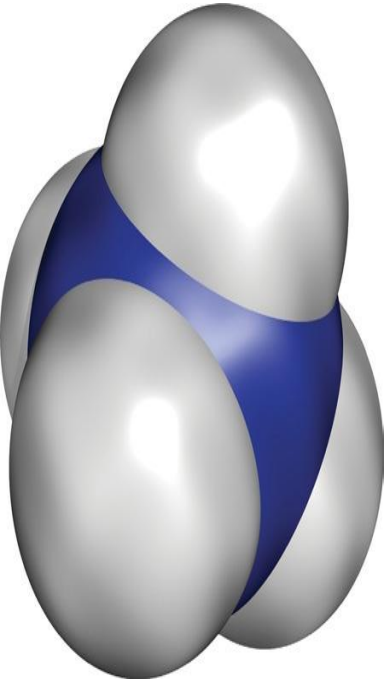
NITROGEN METABOLISM



Nitrogen Fixation and Assimilation
Amino Acid Biosynthesis
Nucleotide Biosynthesis

Amino Acid Catabolism

Nitrogen Disposal: The Urea Cycle

For

Forth stage – Chemistry dept.

Professor

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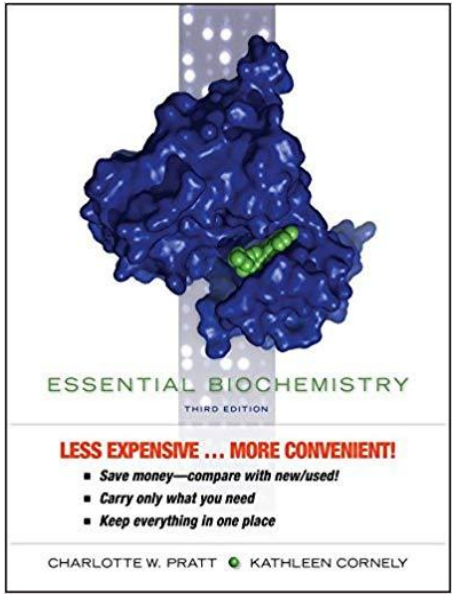


References :

1-Essential Biochemistry, Charlotte W. Pratt , Kathleen Cornely , , Third edition (2014).

Syllabus

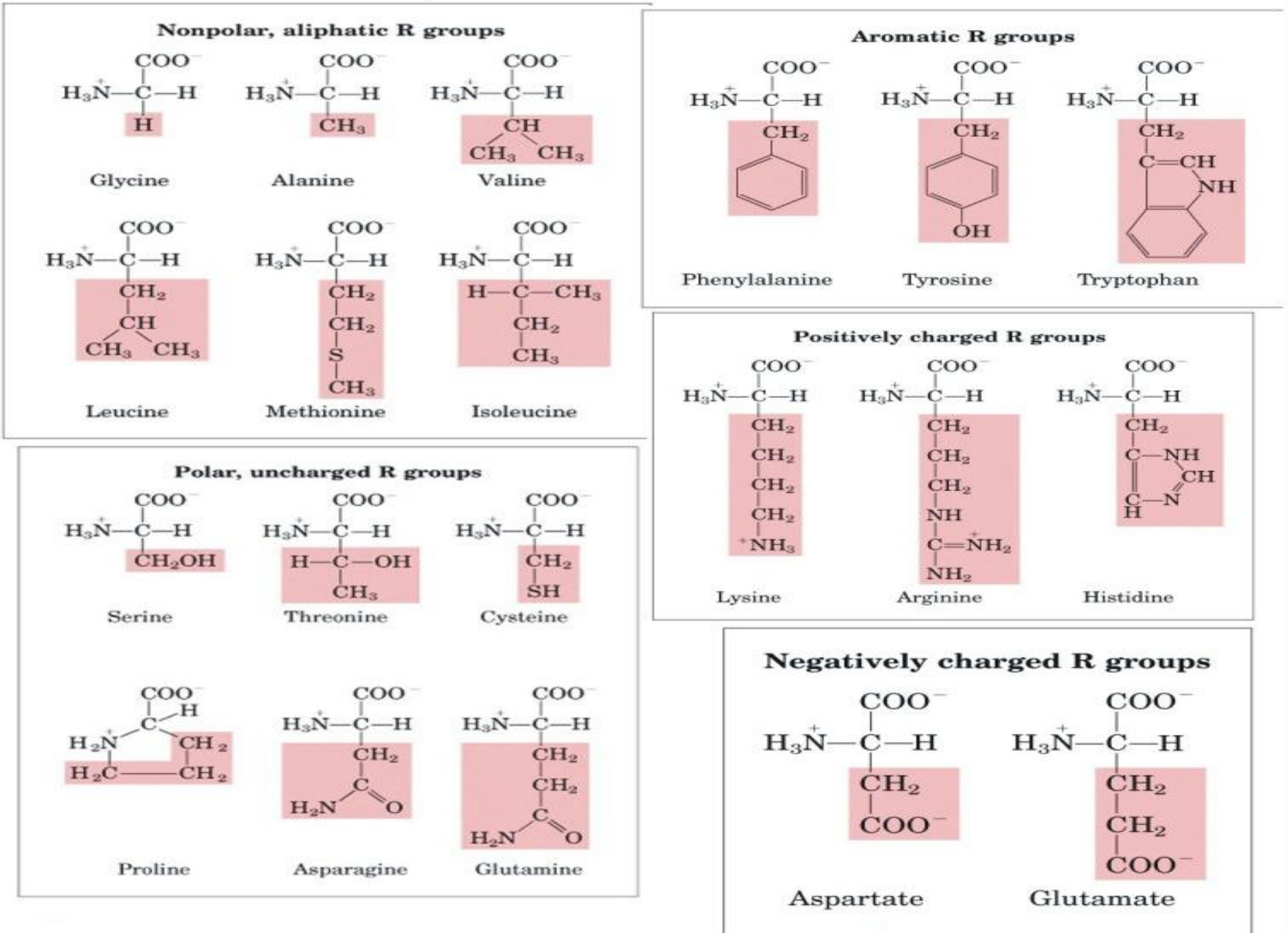
Amino Acid Catabolism

►Amino acids are glucogenic, ketogenic, or both

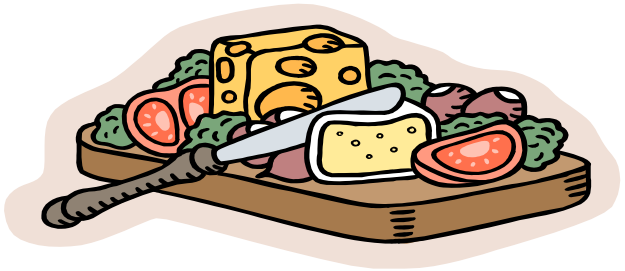
Nitrogen Disposal: The Urea Cycle

►The urea cycle consists of four reactions

Twenty standard Amino Acids



Classification based on nutritional requirements



I. Essential amino acids:

These amino acids cannot be synthesized in the body and have to be present essentially in the diet. Examples-**Valine, Isoleucine, Leucine, Lysine, Methionine, Threonine, Tryptophan and Phenylalanine.**

II. Semi-essential amino acids:

These amino acids can be synthesized in the body but the rate of synthesis is lesser than the requirement(e.g. during growth, repair or pregnancy) Examples-**Arginine and Histidine.**

III. Non-essential amino acids:

These amino acids are synthesized in the body, thus their absence in the diet does not adversely affect the growth.

