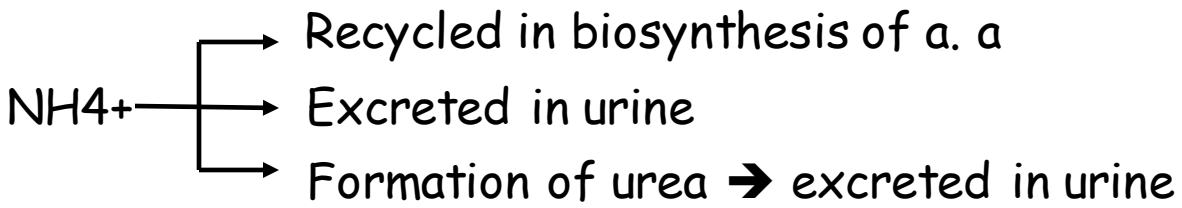
Amino Acids Metabolism: Disposal of Nitrogen.

- a.a **can't be stored**, excess a.a (more than the needs of cells in the protein synthesis or other compound) are catabolised and degraded immediately.

Catabolism of a.a has two phases:

Phase I: removal of a-amino group and forming NH4+ and a-keto acid.

By process called transamination and oxidative deamination.



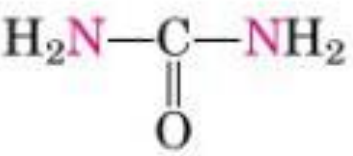
-The most important rout for disposal of N **is urea**.

phase II: is carbon skeleton of the a-ketoacids are converted into common intermediates of energy producing metabolic pathway. So can be metabolized into CO2, H2O, glucose, fatty acids, …

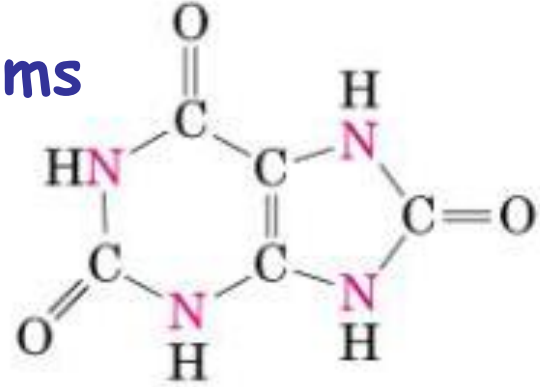
The fate of N_2 in different organisms

NH_4^+

Ammonia (as ammonium ion)



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

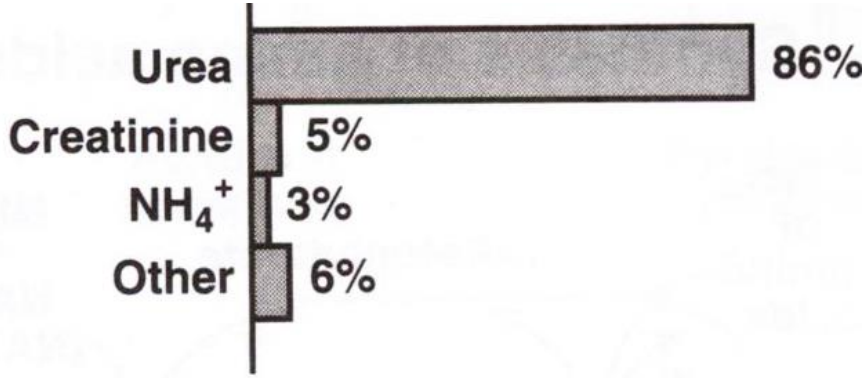


Figure 21.10
Distribution of nitrogen-containing compounds in urine.

Digestion of dietary proteins

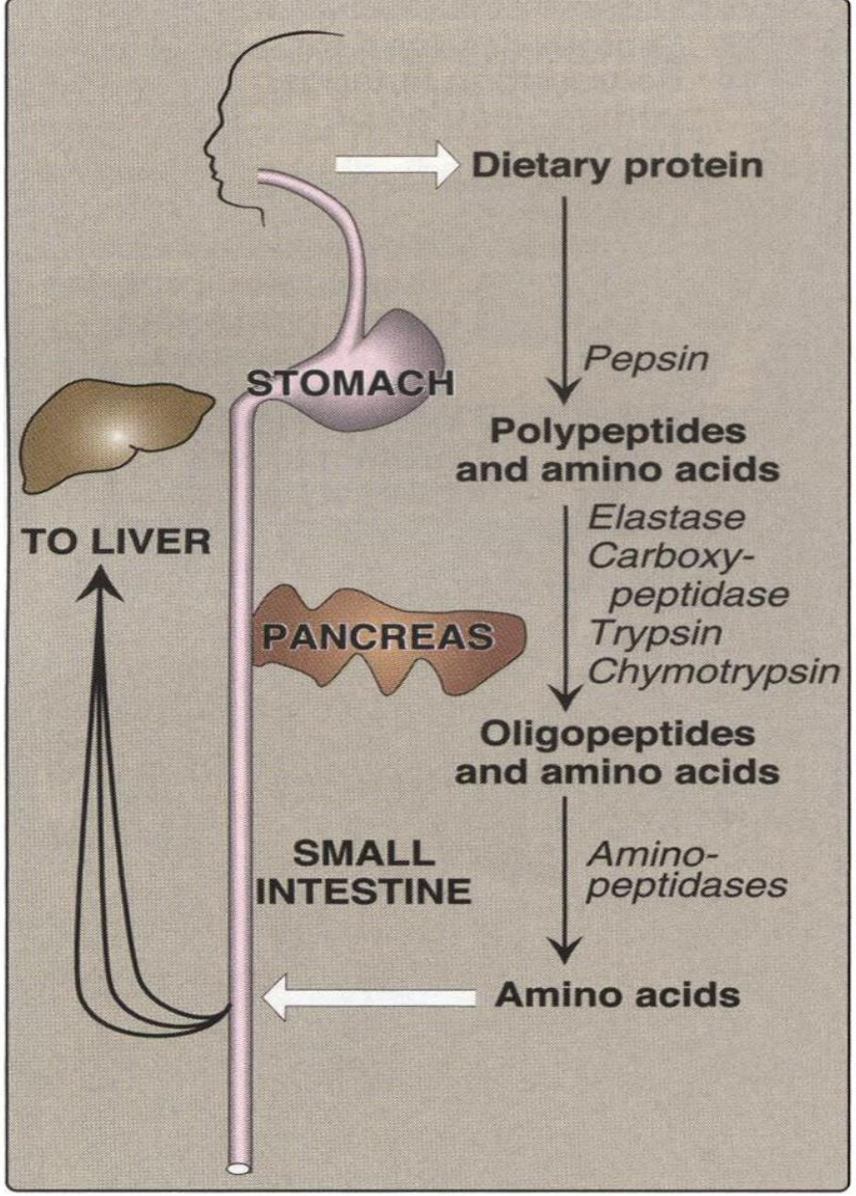


Figure 19.4
Digestion of dietary proteins by the proteolytic enzymes of the gastro-intestinal tract.

After the complete digestion, free a.a and dipeptides are absorbed by the epithelial cells in which dipeptides are hydrolyzed to a.a in the cytosol before entering the portal circulation.

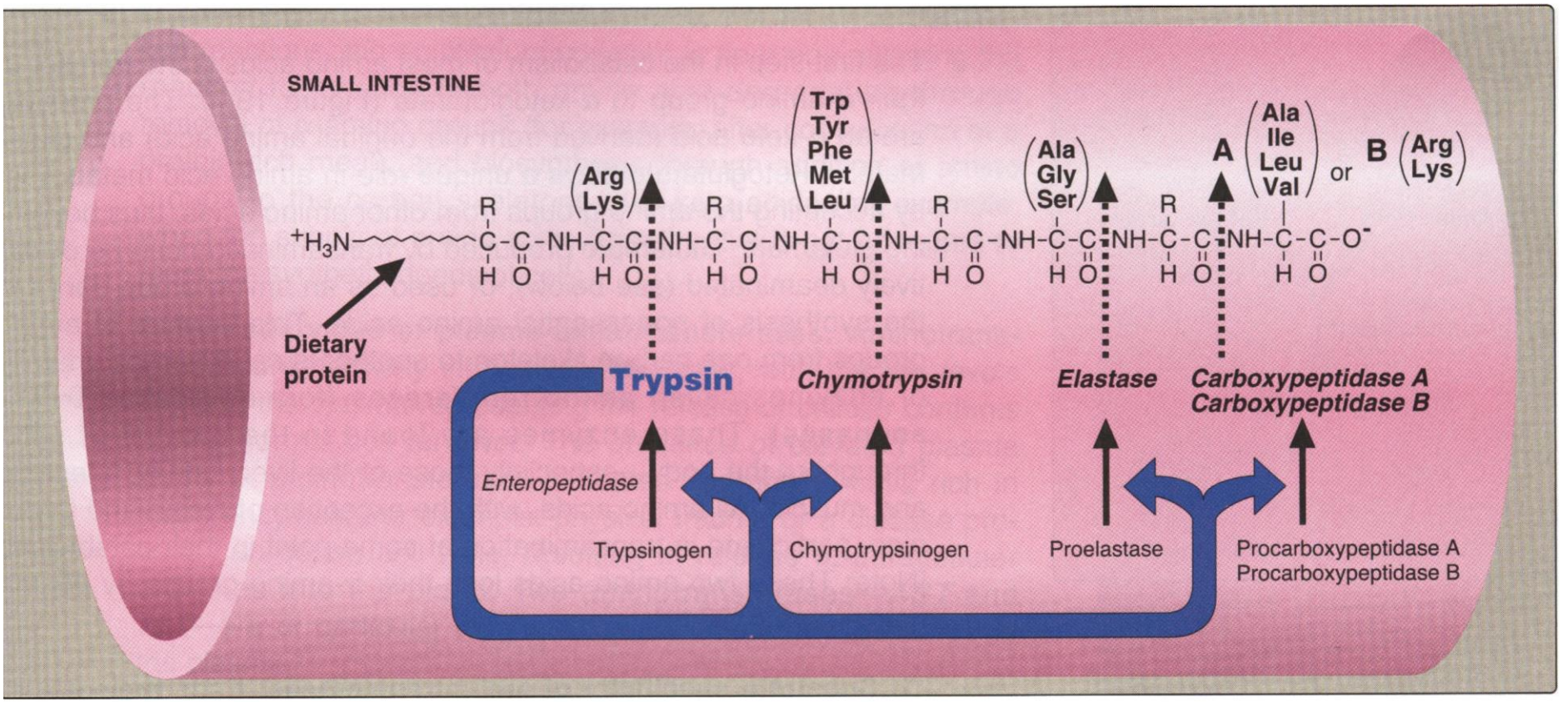


Figure 19.5
Cleavage of dietary protein by proteases from the pancreas. The peptide bonds susceptible to hydrolysis are shown for each of the five major pancreatic proteases. [Note: *Enteropeptidase* is synthesized in the intestine.]

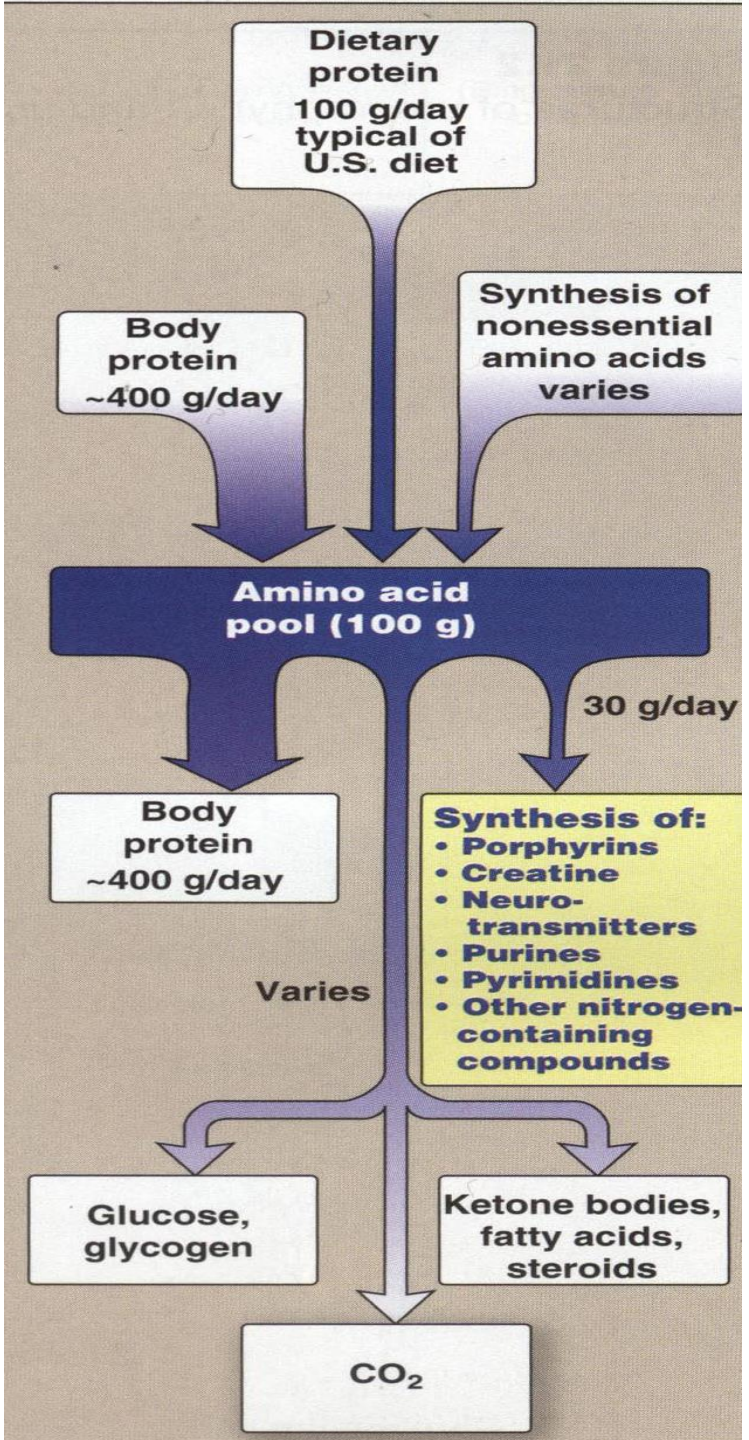
- **Transport of a.a into cells by active transporters**

Over all nitrogen metabolism

Amino acids are precursors of nitrogen-containing compounds

a.a catabolism is part of nitrogen metabolism in the body.

