CATABOLISM OF PROTEINS AND AMINO ACIDS

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- In animals,amino acids undergo oxidative degradation in 3 different metabolic circumstances:
- 1-During normal synthesis and degradation of cellular proteins, some amino acids, that are not needed for new protein synthesis, undergo OXİDATİVE DEGRADATİON
- 2-When a diet is rich in protein, the surplus amino acids are catabolized (in the liver amino acids can't be stored)
- 3-During starvation and uncontrolled DM, when carbohydrates are unavailable or improperly utilized, cellular proteins are used as fuel.

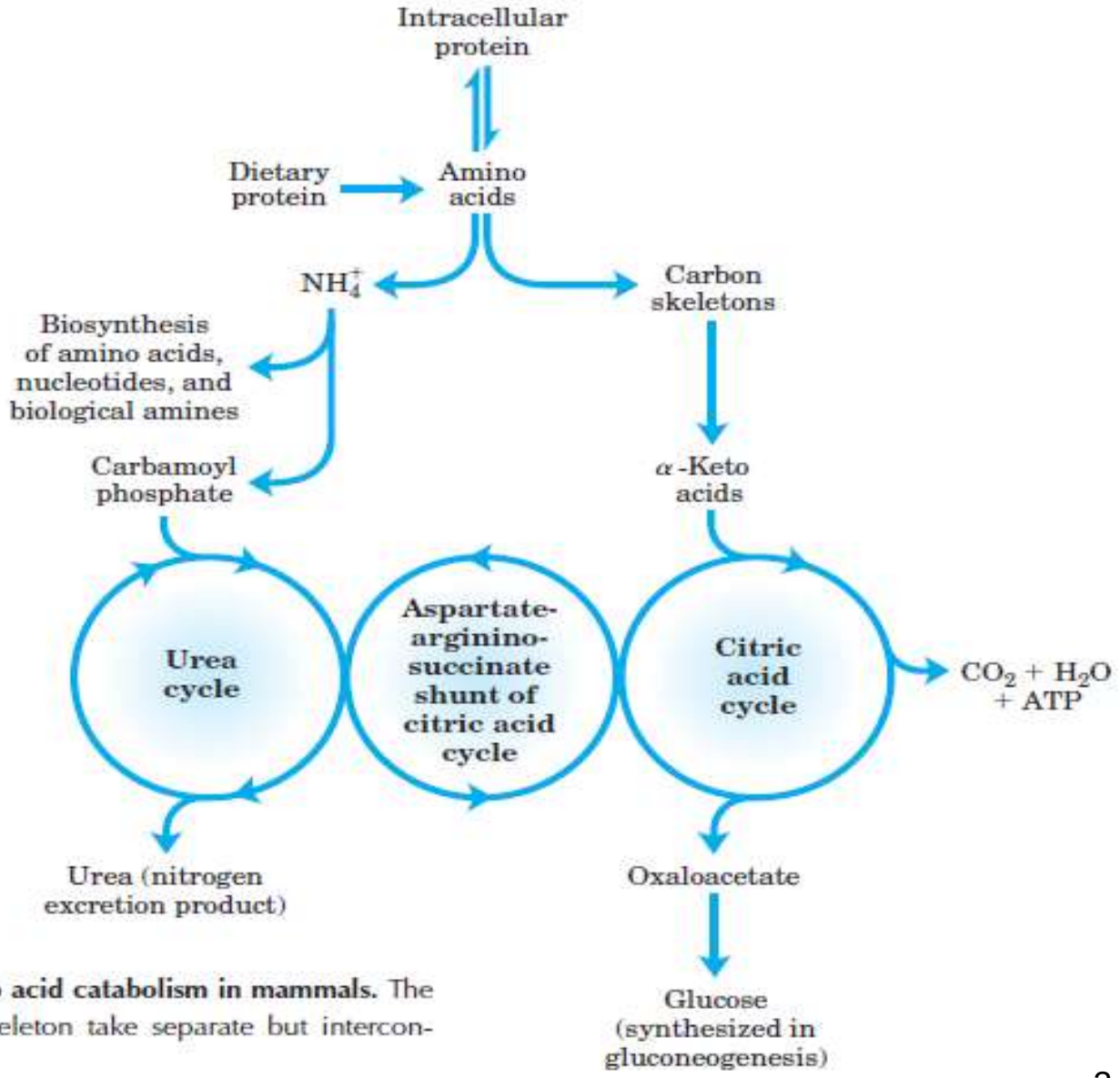


FIGURE 18–1 Overview of amino acid catabolism in mammals. The amino groups and the carbon skeleton take separate but interconnected pathways.

- Animals convert α-amino nitrogen to varied end products as ammonia, uric acid or urea.
- Humans are ureotelic and excrete non-toxic, water-soluble urea.

BİOSYNTHESİS OF UREA

- Urea biosynthesis occurs in 4 states
- 1-Transamination
- 2-Oxidative deamination of glutamate.
- 3-Ammonia transport
- 4-Reactions of urea cycle.

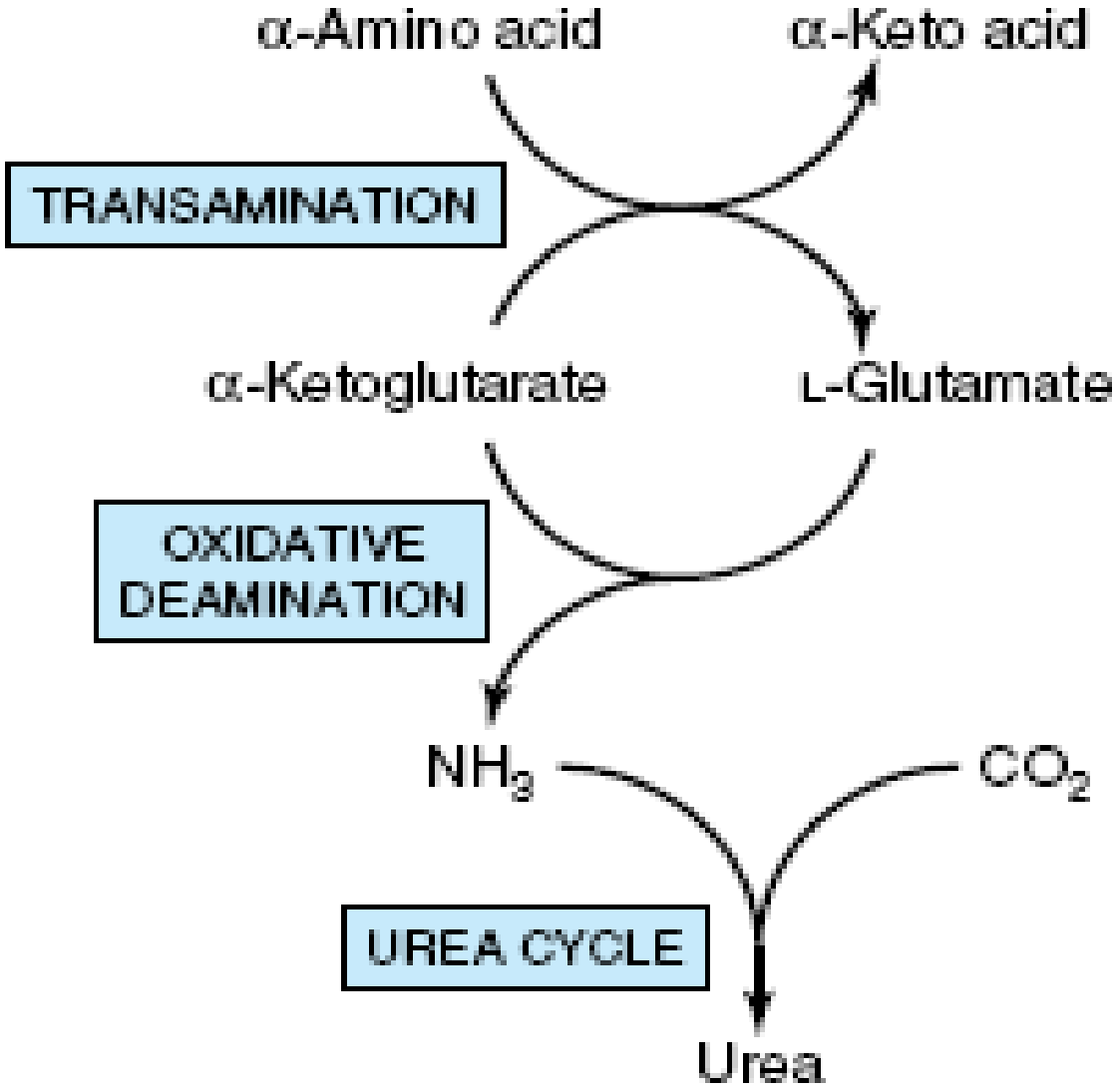


Figure 29–2. Overall flow of nitrogen in amino acid catabolism.

