Amino acid metabolism and Urea Cycle

M.Sc. Biochemistry
1st Yr/2nd Sem

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Amino acid metabolism & Urea cycle

Objectives:

To acquaint the students about:

i) Understanding the metabolic fates of amino acids, transamination, deamination

ii) Glucose-alanine cycle

iii) Urea cycle and its regulation

Amino Acid Metabolism

Amino acids derived from dietary protein are the source of most amino groups. Most amino acids are metabolized in the liver. Some of the ammonia generated in this process is recycled and used in a variety of biosynthetic pathways; the excess is either excreted directly or converted to urea or uric acid for excretion, depending on the organism. Excess ammonia generated in other (extrahepatic) tissues travels to the liver (in the form of amino groups for conversion to the excretory form.

Glutamate and glutamine play especially critical roles in nitrogen metabolism, acting as a kind of general collection point for amino groups. In the cytosol of hepatocytes, amino groups from most amino acids are transferred to -ketoglutarate to form glutamate, which enters mitochondria and gives up its amino group to form NH4. Excess ammonia generated in most other tissues is converted to the amide nitrogen of glutamine, which passes to the liver, then into liver mitochondria. Glutamine or glutamate or both are present in higher concentrations than other amino acids in most tissues.

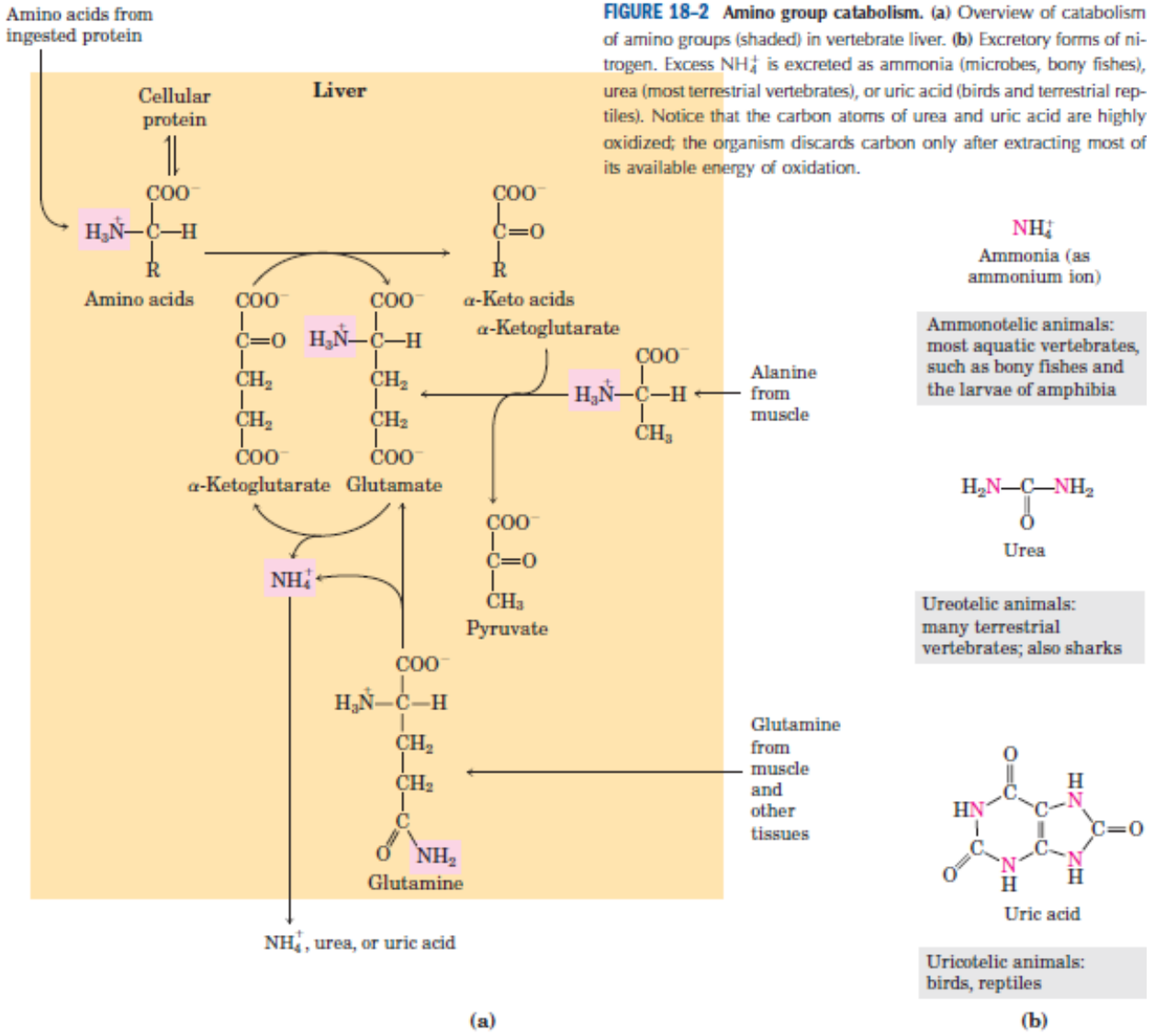


FIGURE 18–2 **Amino group catabolism.** (a) Overview of catabolism of amino groups (shaded) in vertebrate liver. (b) Excretory forms of nitrogen. Excess NH_4^+ is excreted as ammonia (microbes, bony fishes), urea (most terrestrial vertebrates), or uric acid (birds and terrestrial reptiles). Notice that 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.

In skeletal muscle, excess amino groups are generally transferred to pyruvate to form alanine, another important molecule in the transport of amino groups to the liver.

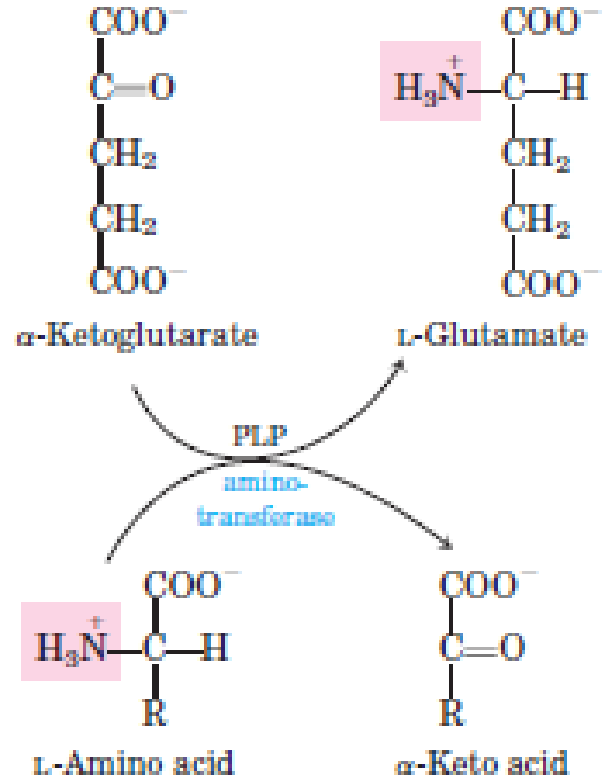
Pyridoxal Phosphate Participates in the Transfer of α-Amino Groups to α-Ketoglutarate

The first step in the catabolism of most L-amino acids, once they have reached the liver, is removal of the α-amino groups, promoted by enzymes called **aminotransferases** or **transaminases.** In these **transamination** reactions, the α-amino group is transferred to the α-carbon atom of α-ketoglutarate, leaving behind the corresponding α-keto acid analog of the amino acid. There is no net deamination (loss of amino groups) in these reactions, because the α–ketoglutarate becomes aminated as the α-amino acid is deaminated.