N2 enter to body (by food) in different forms ➔ converted to a. a and then exist from the body in form of urea and little amounts of NH4+.

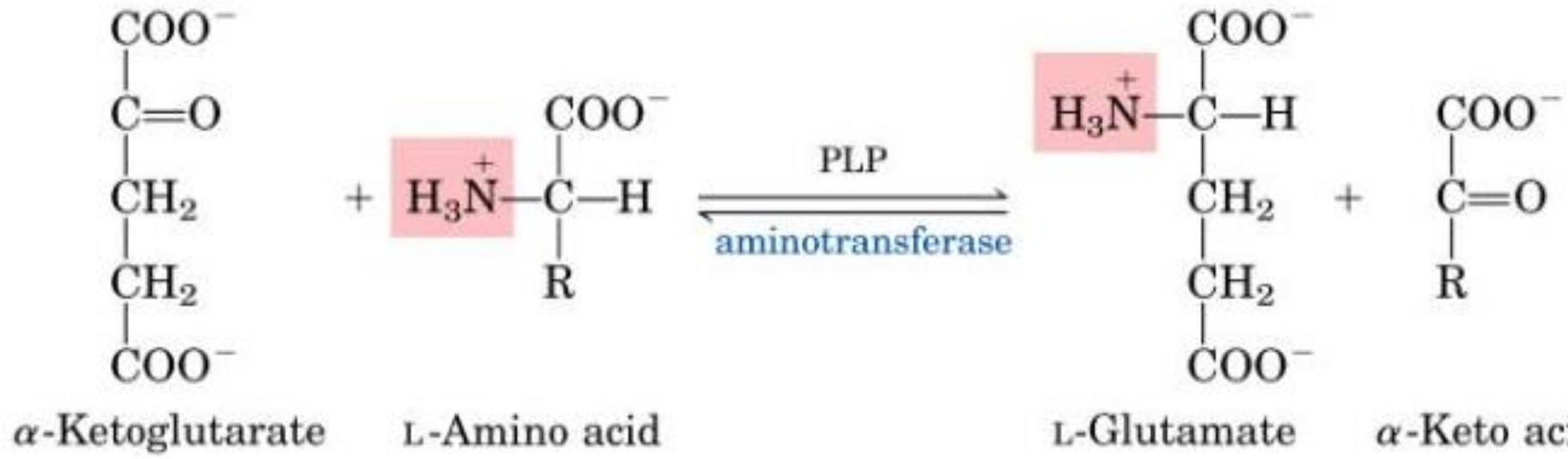


Metabolic fates of amino groups: Transamination and Oxidative deamination

- The first step of a.a catabolism is the transfer of α-amino gp to α-ketoglutarate. The product is **α-keto acid and glutamate**.
- This process is called Transamination and mediated by aminotransferase -
- **Transamination: the funneling of amino groups to gltamate**
- **Occurs in the cytosol of the hepatocytes**

The product Glutamate

- Donor of NH_2 gp in the biosynthesis of non-essential a.a
- Oxidative deamination ➔ release of NH_4^+



***Substrate specificity of aminotransferase:**

Each aminotransferase is specific for one or few a.a , they can be named by a.a donor. Because almost the acceptor is a-ketoglutarate.

***Equilibrium of transamination reactions**

- Most of transamination reactions have equilibrium constant near to 1, allowing the reaction to proceed in both a.a degradation and biosynthesis depending on the relative concentrations.

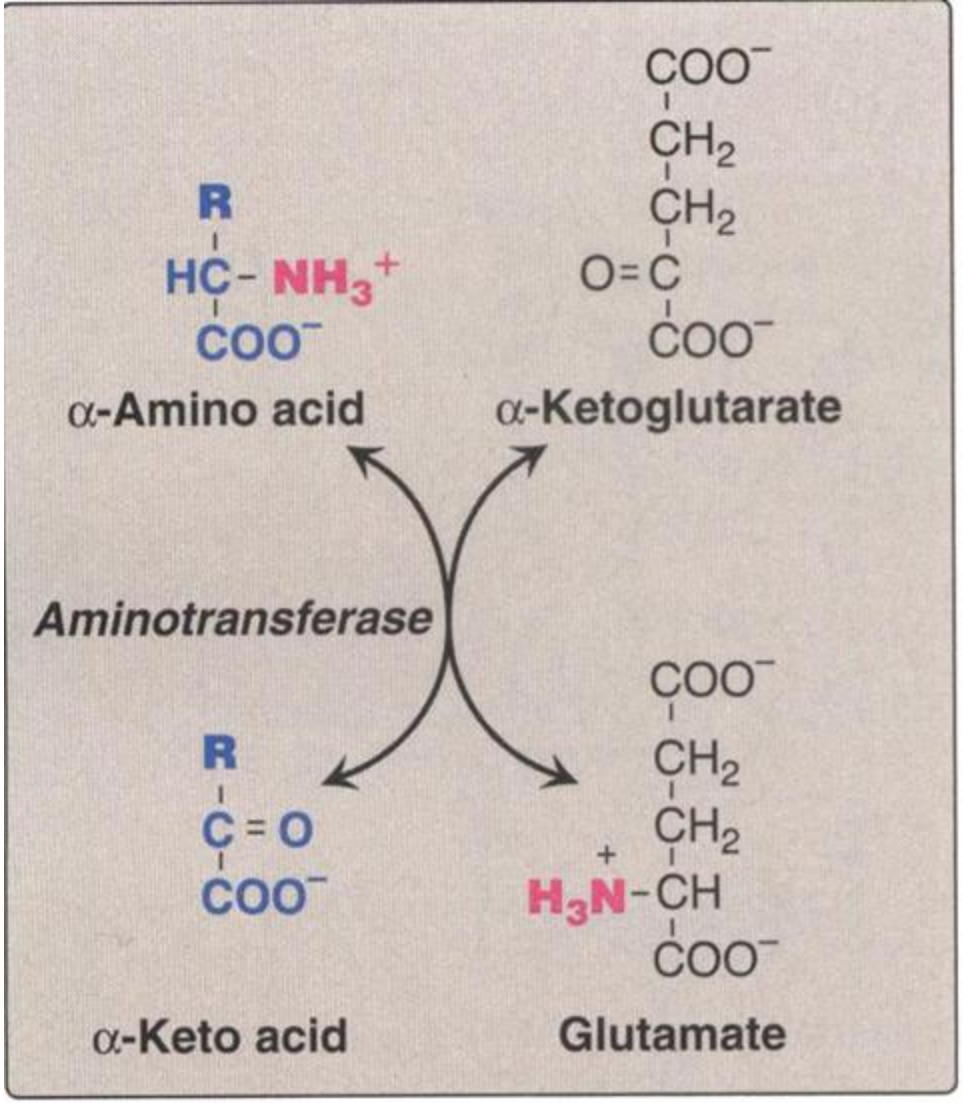


Figure 19.7
Aminotransferase reaction using α-ketoglutarate as the amino-group acceptor.

*Mechanism of action of aminotransferase

- All aminotransferases need **pyridoxal phosphate derivatives of vit B6**

- All a.a except threonine, lysine participate in transamination

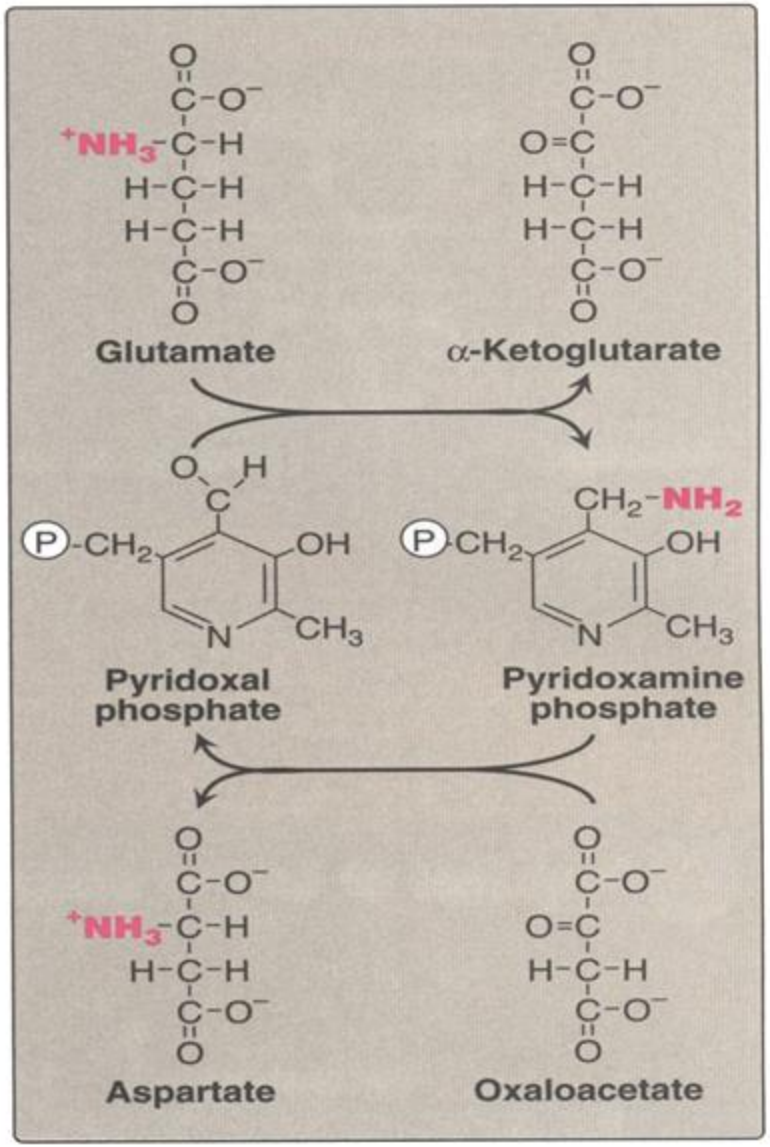


Figure 19.9
Cyclic interconversion of pyridoxal phosphate and pyridoxamine phosphate during the *aspartate aminotransferase* reaction. [Note: Ⓟ = phosphate group.]

Two important transferases:

Alanine aminotransferas (ALT) called also Glutamate – Pyruvate transferase (GPT), found in many tissues catalyzes the transfer of amino gp of **alanine** to produce **pyruvate and glutamate**.

- **Aspartate aminotransferase (AST)** called also Glutamate – Oxaloacetate transferase (GOT),

- During the catabolism of a. a AST takes amino group from glutamate to oxaloacetate forming aspartate. Which used as source of NH4 gp in Urea synthesis

Aspartate ➔ source of NH4+ on the urea cycle.

