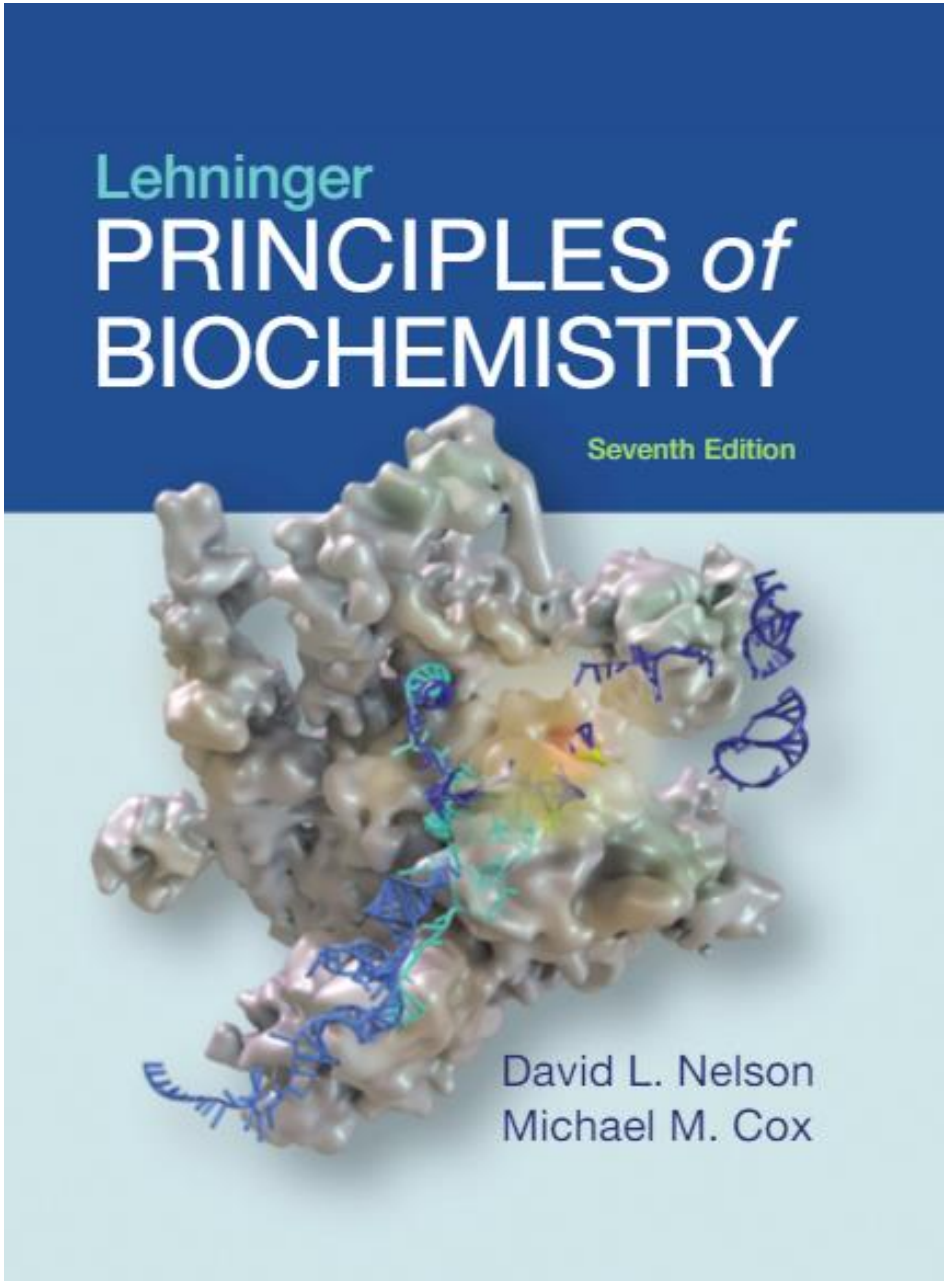
22| Biosynthesis of Amino Acids, Nucleotides, and Related Molecules



Importance of Nitrogen in Biochemistry

- Nitrogen (with H, O, and C) is a major elemental constituent of living organisms.
- Mostly in nucleic acids and proteins
- But also found in:
  - several cofactors (NAD, FAD, biotin …)
  - many small hormones (epinephrine)
  - many neurotransmitters (serotonin)
  - many pigments (chlorophyll)
  - many defense chemicals (amanitin)

Ammonia Is Incorporated into Biomolecules Through Glu and Gln

- Glutamine is made from Glu by glutamine synthetase in a two-step process.
- Phosphorylation of Glu creates a good leaving group that can be easily displaced by ammonia.

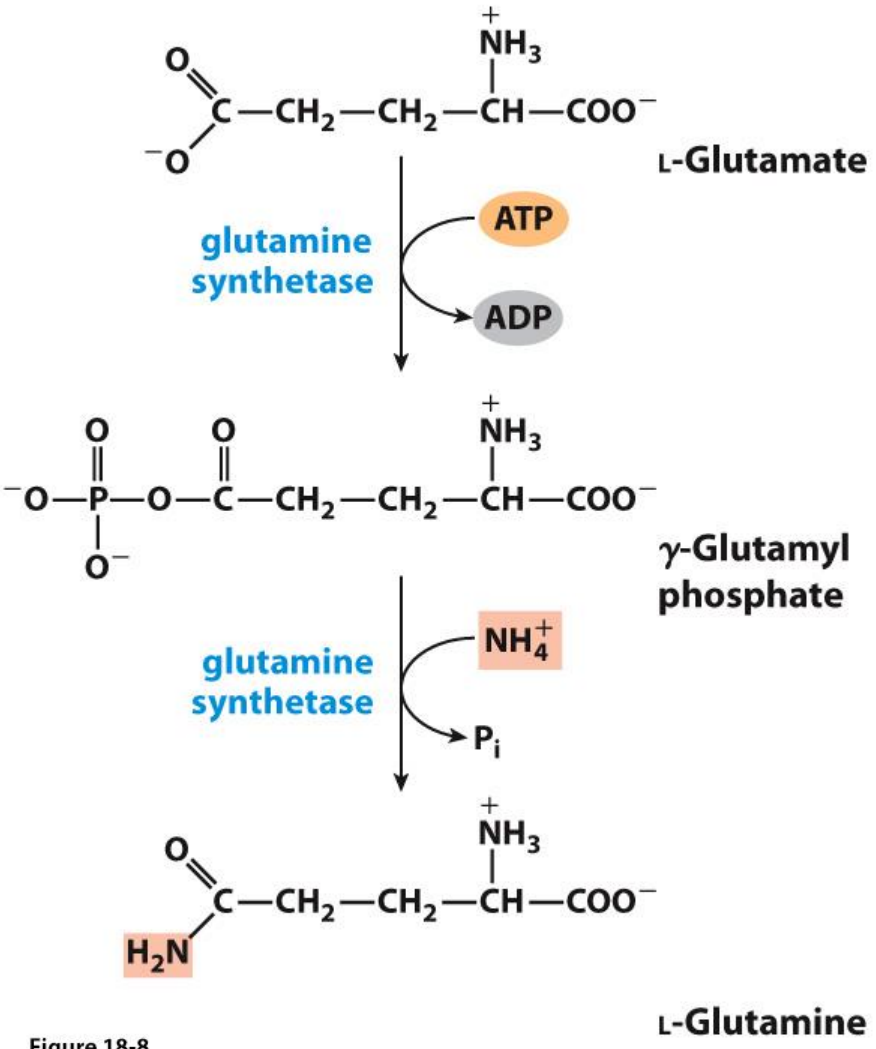


Figure 18-8
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Adenylation of Glutamine Synthetase

Adenylylation (attachment of AMP) to Tyr-397 assists in inhibition.

- Increases sensitivity to inhibitors
- Part of complex cascade that is dependent on [Glu], [α-ketoglutarate], [ATP], and [P_i]
- Activity of adenylyltransferase regulated by binding to regulatory protein P_{II}

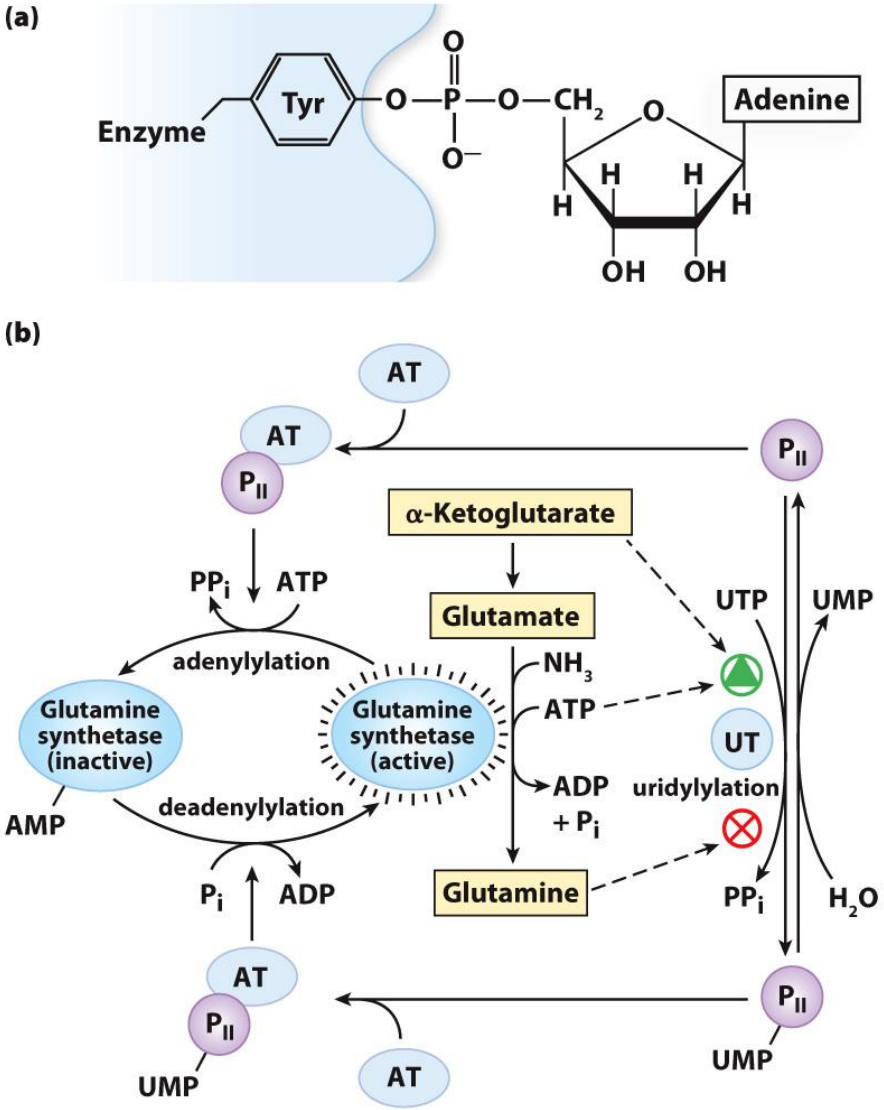

Figure 22-9
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P_{II} Is Regulated by Uridylylation

(Remember that P_{II} regulates adenylyltransferase, which helps inhibit Gln synthetase.)

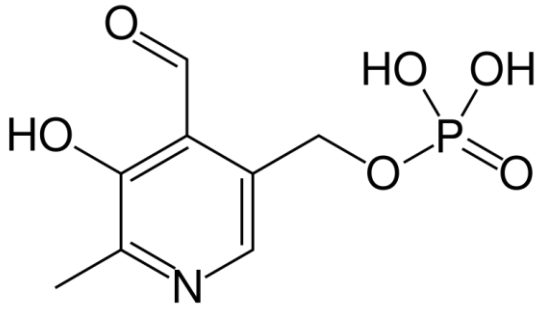
- When P_{II} is uridylylated, adenylyltransferase stimulates deadenylylation of Gln synthetase (increasing the latter's activity).

- ALSO, uridylylated P_{II} upregulates transcription of Gln synthetase.

Biosynthesis of Amino Acids and Nucleotides— MultipleTransaminations

Transaminations and rearrangements using pyridoxal phosphate (PLP)

- PLP is active form of vitamin B_6
- Catalyzed by ***amidotransferases***
- PLP has aldehyde group that forms Schiff base with Lys of aminotransferase



Amino Acid Synthesis Overview

- Source of N is Glu or Gln
- Derived from intermediates of:
  - glycolysis
  - citric acid cycle
  - pentose phosphate pathway
- Bacteria can synthesize all 20.
- Mammals require some in diet.

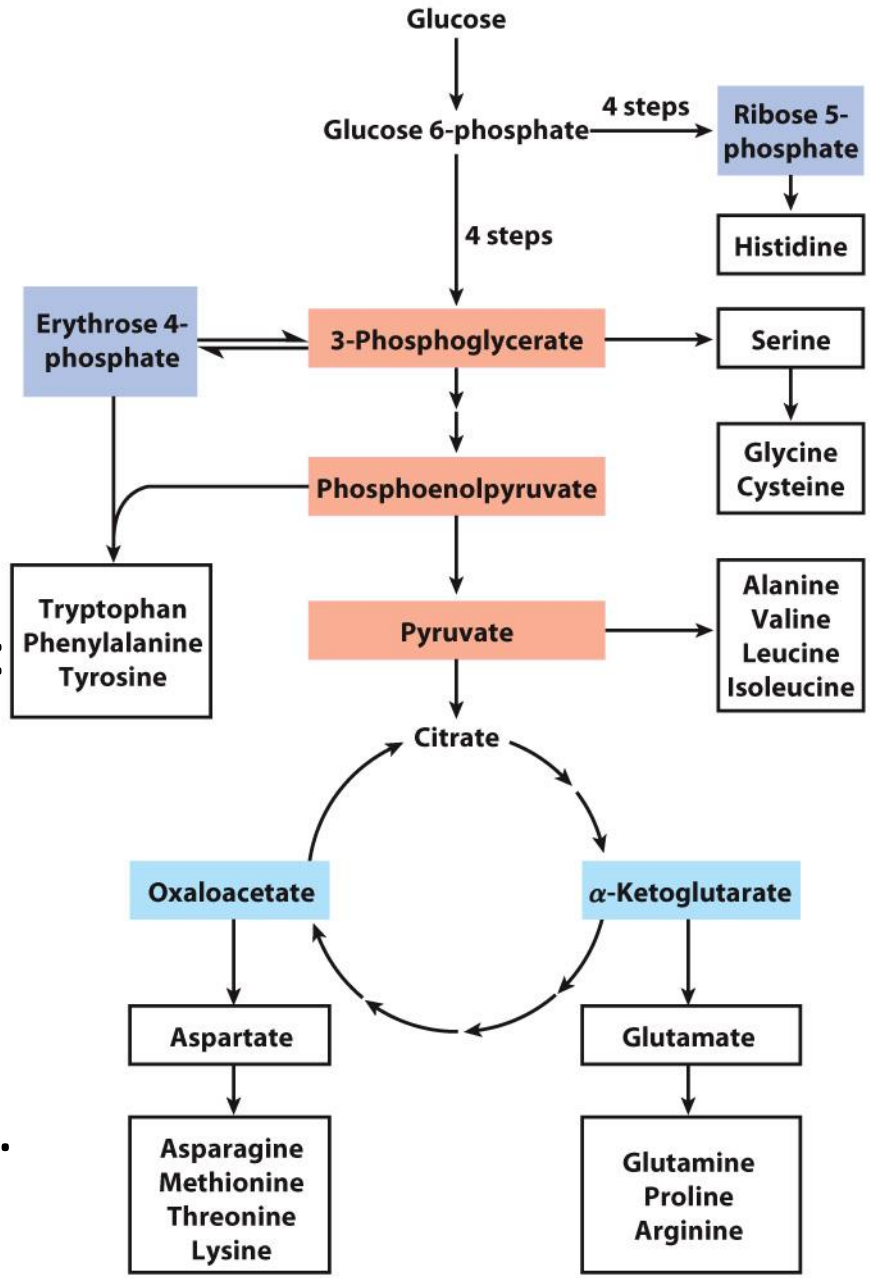


Figure 22-11
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All Amino Acids Derive from One of Seven Precursors

