37

Bio chemistry

Doctor 2017 | Medicine | JU

Sheet ○ Slides

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DOCTOR

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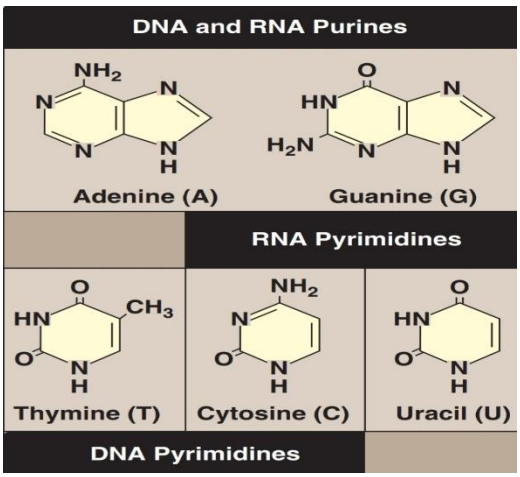
Nucleotides Metabolism

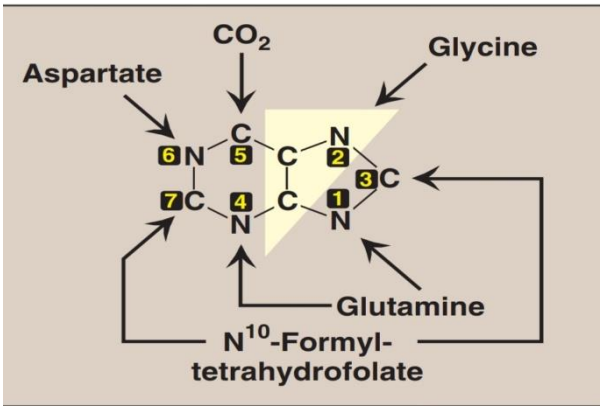


- Purines and Pyrimidines are two families of nitrogen-containing bases.
- Nucleoside is a nitrogenous base (purine or pyrimidine) bound to a pentose sugar.

- Nucleotide is composed of a nitrogenous base, a pentose monosaccharide, and one, two, or three phosphate groups. (More details about their structure and roles are found in sheet 35).
- Don't memorize all names of intermediates and enzymes from the figures ! The important ones are mentioned here in the steps or in the clinical applications.

SYNTHESIS OF PURINE NUCLEOTIDES

- The atoms of the purine ring are contributed by a number of compounds, including: *Amino acids: aspartic acid, glycine, and glutamine, CO2, N10-formyltetrahydrofolate.*
- The purine ring is constructed primarily in the *liver* by a series of reactions that *add the donated carbons and nitrogens to a preformed ribose 5-phosphate.*



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

I. PRPP is an "activated pentose" that participates in the *synthesis* and *salvage* of *purines* and *pyrimidines*. It gives the cell a signal to start synthesis reactions.

II. Synthesis of PRPP from ATP and *ribose 5-phosphate* is catalyzed by *PRPP synthetase* (*ribose phosphate pyrophosphokinase* because it adds a pyrophosphate group on carbon 1 of the ribose 5-phosphate).

➔ This X-linked enzyme is activated by inorganic phosphate and inhibited by purine nucleotide (end-product inhibition).

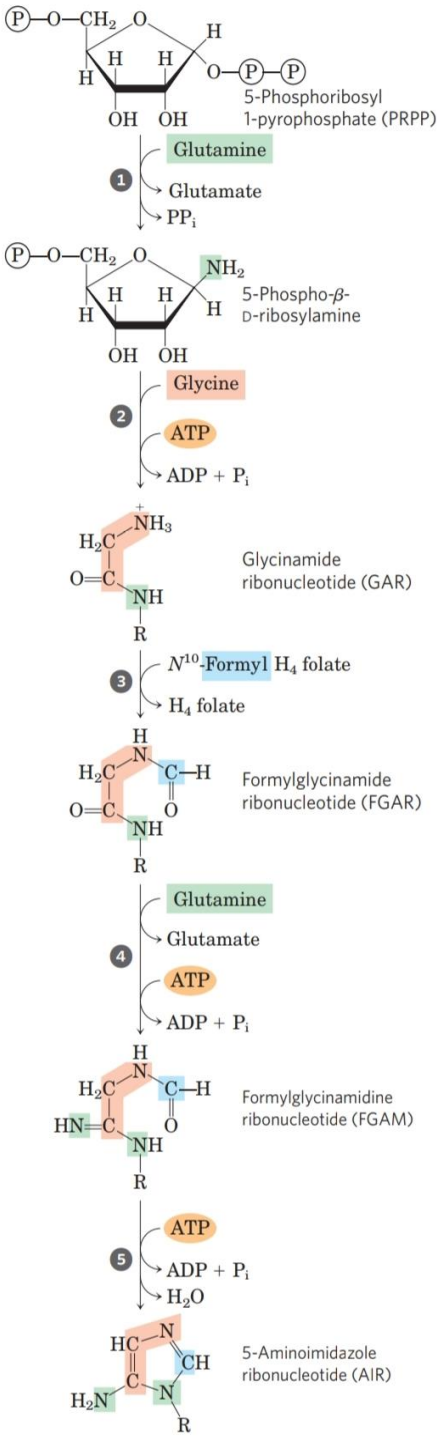
III. Ribonucleotides are the end products of de novo purine synthesis. When deoxyribonucleotides are required for DNA synthesis, the ribose sugar moiety is reduced.

2. Synthesis of 5'-phosphoribosylamine

I. Synthesis of 5'-phosphoribosylamine from *PRPP* and *glutamine* is catalyzed by *glutamine phosphoribosyl pyrophosphate amidotransferase*. It's inhibited by the purine 5'-nucleotides AMP and GMP.