Ex. :- **Glycine, Alanine, and the other remaining amino acids.**

[TABLE 1]

Essential and Nonessential Amino Acids

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

Part -1

KEY CONCEPT

Degradation of the carbon skeletons of amino acids produces acetyl-CoA and precursors for gluconeogenesis.

4-Amino Acid Catabolism

Like monosaccharides and fatty acids, *amino acids are metabolic fuels that can be broken down to release free energy.* In fact, amino acids, not glucose, are the major fuel for the cells lining the **small intestine**. These cells absorb dietary amino acids and break down almost all of the available glutamate and aspartate and a good portion of the glutamine supply (**note that these are all nonessential amino acids**).

Other tissues, mainly the **liver**, also catabolize amino acids originating from the diet and from the normal turnover of intracellular proteins. During periods when dietary amino acids are not available, such as during a prolonged fast, amino acids are mobilized through the breakdown of muscle tissue, which accounts for about 40% of the total protein in the body. **The amino acids undergo transamination reactions to remove their α-amino groups, and their carbon skeletons then enter the central pathways of energy metabolism (principally the citric acid cycle).**

The catabolism of amino acids in the liver is not complete. There is simply not enough oxygen available for the liver to completely oxidize all the carbon to CO_2. And even if there were, the liver would not need all the ATP that would be produced as a result. Instead, the amino acids are partially oxidized to substrates for **gluconeogenesis (or ketogenesis**). Glucose can then be exported to other tissues or stored as glycogen. مهم

The reactions of amino acid catabolism, like those of amino acid synthesis, are too much to describe in full here, and the **catabolic pathways do not necessarily mirror the anabolic pathways**, as they do in carbohydrate and fatty acid metabolism. In this section, we will focus on some general principles and a few interesting chemical aspects of amino acid catabolism. In the following section we will see how organisms dispose of the nitrogen component of catabolized amino acids كيف تتخلص الكائنات الحية من مكونات النيتروجين الناتجة من الأحماض الأمينية المحطمة.

Amino acids are glucogenic, ketogenic, or both

It is useful to classify amino acids in humans as **glucogenic (giving rise to gluconeogenic** precursors such as citric acid cycle intermediates) or **ketogenic (giving rise to** acetyl-CoA, which can be used for ketogenesis or fatty acid synthesis, but not gluconeogenesis). As shown in Table 2, ***all but leucine and lysine are at least partly glucogenic, most of the nonessential amino acids are glucogenic, and the large skeletons of the aromatic amino acids are both glucogenic and ketogenic.***

Ketogenic amino acids : The amino acids that are degraded entirely or in part to acetoacetyl-CoA and/or acetyl-CoA.

can yield ketone bodies in the liver, where acetoacetyl-CoA is converted to acetoacetate and then to acetone and -hydroxybutyrate.

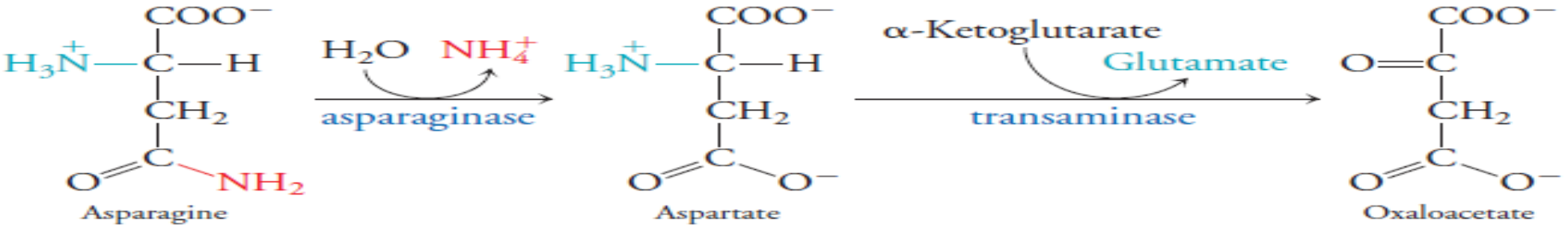
Glucogenic amino acids: The amino acids that are degraded to pyruvate, -ketoglutarate, succinyl-CoA, fumarate, and/or oxaloacetate can be converted to glucose and glycogen.

Five amino acids—tryptophan, phenylalanine, tyrosine, threonine, and isoleucine—are both ketogenic and glucogenic.

TABLE .2 Catabolic Fates of Amino Acids

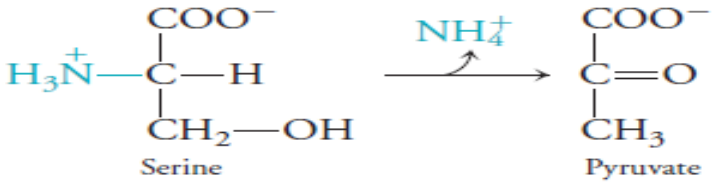
Glucogenic	Both Glucogenic and Ketogenic	Ketogenic
Alanine	Isoleucine	Leucine
Arginine	Phenylalanine	Lysine
Asparagine	Threonine	
Aspartate	Tryptophan	
Cysteine	Tyrosine	
Glutamate		
Glutamine		
Glycine		
Histidine		
Methionine		
Proline		
Serine		
Valine		

Three amino acids are converted to gluconeogenic substrates by simple transamination (the reverse of their biosynthetic reactions): **alanine to pyruvate, aspartate to oxaloacetate, and glutamate to α-ketoglutarate**. Glutamate can also be deaminated in an oxidation reaction that we will examine in the following section. **Asparagine** undergoes a simple hydrolytic deamidation to aspartate, which is then transaminated to oxaloacetate:



Similarly, **glutamine** is deamidated by a glutaminase to glutamate, and the glutamate dehydrogenase reaction yields α -ketoglutarate.

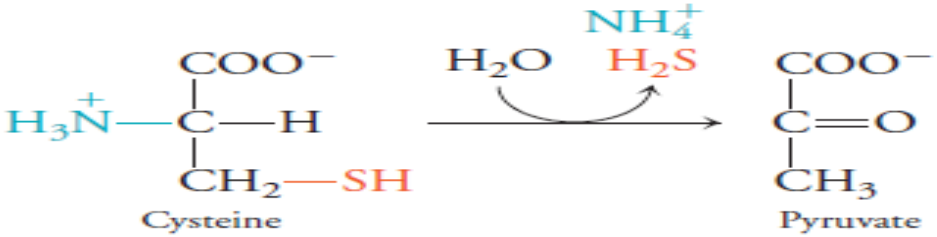
Serine is converted to pyruvate:



Note that in this reaction and in the conversion of asparagine and glutamine to their acid counterparts, the amino group is released as NH4+ rather than being transferred to another compound.

