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Amino acid metabolism - 2

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Objectives

- *Essential and nonessential amino acids*
- *Transmination is an important step*
- Amino acid families
- Regulation of amino acid biosynthesis

Textbook Chap 18

Essential and Nonessential Amino acids (recap)

- Plants and microorganisms can synthesize all 20 amino acids.
- Humans synthesize only 10/20 common amino acids – “nonessential”
- What we cannot make comes from food – “essential”
  - Pathways to synthesize these have been lost due to evolution – we can get them anyway, so why make them?
  - Making them would probably be inefficient.

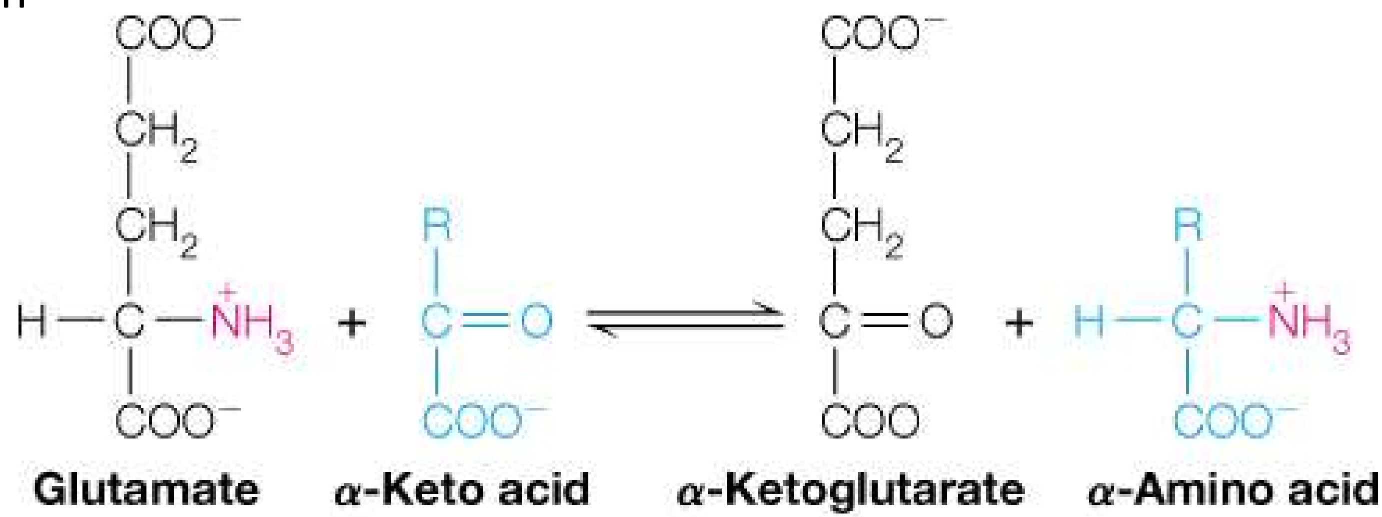
TABLE 18.1 Nutritional requirements for amino acids in mammals

Essential
Arginine,* histidine, isoleucine, leucine, lysine, methionine,* phenylalanine, threonine, tryptophan, valine
Nonessential
Alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, tyrosine**

* Although mammals can synthesize arginine and methionine, the use of these amino acids for the production of urea and methyl groups, respectively, is greater than the capacity of their biosynthetic pathways.
** Tyrosine is considered nonessential because mammals can produce it during phenylalanine degradation via the phenylalanine hydroxylase reaction.

Transamination Reactions in Amino Acid Synthesis

- Amino acid degradation usually begins with conversion to the corresponding α-keto acid by transamination or oxidative deamination
- The α-amino group usually from **glutamate**, is transferred by **transamination** of the corresponding **α-keto acid** to form **α-ketoglutarate** and the **α-amino acid**
- Aminotransferases use a **PLP cofactor**; the reaction has a K_{eq} near 1
- Transamination plays a central role in the citric acid cycle and in ammonia assimilation



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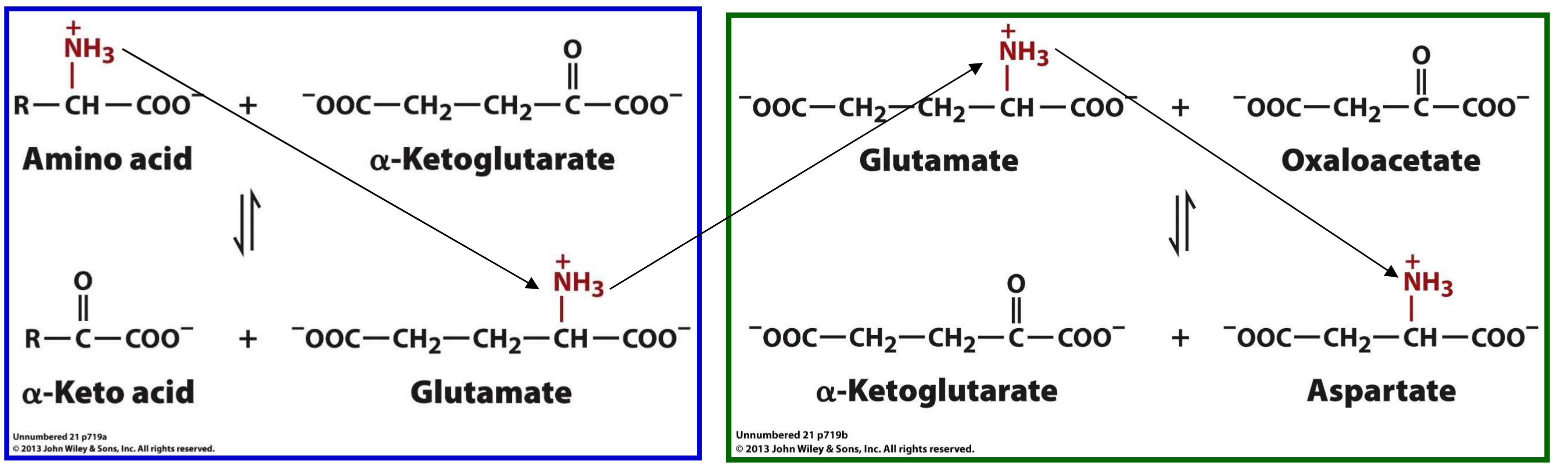
Transaminases use PLP (from Vitamin B_6)

- aka **aminotransferases**: transfer amino group to α-keto acid (predominantly α-ketoglutarate) forming glutamate and an α-keto acid from the aa



- PLP (pyridoxal-5'-phosphate) cofactor – derived from pyridoxine (Vitamin B_6)
- **<u>Plants and microorganisms can synthesize all 20 amino acids</u>** from inorganic forms: NO_3^- and NH_4^+
- ***Animals need preformed glutamate***!

Metabolic Fate of Ammonium Ions

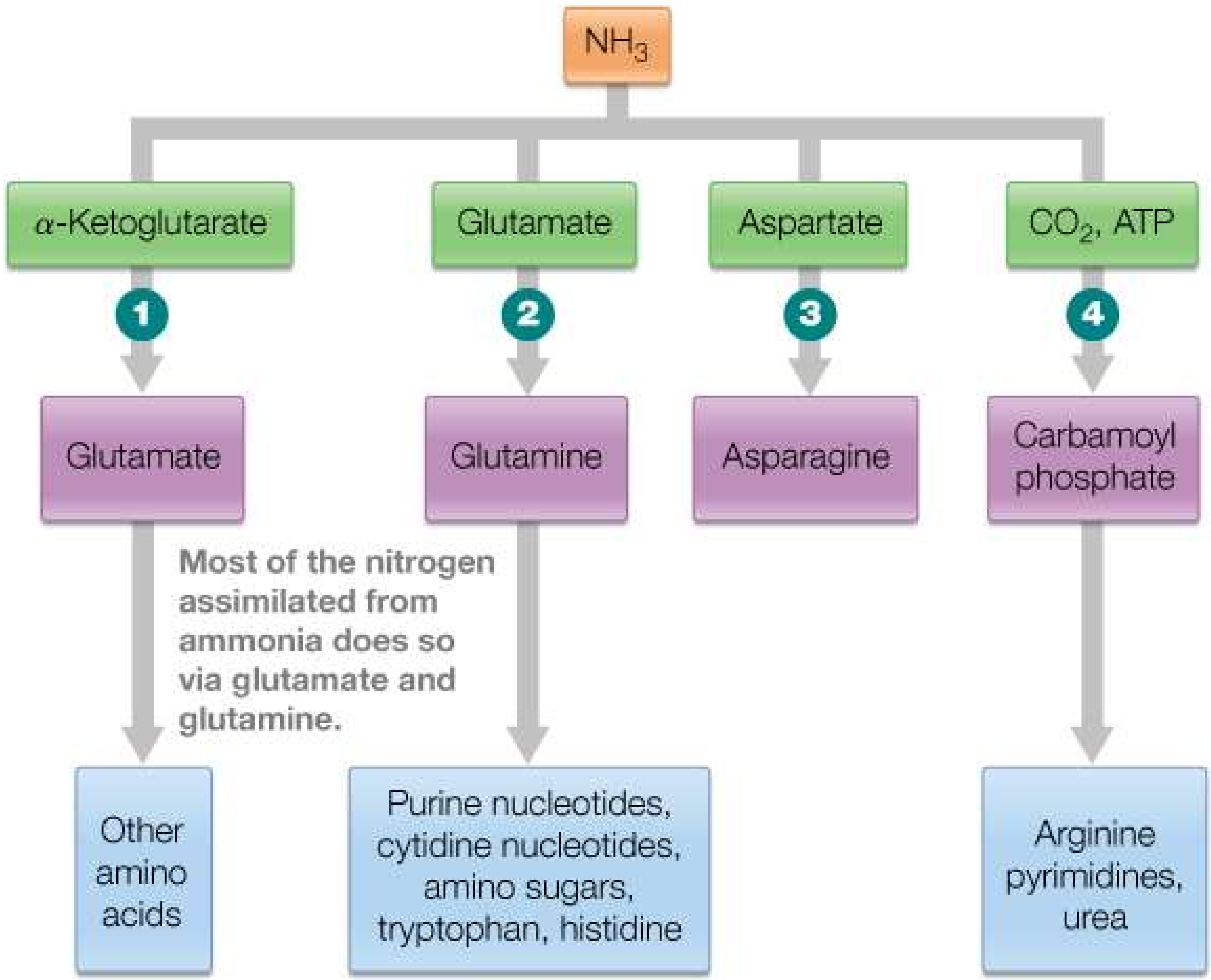
Ammonia is toxic in high concentrations and needs to immediately converted into non-toxic forms.

