

MCB 354 (Fall 2022)

Biochemical and Physical Basis of Life

A. Kalsotra

Lecture 35:

Amino Acid Catabolism/Urea Cycle

Reading in Lehninger, 8th edition:

- Chapter 18

Learning objectives for this lecture

- Describe a typical transamination reaction (including vitamin-derived cofactor); identify the corresponding α -keto acids for alanine and glutamate
- What is the diagnostic value of plasma aminotransferases (AST and ALT)?
- Explain how excess “ammonia” is safely carried through the blood to the liver
- Explain the special role of intracellular glutamate in Nitrogen metabolism
- Describe the reaction catalyzed by carbamoyl phosphate synthetase I, and its regulation by *N*-acetylglutamate
- Identify the sources of the 2 Nitrogen atoms in urea
- Identify the cellular compartments in which the urea cycle takes place, and explain its overall stoichiometry.
- Describe urea cycle regulation by allosteric effectors, substrate availability, and enzyme levels
- Describe the mechanism and treatment of ammonia toxicity (hyperammonemia)

Catabolism (oxidation) of amino acids

When are amino acids oxidized in animals?

- During **normal protein turnover** (of any released amino acids that are not immediately needed for protein synthesis)
- After **protein-rich meal** (if the ingested amino acids exceed the need for protein synthesis)
- During prolonged **fasting, starvation**, and uncontrolled **diabetes** (mostly from degradation of muscle protein)

Digestion of dietary protein

- In stomach, **HCl** secretion lowers pH to ~1 to 2.5
- Low pH denatures proteins; **pepsin** hydrolyzes peptide bonds in proteins
- Low pH also helps kill microorganisms

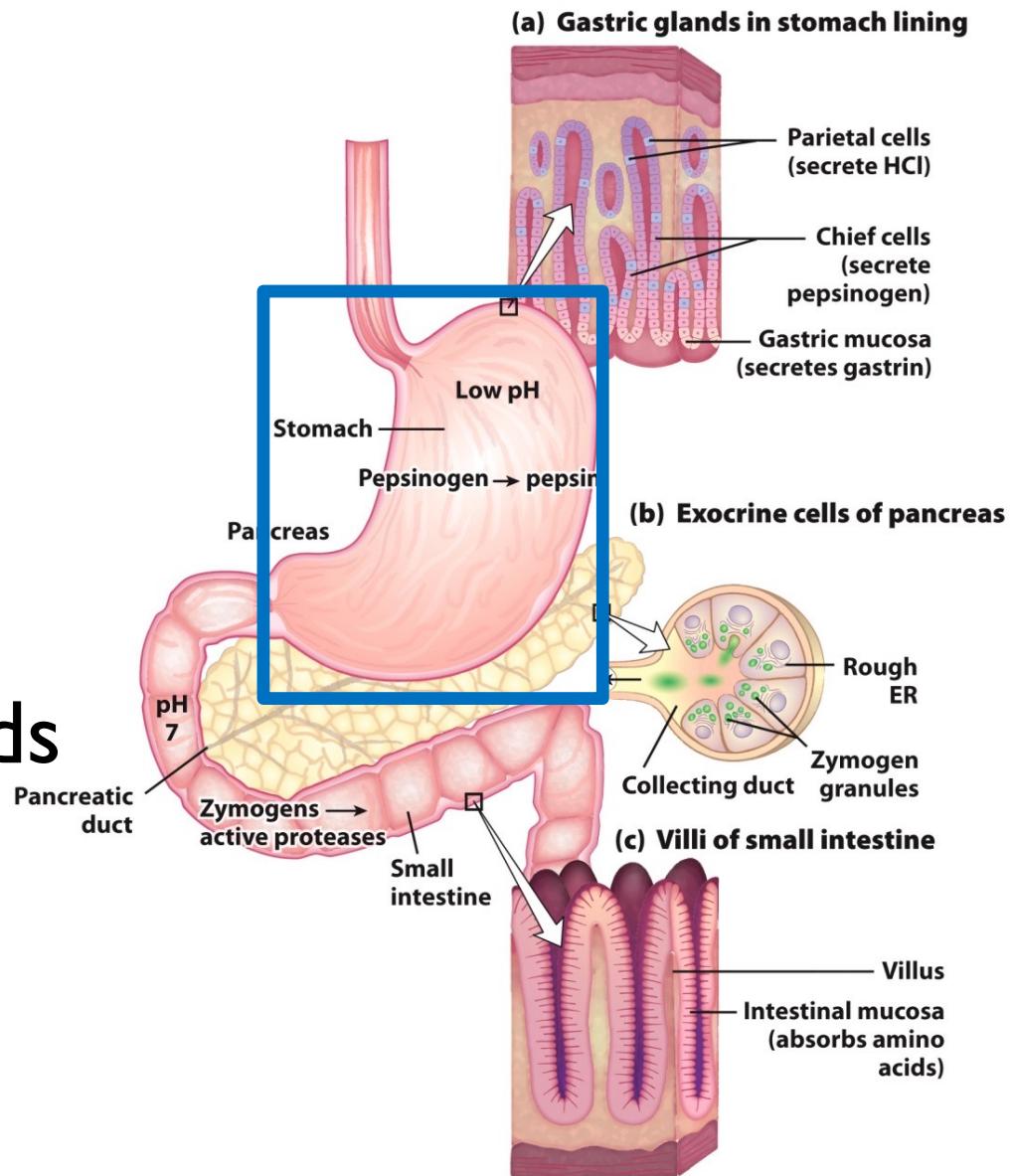


Figure 18-3

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Digestion of dietary protein

- In intestine, pH is raised to ~7 by **bicarbonate** secreted from pancreas
- **Pancreatic proteases** (trypsin, chymotrypsin, carboxypeptidases) and intestinal proteases (aminopeptidase) digest proteins to amino acids
- Amino acids are absorbed by intestinal epithelia; released into bloodstream (**hepatic portal vein**)

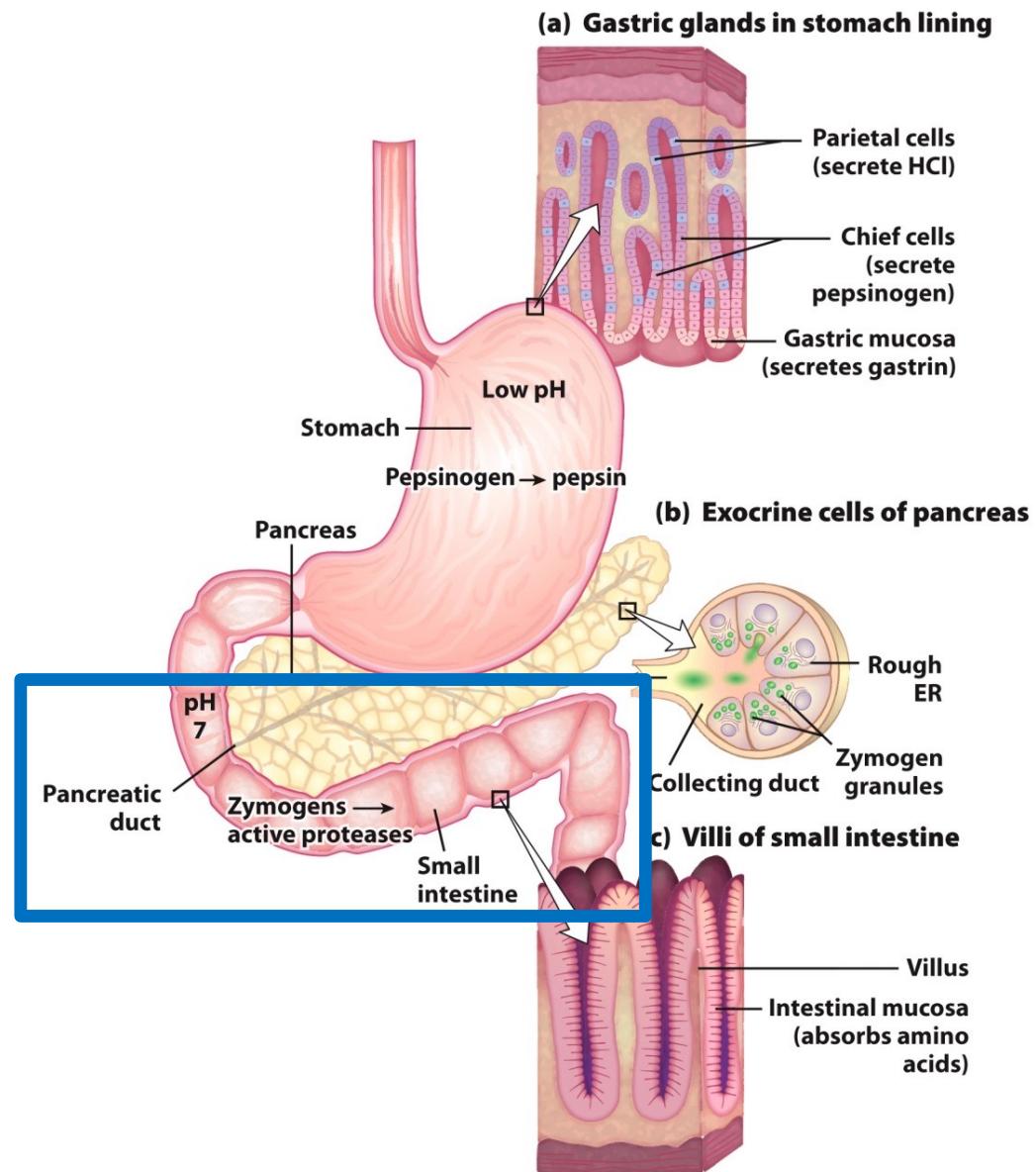


Figure 18-3

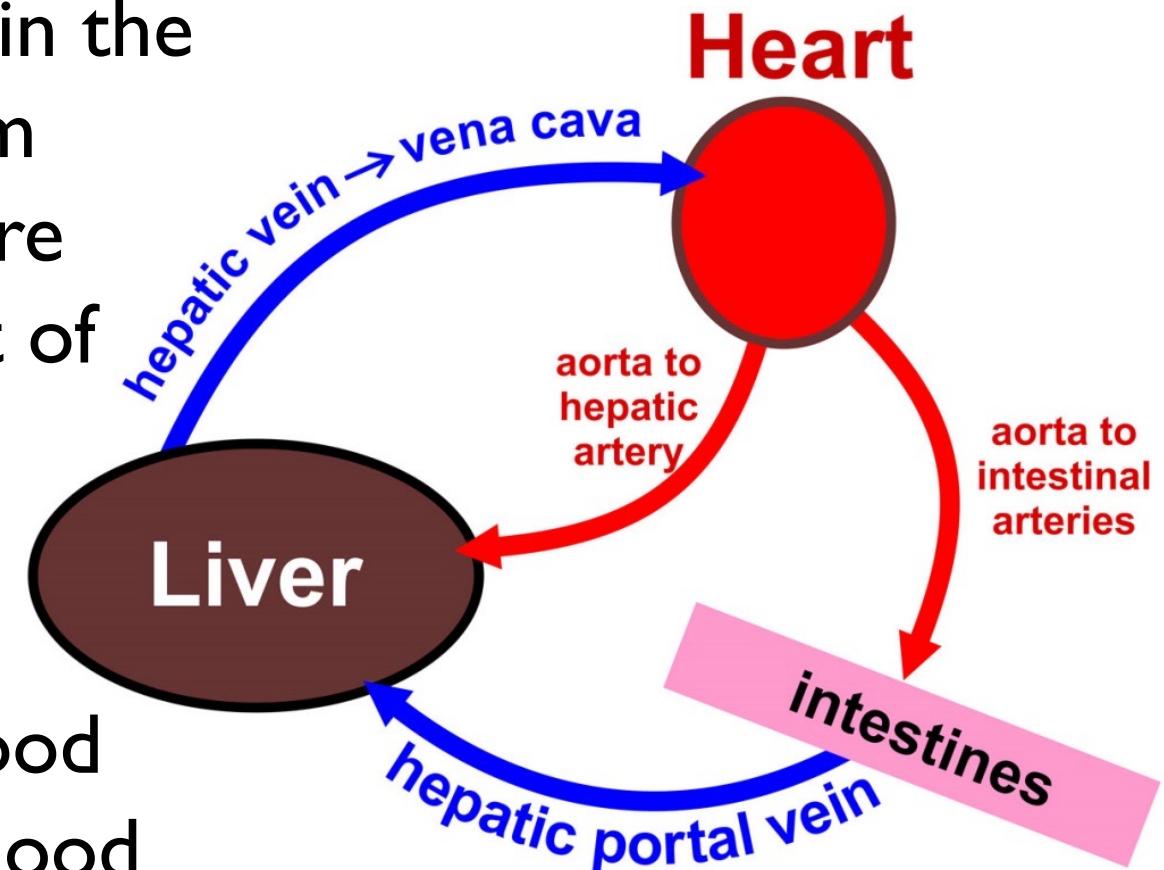
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Hepatic portal circulation

- Substances absorbed in the gastrointestinal system perfuse the liver before continuing to the rest of the circulation

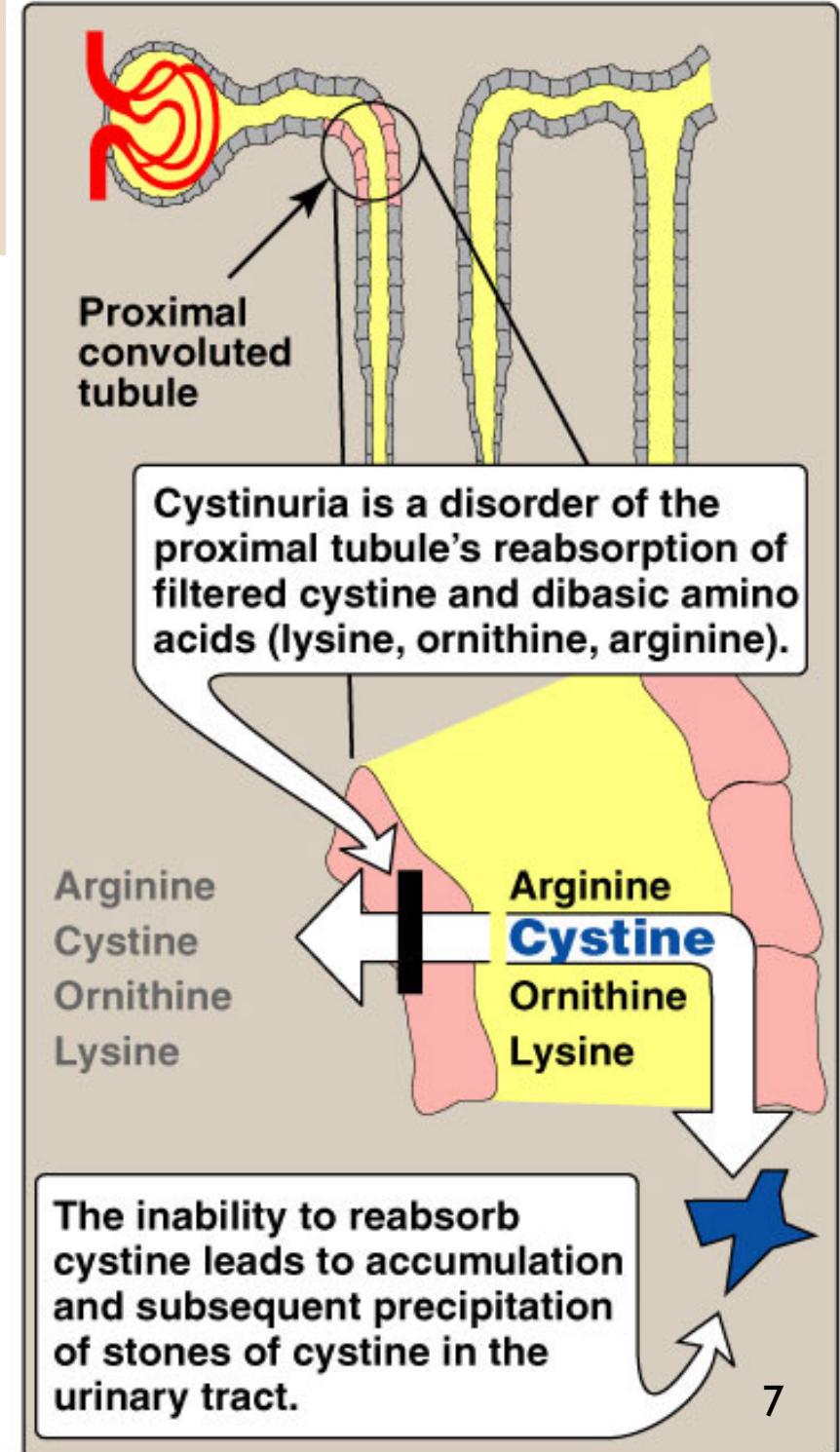
- “First pass effect”

- Liver has a double blood supply: some of the blood comes directly from the heart; the balance from the **hepatic portal vein**



Active transport of amino acids into cells

- Cells take up amino acids by active transport (i.e., coupled to ATP hydrolysis)
- This leads to higher amino acid concentration in cytoplasm than outside cell
- **Cystinuria**: defect in a specific AA transporter



Amino acid degradation

- Catabolized amino acids are **deaminated**
- Different fates for **amino groups** and **carbon skeletons**
- Amino groups are used for biosynthetic reactions or are excreted
- Method of excretion of nitrogen from excess amino groups varies by organism