- CAC:
  - α-ketoglutarate, oxaloacetate
- Glycolysis
  - pyruvate, 3-phosphoglycerate, phosphoenolpyruvate
- Pentose phosphate pathway
  - ribose 5-phosphate, erythrose 4-phosphate

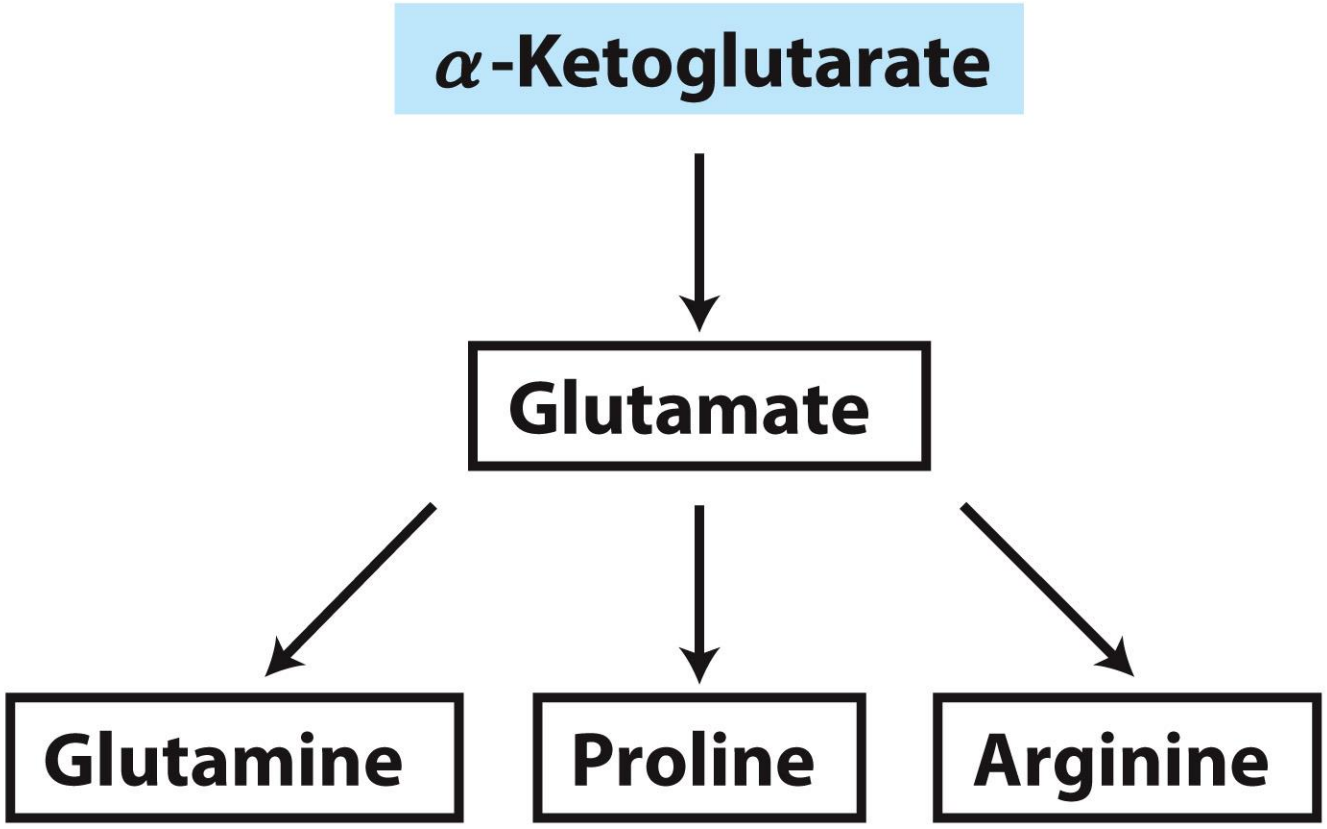
TABLE 22-1 Amino Acid Biosynthetic Families, Grouped by Metabolic Precursor

α-Ketoglutarate Glutamate Glutamine Proline Arginine	**Pyruvate** Alanine Valine[a] Leucine[a] Isoleucine[a]
3-Phosphoglycerate Serine Glycine Cysteine	**Phosphoenolpyruvate and erythrose 4-phosphate** Tryptophan[a] Phenylalanine[a] Tyrosine[b]
Oxaloacetate Aspartate Asparagine Methionine[a] Threonine[a] Lysine[a]	**Ribose 5-phosphate** Histidine[a]

[a]Essential amino acids in mammals.
[b]Derived from phenylalanine in mammals.

Proline and Arginine Derive from Glutamate

- Glutamate is derived from transamination of α-ketoglutarate, as seen in Chapter 18.



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In Animals, Proline Can ALSO Be Synthesized from Arginine

- Ornithine is derived from the urea cycle or degradation of arginine.
- Ornithine δ-aminotransferase converts ornithine to glutamate γ-semialdehyde that cyclizes and converts to Pro.

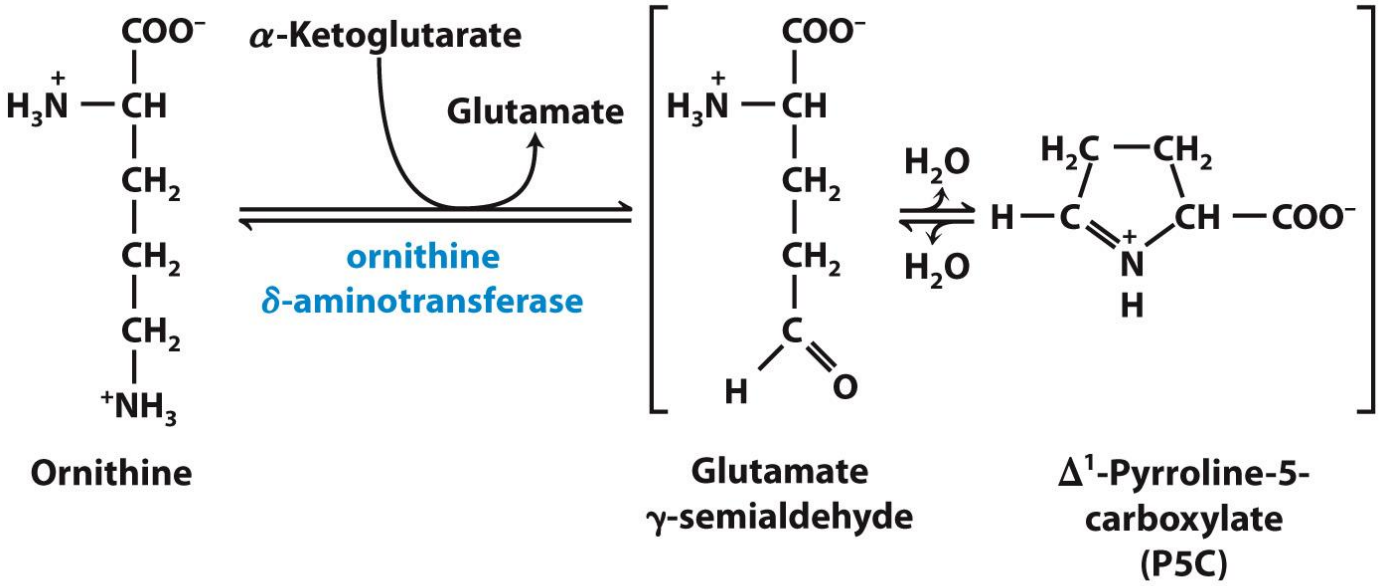

Figure 22-13
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Arginine Is Synthesized from Ornithine in Animals

- Ornithine comes from the urea cycle.
- In bacteria, ornithine has a special synthesis pathway.

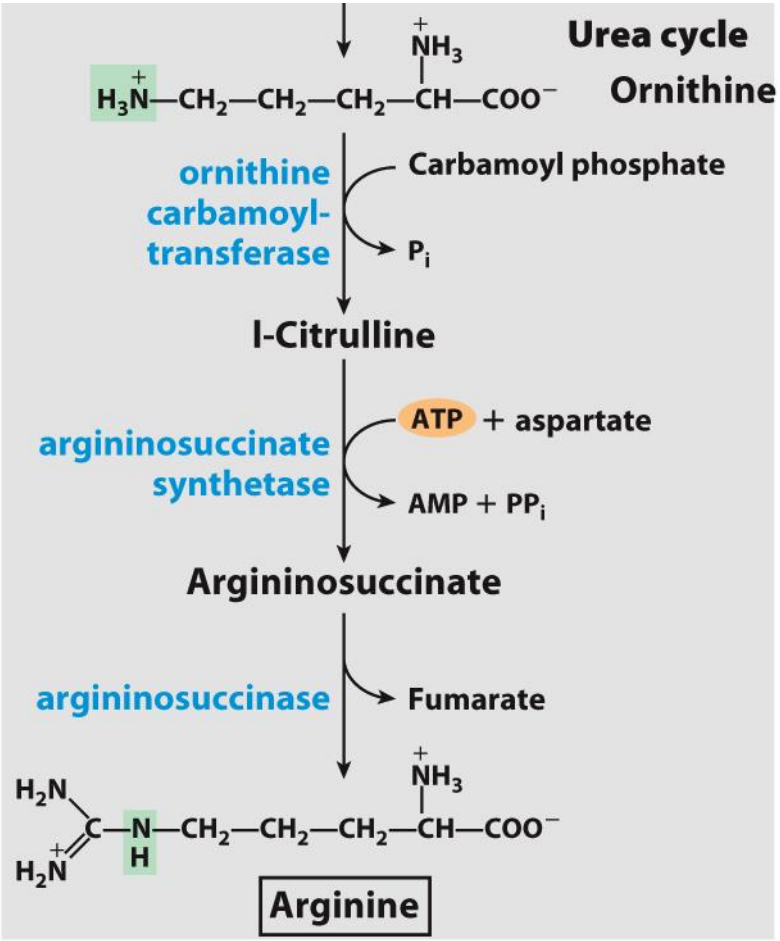

Figure 22-12 part 3
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Serine Derives from 3-Phosphoglycerate of Glycolysis

- Same pathway in all organisms so far
- Requires Glu as source of NH_2 group
- Oxidation → transamination → dephosphorylation to yield serine

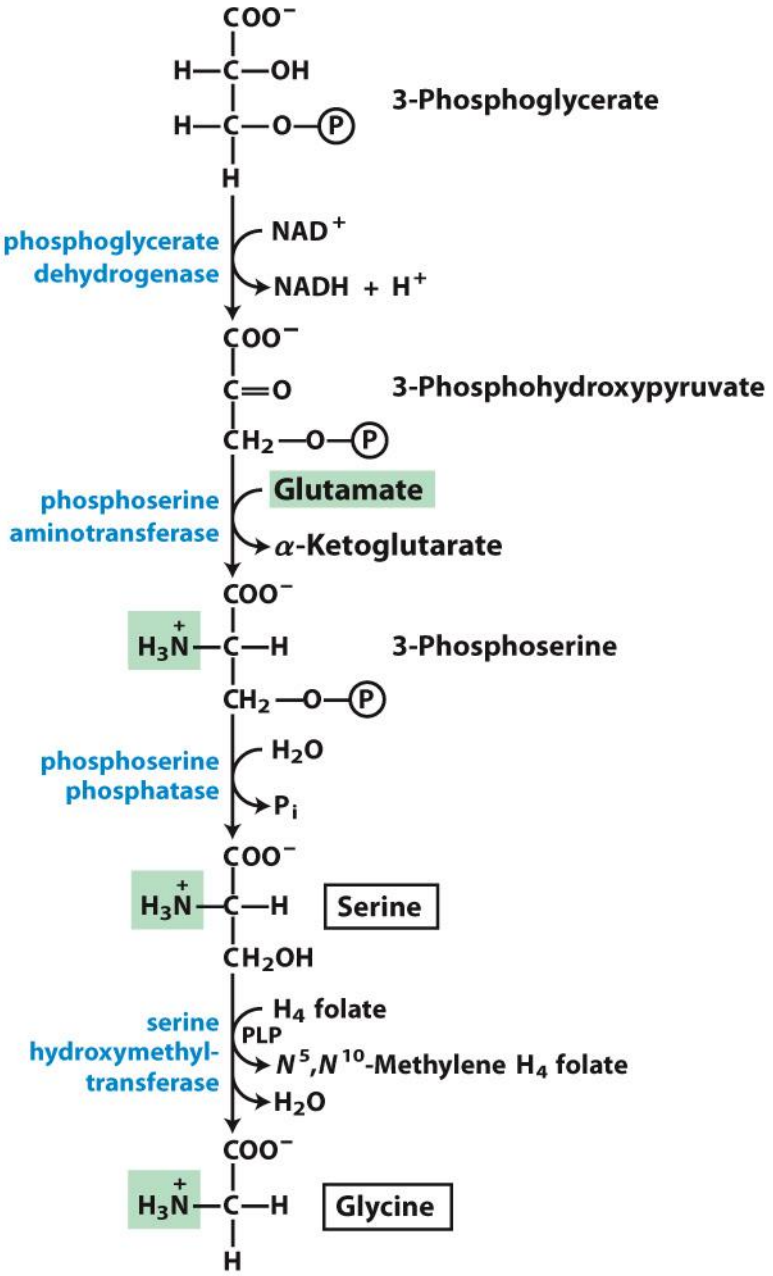

Figure 22-14
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Glycine Derives from Serine

- Carbon removed using tetrahydrofolate (H_4 folate) to accept the C atom and pyridoxal phosphate (PLP)
- Reaction uses serine hydroxymethyltransferase