II. The amide group of glutamine replaces the pyrophosphate group attached to carbon 1 of PRPP.

III. This is the committed step in purine nucleotide biosynthesis. The rate of the reaction is also controlled by the intracellular concentration of PRPP (It's an activator).



3. Synthesis of inosine monophosphate, the "parent" purine nucleotide

I. Addition of *glycine* to the *amino group* of 5-phosphoribosylamine (which was added from glutamine) is catalyzed by *synthetase* (using ATP as a source of energy).

II. Addition of a *formyl group* (HC=O) from *N10-formyltetrahydrofolate* to the *amine group* (of the glycine). It is catalyzed by *formyltransferase*.

III. Another addition of an *amino group* from *glutamine* to the *Carboxyl group*. It is catalyzed by synthetase.

IV. *Cyclization* to make a five-member ring, is catalyzed by *synthetase*. The last added amino group is outside the five-member ring. Also, the oxygen atom of the formyl group is eliminated.

V. *Carboxylation* on alpha carbon (of glycine) using ATP. It is catalyzed by *carboxylase*.

VI. Addition of aspartate from its amino group to C=O which remains from Carboxyl group. It's catalyzed by *synthetase* (using ATP).

VII. Elimination of fumarate from aspartate, so only NH2 remains.

VIII. Addition of a *formyl group (HC=O) from N10-formyltetrahydrofolate to the amine group (of the glutamine)*. It is catalyzed by *formyltransferase*.

IX. *Cyclization* by *elimination of H2O*, which is catalyzed by *IMP cyclohydrolase*.

- These nine steps leading to the synthesis of inosine monophosphate (IMP, whose base is hypoxanthine). It proceeds to the production of AMP or GMP.
- This pathway requires ATP as an energy source.
- Two steps require N10-formyltetrahydrofolate. Another two steps require glutamine.

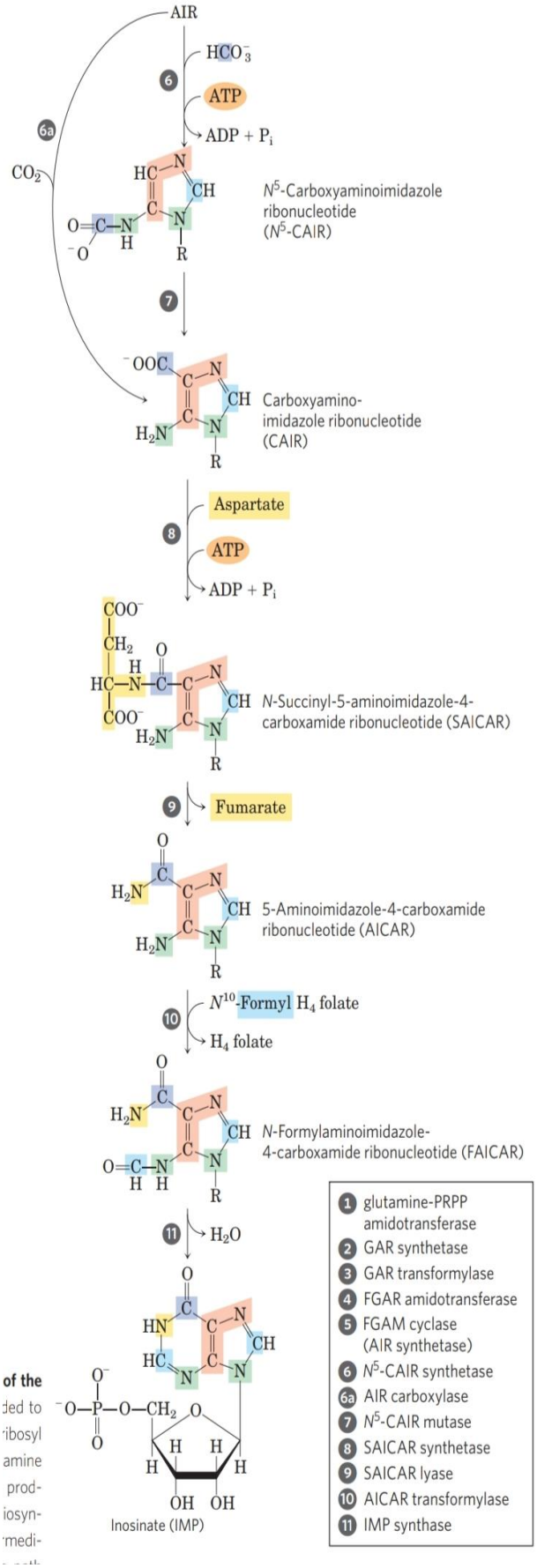
4. Conversion of IMP to AMP and GMP

- The conversion of IMP to either AMP or GMP uses a two-step, energy-requiring pathway.
- The synthesis of AMP requires guanosine triphosphate (GTP) as an energy source, whereas the synthesis of GMP requires ATP. This provides a mechanism for diverting IMP to the synthesis of the species of purine present in lesser amounts.

- **To form GMP :**

I. IMP is oxidized to XMP (xanthosine monophosphate) by reducing NAD+ to NADH. This step is catalyzed by IMP dehydrogenase (inhibited by GMP).