- Transamination
- Transamination transfers α-amino nitrogen to α-ketoglutarate, forming glutamate.
- Transamination interconverts pairs of α-amino acids and α-ketoacids.

- Amino acids that don't participate in transamination:
- Lysine, threonine, proline, hydroxyproline.
- Reversible
- Aminotransferases (transferases) remove the amino group from most amino acids and produce the corresponding α - ketoacid

- Cofactor:Pyridoxal phosphate
- Pyridoxamine is the intermediate in the reaction.
- Alanine-pyruvate amino transferase (alanine aminotransferase) and glutamate α-ketoglutarate amino transferase (glutamate aminotransferase) catalyze the transfer of amino groups to pyruvate (forming alanine) or to α-ketoglutare (forming glutamate)

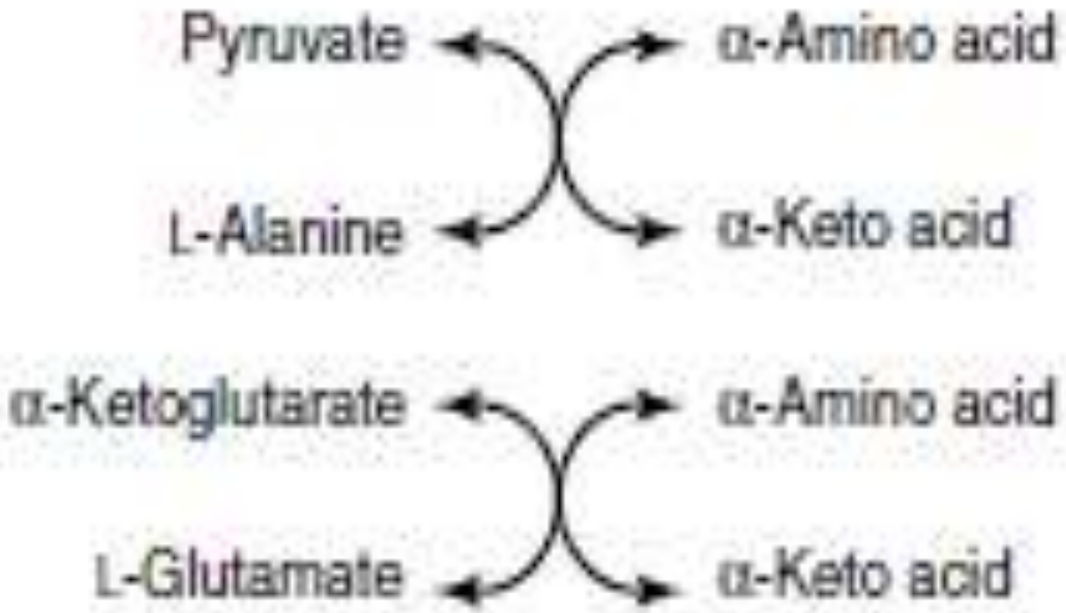


Figure 29–4. Alanine aminotransferase *(top)* and glutamate aminotransferase *(bottom)*.

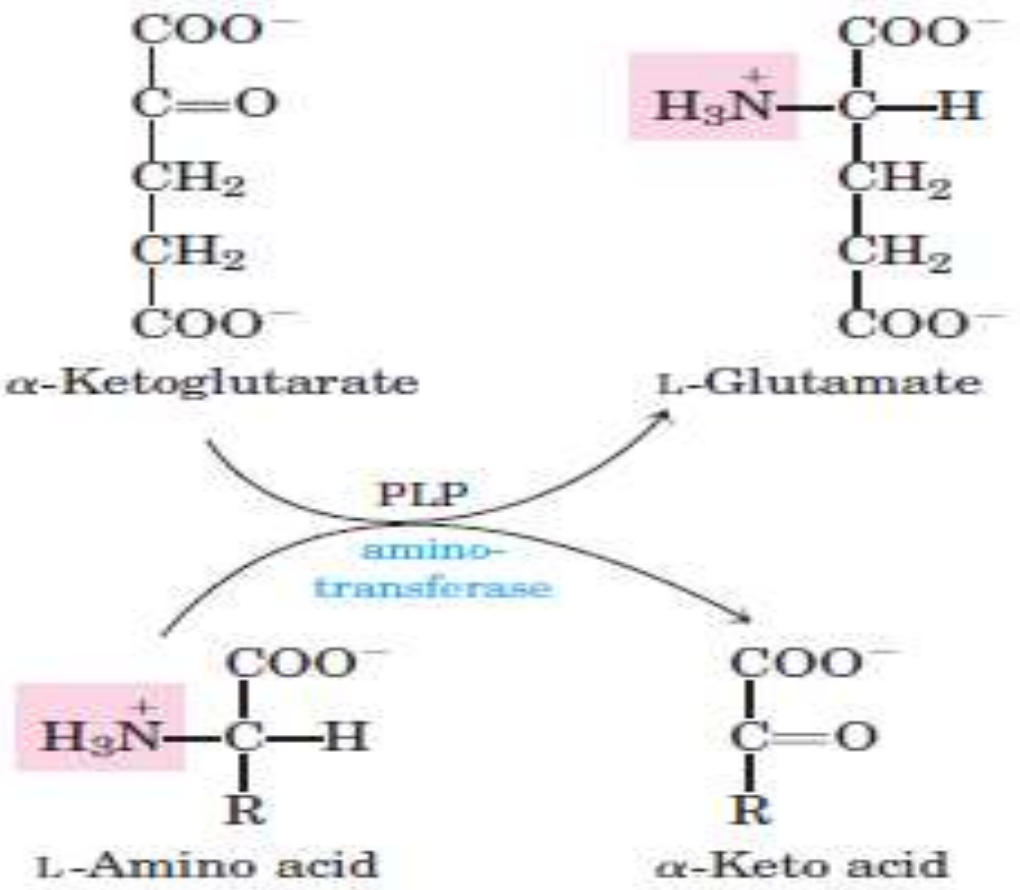


FIGURE 18–4 Enzyme-catalyzed transaminations. In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. Although the reaction is shown here in the direction of transfer of the amino group to α-ketoglutarate, it is readily reversible.

- Each aminotransferase is specific for one pair of substrates but nonspecific for the other.
- Since alanine is also a substrate for glutamate aminotransferase, all the amino nitrogen from amino acids that undergo transamination can be concentrated in glutamate

- The effect of transamination reaction is to collect the amino groups from many different amino acids in the form of L-glutamate.
- L-glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the eliminaton of nitrogenous waste products.

- Glutamate releases its amino qroup as ammonia in the liver.
- In hepatocytes, glutamate is transported from cytosol into mitochondria, where it undergoes OXİDATİVE DEAMİNATİON by glutamate dehydrogenase.

