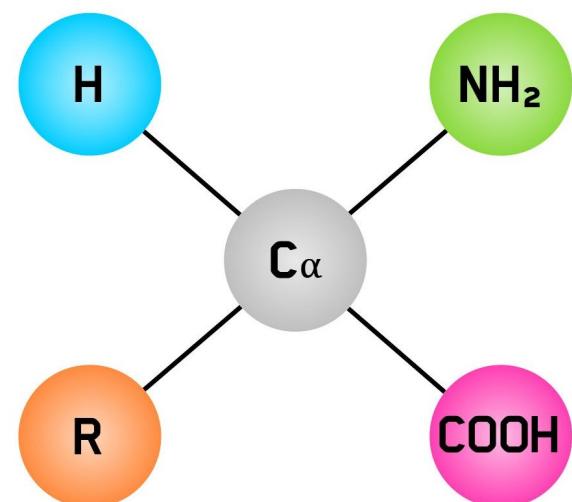


Lecture 6: I. Methods to study proteins II. Amino acid catabolism



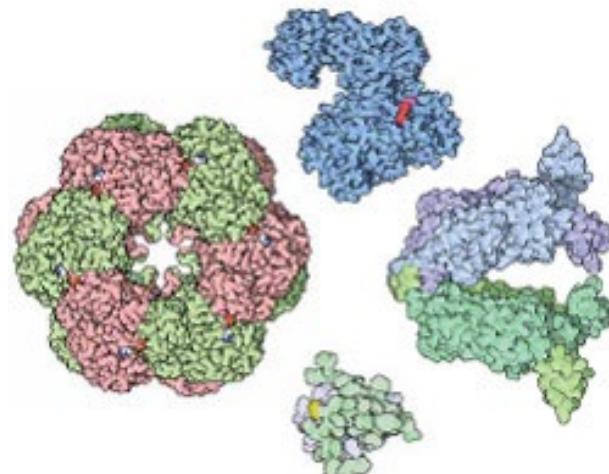
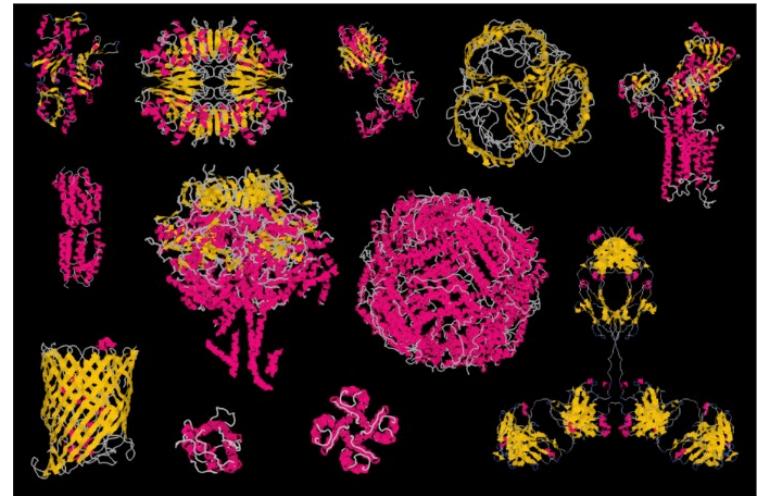
Kwok-On LAI
Department of Neuroscience

Learning outcomes

- To introduce the methods of studying proteins (the principles of protein purification and gel electrophoresis analysis)
- To describe the cleavage of proteins in the digestive system and the overview of amino acid catabolism in the body
- To describe the ammonia catabolism by the urea cycle
- To explain the degradation of the carbon skeleton of the amino acid
- To recognize some diseases associated with gene mutations in amino acid catabolism pathway

Study proteins: need to isolate them first

- Each typical mammalian cell contains 10 billion (1×10^{10}) molecules of 10,000 different varieties
- The whole collection of proteins in a cell is called “proteome”
- **How can you isolate one particular protein from the numerous others to study?**



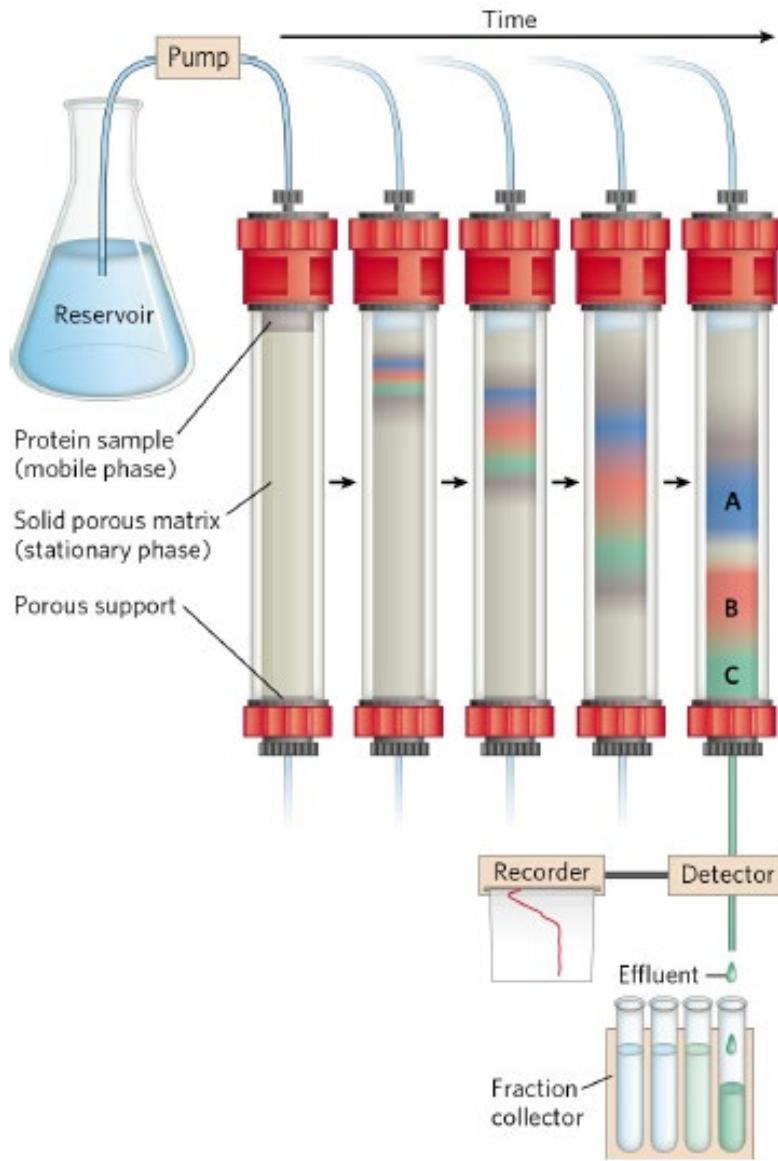
Principles of protein separation

- Proteins in the cells are a complex mixture and they are very heterogeneous in size and shapes
- Isoelectric points (pl) of proteins, defined as the pH at which a protein has no net charge, are also different
- Mixture of proteins can be separated (divided into different fractions) and purified by different means
 - Size (gel filtration or size-exclusion chromatography)
 - Charge (ion-exchange chromatography)
 - Binding properties (affinity chromatography)



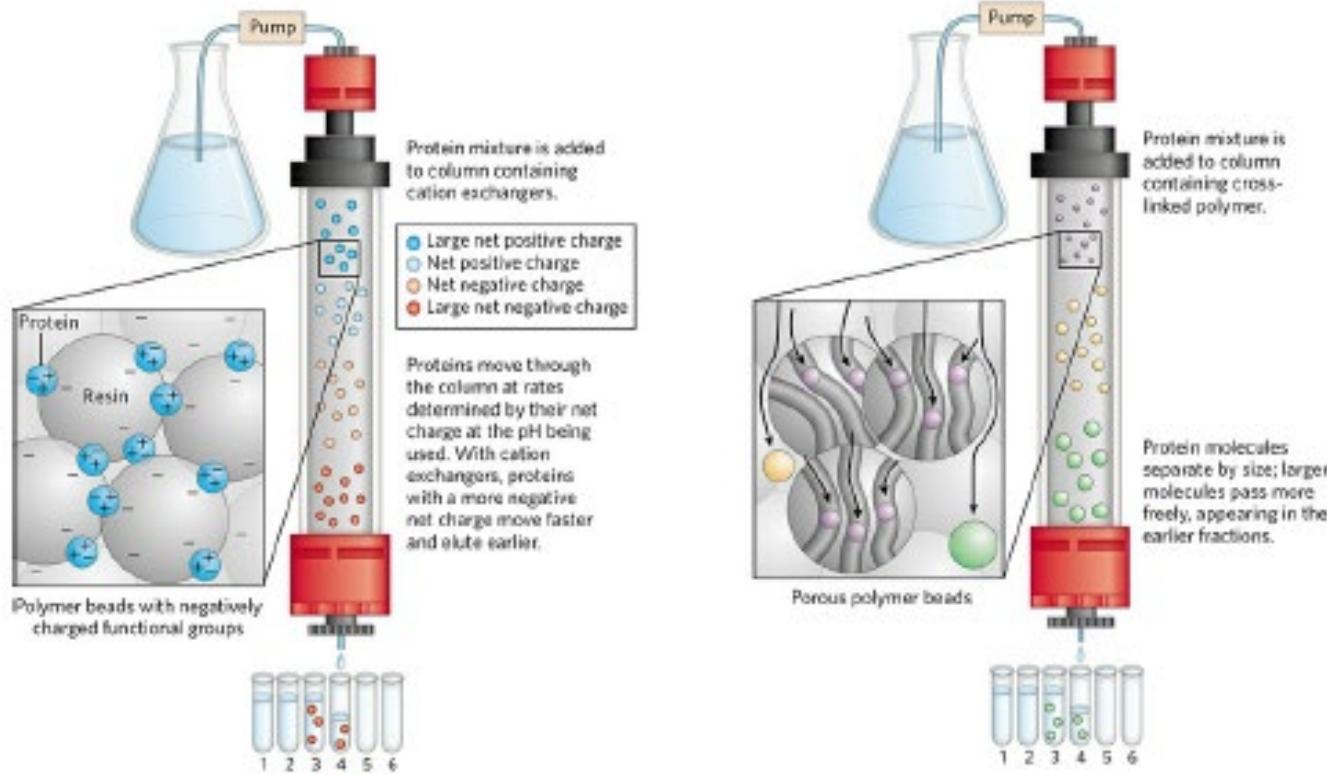
Homogenizer to break up tissues

Fractionation by column chromatography



- A porous solid material with appropriate chemical properties (the stationary phase) in a column
- A buffered solution (the mobile phase) containing the proteins migrates through it
- Individual proteins migrate faster or slower through the column, depending on their properties

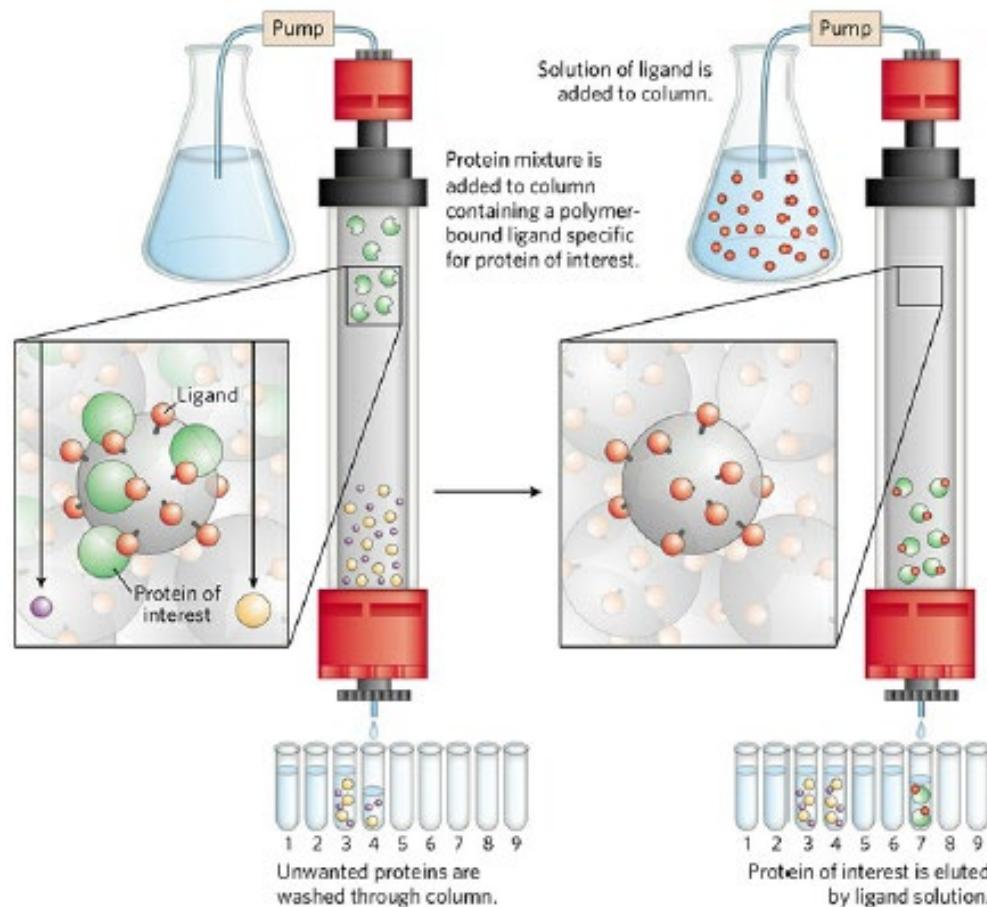
Different types of column chromatography



Ion exchange chromatography
Negatively-charged matrix will
retard the mobility of
positively-charged proteins
(and vice versa)

Size-exclusion chromatography
Proteins are separated by sizes
(large or small proteins come
out from the column first?)