The effect of transamination reactions is to ***collect amino groups from many different amino acids in form of L-glutamate***. The glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways leading to elimination of nitrogenous waste products.

Cells contain different types of aminotransferases. Many are specific for α-ketoglutarate as the amino group acceptor but differ in their specificity for the L-amino acid. The enzymes are named for the amino group donor (alanine aminotransferase, aspartate aminotransferase, for example). The reactions catalyzed by aminotransferases are freely reversible, having an equilibrium constant of about 1.0 (G 0 kJ/mol).

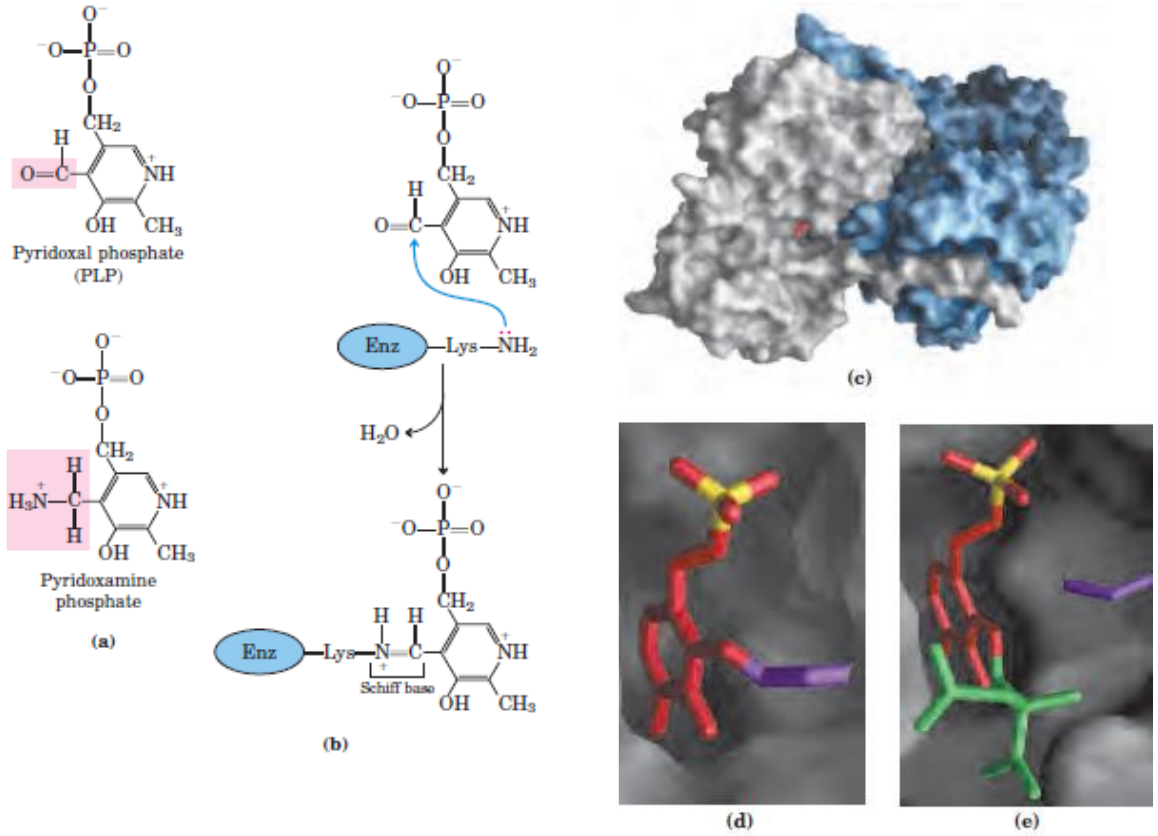
All aminotransferases have the same prosthetic group and the same reaction mechanism. The prosthetic group is **pyridoxal phosphate (PLP),** the coenzyme form of pyridoxine, or vitamin B6. Its primary role in cells is in the metabolism of molecules with amino groups.



Enzyme-catalyzed transaminations. In many aminotransferase reactions, α-ketoglutarate is the amino group acceptor. All aminotransferases have pyridoxal phosphate (PLP) as cofactor. The reaction is readily reversible.

Pyridoxal phosphate functions as an intermediate carrier of amino groups at the active site of aminotransferases. It undergoes reversible transformations between its aldehyde form, pyridoxal phosphate, which can accept an amino group, and its aminated form, pyridoxamine phosphate, which can donate its amino group to an α-keto acid (Fig. a). Pyridoxal phosphate is generally covalently bound to the enzyme's active site through an aldimine (Schiff base) linkage to the α-amino group of a Lys residue (Fig. b, d).

Pyridoxal phosphate participates in a variety of reactions at the α, β, and γ carbons (C-2 to C-4) of amino acids. Reactions at the carbon include racemizations (interconverting L- and D-amino acids) and decarboxylations, as well as transaminations. Pyridoxal phosphate plays the same chemical role in each of these reactions. A bond to the carbon of the substrate is broken, removing either a proton or a carboxyl group. The electron pair left behind on the carbon would form a highly unstable carbanion, but pyridoxal phosphate provides resonance stabilization of this intermediate. The highly conjugated structure of PLP (an electron sink) permits delocalization of the negative charge.



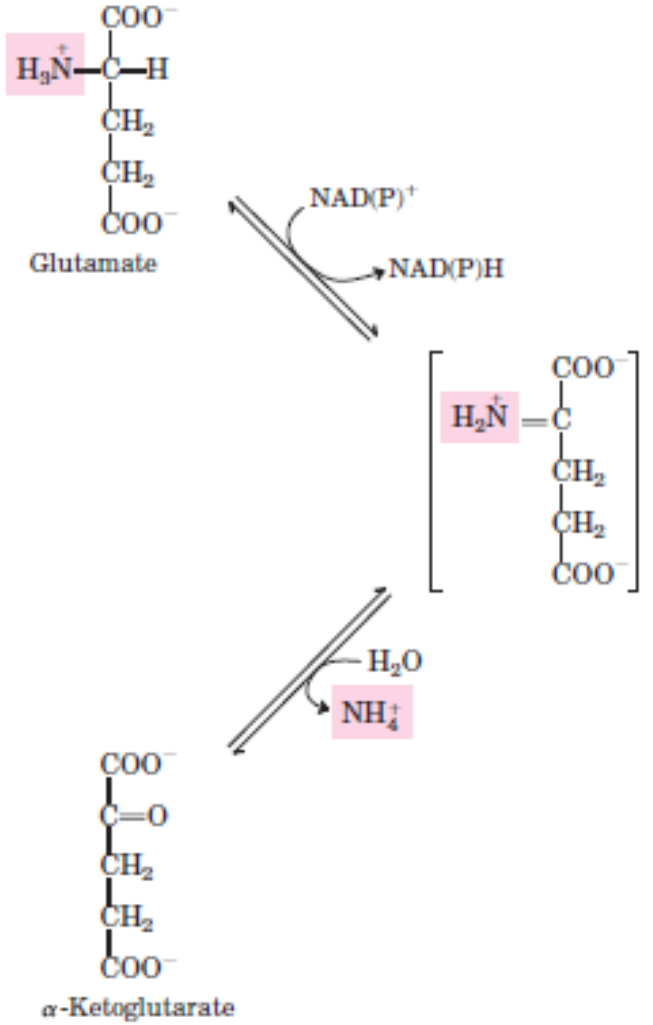
Pyridoxal phosphate, the prosthetic group of aminotransferases.
(a) Pyridoxal phosphate (PLP) and its aminated form, pyridoxamine phosphate, are the tightly bound coenzymes of aminotransferases. The functional groups are shaded. **(b)** Pyridoxal phosphate is bound to the enzyme through noncovalent interactions and a Schiffbase linkage to a Lys residue at the active site. The steps in the formation of a Schiff base from a primary amine and a carbonyl group are detailed in Figure 14–5. **(c)** PLP (red) bound to one of the two active sites of the dimeric enzyme aspartate aminotransferase, a typical aminotransferase; **(d)** close-up view of the active site, with PLP (red, with yellow phosphorus) in aldimine linkage with the side chain of Lys258 (purple); **(e)** another close-up view of the active site, with PLP linked to the substrate analog 2-methylaspartate (green) via a Schiff base (PDB ID 1AJS).

