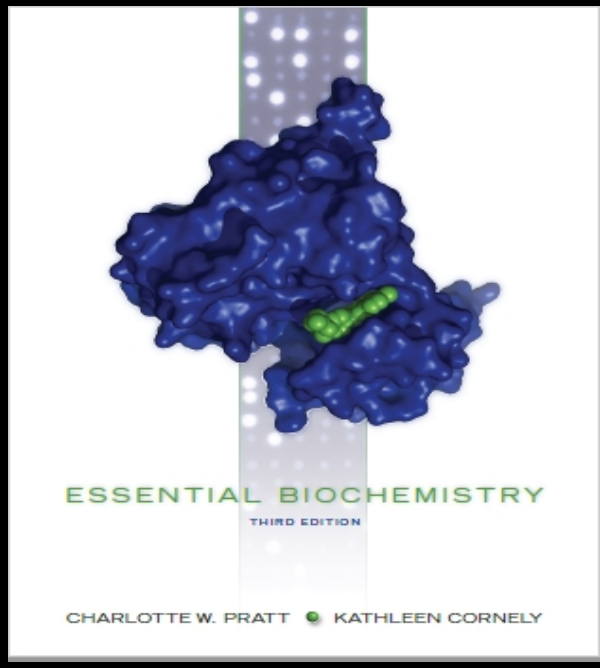
METABOLISM



Fourth Class
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M Sc. & Ph. D. In Clinical Biochemistry

2016-2017

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NITROGEN METABOLISM

Syllabus

NITROGEN METABOLISM

Nitrogen Fixation and Assimilation

►Nitrogenase converts N_2 to NH_3

►Ammonia is assimilated by glutamine synthetase and glutamate synthase

►Transamination moves amino groups between compounds

Amino Acid Biosynthesis

► Several amino acids are easily synthesized from common metabolites

► Amino acids with sulfur, branched chains, or aromatic groups are more difficult to synthesize

► Amino acids are the precursors of some signaling molecules

Nucleotide Biosynthesis

► Purine nucleotide synthesis yields IMP and then AMP and GMP

► Pyrimidine nucleotide synthesis yields UTP and CTP

► Ribonucleotide reductase converts ribonucleotides to deoxyribonucleotides

► Thymidine nucleotides are produced by methylation

► Nucleotide degradation produces uric acid or amino acids

Amino Acid Catabolism

►Amino acids are glucogenic, ketogenic, or both

Nitrogen Disposal: The Urea Cycle

►The urea cycle consists of four reactions

Nitrogen Fixation and Assimilation

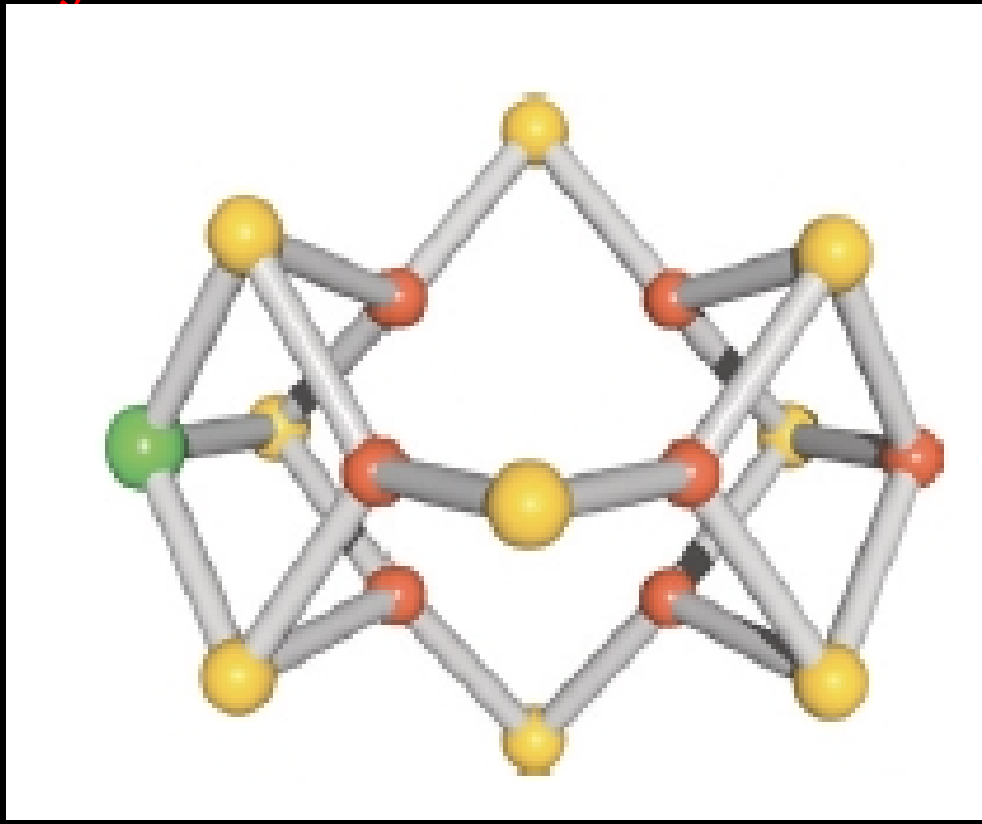
CONCEPTS

- Nitrogen fixation by the activity of nitrogenase is part of the nitrogen cycle.
- Other enzymes incorporate amino groups into glutamine and glutamate.
- Transaminases transfer amino groups to interconvert amino acids and **α-keto acids.**

- Approximately 80 % of the air breathe is nitrogen (N_2), but cannot use N_2 form for the synthesis of amino acids, nucleotides, and other nitrogen containing compounds.
- life-forms depend on the activity of a few types of microorganisms that can "fix" gaseous N_2 by transforming it into biologically useful forms.
- The availability of fixed nitrogen as (nitrite (NO_{2-}), nitrate (NO_3^-), and ammonia(NH_3)) is believed to limit the biological productivity in much of the world's oceans.
- It also limits the growth of terrestrial organisms, which is why farmers use fertilizer (a source of fixed nitrogen, among other things) to promote crop growth.

Nitrogenase converts N_2 to NH_3

Some of bacteria make nitrogenase enzyme , which carries out the energetically expensive reduction of N_2 to NH_3. Nitrogenase is an enzyme contains iron–sulfur centers and a cofactor with both iron and molybdenum, which resembles an elaborate Fe–S cluster .



Model of the FeMo cofactor of Nitrogenase. This prosthetic group in the enzyme Nitrogenase consists of iron atoms (orange), sulfur atoms (yellow), and a molybdenum atom (green). The central cavity includes a carbon atom coordinated with the six iron atoms. The manner in which N_2 interacts with the Fe-Mo cofactor is not understood.

The industrial fixation of Nitrogen also involves metal catalysts, but this non-biological process requires temperatures of 300 to 500C and pressures of over 300 atm in order to break the triple bond between the two nitrogen atoms. Biological N_2 reduction consumes large amounts of ATP and requires a strong reducing agent such as ferredoxin to donate electrons. The net reaction is :

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP + 16\,H_2O \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

Note : Eight electrons are required for the Nitrogenase reaction, although N_2 reduction formally requires only six electrons; the two extra electrons are used to produce H_2. In vivo, the inefficiency of the reaction cosumes the ATP to about 20 or 30 per **N_2 reduced.**

Oxygen inactivates Nitrogenase , many nitrogen-fixing bacteria are confined to anaerobic habitats or carry out nitrogen fixation when O_2 is scarce. Biologically useful nitrogen also originates from nitrate (NO_3^-), which is naturally present in water and soils. *Nitrate is reduced to NH_3 by plants, fungi, and many bacteria. First,* ***nitrate reductase catalyzes the two-electron reduction of nitrate to nitrite*** (NO_2^-)

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$$

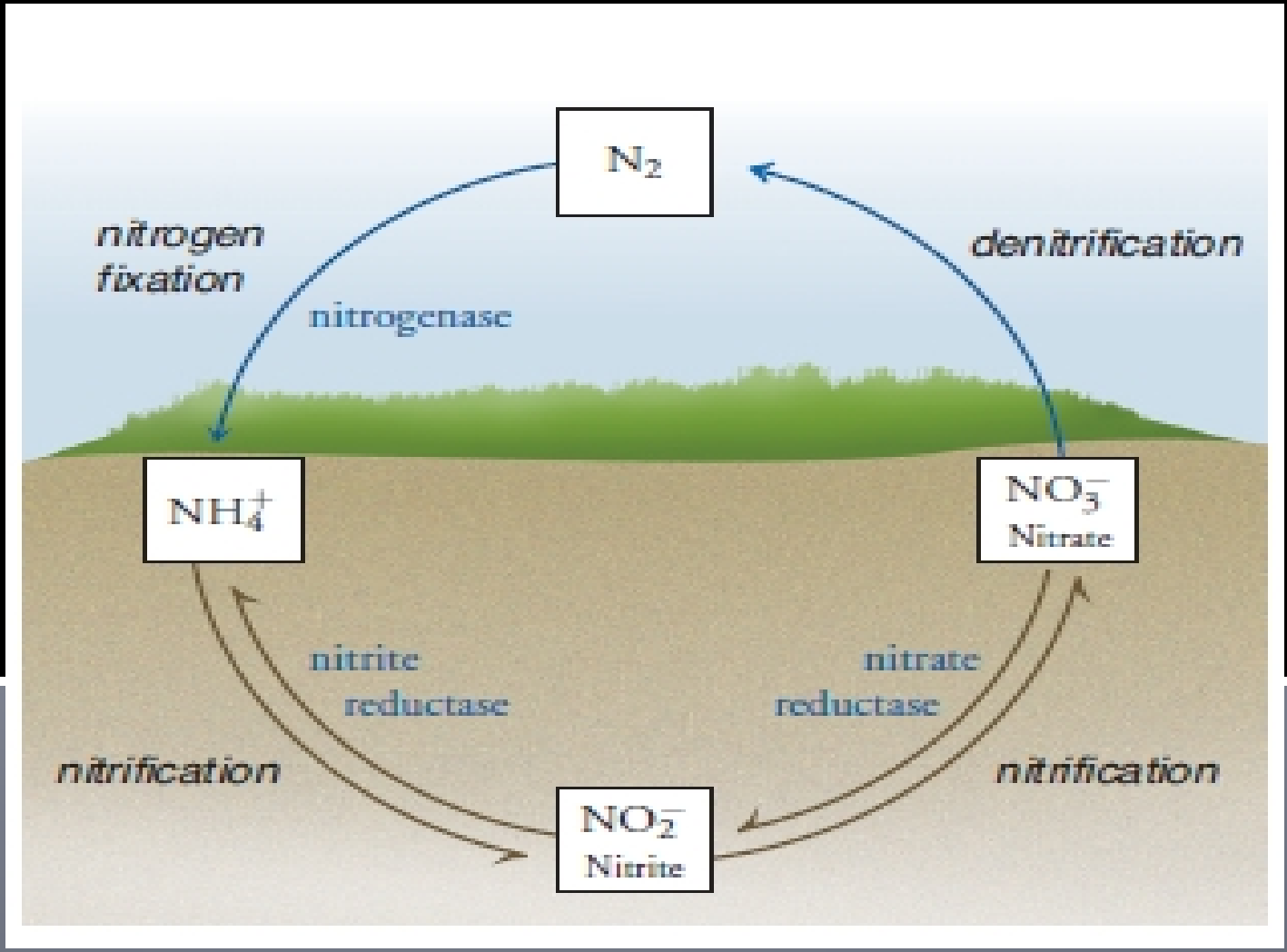
Next, nitrite reductase converts nitrite to ammonia:

$$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$$

Under physiological conditions, ammonia exists primarily in the protonated form, $NH4^+$ (the ammonium ion), which has a p*K of 9.25*. Nitrate is also produced by certain bacteria that oxidize $NH4^+$ to NO_2^- and then NO_3^- **back to N_2 ,** which is called **denitrification .**

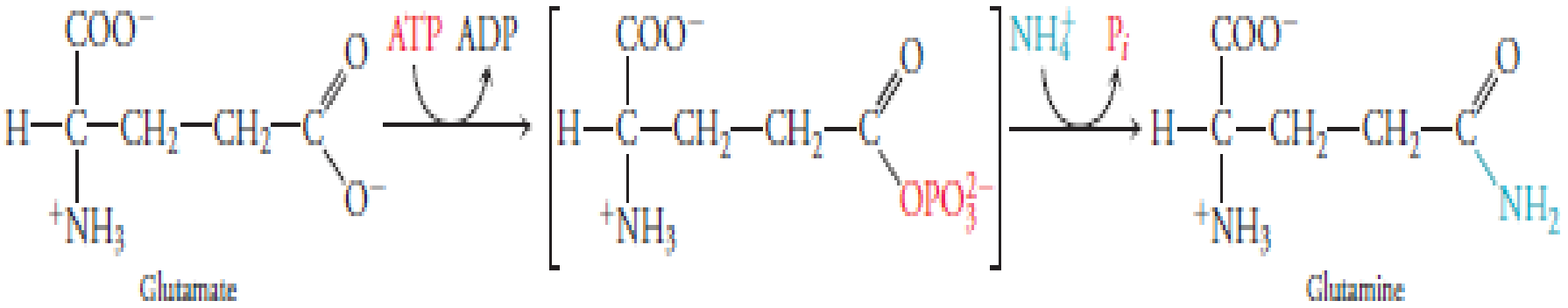
The nitrogen cycle.

Nitrogen fixation converts N_2 to the biologically useful NH_4^+. Nitrate can also be converted to NH_4^+. Ammonia is transformed back to N_2 by nitrification followed by denitrification.



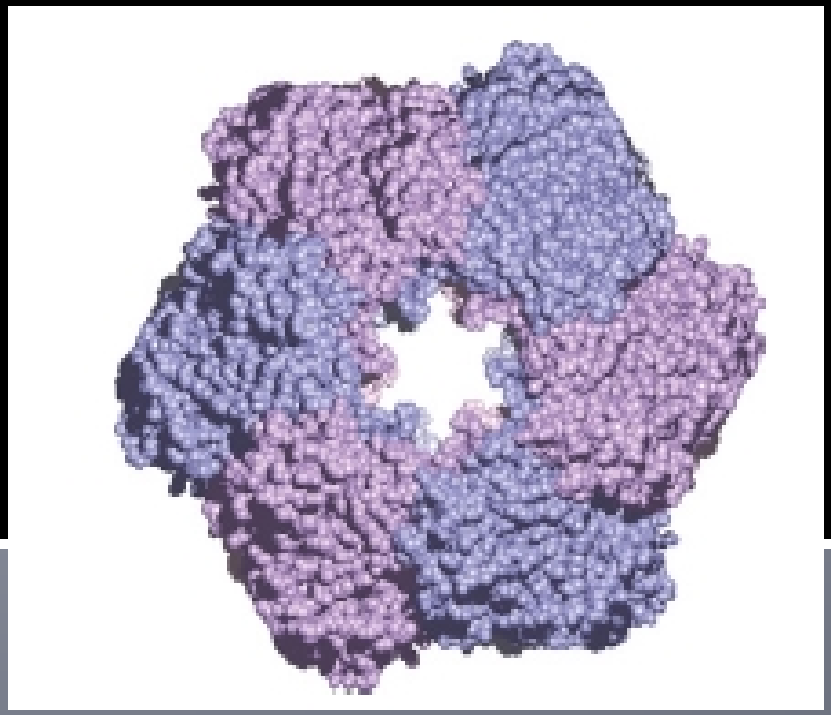
Ammonia is assimilated by glutamine synthetase and glutamate synthase

The enzyme glutamine synthetase is found in all organisms. In microorganisms, it is a metabolic entry point for fixed nitrogen. In animals, it helps mop up excess ammonia, which is toxic. In the first step of the reaction, ATP donates a phosphoryl group to glutamate. Then ammonia reacts with the reaction intermediate, ***displacing Pi to produce glutamine:***



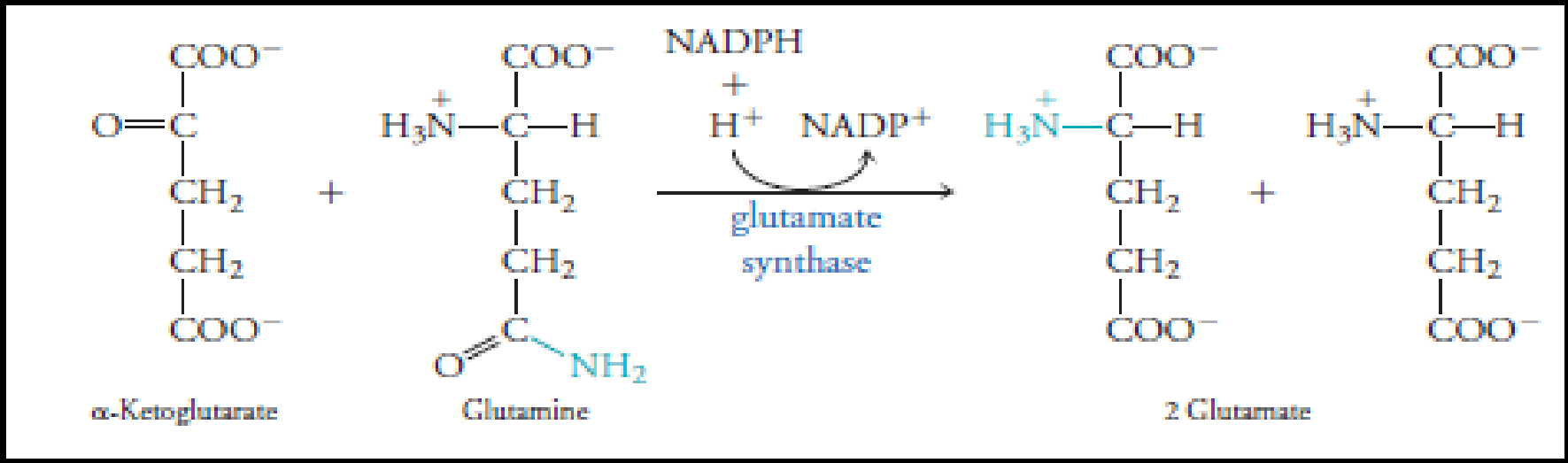
The name *synthetase indicates that ATP is consumed in the reaction.*

Glutamine, along with glutamate, is usually present in organisms at much higher concentrations than the other amino acids, which is consistent with its role as a carrier of amino groups. Not surprisingly, ***the activity of glutamine synthetase is tightly regulated to maintain a supply of accessible amino groups. For example, the dodecameric*** **glutamine synthetase from** ***E. coli is regulated allosterically and by covalent*** **modification .**



E. coli glutamine **synthetase. The 12 identical subunits** of this enzyme are arranged in two stacked rings of 6 subunits (only the upper ring is visible).The symmetrical arrangement of subunits is a general feature of enzymes that are regulated by allosteric effectors: Changes in activity at one of the active sites can be efficiently communicated to the other active sites.

The glutamine synthetase reaction that introduces fixed nitrogen (ammonia) into biological compounds requires a nitrogen-containing compound (glutamate) as a substrate.



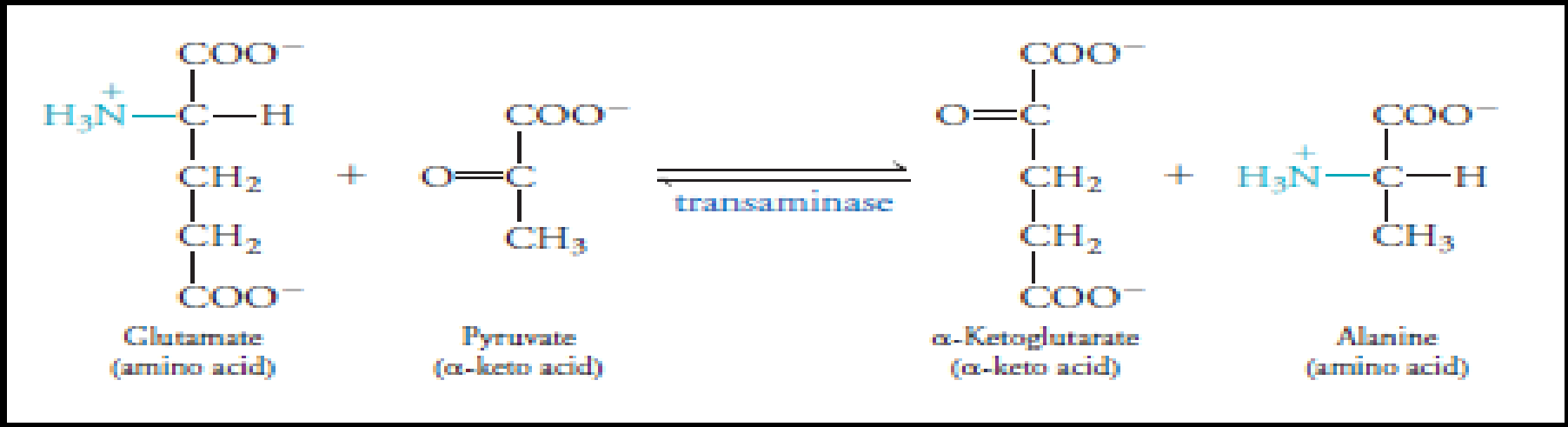
The net result of the glutamine synthetase and glutamate synthase reactions is :

$$\alpha\text{-ketoglutarate} + NH_4^+ + NADPH + ATP \rightarrow \text{glutamate} + NADP^+ + ADP + P_i$$

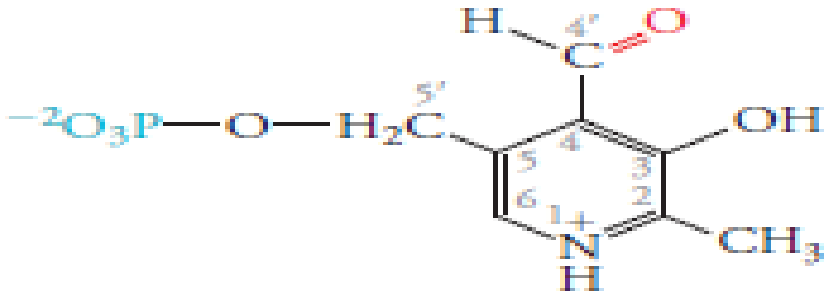
In other words, ***the combined action of these two enzymes assimilates fixed nitrogen*** ***(NH_4^+) into an organic compound (α-ketoglutarate, a citric acid cycle intermediate) to produce an amino acid (glutamate) . Mammals lack glutamate synthase , but glutamate*** **concentrations are relatively high because glutamate is produced by other reactions.**

Transamination moves amino groups between compounds

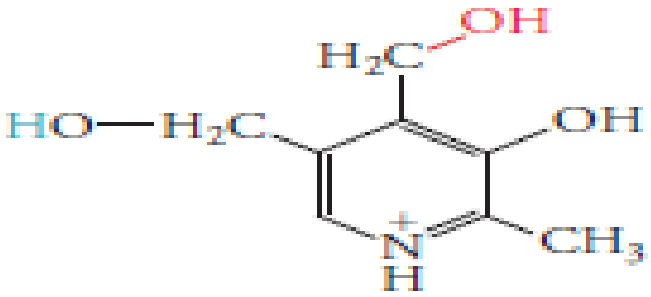
Because reduced nitrogen is so precious but free ammonia is toxic, amino groups are transferred from molecule to molecule, with glutamate often serving as an amino group donor. *A transaminase (also called an aminotransferase) catalyzes the transfer of an amino group to an α-keto acid. For example,*