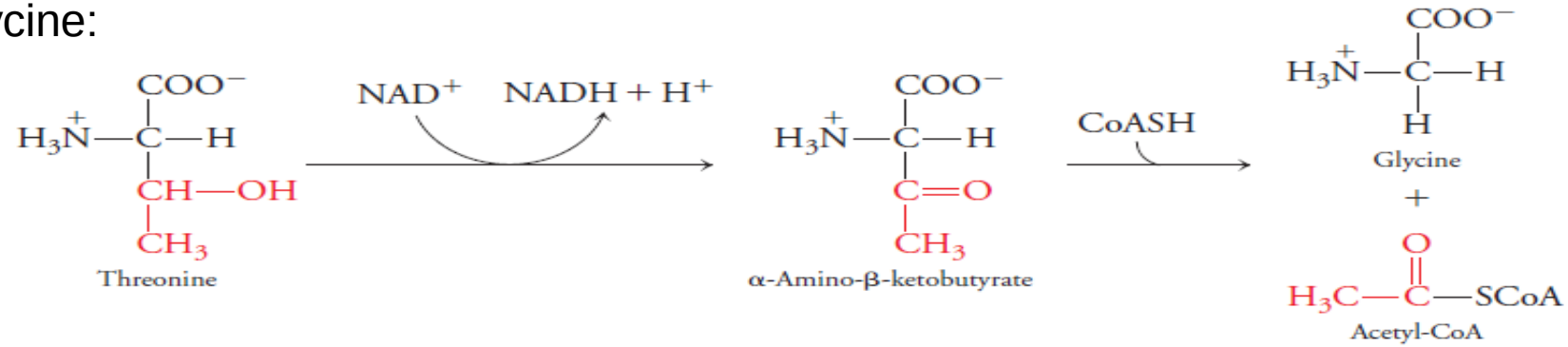
Arginine and **proline** (which are synthesized from glutamate) as well as **histidine** are catabolized to glutamate, which is then converted to α -ketoglutarate. **Amino acids of the glutamate "family," namely arginine, glutamine, histidine, and proline, constitute about 25% of dietary amino acids**, so their potential contribution to energy metabolism is significant.

Cysteine is converted to pyruvate by a process that releases ammonia as well as sulfur:

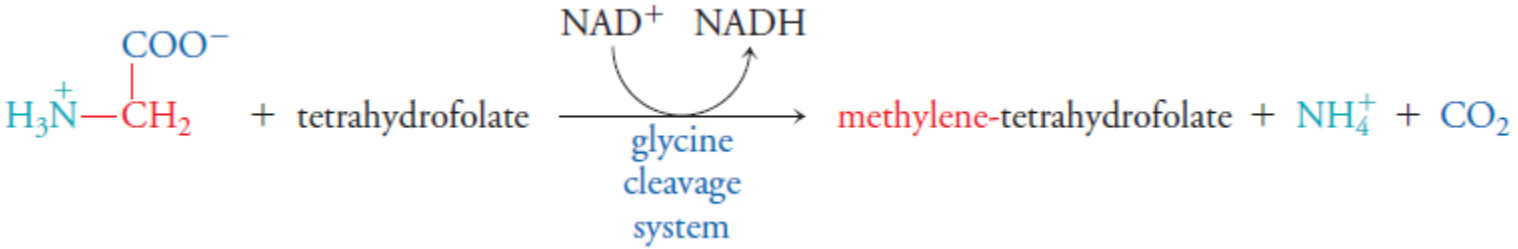


The products of the reactions listed so far-pyruvate, oxaloacetate, and α-ketoglutarate- are all gluconeogenic precursors.

Threonine is both glucogenic and ketogenic because it is broken down to acetyl-CoA and glycine:



The acetyl-CoA is a precursor of ketone, and the **glycine** is potentially glucogenic—if it is first converted to serine by the action of serine hydroxymethyltransferase. The major route for glycine disposal التخلص, however, is catalyzed by a multiprotein complex known as the glycine cleavage system:



The degradation pathways for the remaining amino acids are more complicated. For example, **the branched-chain amino acids— valine, leucine, and isoleucine —** undergo transamination to their α-keto-acid forms and are then linked to coenzyme A in an oxidative decarboxylation reaction. This step is catalyzed by the branched chain α-keto-acid dehydrogenase complex, a multienzyme complex that resembles the pyruvate dehydrogenase complex and even shares some of the same subunits.

The initial reactions of **valine** catabolism are shown in Fig. 18.11. Subsequent steps yield the citric acid cycle intermediate succinyl-CoA. **Isoleucine** is degraded by a similar pathway that yields succinyl-CoA and acetyl-CoA. **Leucine** degradation yields acetyl-CoA and the ketone body acetoacetate. **Lysine** degradation, which follows a different pathway from the branched-chain amino acids, also yields acetyl-CoA and acetoacetate. The degradation of **methionine** produces succinyl-CoA.

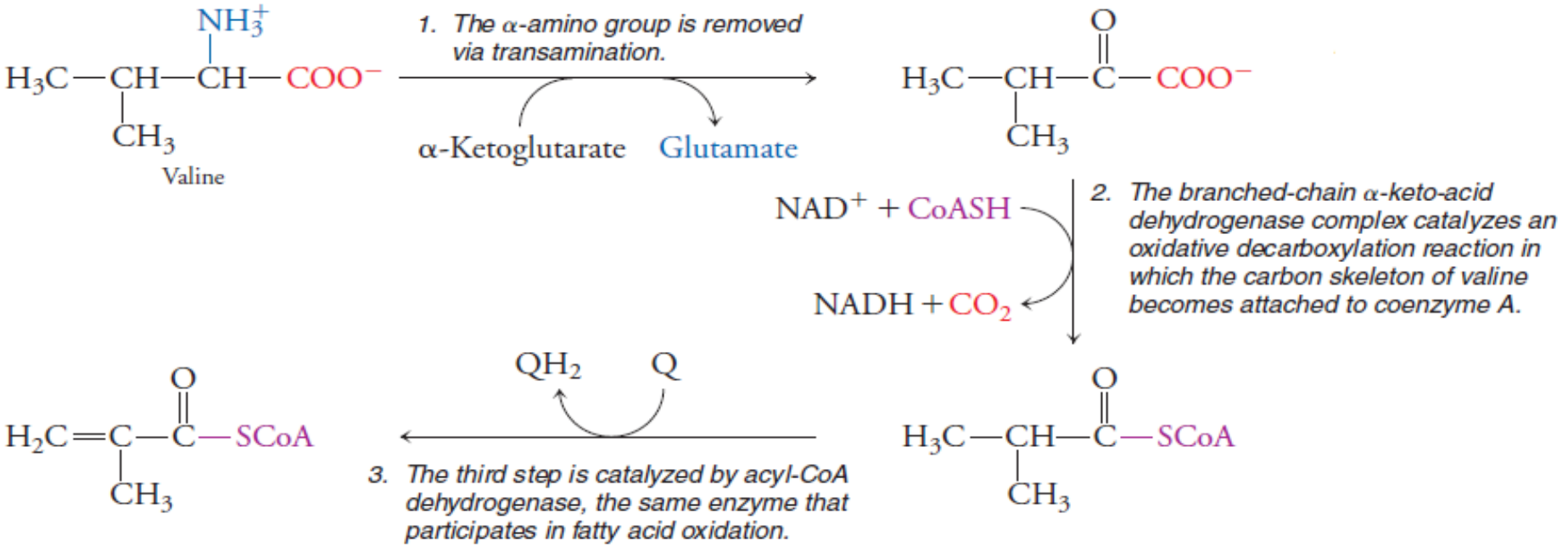
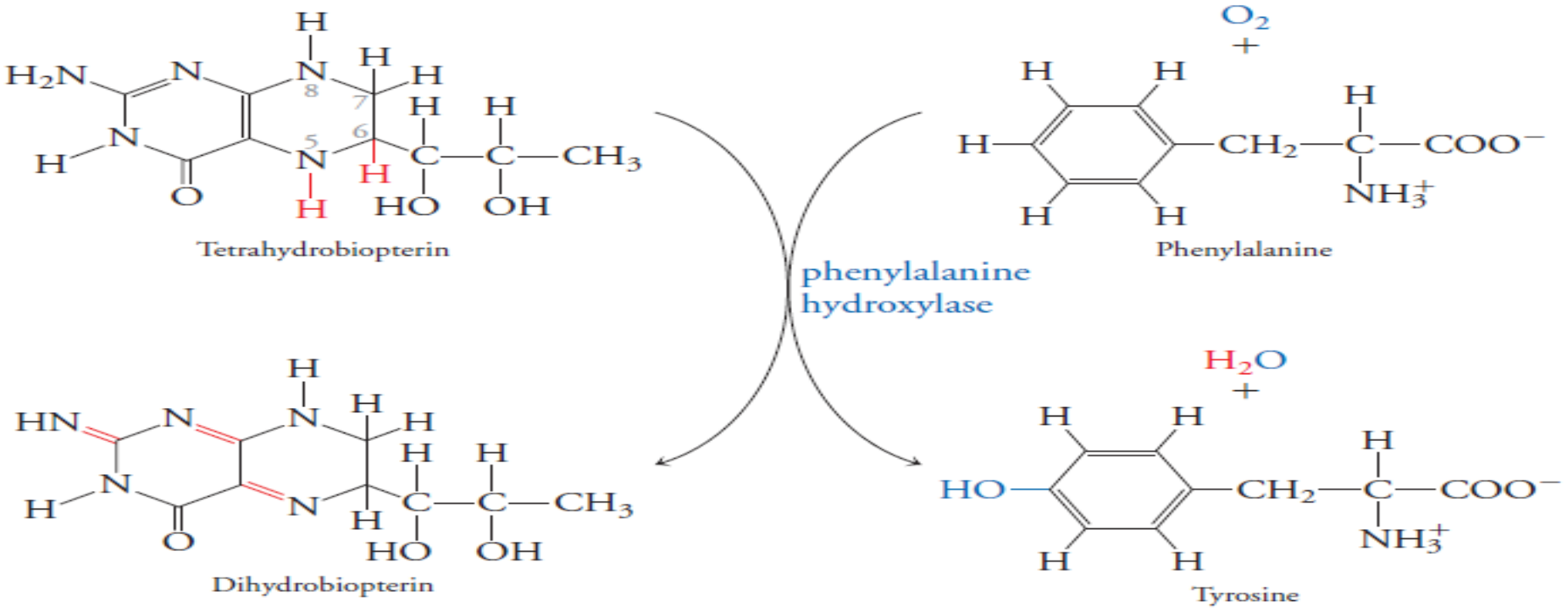


