ADVANCES IN METABOLISM
BCH 540

BIOSYNTHESIS OF UREA

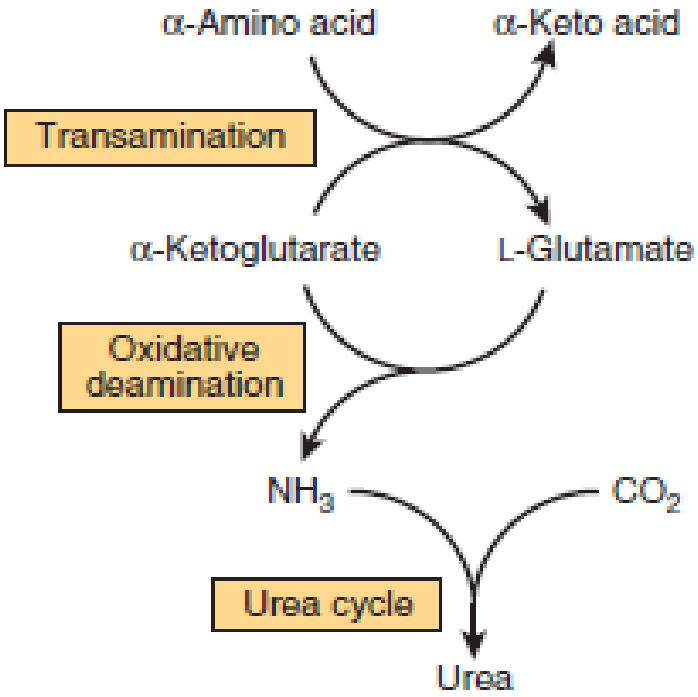
Four stages for urea biosynthesis:

(1) Transamination

(2) Oxidative deamination of glutamate

(3) Ammonia transport

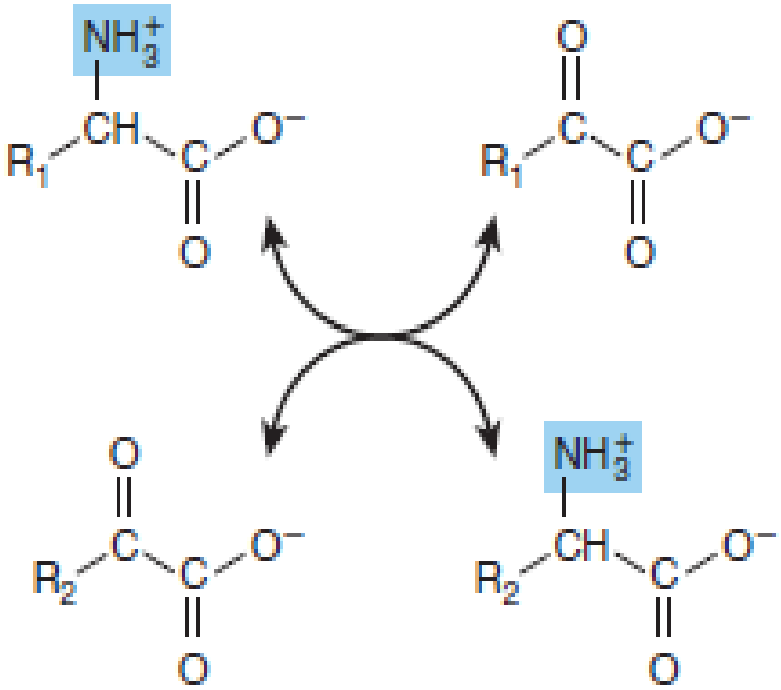
(4) Reactions of the urea cycle



Overall flow of nitrogen in amino acid catabolism.

Transamination

Interconversion pairs of α-amino acids and α-keto acids are reversible, and also function in amino acid biosynthesis. Of the protein amino acids, all except lysine, threonine, proline, and hydroxyproline participate in transamination.



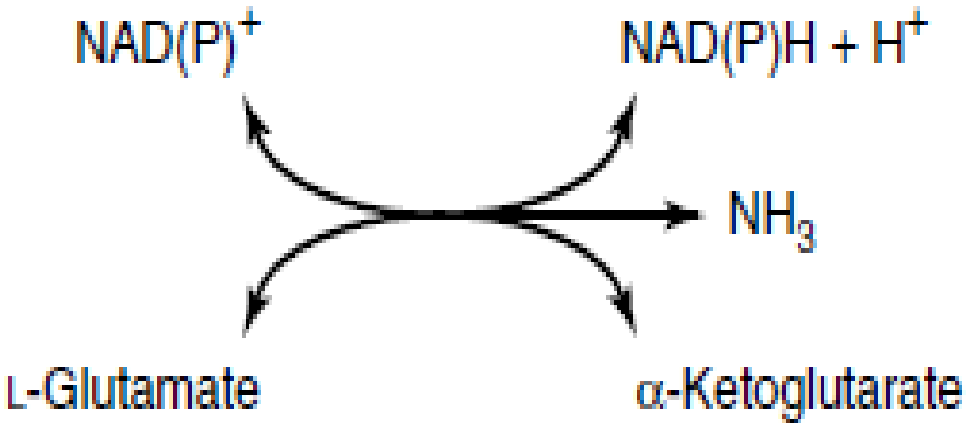
Transamination. The reaction is freely reversible with an equilibrium constant close to unity

Transfers -Amino Nitrogen to -Ketoglutarate, Forming Glutamate

Alanine-pyruvate aminotransferase (alanine aminotransferase) and glutamate-α-ketoglutarate aminotransferase (glutamate aminotransferase) catalyze the transfer of amino groups to pyruvate (forming alanine) or to α–ketoglutarate. The formation of ammonia from α-amino groups occurs mainly via the -amino nitrogen of L-glutamate.