During such an amino-group transfer reaction, the amino group is transiently attached to a prosthetic group of the enzyme. This group is pyridoxal-5"-phosphate (PLP), a derivative of pyridoxine (an essential nutrient also known as vitamin B6):



Pyridoxal-5′-phosphate (PLP)



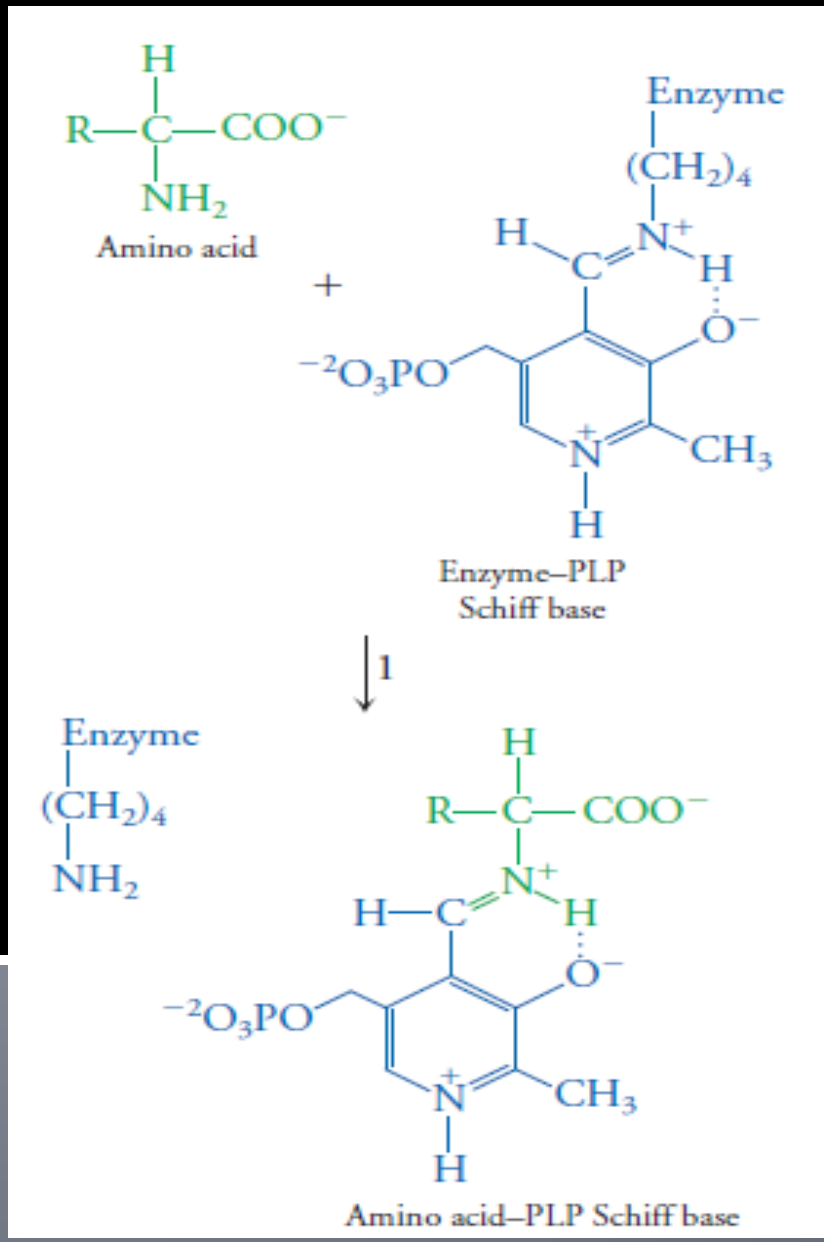
Pyridoxine (vitamin B_6)

PLP is covalently attached to the enzyme via a Schiff base (imine) linkage to the 5′-amino group of a Lys residue (*left). The amino acid substrate of the transaminase* displaces Lys amino group, which then acts as an acid–base catalyst. The steps of the reactions show as next diagramme.

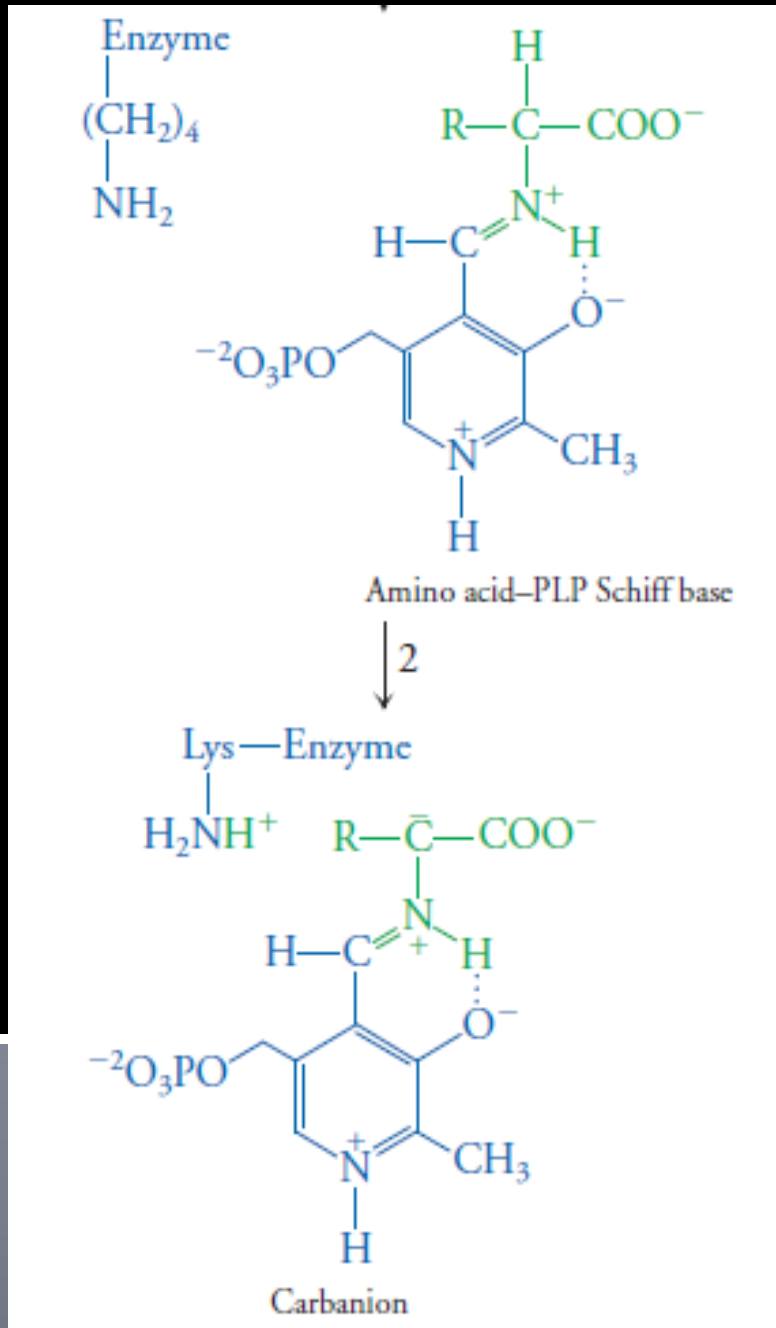
The transamination reaction is freely reversible, so transaminases participate in pathways for amino acid synthesis as well as degradation. If the α keto acid produced in step 4 reenters the active site, then the amino group that was removed from the starting amino acid is restored. However, most transaminases accept only α -ketoglutarate or oxaloacetate as the α-keto acid substrate for the second step.

The Main Steps of PLP-catalyzed transamination.

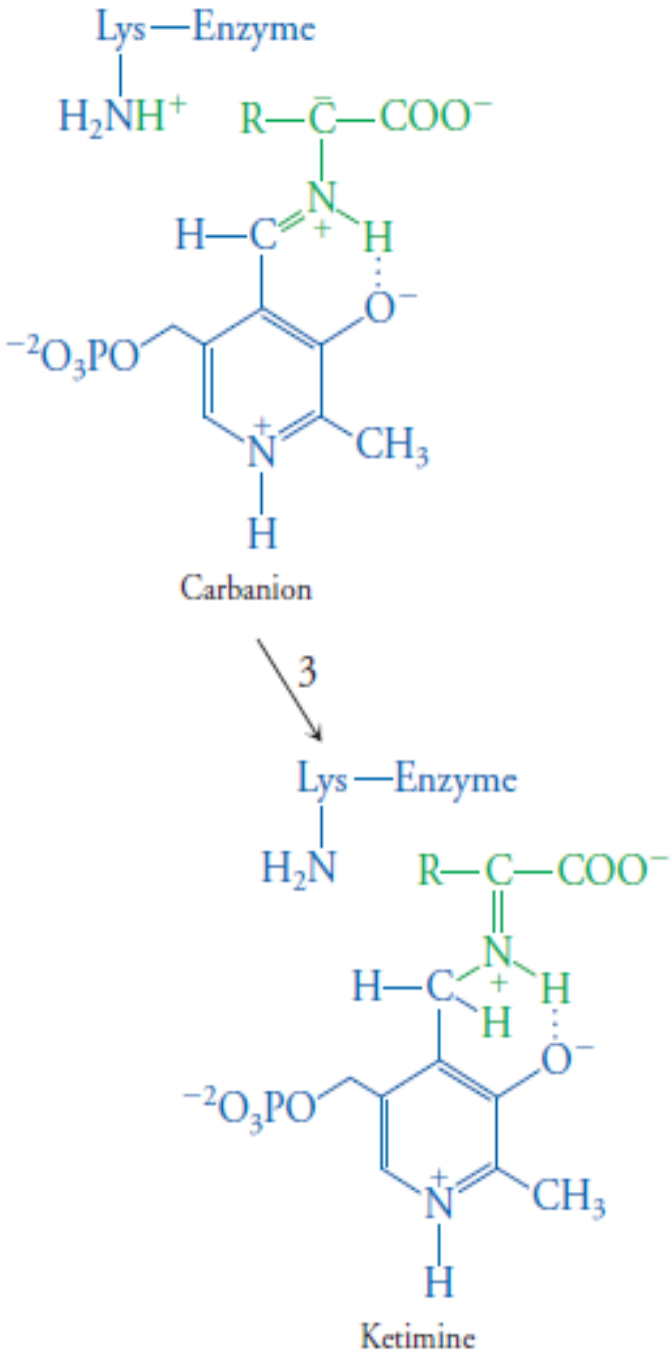
1. The -amino group of an amino acid attacks the enzyme –PLP Schiff base. This transamination reaction forms an amino acid – PLP Schiff base and releases the enzyme's Lys -amino group.



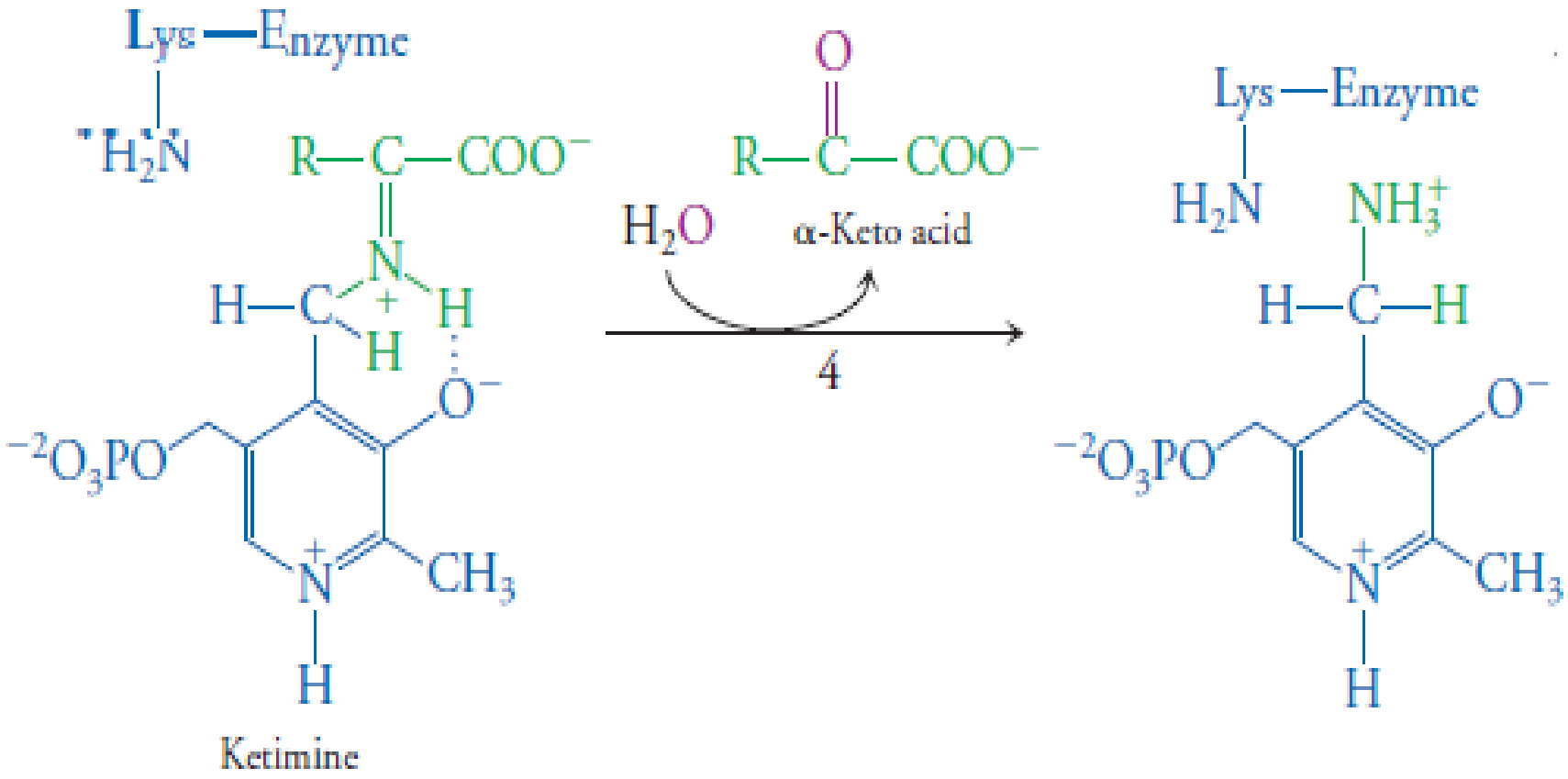
2. The Lys amino group, acting as a base, removes the hydrogen from the substrate
amino acid's carbon. The negative charge of the resulting carbanion is stabilized by the PLP group, which acts as an electron sink.



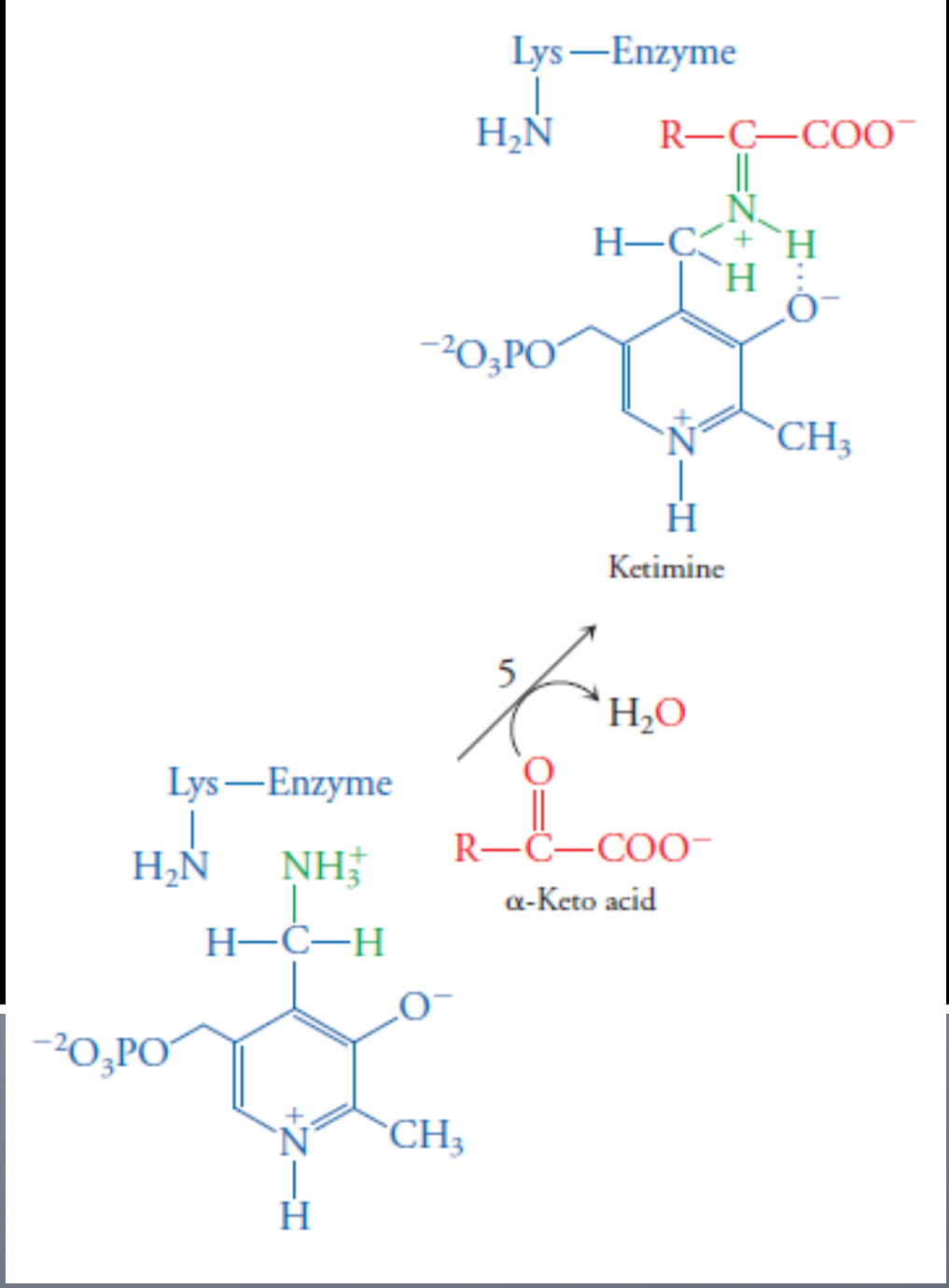
3. The protonated Lys residue, now acting as an acid, donates the proton to the PLP group, generating a ketimine. The molecular rearrangement resulting from the movement of an H atom is known as tautomerization.



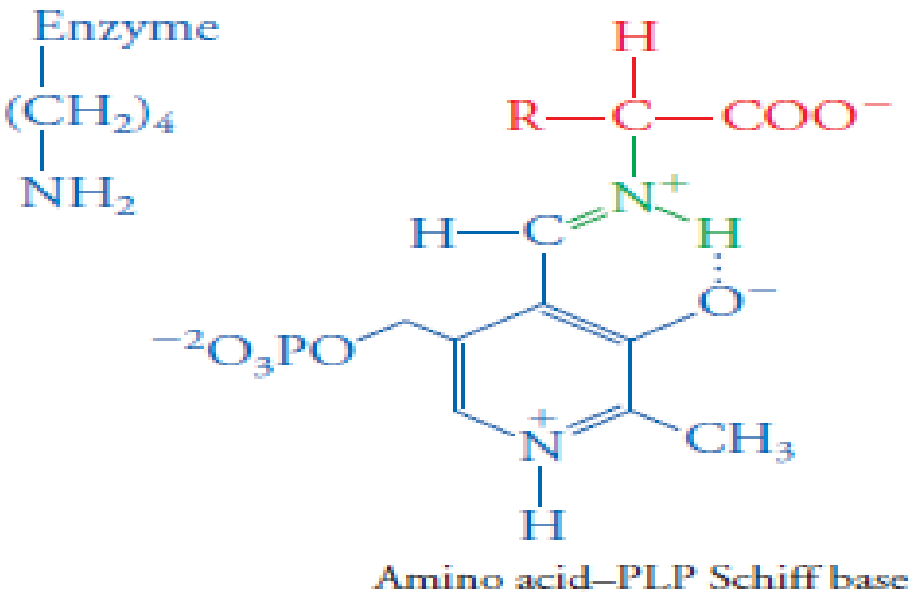
4. Hydrolysis frees the -keto acid and leaves the amino group bound to the PLP group.



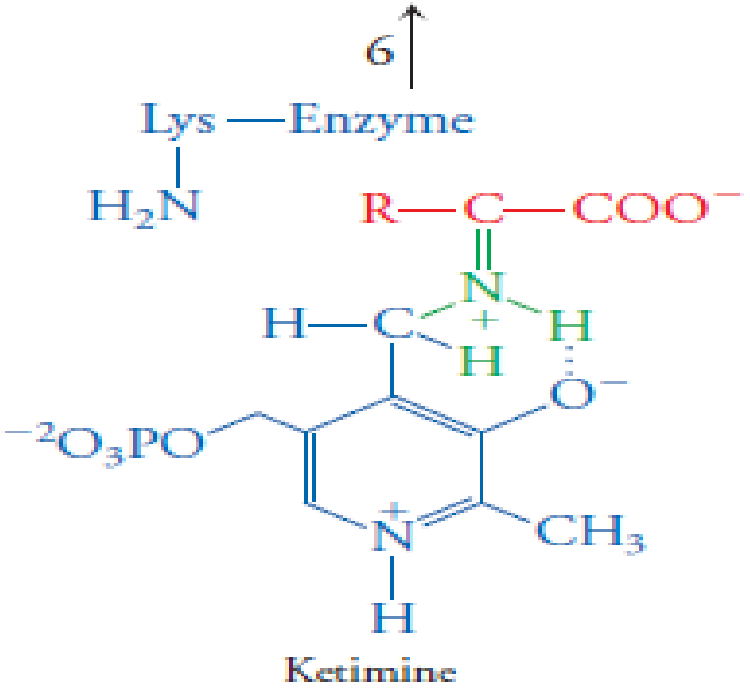
5. Another α - keto acid enters the active site to reform a ketimine (this is the reverse of step 4).