Four major reactions are

1. **Glutamate dehydrogenase**
   - reductive amination of α-ketoglutarate to form glutamate (reverse reaction to the one in slide 9)
2. **Glutamine synthetase**
   - ATP-dependent amidation of γ-carboxyl of glutamate to glutamine
   - **1** and **2** mostly account for ammonium assimilation into carbon compounds.
3. **Asparagine synthetase**
   - Similar to glutamine synthetase, needing ATP
   - In humans, in organs but is relatively low in tissues other than the exocrine pancreas
4. **Carbamoyl phosphate synthetase I (same enzyme as in urea cycle)**
   - Ammonium is converted to carbamoyl-P
   - two ATPs required - one to activate bicarbonate, one to phosphorylate carbamate

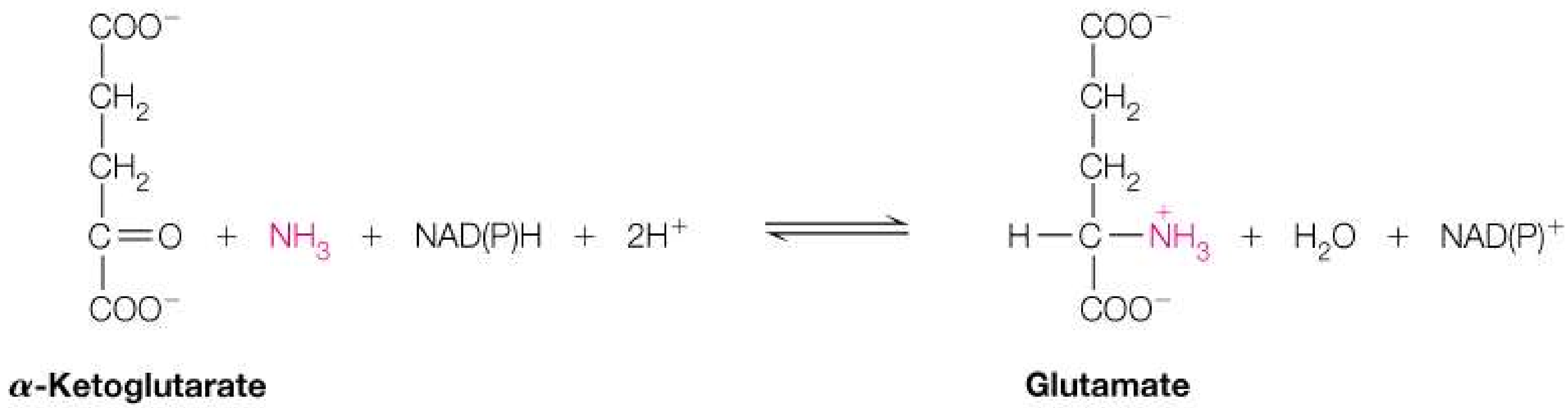
Biogenesis of Organic Nitrogen

1. Glutamate dehydrogenase
2. Glutamine synthetase
3. Asparagine synthetase
4. Carbamoyl phosphate synthetase



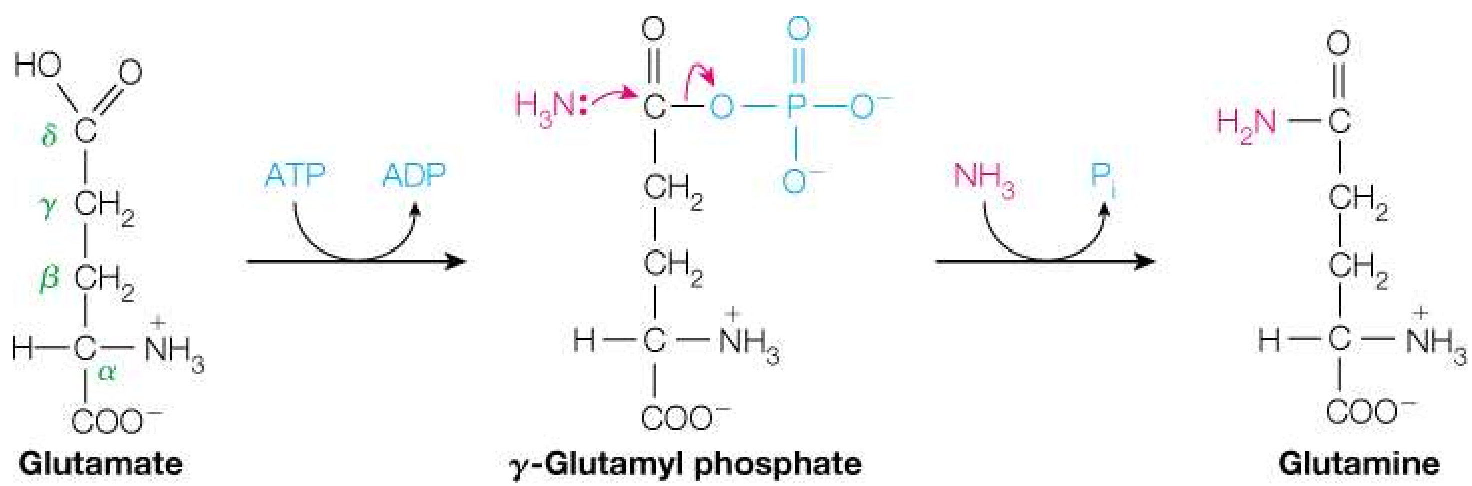
Glutamate Dehydrogenase

- catalyzes a readily reversible reductive amination of α-ketoglutarate
- **in bacteria, it favours the formation of glutamate**
- in **animals**, where the intracellular concentration of NH_3 is low, **the formation of α-ketoglutarate is favoured**



Glutamine Synthetase

- generates a carboxamide using the amino acid side chain carboxyl group of glutamate
- is called a synthetase (rather than a synthase) because it couples bond formation to ATP hydrolysis
- Asparagine synthase catalyzes a similar reaction from aspartate



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Carbamoyl Phosphate Synthetase (recap)

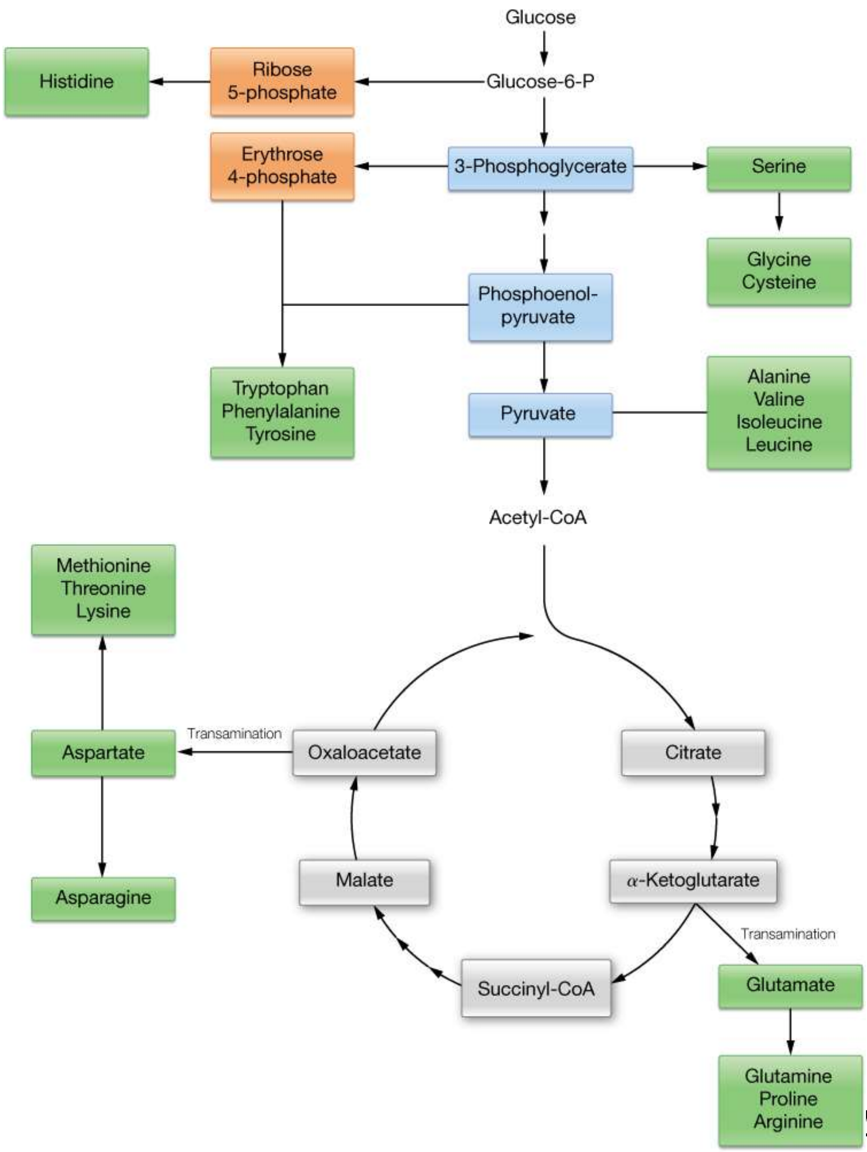
- generates carbamoyl phosphate, an intermediate for arginine (urea cycle) and pyrimidine synthesis (later lectures)
- either NH_3 or glutamine can serve as the nitrogen donor in this reaction

$$\mathrm{NH_3 + HCO_3^- + 2\ ATP \rightarrow carbamoyl\ phosphate + 2\ ADP + P_i}$$

$$\mathrm{Glutamine + H_2O + HCO_3^- + 2\ ATP \rightarrow}$$
$$\mathrm{carbamoyl\ phosphate + 2\ ADP + P_i + glutamate}$$

Precursors for amino acids

- Only **glutamate** and **glutamine** come from nitrogen fixation.
- Other amino acids are made from just a few sources of organic compounds within the cell:
  - Intermediates of glycolysis
  - Intermediates of citric acid (CAC) cycle
  - Intermediates of the pentose phosphate pathway.