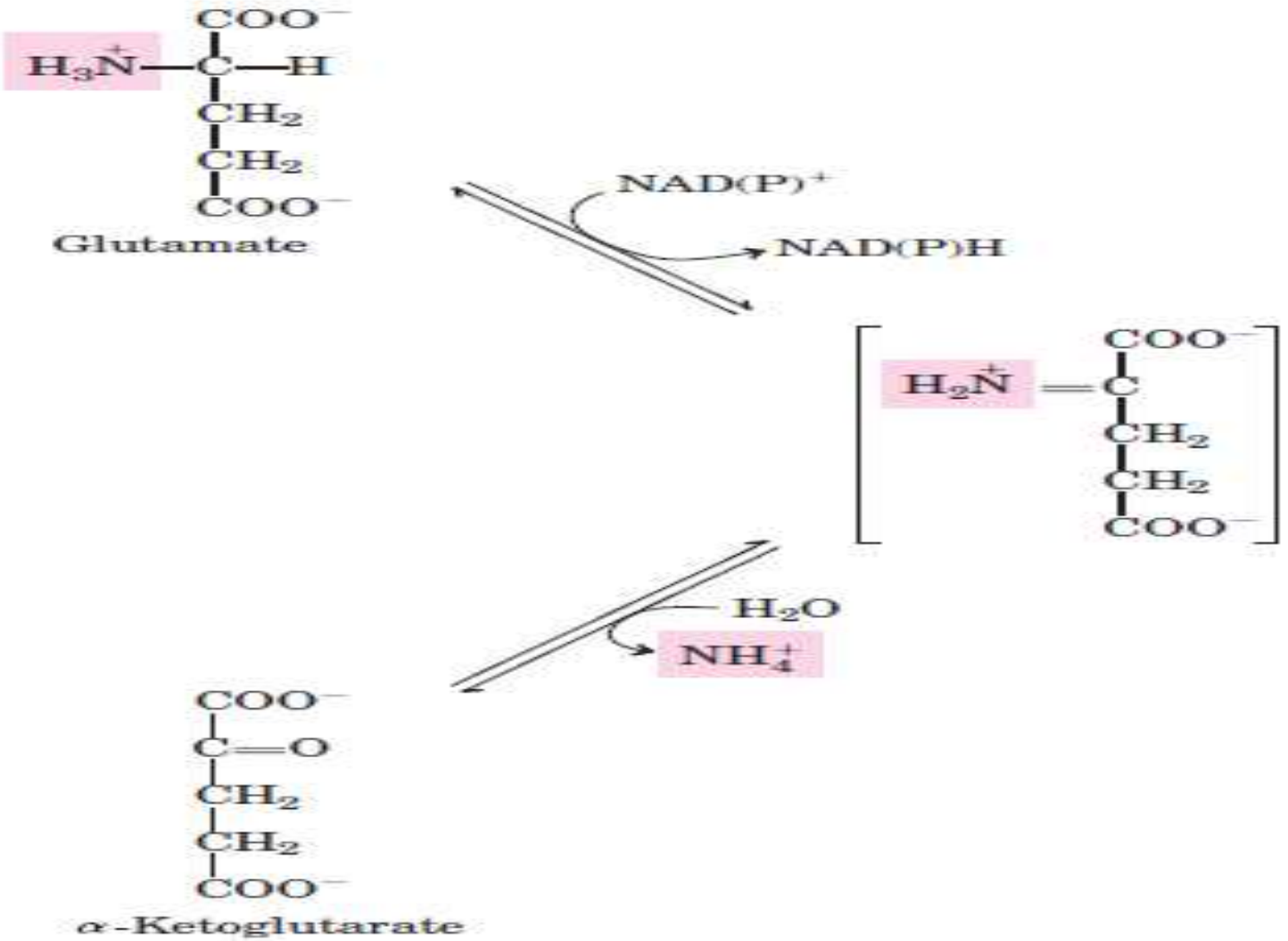


FIGURE 18–7 Reaction catalyzed by glutamate dehydrogenase. The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD^+ or $NADP^+$ as cofactor. The glutamate dehydrogenases of plants and microorganisms are generally specific for one or the other. The mammalian enzyme is allosterically regulated by GTP and ADP.

- L-glutamate is the only amino acid that undergoes oxidative deamination at an appreciable rate in mammalian tissues.
- L-glutamate dehydrogenase (GDH) occupies a central position in nitrogen metabolism.(mitochondrial matrix)

- GDH reaction is a reversible reaction that can produce glutamate from α-ketoglutarate or convert glutamate to α-ketoglutarate and NH3
- Hepatic GDH can use either NAD+ or NADP+, as the acceptor of reducing equivalents.
- Glutamate serves as a precursor of ammonia.Mitochondrial glutamine synthetase catlyses this energy requiring reaction (ATP), consuming a molecule of ammonia.

$$\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + \text{H}^+ \longrightarrow 2\ \text{glutamate} + \text{NADP}^+ \qquad (22\text{–}2)$$

The net reaction of glutamine synthetase and glutamate synthase (Eqns 22–1 and 22–2) is

$$\alpha\text{-Ketoglutarate} + \text{NH}_4^+ + \text{NADPH} + \text{ATP} \longrightarrow \text{L-glutamate} + \text{NADP}^+ + \text{ADP} + \text{P}_i$$

(1) $\text{Glutamate} + \text{ATP} \longrightarrow \gamma\text{-glutamyl phosphate} + \text{ADP}$

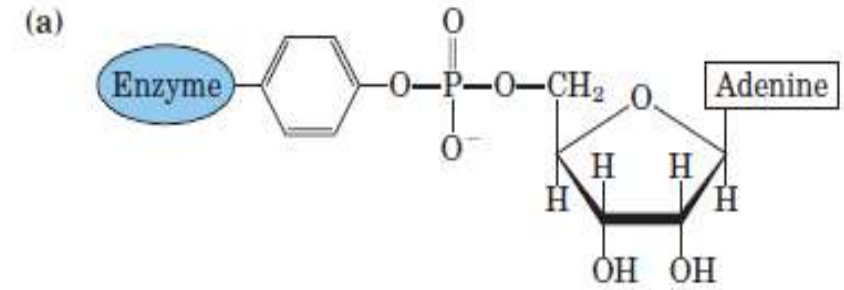
(2) $\gamma\text{-Glutamyl phosphate} + \text{NH}_4^+ \longrightarrow \text{glutamine} + \text{P}_i + \text{H}^+$

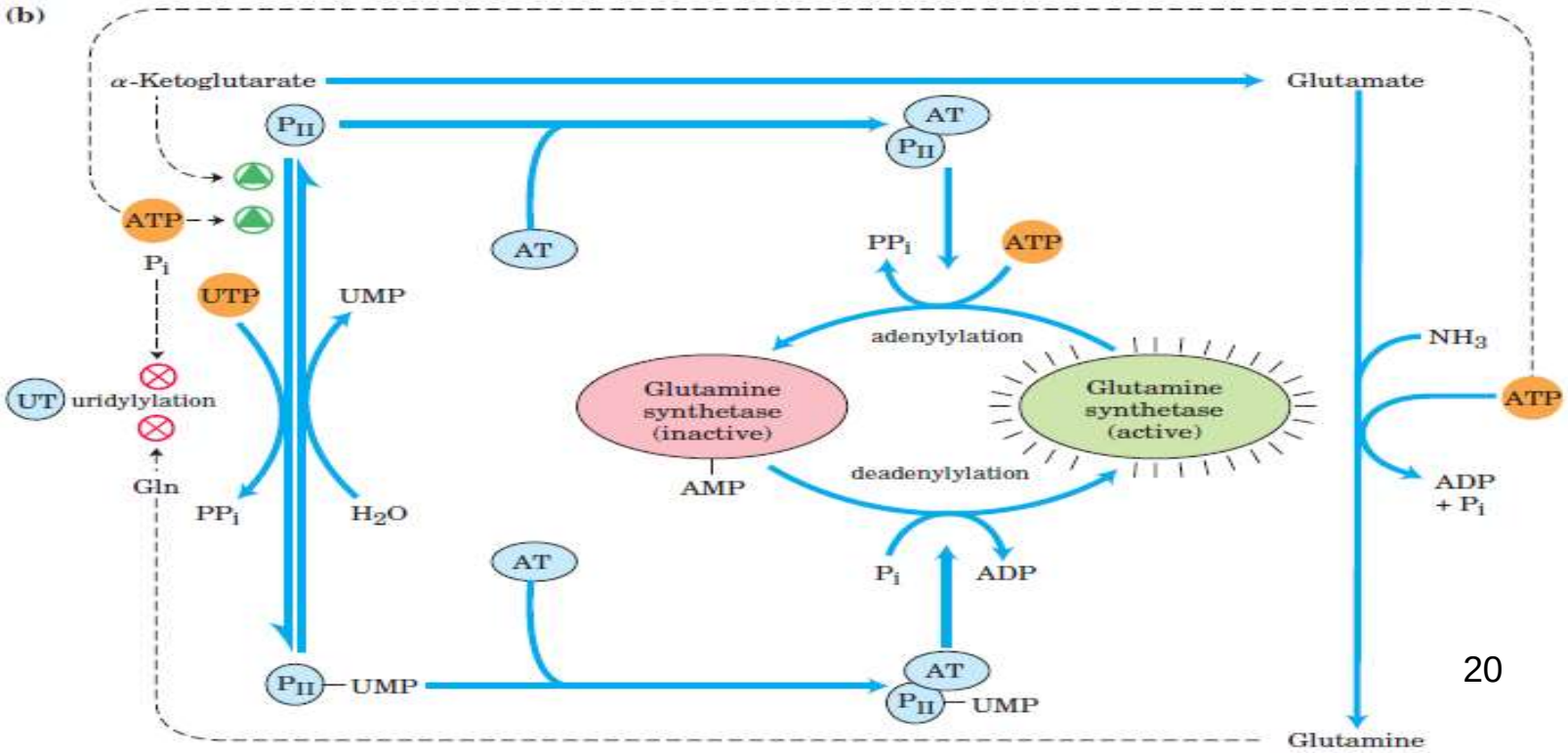
Sum: $\text{Glutamate} + \text{NH}_4^+ + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + \text{Pi} + \text{H}^+ \qquad (22\text{–}1)$

$$\alpha\text{-Ketoglutarate} + \text{NH}_4^+ + \text{NADPH} \longrightarrow \text{L-glutamate} + \text{NADP}^+ + \text{H}_2\text{O}$$