6. Lysine-catalyzed tautomerization yields an amino acid –Schiff base (the reverse of steps 2 and 3).

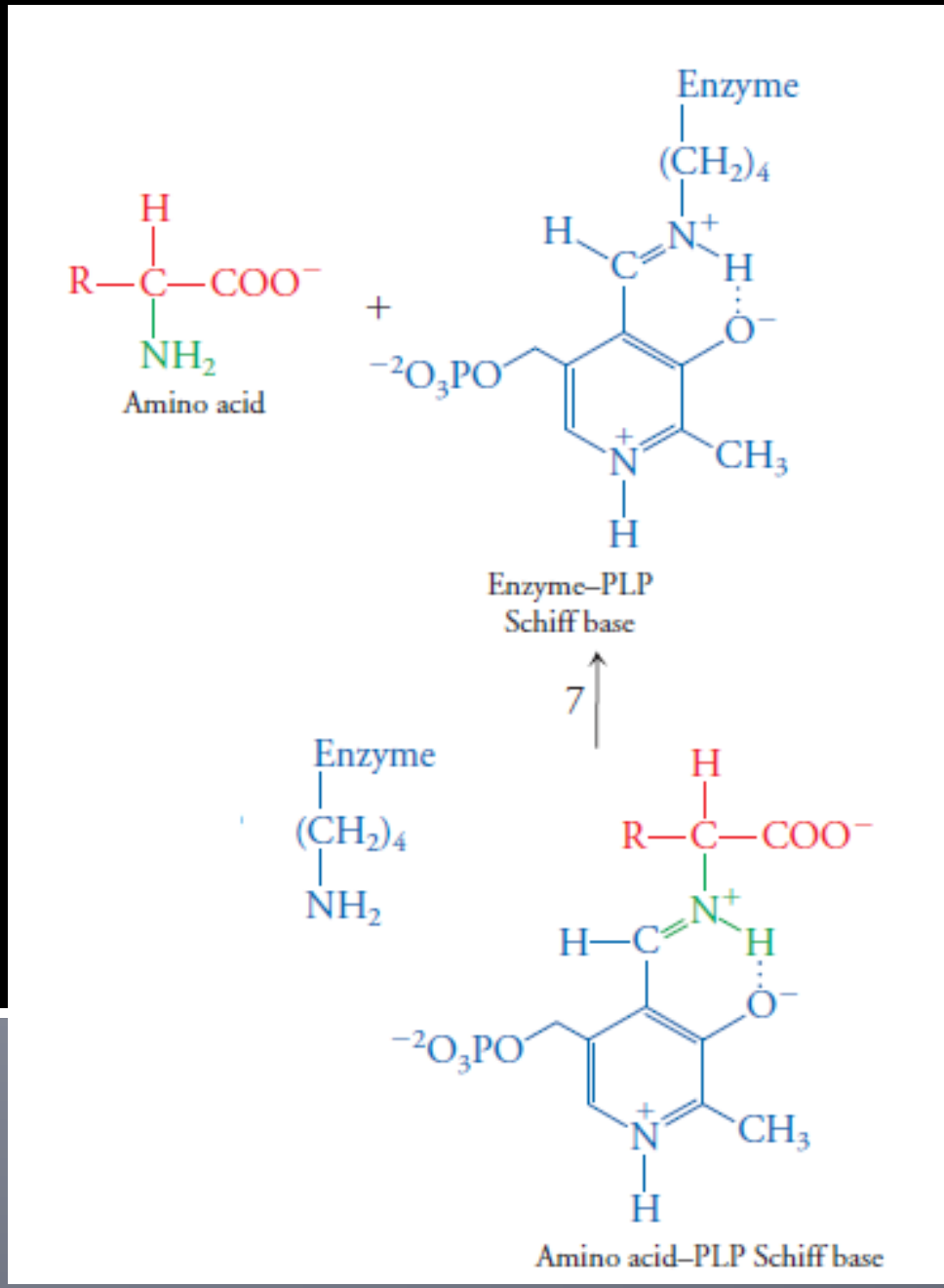


Amino acid–PLP Schiff base



Ketimine

7. In a transamination reaction, the α-amino group of the Lys residue displaces the amino acid and regenerates the enzyme – PLP Schiff base (the reverse of step 1).



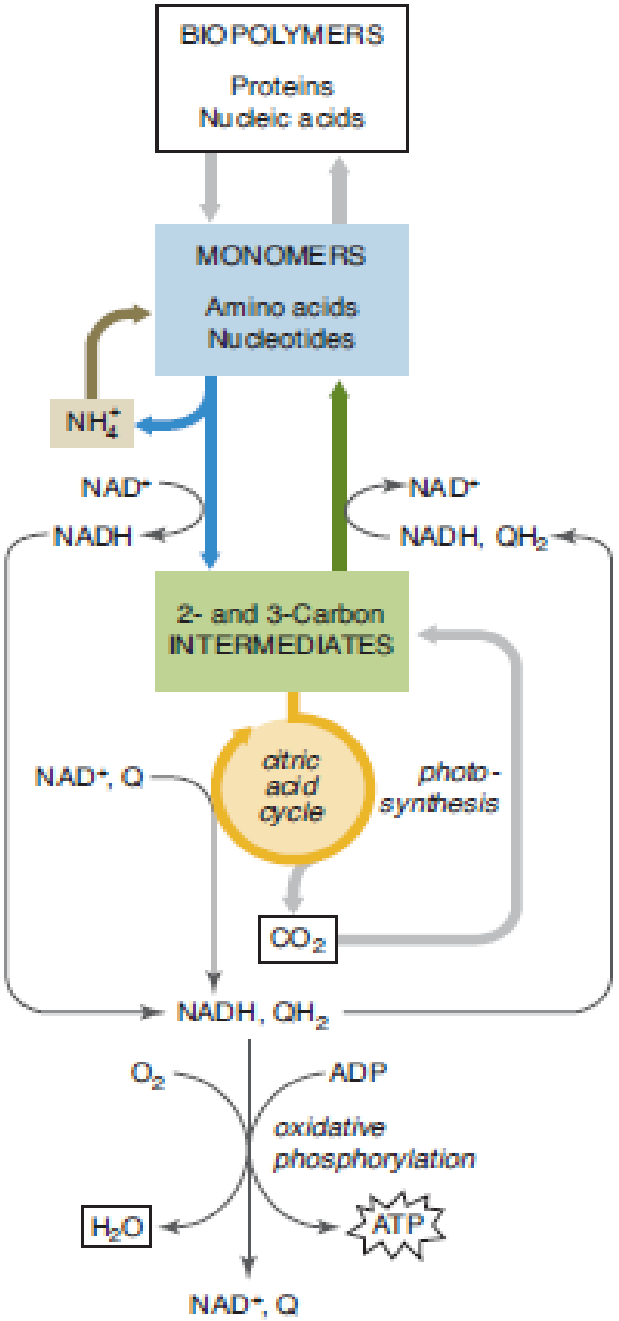
Amino Acid Biosynthesis

CONCEPTS

- Alanine, arginine, asparagine, aspartate, glutamate, glutamine, glycine, proline, and serine are synthesized from intermediates of glycolysis and the citric acid cycle.
- Bacteria and plants synthesize amino acids with sulfur (cysteine and methionine), branched chains (isoleucine, leucine, and valine), and aromatic groups (phenylalanine, tryptophan, and tyrosine) as well as histidine, lysine, and threonine.
- Glutamate and tyrosine are modified to generate neurotransmitters and hormones.

Amino acids are synthesized from intermediates of glycolysis, citric acid cycle, and pentose phosphate pathway. The amino groups are derived from the nitrogen carrier molecules glutamate and glutamine. The relation ships of amino acid biosynthesis and other reactions of nitrogen metabolism with other pathways show as follows.

Amino acids are synthesized mostly from three-carbon intermediates of glycolysis and from intermediates of the citric acid cycle. Amino acid catabolism yields some of the same intermediates, as well as the two-carbon acetyl-CoA. Amino acids are also the precursors of nucleotides.



Humans can synthesize only some of the 20 amino acids that are commonly found in proteins. These are known as **nonessential amino acids. The other amino** acids are said to be **essential because humans cannot synthesize them and must obtain** them from their food. The ultimate sources of the essential amino acids are plants and microorganisms, which produce all the enzymes necessary to undertake the synthesis of these compounds.

The essential and nonessential amino acids for humans are listed in opposite Table. This classification scheme can be somewhat ***confusing***. For example, some nonessential amino acids, such as ***Arginine*** , may be essential for young children; that is, dietary sources must supplement what the body can produce on its own. Human cells cannot synthesize ***Histidine***, so it is classified as an essential amino acid, even though a dietary

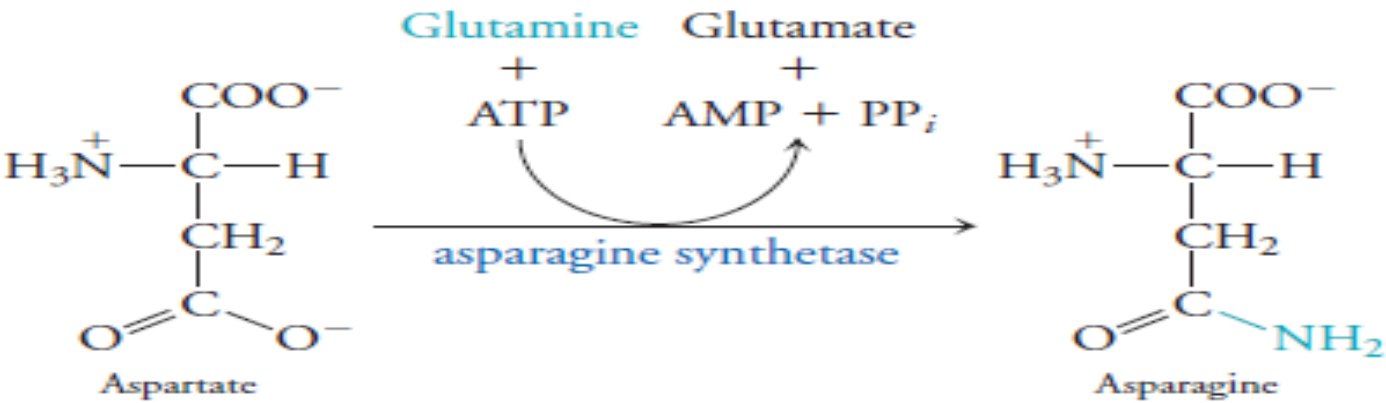
Essential and Nonessential Amino Acids

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

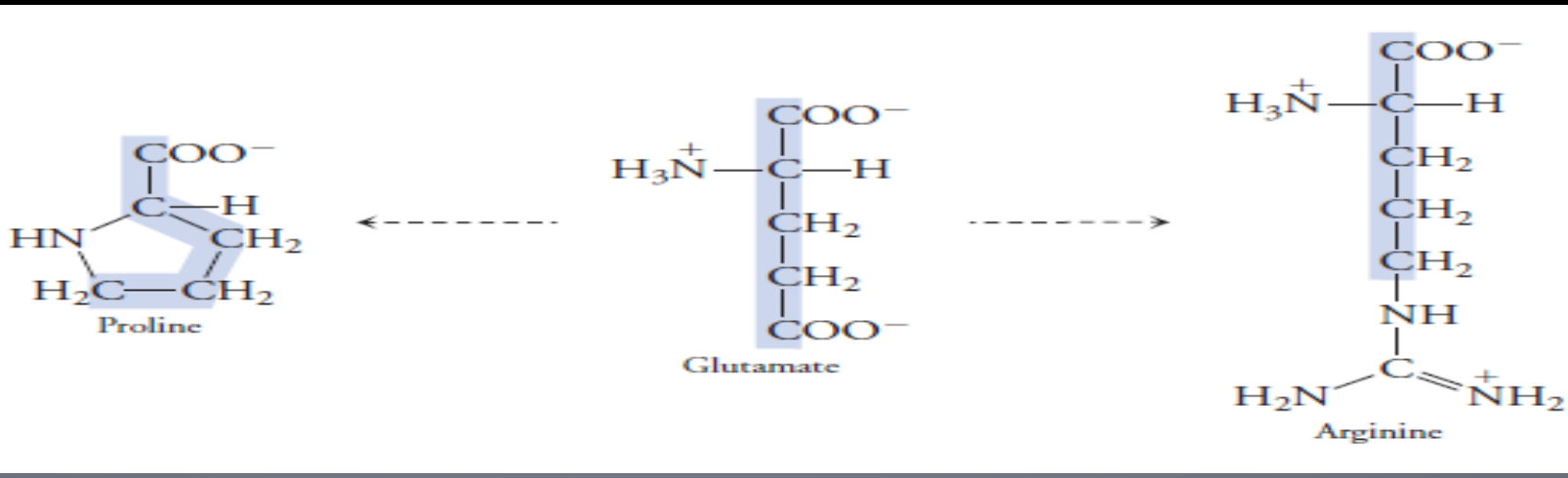
requirement has never been defined (probably because sufficient quantities are naturally supplied by intestinal microorganisms). ***Tyrosine*** can be considered essential in that it is synthesized directly from the essential amino acid phenylalanine. Likewise, ***cysteine*** synthesis depends on the availability of sulfur provided by the essential amino acid ***methionine.***

Several amino acids are easily synthesized from common metabolites

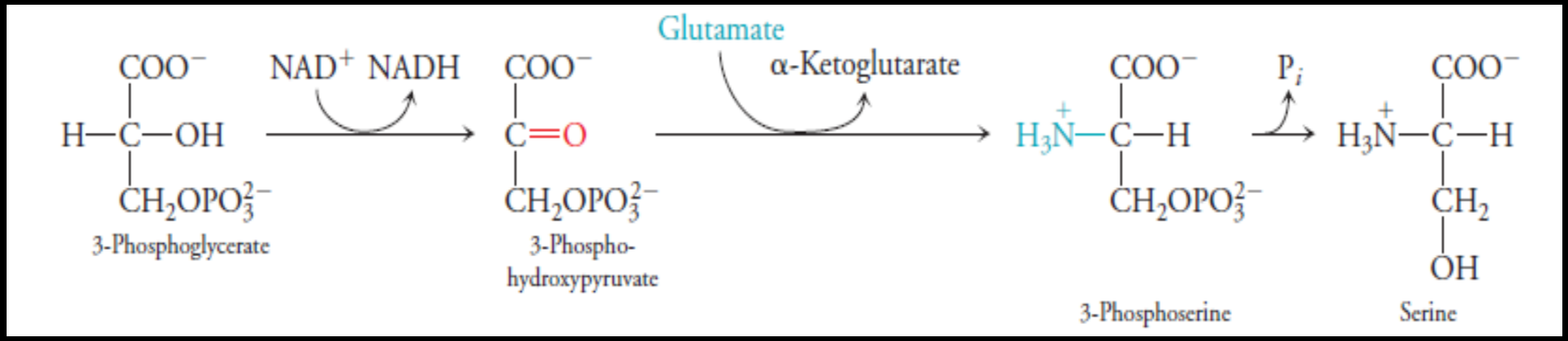
Some amino acids can be produced by transamination reactions. In this way, ***alanine*** *is produced from pyruvate,* ***aspartate*** *from oxaloacetate,* and ***glutamate*** from α-ketoglutarate. glutamine synthetase catalyzes the amidation of glutamate to produce glutamine. Asparagine synthetase, which uses glutamine as an amino-group donor rather than ammonia, converts ***aspartate to asparagine:***



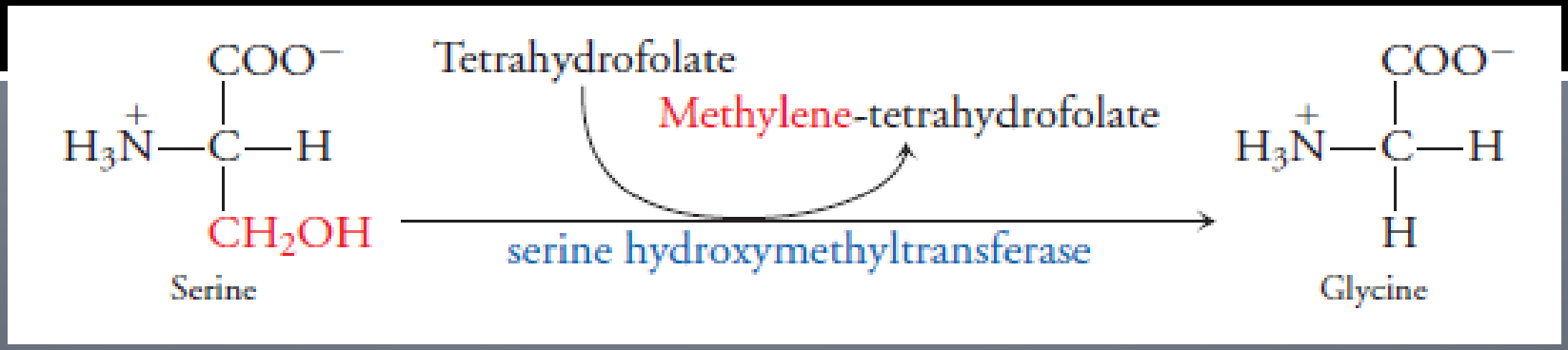
Three common metabolic intermediates (pyruvate, oxaloacetate, and α-ketoglutarate) give rise to five nonessential amino acids by simple transamination and amidation reactions. Slightly longer pathways convert glutamate to proline and arginine, which each have the same five-carbon core:



Serine is derived from the glycolytic intermediate 3-phosphoglycerate in three steps:



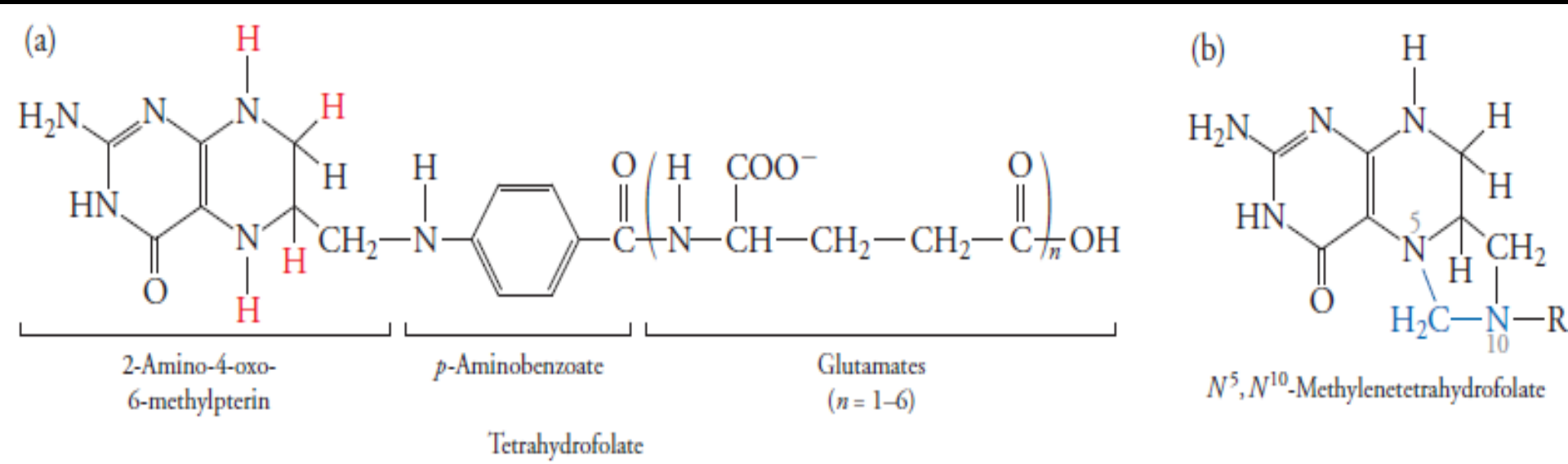
Serine, a three-carbon amino acid, gives rise to the two-carbon glycine in a reaction catalyzed by serine hydroxymethyltransferase (the reverse reaction converts glycine to serine). This enzyme uses a PLP-dependent mechanism to remove the hydroxymethyl (OCH2OH) group attached to the a carbon of serine; this one-carbon fragment is then transferred to the cofactor tetrahydrofolate:



Tetrahydrofolate functions as a carrier of one-carbon units in several reactions of amino acid and nucleotide metabolism ***. mammals cannot synthesize folate (the*** oxidized form of tetrahydrofolate) and must therefore obtain it as a vitamin from their diet. Folate is abundant in foods such as fortifi ed cereal, fruits, and vegetables. The requirement for folate increases during the fi rst few weeks of pregnancy, when the fetal nervous system begins to develop. Supplemental folate appears to revent certain neural tube defects such as spina bifi da, in which the spinal cord remains exposed.

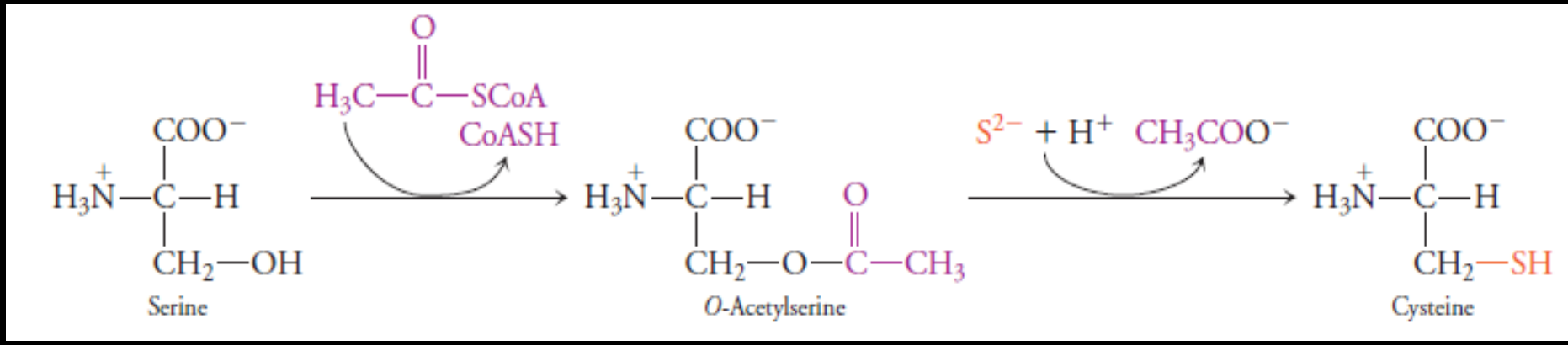
Amino acids with sulfur, branched chains, or aromatic groups are more difficult to synthesize.

We have just described how a few metabolites—pyruvate, 3-phosphoglycerate, oxaloacetate, and α-ketoglutarate—are converted in a few enzyme-catalyzed steps to nine different amino acids. Synthesis of the other amino acids (the essential amino acids and those derived directly from them) also begins with common metabolites. However, these biosynthetic pathways tend to be more complicated. At some point in their evolution, animals lost the ability to synthesize these amino acids, probably because the pathways were energetically expensive and the compounds were already available in food. In general, humans cannot synthesize branched-chain amino acids

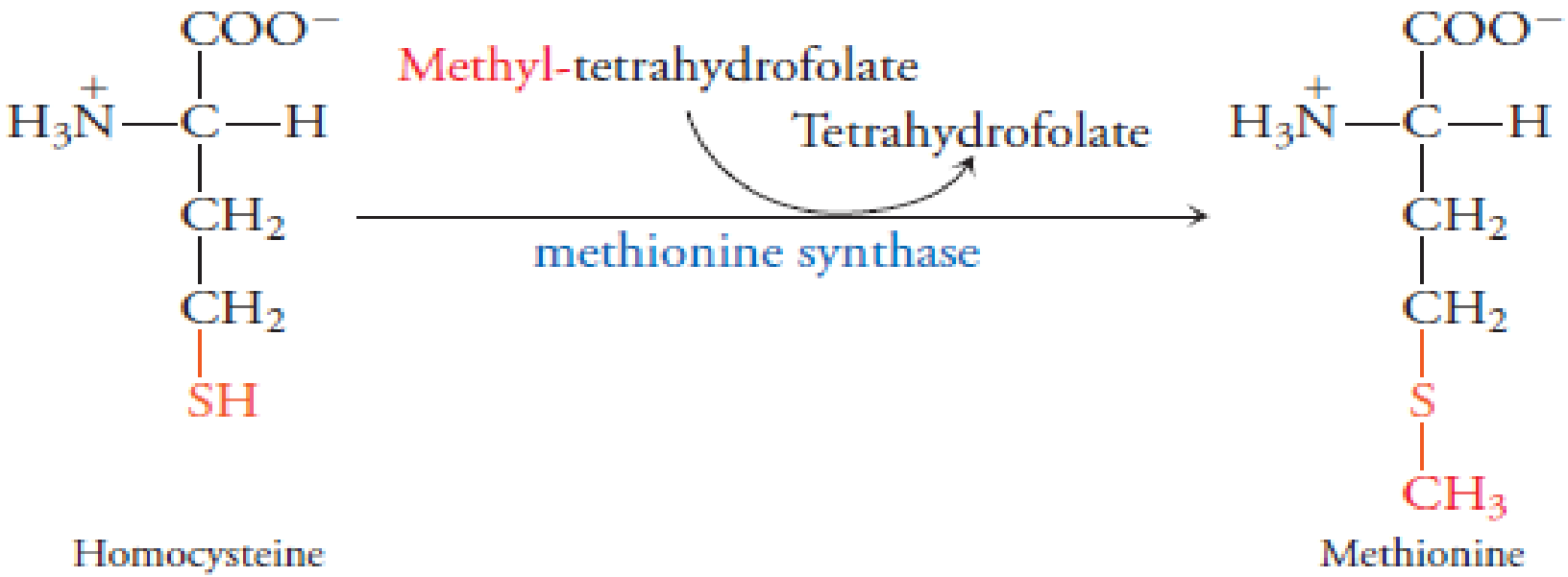


Tetrahydrofolate. (a) This cofactor consists of a pterin derivative, a *p-aminobenzoate residue, and up to six* glutamate residues. It is a reduced form of the vitamin folate. The four H atoms of the tetrahydro form are colored red. (b) In the conversion of serine to glycine, a methylene group (blue) becomes attached to both N5 and N10 of tetrahydrofolate. Tetrahydrofolate can carry carbon units of different oxidation states. For example, a methyl group can attach to N5, and a formyl group (OHCO) can attach at N5 or N10.