Glutamate Releases Its Amino Group as Ammonia in the Liver

As we have seen, the amino groups from many of the α-amino acids are collected in the liver in the form of the amino group of L-glutamate molecules. These amino groups must next be removed from glutamate to prepare them for excretion. In hepatocytes, glutamate is transported from the cytosol into mitochondria, where it undergoes **oxidative deamination** catalyzed by **L-glutamate dehydrogenase** (Mr 330,000). In mammals, this enzyme is present in the *mitochondrial matrix* and can use either NAD or NADP as the acceptor of reducing equivalents.

The combined action of an aminotransferase and glutamate dehydrogenase is referred to as **transdeamination**. A few amino acids bypass the transdeamination pathway and undergo direct oxidative deamination. The fate of the NH4 produced by any of these deamination processes is discussed in detail in Section 18.2. The α-ketoglutarate formed from glutamate deamination can be used in the citric acid cycle and for glucose synthesis.

Glutamate dehydrogenase operates at an important intersection of carbon and nitrogen metabolism. An allosteric enzyme with six identical subunits, its activity is influenced by a complicated array of allosteric modulators. The best-studied of these are the positive modulator ADP and the negative modulator GTP. The metabolic rationale for this regulatory pattern has not been elucidated in detail. Mutations that alter the allosteric binding site for GTP or otherwise cause permanent activation of glutamate dehydrogenase lead to a human genetic disorder called hyperinsulinism-hyperammone syndrome, characterized by elevated levels of ammonia in the bloodstream and hypoglycemia.

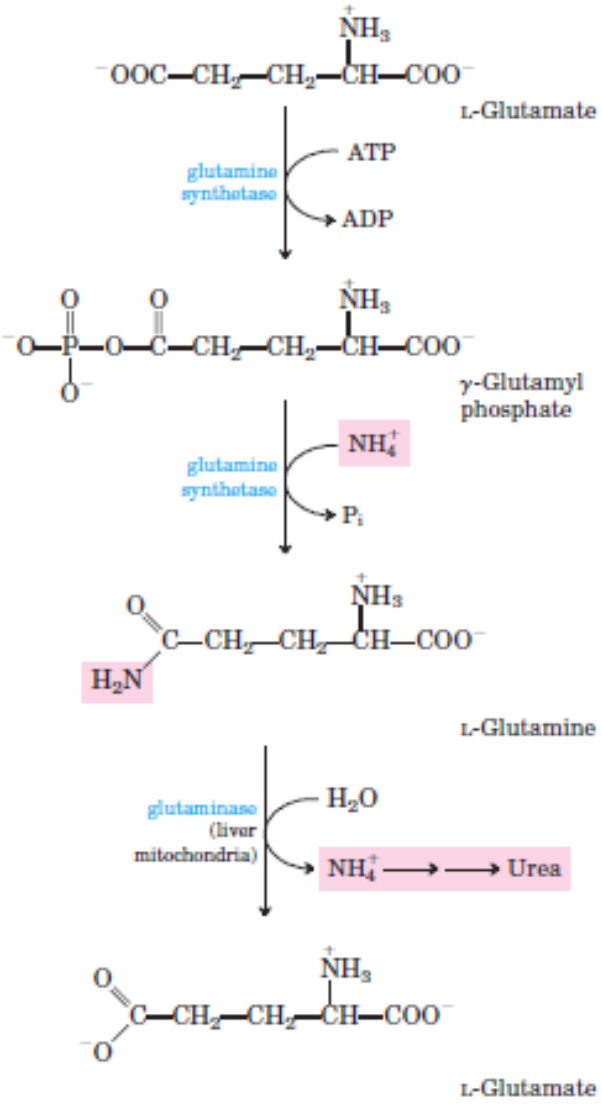


Reaction catalyzed by glutamate dehydrogenase. The glutamate dehydrogenase of mammalian liver has the unusual capacity to use either NAD or NADP as cofactor. The glutamate dehydrogenases of plants and microorganisms are generally specific for one or the other. The mammalian enzyme is allosterically regulated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

Glutamine Transports Ammonia in the Bloodstream

Ammonia is toxic to animal tissues , and the levels present in blood are regulated. In many tissues, including brain, some processes as nucleotide degradation generate free ammonia. In most animals ammonia is converted to a nontoxic compound before export from extrahepatic tissues into blood and transport to liver or kidneys. For this transport, glutamate, critical to intracellular amino group metabolism, is supplanted by L-glutamine. The free ammonia produced in tissues is combined with glutamate to yield glutamine by the action of **glutamine synthetase**. This reaction requires ATP and occurs in 2 steps. Ist, glutamate and ATP react to form ADP and a γ-glutamyl phosphate intermediate, which then reacts with ammonia to produce glutamine and inorganic phosphate. *Glutamine is a nontoxic transport form of ammonia*; it is normally present in blood in much higher concentrations than other amino acids, and also serves as a source of amino groups in various biosynthetic reactions. Glutamine synthetase is found in all organisms, playing a central metabolic role. In microorganisms, the enzyme serves as an essential portal for the entry of fixed N_2 into biological systems.

In most terrestrial animals, excess glutamine (more than required for biosynthesis) is transported in blood to intestine, liver, and kidneys for processing, where, the amide nitrogen is released as NH_4^+ in mitochondria, where the enzyme **glutaminase** converts glutamine to glutamate and NH_4^+. The NH_4^+ from intestine and kidney is transported in blood to liver. In the liver, ammonia from all sources is disposed of by urea synthesis. Some of the glutamate produced in the glutaminase reaction may be further processed in the liver by glutamate dehydrogenase, releasing more ammonia and producing carbon skeletons for metabolic fuel. However, most glutamate enters the transamination reactions required for amino acid biosynthesis and other processes.