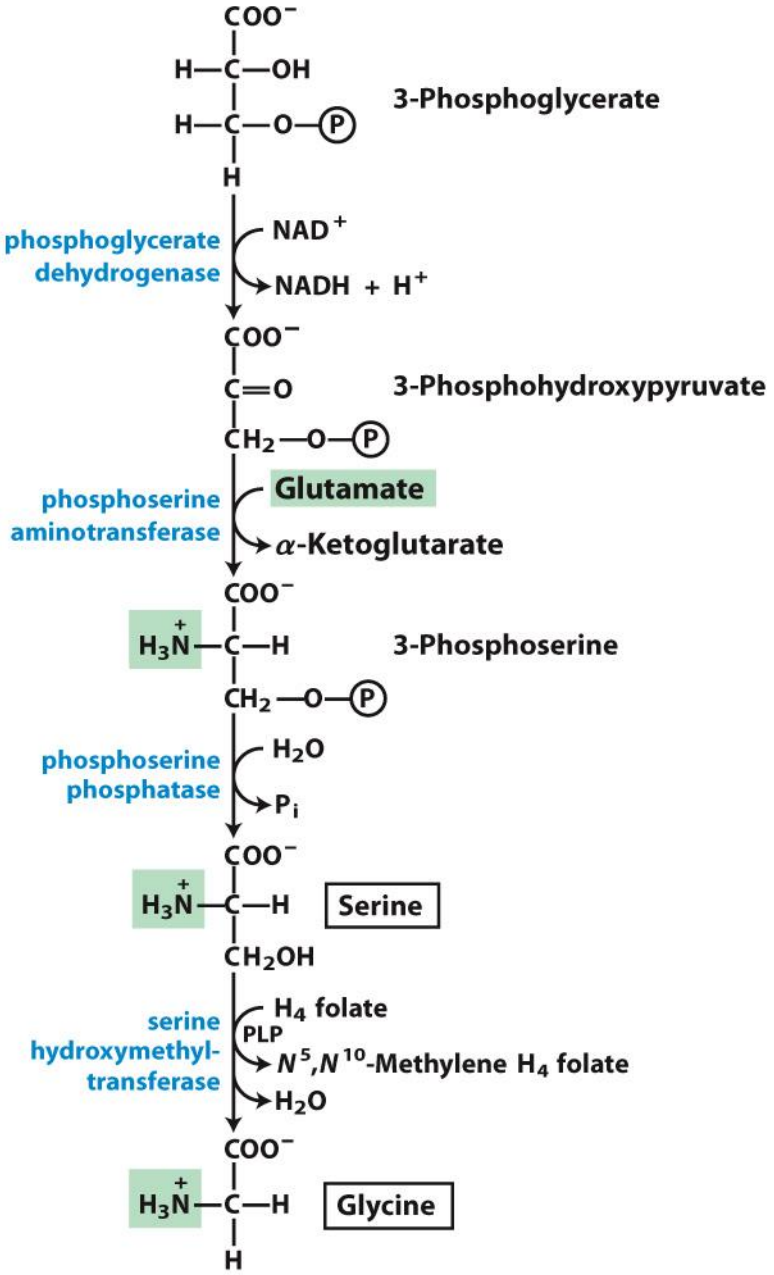

Figure 22-14
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Biosynthesis of Cys from Homocysteine and Ser in Mammals

In mammals, sulfur is recycled from methionine degradation.

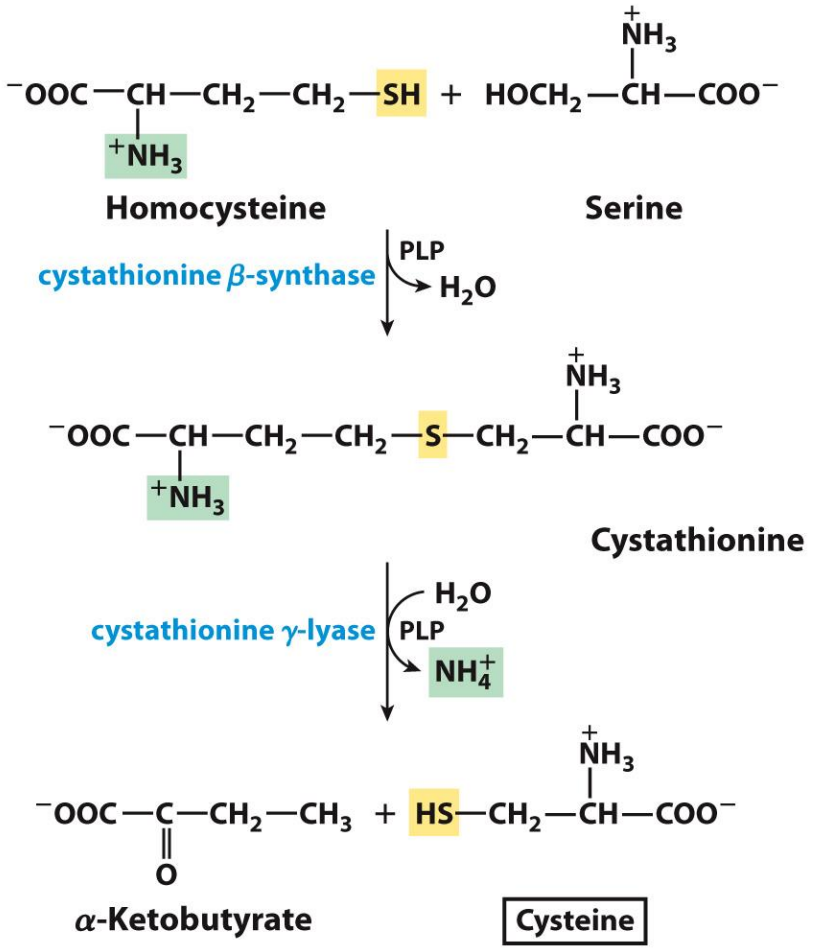

Figure 22-16
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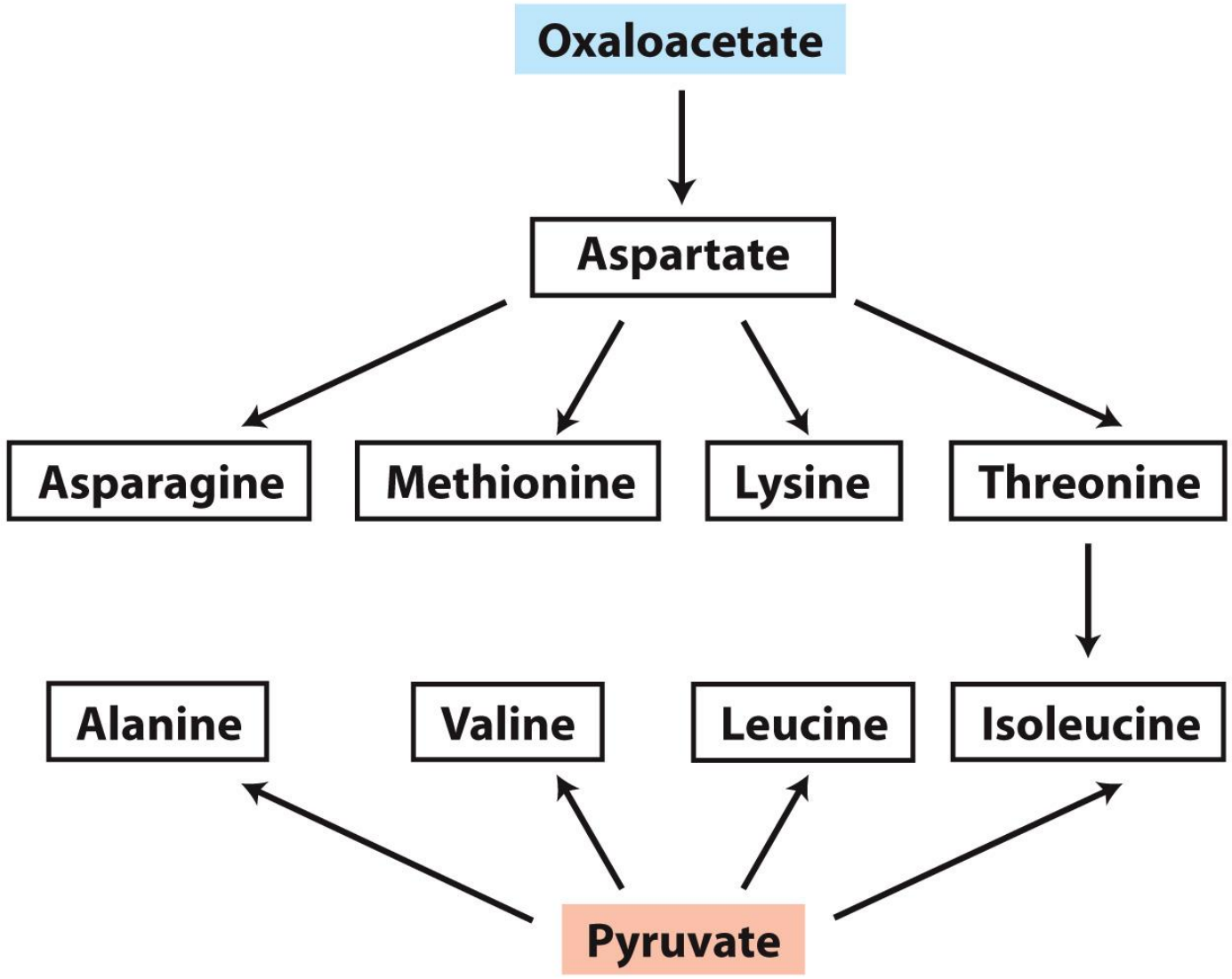
Oxaloacetate yields Asp and Pyruvate Yields Ala, Val, Leu, and Ile



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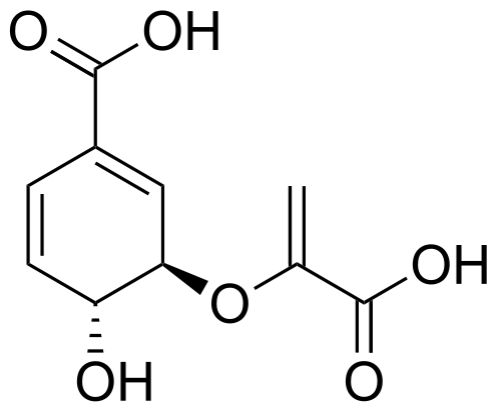
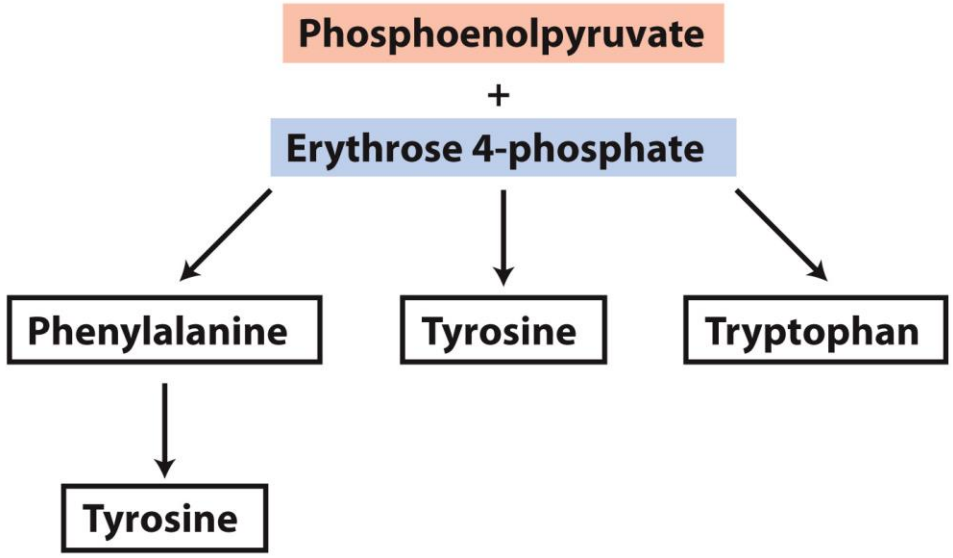
Aromatic Amino Acids Derive from Phosphoenolpyruvate and Erythrose 4-Phosphate

- Very complicated chemistry!
- Rings must be synthesized and closed and then oxidized to create double bonds.
- **Chorismate** is a common intermediate.





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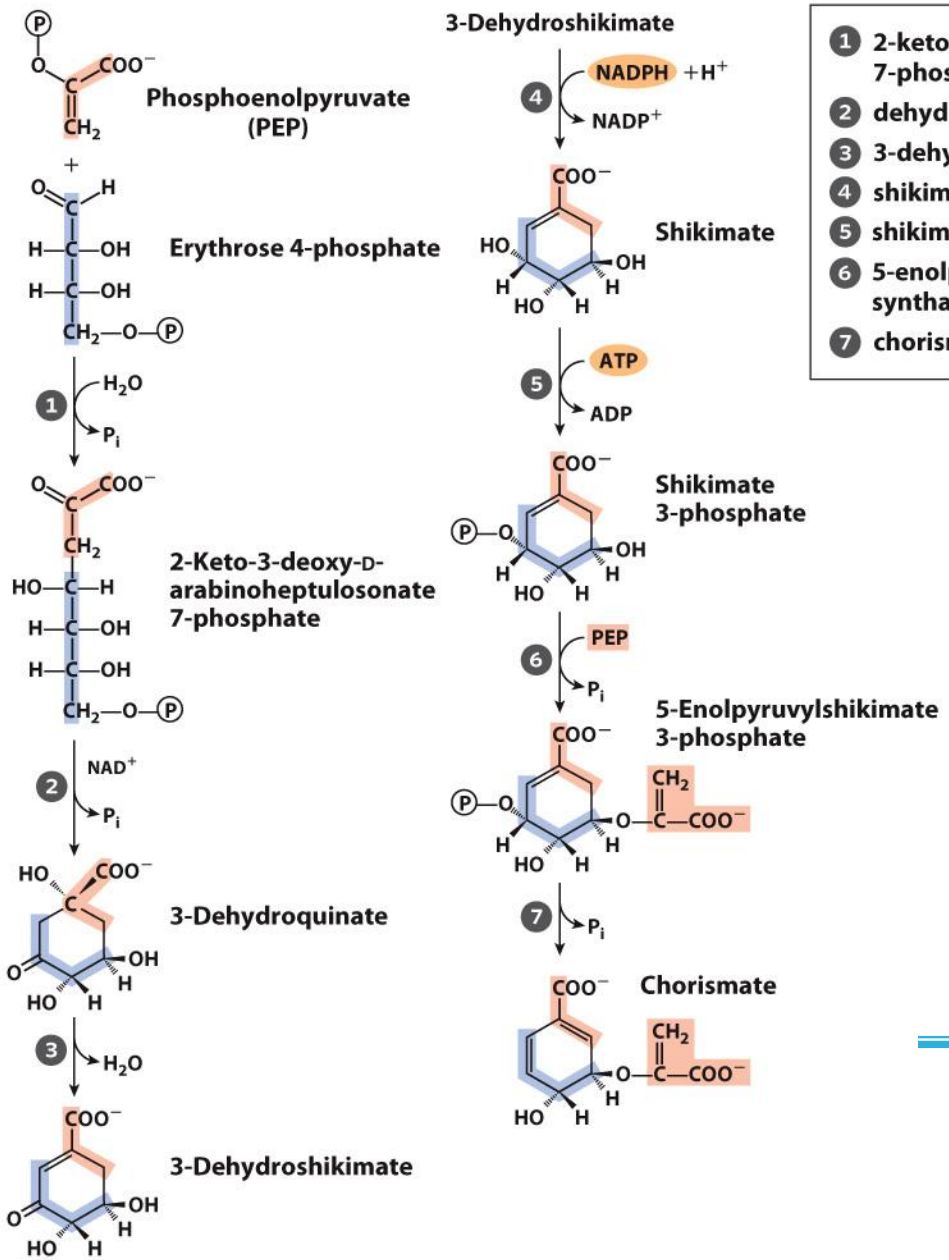

