ENZYMES

Biomedical Importance:

Enzymes, which catalyze the biochemical reactions, are essential for life. They participate in the *breakdown of nutrients* to supply energy and chemical building blocks; the *assembly of those building blocks* into proteins, DNA, membranes, cells, and tissues; and the *harnessing of energy* to power cell motility, neural function, and muscle contraction.

The vast majority of enzymes are proteins. Notable exceptions include ribosomal RNAs and a handful of RNA molecules imbued with endonuclease or nucleotide ligase activity known collectively as ribozymes.

The ability to detect and to quantify the activity of specific enzymes in blood, other tissue fluids, or cell extracts provides information that complements the physician's ability to *diagnose and predict the prognosis of many diseases*. Further medical applications include changes in the quantity or in the catalytic activity of key enzymes that can result from genetic defects, nutritional deficits, tissue damage, toxins, or infection by viral or bacterial pathogens (eg, *Vibrio cholerae*). Medical scientists address imbalances in enzyme activity by using *pharmacologic agents to inhibit specific enzymes* and are investigating gene therapy as a means to remedy deficits in enzyme level or function.

In addition to serving as the catalysts for all metabolic processes, their impressive catalytic activity, substrate specificity, and stereospecificity enable enzymes to fulfill key roles in additional processes related to human health and well-being. Proteases and amylases *augment the capacity of detergents* to remove dirt and stains, and enzymes play important roles in *producing or enhancing the nutrient value of food* products for both humans and animals. The protease rennin, for example, is utilized in the production of cheeses while lactase is employed to remove lactose from milk for the benefit of lactose-intolerant persons deficient in this hydrolytic enzyme. Finally, stereospecific enzyme catalysts can be of particular value in the *biosynthesis of complex drugs or antibiotics*.

The Nature of Enzymes:

Enzymes may be defined as *biocatalysts synthesized by living cells. They are protein in nature (exception: ribozymes which are RNA in nature), colloidal and thermolabile in character, and specific in their action*.

Most enzymes are ***globular proteins*** that exhibit at least tertiary structure with a complex 3-D configuration, capable of binding substrate molecules to a part of their surface. The enzymes that ***catalyze*** the conversion of one or more compounds (substrates) into one or more different compounds (products) generally enhance the rates of the corresponding noncatalyzed reaction by factors of 10^{16} or more. Like almost all catalysts, enzymes are neither consumed nor permanently altered as a consequence of their participation in a reaction.

Unlike most catalysts used in synthetic chemistry, enzymes are ***specific*** not simply for the type of *reaction* catalyzed, but also for a single *substrate* or a small set of closely related substrates.

Enzymes are also stereospecific catalysts that typically catalyze reactions of only one *stereoisomer* of a given compound (for example, D- but not L-sugars, L- but not D-amino acids).

Enzymes Nomenclature and Classification:

The early discovered enzymes were designated by first appending the suffix ***–ase*** to a ***descriptor*** for the type of reaction catalyzed. For example, enzymes that remove hydrogen atoms are generally referred to as dehydrogen*ases*, enzymes that hydrolyze proteins as prote*ases*, and enzymes that catalyze rearrangements in configuration as isomer*ases*, and so on. These general descriptors to be preceded with terms indicating the **substrate** on which the enzyme acts (*xanthine* oxidase), its **source** (*pancreatic* ribonuclease), its **mode of regulation** (*hormone-sensitive* lipase). Where needed, ***alphanumeric designators*** are added to the end of the enzyme name to identify multiple forms of an enzyme (eg, RNA polymerase III; protein kinase Cb).

While simple and straightforward, as more enzymes were discovered these early naming conventions increasingly resulted in the appearance of multiple names for the same enzyme and duplication in the naming of enzymes exhibiting similar catalytic capabilities. To address these problems, the International Union of Biochemistry (IUB) developed a definite system of enzyme nomenclature in which each enzyme has a unique name and code number (the enzyme commission (E.C.) number) that identify the type of reaction catalyzed by the enzyme and the substrates involved. E.C. number is a unique identifier for each enzyme classified according to IUB system.

According to IUB system enzymes are grouped into the following six classes:

1. Oxidoreductases - enzymes that catalyze oxidations and reductions.

2. Transferases - enzymes that catalyze transfer of moieties such as glycosyl, methyl, or phosphoryl groups.

3. Hydrolases - enzymes that catalyze *hydrolytic* cleavage of C-C, C-O, C-N, and other covalent bonds.

4. Lyases - enzymes that catalyze non-hydrolytic cleavage of C-C, C-O, C-N, and other covalent bonds by *atom (functional group) elimination,* generating double bonds.

5. Isomerases - enzymes that catalyze geometric or structural changes *within* a molecule.

6. Ligases - enzymes that catalyze the joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP.

The IUB name of hexokinase is ATP:D-hexose 6-phosphotransferase E.C. 2.7.1.1. This name identifies hexokinase as a member of class 2 (transferases), subclass 7 (transfer of a phosphoryl group), sub-subclass 1 (alcohol is the phosphoryl acceptor), and sub-class 1 (alcohol phosphorylated is on carbon six of a hexose). Despite their clarity, IUB names are lengthy and relatively cumbersome, so we generally continue to refer to hexokinase and many other enzymes by their traditional, albeit sometimes ambiguous names. On the other hand, E.C. numbers are particularly useful to differentiate enzymes with similar functions or catalytic activities.

Prosthetic Groups, Cofactors, and Coenzymes Role in Catalysis:

Many enzymes contain small organic molecules or metal ions that participate directly in substrate binding or in catalysis. Termed **prosthetic groups**, **cofactors**, and **coenzymes**. The term ***holoenzyme*** refers to the *active* enzyme with its non-protein component, whereas the enzyme without its non-protein moiety is termed an ***apoenzyme*** and is *inactive*.

Prosthetic groups are *tightly* and stably *incorporated* into a protein's structure by covalent or noncovalent forces. They are either small organic molecules or inorganic metal ions. Examples include pyridoxal phosphate, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), thiamin pyrophosphate, and biotin. Metal ions constitute the most common type of prosthetic group. The roughly one-third of all enzymes that contain tightly bound Fe, Co, Cu, Mg, Mn, and Zn are termed **metalloenzymes.**
Prosthetic groups may facilitate the binding and orientation of substrates, or by acting as Lewis acids or bases to render substrates more **electrophilic** (electron-poor) or **nucleophilic** (electron-rich), and hence more reactive.

Cofactors can *associate reversibly* with the *enzymes* or with the *substrates*. While cofactors serve functions similar to those of prosthetic groups, they bind in a transient, dissociable manner. Therefore, unlike associated prosthetic groups, cofactors must be present in the medium surrounding the enzyme for catalysis to occur. The most common cofactors are metal ions. Enzymes that require a metal ion cofactor are termed **metal-activated enzymes** to distinguish them from the **metalloenzymes** for which bound metal ions serve as prosthetic groups.

Coenzymes are small organic molecules that serve as *recyclable shuttles* (transporters) that transport many substrates from one point within the cell to another. The function of these shuttles is twofold. ***First***, they *stabilize species* such as hydrogen atoms (FADH) or hydride ions (NADH) that are too reactive to persist for any significant time in the presence of the water or organic molecules that permeate cells. ***Second***, they serve as an adaptor or handle that *facilitates the recognition* and binding of small chemical groups, such as acetate (coenzyme A) or glucose (UDP), by their target enzymes.

The Active Site:

An **active site** is a cleft or pocket on the surface of the enzyme. It is the **recognition site** for binding substrates; the specificity of binding depends on the precisely defined arrangement of atoms in an active site. Because the enzyme and the substrate interact by means of short-range forces that require close contact, a substrate must have a matching shape to fit into the site.
An active site also **xc**. Within the active site, substrates are brought into *close proximity* to one another in *optimal alignment* with the cofactors, prosthetic groups, and amino acid side chains that participate in catalyzing the transformation of substrates into products. Catalysis is further enhanced by the capacity of the active site to *shield substrates* from water and generate an environment whose polarity, hydrophobicity, acidity, or alkalinity can differ markedly from that of the surrounding cytoplasm.

Enzymes Mechanisms to Facilitate Catalysis:

Enzymes use combinations of *four* general mechanisms to achieve dramatic enhancements of the rates of chemical reactions.

1. **Catalysis by Proximity**

For molecules to interact, they must come within bond-forming distance of one another. The higher their concentration, the more frequently they will encounter one another, and the greater will be the rate of their reaction. When an enzyme binds substrate molecules at its active site, it creates a region of high local substrate concentration in which the substrate molecules reactive groups are oriented in a position ideal for them to chemically interact. This results in rate enhancements of at least a thousand fold over the same non-enzyme-catalyzed reaction.

2. **Acid-Base Catalysis**

In addition to contributing to the ability of the active site to bind substrates, the ionizable functional groups of aminoacyl side chains, and where present of prosthetic groups, can contribute to catalysis by acting as acids or bases. We distinguish two types of acid-base catalysis. **Specific acid or base catalysis** refers to reactions for which the only *participating* acid or base are protons or hydroxide ions. The rate of reaction thus is sensitive to changes in the concentration of protons or hydroxide ions, but is *independent* of the concentrations of other acids (proton donors) or bases (proton acceptors) present in the solution or at the active site. Reactions whose rates are responsive to *all* the acids or bases present are said to be subject to **general acid or base catalysis.**

Enzymes of the ***aspartic protease family***, which includes the digestive enzyme *pepsin*, the *lysosomal cathepsins*, and the *protease produced by the human immunodeficiency virus (HIV)* share a common mechanism that employs two conserved aspartyl residues as acid-base catalysts. Catalysis by aspartic proteases involves the direct hydrolytic attack of water on a peptide bond.

3. **Catalysis by Strain**

Enzymes that catalyze *lytic* reactions, chemical transformations that involve breaking a covalent bond, typically bind their substrates in a conformation that is somewhat unfavorable for the bond targeted for cleavage. This strained conformation represents the transition state, or midway point, in the transformation of substrates to products. The resulting strain selectively stretches or distorts the targeted bond, weakening it and making it more vulnerable to cleavage.

4. **Covalent Catalysis**

The process of **covalent catalysis** involves the formation of a covalent bond between the enzyme and one or more substrates. The modified enzyme thus becomes a reactant. Covalent catalysis introduces a new reaction pathway whose activation energy is lower—and the reaction therefore is faster—than the reaction pathway in homogeneous solution. The chemically modified state of the enzyme is, however, transient. Covalent catalysis often follows a "ping-pong" mechanism in which the first substrate is bound and its product released prior to the binding of the second substrate. Completion of the reaction returns the enzyme to its original, unmodified state. Its role thus remains catalytic. Covalent catalysis is particularly common among enzymes that catalyze **group transfer reactions**.

Enzyme-Substrate Interaction Models:

Two models have been proposed to explain how an enzyme binds its substrate. The old model **"lock and key model"** accounted for the specificity of enzyme-substrate interactions, the implied rigidity of the enzyme's active site failed to account for the dynamic changes that accompany substrate binding and catalysis (Figure 1). The other model **"induced fit model"**, which states that when substrates approach and bind to an enzyme they induce a conformational change that is analogous to placing a hand (substrate) into a glove (enzyme). The enzyme in turn induces reciprocal changes in its substrates, joining the energy of binding to facilitate the transformation of substrates into products (Figure 1). The induced fit model has been fully confirmed by biophysical studies of enzyme motion during substrate binding.

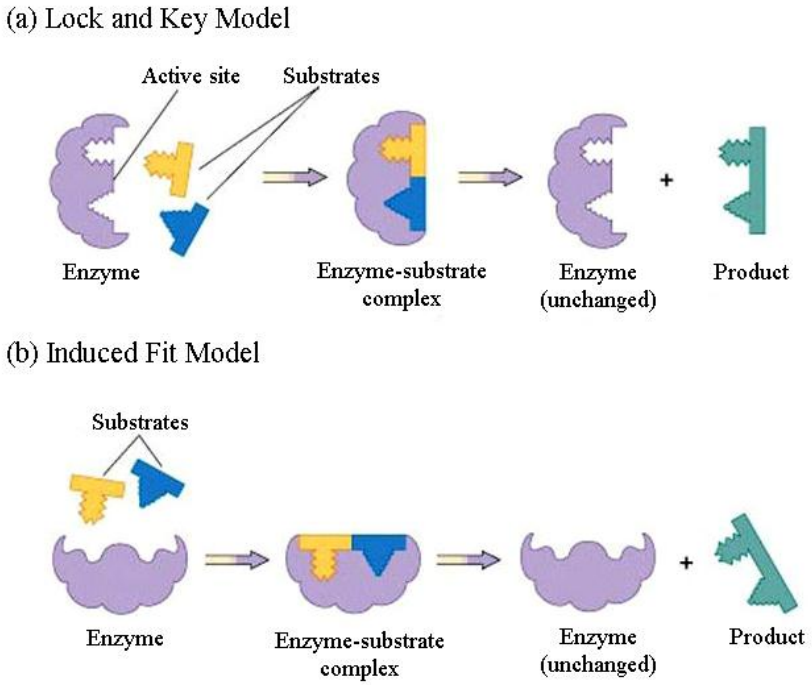


Figure 1: Enzyme-substrate binding models. (a) Lock and Key Model (b) Induced Fit Model.

Isoenzymes:

Isoenzymes are ***physically distinct*** versions of a given enzyme, each of which catalyzes the ***same reaction***. They are sometimes referred to as isozymes. Isoenzymes are produced by ***different genes*** and are not redundant despite their similar functions. They occur in many tissues throughout the body and are important for different developmental and metabolic processes.

Isoenzymes have ***different substrates***, and they may also possess ***differences in properties*** such as *sensitivity to particular regulatory factors* or *substrate affinity* (eg, hexokinase and glucokinase) that adapt them to specific tissues or circumstances rather than distinct substrate specificities.

Detection of Enzymes:

The relatively small quantities of enzymes present in cells hinder determination of their presence and concentration. However, enzymes ability to rapidly transform thousands of molecules of a specific substrate into products had enabled their detection and quantification. Under appropriate conditions, the rate of the catalytic reaction being monitored is proportionate to the amount of enzyme present, which allows its concentration to be calculated. Assays of the catalytic activity of enzymes are frequently used in research and clinical laboratories.

Overview of amino acids metabolism

- Amino acids serve as substrates for the synthesis of protein,
- Amino acids provide nitrogen for the synthesis of other nitrogen-containing compounds,
- Amino acids are catabolized as fuels.

Classification of amino acids:

1. Chemical classification:

- ✓ According to the **chemistry** of the side chains.
- ✓ According to **polarity** of side chains.

2. Nutritional classification:

- ✓ Essential
- ✓ Non-essential

NOTE:

- ✓ All of the 20 amino acids present in proteins are essential for health.
- ✓ Some clinical conditions are associated with amino acid deficiency states, such as Kwashiorkor and Marasmus diseases.
- ✓ Kwashiorkor is protein deficiency with adequate energy intake whereas Marasmus is inadequate energy intake in all forms, including protein.

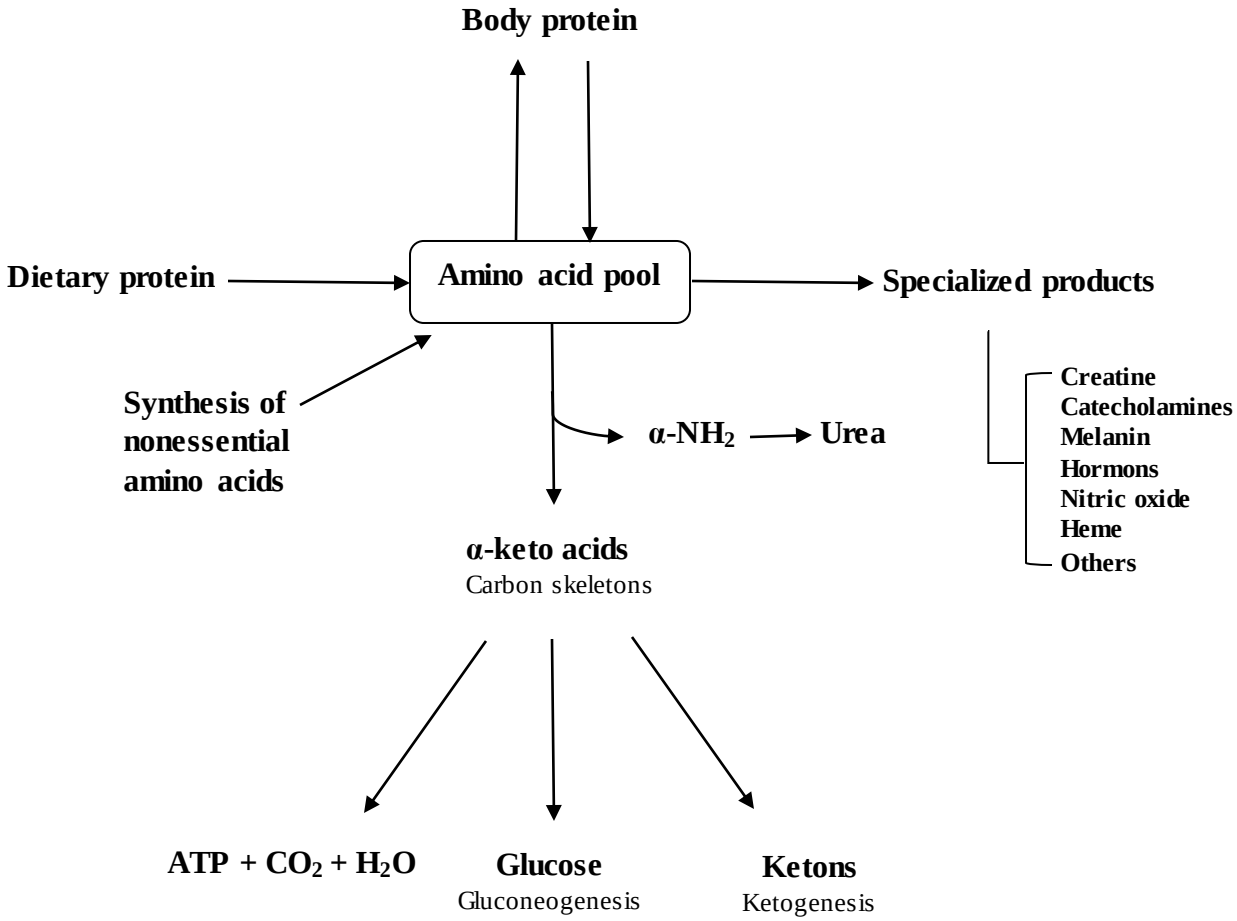
Amino acid requirements of humans

Nutritionally Essential	Nutritionally Nonessential
Arginine[1]	Alanine
Histidine[1]	Asparagine
Isoleucine	Aspartate
Leucine	Cysteine
Lysine	Glutamate
Methionine	Glutamine
Phenylalanine	Glycine
Threonine	Hydroxyproline[2]
Tryptophan	Hydroxylysine[2]
Valine	Proline
	Serine
	Tyrosine

[1] *"Nutritionally semiessential." Synthesized at rates inadequate to support growth of children.*
[2] *Not necessary for protein synthesis but formed during posttranslational processing of collagen.*

3. Metabolic classification:

- ✓ Ketogenic (Leucine and Lysine)
- ✓ Both glucogenic and ketogenic (Isoleucine, Phenylalanine, Tryptophan, Tyrosine and Threonine)
- ✓ Glucogenic (all the remaining)



Overview of amino acid metabolism

Amino acids pool

The amount of free amino acids distributed throughout the body is called amino acid pool. Plasma level for most amino acids varies widely throughout the day. It ranges between 4–8 mg/dl. It tends to increase in the fed state and tends to decrease in the post absorptive state.

Sources of amino acid pool

1. Dietary protein.
2. Breakdown of tissue proteins.
3. Biosynthesis of nonessential amino acids.

NOTE:

In general, the rate of protein synthesis equals the rate of degradation (**steady-state**). However, there is a constant need for dietary intake of protein because:

- ✓ Some amino acids are also used for energy production and storage and for synthesis of non-protein molecules.
- ✓ There are situations where protein synthesis must exceed protein degradation, such as during growth, pregnancy, and recovery from illness.

Digestion of dietary proteins

- ✓ Protein digestion begins in the stomach.
- ✓ The highly acidic environment of the stomach denatures proteins. Denatured proteins are susceptible to proteolytic digestion.
- ✓ The primary enzyme involved in proteolytic digestion is **pepsin**, which catalyzes the nonspecific hydrolysis of peptide bonds at an optimal pH of 2.
- ✓ In the lumen of the small intestine, the pancreas secretes zymogens of **trypsin (**Trypsin cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline.), **chymotrypsin (**Chymotrypsin prefers large hydrophobic residues. Chymotrypsin preferentially catalyzes the hydrolysis of peptide bonds involving tyrosine, phenylalanine, and tryptophan), **elastase** etc.
- ✓ Proteolytic enzymes break the proteins down into free amino acids as well as dipeptides and tripeptides, which in turn are absorbed by the intestinal mucosa cells and subsequently are released into the blood stream where they are absorbed by other tissues.