Ammonia transport in the form of glutamine. Excess ammonia in tissues is added to glutamate to form glutamine, a process catalyzed by glutamine synthetase. After transport in the bloodstream, the glutamine enters the liver and NH4 is liberated in mitochondria by the enzyme glutaminase.

In metabolic acidosis there is an increase in glutamine processing by the kidneys. Not all the excess NH_4^+ thus produced is released into the bloodstream or converted to urea; some is excreted directly into the urine. In the kidney, the NH_4^+ forms salts with metabolic acids, facilitating their removal in the urine. Bicarbonate produced by the decarboxylation of α-ketoglutarate in the citric acid cycle can also serve as a buffer in blood plasma. Taken together, these effects of glutamine metabolism in the kidney tend to counteract acidosis. ■

BOX 18–1 BIOCHEMISTRY IN MEDICINE

Assays for Tissue Damage

Analyses of certain enzyme activities in blood serum give valuable diagnostic information for a number of disease conditions.

Alanine aminotransferase (ALT; also called glutamate-pyruvate transaminase, GPT) and aspartate aminotransferase (AST; also called glutamate-oxaloacetate transaminase, GOT) are important in the diagnosis of heart and liver damage caused by heart attack, drug toxicity, or infection. After a heart attack, a variety of enzymes, including these aminotransferases, leak from the injured heart cells into the bloodstream. Measurements of the blood serum concentrations of the two aminotransferases by the SGPT and SGOT tests (S for serum)—and of another enzyme, **creatine kinase,** by the SCK test—can provide information about the severity of the damage. Creatine kinase is the first heart enzyme to appear in the blood after a heart attack; it also disappears quickly from the blood. GOT is the next to appear, and GPT follows later. Lactate dehydrogenase also leaks from injured or anaerobic heart muscle.

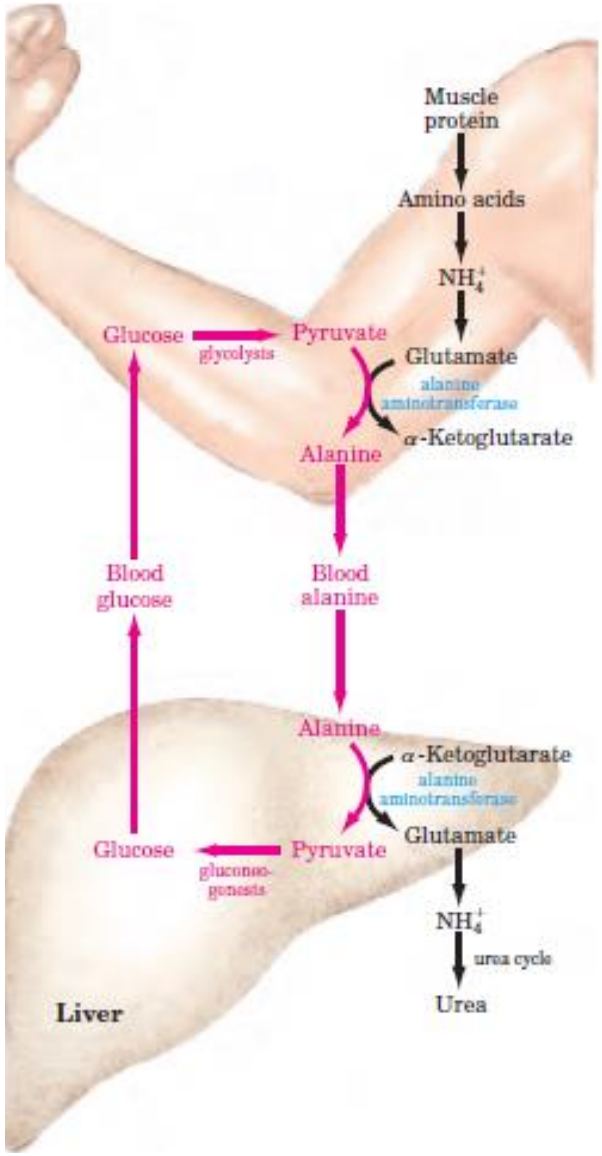
The SGOT and SGPT tests are also important in occupational medicine, to determine whether people exposed to carbon tetrachloride, chloroform, or other industrial solvents have suffered liver damage. Liver degeneration caused by these solvents is accompanied by leakage of various enzymes from injured hepatocytes into the blood. Aminotransferases are most useful in the monitoring of people exposed to these chemicals, because these enzyme activities are high in liver and can be detected in very small amounts.

Alanine Transports Ammonia from Skeletal Muscles to the Liver

Alanine also plays a special role in transporting amino groups to the liver in a nontoxic form, via a pathway called the glucose-alanine cycle. In muscle and certain other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can be converted to glutamine for transport to the liver, as described above, or it can transfer its -amino group to pyruvate, a readily available product of muscle glycolysis, by the action of alanine aminotransferase. The alanine so formed passes into the blood and travels to the liver. In the cytosol of hepatocytes, alanine aminotransferase transfers the amino group from alanine to -ketoglutarate, forming pyruvate and glutamate. Glutamate can then enter mitochondria, where the glutamate dehydrogenase reaction releases $NH4^+$, or can undergo transamination with oxaloacetate to form aspartate, another nitrogen donor in urea synthesis.

The use of alanine to transport ammonia from skeletal muscles to the liver is another example of the intrinsic economy of living organisms. Vigorously contracting skeletal muscles operate anaerobically, producing pyruvate and lactate from glycolysis as well as ammonia from protein breakdown. These products must find their way to the liver, where pyruvate and lactate are incorporated into glucose, which is returned to the muscles, and ammonia is converted to urea for excretion.

The glucose-alanine cycle, in concert with the Cori cycle, accomplishes this transaction. The energetic burden of gluconeogenesis is thus imposed on the liver rather than the muscle, and all available ATP in muscle is devoted to muscle contraction.



Glucose-alanine cycle. Alanine serves as a carrier of ammonia and of the carbon skeleton of pyruvate from skeletal muscle to liver. The ammonia is excreted and the pyruvate is used to produce glucose, which is returned to the muscle.

SUMMARY Metabolic Fates of Amino Groups

- An early step in the catabolism of amino acids is the separation of the amino group from the carbon skeleton. In most cases, the amino group is transferred to α-ketoglutarate to form glutamate. This transamination reaction requires the coenzyme pyridoxal phosphate.