1. 2-keto-3-deoxy-D-arabinoheptulosonate 7-phosphate synthase
2. dehydroquinate synthase
3. 3-dehydroquinate dehydratase
4. shikimate dehydrogenase
5. shikimate kinase
6. 5-enolpyruvylshikimate 3-phosphate synthase
7. chorismate synthase

Biosynthesis of Chorismate, a Key Intermediate in Aromatic Amino Acid Biosynthesis

Figure 22-18
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His Derives from PPP Metabolite Ribose 5-Phosphate

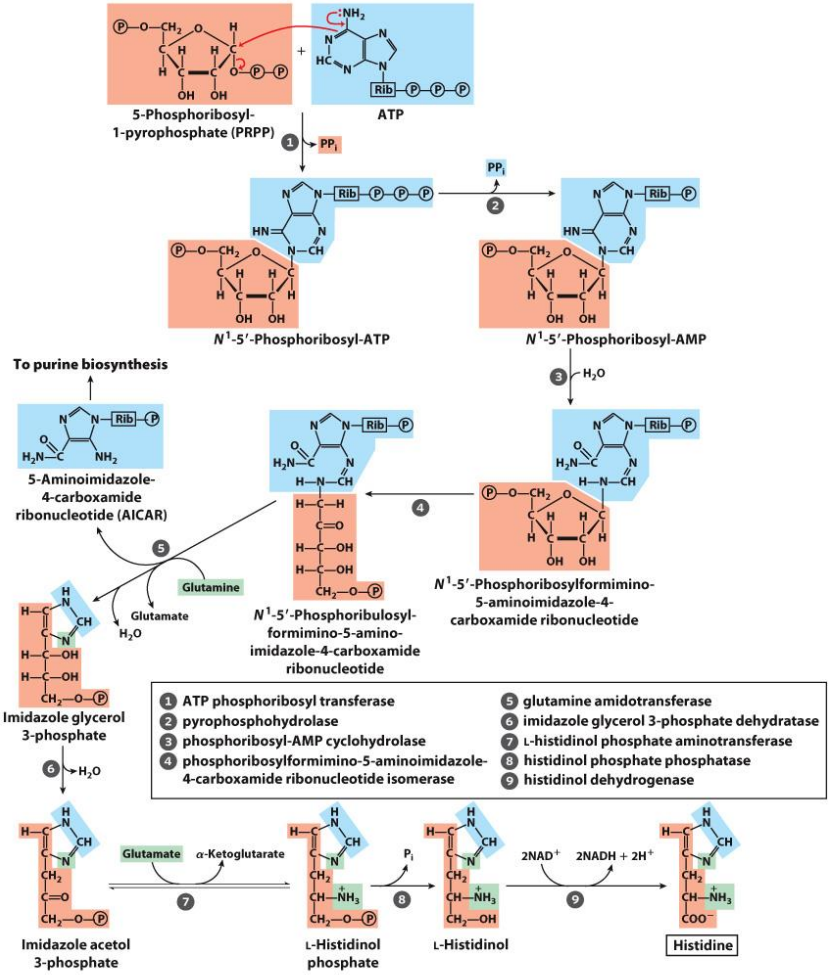

Figure 22-22

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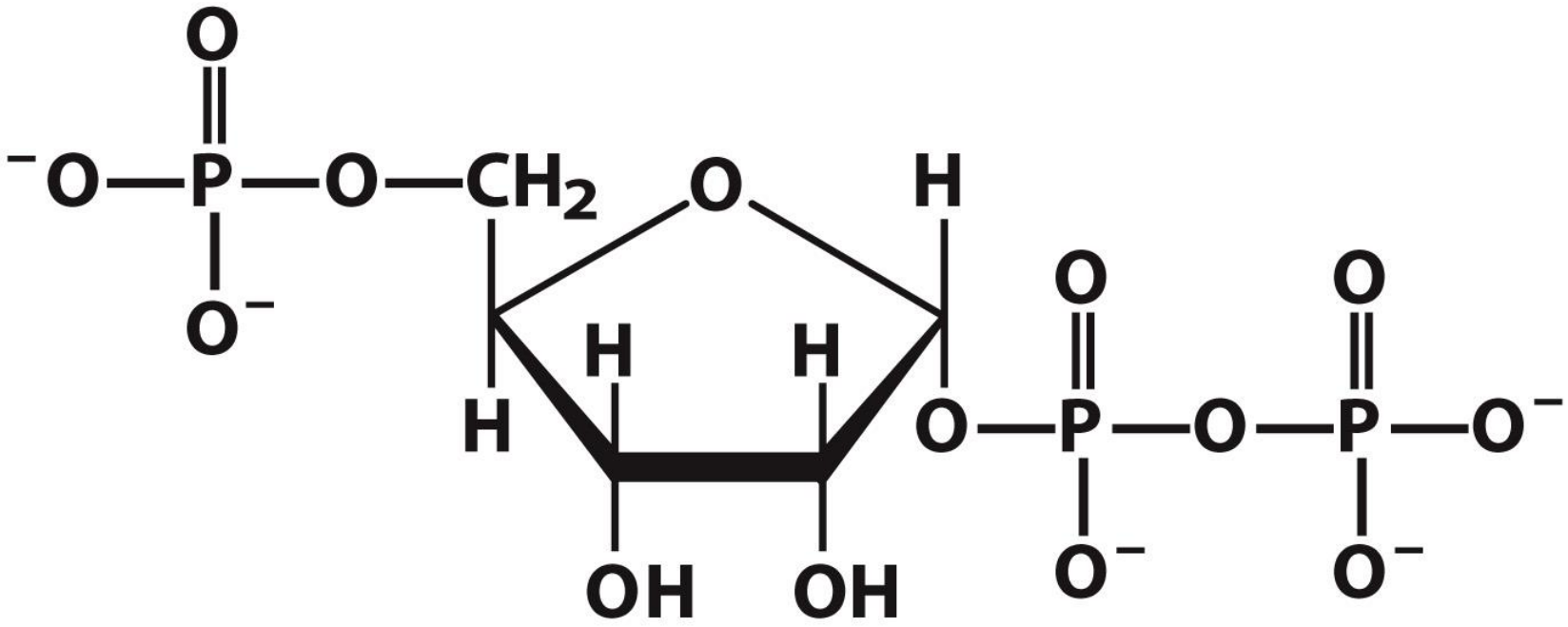
Several Pathways Share 5-Phosphoribosyl-1-Pyrophosphate (PRPP) as an Intermediate

- Synthesized from ribose 5-phosphate of PPP via *ribose phosphate pyrophosphokinase*
  - a highly regulated allosteric enzyme



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Regulation of Amino Acid Biosynthesis

- Multilayered approach: Often, more than one mechanism of regulation is utilized.
  - feedback inhibition of products
  - use of isozymes for regulation of specific pathways

Feedback Inhibition in Ile Synthesis from Thr

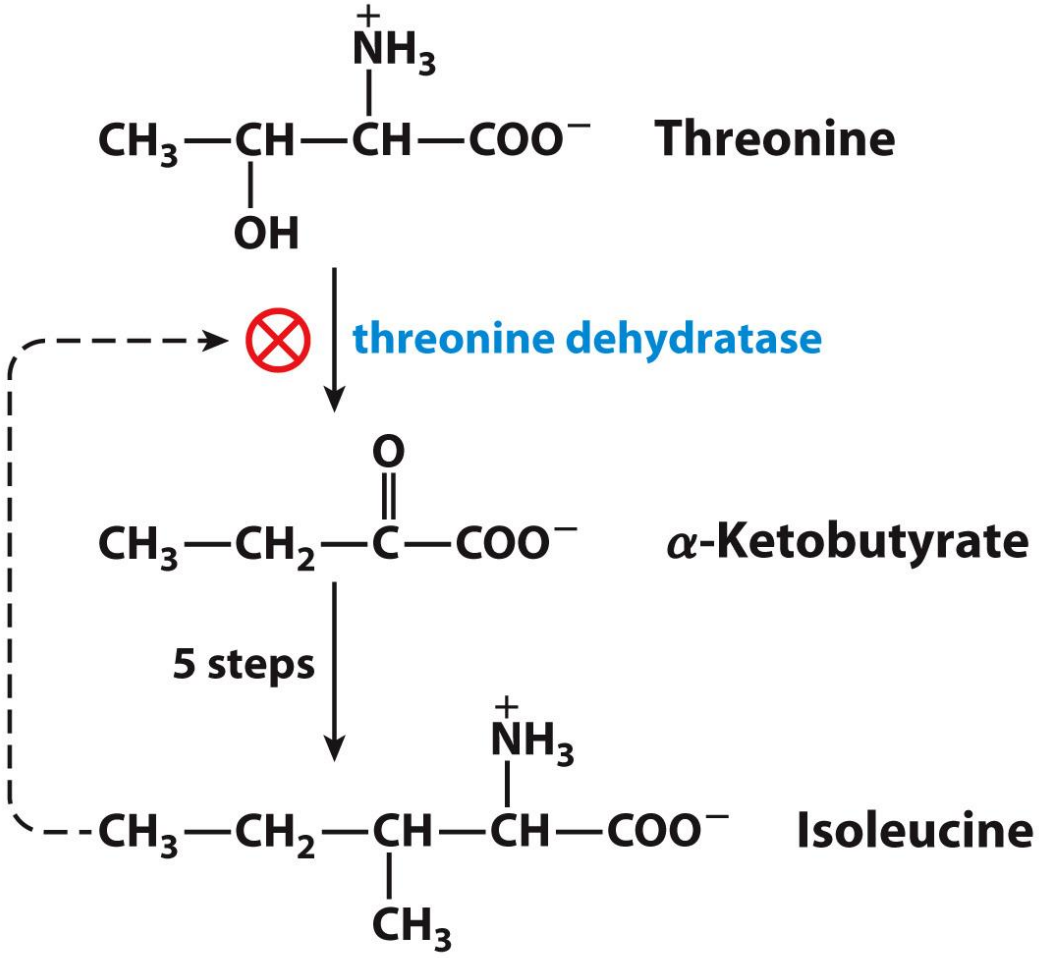

Figure 22-23
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Use of Isozymes Is Another Important Means of Regulation

Example: Asp can lead to Lys, Met, Thr, and Ile. Use of isozymes, all regulated by different effectors, allows *E. coli* to produce the amino acids when needed.

- Example: At step 1, isozyme A1 is inhibited if Ile is high, but not if Met or Thr are high.
- Only the A1 isozyme is inhibited by Ile at this step.

Important Metabolites Are Derived From Amino Acids

- Porphyrin rings (e.g., heme)
- Phosphocreatine
- Glutathione
- Neurotransmitters and signaling molecules
- Cell-wall constituents

Glycine or Glutamate Is the Precursor to Porphyrins

- Porphyrin makes up the heme of hemoglobin, cytochromes, myoglobin.
- In higher animals, porphyrin arises from reaction of glycine with succinyl-CoA.
  - In plants and bacteria, glutamate is the precursor.
- The pathway generates two molecules of the important intermediate ***δ-aminolevulinate***.
- ***Porphobilinogen*** is another important intermediate.

Synthesis of δ-Aminolevulinate in Higher Eukaryotes

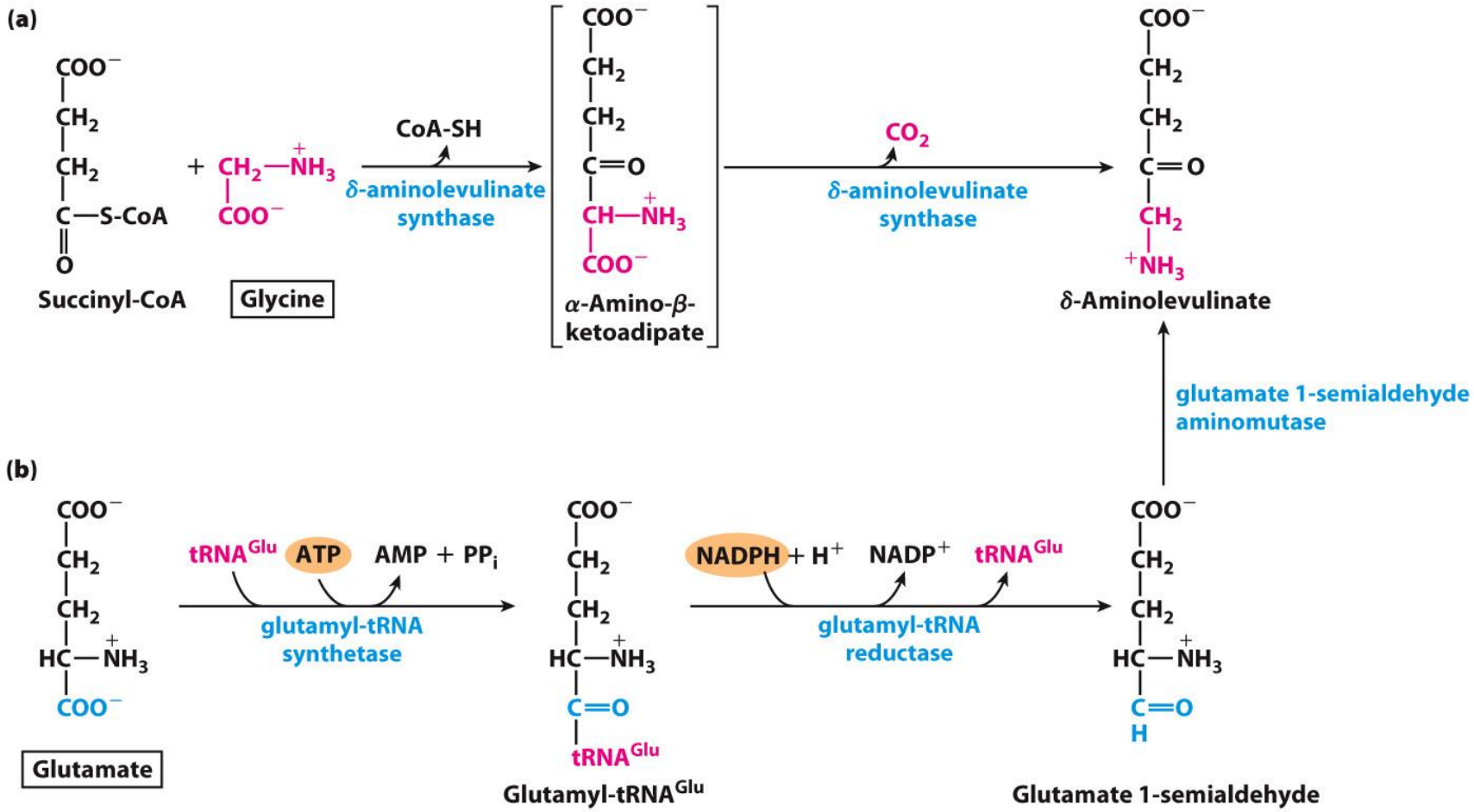

Figure 22-25
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Synthesis of Heme from δ-Aminolevulinate

1. Two molecules of δ-aminolevulinate condense to form porphobilinogen.
2. Four molecules of porphobilinogen combine to form protoporphyrin.
3. Fe ion is inserted into protoporphyrin with the enzyme *ferrochelatase*.