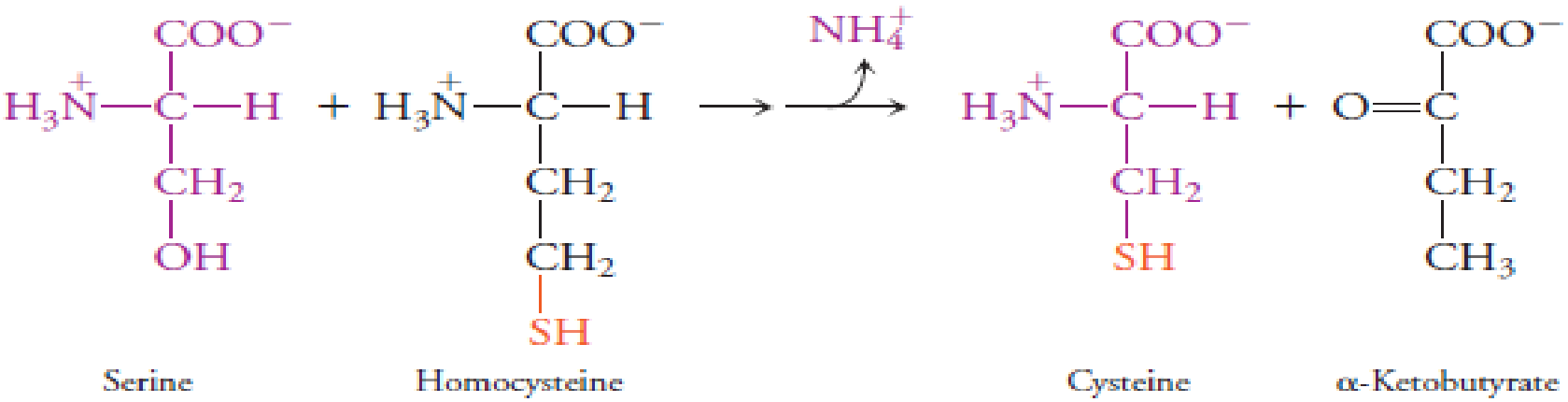
or aromatic amino acids and cannot incorporate sulfur into compounds such as methionine. In this section, we will focus on a few interesting points related to the synthesis of essential amino acids. The bacterial pathway for producing sulfur-containing amino acids begins with serine and uses sulfur that comes from inorganic sulfide:



Cysteine can then donate its sulfur atom to a four-carbon compound derived from aspartate, forming the nonstandard amino acid homocysteine. The final step of methionine synthesis is catalyzed by methionine synthase, which adds to homocysteine a methyl group carried by tetrahydrofolate:

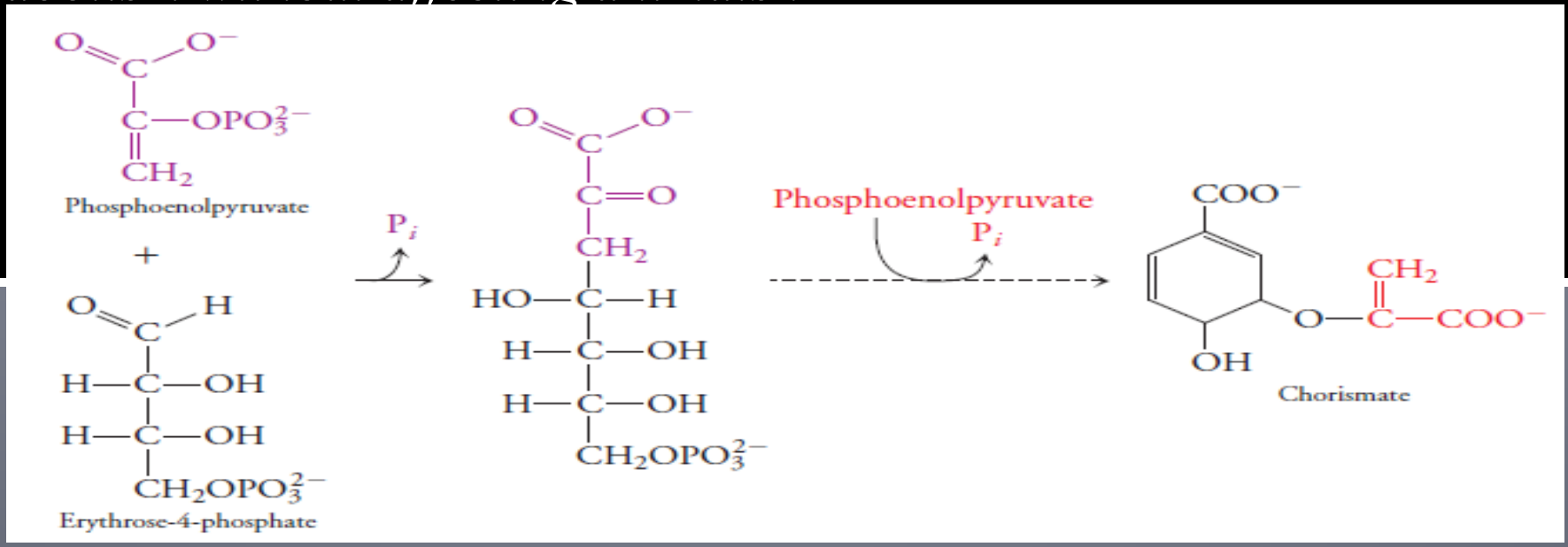


In humans, serine reacts with homocysteine to yield cysteine:

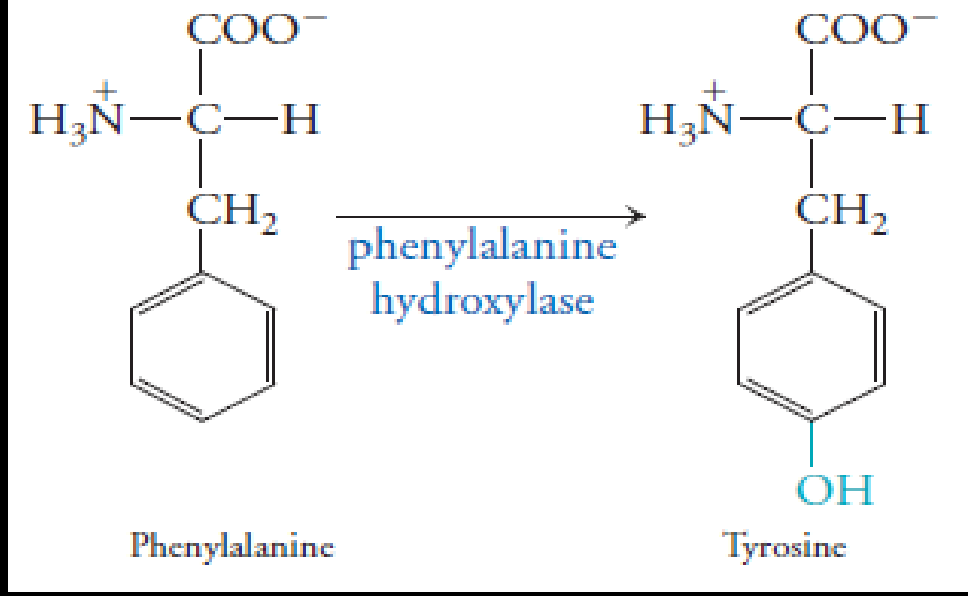


This pathway is the reason why cysteine is considered a nonessential amino acid, although its sulfur atom must come from another amino acid. High levels of homocysteine in the blood are associated with cardiovascular disease. The link was first discovered in individuals with homocystinuria, a disorder in which excess homocysteine is excreted in the urine. These individuals develop atherosclerosis as children, probably because the homocysteine directly damages the walls of blood vessels even in the absence of elevated LDL levels. Increasing the intake of folate, the vitamin precursor of tetrahydrofolate, helps decrease the level of homocysteine by promoting its conversion to methionine. Aspartate, the precursor of methionine, is also the precursor of the essential amino acids threonine and lysine. Since these amino acids are derived from another amino acid, they already have an amino group. The branched-chain amino acids (valine, leucine, and isoleucine) are synthesized by pathways that use pyruvate as the starting substrate. These amino acids require a step catalyzed by a transaminase (with glutamate as a substrate) to introduce an amino group. In plants and bacteria, the pathway for synthesizing the aromatic amino acids

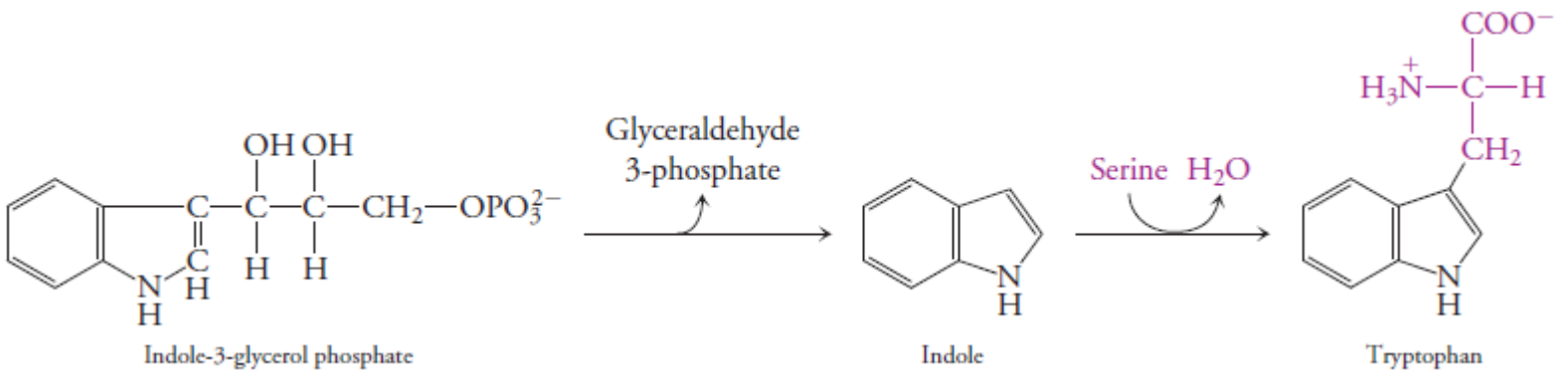
(phenylalanine, tyrosine, and tryptophan) begins with the condensation of the C_3 compound phosphoenolpyruvate (a glycolytic intermediate) and erythrose-4-phosphate(a four-carbon intermediate of the pentose phosphate pathway). The seven-carbon reaction product then cyclizes and undergoes additional modifi cations, including the addition of three more carbons from phosphoenolpyruvate, before becoming chorismate, the last common intermediate in the synthesis of the three aromatic amino acids. *Because animals do not synthesize chorismate, this pathway is an obvious target for agents that can inhibit plant metabolism without affecting animals .*



Phenylalanine and tyrosine are derived from chorismate by diverging pathways. In humans, tyrosine is generated by hydroxylating phenylalanine, which is why tyrosine is not considered an essential amino acid.

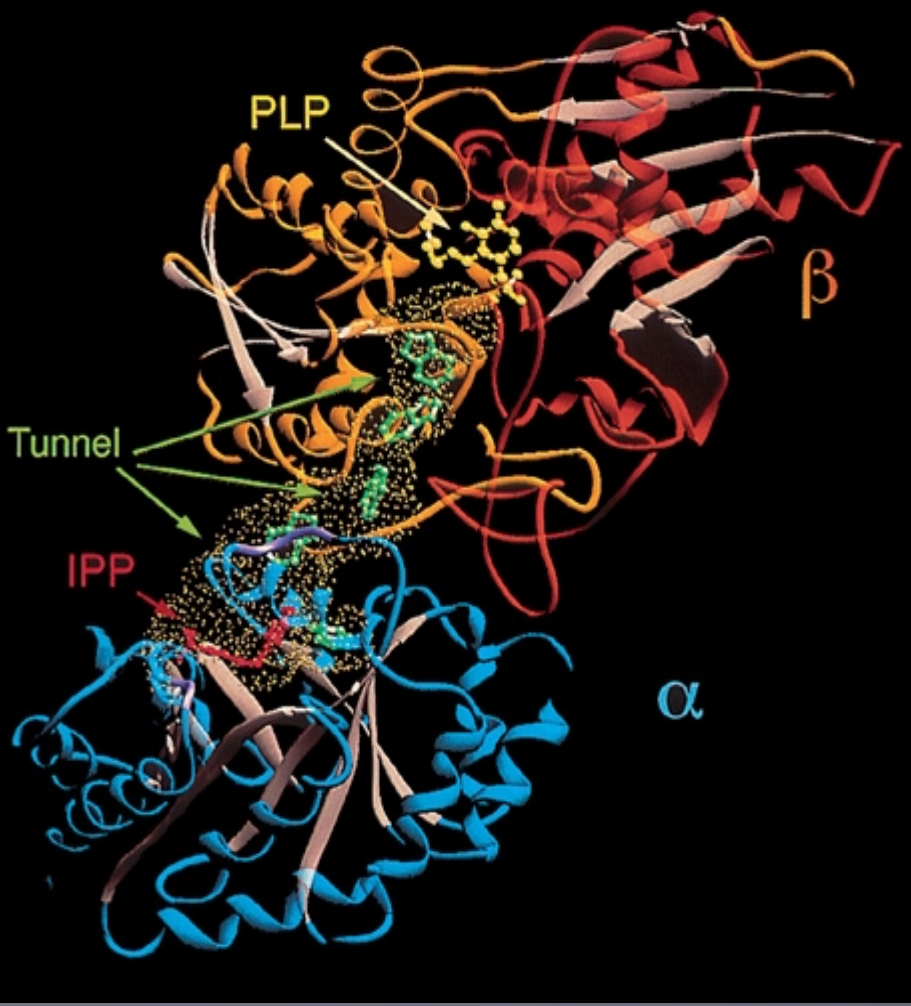


The final two reactions of the tryptophan biosynthetic pathway (which has 13 steps altogether) are catalyzed by tryptophan synthase, a bifunctional enzyme with an $\alpha 2 \beta 2$ quaternary structure. The α subunit cleaves indole-3-glycerol phosphate to indole and glyceraldehyde-3-phosphate, then the β subunit adds serine to indole to produce tryptophan:



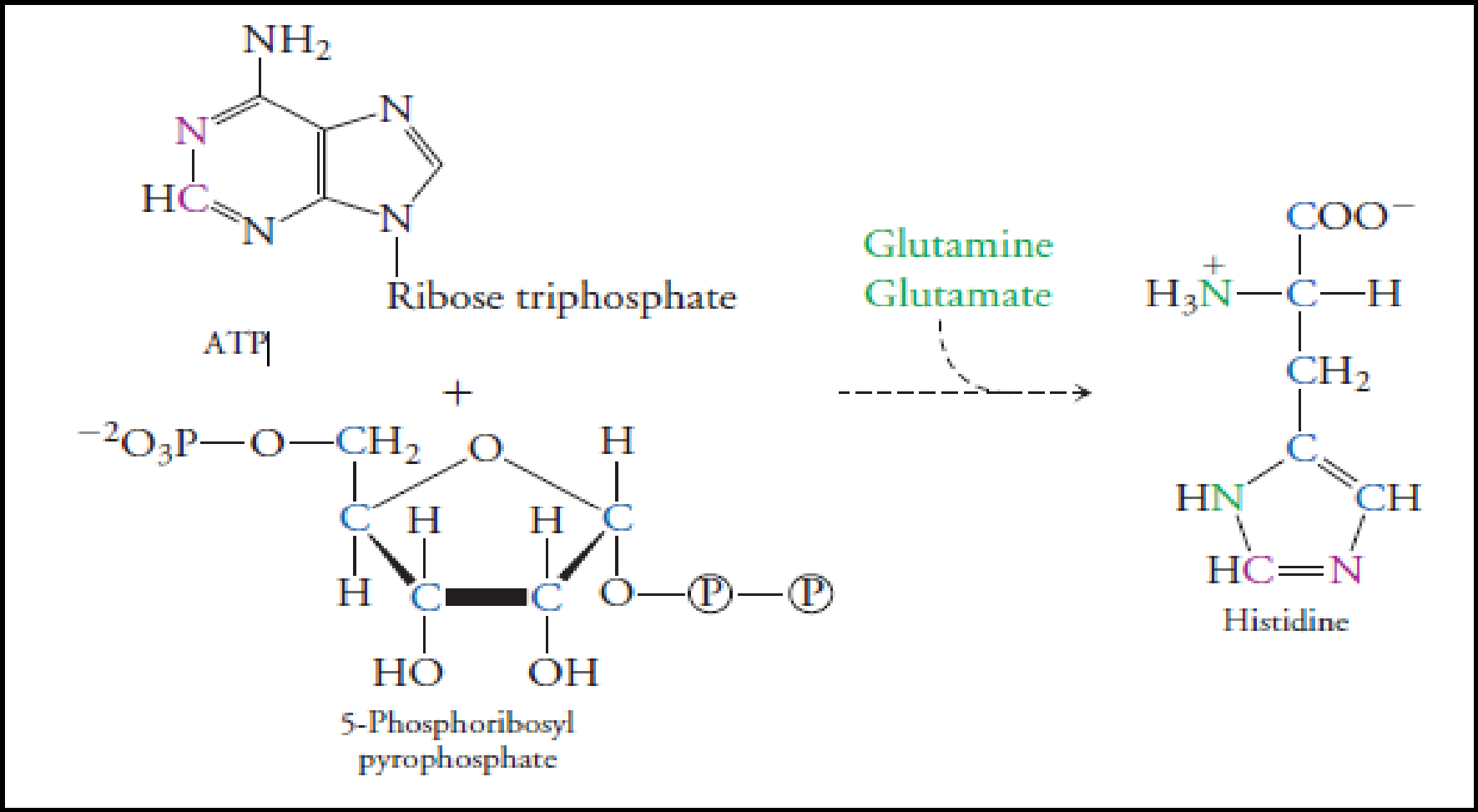
Indole, the product of the a-subunit reaction and the substrate for the β-subunit reaction, never leaves the enzyme. Instead, it diffuses directly from one active site to the other without entering the surrounding solvent. The X-ray structure of the enzyme reveals that the active sites in adjacent a and β subunits are 25 Å apart but are connected by a tunnel through the protein that is large enough to accommodate indole.

Figure :Tryptophan synthase. Only one a subunit (blue and tan) and one b subunit (yellow, orange, and tan)are shown. Indolepropanol phosphate (IPP; red) marks the active site of the a subunit. The b active site is marked by its PLP cofactor (yellow). The surface of the tunnel between the two active sites is outlined with yellow dots. Several indole molecules (green) are included in the model to show how this interme iate can pass between the active sites. [Courtesy of Craig Hyde, National Institutes of Health.] **See Interactive Exercise. The bifunctional enzyme tryptophan synthase.**



The movement of a reactant between two active sites is called ***channeling,*** *and it increases the rate of a metabolic process by preventing the loss of intermediates.*

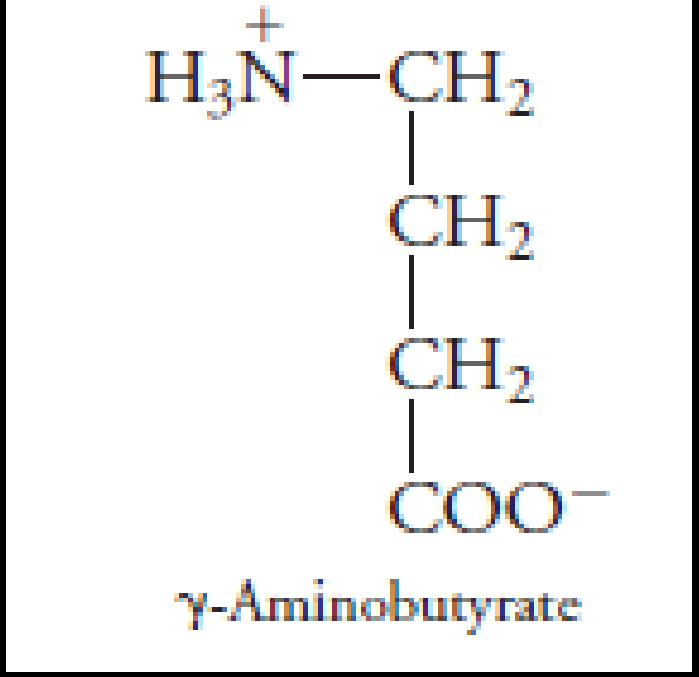
Channeling is known to occur in a few other multifunctional enzymes. All but one of the 20 standard amino acids are synthesized entirely from precursors produced by the main carbohydrate-metabolizing pathways. The exception is histidine, to which ATP provides one nitrogen and one carbon atom. Glutamate and glutamine donate the other two nitrogen atoms, and the remaining five carbons are derived from a phosphorylated monosaccharide, 5- phosphoribosyl pyrophosphate (PRPP):



5-Phosphoribosyl-pyrophosphate is also the source of the ribose group of nucleotides. This suggests that histidine might have been one of the first amino acids synthesized by an early life-form making the transition from an all-RNA metabolism to an RNA-and protein- based metabolism.

