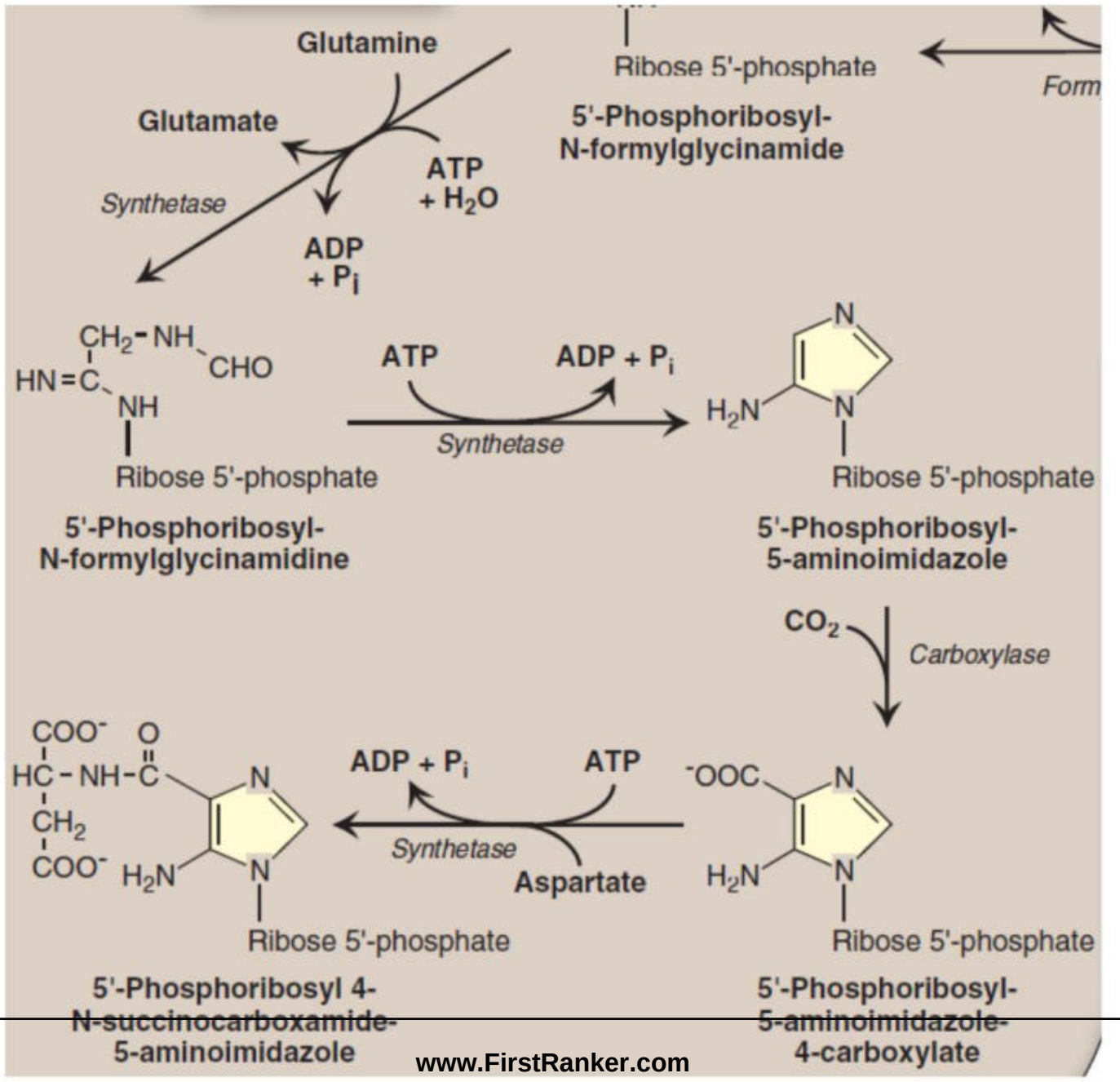


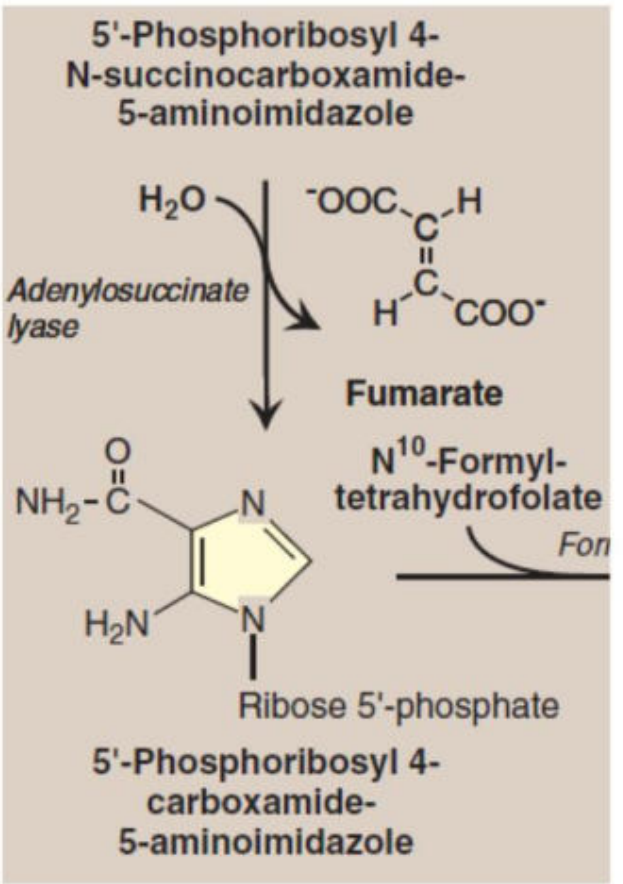
Synthesis of 5-phosphoribosyl-1-pyrophosphate (PRPP)