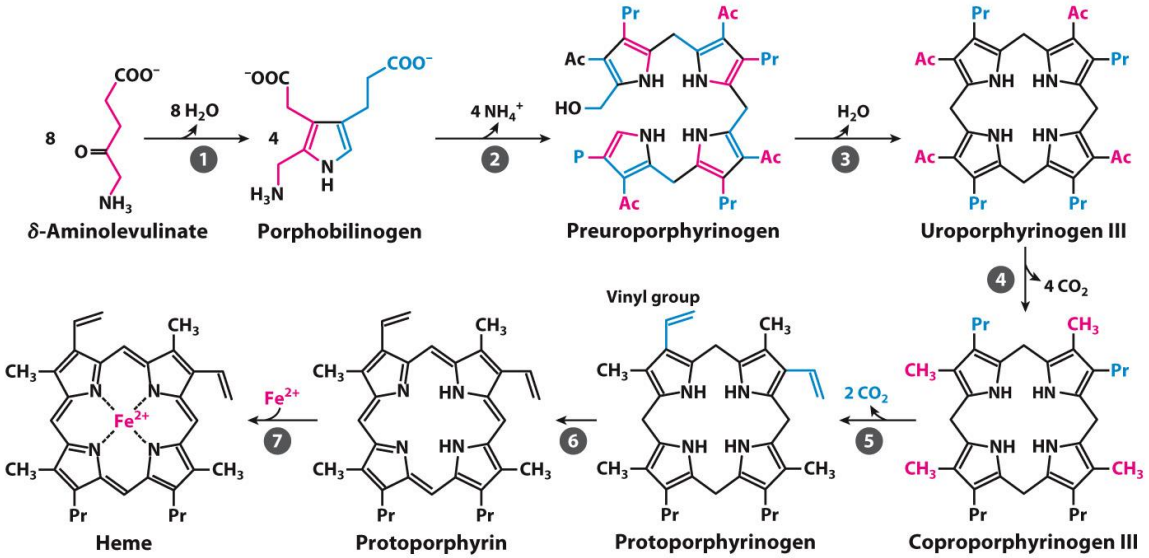

Figure 22-26
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Defects in Heme Biosynthesis

- Most animals synthesize their own heme.
- Mutations or misregulaton of enzymes in the heme biosynthesis pathway lead to porphyrias.
  - Precursors accumulate in red blood cells, body fluids, and liver.
- Accumulation of precursor uroporphyrinogen I
  - Urine becomes discolored (pink to dark purplish depending on light, heat exposure).
  - Teeth may show red fluorescence under UV light.
  - Skin is sensitive to UV light.
  - There is a craving for heme.
- Explored as possible biochemical basis for vampire myths

Heme Is the Source of Bile Pigments

- Heme from degradation of erythrocytes is degraded to **bilirubin** in two steps:
  1. *Heme oxygenase* linearizes heme to create **biliverdin**, a green compound (seen in a bruise).
  2. *Biliverdin reductase* converts biliverdin to **bilirubin,** a yellow compound that travels bound to serum albumin in the bloodstream.
     - major pigment of urine (degradation to urobilin)
     - further degraded by intestinal microbiota to stercobilin

Formation and Breakdown of Bilirubin

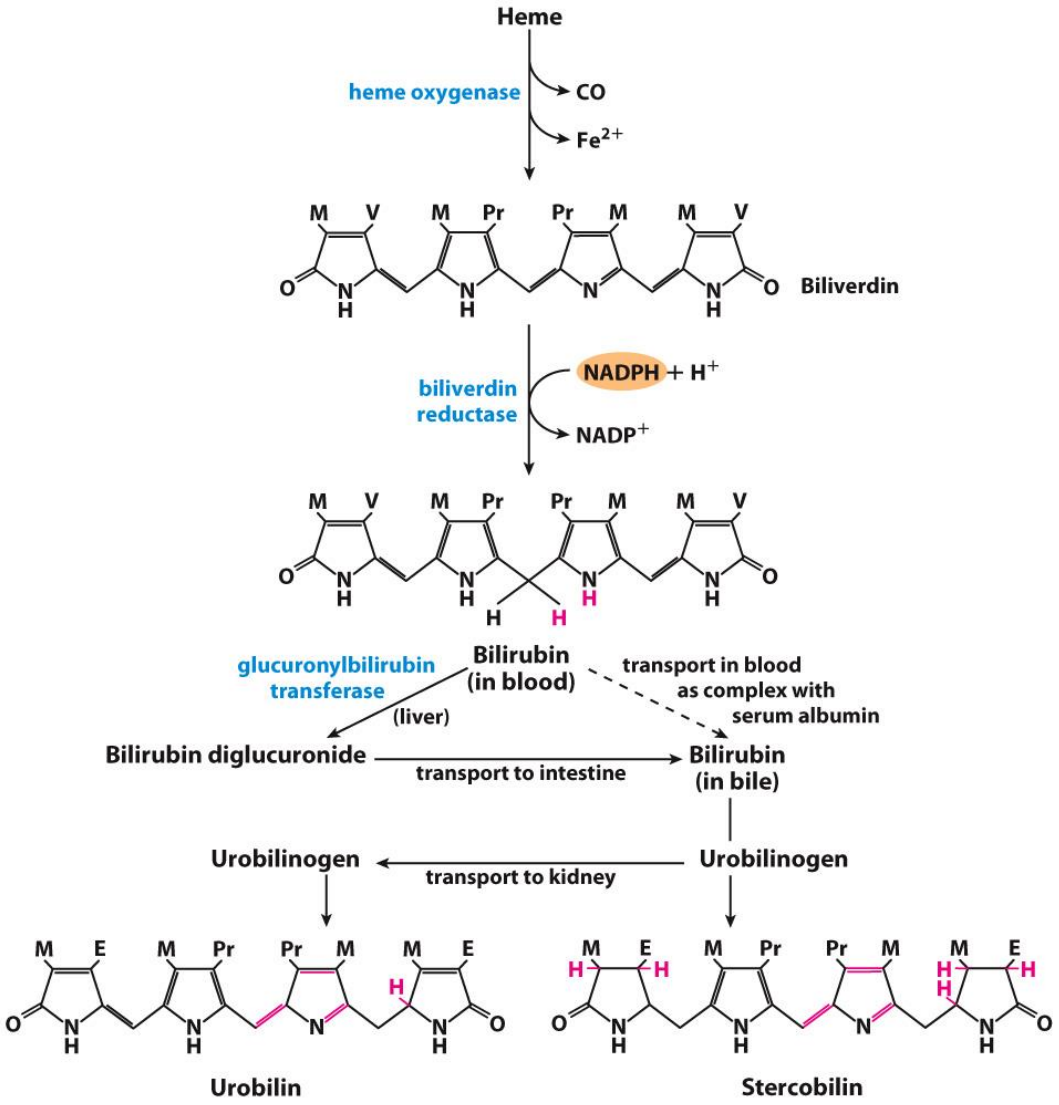

Figure 22-27

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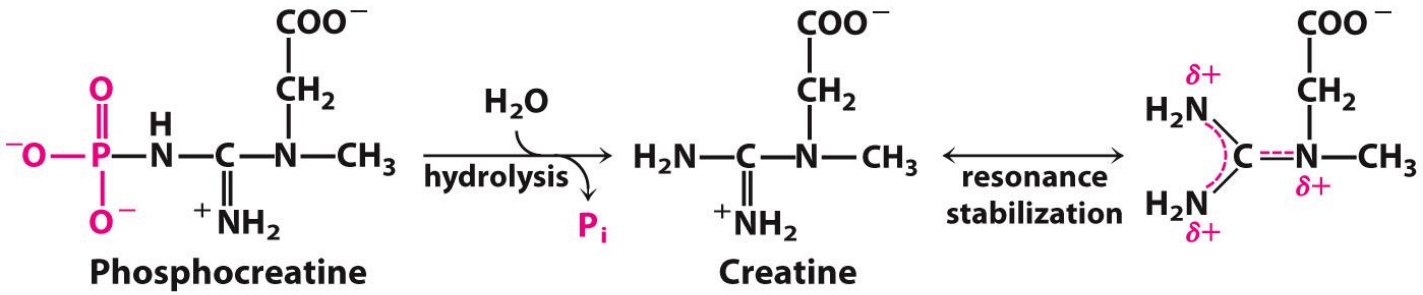
Jaundice Is Caused by Bilirubin Accumulation

- Jaundice (yellowish pigmentation of skin, whites of eyes, etc.) can result from:
  - impaired liver (in liver cancer, hepatitis)
  - blocked bile secretion (due to gallstones, pancreatic cancer)
  - insufficient *glucouronyl bilirubin transferase* to process bilirubin (occurs in infants)
    - treated with UV to cause photochemical breakdown of bilirubin

Gly and Arg Are Precursors of Creatine and Phosphocreatine

- Phosphocreatine is hydrolyzed for energy in muscle.



$$\text{Phosphocreatine}^{2-} + H_2O \longrightarrow \text{creatine} + HPO_4^{2-}$$
$$\Delta G'^{\circ} = -43.0\ \text{kJ/mol}$$

Figure 13-15
Lehninger Principles of Biochemistry, Seventh Edition

- Gly and Arg combine, then *S*-adenosyl-methionine (Ado-Met) acts as a methyl donor.

Biosynthesis of Creatine and Phosphocreatine

Requires glycine, arginine, and *S*-adenosyl-methionine

Phosphocreatine can be phosphorylated by ATP for use as a stored energy source.

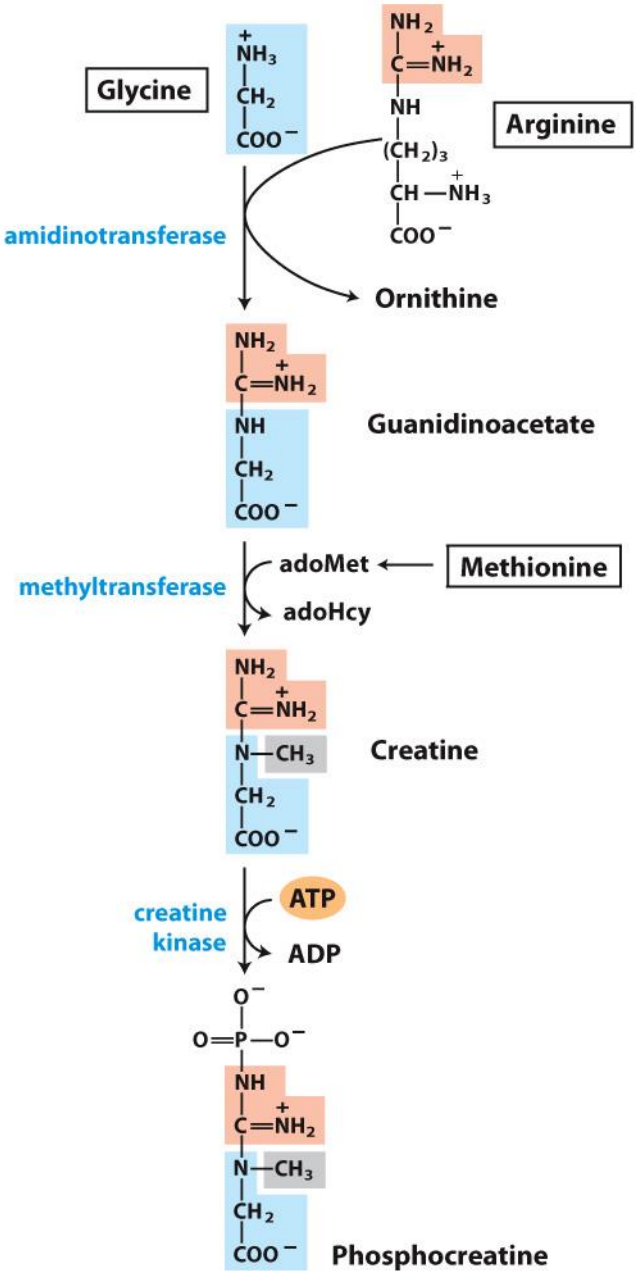


Figure 22-28
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Glutathione (GSH) Derives from Glu, Cys, and Gly

- GSH is present in most cells at high amounts.
- Reducing agent/antioxidant
  - keeps proteins, metal cations reduced
  - keeps redox enzymes in reduced state
  - removes toxic peroxides
- Oxidized to a dimer using disulfide bond (GSSG)