Different types of column chromatography



A two-step process

Need to add ligands to remove them from the column afterwards

Affinity chromatography

Proteins are bound to the column by selective binding to their ligands in the matrix

Keep your protein samples cold

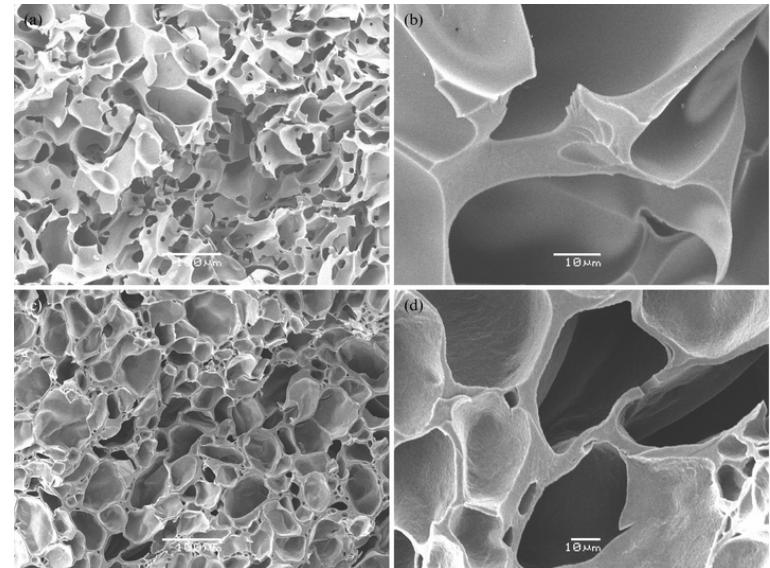


Why?

Separation of proteins in a gel for analysis

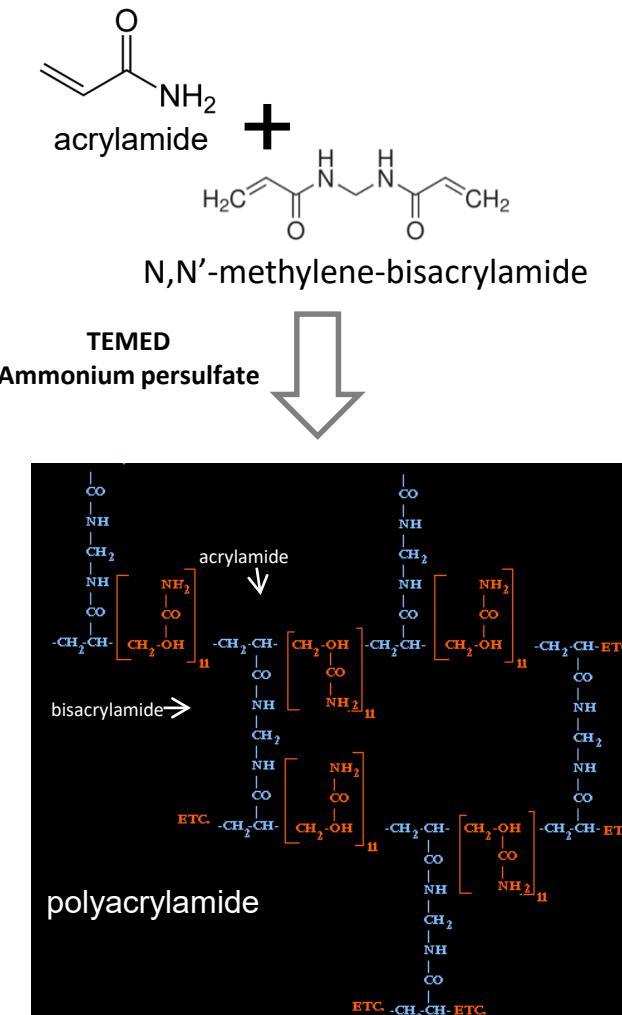
After running chromatography, how to know you have isolated your protein of interest in enough purity?

- The proteins need to be retarded by some **frictions or drags**. Porous materials provide the drags and help prevent diffusion in solution
- **Agarose** and **polyacrylamide** gel are common gel materials. Agarose gel (of larger pores) is mainly used in separating nucleic acids and polyacrylamide gel (of much smaller pores) separates proteins and smaller nucleic acids



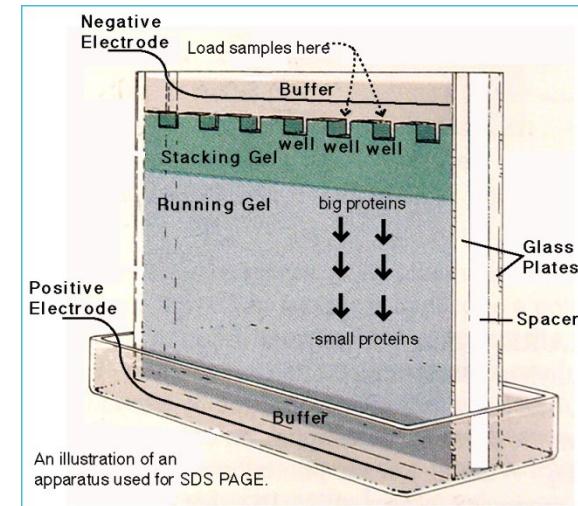
Polyacrylamide gel

- It provides a higher resolving power than agarose gel
- Advantages:
 - 1. separating a wide range of proteins and nucleic acids
 - 2. accommodate large sample size
- It is made by cross-linking **acrylamide** and **N,N'-methylene-bisacrylamide** (the cross-linker) The pore size of a gel is determined by the total amount of acrylamide and cross-linker
- Acrylamide is a neurotoxin and carcinogen – **handle with care**



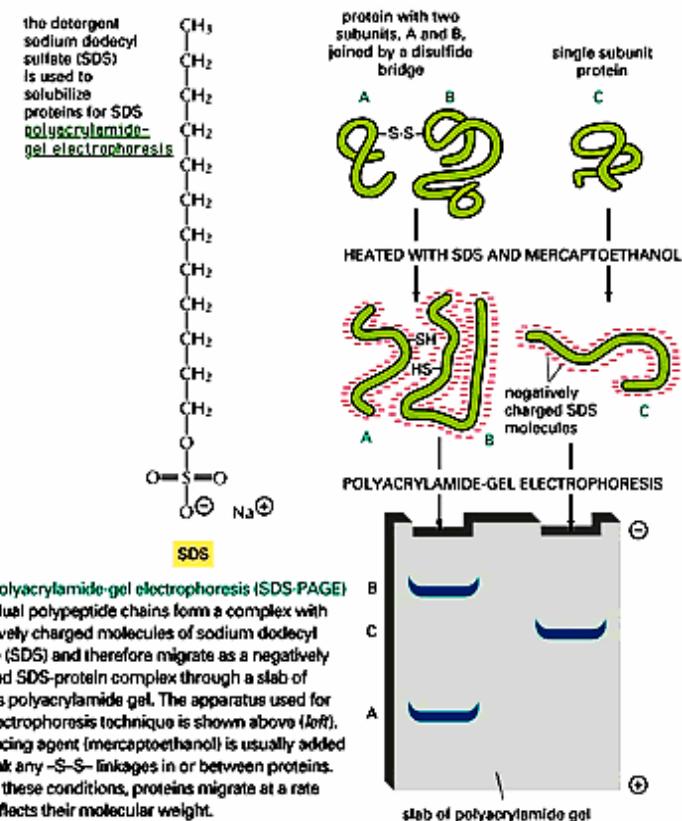
SDS polyacrylamide gel electrophoresis (SDS-PAGE)

- It is the most commonly used analytical protein separation method
- Proteins of different electrophoretic mobility can be separated by SDS-PAGE
- After SDS denaturing treatment, negatively charged SDS binds to proteins proportionately to the length of the proteins so the proteins can be **fractionated based on their sizes**



The principles of SDS-PAGE

- SDS (Sodium dodecyl sulfate) is a strong detergent
- SDS **denatures** (or unfold) the proteins and confers **negative charge** to the proteins in a fixed ratio proportional to the molecular weight of the protein, giving a constant negative charge to length/mass ratio
- Before loading into the gel, the samples are **boiled** in a sample buffer containing **SDS and a reducing agent** (mercaptoethanol to remove the disulfide bonds)

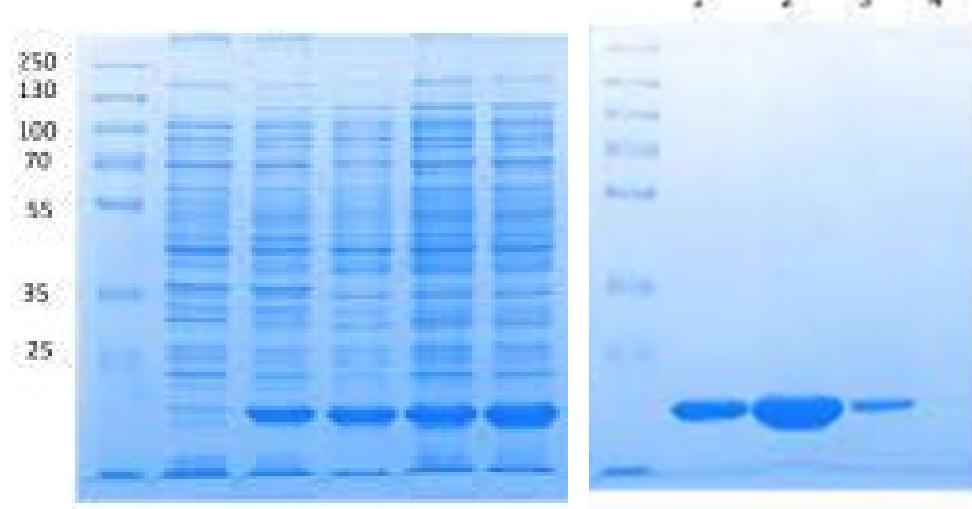


SDS- Sodium dodecyl sulfate

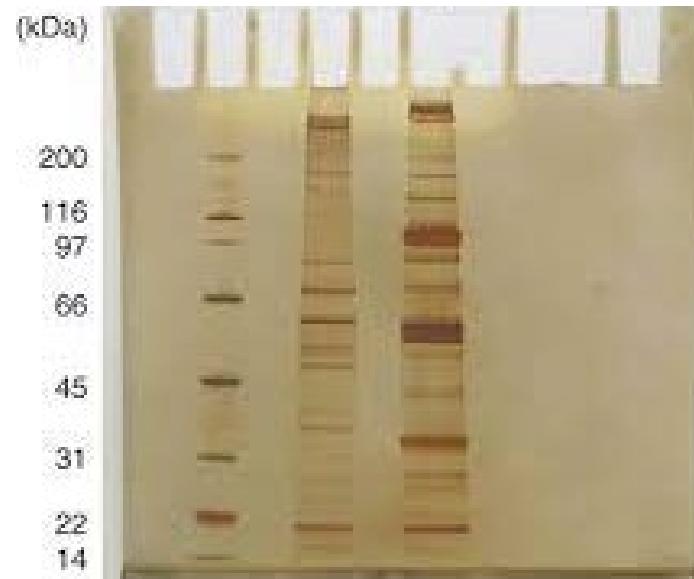
Staining of proteins on gel

There are two ways of staining for SDS PAGE:

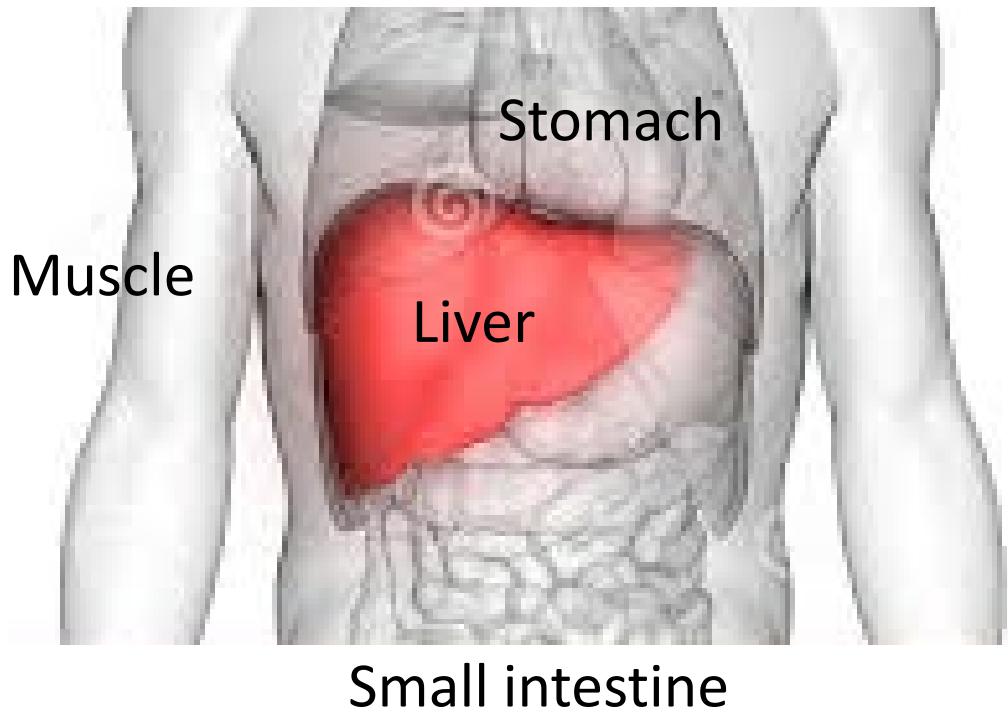
- **Coomassie staining:** The dye used is Coomassie Brilliant Blue dyes (also the dye used in Bradford method). It binds to proteins by adsorption