- Diagnostic value of plasma aminotransferases

Plasma AST = SGOT

Plasma ALT = SGPT

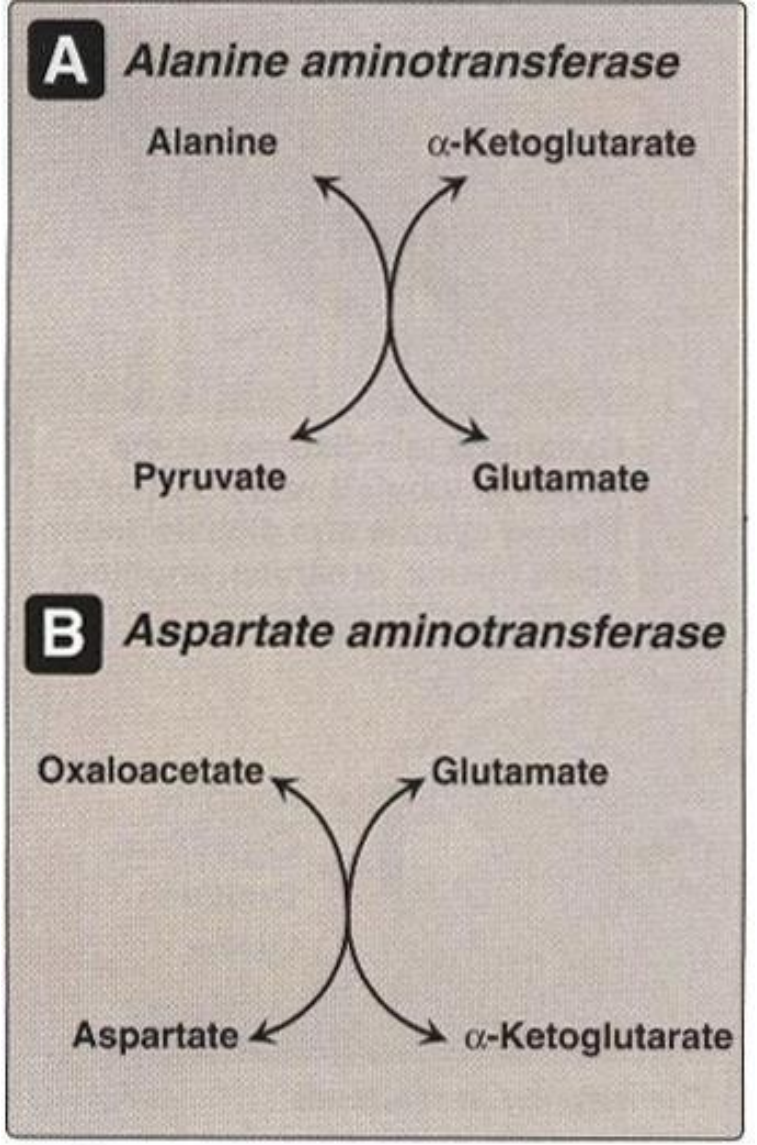
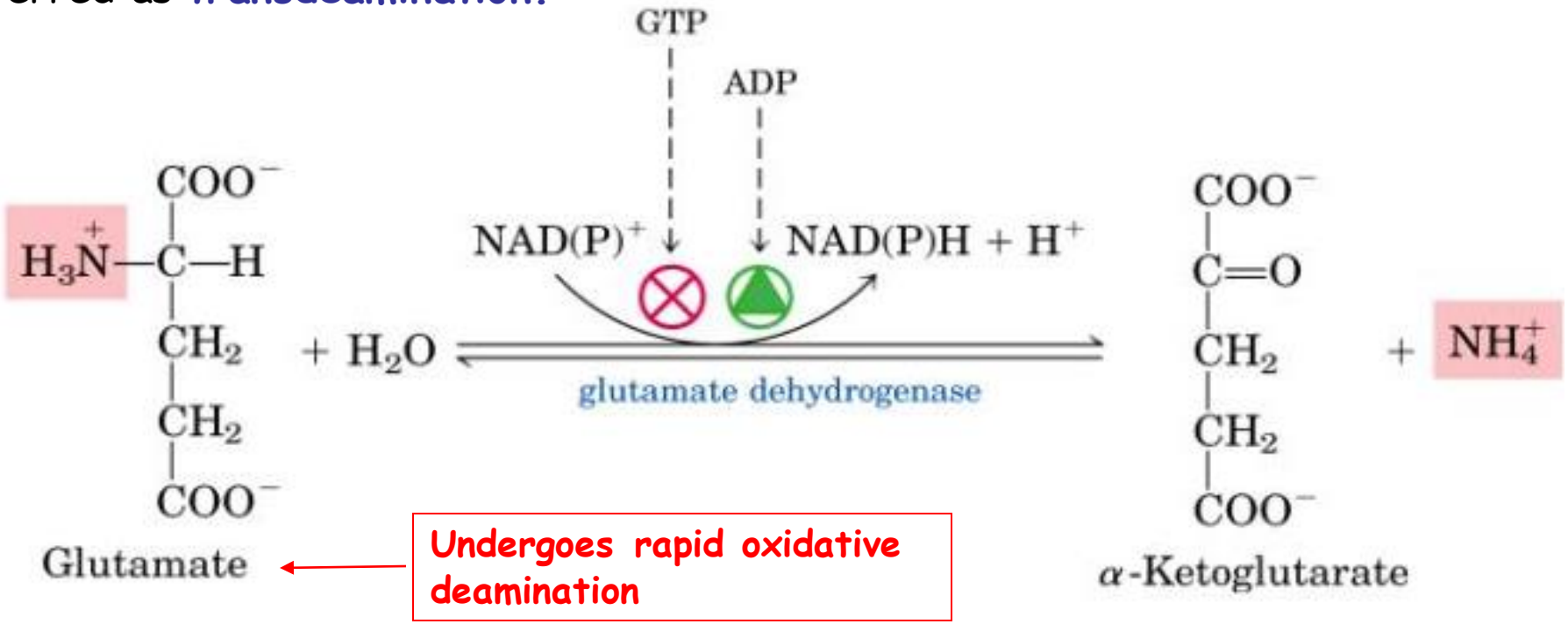


Figure 19.8
Reactions catalyzed during amino acid catabolism.
A. *Alanine aminotransferase.*
B. *Aspartate aminotransferase.*

Oxidative deamination

- Amino groups of many a.a are collected in the liver in the form of the amino group of L-glutamate. Glutamate can be used as a donor of amino group in the biosynthesis of non-essential a.a
- In hepatocytes, glutamate is transported from the cytosol into **mitochondria**, where it undergoes Oxidative deamination catalyzed by **L-glutamate dehydrogenase**.
- The combined action of aminotransferase and glutamate dehydrogenase is referred as **transdeamination**.

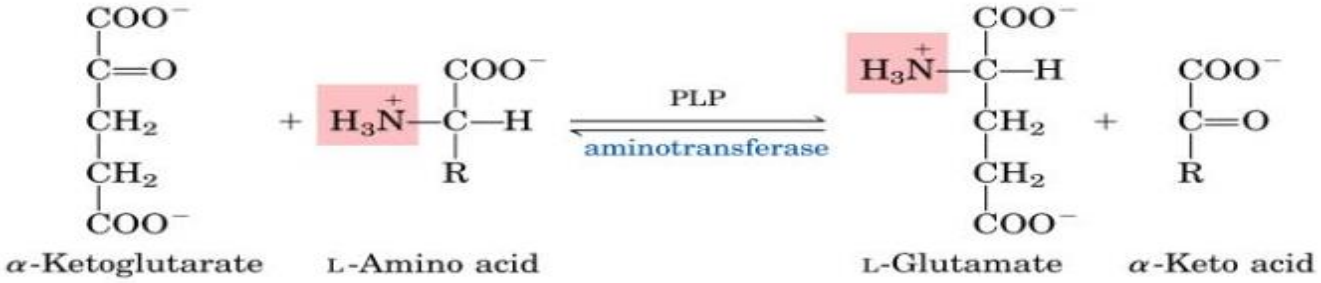


Transdeamination

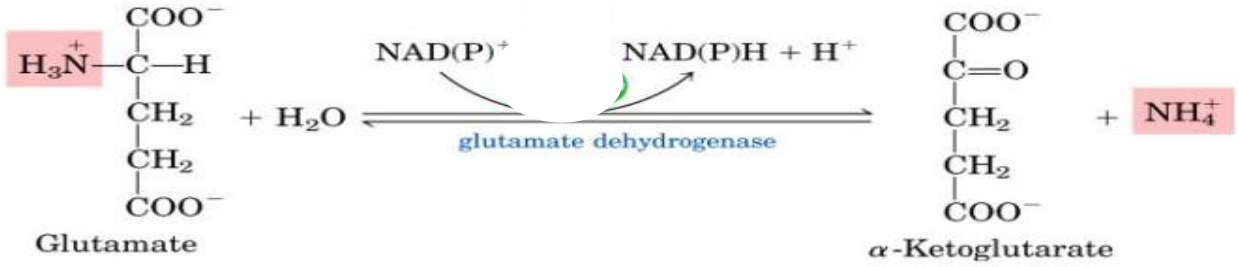
-The combined action of aminotransferase and glutamate dehydrogenase is referred as **transdeamination.**

- The over all reaction of a.a catabolism

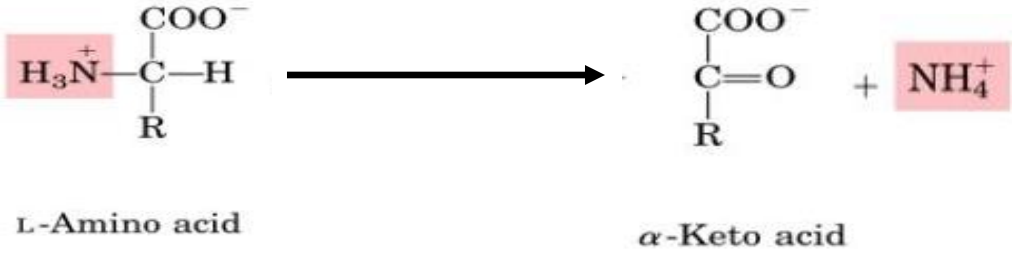
Transamination Reaction



Oxidative Deamination



Transdeamination



Oxidative deamination

***Regulation of oxidative deamination**

Direction of reaction:

1) Depends on the relative concentration of glutamate, α-ketoglutarate and ammonia, the ratio of oxidized to reduced coenzymes.

After high protein meal ➔ increase glutamate ➔ increase ammonia production

2) Allosteric regulation of Glutamate-dehydrogenase

ATP, GTP = inhibitors

ADP, GDP = activators

-Low level of energy (decrease ATP) ➔ increase catabolism of a.a ➔α-ketoglutarate as substrate for TCA cycle.

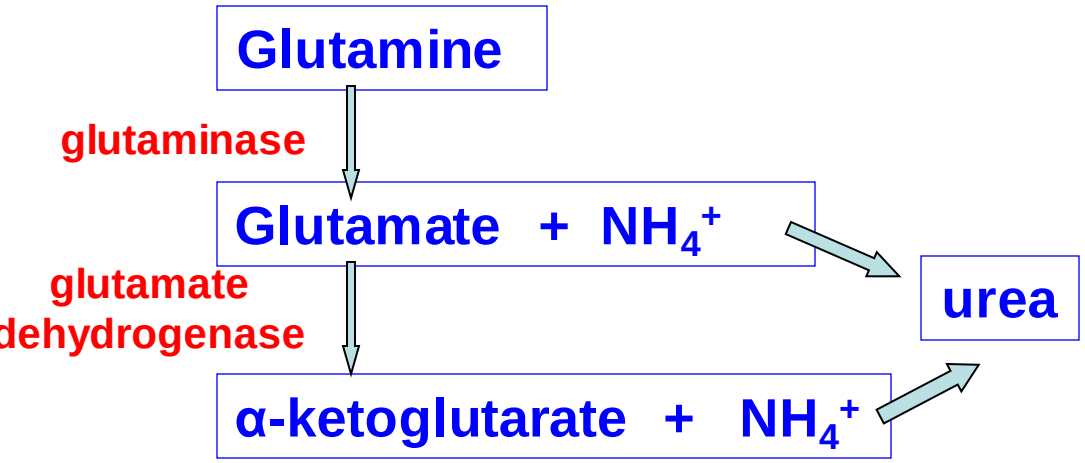
- The enzyme glutamate dehydrogenase presents in mitochondrial matrix and can use either NAD+ or NADP+ as oxidants.

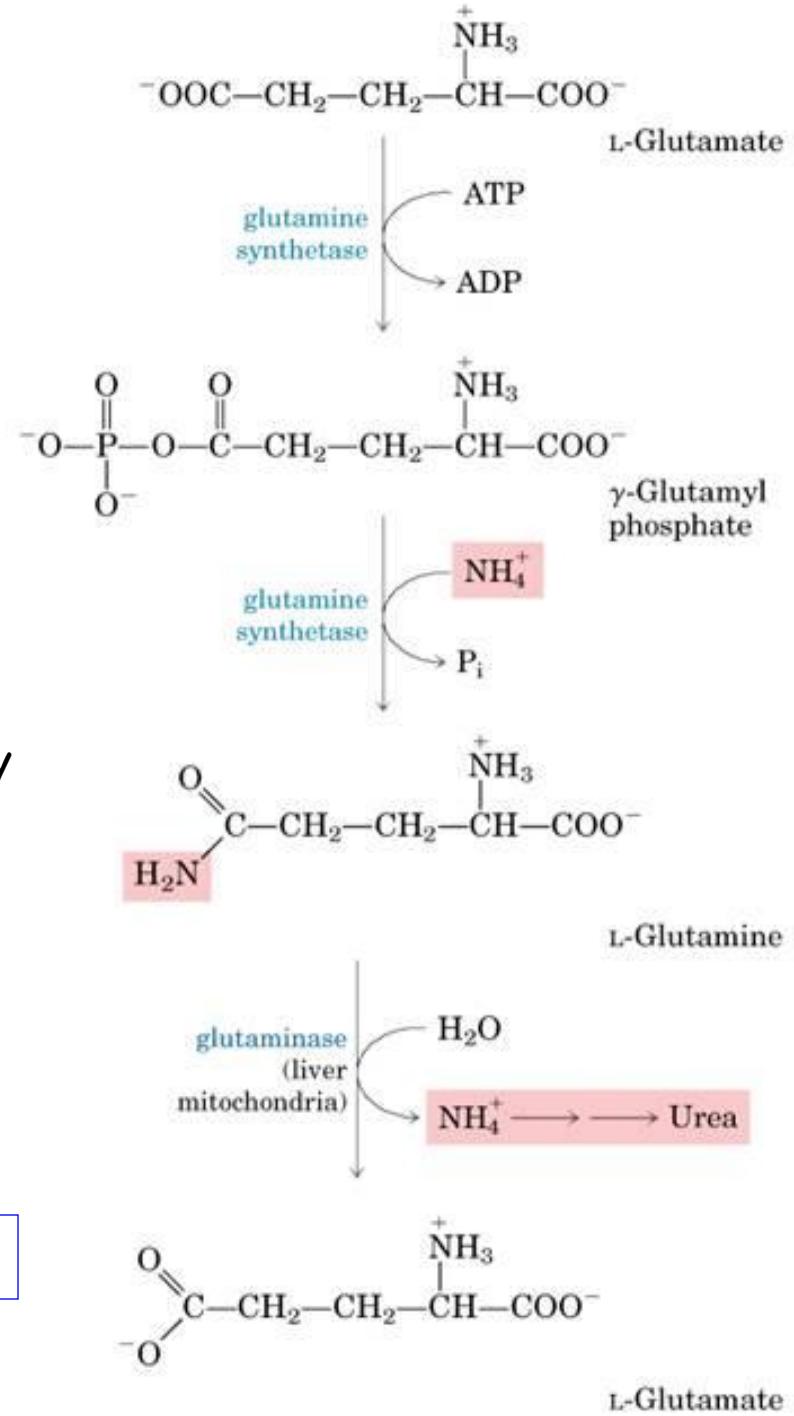
***The oxidative deamination results in:**

- Liberation of the amino group as free ammonia.
- Occur primarily in the **mitochondria of liver and kidney** and provide α-ketoacid

Glutamine transports ammonia

- Many extrahepatic tissues (brain) produce NH4+ from metabolic processes as nucleotide degradation.
-This toxic ammonia is converted into amino group of glutamine that transported to liver or kidneys.
- **Glutamine**: non-toxic transport form of NH4+ and also source of amino group in many biosynthesis reactions.
- The amide nitrogen of glutamine is released as ammonia only in liver and kidney's mitochondria by the enzyme "Glutaminase" which convert glutamine into glutamate + NH4+