- Glutamine synthetase is a primary regulatory point in nitrogen metabolism.
- It is regulated both allosterically and by covalent modification (adenylation inactivation).

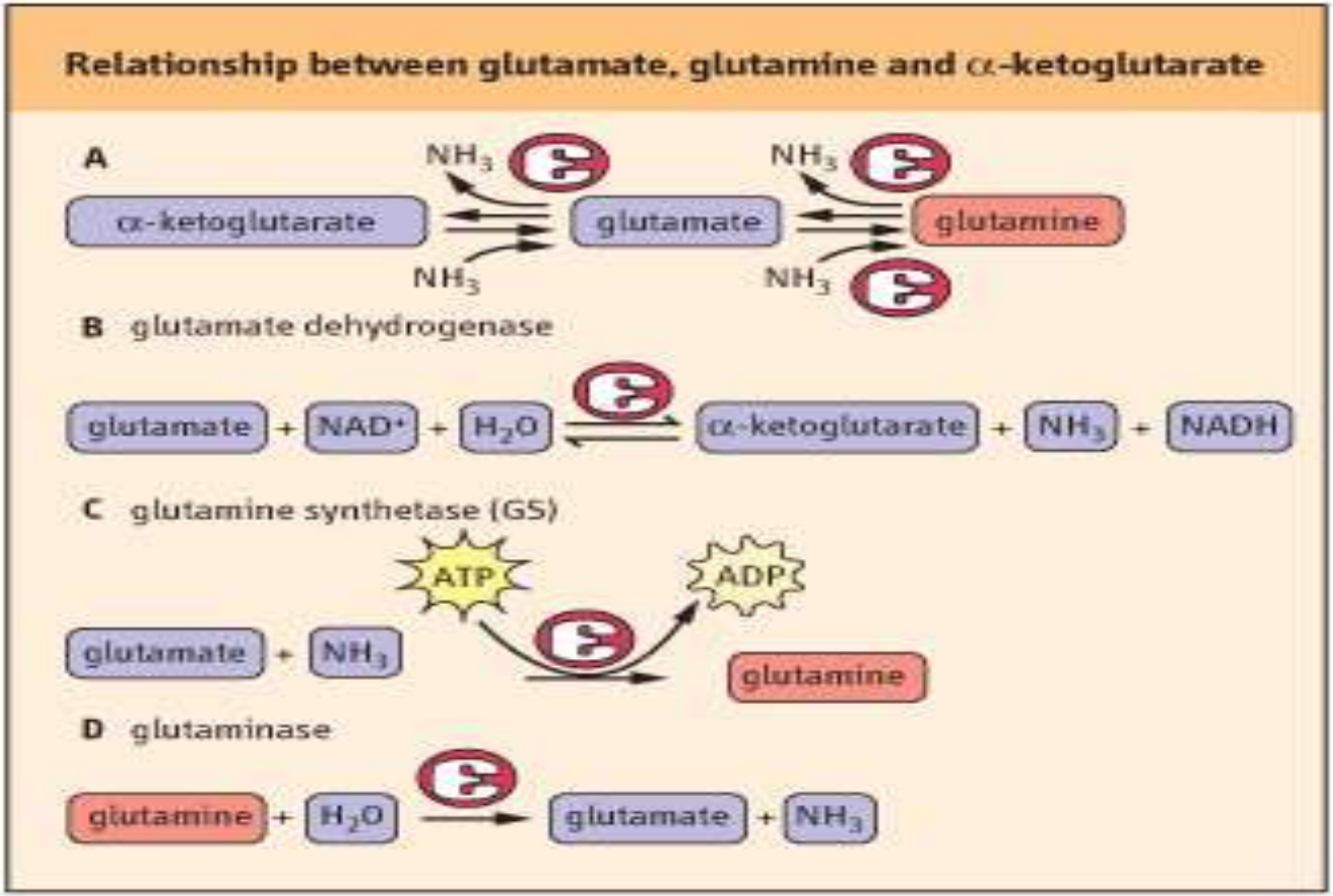
FIGURE 22-6 Allosteric regulation of glutamine synthetase. The enzyme undergoes cumulative regulation by six end products of glutamine metabolism. Alanine and glycine probably serve as indicators of the general status of amino acid metabolism in the cell.





- Glutamine can serve as a buffer for ammonia utilization as a source of ammonia and carrier of amino groups. Glutamine, along with alanine, is a key transporter of amino groups between various tissues and liver and is present in greater concentrations than most amino acids in blood.

- Glutaminase hydrolyses glutamine to glutamate and NH_{4+}.
- This reaction is important in the kidney for the management of proton transport and pH control.


Relationship between glutamate, glutamine and α-ketoglutarate
A
NH3
α-ketoglutarate
glutamate
glutamine
B glutamate dehydrogenase
glutamate + NAD+ + H2O
α-ketoglutarate + NH3 + NADH
C glutamine synthetase (GS)
ATP
ADP
glutamate + NH3
glutamine
D glutaminase
glutamine + H2O
glutamate + NH3

Aminotransferase + GDH action ⟶ TRANSDEAMiNATiON

- GDH operates at an important intersection of carbon and nitrogen metabolism.
- The mammalian GDH is allosterically regulated by GTP (-modulator) and ADP (+ modulator)