- **Silver staining:** Silver metal attached to the protein molecules. It is a sensitive method- a protein band of 50 ng can be detected (50 times more sensitive than Coomassie staining)



Protein Digestion & Amino acid Catabolism

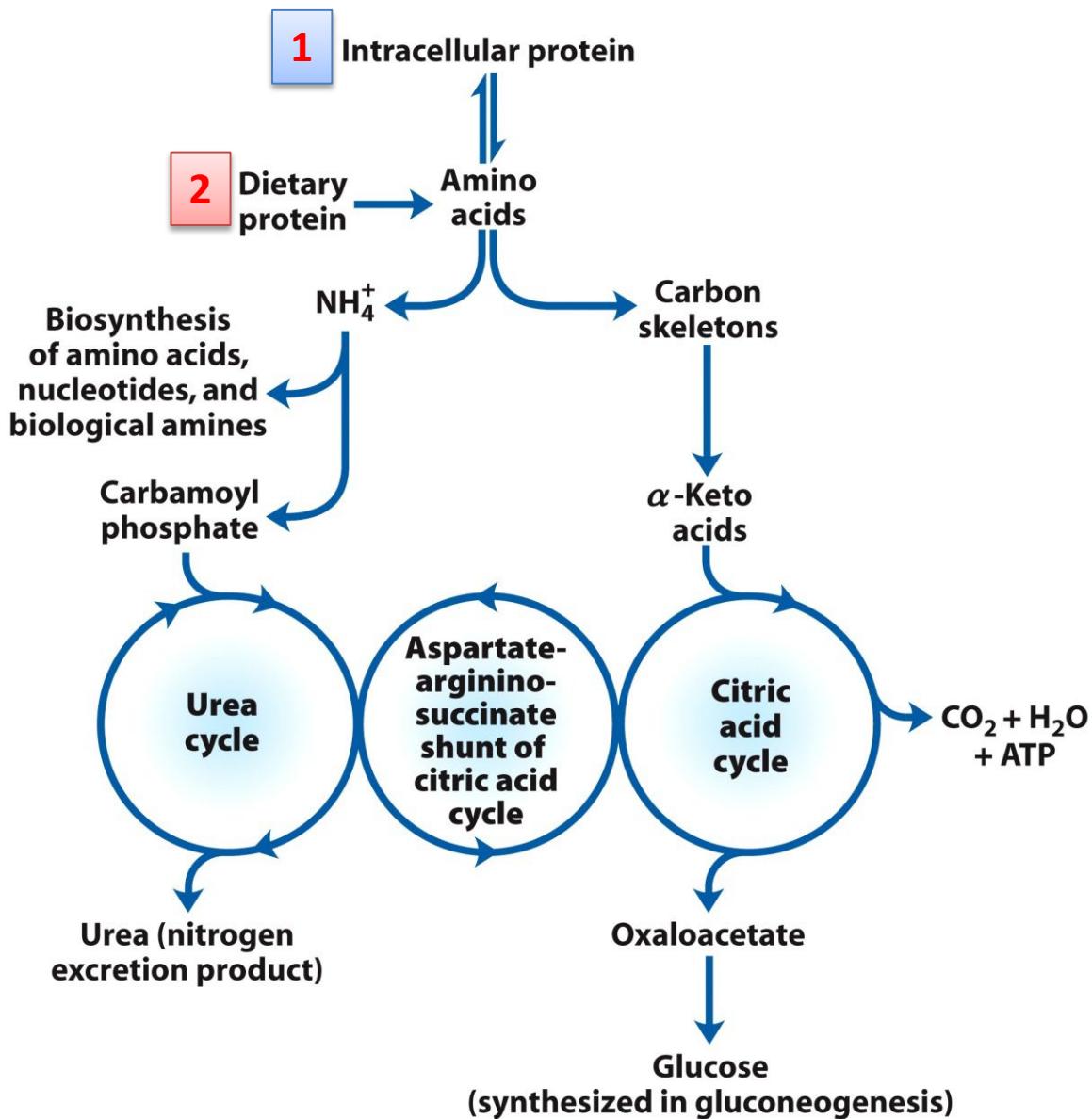


Why we need amino acids catabolism?

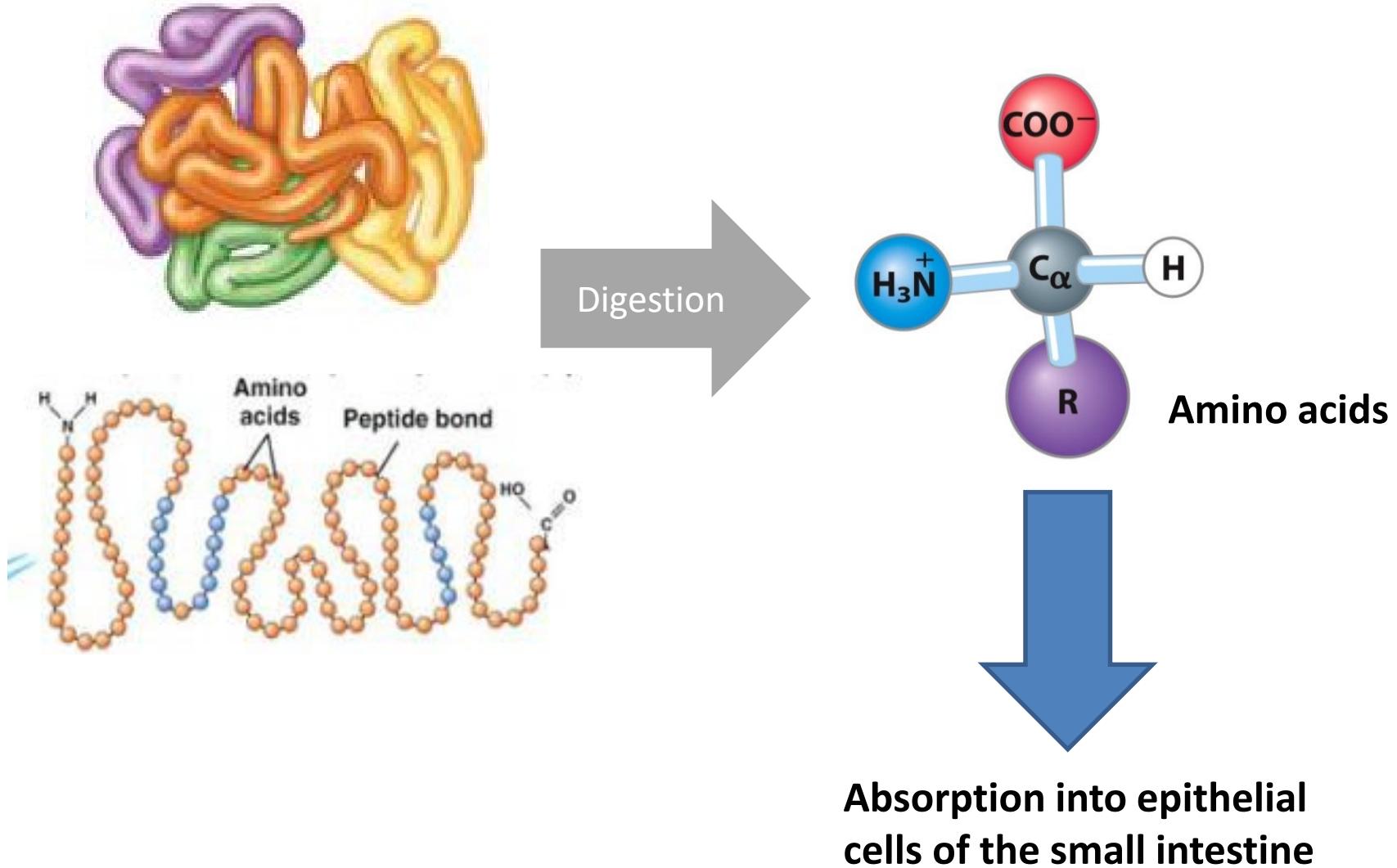
- During the normal degradation (turnover) of cellular proteins, if the released amino acids are not needed for synthesis of new proteins, they are degraded and metabolized
- When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus is catabolized
- During starvation or in uncontrolled diabetes mellitus, when carbohydrates are either unavailable or not properly utilized, cellular proteins are used as fuel.

Overview of amino acids catabolism

- Breakdown large proteins into peptides and then free amino acids by digestion
- Catabolize the amino groups (nitrogen catabolism)
- Catabolize the carbon skeletons



Digestion of proteins



Human digestive tract: Stomach

- **Gastrin**, secreted by gastric mucosa, stimulates secretion of **HCl** (by parietal cells) and **pepsinogen** (by chief cells)
- Pepsinogen (a zymogen) is converted to active **pepsin** by autocatalytic cleavage (cleaves off 44 amino acids from pepsinogen); the cleavage occurs only at low pH
- Pepsin hydrolyzes peptide bonds on the amino-terminal side of the aromatic amino acids **Phe**, **Trp** and **Tyr**

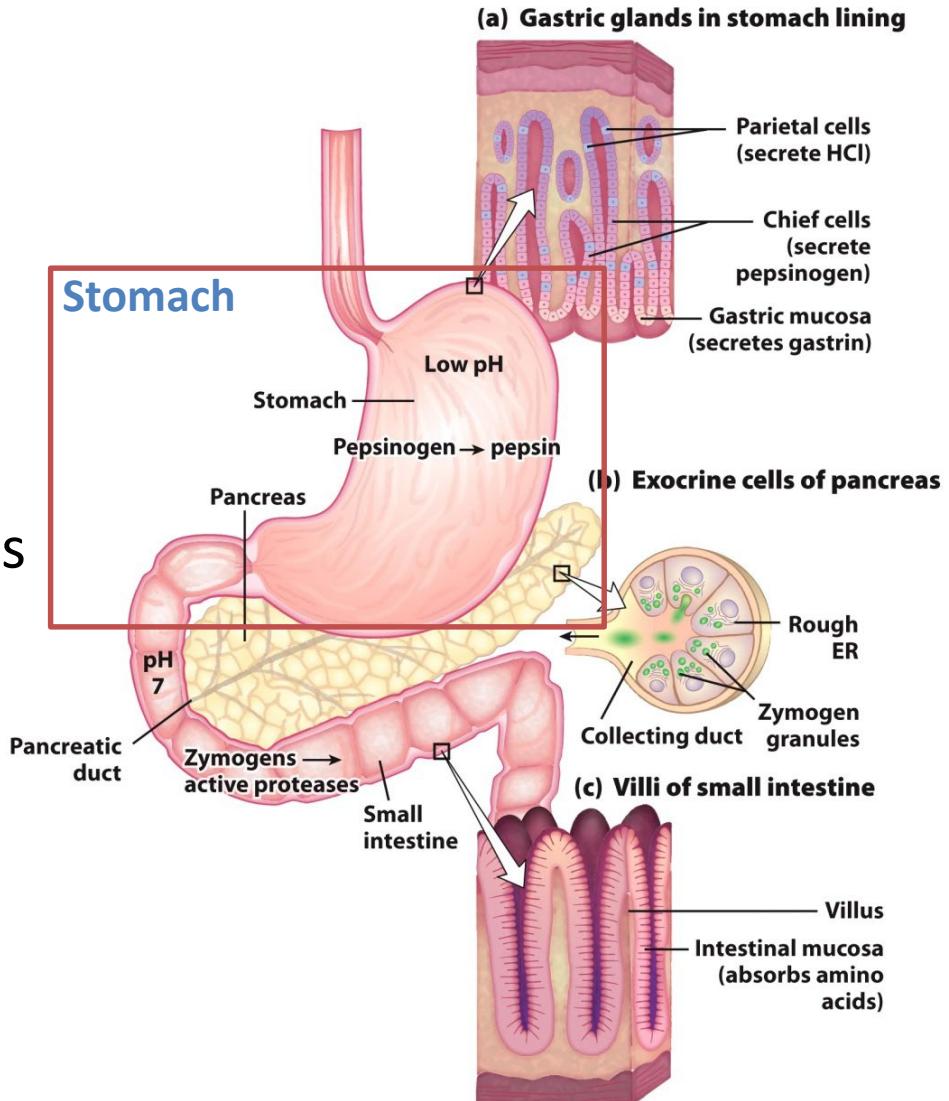


Figure 18-3

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Small intestine

- Low pH triggers the release of the hormone **secretin** from the small intestine to the blood
- Secretin stimulates the pancreas to secrete **bicarbonate (HCO_3^-)** into small intestine to neutralize the pH
- Amino acids arrival causes release of blood hormone **cholecystokinin**, which stimulates secretion of several pancreatic enzymes