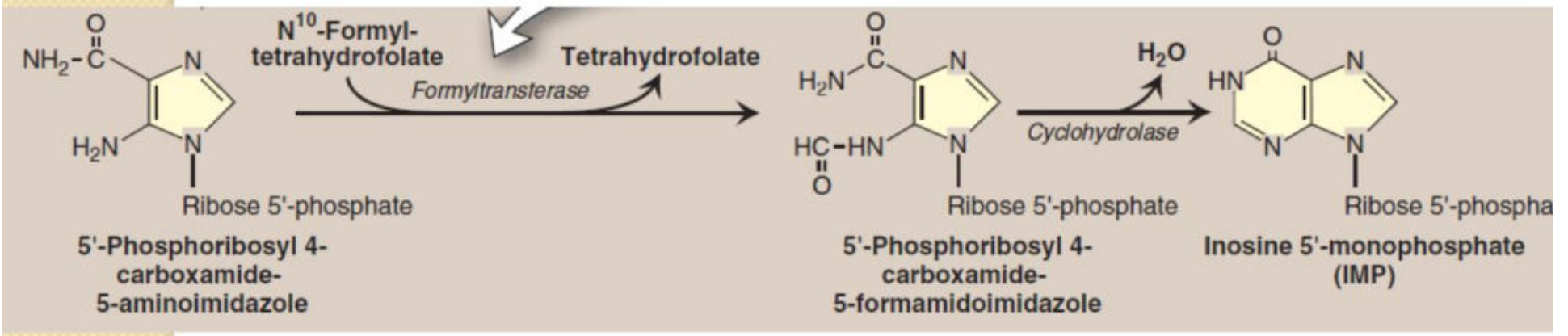


Synthesis of purine nucleotides

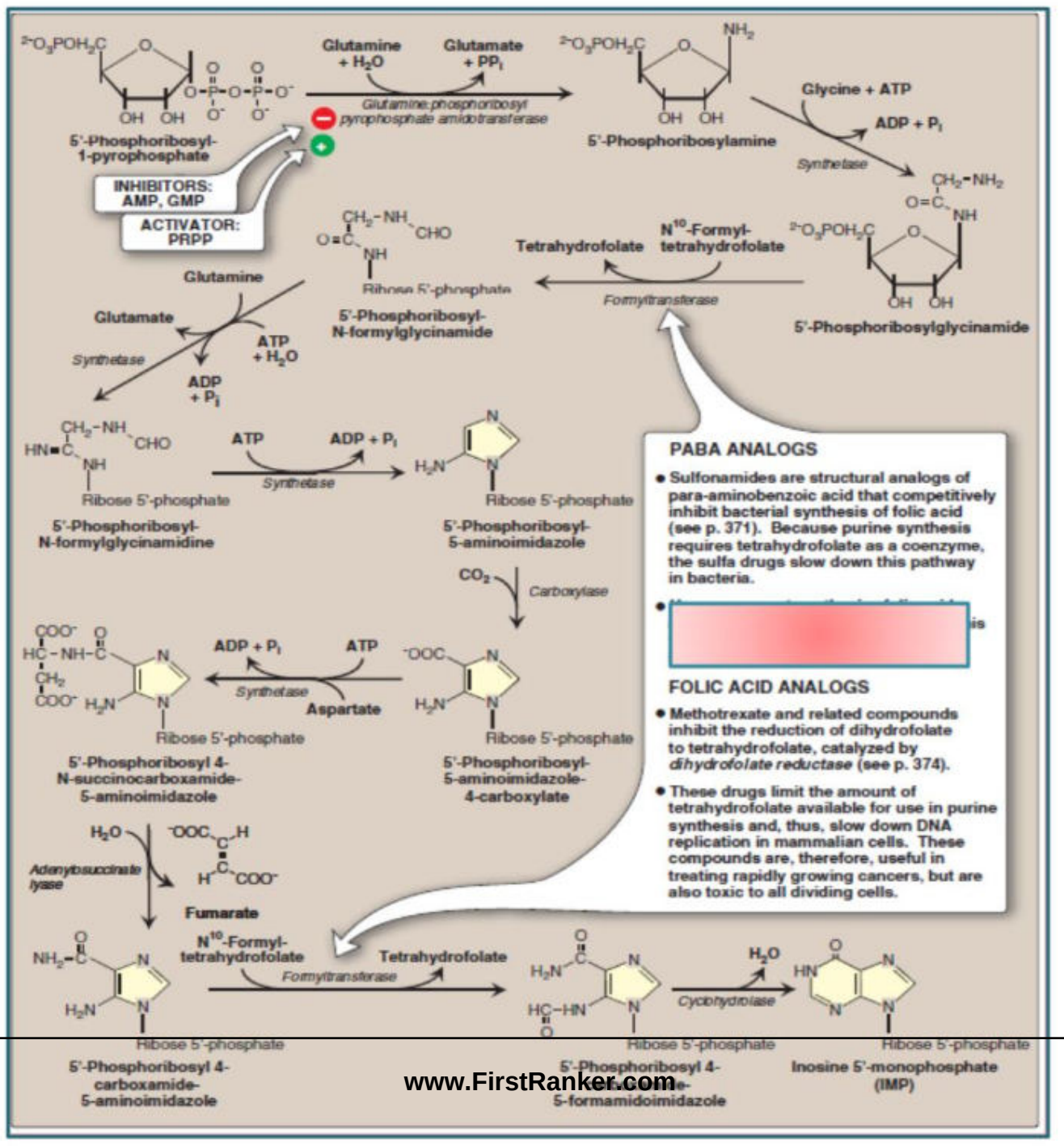




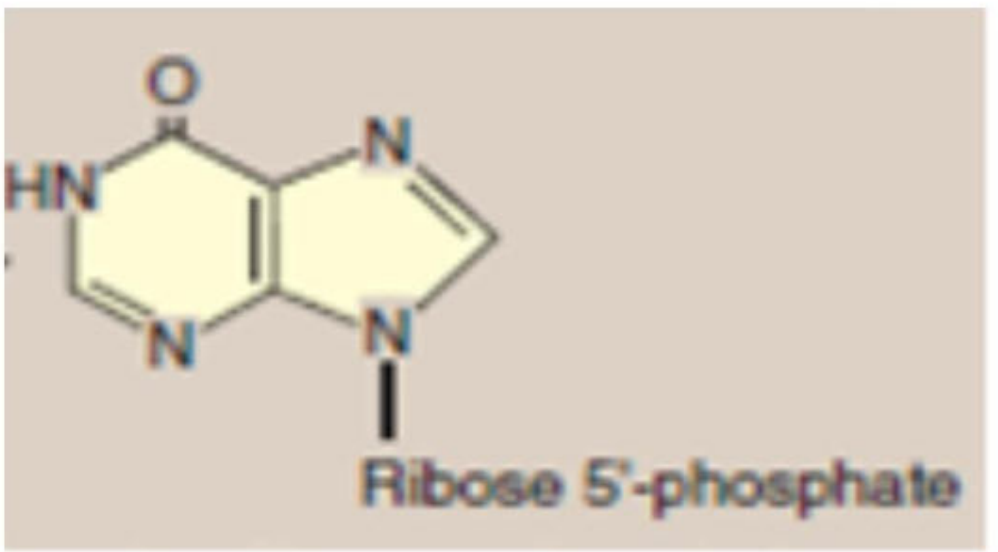


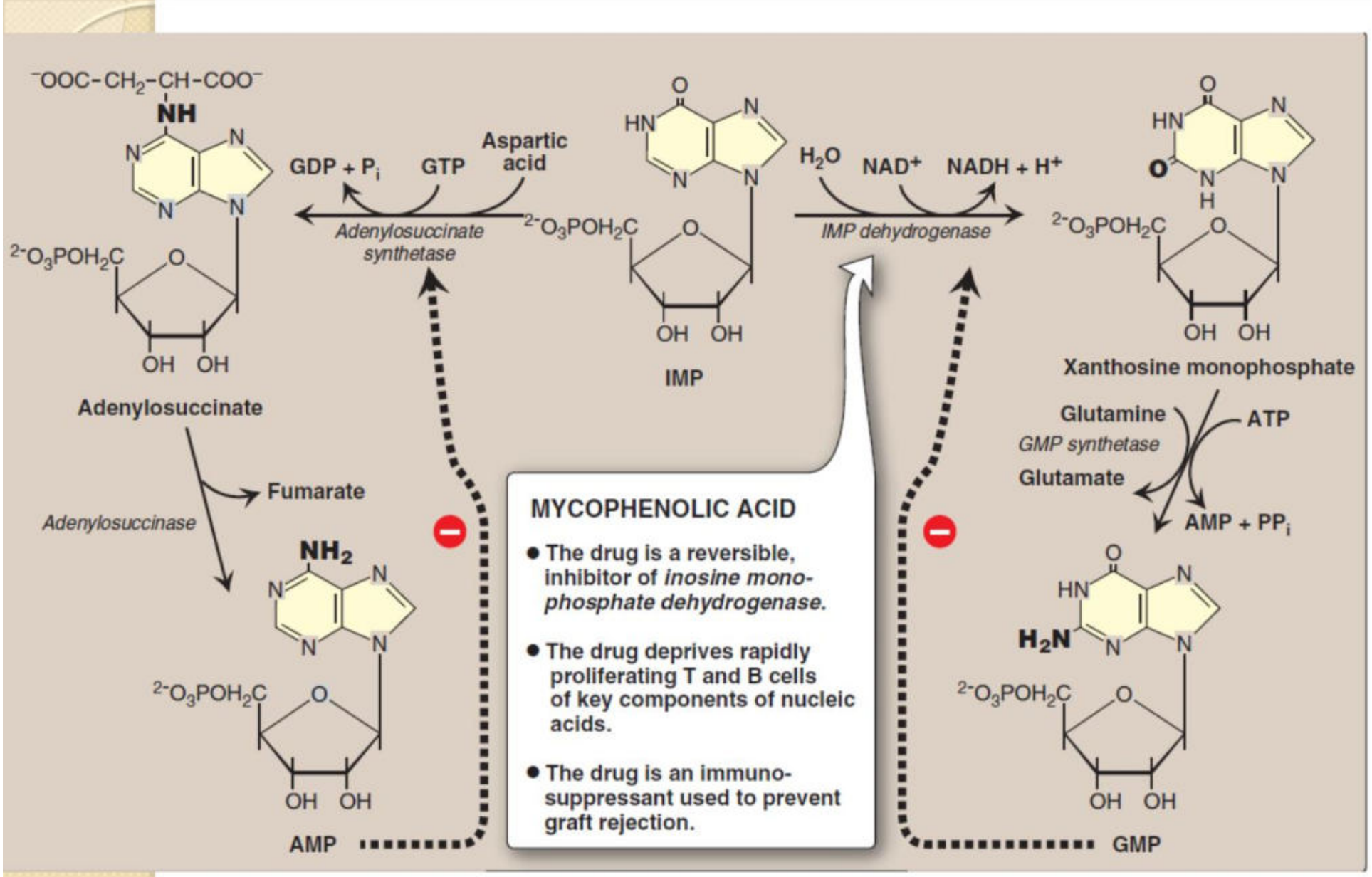


- **Clinical Importance/ Therapeutic implications**



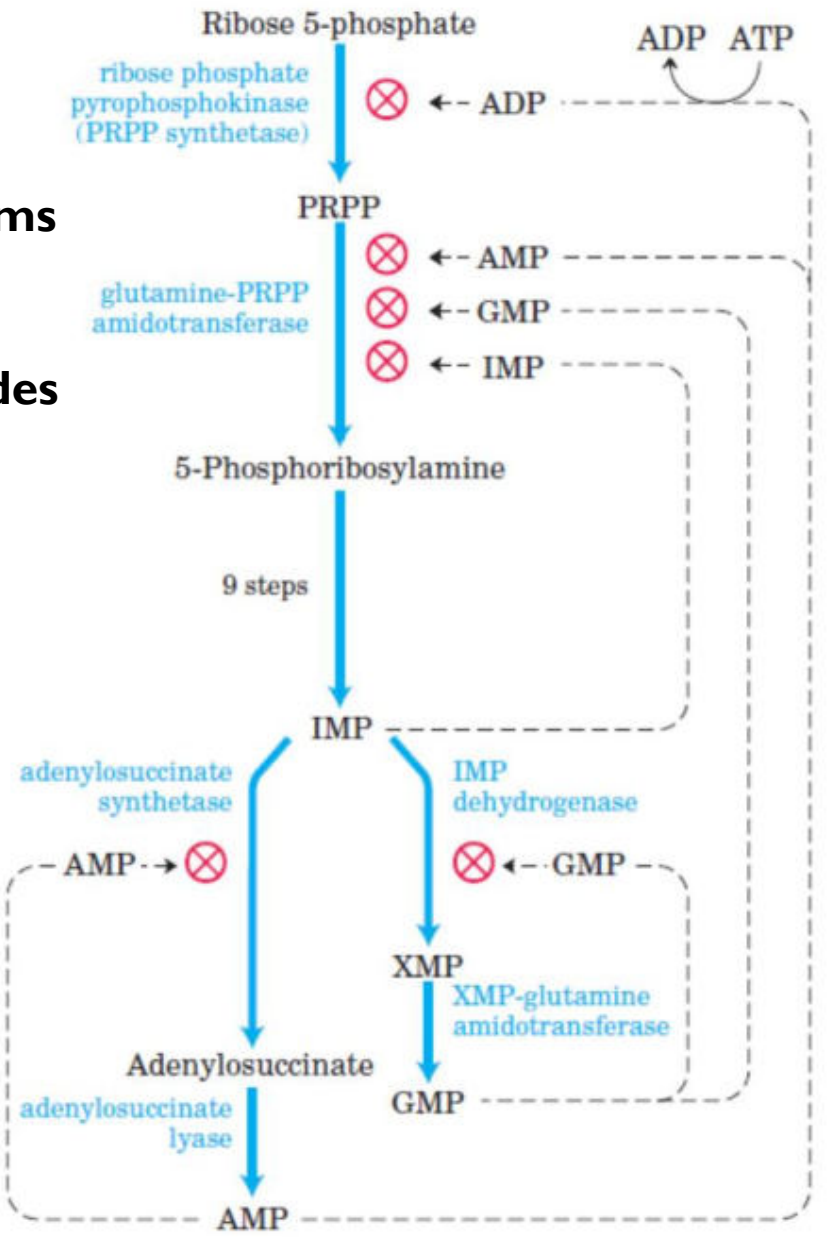
Conversion of IMP to AMP and GMP





Conversion of IMP to AMP and GMP

Regulatory mechanisms in the biosynthesis of adenine and guanine nucleotides

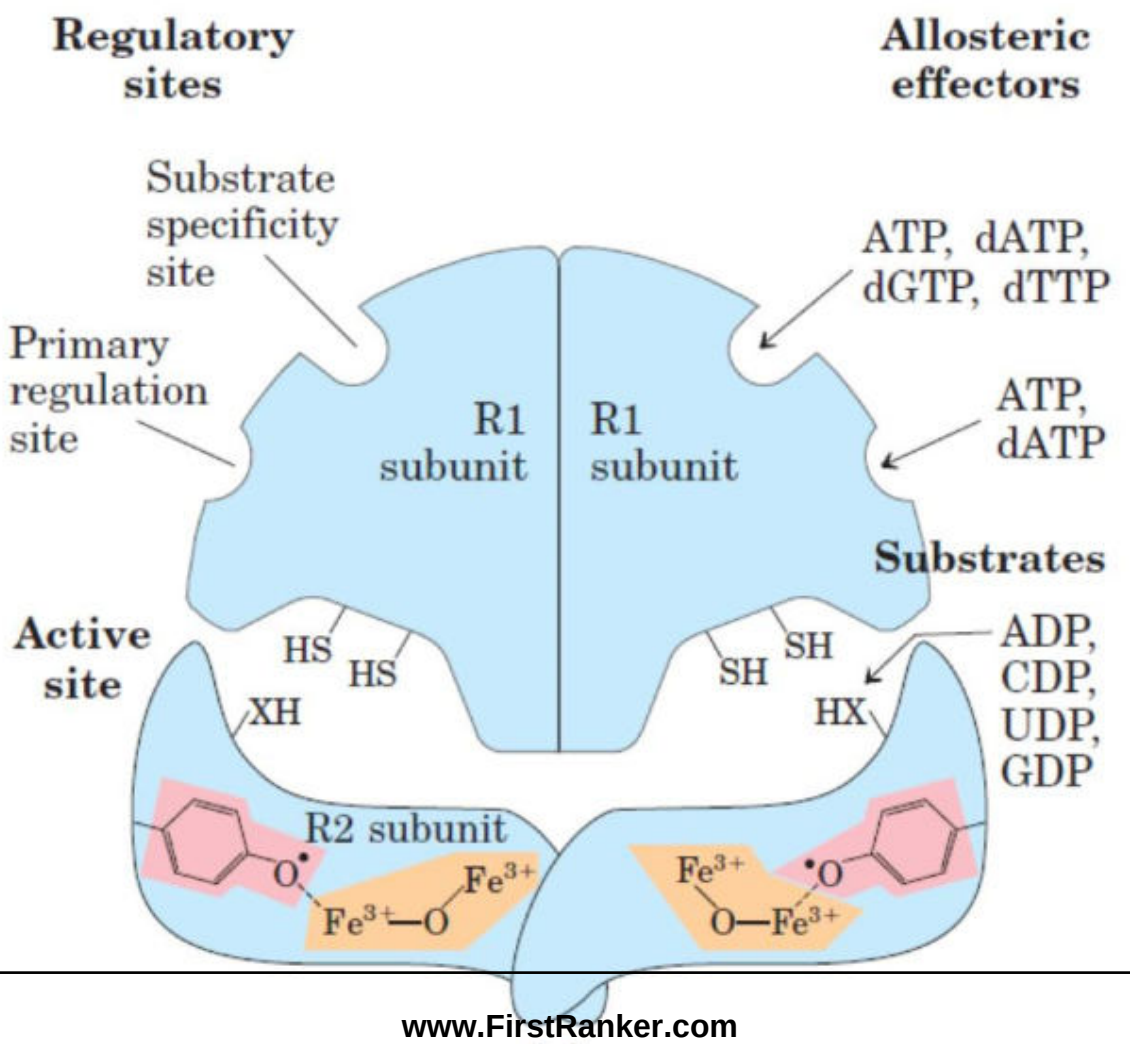


Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates

Base-specific nucleoside monophosphate kinases	
AMP + ATP ⇌ (Adenylate kinase)	2 ADP
GMP + ATP ⇌ (Guanylate kinase)	GDP + ADP
Nucleoside diphosphate kinase	
GDP + ATP ⇌	GTP + ADP
CDP + ATP ⇌	CTP + ADP

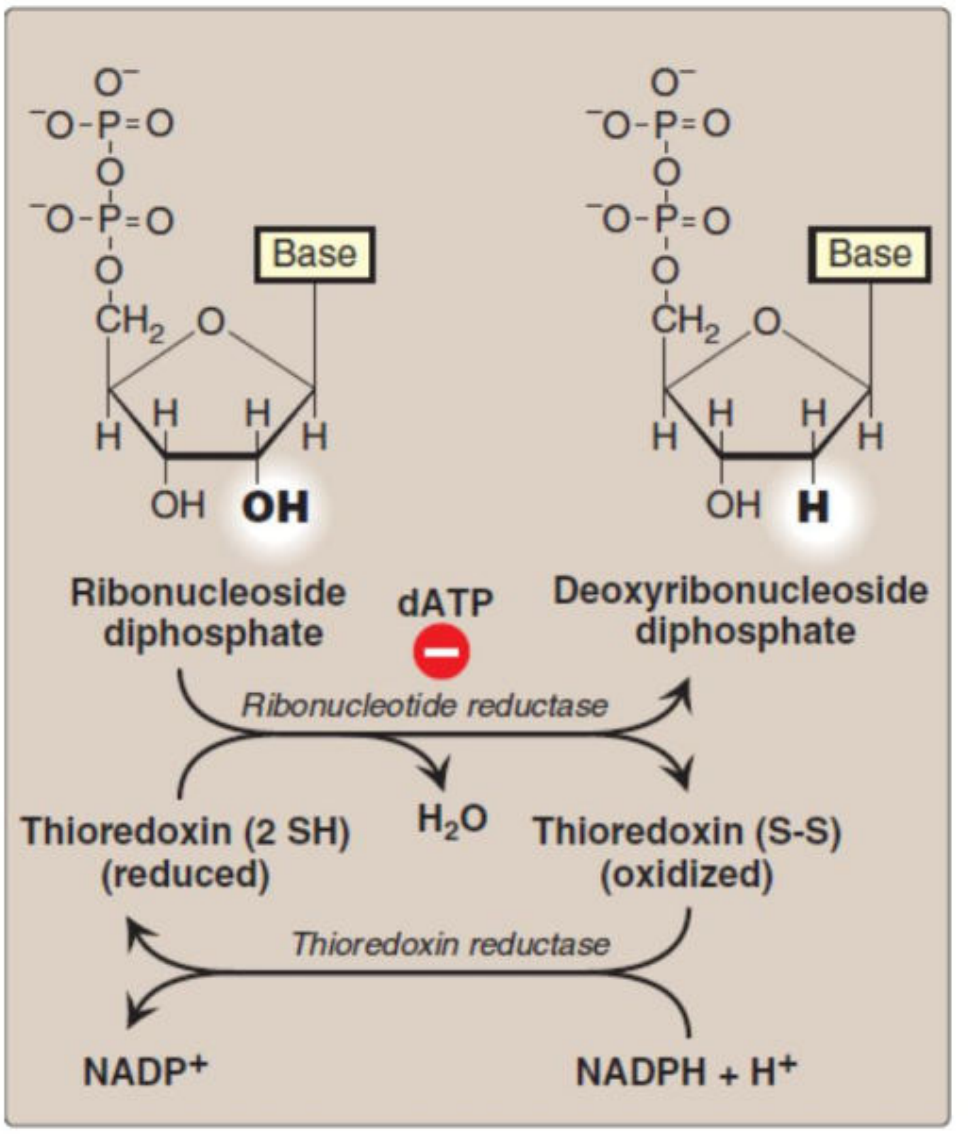
- **Conversion of ribonucleotides to deoxyribonucleotides**