Figure 18-11 The initial steps of valine degradation.

Finally, the cleavage of the **aromatic amino acids-phenylalanine, tyrosine, and tryptophan**-yields the ketone body acetoacetate as well as a glucogenic compound (alanine or fumarate).The first step of phenylalanine degradation is a hydroxylation reaction that produces tyrosine, as we have already seen.



The tetrahydrobiopterin (cofactor) is oxidized to dihydrobiopterin in the **phenylalanine hydroxylase** reaction. This cofactor must be subsequently reduced to the tetrahydro form by a separate NADH-dependent enzyme. Another step of the phenylalanine (and tyrosine) degradation pathway is also notable because a deficiency of the enzyme was one of the first-characterized “inborn errors of metabolism أخطاء فطرية او وراثية في عملية التمثيل الغذائي”

Inborn Errors of Metabolism

many “inborn errors” are catastrophic كارثي. For example, **phenylketonuria (PKU) results from a deficiency of phenylalanine hydroxylase, the first enzyme in the pathway shown in figure 3**. Phenylalanine cannot be broken down, although it can undergo transamination. The resulting α-keto-acid derivative phenylpyruvate accumulates and is excreted in the urine, giving it a mousy odor رائحة عفن. If not treated, PKU causes **mental retardation التخلف العقلي** . Fortunately, the disease can be detected in newborns. Afflicted individuals الأفراد المصابين develop normally if they consume a diet that is low in phenylalanine. Sadly, the biochemical defects behind many other diseases are not as well understood, making them difficult to identify and treat.

Question: Individuals with PKU must consume a certain amount of phenylalanine.

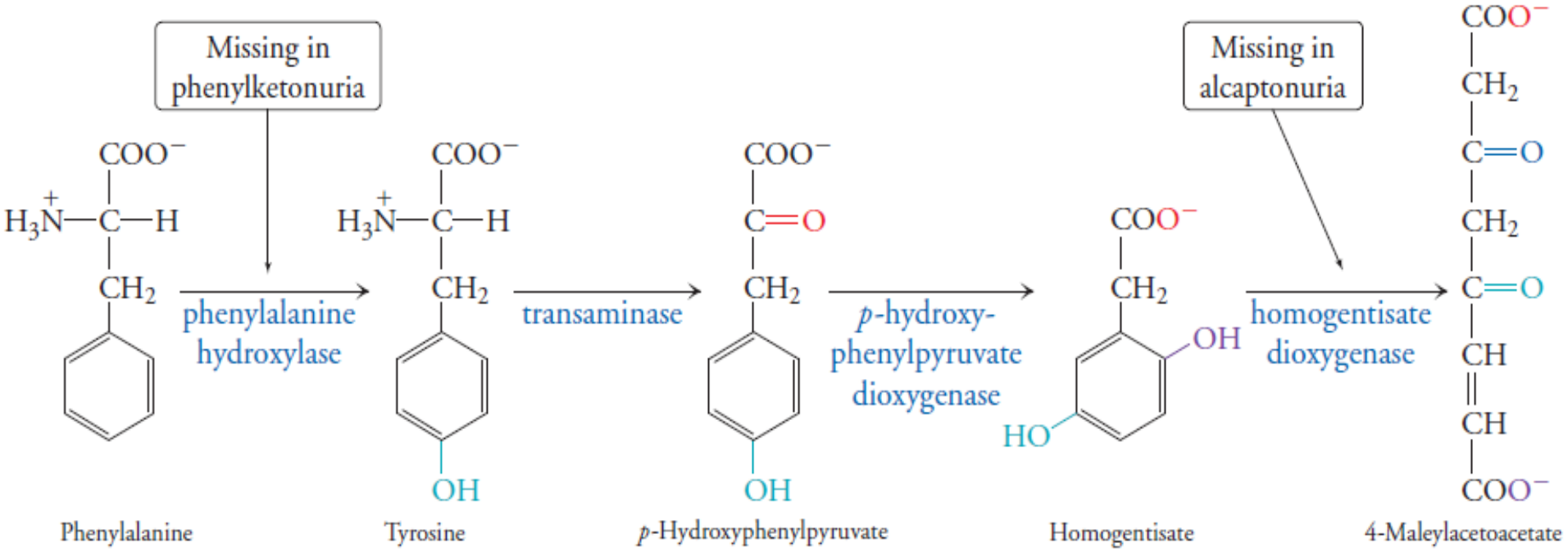


Figure 3. Example for “Inborn Errors”

Part -2
Nitrogen Disposal: The Urea Cycle

KEY CONCEPTS :

• Ammonia released by the glutamate dehydrogenase reaction is incorporated into carbamoyl phosphate.