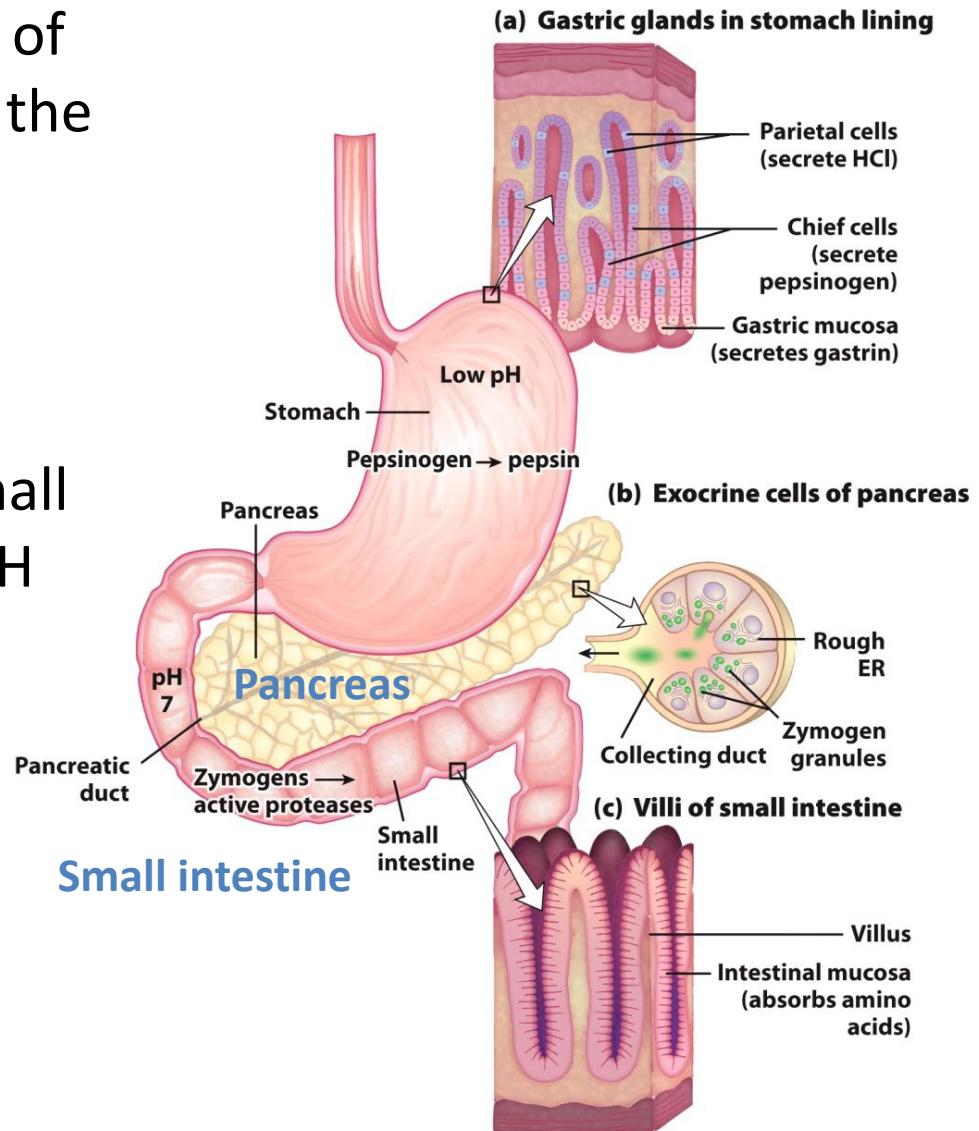
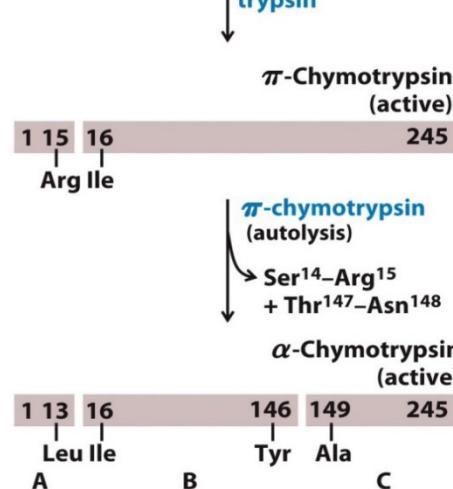
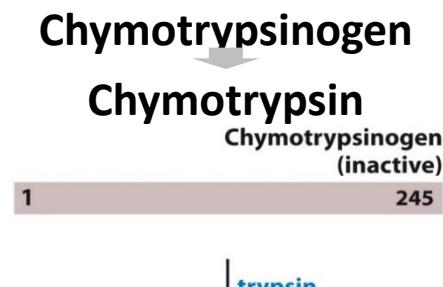
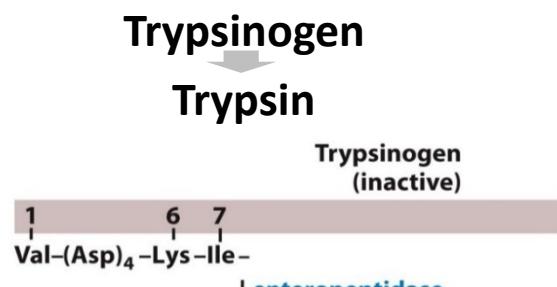


Figure 18-3

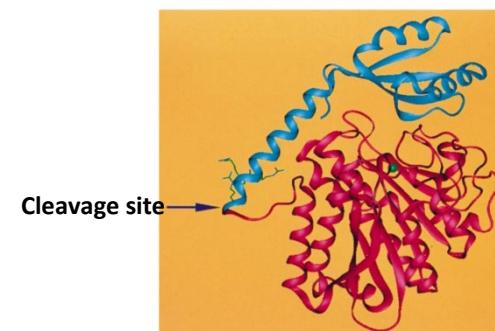
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Proteolytic enzymes are zymogens

- **Zymogen:** an inactive precursor of an enzyme; e.g. proteases in stomach and pancreas; activated by specific cleavage
- Protects exocrine cells from destructive proteolytic attack
- **Acute pancreatitis:** obstruction of pancreatic secretion; premature activation of proteases inside pancreas causes excruciating pain and damage to organ that can prove fatal

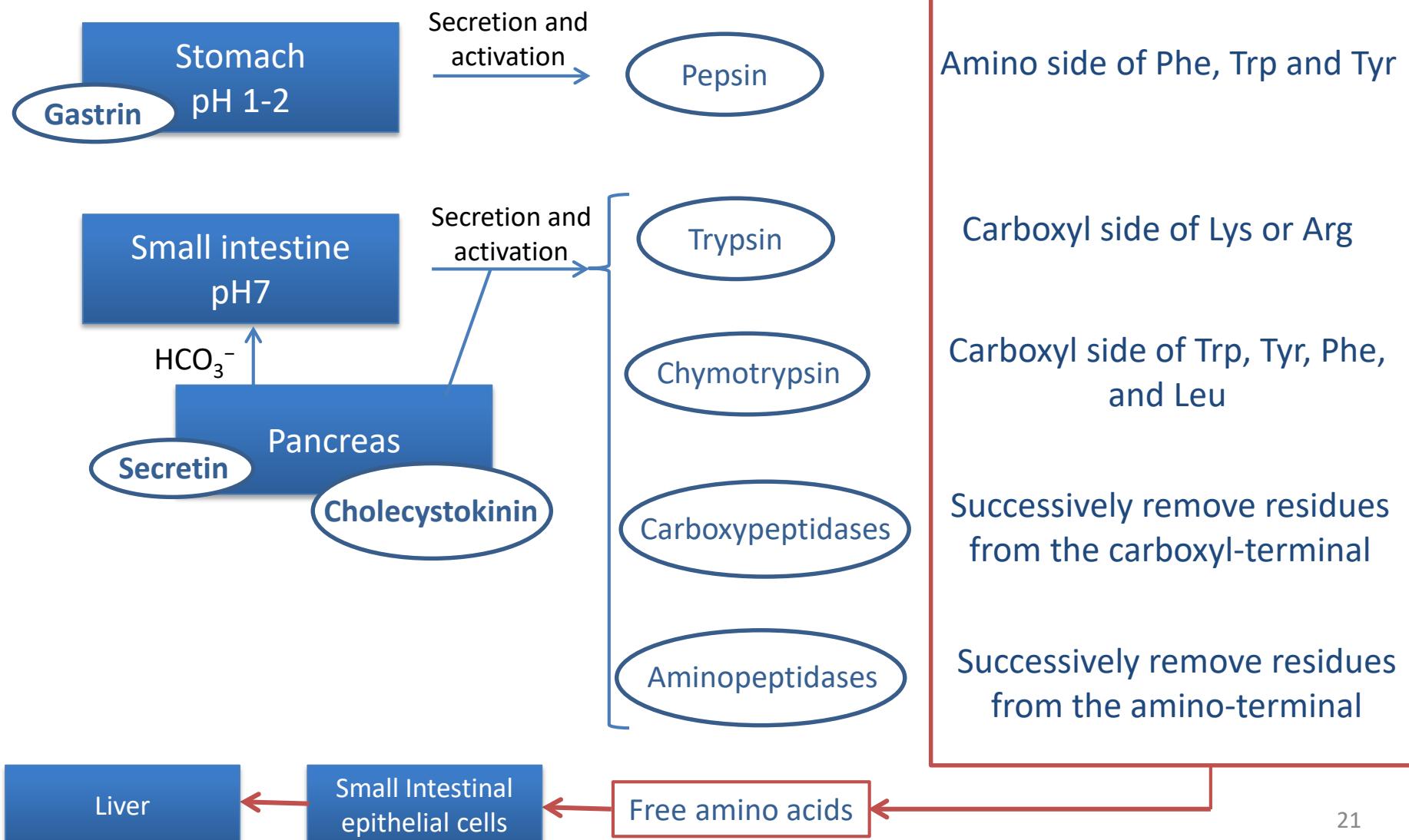


releases ~94-95 residues from C-ter



Human procarboxypeptidase A2
Red: active fragment

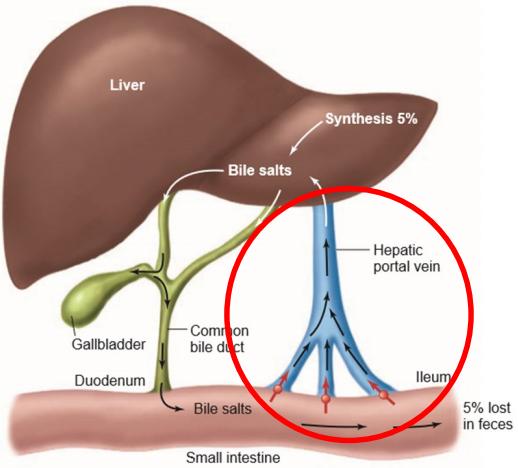
Summary of the proteolytic processes



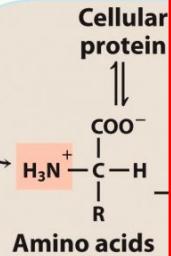
Catabolism of the amino group: 3 routes



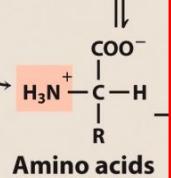
Amino acids directly transported to liver



Amino acids from ingested protein



Cellular protein

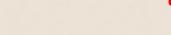


Liver

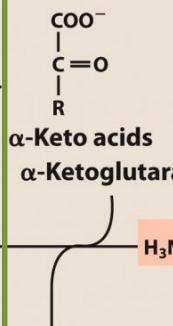
Amino acids



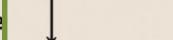
α -Ketoglutarate



Glutamate



α -Keto acids
 α -Ketoglutarate



Alanine



Ammonia transport by glucose-alanine cycle



Ammonia transport by glutamine

NH_4^+ , urea, or uric acid

1. Transdeamination of amino acids in the liver

- First step of amino acid catabolism in liver
- Amino-transferases (transaminases), which contain different specificities to different amino acids, catalyze this step
- **α -ketoglutarate** is often the amino group acceptor
- All amino groups are collected in the form of **L-glutamate**