Turnover of cellular proteins

- ✓ Turnover of cellular proteins (continuous degradation and synthesis) occur in all forms of life.
- ✓ Each day, humans turn over 1% to 2% of their total body protein, principally muscle protein.
- ✓ Approximately 75% of the amino acids liberated by protein degradation are reutilized, the remaining excess free amino acids are not stored for future use (i.e. amino acids not immediately incorporated into new protein are rapidly degraded).
- ✓ The relative susceptibility of a protein to degradation is expressed as its half-life ($t_{1/2}$).
- ✓ Half-lives of proteins may range from under 30 minutes to over 150 hours, or even the life time of an organ.

Cellular functions of protein degradation

1. The recycling of amino acids.
2. Elimination of misfolded and damaged proteins (due to environmental toxins, translation errors and genetic mutations) when cannot be repaired.
3. Regulation of cellular metabolism and cellular growth (increase or decrease the number of enzyme molecules and regulatory substances).
4. The generation of active proteins (the proteolytic cleavage of the precursor generates an active enzyme).
5. The regulation of cell division (degradation of **cyclins**)

Pathways of protein degradation

A. General "non-specific" protein degradation takes place in **lysosomes** (specialized organelles that operate at low pH (to denature proteins) and contain proteases for proteins, lipases for lipids, and many other hydrolases (~ 50 total)). By this pathway extracellular, membrane-associated, and long-lived intracellular proteins are degraded in lysosomes by **ATP-independent** processes.

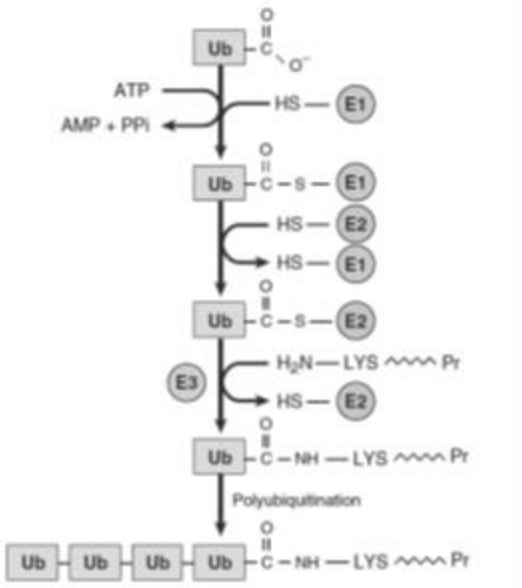
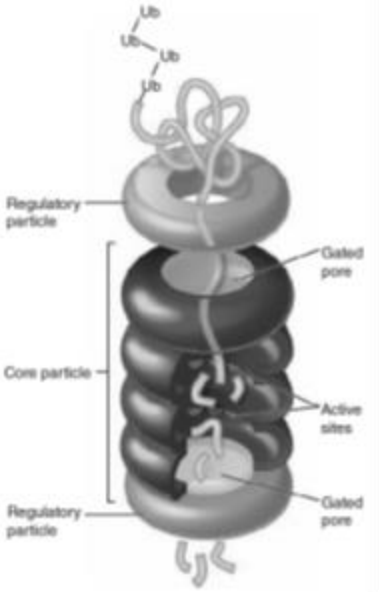
Many normal and pathological processes involve **increased lysosomal activity**, including:

- ✓ Disuse atrophy of muscles and regression of the uterus after childbirth (the muscular mass of the uterus is reduced from about 2 kg to about 50 g in just nine days).
- ✓ Chronic inflammatory diseases such as rheumatoid arthritis involve extracellular release lysosomal enzymes, which attack surrounding tissues.

B. Controlled or programmed protein degradation involves the **Ubiquitin-Proteasome system.**

Degradation of regulatory proteins with short half-lives and of abnormal or misfolded proteins occurs in the cytosol within the **proteasomes**, and **requires ATP** and **ubiquitin (Ub) (**a small polypeptide found in all eukaryotic cells, by which the cell distinguish between functional proteins and intracellular proteins that need to be degraded).

- ✓ Three different enzymes add progressively more Ub molecules, in tandem chains, an energy-requiring process (ATP). The more Ub molecules attached, the more rapid the degradation.
- ✓ Carboxyl terminal of Ub is attached to the ε-amino groups of lysyl residues in the target protein (isopeptide bond).
- ✓ The residue present at its amino terminus affects whether a protein is ubiquitinated. Amino terminal Met, or Ser residues retard, whereas Asp, or Arg accelerate ubiquitination.
- ✓ Subsequent degradation of Ub-tagged proteins takes place in the **proteasome**, a macromolecule that also is ubiquitous in eukaryotic cells.

	
Reactions involved in the attachment of ubiquitin (Ub) to proteins	**Representation of the structure of a proteasome** *The upper ring is gated to permit only polyubiquitinated proteins to enter the proteosome, where immobilized internal proteases degrade them to peptides.*

What controls the rate of protein degradation?

Different proteins are degraded at different rates. Abnormal proteins are quickly degraded, whereas the rate of degradation of normal proteins may vary widely depending on their functions. Enzymes at important metabolic control points may be degraded much faster than those enzymes whose activity is largely constant under all physiological conditions.

Degradation of normal proteins with comparable functions depends on:

- ✓ **N-terminal residue**, the residue present at its amino terminal affects whether a protein is ubiquitinated and subsequently degraded. Amino terminal Met or Ser retards whereas Asp or Arg accelerates ubiquitination.
- ✓ **PEST sequences**, regions rich in proline (P), glutamate (E), serine (S), and threonine (T), target **some** proteins for rapid degradation.

Interorgan exchange of amino acids

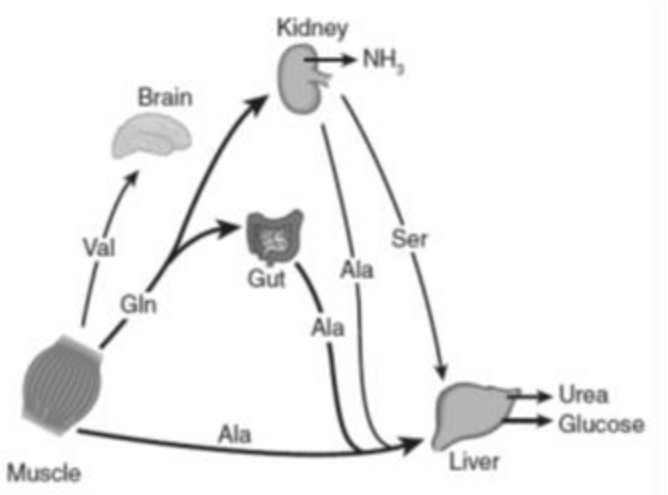
The net balance between release from endogenous protein stores and utilization by various tissues keeps the steady-state concentrations of circulating plasma amino acids between meals.

- In the **postabsorptive state**, free amino acids, particularly alanine and glutamine, are released from **muscle** into the circulation.

Alanine is extracted primarily by the liver, and glutamine is extracted by the gut and the kidney, both of which convert a significant portion to alanine. Alanine is a key **gluconeogenic amino acid**, and the rate of hepatic gluconeogenesis from alanine is far higher than from all other amino acids. Glutamine also serves as a source of ammonia for excretion by the kidney.

Branched-chain amino acids, particularly valine, are released by muscle and taken up predominantly by the brain.

The **kidney** provides a major source of serine for uptake by peripheral tissues, including liver and muscle.



Interorgan amino acid exchange in normal postabsorptive humans

- In the **fed state (**following a protein-rich meal), the splanchnic tissues release amino acids while the peripheral muscles extract amino acids.

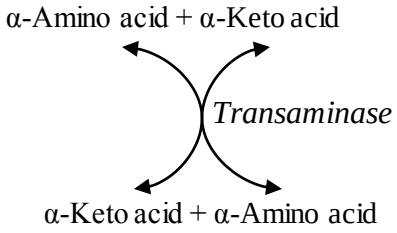
Biosynthesis of Nonessential Amino Acids

Humans do not have the ability to synthesize 10 of the necessary 20 amino acids and must obtain them from the diet. These 10 are termed the nutritionally essential amino acids.

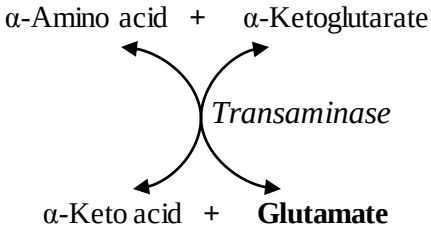
The number of enzymes required by cells to synthesize the nutritionally essential amino acids is large in relation to the number of enzymes required to synthesize the nutritionally nonessential amino acids. This suggests that there is a positive survival advantage in retaining the ability to manufacture 'easy' amino acids while losing the ability to make 'difficult' amino acids.

The 10 nonessential amino acids are formed by 3 general mechanisms:

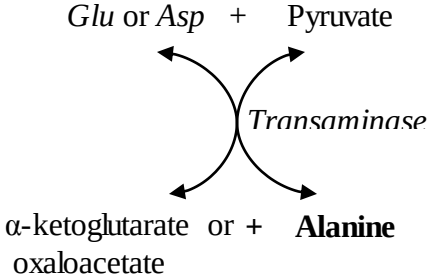
I) Transamination:



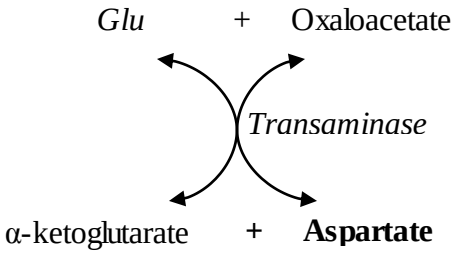
- **Glutamate:** can be synthesized by transamination of the corresponding α -keto acid, α – ketoglutarate. The amino donor may be any amino acid (except lysine, threonine, proline and hydroxyproline).



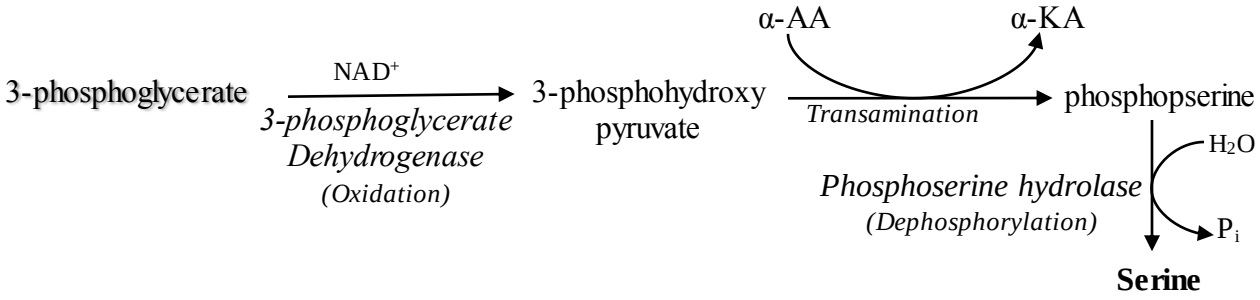
- **Alanine:** transamination of pyruvate forms alanine. The amino donor may be glutamate or aspartate. The other product thus is α-ketoglutarate or oxaloacetate.



- **Aspartate:** can be synthesized by transamination of oxaloacetate to form aspartate and α-ketoglutarate. The amino donor is glutamate.



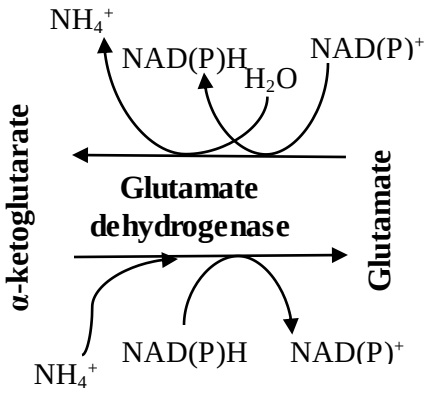
- **Serine** is synthesized by the oxidation, transamination and subsequent dephosphorylation of 3-phosphogylcerate, an intermediate of glycolysis.



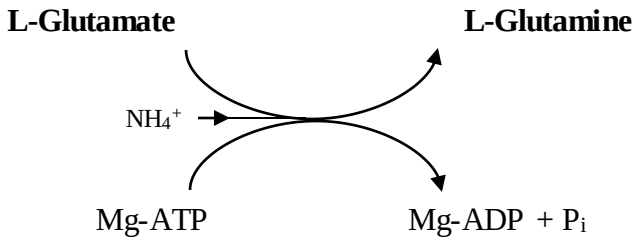
NOTE
In all of the transamination reactions, pyridoxal phosphate PLP (**vitamin B6**) acts as an intermediate carrier of the amino group that is being transferred.

II) Assimilation of free ammonia:

- **Glutamate:** Formation of glutamate from free ammonia and α-ketoglutarate is catalyzed by glutamate dehydrogenase. This reaction is reversible and plays a role in both synthesis and breakdown of glutamate. Both NADPH and NADH can serve as the source of reducing equivalents used in this reaction.

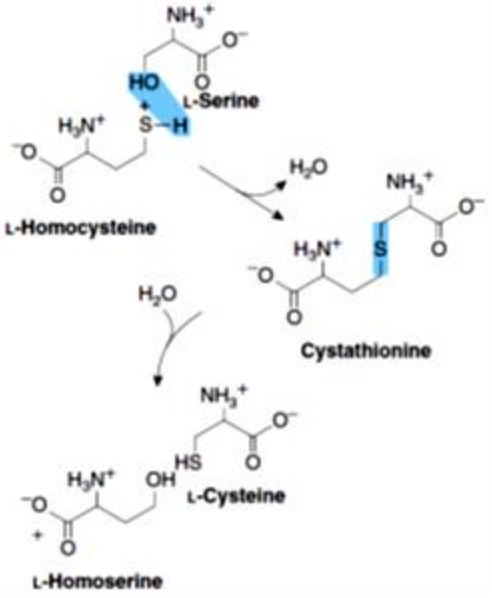


- **Glutamine:** Glutamine synthetase catalyzes the ATP-dependent formation of glutamine, using glutamate and ammonia as substrates.



III) Modification of the carbon skeletons of existing amino acids.

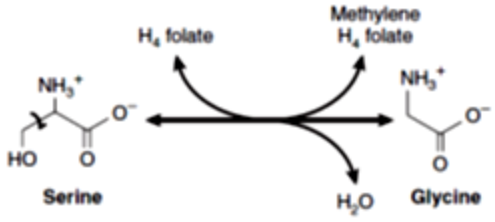
- **Cysteine**: Cysteine contains atoms donated by both methionine and serine. Following conversion of methionine to homocysteine, homocysteine and serine form cystathionine, a thioether (RSR′), the reaction is catalyzed by cystathionine β-synthase. Hydrolysis of cystathionine by cystathionine lyase forms cysteine.



While cysteine is nutritionally nonessential, it is formed from methionine, which is nutritionally essential.

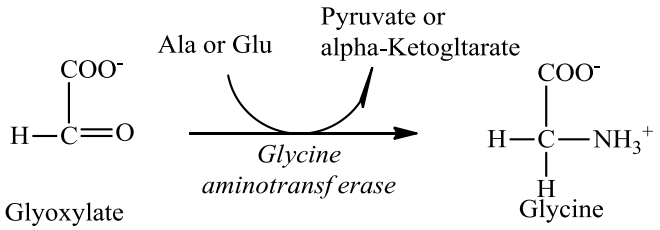
- **Glycine:** three mammalian routes for glycine formation

✓ From serine which is converted to glycine by the removal of its hydroxymethyl group. The reaction is freely **reversible** and catalyzed by **serine hydroxymethyl transferase**



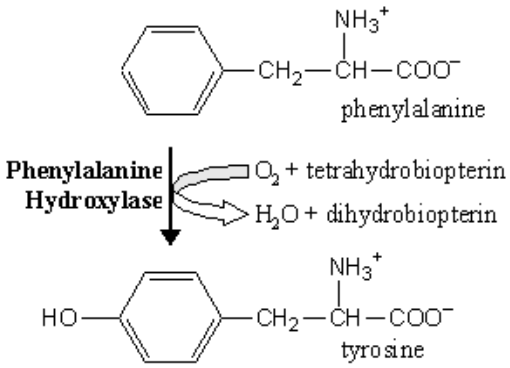
Interconversion of serine and glycine, catalyzed by serine hydroxymethyltransferase (H4 folate:tetrahydrofolate, cofactor)

- ✓ From glyoxylate and glutamate or alanine catalyzed by **glycine aminotransferase** (**irreversible**, unlike other transamination reactions)



- ✓ From choline

- **Tyrosine:** Phenylalanine is hydroxylated to form tyrosine a reaction catalyzed by phenylalanine hydroxylase.

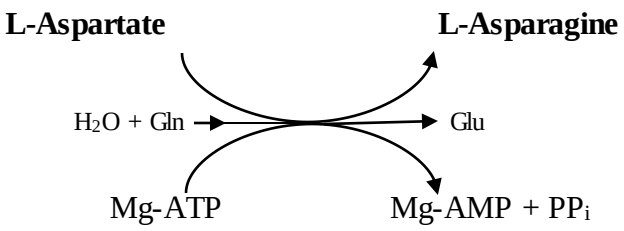


NOTE

- ✓ Provided that the diet contains adequate nutritionally essential phenylalanine, tyrosine is nutritionally nonessential.
- ✓ Since the reaction is irreversible, dietary tyrosine cannot replace phenylalanine.

- **Proline:** Glutamate is reduced and cyclized to form proline.

- **Asparagine:** Asparagine is synthesized from aspartate catalyzed by asparagine synthetase. Coupled hydrolysis of PP_i to P_i by pyrophosphatase ensures that the reaction is strongly favored. (Note similarities to and differences from the glutamine synthetase reaction)

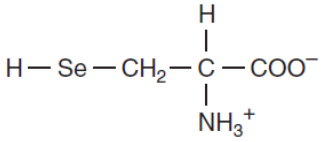


Hydroxyproline and Hydroxylysine

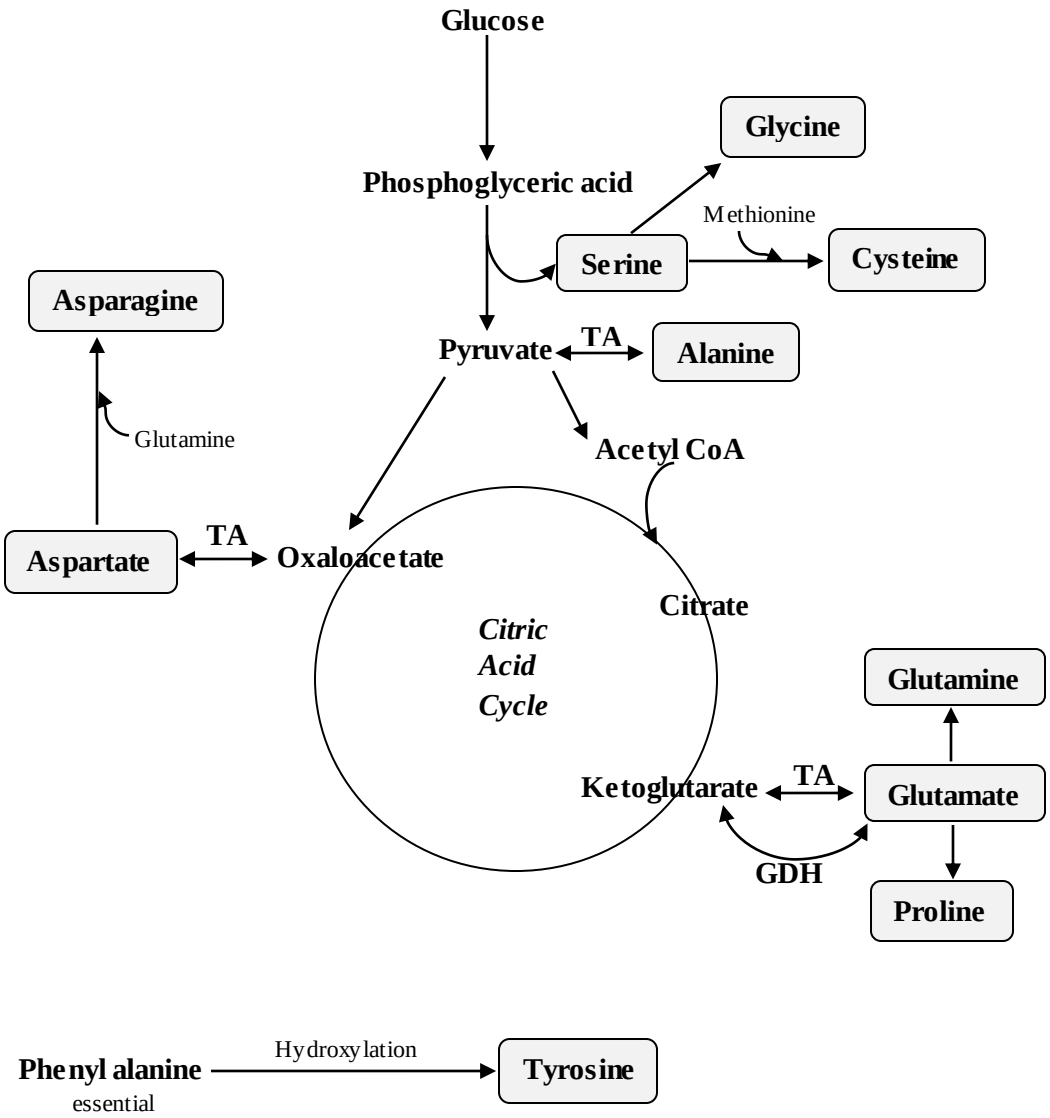
- ✓ They are present principally in **collagen**.
- ✓ Since there is no tRNA for either hydroxylated amino acid, neither dietary hydroxyproline nor hydroxylysine is incorporated into protein.
- ✓ They arise from proline and lysine, but only after these amino acids have been incorporated into peptides.
- ✓ Hydroxylation of prolyl and lysyl residues is catalyzed by **prolyl hydroxylase** and **lysyl hydroxylase** of tissues, including skin and skeletal muscle, and of granulating wounds.
- ✓ These hydroxylases require **ascorbate** as a **cofactor**, thus, a deficiency of the vitamin C results in scurvy (impaired hydroxylation of peptidyl proline and peptidyl lysine results in a failure to provide the substrates for cross-linking of maturing collagens).