Ribonucleotide reductase

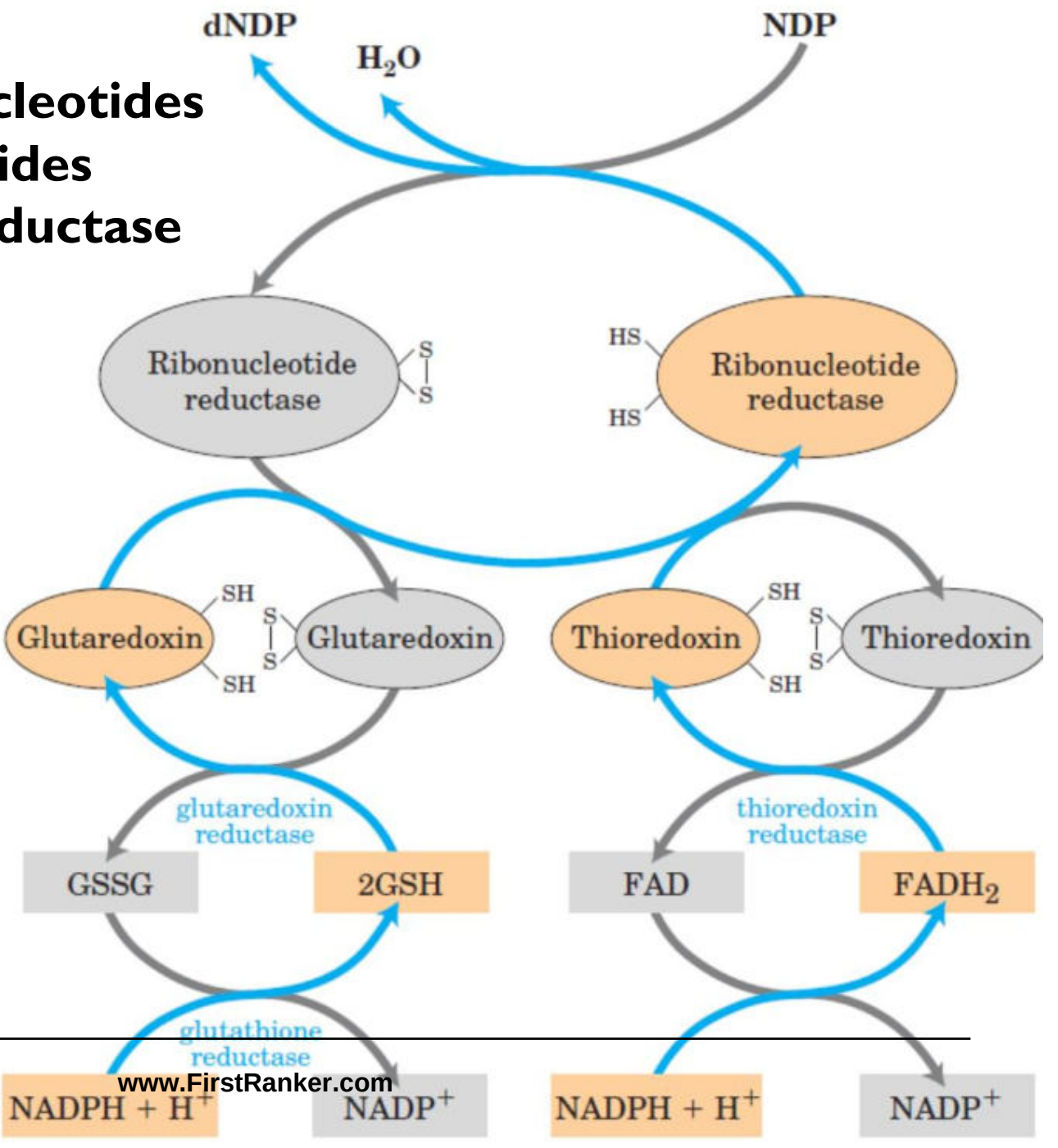


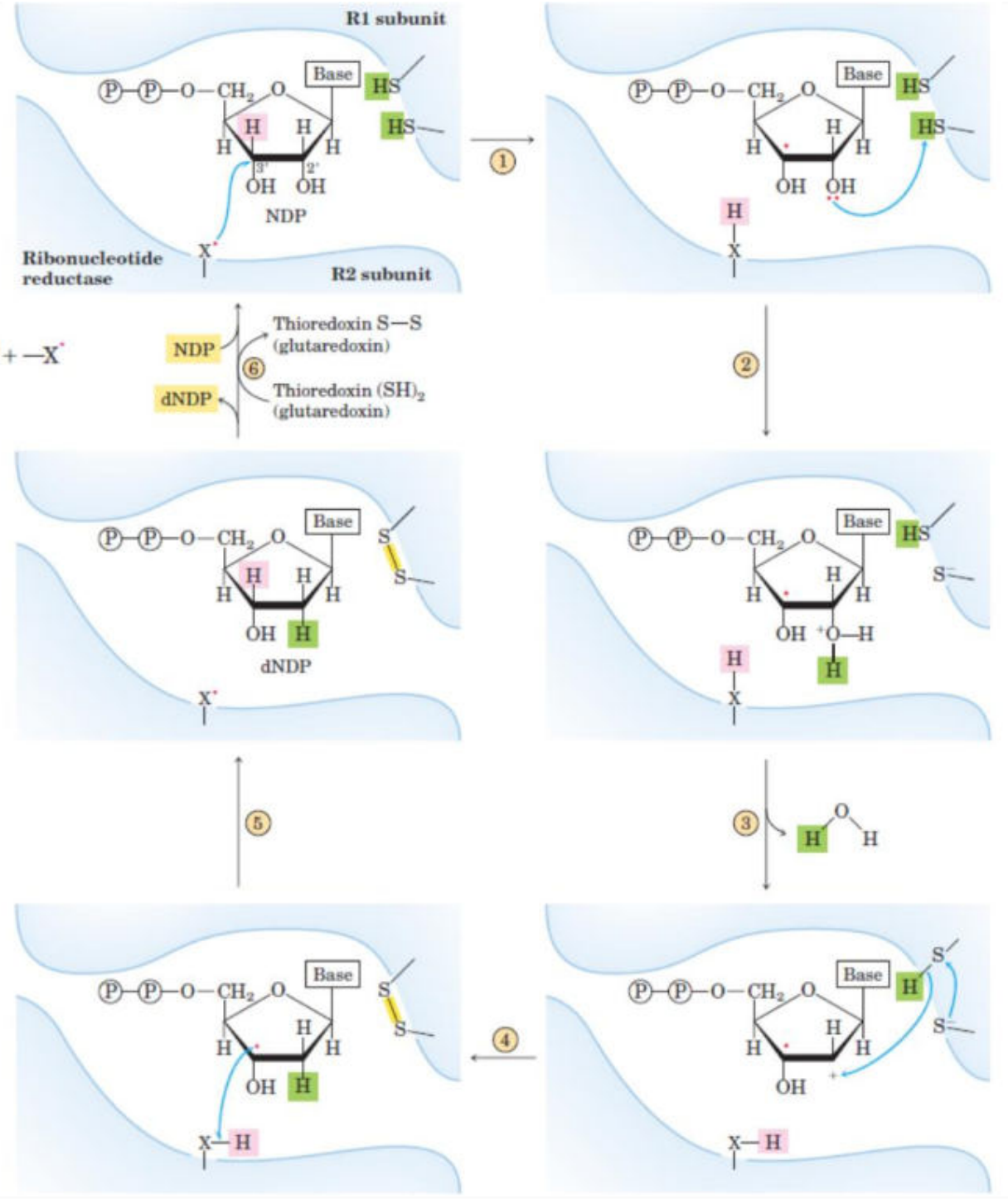
(a)

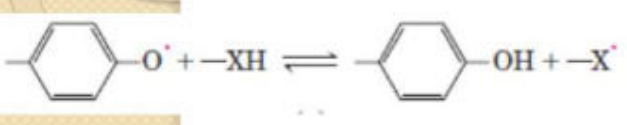
Conversion of ribonucleotides to deoxyribonucleotides



Reduction of ribonucleotides to deoxyribonucleotides by ribonucleotide reductase







Generation of active site radical —X˙

Mechanism for ribonucleotide reductase

mechanism for the ribonucleotide reductase reaction

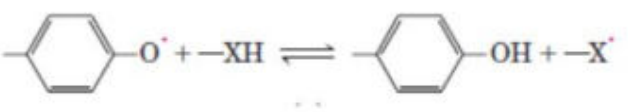
Step 1:The 3'-ribonucleotide radical formation

Step 2 and 3: cation formation at the 2' carbon after the loss of H2O

Step 4: Two one-electron transfers accompanied by oxidation of the dithiol reduce the radical cation

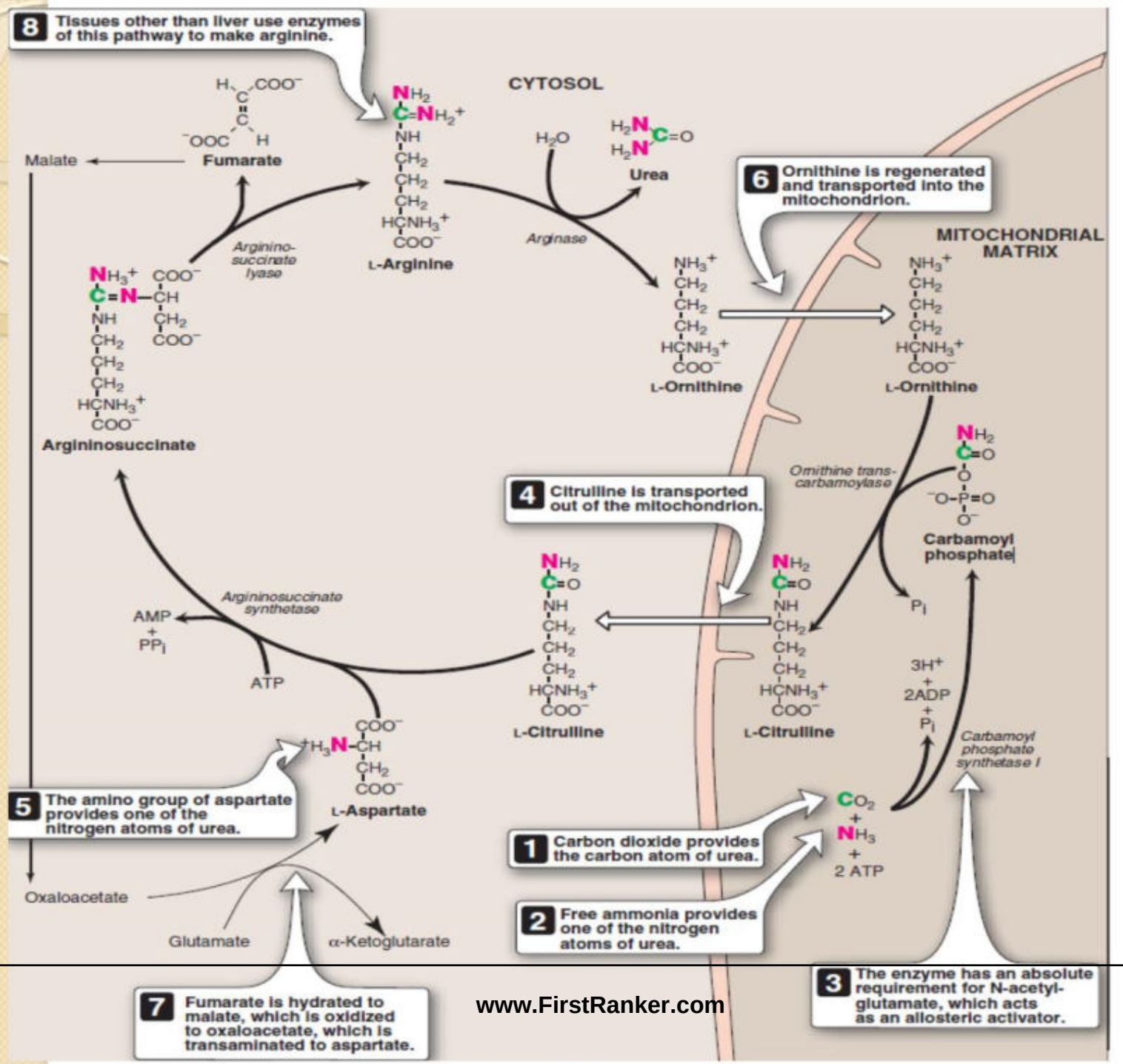
Step 5: (reverse of 1): regenerating the active site radical (ultimately, the tyrosyl radical) and forming the deoxy product

Step 6:The oxidized dithiol is reduced to complete the cycle

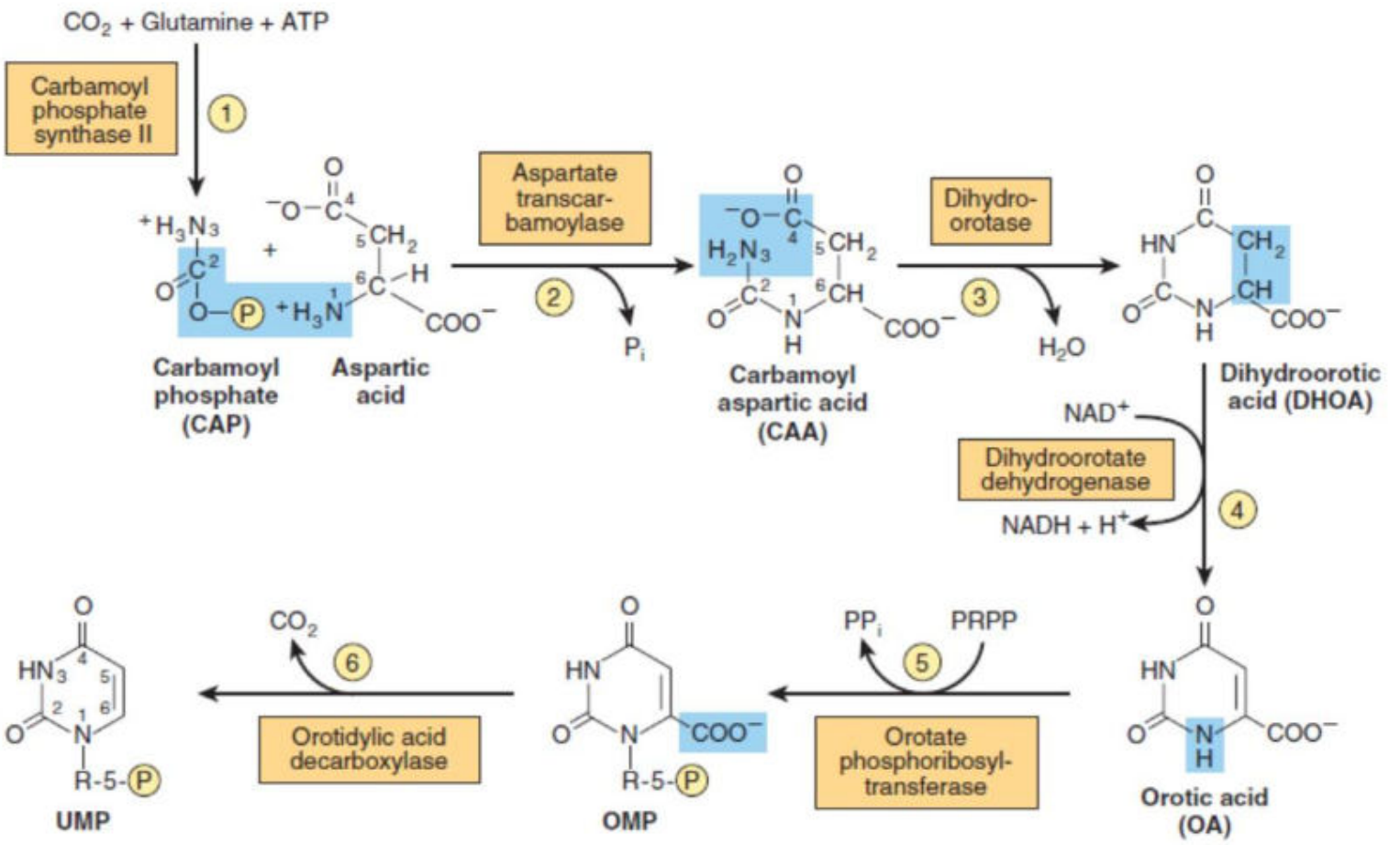


The tyrosyl radical functions to generate the active-site radical (OX), which is used in the mechanism

