Biochemistry of Proteins and Nucleic Acids

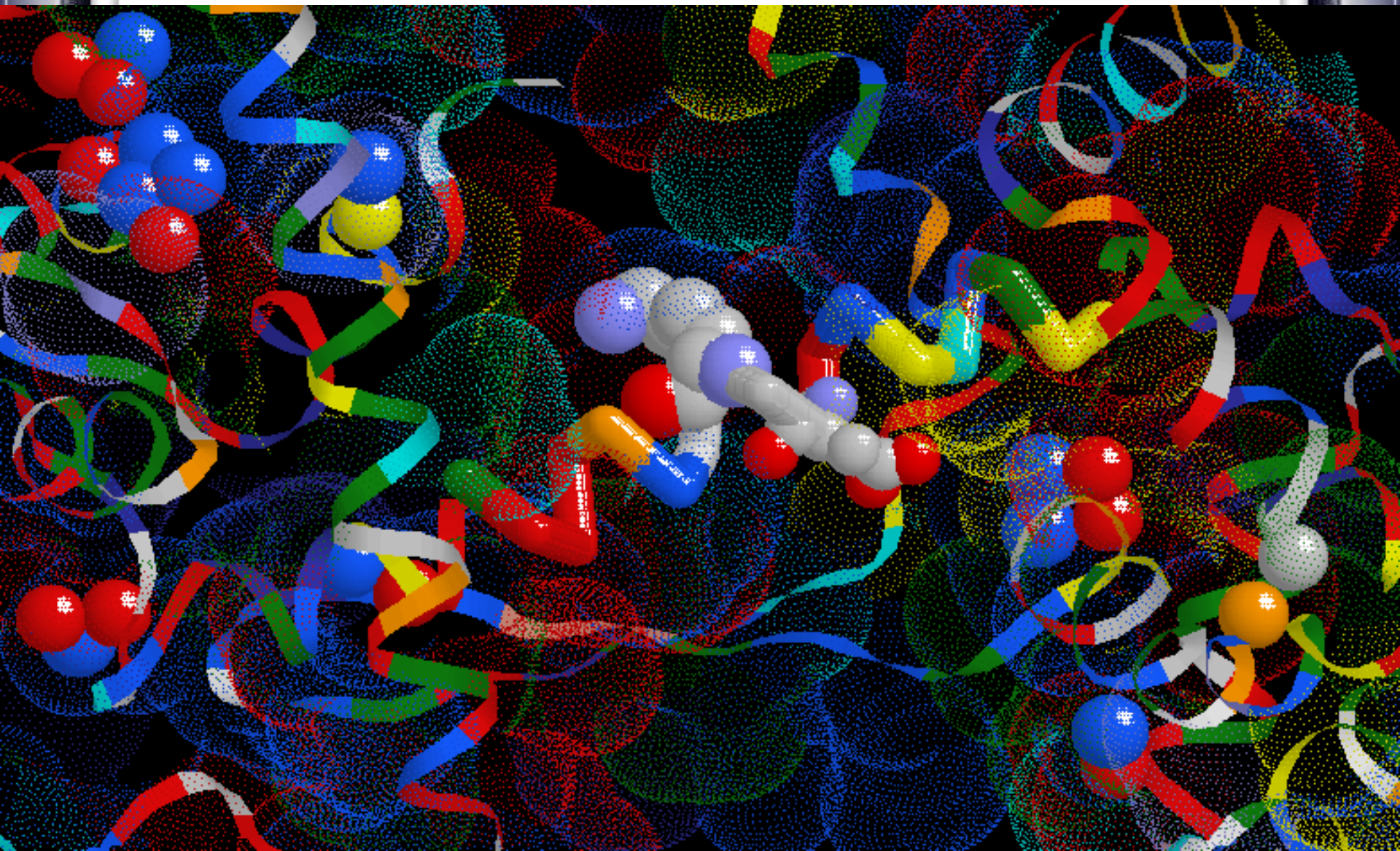
1

Digestion and absorption

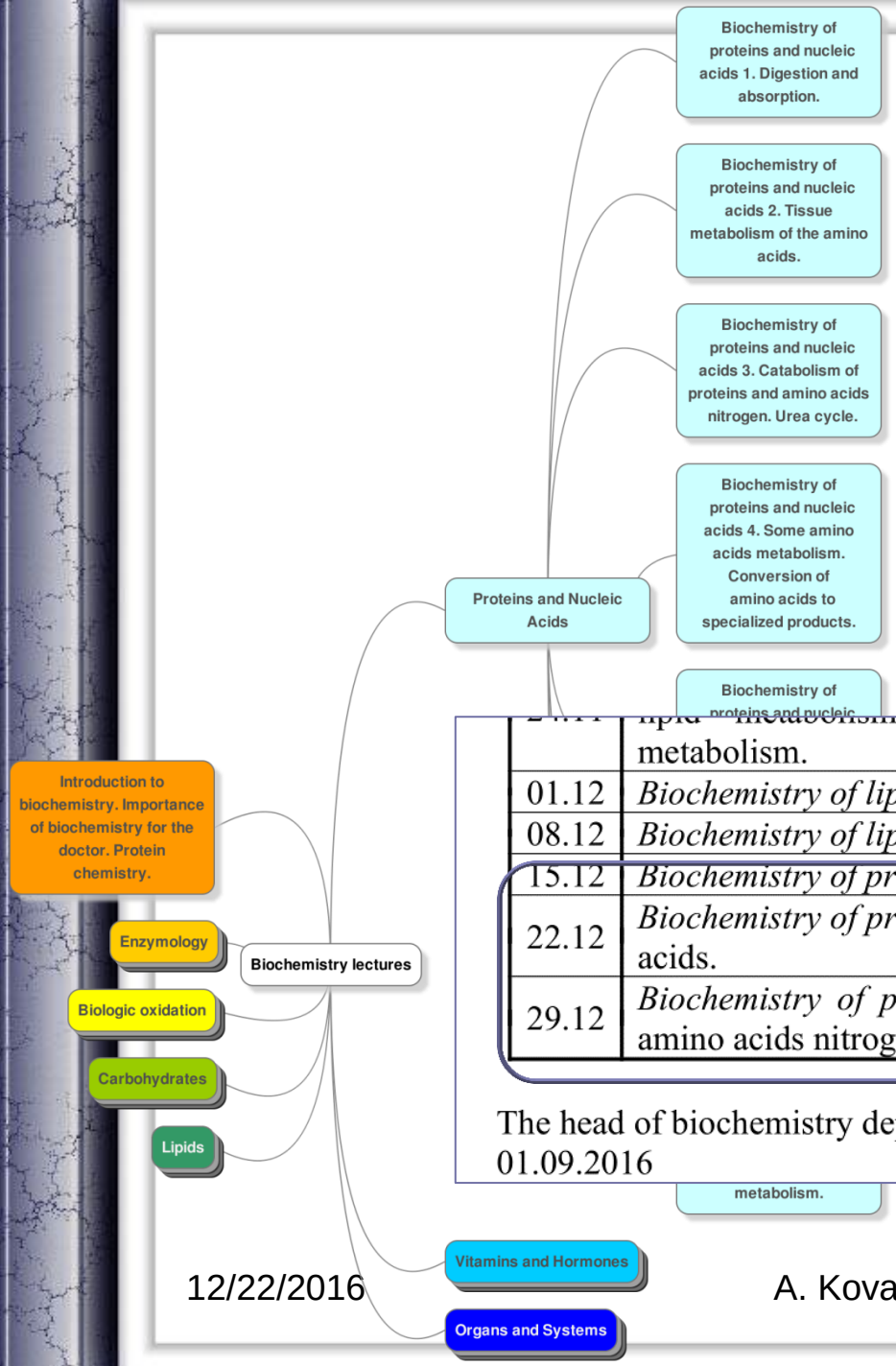
Lecture #14

Lecturer: PhD Alexander N. Koval

Presentation of Protein



Lectures plan



	metabolism.
01.12	*Biochemistry of lipids 3.* Tissue lipid metabolism: lipid biosynthesis.
08.12	*Biochemistry of lipids 4.* Lipid metabolism regulation and pathology.
15.12	*Biochemistry of proteins and nucleic acids 1.* Digestion and absorption.
22.12	*Biochemistry of proteins and nucleic acids 2.* Tissue metabolism of the amino acids.
29.12	*Biochemistry of proteins and nucleic acids 3.* Catabolism of proteins and amino acids nitrogen. Urea cycle.

The head of biochemistry department,Dr. Med (Sci), professor A.I. Gritsuk
01.09.2016

Overview of Protein Metabolism

- Proteins: primary, secondary, tertiary and quaternary structure of proteins
- Complex protein structures
- Clinical significances
- Analysis of protein structure
  - N-terminal analysis of proteins
  - Protease digestion for peptide generation
  - C-terminal analysis of proteins
  - Chemical digestion of proteins
  - Size exclusion chromatography
  - Ion exchange chromatography
  - Affinity chromatography
  - High performance/(pressure) liquid chromatography
  - Electrophoresis of proteins
  - Centrifugation of proteins
- Myoglobin and hemoglobin
  - *Look: kvl\06112004\proteins.pdf*

Introduction

- All tissues have some capability for synthesis of the **non-essential** amino acids, amino acid remodeling, and conversion of non-amino acid carbon skeletons into amino acids and other derivatives that contain nitrogen.
- **Liver** is the major site of nitrogen metabolism in the body. In times of dietary surplus, the potentially toxic nitrogen of amino acids is eliminated via transaminations, deamination, and **urea** formation; the carbon skeletons are generally conserved as carbohydrate, via *gluconeogenesis*, or as fatty acid via *fatty acid synthesis pathways*.

Glucogenic and Ketogenic Amino Acids

- In this respect amino acids fall into three categories:
  - **Glucogenic**: those that give rise to a net production of *pyruvate* or *TCA cycle intermediates* (such as α-ketoglutarate or oxaloacetate), all of which are precursors to glucose via **gluconeogenesis.** All amino acids except *lysine* and *leucine* are at least partly glucogenic.
  - **Ketogenic:** *Lysine* and *leucine* are the only amino acids that are solely ketogenic, giving rise only to **acetyl-CoA** or **acetoacetyl-CoA**, neither of which can bring about net glucose production.
  - **Glucogenic and ketogenic**: *Isoleucine, phenylalanine, threonine, tryptophan,* and *tyrosine* give rise to both glucose and fatty acid precursors.
- Finally, it should be recognized that amino acids have a third possible fate. During times of starvation the reduced carbon skeleton is used for energy production, with the result that it is oxidized to CO_2 and H_2O.