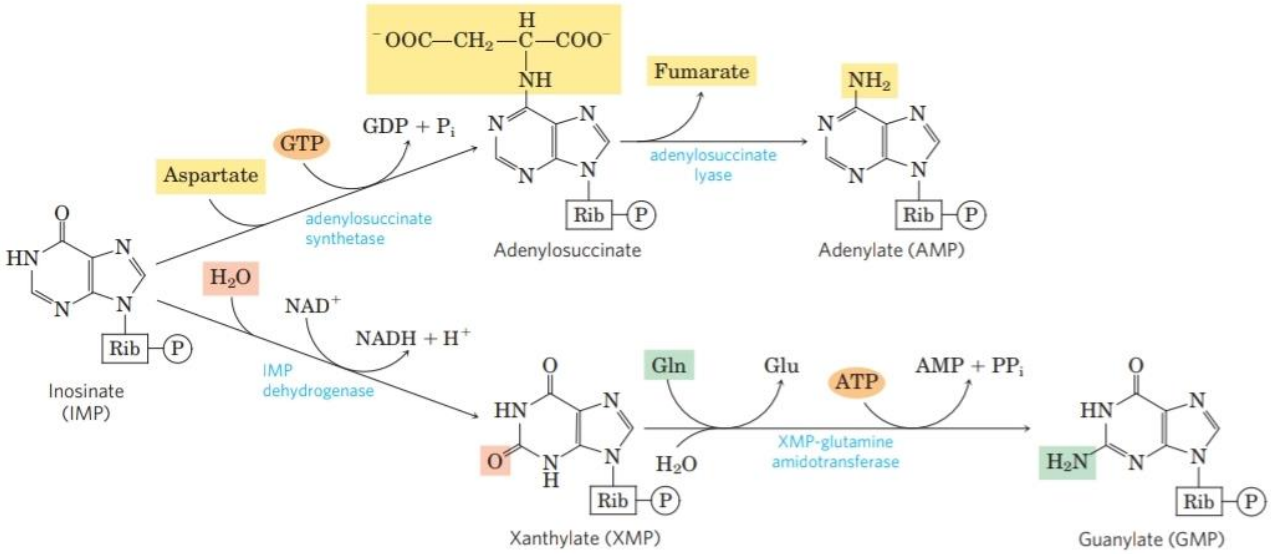
II. An amino group (from glutamine) replaces oxygen which is added in the previous reaction. That is catalyzed by GMP synthetase (but it converts ATP to AMP).



- **To form AMP :**

I. Aspartate replaces the oxygen atom of IMP, using GTP. That is catalyzed by adenylosuccinate synthetase (inhibited by AMP).

II. Fumarate is elaborated from aspartate, leaving an amino group. That is catalyzed by adenylosuccinate lyase.



5. Conversion of nucleoside monophosphates to nucleoside diphosphates and triphosphates (phosphorylation modification)

I. Nucleoside diphosphates are synthesized from the corresponding nucleoside monophosphates by *base-specific nucleoside monophosphate kinases*. [These kinases do not discriminate between ribose or deoxyribose in the substrate.]

II. Adenylate kinase phosphorylates AMP to ADP, while Guanylate kinase phosphorylates GMP to GDP.

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

III. ATP is generally the source of the transferred phosphate, because it is present in higher concentrations than the other nucleoside triphosphates. Adenylate kinase is particularly active in liver and muscle, where the turnover of energy from ATP is high. Its function is to maintain an equilibrium among AMP, ADP, and ATP.

IV. Nucleoside triphosphates are synthesized by *nucleoside diphosphate kinase*—an enzyme that, unlike the monophosphate kinases, has broad specificity (works on purines and pyrimidines).

Synthetic inhibitors of purine synthesis

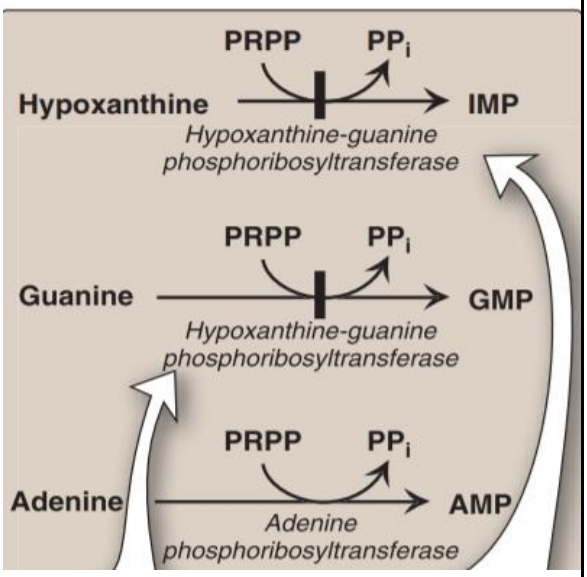
1) The Sulfonamides1(antibiotics) are designed to inhibit the growth of rapidly dividing microorganisms without interfering with human cell functions.

2) Structural analogs of folic acid (for example, methotrexate2), are used pharmacologically to control the spread of cancer by interfering with the synthesis of nucleotides and, therefore, of DNA and RNA.

❖ Inhibitors of human purine synthesis are extremely toxic to tissues, especially to developing structures such as in a fetus, or to cell types that normally replicate rapidly, including those of bone marrow, skin, gastrointestinal (GI) tract, immune system, or hair follicles. As a result, individuals taking such anticancer drugs can experience adverse effects, including anemia, scaly skin, GI tract disturbance, immunodeficiencies, and hair loss.

Salvage pathway for purines

- Purines that result from **The normal turnover of cellular nucleic acids and The small amount that is obtained from the diet and not degraded,** can be converted to nucleoside triphosphates and used by the body. This is referred to as the "salvage pathway" for purines.



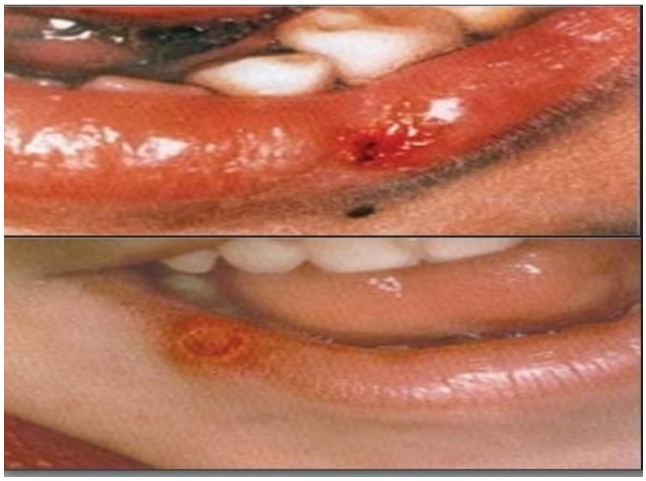
- Two enzymes are involved in Salvage Pathway :
  - ✓ *Adenine Phosphoribosyltransferase (APRT), which adds adenine to PRPP , producing AMP.*
  - ✓ *Hypoxanthine-Guanine phosphoribosyltransferase (HGPRT), which adds Hypoxanthine or Guanine to PRPP, producing IMP and GMP respectively.*