- **Pyrimidine Synthesis**



The biosynthetic pathway for pyrimidine nucleotides



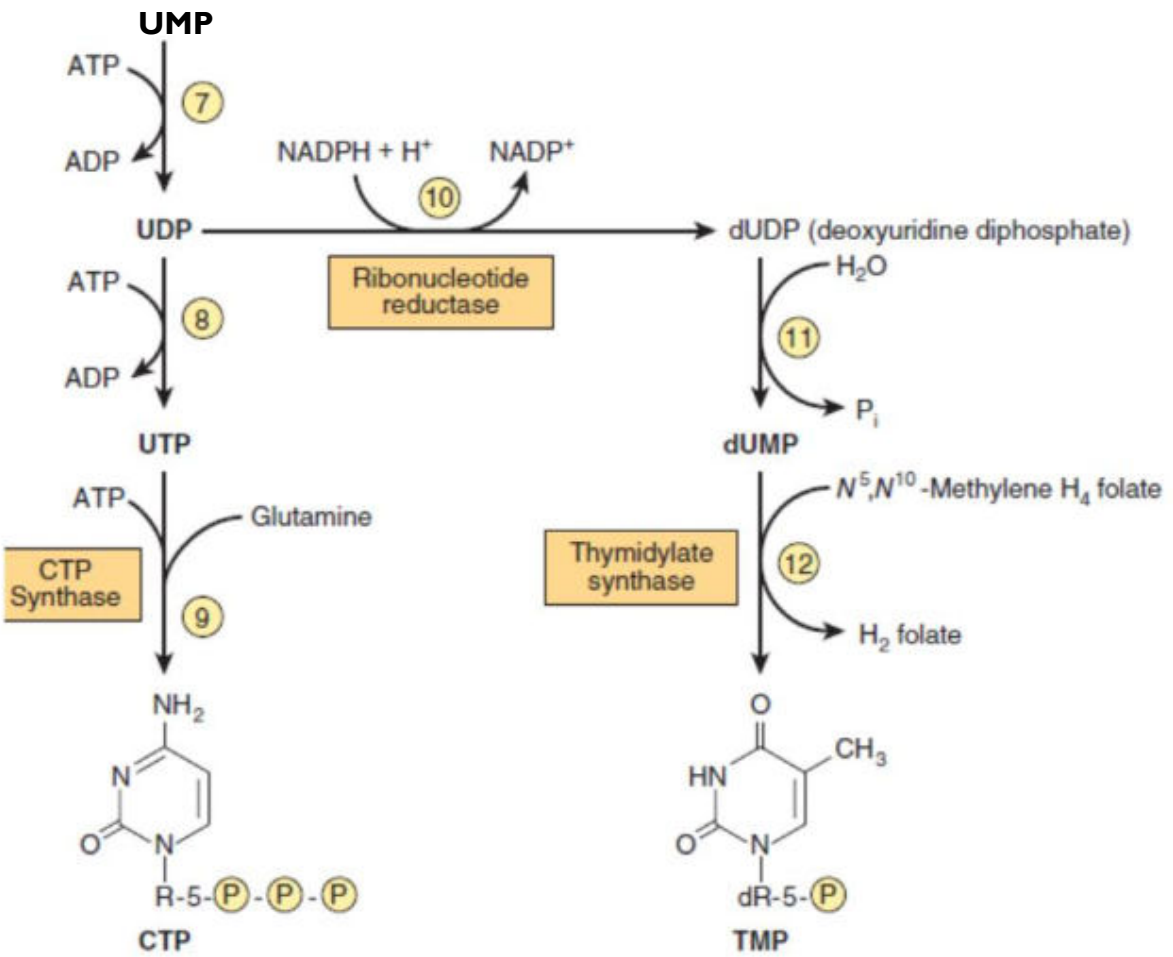
Orotic aciduria

Megaloblastic anaemia not responsive to Fe and folic acid

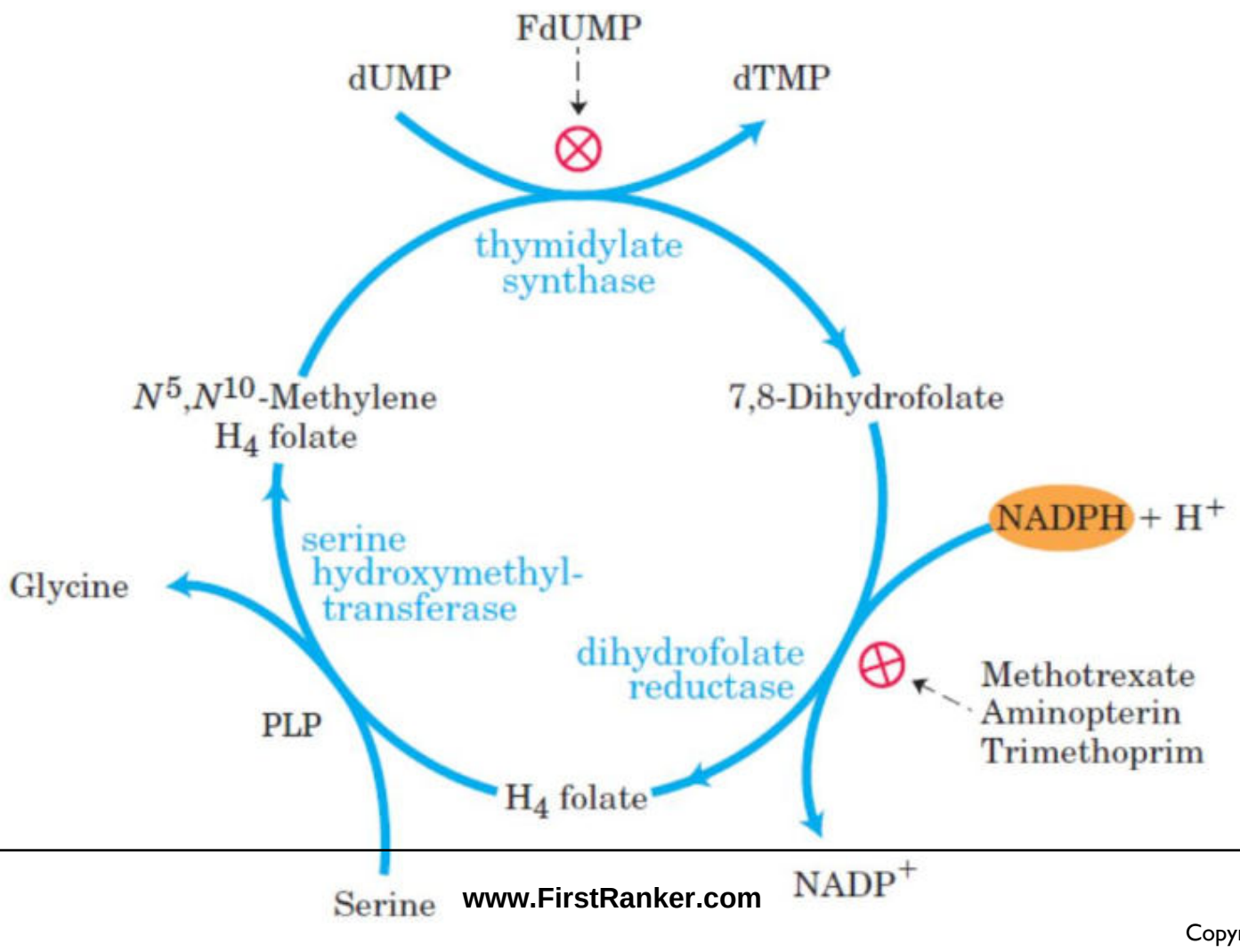
Deficiency of Orotate phosphoribosyl transferase

Deficiency of OMP decarboxylase

Typel
Typell



Thymidylate synthesis and folate metabolism as targets of chemotherapy.



Thymidylate Is Derived from dCDP and dUMP

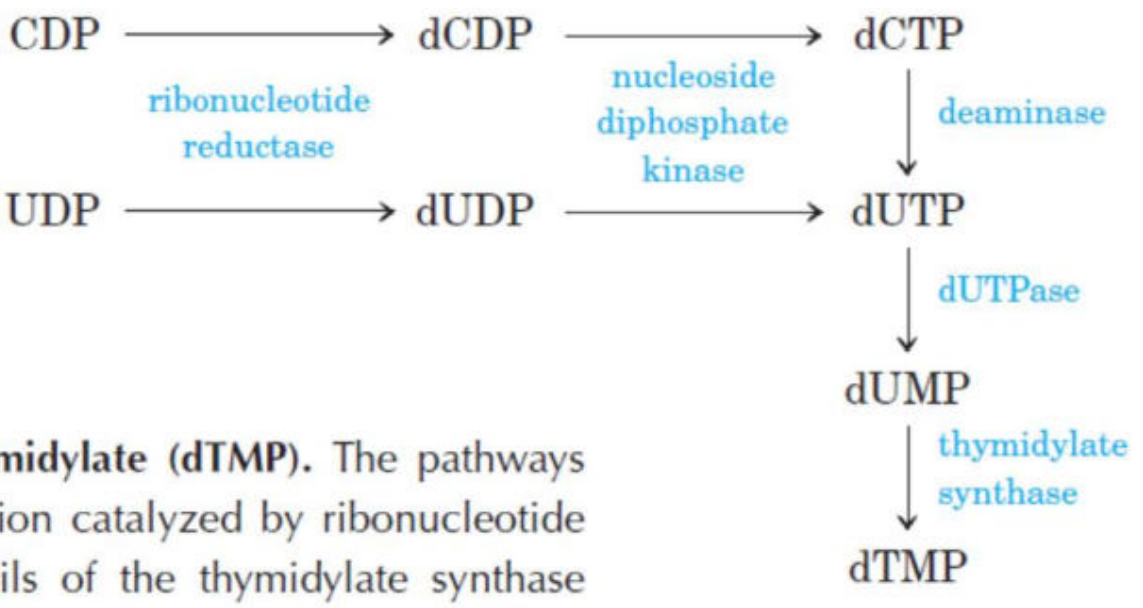


FIGURE 22–43 Biosynthesis of thymidylate (dTMP). The pathways are shown beginning with the reaction catalyzed by ribonucleotide reductase. Figure 22–44 gives details of the thymidylate synthase reaction.

Clinical Case 3

A 4 year old girl presented to the clinic with Megaloblastic anaemia, growth failure and mental retardation. History revealed that the child was born normally. The RBC count was 2.55 million /cmm and Hb was 6 g/dl, She was given antibiotics and transfusion. Despite that the anaemia worsened. There was no response following treatment with B12, Folic acid and , or Pyridoxine.

A prominent feature of the child's urine was a crystalline sediment, which was found to be orotic acid. Orotic acid in amounts as high as 1500 mg (9.6 mmol) was excreted daily (Normal 1.4mg/day, 9µmol).

- **What is the likely diagnosis?**
- **How would you make a definite diagnosis?**
- **What is the pathophysiology of this disorder?**
- **What could be the treatment?**

- Secondary Orotic aciduria?

- Ornithine transcarbamylase deficiency

MCQ

- Methotrexate a dihydrofolate reductase inhibitor used for the treatment of rapidly growing cancer, incorporation of which of the ring carbons in the generic purine structure would ~~**most likely**~~ be affected by methotrexate?
- A. 2
- B. 4
- C.6
- D.8

- Difference between De novo pathway and salvage pathway
- What is the Requirement of salvage pathway
  - Brain---Low levelof PRPP glutamyl transferase
  - RBC, PMN-lack 5 phospho ribosyl amine