• The four reactions of the urea cycle incorporate two amino groups into urea, a highly water-soluble waste product.

5- Nitrogen Disposal: The Urea Cycle

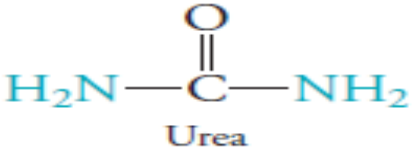
When the supply of amino acids exceeds the cell's immediate needs for protein synthesis or other amino acid–consuming pathways, the carbon skeletons are broken down and the nitrogen disposed of. **All amino acids except lysine can be deaminated by the action of transaminases, but this just transfers the amino group to another molecule; it does not eliminate it from the body**.

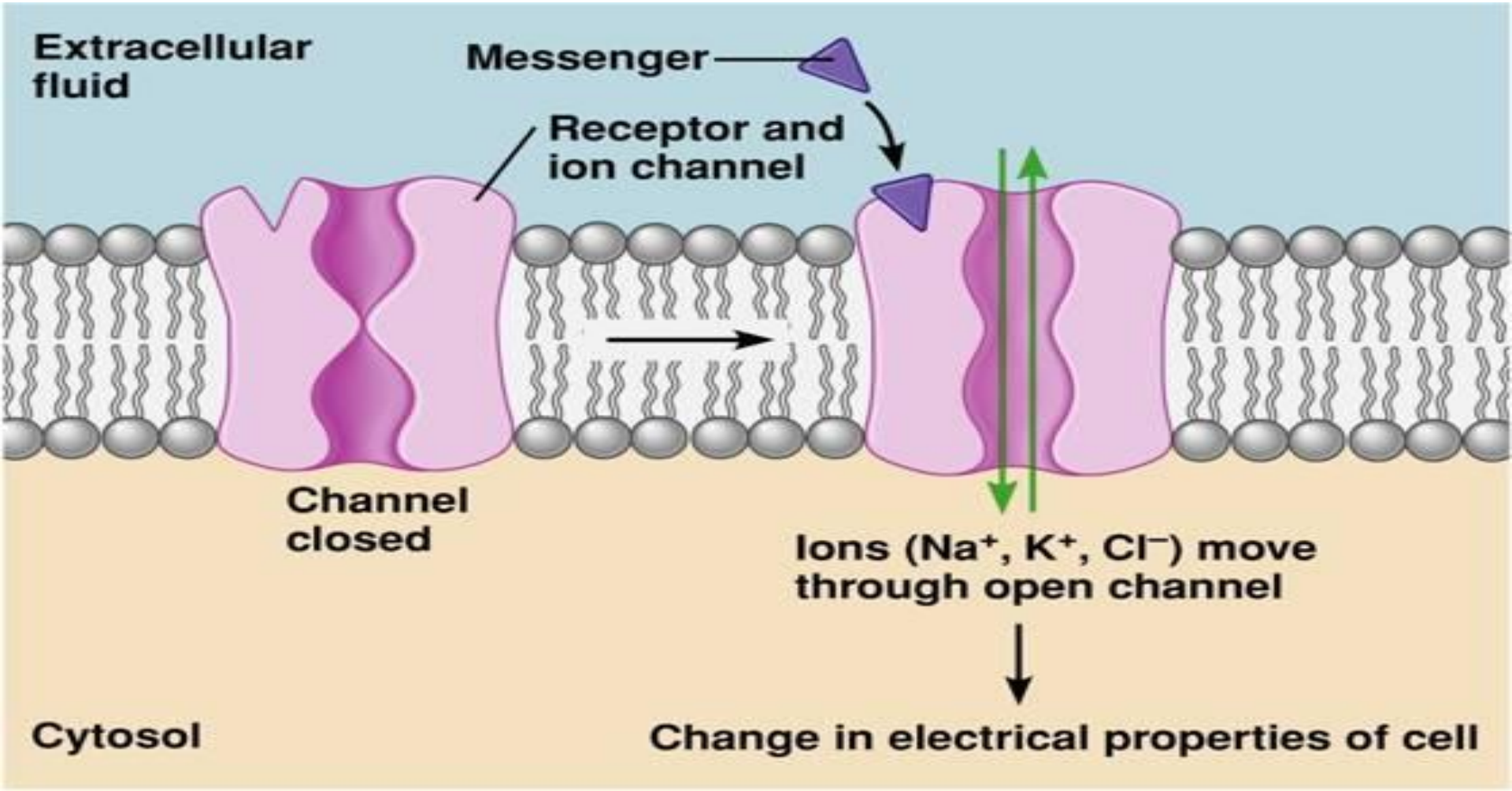
Some catabolic reactions do release free ammonia, which can be excreted as a waste product in the urine. In fact, the kidney is a major site of glutamine catabolism, and the resulting NH_4^+ facilitates the excretion of metabolic acids such as H_2SO_4 that arise from the catabolism of methionine and cysteine. **However, ammonia production is not feasible for disposing of large amounts of excess nitrogen. انتاج الامونيا لايمكن من خلالة التخلص من الكميات الكبيرة من النتروجين .**

First, high concentrations of NH_4^+ in the blood cause alkalosis. **Second**, ammonia is highly toxic. It easily enters the brain, where it activates the NMDA receptor. The activated receptor is an ion channel that normally opens to allow Ca^{2+} and Na^+ ions to enter the cell and K^+ ions to exit the cell. However, the large Ca^{2+} influx triggered آثار by ammonia binding to the receptor results in neuronal cell death موت الخلايا العصبية, a phenomenon called **excitotoxicity التحفيز الزائد** . Humans and many other organisms have therefore evolved safer ways to deal with excess amino groups.

التحفيز الزائد هو إجراء أيضي حيث يتم إتلاف أو القضاء على الخلايا العصبية عبر تعريضها لحث زائد من قِبَل الناقلات العصبية مثل الغلوتومات أو غيرها من الأجسام المشابهة.