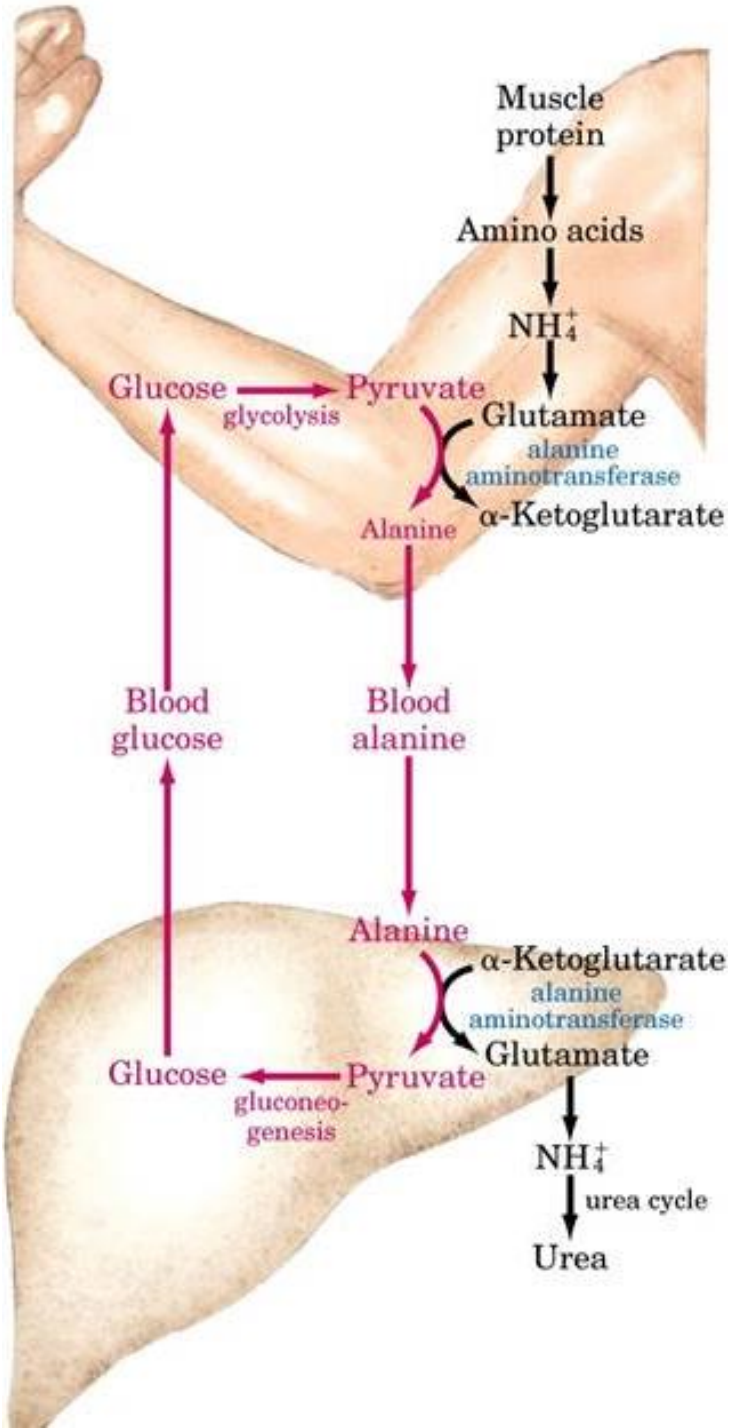


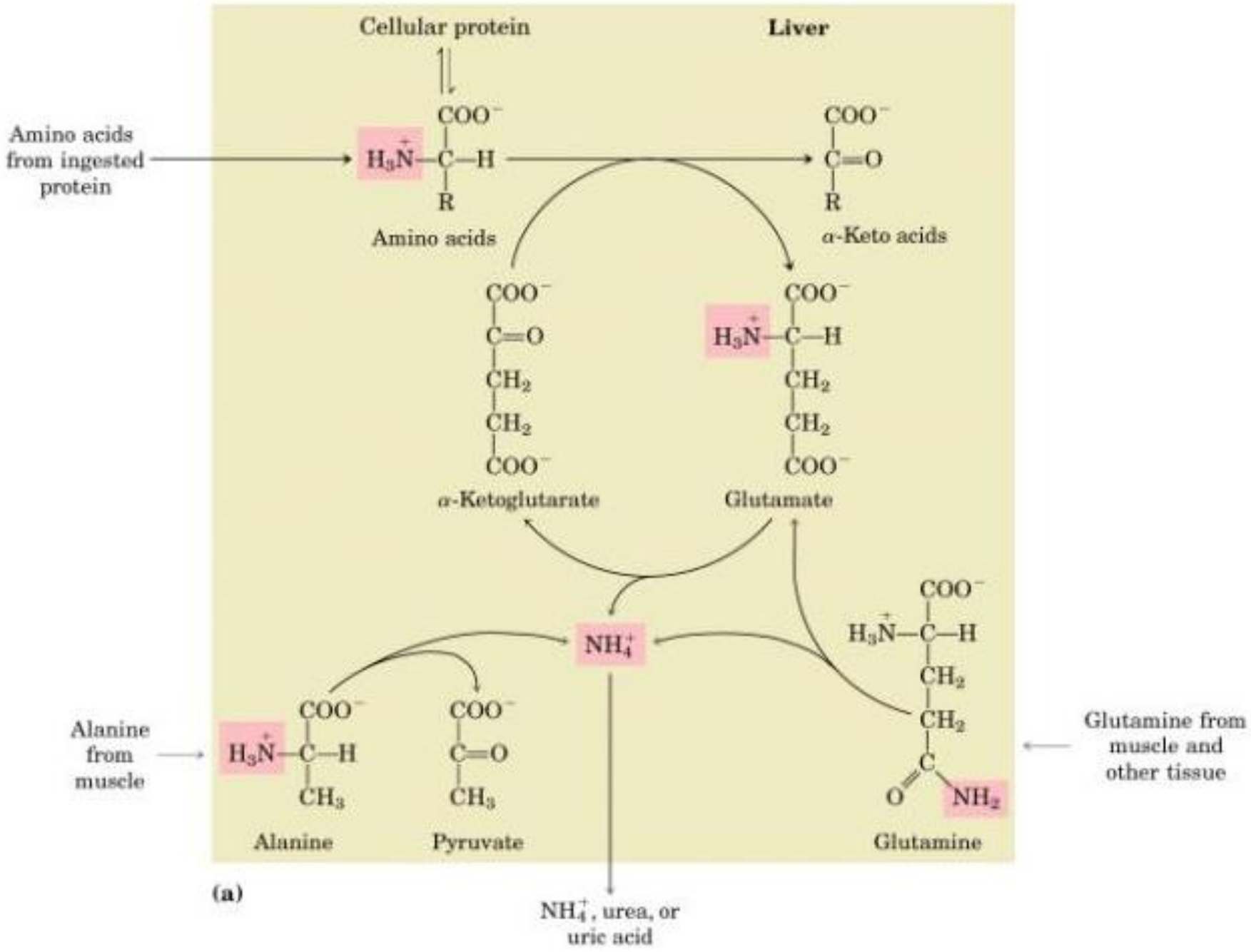


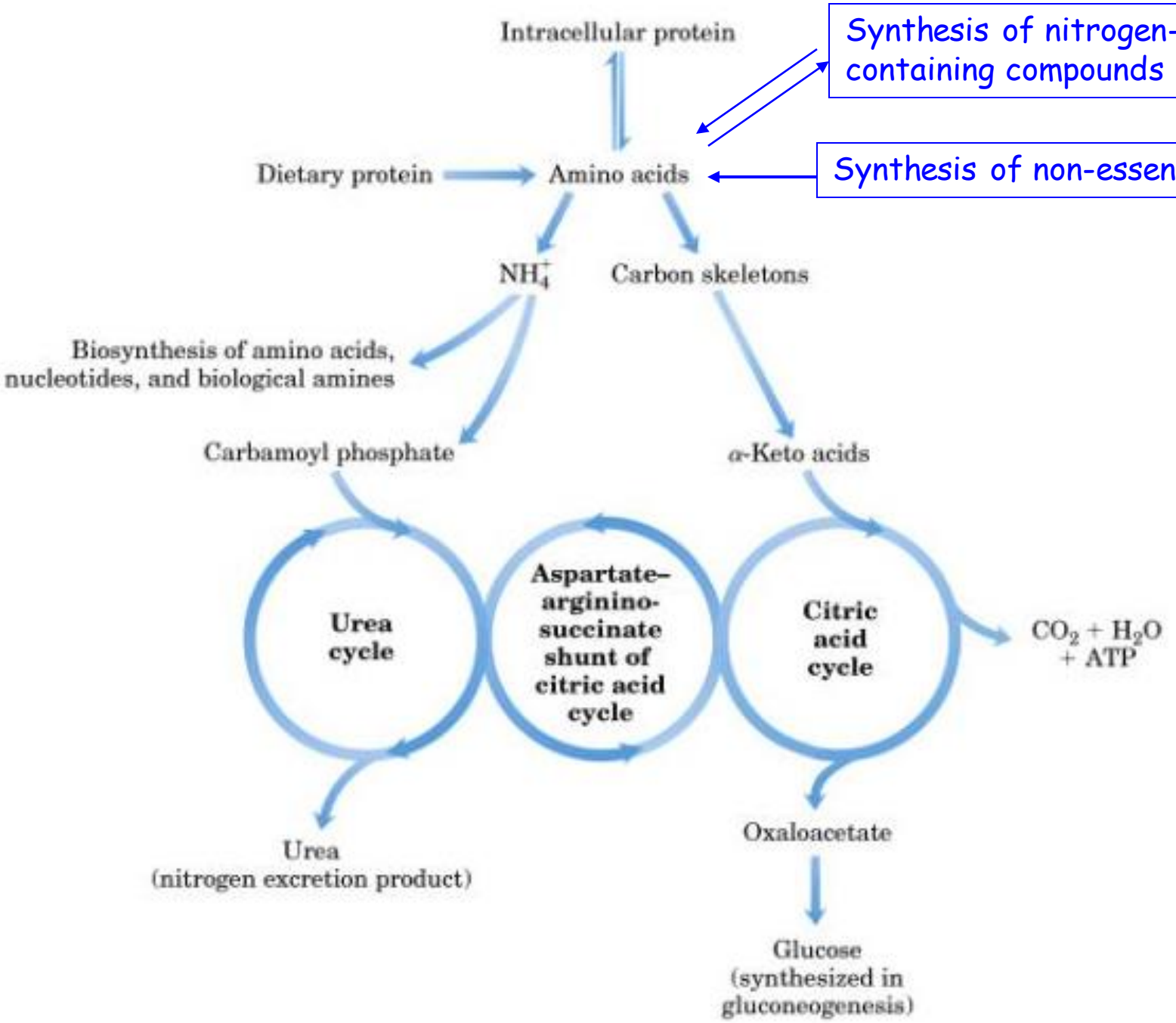
"Glucose-alanine cycle"

Alanine transports ammonia from muscles to liver.

- In muscle, a.a are degraded, the amino groups are collected in form of glutamate by transamination.
- α-amino group can transferred to pyruvate (resulted from glycolysis) by enzyme Alanine Amino Transferase (ALT)
- Alanine is reconverted into pyruvate in the cytosol of hepatocytes and enters the gluconeogenic pathaway to produce glucose
- In the liver the formed glutamate enters the mitochondria where glutamate dehydrogenase releases NH4+




Cellular protein
Liver
Amino acids from ingested protein
Amino acids
α-Keto acids
α-Ketoglutarate
Glutamate
NH4+
Alanine from muscle
Alanine
Pyruvate
Glutamine from muscle and other tissue
Glutamine
(a)
NH4+, urea, or uric acid


Intracellular protein
Synthesis of nitrogen-containing compounds
Dietary protein
Amino acids
Synthesis of non-essential a.a
NH_4^+
Carbon skeletons
Biosynthesis of amino acids, nucleotides, and biological amines
Carbamoyl phosphate
α-Keto acids
Urea cycle
Aspartate–arginino-succinate shunt of citric acid cycle
Citric acid cycle
$CO_2 + H_2O$ + ATP
Urea (nitrogen excretion product)
Oxaloacetate
Glucose (synthesized in gluconeogenesis)