L-Glutamate Dehydrogenase Occupies a Central Position in Nitrogen Metabolism

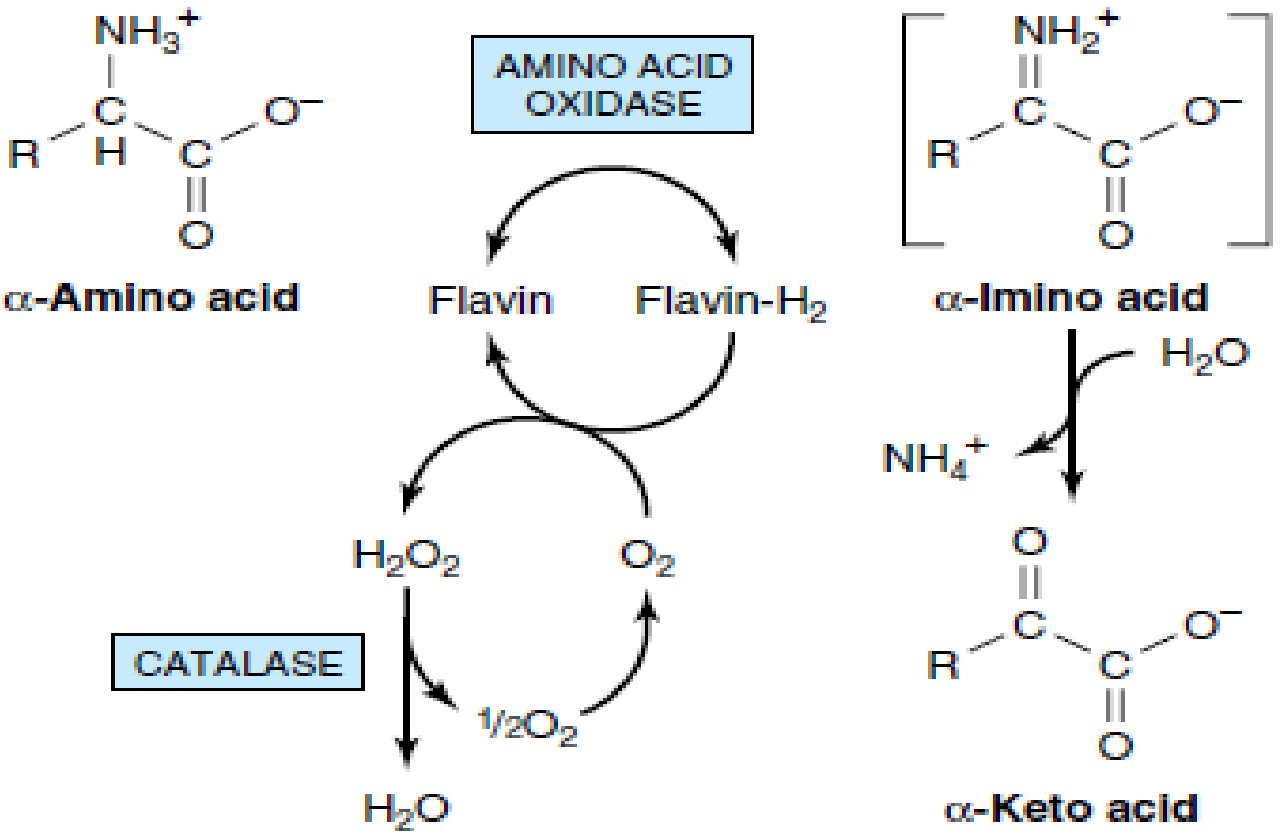
- Transfer of amino nitrogen to α-ketoglutarate forms L -glutamate. Hepatic L -glutamate dehydrogenase (GDH), which can use either NAD^+ or $NADP^+$, releases this nitrogen as ammonia.
- Conversion of α-amino nitrogen to ammonia by the concerted action of glutamate aminotransferase and GDH is often termed "transdeamination."
- Liver GDH activity is inhibited by ATP, GTP, and NADH, and is activated by ADP.



The L-glutamate dehydrogenase reaction. $NAD(P)^+$ means that either NAD^+ or $NADP^+$ can serve as the oxidoreductant. The reaction is reversible, but favors glutamate formation

Amino Acid Oxidases Also Remove Nitrogen as Ammonia

- L -amino acid oxidases of liver and kidney convert an amino acid to an α-imino acid that decomposes to an α-keto acid with release of ammonium ion.
- The reduced flavin is reoxidized by molecular oxygen, forming hydrogen peroxide (H_2O_2), which then is split to O_2 and H_2O by catalase.



Oxidative deamination catalyzed by L-amino acid oxidase (L-α-amino acid:O2 oxidoreductase). The α-imino acid, shown in brackets, is not a stable intermediate.

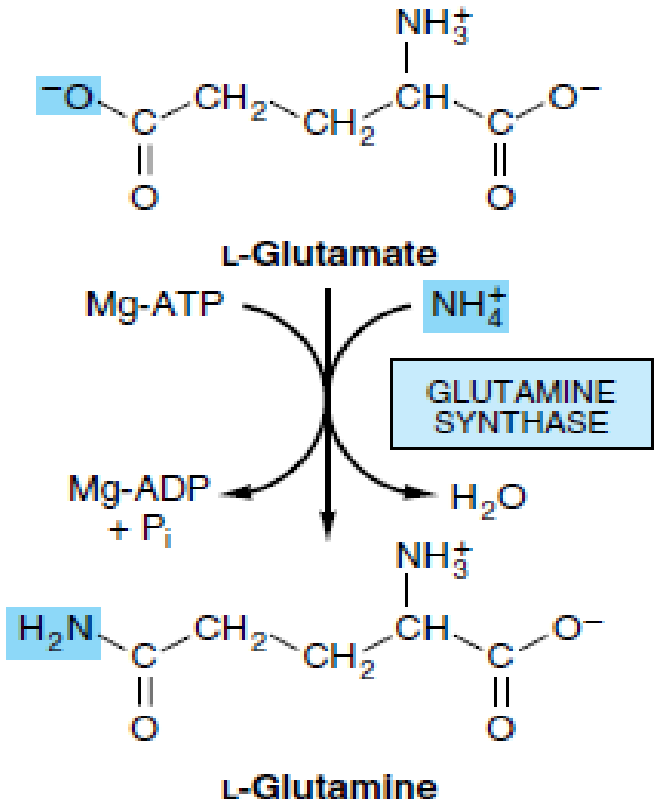
AMMONIA INTOXICATION IS LIFE-THREATENING

- **The ammonia produced by enteric bacteria and absorbed into portal venous blood and the ammonia produced by tissues are rapidly removed from circulation by the liver and converted to urea.**
- **Only traces (10–20 g/dL) normally are present in peripheral blood. This is essential, since ammonia is toxic to the central nervous system.**

- **Systemic blood ammonia levels may attain toxic levels. This occurs in severely impaired hepatic function or the development of collateral links between the portal and systemic veins in cirrhosis. Symptoms of ammonia intoxication include tremor, slurred speech, blurred vision, coma, and ultimately death.**
- **Ammonia may be toxic to the brain in part because it reacts with α-ketoglutarate to form glutamate. The resulting depletion of levels of α-ketoglutarate then impairs function of the tricarboxylic acid (TCA) cycle in neurons.**