Selenocysteine, the 21st amino acid

- ✓ While the occurrence of selenocysteine in proteins is uncommon, at least 25 human selenoproteins are known.
- ✓ Selenocysteine occurs at the active sites of several enzymes that catalyze redox reactions. Examples include the human enzymes thioredoxin reductase, glutathione peroxidase, and the deiodinase that converts thyroxine to triiodothyronine.
- ✓ The replacement of selenocysteine by cysteine in these enzymes can actually impair their catalytic activity.
- ✓ Impairments in human selenoproteins have been implicated in tumorigenesis and atherosclerosis, and are associated with selenium deficiency cardiomyopathy (Keshan disease).
- ✓ Unlike hydroxyproline or hydroxylysine, selenocysteine arises co-translationally during its incorporation into peptides.



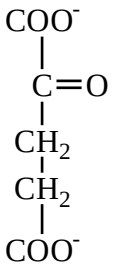
Selenocysteine



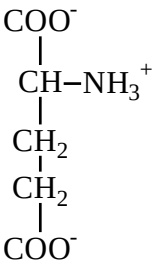
Biosynthesis of nonessential amino acids

TA: Transamination

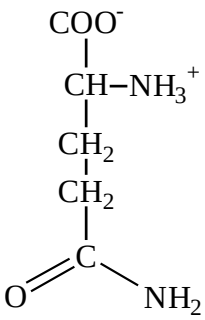
GDH: Glutamate dehydrogenase



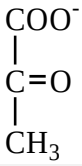
α-Ketoglutarate



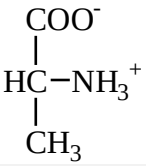
Glutamate



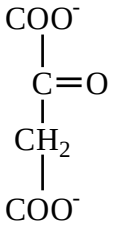
Glutamine



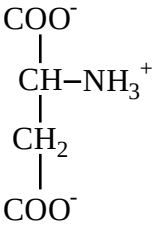
Pyruvate



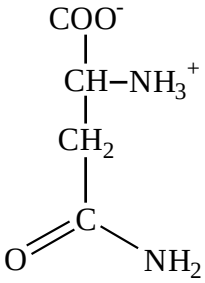
Alanine



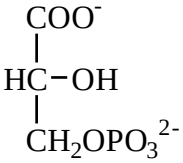
Oxaloacetate



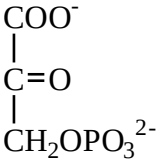
Aspartate



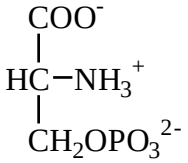
Asparagine



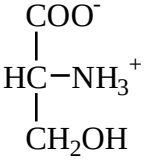
3-phosphoglycerate



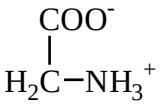
3-phosphohydroxypyruvate



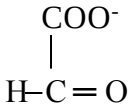
Phosphoserine



Serine



Glycine



Glyoxylate

How Enzymes Work

Energy changes occurring during the reaction

Virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product. Figure 1 shows the changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T*:

$$A \leftrightarrow T^* \longrightarrow B$$

Free energy of activation is the difference in free energy between the reactant and the high-energy intermediate formed during the conversion of reactant to product (T*). Because of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.

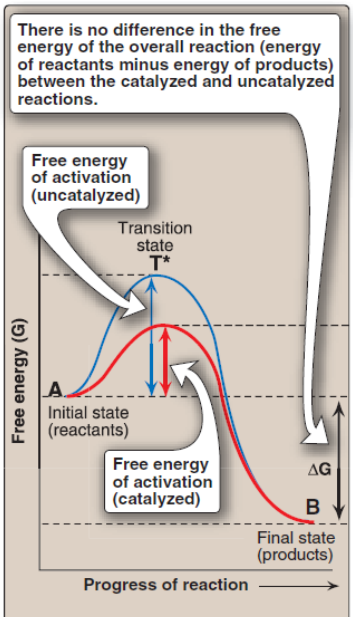


Figure 1: Effect of an enzyme on the activation energy of a reaction.

For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess that sufficient energy. ***The rate of reaction*** is determined by the number of such energized molecules. In general, *the lower the free energy of activation, the faster the rate of the reaction*.

An enzyme allows a reaction to proceed *rapidly* under conditions prevailing in the cell by providing an *alternate reaction pathway with a lower free energy of activation*. The enzyme *does not change the free energies of the reactants or products* and, ***therefore***, *does not change the equilibrium of the reaction*. It does, however, accelerate the rate with which equilibrium is reached.

Factors Affecting the Rates of Enzyme-Catalyzed Reactions

Temperature

Raising the temperature increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules. However, heat energy can disturb enzyme's (protein) three-dimensional structure leading to unfolding the polypeptide chain or ***denaturation***, with an accompanying loss of the catalytic activity (Figure 2). The temperature range over which an enzyme maintains a stable, catalytically competent conformation depends upon (and typically moderately exceeds) the normal temperature of the cells in which it resides. Enzymes from humans generally exhibit stability at temperatures up to 45 to 55°C. By contrast, enzymes from the thermophilic microorganisms that reside in volcanic hot springs or undersea hydrothermal vents may be stable at temperatures up to or even above 100°C.

The **temperature coefficient (Q_{10})** is the factor by which the rate of a biologic process increases for a 10°C increase in temperature. For the temperatures over which enzymes are stable, the rates of most biological processes typically double for a 10°C rise in temperature (Q_{10} = 2). For mammals and other homeothermic organisms (organisms that maintain a constant body temperature despite fluctuating environmental temperatures), changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.

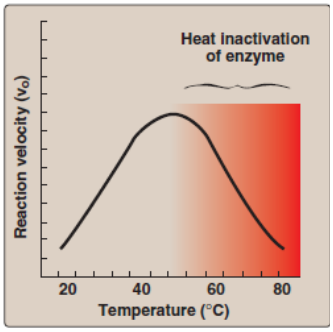


Figure 2: Effect of temperature on an enzyme-catalyzed reaction.

Hydrogen Ion Concentration

The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration. Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9. The relationship of activity to hydrogen ion concentration (**Figure 3**) reflects the balance between enzyme *denaturation* at high or low pH and *effects on the charged state* of the enzyme, the substrates, or both. For enzymes whose mechanism involves acid-base catalysis, the residues involved must be in the appropriate state of protonation for the reaction to proceed. The binding and recognition of substrate molecules with dissociable groups also typically involves the formation of salt bridges with the enzyme. The most common charged groups are carboxylate groups (negative) and protonated amines (positive). Gain or loss of critical charged groups adversely affects substrate binding and thus will retard or abolish catalysis.

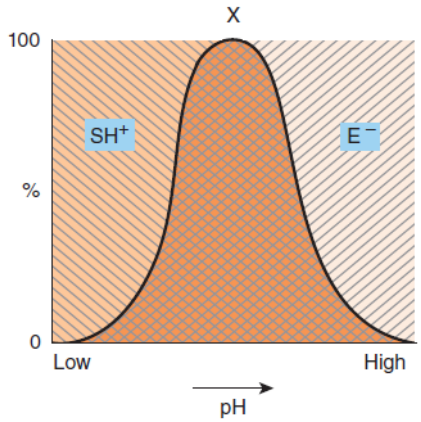


Figure 3: Effect of pH on enzyme activity.

A negatively charged enzyme (E−) that binds a positively charged substrate (SH^+). Shown is the proportion (%) of SH^+ [\\\] and of E− [////] as a function of pH. Only in the cross-hatched area do both the enzyme and the substrate bear an appropriate charge.

The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the [H^+] at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment (Figure 4).

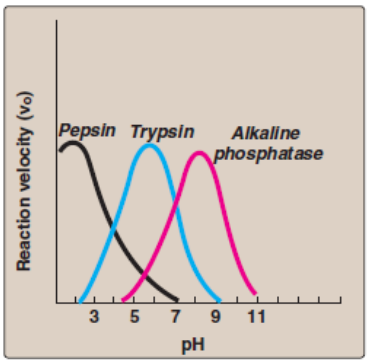


Figure 4: Effect of pH on enzyme-catalyzed reactions.

Substrate Concentration

For a typical enzyme, as substrate concentration is increased, v_i increases until it reaches a maximum value *V*max (**Figure 5**). When further increases in substrate concentration fail to increase v_i, the enzyme is said to be "saturated" with the substrate. Note that the shape of the curve that relates activity to substrate concentration (Figure 5) is *hyperbolic*.

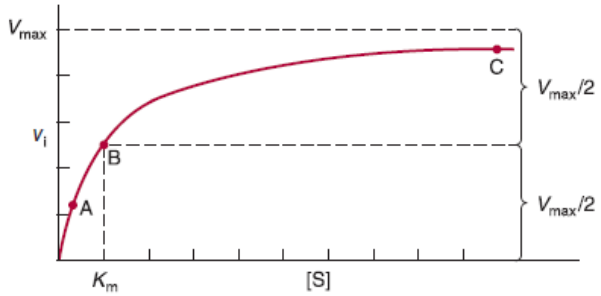


Figure 5: Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction.

At any given instant, only substrate molecules that are combined with the enzyme as an enzyme-substrate (ES) complex can be transformed into a product. The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present. Figure 6 shows that, at points A or B, increasing or decreasing [S] therefore will increase or decrease the number of ES complexes with a corresponding change in v_i. At point C, however, essentially all the enzyme is present as the ES complex. Since no free enzyme remains available for forming ES, further increases in [S] cannot increase the rate of the reaction. ***Under these saturating conditions, v_i depends solely on—and thus is limited by—the rapidity with which product dissociates from the enzyme so that it may combine with more substrate.***

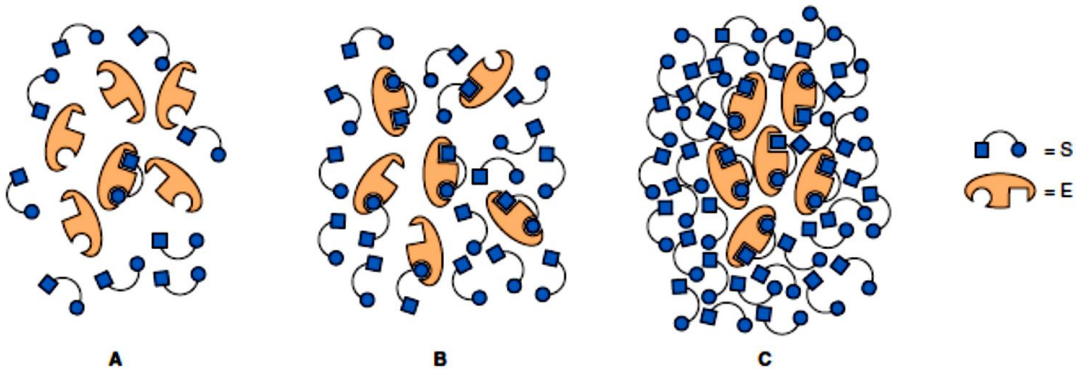


Figure 6: Representation of an enzyme in the presence of different substrate concentration.

THE MICHAELIS-MENTEN EQUATION MODEL THE EFFECTS OF SUBSTRATE CONCENTRATION

The Michaelis-Menten Equation

The Michaelis-Menten equation illustrates in mathematical terms the relationship between initial reaction velocity *v*i and substrate concentration [S], shown graphically in Figure 5:

$$v_i = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$$

The *Michaelis constant* ***K***m **is the substrate concentration at which v_i is half the maximal velocity (*V*max/2) attainable at a particular concentration of the enzyme.** The dependence of initial reaction velocity on [S] and *K*m may be illustrated by evaluating the Michaelis-Menten equation under three conditions.

1. When [S] is much less than *K*m (point A in Figures 5 and 6), the term *K*m + [S] is essentially equal to *K*m. Replacing *K*m + [S] with *K*m reduces Michaelis-Menten equation to:

$$v_i \approx \frac{V\text{max}\,[S]}{K\text{m}} \approx \frac{V\text{max}}{K\text{m}} * [S]$$

Since *V*max and *K*m are both constants, their ratio is a constant. In other words, when [S] is considerably below *K*m, *v*i is proportionate to *K*[S]. The initial reaction velocity therefore is directly proportional to [S].

2. When [S] is much greater than *K*m (point C in Figures 5 and 6), the term *K*m + [S] is essentially equal to [S].

$$v_i \approx \frac{V\text{max}\,[S]}{[S]} \approx V\text{max}$$

Thus, when [S] greatly exceeds *K*m, the reaction velocity is maximal (*V*max) and unaffected by further increases in the substrate concentration.

3. When [S] = *K*m (point B in Figures 5 and 6):

$$v_i = \frac{V\text{max}\,[S]}{K\text{m}+[S]} = \frac{V\text{max}\,[S]}{2[S]} = \frac{V\text{max}}{2}$$

Thus, when [S] equals *K*m, the initial velocity is half-maximal. Moreover, *K*m is (and may be determined experimentally from) the substrate concentration at which the initial velocity is half-maximal.

Important conclusions about Michaelis-Menten kinetics

1. Characteristics of *K*m: *K*m or the Michaelis constant is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate. *K*m does not vary with the concentration of enzyme.

a. Small *K*m: A numerically small (low) *K*m reflects a high affinity of the enzyme for substrate, because a low concentration of substrate is needed to half-saturate the enzyme, that is, to reach a velocity that is 1⁄2*V*max.

b. Large *K*m: A numerically large (high) *K*m reflects a low affinity of enzyme for substrate because a high concentration of substrate is needed to half-saturate the enzyme.

2. Relationship of velocity to enzyme concentration: The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v_i), as well as that of *V*max, are reduced to half that of the original.

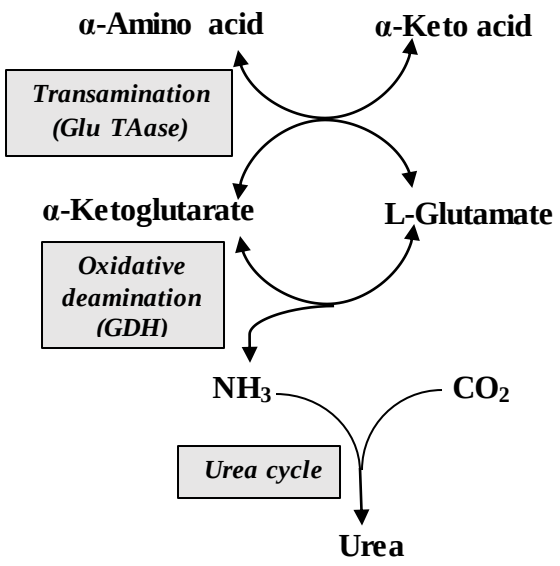
3. Order of reaction: When [S] is much less than *K*m, the velocity of the reaction is approximately proportional to the substrate concentration. The rate of reaction is then said to be first order with respect to substrate. When [S] is much greater than *K*m, the velocity is constant and equal to *V*max. The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate.

Amino Acid Catabolism

Catabolism of Amino Acid Nitrogen

In normal adults, nitrogen intake matches nitrogen excreted (**nitrogen equilibrium**). **Positive nitrogen balance**, means an excess of ingested over excreted nitrogen, and usually accompanies growth and pregnancy. **Negative nitrogen balance**, where output exceeds intake, may follow surgery, advanced cancer, and the nutritional disorders kwashiorkor and marasmus.

Depending on their environment and physiology, different animals excrete excess nitrogen as *ammonia*, as *uric acid*, or as *urea*. Teleostean fish are **ammonotelic** (excrete ammonia as ammonium ion). Birds are **uricotelic,** excrete nitrogen-rich uric acid as semisolid guano. Many land animals, including humans, are **ureotelic**, excrete nontoxic, highly water-soluble urea in urine.



Biosynthesis of urea:

Urea biosynthesis occurs in four stages:
(1) Transamination,
(2) Oxidative deamination of glutamate (major),
(3) Ammonia transport, and
(4) Reactions of the urea cycle

1. Transamination of Amino Acids:

Transamination reactions serve two purposes, they help maintain adequate levels of non-essential amino acids required for protein synthesis; and importantly, they also funnel amino groups from catabolized amino acids to glutamate for eventual excretion as urea.

- ✓ There are at least 17 different transaminases, all of them require **pyridoxal phosphate**.
- ✓ Alanine aminotransferase (ALT), catalyze the transfer of amino group to pyruvate (forming alanine).
- ✓ Aspartate aminotransferase (AST) catalyzes the transfer of amino group to oxaloacetic acid (forming aspartic acid).
- ✓ Glutamate aminotransferase, catalyze the transfer of amino groups to α-ketoglutarate (forming glutamate).
- ✓ Since most of amino acids (except those do not participate in transamination) are also substrate for glutamate aminotransferase, the α-amino nitrogen from nearly all amino acids that undergo transamination can be concentrated in glutamate.

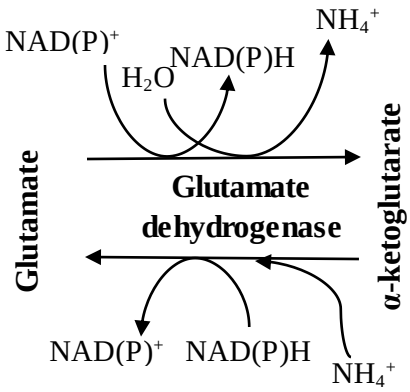
Levels of ALT and AST in serum are of diagnostic value. Aminotransferases are normally intracellular enzymes, with normal low levels in serum indicating release of cellular contents during normal cell turnover. Elevated levels in serum indicate damage to tissues rich in a particular enzyme.

ALT is present mainly in the cytoplasm of liver cells, while AST is present in both cytoplasm and mitochondria in liver, heart and skeletal muscles.

- ✓ In liver diseases, there is an increase in both serum ALT and AST levels. In acute liver diseases, e.g. acute viral hepatitis, the increase is more in ALT. In chronic liver diseases, e.g. liver cirrhosis the increase is more in AST.
- ✓ In heart diseases, e.g. myocardial infarction, there is an increase in AST only.
- ✓ In skeletal muscle diseases, e.g. myasthenia gravis, there is an increase in AST only.

2. Oxidative deamination of glutamate:

By transamination the α-amino nitrogen from nearly all amino acids can be concentrated in glutamate. This is important because **L-glutamate** is the **only** amino acid that undergoes oxidative deamination at an **appreciable** rate in mammalian tissues. The formation of ammonia from α-amino groups thus occurs mainly via the α-amino nitrogen of L-glutamate.