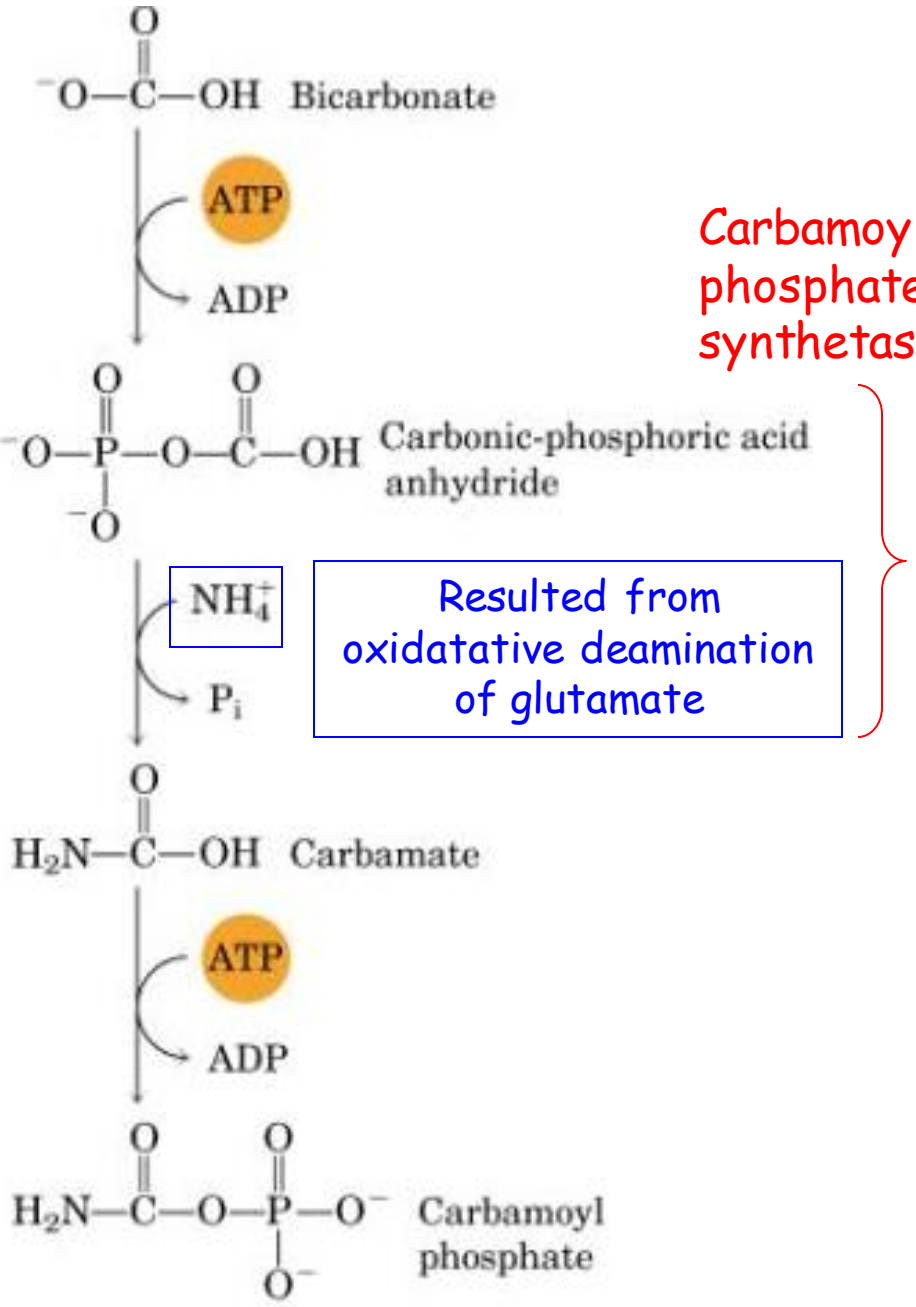
Urea Cycle

- Urea is the major disposal form of amino group derived from a.a
- One nitrogen is supplied by **free NH4+** and the other from **Aspartate.**
- **Glutamate is the immediate precursor** of both ammonia through oxidative deamination and by aspartate aminotransferase
- Carbon and Oxygen are derived from **CO2**
-Urea is produced in the **liver** then transported in the blood to the **kidneys** for excretion in the urine.
-The first two reactions lead to the synthesis of urea occur in mitochondria where the remaining cycle enzymes are located in cytosol.

*Formation of carbamoyl phosphate

The enzyme has an absolute requirement for N-acetylglutamate which act as an allosteric activator.

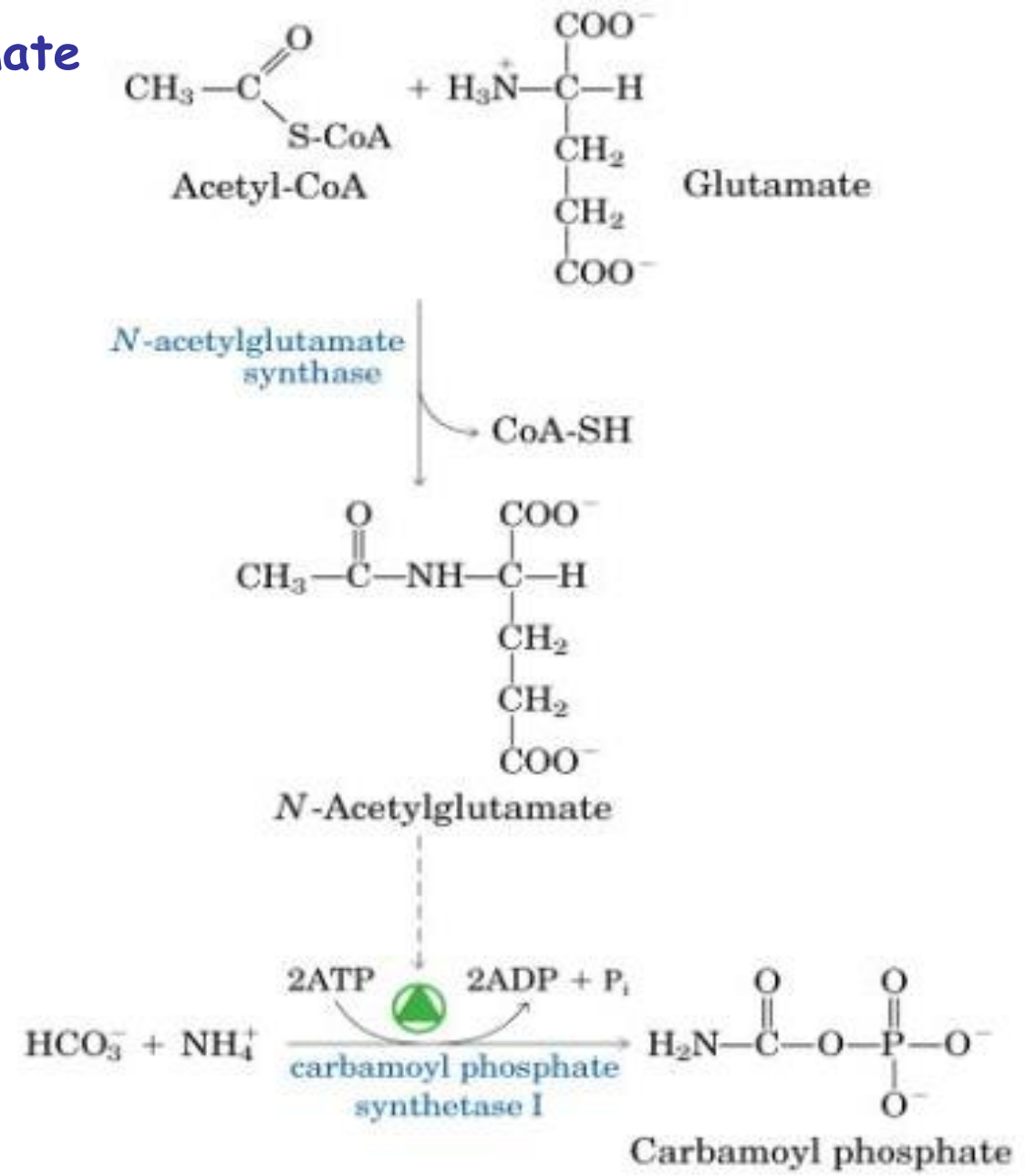
***Carbamoyl phosphate synthetase II**, participates in biosynthesis of pyrimidines, does not require N-acetylglutamate and located in the cytosol.



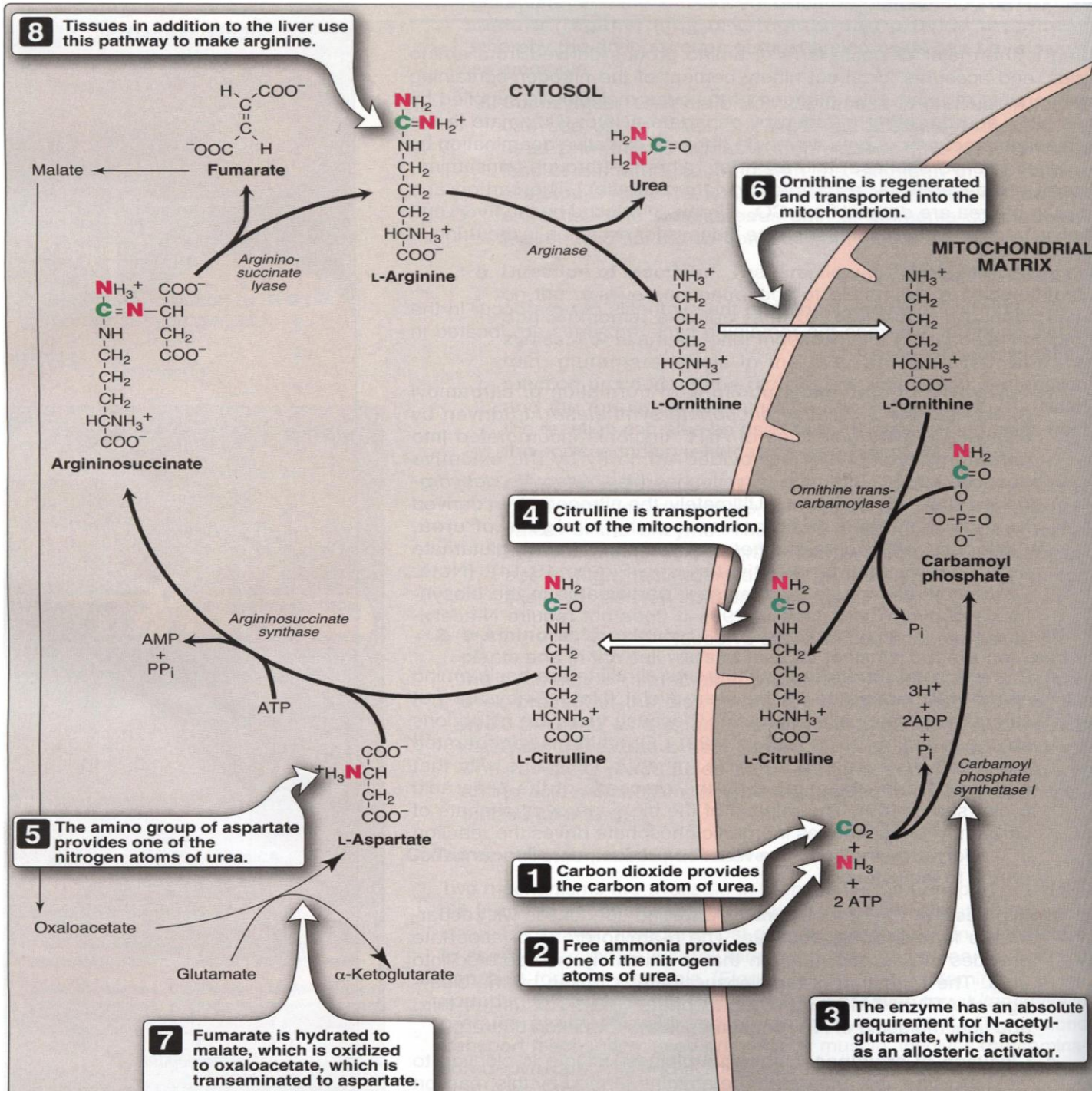
Formation of N-acetylglutamate

N-acetylglutamate is essential activator of carbamoyl phosphate synthetase I,which catalyzes **the rate limiting step in urea cycle.**

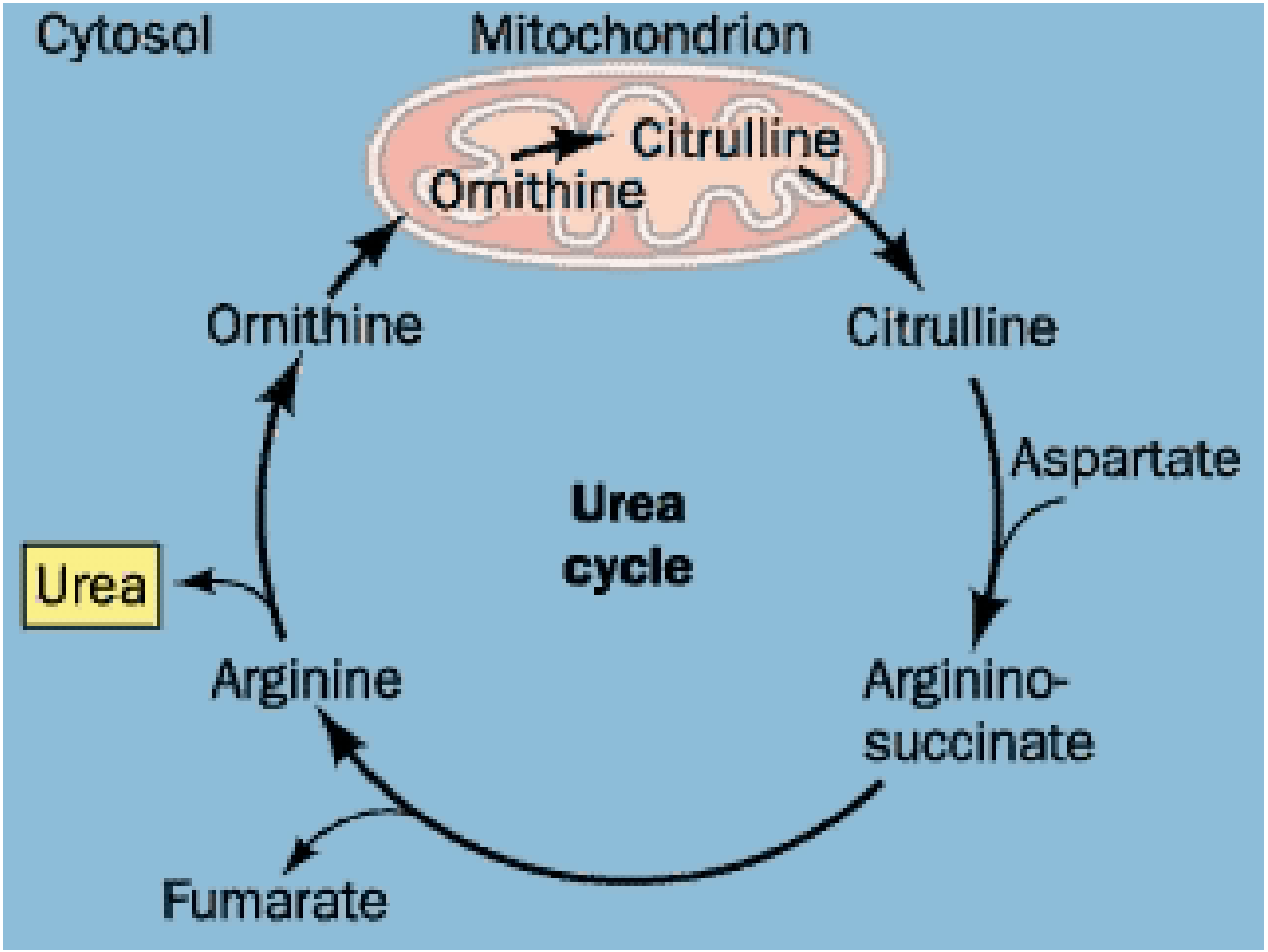
The intrahepatic concentration of N-acetylglutamate increases after ingestion of a protein-rich meal ➔ increase of urea synthesis.