Glutamine Synthetase Fixes Ammonia as Glutamine

- **Formation of glutamine is catalyzed by mitochondrial glutamine synthetase. Since amide bond synthesis is coupled to the hydrolysis of ATP to ADP and Pi, the reaction strongly favors glutamine synthesis. One key function of glutamine is to sequester ammonia in a nontoxic form**

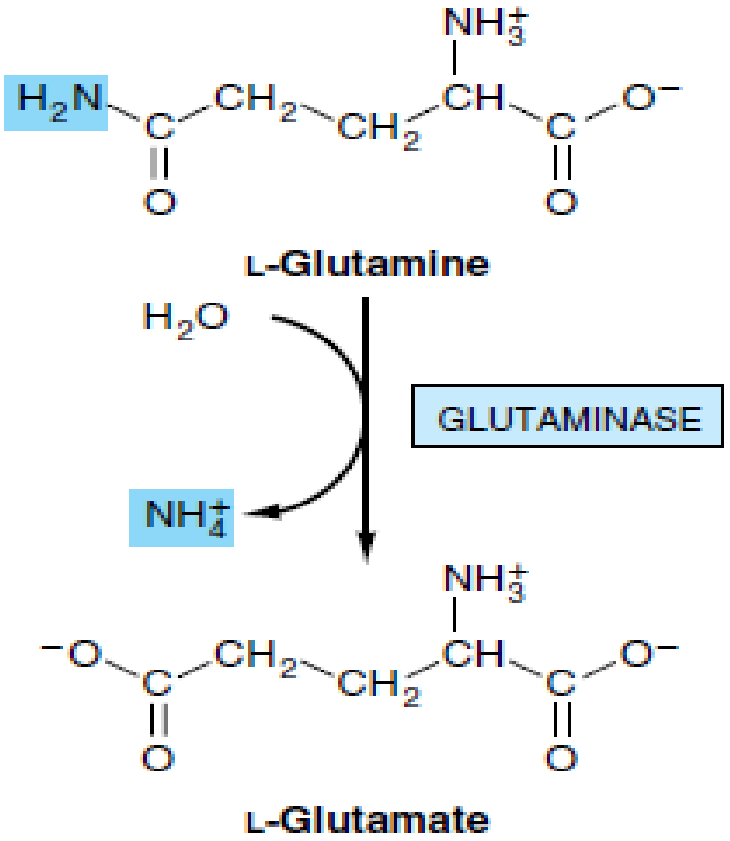


The glutamine synthetase reaction strongly favors glutamine synthesis.

Glutaminase & Asparaginase Deamidate Glutamine & Asparagine

- **Glutamine synthetase plays a major role in ammonia detoxification, interorgan nitrogen flux, and acid-base homeostasis.**
- **Hydrolytic release of the amide nitrogen of glutamine as ammonia, catalyzed by glutaminase, strongly favors glutamate formation.**

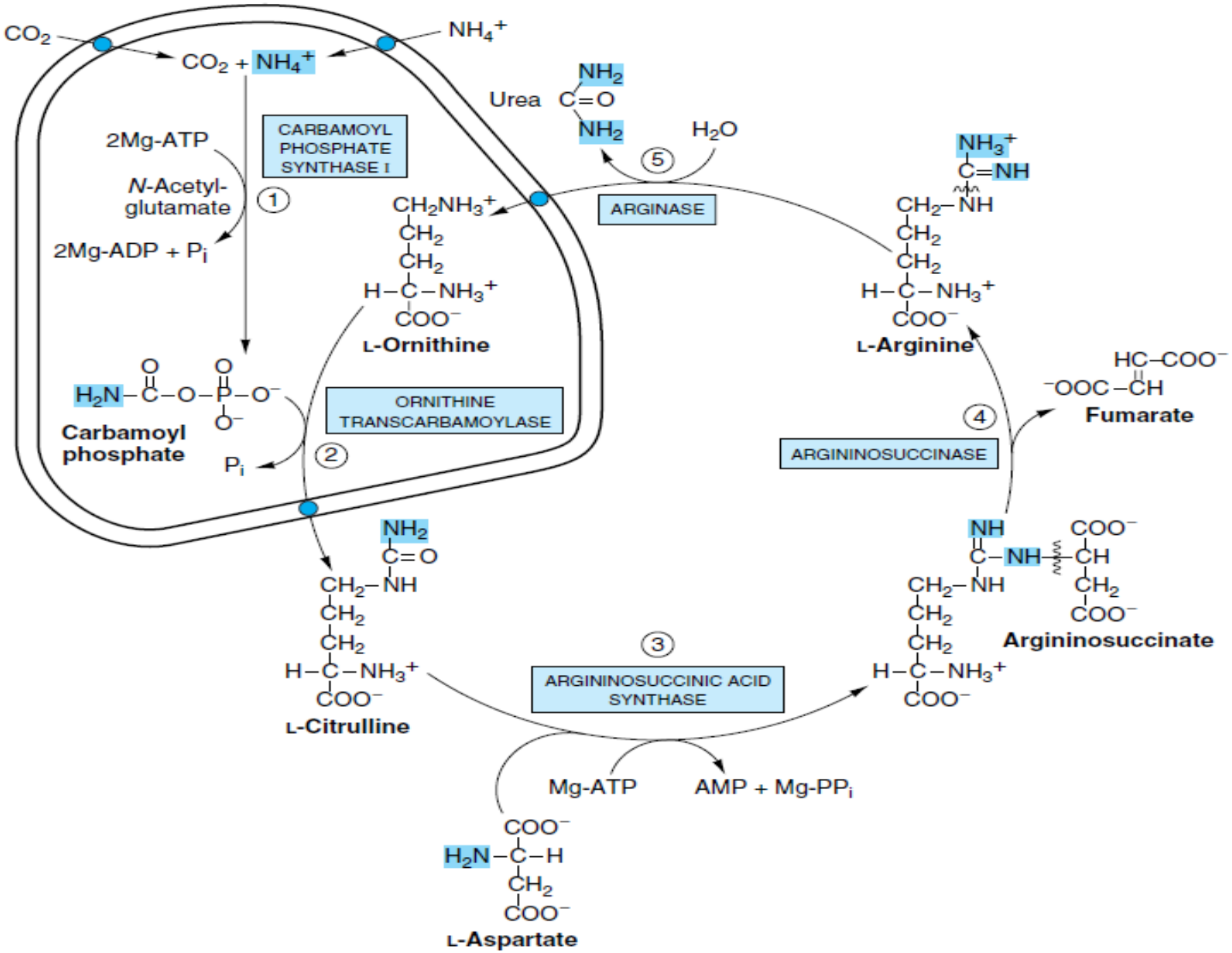
- **An analogous reaction is catalyzed by L-asparaginase. The concerted action of glutamine synthetase and glutaminase thus catalyzes the interconversion of free ammonium ion and glutamine.**
- **A rare deficiency in neonate glutamine synthetase results in severe brain damage, multi-organ failure, and death.**