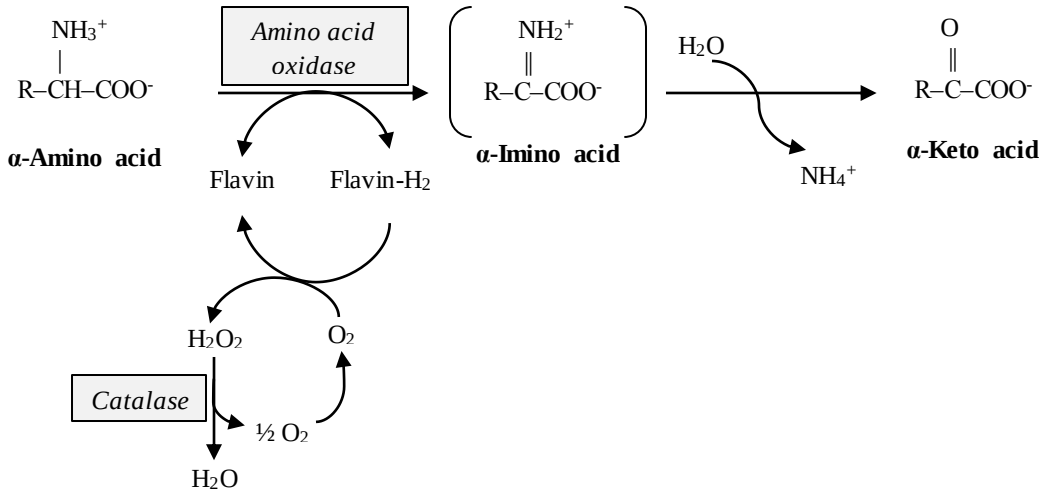
- ✓ In contrast to transaminase reactions, oxidative deamination yields an α-keto acid with release of the amino group as free ammonia.
- ✓ **Glutamate dehydrogenase** in liver, catalyzes oxidative deamination of glutamate.
- ✓ The released ammonia provides one of the two NH_3 groups for urea synthesis.

Liver GDH is allosterically regulated by the cell's energy state. During the formation of α-ketoglutarate GDP and ADP positively regulate GDH in mammals, and GTP, ATP and NADH inhibit the enzyme. Therefore, when the level of ATP is high, conversion of glutamate to α-ketoglutarate is limited; however when the cellular energy charge is low, glutamate is converted to ammonia and α-ketoglutarate

Conversion of α-amino nitrogen to ammonia by the **combined** action of aminotransferases and GDH is often termed "**transdeamination**."

Minor pathway of oxidative deamination of Amino Acids

Oxidative deamination of L-amino acid in the **liver** and **kidney** may be catalyzed by **L-amino acid oxidase** to form 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.**

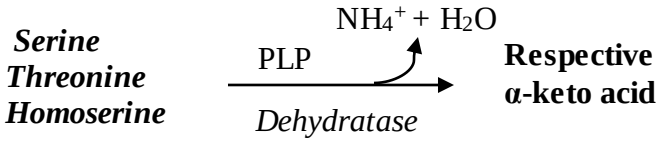


L-amino acid oxidase does not act on glycine and dicarboxylic acids. This enzyme, due to its very low activity, does not appear to play any significant role in the amino acid metabolism.

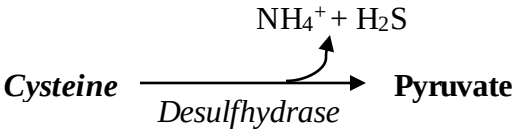
Non-oxidative Deamination of Amino Acids

Some of the amino acids can be deaminated to liberate NH_3 without undergoing oxidation, such as:

- Serine, threonine and homoserine are the hydroxy amino acids. They undergo non-oxidative deamination catalysed by PLP-dependent dehydratases. forming pyruvate and α-ketobutyrate respctively



- The sulfur amino acids, namely cysteine and homocysteine, undergo deamination coupled with desulfhydration to give keto acids.



- The enzyme histidase acts on histidine to liberate NH_4^+ by a non-oxidative deamination process.

3. Transport of ammonia to the liver (and kidney):

All tissues produce some ammonia from a variety of compounds. The level of ammonia in blood must be kept very low, because even slightly elevated concentrations (**hyperammonemia**) are toxic to the central nervous system.

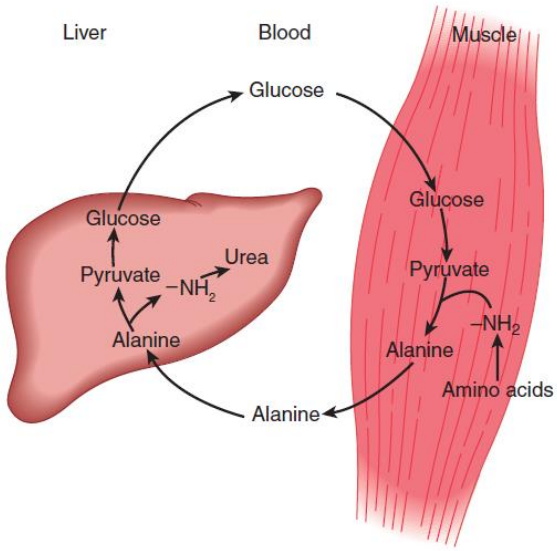
There are **two** major mechanisms to **transport** ammonia to liver for its conversion to urea and ultimate excretion in the urine.

Glutamine synthetase in many tissues assimilates free ammonia on glutamate to form ***glutamine***, an energy requiring process. Glutamine acts as a non-toxic transport and storage form of ammonia (carries 2 NH_3, actually). Glutamine is **freely diffusible** in tissues, hence easily transported. Glutamine travels in the blood to the liver, where **L-glutaminase** releases free ammonia which can enter the urea cycle. An analogous reaction is catalyzed by **L-asparaginase** that deaminates asparagine.

The kidneys can also form ammonia from glutamine by action of **renal glutaminase**. This ammonia is excreted into the urine as ammonium ion (NH_4^+), an important mechanism for maintaining whole-body acid-base balance.

Hepatic glutaminase levels rise in response to high protein intake while renal glutaminase increases in metabolic acidosis.

The second mechanism involves the *glucose-**alanine** cycle*. Amino acids derived from skeletal muscle protein breakdown, in the postabsorptive state, are converted to ***alanine***, which is transported to liver where it is deaminated to form pyruvate. Waste NH_3 groups enter the urea cycle, while pyruvate is used for gluconeogenesis.



The glucose-alanine cycle

*Alanine is synthesized in muscle by **transamination** of glucose-derived pyruvate, released into the bloodstream, and taken up by the liver. In the liver, the carbon skeleton of alanine is reconverted to glucose and released into the bloodstream, where it is available for uptake by muscle and resynthesis of alanine.*

4. Urea Cycle:

The urea cycle is **how mammals get rid of excess nitrogen** arising mostly from metabolism of amino acids. In the **liver**, ammonia, combine with carbon dioxide (actually a HCO_3^- ion) to form

urea. The urea is carried through the blood to the **kidney**, which isolates it for excretion in the **urine**. Urea accounts for about 90% of N-containing compounds in urine

The urea cycle is the first metabolic pathway to be elucidated, by Hans Krebs and Kurt Henseleit. Hence, the cycle is known as **Krebs-Henseleit** cycle. As ornithine is the first member of the reaction, it is also called as **Ornithine cycle**.

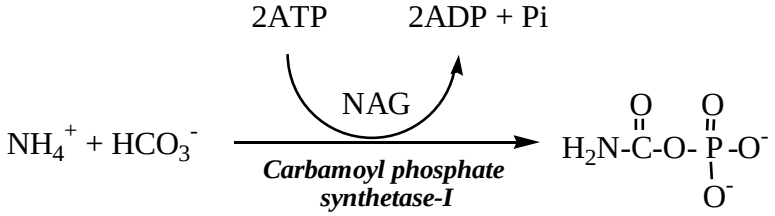
The two nitrogen atoms of urea are derived from two different sources, one from ammonia and the other directly from the alpha amino group of aspartic acid. Carbon atom is supplied by CO_2.

Urea cycle occurs in five steps:

Step 1: Formation of Carbamoyl Phosphate

One molecule of ammonia condenses with CO_2 (HCO_3^-) in the presence of **two molecules of ATP** to form carbamoyl phosphate. The reaction is catalyzed by the mitochondrial enzyme **carbamoyl phosphate synthetase-I (CPS-I).** CPS I requires **N-acetylglutamate (NAG)** for its activity; which acts as an allosteric activator that enhances the affinity of the CPS-I for ATP. CPS-I reaction is the **rate-limiting step** in urea formation.

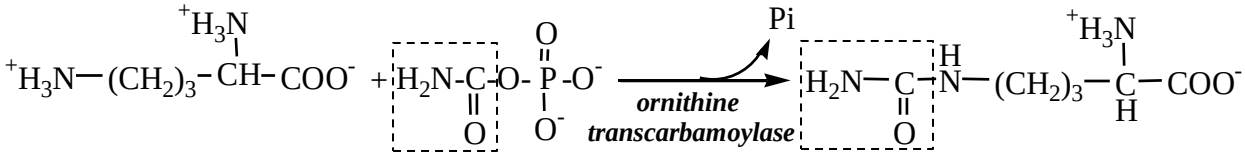
A cytosolic form of carbamoyl phosphate synthetase, **carbamoyl phosphate synthetase II (CPS-II)**, uses glutamine rather than ammonia as the nitrogen donor and functions in pyrimidine biosynthesis.



Carbamoyl Phosphate

Step 2: Formation of Citrulline

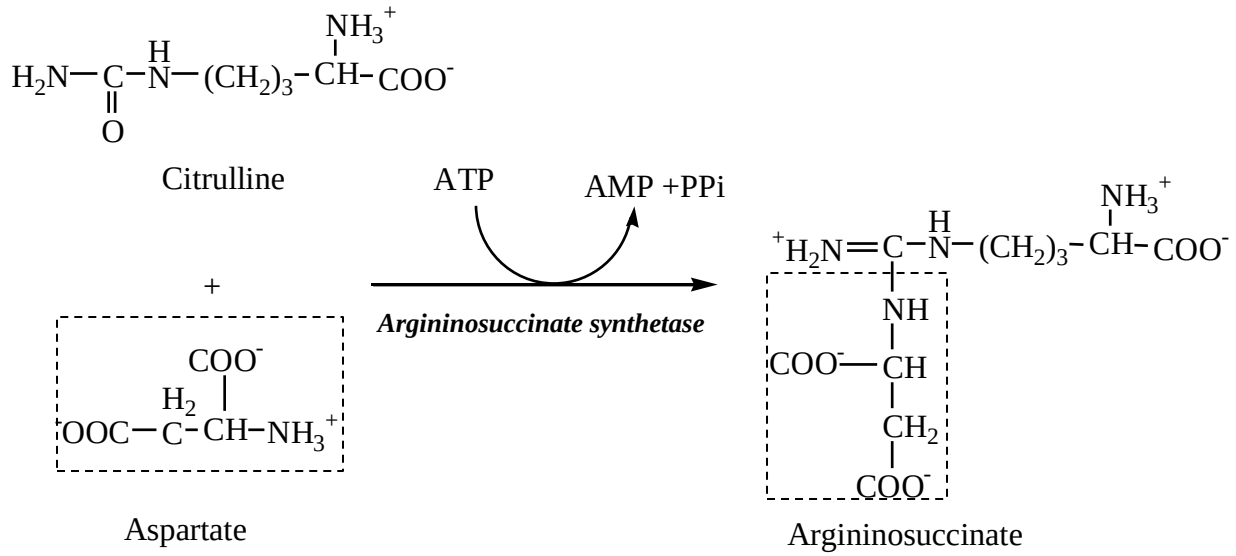
The second reaction is also **mitochondrial.** The carbamoyl group is transferred to the NH_2 group of ornithine by **ornithine transcarbamoylase** (also called *citrulline synthase*) forming citrulline, which leaves the mitochondria and further reactions of the cycle are taking place in cytoplasm. Entry of ornithine into mitochondria and exodus of citrulline from mitochondria therefore involve mitochondrial inner membrane **permeases.**



Ornithine Carbamoyl Phosphate Citrulline

Step 3: Formation of Argininosuccinate

Argininosuccinate synthetase links aspartate and citrulline via the amino group of aspartate, and thus aspartate provides the second nitrogen of urea. The reaction requires hydrolysis of ATP to AMP level, so **two high energy phosphate bonds** are utilized.



Step 4: Formation of Arginine

Argininosuccinate is cleaved by **argininosuccinate lyase** (argininosuccinase) to arginine and fumarate. Subsequent addition of water to fumarate forms malate, whose subsequent oxidation forms oxaloacetate. 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.

Thus, the **urea cycle is linked to citric acid cycle through fumarate,** and so, it is called as "**urea bicycle**"

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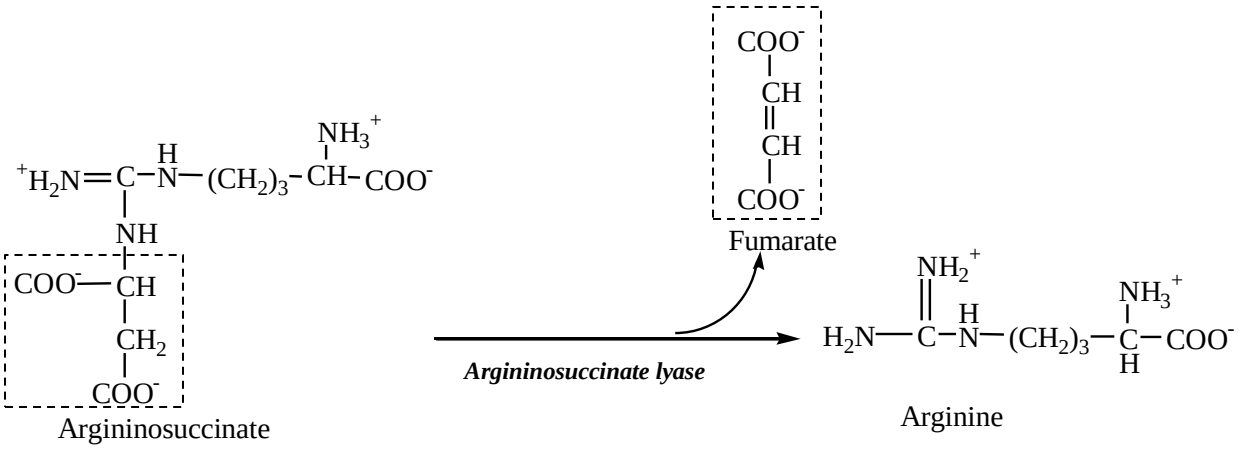
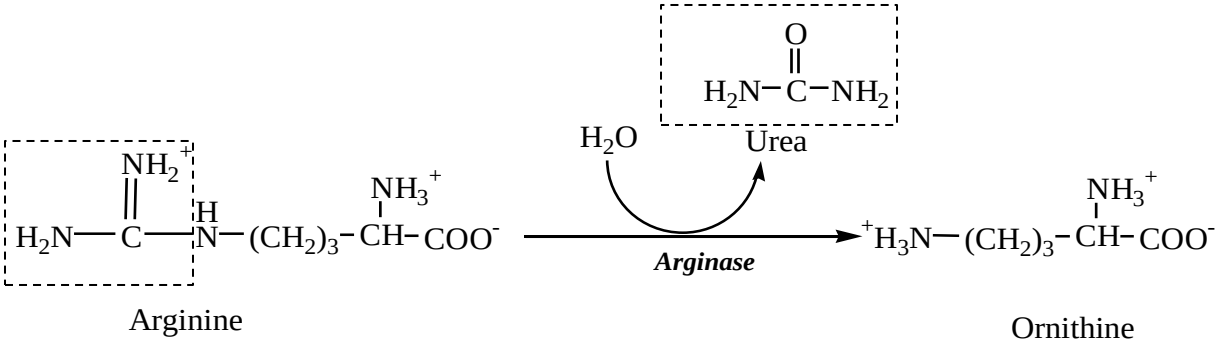


Step 5: Formation of Urea

The final reaction of the cycle is the hydrolysis of arginine to urea and ornithine by **arginase**. The ornithine returns to the mitochondria to react with another molecule of carbamoyl phosphate

so that the cycle will proceed. Ornithine and lysine are potent inhibitors of arginase, and compete with arginine.



Arginase is mostly found in the liver, while the rest of the enzymes (four) of urea cycle are also present in other tissues. For this reason, arginine synthesis may occur to varying degrees in many tissues. But only the liver can ultimately produce urea.

Overall Reaction and Energetics of Urea Cycle

Synthesis of 1 mol of urea requires 3 mol of ATP (utilized as 4 high energy phosphates), 1 mol each of ammonium ion and of aspartate, and employs five enzymes. Urea synthesis is a cyclic process. While ammonium ion, CO_2, ATP, and aspartate are consumed, the ornithine consumed in reaction 2 is regenerated in reaction 5. There thus is no net loss or gain of ornithine, citrulline, argininosuccinate, or arginine.

NH_4^+ + CO_2 + Aspartate + 3ATP ⟶ Urea + Fumarate + 2 ADP + 2 Pi + AMP + PPi

Regulation of Urea Cycle

- ✓ The first reaction catalysed by **carbamoyl phosphate synthetase I** (CPS I) is **rate-limiting** reaction in urea synthesis.
- ✓ CPS I is allosterically activated by N-acetylglutamate (NAG).
- ✓ NAG is synthesized from glutamate and acetyl CoA by NAG synthase and degraded by a NAG hydrolase.
- ✓ High concentrations of **arginine** (an allosteric activator of NAG Synthase), and the consumption of a **protein-rich meal** increases the level of NAG in liver, leading to enhanced urea synthesis.
- ✓ During **starvation** the expression of all the enzymes of the urea cycle in liver increases several fold, secondary to enhanced protein degradation to provide energy.
- ✓ The last four enzymes of urea cycle are mostly controlled by the concentration of their respective substrates.

Disposal of Urea

Urea produced in the liver is transported in blood to **kidneys**, and excreted in urine.

- ✓ A small amount of urea synthesized in the liver enters, via circulation, the lumen of the intestine (primarily the small intestine), where urea is hydrolyzed by microbial urease into ammonia and CO_2. This ammonia is either lost in the feces or absorbed into the blood.
- ✓ In renal failure, the blood urea level is elevated (uremia), resulting in diffusion of more urea into intestine and its breakdown to NH_3.
- ✓ Hyperammonemia (increased blood NH_3) is commonly seen in patients of kidney failure. For these patients, oral administration of antibiotics (neomycin) to kill intestinal bacteria is advised.

Metabolic Disorders of Urea Cycle

Defects in each enzyme of the urea cycle function or synthesis can lead to metabolic disorders.

- ✓ Urea cycle disorders are characterized by *hyperammonemia*, *encephalopathy*, and *respiratory alkalosis*. Ammonia intoxication is most severe when the metabolic block occurs at reactions 1 or 2, for if citrulline can be synthesized, some ammonia has already been removed by being covalently linked to an organic metabolite.
- ✓ Clinical symptoms common to all urea cycle disorders include vomiting, avoidance of high-protein foods, intermittent ataxia, irritability, lethargy, and severe mental retardation.
- ✓ The clinical features and treatment of all five disorders are similar. Significant improvement and minimization of brain damage can accompany a low-protein diet ingested as frequent small meals to avoid sudden increases in blood ammonia levels. The goal of dietary therapy is to provide sufficient protein, arginine, and energy to promote growth and development while simultaneously minimizing the metabolic perturbations.

Urea cycle disorders with the corresponding defective enzyme or transporter

Disorder	Defective Enzyme or Transporter
Hyper ammonemia Type I	Carbamoyl Phosphate Synthetase I
Hyper ammonemia Type II	Ornithine Transcarbamoylase
Citrullinemia Type I (Classic Citrullinemia)	Argininosuccinate synthetase
Argininosuccinic aciduria	Argininosuccinate lyase
Hyperargininemia	Arginase
Citrullinemia Type II	Citrulline permease
Hyperammonemia Hyperornithinemia Homocitrullinuria (HHH) Syndrome	Ornithine permease

NOTE:

N-Acetyl Glutamate Synthase Deficiency: The sixth enzyme deficiency which lead to a urea cycle disorder. The condition is almost similar to Hyperammonemia Type I. But, arginine, an allosteric activator of NAG Synthase improves CPS-I defect as NAG activates CPS-I; while arginine does not improve NAG deficiency, as the enzyme itself is defective.

Newborn Screening for Metabolic Diseases

- ✓ Congenital metabolic diseases caused by the absence or functional impairment of metabolic enzymes have serious consequences.
- ✓ Early dietary intervention can in many instances ameliorate the otherwise expected dire effects. The early detection of such metabolic diseases is thus is of primary importance.
- ✓ Newborn screening using the powerful and sensitive technique of **tandem mass spectrometry** (which can in a few minutes detect over 40 analytes), is of great importance in the early detection of metabolic disorders.