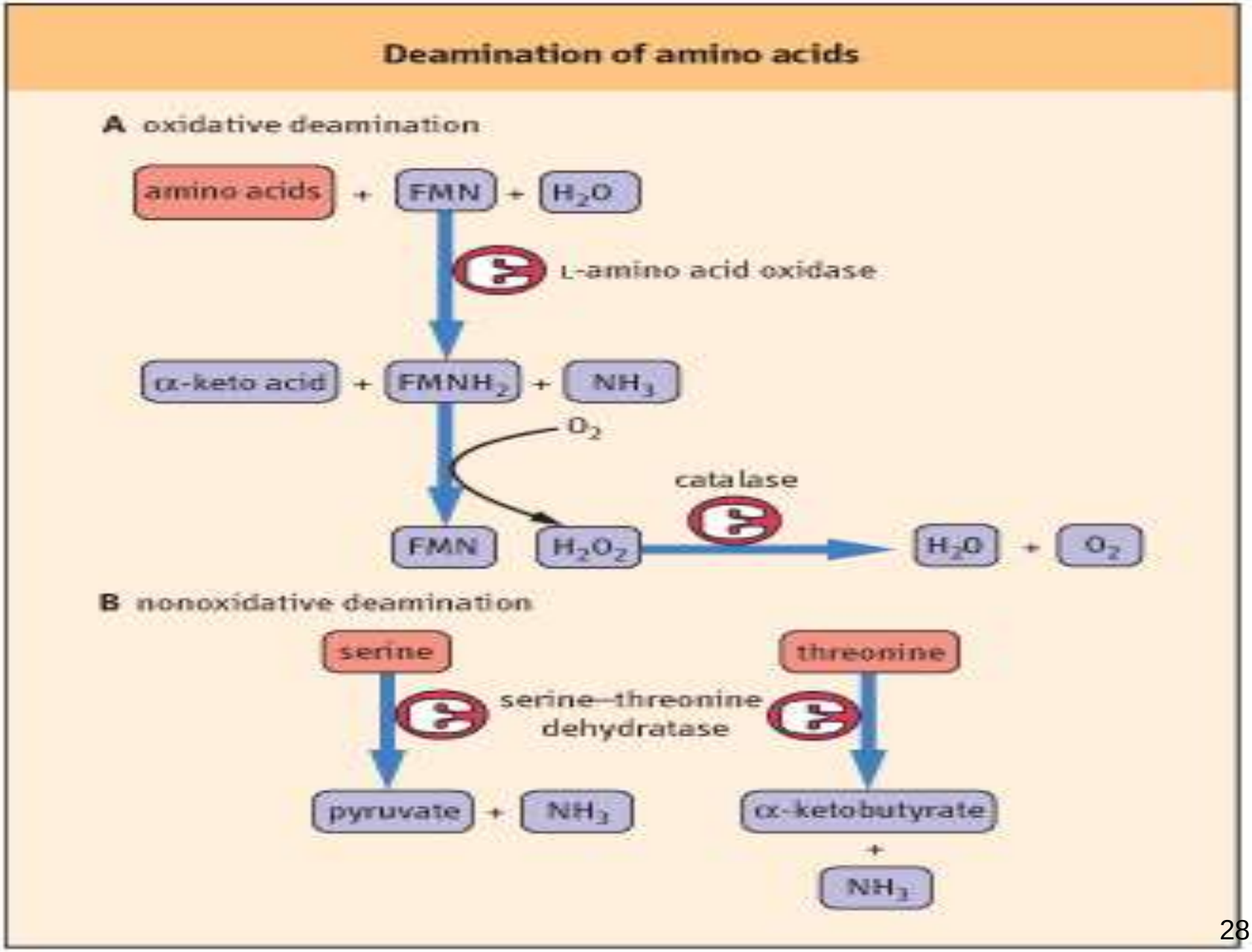
Hyperinsulinism-hyperammonemia syndrome:

- Mutations that alter the allosteric binding site for GTP
- Permanent activation of GDH
- Genetic disorder
- NH_3 increase (in blood)
- Hypoglycemia

- Oxidative deamination of amino acids
- L-amino acid oxidases of liver and kidney produces NH_3 and α-keto acid directly, using FMN as a cofactor.(through α-imino acid)
- $FMNH_2$ is converted to FMN, using O_2 and produces H_2O_2 which is decomposed by catalase.

Non-oxidative deamination

- Hydroxyaminoacids (serine, threonine) are non-oxidatively deaminated by dehydratase to form keto acids (pyruvate, and α-ketobutyrate) and NH_3.


Deamination of amino acids
A oxidative deamination
amino acids
+
FMN
+
H_2O
L-amino acid oxidase
α-keto acid
+
$FMNH_2$
+
NH_3
O_2
catalase
FMN
H_2O_2
H_2O
+
O_2
B nonoxidative deamination
serine
threonine
serine–threonine dehydratase
pyruvate
+
NH_3
α-ketobutyrate
+
NH_3

- The NH_4^+ ,from intestine and kidney, is transported in the blood to liver.
- In the liver, the ammonia from all sources is disposed of by urea synthesis.

Ammonia Transport

- Ammonia produced by enteric bacteria and absorbed into portal venous blood and ammonia produced by tissues are rapidly removed from circulation by liver and converted to urea.
- Only traces (10-20 µg/dl) are normally present in peripheral blood.

- This is essential since NH_3 is toxic to central nervous system.
- In severely impaired hepatic function and in the development of collateral links between portal and systemic veins ,cirrhois,ammonia intoxication develops.

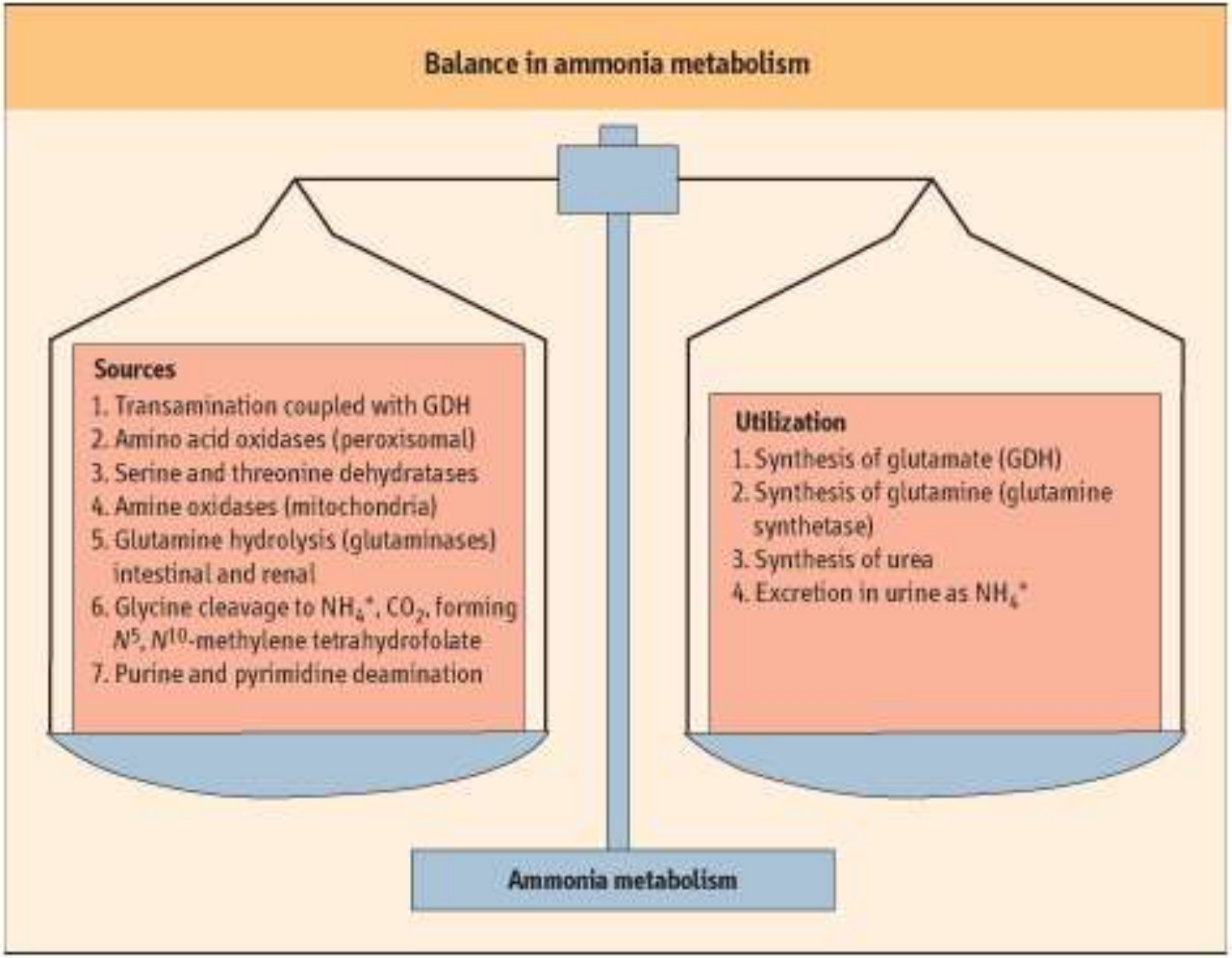
- Symptoms:Tremor, slurred speech, blurred vision, coma
- Ammonia Encephalopathy
- When ammonia concentration increases in blood and other biological fluİds, it diffuses across blood-brain barrier.

- Increased synthesis of glutamate from α-ketoglutarate leads to α-ketoglutarate depletion in CNS cells, resulting in TCA cycle inhibition and ATP decrease.
- Glutamate, a major inhibitory neurotransmitter, or its derivative GABA, may also contribute to CNS effects.

- The sensitivity of brain to ammonia may reflect the depletion of neurotransmitters as well as changes in cellular osmotic balance.
- GDH and glutamine synthetase are present at high levels in the brain, although glutamine synthetase reaction is the more important pathway for removal of NH_4^+.
- High levels of NH_4^+ lead to increased levels of

glutamine, which acts as an osmotically active solute (osmolyte) in brain astrocytes.