- Both enzymes use PRPP as the source of the ribose 5-phosphate group. The release of pyrophosphate and its subsequent hydrolysis by pyrophosphatase makes these reactions *irreversible*.
- *Adenosine* is the only *purine nucleoside* to be salvaged. It is phosphorylated to AMP by *adenosine kinase.*

Lesch-Nyhan syndrome

A rare, X-linked, recessive disorder associated with a virtually complete deficiency of HGPRT. This deficiency of salvage pathway causes :

a) Inability to salvage hypoxanthine or guanine, that results in excessive amounts of uric acid, the end product of purine degradation.
b) *Increased PRPP* levels and *decreased IMP and GMP* levels. As a result, glutamine-phosphoribosyl pyrophosphate amidotransferase (the committed step in purine synthesis) has *excess substrate and decreased inhibitors* available, and de novo purine synthesis is increased, and IMP can produce both AMP and GMP (there is no salvage production of GMP and AMP is produced de novo and from salvage pathway) meaning there will be more production of AMP compared to GMP.



c) Hyperuricemia (which causes *Urolithiasis and gouty arthritis*).
d) *Many characteristics* such as: Motor dysfunction, cognitive deficits, and behavioral disturbances that include self-mutilation (biting of lips and fingers).

SYNTHESIS OF DEOXYRIBONUCLEOTIDES

The nucleotides required for DNA synthesis are 2'-deoxyribonucleotides, which are produced from ribonucleoside diphosphates by the enzyme ribonucleotide reductase during the S-phase of the cell cycle. [Note: The same enzyme acts on pyrimidine ribonucleotides.]

1. Ribonucleotide reductase

Ribonucleotide reductase (ribonucleoside diphosphate reductase) is composed of two non-identical dimeric subunits, R1 and R2, and is *specific for the reduction of* :

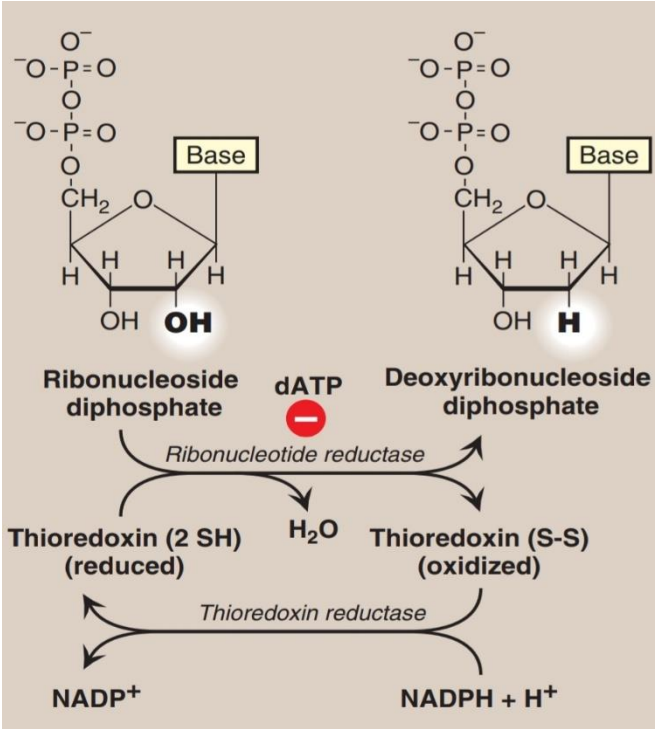
- **Purine nucleoside diphosphates (ADP and GDP) to their deoxy forms (dADP and dGDP).**
- **Pyrimidine nucleoside diphosphates, cytidine diphosphate (CDP) and uridine diphosphate (UDP) to their deoxy forms (dCDP, and dUDP).**

The immediate donors of the hydrogen atoms needed for the reduction of the 2'-hydroxyl group are two sulfhydryl groups on the enzyme itself, which, during the reaction, form a disulfide bond.

2. Regeneration of reduced enzyme:

- To continue in producing deoxyribonucleotides, Thioredoxin, which is a peptide coenzyme of ribonucleotide reductase, provides the reducing equivalents for ribonucleotide reductase.

- Thioredoxin contains two cysteine residues separated by two amino acids in the peptide chain. The two sulfhydryl groups of thioredoxin donate their hydrogen atoms to ribonucleotide reductase, in the process forming a disulfide bond.