Ammonia

- ✓ Ammonia is toxic and its accumulation in the body is fatal. Only traces (10-20 µg/dl) normally are present in peripheral blood.
- ✓ It is removed by the liver that converts it to urea, which is nontoxic, water soluble and easily excreted in the urine.
- ✓ Since urea is nontoxic to humans, high blood levels in renal disease are a consequence, not a cause, of impaired renal function.

Sources of ammonia

- ✓ **Deamination and transamination** of amino acids.
- ✓ Ammonia produced by the action of intestinal bacteria on the **non-absorbed dietary amino acids** or from **urea** reaching the intestine via circulation which is hydrolyzed to NH_3 and CO_2.
- ✓ Ammonia is released from **monoamines** (e.g. epinephrine, norepinephrine and dopamine) by the action of monoamine oxidase enzyme.
- ✓ Ammonia is released during **purine and pyrimidine catabolism**.

Fate of ammonia

- ✓ Biosynthesis of urea is the main fate of ammonia
- ✓ Biosynthesis of nonessential amino acids
- ✓ Small amounts of ammonia are excreted in urine

Causes of hyperammonemia

1-Acquired hyperammonemia

- ✓ Liver cell failure
- ✓ Shunts between portal and systemic circulation

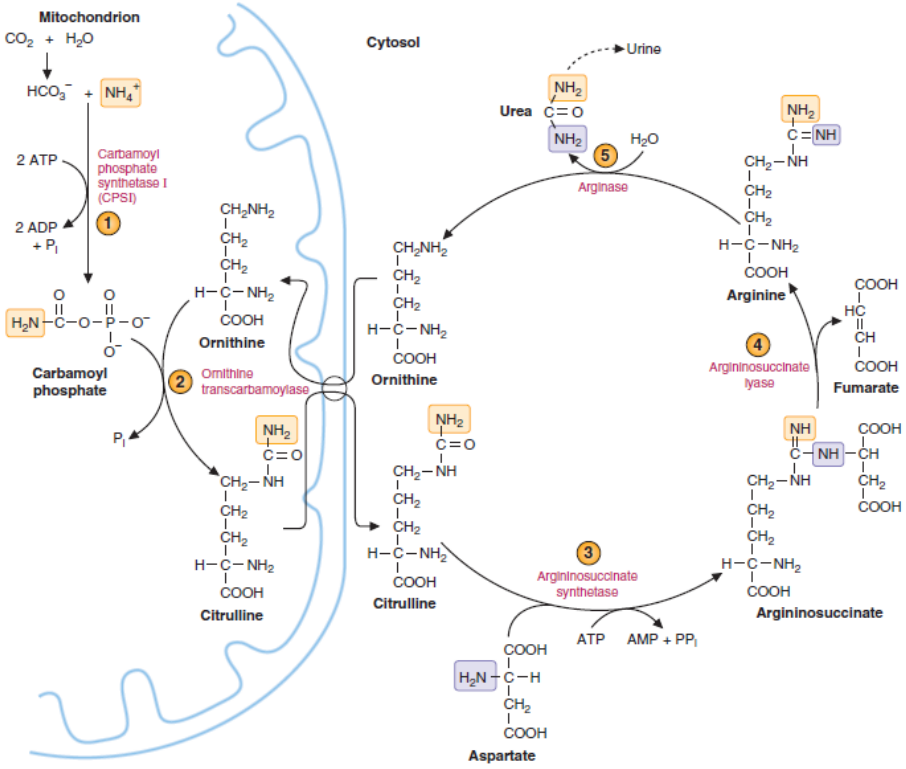
2-Congenital hyperammonemia

- ✓ Defect in any one of the enzymes that catalyze urea cycle steps, or in **ornithine** and **citrulline permeases** or **NAG-synthase**

Why is ammonia toxic?

$$\alpha\text{-ketoglutarate} + NH_3 + NADPH \xleftrightarrow{\textit{GDH}} \text{glutamate} + NADP^+$$

Accumulation of NH_3 shifts the equilibrium to the right with more glutamate formation, hence more utilization of α-ketoglutarate. α-Ketoglutarate is a key intermediate in citric acid cycle and its depleted levels impair the citric acid cycle. The net result is that production of energy (ATP) by the brain is reduced, leading to neurological manifestations (flapping tremors, slurred speech, blurred vision, vomiting, coma, and death may occur if hyperammonemia not corrected).



Urea Cycle Reactions

Enzymes Kinetics (Cont'd.)

A Linear Form of the Michaelis-Menten Equation Is Used to Determine *K*m & *V*max

The direct measurement of the numeric value of *V*max, and therefore the calculation of *K*m, often requires impractically high concentrations of substrate to achieve saturating conditions. A linear form of the Michaelis-Menten equation circumvents this difficulty and permits *V*max and *K*m to be extrapolated from initial velocity data obtained at less than saturating concentrations of the substrate.

$$v_i = \frac{V\text{max}\,[S]}{K\text{m}+[S]}$$

invert:

$$\frac{1}{vi} = \frac{K\text{m}+[S]}{V\text{max}\,[S]}$$

$$= \frac{K\text{m}}{V\text{max}\,[S]} + \frac{[S]}{V\text{max}\,[S]}$$

$$\frac{1}{vi} = \frac{K\text{m}}{V\text{max}} * \frac{1}{[S]} + \frac{1}{V\text{max}}$$

This equation is an equation for a straight line, $y = ax + b$, where $y = 1/v_i$ and $x = 1/[S]$. A plot of 1/*v*i as *y* as a function of 1/[S] as *x* therefore gives a straight line whose *y* intercept is 1/*V*max and whose slope is *K*m/*V*max. Such a plot is called a **double reciprocal** or **Lineweaver-Burk plot** (Figure 1). Setting the *y* term of the last equation equal to zero and solving for *x* reveals that the *x* intercept is −1/*K*m.

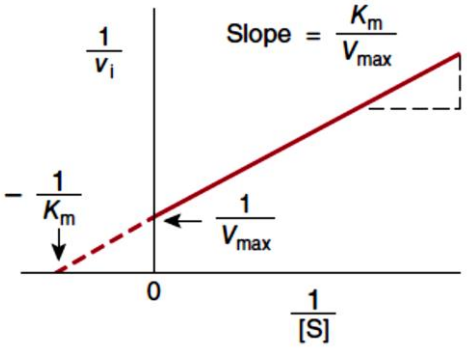


Figure 1: Double-reciprocal or Lineweaver-Burk plot of 1/vi versus 1/[S] used to evaluate Km and Vmax.

The Catalytic Constant, *k*cat

Several parameters may be used to compare the relative activity of different enzymes or of different preparations of the same enzyme. The activity of impure enzyme preparations typically is expressed as a *specific activity* (*V*max divided by the protein concentration). For a homogeneous enzyme, one may calculate its *turnover number* (*V*max divided by the moles of enzyme present). But if the number of active sites present is known, the catalytic activity of a homogeneous enzyme is best expressed as its *catalytic constant, k*cat (*V*max divided by the number of active sites, St):

$$k_{\text{cat}} = \frac{V\text{max}}{S\text{t}}$$

The units of *k*cat are reciprocal time (sec^{-1}).

Catalytic Efficiency, *k*cat/*K*m

While the maximum capacity of a given enzyme to convert substrate to product is important, the benefits of a high *k*cat can only be realized if *K*m is sufficiently low. Thus, *catalytic efficiency* of enzymes is best expressed in terms of the *ratio* of these two kinetic constants, *k*cat/*K*m.

For certain enzymes, once substrate binds to the active site, it is converted to product and released so rapidly as to render these events effectively instantaneous. For these exceptionally efficient catalysts, the rate-limiting step in catalysis is the formation of the ES complex. Such enzymes are said to be *diffusion-limited*, or catalytically perfect, since the fastest possible rate of catalysis is determined by the rate at which molecules move or diffuse through the solution.

The Hill Equation Describes the Behavior of Enzymes That Exhibit Cooperative Binding of Substrate

While most enzymes display the simple **saturation kinetics** and are adequately described by the Michaelis-Menten expression, some enzymes bind their substrates in a **cooperative** fashion. Cooperative behavior is an *exclusive* property of multimeric enzymes that bind substrate at multiple sites.

For enzymes that display positive cooperativity in binding the substrate, the shape of the curve that relates changes in v_i to changes in [S] is sigmoidal (**Figure 2**).

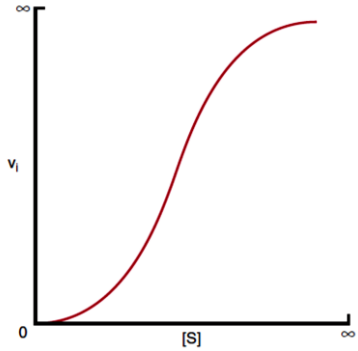


Figure 2: Representation of sigmoid substrate saturation kinetics.

Neither the Michaelis-Menten expression nor its derived plots can be used to evaluate cooperative kinetics.

Hill equation which is originally derived to describe the cooperative binding of O_2 by hemoglobin can be used for this purpose.

$$\frac{\log v_i}{V_{max} - v_i} = n \log[S] - \log k'$$

where k' is a complex constant.
Hill equation states that when [S] is low relative to k', the initial reaction velocity increases as the n^{th} power of [S]. A graph of log $v_i/(V\text{max} - v_i)$ versus log[S] gives a straight line (**Figure 3**).

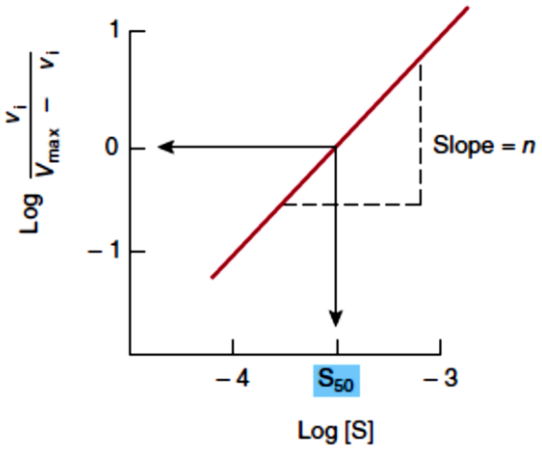


Figure 3: A graphical representation of a linear form of the Hill equation is used to evaluate S_{50}, the substrate concentration that produces half-maximal velocity, and the degree of cooperativity n.

The slope of the line, ***n,*** is the **Hill coefficient,** an empirical parameter whose value is a function of the number, kind, and strength of the interactions of the multiple substrate binding sites on the enzyme. When $n = 1$, all binding sites behave independently and simple Michaelis-Menten kinetic behavior is observed. If n is greater than 1, the enzyme is said to exhibit **positive cooperativity**. Binding of substrate to one site then enhances the affinity of the remaining sites to bind additional substrate. The greater the value for n, the higher the degree of cooperativity and the more markedly sigmoidal will be the plot of v_i versus [S]. A perpendicular dropped from the point where the y term log $v_i/(V\text{max} - v_i)$ is zero intersects the x-axis at a substrate concentration termed **S_{50},** the substrate concentration that results in half-maximal velocity.

Amino Acid Catabolism

Catabolism of the carbon skeleton

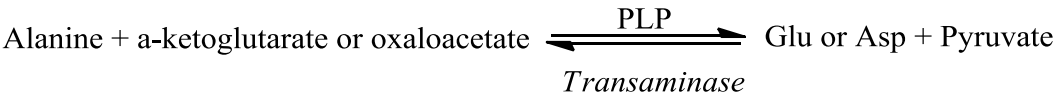
Transamination typically **initiates** amino acids catabolism. Removal of α-amino nitrogen by transamination, catalyzed by an **aminotransferase**, is the first catabolic reaction of most of the protein amino acids. The exceptions are proline, hydroxyproline, threonine, and lysine, whose α-amino groups do not participate in transamination. Then, **oxidative deamination** (major) and non-oxidative deamination remove the nitrogen atom; and the resultant hydrocarbon skeletons are then degraded to metabolic intermediates.

The carbon atoms of fat, carbohydrate, and protein are interconvertible. All or a portion of the carbon skeleton of every amino acid is convertible either to carbohydrate, fat, or both fat and carbohydrate.

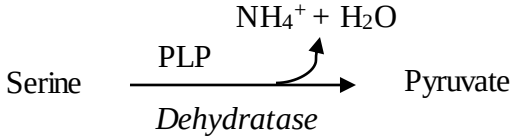
The amino acids may be grouped for discussion on the basis of the specific keto acid products of their deamination.

- The **3-carbon** α-keto acid **pyruvate** is produced from **alanine, cysteine, glycine, serine,** and **threonine**.

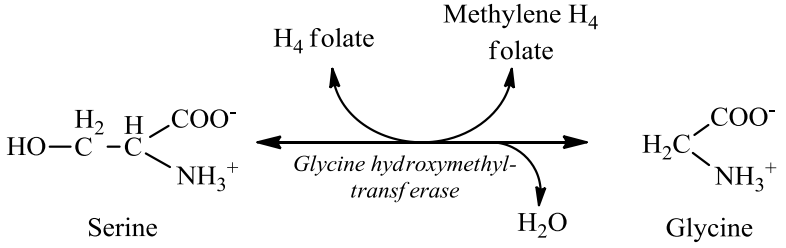
✓ **Alanine** deamination via **transaminase** directly yields pyruvate.



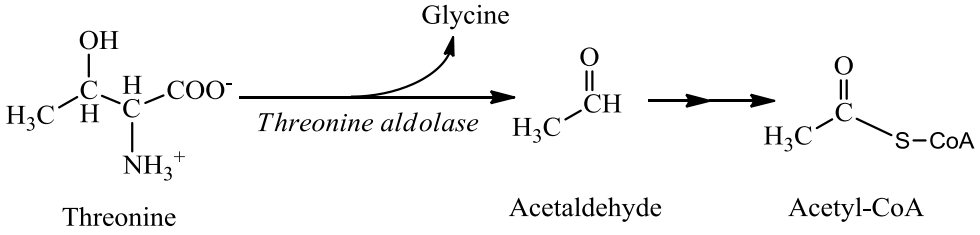
✓ **Serine** is deaminated to form pyruvate via **serine dehydratase**.



Also, serine following conversion to glycine, is catalyzed by **glycine hydroxymethyltransferase**, then catabolism merges with that of glycine.



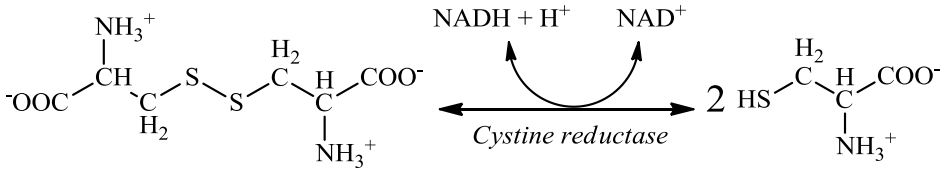
✓ **Threonine aldolase** cleaves threonine to glycine and acetaldehyde. Oxidation of acetaldehyde to acetate is followed by formation of **acetyl-CoA**.



- ✓ **Glycine**, which is also a product of **threonine** catabolism, The **glycine cleavage complex** of liver mitochondria splits glycine to CO_2 and NH_4^+ and forms *N*5, *N*10-methylene tetrahydrofolate.

$$\text{Glycine} + H_4\text{folate} + NAD^+ \longrightarrow CO_2 + NH_4^+ + 5,10\text{-}CH_2\text{-}H_4\text{folate} + NADH + H^+$$

- ✓ **Cystine** is first reduced to cysteine by **cystine reductase**. Two different reactions (deamination coupled with desulfhydration) then convert cysteine to pyruvate

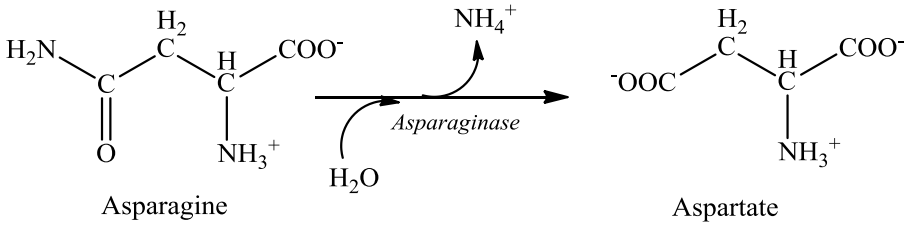


- The **4-carbon** Krebs Cycle intermediate **oxaloacetate** is produced from **aspartate** and **asparagine**.
- ✓ **Aspartate** deamination via transaminase directly yields **oxaloacetate**.

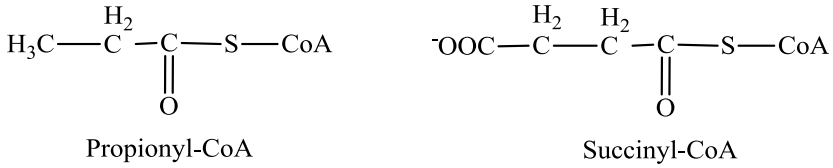
$$\text{Aspartate} + \text{a-ketoglutarate} \underset{\textit{Transaminase}}{\overset{PLP}{\rightleftharpoons}} \text{Glutamate} + \text{oxaloacetate}$$

Aspartate also is converted to **fumarate** in the Urea Cycle. Fumarate is then converted in Krebs Cycle to oxaloacetate.

- ✓ **Asparagine** loses the amino group from its R-group by hydrolysis catalyzed by **Asparaginase**. This yields aspartate, which can then be converted to oxaloacetate by transamination

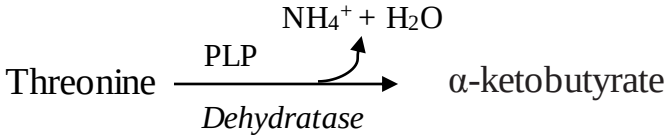


- The **4-carbon** Krebs Cycle intermediate **succinyl-CoA** is produced from **isoleucine, valine, methionine,** and **threonine**. **Propionyl-CoA**, is the intermediate in these pathways.



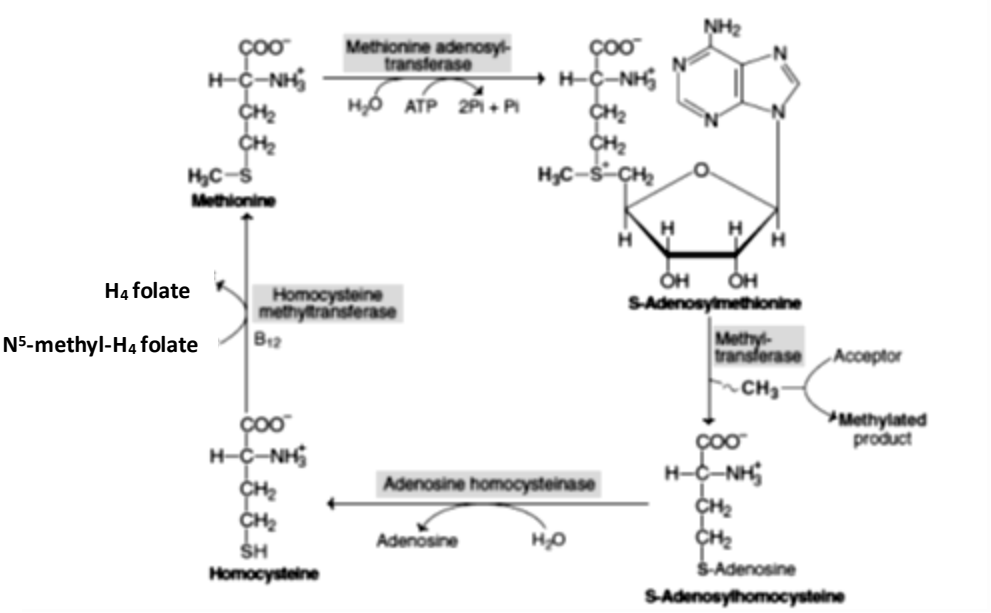
- ✓ The **branched chain amino acids**, isoleucine and valine, initially share in part a common pathway, catalyzed by a multi-subunit complex, **branched chain α-keto acid dehydrogenase**.

- ✓ **Threonine** undergoes deamination by **threonine dehydratase** to α-ketobutyrate which is decarboxylated to propionyl-CoA.