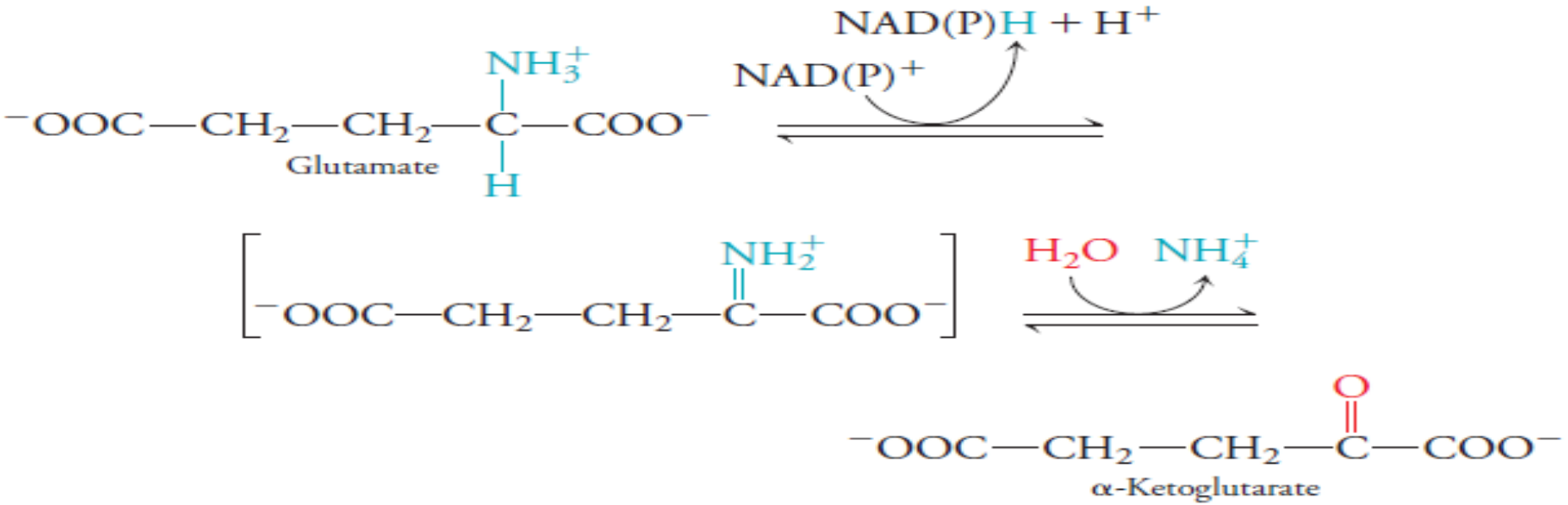
Approximately 80% of the body's excess nitrogen is excreted in the form of urea, which is produced in the liver by the reactions of the urea cycle.




Extracellular fluid
Messenger
Receptor and ion channel
Channel closed
Ions (Na^+, K^+, Cl^-) move through open channel
Cytosol
Change in electrical properties of cell

Glutamate supplies nitrogen to the urea cycle

Because many transaminases use α-ketoglutarate as the amino-group acceptor, glutamate is one of the most abundant amino acids inside cells. Glutamate can be deaminated to regenerate α-ketoglutarate and release NH_4^+ in an oxidation–reduction reaction catalyzed by **glutamate dehydrogenase**:



This mitochondrial enzyme is unusual: It is the only known enzyme that can use either NAD^+ or $NADP^+$ as a cofactor. The glutamate dehydrogenase reaction is a major route for feeding amino acid–derived amino groups into the urea cycle and, not surprisingly, is subject to allosteric activation and inhibition.

The starting substrate for the urea cycle is an "**activated**" molecule produced by the condensation of bicarbonate and ammonia, as catalyzed by carbamoyl phosphate synthetase **(Fig. 4).** The NH_4^+ may be contributed تساهم by the glutamate dehydrogenase reaction or another process that releases ammonia. **The bicarbonate is the source of the urea carbon**. Note that the phosphoanhydride bonds of two ATP molecules are consumed in the energetically costly production of carbamoyl phosphate.

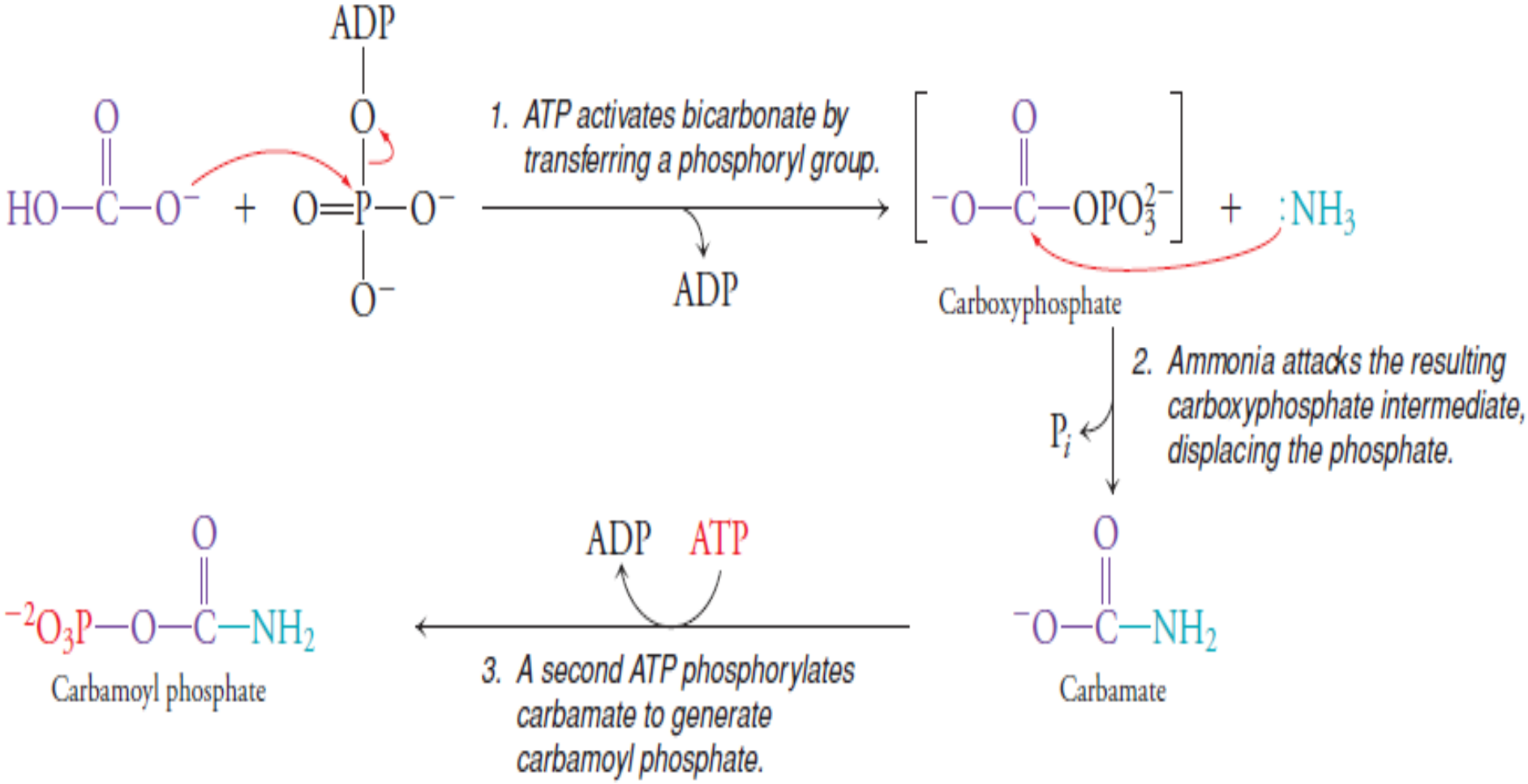


Figure 4 The carbamoyl phosphate synthetase reaction.