The glutaminase reaction proceeds essentially irreversibly in the direction of glutamate and NH_4^+ formation. Note that the amide nitrogen, not the α-amino nitrogen, is removed.

Formation & Secretion of Ammonia Maintains Acid-Base Balance.

Excretion into urine of ammonia produced by renal tubular cells facilitates cation conservation and regulation of acid-base balance. Ammonia production from intracellular renal amino acids, especially glutamine, increases in metabolic acidosis and decreases in metabolic alkalosis.


CO2
NH4+
CO2 + NH4+
2Mg-ATP
N-Acetyl-glutamate
2Mg-ADP + Pi
CARBAMOYL PHOSPHATE SYNTHASE I
1
Carbamoyl phosphate
Pi
ORNITHINE TRANSCARBAMOYLASE
2
L-Ornithine
Urea
H2O
5
ARGINASE
L-Arginine
HC–COO⁻
⁻OOC–CH
Fumarate
4
ARGININOSUCCINASE
Argininosuccinate
3
ARGININOSUCCINIC ACID SYNTHASE
L-Citrulline
Mg-ATP
AMP + Mg-PPi
L-Aspartate

- **Synthesis of 1 mol of urea requires 3 mol of ATP plus 1 mol each of ammonium ion and of the α-amino nitrogen of aspartate.**
- **Five enzymes catalyze the numbered reactions of the six participating amino acids, N α-acetylglutamate functions solely as an enzyme activator. The others serve as carriers of the atoms that ultimately become urea.**

- The major metabolic role of ornithine, citrulline, and argininosuccinate in mammals is urea synthesis.
- The ornithine consumed in reaction 2 is regenerated in reaction 5, and so there is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine.
- Ammonium ion, CO_2, ATP, and aspartate are, however, consumed. Some reactions of urea synthesis occur in the matrix of the mitochondrion, and other reactions in the cytosol.

Carbamoyl Phosphate Synthetase I Initiates Urea Biosynthesis

- Condensation of CO_2, ammonia, and ATP to form carbamoyl phosphate is catalyzed by mitochondrial carbamoyl phosphate synthetase I.
- A cytosolic form of this enzyme, carbamoyl phosphate synthetase II, uses glutamine rather than ammonia as the nitrogen donor and functions in pyrimidine biosynthesis.

- The action of GDH and carbamoyl phosphate synthetase 1 thus shuttle amino nitrogen into carbamoyl phosphate (a compound with high group transfer potential).
- Carbamoyl phosphate synthetase I, the rate-limiting enzyme of the urea cycle, is active only in the presence of N -acetylglutamate, an allosteric activator that enhances the affinity of the synthetase for ATP.

- **Synthesis of one mol of carbamoyl phosphate requires 2 mol of ATP. One ATP serves as the phosphoryl donor for formation of the mixed acid anhydride bond of carbamoyl phosphate. The second ATP provides the driving force for synthesis of the amide bond of carbamoyl phosphate.**
- **The other products are two mol of ADP and one mol of Pi.**

- **Reaction of bicarbonate with ATP forms carbonyl phosphate and ADP. Ammonia then displaces ADP, forming carbamate and orthophosphate. Phosphorylation of carbamate by the second ATP then forms carbamoyl phosphate.**

Carbamoyl Phosphate Plus Ornithine Forms Citrulline

- L-Ornithine transcarbamoylase catalyzes transfer of the carbamoyl group of carbamoyl phosphate to ornithine, forming citrulline and orthophosphate.
- While the reaction occurs in the mitochondrial matrix, both the formation of ornithine and the subsequent metabolism of citrulline take place in the cytosol.
- Entry of ornithine into mitochondria and exodus of citrulline from mitochondria therefore involve mitochondrial inner membrane transport systems.

Citrulline Plus Aspartate Forms Argininosuccinate

Argininosuccinate synthetase links aspartate and citrulline via the amino group of aspartate and provides the second nitrogen of urea. The reaction requires ATP and involves intermediate formation of citrullyl-AMP. Subsequent displacement of AMP by aspartate then forms arginosuccinate.

Cleavage of Argininosuccinate Forms Arginine & Fumarate

- Cleavage of argininosuccinate, catalyzed by argininosuccinase, proceeds with retention of nitrogen in arginine and release of the aspartate skeleton as fumarate.
- Addition of water to fumarate forms L -malate, which subsequent NAD^+-dependent oxidation converts to oxaloacetate. These two reactions are analogous to reactions of the citric acid cycle, but are catalyzed by cytosolic fumarase and malate dehydrogenase.

- **Transamination of oxaloacetate by glutamate aminotransferase then re-forms aspartate. The carbon skeleton of aspartate-fumarate thus acts as a carrier of the nitrogen of glutamate into a precursor of urea.**

Cleavage of Arginine Releases Urea & Re-Forms Ornithine

- **Hydrolytic cleavage of the guanidino group of arginine, catalyzed by liver arginase, releases urea. The other product, ornithine, reenters liver mitochondria and participates in additional rounds of urea synthesis.**
- **Ornithine and lysine are potent inhibitors of arginase, and compete with arginine. Arginine also serves as the precursor of the potent muscle relaxant nitric oxide (NO) in a Ca^{2+}-dependent reaction catalyzed by NO synthase.**

Carbamoyl Phosphate Synthetase I Is the Pacemaker Enzyme of the Urea Cycle

- The activity of carbamoyl phosphate synthetase I is determined by N-acetylglutamate, whose steady-state level is dictated by its rate of synthesis from acetyl-CoA and glutamate and its rate of hydrolysis to acetate and glutamate. These reactions are catalyzed by N-acetylglutamate synthase and N-acetylglutamate hydrolase, respectively.