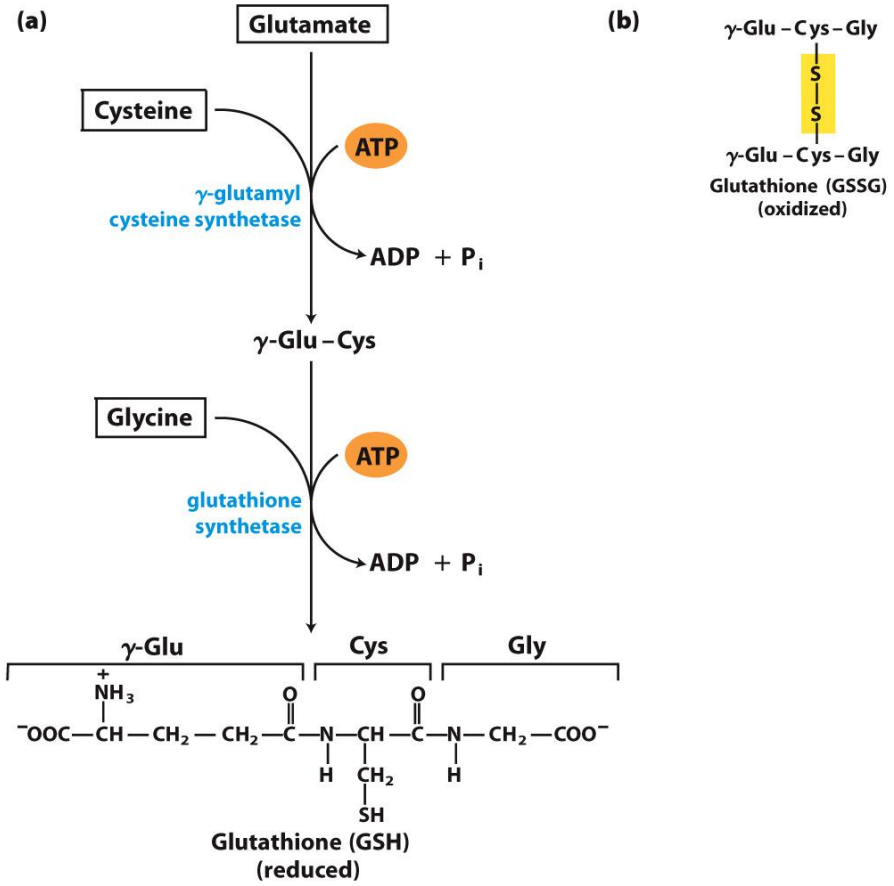

Figure 22-29
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Some Neurotransmitters Are Derived from Amino Acids

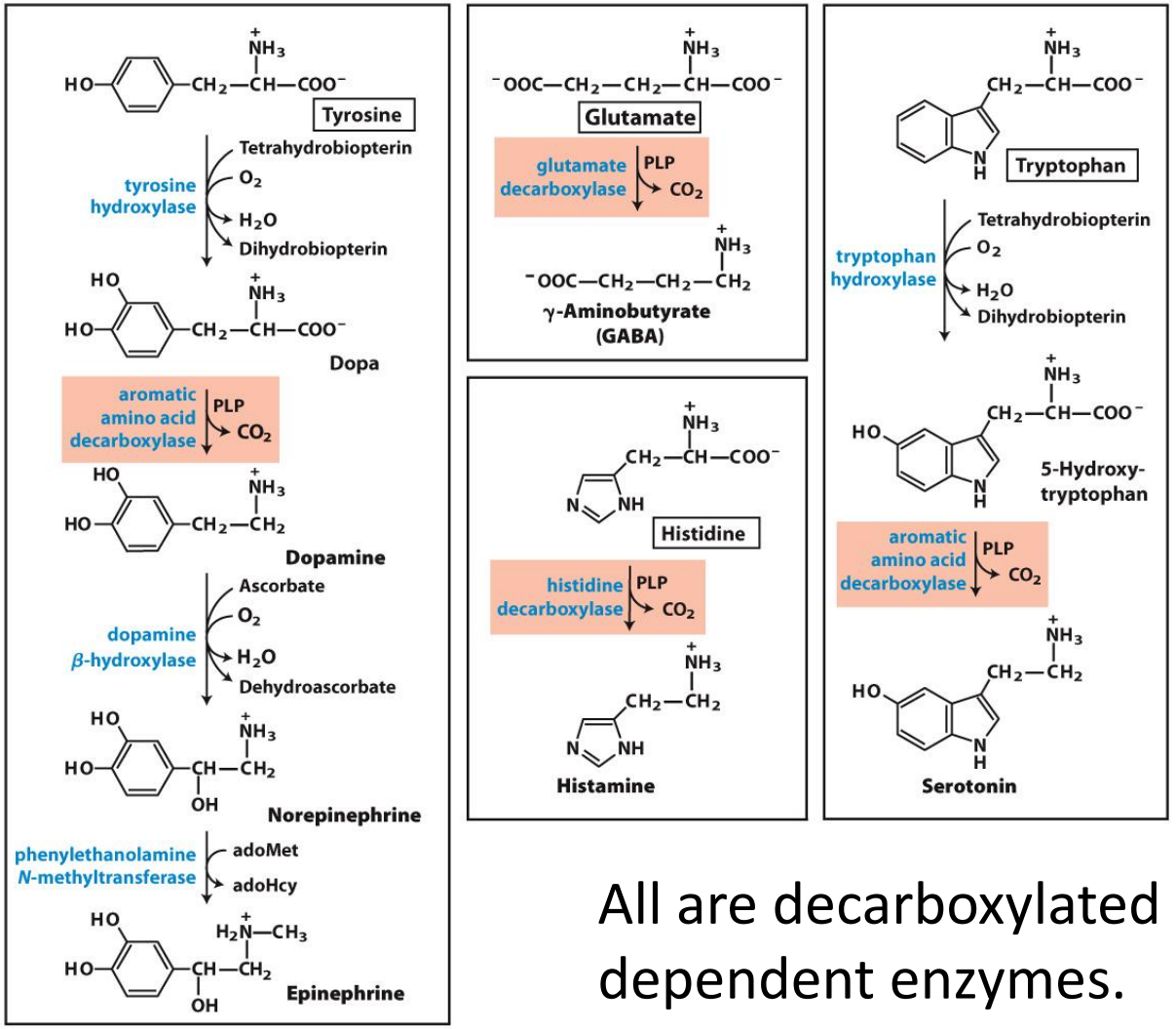

Figure 22-31
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All are decarboxylated using PLP dependent enzymes.

Nucleotide Biosynthesis

- Nucleotides can be synthesized de novo ("from the beginning") from amino acids, ribose-5-phosphate, CO_2, and NH_3.
- Nucleotides can be salvaged from RNA, DNA, and cofactor degradation.
- Many parasites (e.g., malaria) lack de novo biosynthesis pathways and rely exclusively on salvage.
  - Compounds that inhibit salvage pathways are promising antiparasite drugs.

De Novo Biosynthesis of Nucleotides

- Approximately the same in all organisms studied
- Bases synthesized *while* attached to ribose
- Glu provides most amino groups.
- Gly is precursor for purines
- Asp is precursor for pyrimidines
- Nucleotide pools are kept low, so cells must continually synthesize them.
  - This synthesis may actually limit rates of transcription and replication.

Origin of Ring Atoms in Purines

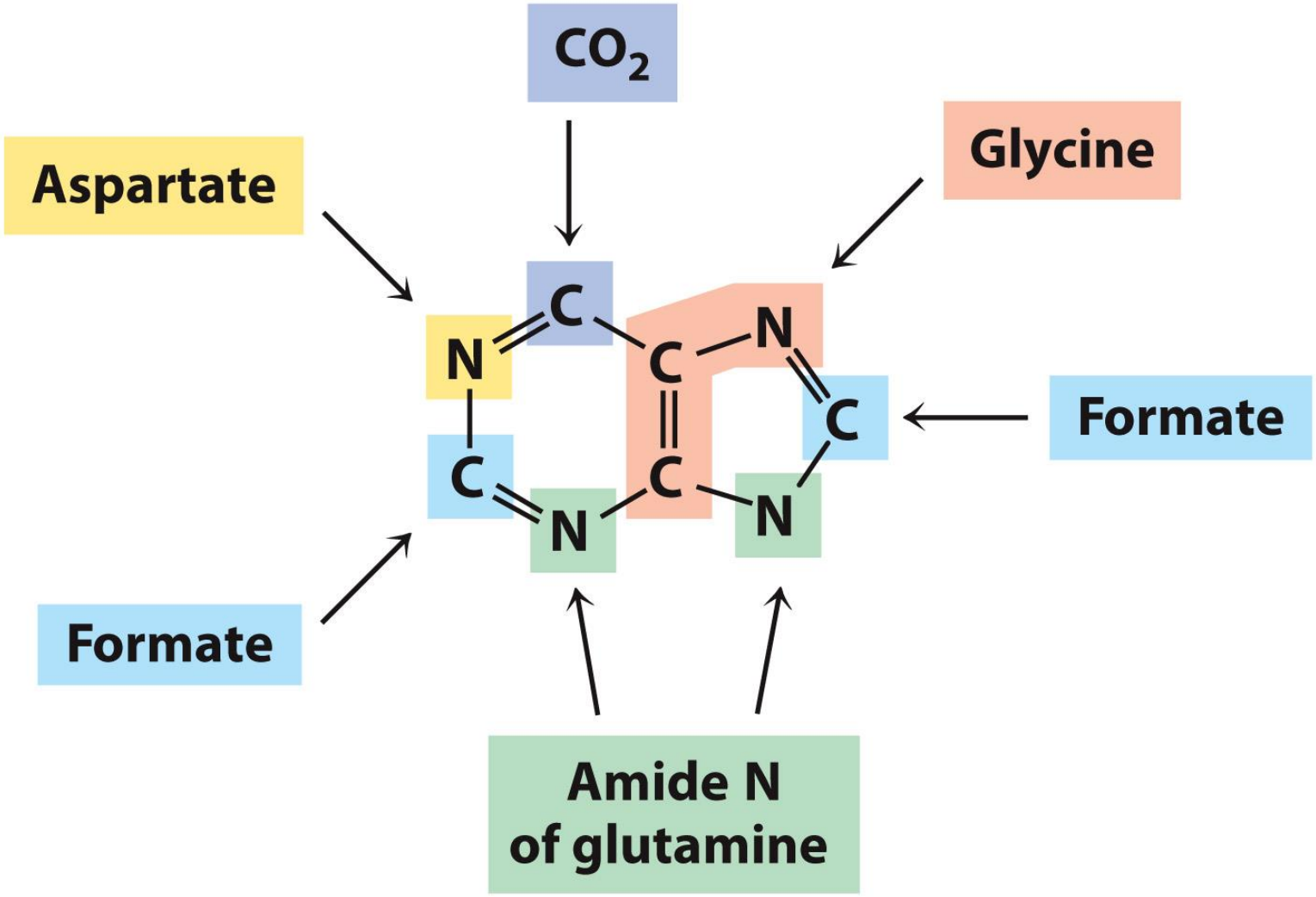

Figure 22-34
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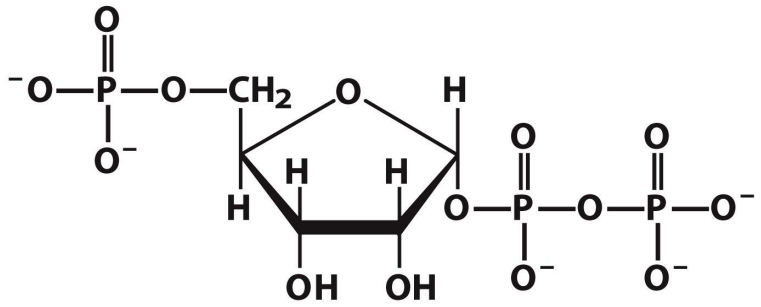
De Novo Biosynthesis of Purines Begins with PRPP

- Adenine and guanine are synthesized as AMP and GMP.
- Synthesis begins with reaction of 5-phosphoribosyl 1-pyrophosphate (PRPP) with Glu.
- Purine ring builds up following the addition of three carbons from glycine.
- The first intermediate with a full purine ring is **inosinate (IMP).**



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Regulation of Purine Biosynthesis in *E. coli* Largely Consists of Feedback Inhibition

Four Major Mechanisms

1. *Glutamine-PRPP amidotransferase* is inhibited by end-products IMP, AMP, and GMP.
2. Excess GMP inhibits formation of xanthylate from inosinate by *IMP dehydrogenase.*
3. GMP and AMP concentrations inhibit phosphorylation steps.
4. PRPP synthesis is inhibited by ADP and GDP.

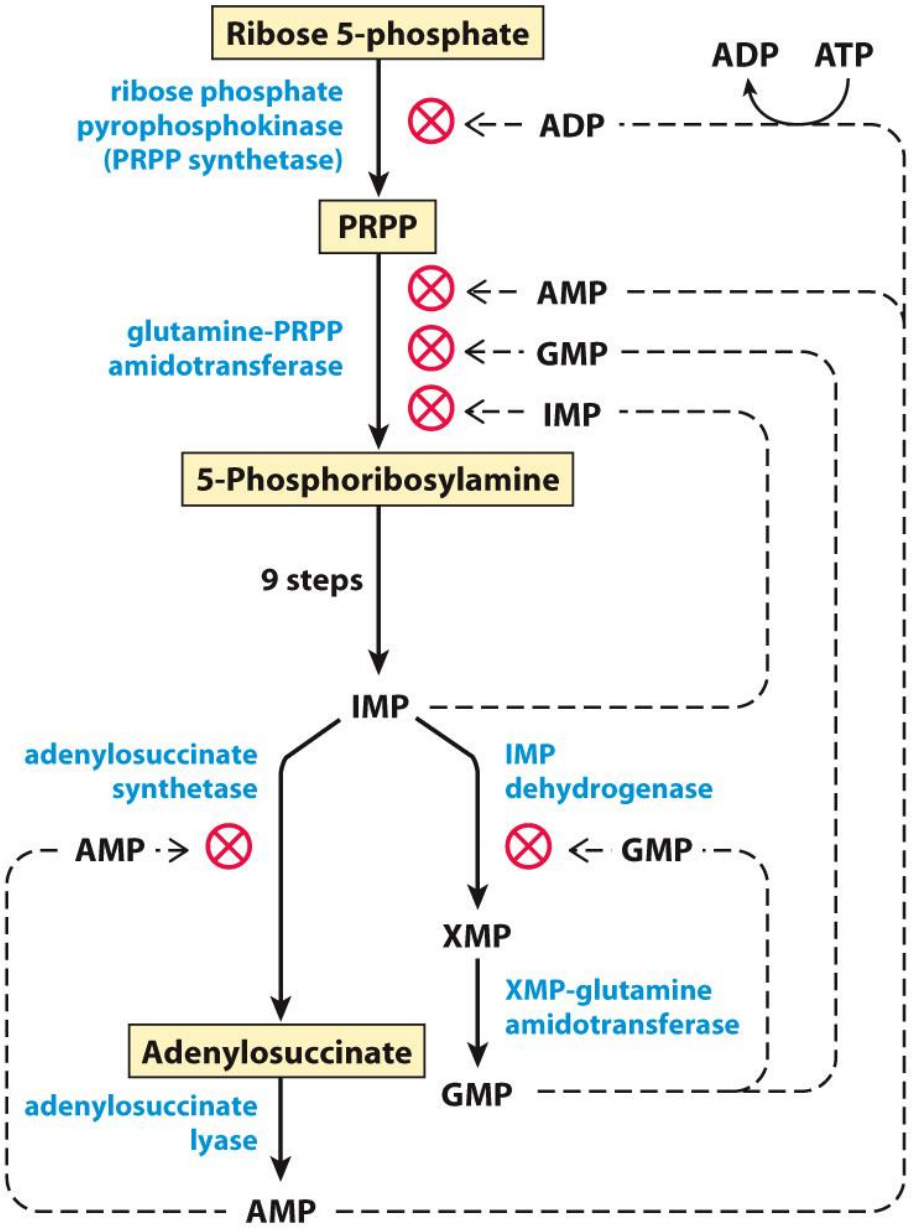

Figure 22-37
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De Novo Synthesis of Pyrimidine Nucleotides (1)

Unlike purine synthesis, pyrimidine synthesis proceeds *by first making the pyrimidine ring* (in the form of orotate) and *then* attaching it to ribose 5-phosphate.