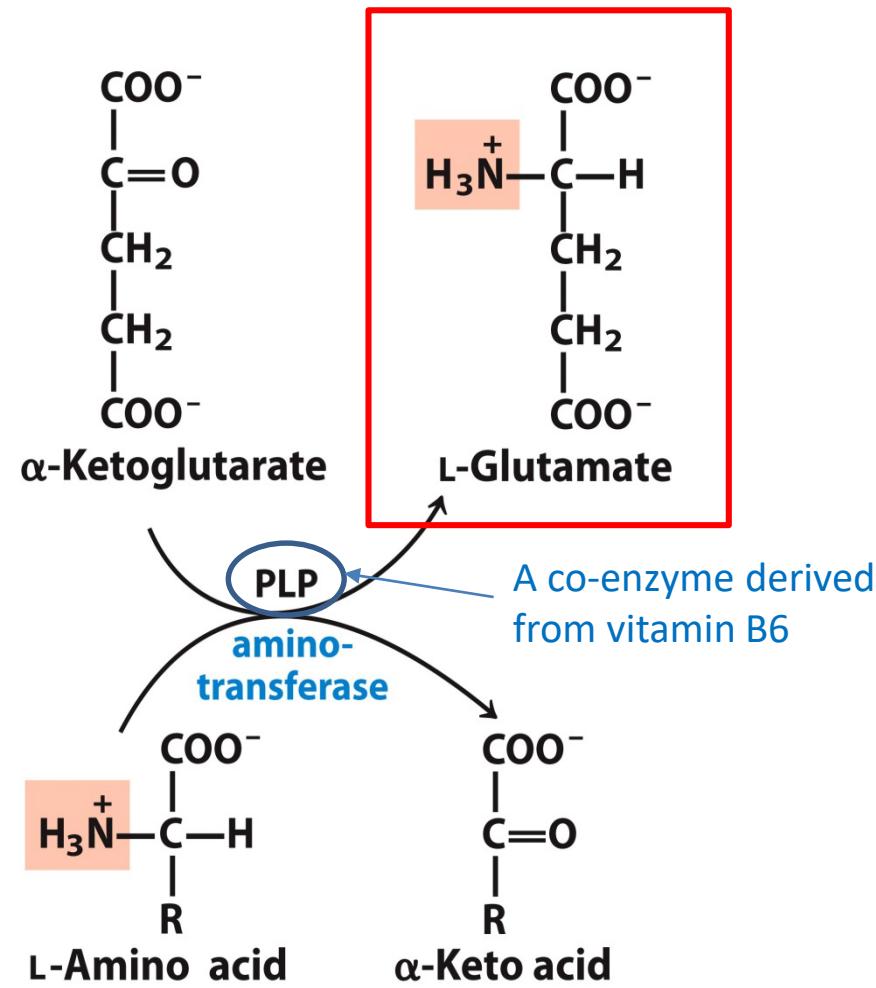
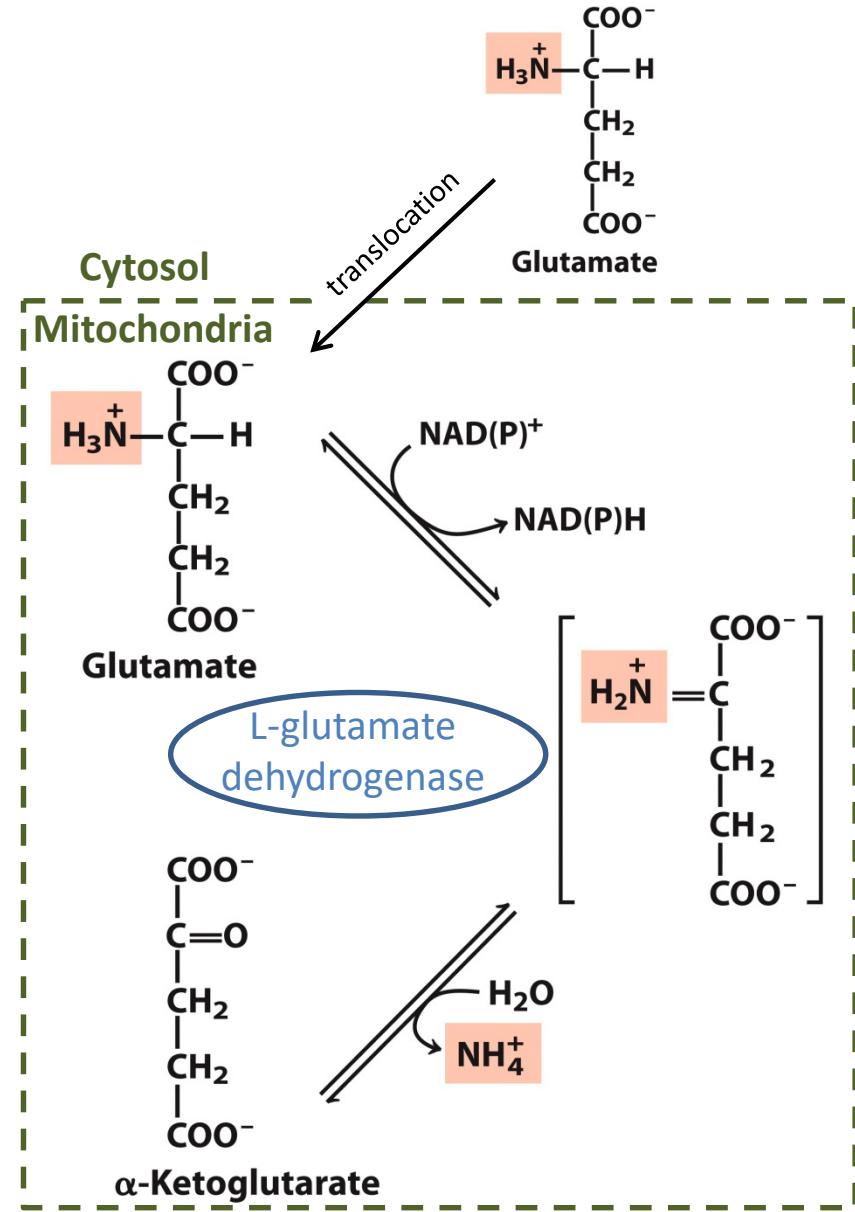


Figure 18-4
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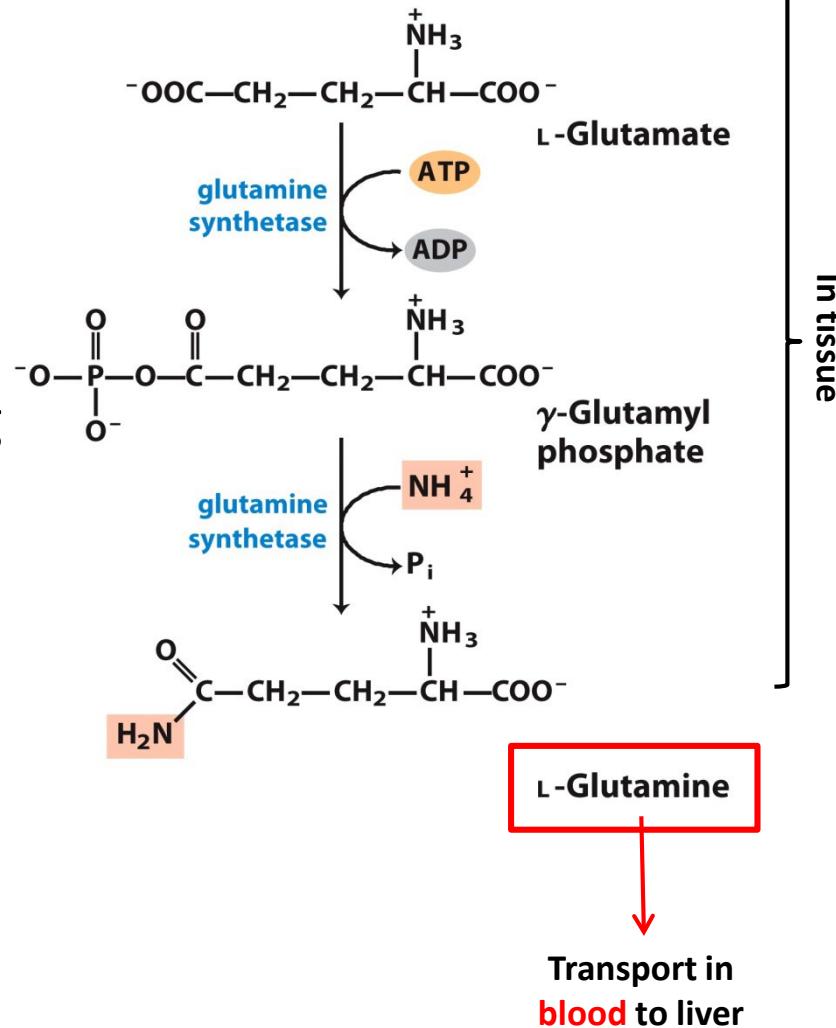
1. Transdeamination in the liver

- Second step: L-glutamate are transferred from cytosol into mitochondria
- Undergoes oxidative deamination catalyzed by L-glutamate dehydrogenase and produces NH_4^+
- L-glutamate dehydrogenase in mammals can use both NAD^+ and NADP^+



2. Ammonia (NH_3) generation in extrahepatic tissues

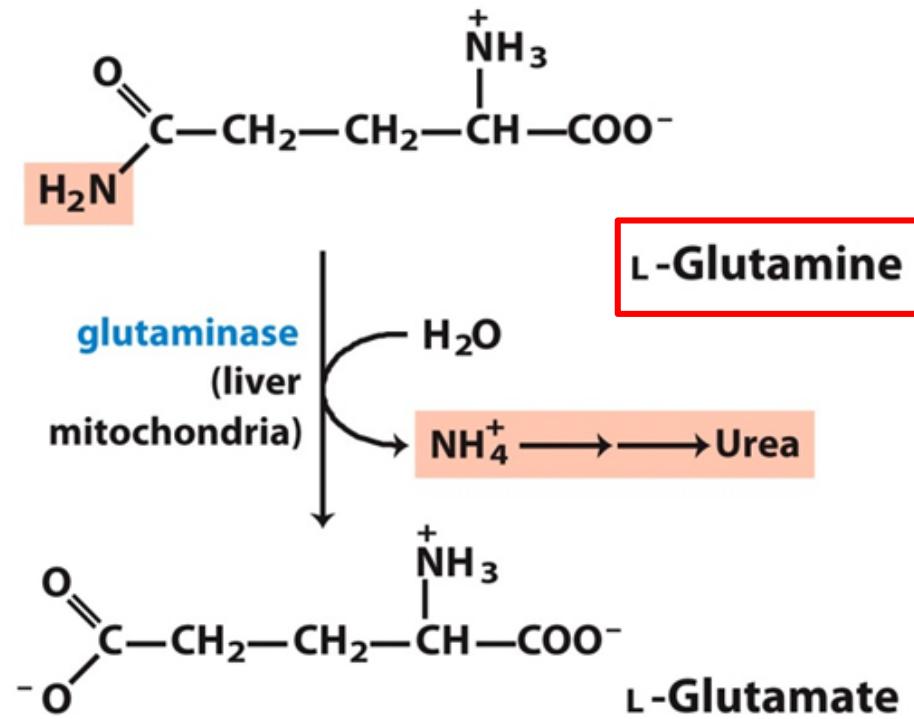
- In many tissues, ammonia (ionic form: ammonium NH_4^+) are generated in the process of nucleotide degradation
- Ammonia is toxic and can damage the brain after crossing the blood-brain barrier
- Glutamine is the nontoxic transport form of ammonia in the blood; **glutamine synthetase** converts glutamate and NH_4^+ to **glutamine**



2. Ammonia (NH_3) generation in extrahepatic tissues

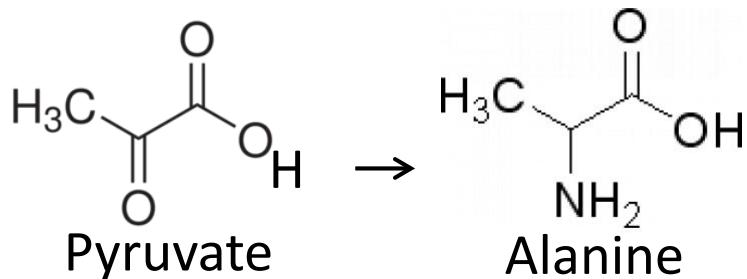
- When the glutamine from extrahepatic tissue come to the liver, NH_4^+ is released from glutamine by the enzyme **glutaminase**
- The L-glutamine will be converted to L-glutamate