Urea Cycle


8 Tissues in addition to the liver use this pathway to make arginine.
CYTOSOL
MITOCHONDRIAL MATRIX
Malate
Fumarate
L-Arginine
Urea
Arginino-succinate lyase
Arginase
6 Ornithine is regenerated and transported into the mitochondrion.
L-Ornithine
L-Ornithine
Argininosuccinate
Ornithine trans-carbamoylase
Carbamoyl phosphate
4 Citrulline is transported out of the mitochondrion.
Argininosuccinate synthase
AMP + PPi
ATP
Pi
L-Citrulline
L-Citrulline
3H+ + 2ADP + Pi
Carbamoyl phosphate synthetase I
5 The amino group of aspartate provides one of the nitrogen atoms of urea.
L-Aspartate
CO2 + NH3 + 2 ATP
1 Carbon dioxide provides the carbon atom of urea.
Oxaloacetate
2 Free ammonia provides one of the nitrogen atoms of urea.
Glutamate
α-Ketoglutarate
3 The enzyme has an absolute requirement for N-acetyl-glutamate, which acts as an allosteric activator.
7 Fumarate is hydrated to malate, which is oxidized to oxaloacetate, which is transaminated to aspartate.

Urea Cycle


Cytosol
Mitochondrion
Citrulline
Ornithine
Ornithine
Citrulline
Aspartate
Urea cycle
Urea
Arginine
Arginino-
succinate
Fumarate

Urea Cycle

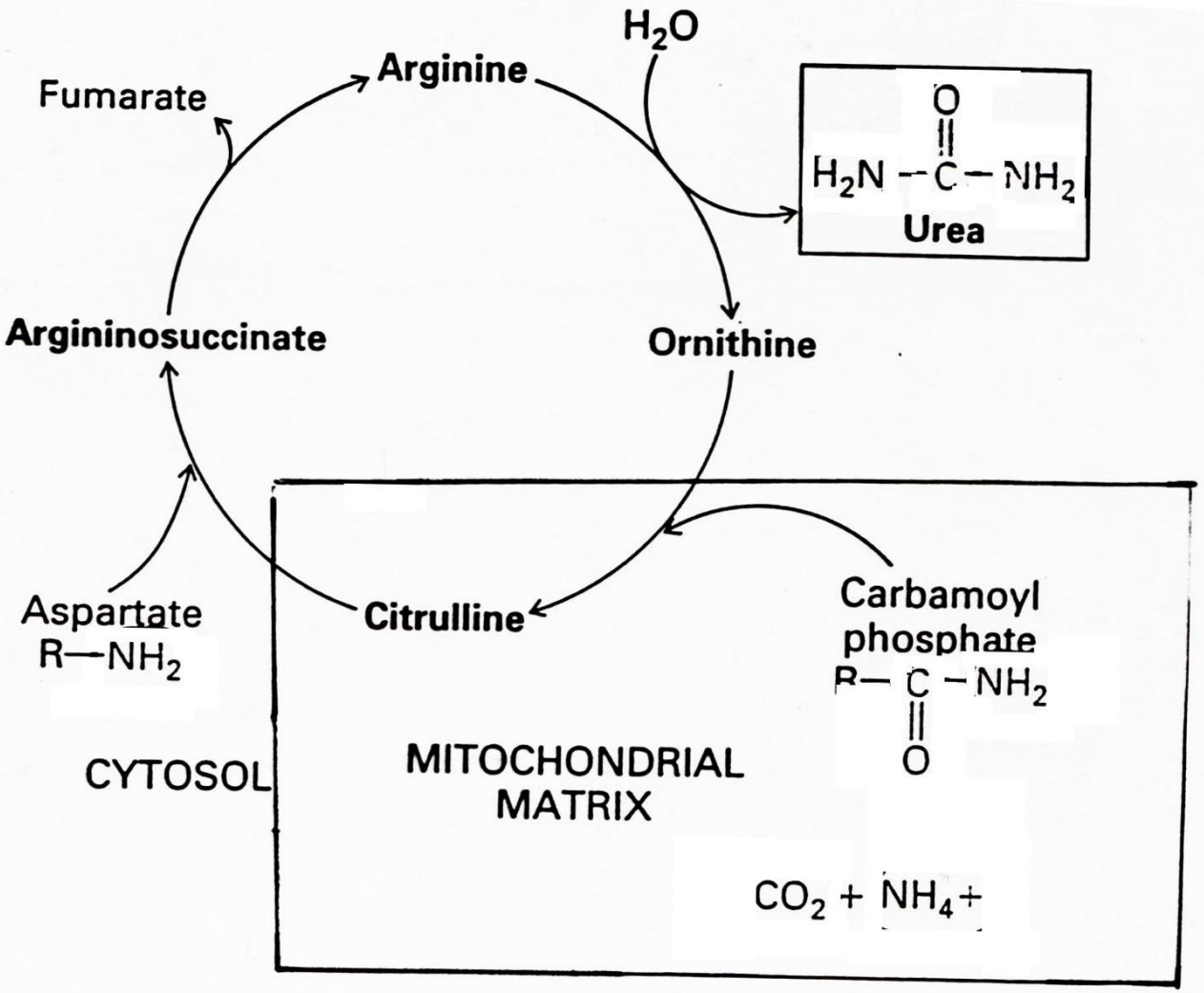
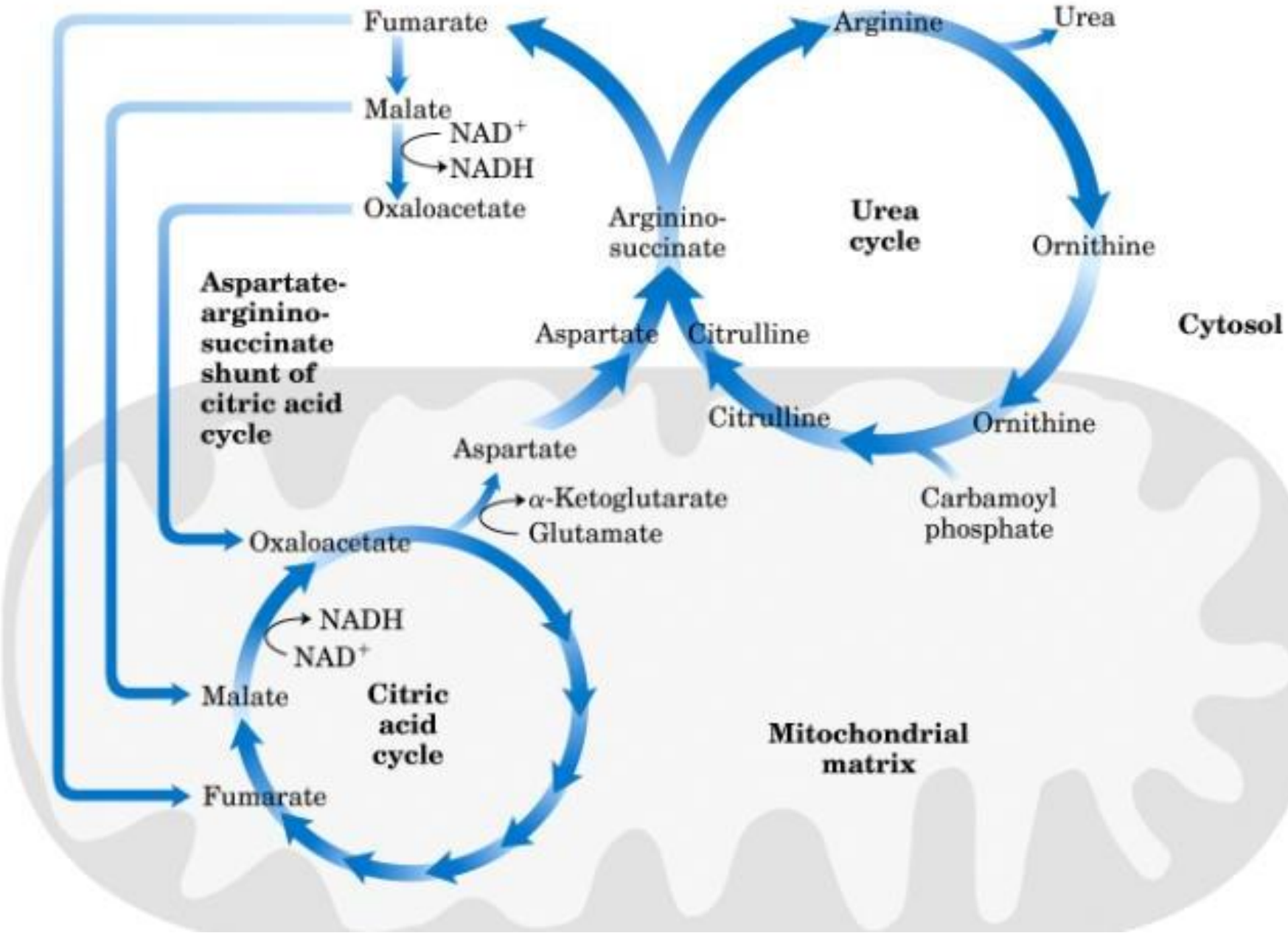


Figure 25-6
The urea cycle.

The citric acid and urea cycles are linked



The citric acid and urea cycles are linked

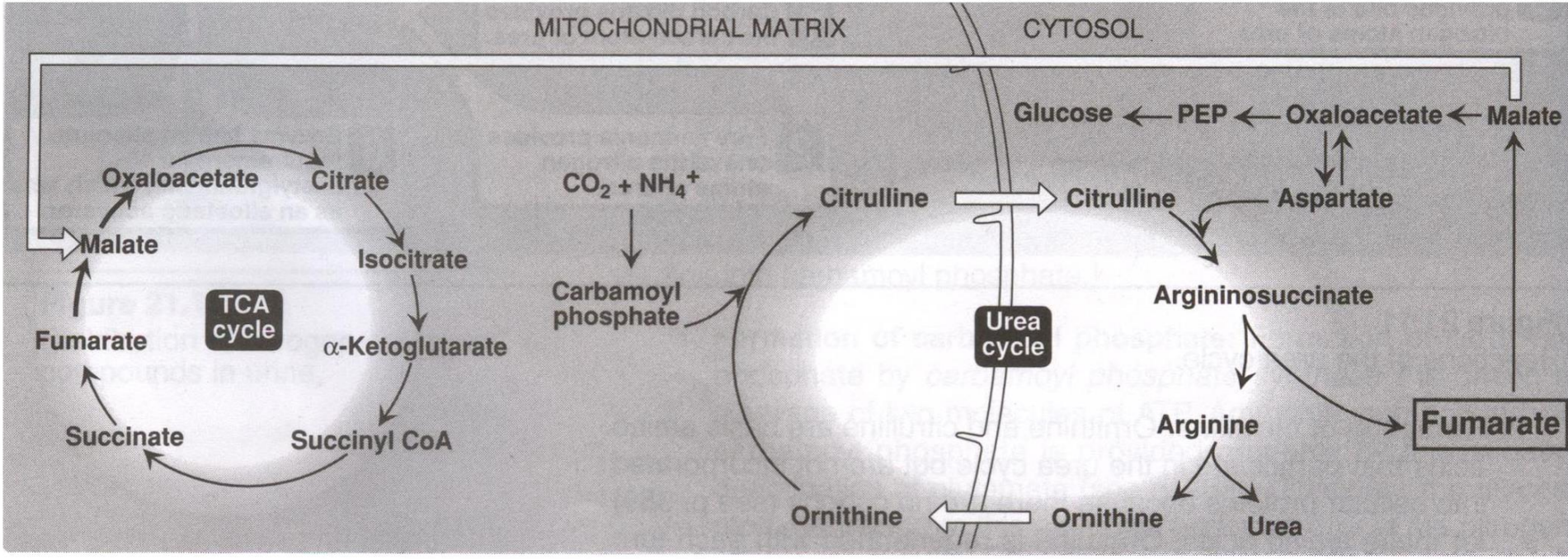


Figure 21.12
Fate of fumarate produced by the urea cycle.