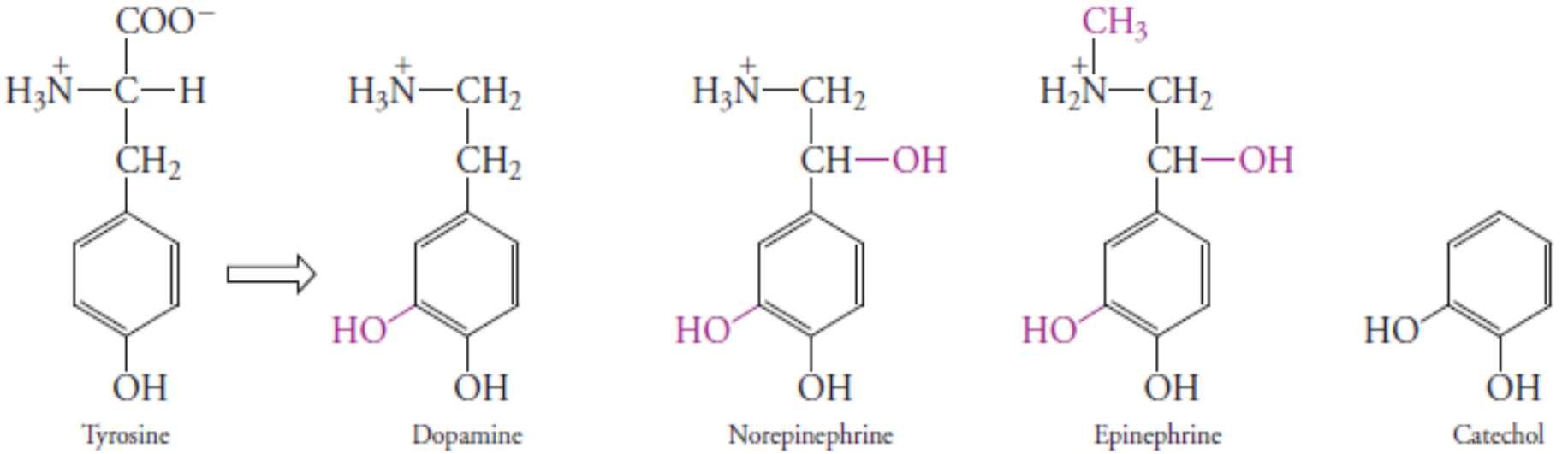
Amino acids are the precursors of some signaling molecules

Many amino acids that are ingested or built from scratch find their way into a cell's proteins, but some also have essential functions as precursors of other compounds, including **neurotransmitters.** Communication in the complex neuronal circuitry of the nervous system relies on small chemical signals that are released by one neuron and taken up by another . Common neurotransmitters include the amino acids glycine and glutamate and a glutamate derivative (its carboxylate group has been removed) known as γ - aminobutyric acid (GABA) or γ - aminobutyrate.

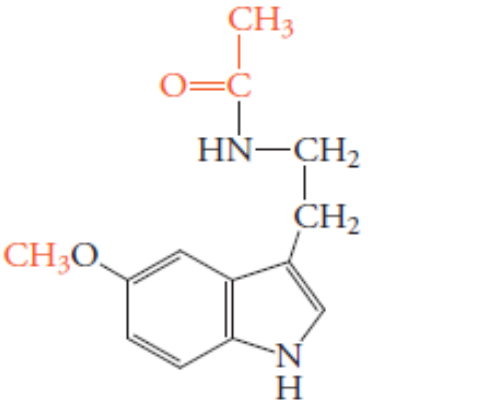


γ-Aminobutyrate

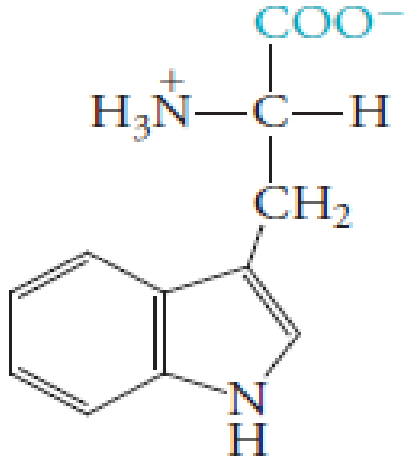
Several other amino acid derivatives also function as neurotransmitters. For example, tyrosine gives rise to dopamine, norepinephrine, and epinephrine. These compounds are called catecholamines, reflecting their resemblance to catechol.



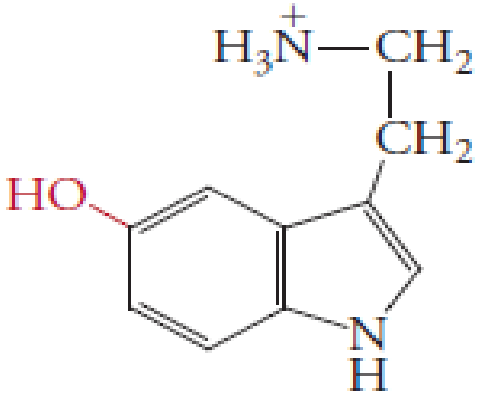
A deficiency of dopamine produces the symptoms of Parkinson's disease: tremor, rigidity, and slow movements. catecholamines are also produced by other tissues and function as hormones. Tryptophan is the precursor of the neurotransmitter serotonin:



Melatonin



Tryptophan



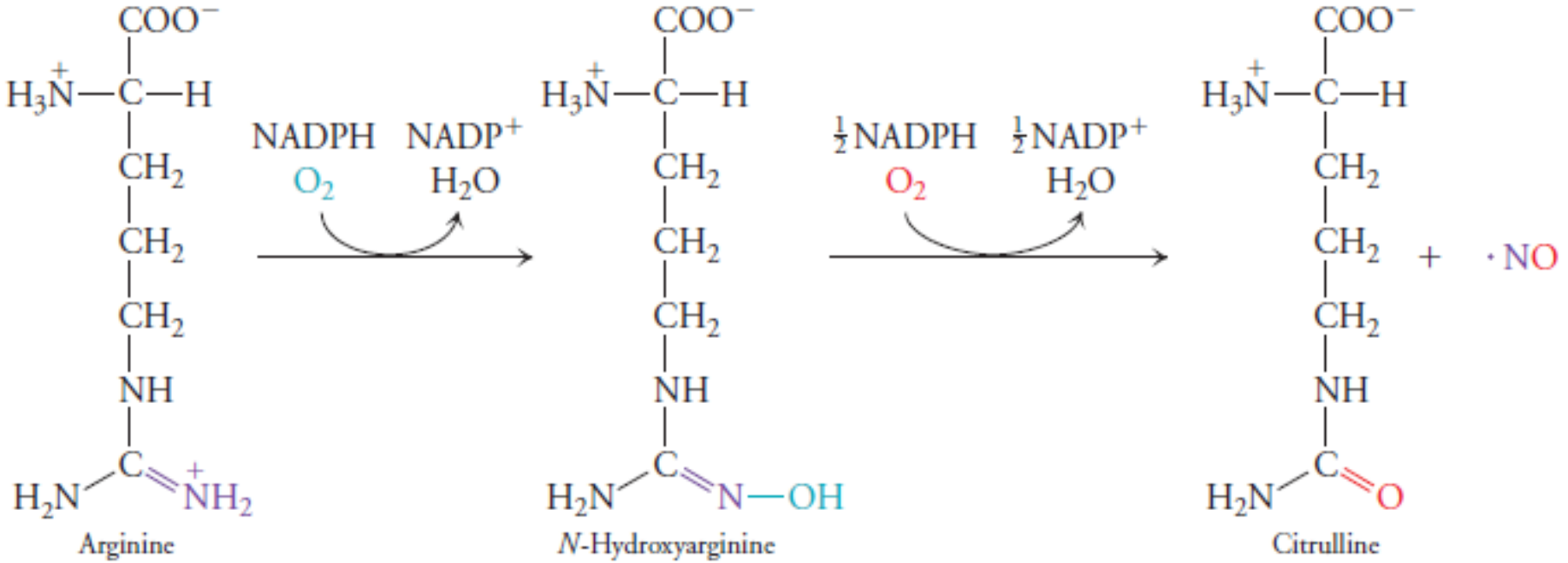
Serotonin

Low levels of serotonin in the brain have been linked to conditions such as depression, aggression, and hyperactivity. The antidepressive effect of drugs such as Prozac® results from their ability to increase serotonin levels by blocking the reabsorption of the released neurotransmitter. Serotonin is the precursor of melatonin (*right).* This tryptophan derivative is synthesized in the pineal gland and retina. Its concentration is low during the day, rising during darkness. Because melatonin appears to govern the synthesis of some other neurotransmitters that control circadian (daily) rhythms, it has been touted as a cure for sleep disorders and jet lag. Arginine is also the precursor of a signaling molecule that was discovered only a few years ago to be the free radical gas nitric

Nitric Oxide

In the 1980s, vascular biologists were investigating the nature of an endothelial cell–derived "relaxation factor" that caused blood vessels to dilate. This substance diffused quickly, acted locally, and disappeared within seconds. To the surprise of many, the mysterious factor turned out to be the free radical nitric oxide (NO). Although NO was known to elicit vasodilation , it had not been considered a good candidate for a biological signaling molecule because its unpaired electron makes it extremely reactive and it breaks down to yield the corrosive nitric acid.

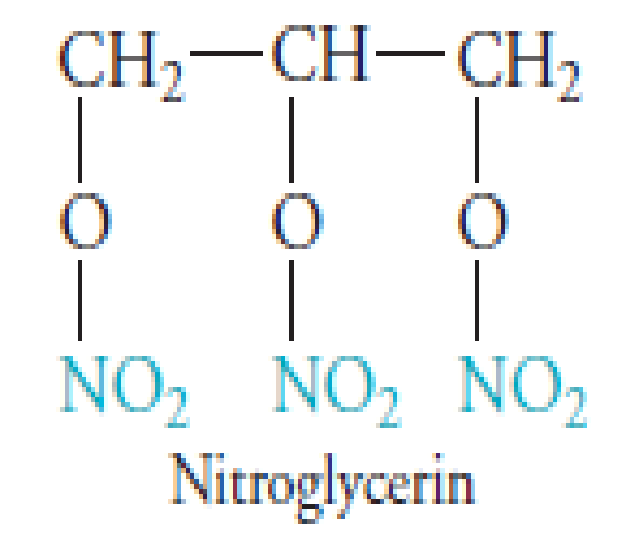
NO is a signaling molecule in a wide array of tissues. At low concentrations it induces blood vessel dilation; at high concentrations (along with oxygen radicals) it kills pathogens. NO is synthesized from Arginine by nitric oxide synthase , an enzyme whose cofactors include FMN, FAD, tetrahydrobiopterin (discussed in Section 18-4), and a heme group. The fi rst step of NO production is a hydroxylation reaction. In the second step, one electron oxidizes *N-hydroxyarginine*.



NO is unusual among signaling molecules for several reasons: It cannot be stockpiled for later release; it diffuses into cells, so it does not need a cell-surface receptor; and it needs no degradative enzyme because it breaks down on its own. NO is produced only when and where it is needed. A free radical gas such as NO cannot be directly introduced into the body, but an indirect source of NO has been clinically used for over a century. Individuals who suffer from angina pectoris, a painful condition caused by obstruction of the coronary blood vessels, can relieve their symptoms by taking nitroglycerin:

In vivo, nitroglycerin yields NO , which rapidly stimulates vasodilation , temporarily relieving the symptoms of angina.

Question: ***Explain why blood vessels express constant amounts of nitric oxide synthase whereas white blood cells must be induced to produce the enzyme.***



Nitroglycerin

Nucleotide Biosynthesis

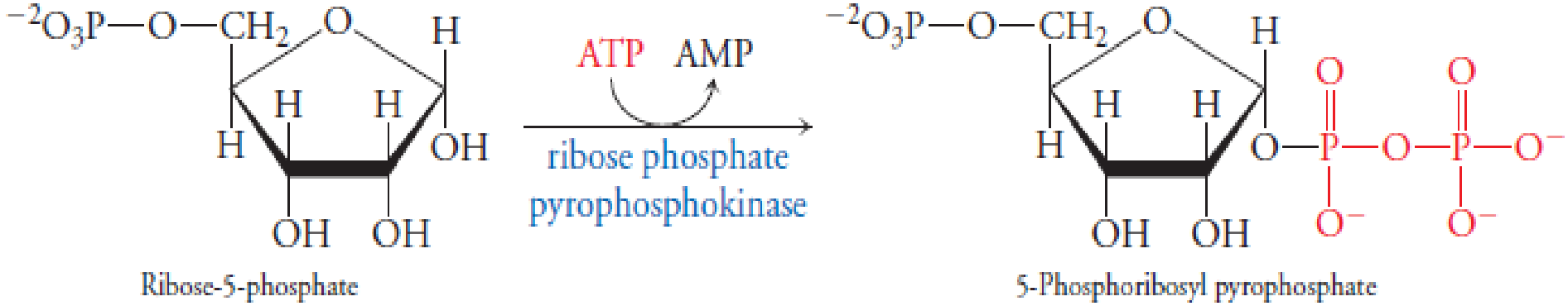
CONCEPTS

- AMP and GMP are derived from the purine nucleotide IMP.
- Pyrimidine nucleotide synthesis produces UTP and then CTP.
- Ribonucleotide reductase converts NDPs to dNDPs using a free radical mechanism.
- dUMP is methylated to produce dTMP.
- Nucleotides are degraded for excretion and to supply materials for salvage or other pathways.

Nucleotides are synthesized from precursors that include several amino acids. *The human* body can also recycle nucleotides from nucleic acids and nucleotide cofactors that are broken down. Although food supplies nucleotides, the biosynthetic and recycling pathways are so efficient that there is no true dietary requirement for purines and pyrimidines The biosynthetic pathways for purine and pyrimidine nucleotides in mammals disccase here.

Purine nucleotide synthesis yields IMP and then AMP and GMP

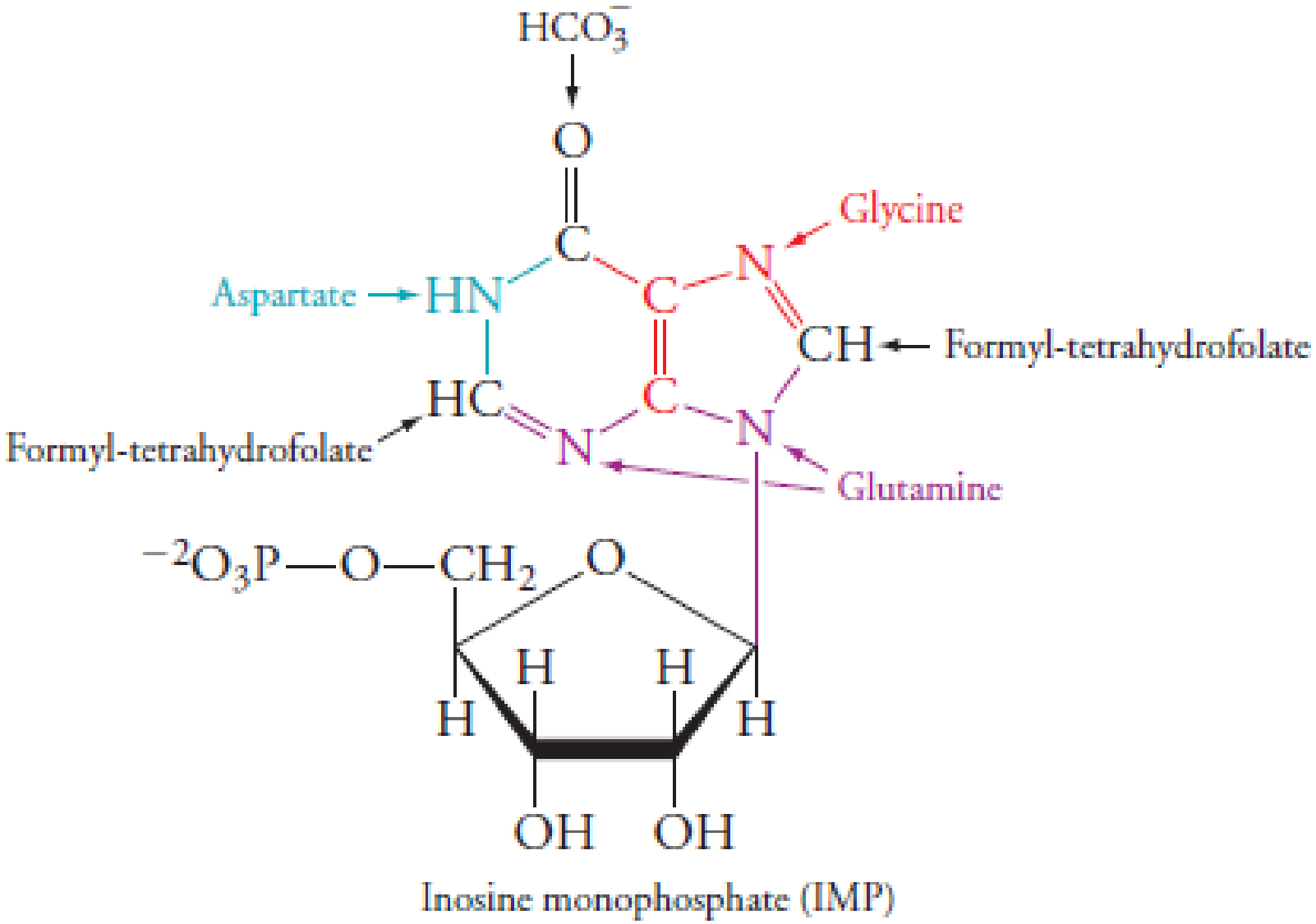
Purine nucleotides (AMP and GMP) are synthesized by building the purine base on to a ribose-5-phosphate molecule. In fact, the first step of the pathway is the production of 5-phosphoribosyl pyrophosphate (which is also a precursor of Histidine):



Ribose-5-phosphate

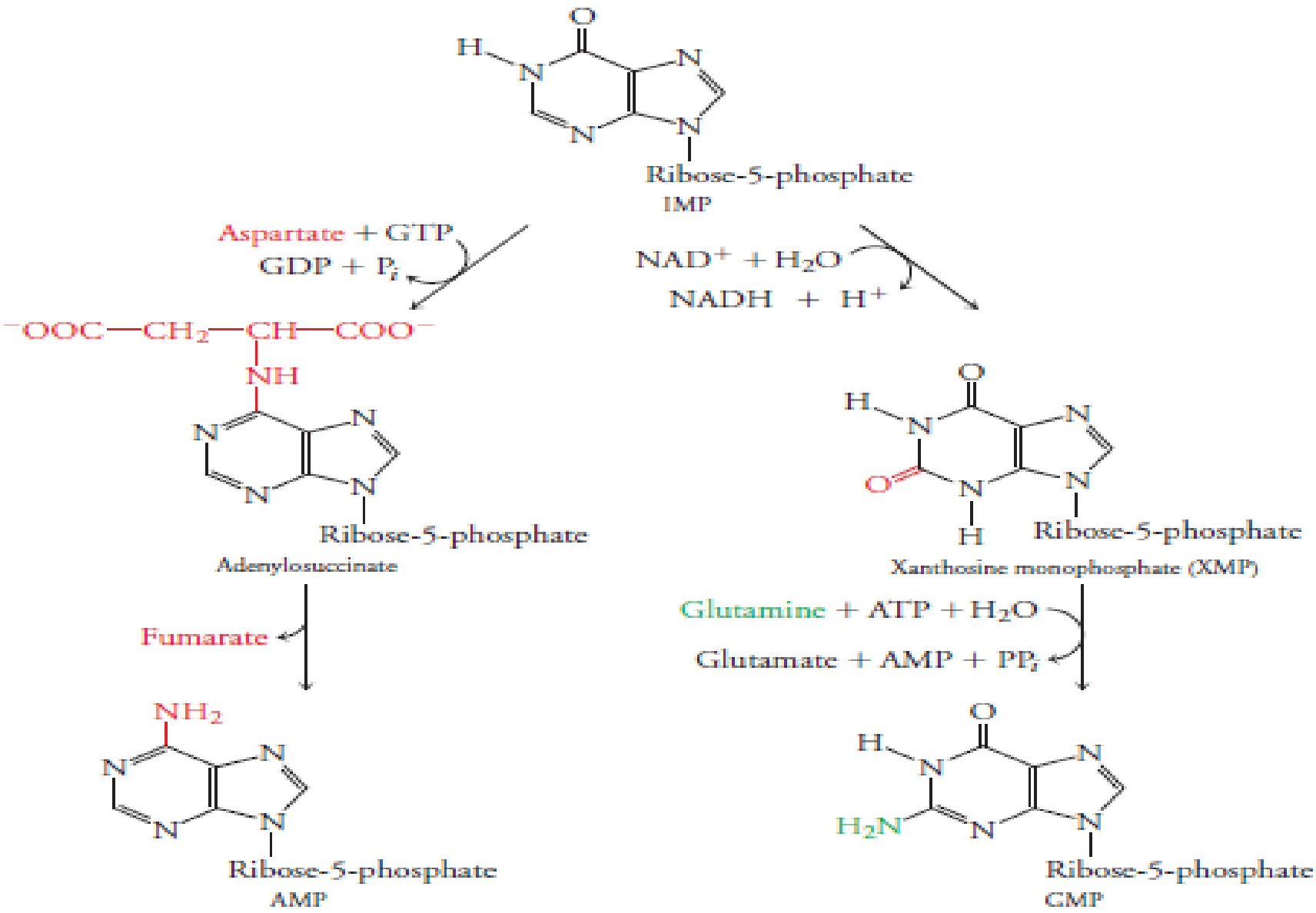
5-Phosphoribosyl pyrophosphate

The subsequent ten steps of the pathway require as substrates glutamine, glycine, aspartate, bicarbonate, plus one-carbon formyl (OHCPO) groups donated by tetrahydrofolate . The product is inosine monophosphate (IMP), a nucleotide whose base is the purine hypoxanthine:



Inosine monophosphate (IMP)

AMP and GMP synthesis from IMP.