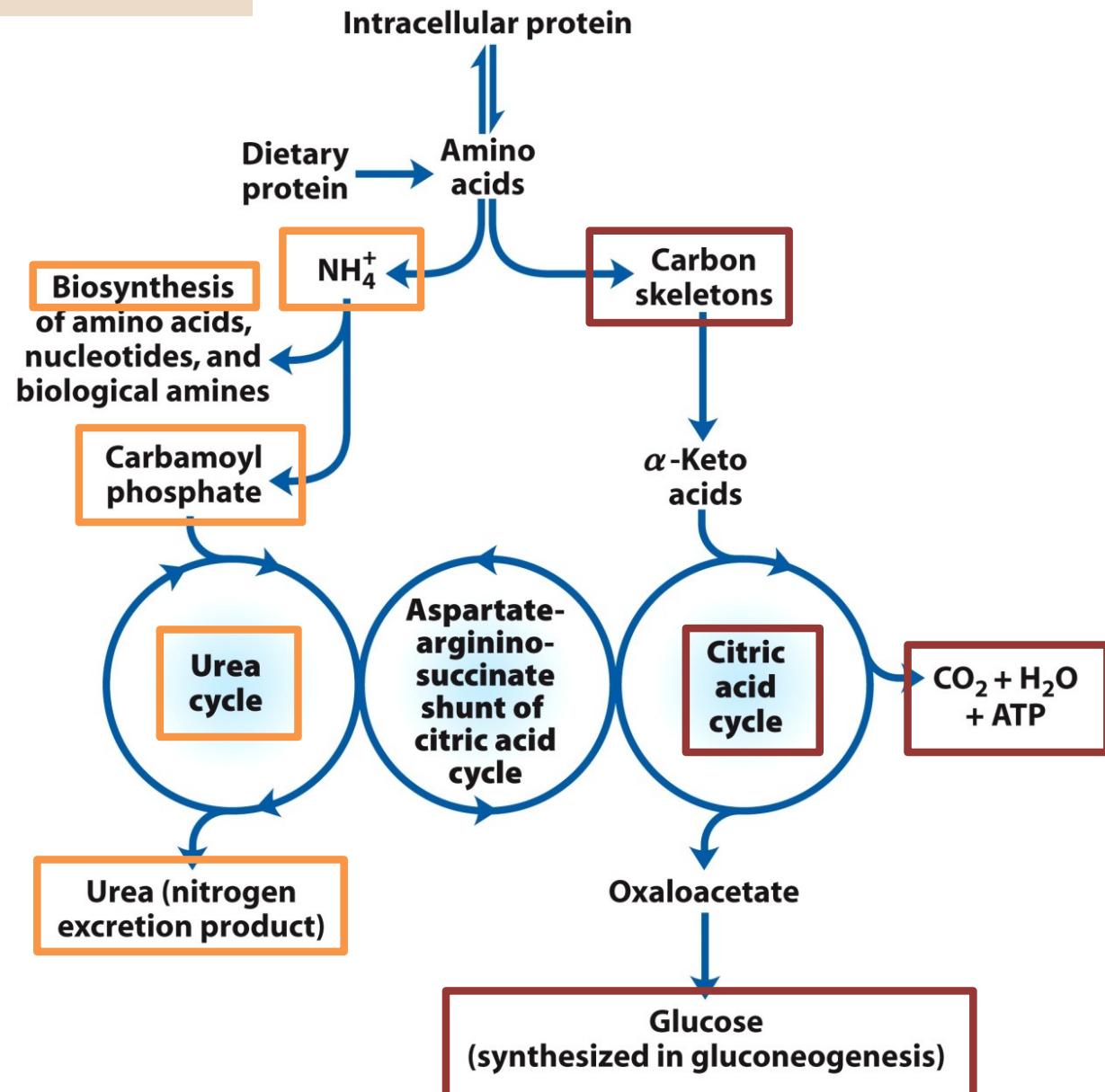
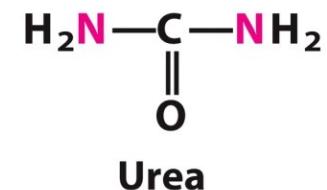


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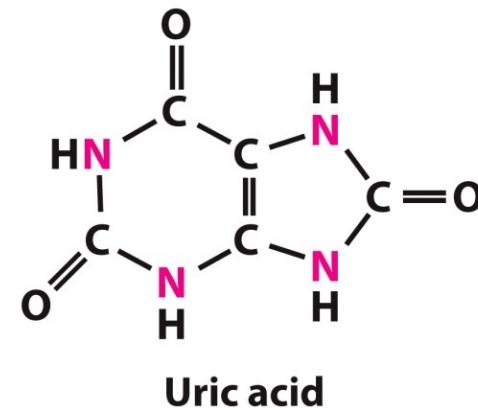
Excretory forms of nitrogen in different organisms

- Aquatic animals can excrete NH_4^+ directly, but this is too toxic for terrestrial organisms to accumulate
- Terrestrial animals typically excrete nitrogen as **urea** or **uric acid**
- Note the highly oxidized carbons in urea & uric acid



Ammonotelic animals:
most aquatic vertebrates,
such as bony fishes and
the larvae of amphibia

Ureotelic animals:
many terrestrial
vertebrates; also sharks



Uricotelic animals:
birds, reptiles

Figure 18-2b
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Amino acid degradation in the liver

- In vertebrates, most amino acid degradation occurs in the liver
- Amino acids are **deaminated** via **transamination** reactions in the **cytosol**
- Amino group is moved to an acceptor molecule, converting the starting amino acid into its corresponding **α -keto acid**
- Examples:

alanine becomes pyruvate

glutamate becomes α -ketoglutarate

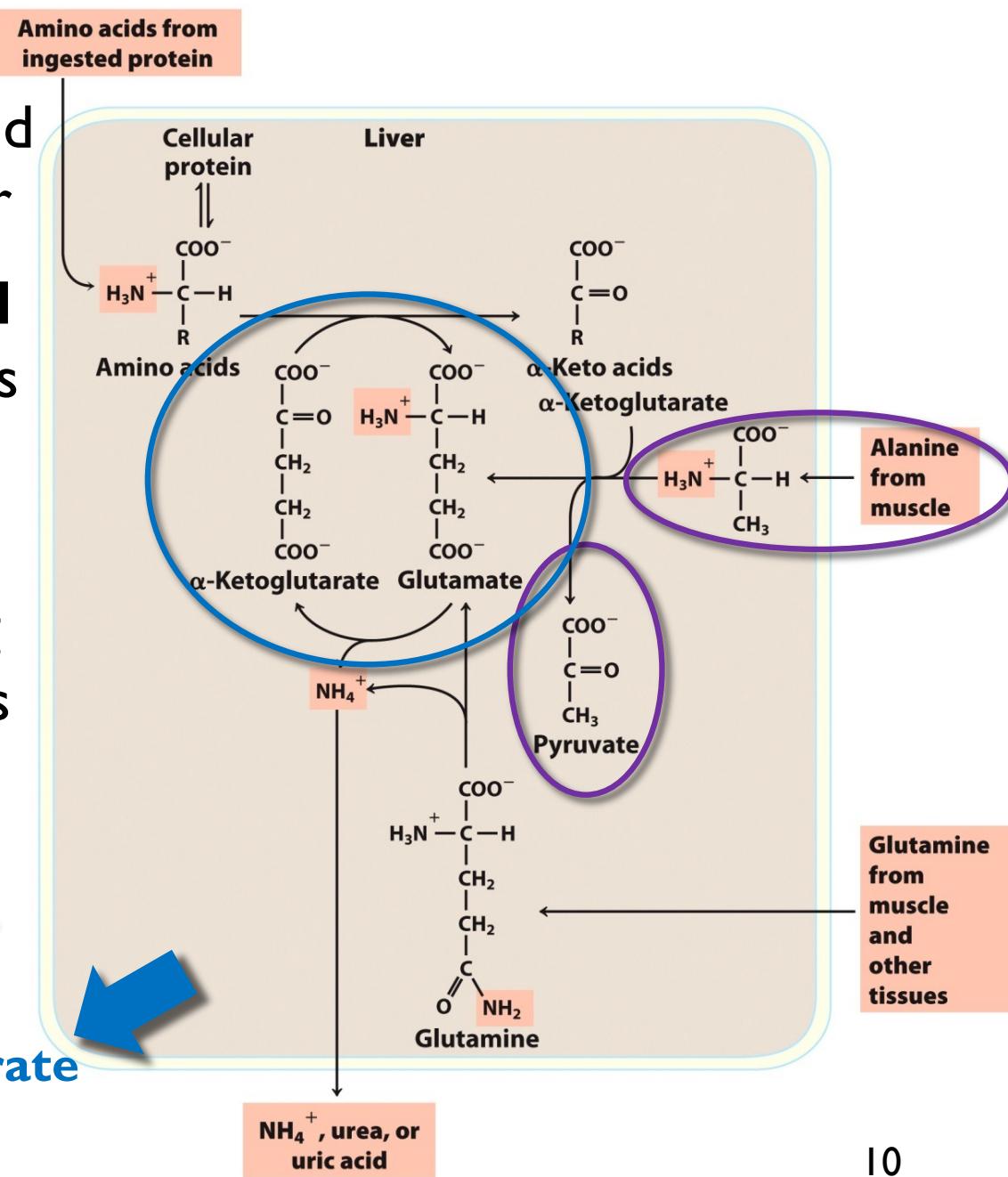


Figure 18-2a

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Transamination reactions

- For many amino acids, **α -keto-glutarate** is the amino group acceptor for transamination
- Enzyme:** aminotransferase
- Products:**
 - glutamate
 - α -keto analog of amino acid
- Glutamate** can then act as the amino group donor for biosynthetic reactions, or for nitrogen excretion

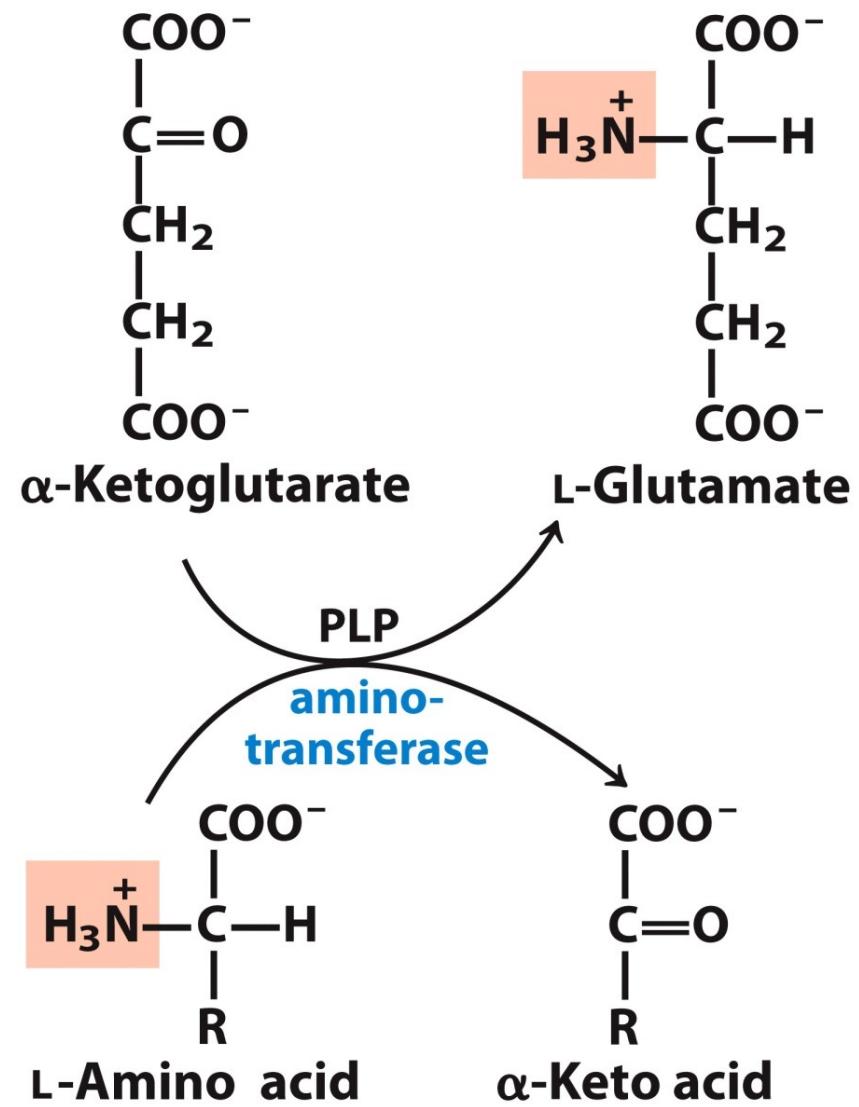


Figure 18-4
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Glutamate and glutamine

- In transamination, the amino group is not released as ammonia; it is exchanged (& these reactions are reversible)
- **Glutamate and glutamine** have central roles in the metabolism of the amino groups of amino acids
- Glutamate & glutamine are present at higher concentrations than most other amino acids

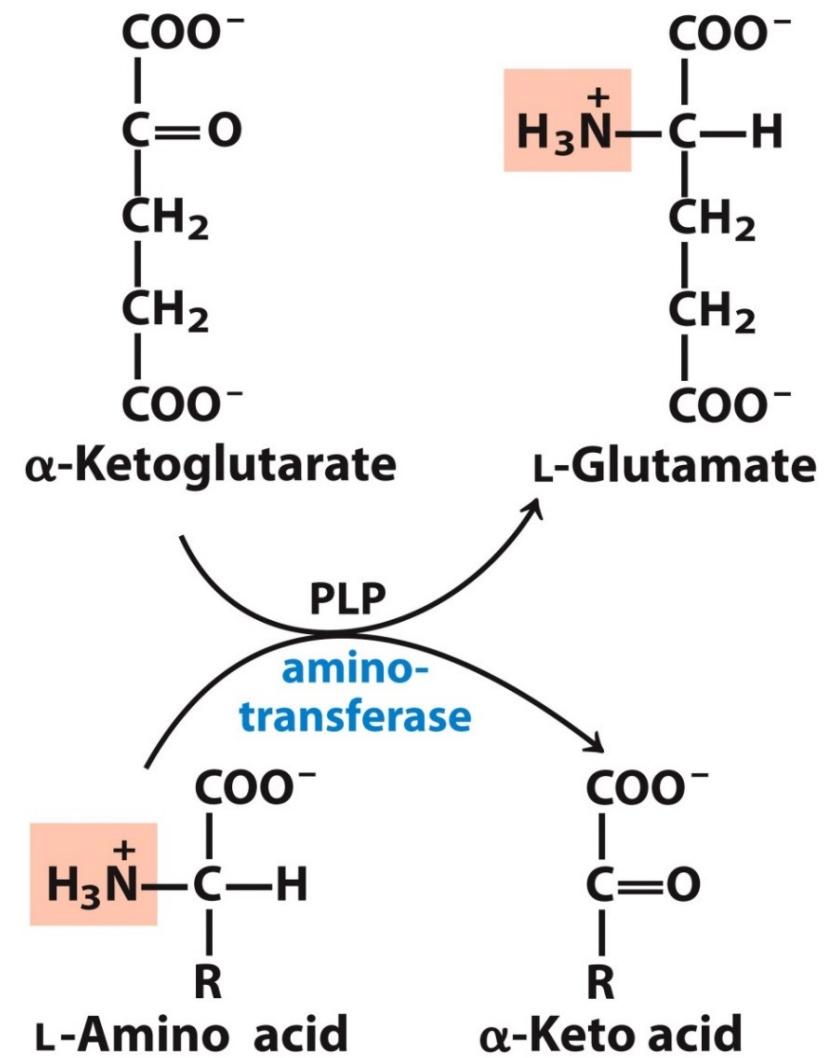


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Transport of excess nitrogen to liver

- Most tissues transfer excess amino groups to **glutamine** → blood → liver cells
- In liver cells, **Glutaminase** deaminates **glutamine** → **glutamate + NH₄⁺**
- **NH₄⁺** is converted into **urea** via **urea cycle**

-
- **Muscles** also transfer excess amino groups to **alanine** → blood → liver cells
 - In liver cells, amino groups are transferred from alanine to **glutamate**

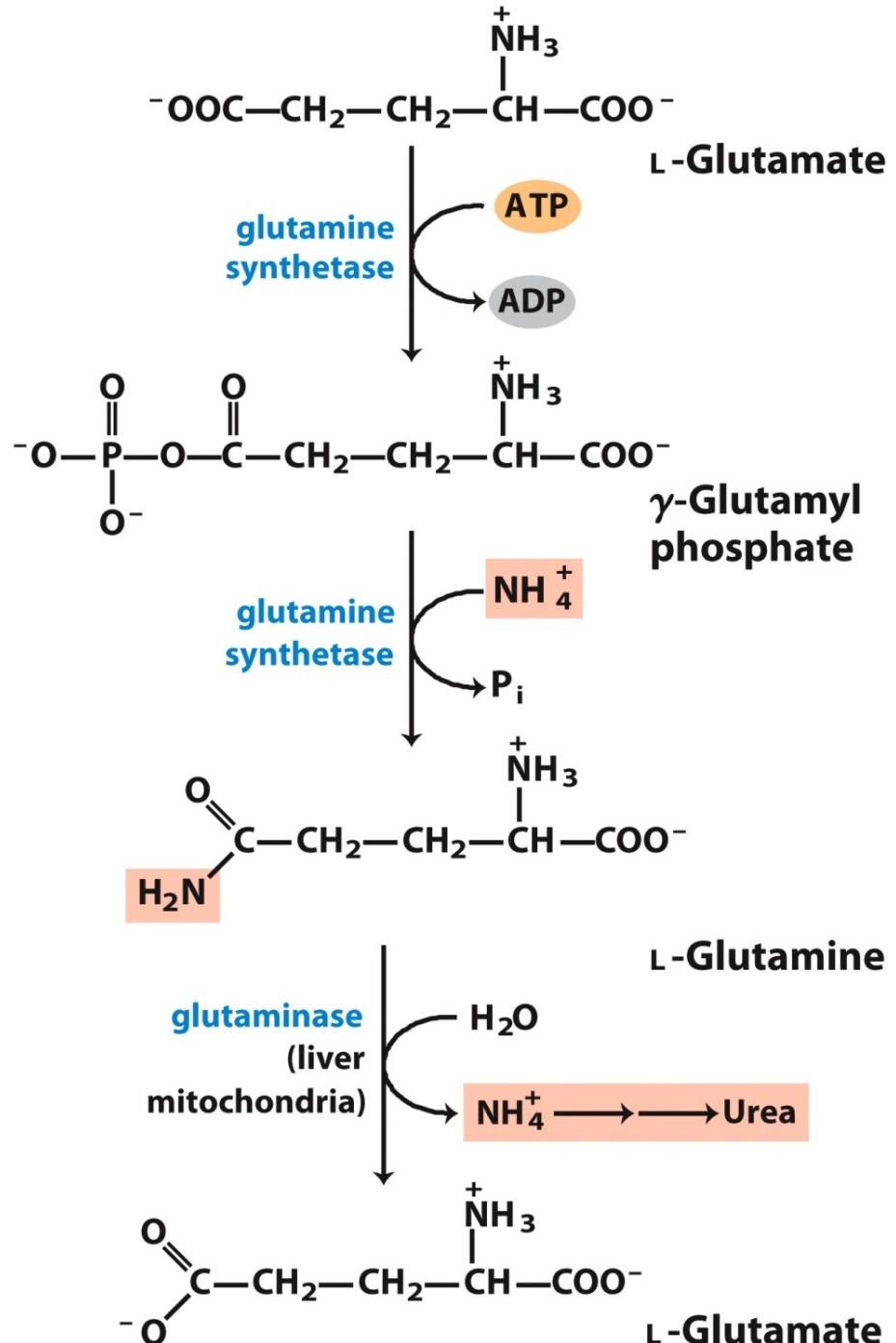


Figure 18-8

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Glucose-alanine cycle

- Muscles (and some other tissues) catabolize amino acids for fuel
- Resulting NH_4^+ in muscle cells is transferred to pyruvate to make **alanine**, which is sent via blood to liver
- Liver deaminates alanine; uses pyruvate to re-synthesize glucose via gluconeogenesis