Essential vs. Nonessential Amino Acids

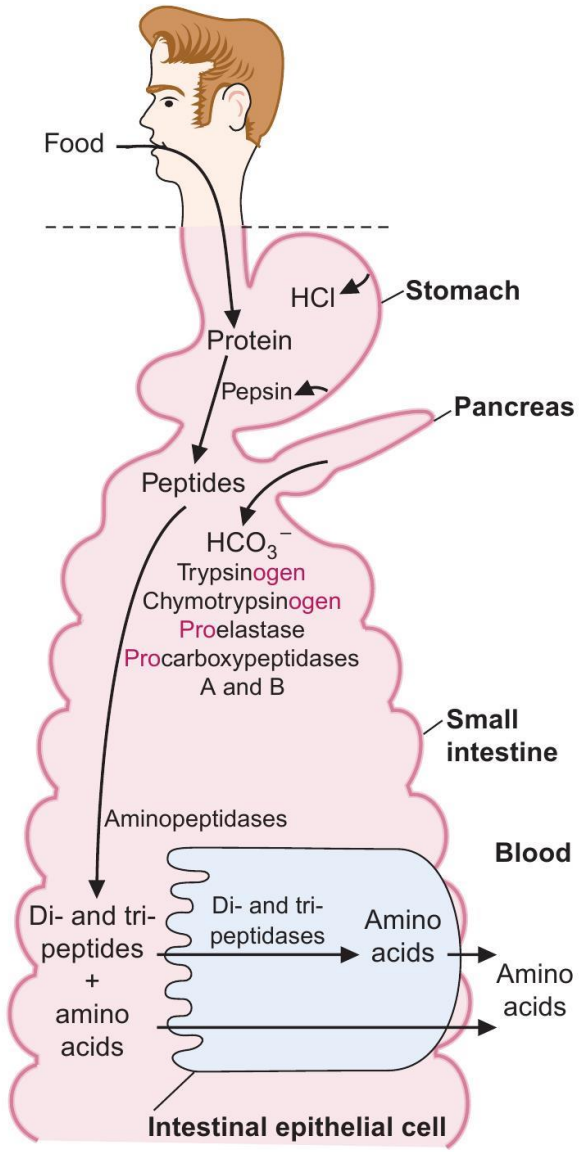
Nonessential	Essential
Alanine	Arginine*
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine	Methionine*
Glycine	Phenylalanine*
Proline	Threonine
Serine	Tyrptophan
Tyrosine	Valine

Arginine, Methionine and Phenylalanine

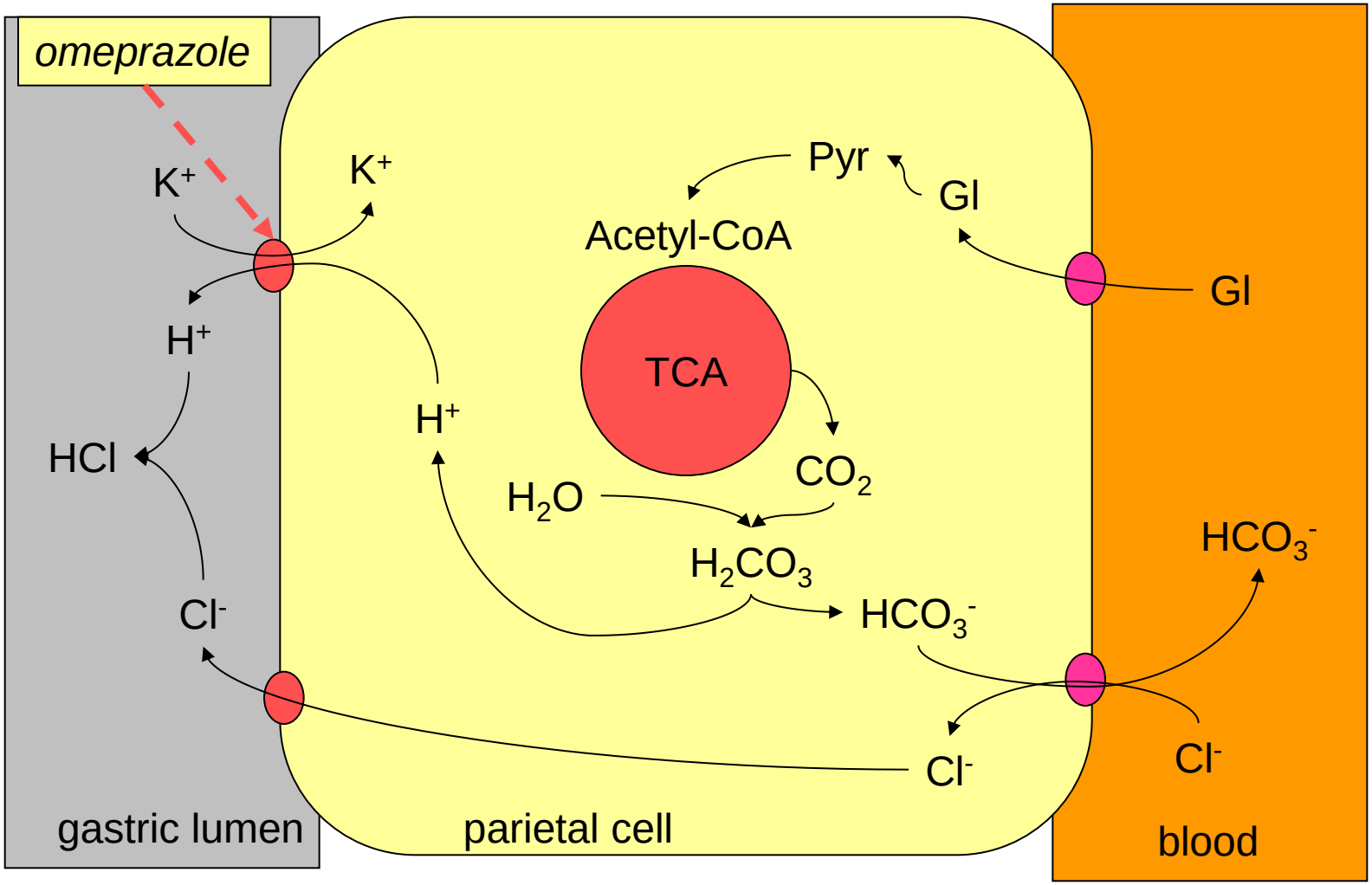
- The amino acids arginine, methionine and phenylalanine are considered essential for reasons not directly related to lack of synthesis.
  - Arginine is synthesized by mammalian cells but at a rate that is insufficient to meet the growth needs of the body and the majority that is synthesized is cleaved to form urea.
  - Methionine is required in large amounts to produce cysteine if the latter amino acid is not adequately supplied in the diet.
  - Similarly, phenylalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet.
    - *See: kvl\06112004\UpdateCellular Structure and FunctionLecture1.pdf*

Protein Digestion

- The proteolytic enzymes, pepsin, trypsin, chymotrypsin, elastase, and the carboxypeptidases, are produced as **zymogens** (the *pro-* and *-ogen* acompanying the enzyme name) that are activated by cleavage after they enter the gastrointestinal lumen.



HCl Secretion Mechanism



Digestion: Proteases

Enzyme	Source	Specificity	Additional Points
Pepsin	Gastric mucosa	peptide bond N-terminal to L, F, W, Y, but when next to P	exhibits little specificity, requires low pH
Trypsin	Pancreas	peptide bond C-terminal to R, K, but not if next to P	highly specific for positively charged residues
Chymo-trypsin	Pancreas	peptide bond C-terminal to F, Y, W but not if next to P	prefers bulky hydrophobic residues, cleaves slowly at N, H, M, L
Elastase	Pancreas	peptide bond C-terminal to A, G, S, V, but not if next to P	

Exopeptidases: Carboxypeptidases

•Exopeptidases cleave peptides at the C-terminal residue which can then be analyzed chromatographically and compared to standard amino acids.

- •This class of exopeptidases are called, **carboxypeptidases**.
- •Contain Zn^{2+} - ***metalloenzymes***.

Enzyme	Source	Specificity
Carboxypeptidase A	Pancreas	Will cleave when C-terminal residue = F, Y, W
Carboxypeptidase B	Pancreas	Will cleave when C-terminal residue = R, K or P or if P resides next to terminal residue

Exopeptidases: Aminopeptidases. Dipeptidases

- The luminal surface of *intestinal* epithelial cells contains **aminopeptidases** and **dipeptidases**.
- **Aminopeptidases** are non-specific *exopeptidases* which repeatedly cleaves N-terminal amino acids one by one to produce free amino acids and smaller peptides.
  - **Alanineaminopeptidase**: cleaves *ala* from N-terminus.
  - **Leucineaminopeptidase**: cleaves any AA from N-terminus (not only *leu*).
- The **dipeptidases** act on different dipeptides to liberate amino acids:
  - **Prolinase**: cleaves Pro-X,
  - **Prolidase**: cleaves X-Pro (X – any AA).

Amino Acid Absorption: Transporters

- There are at least 6 transport systems for AA
  - For short chain neutral amino acids (Ala, Ser, Thr)
  - For long chain, neutral and aromatic amino acids (Val, Leu, Ile, Met, Phe, Tyr)
  - For acidic amino acids (Asp, Glu)
  - For basic amino acids (Lys, Arg, Cys-Cys)
  - For imino acids (Pro, Hyp)
  - For β-amino-acids (β-Ala, taurine).

From Danchenko, 2004

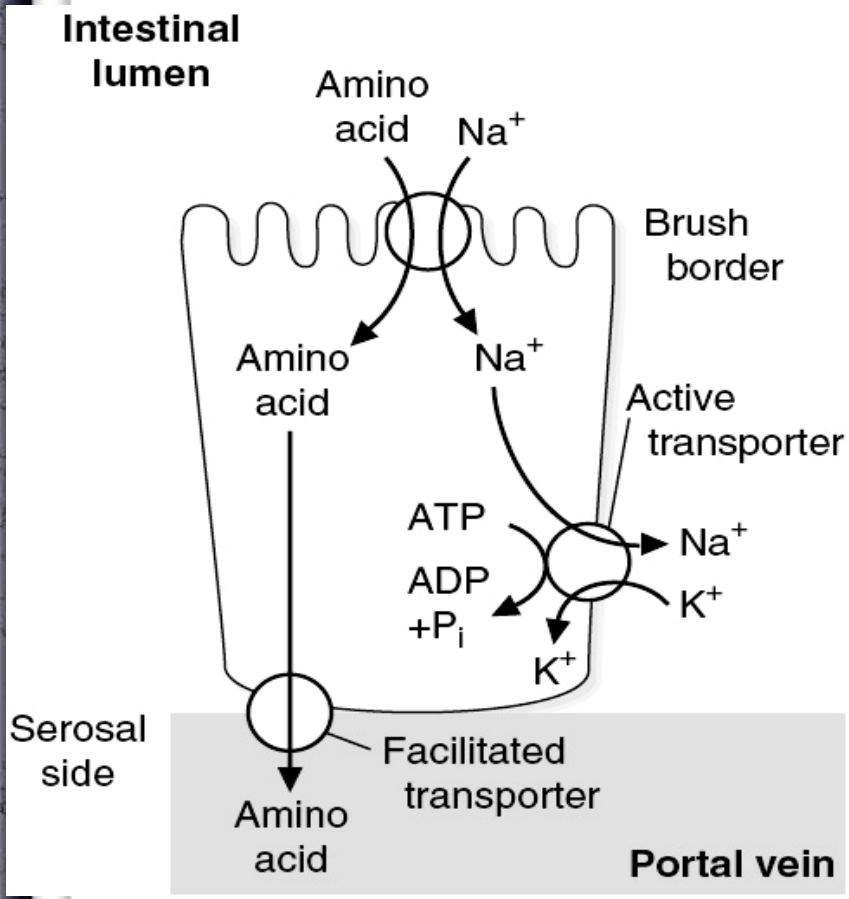
Partial List of Amino Acids Transport Systems

System Name	Sodium-dependent?	Specificity	Tissues Expressed
A	Yes	Small amino acids (ala, ser, gln)	Many
ASC	Yes	Small amino acids (ala, ser, cys)	Many
N	Yes	Gln, asn, his	Liver
L	No	Branched and aromatic amino acids	Many
$B^{0,+}$	Yes	Basic amino acids	Intestine (brush border)[b]
ATB°	Yes	Zwitterionic amino acids (monoamino, monocarboxylic acid amino acids)	Intestine and kidney[c]
X_{AG}^-	Yes	Anionic amino acids	Intestine (brush border)
Imino	Yes	Pro, hypro, gly	Intestine (brush border)

[a] Not all transport systems are listed.
[b] This system is most likely defective in cystinuria.
[c] This system is most likely defective in Hartnup disease.