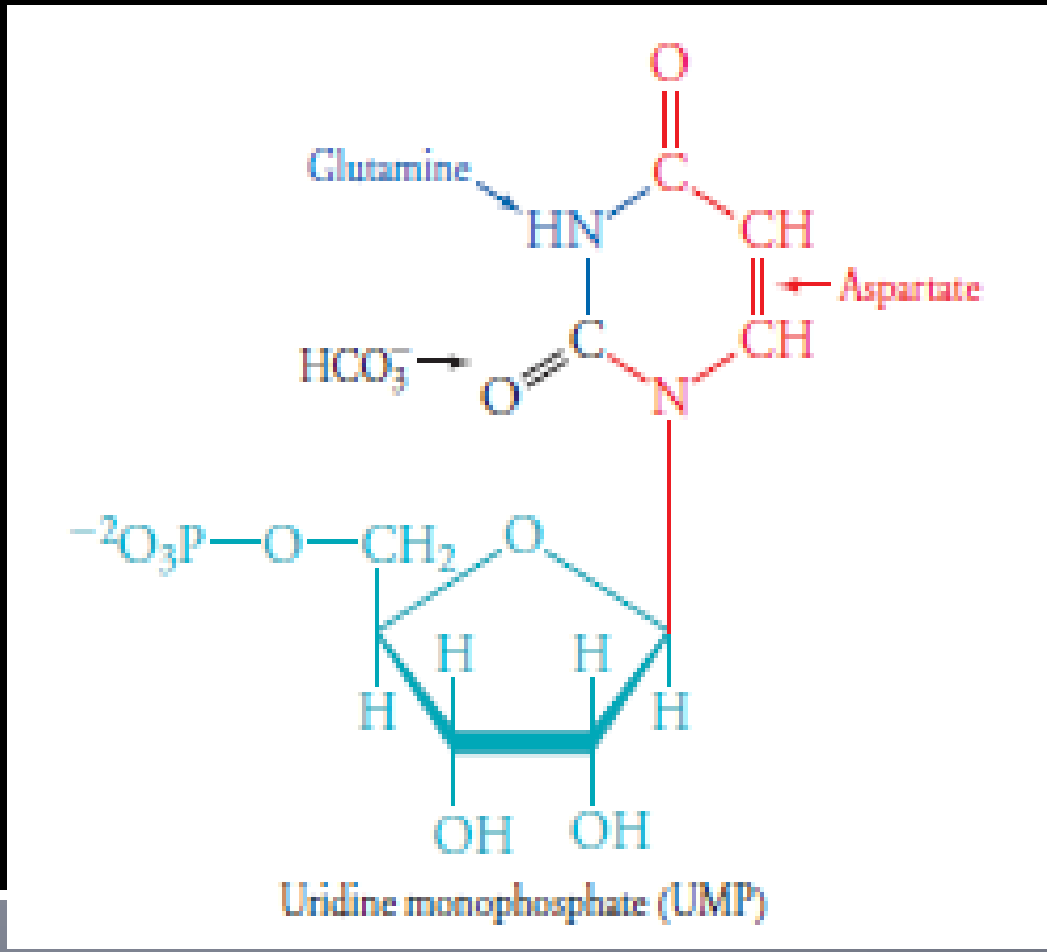


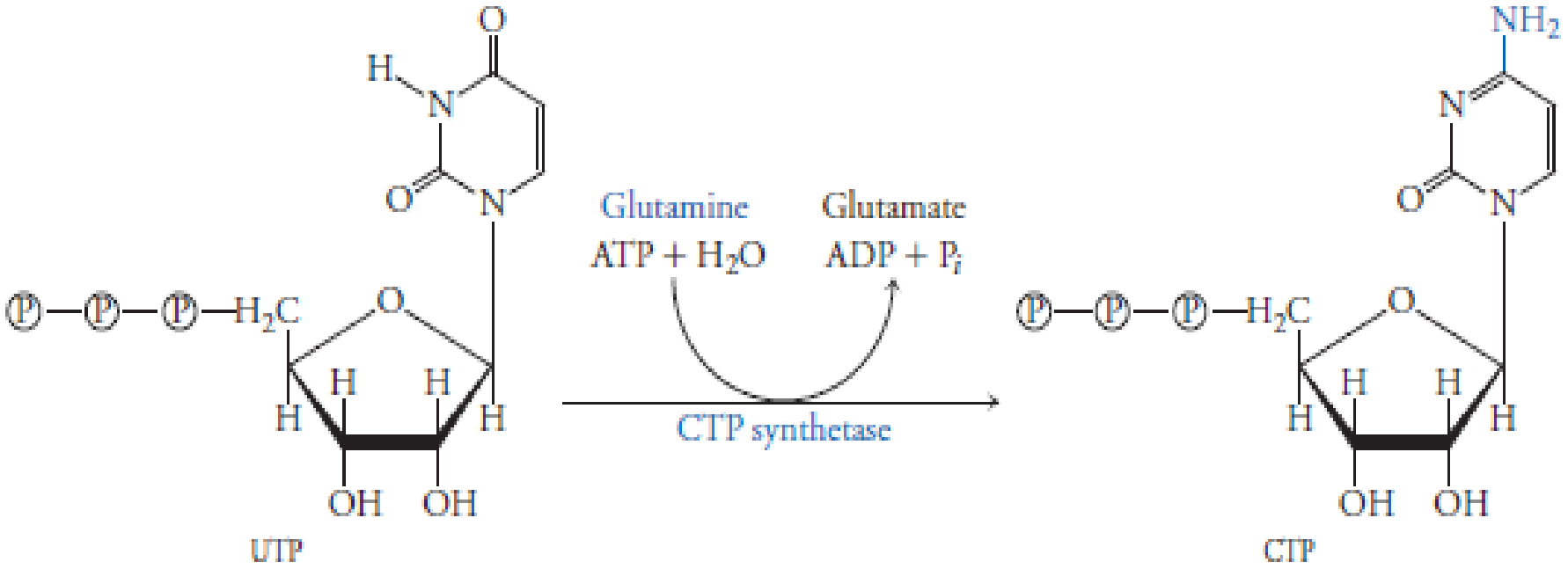
IMP is the substrate for two short pathways that yield AMP and GMP. In AMP synthesis, an amino group from aspartate is transferred to the purine; in GMP synthesis, glutamate is the source of the amino group. **Kinases then catalyze** phosphoryl-group transfer reactions to convert the nucleoside monophosphates to diphosphates and then triphosphates (ATP and GTP).

The pathway indicates that GTP participates in AMP synthesis and ATP participates in GMP synthesis. High concentrations of ATP therefore promote GMP production, and high concentrations of GTP promote AMP production. *This reciprocal relationship is one mechanism for balancing the production of adenine and guanine nucleotides. (Because most nucleotides are destined for DNA or RNA synthesis, they are required in roughly equal amounts.)* The pathway leading to AMP and GMP is also regulated by feedback inhibition at several points, including the first step, the production of 5-phosphoribosyl pyrophosphate from ribose-5-phosphate, which is inhibited by both ADP and GDP.

Pyrimidine nucleotide synthesis yields UTP and CTP

In contrast to purine nucleotides, pyrimidine nucleotides are synthesized as a base that is subsequently attached to 5-phosphoribosyl pyrophosphate to form a nucleotide. The six-step pathway that yields uridine monophosphate (UMP) requires glutamine, aspartate, and bicarbonate. UMP is phosphorylated to yield UDP and then UTP. CTP synthase catalyzes the amination of UTP to CTP, using glutamine as the donor:

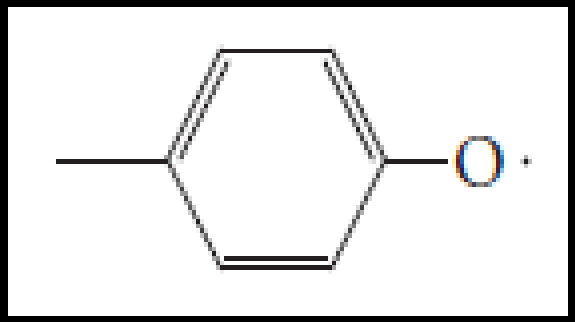




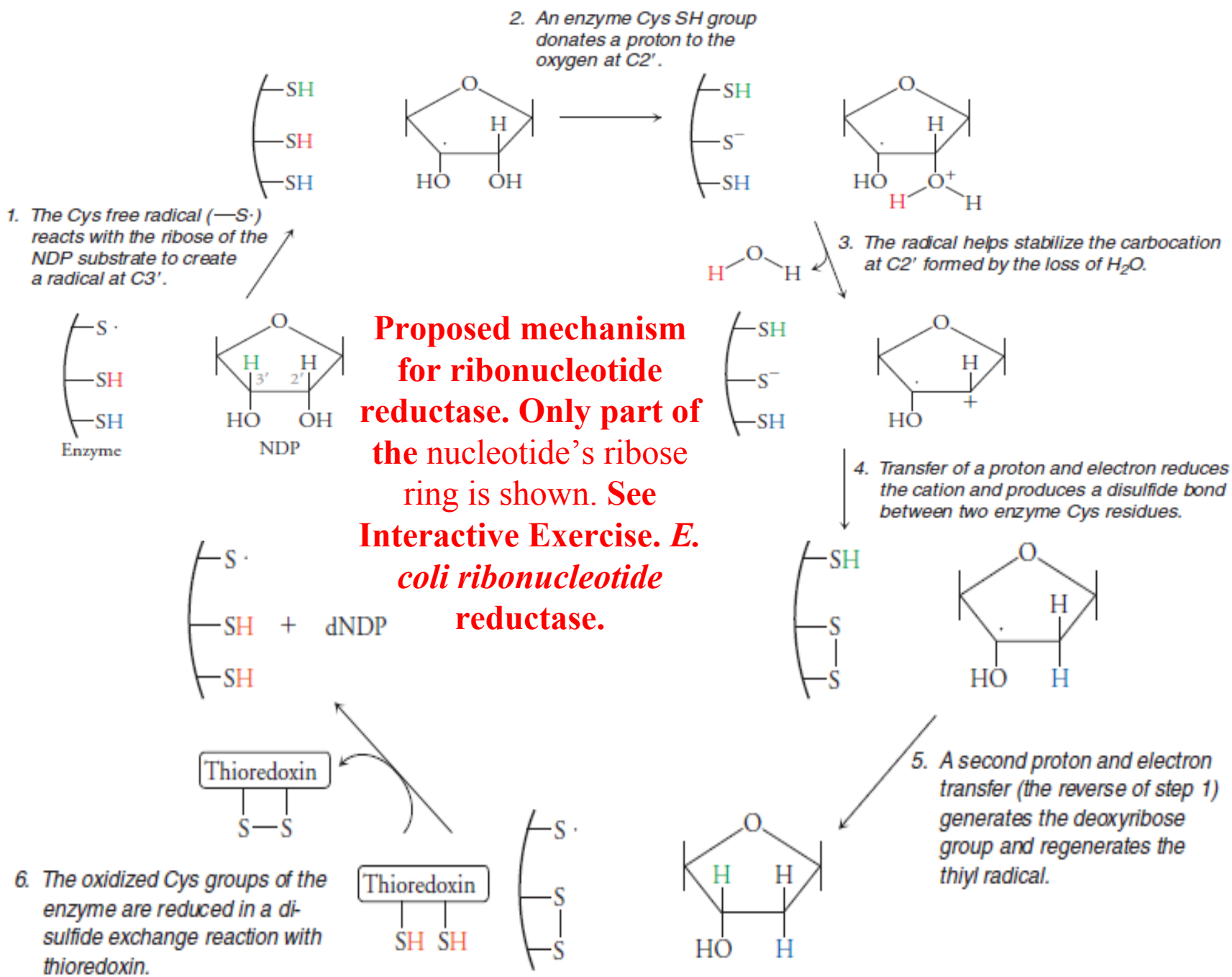
The UMP synthetic pathway in mammals is regulated primarily through feedback inhibition by UMP, UDP, and UTP. ATP activates the enzyme that catalyzes the first step; this helps balance the production of purine and pyrimidine nucleotides.

Ribonucleotide reductase converts ribonucleotides to deoxyribonucleotides

The synthesis of ATP, GTP, CTP, and UTP, which are substrates for the synthesis of RNA. DNA, of course, is built from deoxynucleotides. In deoxynucleotide synthesis, each of the four nucleoside triphosphates (NTPs) is converted to its diphosphate (NDP) form, ribonucleotide reductase replaces the 29 OH group with H, then the resulting deoxynucleoside diphosphate (dNDP) is phosphorylated to produce the corresponding triphosphate (dNTP). Ribonucleotide reductase is an essential enzyme that carries out a chemically difficult reaction using a mechanism that involves free radicals. Three types of ribonucleotide reductases, which differ in their catalytic groups, have been described. Class I enzymes (the type that occurs in mammals and most bacteria) have two Fe_3^+ ions and an unusually stable tyrosine radical (most free radicals, which have one unpaired electron, are highly reactive and short-lived).



Tyrosine radicals are also features of the active sites of cytochrome *c oxidase (mitochondrial* Complex IV) and Photosystem II in plants. Class II ribonucleotide reductases use adenosylcobalamin (the cofactor used in the isomerization of methylmalonyl-CoA), and class III enzymes use a glycyl radical. The job of all these groups is to interact with a Cys side chain to generate a thiyl radical that attacks the ribonucleotide substrate. A possible reaction mechanism is shown in follows figure.

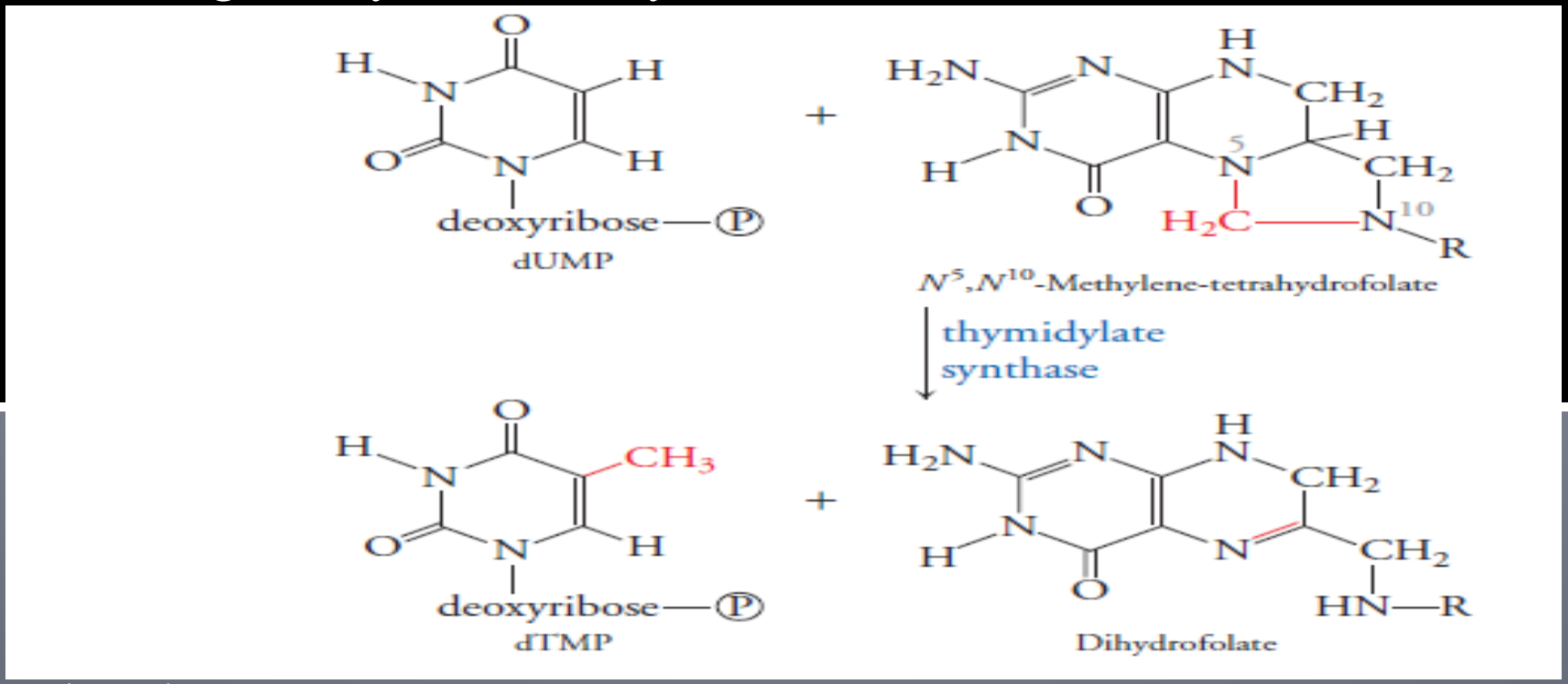

2. An enzyme Cys SH group donates a proton to the oxygen at C2′.
1. The Cys free radical (—S·) reacts with the ribose of the NDP substrate to create a radical at C3′.
3. The radical helps stabilize the carbocation at C2′ formed by the loss of H_2O.
Enzyme
NDP
Proposed mechanism for ribonucleotide reductase. Only part of the nucleotide's ribose ring is shown. See Interactive Exercise. E. coli ribonucleotide reductase.
4. Transfer of a proton and electron reduces the cation and produces a disulfide bond between two enzyme Cys residues.
dNDP
5. A second proton and electron transfer (the reverse of step 1) generates the deoxyribose group and regenerates the thiyl radical.
Thioredoxin
6. The oxidized Cys groups of the enzyme are reduced in a di-sulfide exchange reaction with thioredoxin.

The final step of the reaction, which regenerates the enzyme, requires the small protein thioredoxin. The oxidized thioredoxin must then undergo reduction to return to its original state. *This reaction uses NADPH, which is therefore the ultimate source of reducing power for the synthesis of deoxyribonucleotides. Recall that the pentose* phosphate pathway, which provides the ribose-5-phosphate for nucleotide synthesis, also generates NADPH . Not surprisingly, the activity of ribonucleotide reductase is tightly regulated so that the cell can balance the levels of ribo- and deoxyribonucleotides as well as the proportions of each of the four deoxyribonucleotides. Control of the enzyme involves two regulatory sites that are distinct from the substrate-binding site. For example, ATP binding to the so-called activity site activates the enzyme. Binding of the deoxyribonucleotide dATP decreases enzyme activity. Several nucleotides bind to the so-called substrate specificity site. Here, ATP binding induces the enzyme to act on pyrimidine nucleotides, and dTTP binding causes the enzyme to prefer GDP as a substrate. These mechanisms, in concert with other mechanisms for balancing the amounts of the various nucleotides, help make all four deoxynucleotides available for DNA synthesis

Thymidine nucleotides are produced by methylation

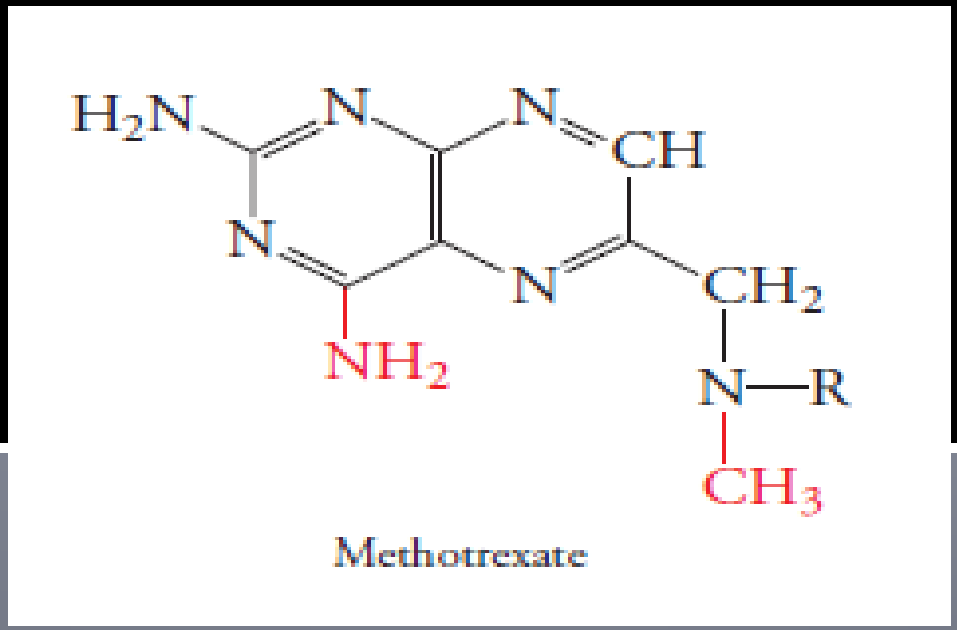
The ribonucleotide reductase reaction, followed by kinase-catalyzed phosphorylation, generates dATP, dCTP, dGTP, and dUTP. However, dUTP is not used for DNA synthesis. Instead, *it is rapidly converted to thymine nucleotides (which helps prevent the* accidental incorporation of uracil into DNA). First, dUTP is hydrolyzed to dUMP.

Next, thymidylate synthase adds a methyl group to dUMP to produce dTMP, using methylene-tetrahydrofolate as a one-carbon donor.



The serine hydroxymethyltransferase reaction, which converts serine to glycine , is the main source of methylene-tetrahydrofolate.
In converting the methylene group (OCH_2O) of the cofactor to the ethyl group (OCH_3) attached to thymine, thymidylate synthase oxidizes the tetrahydrofolate cofactor to dihydrofolate. An NADPH-dependent enzyme called dihydrofolate reductase must then regenerate the reduced tetrahydrofolate cofactor. Finally, dTMP is phosphorylated to produce dTTP, the substrate for DNA polymerase. Because cancer cells undergo rapid cell division, the enzymes of nucleotide synthesis, including thymidylate synthase and dihydrofolate reductase, are highly active. Compounds that inhibit either of these reactions can therefore act as anticancer agents.

For example, the dUMP analog 5-fluoro -deoxyuridylate, inactivates thymidylate synthase. "Antifolates" such as ***methotrexate*** *are competitive inhibitors of dihydrofolate reductase because they compete* with dihydrofolate for binding to the enzyme. In the presence of methotrexate, a cancer cell cannot regenerate the tetrahydrofolate required for dTMP production, and the cell dies. Most noncancerous cells, which grow much more slowly, are not as sensitive to the effect of the drug.

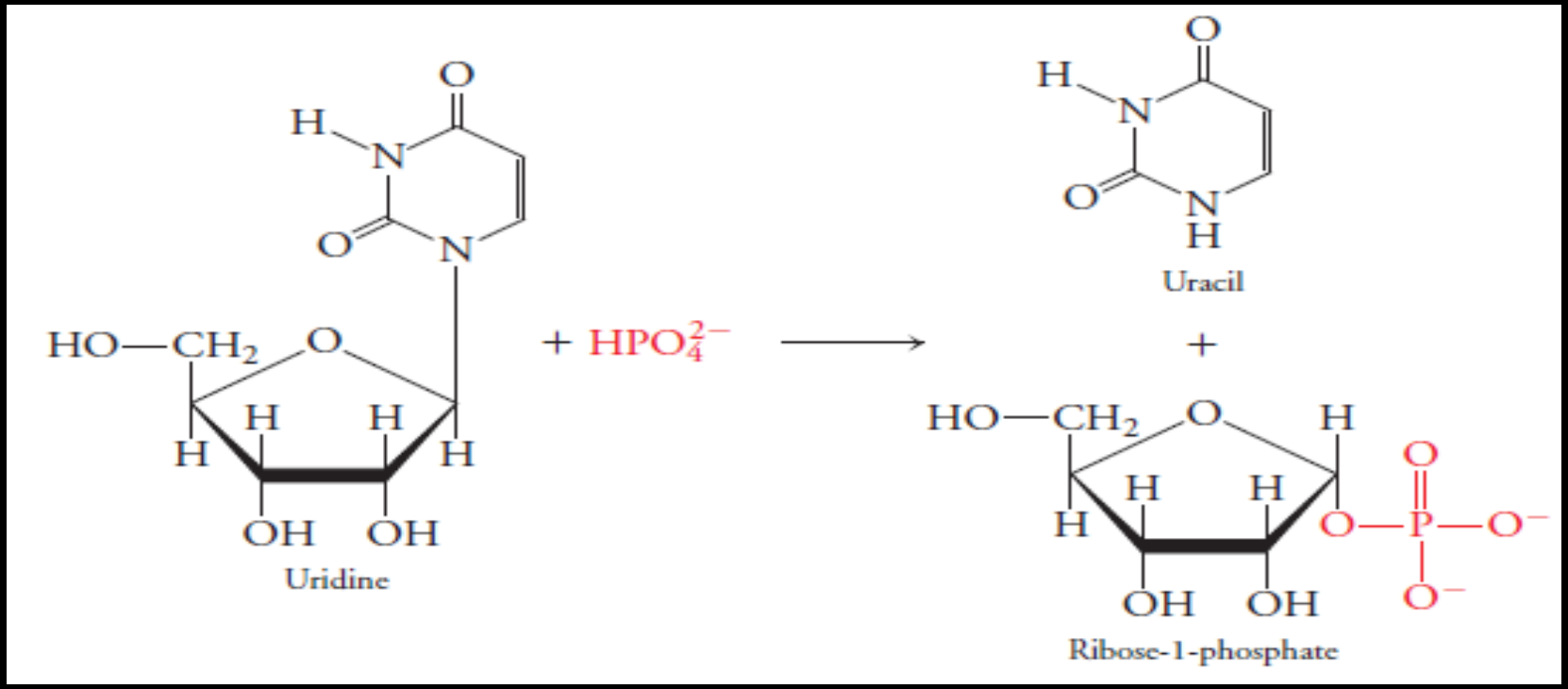


Methotrexate

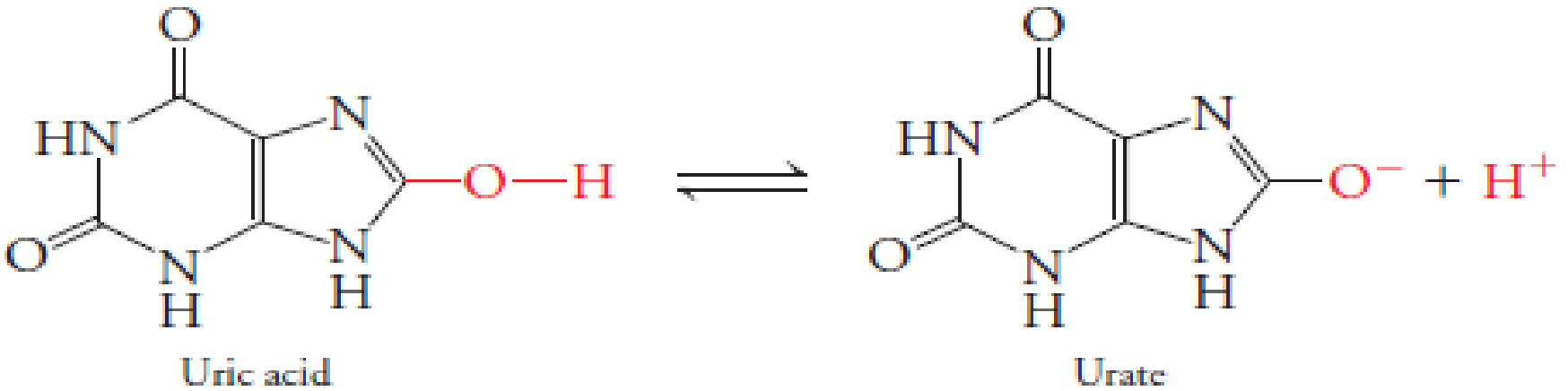
Nucleotide degradation produces uric acid or amino acids

Nucleotides that are obtained from food or synthesized by cells can be broken down, releasing ribose groups and a purine or pyrimidine that can be further catabolized and excreted (purines) or used as a metabolic fuel (pyrimidines). At several points in the degradation pathways, intermediates may be redirected toward the synthesis of new nucleotides by so-called ***salvage pathways*****. For example,** ***a free adenine base can be reattached to ribose by the reaction :***

Degradation of a nucleoside monophosphate begins with dephosphorylation to produce a nucleoside. In a subsequent step, a phosphorylase breaks the glycosidic bond between the base and the ribose by adding phosphate (a similar phosphorolysis reaction occurs during glycogenolysis).