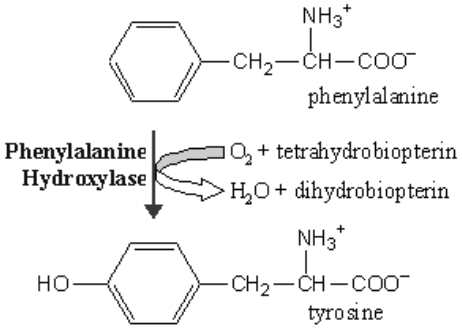
- ✓ **Methionine** is converted to ***S*-Adenosylmethionine (SAM)** by an ATP-dependent reaction. **SAM** serves as a methyl group donor in various synthetic reactions. The resulting adenosylhomocysteine is hydrolyzed to **homocysteine**, which may be catabolized via a complex pathway to **cysteine** and **homoserine** (please refer to the biosynthetic reaction of cysteine in lecture 2); homoserine in turn is deaminated by dehydratase to α-ketobutyrate (lecture 3) which is decarboxylated to propionyl-CoA, and thus, **succinyl-CoA**.

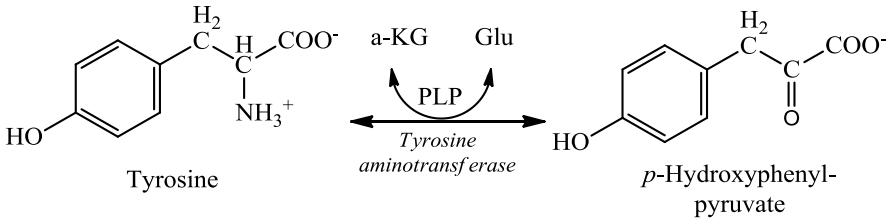
Or **methionine** may be regenerated from homocysteine by methyl transfer from ***N*^{5}-methyl-tetrahydrofolate (H_4 folate)**, via a methyltransferase enzyme that utilizes **B_{12}** as prosthetic group. The methyl group is transferred from tetrahydrofolate to B_{12} to homocysteine.
Another pathway converts homocysteine to glutathione.



- The **4-carbon** Krebs Cycle intermediate **fumarate** is produced from the aromatic amino acids **phenylalanine** and **tyrosine** are catabolized to **fumarate** and **acetoacetate**.

  Following transamination of tyrosine to *p*-hydroxyphenylpyruvate, successive reactions form maleylacetoacetate, fumarylacetoacetate, fumarate, acetoacetate, and ultimately acetyl-CoA and acetate.



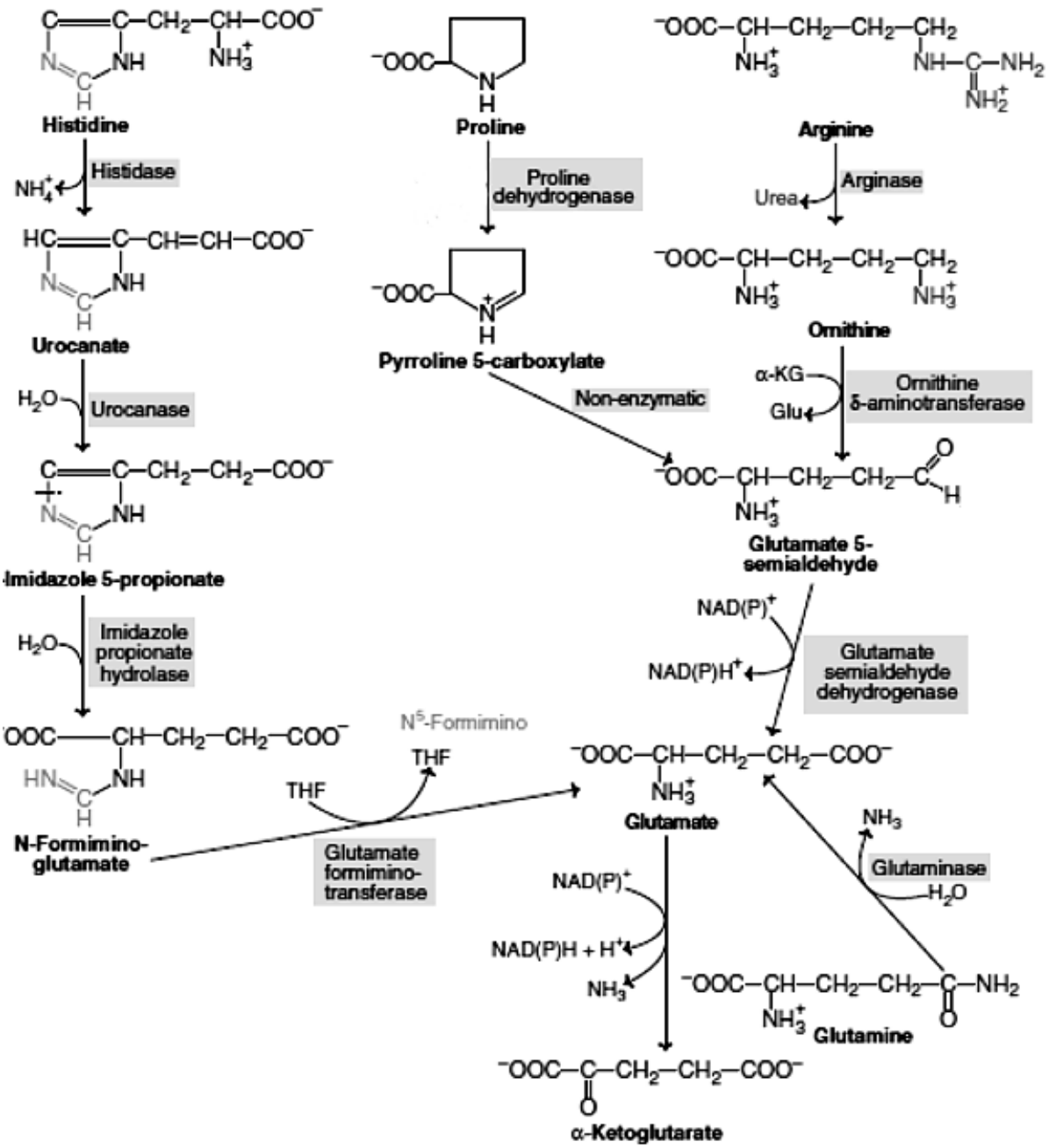


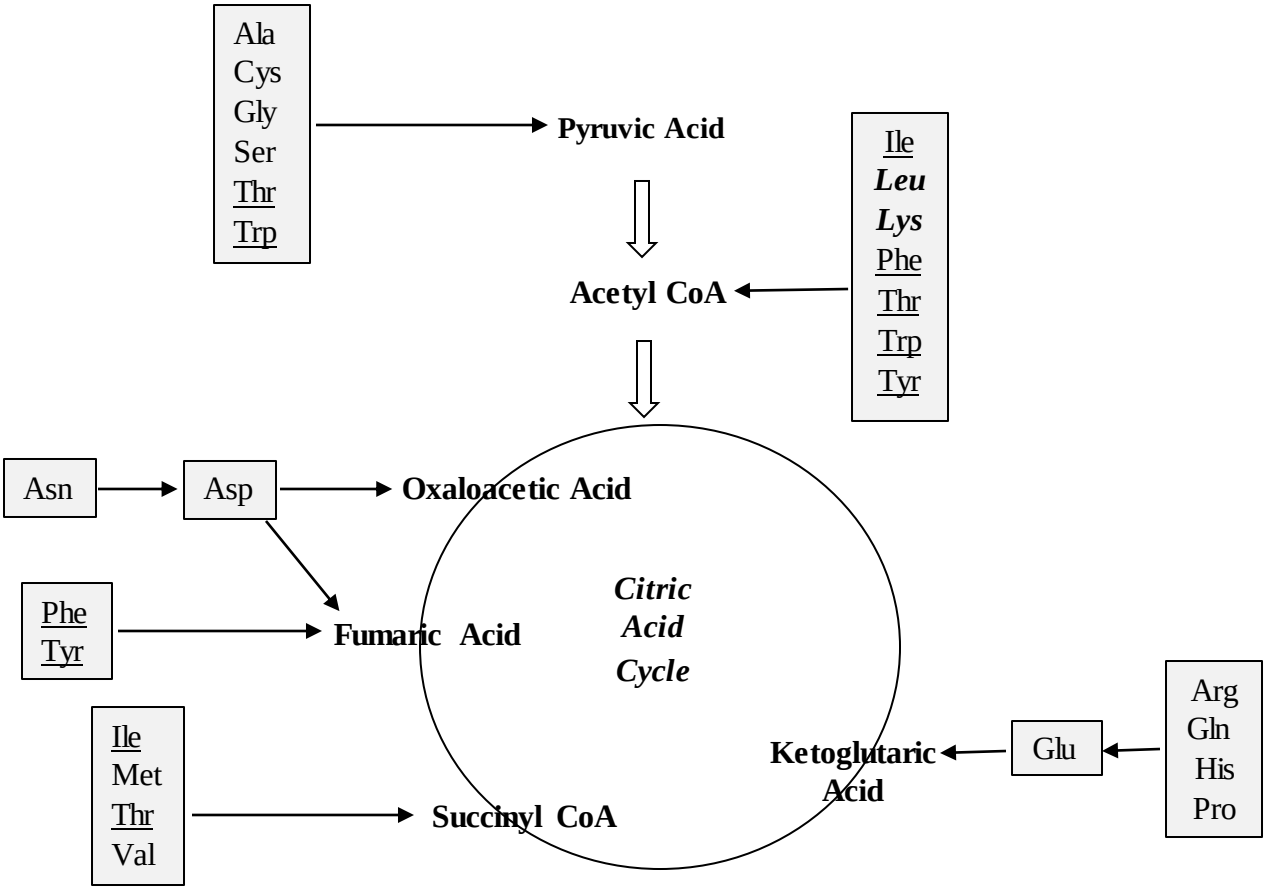
- The **5-carbon** Krebs Cycle intermediate **α-ketoglutarate** is produced from **arginine, glutamate, glutamine, histidine,** and **proline**.

✓ **Glutamate** deamination, via **Glutamate Dehydrogenase** or **Transaminase**, directly yields **α-ketoglutarate**.

Note: *Glutamine, Histidine, Proline, and Arginine are converted to glutamate first and then to α-ketoglutarate.*

✓ **Glutamine** is deaminated by **glutaminase** to glutamate.
✓ The last step in the pathway of **histidine** conversion to glutamate involves the transfer of single-carbon unit to a cofactor **tetrahydrofolate.**
✓ Since proline does not participate in transamination, its α-amino nitrogen is retained throughout a two-stage oxidation to glutamate. Oxidation to Δ^1-pyrroline-5-carboxylate is catalyzed by **proline dehydrogenase**. Subsequent oxidation to glutamate is catalyzed by **Δ^1-pyrroline-5-carboxylate dehydrogenase** (also called glutamate-δ-semialdehyde dehydrogenase).
✓ **Arginine** is cleaved by arginase to liberate urea and produce ornithine. Ornithine undergoes transamination of δ-amino group to form glutamate δ-semialdehyde which is converted to glutamate.


Histidine
Histidase
Urocanate
Urocanase
Imidazole 5-propionate
Imidazole propionate hydrolase
N-Formimino-glutamate
Glutamate formimino-transferase
THF
N5-Formimino
Proline
Proline dehydrogenase
Pyrroline 5-carboxylate
Non-enzymatic
Arginine
Arginase
Urea
Ornithine
α-KG
Glu
Ornithine δ-aminotransferase
Glutamate 5-semialdehyde
NAD(P)+
NAD(P)H+
Glutamate semialdehyde dehydrogenase
Glutamate
NAD(P)+
NAD(P)H + H+
NH3
Glutaminase
H2O
Glutamine
α-Ketoglutarate



Overview of the metabolic intermediates that result from amino acid catabolism

Where:

The amino acids ***bold italic*** are ketogenic

Underlined amino acids are both glucogenic and ketogenic

The remaining amino acids are glucogenic only

ENZYME INHIBITION

Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an **inhibitor**. Inhibitors of the catalytic activities of enzymes provide both pharmacologic agents and research tools for the study of the mechanism of enzyme action.

Inhibitors can be classified on the basis of their site of action on the enzyme, on whether they chemically ***modify the enzyme***, or on the ***kinetic parameters*** they influence. Compounds that mimic the transition state of an enzyme-catalyzed reaction (**transition state analogs**) or that take advantage of the catalytic machinery of an enzyme (**mechanism-based inhibitors**) can be particularly potent inhibitors. Kinetically, we distinguish two classes of inhibitors based upon whether raising the substrate concentration does or does not overcome the inhibition.

Competitive Inhibitors:

The effects of competitive inhibitors can be overcome by raising the concentration of substrate. Most frequently, in competitive inhibition the inhibitor **(I)** binds to the substrate-binding portion of the active site thereby blocking access by the substrate.

The structures of most classic competitive inhibitors therefore tend to ***resemble*** the structure of a substrate, and thus are termed ***substrate analogs***. Both substrate and its structural analog (inhibitor) can bind to the active site of the enzyme, forming an ES or an EI complex, respectively. However, EI complex will not proceed to form the product.

For example, malonate, a structural analog of succinate, can competitively inhibit succinate dehydrogenase. Succinate dehydrogenase catalyzes the removal of one hydrogen atom from each of the two methylene carbons of succinate ($^{-}OOC\text{-}CH_2\text{-}CH_2\text{-}COO^{-}$). Both succinate and malonate ($^{-}OOC\text{-}CH_2\text{-}COO^{-}$) can bind to the active site of succinate dehydrogenase, forming an ES or an EI complex, respectively. However, since malonate contains only one methylene carbon, it cannot undergo dehydrogenation.

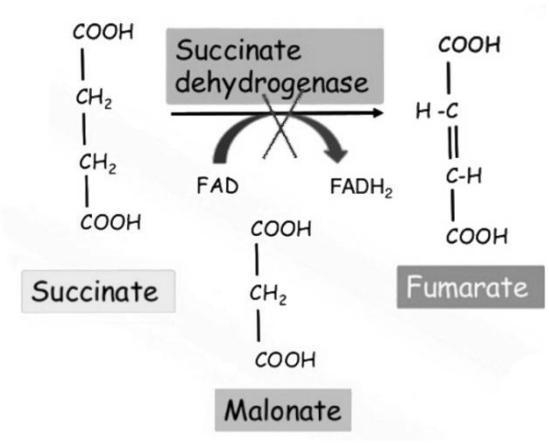


Figure 1: Malonate as competitive inhibitor of succinate dehydrogenase

The formation and dissociation of the EI complex is a dynamic process described by:

$$\mathbf{E\text{-}I} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathbf{E + I}$$

for which the equilibrium constant $\boldsymbol{K_i}$ is

$$\boldsymbol{K_i = \frac{[E][I]}{[E-I]} = \frac{k_1}{k_{-1}}}$$

In other words, ***a competitive inhibitor acts by decreasing the number of free enzyme molecules available to bind substrate, that is, to form ES, and thus eventually to form product***.

Increasing the substrate concentration will increase the formation of ES complexes. Since the formation of ES complexes removes free enzyme available to combine with the inhibitor, thus, *decreasing* the concentration of the EI complex and *raising* the reaction velocity. The extent to which [S] must be increased to completely overcome the inhibition depends upon:

- ✓ the *concentration of the inhibitor* present,
- ✓ the *affinity of the inhibitor* ($\boldsymbol{K_i}$) for the enzyme, and
- ✓ the *affinity*, ***Km****, of the enzyme* for its substrate.

Double-Reciprocal Plots Facilitate the Evaluation of Inhibitors

Double-reciprocal plots typically are used both to distinguish between competitive and noncompetitive inhibitors and to simplify evaluation of inhibition constants. v_i is determined at several substrate concentrations both in the presence and in the absence of the inhibitor. For classic competitive inhibition, the lines that connect the experimental data points converge at the *y*-axis (**Figure 2**). Since the *y* intercept is equal to 1/*V*max, this pattern indicates that **when 1/[S] approaches 0, v_i is independent of the presence of inhibitor.**

The effect of a competitive inhibitor on *V*max is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the *V*max observed in the absence of inhibitor.

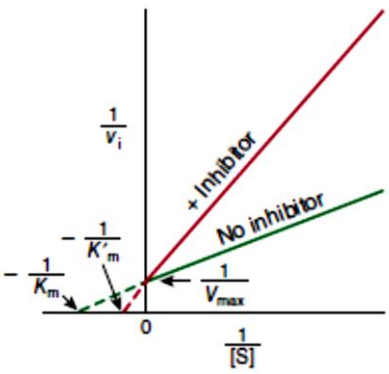


Figure 2: Lineweaver-Burk plot of simple competitive inhibition

Note, however, that the intercept on the *x*-axis *does* vary with inhibitor concentration and that, since $-1/K'm$ is smaller than $-1/Km$, $K'm$ (the "apparent *K*m") becomes larger in the presence of increasing concentrations of the inhibitor. Thus, **a competitive inhibitor has no effect on *V*max but raises *K*′m, the apparent *K*m for the substrate.**

*K*i values are used to compare different inhibitors of the same enzyme. The *lower* the value for *K*i, the more effective the inhibitor. For example, the statin drugs that act as competitive inhibitors of HMG-CoA reductase have *K*i values several orders of magnitude lower than the *K*m for the substrate, HMG-CoA.

Simple Noncompetitive Inhibitors:

In noncompetitive inhibition, the inhibitor and substrate bind at different sites on the enzyme. The noncompetitive inhibitor can bind either free enzyme (forming EI), or the ES complex (forming EIS). However, while the enzyme-inhibitor complex can still bind the substrate, its efficiency at transforming substrate to product, reflected by *V*max, is decreased **(Figure 3**). Noncompetitive inhibitors *bind enzymes at sites distinct from the substrate-binding site* and generally bear *little or no structural resemblance to the substrate*.

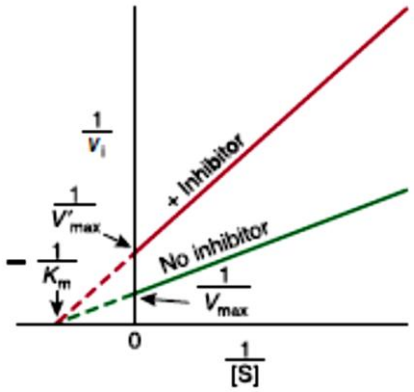


Figure 3: Lineweaver-Burk plot of simple noncompetitive inhibition

An example of noncompetitive inhibitors is lead which noncompetitively forms covalent bonds with the sulfhydryl side chains of cysteine in ferrochelatase, an enzyme that catalyzes the insertion of Fe^{2+} into protoporphyrin (a precursor of heme).

Note:

	Competitive Inhibitor	**Noncompetitive Inhibitor**
Structure	Resemble the structure of a substrate (substrate analogs).	Little or no structural resemblance to the substrate.
Binding Site on Enzyme	Specifically at the catalytic site, where it competes with substrate for binding in a dynamic equilibrium- like process. Inhibition is reversible by substrate.	Binds E or ES complex other than at the catalytic site. Substrate binding unaltered, but EIS complex cannot form products. Inhibition cannot be reversed by substrate.
Kinetic effect	*V*max is unchanged; *K*m is apparently increased.	*K*m appears unaltered; *V*max is apparently decreased, proportionately to inhibitor concentration.

IC_{50}

A less rigorous alternative to *K*i as a measure of inhibitory potency is the concentration of inhibitor that produces 50% inhibition, **IC_{50}**. Unlike the equilibrium dissociation constant *K*i, the numeric value of IC_{50} varies as a function of the specific circumstances of substrate concentration, etc. under which it is determined.

Irreversible Inhibitors "Poison" Enzymes

In the above examples, the inhibitors form a dissociable, dynamic complex with the enzyme. Fully active enzyme can therefore be recovered simply by removing the inhibitor from the surrounding medium. However, a variety of other inhibitors act *irreversibly* by chemically modifying the enzyme. These modifications generally involve making or breaking covalent bonds with aminoacyl residues essential for substrate ***binding***, ***catalysis***, or maintenance of the enzyme's ***functional conformation***. Since these covalent changes are relatively stable, an enzyme that has been "poisoned" by an irreversible inhibitor such as a heavy metal atom or an acylating reagent remains inhibited even after the removal of the remaining inhibitor from the surrounding medium.

Mechanism-Based Inhibition

It is an irreversible form of enzyme inhibition. "Mechanism-based" or "suicide" inhibitors are specialized substrate analogs that contain a chemical group that can be transformed by the catalytic machinery of the target enzyme (i.e. during the "normal" catalysis reaction*)*. After binding to the active site, catalysis by the enzyme generates a highly reactive group that forms a covalent bond to and **blocks the function of a catalytically essential residue**. The specificity and persistence of suicide inhibitors, which are both enzyme-specific and unreactive outside the confines of the enzyme's active site, render them promising leads for the development of enzyme-specific drugs.