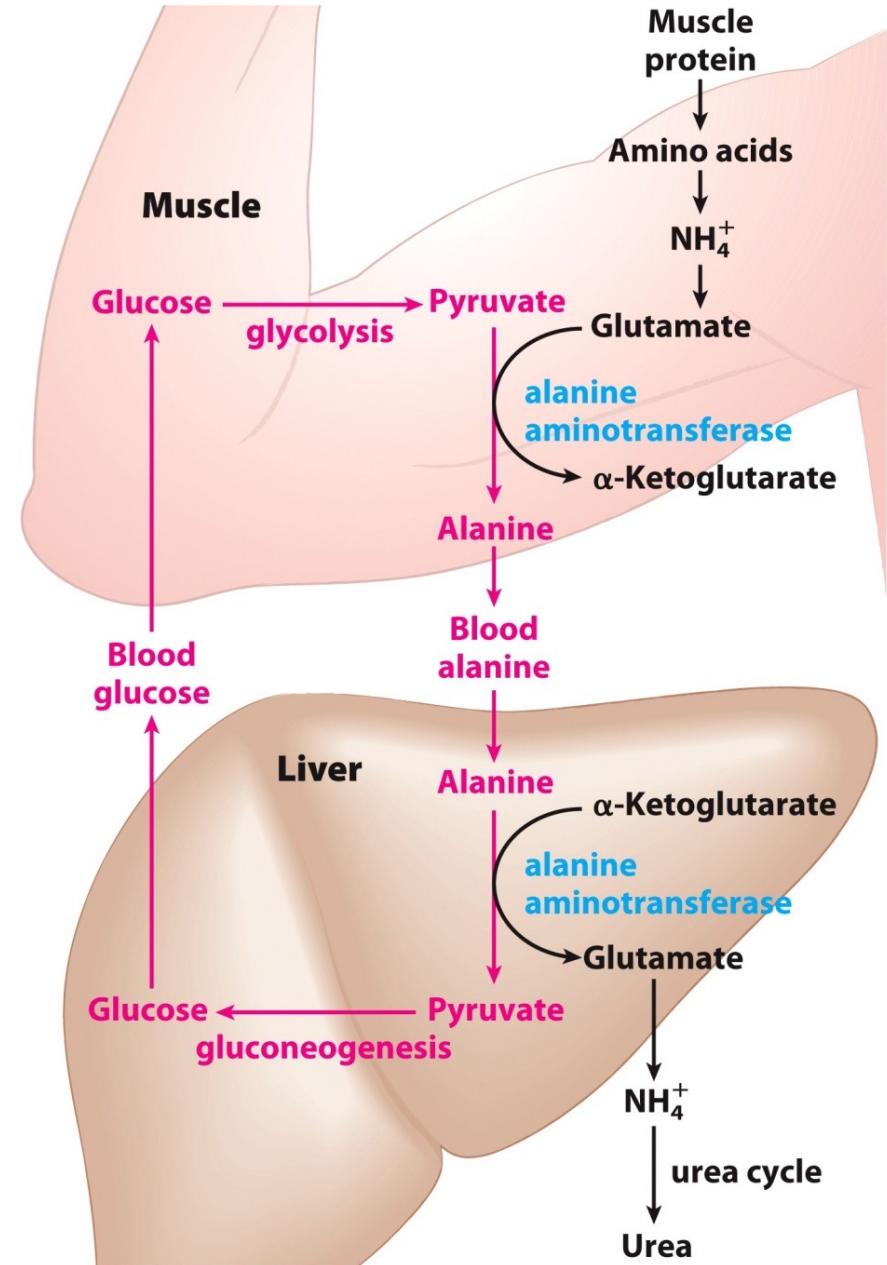
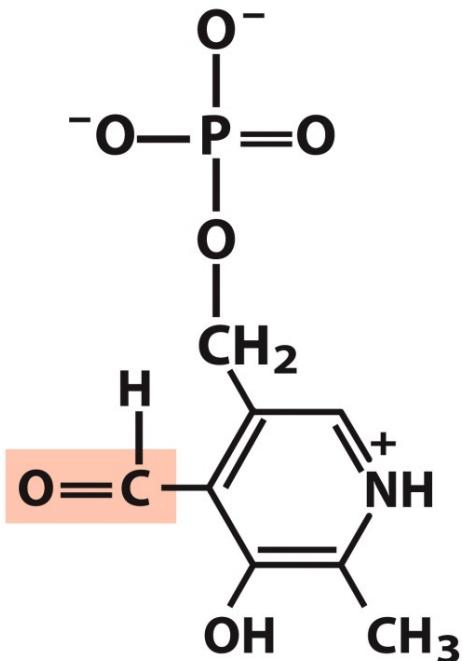


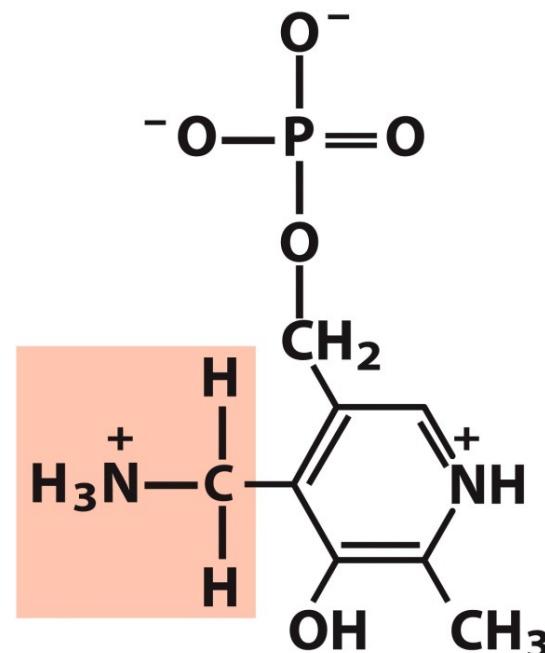
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Transaminases (aminotransferases)

Pyridoxal phosphate cofactor
(derived from vitamin B₆ — pyridoxine)



Pyridoxal phosphate
(PLP)



Pyridoxamine
phosphate

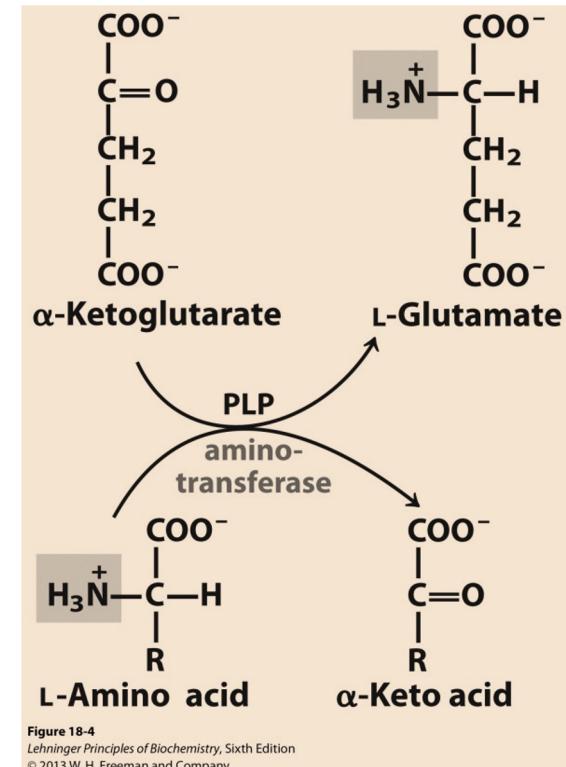


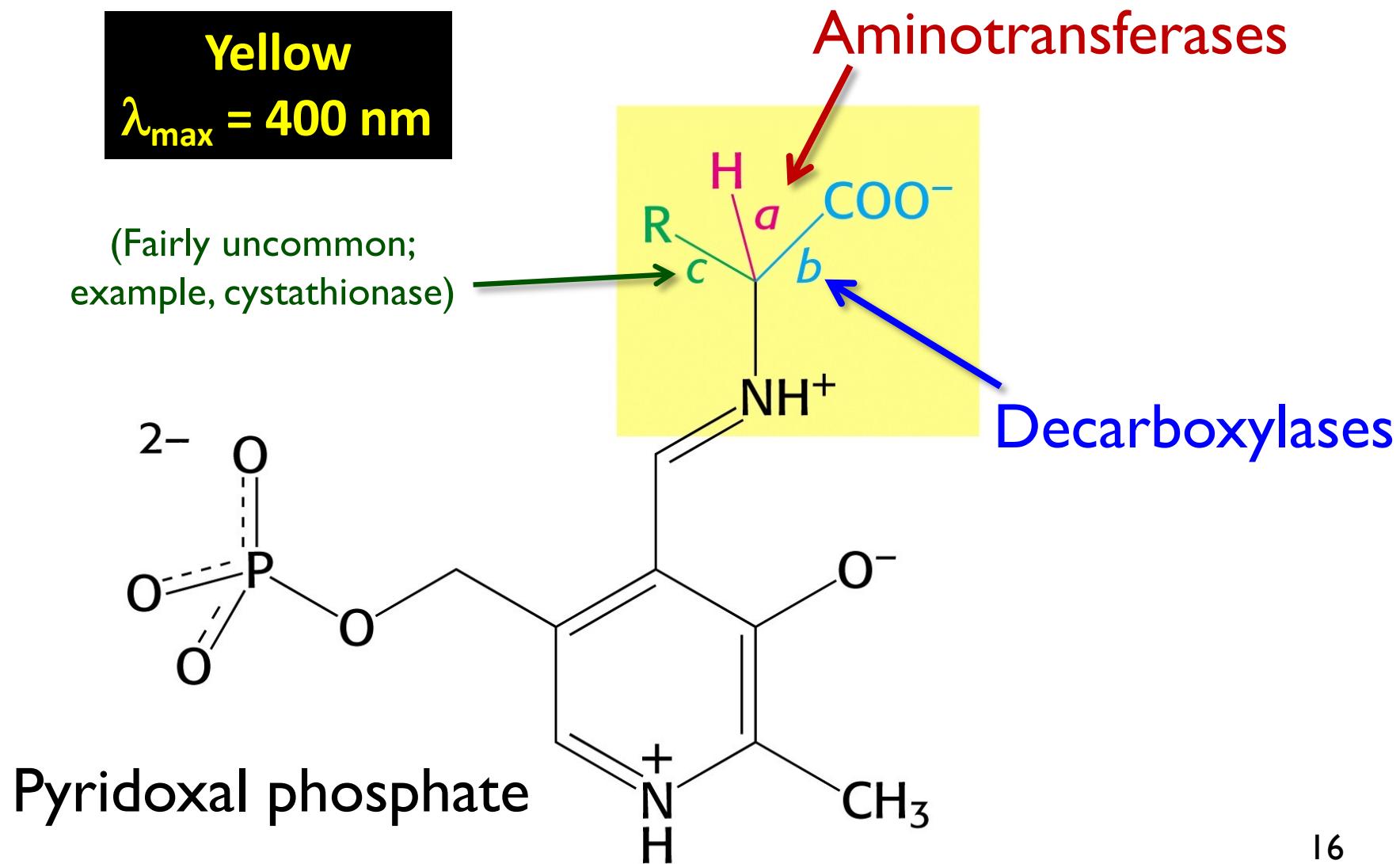
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Irwin Gunsalus

Pyridoxal phosphate (PLP) is a versatile cofactor

Many fates for amino acids linked to PLP via Schiff base



Transaminase mechanism

The **pyridoxal phosphate** (PLP) cofactor forms a **Schiff base** (aldimine) with the ϵ -amino group of a lysine from the enzyme

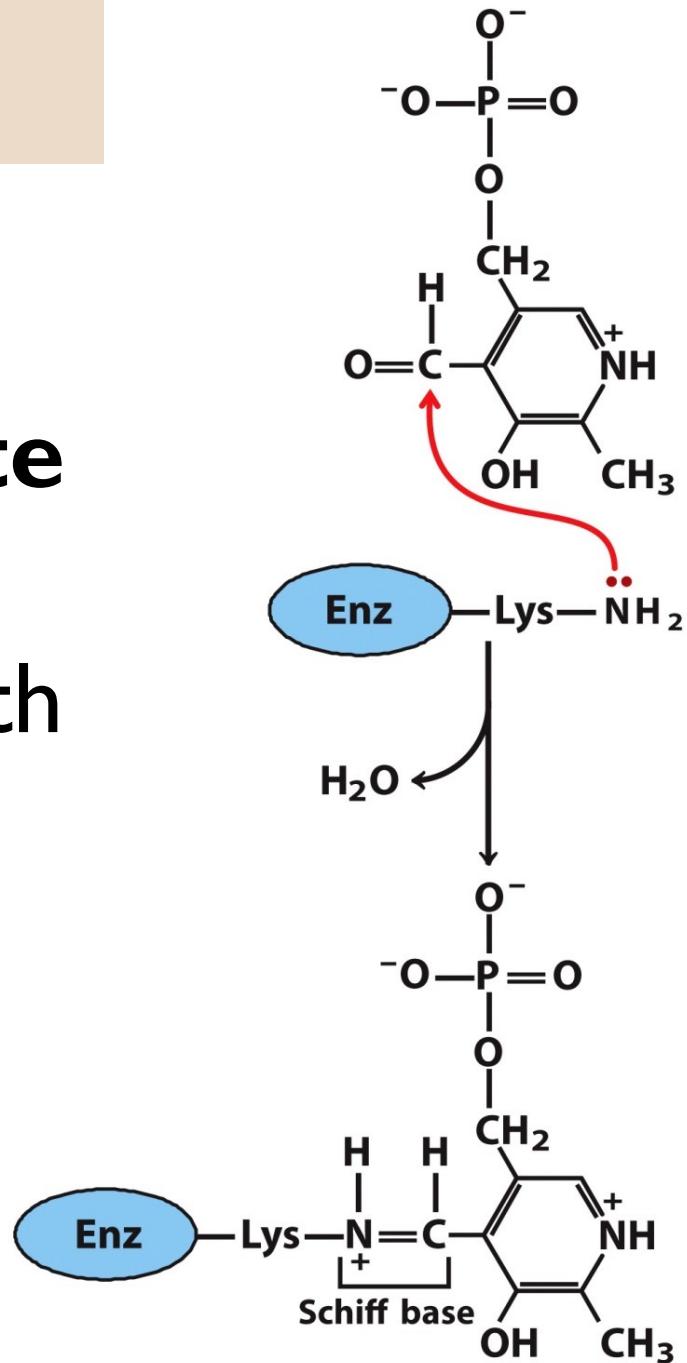
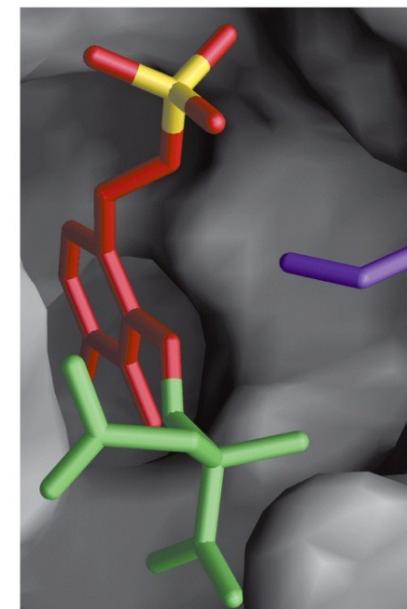
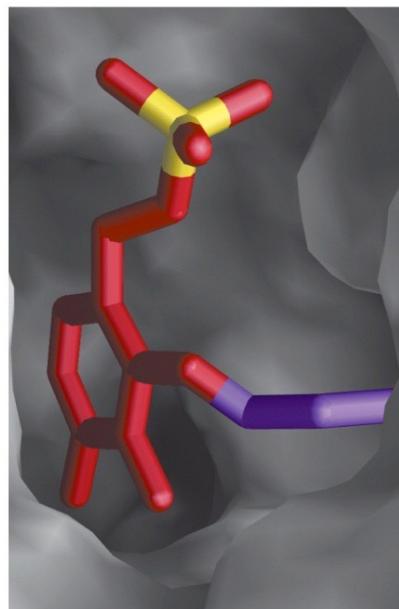
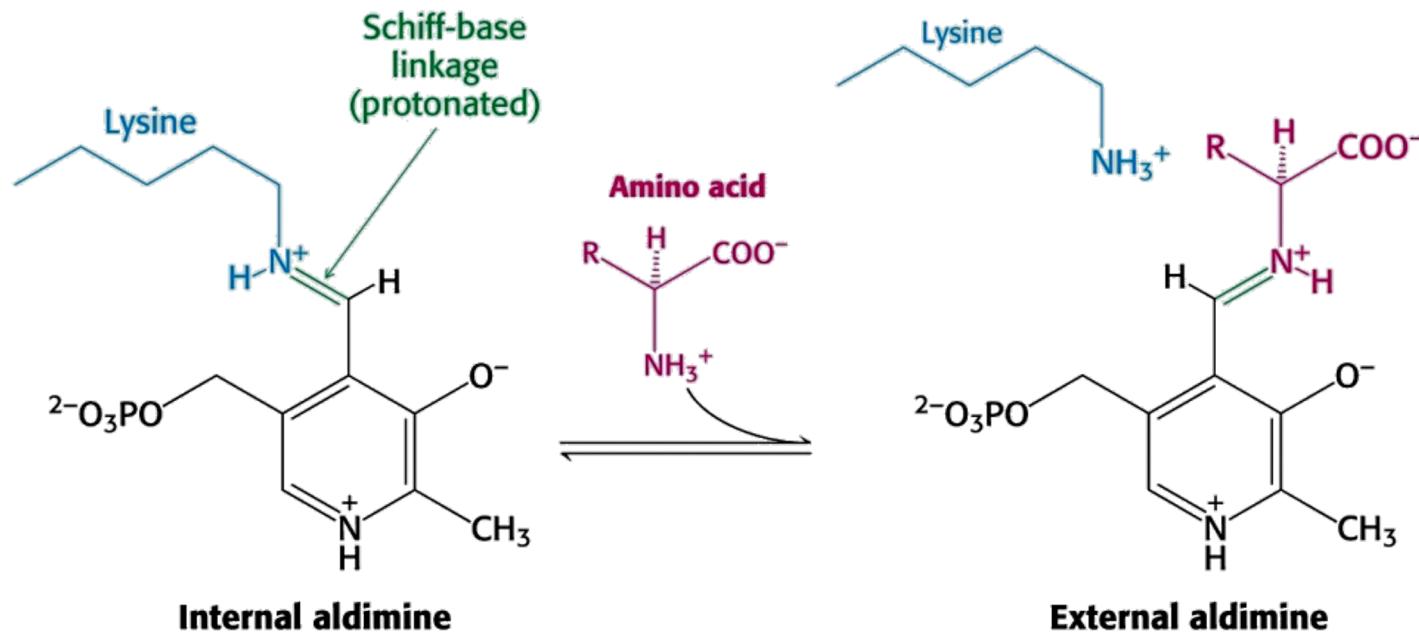