The urea cycle consists of four reactions

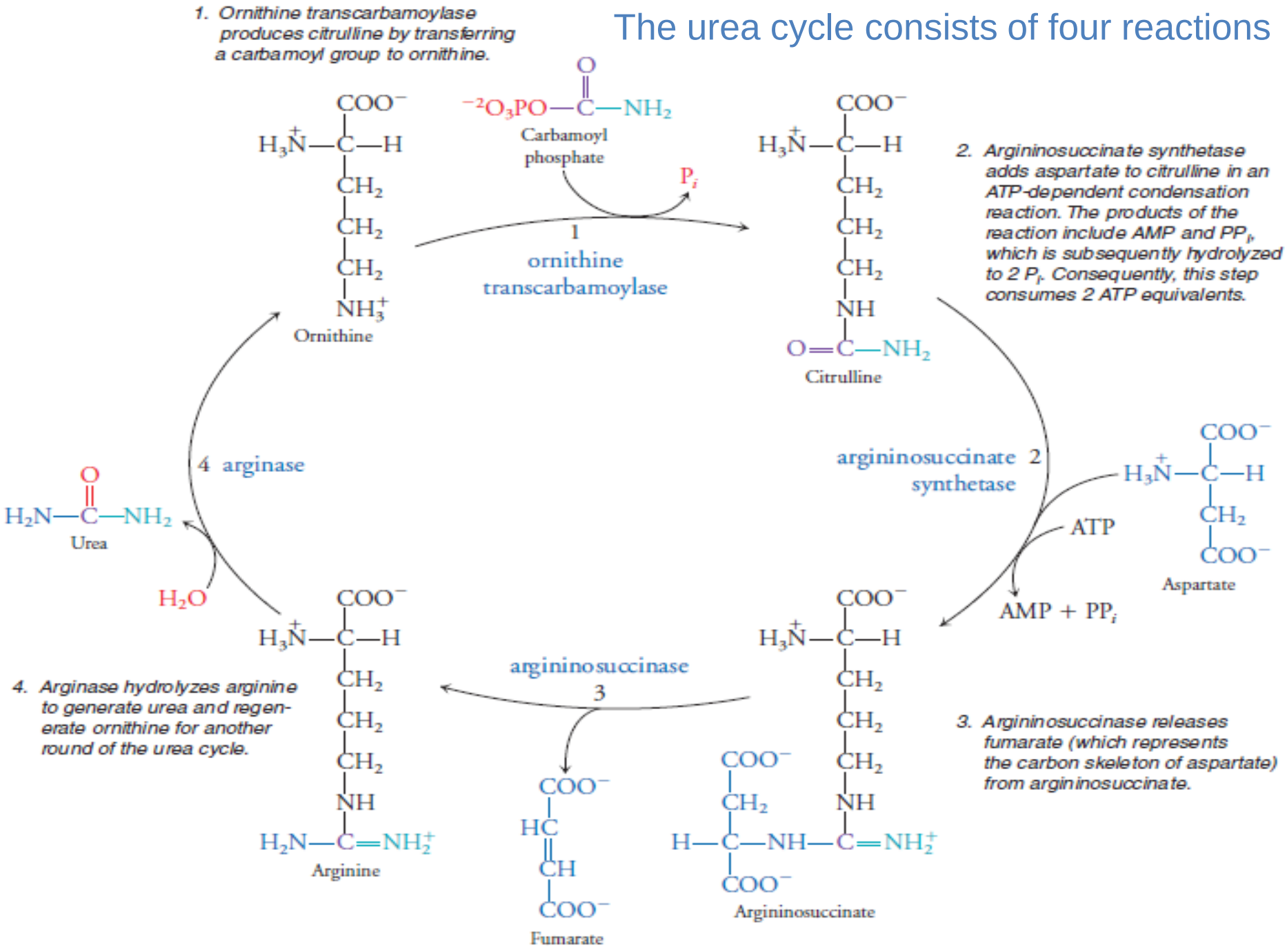


Figure 5 The four reactions of the urea cycle.

The urea cycle consists of four reactions

The four enzyme-catalyzed reactions of the urea cycle proper are shown in **Figure 5.** The cycle also provides a means for synthesizing arginine: **The five-carbon ornithine is derived from glutamate**, and the **urea cycle converts it to arginine**. However, the **arginine needs of children exceed the biosynthetic capacity** of the urea cycle, so **arginine is classified as an essential amino acid.**

The fumarate generated in step 3 of the urea cycle is converted to malate and then oxaloacetate, which is used for gluconeogenesis. The aspartate substrate for Reaction 2 may represent oxaloacetate that has undergone transamination. Combining these ancillary reactions التفاعلات الاضافية او الملحقة with those of the urea cycle, the carbamoyl phosphate synthetase reaction, and the glutamate dehydrogenase reaction yields the pathway outlined in **Figure 6.**