- **Major changes in diet can increase the concentrations of individual urea cycle enzymes 10- to 20-fold. For example, starvation elevates enzyme levels, presumably to cope with the increased production of ammonia that accompanies enhanced starvation-induced degradation of protein.**

CONVERSION OF AMINO ACIDS TO SPECIALIZED PRODUCTS

- Amino acid serving as building blocks for proteins.
- Amino acids act as a precursors of many nitrogen containing compounds that have important physiologic functions.
- Example of these products (porphyrins, neurotransmitters, hormones, purines, and pyrimidines).

Catecholamines

- **Biologically active (biogenic) amines (Dopamine, norepinephrine, and epinephrine).**
- **Dopamine and norepinephrine are synthesized in the brain and function as neurotransmitters. Norepinephrine is also synthesized in the adrenal medulla, as is epinephrine.**

Function:

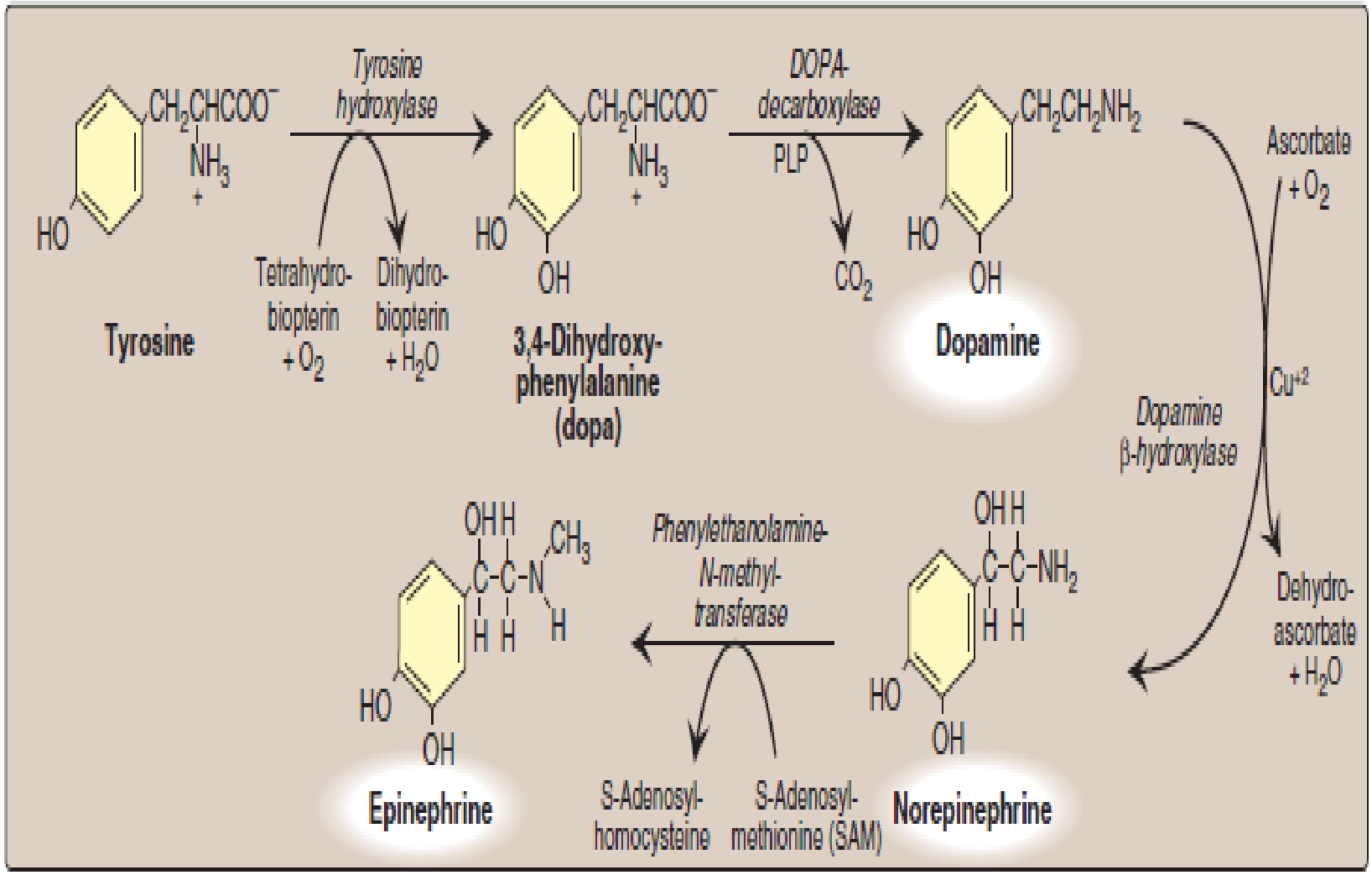
Outside the nervous system, norepinephrine and its methylated derivative, epinephrine, are hormone regulators of carbohydrate and lipid metabolism. Norepinephrine and epinephrine are released from storage vesicles in the adrenal medulla in response to fright, exercise, cold, and low levels of blood glucose.

They increase the degradation of glycogen and triacylglycerol, as well as increase blood pressure and the output of the heart. These effects are part of a coordinated response to prepare the individual for stress, and are often called the “fight-or-flight” reactions.

Synthesis of catecholamines: The catecholamines are synthesized from tyrosine, as following.

- Tyrosine is first hydroxylated by tyrosine hydroxylase to form 3,4-dihydroxy phenyl - alanine (DOPA).
- DOPA is decarboxylated in a reaction requiring pyridoxal phosphate (PLP) to form dopamine.
- Dopamine is hydroxylated by dopamine β-hydroxylase to yield norepinephrine in a reaction that requires ascorbate (vitamin C) and copper.