Marks, 2000

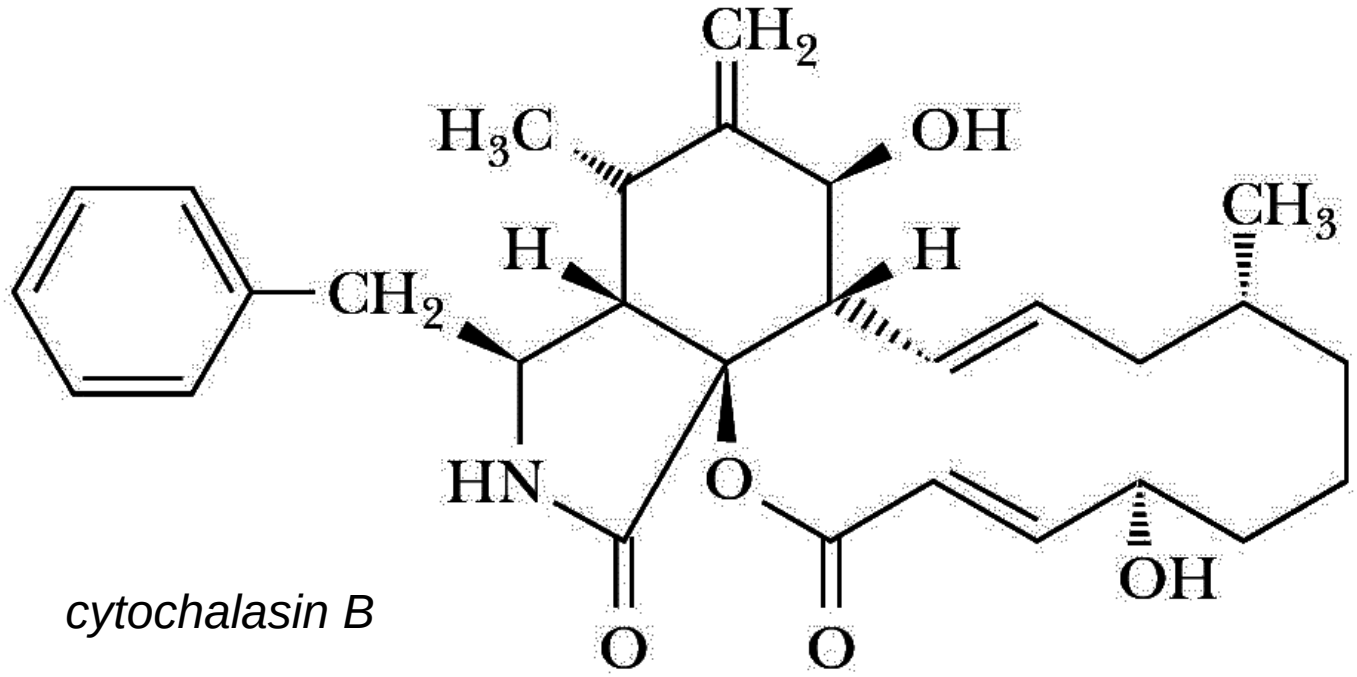
Amino Acids Absorption: Na^+-dependent Transporters Prevail



- Na^+-dependent carriers transport both Na^+ and an AA into the intestinal epithelial cell from the intestinal lumen.
  - Na^+ is pumped out on the serosal side (across the basolateral membrane) in exchange for K^+ by the **Na^+,K^+-ATPase**.
  - On the serosal side, the AA is carried by a facilitated transporter down its concentration gradient into the blood.
- This process is an example of **secondary active transport**.

From Marks, 2000

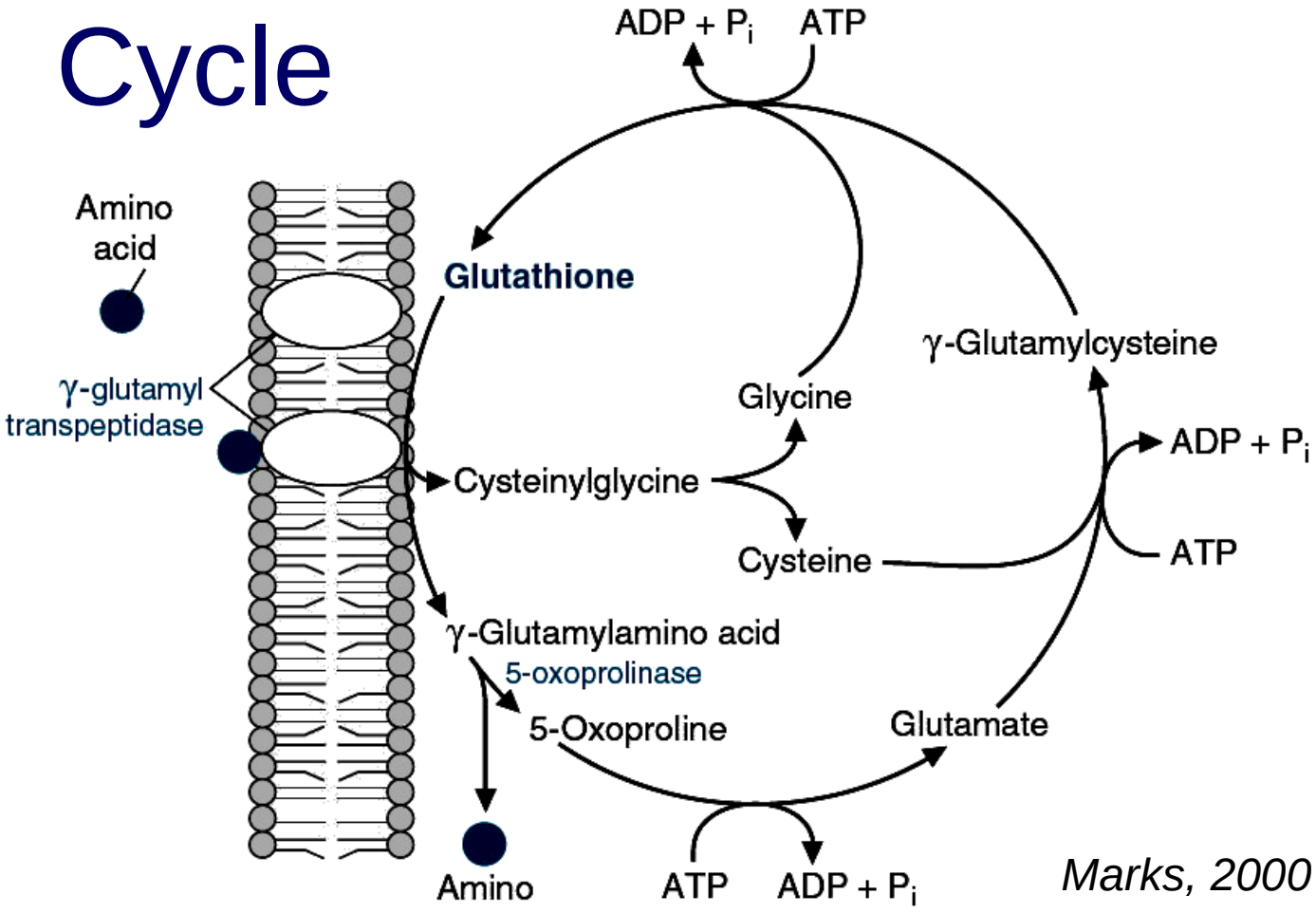
Amino Acids Absorption: Na^+-independent Transport



cytochalasin B

- Na^+-independent system of AA transport across intestinal cells is inhibited by **cytochalasin B**.
  - *Cytochalasin B*, a fungal metabolite, is also a competitive inhibitor of glucose transport.