Salvage pathway

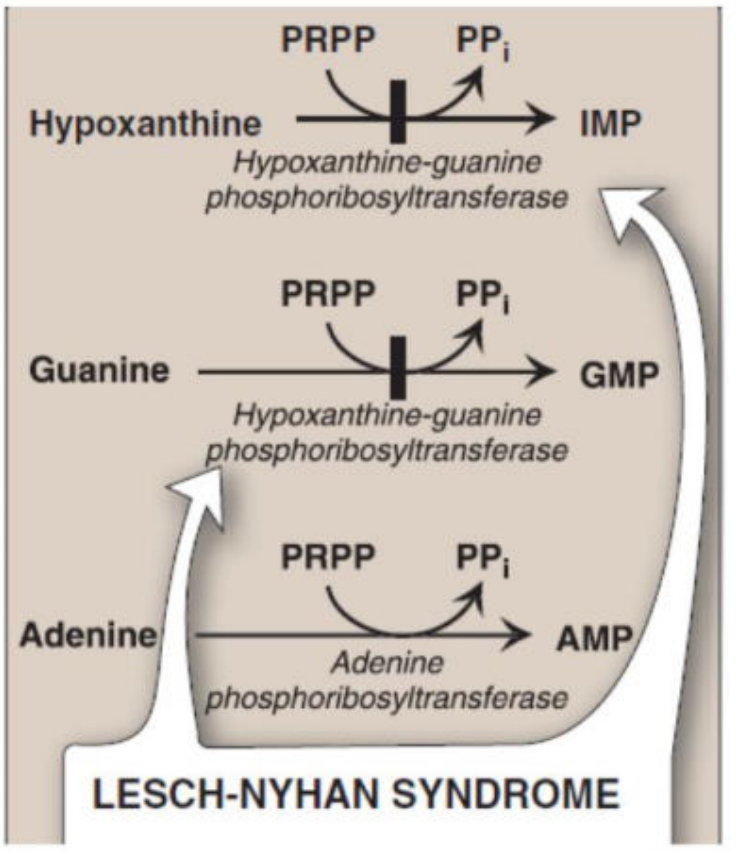
Adenosine and hypoxanthine-phosphoribosyl transferases

$$Pu + PR\text{-}PP \rightarrow Pu\text{-}RP + PPi$$

Adenosine kinase

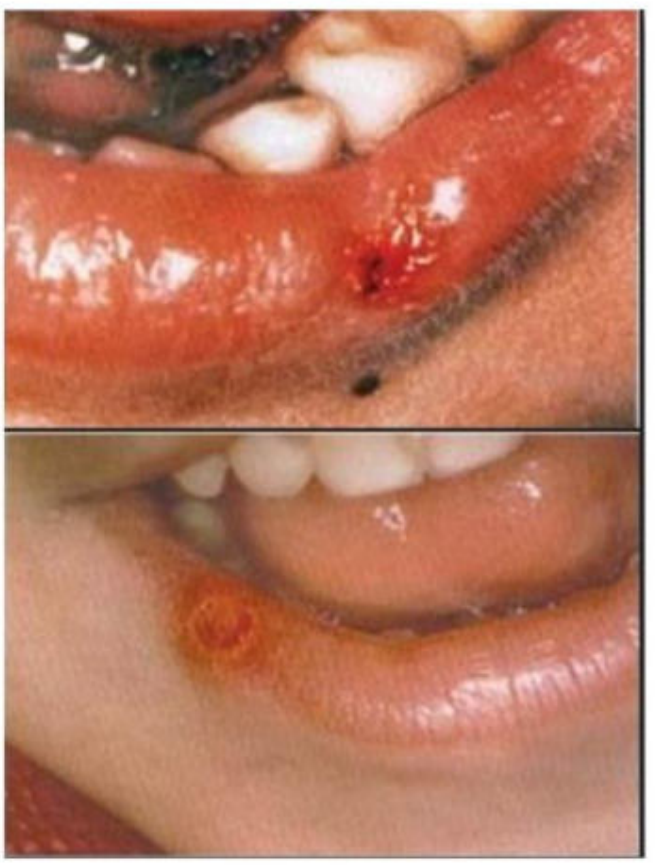
$$Pu\text{-}R + ATP \rightarrow PuR\text{-}P + ADP$$

Salvage pathways of purine nucleotide synthesis



Lesions on the lips of Lesch-Nyhan patients caused by self-mutilation.

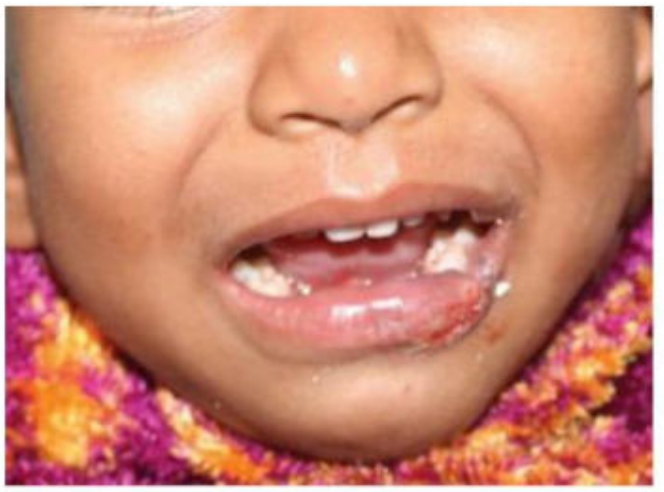
X linked recessive disorder
Gouty arthritis
Urolithiasis
Motor dysfunction
Cognitive defects
Behavioural disturbances



Clinical case

- A one-and-a-half-year-old male child born to a consanguineously married couple was brought to the Department of Pedodontics, Sinhgad Dental College, for dermatological assessment of failure to thrive and lacerations over lower lip, thumbs, and index finger due to self-biting (Fig. 1). After recording the history from the parents, it was revealed that the child had developed the habit of self-biting at 10 months of age. He was the only child born to the parents after a normal gestation/pregnancy. He was unable to hold his head yet. He measured 58 cm (50th centile 74.6 cm) in height, weighed 6.3 kg (50th centile 9.08 kg), and had occipitofrontal circumference of 44.9 cm (50th centile 45.3 cm). His bone age was consistent with his chronological age and mental age was 8 months. On examination, welldefined ulcers/lacerations with crusting and scarring at places involving the left thumb and left index finger were observed (Fig. 2). Also a gauze piece was seen rapped around the child's right thumb

, with the parents giving the history of ulcer wound healing spontaneously after bandaging but recurrence within a day after removal (Fig. 3). Nails of the involved fingers were dystrophic. A single, well-defined, deep ulcer with ragged margins and some scarring was present over the lower lip (Fig. 4). Chorea, hyperreflexia, and positive Babinski's sign were evident on neurological examination. All deep tendon reflexes were brisk and plantar reflex were extensor on either side. The systemic examination and laboratory investigations (blood cell counts, hepatorenal functions, urinalysis, and chest radiograph) were normal. The serum uric acid levels were 6.5 mg/dl (normal 1.7–5.8 mg/dl), and the urine uric acid: Creatinine ratio was 3:4 (normal 2:5–3:5).



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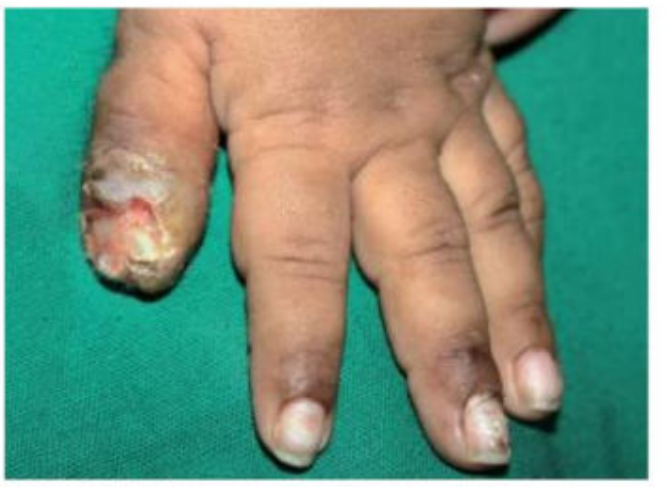


Fig. 2: Mutilated thumb of left hand

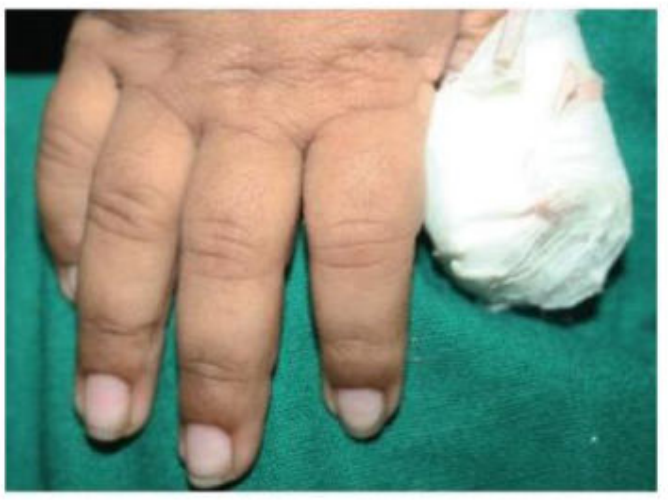


Fig. 3: Bandaged thumb on right hand

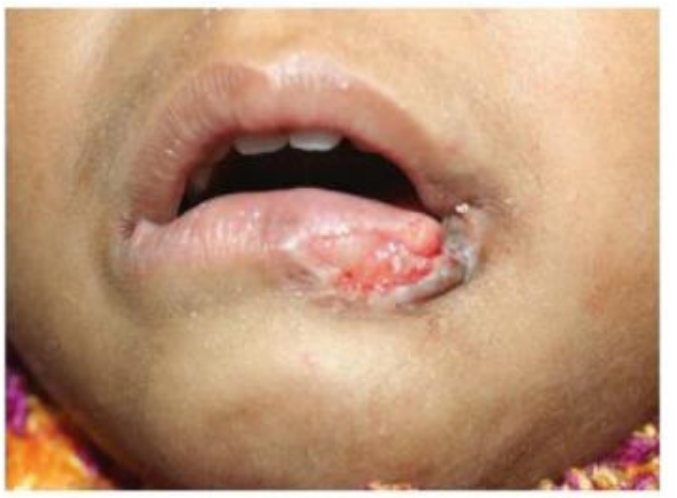


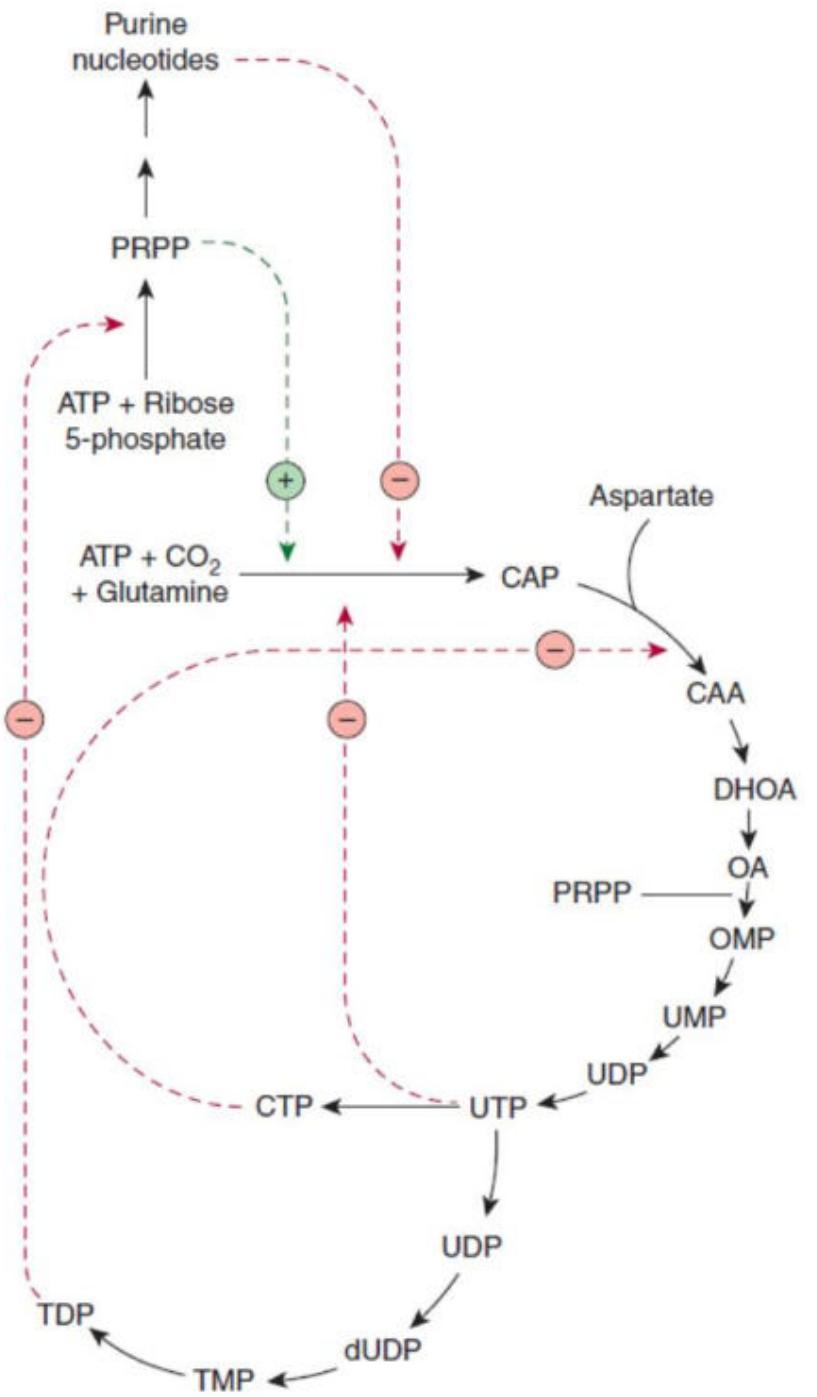
Fig. 4: Severely injured lower lip

- **What is the likely diagnosis?**
- **How would you make a definite diagnosis?**
- **What is the pathophysiology of this disorder?**