Figure 18-5b

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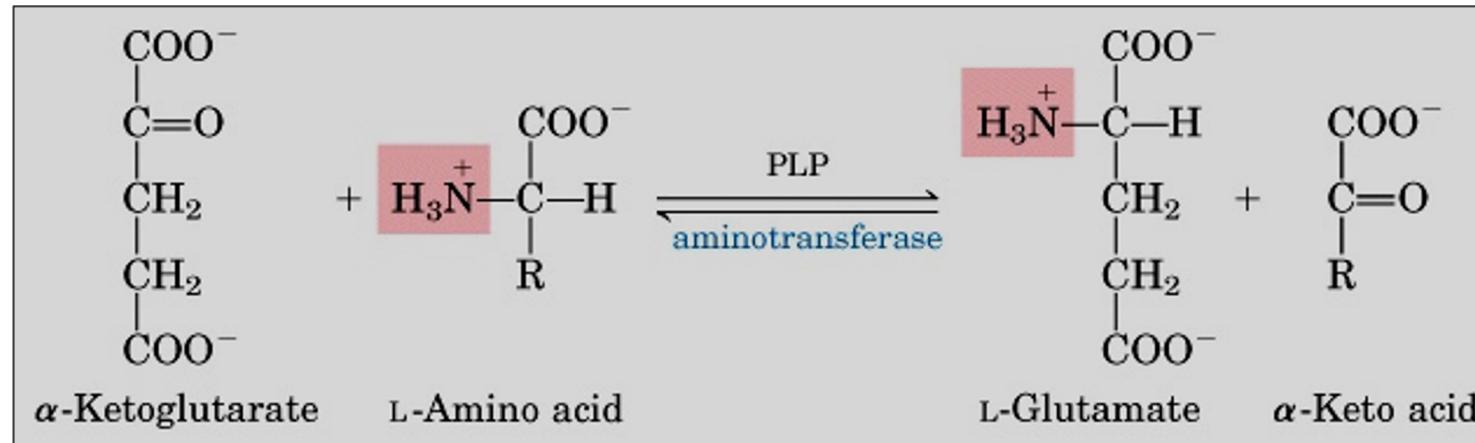
Transaminase mechanism

PLP-enzyme Schiff base exchanges w/ incoming amino acid -NH₂



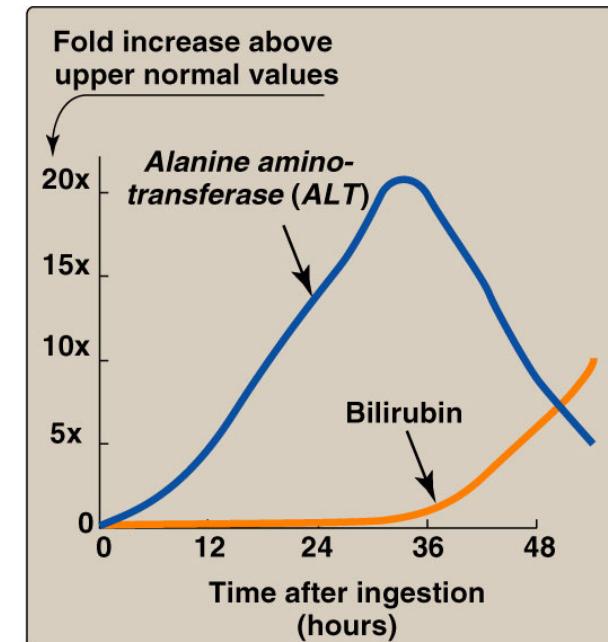
Transaminases — overall reaction

Interconversion of **amino** acid and **keto** acid with concomitant transfer of **amino** group

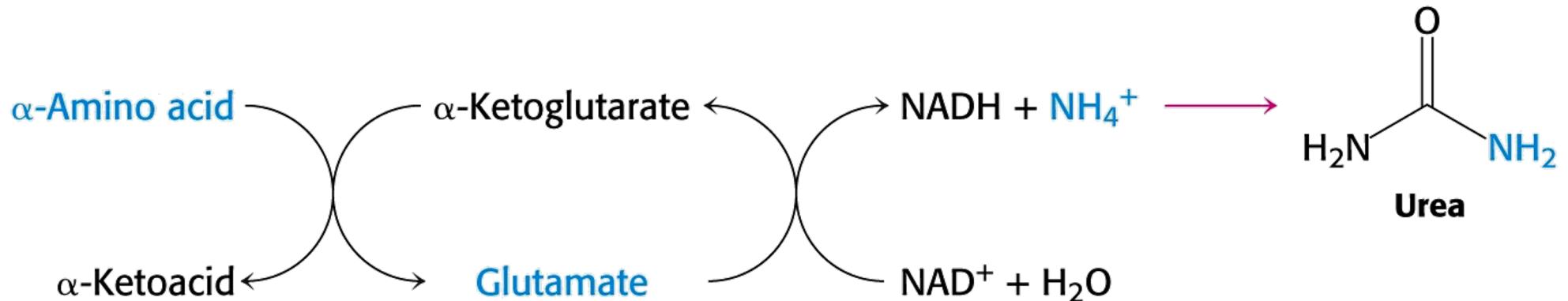


Clinical tests — transaminases released from damaged cells (such as in **liver failure**) can be quantified in blood samples; examples:

- **AST**: aspartate aminotransferase (also called serum glutamate-oxaloacetate transaminase; SGOT)
 - **ALT**: alanine aminotransferase (also called serum glutamate-pyruvate transaminase; SGPT)



Ammonia from glutamate → urea



- In liver, glutamate is also converted to α-keto-glutarate via **oxidative deamination**, releasing the amino group as **ammonia**
- Ammonia is converted ultimately to urea via the **urea cycle**

Oxidative deamination: Glutamate dehydrogenase

- Oxidative deamination of glutamate yields α -ketoglutarate, ammonia and NADH
- Occurs in mitochondrial matrix in liver cells

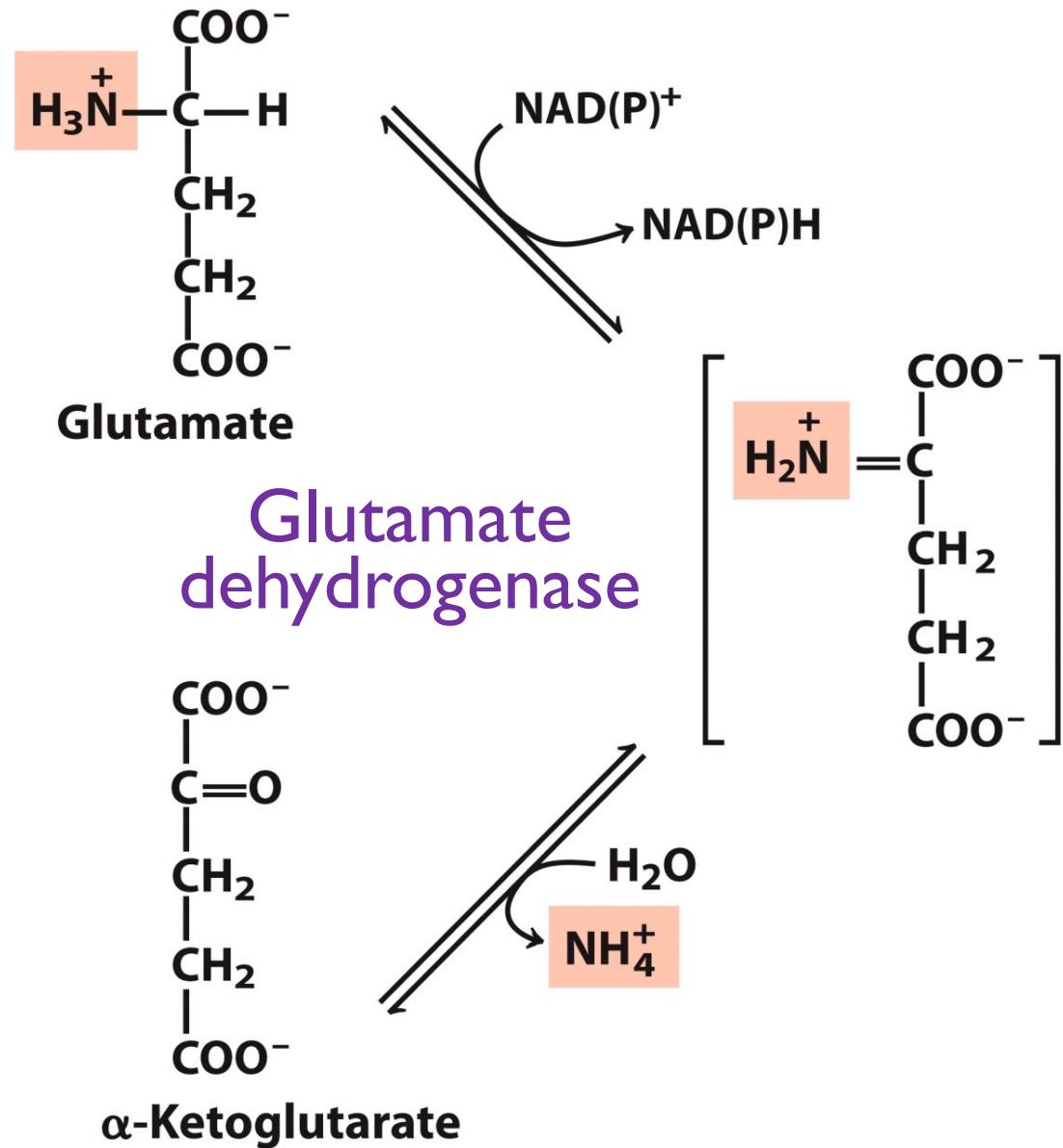


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*The glutamate dehydrogenase has the unusual capacity to use either NAD^+ or NADP^+ as cofactor.

Reprise: Nitrogen transport to liver

- In hepatocyte cytosol, most excess amino groups transferred to α -ketoglutarate
- Resulting glutamate imported into mitochondria; converted into ammonia $\rightarrow \rightarrow$ urea

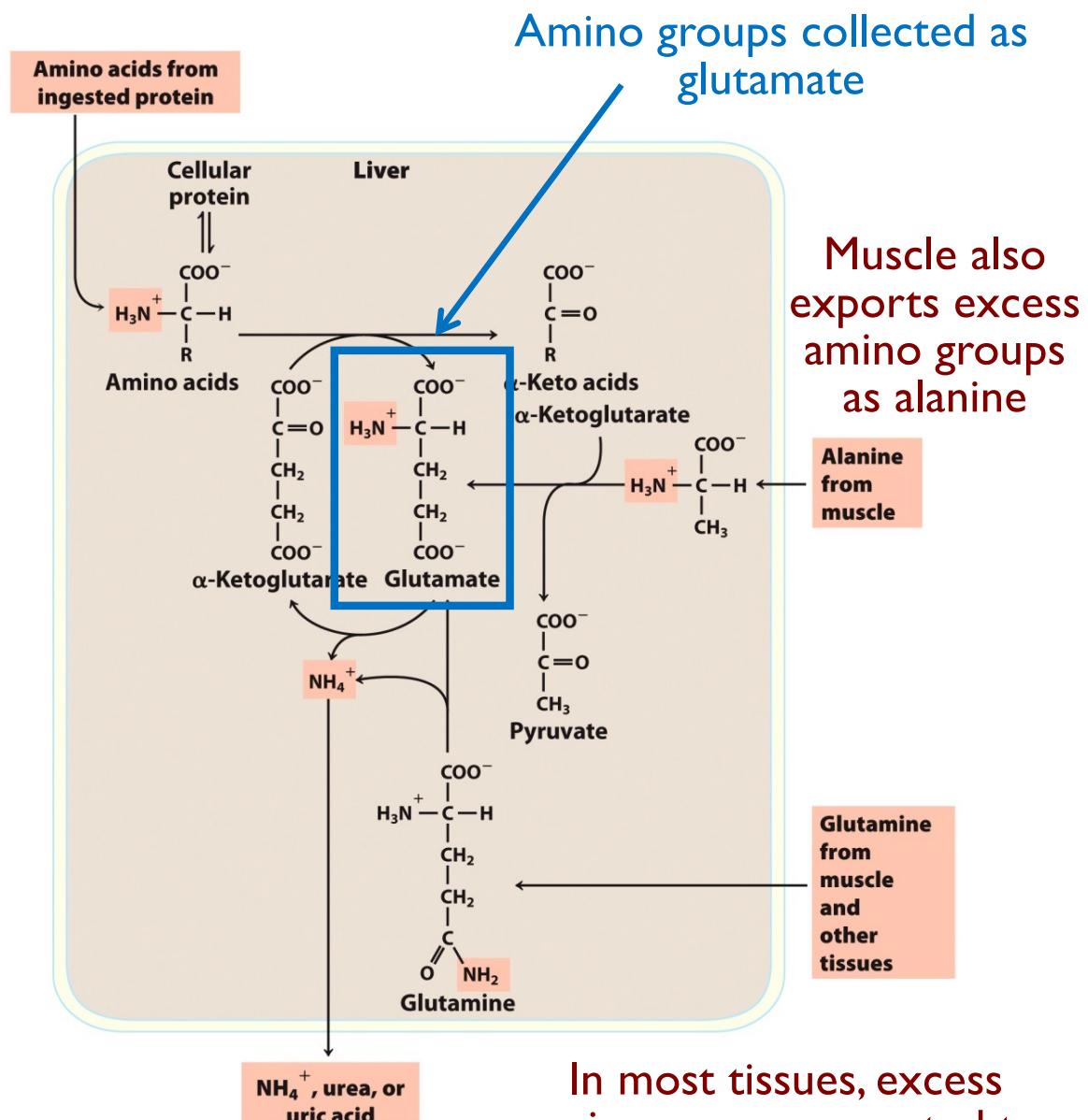


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Urea cycle

- **Urea** is produced in **liver**
- Ammonia is converted into urea via 5 enzymatic steps (4 of which comprise the urea cycle)
- Note: portions of the urea cycle occur in the **cytosol** and in the **mitochondrial matrix**
- **2 nitrogens in urea: one from NH_3 & one from Asp**
- **Ornithine is a “true” catalyst, in contrast to oxaloacetate in the TCA cycle**