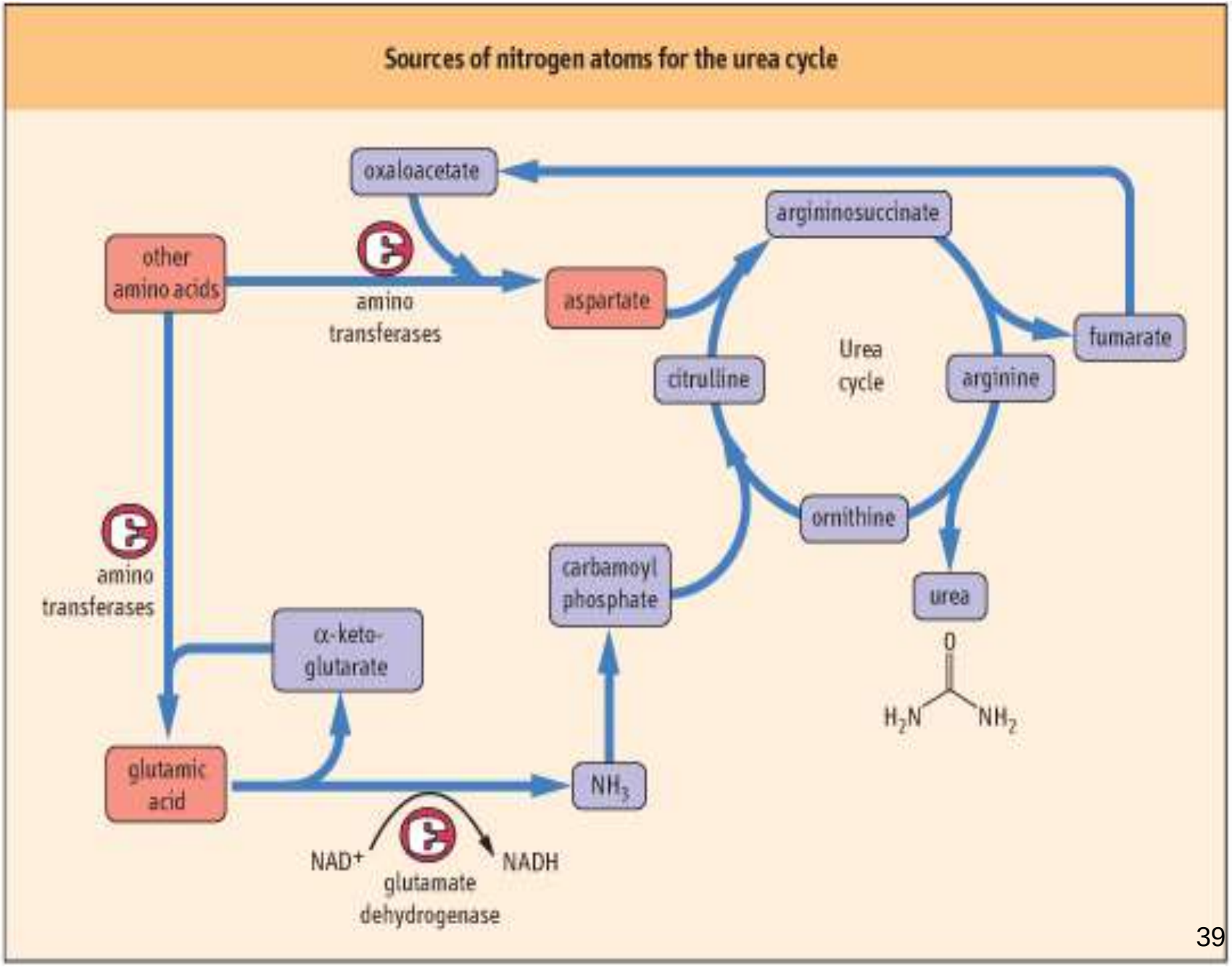
- Uptake of water into astroyctes to maintain osmotic balance leads to swelling of cells and coma.


Balance in ammonia metabolism
Sources
1. Transamination coupled with GDH
2. Amino acid oxidases (peroxisomal)
3. Serine and threonine dehydratases
4. Amine oxidases (mitochondria)
5. Glutamine hydrolysis (glutaminases) intestinal and renal
6. Glycine cleavage to NH_4^+, CO_2, forming N^5, N^{10}-methylene tetrahydrofolate
7. Purine and pyrimidine deamination
Utilization
1. Synthesis of glutamate (GDH)
2. Synthesis of glutamine (glutamine synthetase)
3. Synthesis of urea
4. Excretion in urine as NH_4^+
Ammonia metabolism

- NH_3 may be toxic to brain because it reacts with α-ketoglutarate to form glutamate.
- Depleted levels of α-ketoglutarate impair TCA cycle function.
- Excretion into urine of ammonia produced by renal tubular cells facilitates cation conservation and regulation of acid-base balance.
- NH_3 production from intracellular renal glutamine increases in metabolic acidosis, decreases in metabolic alkalosis.

Urea Cycle

- Urea is the principal nitrogenous excretion product in humans.
- The urea cycle was the first metabolic cycle to be well defined.
- Its description preceded that of TCA cycle.


Sources of nitrogen atoms for the urea cycle
oxaloacetate
argininosuccinate
other amino acids
amino transferases
aspartate
fumarate
Urea cycle
citrulline
arginine
ornithine
amino transferases
carbamoyl phosphate
urea
α-keto-glutarate
glutamic acid
NH3
NAD+
NADH
glutamate dehydrogenase

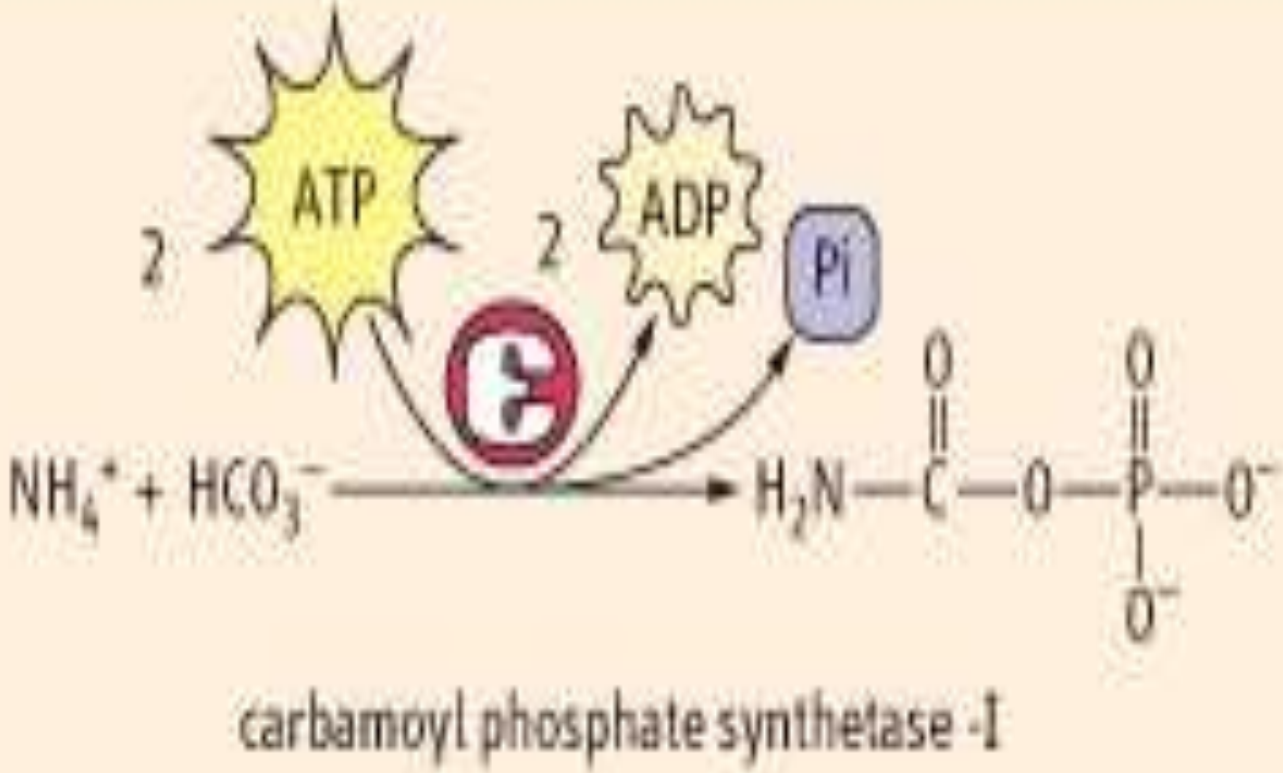
- Synthesis of 1 mol. of urea requires 3 mol. of ATP (4 high energy phosphate groups)

  plus 1 mol. of ammonium and of α-amino nitrogen of aspartate. (Source of nitrogen atom)
- Of the 6 participating amino acids, N-acetylglutamate functions only as an enzyme activator.