Carbon Skeletons for Amino Acid Biosynthesis

- Amino acids can be synthesized from intermediates in glycolysis, the pentose phosphate pathway, or the citric acid cycle

Amino acid families

TABLE 25.1 The Grouping of Amino Acids into Families According to the Metabolic Intermediates That Serve as Their Progenitors		
α-Ketoglutarate Family	**(CAC cycle)**	Aspartate Family
Glutamate		Aspartate
Glutamine		Asparagine
Proline		Methionine
Arginine		Threonine
		Lysine*
Pyruvate Family	**(glycolysis)**	3-Phosphoglycerate Family
Alanine		Serine
Valine		Glycine
Leucine		Cysteine
Phosphoenolpyruvate and Erythrose-4-P Family **(PPP)**		
The aromatic amino acids		
Phenylalanine		
Tyrosine		
Tryptophan		
The remaining amino acid, *histidine*, is derived from PRPP (5-phosphoribosyl-1-pyrophosphate) and ATP.		

PRPP comes from R5P (PPP)

- Based on the metabolic intermediates serving as synthetic precursors.

α-ketoglutarate family of aa's

- Comprises **Glu, Gln, Pro and Arg** (precursor in yellow; essential underlined)
- **Glu** and **Gln**: from N metabolism (last lecture)
- **Pro**: from Glu, by NADPH-dependent reduction, following activation by ATP.
- **Arg**: *via* ornithine - both are involved in the urea cycle (previous lecture).

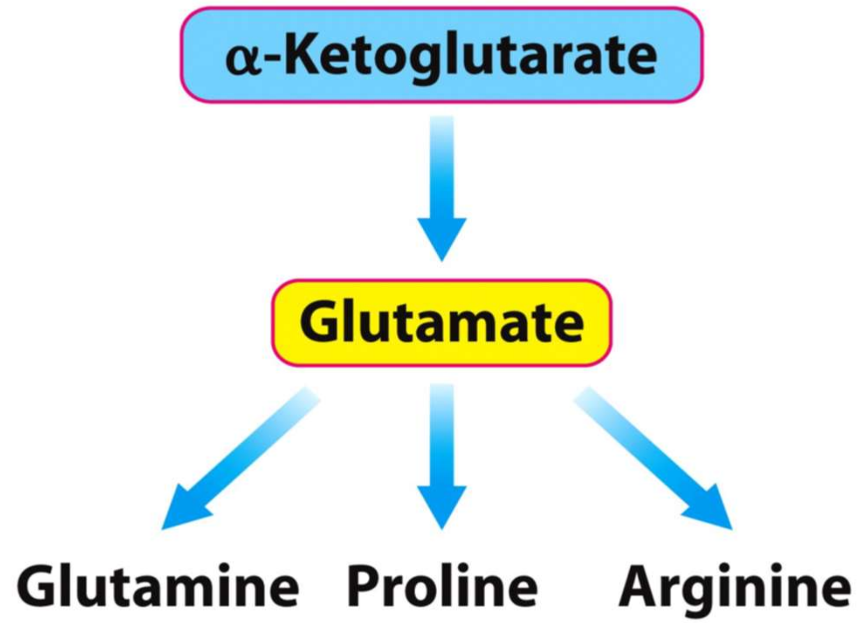
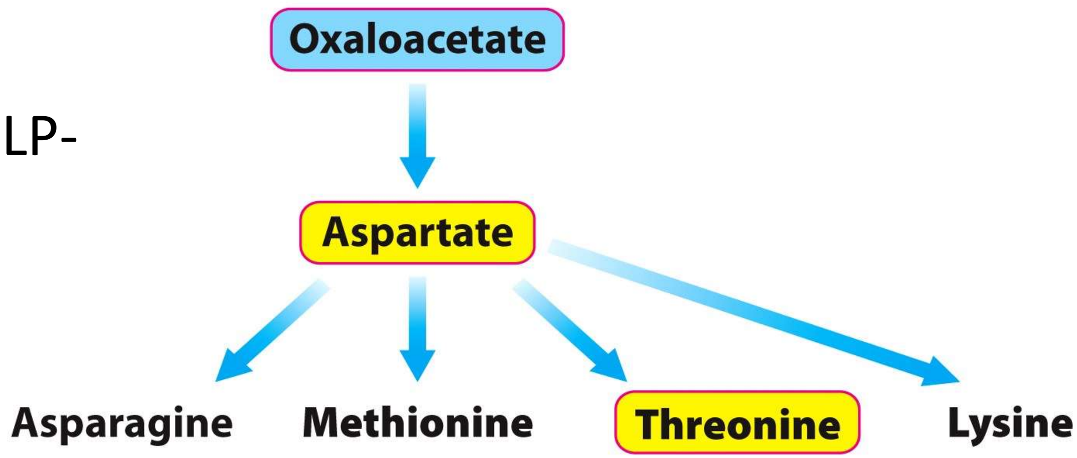


Figure 29.5 part 5
Biochemistry: A Short Course, First Edition

Aspartate family of aa's

- Comprises Asp, Asn, Met, Thr, Lys (precursor aa in yellow, essential underlined)
- **Asp**: from CAC intermediate oxaloacetate by transamination (PLP-dependent).
- **Asn**: from Asp, by amidation:
  1. Bacteria: directly by NH_4^+
  2. Other organisms: by asparagine synthetase and ATP
- **Met, Thr** and **Lys**: in bacteria from Asp by phosphorylation and reduction followed by aa-specific enzymes.



Pyruvate family of aa's

- Comprises **Ala, Val, Leu, Ile** (precursor aa in yellow, essential underlined)
- **Ala**: from pyruvate by transamination (Glu is the amino group donor) – occurs in all organisms.

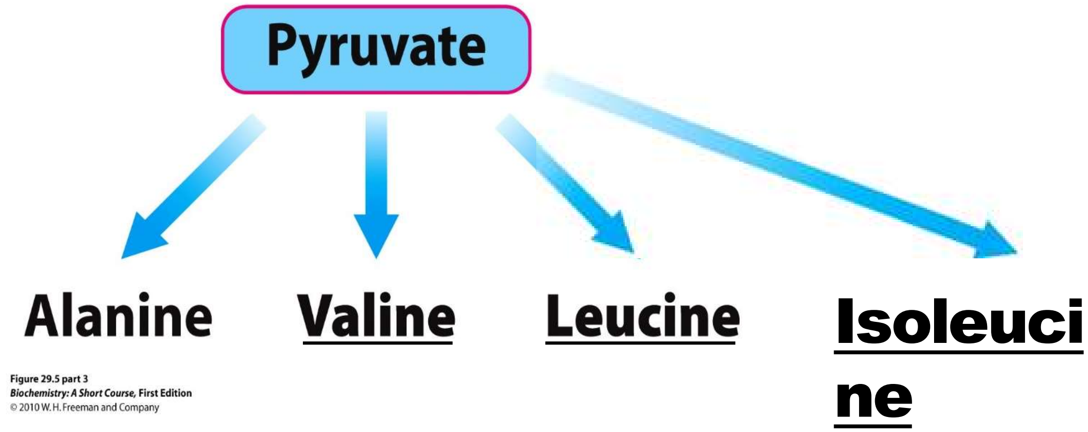
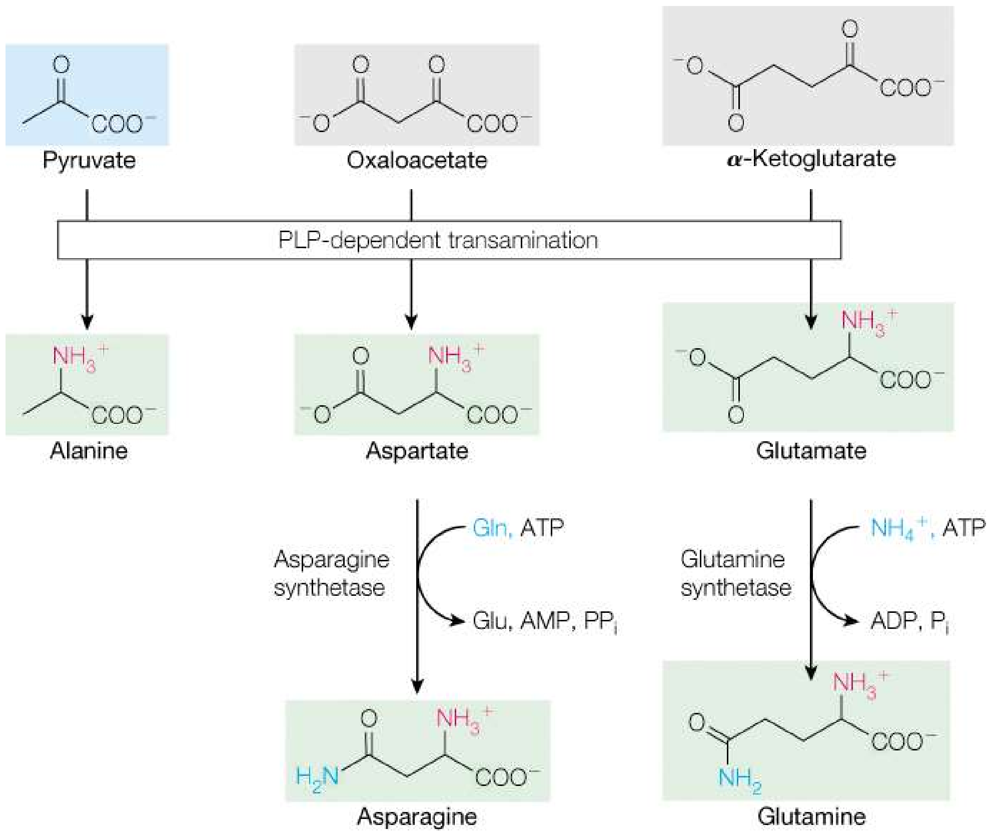


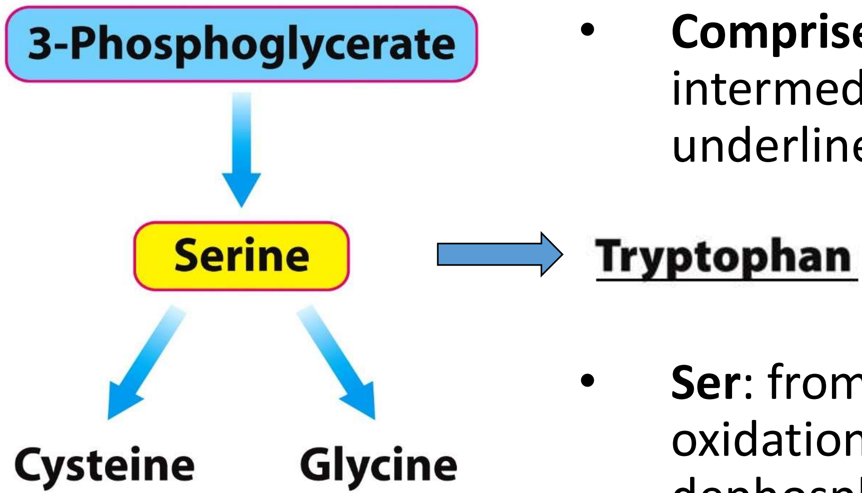
Figure 29.5 part 3
Biochemistry: A Short Course, First Edition

- **Val and Leu (and Ile)**: are essential aa's – not synthesized in mammals.
  - Val and Ile: similar pathways with 4 common enzymes, from appropriate α-keto acid precursor.
    - Both contain branched chains at the β-carbon atom
    - Require a special enzyme, the branched chain amino acid aminotransferase for the last step.
- **Leu**: follows Val biosynthesis from pyruvate upto transamination, when three additional steps are introduced, with acetyl-CoA serving to extend the carbon chain.