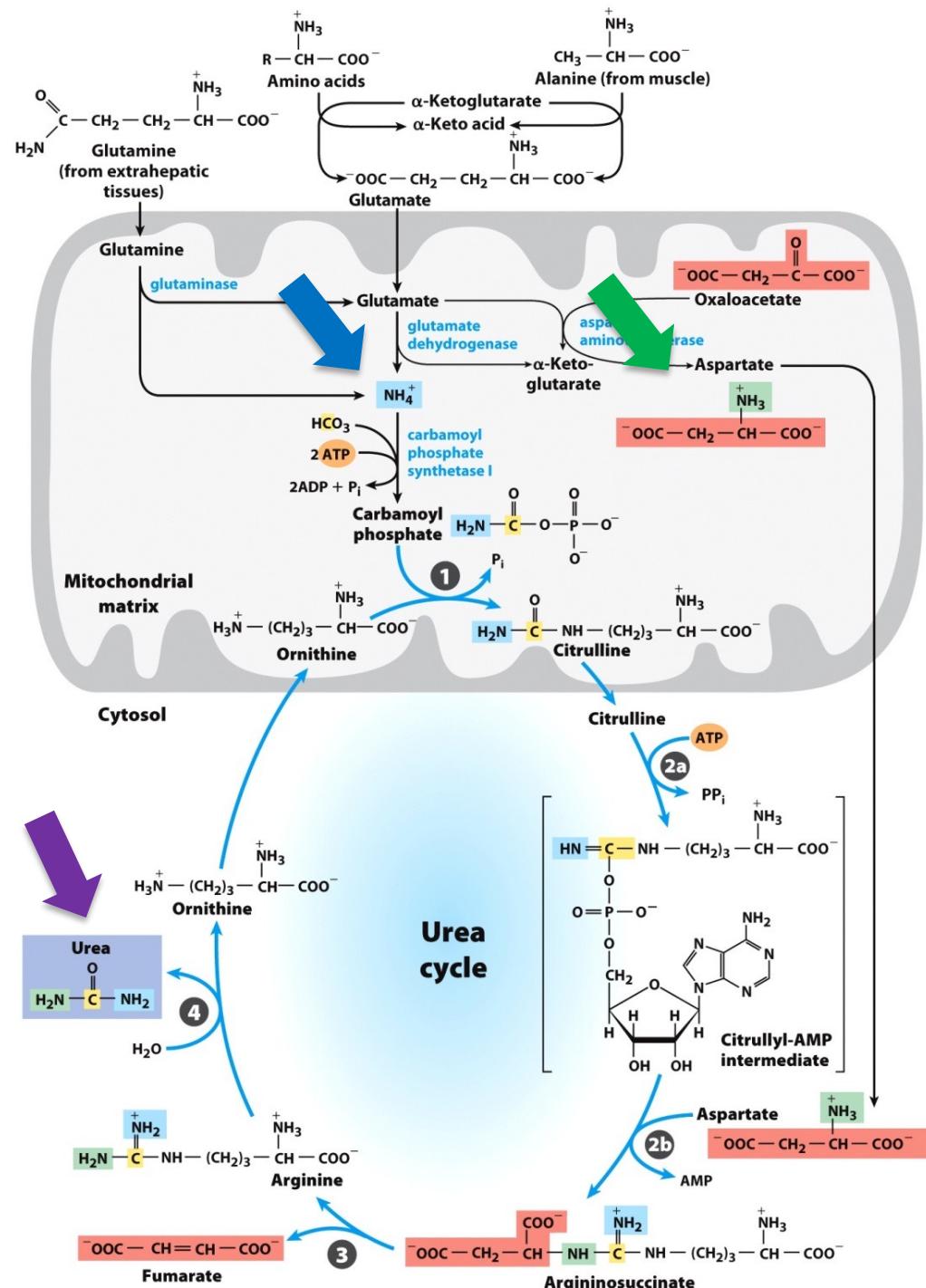
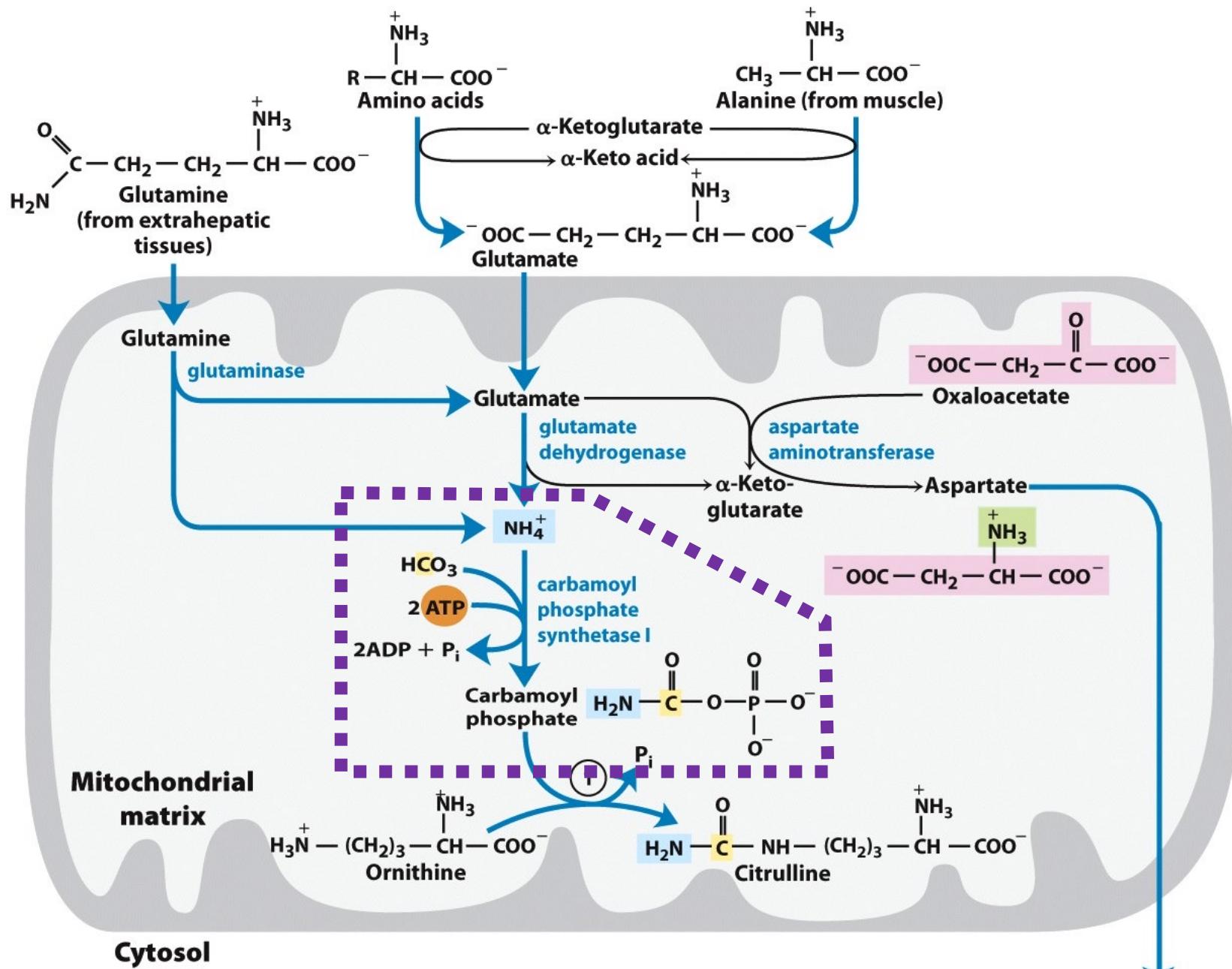


Figure 18-10

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Step before the urea cycle: Synthesis of carbamoyl phosphate in mitochondrial matrix



Carbamoyl phosphate synthetase I

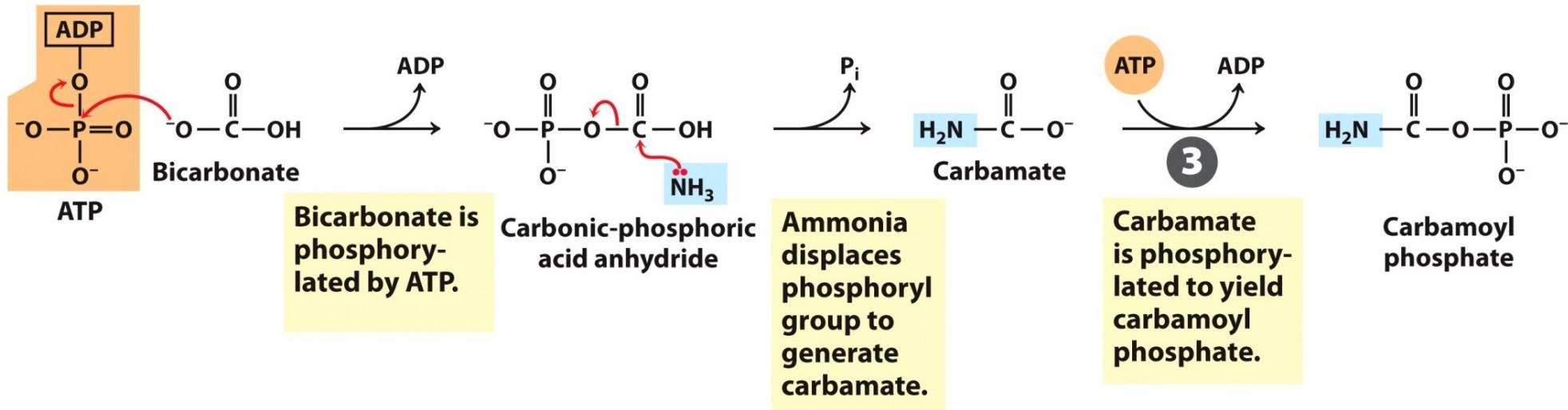


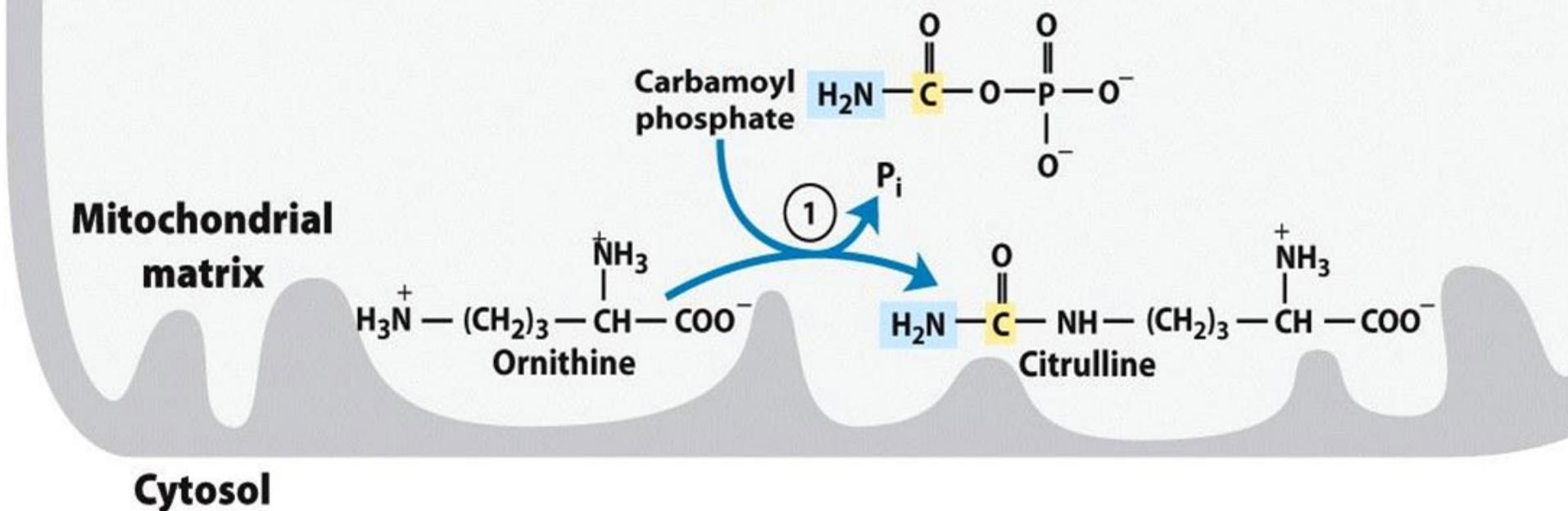
Figure 18-11a

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The enzyme has 3 active sites, connected to each other. Through “Substrate Channeling”, product from one active site is channeled to the other active site...

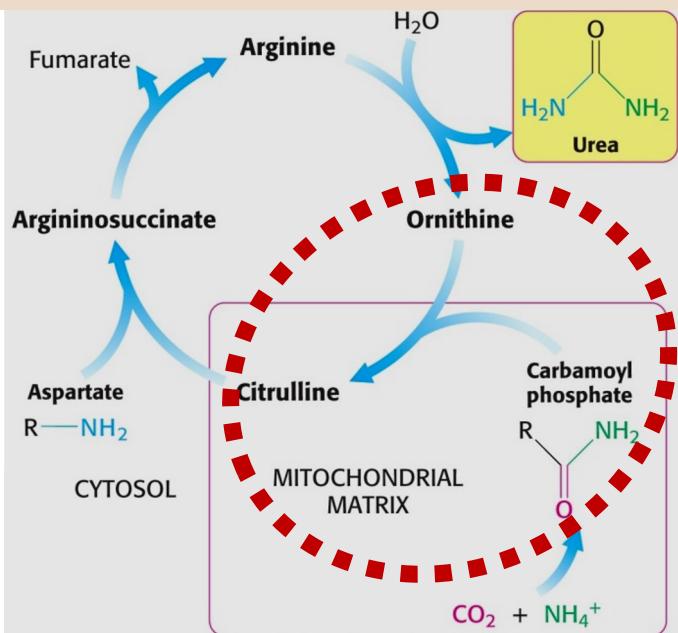
- In the **mitochondrial matrix**
- Note two activation steps (phosphorylations via ATP) are used by this enzyme to generate carbamoyl phosphate
- Does not employ biotin.
- Phosphates are good leaving groups

Step I: Ornithine transcarbamoylase

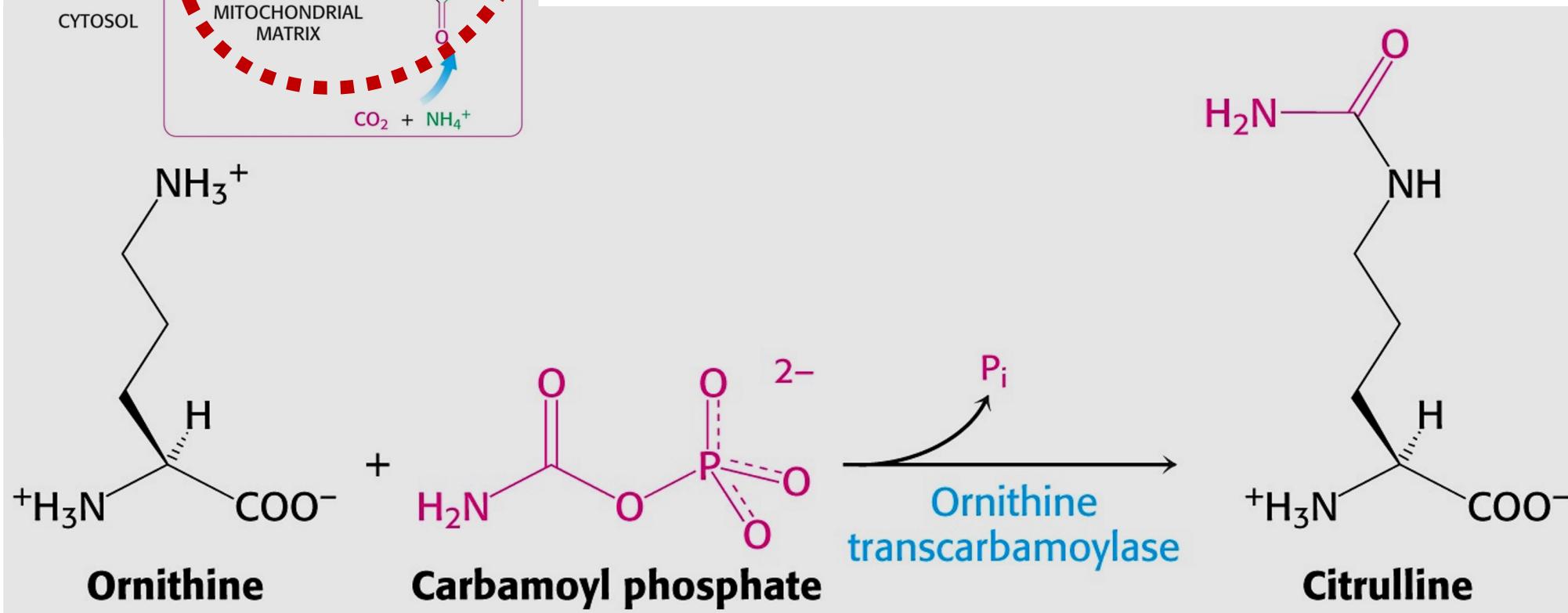


- In the **mitochondrial matrix**
- (Also spelled “ornithine transcarbamylase”)
- Ornithine + carbamoyl phosphate \rightarrow citrulline + P_i

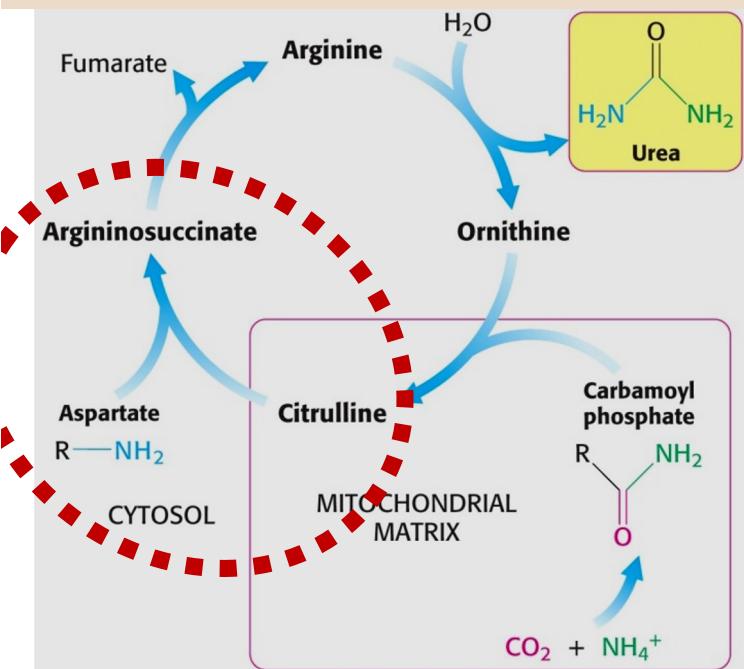
Ornithine transcarbamoylase



- Another view of this reaction
- Nucleophilic attack of NH₂ group of ornithine on the carbonyl of carbamoyl phosphate
- Citrulline is produced and transported out to the cytosol.