3. Regeneration of reduced thioredoxin:

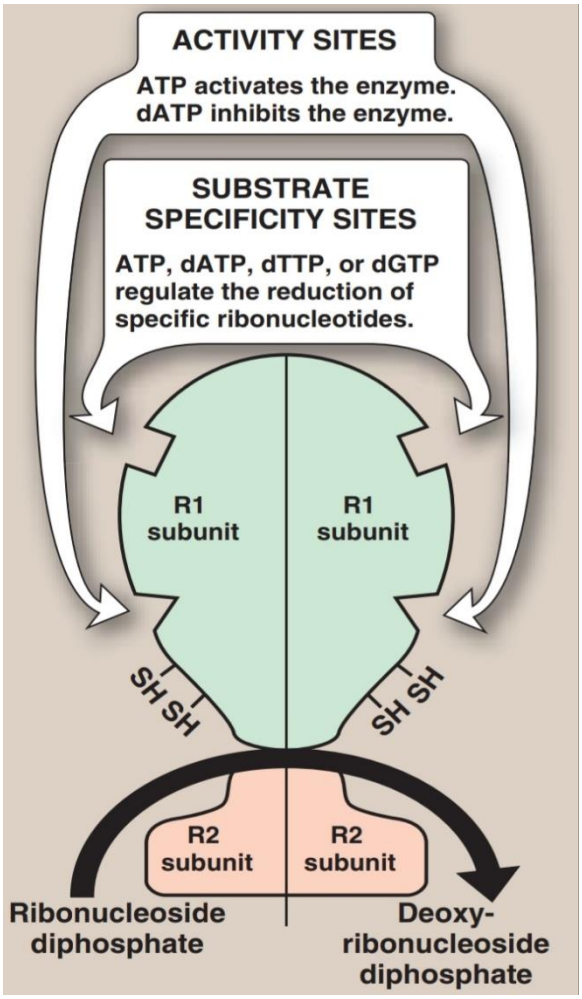
The necessary reducing equivalents are provided by NADPH + H+, and the reaction is catalyzed by thioredoxin reductase.

Regulation of deoxyribonucleoside synthesis

Ribonucleotide reductase is responsible for maintaining a balanced supply of the deoxy ribonucleotides required for DNA synthesis. In addition to the catalytic (active) site, there are allosteric sites on the enzyme involved in regulating its activity. It is composed of 4 subunits (2 dimeric subunits), and these subunits have different allosteric regulatory sites.

A. Activity sites:

- The binding of dATP to allosteric sites (known as the activity sites) on the enzyme inhibits the overall catalytic activity of the enzyme and, therefore, prevents the reduction of any of the four nucleoside diphosphates. This effectively prevents DNA synthesis, and explains the toxicity of increased levels of dATP seen in conditions such as adenosine deaminase deficiency.
- ATP bound to these sites activates the enzyme.



B. Substrate specificity sites:

- The binding of deoxy-nucleoside triphosphates to additional allosteric sites (known as the substrate specificity sites) on the enzyme regulates substrate specificity, causing an increase in the conversion of different species of ribonucleotides to deoxyribonucleotides as they are required for DNA synthesis (the binding of one type of nucleotide activates the formation of another type of nucleotide).
- For example, deoxythymidine triphosphate (dTTP) binding at the specificity sites causes a conformational change that allows reduction of GDP to dGDP at the catalytic site.
- ***The drug Hydroxyurea***

✓ It destroys the *free radical* required for enzymic activity of *ribonucleotide reductase*, and, thus *inhibits the generation of substrates for DNA synthesis.*

✓ It has been used in the treatment of cancers such as *chronic myelogenous leukemia (CML)*.

DEGRADATION OF PURINE NUCLEOTIDES

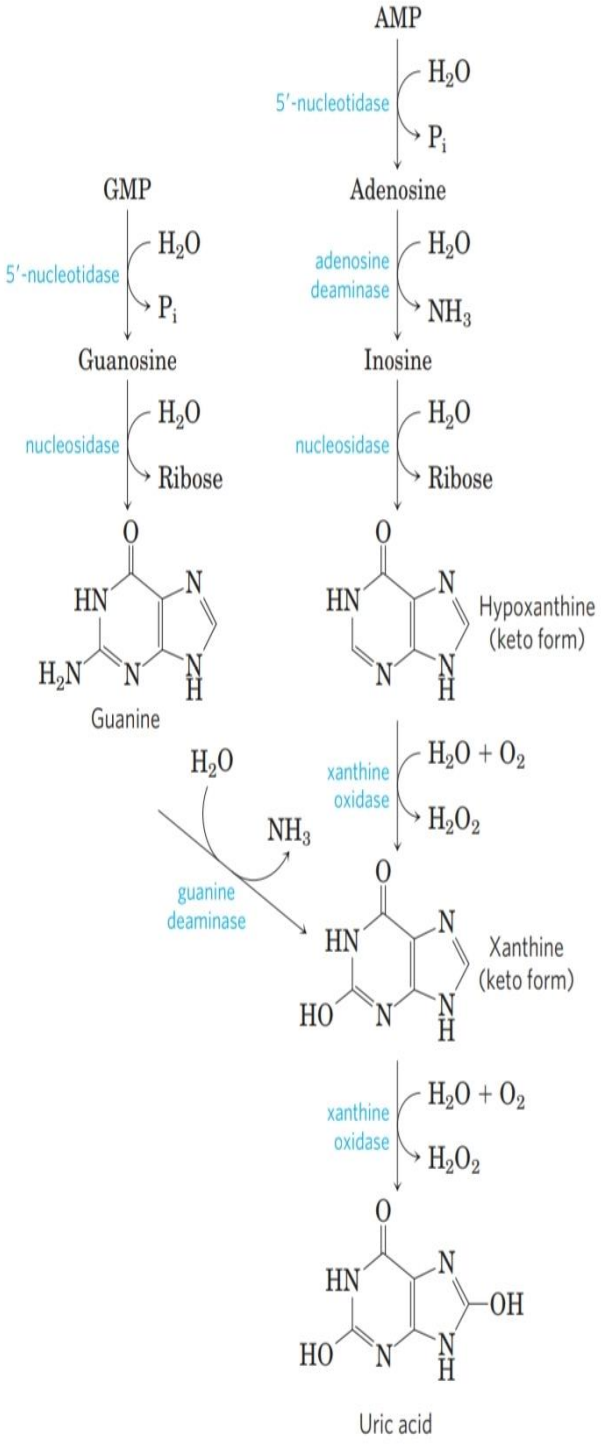
- Degradation of dietary nucleic acids occurs in the small intestine by a family of pancreatic enzymes hydrolyzes the nucleic acids to nucleotides.
- Inside the intestinal mucosal cells, purine nucleotides are sequentially degraded by specific cellular enzymes to nucleosides and free bases, with uric acid as the end product.
- Purine nucleotides from de novo synthesis are degraded in the liver primarily. The free bases are sent out from liver and salvaged by peripheral tissues.