In the liver

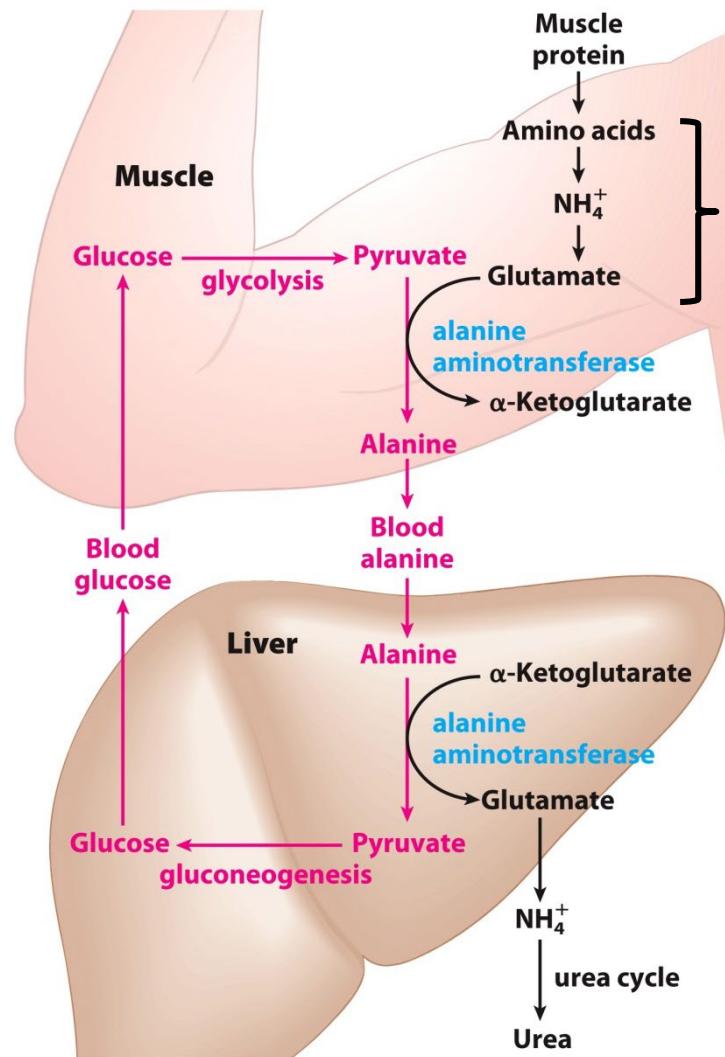


3. Ammonia transport in glucose-alanine cycle

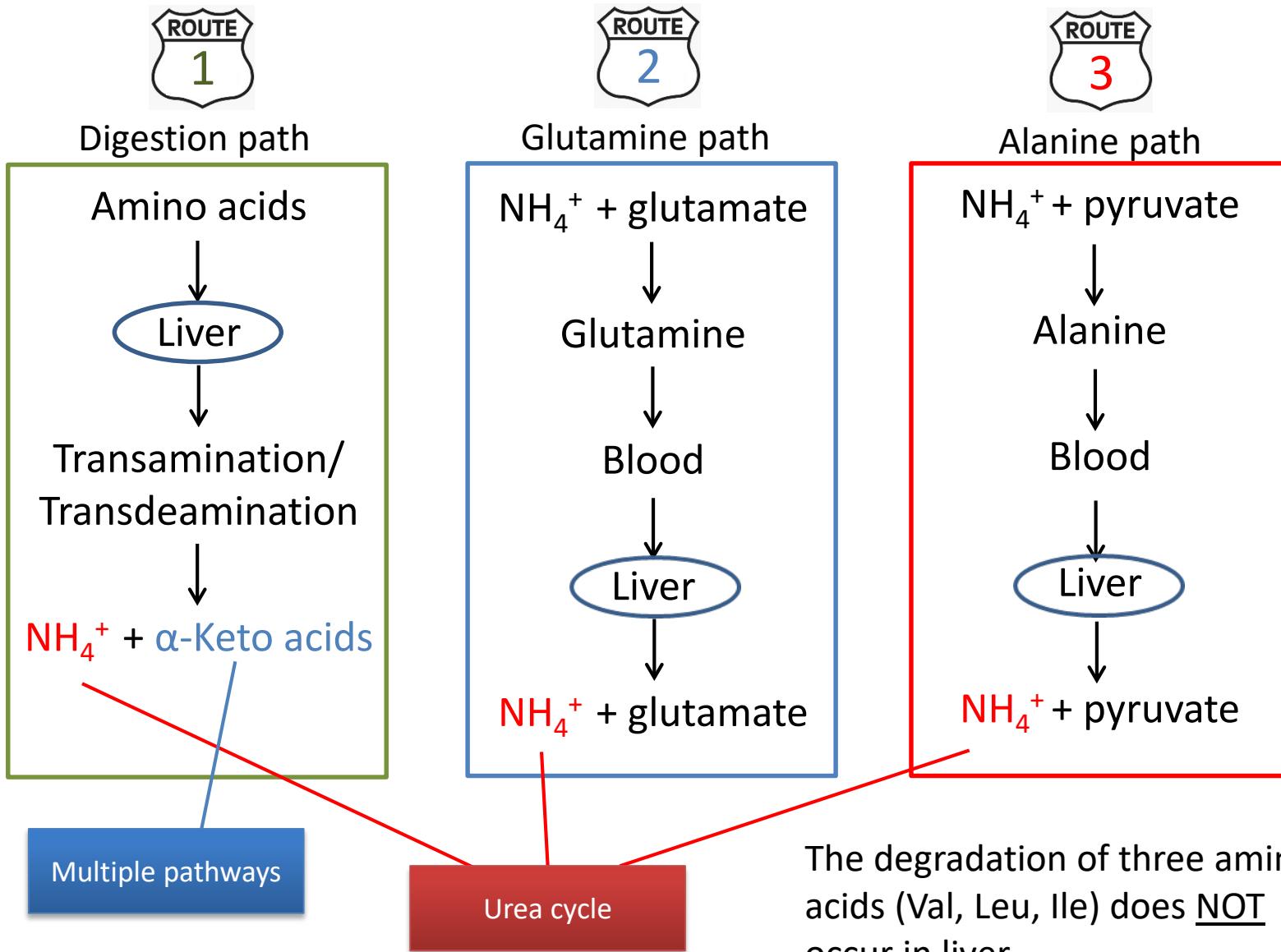
- In muscle and certain other tissues, the α -amino group of glutamate can be transferred to **pyruvate** to form **alanine** by alanine aminotransferase



- Alanine is converted back to pyruvate and releases NH_4^+ after being transported to liver through blood circulation; pyruvate is converted to glucose and transported back to muscle



Summary of the ammonia production pathways

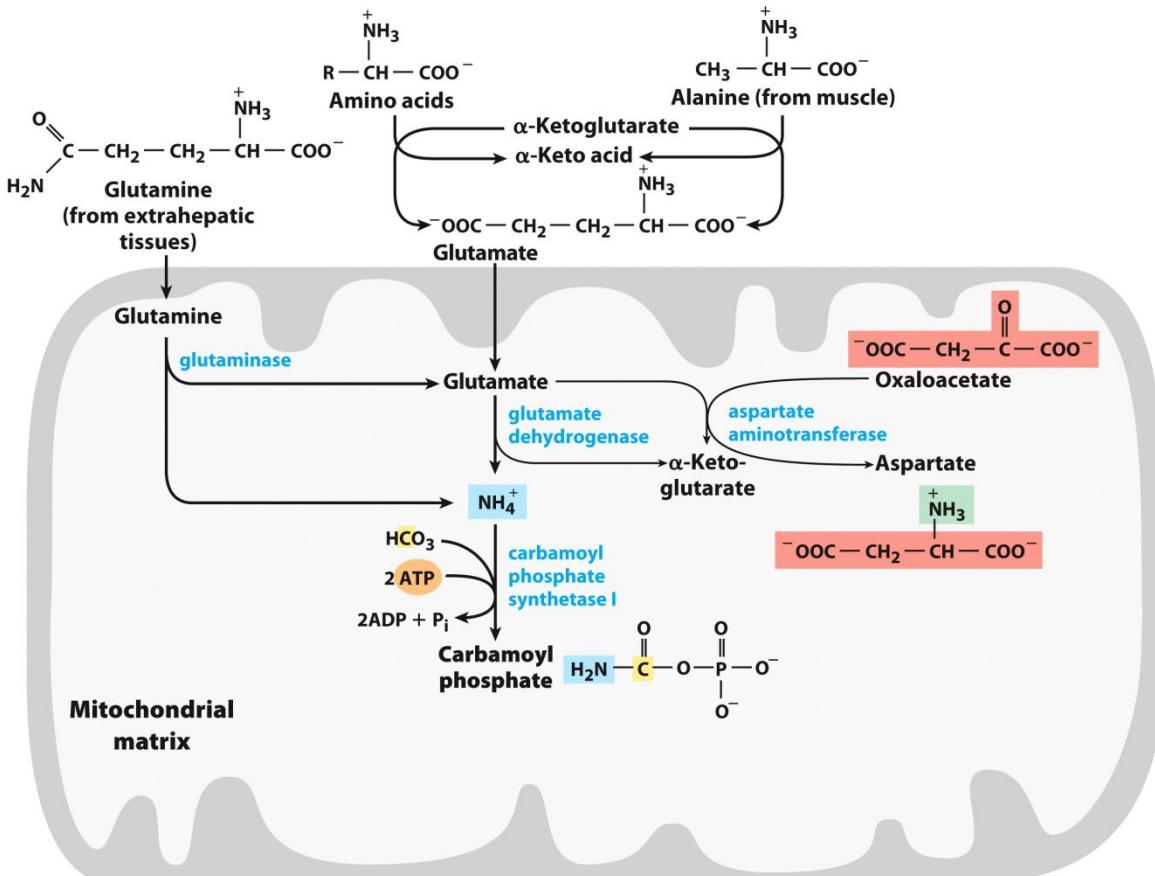
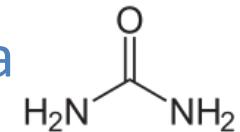


Study Question

- **Which of the following amino acids has much higher concentration in the blood than most other amino acids?**
 - A. Glycine
 - B. Glutamine
 - C. Leucine
 - D. Lysine

Nitrogen excretion and urea cycle

- Most animals excrete amino nitrogen in the form of urea
- The ammonia deposited in the mitochondria of hepatocytes is converted to urea in the **urea cycle**
- NH_4^+ is immediately used together with CO_2 (as HCO_3^-) to form **carbamoyl phosphate**, which enters the urea cycle

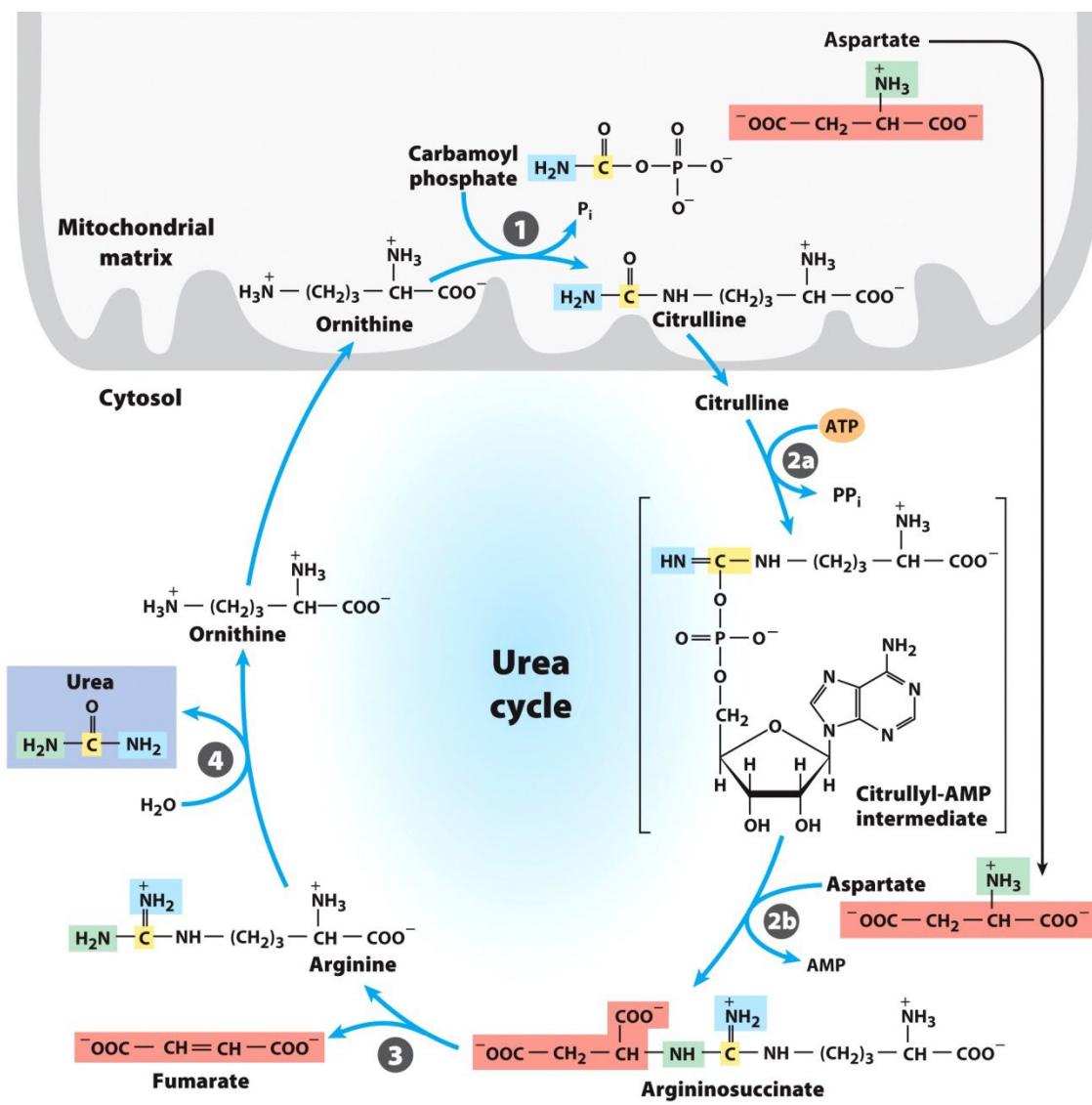


The urea cycle in 5 steps

1. Ornithine is a rare amino acid that is synthesized from glutamate. It is the key molecule accepting material at each cycle

4. Arginine is cleaved by arginase to yield the urea

3. Fumarate is also an intermediate of the citric acid cycle



2. The second amino group comes from an aspartate generated in mitochondria

Connection between urea cycle and citric acid cycle - “Krebs bicycle”

1. Fumarate is converted to malate in the cytosol which is then transported into the mitochondria to participate in the citric acid cycle

2. Oxaloacetate produced from citric acid cycle is converted to aspartate, which is transferred out of mitochondria to provide a nitrogen donor in the urea cycle