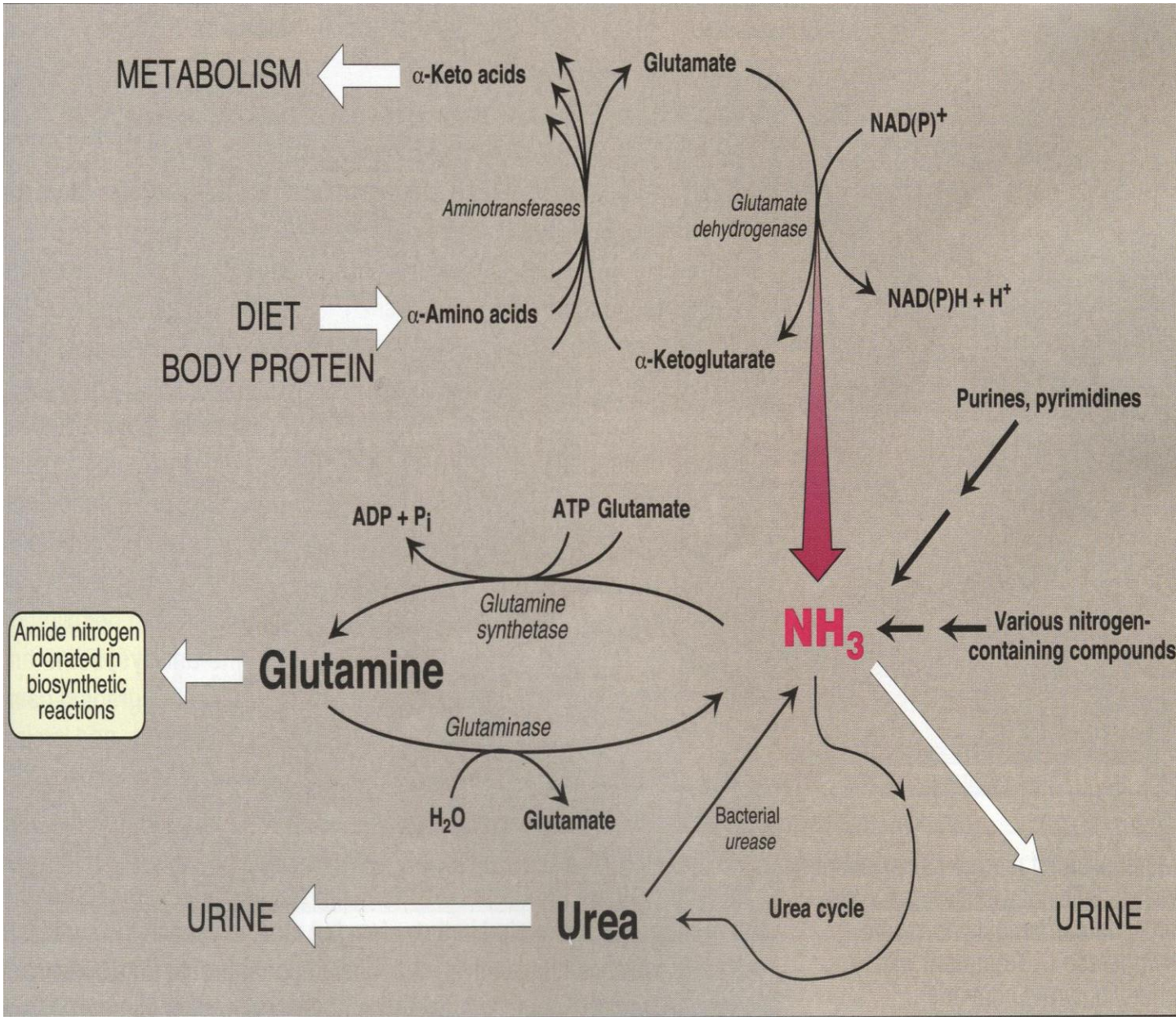
Fate of urea

Urea diffuses from liver and transported to kidneys ➔ excreted to urine.

-Portion of the urea diffuses from blood to intestine and is cleaved to CO2 + NH3 by **bacterial urease**.

-Ammonia is lost by feces and little is reabsorbed by blood and excreted by kiddney in urine.

-Kidney failur: NH4+ in blood is elevated. ➔ Ammonia toxicity.



Metabolism of Ammonia

If not used in the synthesis of new a.a or other nitrogenous compound it should exit from the body because it is very toxic to the CNS.

***Sources of ammonia**

1) Liver produces ammonia from a.a by aminotransferases and glutamate dehydrogenase.
2) Renal glutaminase produces from glutamine ➔ NH_4^+ is released.
3) Bacteria action in the intestinal.
4) From amines: amines obtained from diet and ammonia can be produced by amine oxidase.
5) The catabolism of purines and pyrimidines: in of purines and pyrimidines,

***Transport of ammonia in the circulation**

- Ammonia is continuously produced by tissues, but it is rapidly removed from the body in form of urea which is the most important disposal route for ammonia travels from liver to kidneys

-Glutamine: provides non-toxic storage and transport form of ammonia.
glutamine occurs in skeletal muscle, liver and brain and hydrolyzed to give NH_4^+ in the kidney by "glutaminase"

Hyperammonemia

-Aquired Hyperammonemia
-Hereditary Hyperammonemia

The catabolism of the branched-chain amino acids

- Isolecine, Leucine, Valine are essential a.a
- Can be metabolized by peripheral tissues mainly skeletal muscles rather than by the liver.

• **Catabolism of these a.a**

1) Transamination: catabolized by branched-chain α-amino acid transferase to produce α-keto acids.

2) Oxidative decarboxylation: removing the carboxyl group (COO-) derived from the a.a

- Catalyzed by branched-chain α-ketoacid dehydrogenase.
-Deficiency of this enzyme ➔ accumulation of α-acids ➔ maple-syrup urine disease.

3) Dehydrogenation

4) End product

Isoleucine ➔ acetyl CoA + succinyl CoA

Leucine ➔ acetyl CoA + acetoacetate

Valine ➔ succinyl CoA

The catabolism Carbon Skeleton Amino Acids

- According to the nature of metabolic end product amino acids are classified into Glucogenic and ketogenic amino acids

Ketogenic: acetoacetate or acetyl CoA

- **Leucine and lysine** are the only exclusively ketogenic amino acids.

Glucogenic: pyruvate or one of the intermediates of citric acid cycle, and these intermediates are also substrate for gluconeogenesis

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

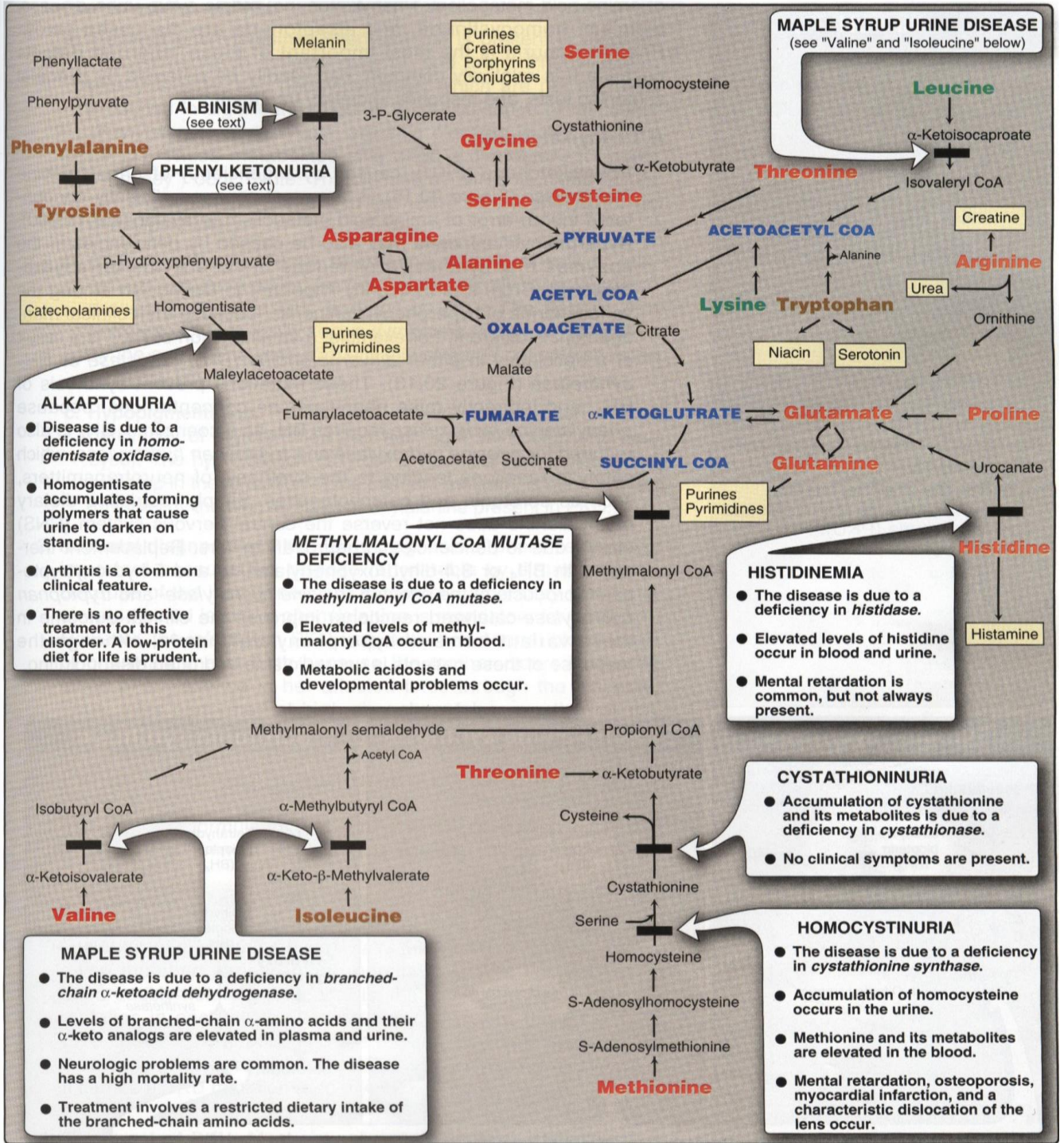
The catabolism of carbon skeleton of amino acids

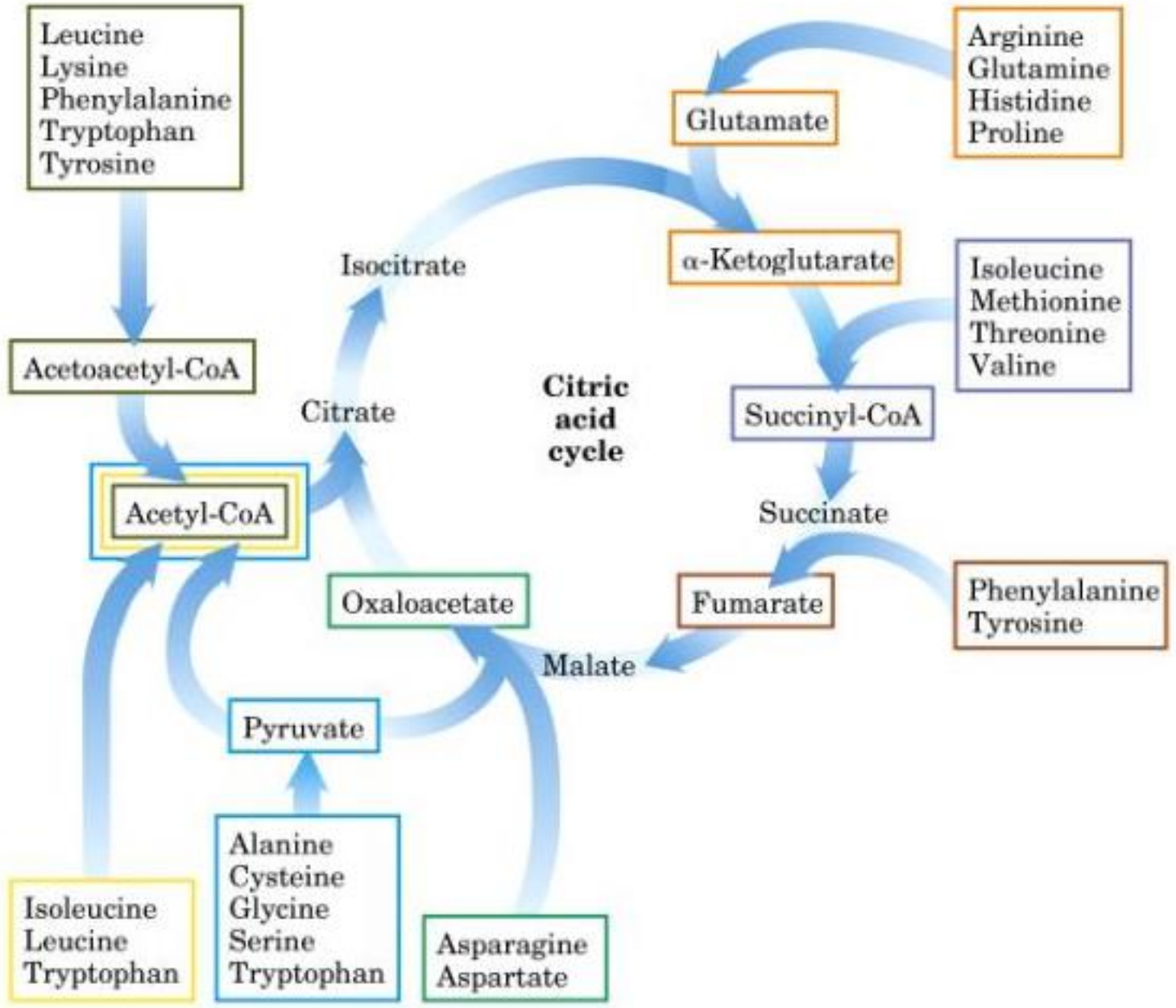
- Normally 10 – 15% of energy is from proteins.

***The catabolism of carbon skeletons of a.a can forms seven products:**

1- Oxaloacetate
2- a-ketoglutarate
3- Pyruvate
4- Fumarate
5- Acetyl CoA
6- Acetoacetyl CoA
7- Succinyl CoA

- These products end with production of glucose or fat or energy by entering citric acid cycle.