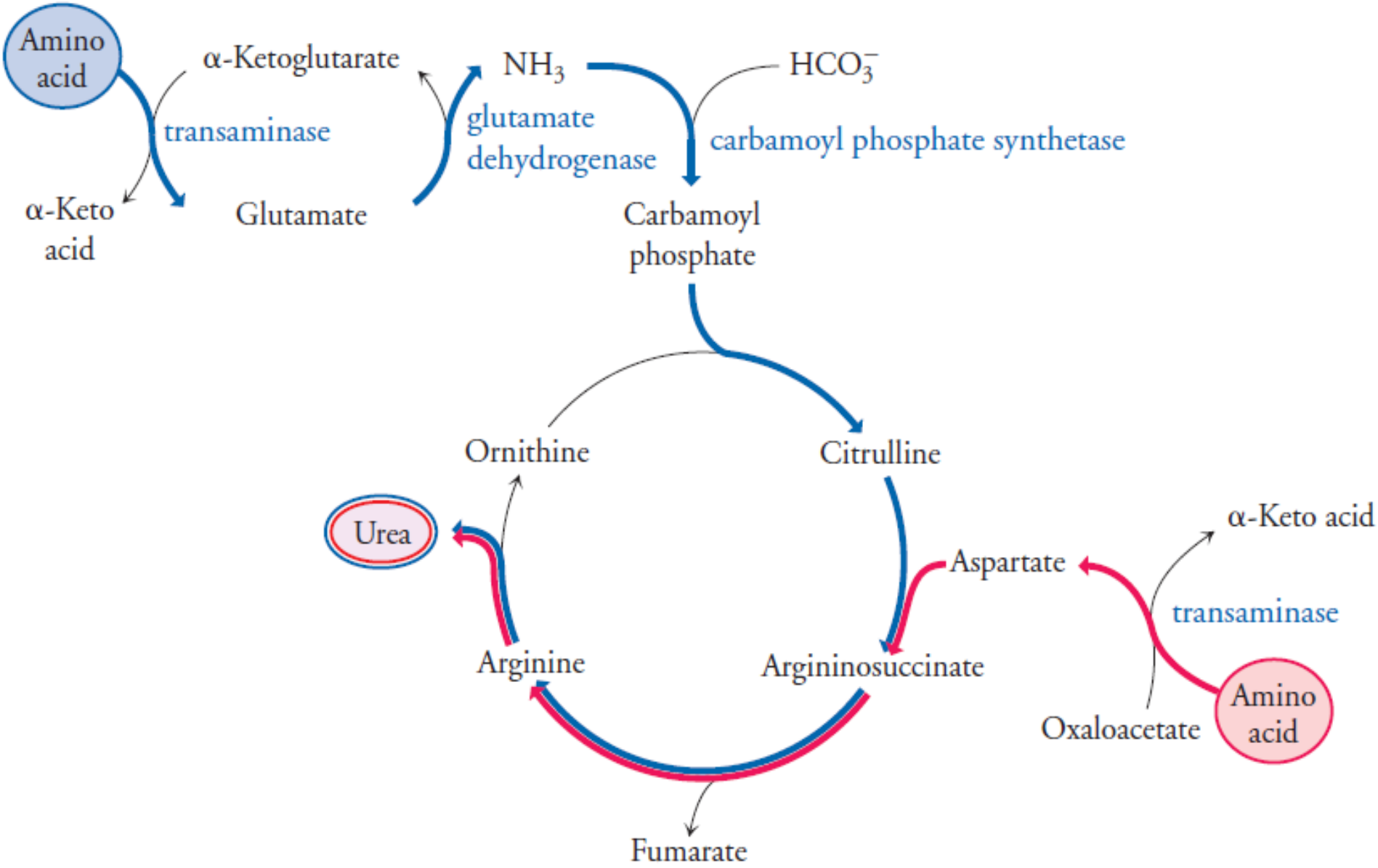


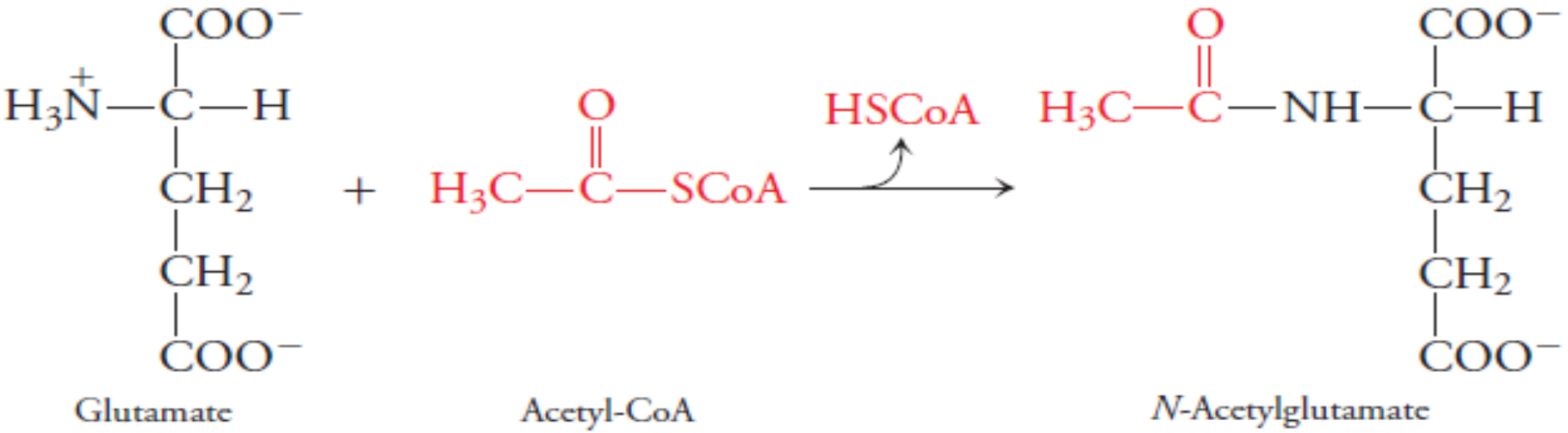
Figure . 6 **The urea cycle and related reactions.** Two routes for the disposal of amino groups are highlighted. The blue pathway shows how an amino group from an amino acid enters the urea cycle via glutamate and carbamoyl phosphate. The red pathway shows how an amino group from an amino acid enters via aspartate.

The overall effect is that transaminated amino acids donate amino groups, via glutamate and aspartate, to urea synthesis. Because the liver is the only tissue that can carry out urea synthesis, amino groups to be eliminated travel through the blood to the liver mainly as glutamine, which accounts for up to one-quarter of circulating amino acids. الذي يمثل ما يصل إلى ربع الأحماض الأمينية المتداولة.

Like many other metabolic loops, the urea cycle involves enzymes located in both the mitochondria and cytosol. **Glutamate dehydrogenase, carbamoyl phosphate synthetase, and ornithine transcarbamoylase are mitochondrial**, whereas **argininosuccinate synthetase, argininosuccinase, and arginase are cytosolic**. Consequently, citrulline is **produced in the mitochondria but must be transported to the cytosol** for the next step, and ornithine **produced in the cytosol must be imported into the mitochondria** to begin a new round of the cycle.

The **carbamoyl phosphate synthetase** reaction and the **argininosuccinate synthetase** reactions **each consume 2 ATP equivalents, so the cost of the urea cycle is 4 ATP per urea**. However, when considered in context, operation of the urea cycle is often accompanied by ATP synthesis. The **glutamate dehydrogenase reaction produces NADH (or NADPH),** whose free energy is conserved in the synthesis of **2.5 ATP by oxidative phosphorylation**. **Catabolism of the carbon skeletons** of the amino acids that donated their amino groups via transamination also **yields ATP**.

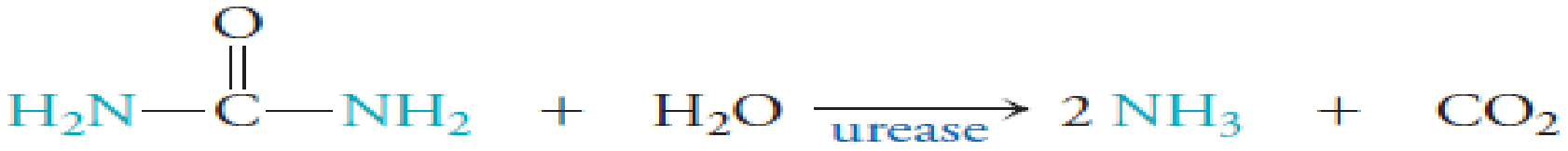
The rate of **urea production is controlled** largely by the activity of **carbamoyl phosphate synthetase**. This enzyme is allosterically activated by *N-acetylglutamate,* which is synthesized from glutamate and acetyl-CoA:



When amino acids are undergoing transamination and being catabolized, the resulting increases in the cellular glutamate and acetyl-CoA concentrations boost يعززproduction of N-acetylglutamate. **This stimulates carbamoyl phosphate synthetase activity, and flux through the urea cycle increases. Such a regulatory system allows the cell to efficiently dispose of the nitrogen released from amino acid degradation.** مهم

Urea is relatively nontoxic and easily transported through the bloodstream to the kidneys for excretion in the urine. However, the polar urea molecule requires large amounts of water for its efficient excretion. This presents a problem for flying vertebrates الفقريات الطائرةsuch as birds and for reptiles الزواحفthat are adapted to. arid habitats البيئات الجافة. **These organisms deal with waste nitrogen by converting it to uric acid via purine synthesis**.

The relatively **insoluble uric acid is excreted as a semisolid paste, which conserves water للحفاظ على الماء**. Bacteria, fungi, and some other organisms use an enzyme called urease to break down urea, a reaction that completes our story of nitrogen disposal.



Urease has the distinction of being the first enzyme to be crystallized (in 1926). It helped promote the theory that catalytic activity was a property of proteins. This premise is only partly true, as we have seen, since many enzymes contain metal ions or inorganic cofactors (urease itself contains two catalytic nickel atoms).

SUMMARY

Amino Acid Catabolism

• Following removal of their amino groups by transamination, amino acids are broken down to intermediates that can be converted to glucose or acetyl-CoA for use in the citric acid cycle, fatty acid synthesis, or ketogenesis.

Nitrogen Disposal: The Urea Cycle

• In mammals, excess amino groups are converted to urea for disposal. The urea cycle is regulated at the carbamoyl phosphate synthetase step, an entry point for ammonia. Other organisms convert excess nitrogen to compounds such as uric acid.