MOST ENZYME-CATALYZED REACTIONS INVOLVE TWO OR MORE SUBSTRATES

While several enzymes have a single substrate, many others have two—and sometimes more—substrates and products. The fundamental principles discussed earlier, while illustrated for single-substrate enzymes, apply also to multisubstrate enzymes. The mathematical expressions used to evaluate multisubstrate reactions are, however, complex. The two-substrate, two-product reactions are termed "Bi-Bi" reactions.

Sequential or Single-Displacement Reactions

In **sequential reactions,** both substrates must combine with the enzyme to form a ternary complex before catalysis can proceed. Sequential reactions are sometimes referred to as single-displacement reactions because the group undergoing transfer is usually passed directly, in a single step, from one substrate to the other. Sequential Bi-Bi reactions can be further distinguished on the basis of whether the two substrates add in a **random** or in a **compulsory** order. For random-order reactions, either substrate A or substrate B may combine first with the enzyme to form an EA or an EB complex (**Figure 4**, center). For compulsory-order reactions (**Figure 4**, top), A must first combine with E before B can combine with the EA complex. One explanation for why some enzymes follow a compulsory-order mechanism can be found in **the induced fit hypothesis**: the addition of A induces a conformational change in the enzyme that aligns residues that recognize and bind B.

Ping-Pong Reactions

The term **"ping-pong"** applies to mechanisms in which one or more products are released from the enzyme before all the substrates have been added. Ping-pong reactions involve covalent catalysis and a transient, modified form of the enzyme. Ping-pong Bi-Bi reactions are often referred to as **double displacement reactions.** The group undergoing transfer is first displaced from substrate A by the enzyme to form product P and a modified form of the enzyme (F). The subsequent group transfer from F to the second substrate B, forming product Q and regenerating E, constitutes the second displacement (**Figure 4**, bottom).

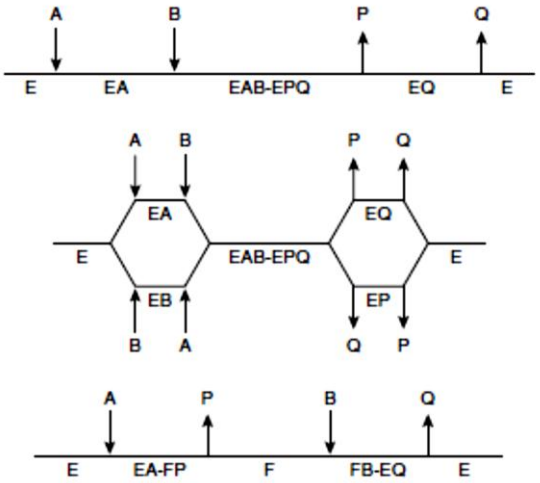


Figure 4: Representations of three classes of Bi-Bi reaction mechanisms. *Horizontal lines represent the enzyme. Arrows indicate the addition of substrates and departure of products.* ***Top:*** *an ordered Bi-Bi reaction,* ***Center:*** *a random Bi-Bi reaction,* ***Bottom:*** *a ping-pong reaction*

Most Bi-Bi Reactions Follow Michaelis-Menten Kinetics

Most Bi-Bi reactions conform to a ***somewhat more complex form*** of Michaelis-Menten kinetics in which ***V*max** refers to the reaction rate attained when ***both*** substrates are present at saturating levels. ***Each substrate has its own characteristic K*m** value, which corresponds to the concentration that yields half-maximal velocity when the second substrate is present at saturating levels. As for single-substrate reactions, doublereciprocal plots can be used to determine *V*max and *K*m. v_i is measured as a function of the concentration of one substrate (the variable substrate) while the concentration of the other substrate (the fixed substrate) is maintained constant. If the lines obtained for several fixed-substrate concentrations are plotted on the same graph, it is possible to distinguish a ping–pong mechanism, which yields parallel lines (**Figure 5**), from a sequential mechanism, which yields a pattern of intersecting lines.

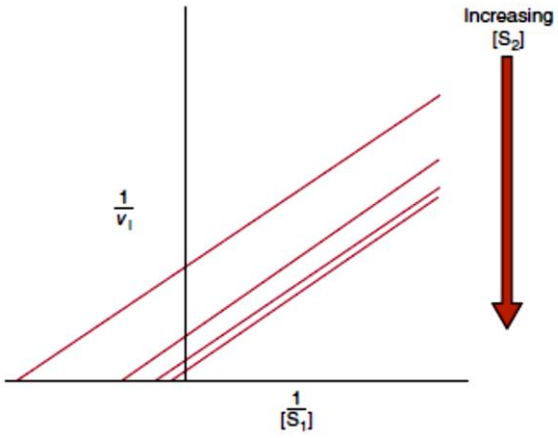


Figure 5: Lineweaver-Burk plot for a two-substrate ping-pong reaction.
Increasing the concentration of one substrate (S1) while maintaining that of the other substrate (S2) constant alters both the x and y intercepts, but not the slope.

Knowledge of enzyme kinetics, mechanism and inhibition as an aid in drug development:

By virtue of their diverse physiologic roles and high degree of substrate selectivity, enzymes constitute natural targets for the development of pharmacologic agents that are both potent and specific. Statin drugs, for example, lower cholesterol production by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase. Pharmacologic treatment of hypertension often includes the administration of an inhibitor of angiotensin-converting enzyme, thus lowering the level of angiotensin II, a vasoconstrictor.

Applied enzyme kinetics facilitate the identification, characterization and elucidation of the mode of action of drugs that selectively inhibit specific enzymes.

Enzyme kinetics plays a central role in the analysis and optimization of drug metabolism, a key determinant of drug efficacy. Metabolic transformation is sometimes required to convert an inactive drug precursor, or **prodrug,** into its biologically active form. The effective design and administration of prodrugs requires knowledge of the kinetics and mechanisms of the enzymes responsible for transforming them into their biologically active forms.

Enzymes Regulation

Why regulation is important?

The regulation of the reaction velocity of enzymes is essential if an organism is to coordinate its numerous metabolic processes. In the cell, enzymes do not work alone but often work together in groups. These sets of reactions are called **metabolic pathways**. Given the fact the enormous amount of energy and resources are dedicated for each pathway to carry out different metabolic functions, the cells have to regulate the activities of the enzymes very precisely. Regulation will allow the changing needs of the cell to meet its energy and resource demands. If a product is available in excess, it could then divert the resources to other needy reactions. If a product is in demand, it could activate the pathway to produce more of the biomolecule that is needed. Thus, regulation is the process by which cells can turn on, turn off, or modulate the activities of various metabolic pathways.

The rates of most enzymes are responsive to changes in substrate concentration, because the intracellular level of many substrates is in the range of the *K*m. Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal. (**Figure 1**).

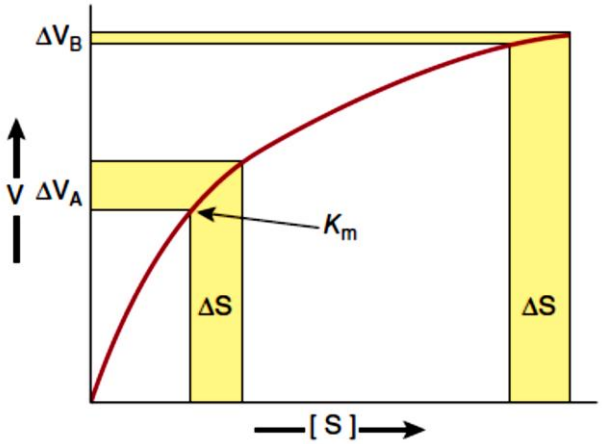


Figure 1: Differential response of the rate of an enzyme catalyzed reaction, DV, to the same incremental change in substrate concentration at a substrate concentration close to Km (DV_A) or far above Km (DV_B).

Responses to changes in **substrate** level represent an important but *passive* means for coordinating metabolite flow. However, their capacity for responding to changes in environmental variables is *limited*.

While all chemical reactions are to some extent reversible, in living cells the reaction products serve as substrates for—and are removed by—other enzyme-catalyzed reactions. Many "reversible" reactions in living cells thus occur unidirectionally. Because this sequence of coupled metabolic reactions is accompanied by an *overall* change in free energy that favors unidirectional metabolite flow.

COMPARTMENTATION ENSURES METABOLIC EFFICIENCY & SIMPLIFIES REGULATION

In eukaryotes, the anabolic and catabolic pathways that synthesize and break down common biomolecules often are physically separated from one another. Certain metabolic pathways reside only within specialized cell types or, within a cell, inside distinct subcellular compartments. For example, many of the enzymes that degrade proteins and polysaccharides reside inside organelles called lysosomes. Similarly, fatty

acid biosynthesis occurs in the cytosol, whereas fatty acid oxidation takes place within mitochondria.

Compartmentation means that enzymes needed for specific processes can be kept in the places where they act, ensuring they can *find their substrates readily*, *don't damage the cell*, and *have the right microenvironment to work well*. For instance, digestive enzymes of the lysosome work best at a pH around 5.0 which is found in the acidic interior of the lysosome (but not in the cytosol, which has a pH of about 7.2). Lysosomal enzymes have low activity at the pH of the cytosol, which may serve as "insurance" for the cell that even if a lysosome bursts and spills its enzymes, the enzymes will not begin digesting the cell, because they will no longer have the right pH to function.

Controlling an Enzyme That Catalyzes a Rate-Limiting Reaction Regulates an Entire Metabolic Pathway

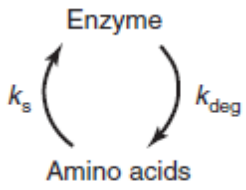
While the flux of metabolites through metabolic pathways involves catalysis by numerous enzymes, active control of homeostasis is achieved by the regulation of only a select subset of these enzymes. The ideal enzyme for regulatory intervention is one whose quantity or catalytic efficiency dictates that the reaction it catalyzes is slow relative to all others in the pathway. Decreasing the catalytic efficiency or the quantity of the catalyst responsible for the "bottleneck" or **rate-limiting reaction** will immediately reduce metabolite flux through the entire pathway. Conversely, an increase in either its quantity or catalytic efficiency will enhance flux through the pathway as a whole. For example, acetyl-CoA carboxylase catalyzes the synthesis of malonyl-CoA, the first committed reaction of fatty acid biosynthesis. When synthesis of malonyl-CoA is inhibited, subsequent reactions of fatty acid synthesis cease for lack of substrates. As natural "governors" of metabolic flux, the enzymes that catalyze rate-limiting steps also constitute efficient targets for regulatory intervention by drugs. For example, "statin" drugs limit synthesis of cholesterol by inhibiting HMG-CoA reductase, catalyst of the rate-limiting reaction of cholesterogenesis.

REGULATION OF ENZYME QUANTITY

The catalytic capacity of the rate-limiting reaction in a metabolic pathway is the product of the concentration of enzyme molecules and their intrinsic catalytic efficiency. It therefore follows that catalytic capacity can be controlled by changing the quantity of enzyme present, altering its intrinsic catalytic efficiency, or a combination thereof.

Proteins Are Continuously Synthesized and Degraded

Proteins exist in a state of "dynamic equilibrium" within our bodies where they are continuously synthesized and degraded—a process referred to as **protein turnover.** This holds even for **constitutive** proteins, those whose concentrations remain essentially constant over time. On the other hand, the concentrations of many enzymes are influenced by a wide range of physiologic, hormonal, or dietary factors. The absolute quantity of an enzyme reflects the net balance between its rate of synthesis and its rate of degradation. In human subjects, alterations in the levels of specific enzymes can be effected by a change in the rate constant for the overall processes of synthesis (*k*s), degradation (*k*deg), or both.



Control of Enzyme Synthesis

The synthesis of certain enzymes depends upon the presence of **inducers,** typically substrates or structurally related compounds that stimulate the transcription of the gene that encodes them. *Escherichia coli* grown on glucose will, for example, only catabolize lactose after addition of a β-galactoside, an inducer that triggers synthesis of a β-galactosidase and a galactoside permease. Inducible enzymes of humans include tryptophan pyrrolase, threonine dehydratase, tyrosine-α-ketoglutarate aminotransferase, enzymes of the urea cycle, HMG-CoA reductase, δ-aminolevulinate synthase, and cytochrome P_{450}. Conversely, an excess of a metabolite may limit synthesis of its related enzyme via **repression.** Both induction and repression involve *cis* elements, specific DNA sequences located upstream of regulated genes, and *trans*-acting regulatory proteins.

Control of Enzyme Degradation

In animals many proteins are degraded by the ubiquitin proteasome pathway. Degradation takes place in the proteasome, a large macromolecular complex made up of more than 30 polypeptide subunits arranged in the form of a hollow cylinder. The active sites of its proteolytic subunits face the interior of the cylinder, thus preventing indiscriminate degradation of cellular proteins. Proteins are targeted to the interior of the proteasome by "ubiquitination," the covalent attachment of one or more ubiquitin molecules. Ubiquitin is a small, approximately 8.5 kDa protein that is highly conserved among eukaryotes. Ubiquitination is catalyzed by a large family of enzymes called E3 ligases, which attach ubiquitin to the sidechain amino group of lysyl residues.

The ubiquitin-proteasome pathway is responsible both for the regulated degradation of selected cellular proteins, for example, cyclins, and for the removal of defective or aberrant protein species. The key to the versatility and selectivity of the ubiquitin-proteasome system resides in the variety of intracellular E3 ligases and their ability to discriminate between the different physical or conformational states of target proteins. Thus, the ubiquitin-proteasome pathway can selectively degrade proteins whose physical integrity and functional competency have been compromised by the loss of or damage to a prosthetic group, oxidation of cysteine or histidine residues, or deamidation of asparagine or glutamine residues.

Recognition by proteolytic enzymes also can be regulated by covalent modifications such as phosphorylation; binding of substrates or allosteric effectors; or association with membranes, oligonucleotides, or other proteins. A growing body of evidence suggests that dysfunctions of the ubiquitin-proteasome pathway contribute to the accumulation of the misfolded proteins characteristic of several neurodegenerative diseases.

MULTIPLE OPTIONS ARE AVAILABLE FOR REGULATING CATALYTIC ACTIVITY

In humans the induction of protein synthesis is a complex multistep process that typically requires hours to produce significant changes in overall enzyme level. By contrast, changes in intrinsic catalytic efficiency effected by binding of dissociable ligands **(allosteric regulation)** or by **covalent modification** achieve regulation of enzymic activity within seconds. Consequently, changes in protein level generally dominate when meeting long-term adaptive requirements, whereas changes in catalytic efficiency are favored for rapid and transient alterations in metabolite flux.

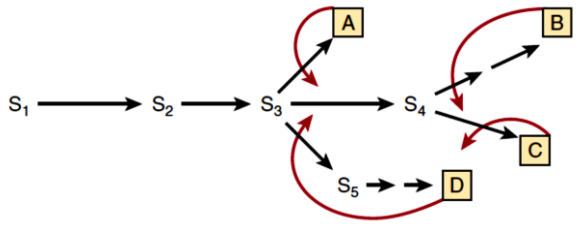
ALLOSTERIC REGULATION

Feedback inhibition refers to the process by which the end product of a multistep biosynthetic pathway binds to and inhibits an enzyme catalyzing one of the early steps in that pathway. In most cases, feedback inhibitors inhibit the enzyme that catalyzes the first committed step in a particular biosynthetic sequence. In the following example, for the biosynthesis of D from A is catalyzed by enzymes Enz1 through Enz3:

$$\mathbf{A} \xrightarrow{Enz.1} \mathbf{B} \xrightarrow{Enz.2} \mathbf{C} \xrightarrow{Enz.3} \mathbf{D}$$

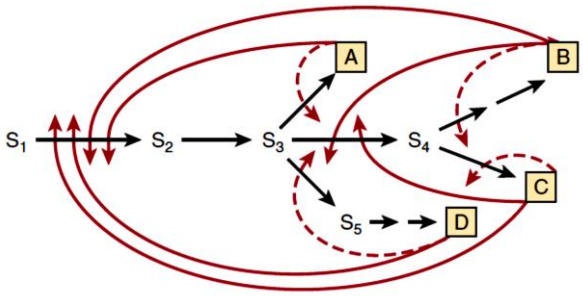
High concentrations of D inhibit the conversion of A to B. In this example, the feedback inhibitor D acts as a **negative allosteric effector** of Enz1. Inhibition results, not from the "backing up" of intermediates, but from the ability of D to bind to and inhibit Enz1. Generally, D binds at an **allosteric site,** one spatially distinct from the catalytic site of the target enzyme. Feedback inhibitors thus typically bear little or no structural similarity to the substrates of the enzymes they inhibit. For example, NAD^+ and 3-phosphoglycerate, the substrates for 3-phosphoglycerate dehydrogenase, which catalyzes the first committed step in serine biosynthesis, bear no resemblance to the feedback inhibitor serine.

In branched biosynthetic pathways, such as those responsible for nucleotide biosynthesis, the initial reactions supply intermediates required for the synthesis of multiple end products. **Figure 2** shows a hypothetical branched biosynthetic pathway in which curved arrows lead from feedback inhibitors to the enzymes whose activity they inhibit.



The sequences $S_3 \rightarrow A$, $S_4 \rightarrow B$, $S_4 \rightarrow C$, and $S_3 \rightarrow \rightarrow D$ each represent linear reaction sequences that are feedback-inhibited by their end products. Branch point enzymes thus can be targeted to direct later stages of metabolite flow.

The kinetics of feedback inhibition may be competitive, noncompetitive, partially competitive, or mixed. Layering multiple feedback loops can provide additional fine control. For example, as shown in **Figure 3**, the presence of excess product B decreases the requirement for substrate S_2. However, S_2 is also required for synthesis of A, C, and D. Therefore, for this pathway, excess B limits synthesis of all four end products, regardless of the need for the other three. To circumvent this potential difficulty, each end product may only *partially* inhibit catalytic activity. The effect of an excess of two or more end products may be strictly additive or, alternatively, greater than their individual effect (cooperative feedback inhibition).



Alternatively, for example the branched pathway responsible for the synthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan in bacteria, multiple isoforms of an enzyme may evolve, each of which is sensitive to a different pathway end product. High levels of any one end product will inhibit catalysis by only a single isoform, reducing but not eliminating flux through the shared portion of the pathway.

Aspartate Transcarbamoylase Is a Model Allosteric Enzyme

Aspartate transcarbamoylase (ATCase), the catalyst for the first reaction unique to pyrimidine biosynthesis, is a target of feedback regulation by two nucleotide triphosphates: cytidine triphosphate (CTP) and adenosine triphosphate (ATP). CTP, an end product of the pyrimidine biosynthetic pathway, inhibits ATCase, whereas the purine nucleotide ATP activates it. Moreover, high levels of ATP can overcome inhibition by CTP, enabling synthesis of *pyrimidine* nucleotides to proceed when *purine* nucleotide levels are elevated.

Allosteric & Catalytic Sites Are Spatially Distinct

Allosteric as a word means "occupy another space". **Allosteric enzymes thus are those for which catalysis at the active site may be modulated by the presence of effectors at an allosteric site.** The existence of spatially distinct active and allosteric sites has since been verified in several enzymes using many lines of evidence. In general, binding of an allosteric regulator influences catalysis by inducing a conformational change that encompasses the active site.

Allosteric Effects May Be on *K*m or on *V*max

To refer to the kinetics of allosteric inhibition as "competitive" or "noncompetitive" with substrate carries misleading mechanistic implications. Instead, two classes of allosterically regulated enzymes are called **K-series** and **V-series** enzymes. For K-series allosteric enzymes, the substrate saturation kinetics is competitive in the sense that *K*m is raised without an effect on *V*max. For V-series allosteric enzymes, the allosteric inhibitor lowers *V*max without affecting the *K*m. Alterations in *K*m or *V*max often are the product of conformational changes at the catalytic site induced by binding of the allosteric effector at its site. For a K-series allosteric enzyme, this conformational change may weaken the bonds between substrate and substrate-binding residues. For a V-series allosteric enzyme, the primary effect may be to alter the orientation or charge of catalytic residues, lowering *V*max. Intermediate effects on *K*m and *V*max, however, may be observed consequent to these conformational changes.