A. Degradation of dietary nucleic acids in the small intestine

- Ribonucleases and deoxyribonucleases, secreted by the pancreas, hydrolyze dietary RNA and DNA primarily to oligonucleotides.
- Oligonucleotides are further hydrolyzed by pancreatic *phosphodiesterases*, producing a mixture of 3'- and 5'-mononucleotides.
- In the intestinal mucosal cells, a family of nucleotidases removes the phosphate groups hydrolytically, releasing nucleosides that are further degraded by nucleosidases to free bases.

B. Formation of uric acid

- An amino group is removed from AMP to produce IMP by AMP deaminase, or from adenosine to produce inosine (hypoxanthine-ribose) by adenosine deaminase.
- IMP, GMP, and AMP are converted into their nucleoside forms inosine, guanosine, and adenosine by the action of 5'-nucleotidase.



- **Purine nucleoside phosphorylase** converts inosine and guanosine into their respective purine bases, *hypoxanthine* and *guanine* by removing the sugar and phosphorylating it. [Note: A mutase interconverts ribose 1- and ribose 5-phosphate.]
- Guanine is deaminated to form xanthine by Guanine deaminase.
- Hypoxanthine is oxidized by xanthine oxidase to xanthine.
- Xanthine is further oxidized by xanthine oxidase to uric acid, which is excreted primarily in the urine.

C. Diseases associated with purine degradation

1) Gout: النقرس

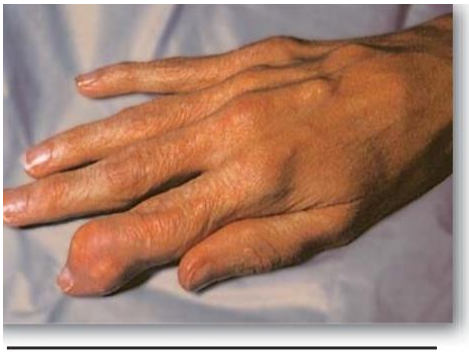


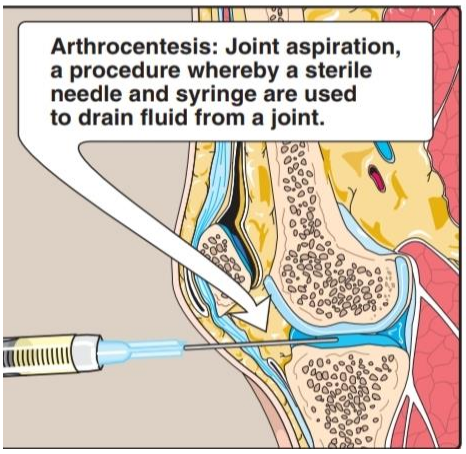
Figure 22.16
Tophaceous gout.

- Gout is a disorder characterized by high levels of uric acid—the end product of purine catabolism—in blood (hyperuricemia), as a result of either the overproduction or underexcretion of uric acid (most of the time it's underexcretion).
- The hyperuricemia can lead to:

✓ The formation of *uric acid stones* in the kidneys (*Urolithiasis*).

✓ The deposition of *monosodium urate crystals* in the joints, which are identified by the immune system as a foreign body causing an inflammation (*gouty arthritis*).

✓ The deposition of *nodular masses of monosodium urate crystals* (tophi) in the soft tissues, resulting in *chronic tophaceous gout.*



- The definitive diagnosis of gout requires aspiration and examination of synovial fluid from an affected joint (or material from a tophus) using polarized light microscopy to confirm the presence of needle-shaped monosodium urate crystals.

- Hyperuricemia is typically asymptomatic and does not lead to gout, but gout is preceded by hyperuricemia.

Causes of hyperuricemia (from slides)

a. Underexcretion of uric acid:

In the vast majority of patients, the hyperuricemia leading to gout is caused by underexcretion of uric acid. Underexcretion can be :

- Primary, due to as-yet-unidentified inherent excretory defects
- Secondary, due to:
  1) Known disease processes that affect how the kidney handles urate, for example lactic acidosis (lactate and urate compete for the same renal transporter)
  2) Environmental factors such as the use of drugs, for example, thiazide diuretics
  3) Exposure to lead (saturnine gout).

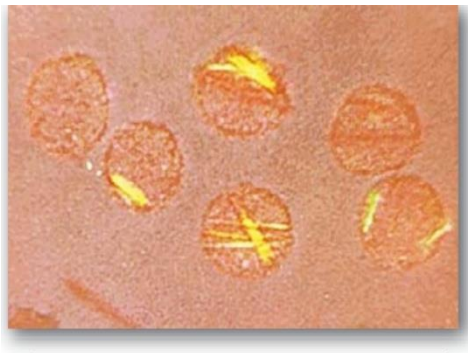


Figure 22.18
Gout can be diagnosed by the presence of negatively birefringent monosodium urate crystals in aspirated synovial fluid examined by polarized-light microscopy. Here, crystals are within polymorphonuclear leukocytes.

b. Overproduction of uric acid:

A less common cause of gout is hyperuricemia from the overproduction of uric acid. Several identified mutations in the gene for X-linked PRPP synthetase that results in increased availability of PRPP increases purine production, resulting in elevated levels of plasma uric acid.

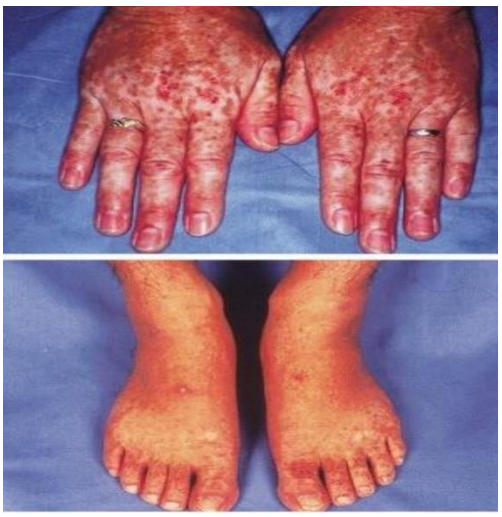
Lesch-Nyhan syndrome also causes hyperuricemia as a result of the decreased salvage of hypoxanthine and guanine, and the subsequent increased availability of PRPP.