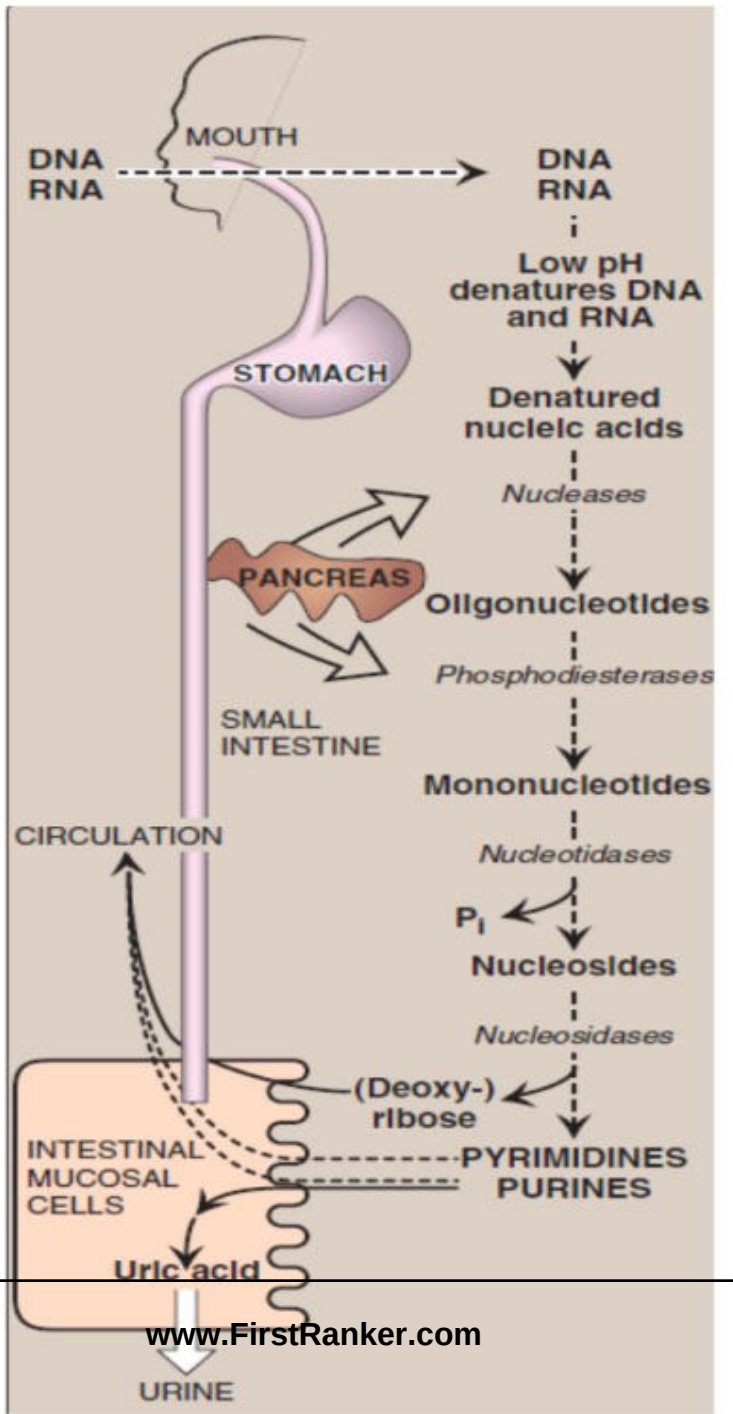
Causes of Hyperuricemia in LNS

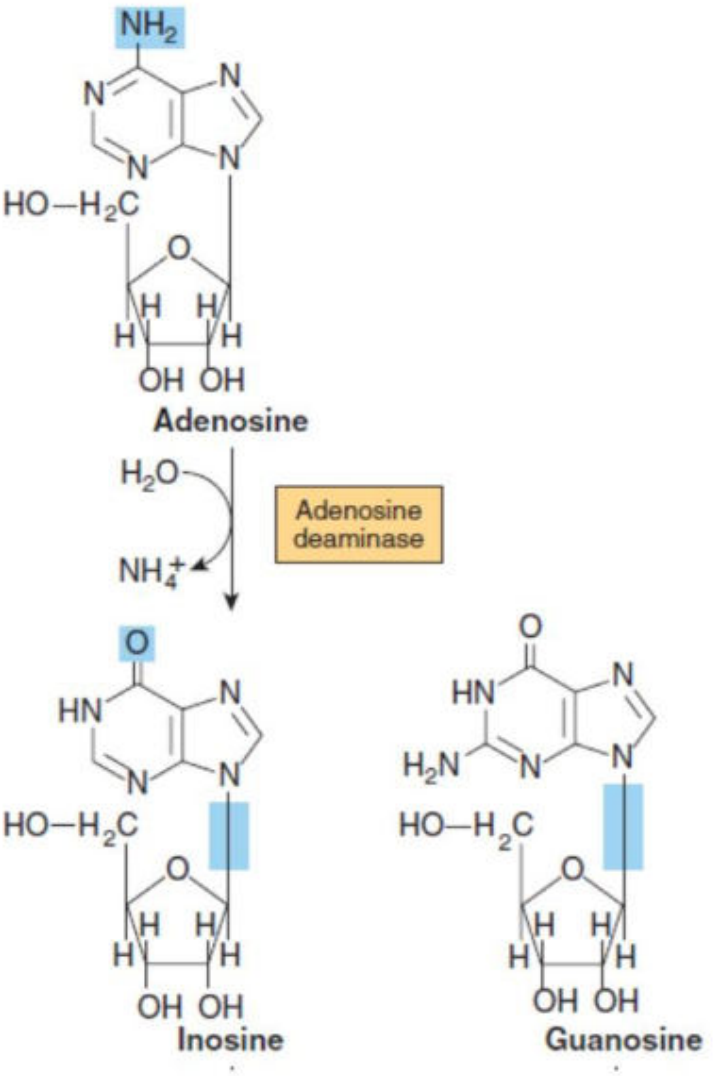
- Increased availability of PRPP
- Loss of feed back inhibition by AMP and GMP
- Increased degradation of Hypoxanthine and Guanine

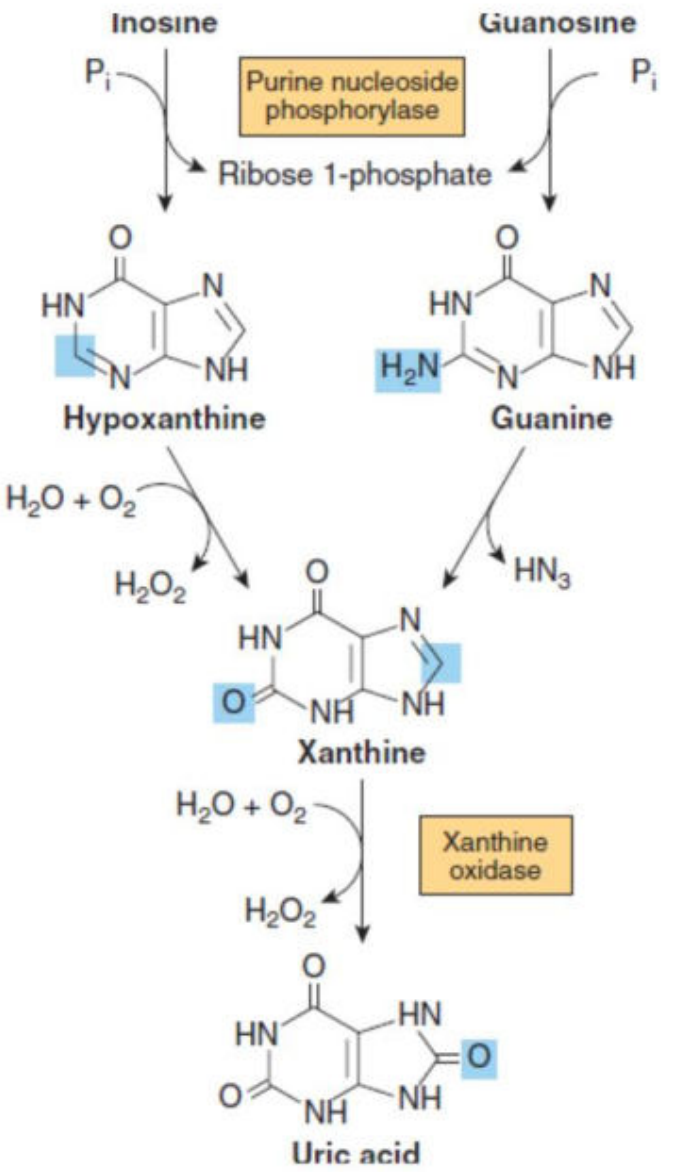
Regulatory aspects of the biosynthesis of purine and pyrimidine ribonucleotides