- Ornithine, consumed in reaction 2, is regenerated in reaction 5.
- There is no net loss or gain of ornithine, citrulline, argininosuccinate or arginine.
- Ammonium ion, CO2 , ATP and aspartate are consumed.
- Some reactions occur in the matrix of mitochondrion and others in the cytosol of the liver.

- The start of urea cycle is the synthesis of carbamoyl phosphate from an ammonium, derived primarily from glutamate via GDH, and CO2 (as bicarbonate) produced by mitochondrial respiration in liver.
- This reaction requires 2 molecules of ATP and is catalyzed by carbamoyl phosphate synthetase I (CPS I) ,rate limiting enzyme of the urea cycle.

Synthesis of carbamoyl phosphate



- .CPS 1 requires N-acetylglutamate as a cofactor.
- CPS 2, found in the cytosol, is involved in pyrimidine biosynthesis and does not require N-acetylglutamate, uses glutamine rather than ammonia as the nitrogen donor
- 1 mol of ATP serves as a phosphate donor

- Conversion of the second ATP to AMP and pyrophosphate, with the hydrolysis of pyrophosphate to ortophosphate,
- provides the driving force for the synthesis of the amide bond and the mixed acid anhyride bond of carbamayl phosphate.
- (high group transfer potential)

- Ornithine transcarbamoylase catalyses the condensation of carbamoyl phosphate with amino acid ornithine to form citrulline.
- Ornıthine plays a role resembling that of oxaloacetate in citric acid cycle, accepting material at each turn of cycle.

- Citrulline passes from mitochondrion to cyctosol and condenses with
- aspartate to form argininosuccinate.
- This step is catalyzed by argininosuccinate synthetase and requires ATP.
- The reaction cleaves ATP to AMP and PP
- which is hydrolyzed to two phosphate.

- The formation of argininosuccinate provides the second nitrogen of urea.
- Argininusuccinate is cleaved by argininosuccinase (reversible) to arginine and fumarate.

- Cleavage of arginine by arginase (cytosolic) releases urea and reforms ornithine.

- Ornithine reenters liver mitochondria for new urea synthesis.
- Ornithine and lysine are potent inhibitors of arginase.
- Urea diffuses into the blood, is transported to kidney and excreted in urine.

- Fumarate ,which enters the mitochondria, may be recycled through TCA cycle to oxaloacetate:

- Addition of H2O to fumarate forms L-malate and subsequent NAD+-dependent oxidation of malate in the mitochondrion forms oxaloacetate (malate dehydrogenase)
- Each NADH molecule can generate up to 2.5 ATP during mitochondrial respiration, greatly reducing the overall energetic cost of urea synthesis.

Aspartate-argininosuccinate shunt.

- Aspartate can be transported into cytosol, where it serves as a nitrogen donor in urea cycle ,reaction catalysed by argininosuccinate synthetase.
- This shunt provides metabolic links between separate pathways by which amino groups and carbon skeletons of AAs are processed.
- Thus the funneling of amino groups from other amino acids into glutamate and aspartate provides the nitrogen for urea synthesis.

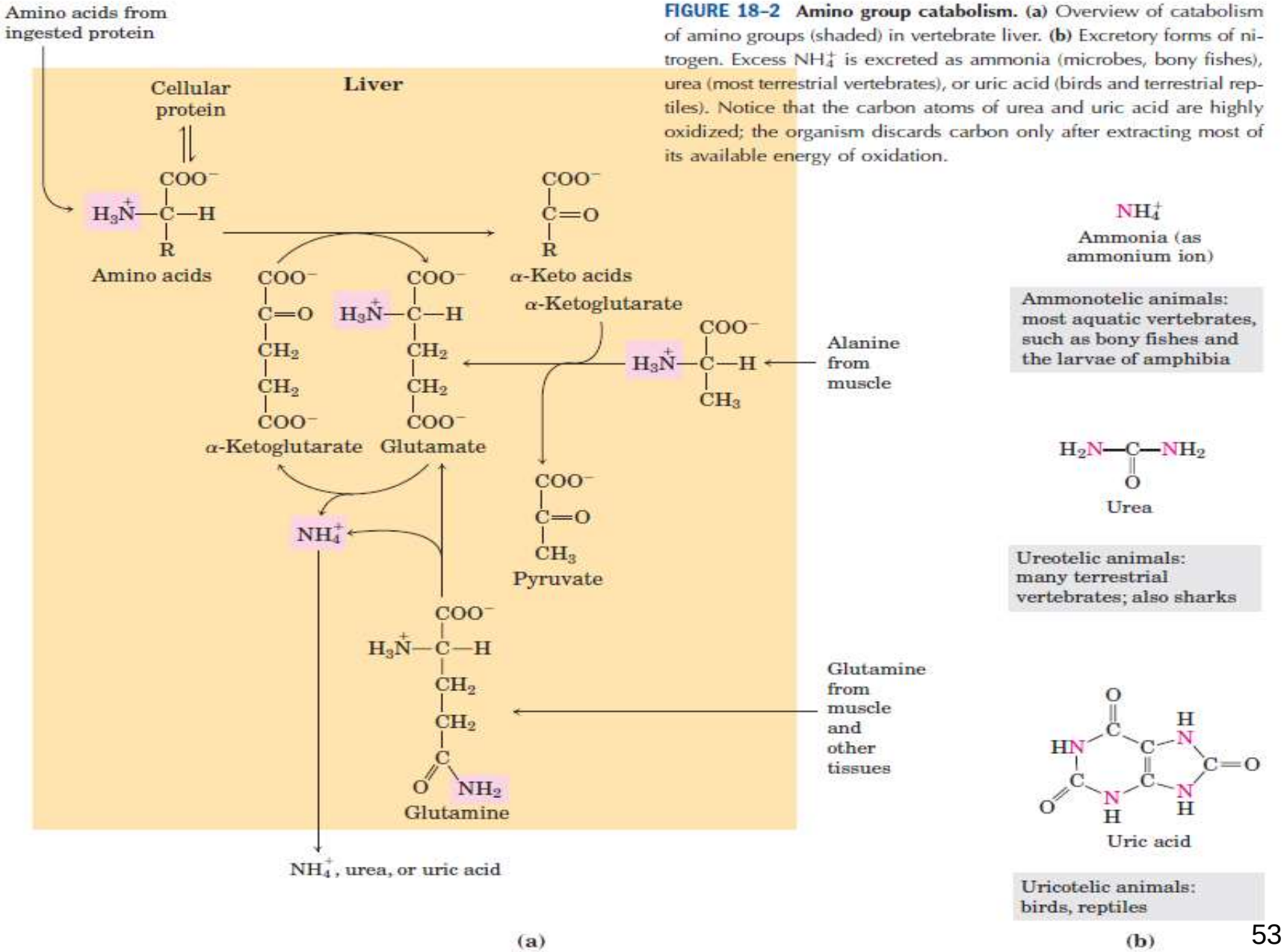
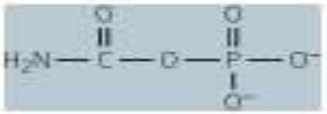
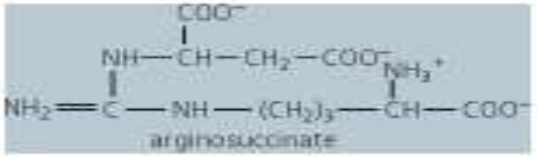
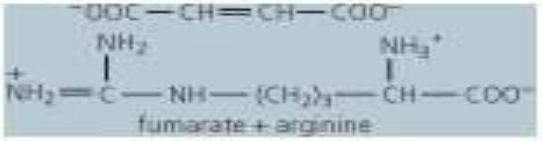
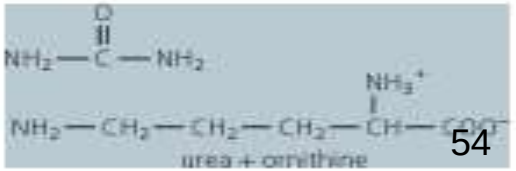


FIGURE 18–2 Amino group catabolism. (a) Overview of catabolism of amino groups (shaded) in vertebrate liver. **(b)** Excretory forms of nitrogen. Excess NH_4^+ is excreted as ammonia (microbes, bony fishes), urea (most terrestrial vertebrates), or uric acid (birds and terrestrial reptiles). Notice that the carbon atoms of urea and uric acid are highly oxidized; the organism discards carbon only after extracting most of its available energy of oxidation.