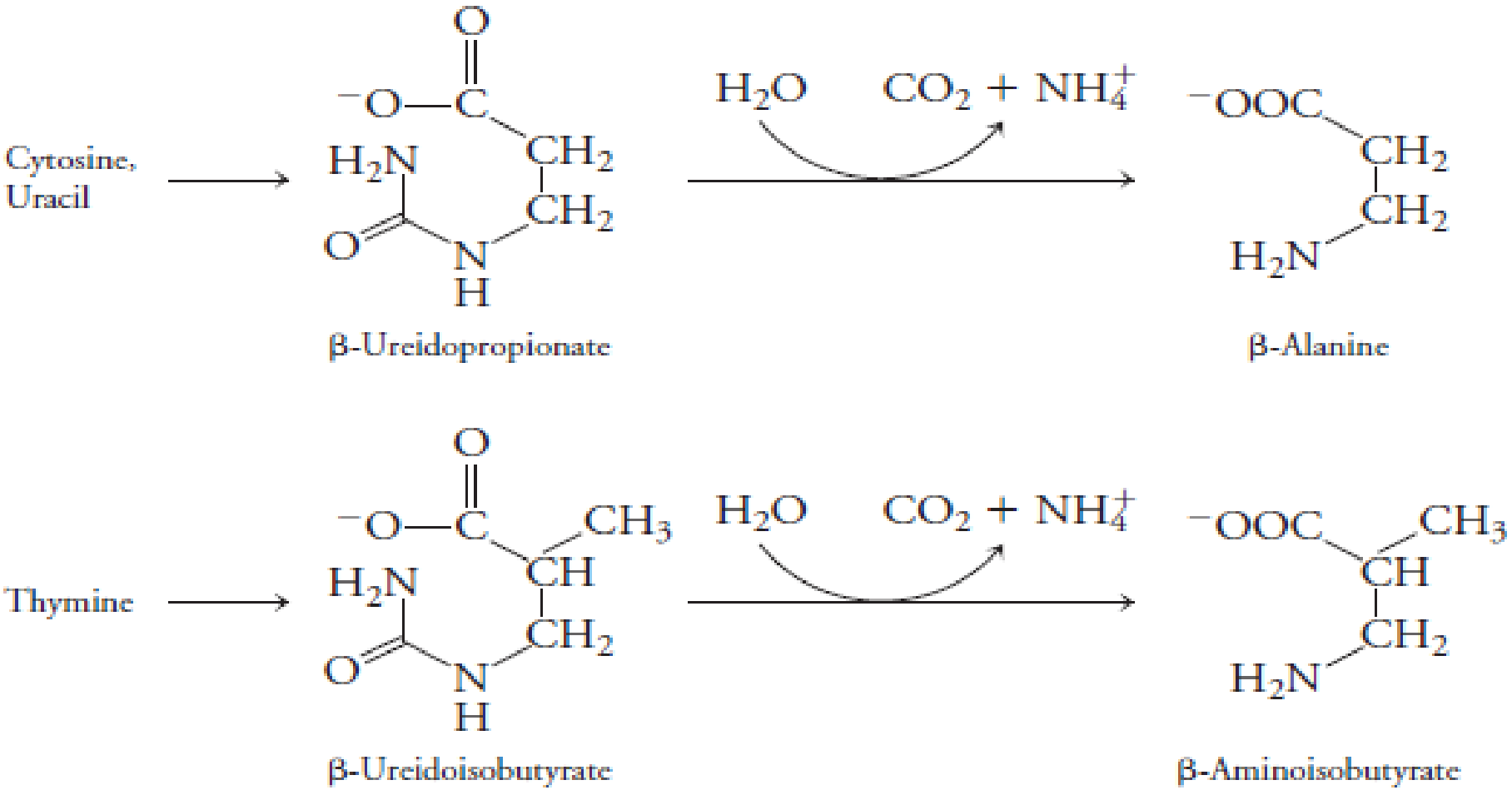


The phosphorylated ribose can be catabolized or ***salvaged*** and converted to 5-phosphoribosyl pyrophosphate for synthesis of another nucleotide. The fate of the base depends on whether, it is a purine or a pyrimidine. The purine bases are eventually converted to uric acid in a process that may require deamination and oxidation, depending on whether the original base was adenine, guanine, or hypoxanthine. Uric acid has a p*K* *of 5.4, so it exists mainly as urate.*



In humans, urate, a poorly soluble compound, is excreted in the urine. Excess urate may precipitate as crystals of sodium urate in the kidneys (kidney "stones"). Deposits of urate in the joints, primarily the knees and toes, cause a painful condition called gout. Other organisms may further catabolize urate to generate more soluble waste products such as urea and ammonia.

The pyrimidines cytosine, thymine, and uracil undergo deamination and reduction, after which the pyrimidine ring is opened. Further catabolism produces the nonstandard amino acid β-alanine (from cytosine and uracil) or β-aminoisobutyrate (from thymine), both of which feed into other metabolic pathways.



Consequently, pyrimidine catabolism contributes to the pool of cellular metabolites for both anabolic and catabolic processes. In contrast, purine catabolism generates a waste product that is excreted from the body.

Amino Acid Catabolism

CONCEPT

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

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 a-amino groups, and their carbon skeletons then enter the central pathways of energy metabolism (principally the citric acid cycle). of catabolized amino acids.

However, *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.

The reactions of amino acid catabolism, like those of amino acid synthesis, are too numerous and the catabolic pathways do not necessarily mirror the anabolic pathways, as they do in carbohydrate and fatty acid metabolism.

Amino acids are glucogenic, ketogenic, or both

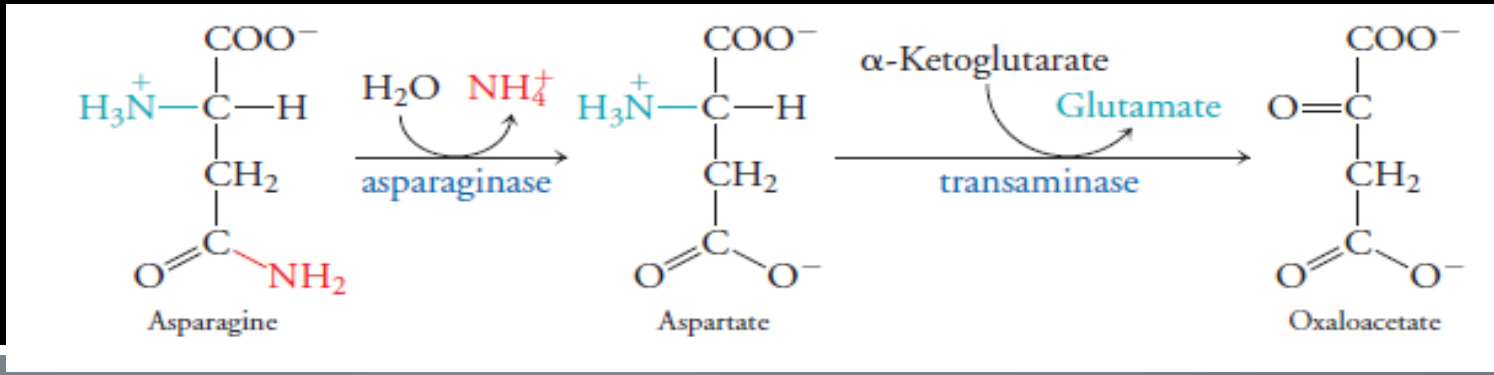
It is useful to classify amino acids in humans as ***glucogenic*** **(giving rise to gluconeogenic** precursors such as citric acid cycle intermediates) or ***ketogenic*** **(giving rise to** acetyl-CoA, which can be used for ketogenesis or fatty acid synthesis, but not gluconeogenesis).

As shown in below table:

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		

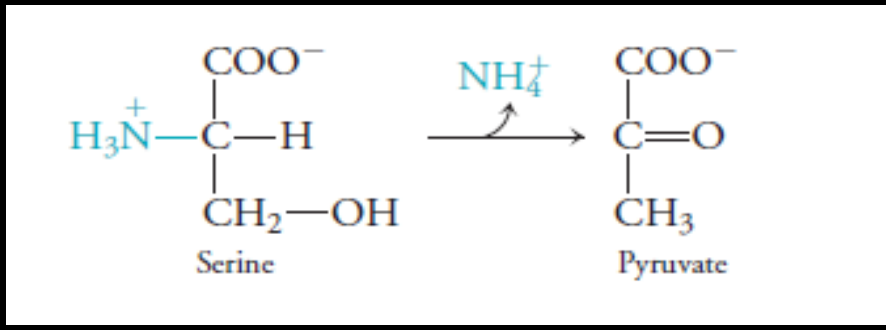
All amino acids but leucine and lysine are at least partly glucogenic, most of the nonessential amino acids are glucogenic, and the large skeletons of the aromatic amino acids are both glucogenic and ketogenic. Three amino acids are converted to gluconeogenic substrates by simple transamination (the 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:

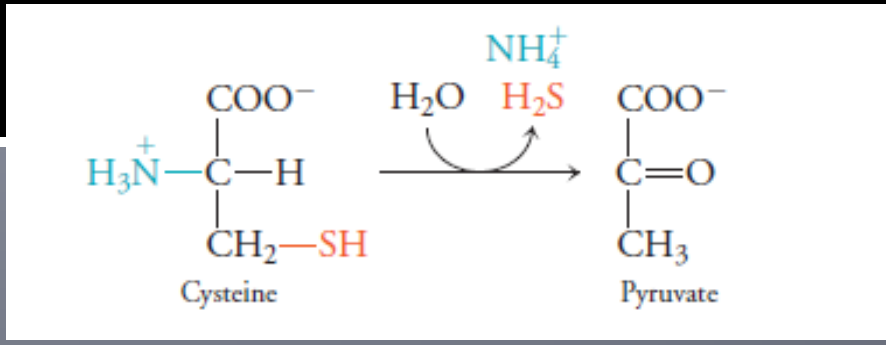


Similarly, glutamine is deamidated by a glutaminase to glutamate, and the glutamate dehydrogenase reaction yields α-ketoglutarate. Serine is converted to pyruvate:

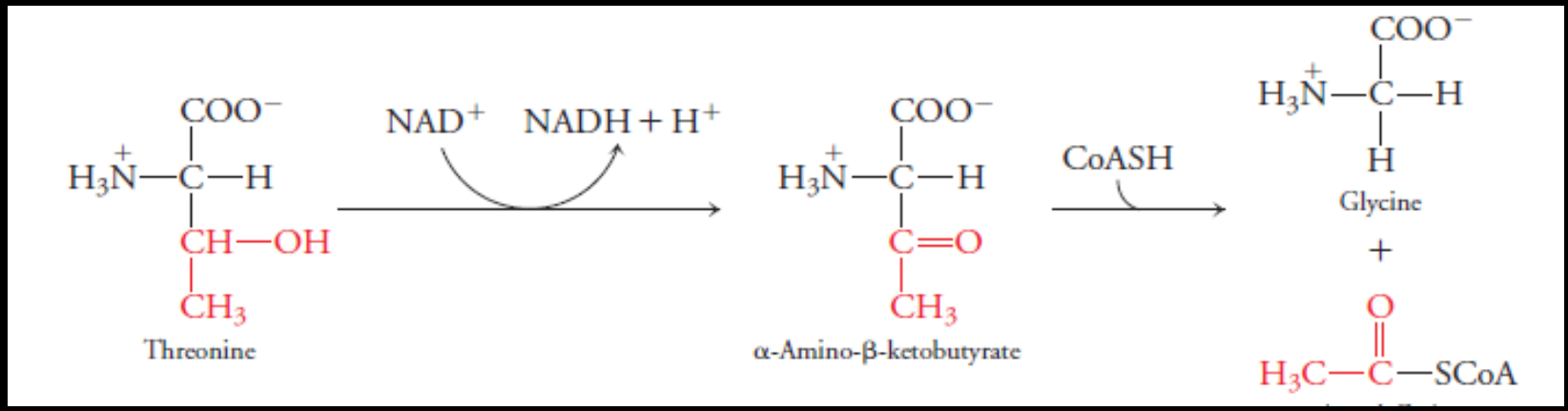
Note that in this reaction and in the conversion of Aasparagine and glutamine to their acid counterparts, the amino group is released as NH_4^+ rather than being transferred to another compound. Arginine and proline (which are synthesized from glutamate) as well as Histidine are catabolized to glutamate, 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:

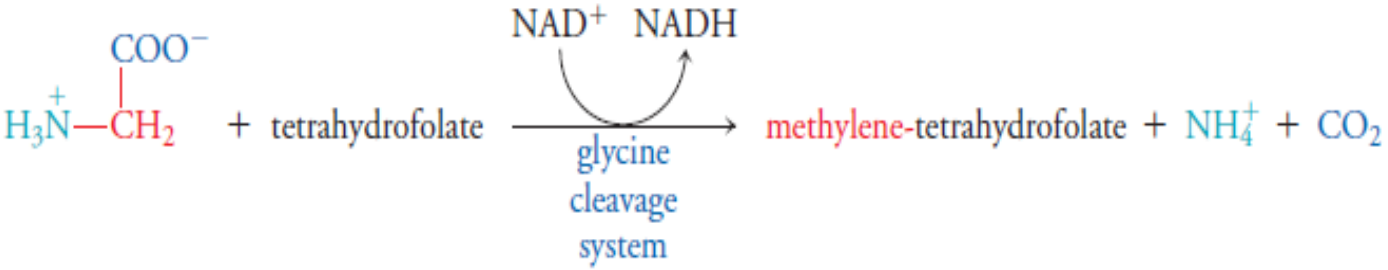




The products of the reactions listed so far pyruvate, oxaloacetate, and α-ketoglutarate are all gluconeogenic precursors. Threonine is both glucogenic and ketogenic because it is broken down to acetyl-CoA and glycine:



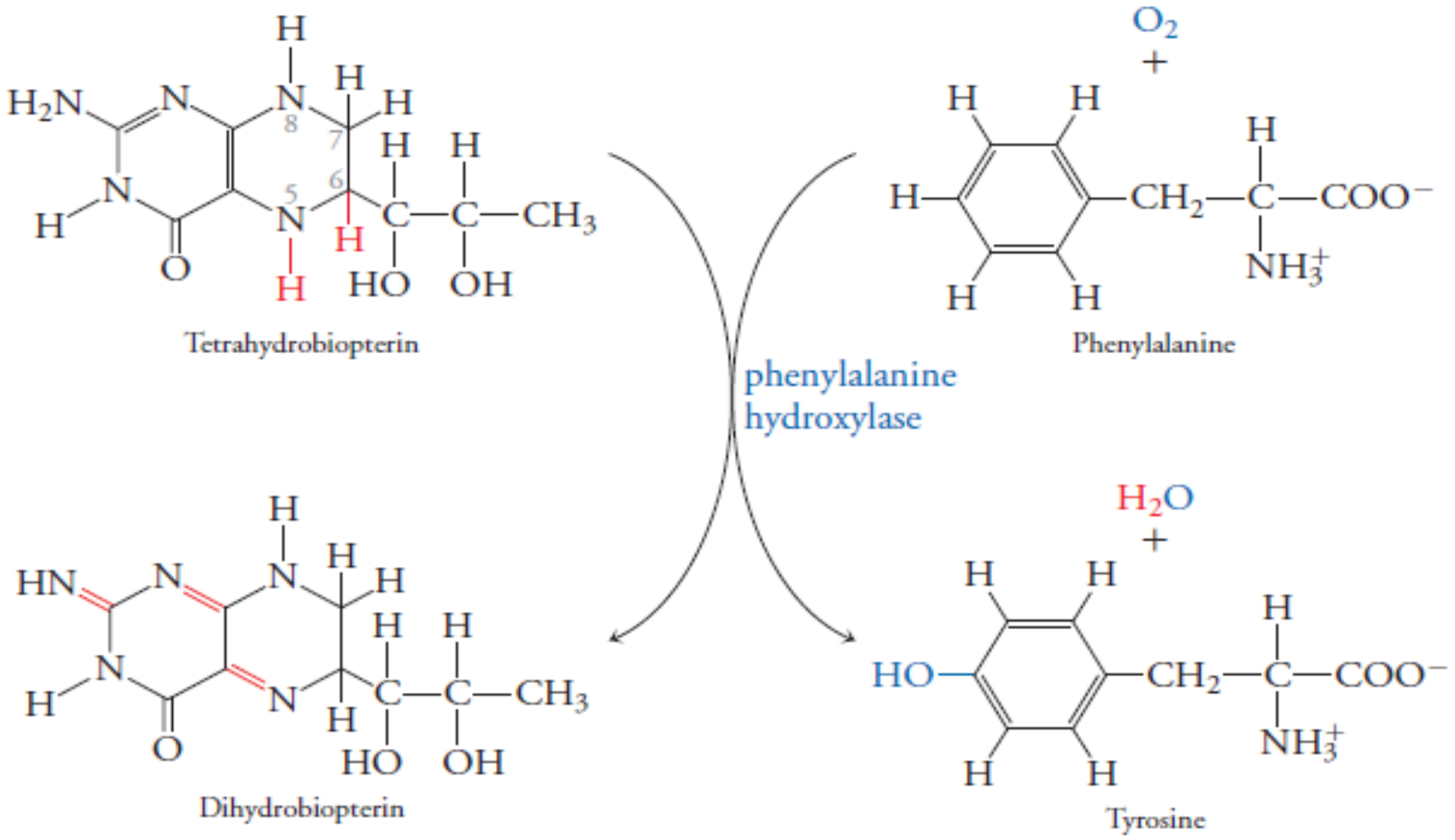
The acetyl-CoA is a precursor of ketone bodies and the glycine is potentially glucogenic , if it is first converted to serine by the action of serine hydroxymethyltransferase. The major route for glycine disposal, however, is catalyzed by a multiprotein complex known as the glycine cleavage system:



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 branchedchain 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 **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.

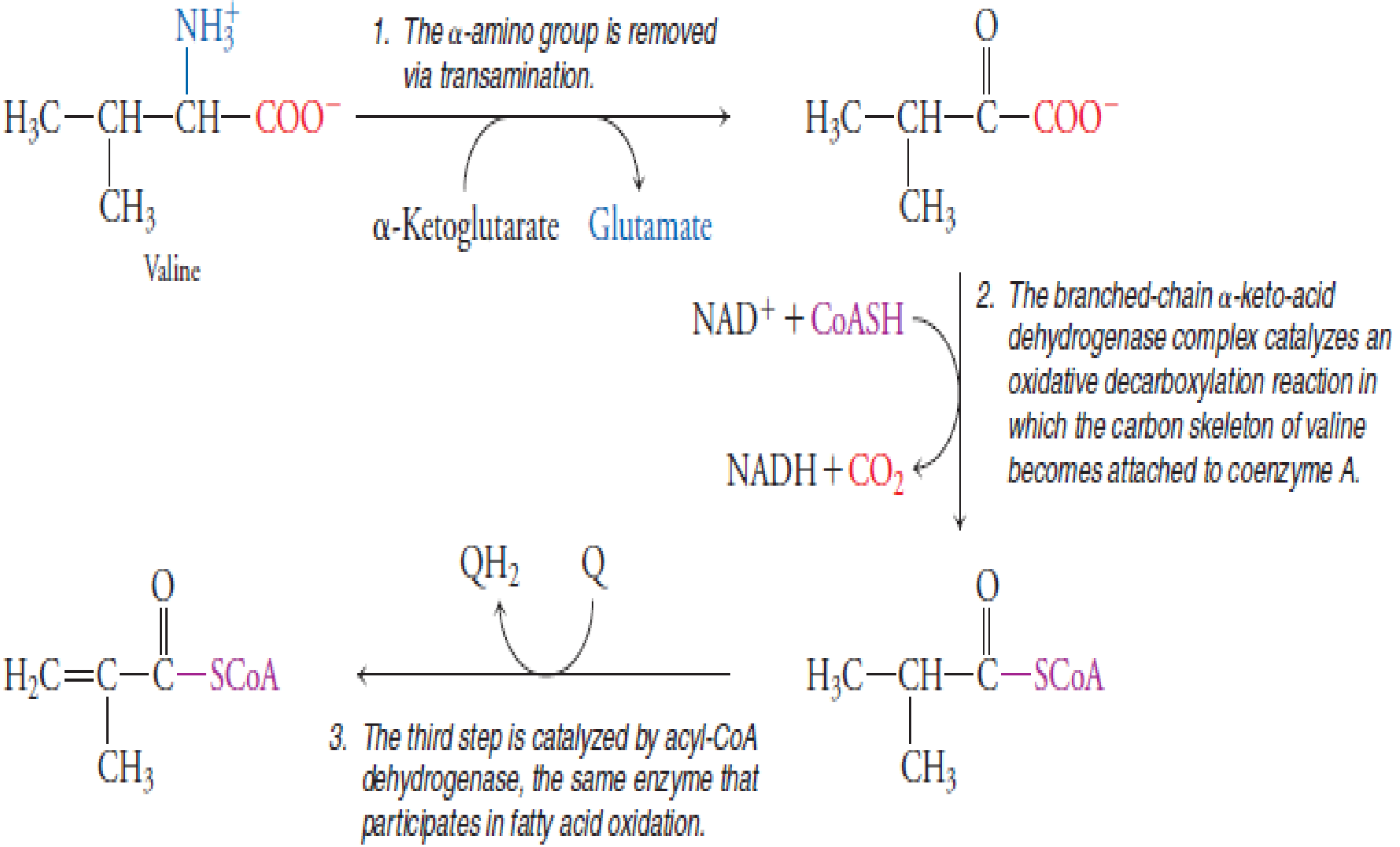
Finally, the cleavage of the aromatic amino acids phenylalanine, tyrosine, and tryptophan yields the ketone body acetoacetate as well as a glucogenic compound (alanine or fumarate).