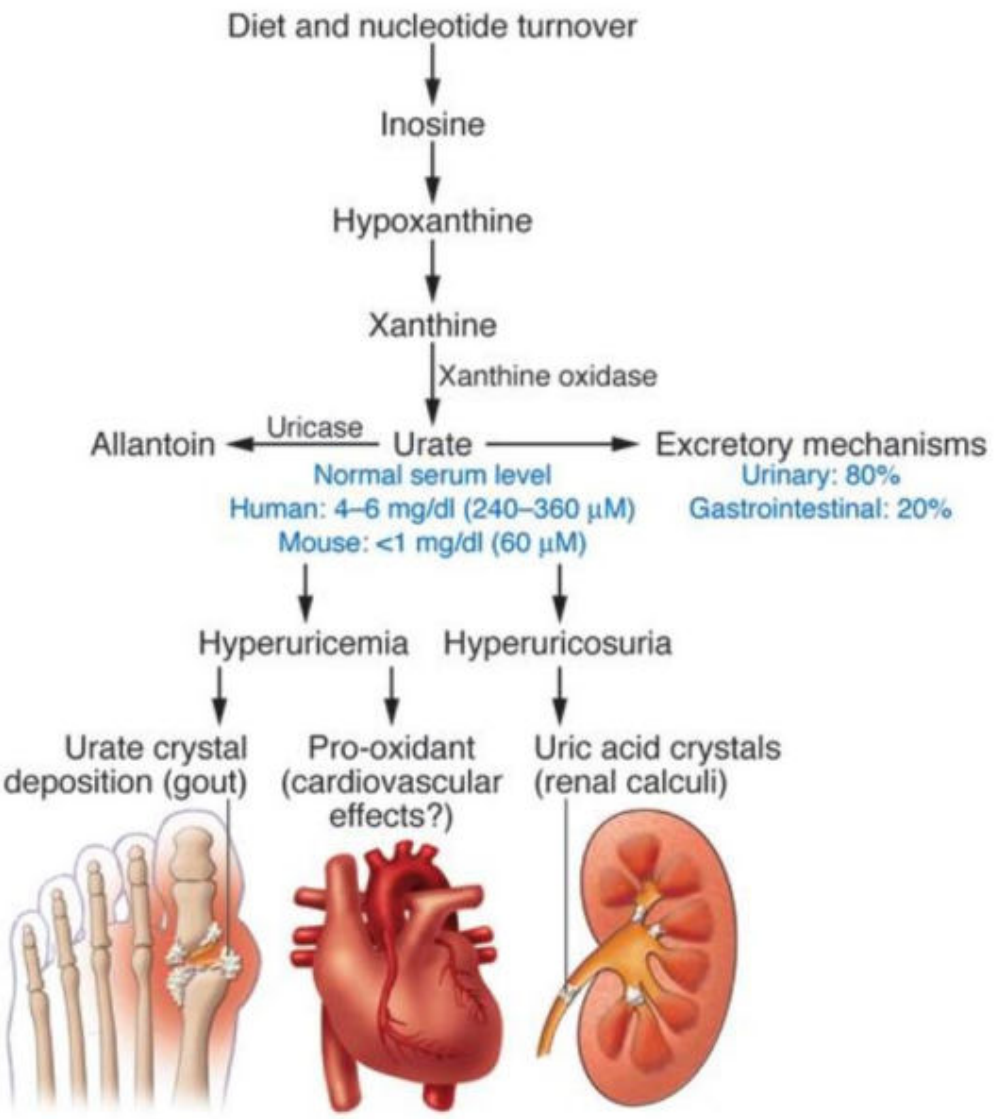


Digestion of dietary nucleic acids

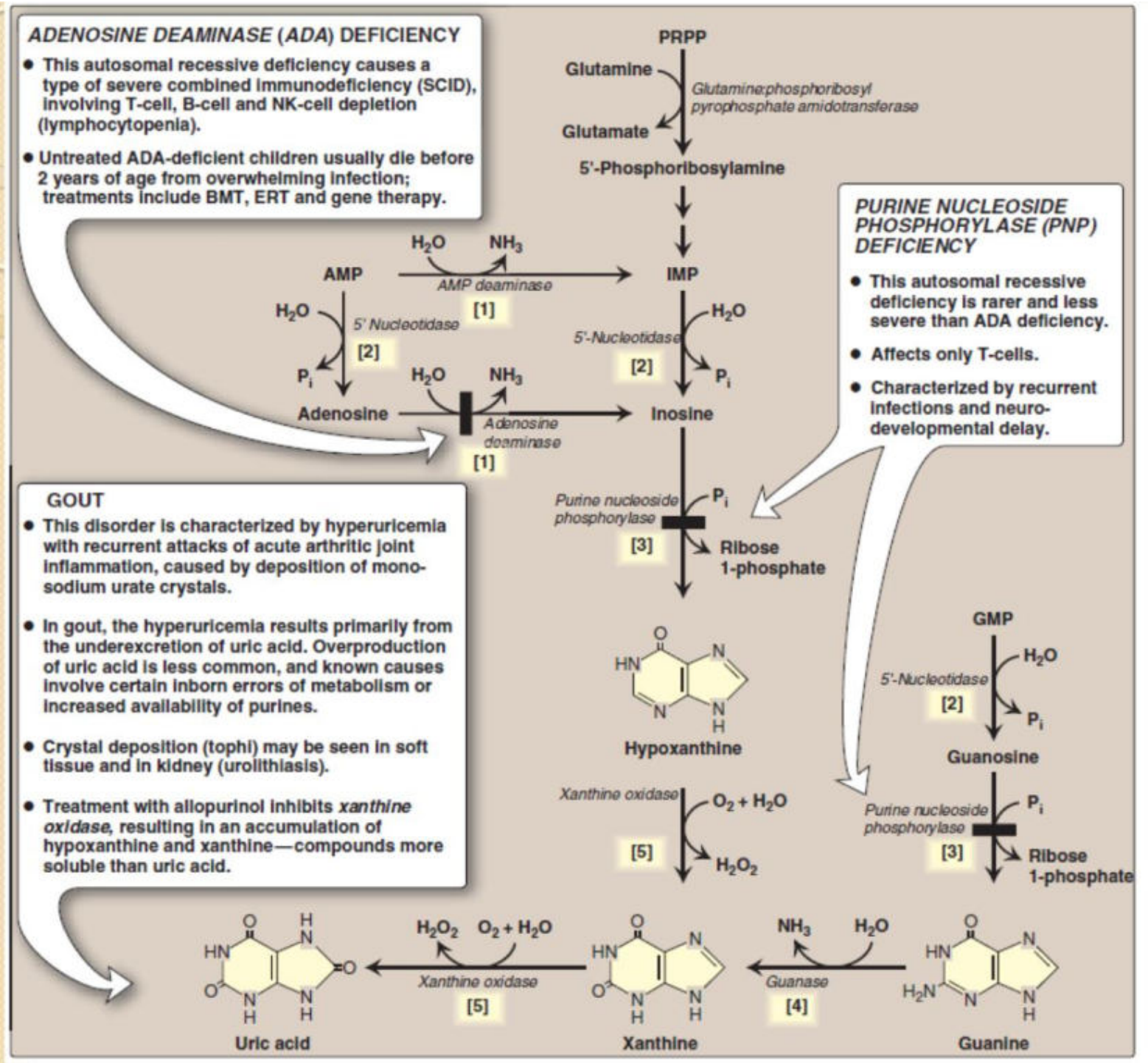




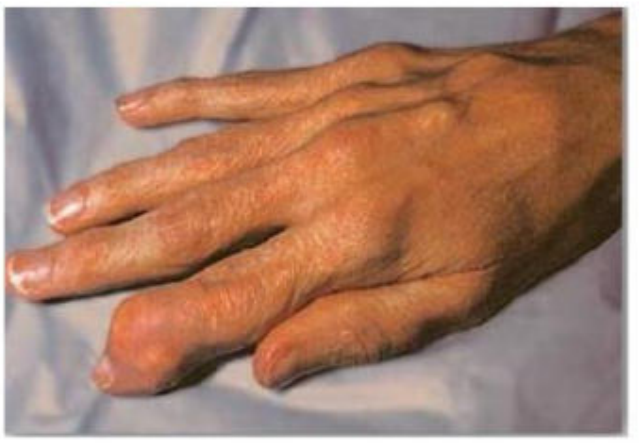




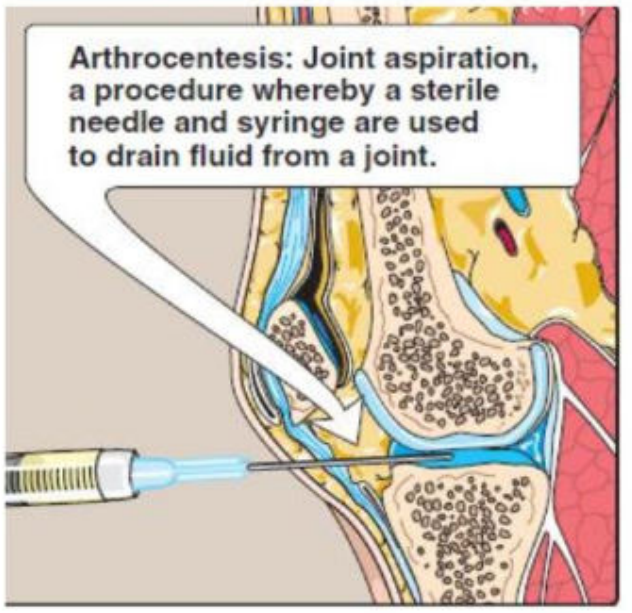
- **Clinical conditions**

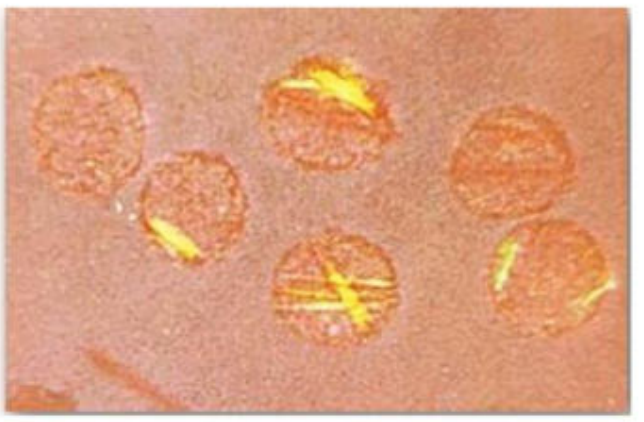


Tophaceous gout



Analysis of joint fluid





Negatively birefringent monosodium urate crystals within polymorphonuclear leukocytes in aspirated synovial fluid examined by **polarized-light microscopy**.

Causes of hyperuricemia

Under excretion

Defects in the excretory system
Lactic acidosis
Drugs
Lead poisoning—Basophilic stippling
---Saturnine gout

Over production

PRPP synthetase mutation
Leschnyhan syndrome
Myeloproliferative disorder
Von Gierkes disease
Fructose intolerance

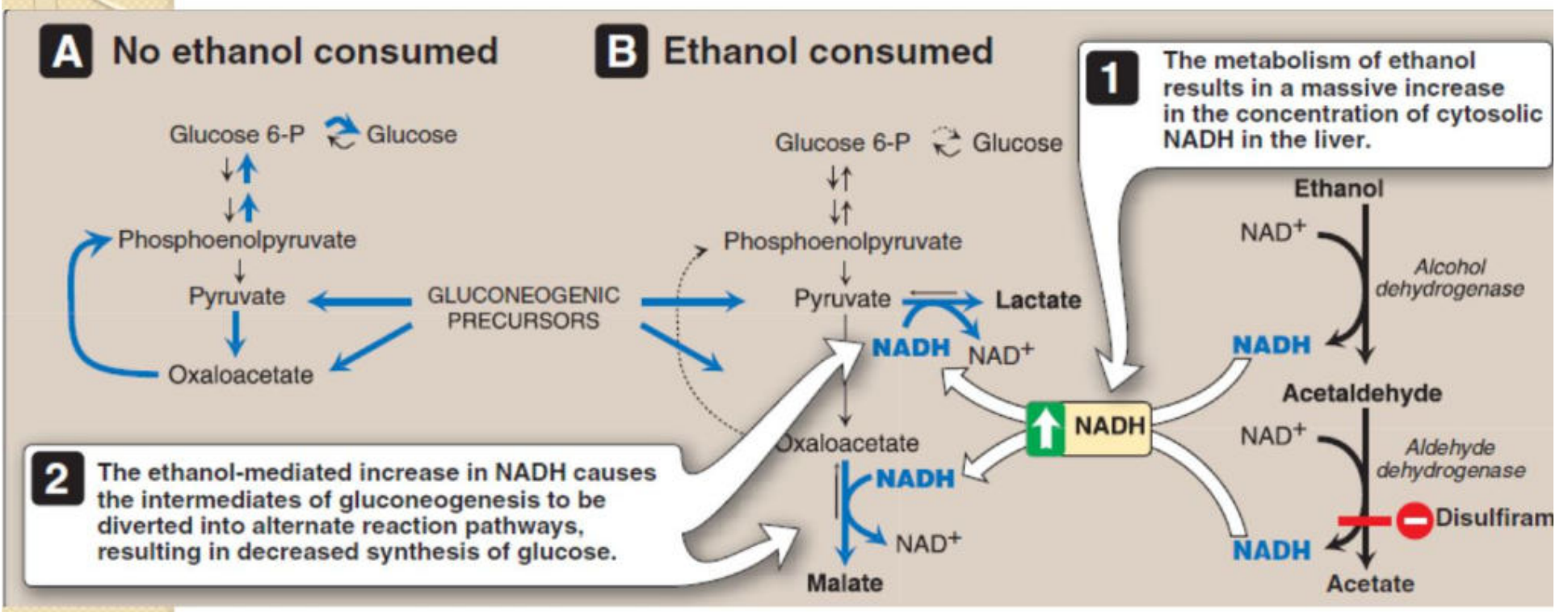
Clinical case-1

A 46-year-old male presents to the emergency department with severe right toe pain. The patient was in usual state of health until early in the morning when he woke up with severe pain in his right big toe. The patient denies any trauma to the toe and no previous history of such pain in other joints. He did say that he had a "few too many" beers with the guys last night. On examination, he was found to have a temperature of 38.2°C (100.8°F) and in moderate distress secondary to the pain in his right toe. The right big toe was swollen, warm, red, and exquisitely tender. The remainder of the examination was normal. Synovial fluid was obtained and revealed rod- or needle-shaped crystals that were negatively birefringent under polarizing microscopy.

- **What is the likely diagnosis?**
- **How would you make a definite diagnosis?**
- **What is the pathophysiology of this disorder?**

- Why Alcohol precipitates gout

Alcohol precipitates gout



Treatment of GOUT

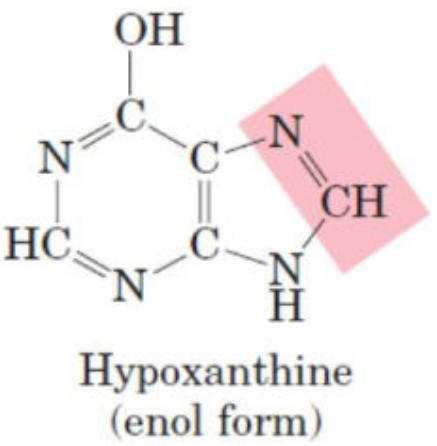
Acute attack

- Colchicine
- Steroids
- Indomethacin

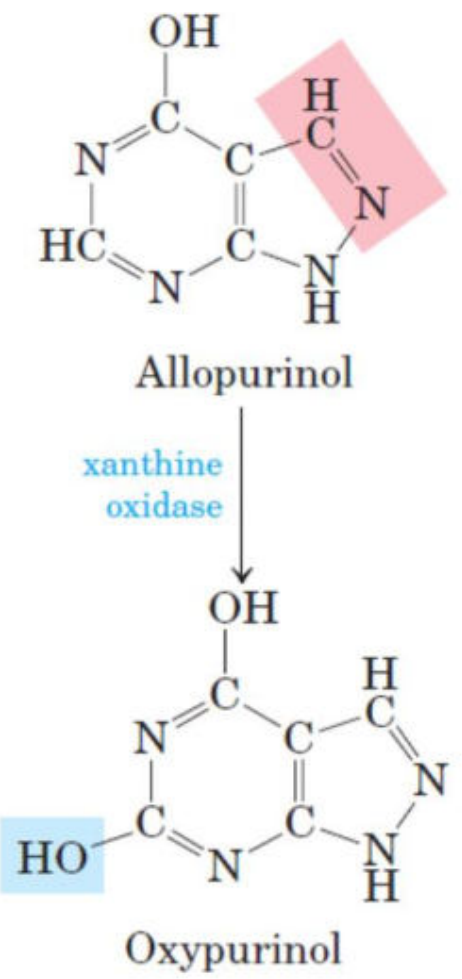
Longterm therapeutic strategy

- Allopurinol
- Febuxostat—Nonpurine inhibitor of Xanthine oxidase

Diet: Avoid diet rich in nucleic acid



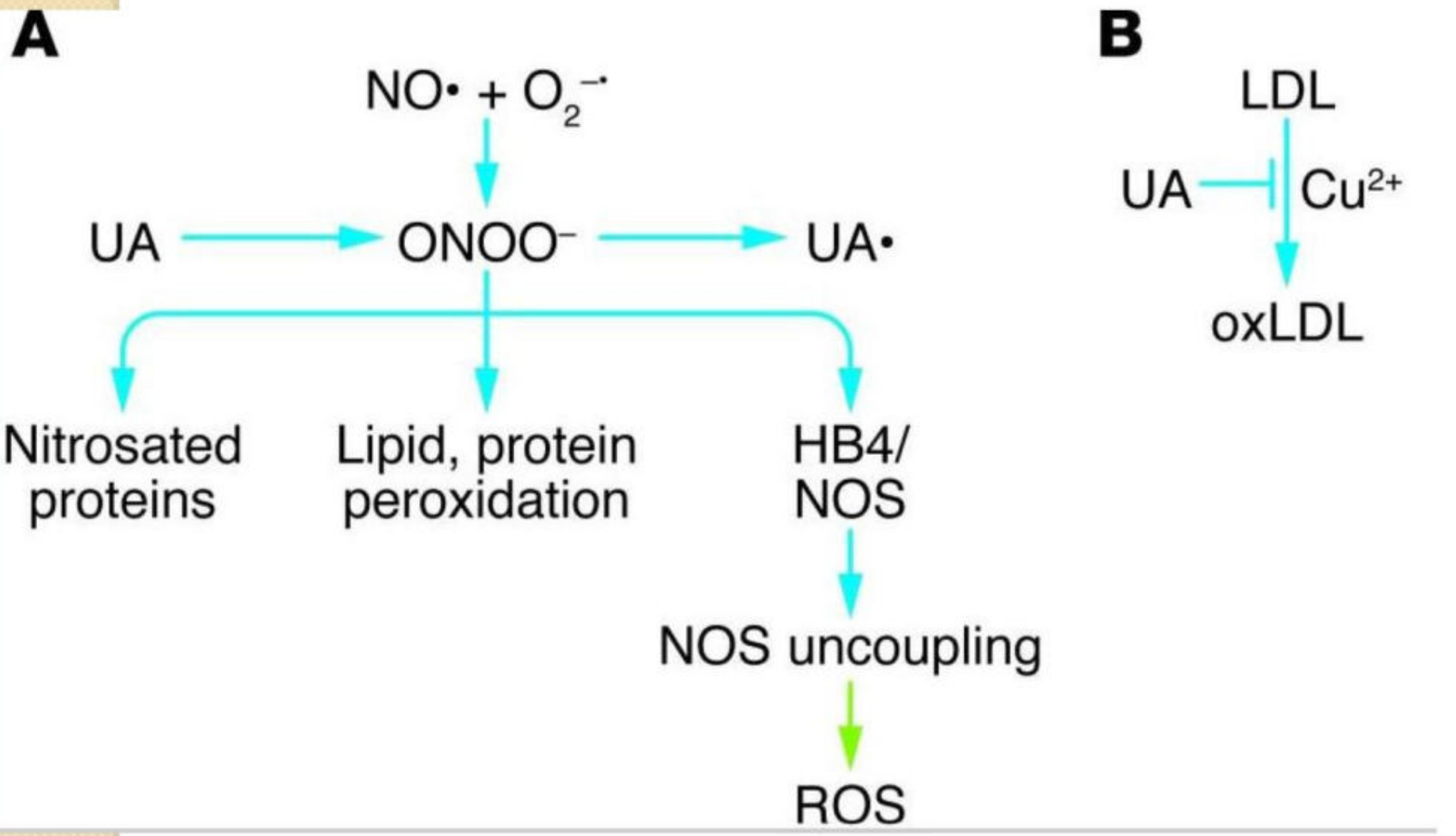
Hypoxanthine
(enol form)