Step 2: Argininosuccinate synthetase



- Cytosolic
- Second amino group enters pathway from aspartate
- 2 “high-energy” bonds are used (PPi is hydrolyzed)

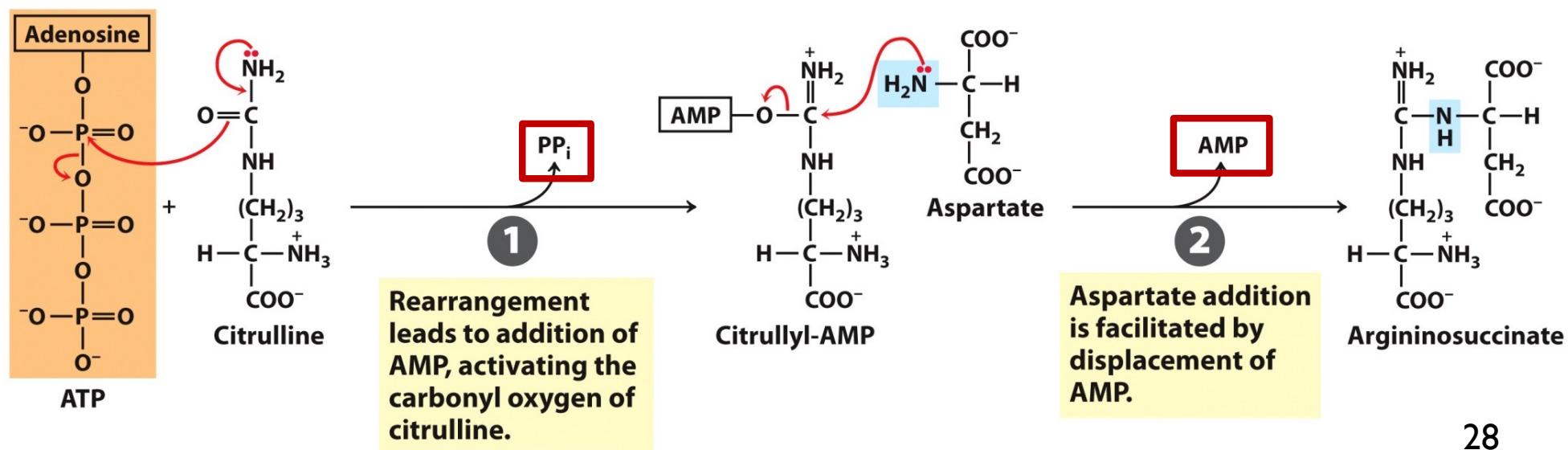
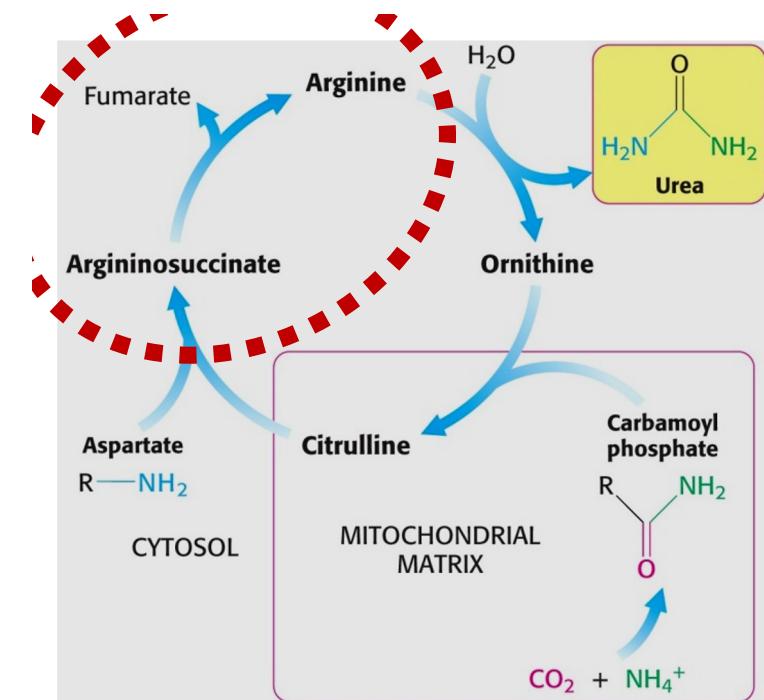


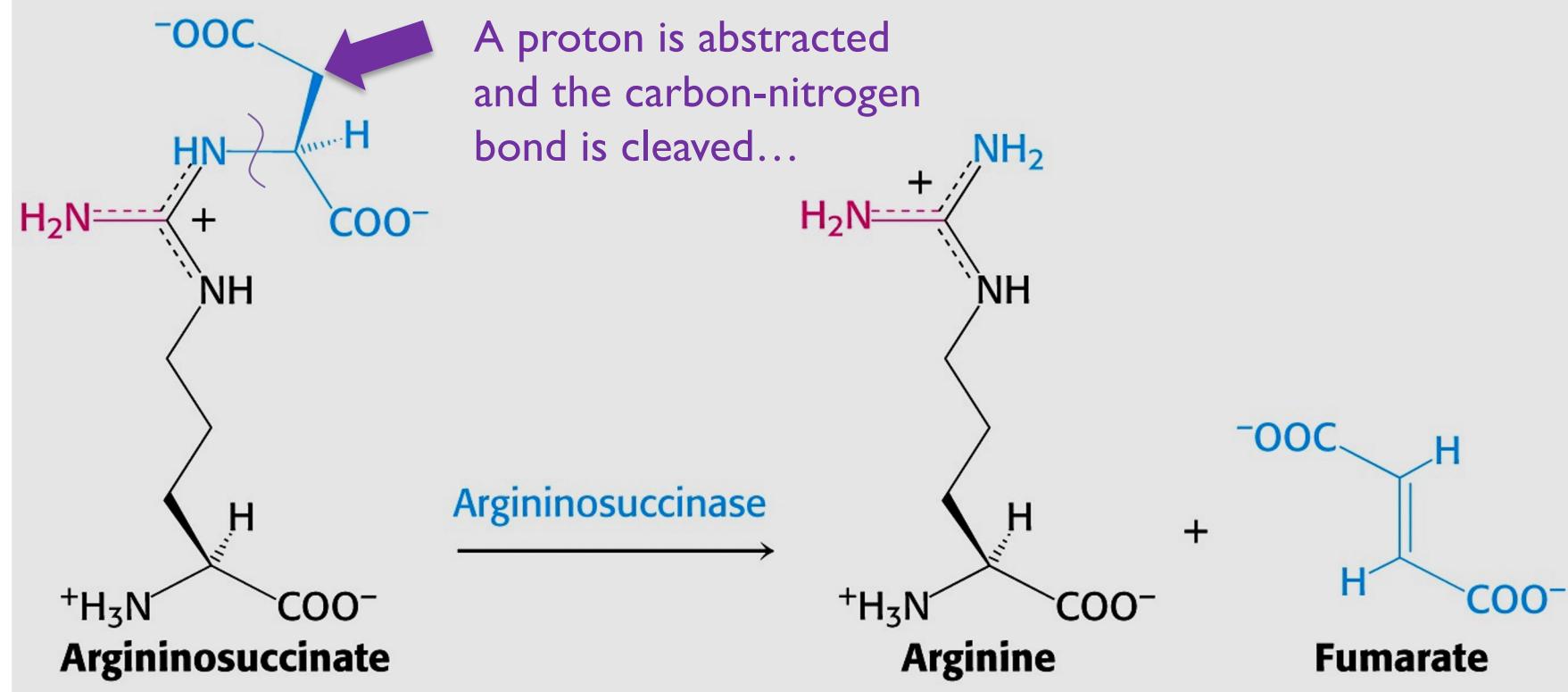
Figure 18-11b

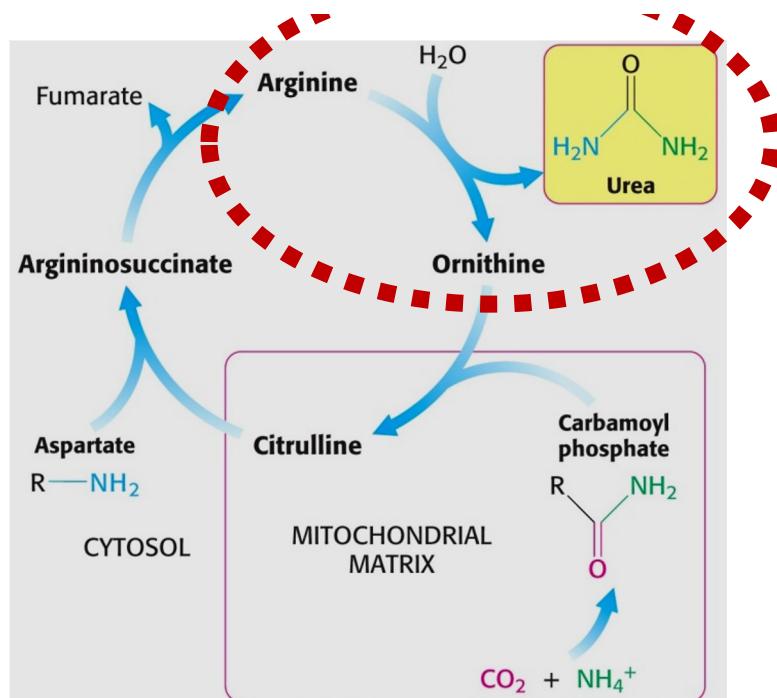
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Step 3: Argininosuccinase

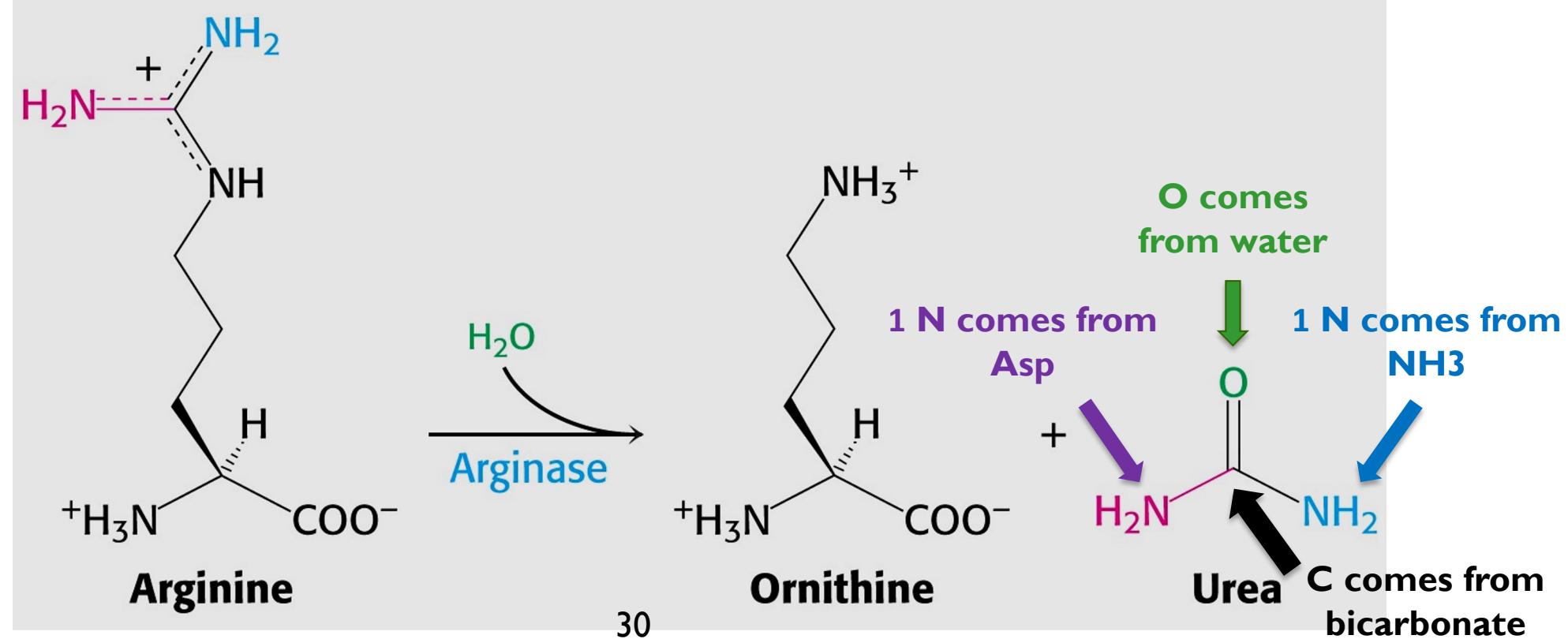
- (Also called argininosuccinate lyase)
- **Cytosolic**
- Arginine now carries both amino groups (one originally from NH_4^+ ; one from aspartate)





Step 4: Arginase

- **Cytosolic**
- Hydrolyses arginine to **urea** + ornithine



Urea cycle

- **Urea** is produced in **liver**
- Ammonia is converted into urea via 5 enzymatic steps (4 of which comprise the urea cycle)
- Note: portions of the urea cycle occur in the **cytosol** and in the **mitochondrial matrix**
- **2 nitrogens in urea: one from NH_3 & one from Asp**
- **Ornithine is a “true” catalyst, in contrast to oxaloacetate in the TCA cycle**

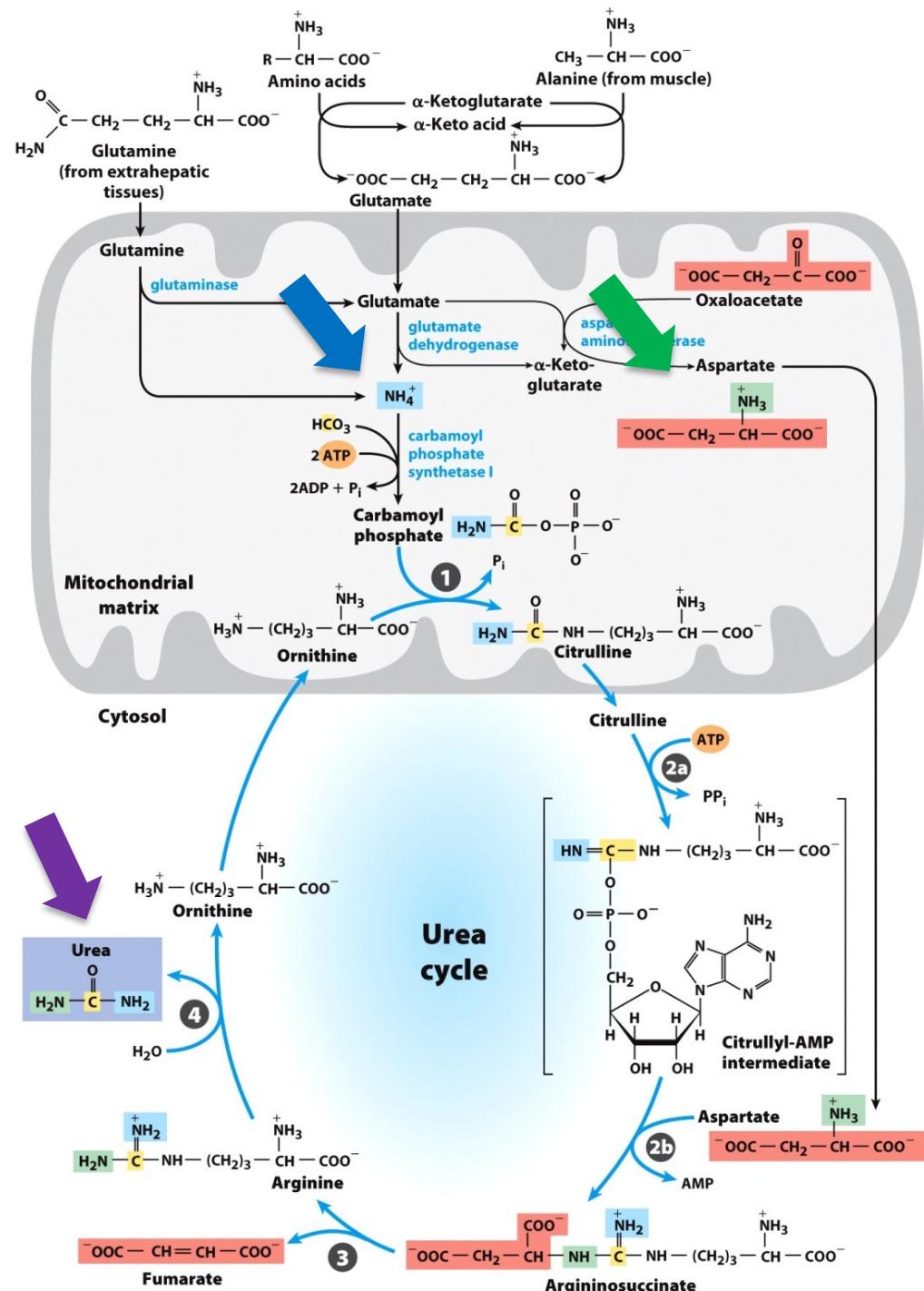
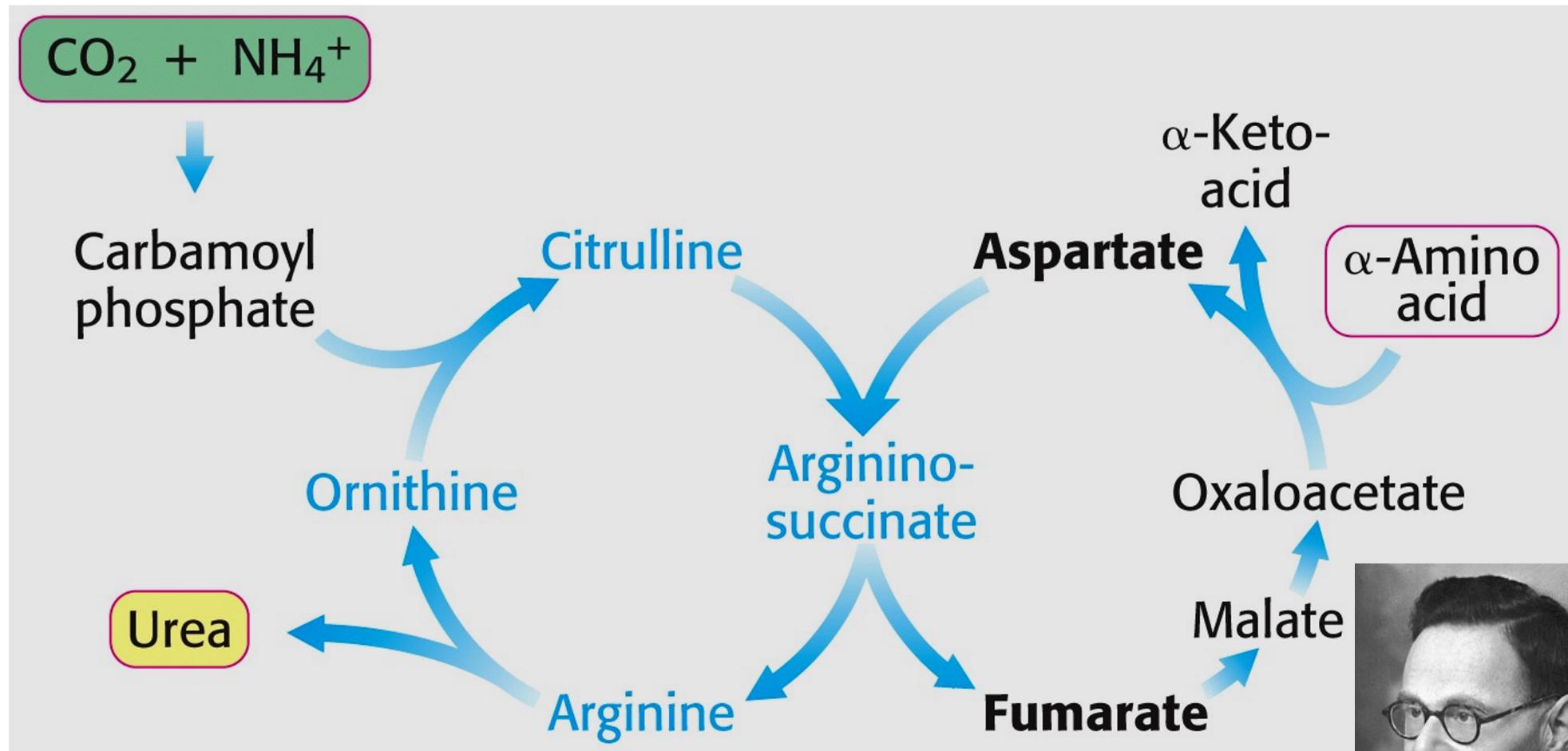