- **Epinephrine is formed from norepinephrine by an N-methylation reaction using S-adenosylmethionine (SAM) as the methyl donor.**

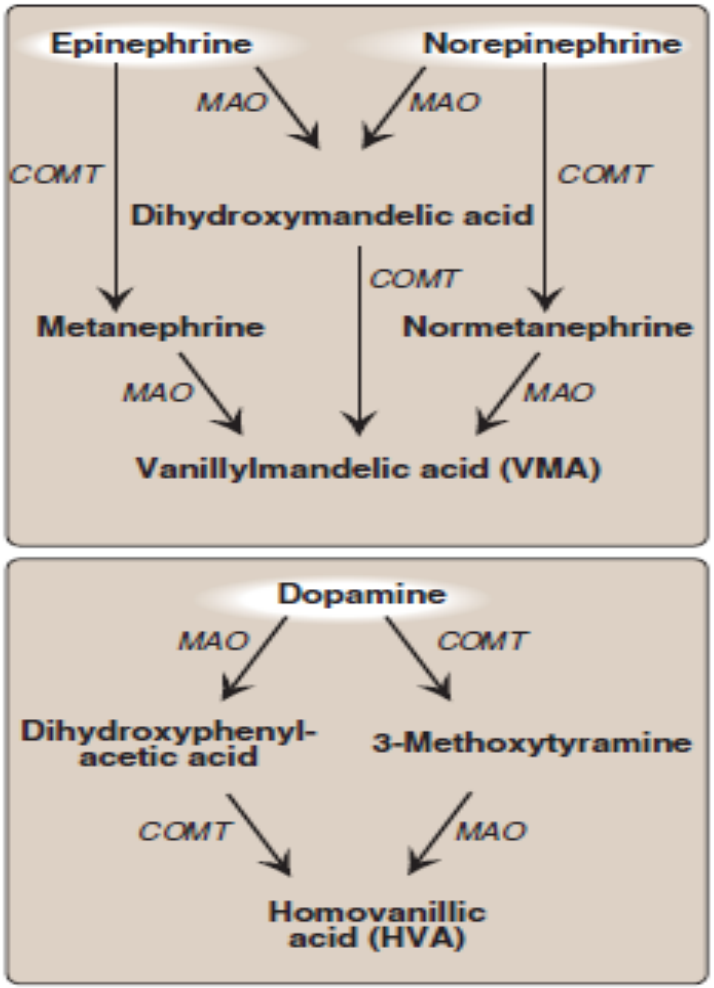


Synthesis of catecholamines.

Degradation of catecholamines:

- The catecholamines are inactivated by oxidative deamination catalyzed by monoamine oxidase (MAO), and by O-methylation carried out by catechol-O-methyltransferase using SAM as the methyl donor.
- The two reactions can occur in either order. The aldehyde products of the MAO reaction are oxidized to the corresponding acids.

- **The metabolic products of these reactions are excreted in the urine as vanillylmandelic acid (VMA) from epinephrine and norepinephrine, and homovanillic acid from dopamine. [Note: VMA is increased with pheochromocytomas, tumors of the adrenal characterized by excessive production of catecholamines.]**



Metabolism of the catecholamines by catechol-O-methyltranferase (COMT) and monoamine oxidase (MAO).

MAO inhibitors:

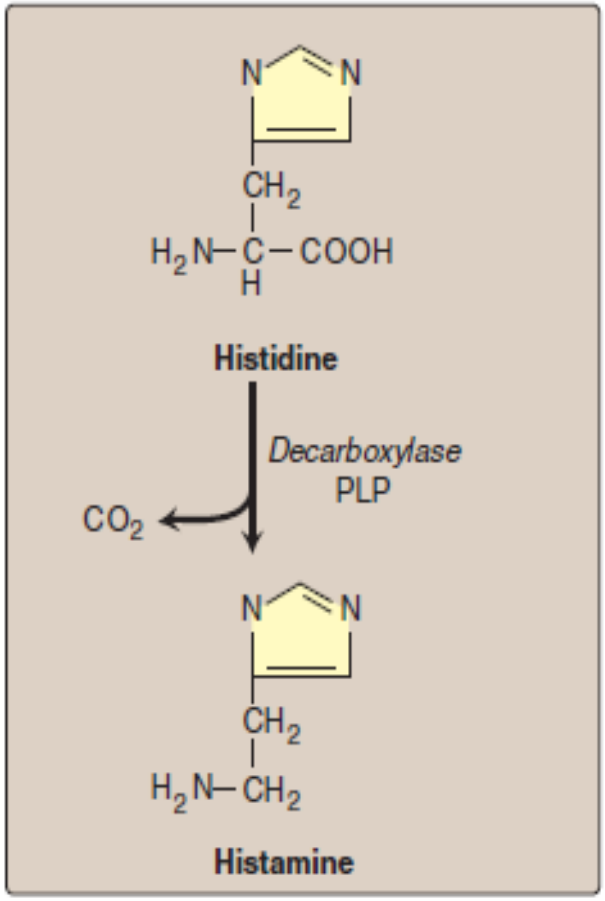
- MAO is found in neural and other tissues, such as the intestine and liver. In the neuron, this enzyme oxidatively deaminates and inactivates any excess neurotransmitter molecules (norepinephrine, dopamine, or serotonin) that may leak out of synaptic vesicles when the neuron is at rest.

- **MAO inhibitors may irreversibly or reversibly inactivate the enzyme, permitting neurotransmitter molecules to escape degradation and, therefore, to both accumulate within the presynaptic neuron and to leak into the synaptic space. This causes activation of norepinephrine and serotonin receptors, and may be responsible for the antidepressant action of these drugs.**

Histamine

- **Histamine is a chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion, and possibly neurotransmission in parts of the brain.**
- **A powerful vasodilator, histamine is formed by decarboxylation of histidine in a reaction requiring PLP.**

- **It is secreted by mast cells as a result of allergic reactions or trauma. Histamine has no clinical applications, but agents that interfere with the action of histamine have important therapeutic applications.**