Enzymes of the urea cycle

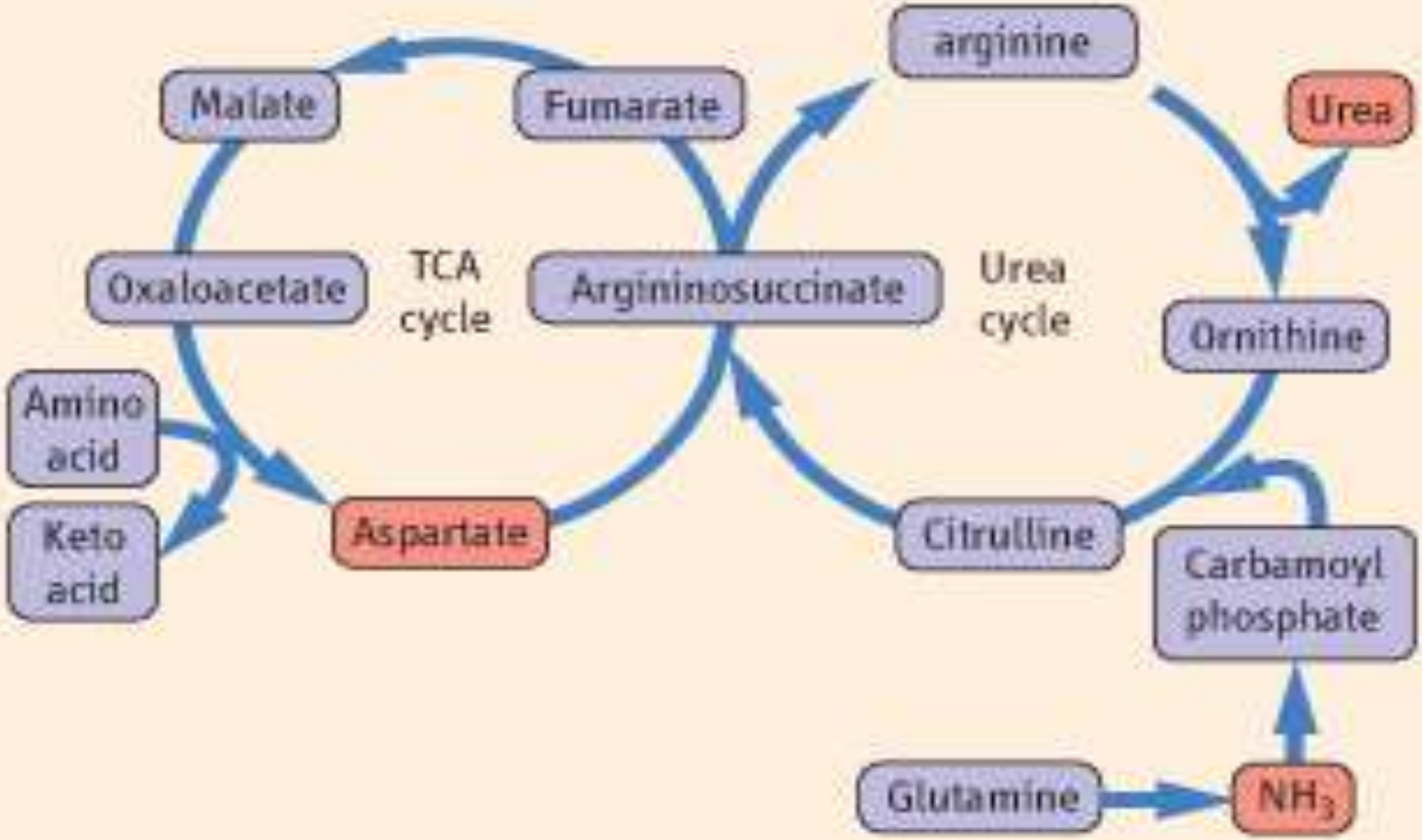
Enzyme	Reaction catalyzed	Remarks	Reaction Product
Carbamoyl phosphate synthetase	formation of carbamoyl phosphate from ammonia and CO_2	fixes ammonia released from amino acids, especially glutamine, uses 2 ATP, located in the **mitochondrion**, deficiency leads to high blood concentrations of ammonia and related toxicity	
Ornithine transcarbamoylase	formation of citrulline from ornithine and carbamoyl phosphate	releases P_i, an example of a transferase, located in the**mitochondrion**, deficiency leads to high blood concentrations of ammonia and orotic acid, as carbamoyl phosphate is shunted to pyrimidine biosynthesis	
Argininosuccinate synthetase	formation of argininosuccinate from citrulline and aspartate	requires ATP, which is cleaved to AMP + PP_i – an example of a ligase, located in the **cytosol**, deficiency leads to high blood concentrations of ammonia and citrulline	
Argininosuccinase	cleavage of argininosuccinate to arginine and fumarate	an example of a lyase, located in **cytosol**, deficiency leads to high blood concentrations of ammonia and citrulline	
Arginase	cleavage of arginine to ornithine and urea	an example of a hydrolase, located in the **cytosol** and primarily in the liver, deficiency leads to moderately increased blood ammonia and high blood concentrations of arginine	

Urea[Rx] synthesis

Component reactions in urea[Rx] synthesis

$CO_2 + NH_3 + 2\ ATP$	→	carbamoyl phosphate + 2 ADP + Pi
Carbamoyl phosphate + ornithine	→	citrulline + Pi
Citrulline + aspartate + ATP	→	arginosuccinate + AMP + PPi
Arginosuccinate	→	arginine + fumarate
Arginine	→	urea[Rx] + ornithine
$CO_2 + NH_3 + 3\ ATP$ + aspartate	→	urea[Rx] + 2 ADP + AMP + 2 Pi + PPi + fumarate

TCA and urea cycles



- The urea cycle is split between the mitochondrial matrix and the cytosol:
- The first 2 steps occur in the mitochondrion.
- Citrulline, which is formed in the mitochondrion, moves into the cytosol by a specific passive transport system.
- The cycle is completed in the cytosol.
- Ornithine, which is regenerated, is transported back across the mitochondrial membrane.

- Regulation of urea cycle:
- The urea cycle is regulated by N-acetylglutamate, the essential allosteric activator of CPS I.
- Arginine is an allosteric activator of N-acetylglutamate synthase and also a source of ornithine(via arginase) for urea cycle.

- The steady state levels of N-acetyl glutamate are determined by glutamate, acetyl coA and arginine
- Urea cycle enzymes increase or decrease in response to high or low-protein diet
- Starvation and high-protein diets elevate enzyme levels to cope with increased ammonia production that accompanies enhanced protein degradation.

- .Urea synthesis and excretion are decreased and NH_4^+ excreton is increased during acidosis to excrete protons into the urine.
- .Infants born with defects in any of the first 4 enzymes may appear normal at birth, but rapidly become lethargic, lose body temperature and may have difficulty in breathing.

- Blood NH_3 concentrations increase quickly, followed by cerebral edema.
- Clinical symptoms include vomiting intermittent ataxia, irritability, lethargy and mental retardation.
- Ornithine transcarbamoylase deficiency is the most common defect and shows x-linked inheritence pattern.

- Other enzyme deficiencies are autosomal recessive.
- Hemodialysis must be applied to individuals with high ammonia concentrations, followed by IV Na benzoate and phenyllactate administration.

- These compounds are conjugated with glycine and glutamine respectively to form water-soluble adducts, trapping ammonia in a nontoxic form that can be excreted in the urine(hippurate)