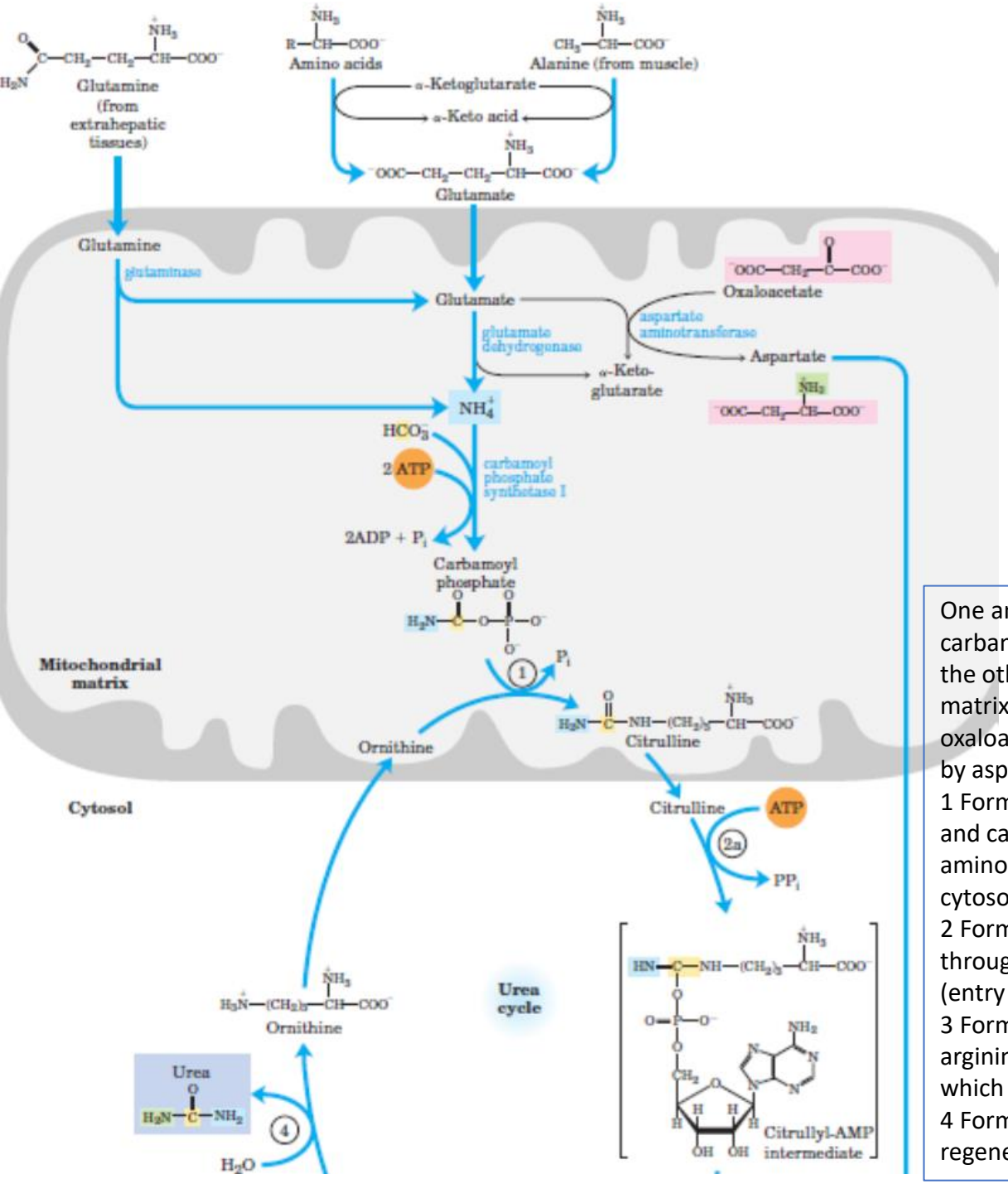
- Glutamate is transported to liver mitochondria, where glutamate dehydrogenase liberates the amino group as ammonium ion (NH_4^+).
Ammonia formed in other tissues is transported to the liver as the amide nitrogen of glutamine or, in transport from skeletal muscle, as the amino group of alanine.

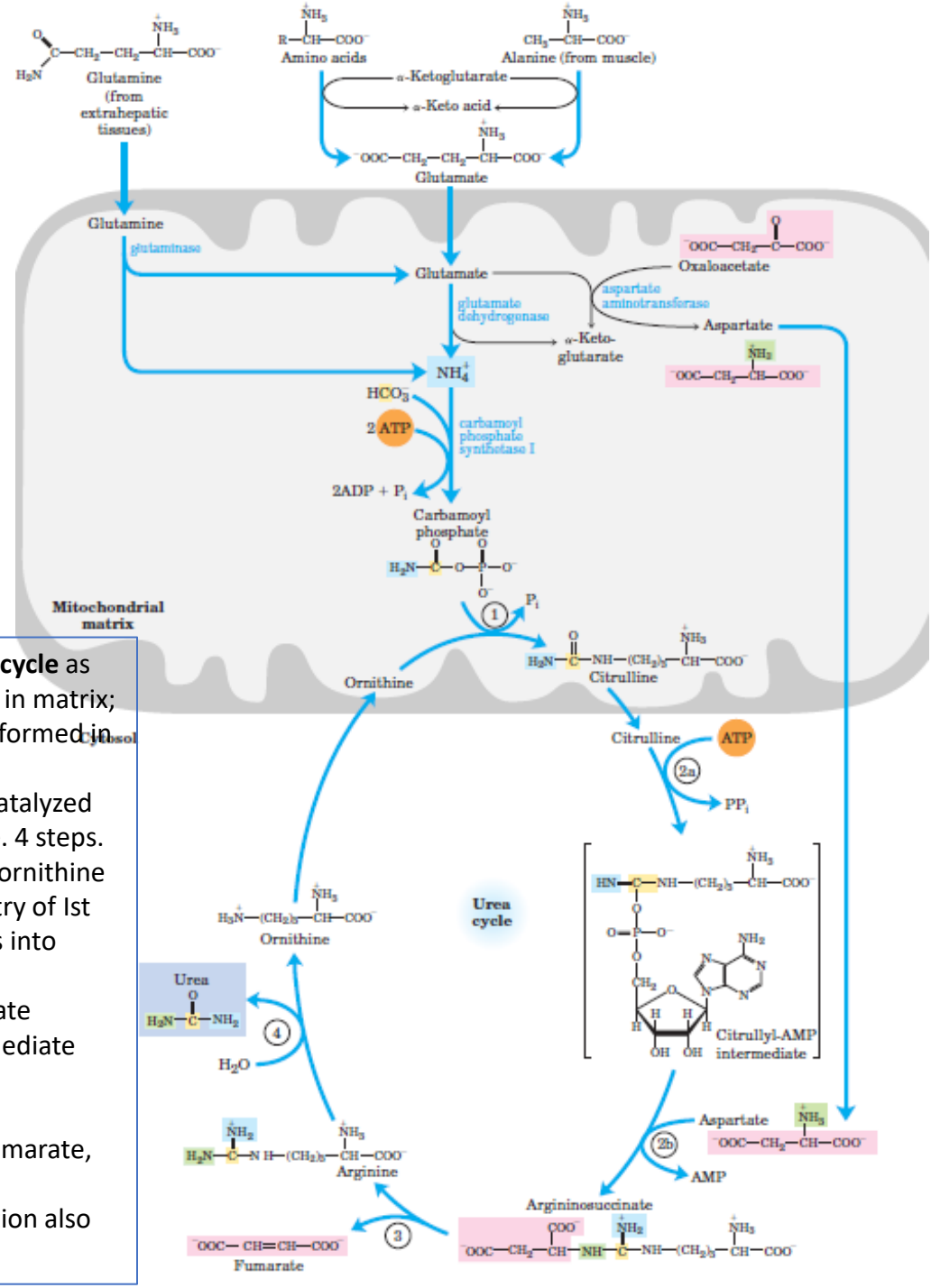
- The pyruvate produced by deamination of alanine in the liver is converted to glucose, which is transported back to muscle as part of the glucose-alanine cycle.