Aspartate and carbamoyl phosphate provide the atoms for the ring structure.

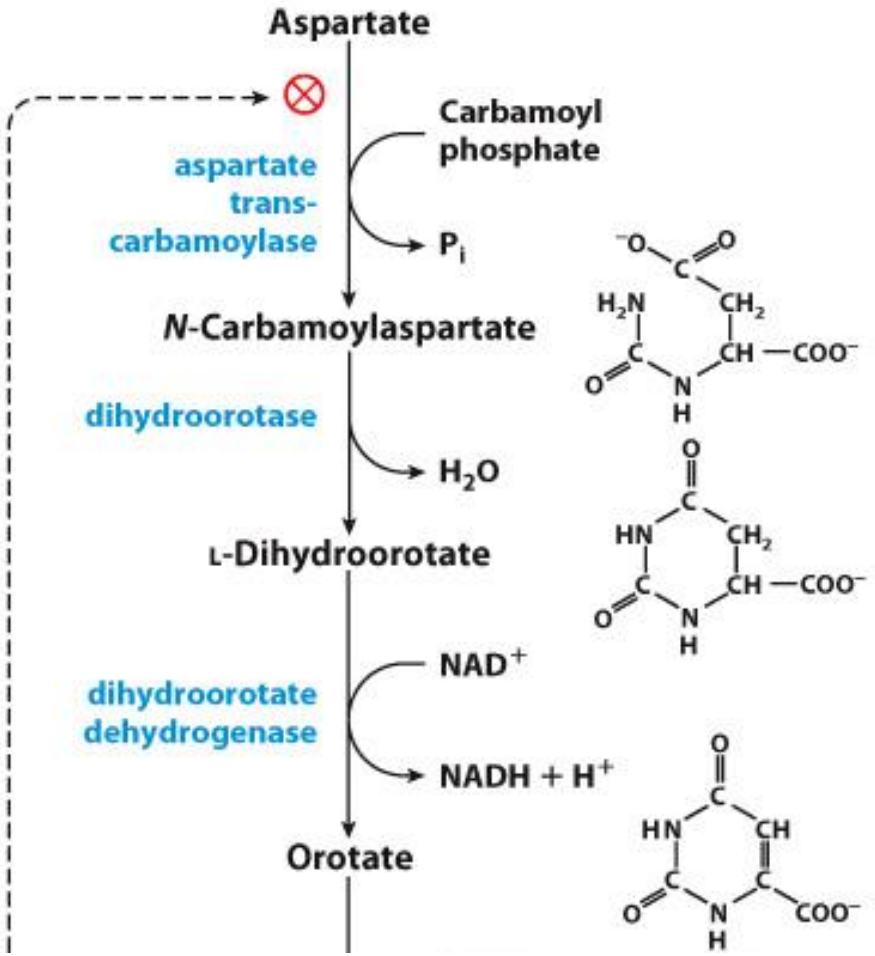

Figure 22-38
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De Novo Synthesis of Pyrimidine Nucleotides (2)

After addition of ribose-5-phosphate via PRPP, the resulting nucleotide (orotidylate) is decarboxylated to form uridylate (UMP), the first possible pyrimidine.

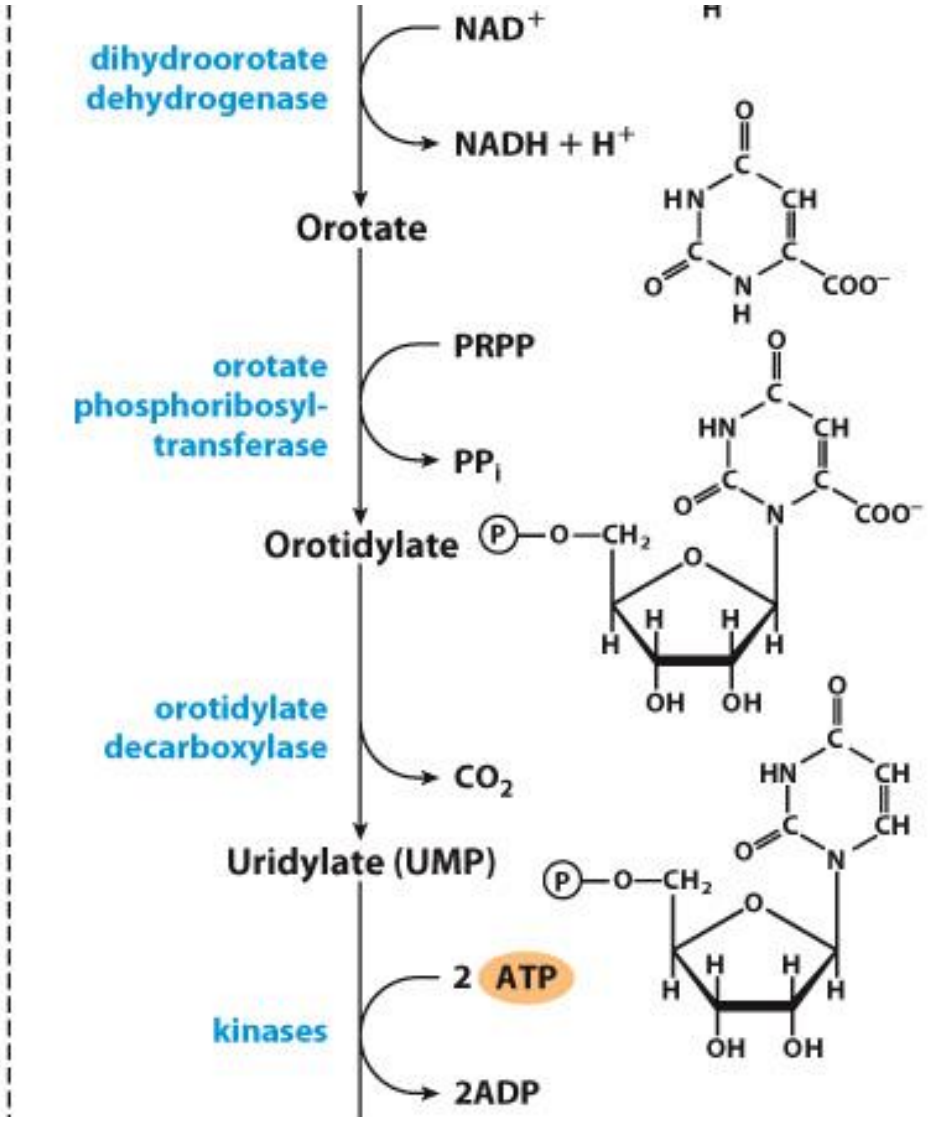

Figure 22-38
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De Novo Synthesis of Pyrimidine Nucleotides (3)

UMP is phosphorylated to UTP.

After formation of UTP, amination can convert UTP to CTP.

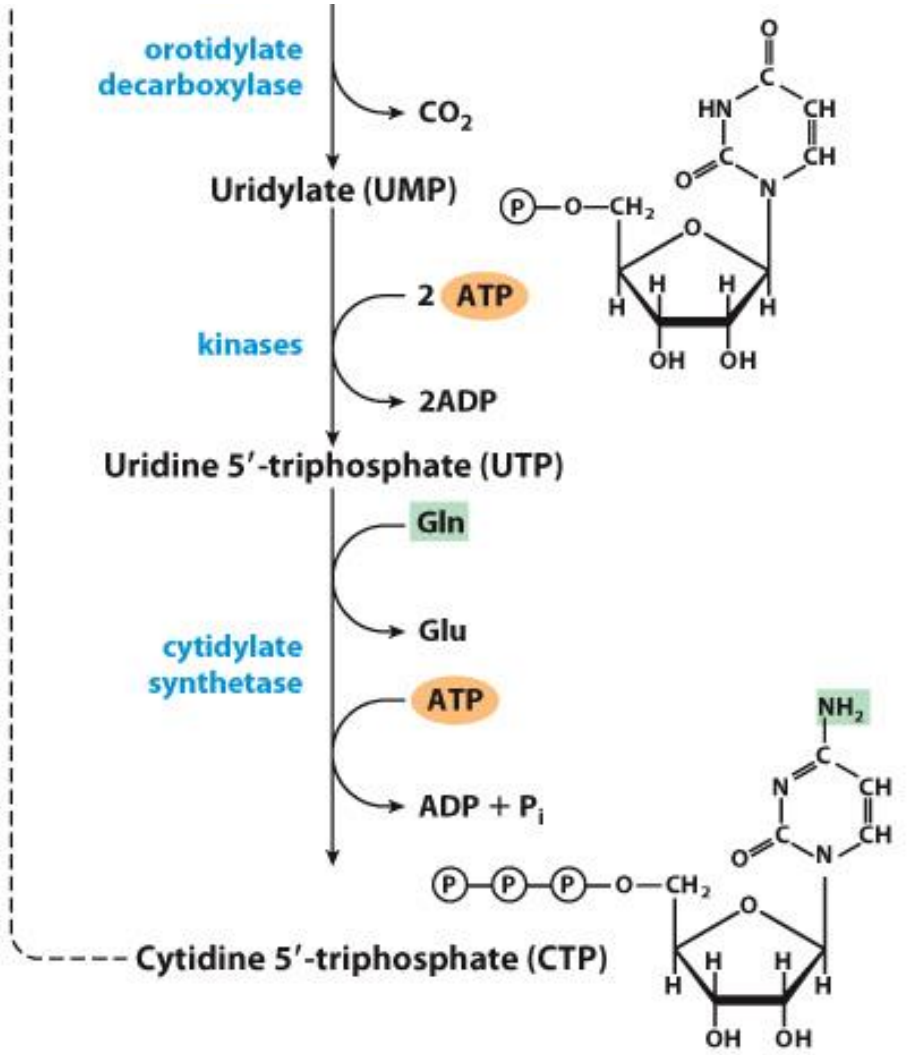

Figure 22-38

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Regulation of Pyrimidine Biosynthesis Is Also via Feedback Inhibition

- ATCase is inhibited by end-product CTP and is accelerated by ATP.

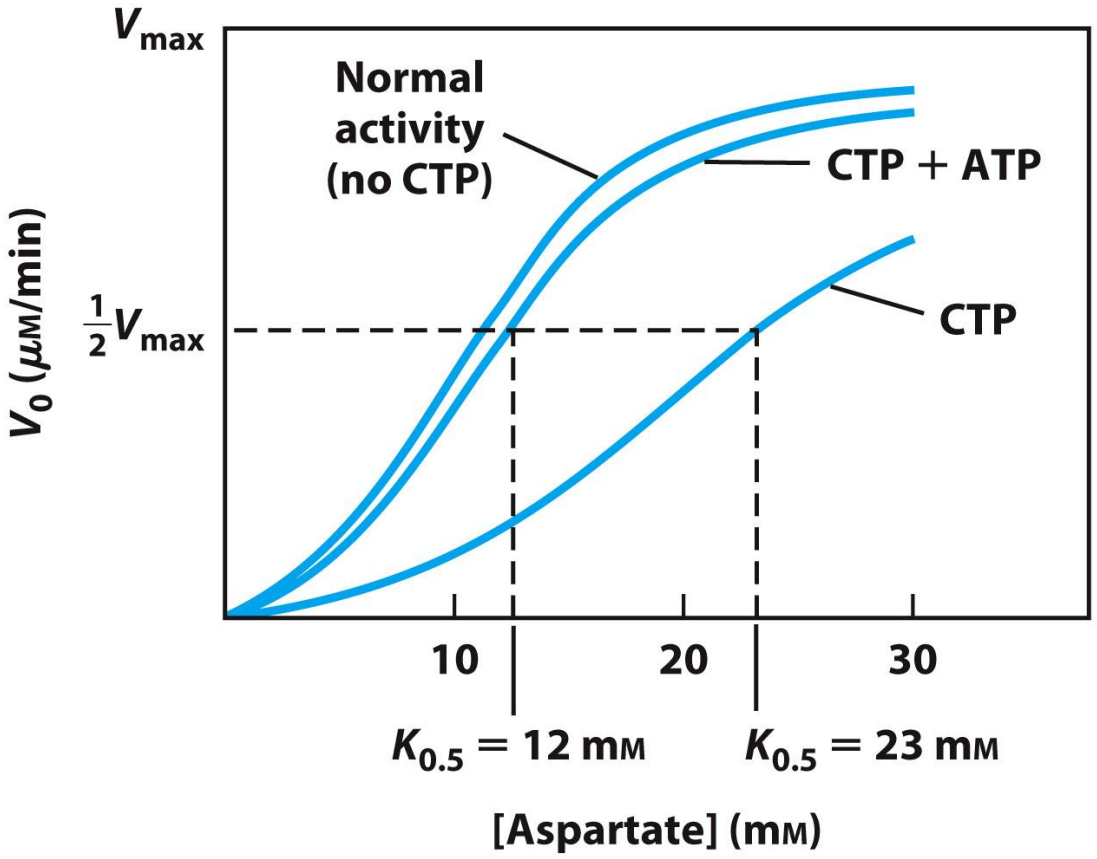

Figure 22-40
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Ribonucleotides Are Precursors to Deoxyribonucleotides

- 2'C-OH bond is directly reduced to 2'-H bond… without activating the carbon!
  - catalyzed by *ribonucleotide reductase*
- Mechanism: Two H atoms are donated by NADPH and carried by proteins thioredoxin or glutaredoxin.

Folic Acid Deficiency Leads to Reduced Thymidylate Synthesis

- Folic acid deficiency is widespread, especially in nutritionally poor populations.
- Reduced thymidylate synthesis causes uracil to be incorporated into DNA.
- Repair mechanisms remove the uracil by creating strand breaks that affect the structure and function of DNA.
  - associated with cancer, heart disease, neurological impairment

Catabolism of Purines

1. Dephosphorylation (via *5'-nucleotidase*)
2. Deamination and hydrolysis of ribose lead to production of xanthine.
3. Hypoxanthine and xanthine are then oxidized into uric acid by ***xanthine oxidase.***

Spiders and other arachnids lack xanthine oxidase.