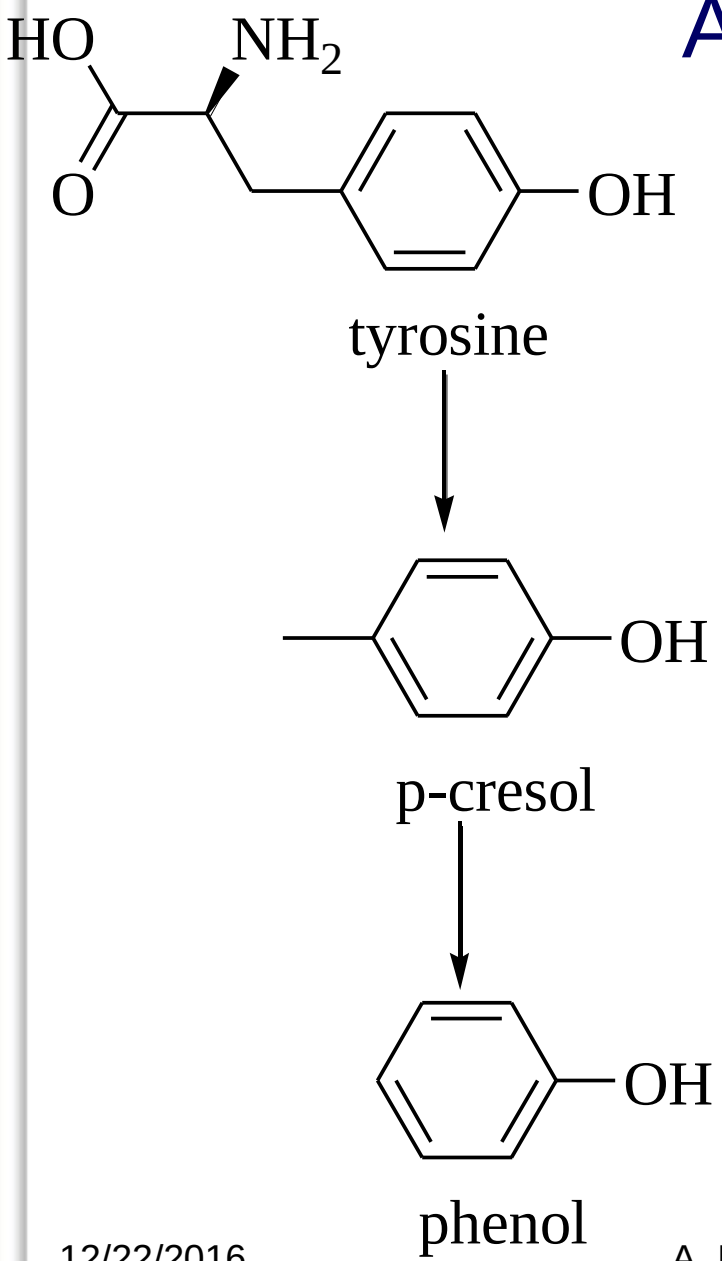
γ-Glutamyl Cycle



Marks, 2000

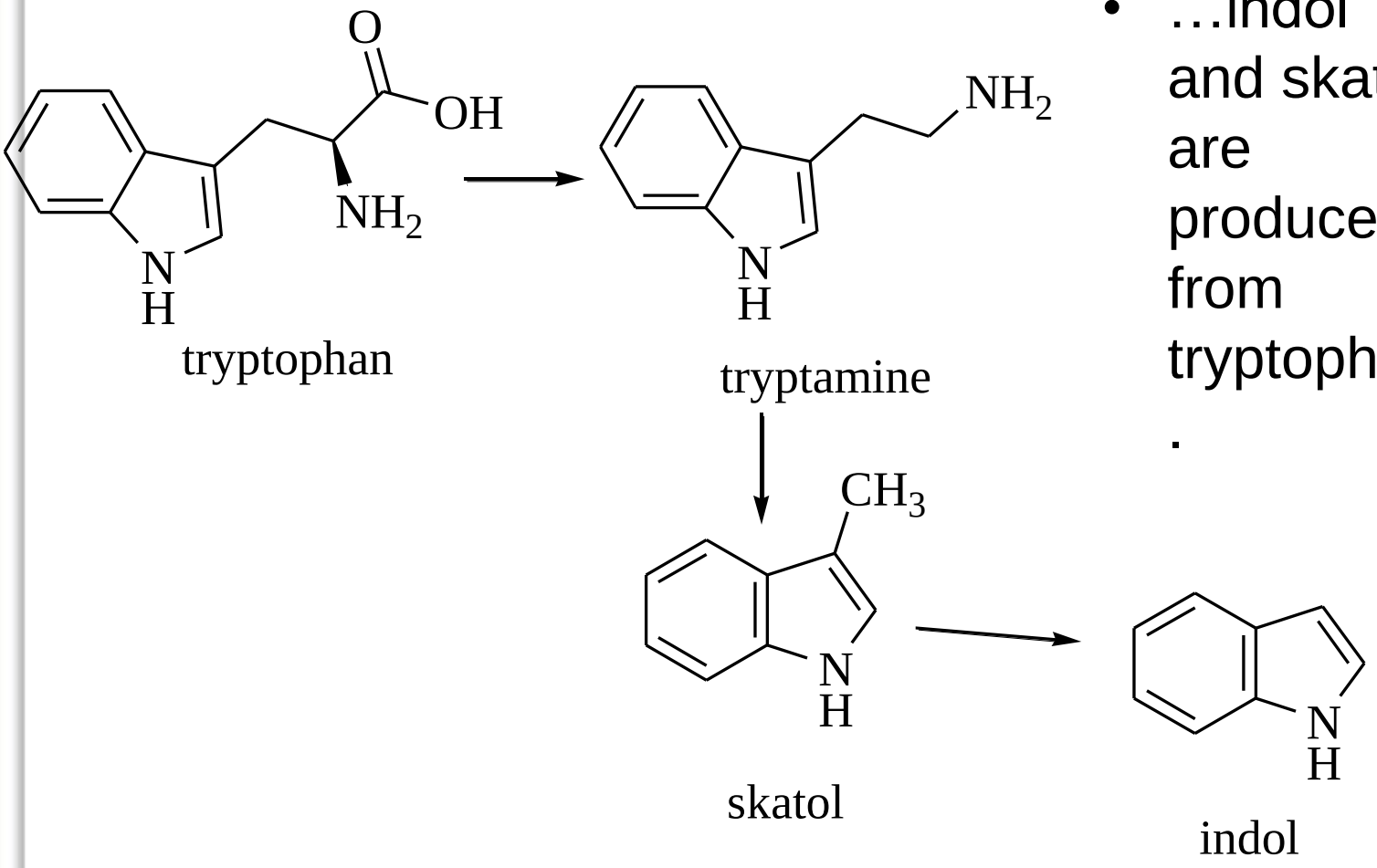
- **γ-glutamlyl cycle** or **Meister cycle** is another transport system of AA.
- It persists in intestine and kidney.

Amino acid putrefaction by microorganisms



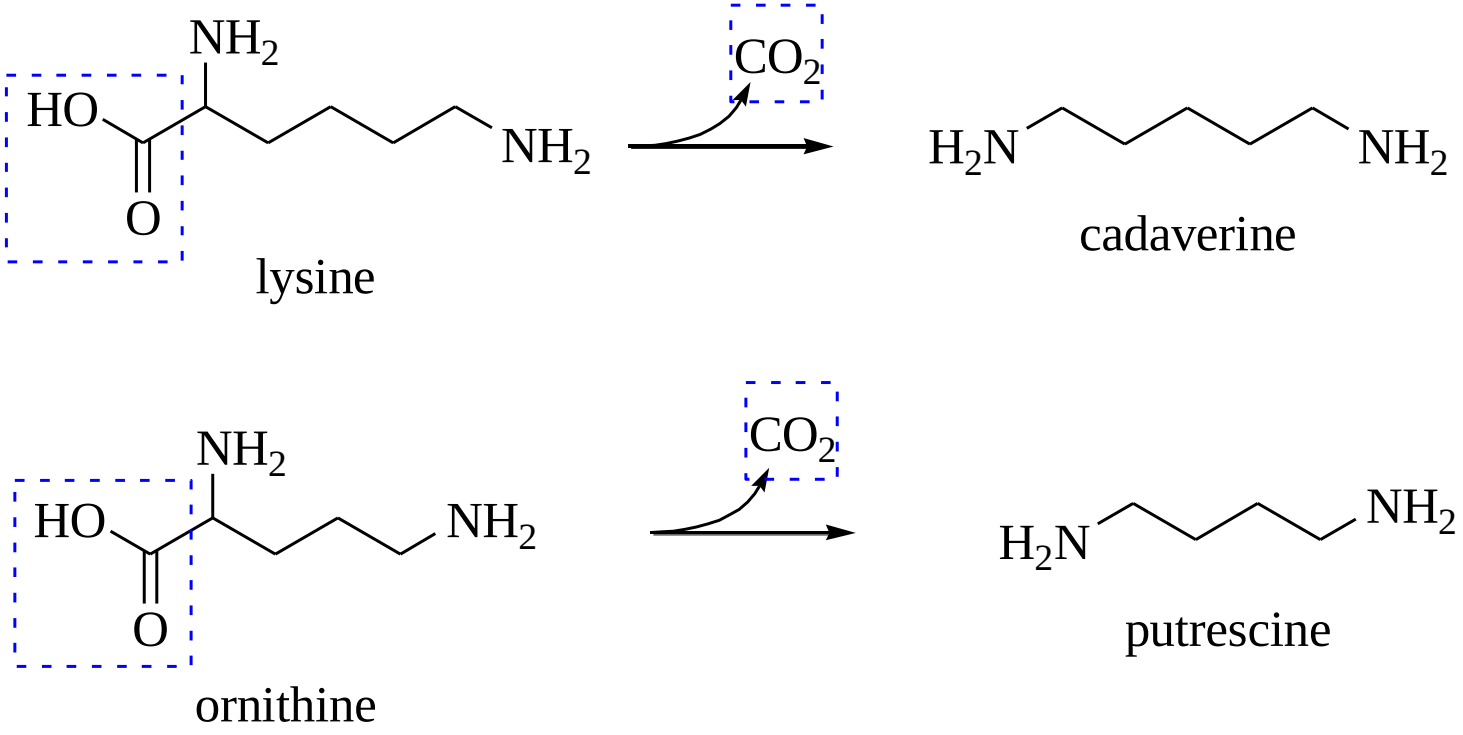
- If some amino acids were not absorbed, they undergo processes of degradation due to intestine microflora – putrefaction.
- The toxic products from tyrosine (cresol, phenol) are the result.

Amino acid putrefaction by microorganisms (cont'd)



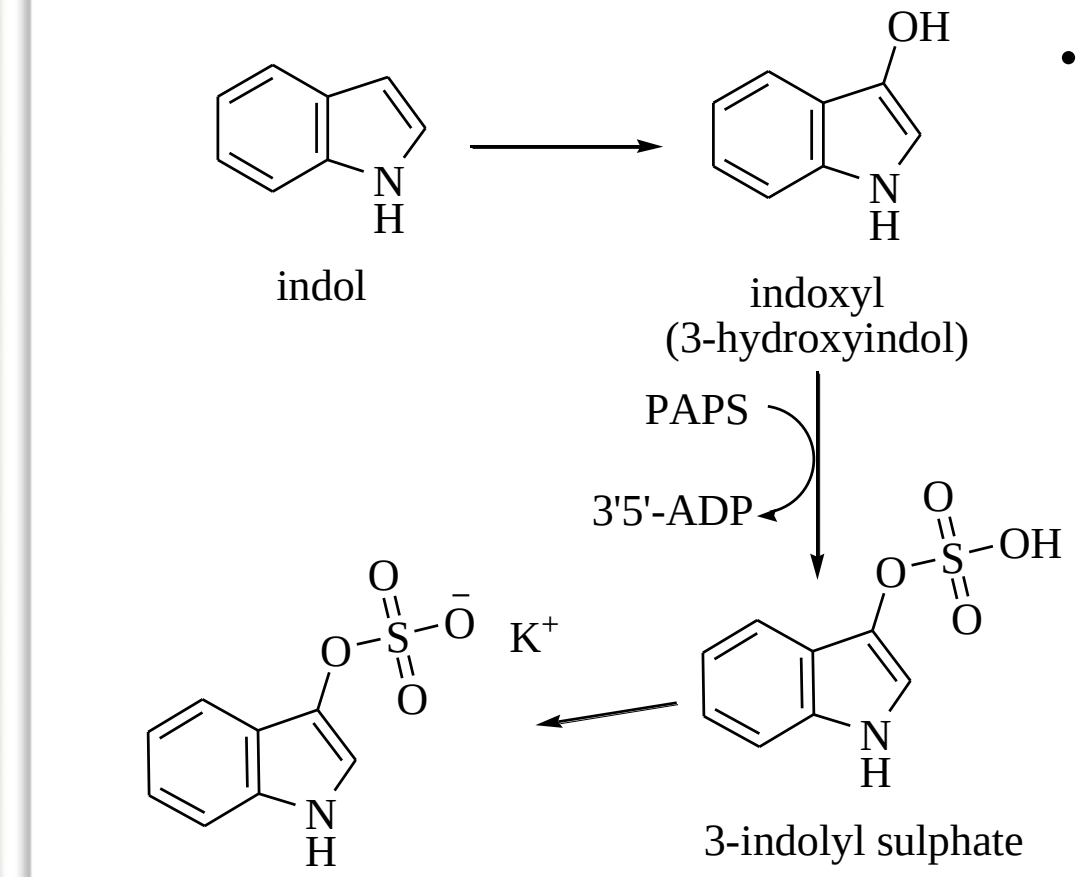
- …indol and skatol are produced from tryptophan .