The first step of phenylalanine degradation is a hydroxylation reaction that produces tyrosine, This reaction is worth noting because it uses the cofactor tetrahydrobiopterin (which, like folate, contains a pterin moiety).



The tetrahydrobiopterin is oxidized to dihydrobiopterin in the phenylalanine hydroxylase reaction. This cofactor must be subsequently reduced to the tetrahydroform by a separate NADH-dependent enzyme. Another step of the phenylalanine (and tyrosine) degradation pathway is also notable because a defi ciency of the enzyme was one of the fi rst-characterized “inborn errors of metabolism”.

The initial steps of valine degradation.



Nitrogen Disposal: The Urea Cycle

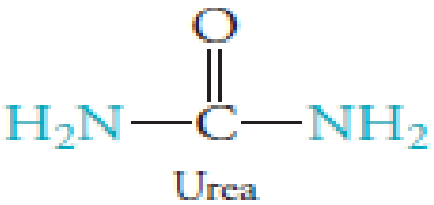
CONCEPTS

- 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.

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 merely 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 1 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 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, whose normal agonist is the neurotransmitter glutamate. 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 by ammonia binding to the receptor results in neuronal cell death, a phenomenon called excitotoxicity. Humans and many other organisms have therefore evolved safer ways to deal with excess amino groups.

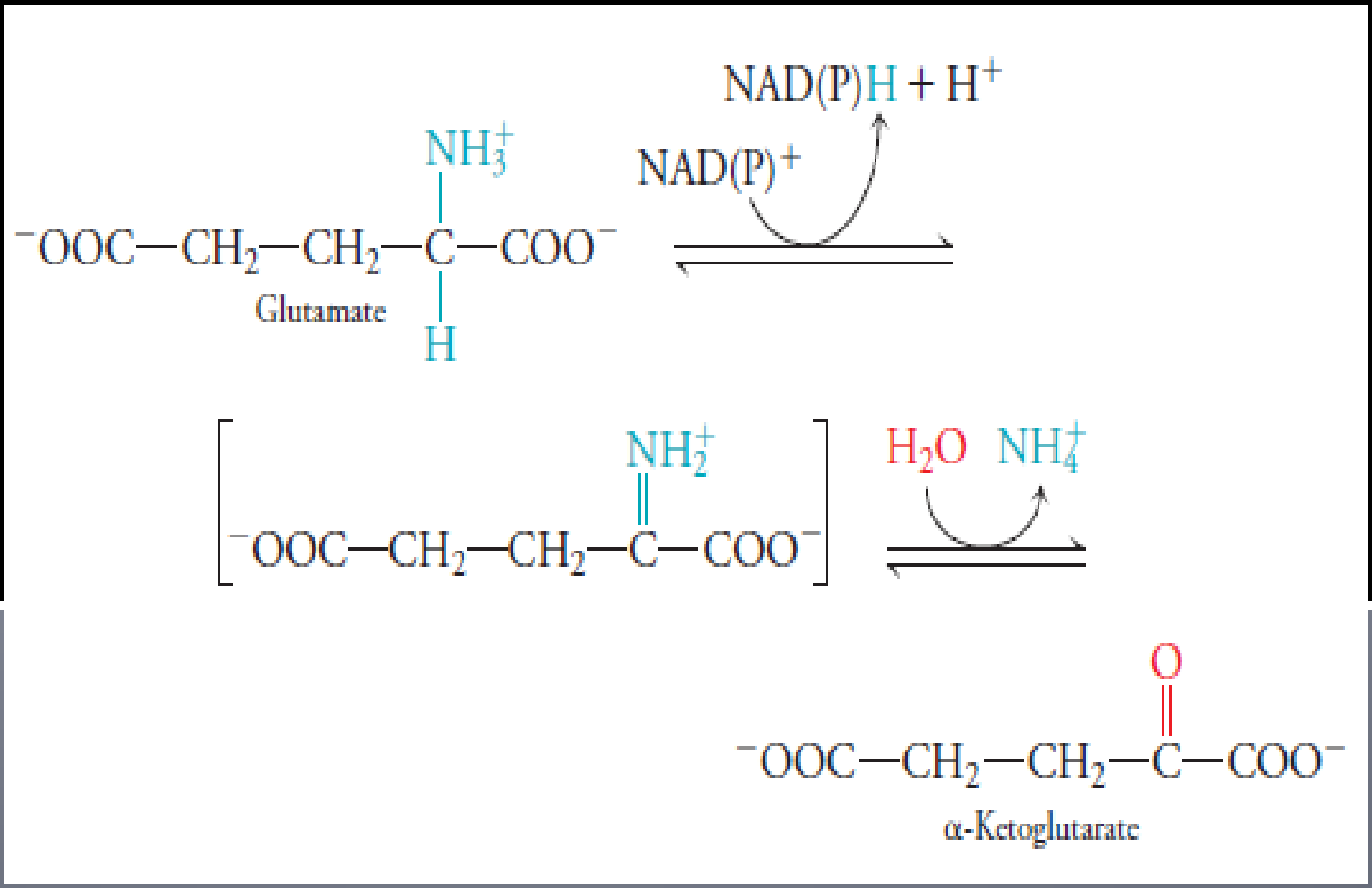
Approximately 80% of the body's excess nitrogen is excreted in the form of urea, which is produced in the liver by the reactions of the ***urea cycle.*** ***This catabolic cycle*** was elucidated in 1932 by Hans Krebs and Kurt Henseleit; Krebs went on to outline another circular pathway the citric acid cycle in 1937.



Urea

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:

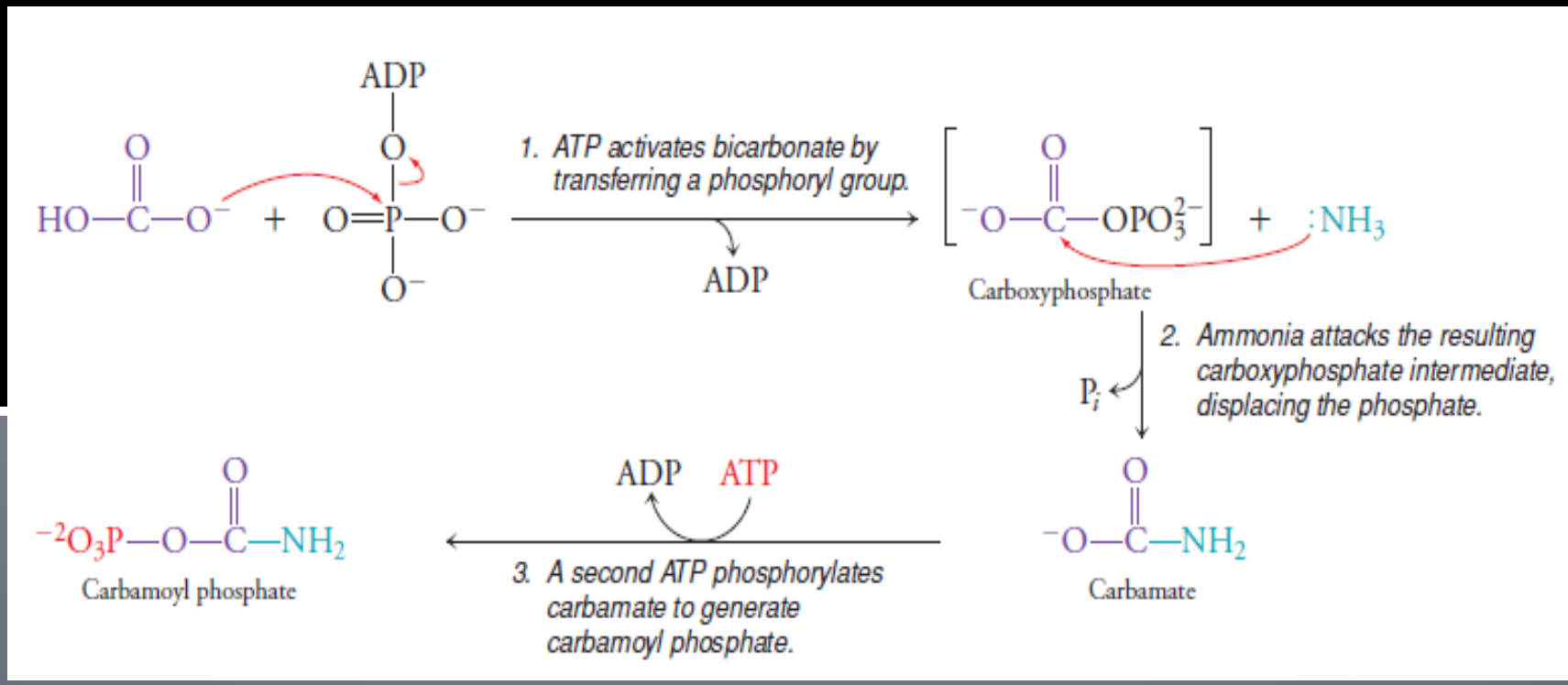


This mitochondrial enzyme is unusual: It is the only known enzyme that can use either NAD^+ or $NADP^+$ as a cofactor. The glutamate dehydrogenase reaction is a major route for feeding amino acid derived amino groups into the urea cycle and, not surprisingly, is subject to allosteric activation and inhibition. 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 . ***The*** NH_4^+ may be contributed by the glutama 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.

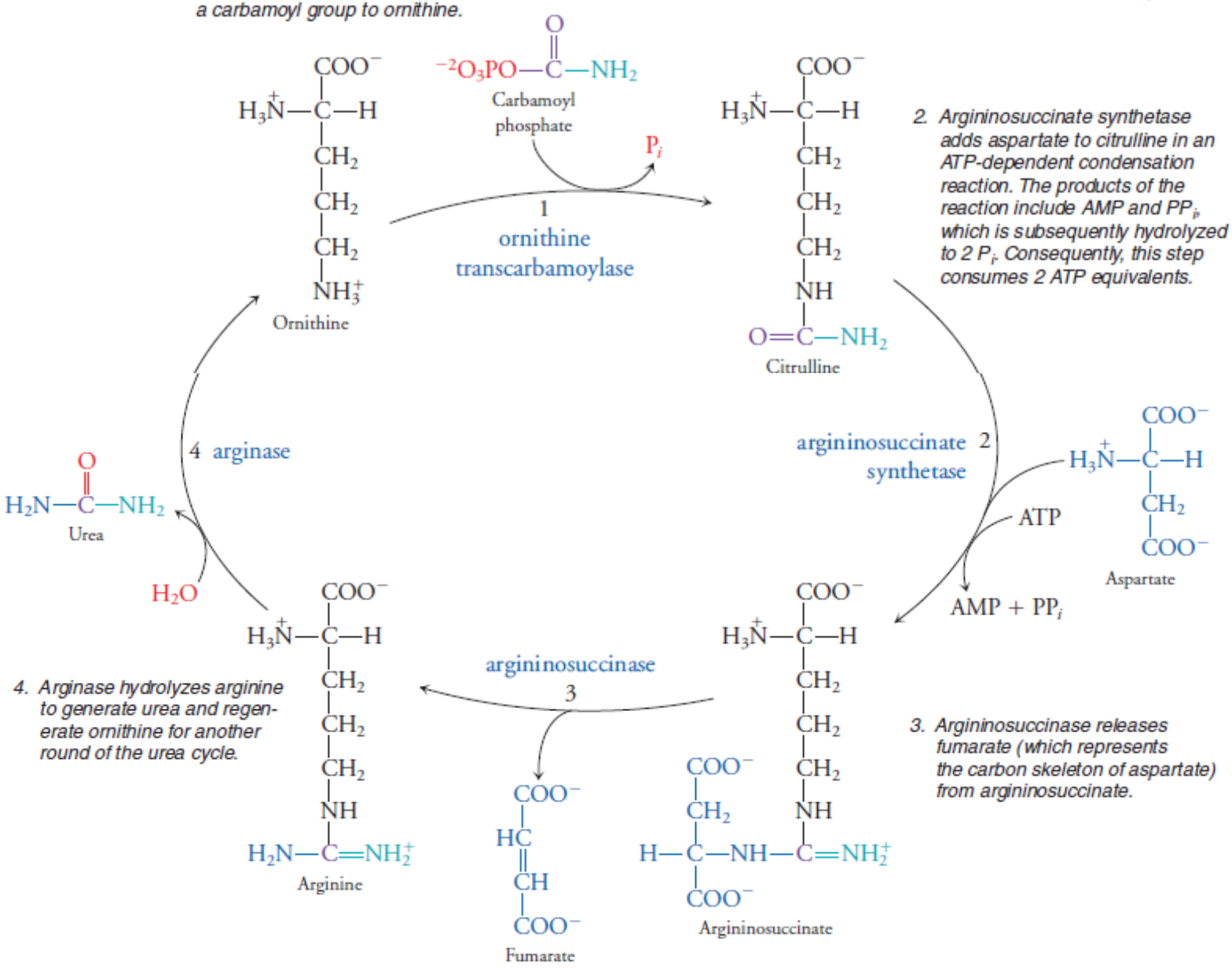
The urea cycle consists of four reactions

The four enzyme-catalyzed reactions of the urea cycle proper are shown below.The cycle also provides a means for synthesizing arginine: The fi ve-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 classifi ed as an essential amino acid.

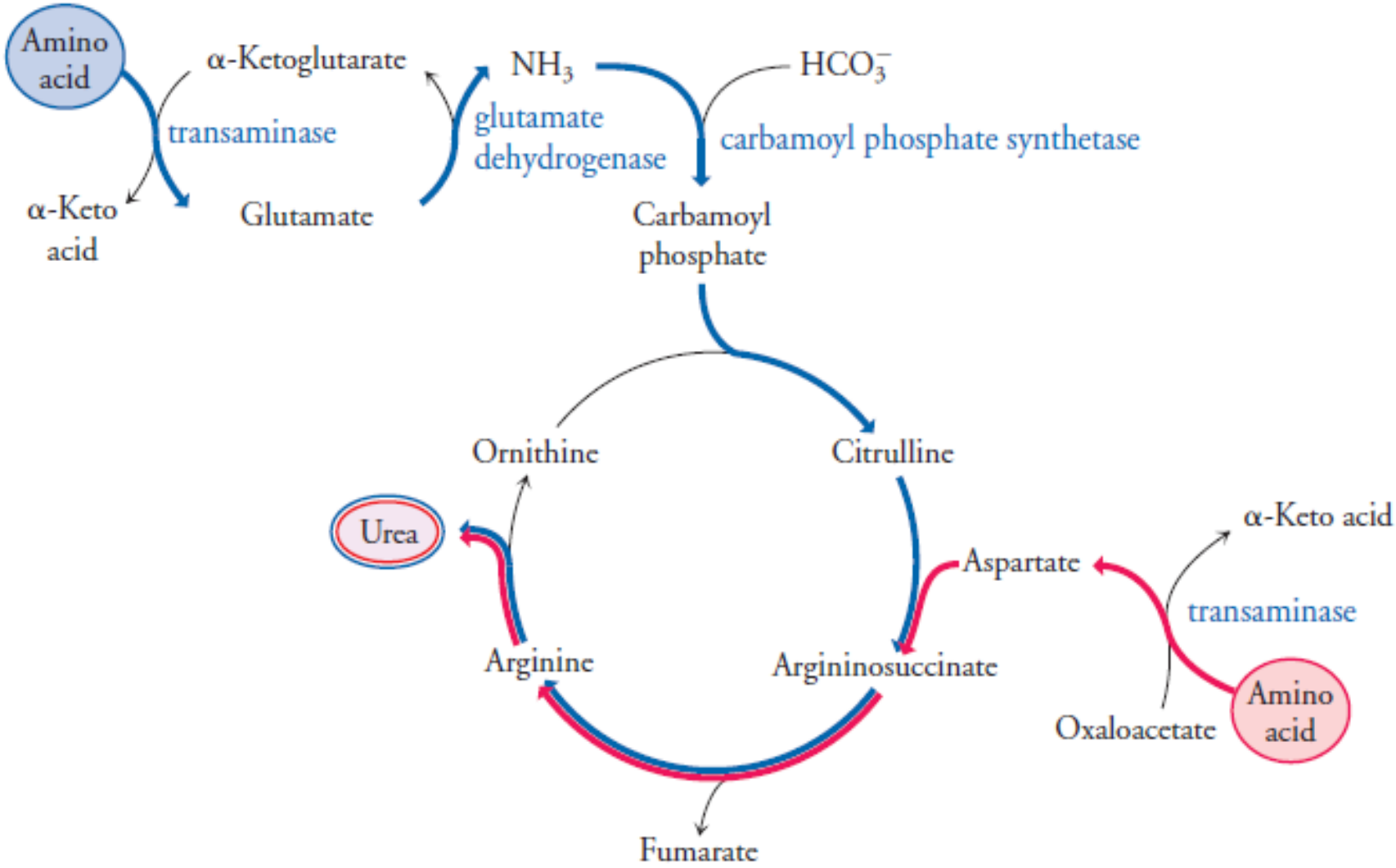
The carbamoyl phosphate synthetase reaction.



The four reactions of the urea cycle.



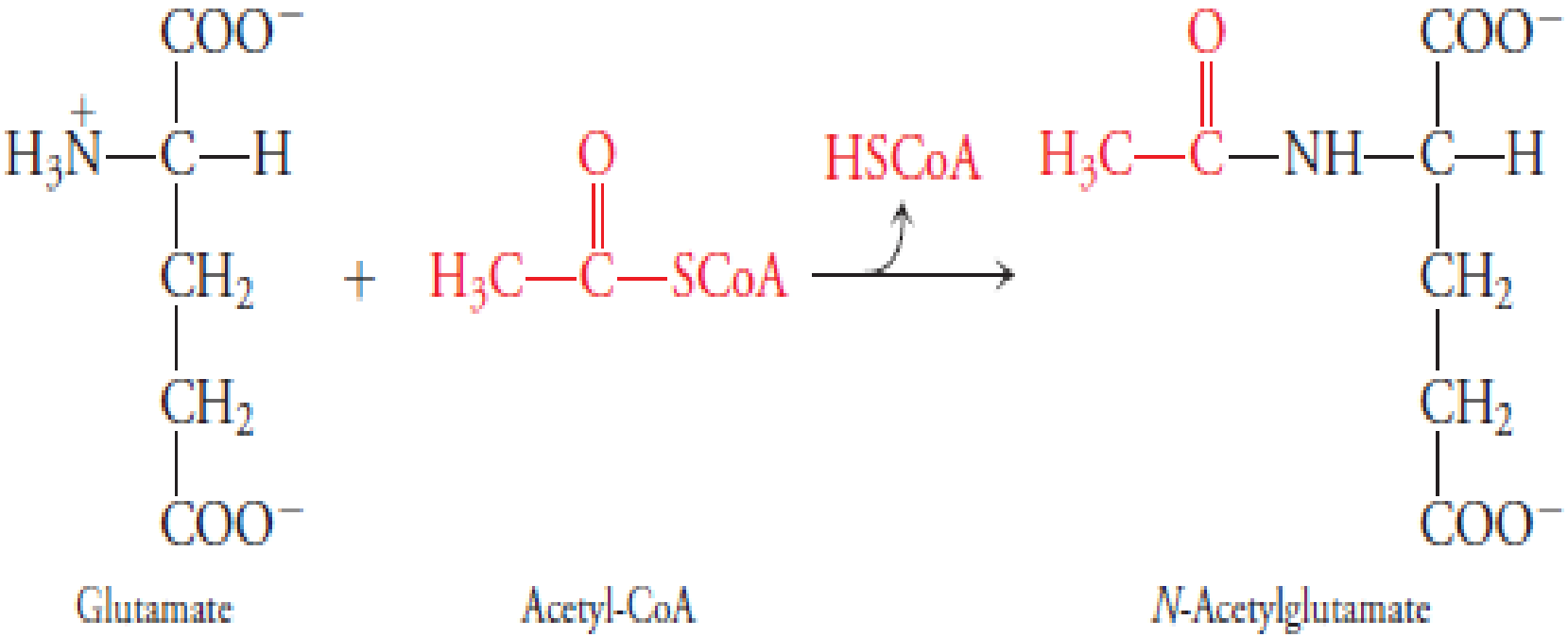
The fumarate generated in step 3 of the urea cycle is converted to malate and then oxaloacetate, which is used for gluconeogenesis. The aspartate substrate for Reaction 2 may represent oxaloacetate that has undergone transamination. Combining these ancillary reactions with those of the urea cycle, the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

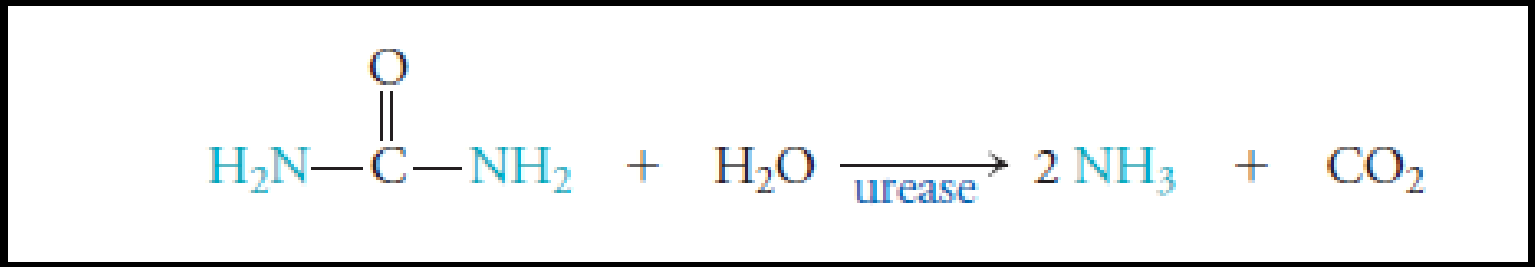


When amino acids are undergoing transamination and being catabolized, the resulting increases in the cellular glutamate and acetyl-CoA concentrations boost production of *N-acetylglutamate. This stimulates carbamoyl phosphate synthetase activity,* and fl ux through the urea cycle increases. Such a regulatory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

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 such as birds and for reptiles that are adapted to arid habitats. 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 . 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 fi rst 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).

GOOD LUCK