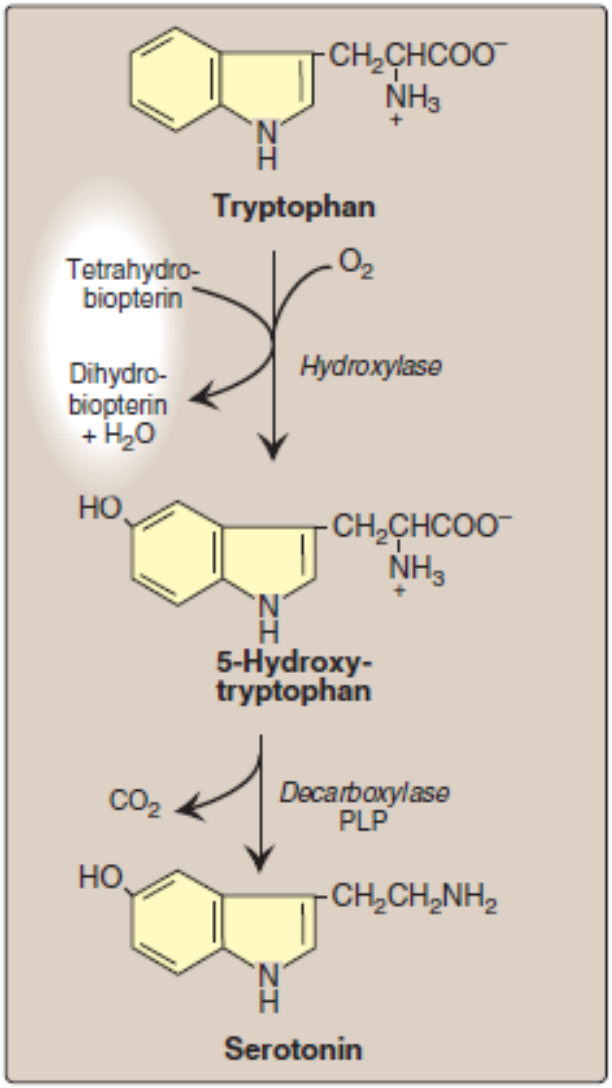


Biosynthesis of histamine

Serotonin

- Serotonin, also called 5-hydroxytryptamine (5HT), is synthesized and stored at several sites in the body.
- The largest amount of serotonin is found in cells of the intestinal mucosa. Smaller amounts occur in the central nervous system, where it functions as a neurotransmitter, and in platelets.

- **Serotonin is synthesized from tryptophan, which is hydroxylated to 5-hydroxy tryptophan, the reaction catalyzed by phenylalanine hydroxylase.**
- **The product, 5-hydroxy tryptophan, is decarboxylated to serotonin, which is also degraded by MAO. Serotonin has multiple physiologic roles, including pain perception, regulation of sleep, appetite, temperature, blood pressure, cognitive functions, and mood (causes a feeling of well-being). [Note: Serotonin is converted to melatonin in the pineal gland via acetylation and methylation.]**



Synthesis of serotonin

Creatine

- **Creatine phosphate (also called phosphocreatine), the phosphorylated derivative of creatine found in muscle, is a high-energy compound that provides a small but rapidly mobilized reserve of high-energy phosphates that can be reversibly transferred to ADP to maintain the intracellular level of ATP during the first few minutes of intense muscular contraction.**

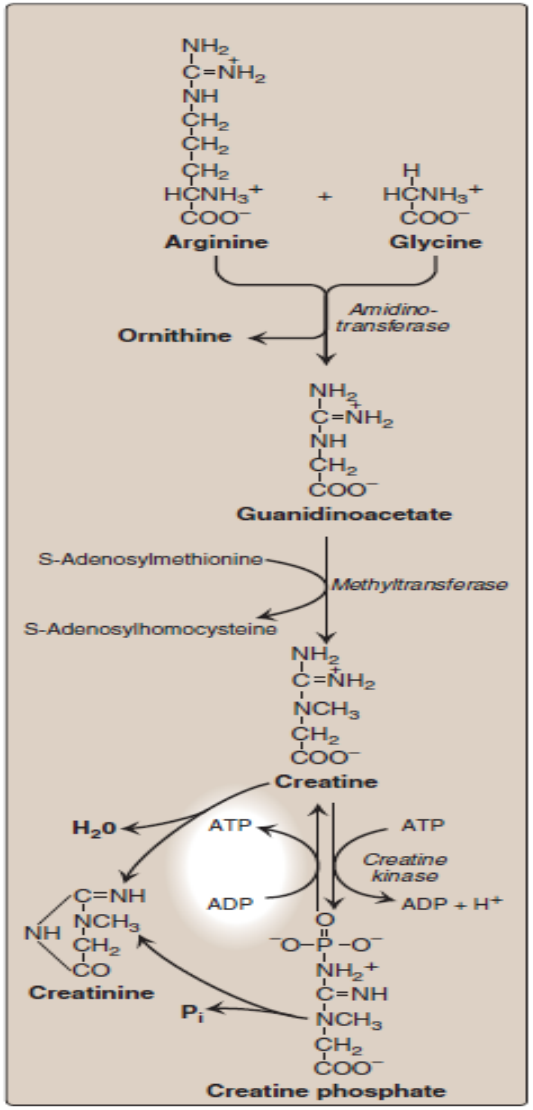
Synthesis:

- Creatine is synthesized from glycine and the guanidine group of arginine, plus a methyl group from SAM.
- Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase, using ATP as the phosphate donor. [Note: The presence of creatine kinase. in the plasma is indicative of heart damage, and is used in the diagnosis of myocardial infarction. 65).]

Degradation:

- Creatine and creatine phosphate spontaneously cyclize at a slow but constant rate to form creatinine, which is excreted in the urine. The amount of creatinine excreted is proportional to the total creatine phosphate content of the body, and thus can be used to estimate muscle mass.

- **When muscle mass decreases for any reason, the creatinine content of the urine falls. In addition, any rise in blood creatinine is a sensitive indicator of kidney malfunction, because creatinine normally is rapidly removed from the blood and excreted. A typical adult male excretes about 15 mmol of creatinine per day.**