NITROGEN EXCRETION AND UREA CYCLE

If not reused for the synthesis of new amino acids or other nitrogenous products, amino groups are channeled into a single excretory end product (Fig. 18–10). Most aquatic species, such as the bony fishes, are ammonotelic, excreting amino nitrogen as ammonia. The toxic ammonia is simply diluted in the surrounding water. Terrestrial animals require pathways for nitrogen excretion that minimize toxicity and water loss. Most terrestrial animals are ureotelic, excreting amino nitrogen in the form of urea; birds and reptiles are uricotelic, excreting amino nitrogen as uric acid. Plants recycle virtually all amino groups, and nitrogen excretion occurs only under very unusual circumstances.

In ureotelic organisms, the ammonia deposited in the mitochondria of hepatocytes is converted to urea in the **urea cycle**. This pathway was discovered in **1932 by Hans Krebs** (who later also discovered the citric acid cycle) and a medical student associate, Kurt Henseleit. Urea production occurs almost exclusively in the **liver** and is the fate of most of the ammonia channeled there. The urea passes into the bloodstream and thus to the kidneys and is excreted into the urine.





One amino group enters **Urea cycle** as carbamoyl phosphate, formed in matrix; the other enters as aspartate, formed in matrix by transamination of oxaloacetate and glutamate, catalyzed by aspartate aminotransferase. 4 steps.

1 Formation of citrulline from ornithine and carbamoyl phosphate (entry of Ist amino group); citrulline passes into cytosol.

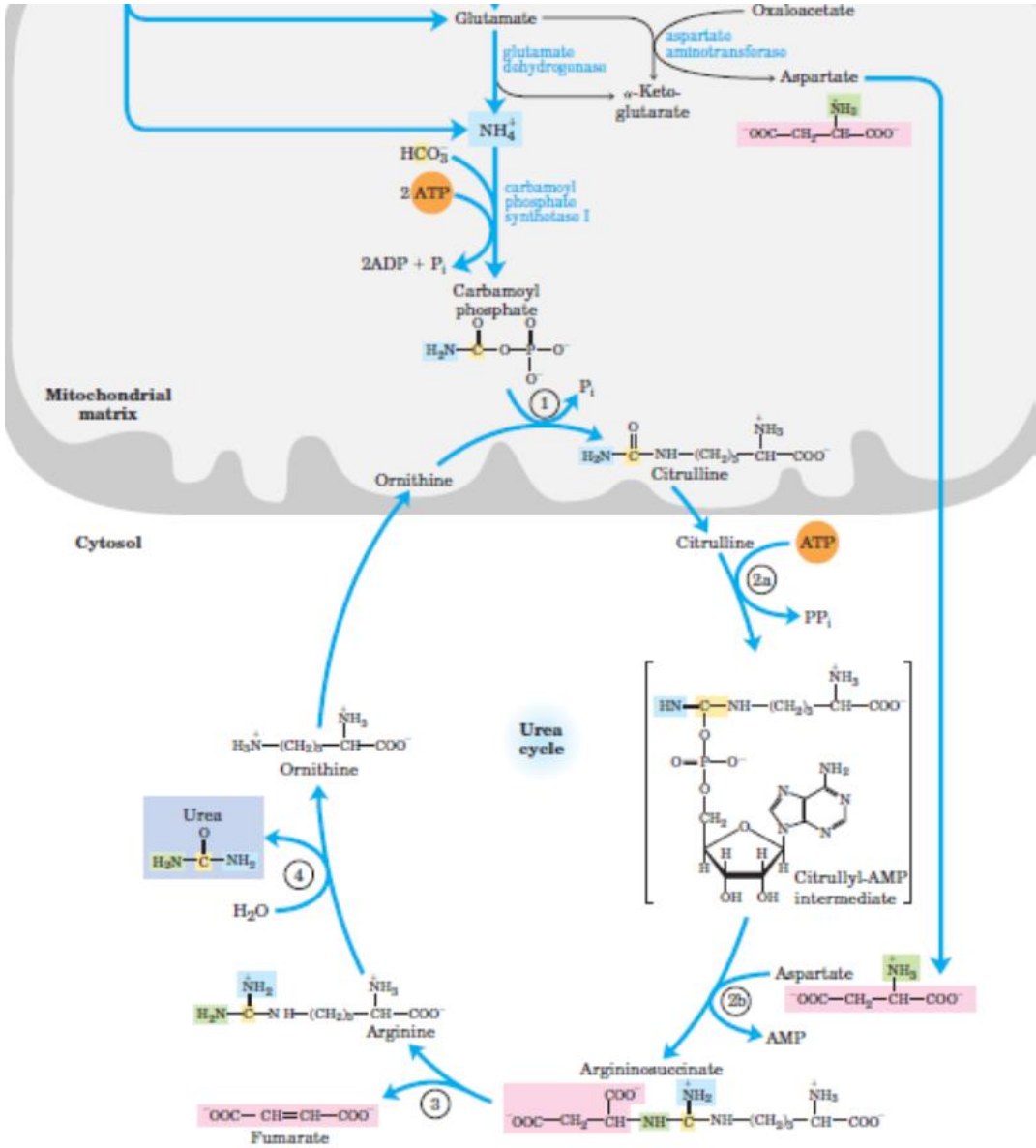
2 Formation of argininosuccinate through a citrullyl-AMP intermediate (entry of 2nd amino group).