Lysine and Ornithine Decarboxylation



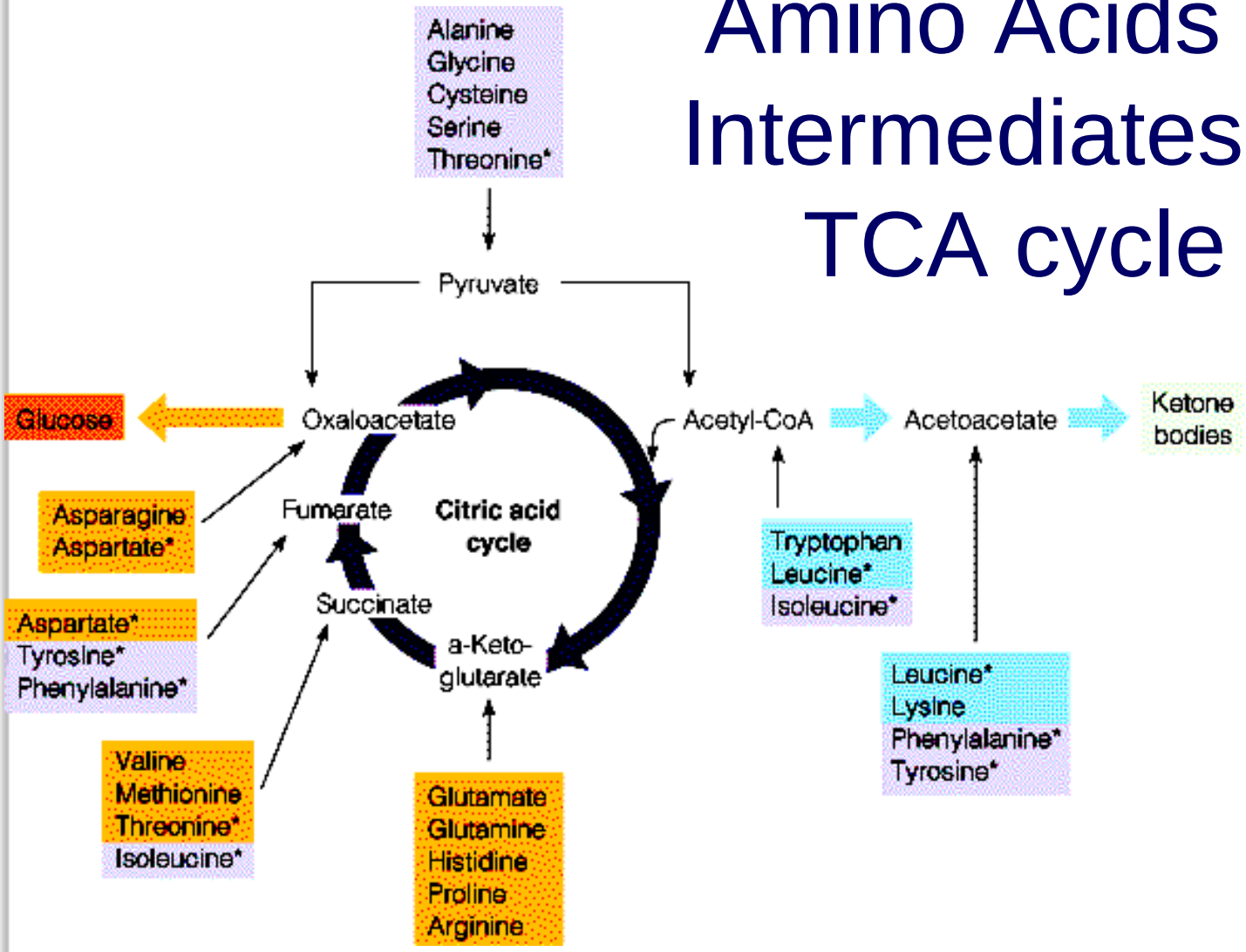
- **Cadaverine** and **putrescine** can be formed from Lys and Orn by decarboxylation.
  - These amines also known as *cadaver toxins*.

Detoxication in the liver



- Detoxication of putrefaction products occurs in the liver.
  - There are 2 main steps in the detoxication:
  - Hydroxylation by microsomal respiratory chain
  - Conjugation with either sulfuric acid or glucuronate.
  - PAPS serves as a donor of sulphate.

Degradation of Amino Acids to Intermediates of TCA cycle



Overview of Amino Acid Catabolism

- Alanine, serine, cysteine, glycine, threonine, and tryptophan are degraded to **pyruvate**.
- Asparagine and aspartate are converted into **oxaloacetate**.
- **α-Ketoglutarate** is the point of entry for glutamate and four amino acids (glutamine, histidine, proline, and arginine) that can be converted into glutamate.
- **Succinyl CoA** is the point of entry for some of the carbon atoms of four amino acids (isoleucine, valine, methionine and threonine) that are degraded through the intermediate ***methylmalonyl CoA***.
- Leucine is degraded to **acetoacetyl CoA** and **acetyl CoA**.
  - The breakdown of valine and isoleucine is like that of leucine. Their α-ketoacid derivatives are oxidatively decarboxylated by the branched-chain α-ketoacid dehydrogenase.

Overview of Amino Acid Catabolism (cont'd)

- The rings of aromatic amino acids are degraded by **oxygenases**.
  - *Phenylalanine hydroxylase*, a monooxygenase, uses **tetrahydrobiopterin** as the reductant. One of the oxygen atoms of O_2 emerges in tyrosine and the other in water.
  - Subsequent steps in the degradation of these aromatic amino acids are catalyzed by **dioxygenases**, which catalyze the insertion of both atoms of O_2 into organic products.
- Four of the carbon atoms of phenylalanine and tyrosine are converted into **fumarate**, and four emerge in **acetoacetate**.

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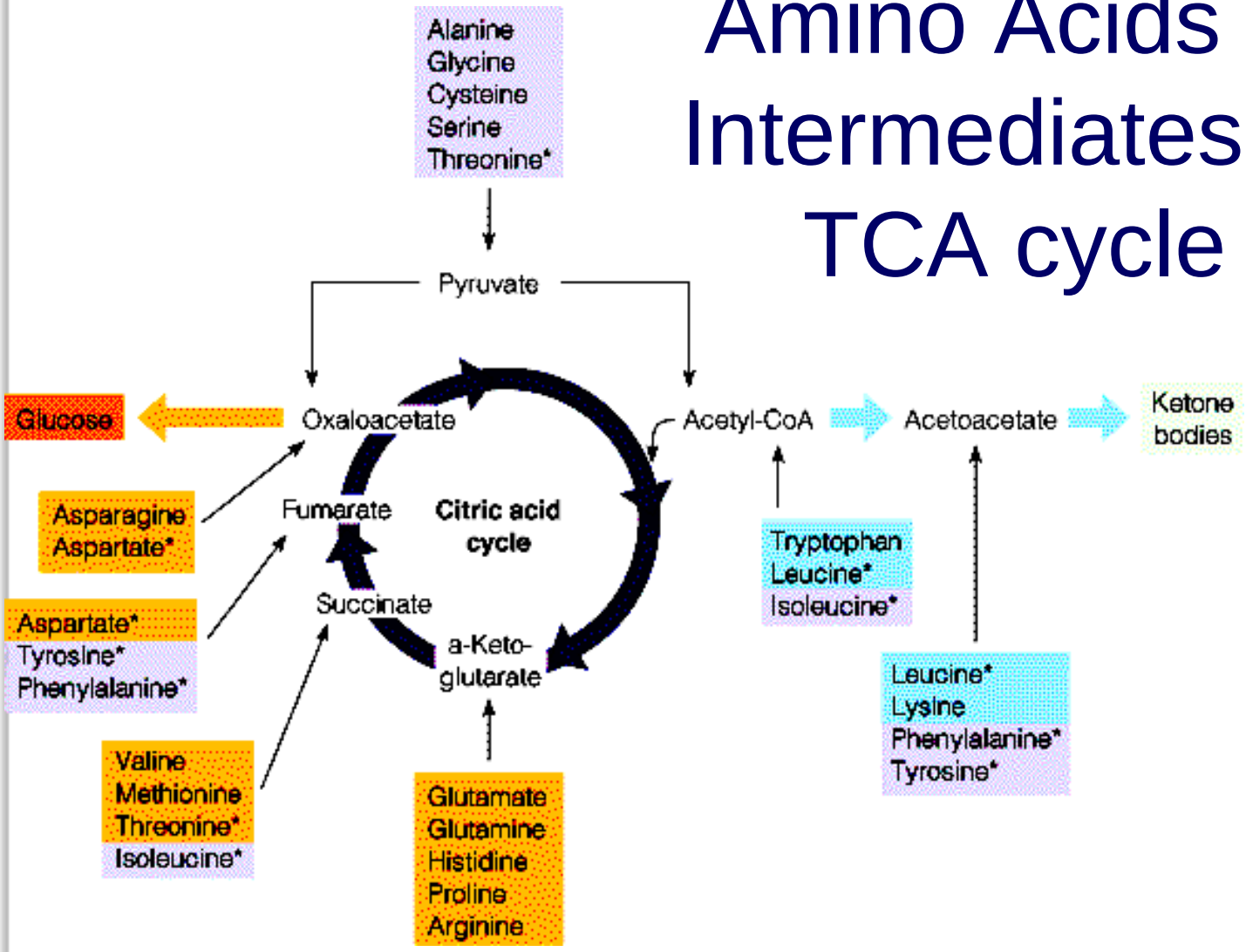
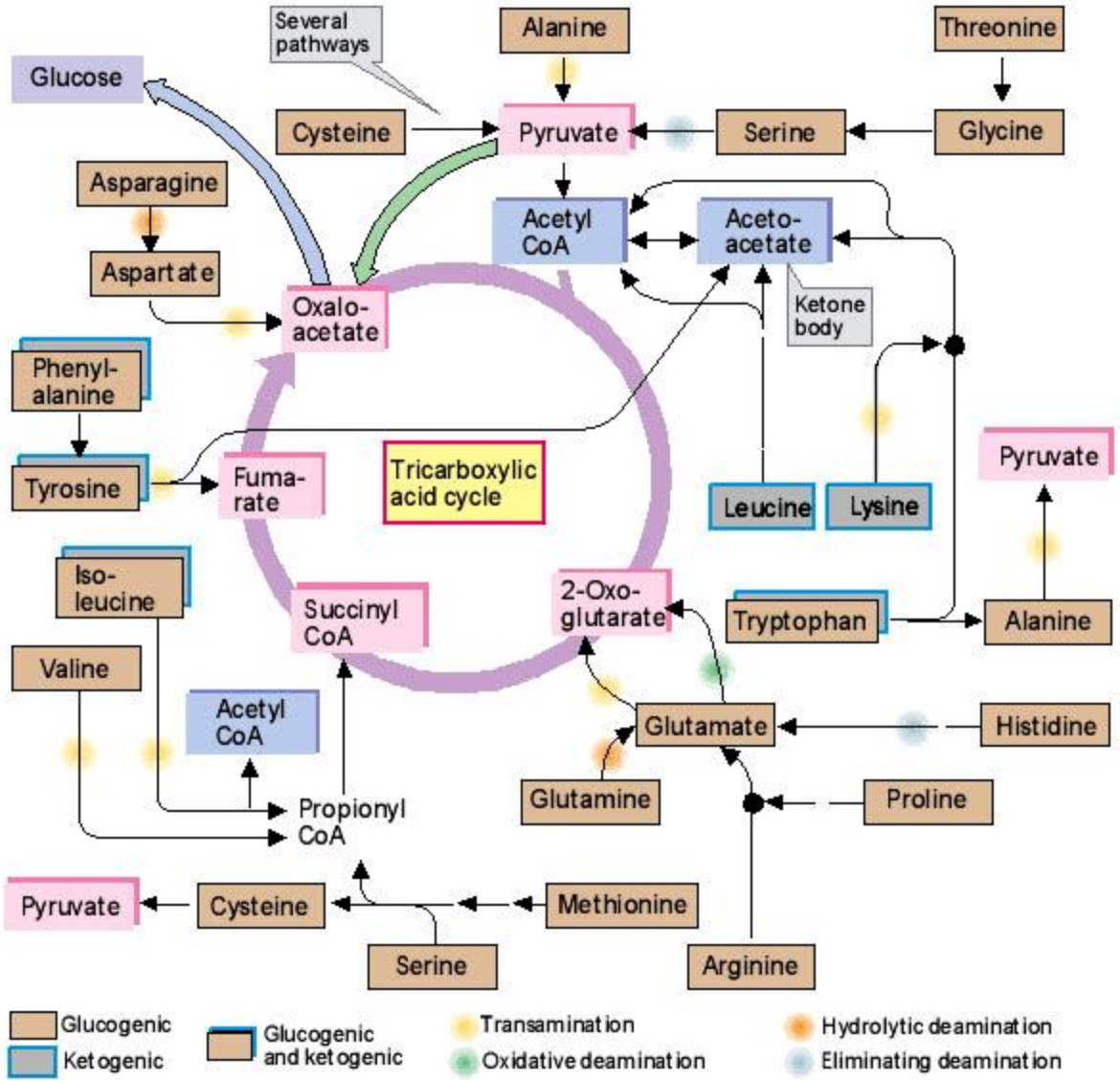