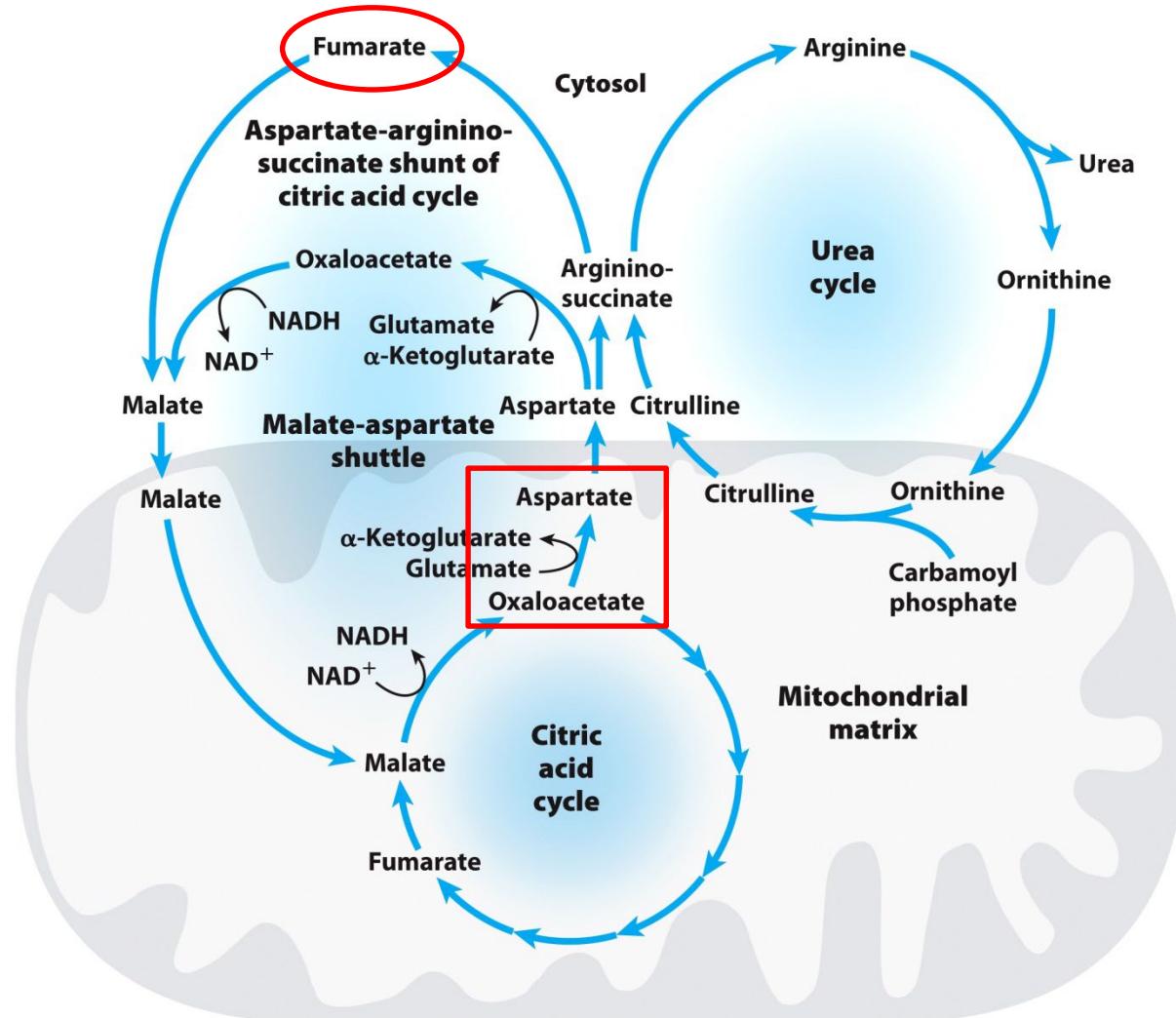
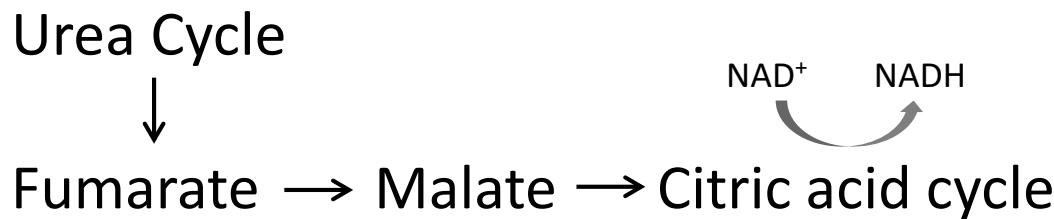
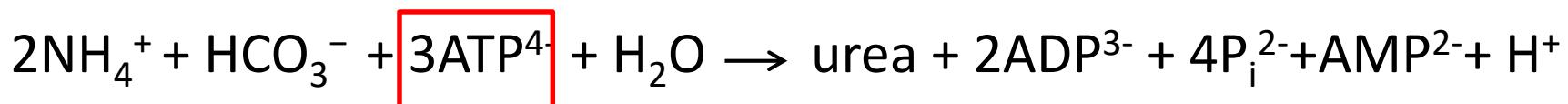


Figure 18-12

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Energy consumption of the urea cycle

Overall equation of urea cycle:



Connection of urea cycle to citric acid cycle causes reduction of energy because **NADH** (equals to 2.5 ATP) is produced in the citric acid cycle

Summary of the urea cycle

- Ammonia is highly toxic to animal tissues. Ammonia, which becomes the cation ammonium (NH_4^+) in mitochondria of liver, forms a part of carbamoyl phosphate, which then enters the urea cycle
- In the end of the urea cycle, ammonia is converted to urea which is then excreted in the urine by kidney
- The urea cycle is interconnected with the citric acid cycle through a net conversion of oxaloacetate (in citric acid cycle) to fumarate (in urea cycle)
- The connection to the citric acid cycle generates NADH, hence reduces the energy consumption of the urea cycle

Degradation of the carbon skeleton of amino acid

- **Glucogenic amino acids:** degraded to gluconeogenic precursors like pyruvate, α -ketoglutarate, succinyl-CoA, fumarate and/or oxaloacetate, that can be converted to glucose and glycogen
- **Ketogenic amino acids:** degraded to acetyl-CoA and/or acetoacetate-CoA that can be used for ketogenesis or fatty acids synthesis

Amino acids are grouped based on their major degradative end products

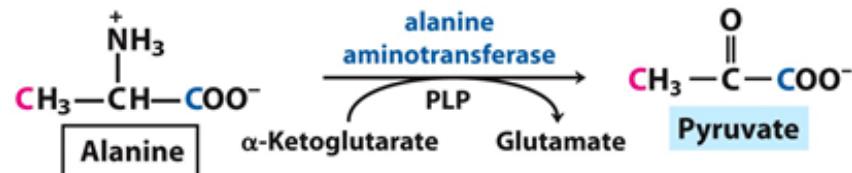
Nonessential	Glucogenic	Glucogenic and Ketogenic	Ketogenic
	Alanine Arginine*Asparagine Aspartate Cysteine Glutamate Glutamine Glycine Histidine*Proline Serine	Tyrosine	
Essential	Methionine Valine	Threonine Isoleucine Phenylalanine Tryptophan	Leucine Lysine

Figure 20.2

Classification of amino acids. *Arginine and histidine are essential under some conditions.

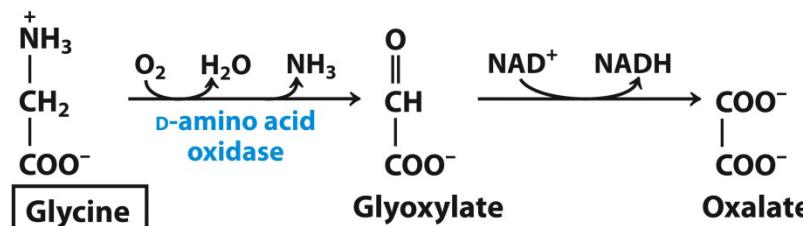
Examples of amino acid degradation pathways

- Alanine → Pyruvate



- Glycine degradation pathways:

- To pyruvate
- Glycine cleavage to CO₂, NH₄⁺ and a methylene group
- Through D-amino acid oxidase to oxalate



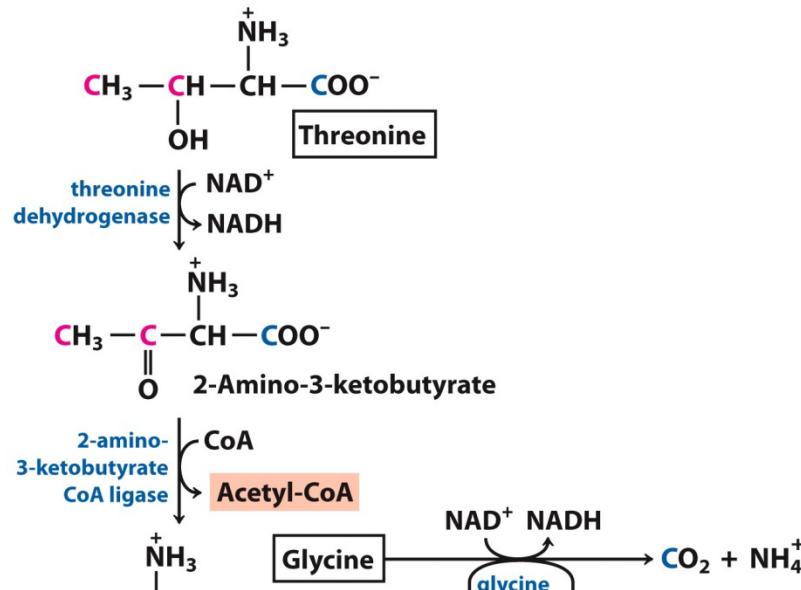
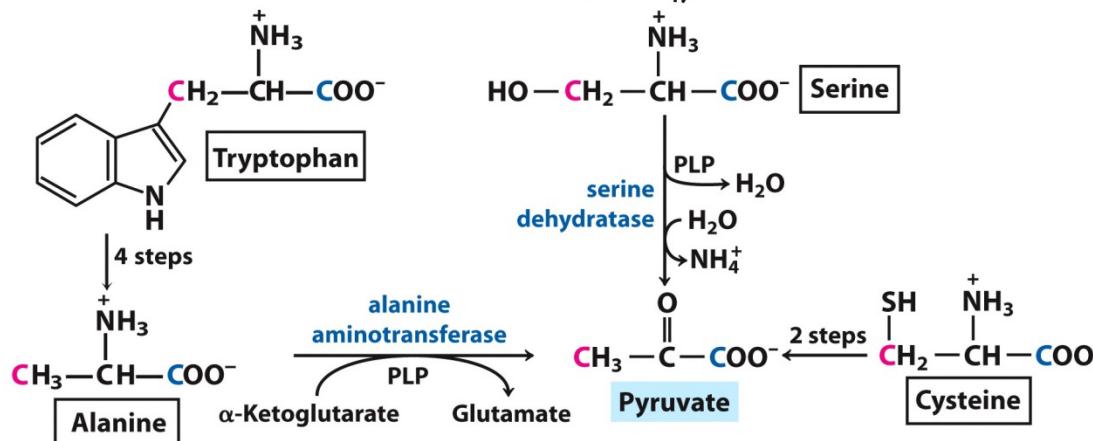
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- Threonine degradation pathways:

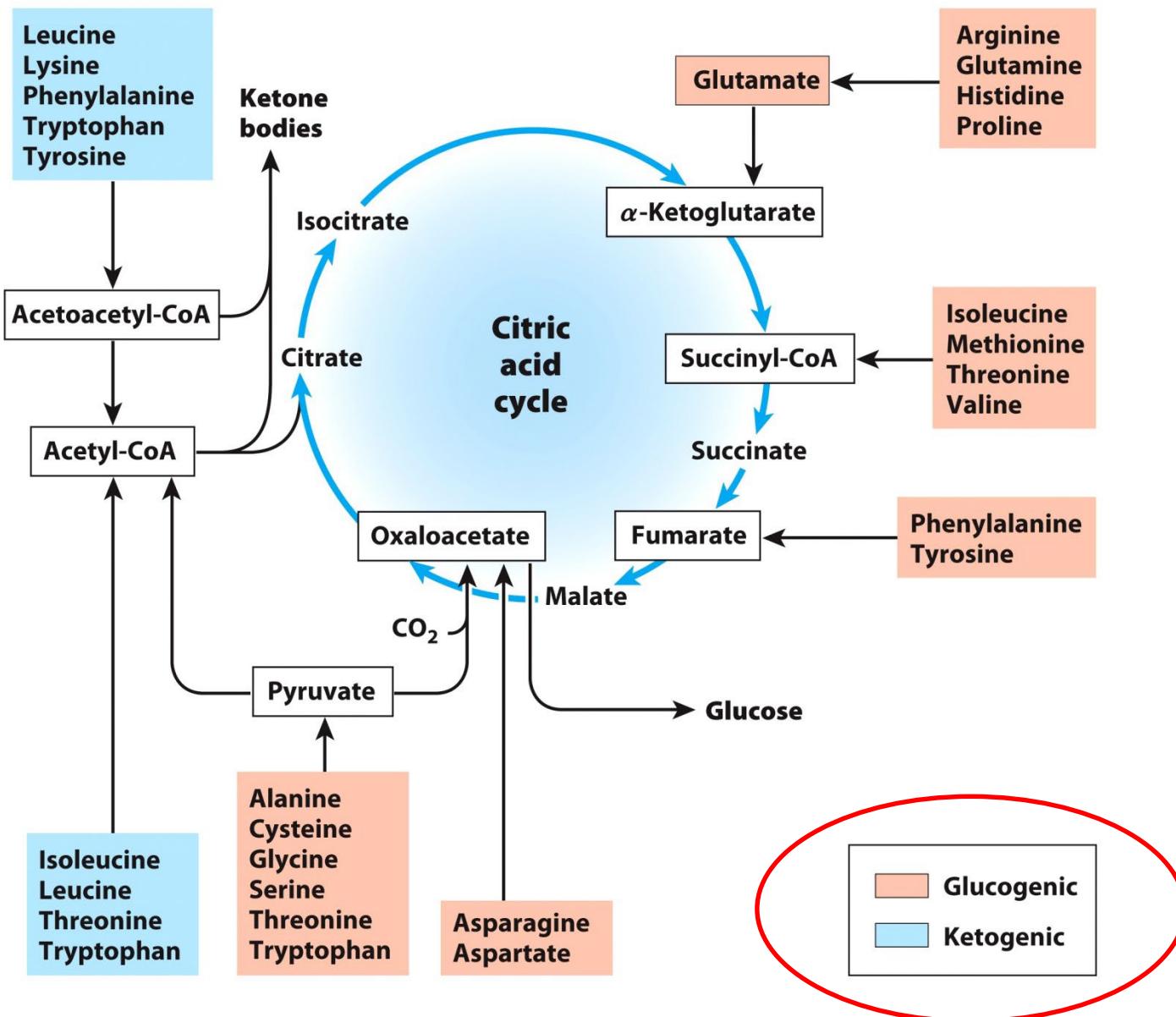
- Via glycine, making acetyl-CoA and pyruvate (minor) Glucogenic and ketogenic
- To succinyl-CoA (major) Glucogenic

Conversion of amino acids to pyruvate

- Six amino acids are converted to pyruvate
- Interconversion between amino acids are important for amino acid degradation