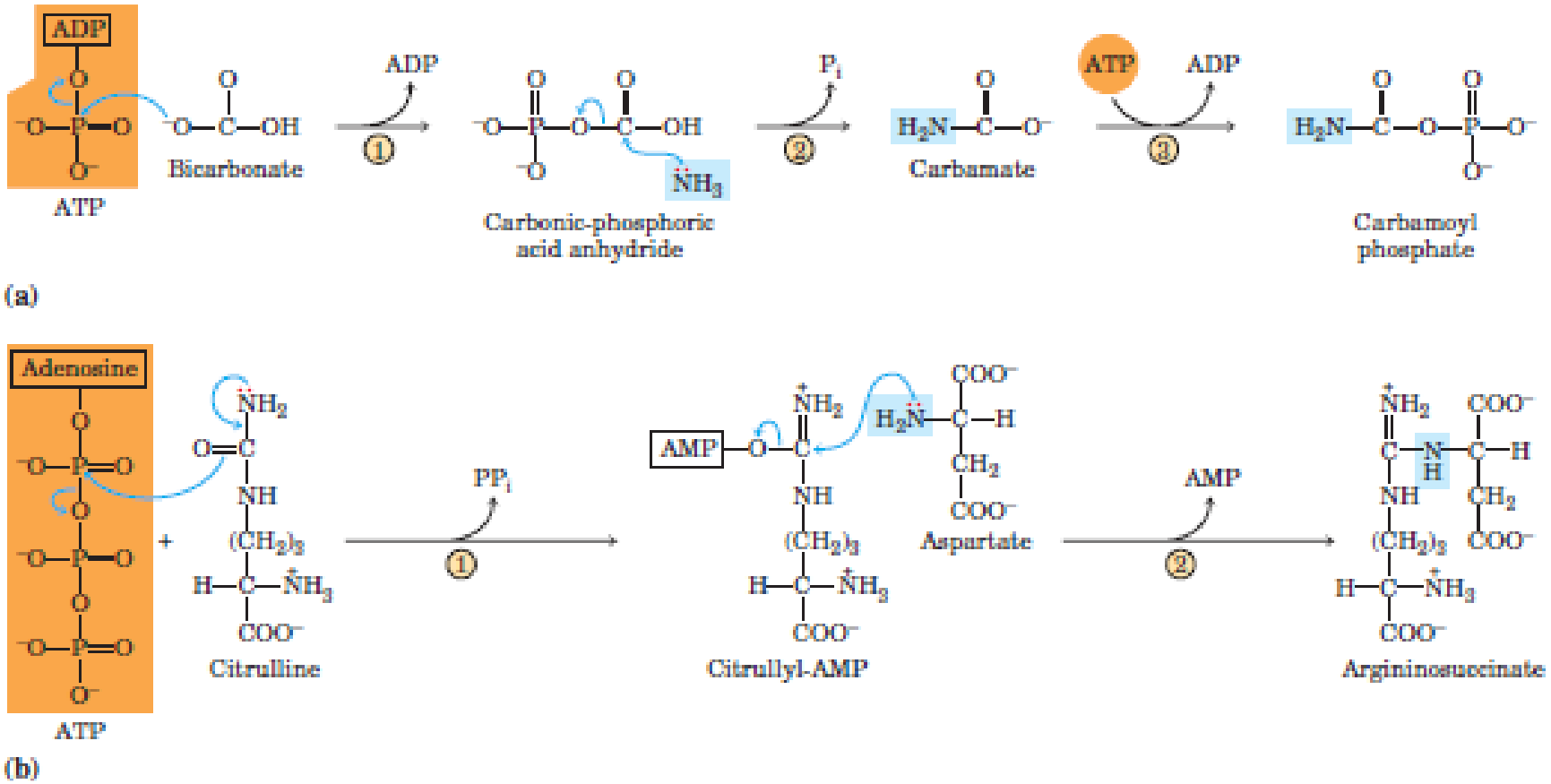
3 Formation of arginine from argininosuccinate; releasing fumarate, which enters citric acid cycle.

4 Formation of urea; this reaction also regenerates, ornithine.

Urea Is Produced from Ammonia in Five Enzymatic Steps: The urea cycle begins inside liver mitochondria, but three subsequent steps take place in cytosol; the cycle thus spans two cellular compartments. The first amino group to enter urea cycle is derived from ammonia in mitochondrial matrix. The liver also receives some ammonia via portal vein from intestine, from bacterial oxidation of amino acids.

NH_4 generated in liver mitochondria is immediately used, together with CO_2 (as HCO_3) produced by mitochondrial respiration, to form carbamoyl phosphate in matrix in an ATP-dependent reaction, catalyzed by **carbamoyl phosphate synthetase I**. (The cytosolic (II) form,functions in pyrimidine biosynthesis.) The carbamoyl phosphate, which functions as an activated carbamoyl group donor, now enters urea cycle, that has four enzymatic steps. Ist, carbamoyl phosphate donates its carbamoyl group to ornithine to form citrulline, with the release of P_i **(1)**, (catalyzed by **ornithine transcarbamoylase**), and citrulline passes from mitochondrion to cytosol. The second amino group now enters from aspartate (generated in mitochondria by transamination and transported into cytosol) by a condensation reaction between amino group of aspartate and ureido (carbonyl) group of citrulline, forming argininosuccinate **(2)**, catalyzed by **argininosuccinate synthetase,** requires ATP and proceeds through a citrullyl-AMPintermediate. The argininosuccinate is then cleaved by **argininosuccinase (3)** to form arginine and fumarate, latter entering mitochondria to join the pool of citric acid cycle intermediates. This is the only reversible step in urea cycle. In last reaction (step **4**), cytosolic **arginase** cleaves arginine to yield **urea** and ornithine. Ornithine is transported into mitochondrion to initiate another round of urea cycle.



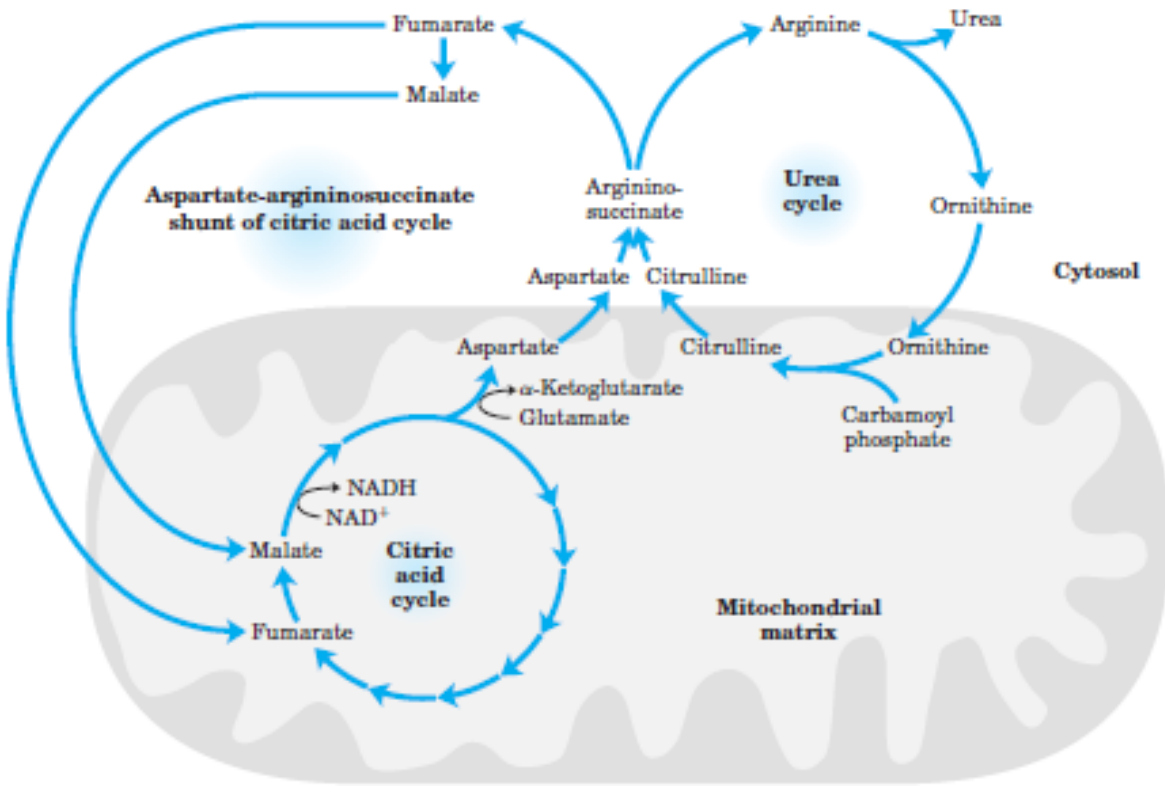


MECHANISM FIGURE 18–11 Nitrogen-acquiring reactions in the synthesis of urea. The urea nitrogens are acquired in two reactions, each requiring ATP. **(a)** In the reaction catalyzed by carbamoyl phosphate synthetase I, the first nitrogen enters from ammonia. The terminal phosphate groups of two molecules of ATP are used to form one molecule of carbamoyl phosphate. In other words, this reaction has two activation steps (1 and 3). Carbamoyl Phosphate Synthetase I Mechanism **(b)** In the reaction catalyzed by argininosuccinate synthetase, the second nitrogen enters from aspartate. The ureido oxygen of citrulline is activated by the addition of AMP in step 1 ; this sets up the addition of aspartate in step 2 , with AMP (including the ureido oxygen) as the leaving group. Argininosuccinate Synthetase Mechanism

The Citric Acid and Urea Cycles Can Be Linked

Because the fumarate produced in the argininosuccinase reaction is also an intermediate of the citric acid cycle, the cycles are, in principle, interconnected—in a process dubbed the "Krebs bicycle" (Fig. 18–12). However, each cycle can operate independently and communication between them depends on the transport of key intermediates between the mitochondrion and cytosol.