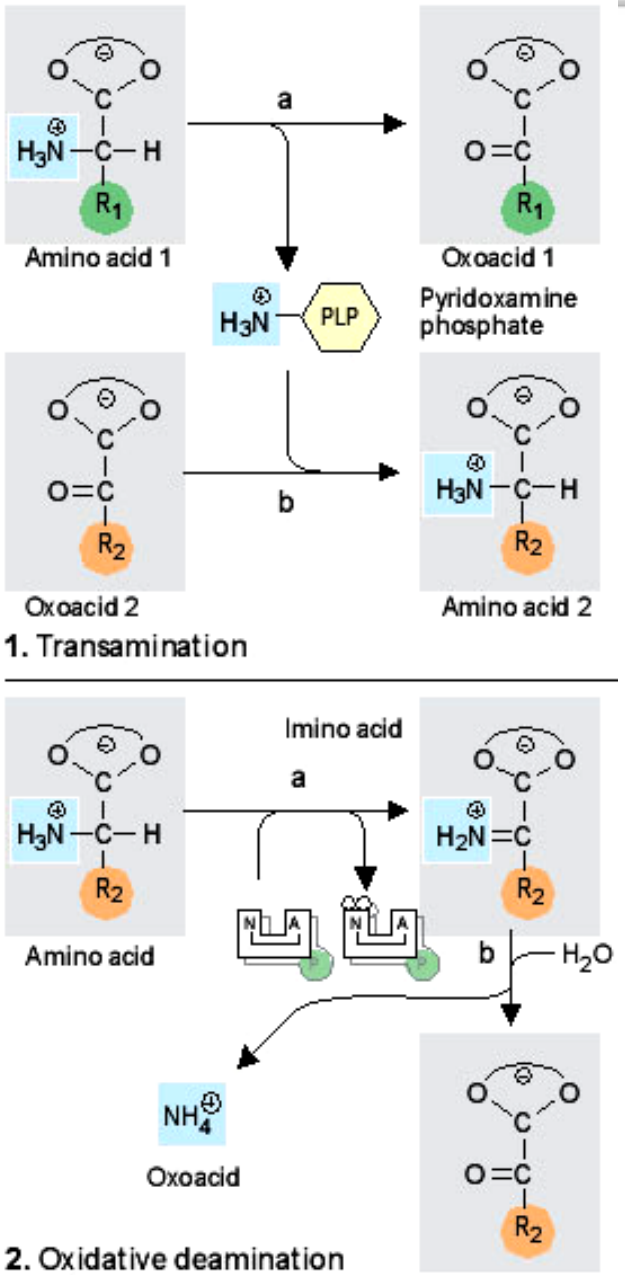
Carbon Atoms of Degraded Amino Acids Emerge as Major Metabolic Intermediates

- The carbon atoms of degraded amino acids are converted into **pyruvate**, **acetyl CoA**, **acetoacetate**, or an intermediate of the **citric acid cycle**.
- Most amino acids are solely **glucogenic**, two are solely **ketogenic**, and a few are both **ketogenic and glucogenic**.

Amino Acid Degradation: Overview

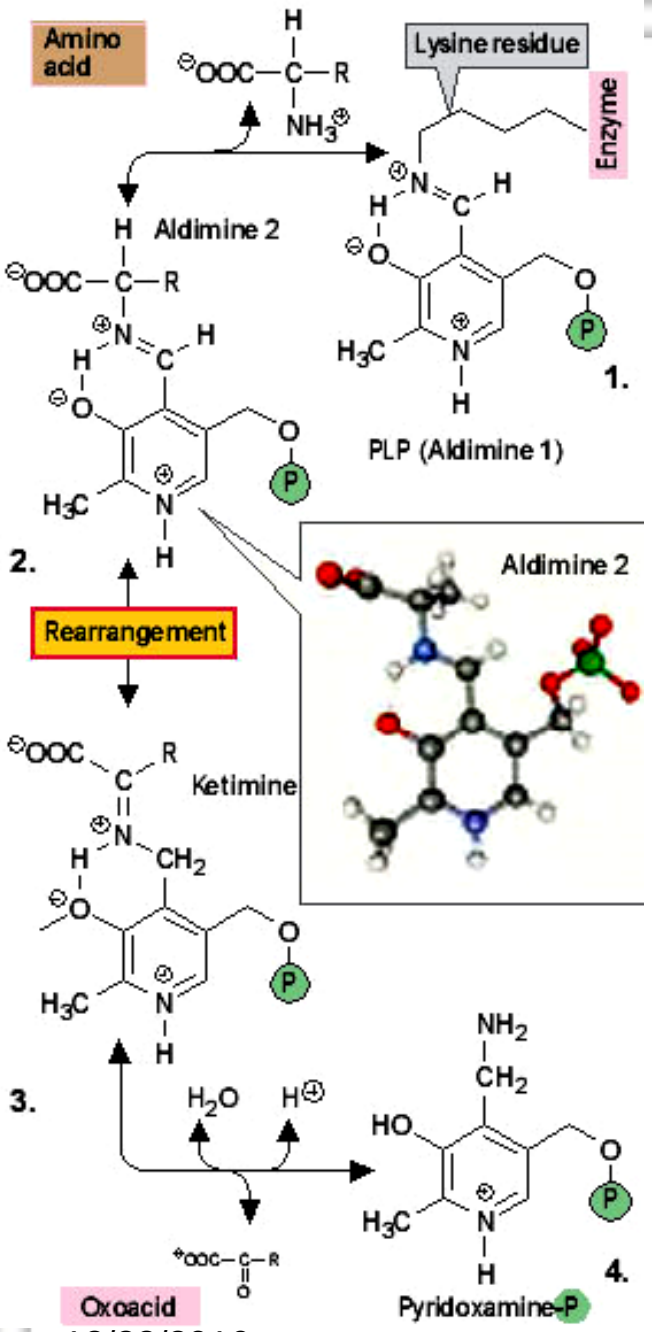


The First Step in Amino Acid Degradation Is the Removal of Nitrogen



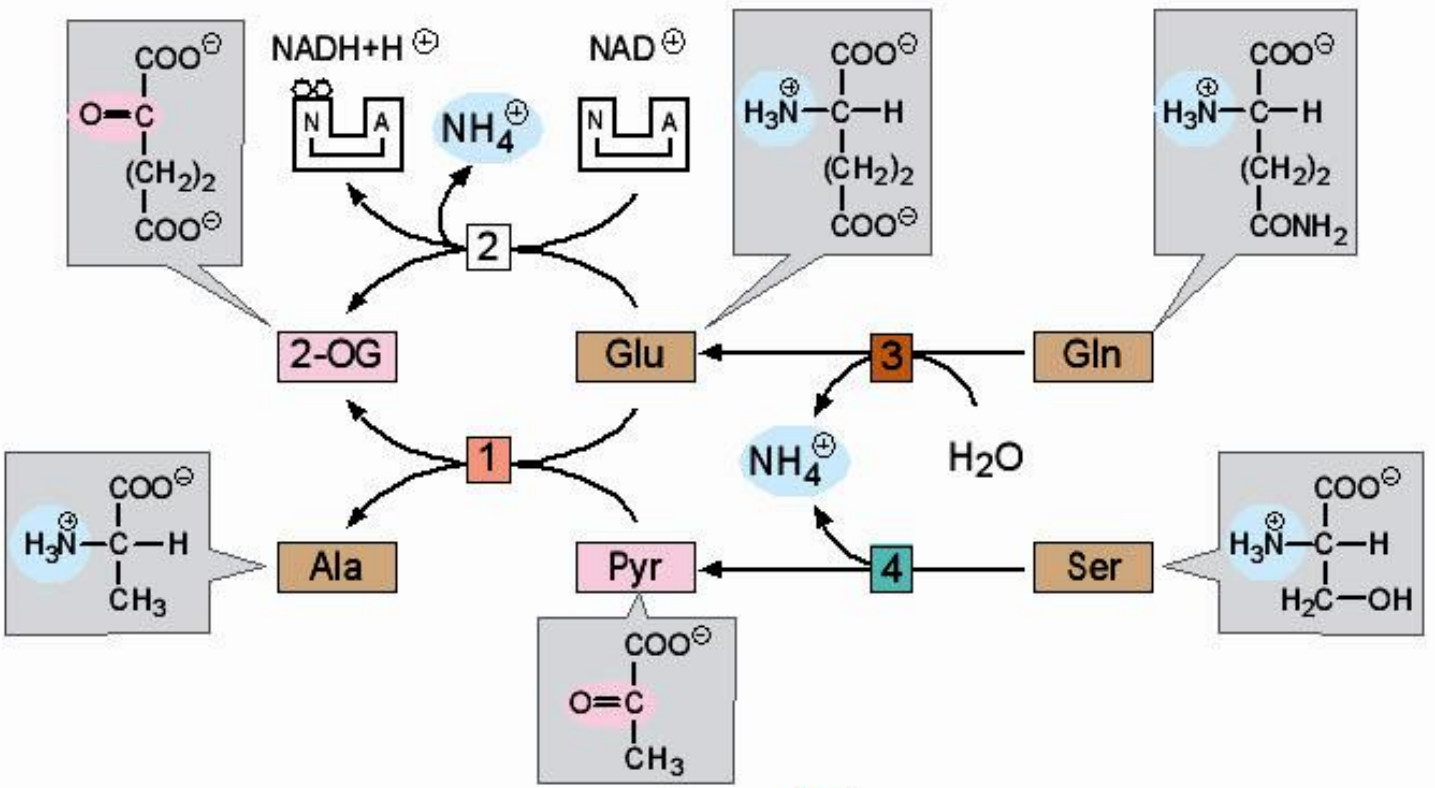
- The first step in their degradation is the removal of their α-amino groups by transamination to an α-ketoacid.
  - The α-amino group funnels into α-ketoglutarate to form glutamate, which is then oxidatively deaminated by glutamate dehydrogenase to give NH_4+and α-ketoglutarate.
  - NAD^+ or $NADP^+$ is the electron acceptor in this reaction.