Treatment of gout: (you won't be asked about the drugs)

- ✓ Acute attacks of gout are treated with anti-inflammatory agents. anti-inflammatory drugs have no effect on uric acid levels.
- ✓ Long-term therapeutic strategies for gout involve lowering the uric acid level below its saturation point, thereby preventing the deposition of urate crystals.
- ✓ Uricosuric agents, such as probenecid or sulfinpyrazone, that increase renal excretion of uric acid, are used in patients who are “underexcretors” of uric acid.
- ✓ Allopurinol, a structural analog of hypoxanthine, inhibits uric acid synthesis and is used in patients who are “overproducers” of uric acid. Allopurinol is converted in the body to oxypurinol, which inhibits xanthine oxidase (XO), resulting in an accumulation of hypoxanthine and xanthine —compounds more soluble than uric acid and, therefore, less likely to initiate an inflammatory response.

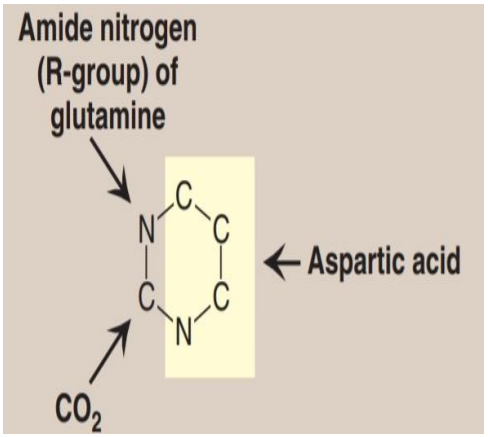
2) Adenosine deaminase (ADA) deficiency

- An autosomal recessive disorder results in an *accumulation of adenosine*, which is *converted to its ribonucleotide or deoxyribonucleotide* forms by cellular kinases.
- As *dATP levels rise*, *ribonucleotide reductase is inhibited*, thus preventing the production of all deoxyribose-containing nucleotides. Consequently, cells cannot make DNA and divide.
- ADA is expressed in a variety of tissues, but, in humans, lymphocytes have the highest activity of this cytoplasmic enzyme.
- The dATP and adenosine that accumulate in ADA deficiency lead to developmental arrest and *apoptosis of lymphocytes.*
- Treatment requires either *bone marrow transplantation* (BMT) or *enzyme replacement therapy* (ERT).
- Without appropriate treatment, *children with this disorder usually die by the age of two.*



PYRIMIDINE SYNTHESIS AND DEGRADATION

- Unlike the synthesis of the purine ring, which is constructed on a preexisting ribose 5-phosphate, the pyrimidine ring is synthesized before being attached to ribose 5-phosphate, which is donated by PRPP.
- The *sources of the atoms* in the pyrimidine ring are :
  - *Amino acids: aspartic acid and glutamine.*
  - *CO2*



1. Synthesis of carbamoyl phosphate

- The regulated step of this pathway is the synthesis of *carbamoyl phosphate* from *glutamine and CO2*, catalyzed by *carbamoyl phosphate synthetase (CPS) II* consuming 2 ATP.
- CPS II is inhibited by UTP (the end product of this pathway) and is activated by PRPP. Carbamoyl phosphate, synthesized by CPS I, is also a precursor of urea.
- Defects in ornithine transcarbamylase of the urea cycle promote pyrimidine synthesis due to increased availability of carbamoyl phosphate.

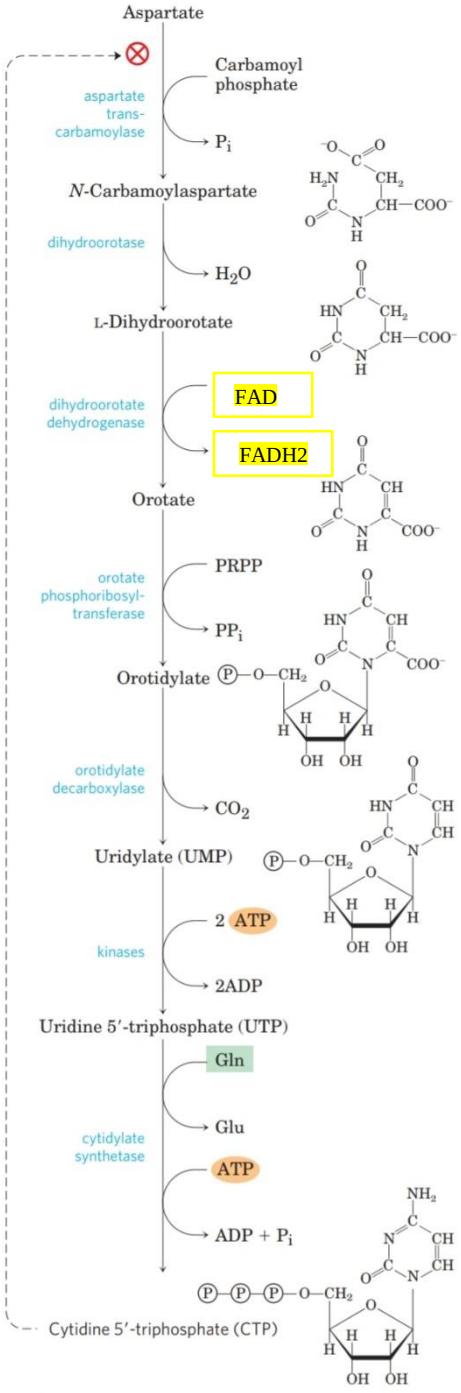
	CPS I	CPS II
Cellular location	Mitochondria	Cytosol
Pathway involved	Urea cycle	Pyrimidine synthesis
Source of nitrogen	Ammonia	γ-Amide group of glutamine
Regulators	Activator: N–acetyl-glutamate	Activator: PRPP Inhibitor: UTP