Similarities in the Synthesis of Alanine, Aspartate, Glutamate, Asparagine, and Glutamine



3-phosphoglycerate family of aa's



- **Comprises Ser, Gly and Cys**, from the glycolytic intermediate, 3-PG (precursor aa in yellow, essential underlined). **Trp** uses **Ser** backbone.

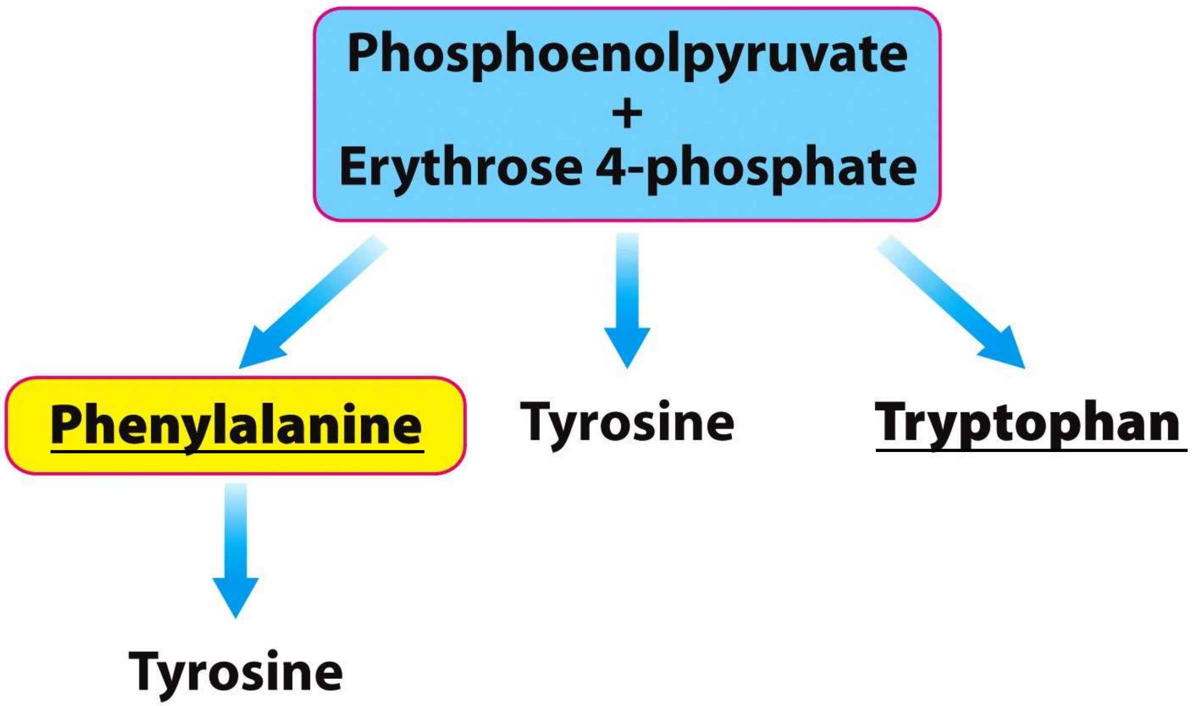
- **Ser**: from 3PG, by 3PG dehydrogenase and NAD^+ oxidation, followed by transamination and dephosphorylation.
- **Gly:** from Ser, *via* two related enzymatic reactions.
- **Cys**: by sulfhydryl (-SH) transfer to Ser.

Aromatic aa's

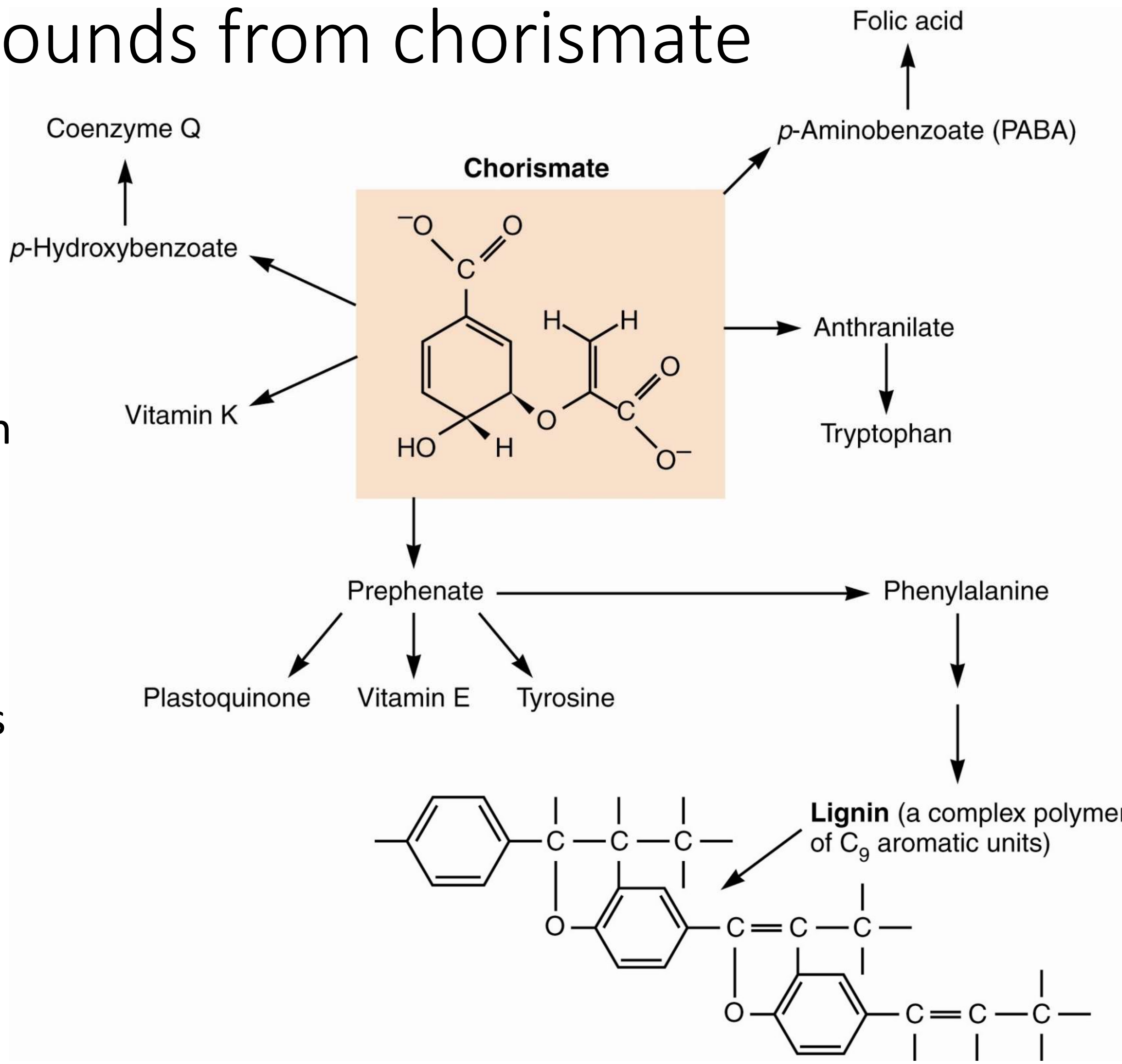
- Comprises **Phe, Tyr and Trp**, all made from glycolysis/PPP derivatives *via* chorismic acid (or chorismate).
  - **Chorismate** is common to the synthesis of cellular compounds having benzene rings, the fat-soluble **vitamins E and K**, **folic acid** as well as **coenzyme Q** and plastoquinone (electron transporters in respiration and photosynthesis).
  - **Chorismate** is made from ***phosphoenol pyruvate (PEP) and erythrose-4-phosphate*** via the shikimate pathway.
  - **Chorismate** is made in plants and bacteria
- Mammals have some of the enzymes after the biosynthesis of chorismate.
  - **Humans can make tyrosine from phenylalanine**
  - **Phenylalanine from phenylpyruvate**.
- **Trp** synthesis uses most of the amino acid, ***serine***

Aromatic aa's:

Phe, Tyr and Trp (precursor aa in yellow, essential underlined)



Aromatic compounds from chorismate



- **Chorismate** is made in prokaryotes (mostly bacteria) and several eukaryotes, including ascomycete fungi, apicomplexans, plants and algae.

Histidine biosynthesis

- **Histidine** biosynthesis and **purine** biosynthesis are connected by common intermediates
- 10 step pathway, catalyzed by nine enzymes
  - 5 of His's 6 C's are from ribose-5-phosphate (R5P), via PRPP (phosphoribosyl-pyrophosphate)
- Transamination step with glutamine yields:
  - Imidazole glycerol phosphate and
  - a purine nucleotide precursor, **AICAR** (5-aminoimidazole-4-carboxamide ribonucleotide) – more in nucleic acid synthesis.