MAPLE SYRUP URINE DISEASE
(see "Valine" and "Isoleucine" below)
Melanin
Purines
Creatine
Porphyrins
Conjugates
Serine
Homocysteine
Leucine
Phenyllactate
Phenylpyruvate
ALBINISM
(see text)
3-P-Glycerate
Glycine
Cystathionine
α-Ketoisocaproate
Phenylalanine
PHENYLKETONURIA
(see text)
α-Ketobutyrate
Threonine
Isovaleryl CoA
Tyrosine
Serine
Cysteine
Creatine
Asparagine
PYRUVATE
ACETOACETYL COA
p-Hydroxyphenylpyruvate
Alanine
Alanine
Arginine
Aspartate
ACETYL COA
Urea
Catecholamines
Homogentisate
Lysine
Tryptophan
Ornithine
Purines
Pyrimidines
OXALOACETATE
Citrate
Niacin
Serotonin
Maleylacetoacetate
Malate
ALKAPTONURIA
Disease is due to a deficiency in homogentisate oxidase.
Homogentisate accumulates, forming polymers that cause urine to darken on standing.
Arthritis is a common clinical feature.
There is no effective treatment for this disorder. A low-protein diet for life is prudent.
Fumarylacetoacetate
FUMARATE
α-KETOGLUTRATE
Glutamate
Proline
Acetoacetate
Succinate
SUCCINYL COA
Glutamine
Urocanate
Purines
Pyrimidines
METHYLMALONYL CoA MUTASE DEFICIENCY
The disease is due to a deficiency in methylmalonyl CoA mutase.
Elevated levels of methylmalonyl CoA occur in blood.
Metabolic acidosis and developmental problems occur.
Methylmalonyl CoA
HISTIDINEMIA
The disease is due to a deficiency in histidase.
Elevated levels of histidine occur in blood and urine.
Mental retardation is common, but not always present.
Histidine
Histamine
Methylmalonyl semialdehyde
Propionyl CoA
Acetyl CoA
Threonine
α-Ketobutyrate
CYSTATHIONINURIA
Accumulation of cystathionine and its metabolites is due to a deficiency in cystathionase.
No clinical symptoms are present.
Isobutyryl CoA
α-Methylbutyryl CoA
Cysteine
α-Ketoisovalerate
α-Keto-β-Methylvalerate
Cystathionine
Valine
Isoleucine
Serine
HOMOCYSTINURIA
The disease is due to a deficiency in cystathionine synthase.
Accumulation of homocysteine occurs in the urine.
Methionine and its metabolites are elevated in the blood.
Mental retardation, osteoporosis, myocardial infarction, and a characteristic dislocation of the lens occur.
Homocysteine
S-Adenosylhomocysteine
MAPLE SYRUP URINE DISEASE
The disease is due to a deficiency in branched-chain α-ketoacid dehydrogenase.
Levels of branched-chain α-amino acids and their α-keto analogs are elevated in plasma and urine.
Neurologic problems are common. The disease has a high mortality rate.
Treatment involves a restricted dietary intake of the branched-chain amino acids.
S-Adenosylmethionine
Methionine


Leucine
Lysine
Phenylalanine
Tryptophan
Tyrosine
Acetoacetyl-CoA
Acetyl-CoA
Citrate
Isocitrate
Glutamate
Arginine
Glutamine
Histidine
Proline
α-Ketoglutarate
Isoleucine
Methionine
Threonine
Valine
Citric acid cycle
Succinyl-CoA
Succinate
Fumarate
Phenylalanine
Tyrosine
Oxaloacetate
Malate
Pyruvate
Alanine
Cysteine
Glycine
Serine
Tryptophan
Isoleucine
Leucine
Tryptophan
Asparagine
Aspartate

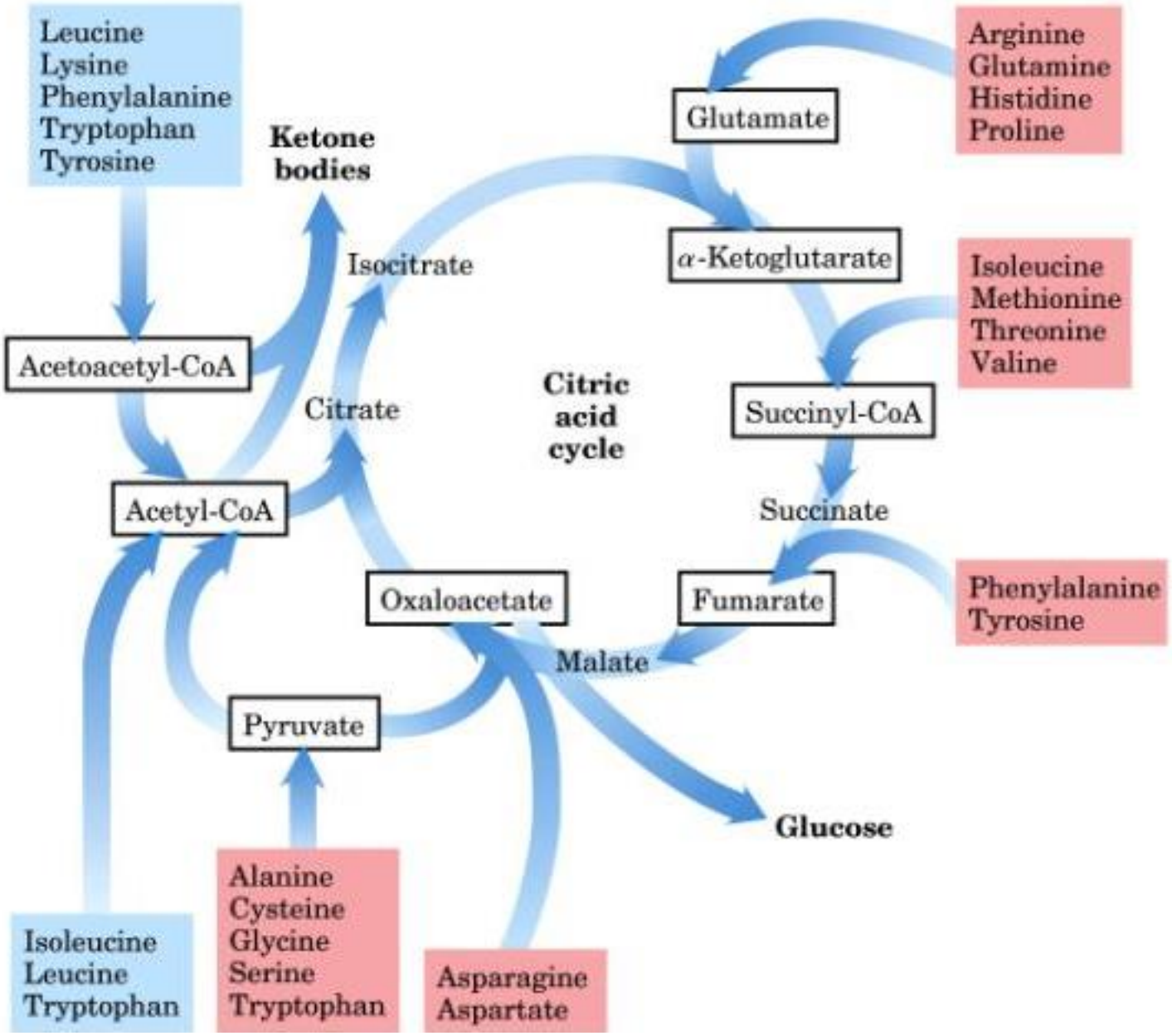

Leucine
Lysine
Phenylalanine
Tryptophan
Tyrosine
Ketone bodies
Glutamate
Arginine
Glutamine
Histidine
Proline
Isocitrate
α-Ketoglutarate
Isoleucine
Methionine
Threonine
Valine
Acetoacetyl-CoA
Citrate
Citric acid cycle
Succinyl-CoA
Acetyl-CoA
Succinate
Oxaloacetate
Fumarate
Phenylalanine
Tyrosine
Malate
Pyruvate
Glucose
Alanine
Cysteine
Glycine
Serine
Tryptophan
Isoleucine
Leucine
Tryptophan
Asparagine
Aspartate

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-mono-oxygenase (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	Argininosuccinate lyase	Vomiting, convulsions
Carbamoyl phosphate synthetase I deficiency	>0.5	Urea synthesis	Carbamoyl phosphate synthetase I	Lethargy, convulsions, early death
Homocystinuria	0.5	Methionine degradation	Cystathionine 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 phenyl-alanine to tyrosine	Phenylalanine hydroxylase	Neonatal vomiting; mental retardation

The End

Hyperammonemia

-Aquired Hyperammonemia

-Hereditary Hyperammonemia

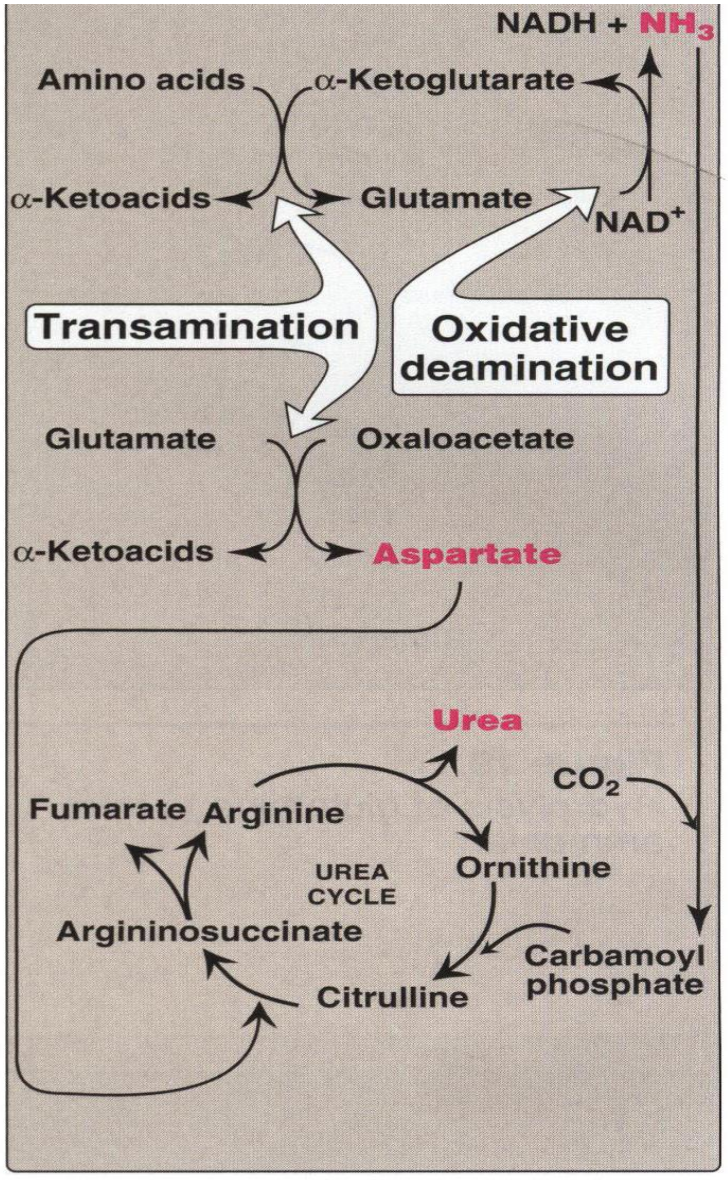


Figure 19.15
Flow of nitrogen from amino acids to urea. Amino groups for urea synthesis are collected in the form of ammonia and aspartate.

Metabolic fates of amino groups:

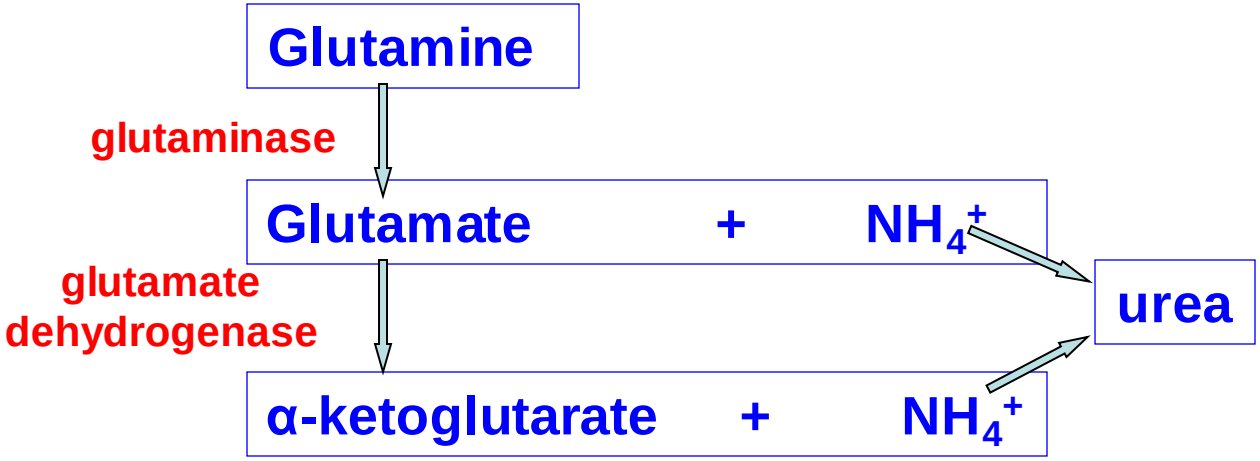
Most amino acids are ammonia generated in extrahepatic tissues travels to the liver in the form of amino group of metabolized in the liver. Some of NH_4 generated is recycled (In the biosynthesis of a.a), the excess is either excreted directly or converted to urea in urine.

- Excess ammonia generated in extrahepatic tissues travels to the liver in the form of amino group of glutamine

- Glutamate and Glutamine: play critical role in the catabolism of a.a
- In the cytosol of hepatocytes, amino groups are transferred from most a.a to α-ketoglutarate to form glutamate which transported to the mitochondria where NH_2 is removed.
- Excess NH_4^+ generated in most of tissues is converted to the amide nitrogen of glutamine which carried to the mitochondria of hepatocytes (liver)
- In most tissues glutamate and glutamine are present in higher concentration other than a.a

Glutamine transports ammonia

- The toxic ammonia that formed in extrahepatic tissue is converted into amino group of glutamine that then transported from extrahepatic tissues to liver or kidneys.
- Many tissues (brain) NH4+ is released resulted from metabolic processes as nucleotide degradation.
- **Glutamine**: non-toxic transport form of NH4+ and also source of amino group in many biosynthesis reactions.
- The amide nitrogen of glutamine is released as ammonia only in liver and kidney's mitochondria by the enzyme "Glutaminase" which convert glutamine into glutamate + NH4+



Over all stoichiometry of urea cycle

Aspartate + NH_3 + CO_2 + 3ATP

Urea + Fumarate + 2ADP + AMP + 2Pi + PPi + $8H_2O$

➔

- 4 High energy phosphates are consumed in the synthesis of each molecule of urea.
- The synthesis of urea is irreversible with large +ve ΔG

$2NH_4^+ + HCO_3^- + H_2O$ ➔ Urea + 2ADP + 4Pi + AMP

- If the urea cycle is isolated 1 urea ➔ 4 ATP molecules.
- Urea cycle causes also net conversion of oxaloacetate to fumarate. The regeneration of oxaloacetate produce NADH in the malate dehydrogenase reaction.
- NADH corresponds to 8 ATP, so this reduce the overall energetic cost of urea synthesis.