Summary of amino acid catabolism



Genetic diseases caused by genes affecting amino acid catabolism

TABLE 18-2 Some Human Genetic Disorders Affecting Amino Acid Catabolism

Medical condition	Approximate incidence (per 100,000 births)	Defective process	Defective enzyme	Symptoms and effects
Albinism	<3	Melanin synthesis from tyrosine	Tyrosine 3-monoxygenase (tyrosinase)	Lack of pigmentation; white hair, pink skin
Alkaptonuria	<0.4	Tyrosine degradation	Homogentisate 1,2-dioxygenase	Dark pigment in urine; late-developing arthritis
Argininemia	<0.5	Urea synthesis	Arginase	Mental retardation
Argininosuccinic acidemia	<1.5	Urea synthesis	Argininosuccinase	Vomiting; convulsions
Carbamoyl phosphate synthetase I deficiency	<0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy; convulsions; early death
Homocystinuria	<0.5	Methionine degradation	Cystathione β -synthase	Faulty bone development; mental retardation
Maple syrup urine disease (branched-chain ketoaciduria)	<0.4	Isoleucine, leucine, and valine degradation	Branched-chain α -keto acid dehydrogenase complex	Vomiting; convulsions; mental retardation; early death
Methylmalonic acidemia	<0.5	Conversion of propionyl-CoA to succinyl-CoA	Methylmalonyl-CoA mutase	Vomiting; convulsions; mental retardation; early death
Phenylketonuria	<8	Conversion of phenylalanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

Table 18-2

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Treating amino acid catabolism diseases

- Restricted food intake of certain amino acids (e.g. avoid phenylalanine for phenylketonuria)
- Supplement certain amino acid or activator of a certain enzymatic pathway
- For defects in urea cycle, careful administration of:
 1. **Benzoate:**
take away NH_4^+ through the removal of **glycine**
 2. **Phenylbutyrate:**
take away NH_4^+ through the removal of **glutamine**

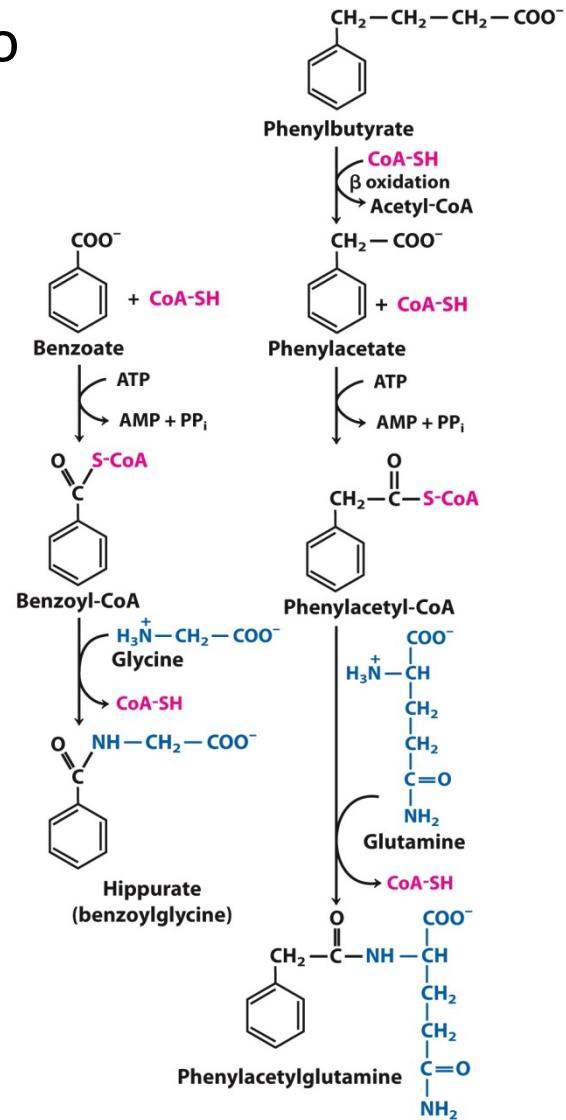
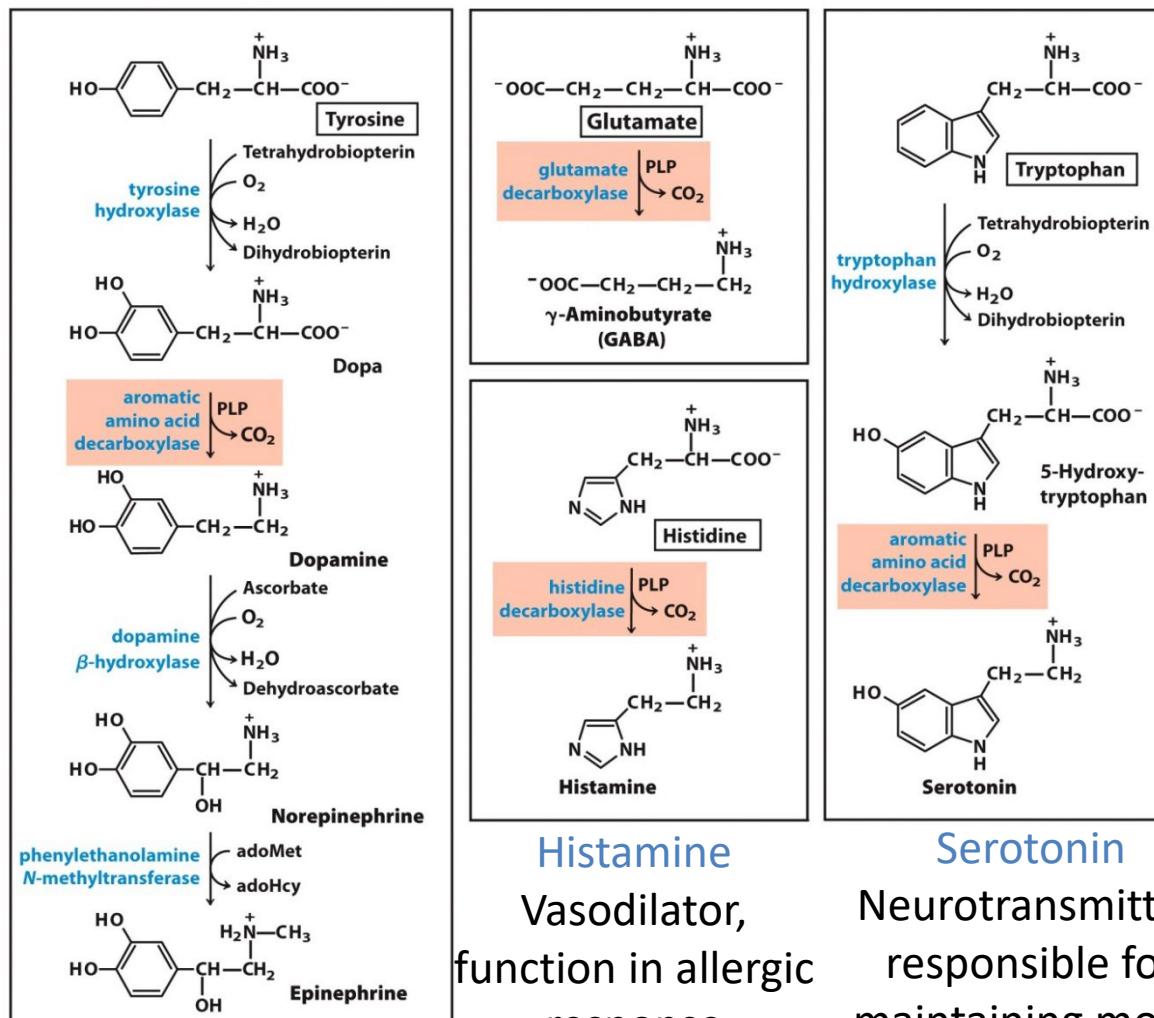


Figure 18-14
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Amino acids are precursors of many neurotransmitters

- Catecholamine, including dopamine, norepinephrine and epinephrine, are derived from tyrosine
- They are neuromodulators or hormones Increase heart rate, blood pressure, blood glucose levels, and induce a general reaction of the sympathetic nervous system
- Function in the “Fight-or-flight” responds



Histamine
Vasodilator,
function in allergic
response

Serotonin
Neurotransmitter,
responsible for
maintaining mood
balance; a deficit leads
to depression

Heme is synthesized from glycine in animals and from glutamate in bacteria and plants

Glycine + succinyl-CoA \rightarrow δ -aminolevulinate \rightarrow Protoporphyrin $\xrightarrow{\text{Fe}^{2+}}$ Heme

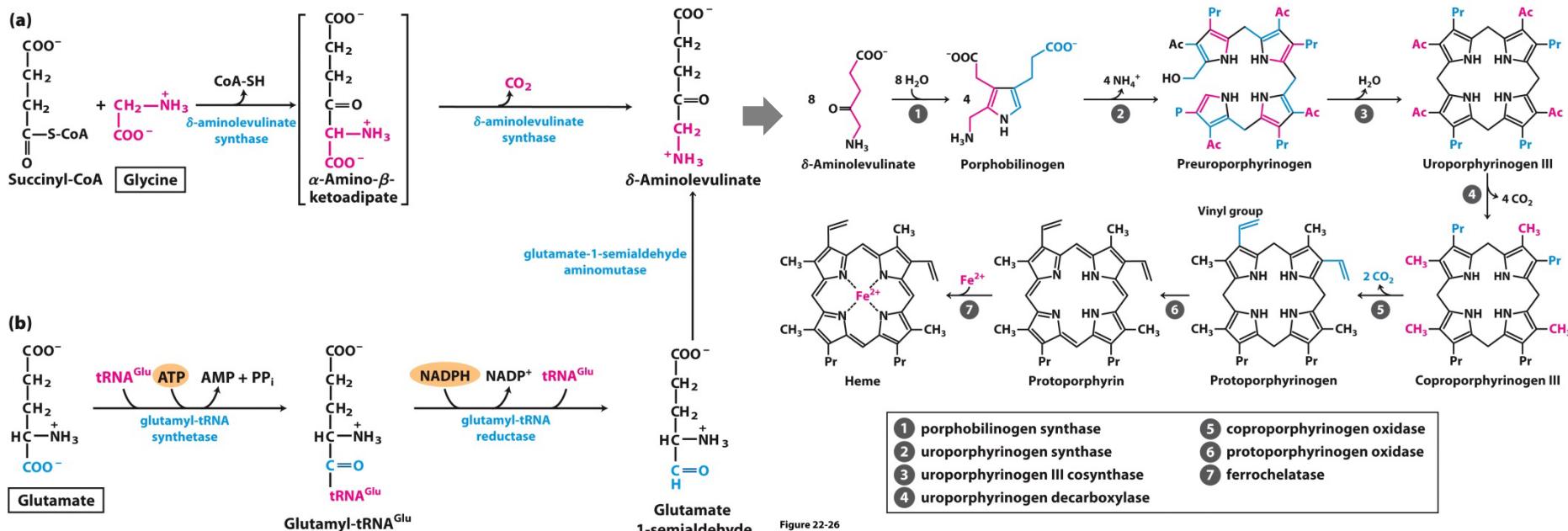


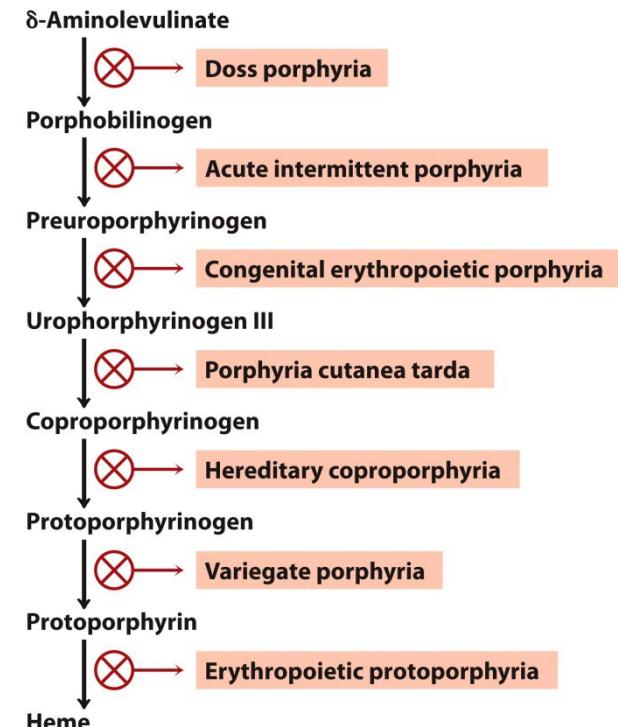
Figure 22-25
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Figure 22-26
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Porphyrias

- A genetic disease results from the defect in glycine to protoporphyrins synthesis pathway
- Can lead to attacks of acute abdominal pain and neurological dysfunctions – possibly the mental illness exhibited by King George III
- A rarer form of porphyrias results in an accumulation of uroporphyrinogen, which stains urine red, causes teeth to fluoresce strongly under UV, makes skin abnormally sensitive to sunlight, causes anemia and

VAMPIRE



Box 22-2
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Porphyria Photosensitivity



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Summary of the lecture

- Proteins are digested into free amino acids in the digestive tract through the concerted functions of a series of proteases
- Amino acids are catabolized in liver. The amino group is converted to ammonia. In the urea cycle, ammonia is converted to urea which is then excreted in the urine by kidney
- The carbon skeleton of amino acids are catabolized into either precursors in the gluconeogenesis pathway (glucogenic amino acids) or the ketogenesis/fatty acid synthesis pathway (ketogenic amino acids)
- Amino acids are also precursors of many important biomolecules (e.g. neurotransmitters and Heme)

Study Question

- In blood test, the activity of the enzyme aspartate aminotransferase can indicate problem of which tissue?
 - A. Brain
 - B. Heart
 - C. Intestine
 - D. Liver