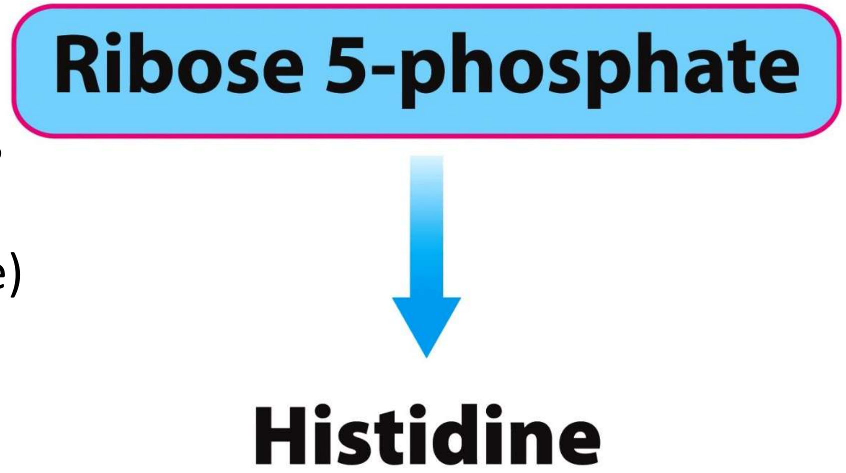


Figure 29.5 part 4
Biochemistry: A Short Course, First Edition

Overview of Amino Acid Biosynthesis

+

Amino Acid Biosynthesis

3

- Essential amino acids
  - Unable to synthesize them
  - Sources are plants and microbes
- Nonessential amino acids
  - Amine groups come from Glu and Gln
  - Some synthesized from essentials
    - Tyrosine from phenylalanine
    - Cysteine depends on S from Methionine

TABLE 18-1 | Essential and Nonessential Amino Acids

Essential	Nonessential
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine
Valine	Proline
	Serine
	Tyrosine

https://www.youtube.com/watch?v=XxNrz65dFJA

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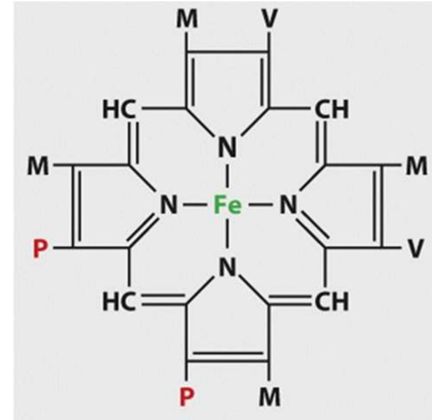
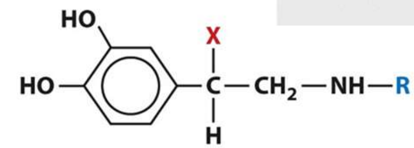
Amino acids are important precursors

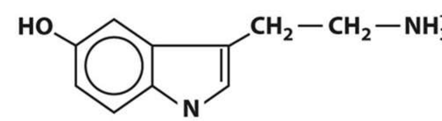
- **Heme** is synthesized from **<u>glycine</u>** and **<u>succinyl-CoA</u>** (from CAC) in the mitochondria.
- The synthesis of **bioactive amines** begins with amino acid decarboxylation: **epinephrine (adrenaline), norepinephrine, dopamine, serotonin, γ-aminobutyric acid (GABA)** and **histamine**.
- **<u>Arginine</u>** gives rise to the hormonally active gas **nitric oxide** (NO).
  - The identification of NO as a vasodilator has led to the therapeutic agent, *nitroglycerin*, to relieve chest pain in **angina pectoris** sufferers.



Heme



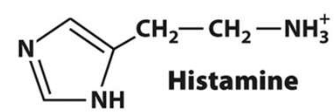
X = OH, R = CH_3 **Epinephrine (adrenalin)**
X = OH, R = H **Norepinephrine**
X = H, R = H **Dopamine**

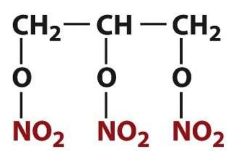


Serotonin (5-hydroxytryptamine)

$^{-}OOC-CH_2-CH_2-CH_2-NH_3^+$

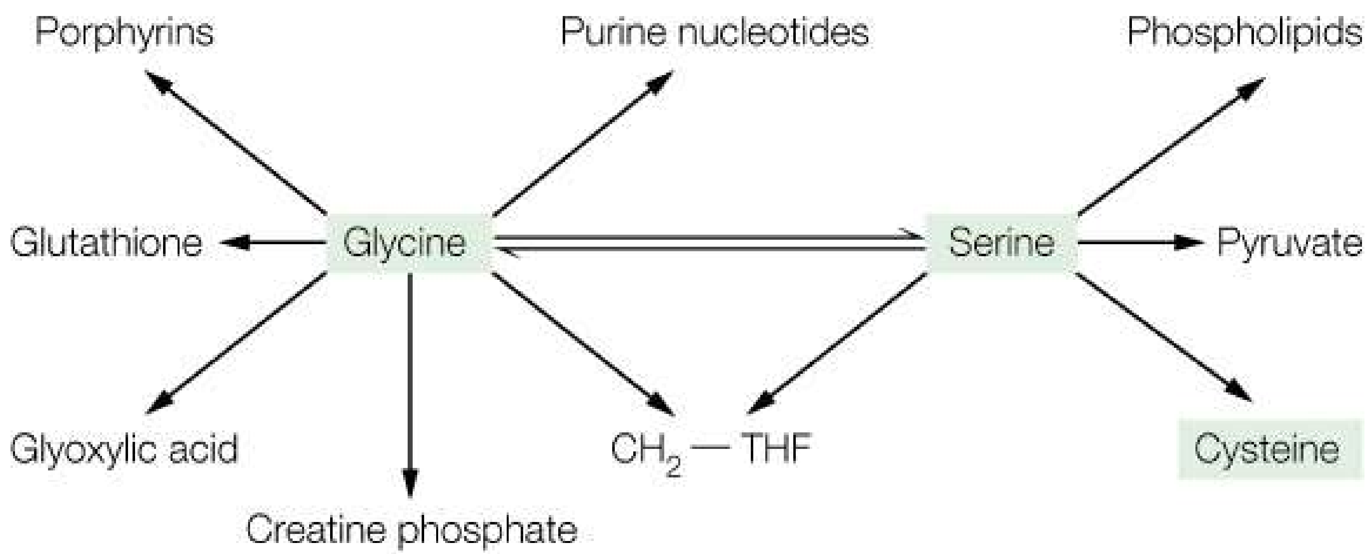
γ-Aminobutyric acid (GABA)





Nitroglycerin

Metabolites from Serine and Glycine

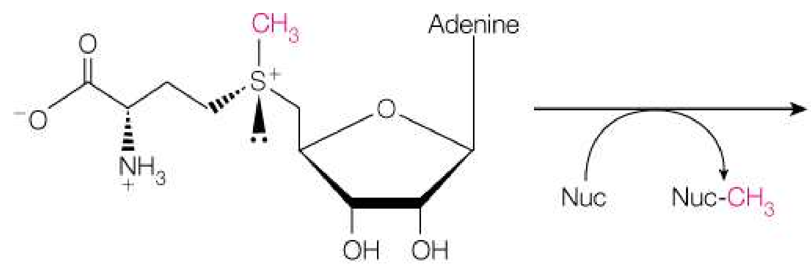


Several important metabolites:

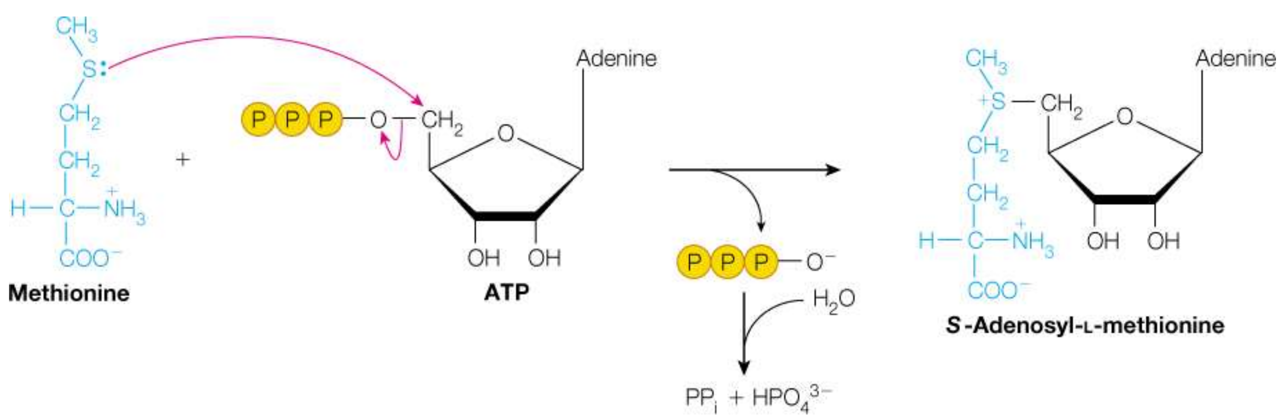
- Creatine measures how much protein in broken down

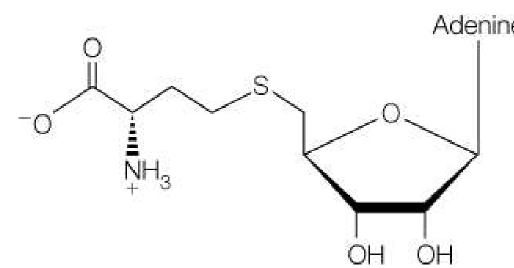
Methionine as a Precursor of *S*-adenosyl-L-Methionine

- *S*-adenosyl-L-methionine (AdoMet), synthesized from methionine
- *S*-Adenosyl-L-methionine is a "universal" methyl donor involved in numerous methyl group transfer reactions