The Role of Pyridoxal Phosphate

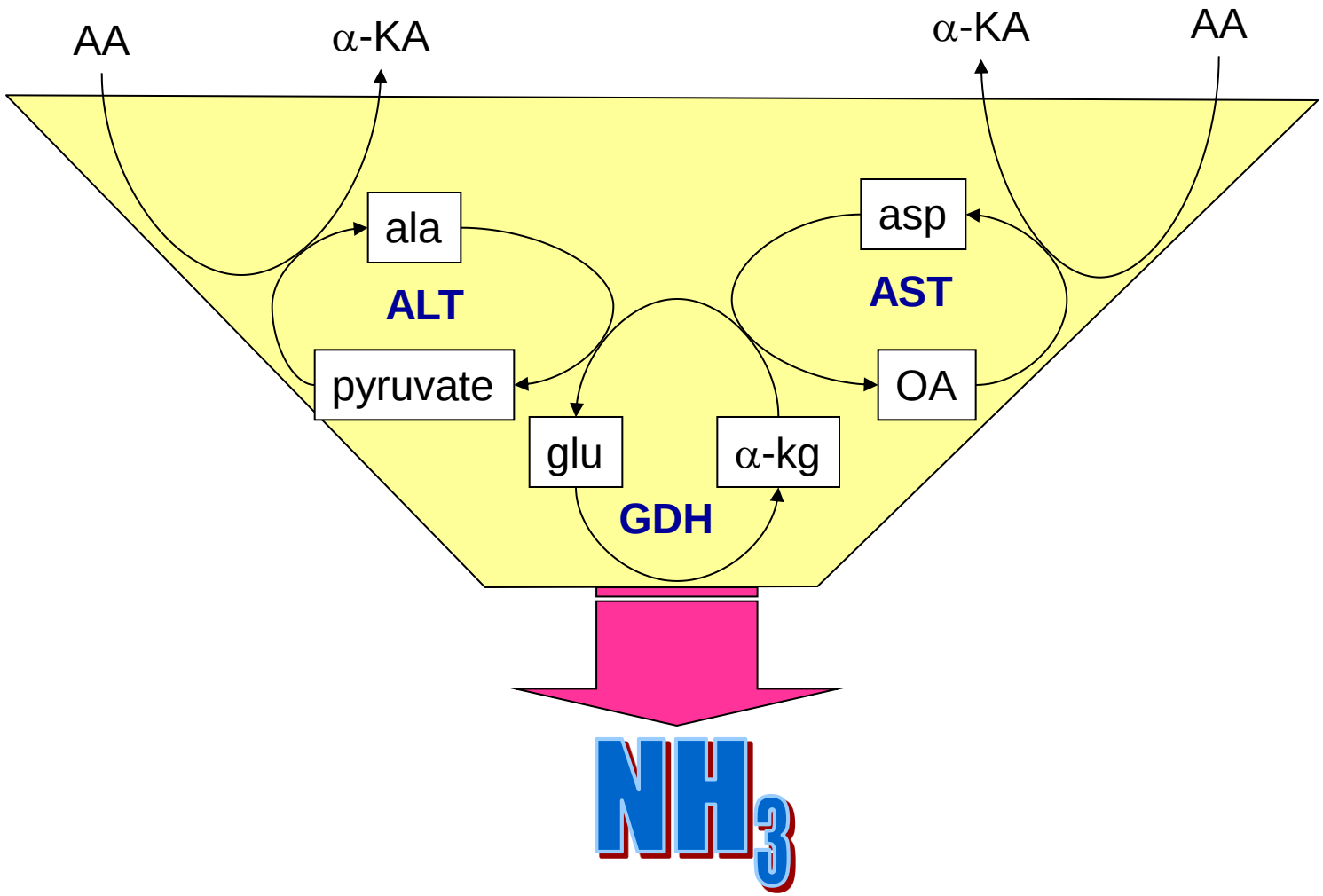


- Pyridoxal phosphate is the coenzyme in all aminotransferases and in many other enzymes catalyzing amino acids transformations.

Deamination

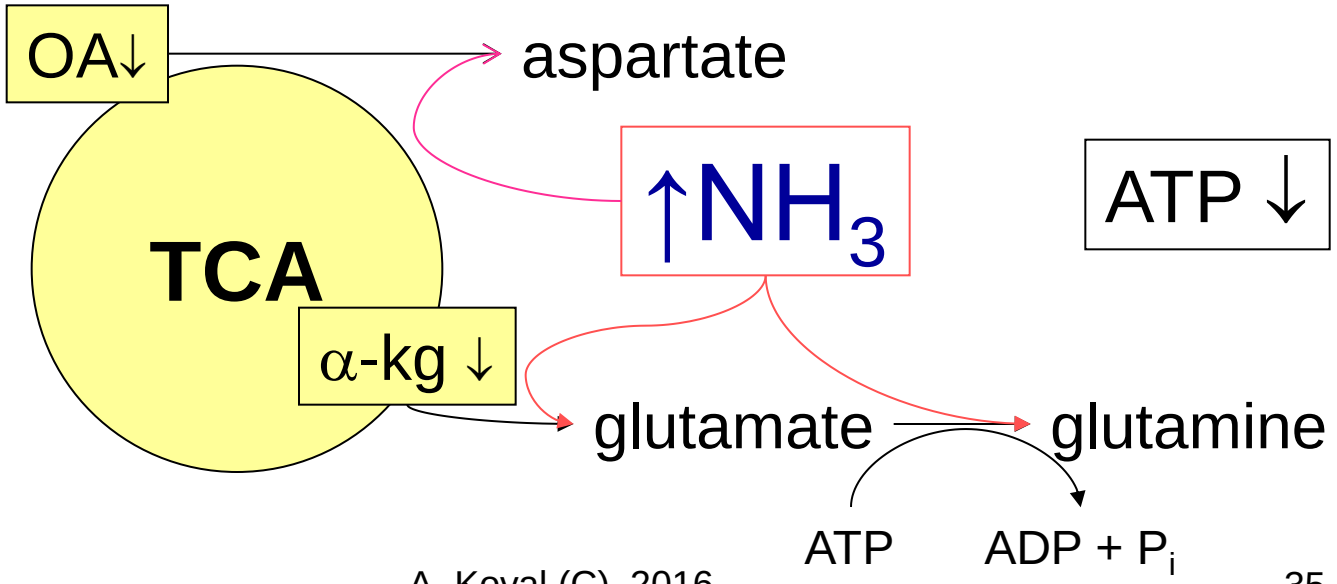


Metabolic Funnel



Toxicity of Ammonia

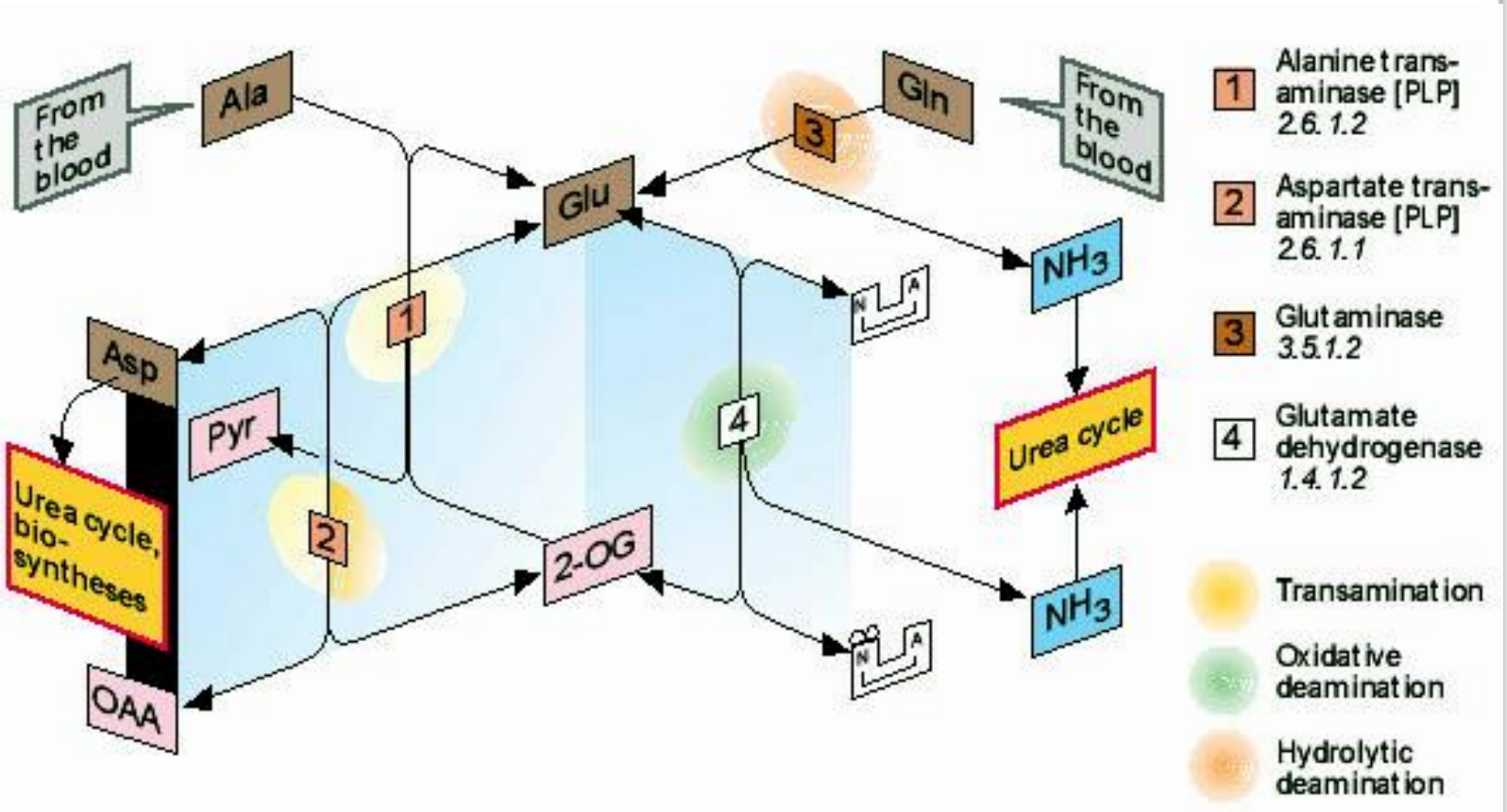
- Degradation of proteins results in ammonia production.
- The toxicity of ammonia is caused by the depletion of TCA intermediates and ATP in the central nervous system.