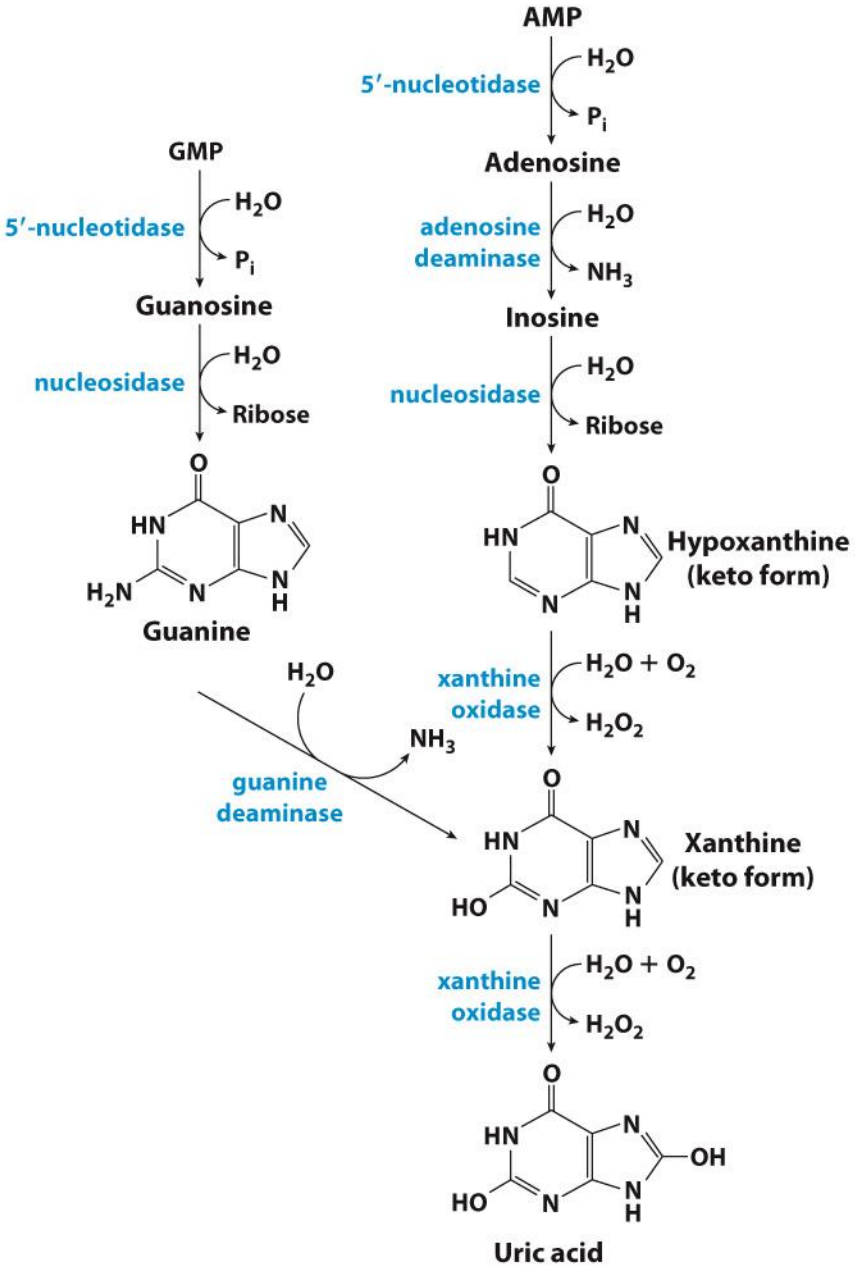

Figure 22-48 part 1
Lehninger Principles of Biochemistry, Seventh Edition

Conversion of Uric Acid to Allantoin, Allantoate, and Urea

Degree of further oxidation of uric acid is organism dependent.

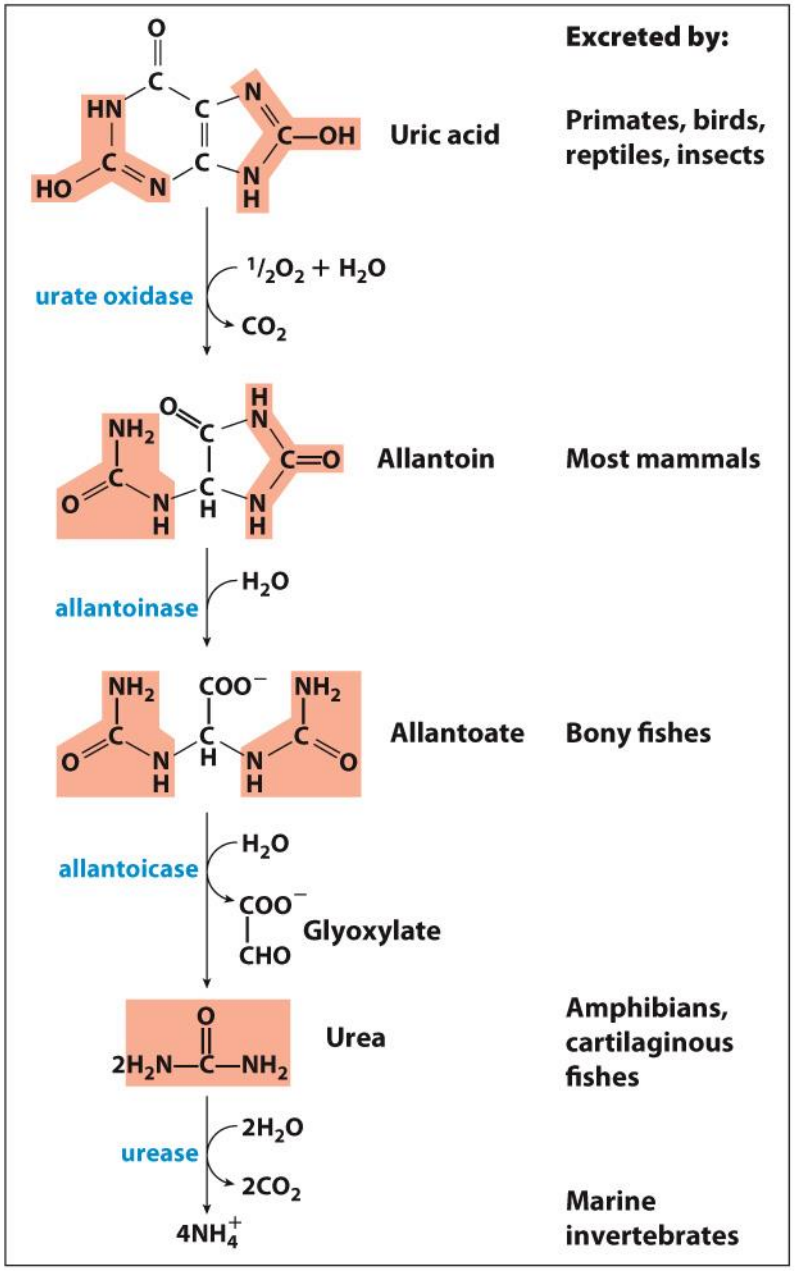

Figure 22-48 part 2
Lehninger Principles of Biochemistry, Seventh Edition

Excess Uric Acid Seen in Gout

- Painful joints (often in toes) due to deposits of sodium urate crystals
- Primarily affects males
- May involve genetic under-excretion of urate and/or may involve overconsumption of fructose
- Treated with avoidance of purine-rich foods (seafood, liver) or avoidance of fructose
- Also treated with xanthine oxidase inhibitor **allopurinol**

Allopurinol Inhibits Xanthine Oxidase

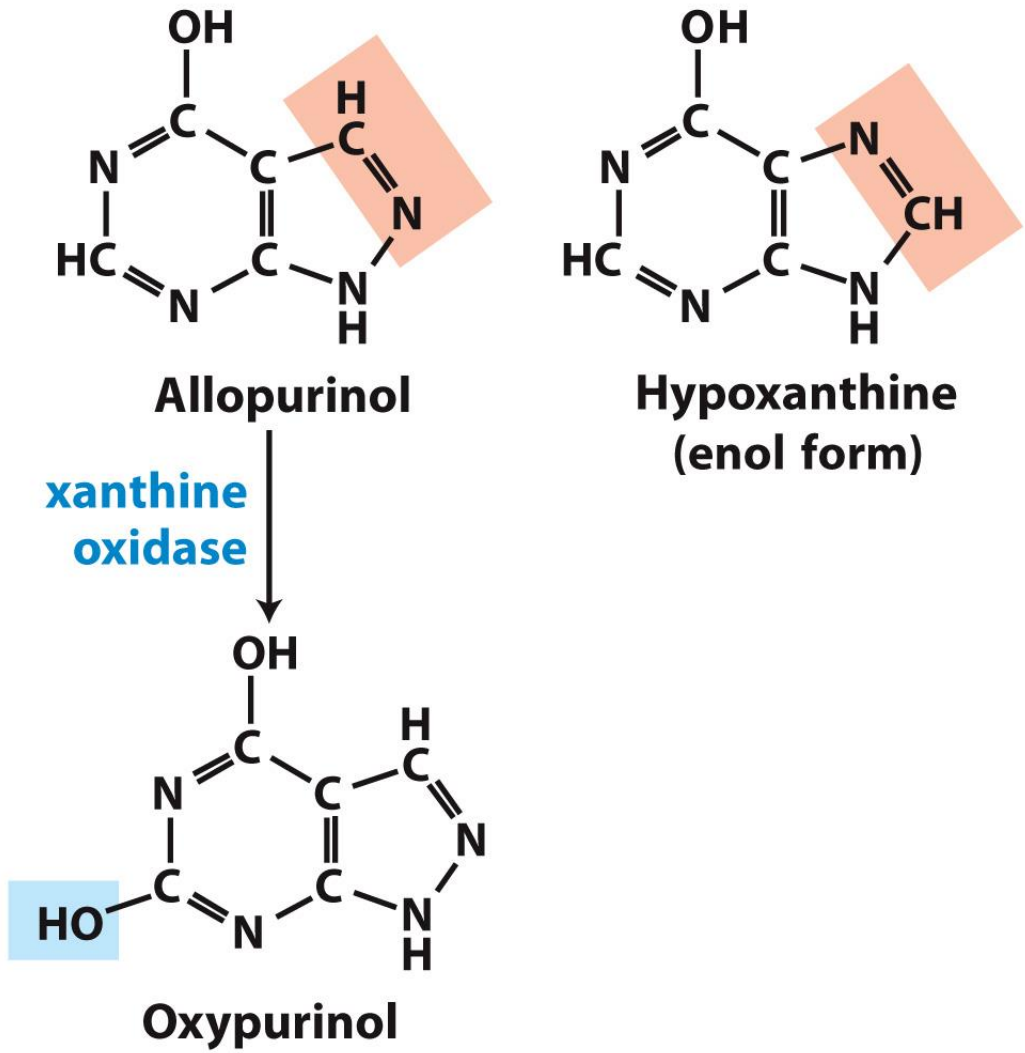

Figure 22-50
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Catabolism of Pyrimidines

- Leads to NH_4^+ and urea
- Can produce intermediates of CAC
  - Example: Thymine is degraded to succinyl-CoA.

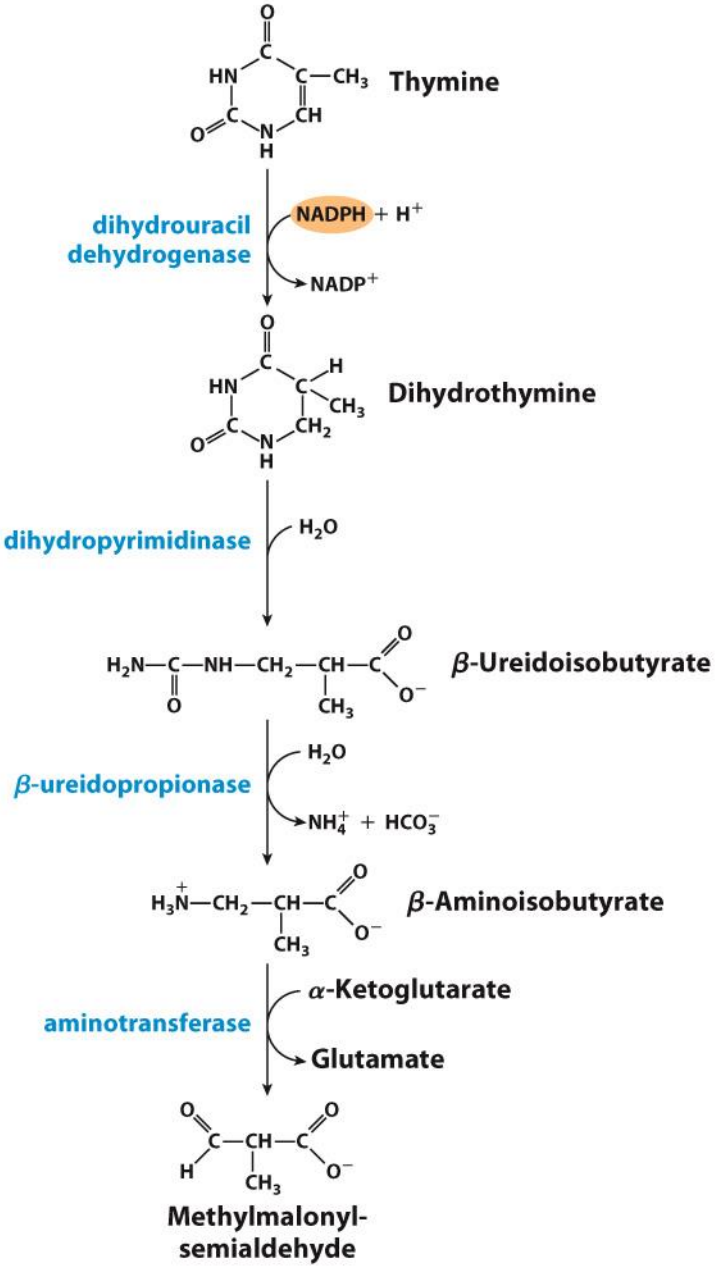

Figure 22-49
Lehninger Principles of Biochemistry, Seventh Edition

Purine and Pyrimidine Bases Are Recycled by Salvage Pathways

- Free bases, released in metabolism, are reused.
  - Example: Adenine reacts with PRPP to form the adenine nucleotide AMP.
    - catalyzed by *adenosine phosphoribosyltransferase*
- The brain is especially dependent on salvage pathways.
- The lack of hypoxanthine-guanine phosphoribosyltransferase leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.

Many Chemotherapeutic Agents Target Nucleotide Biosynthesis

- Glutamine analogs: azaserine, acivicin
  - inhibit glutamine amidotransferases
- Fluorouracil
  - converted by salvage pathway into FdUMP, which inhibits thymidylate synthase
- Methotrexate and aminopterin
  - inhibit dihydrofolate reductase (competitive inhibitors)

Antibiotics Also Target Nucleotide Biosynthesis

- Allopurinol, and so on
  - studied against African sleeping sickness (*trypanosomiasis*) because the trypanosomes lack enzymes for de novo nucleotide synthesis
- Trimethoprim
  - inhibits bacterial dihydrofolate reductase but binds human enzyme several orders of magnitude less strongly