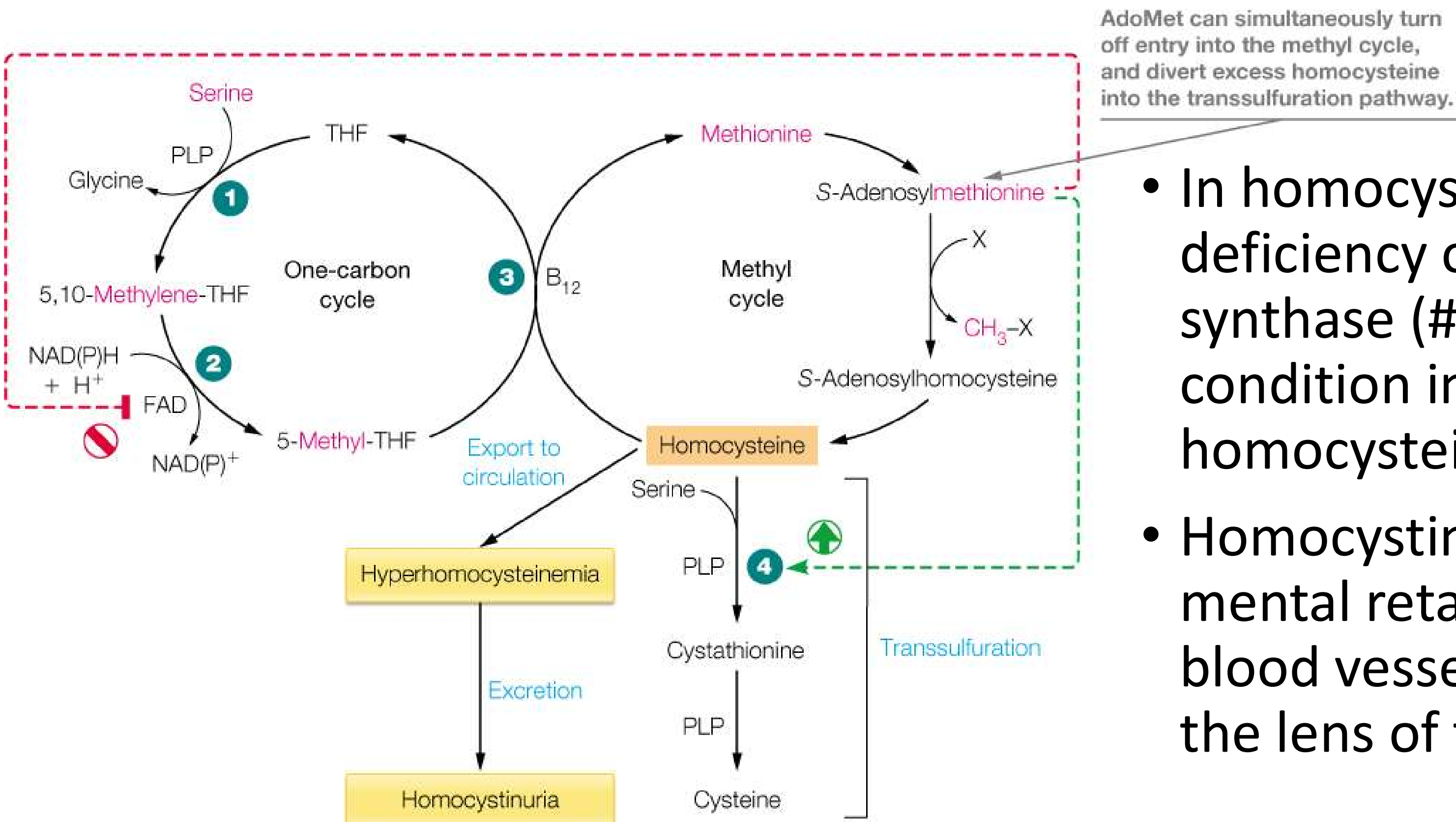
S-Adenosyl-L-methionine (AdoMet)





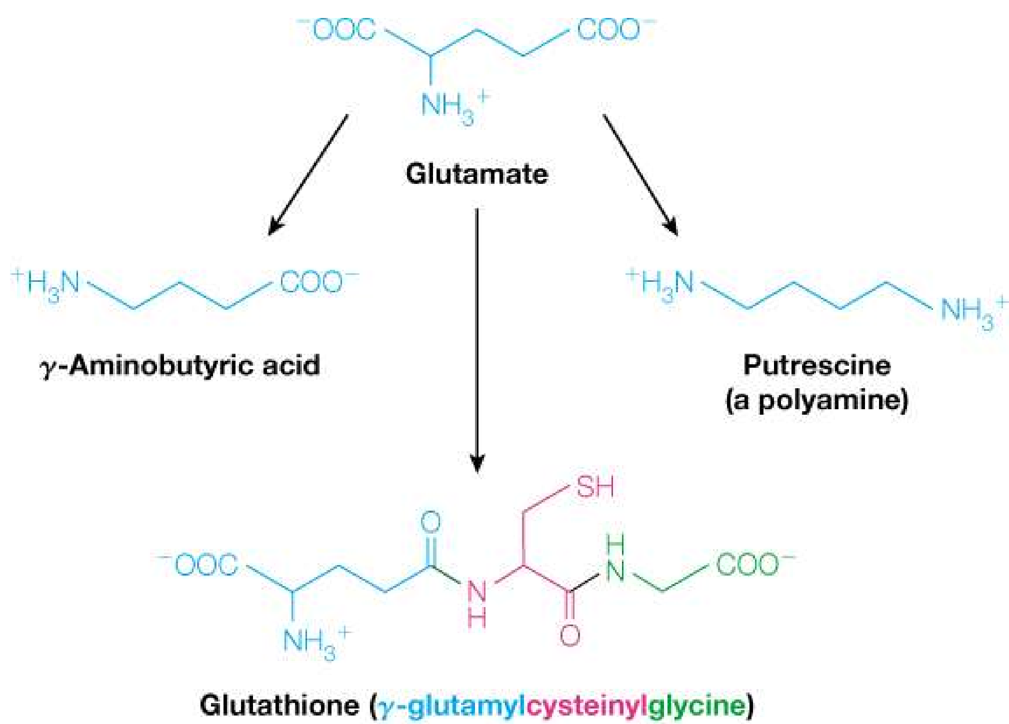
S-Adenosyl-L-homocysteine (AdoHcy)

Methyl Group Metabolism and Homocystinuria

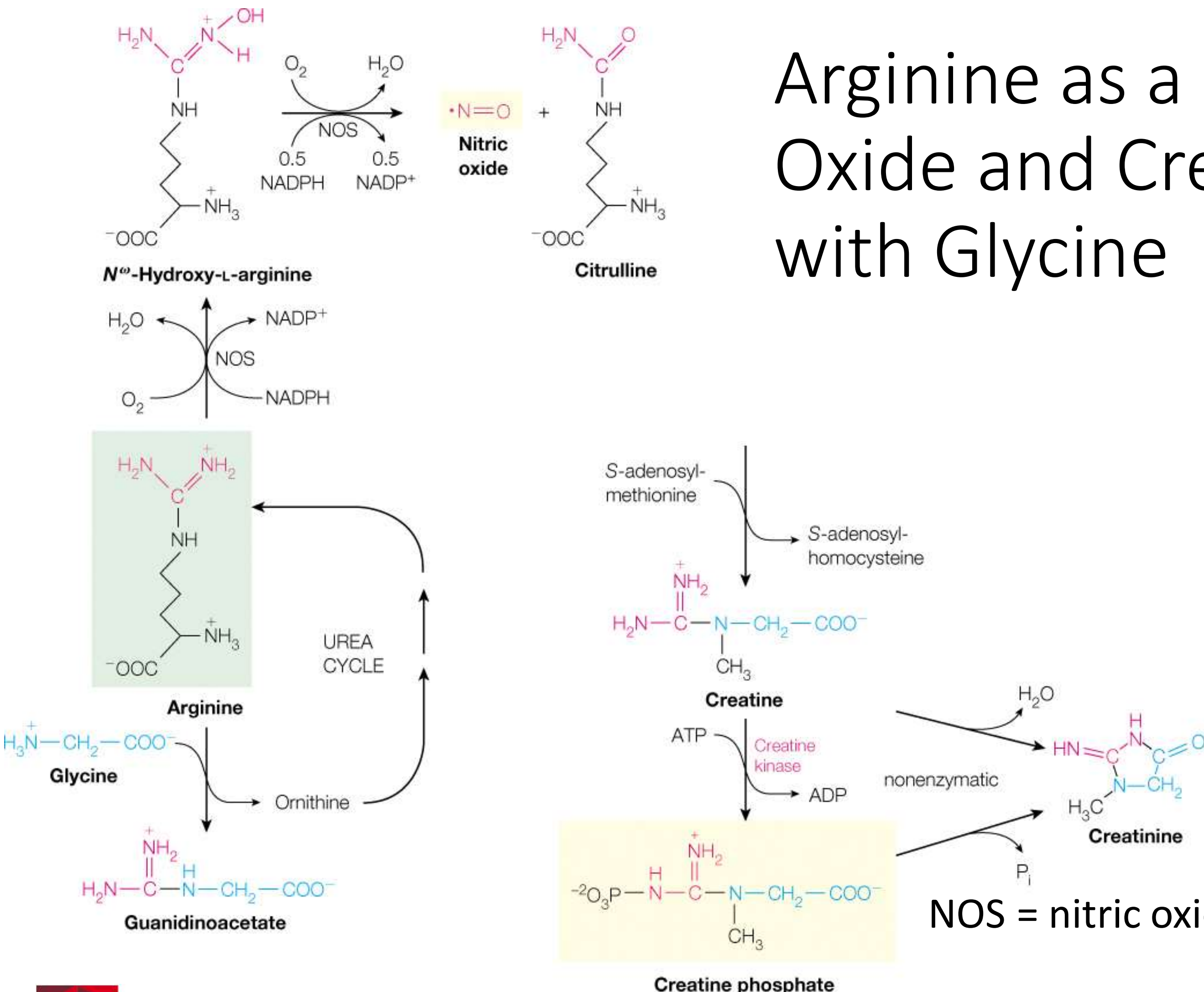


- In homocystinuria, a genetic deficiency of cystathionine β-synthase (#4) leads to a condition in which homocysteine accumulates.
- Homocystinuria results in severe mental retardation, damage to blood vessels, and dislocation of the lens of the eye.