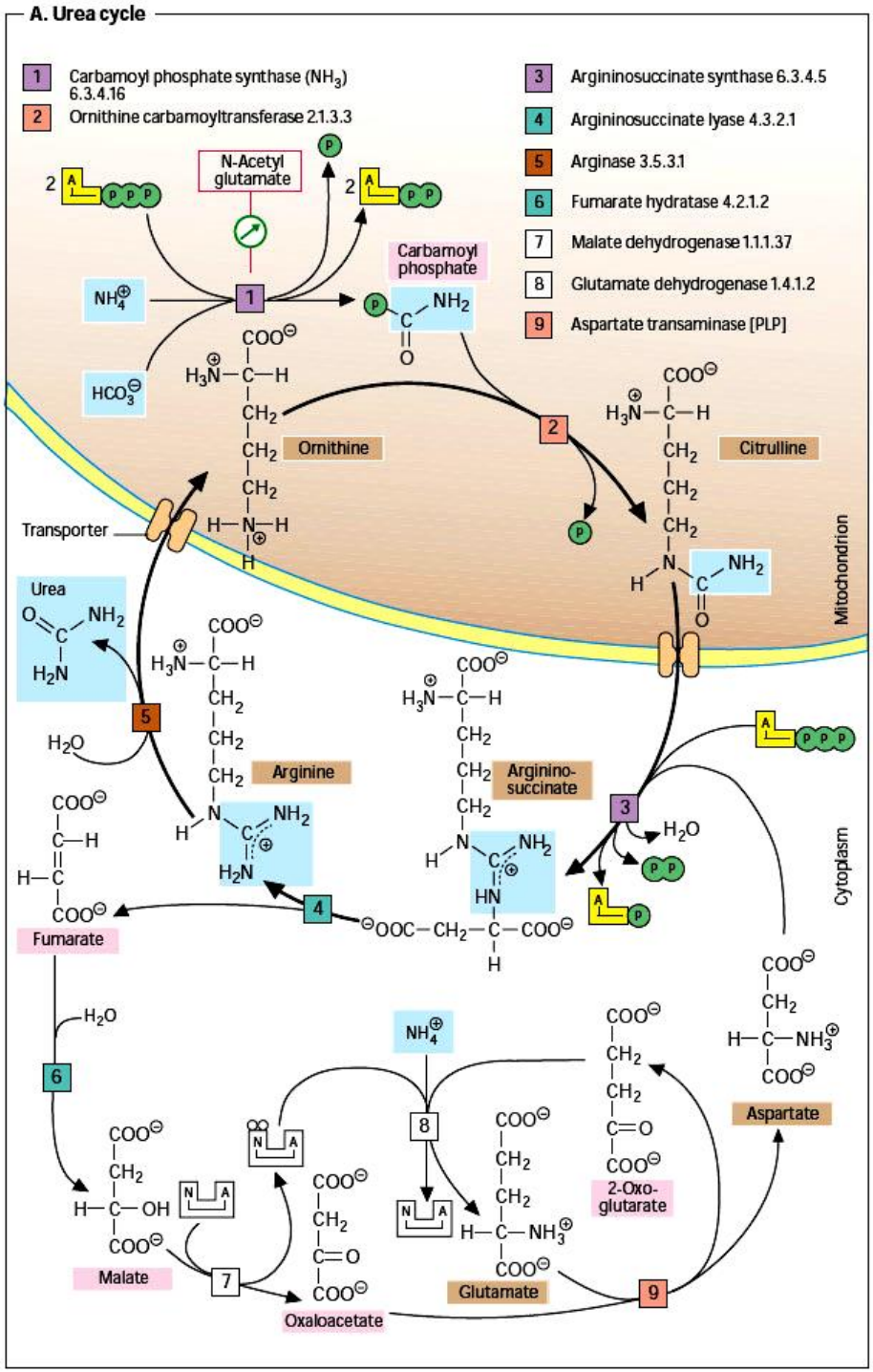


Effects of Hepatic Failure

- γ-aminobutyric acid (GABA), an important inhibitory neurotransmitter in the brain, is also produced in the gut lumen and is shunted into the systemic circulation in increased amounts in patients with hepatic failure.
  - In addition, other compounds (such as aromatic amino acids, false neurotransmitters, and certain short-chain fatty acids) bypass liver metabolism and accumulate in the systemic circulation, adversely affecting central nervous system function.
  - Their relative importance in the pathogenesis of hepatic encephalopathy remains to be determined.

Metabolism of NH_3 in Liver

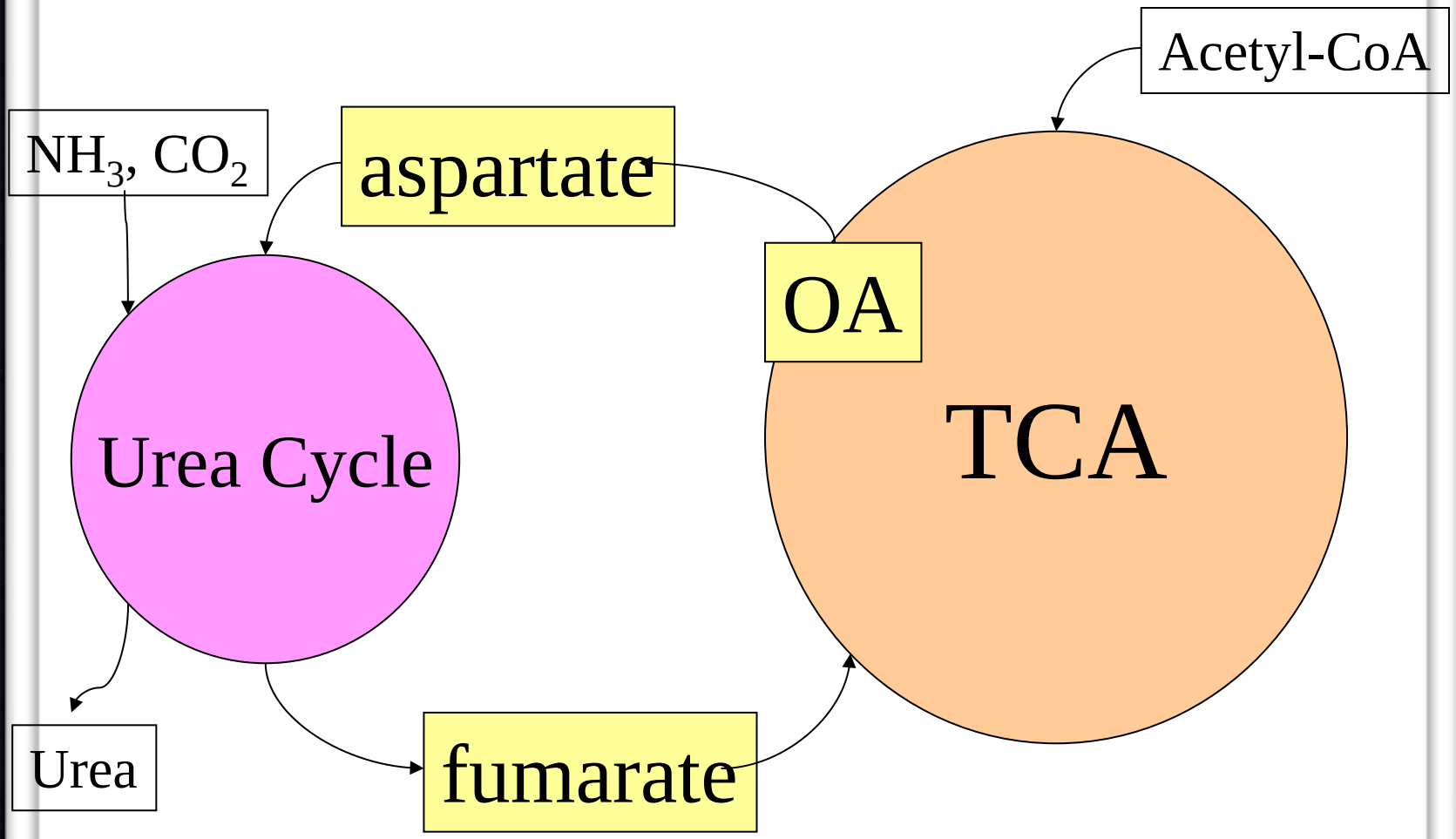




Urea Cycle

- In mitochondria:
  - The first step is the formation of carbamoyl phosphate (from HCO_3^-, NH_4^+, and 2 ATP) by **carbamoyl phosphate synthetase**.
  - **Ornithine** is then carbamoylated to **citrulline** by **ornithine transcarbamoylase**.
- In cytosol:
  - Citrulline leaves the mitochondrion and with *aspartate* forms **argininosuccinate**,
  - the last is cleaved into **arginine** and **fumarate**.
  - The other nitrogen atom of urea comes from *aspartate*.
  - Urea is formed by the hydrolysis of **arginine**, which also regenerates ornithine.
- Some enzymatic deficiencies of the urea cycle can be bypassed by supplementing the diet with arginine or compounds that form conjugates with glycine and glutamine.

Regulatory Role of TCA: "Krebs' bicycle"



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Inborn Errors of Metabolism Can Disrupt Amino Acid Degradation

- Errors in amino acid metabolism served as sources of some of the first insights into the correlation between pathology and biochemistry.
- Although there are many hereditary errors of amino acid metabolism, **phenylketonuria** is the best known.
  - This condition is the result of the accumulation of high levels of phenylalanine in the body fluids.
  - By unknown mechanisms, this accumulation results in *mental retardation* unless the afflicted are placed on low phenylalanine diets immediately after birth.


Thank you
for your attention