Figure 18-10

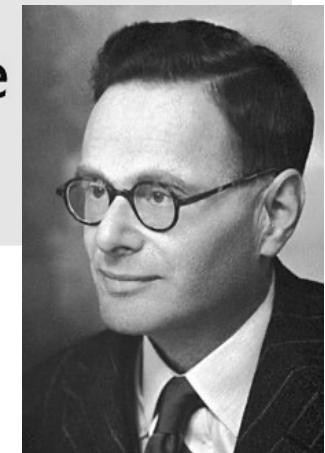
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The urea cycle is linked to the TCA cycle

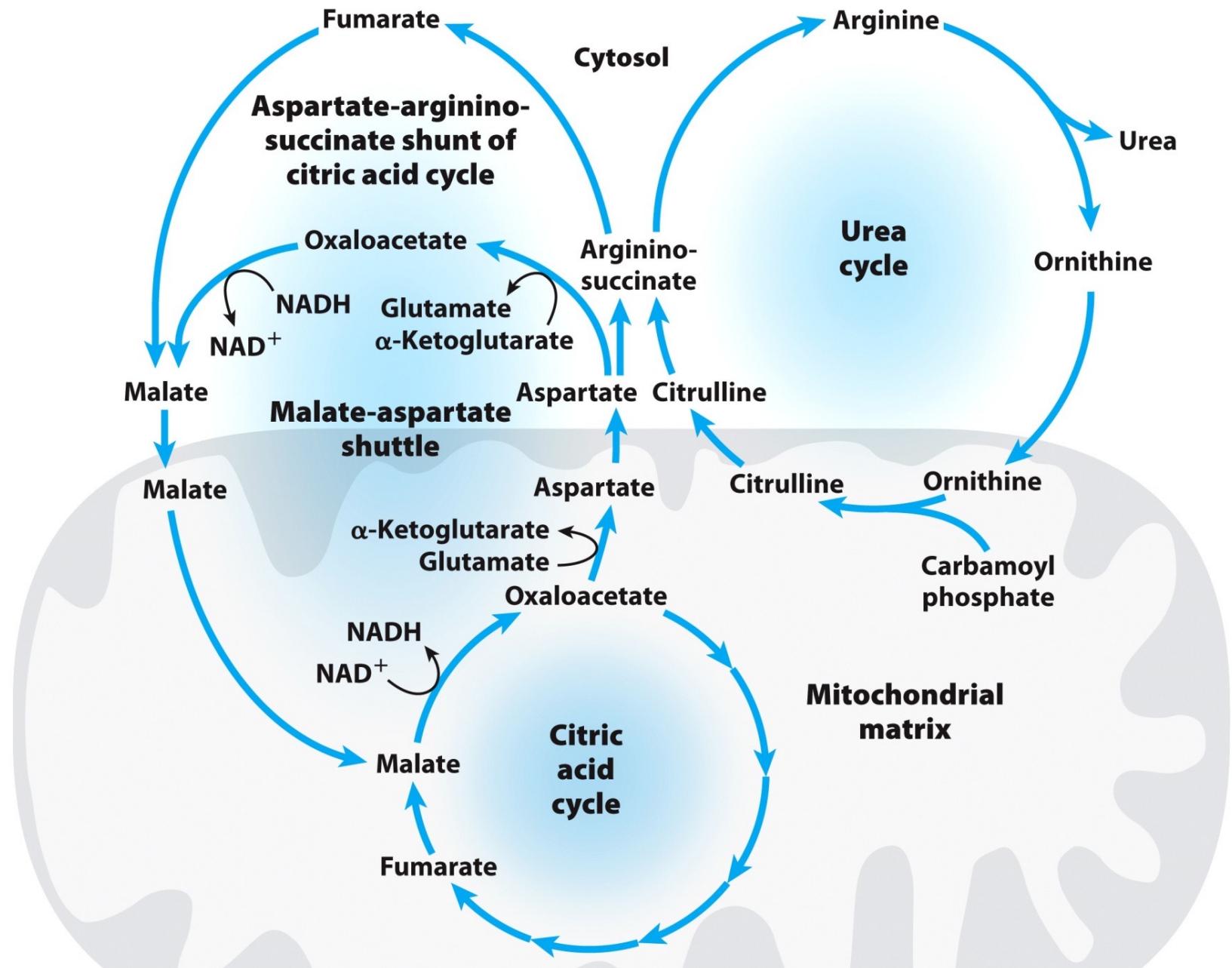


“Krebs bicycle”



Hans Krebs

Urea cycle is linked to TCA cycle through cytosolic isozymes and transport systems



Urea cycle regulation

- **Short term:** allosteric regulation of carbamoyl phosphate synthetase I by **N-acetylglutamate**
- **Long term:** Increased urea cycle enzyme synthesis

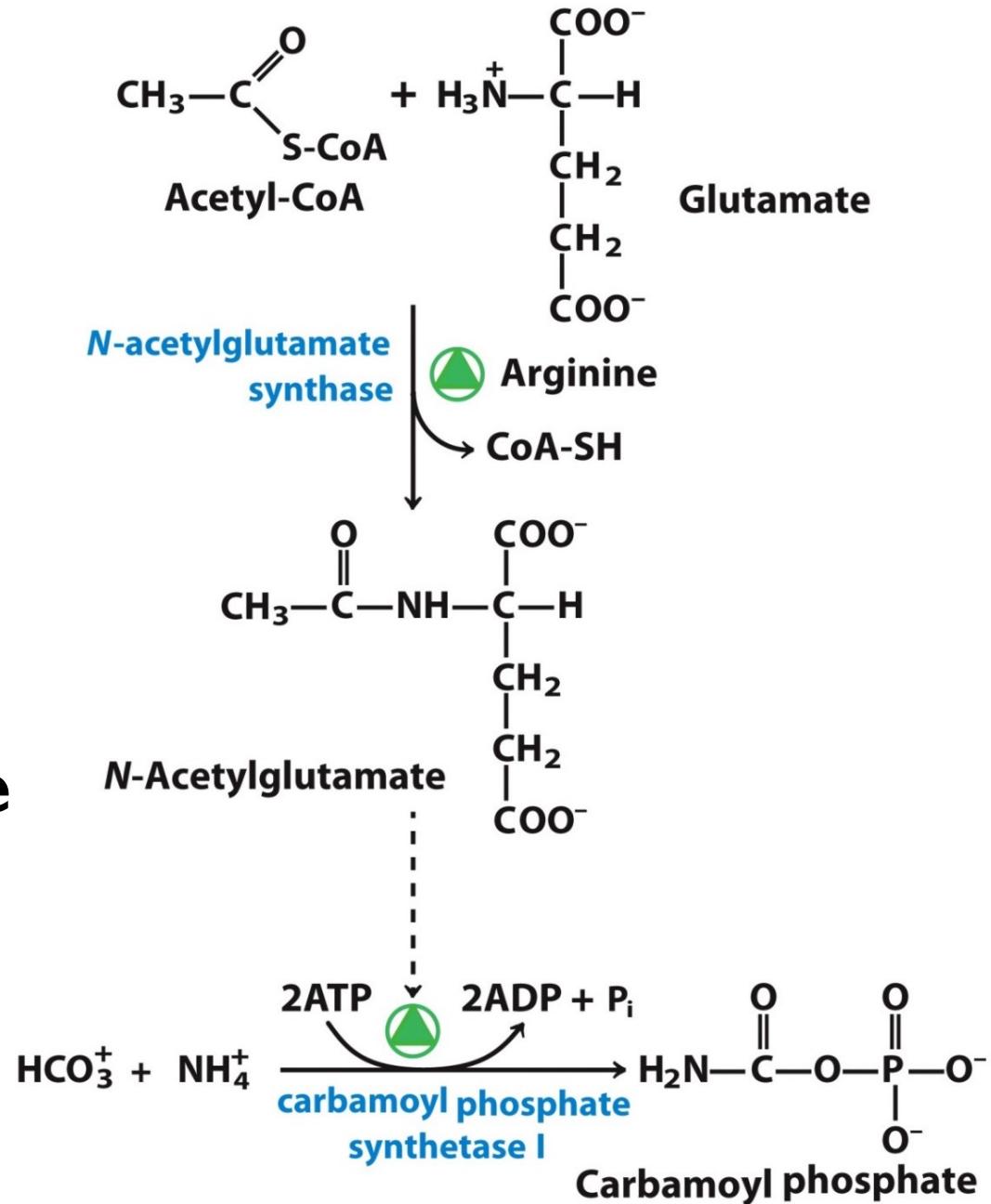
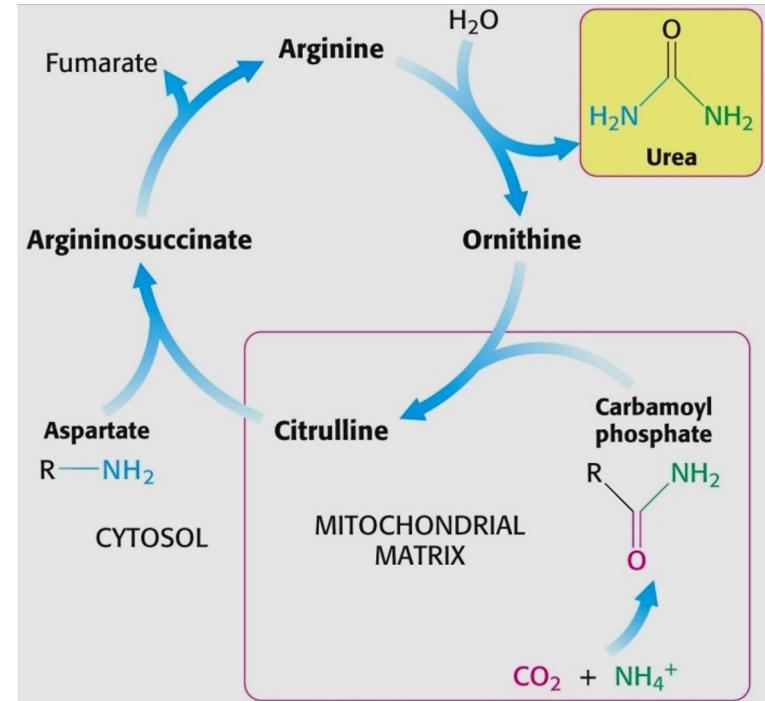


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Urea cycle disorders

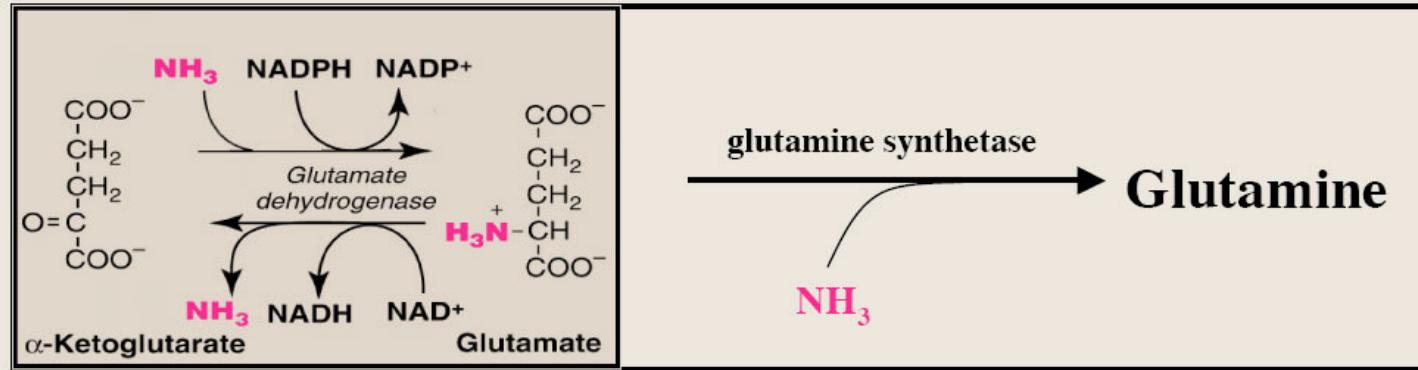
- Compromised liver function or genetic deficiencies in urea cycle enzymes (or in the production of N-acetylglutamate) can lead to **hyperammonemia**
- Ammonia is very toxic ($>60 \mu\text{M}$ ammonia), especially to the CNS:
 - Acute: lethargy, vomiting, disorientation, cerebral edema, coma, death
 - Chronic: developmental delay, mental retardation, progressive liver damage



Normal serum ammonia:
about **10 to 40 μM**
In **hyperammonemia**, the
levels can exceed **1000 μM**

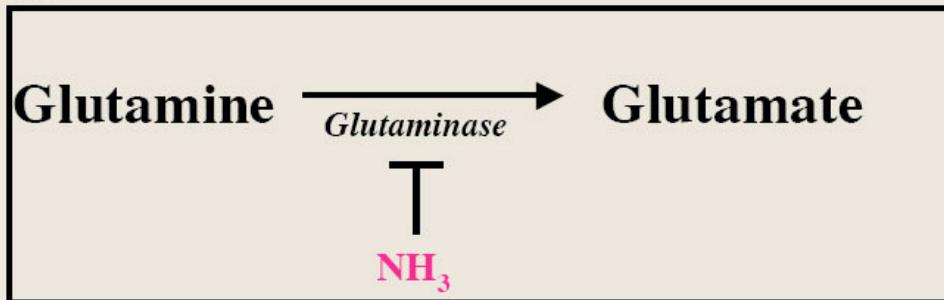
Mechanism of ammonia toxicity

A.



Glutamate dehydrogenase reaction is shifted toward synthesis of glutamate, resulting in the depletion of citric acid cycle intermediates and deprivation of brain energy.

B.

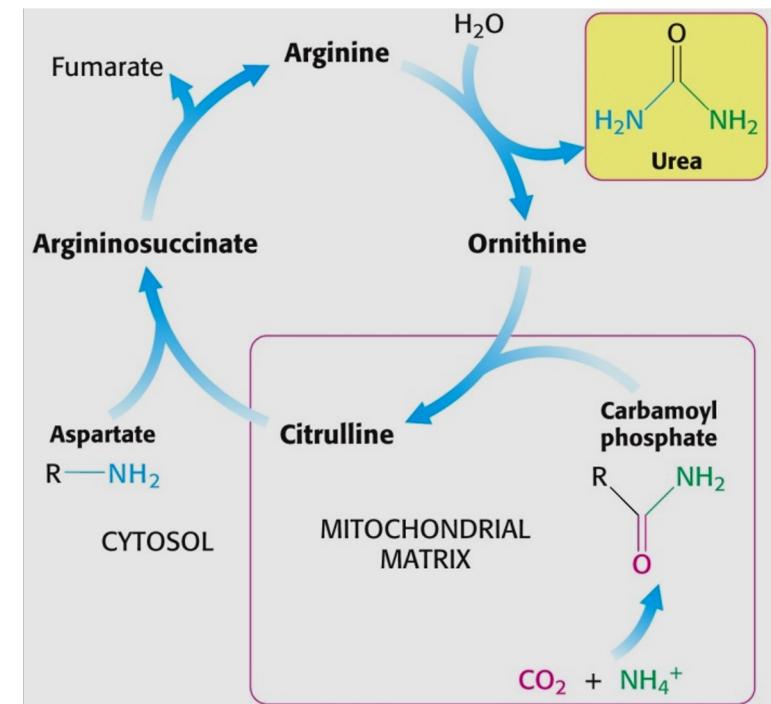


Glutamine is the main source of glutamate (neurotransmitter) and is produced by the glial cells. Ammonia inhibits the activity of glutaminase, resulting in the neurotransmitter deficiency.

Treating urea cycle disorders

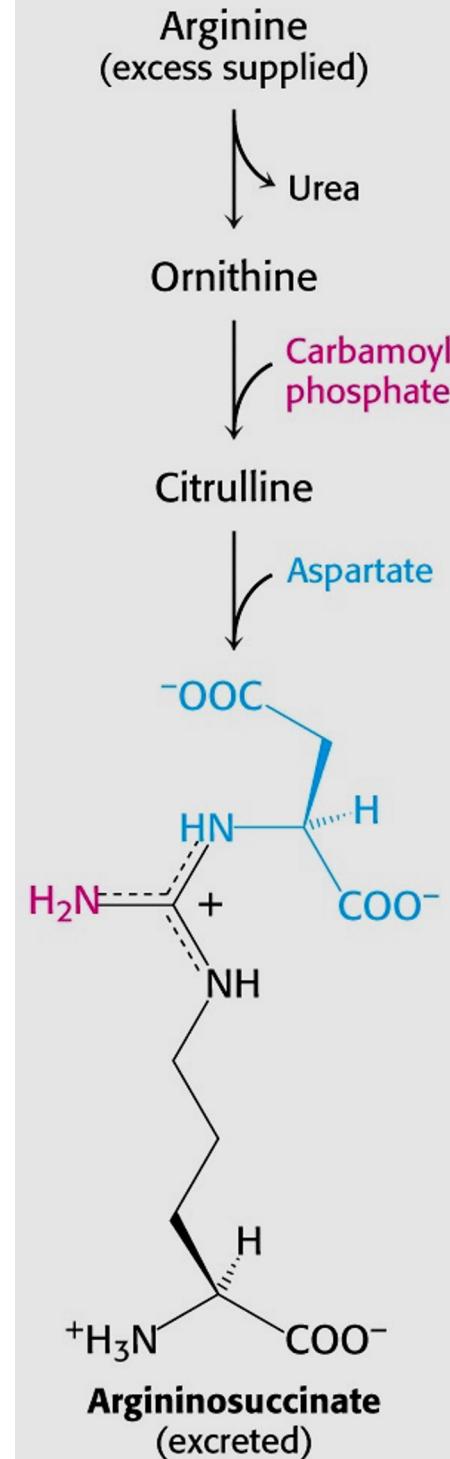
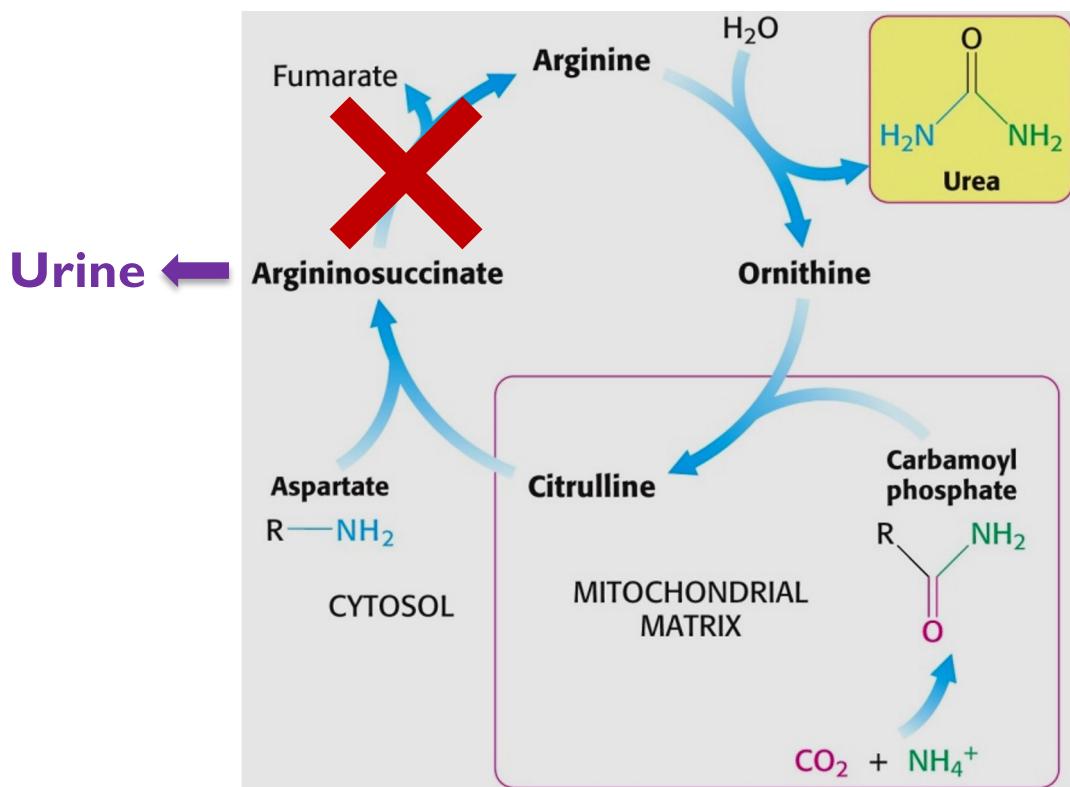
Treatments include:

- Immediate hemodialysis to remove ammonia from blood
- Low-protein diet (but you still have to provide the essential amino acids)
- Administration of substances to promote excretion of excess amino groups (alternatives to urea cycle) — in **stoichiometric amounts**



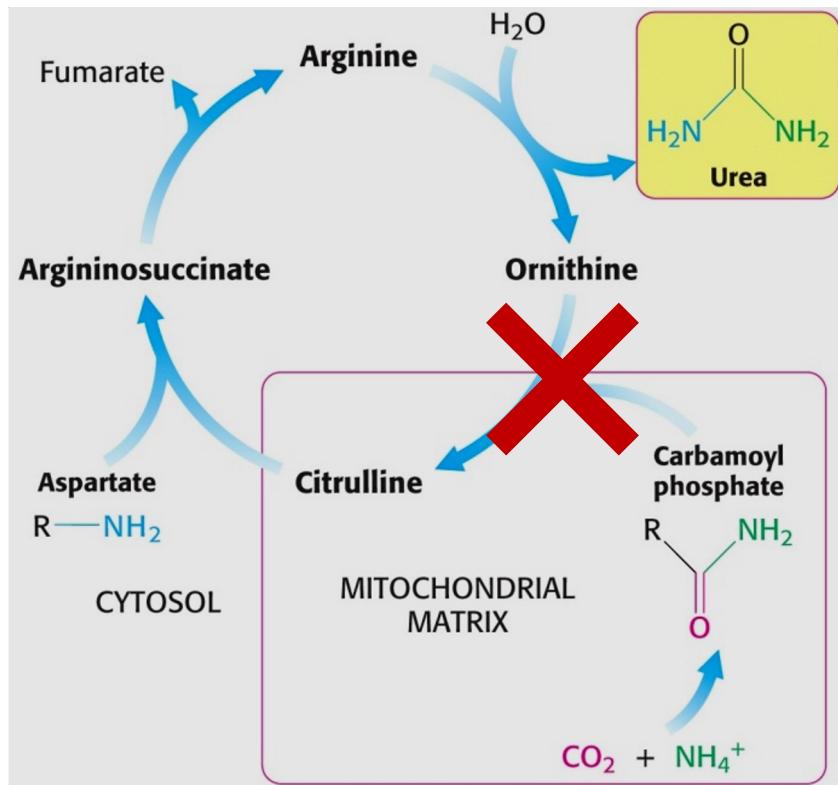
Urea cycle defects: Argininosuccinase deficiency

- Administer stoichiometric amounts of arginine
- Argininosuccinate eliminated in urine

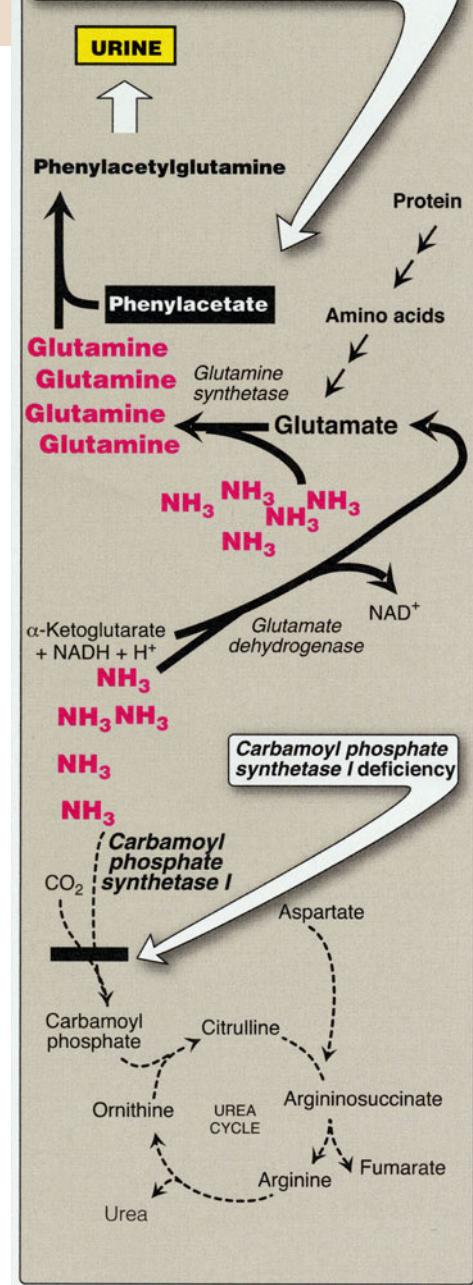


Urea cycle defects: Ornithine transcarbamoylase deficiency

- X-linked disorder
- Administer benzoate + phenylbutyrate
- Hippurate & phenylacetylglutamine eliminated in urine



Phenylbutyrate is a prodrug that is rapidly converted to phenylacetate, which combines with glutamine to form phenylacetylglutamine. The phenylacetylglutamine, containing two atoms of nitrogen, is excreted in the urine, thus assisting in clearance of nitrogenous waste.



Urea cycle, continued

- Stoichiometry:



- Energy cost: 4 high-energy bonds from ATP

- Location: cytoplasm and mitochondrial matrix of liver cells

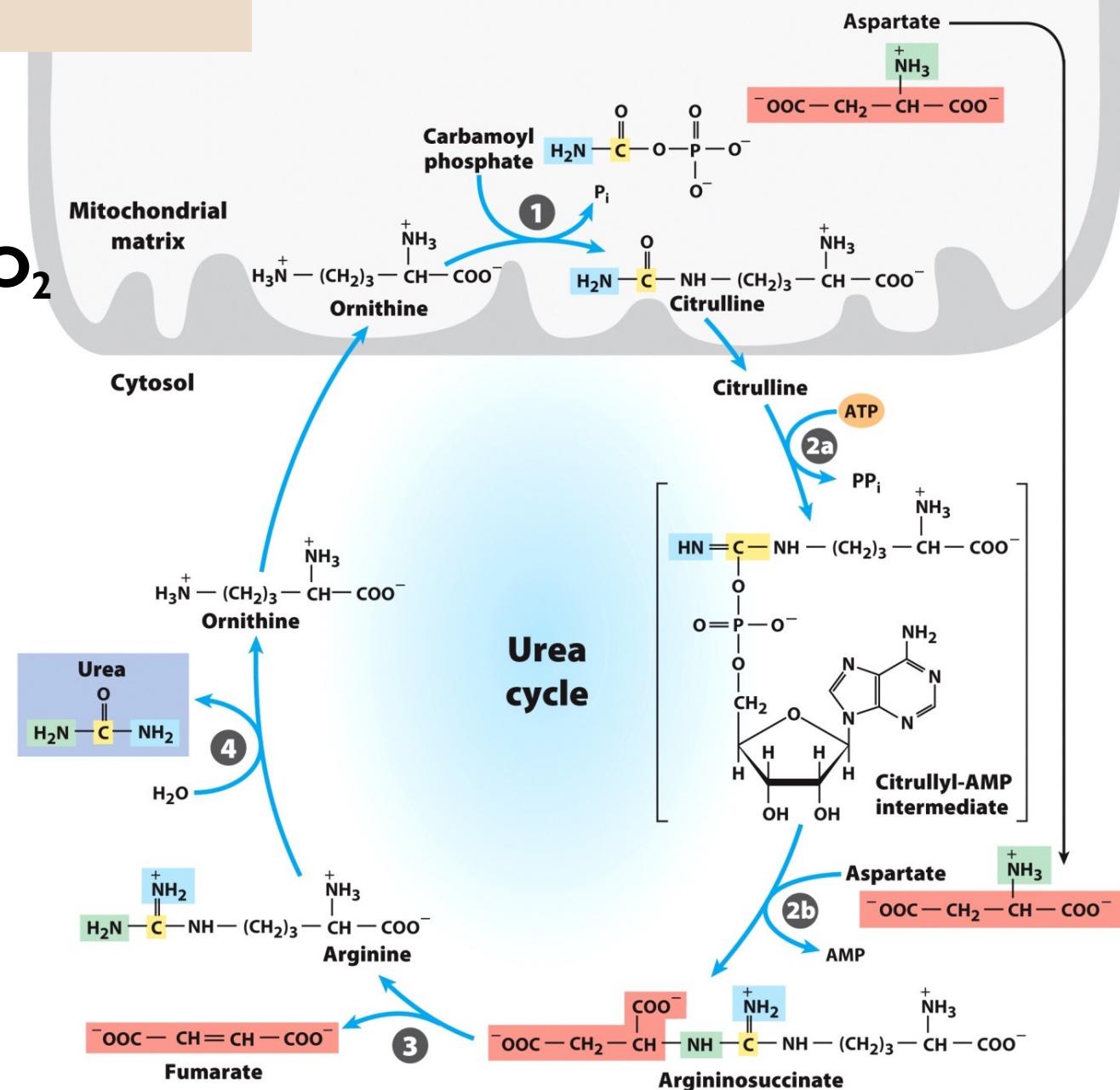


Figure 18-10 part 2
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Carbons from amino acids enter the TCA cycle

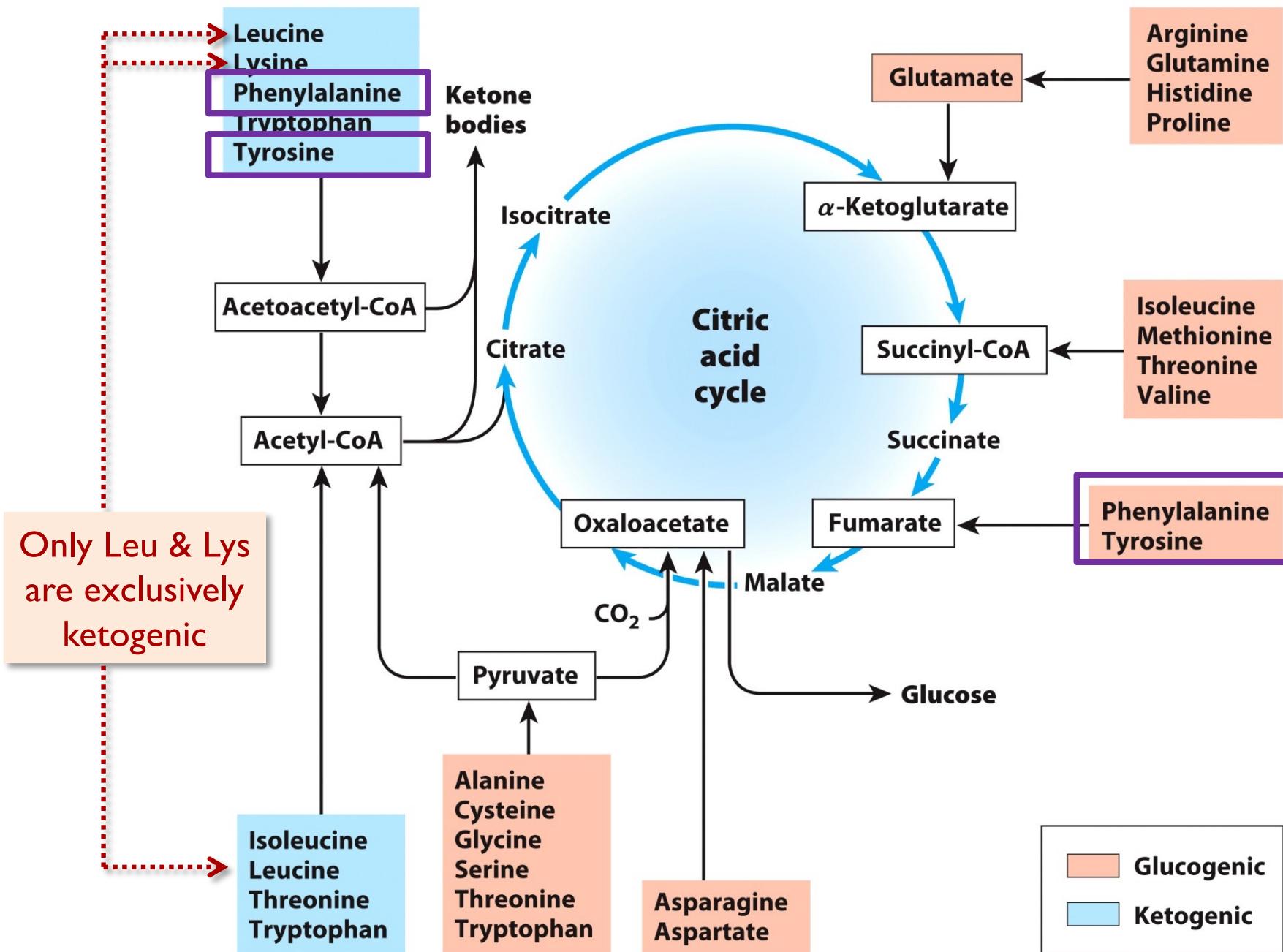
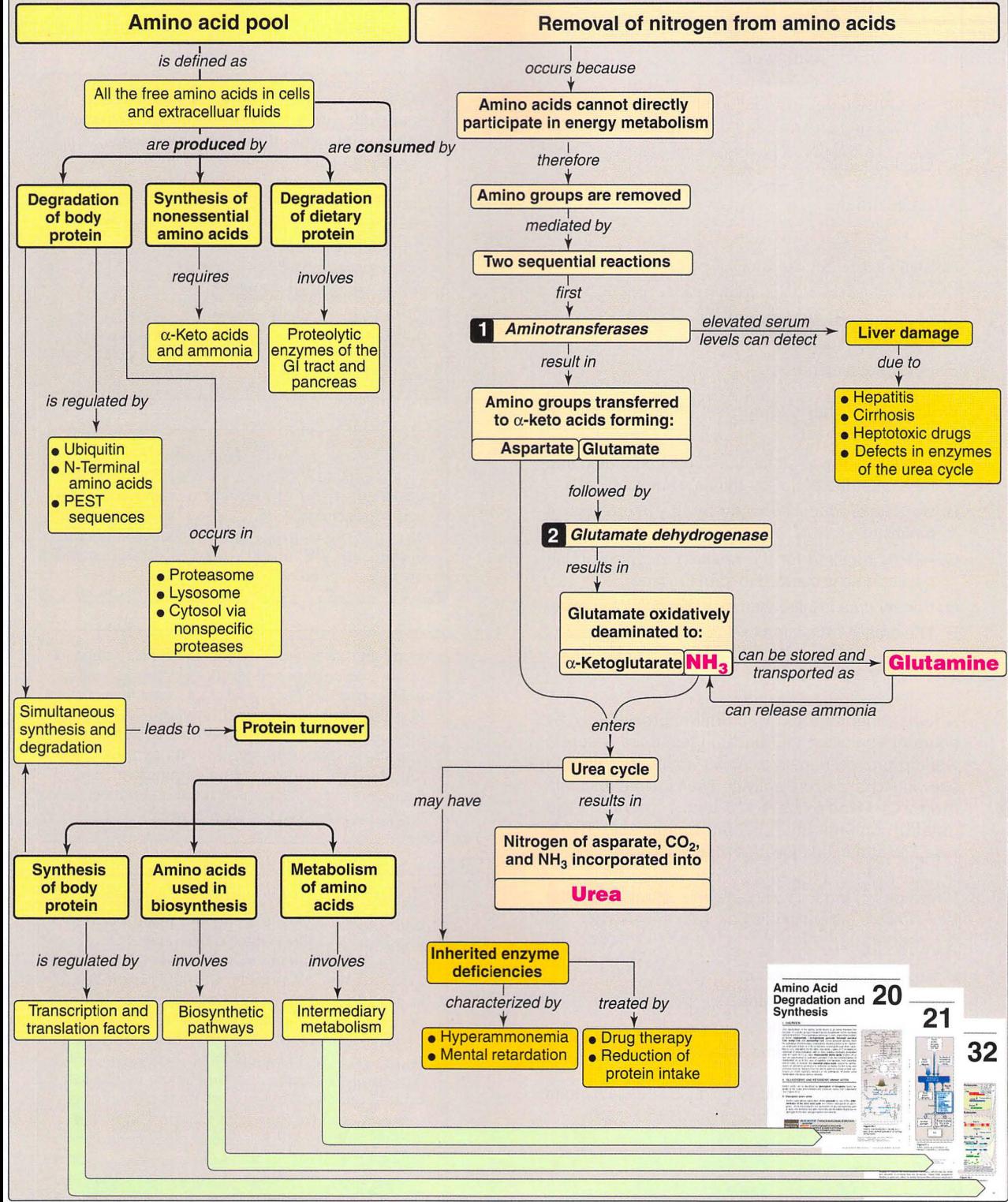


Figure 18-15

Concept map for nitrogen metabolism



Next time:

Lecture 36: Amino acid biosynthesis

Reading in Lehninger, 6th edition:

- Chapter 22