Glutamate as a Precursor of Polyamines, Glutathione, and the Neurotransmitter α-Aminobutyric Acid (GABA)

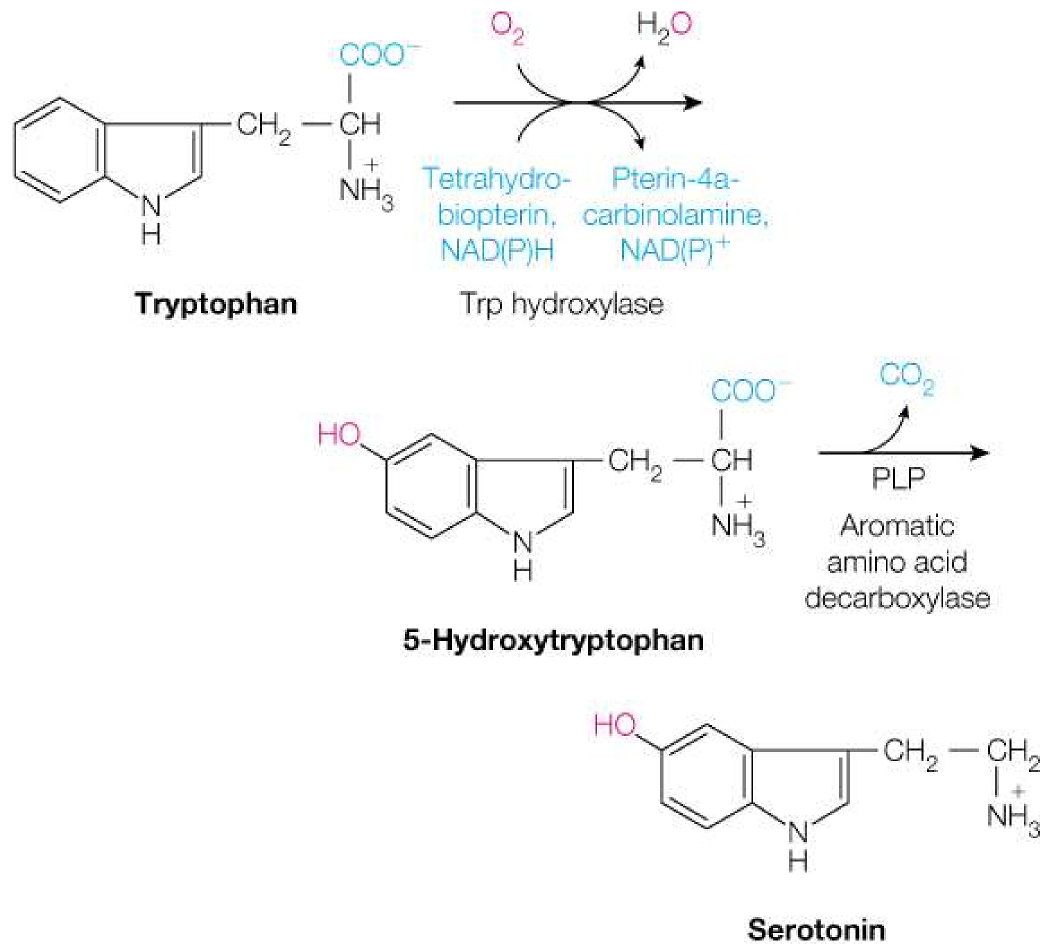


Arginine as a Precursor of Nitric Oxide and Creatine Phosphate, with Glycine

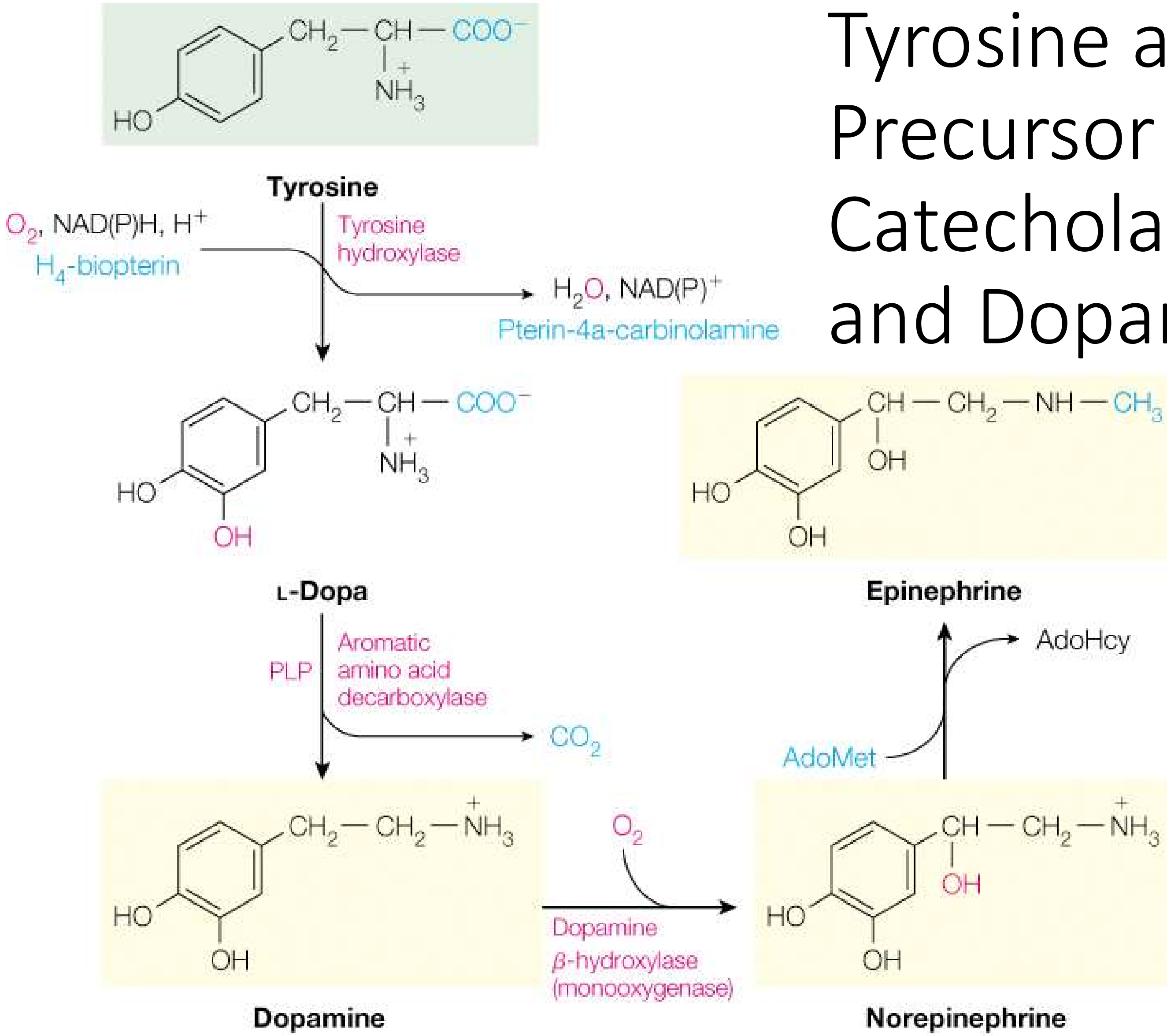


NOS = nitric oxide synthase

Tryptophan as a Precursor of the Neurotransmitter Serotonin

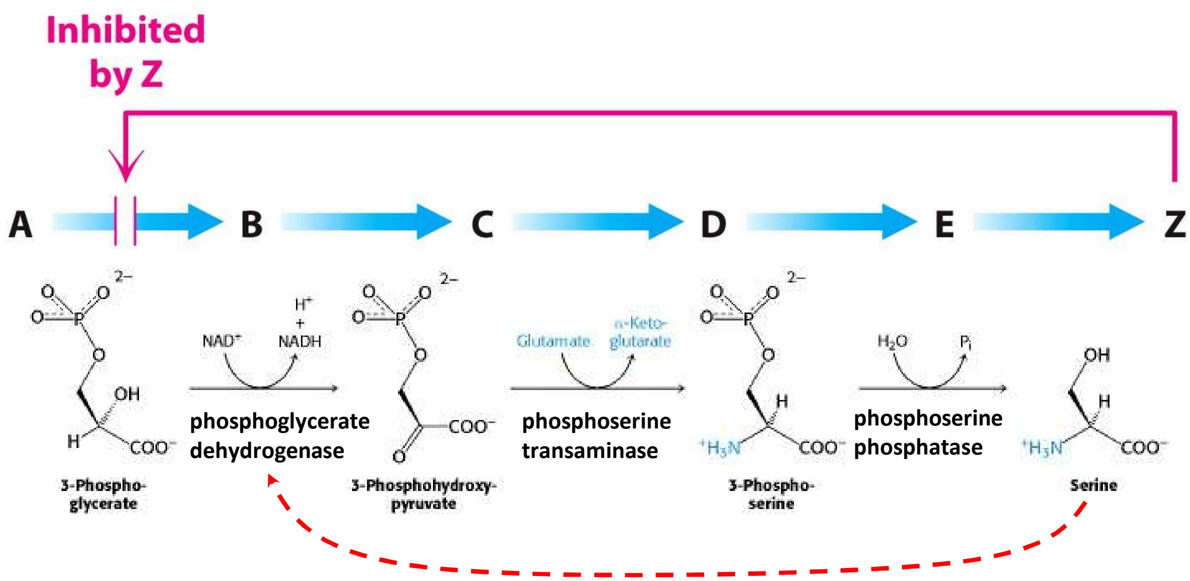


Tyrosine as a Precursor of Catecholamines and Dopamine



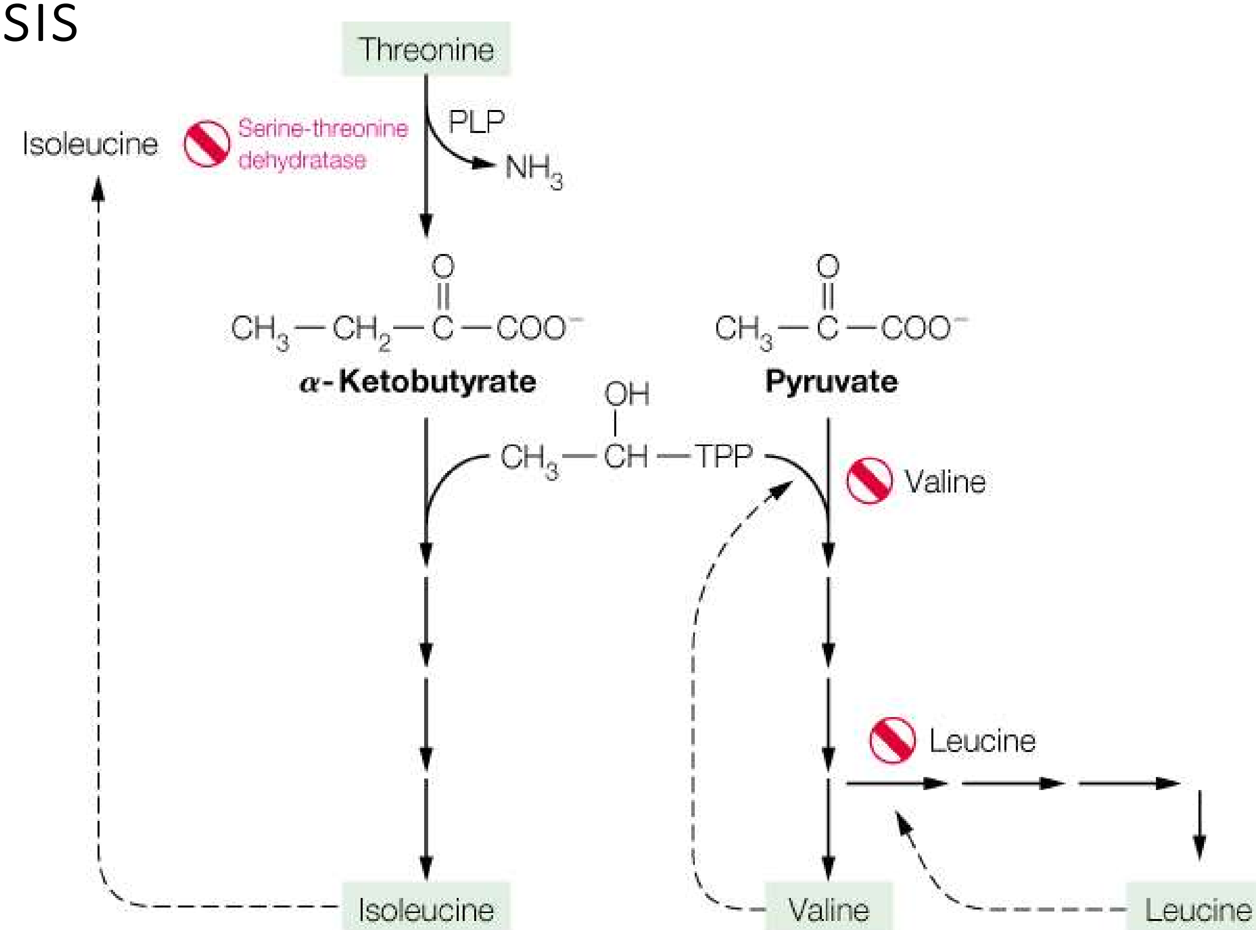
Regulation of amino acid biosynthesis

- Many amino acids follow a linear set of reaction steps.
- Feedback inhibition of the first committed step, with the final product (Z) inhibiting the enzyme that catalyzes the first step.
- End-point inhibition is observed (Lecture 8): e.g. serine biosynthesis



Regulation of branched pathways in amino acid biosynthesis

- Feedback inhibition in branched pathway
- e.g. biosynthesis of Val, Leu and Ile.
- Common intermediate: hydroxyethyl thiamine pyrophosphate
- [pyruvate]:[α-keto butyrate] = [Val/Leu]:[Ile]
- So, Val and Leu inhibit branches leading to their synthesis
- Ile inhibits inhibits Thr's oxidative deamination.



Multiple enzymes can catalyze the committed step in aa biosynthesis

- Independent regulatio of same step by controlling the differer enzymes that catalyze the step.

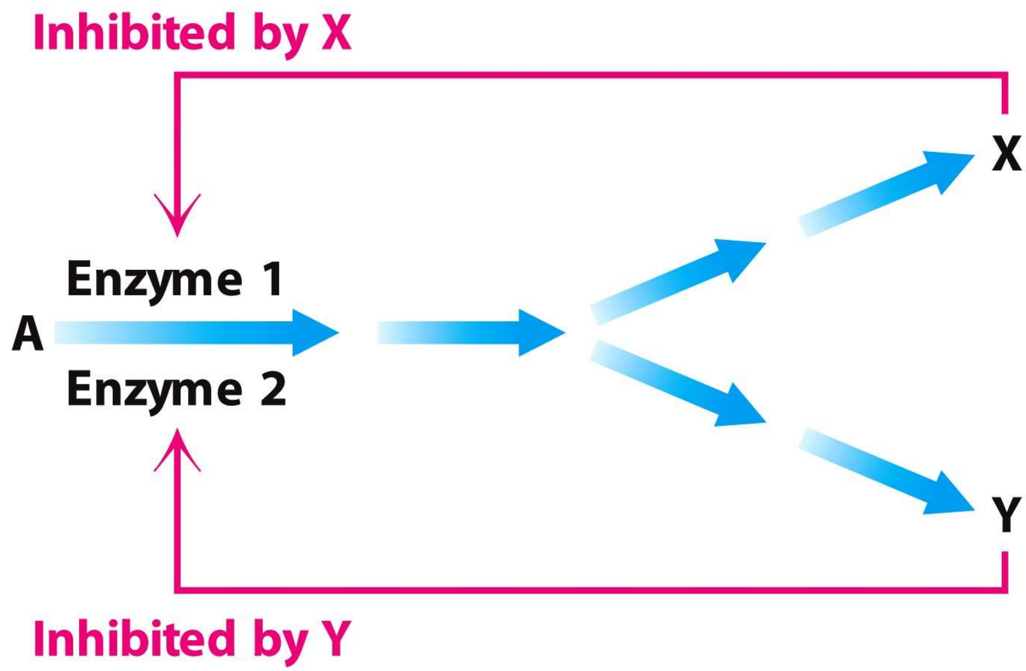


Figure 29.12
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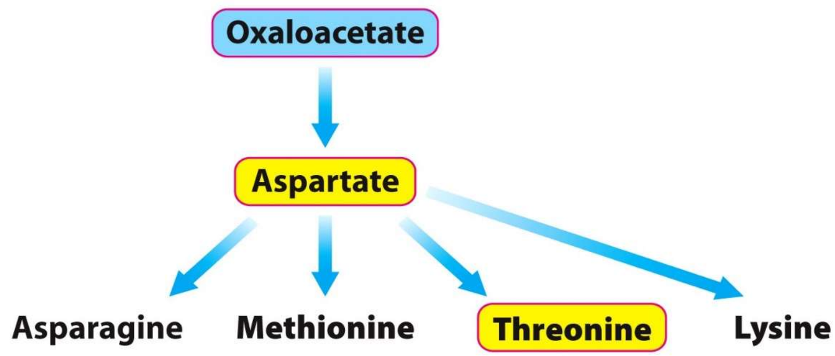
Multiple enzymes can catalyze the committed step in aa biosynthesis

- **Asp** is phosphorylated in the biosynthesis of Thr, Met and Lys.
- Three distinct **aspartokinases** catalyze this step in *E. coli*.
- Same mechanism but regulation is different.

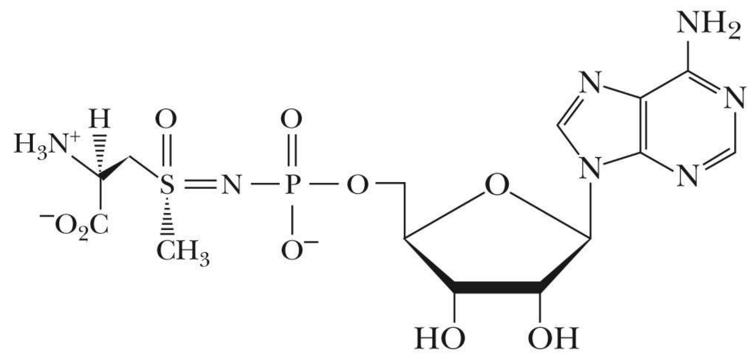
  I: Inhibited by Thr

  II: No inhibition by Met but synthesis repressed by Met

  III: Inhibited by Lys.



Asparagine and Leukemia

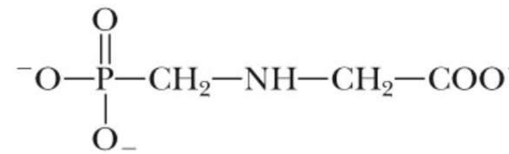