2. Synthesis of orotic acid

a. *Aspartate* replaces the phosphate group to form *carbamoyl aspartate*. That is catalyzed by *aspartate transcarbamoylase.*

b. Cyclization *hydrolytically* by *dihydroorotase*. The resulting *dihydroorotate* is oxidized to produce orotic acid (*orotate*), by *dihydroorotate dehydrogenase*, which is associated with the inner mitochondrial membrane and requires FAD. (All other enzymes in pyrimidine biosynthesis are cytosolic).

⇨ The first three enzymic activities in this pathway (CPS II, aspartate transcarbamoylase, and dihydroorotase) are actually three different catalytic domains of a single polypeptide chain known as CAD from the first letter in the name of each domain. This is an example of a multifunctional or multi-catalytic polypeptide that facilitates the ordered synthesis of an important compound.



3. Formation of a pyrimidine nucleotide

a. Addition of ribose 5-phosphate from PRPP to orotate, converting it to the nucleotide orotidine 5'-monophosphate (OMP), the parent pyrimidine mononucleotide, by *orotate phosphoribosyltransferase* (the reaction biologically irreversible because it releases pyrophosphate).
b. Decarboxylation of OMP to uridine monophosphate (UMP) by *orotidylate decarboxylase*, which removes the acidic carboxyl group. (Orotate phosphoribosyl transferase and orotidylate decarboxylase are also catalytic domains of a single polypeptide chain called UMP synthase).
c. Phosphorylation of UMP to UDP and UTP.
d. Reduction of UDP to dUDP by ribonucleotide reductase.
e. Phosphorylation of dUDP to dUTP, which is rapidly hydrolyzed to dUMP by UTP diphosphatase (dUTPase).

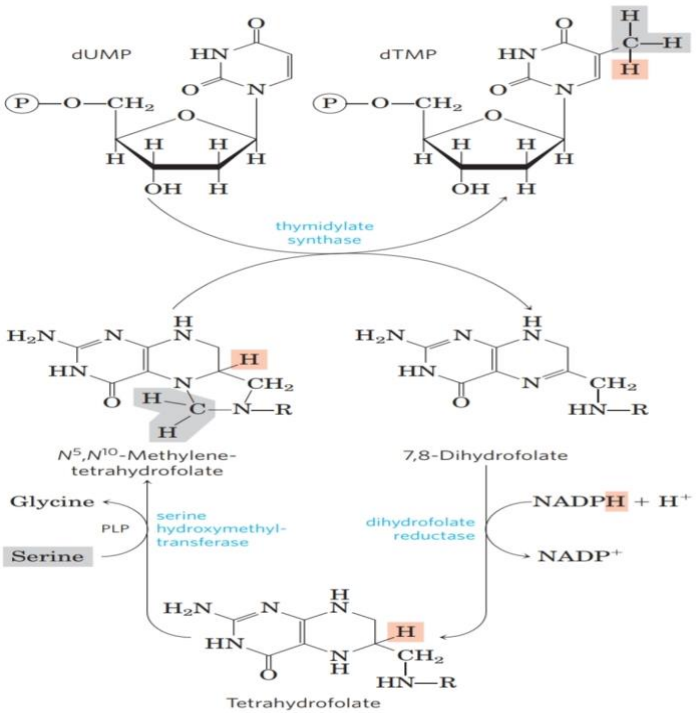
- **dUTPase reduces the available dUTP as a substrate for DNA synthesis, thus preventing incorporation of deoxy-uracil into DNA.**

4. Synthesis of UTP and cytidine triphosphate (CTP)

a. Amination of UTP by CTP synthetase to CTP, with glutamine providing the nitrogen.
b. Dephosphorylating of CTP to CDP, which is a substrate for ribonucleotide reductase.
c. Phosphorylation of dCDP to dCTP for DNA synthesis.

5. Synthesis of thymidine monophosphate (dTMP) from dUMP

a. Addition of a methyl group from N5, N10-methylene tetrahydrofolate to dUMP (UMP is transformed to dUMP by ribonucleotide reductase) to form dTMP by *thymidylate synthase*.
b. Reduction of dihydrofolate (inactive form of folic acid) to tetrahydrofolate (can carry methylene again) by dihydrofolate reductase.



Anti-cancerous agents

1. Inhibitors of thymidylate synthase include thymine analogs such as 5-fluorouracil, which serve as successful antitumor agents.

⇨ 5-Fluorouracil (suicide inhibitor) is metabolically converted to 5-FdUMP, which becomes permanently bound to the inactivated thymidylate synthase.

2. Methotrexate is a drug inhibits dihydrofolate reductase, an enzyme that reduces DHF to THF.

⇨ Decreasing the supply of THF, inhibits purine synthesis and prevents methylation of dUMP to dTMP, they also lower the cellular concentration of this essential component of DNA. DNA synthesis is inhibited, and cell growth slowed.

Salvage of pyrimidines

- Few pyrimidine bases are salvaged in human cells.
- The pyrimidine nucleosides can be salvaged by nucleoside kinases that utilize ATP in the phosphorylation of the nucleosides to nucleotides.

Degradation of pyrimidine nucleotides

The pyrimidine ring is opened and degraded to highly soluble products, *β-alanine* and *β-aminoisobutyrate*, with the production of *NH_3* and *CO_2*. (the purine ring is not cleaved human cells).

The table comparing CPS I and II is important.

The textbook includes a lot of summaries, you can check them.

Without hard work, nothing grows but weeds.

Good MARKS ! ◻