Several enzymes of the citric acid cycle, including fumarase (fumarate hydratase) and malate dehydrogenase, are also present as isozymes in the cytosol. The fumarate generated in cytosolic arginine synthesis can therefore be converted to malate in the cytosol, and these intermediates can be further metabolized in the cytosol or transported into mitochondria for use in the citric acid cycle. Aspartate formed in mitochondria by transamination between oxaloacetate and glutamate can be transported to the cytosol, where it serves as nitrogen donor in the urea cycle reaction catalyzed by argininosuccinate synthetase. These reactions, making up the **aspartate-argininosuccinate shunt,** provide metabolic links between the separate pathways by which the amino groups and carbon skeletons of amino acids are processed.

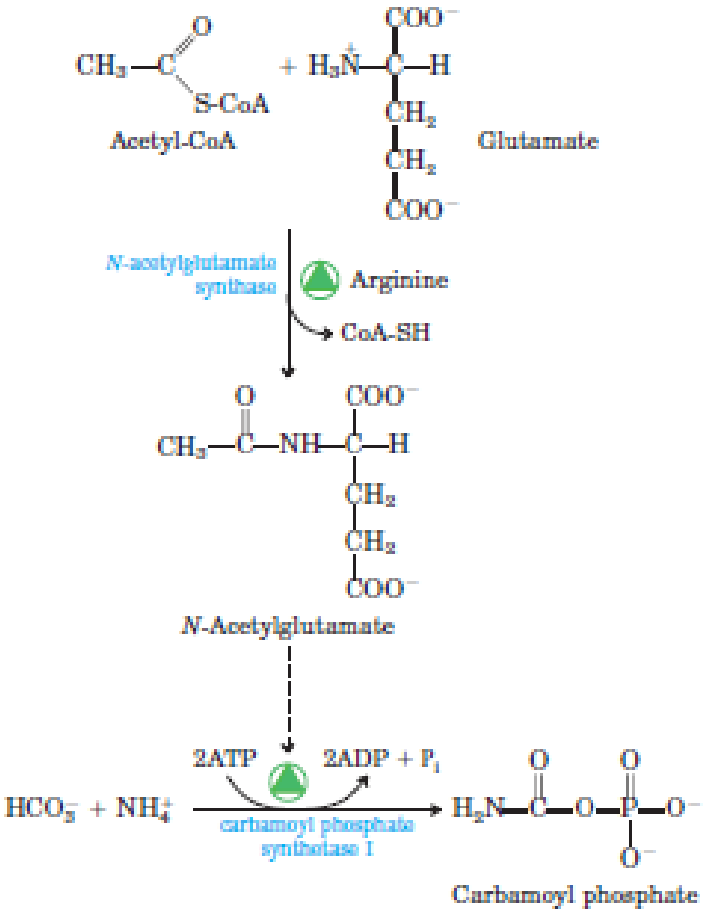


Links between the urea cycle and citric acid cycle.
The interconnected cycles have been called the "Krebs bicycle." The pathways linking the citric acid and urea cycles are called the aspartate-argininosuccinate shunt; these effectively link the fates of the amino groups and the carbon skeletons of amino acids. The interconnections are even more elaborate than the arrows suggest. For example, some citric acid cycle enzymes, such as fumarase and malate dehydrogenase, have both cytosolic and mitochondrial isozymes. Fumarate produced in the cytosol—whether by the urea cycle, purine biosynthesis, or other processes—can be converted to cytosolic malate, which is used in the cytosol or transported into mitochondria (via the malate-aspartate shuttle) to enter the citric acid cycle.

The Activity of the Urea Cycle Is Regulated at Two Levels

The flux of nitrogen through the urea cycle in an individual animal varies with diet. When the dietary intake is primarily protein, the carbon skeletons of amino acids are used for fuel, producing much urea from the excess amino groups. During prolonged starvation, when breakdown of muscle protein begins to supply much of the organism's metabolic energy, urea production also increases substantially. These changes in demand for urea cycle activity are met over the long term by regulation of the rates of synthesis of the four urea cycle enzymes and carbamoyl phosphate synthetase I in the liver. *All five enzymes are synthesized at higher rates in starving animals and in animals on very-high-protein diets than in well-fed animals eating primarily carbohydrates and fats. Animals on protein-free diets produce lower levels of urea cycle enzymes.*

On a shorter time scale, allosteric regulation of at least one key enzyme adjusts the flux through the urea cycle. The first enzyme in the pathway, carbamoyl phosphate synthetase I, is allosterically activated by ***N*-acetylglutamate,** which is synthesized from acetyl-CoA and glutamate by ***N*-acetylglutamate synthase**. In plants and microorganisms this enzyme catalyzes the first step in the *de novo* synthesis of arginine from glutamate, but in mammals *N*-acetylglutamate synthase activity in the liver has a purely regulatory function (mammals lack the other enzymes needed to convert glutamate to arginine). The steady-state levels of *N*-acetylglutamate are determined by the concentrations of glutamate and acetyl-CoA (the substrates for *N*-acetylglutamate synthase) and arginine (an activator of *N*-acetylglutamate synthase, and thus an activator of the urea cycle).



Synthesis of *N*-acetylglutamate and its activation of carbamoyl phosphate synthetase I.

Pathway Interconnections Reduce the Energetic Cost of Urea Synthesis

If we consider the urea cycle in isolation, we see that the synthesis of one molecule of urea requires four high energy phosphate group). Two ATP molecules are required to make carbamoyl phosphate, and one ATP to make argininosuccinate—the latter ATP undergoing a pyrophosphate cleavage to AMP and PP_i, which is hydrolyzed to two P_i. The overall equation of the urea cycle is

$$2NH_4^+ + HCO_3^- + 3ATP^{4-} + H_2O \longrightarrow \text{urea} + 2ADP^{3-} + 4P_i^{2-} + AMP^{2-} + 2H^+$$

However, the urea cycle also causes a net conversion of oxaloacetate to fumarate (via aspartate), and the regeneration of oxaloacetate produces NADH in the malate dehydrogenase reaction. Each NADH molecule can generate up to 2.5 ATP during mitochondrial respiration, greatly reducing the overall energetic cost of urea synthesis.

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

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