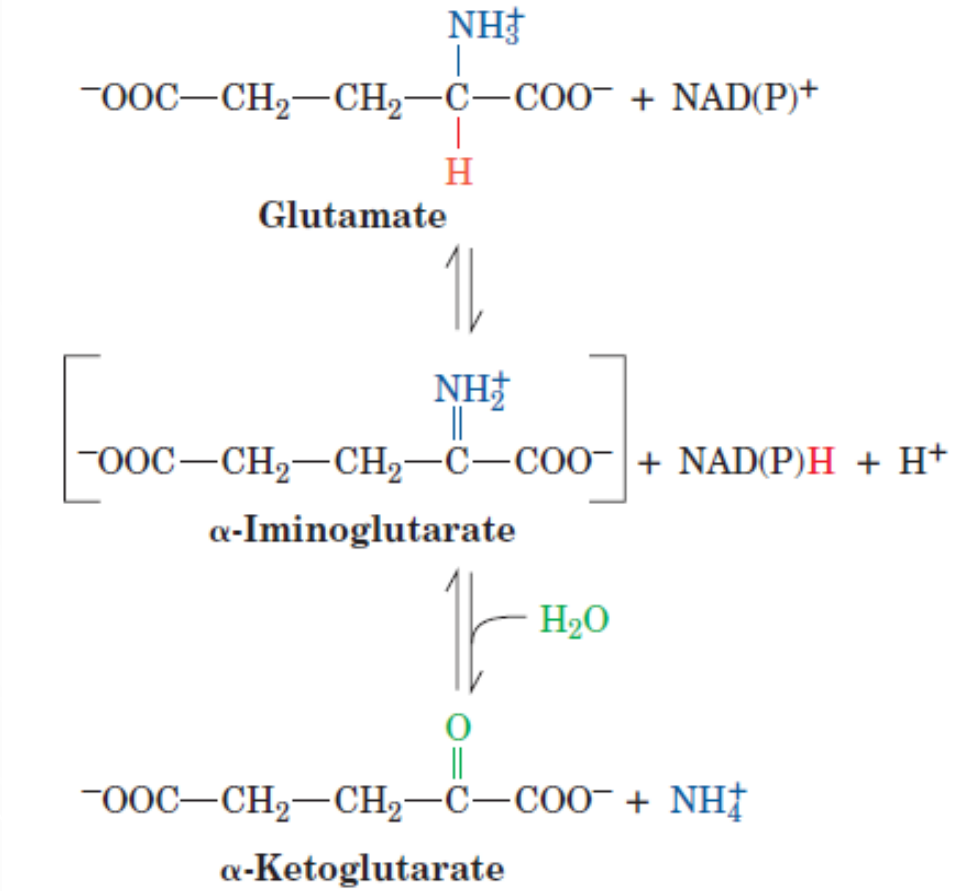
Synthesis of creatine

Melanin

- Melanin is a pigment that occurs in several tissues, particularly the eye, hair, and skin. It is synthesized from tyrosine in the epidermis by pigment-forming cells called melanocytes.
- Its function is to protect underlying cells from the harmful effects of sunlight. [Note: A defect in melanin production results in albinism, the most common form beingdue to defects in copper-containing tyrosinase.

Oxidative Deamination: Glutamate Dehydrogenase

Glutamate is oxidatively deaminated in the mitochondrial matrix by glutamate dehydrogenase (GDH), the only known enzyme that, in at least some organisms, can accept either NAD^+ or $NADP^+$ as its redox coenzyme. Oxidation is thought to occur with transfer of a hydride ion from glutamate's Cα to $NAD(P)^+$, thereby forming α -iminoglutarate, which is hydrolyzed to α -ketoglutarate and ammonia.



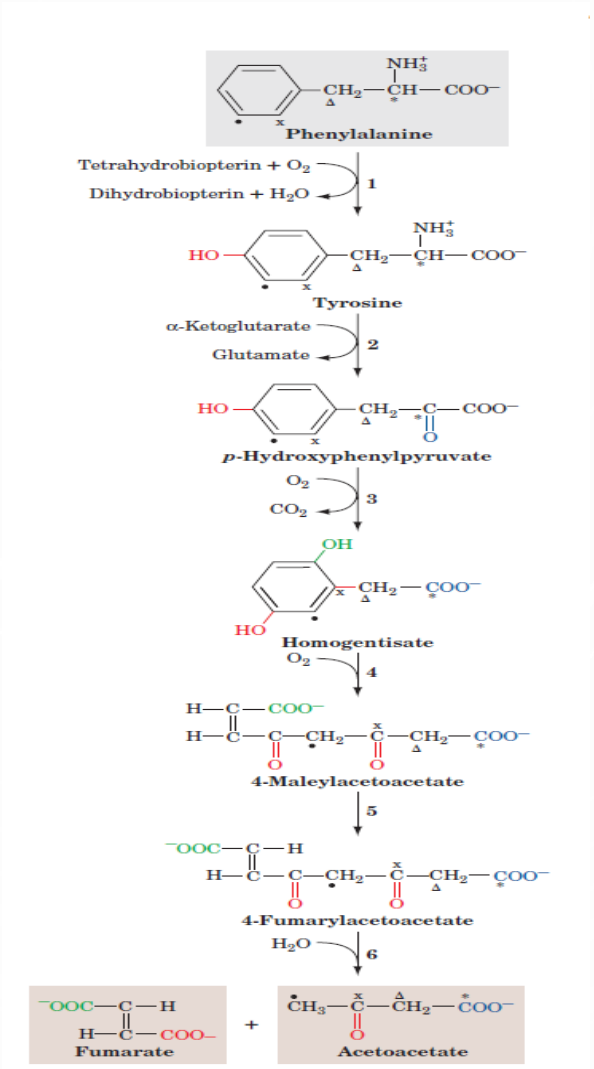
The oxidative deamination of glutamate by glutamate dehydrogenase. This reaction involves the intermediate formation of α-iminoglutarate.

GDH is allosterically inhibited by GTP, NADH, and nonpolar compounds such as palmitoyl-CoA and steroid hormones. It is activated by ADP, NAD^+, and leucine (the most abundant amino acid in proteins.

Hyperinsulinism/Hyperammonemia (HI/HA) Is Caused by Uncontrolled GDH Activity

New form of congenital hyperinsulinism that is characterized by hypoglycemia and hyperammonemia (HI/HA; hyperammonemia is elevated levels of ammonia in the blood) and has shown that it is caused by mutations in GDH. The mutant enzymes have reduced sensitivity to GTP inhibition but retain their ability to be activated by ADP.

The hypoglycemia and hyperammonemia in HI/HA patients arises from the increased activity of the GDH mutants in the breakdown direction, producing increased amounts of α -ketoglutarate and NH3. The increased levels of α -ketoglutarate stimulate the citric acid cycle and oxidative phosphorylation, which has been shown to lead to increased insulin secretion and hypoglycemia, thereby producing the symptoms of the disease. The produced is usually converted to urea.



The pathway of phenylalanine degradation. The enzymes involved are (1) phenylalanine hydroxylase, (2) aminotransferase, (3) p-hydroxyphenylpyruvate dioxygenase, (4) homogentisate dioxygenase, (5) maleylacetoacetate isomerase, and (6) fumarylacetoacetase. The symbols labeling the various carbon atoms serve to indicate the group migration that occurs in Reaction 3 of the pathway

Phenylalanine Hydroxylase Is Controlled by Phosphorylation and by Allosteric Interactions

PAH initiates the detoxification of high concentrations of phenylalanine as well as the synthesis of the catecholamine hormones and neurotransmitters. It is allosterically activated by its substrate, phenylalanine, and by phosphorylation at its Ser 16 by the cAMP-dependent protein kinase A.