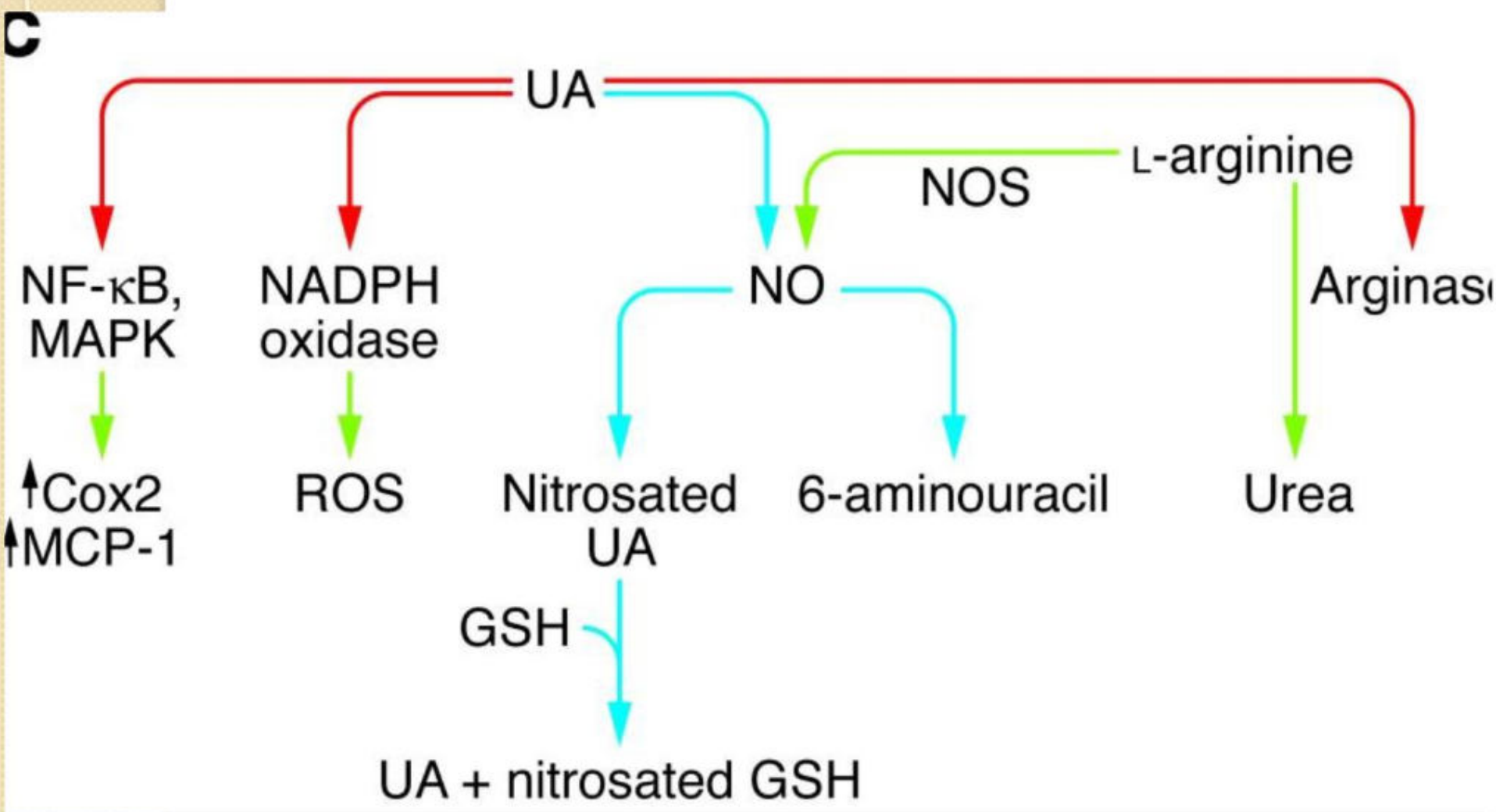
Oxypurinol

Allopurinol, an inhibitor of xanthine oxidase

Antioxidant effect of uric acid



Pro-oxidant effect of uric acid



What is pseudogout?

- Pseudogout is a type of inflammation of joints (arthritis) that is caused by deposits of crystals, called calcium pyrophosphate, in and around the joints. Pseudogout literally means "false gout." It derives its name from its similarity to gout.
- Pseudogout is clearly related to aging
- is associated with degenerative arthritis.
- Acute attacks of the arthritis of pseudogout can be caused by dehydration
- Pseudogout can also be caused by the hormonal effects on calcium metabolism from hyperparathyroidism.
- abnormal calcifications seen in the cartilage of joints on X-rays
- weakly **positively** birefringent rhomboid crystals under polarizing microscope

Adenosine deaminase and Purine nucleoside phosphorylase deficiencey

Toxic effects due to

2’ dATP

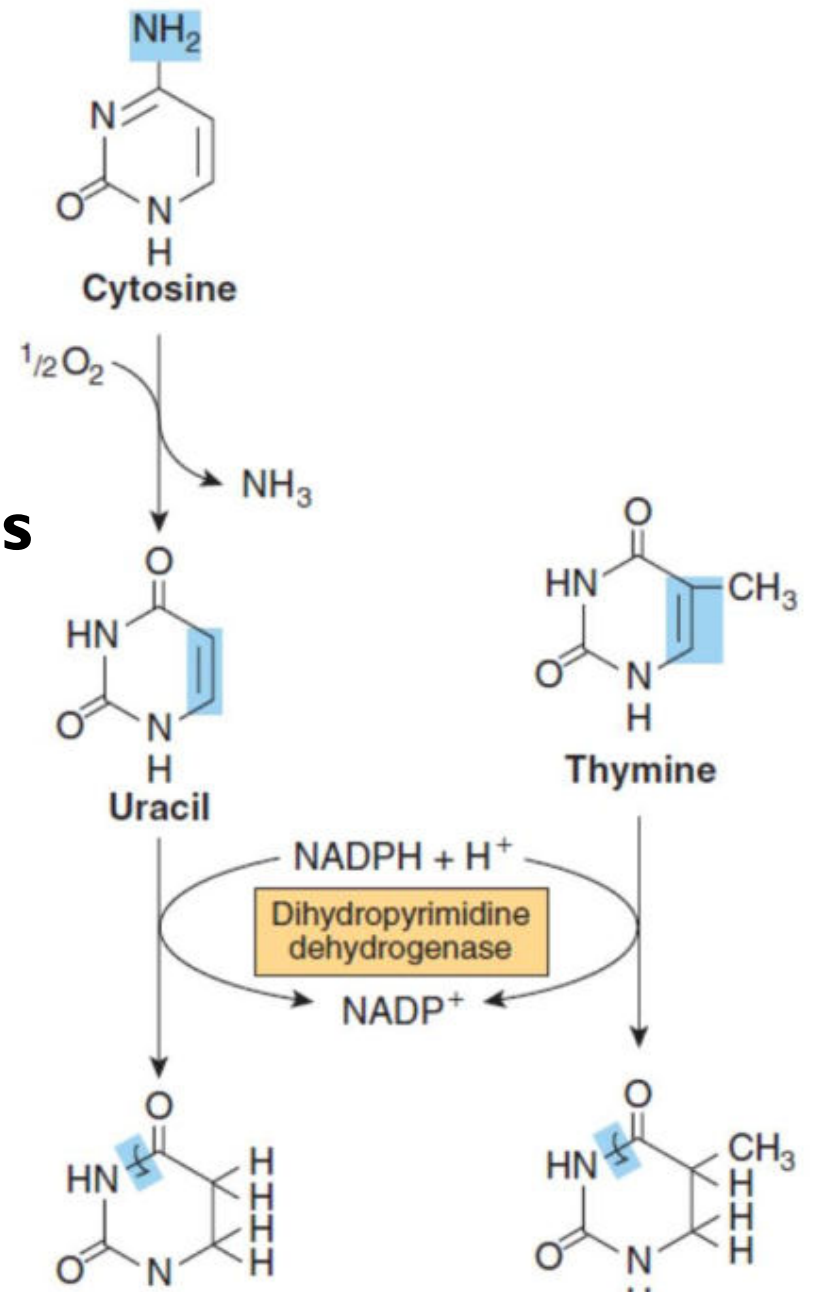
S adenosyl homocystein

Treatment

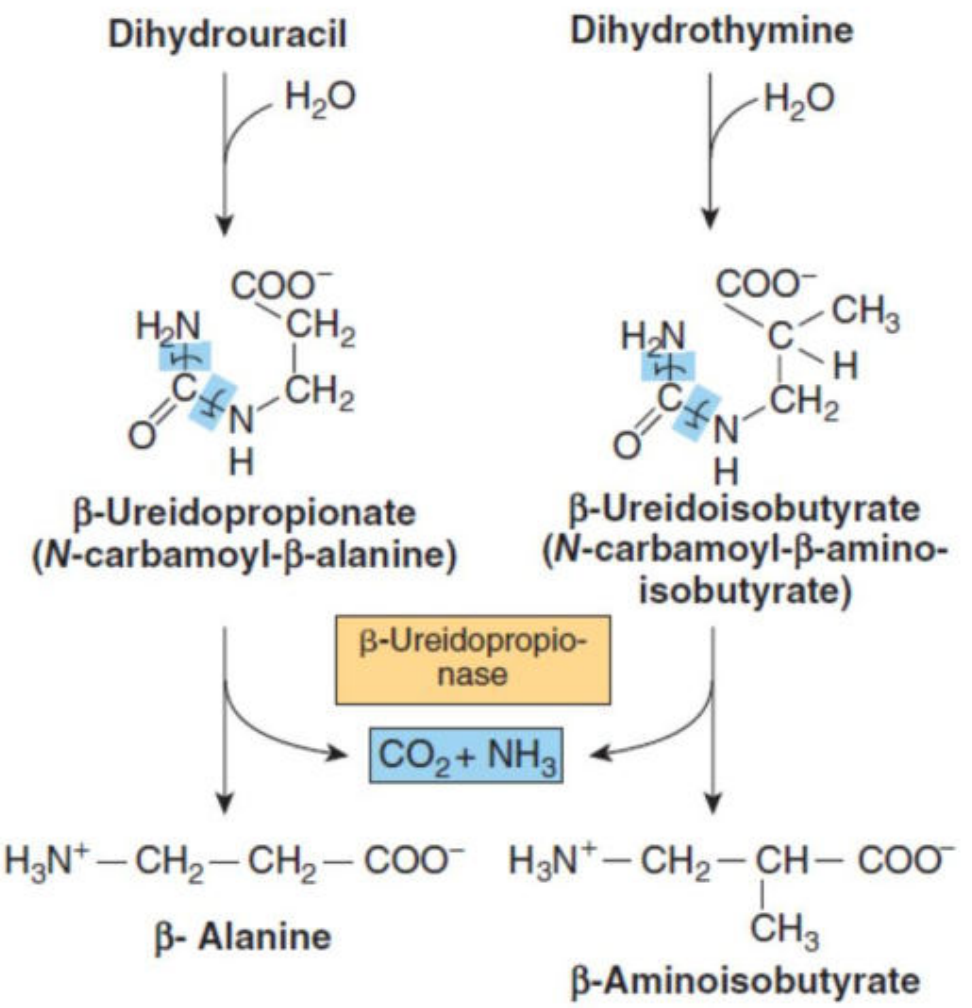
Bonemarrow transplantattion

Enzyme replacement therapy

Catabolism of pyrimidines



Catabolism of pyrimidines contd



Summary

- Purines and pyrimidines are formed from amphibolic intermediates and thus are dietarily nonessential.
- Ingested nucleic acids are degraded to purines and pyrimidines
- Several reactions of IMP biosynthesis require folate derivatives and glutamine. Consequently, antifolate drugs and glutamine analogs inhibit purine biosynthesis.
- IMP is a precursor both of AMP and of GMP. Glutamine provides the 2-amino group of GMP, and aspartate the 6-amino group of AMP.
- Phosphoryl transfer from ATP converts AMP and GMP to ADP and GDP. A second phosphoryl transfer from ATP forms GTP, but ADP is converted to ATP primarily by oxidative phosphorylation.

Summary contd

- Hepatic purine nucleotide biosynthesis is stringently regulated by the pool size of PRPP and by feedback inhibition of PRPP glutamyl amidotransferase by AMP and GMP
- Coordinated regulation of purine and pyrimidine nucleotide biosynthesis ensures their presence in proportions appropriate for nucleic acid biosynthesis and other metabolic needs
- Humans catabolize purines to uric acid (p*Ka 5.8), present as* the relatively insoluble acid at acidic pH or as its more soluble sodium urate salt at a pH near neutrality.
- Urate crystals are diagnostic of gout. Other disorders of purine catabolism include Lesch-Nyhan syndrome, von Gierke disease, and hypouricemias
- Since pyrimidine catabolites are water-soluble, their overproduction does not result in clinical abnormalities
- Excretion of pyrimidine precursors can, however, result from a deficiency of ornithine transcarbamoylase because excess carbamoyl phosphate is available for pyrimidine biosynthesis.

MCQ 1

- A 42-year-old male patient undergoing radiation therapy for prostate cancer develops severe pain in the metatarsal phalangeal joint of his right big toe. Monosodium urate crystals are detected by polarized light microscopy in fluid obtained from this joint by arthrocentesis. Uric acid crystals are present in his urine. This patient's pain is directly caused by the overproduction of the end product of which of the following metabolic pathways?

- A. De novo pyrimidine biosynthesis.
- B. Pyrimidine degradation.
- C. De novo purine biosynthesis.
- D. Purine salvage.
- E. Purine degradation

MCQ 2

- A 1-year-old female patient is lethargic, weak, and anemic. Her height and weight are both low for her age. Her urine contains an elevated level of orotic acid. The administration of which of the following compounds is most likely to alleviate her symptoms?

- A. Adenine.
- B. Guanine.
- C. Hypoxanthine.
- D. Thymidine.
- E. Uridine.

MCQ 3

- The rate of DNA synthesis in a culture of cells could be most accurately determined by measuring the incorporation of which of the following radiolabeled compounds?

- A. Adenine.
- B. Guanine.
- C. Phosphate.
- D. Thymidine.

- .

MCQ 4

A 44-year-old woman who recently lost her job because of absenteeism, presents to her physician complaining of loss of appetite, fatigue, muscle weakness, and emotional depression. The physical examination reveals a somewhat enlarged liver that feels firm and nodular, and there is a hint of jaundice in the sclerae and a hint of alcohol on her breath. The initial laboratory profile included a hematological analysis that showed that she had an anemia with enlarged red blood cells (macrocytic). A bone marrow aspirate confirmed the suspicion that she has a megaloblastic anemia because it showed a greater than normal number of red and white blood cell precursors, most of which were larger than normal. Further analyses revealed that her serum folic acid level was 2.9 ng/mL (normal = 6 to 15), her serum B12 level was 153 pg/mL (normal = 150 to 750), and her serum iron level was normal.

The patient's megaloblastic anemia is most likely caused by which of the following?

- A.A decreased synthesis of methionine
- B.A decreased conversion of dUMP to dTMP
- C.A decrease in the synthesis of phosphatidyl choline
- D.A decrease in the levels of succinyl CoA
- E.A decreased synthesis of dUTP

MCQ 5

Leukemia patients are often given the compound Leucovorin (N5-formyl THF) following treatment with the drug methotrexate. Why is Leucovorin useful as part of this treatment protocol?

- A. It facilitates the uptake of methotrexate by cells
- B. It can be converted to THF by bypassing DHFR
- C. It acts as an activator of thymidylate synthase
- D. It prevents the uptake of methotrexate by normal cells
- E. It stimulates cells of the immune system

- What laboratory test would help in distinguishing an orotic aciduria caused by ornithine transcarbamylase deficiency from that caused by UMP synthase deficiency?