An adenylated sulfoximine

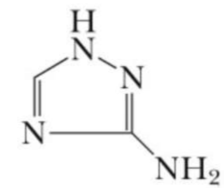
- Leukemia is a cancer of the bone marrow that affects production of lymphocytes
- Both normal and malignant lymphocytes are highly dependent on asparagine uptake from blood
- Administration of **asparaginase** (which converts Asn to Asp) is one therapeutic approach to treat childhood leukemia (recap)
- Inhibition of **asparagine synthetase** presents an alternative way to deprive malignant lymphocytes of essential Asn, and Asn synthetase inhibitors might offer a strategy for treating some leukemias
- Adenylated sulfoximines inhibit **asparagine synthetase** at low concentrations, as an analog of the aspartyl-AMP intermediate formed in the asparagine synthetase reaction.

Amino acid biosynthesis inhibitors as herbicides

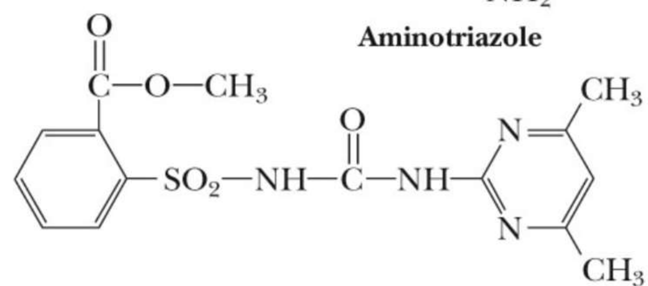
- A variety of herbicides have been developed as inhibitors of plant enzymes that synthesize “essential” amino acids
- These substances show no effect on animals
- For example, glyphosate, sold as **RoundUp**, is a PEP analog that acts as an uncompetitive inhibitor of 3-enolpyruvylshikimate-5-P synthase.



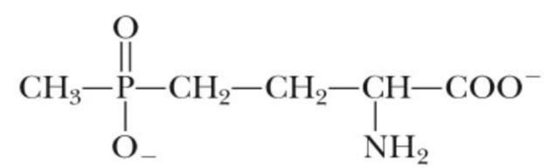
Glyphosate



Aminotriazole



Sulfmeturon methyl



DL-Phosphinothricin (PPT)

Summary

- AA synthesis is by transamination
  - except for Glu and Gln (from nitrogen fixation)
  - Pathways providing precursors
  - Examples
- Amino acid families
- Regulation of amino acid synthesis
  - Mechanism and example
- Applications of amino acid synthesis knowledge in medicine and herbicides

For Test 2 and Exam prep

- Transamination is am important reaction in aa metabolism
  - One example in words
- Urea cycle
  - Products and related pathways
  - Similarities to CAC
- Ketogenic and glucogenic amino acids
  - Two examples of ketogenic, glucogenic and both.
- Essential and non-essential amino acids
  - Two examples of each
  - AA families and 2 examples of each