Notes regarding Allosteric regulation:

Feedback regulation can be either stimulatory or inhibitory

In both mammalian and bacterial cells, some pathway end products *"feedback"* to control their own synthesis, in *many instances by feedback inhibition* of an early biosynthetic enzyme. We must, however, distinguish between **feedback regulation,** a phenomenologic term lacking mechanistic implications, and **feedback inhibition,** a mechanism for regulation of enzyme activity. For example, while dietary cholesterol decreases hepatic synthesis of cholesterol, this feedback **regulation** does not involve feedback **inhibition.** HMG-CoA reductase, the rate-limiting enzyme of cholesterogenesis, is affected, but cholesterol does not inhibit its activity. Rather, regulation in response to dietary cholesterol involves *reduction of the expression* of the gene that encodes HMG-CoA reductase (enzyme repression); by cholesterol or a cholesterol metabolite. As mentioned earlier, ATP, a product of the purine nucleotide pathway, stimulates the synthesis of pyrimidine nucleotides by activating aspartate transcarbamoylase, a process sometimes referred to as *"feed forward"* regulation.

Many hormones act via second messengers

Nerve impulses and the binding of many hormones to cell surface receptors elicit changes in the rate of enzyme-catalyzed reactions within target cells by inducing the release or synthesis of specialized allosteric effectors called **second messengers.** The primary, or "first," messenger is the hormone molecule or nerve impulse. Second messengers include **3′, 5′-cAMP**, synthesized from ATP by the enzyme adenylyl cyclase in response to the hormone epinephrine. Membrane depolarization resulting from a nerve impulse opens a membrane channel that releases **calcium ions** into the cytoplasm, where they bind to and activate enzymes involved in the regulation of muscle contraction and the mobilization of stored glucose from glycogen to supply the increased energy demands of muscle contraction. Other second messengers include **3′,5′-cGMP**, **nitric oxide**, and the **polyphosphoinositols** produced by the hydrolysis of inositol phospholipids by hormone-regulated phospholipases.

REGULATORY COVALENT MODIFICATIONS:

In mammalian cells, a wide range of regulatory covalent modifications occur. These modifications can be either irreversible (**partial proteolysis),** or reversible **(phosphorylation, acetylation, methylation** and **ADP-ribosylation)**.

- **Irreversible Covalent Modification: (proteolytic activation of proenzymes.)**

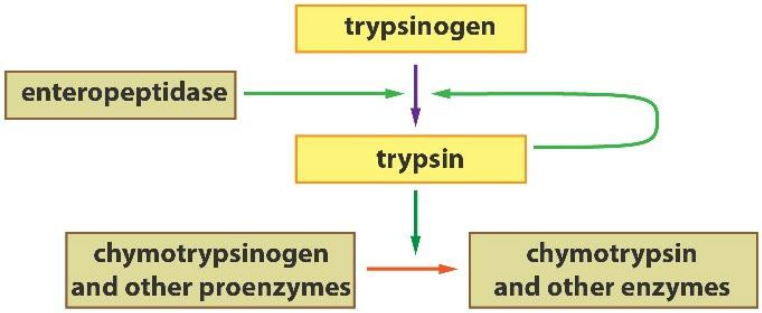
Certain proteins are synthesized as inactive precursor proteins known as **proproteins.** Selective, or "partial," proteolysis of a proprotein by one or more successive proteolytic "clips" converts it to a form that exhibits the characteristic activity of the mature protein, for example, its catalytic activity.

The proprotein forms of enzymes are termed **proenzymes** or **zymogens.** Proteins synthesized as proproteins include the hormone insulin (proprotein = proinsulin), the digestive enzymes pepsin, trypsin, and chymotrypsin (proproteins = pepsinogen, trypsinogen, and chymotrypsinogen, respectively), several factors of the blood clotting and complement cascades, and the connective tissue protein collagen (proprotein = procollagen).

Proteolytic activation of proproteins constitutes a physiologically irreversible modification because reunification of the two portions of a protein produced by hydrolysis of a peptide bond is entropically disfavored. Once a proprotein is activated, it will continue to carry out its catalytic or other functions until it is removed by

degradation or some other means. Zymogen activation thus represents a simple and economical, although one way, mechanism for restraining the latent activity of a protein until the appropriate circumstances are encountered. It is therefore not surprising that partial proteolysis is employed frequently to regulate proteins that work in the gastrointestinal tract or bloodstream rather than in the interior of cells.

Selective proteolysis involves one or more highly specific proteolytic clips that may or may not be accompanied by separation of the resulting peptides. Most importantly, selective proteolysis often results in conformational changes that *properly configure an enzyme's active site*. The conformational changes that accompany selective proteolysis of prochymotrypsin (chymotrypsinogen) align three residues, forming the catalytic site. Contact and catalytic residues can be located on different peptide chains but still be within bond-forming distance of bound substrate.



Importance of existence of some enzymes as catalytically inactive proenzymes

The synthesis and secretion of *proteases as catalytically inactive proenzymes protect the tissue of origin* (eg, the pancreas) from autodigestion, such as can occur in pancreatitis. Certain physiologic processes such as digestion are intermittent but fairly regular and predictable in frequency. Others such as blood clot formation, clot dissolution, and tissue repair are brought "on line" only in response to pressing pathophysiologic need. The processes of blood clot formation and dissolution clearly must be temporally coordinated to achieve homeostasis. *Enzymes needed intermittently but rapidly often are secreted in an initially inactive form since new synthesis and secretion of the required proteins might be insufficiently rapid to respond to a pressing pathophysiologic demand such as the loss of blood.*

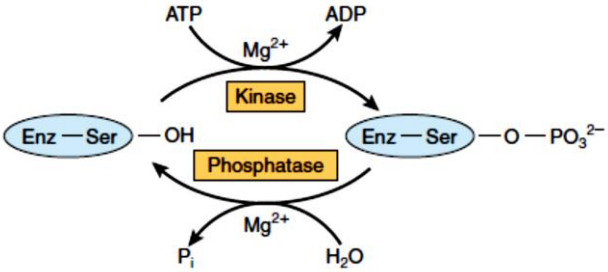
- **Reversible Covalent Modification**

Acetylation, ADP-ribosylation, methylation, and phosphorylation are all examples of "reversible" covalent modifications. In this context, ***reversible*** refers to the fact that the modified protein can be restored to its original, modification-free state, not the mechanism by which restoration takes place.

Mammalian proteins are the targets of a wide range of covalent modification processes. Modifications such as prenylation, glycosylation, and hydroxylation introduce unique structural features into newly synthesized proteins that tend to persist for the lifetime of the protein. Among the covalent modifications that regulate protein function, the most common by far are phosphorylation-dephosphorylation and acetylation-deacetylation.

Phosphorylation

Protein kinases phosphorylate proteins by catalyzing transfer of the terminal phosphoryl group of ATP to the hydroxyl groups of seryl, threonyl, or tyrosyl residues, forming *O*-phosphoseryl, *O*-phosphothreonyl, or *O*-phosphotyrosyl residues, respectively.



Some protein kinases target the side chains of histidyl, lysyl, arginyl, and aspartyl residues. The unmodified form of the protein can be regenerated by hydrolytic removal of phosphoryl groups, catalyzed by ***protein phosphatases*.**

A typical mammalian cell possesses thousands of phosphorylated proteins and several hundred protein kinases and protein phosphatases that catalyze their interconversion. The *ease of interconversion* of enzymes between their phospho- and dephospho- forms accounts, in part, for the frequency with which phosphorylation-dephosphorylation is utilized as a mechanism for regulatory control. Phosphorylation-dephosphorylation permits the functional properties of the affected enzyme to be altered only for as long as it serves a specific need. Once the need has passed, the enzyme can be converted back to its original form, capable to respond to the next stimulatory event. A second factor underlying the widespread use of protein phosphorylation-dephosphorylation lies in the *chemical properties of the phosphoryl group itself*. In order to alter an enzyme's functional properties, any modification of its chemical structure must influence the protein's three-dimensional configuration. The high charge density of protein-bound phosphoryl groups (generally –2 at physiologic pH) and their tendency to form strong salt bridges with arginyl and lysyl residues renders them potent agents for modifying protein structure and function. Phosphorylation generally influences an enzyme's intrinsic catalytic efficiency or other properties by inducing conformational changes. Consequently, the amino acids targeted by phosphorylation can be and typically are **relatively distant from the catalytic site itself**.

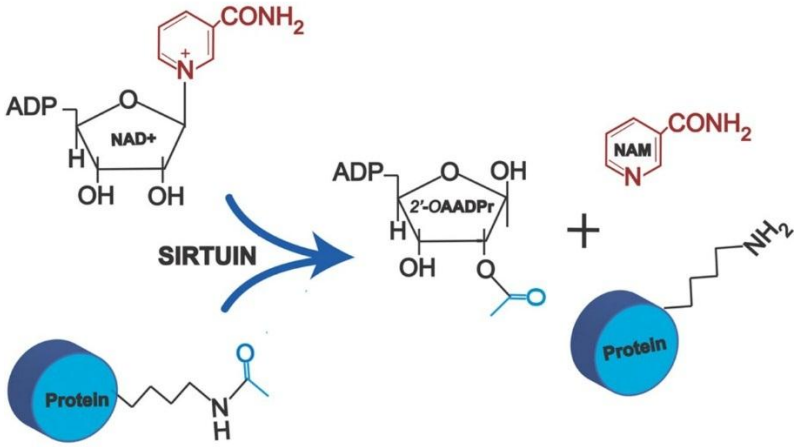
Protein phosphorylation-dephosphorylation is a **highly selective** process. Not all proteins are subject to phosphorylation, and of the many hydroxyl groups on a protein's surface, only one or a small subset are targeted.

The process is also **highly versatile**. While the most common enzyme function affected is the protein's catalytic efficiency, phosphorylation can also alter its location within the cell, susceptibility to proteolytic degradation, or responsiveness to regulation by allosteric ligands. Phosphorylation can increase an enzyme's catalytic efficiency, converting it to its active form in one protein, while phosphorylation of another protein converts it to an intrinsically inefficient, or inactive form. Many proteins can be phosphorylated at multiple sites. Others are subject to regulation both by phosphorylation-dephosphorylation and by the binding of allosteric ligands, or by phosphorylation-dephosphorylation and another covalent modification. Phosphorylation-dephosphorylation at any one site can be catalyzed by multiple protein kinases or protein phosphatases. Many protein kinases and most protein phosphatases act on more than one protein and are themselves interconverted between active and inactive forms by the binding of second messengers or by covalent modification by phosphorylation-dephosphorylation. This interplay provides the basis for regulatory networks that integrate multiple environmental input signals to evoke an appropriate coordinated cellular response.

Acetylation

Covalent acetylation-deacetylation occur in thousands of mammalian proteins, including nearly every enzyme present in key metabolic pathways such as glycolysis, glycogen synthesis, gluconeogenesis, the tricarboxylic acid cycle, β-oxidation of fatty acids, and the urea cycle. **Lysine acetyltransferases** catalyze the transfer of the acetyl group of acetyl-CoA to the ε-amino groups of lysyl residues, forming *N*-acetyl lysine. In addition, some proteins, particularly those in the mitochondria, become acetylated by reacting with acetyl-CoA directly, ie, without the intervention of an enzyme catalyst. Acetylation not only increases the steric bulk of the lysine side chain, it transforms a basic and potentially positively charged primary amine into a neutral, nonionizable amide.

Two classes of protein deacetylases have been identified: **histone deacetylases** and **sirtuins**. Histone deacetylases catalyze the removal by hydrolysis of acetyl groups, regenerating the unmodified form of the protein and acetate as products. Sirtuins, on the other hand, use NAD^+ as substrate, which yields *O*-acetyl ADP-ribose and nicotinamide as products in addition to the unmodified protein.



Reversible modifications of DNA-binding proteins

Histones and other DNA-binding proteins in chromatin are subject to extensive modification by acetylation, methylation, phosphorylation, as well as ADP-ribosylation. These modifications, modulate the manner in which the proteins within chromatin interact with each other as well as the DNA itself, constitute the basis for the "histone code." The resulting changes in chromatin structure within the region affected can render genes more accessible to the proteins responsible for their transcription, thereby enhancing gene expression or, on a larger scale, facilitating replication of the entire genome. On the other hand, changes in chromatin structure that restrict the accessibility of genes to transcription factors, DNA-dependent RNA polymerases, etc, thereby inhibiting transcription, are said to **silence** gene expression.

The Histone Code

The "histone code" represents a classic example of **epigenetics,** the hereditary transmission of information by a means other than the sequence of nucleotides that comprise the genome. In this instance, the pattern of gene expression within a newly formed "daughter" cell will be determined, in part, by the particular set of histone covalent modifications embodied in the chromatin proteins inherited from the "parental" cell.

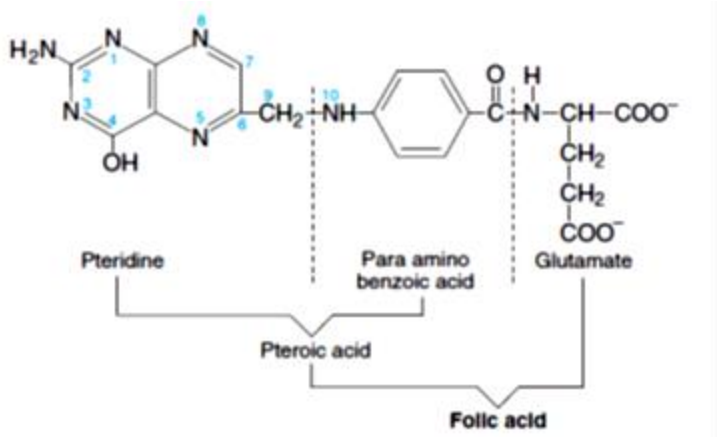
ONE-CARBON METABOLISM

The degradation pathways for several amino acids produce one-carbon units that are transferred to tetrahydrofolate (H_4folate or THF) as an intermediate carrier. Tetrahydrofolate, in turn, donates the single carbons to various biosynthetic intermediates.

The following **one-carbon units** are encountered in the biological reactions, which constitute one-carbon pool

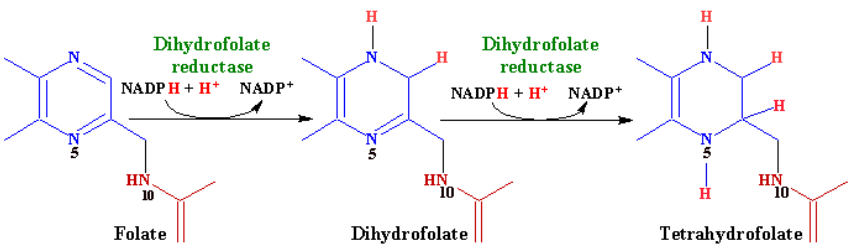
Methyl ($-CH_3$)
Methylene ($=CH_2$)
Methenyl ($-CH=$)
Hydroxymethyl ($-CH_2OH$)
Formyl ($-CH=O$)
Formimino ($-CH=NH$)

Folic acid is the vitamin as it is found in the diet. It consists of three distinct moieties: a **pteridine** moiety, a ***p*-aminobenzoate (PABA)** moiety, and one or several **glutamate residues**. Mammals can synthesize these components, but lack the enzymes necessary for their conjugation. The number of glutamate residues contained in folic acid or THF changes at various stages of transport and utilization. Intestinal uptake and transport through the blood occur with only one glutamate attached. After uptake into liver cells, several more glutamate residues are added.



The metabolically active form of folate is THF, which is formed from folate in two successive NADPH-dependent reductions, both catalyzed by the same enzyme, namely, **dihydrofolate reductase**. The hydrogen atoms are present at positions 5, 6, 7 and 8 of THF.

The one-carbon unit covalently binds with THF at position N^5 or N^{10} or on both N^5 and N^{10} of pteroyl structure of folate.

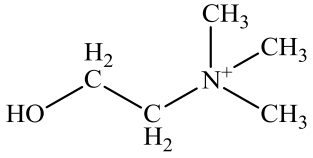


The one-carbon metabolism is rather complex, involving many reactions. For the sake of better understanding, it is divided into generation and utilization of one-carbon units, and the role of methionine and vitamin B_{12}.

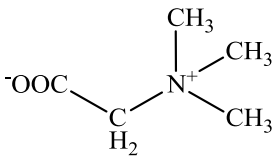
I. Generation of one-carbon units

Many compounds (particularly amino acids) act as donors of one-carbon fragments:

1. When serine is converted to glycine, N^5, N^{10}-methylene THF is formed. This is the most predominant entry of one-carbon units into one-carbon pool.
2. The formate released from glycine and tryptophan metabolism combines with THF to form N^{10}-formyl THF.
3. Histidine contributes formimino fragment to produce N^5-formimino THF.
4. Choline and betaine contribute to the formation of N^5-methyl THF.



Choline



Betaine

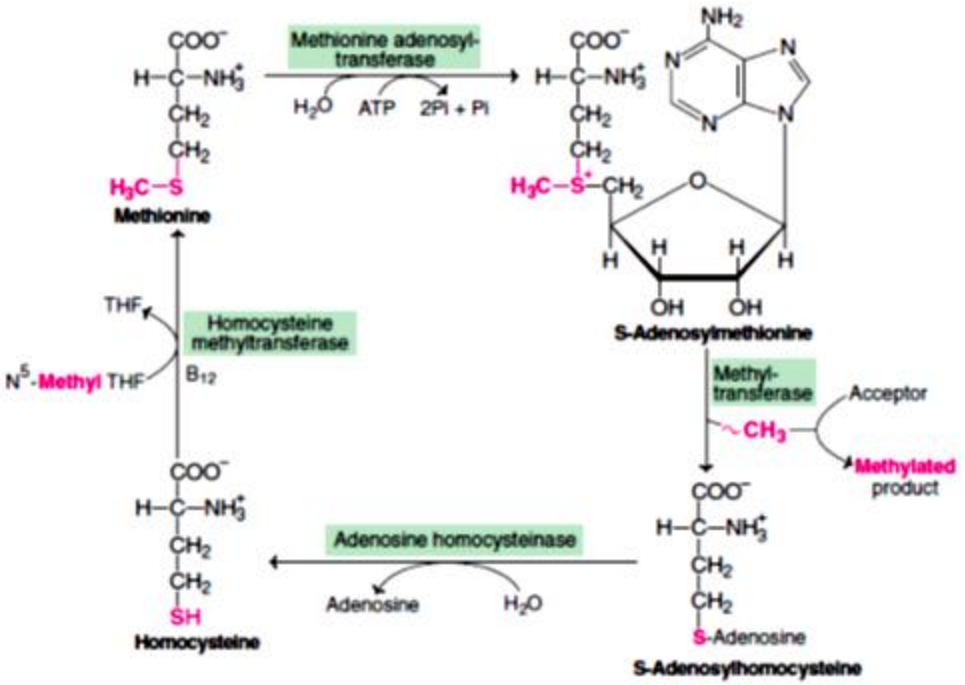
The different derivatives of THF carrying one-carbon units are interconvertible, and this is metabolically significant for the continuity of one-carbon pool.

II. Utilization of one-carbon moieties

One-carbon fragments from THF are used for the synthesis of a wide variety of compounds. These include purines and pyrimidine nucleotide (thymidylate), formylmethionine tRNA (required for initiation of protein synthesis), glycine, etc.

III. Role of methionine and B12 in one-carbon metabolism

Methyl ($-CH_3$) group is an important one-carbon unit in many biosynthetic reactions. The role of active methionine as methyl donor in transmethylation reactions is good example. Although *tetrahydrofolate* can carry a *methyl group* at N-5, the methyl group's *transfer potential is insufficient* for most biosynthetic reactions. **S-Adenosylmethionine** is more commonly used for methyl group transfers. It is synthesized from ATP and methionine by the action of **methionine adenosyl-transferase**. This reaction is unusual in that the nucleophilic sulfur atom of methionine attacks at the 5' carbon of the ribose moiety of ATP, releasing triphosphate, rather than attacking at one of the phosphorus atoms. S-Adenosylmethionine is a potent alkylating agent by virtue of its destabilizing sulfonium ion. The methyl group is subject to attack by nucleophiles and is about 1,000 times more reactive than the methyl group of N^5-methyltetrahydrofolate.



After the release of methyl group, methionine is converted to homocysteine. For the regeneration of methionine, free homocysteine and N^5-methyl THF are required and this reaction is dependent on methylcobalamin (vitamin B_{12}). The one-carbon pool, under the control of THF, is linked with methionine metabolism (transmethylation) through vitamin B_{12}. Hence vitamin B_{12} is also involved in one-carbon metabolism.

- ✓ Folate deficiency impairs DNA synthesis and cell division resulting in many clinical conditions such as macrocytic anemia and neural tube defects in pregnancy (spina bifida or anencephaly).

- ✓ Folic acid antagonists:

Methotrexate (structural analogue of folic acid), competitively inhibits dihydrofolate reductase, is used in the treatment of leukemia.
Sulfonamides (structural analogues of PABA), competitively inhibit the enzyme responsible for the conjugation of PABA in folic acid molecule, are antibacterial agents.

Note:

Some authors consider CO_2 as a one-carbon unit, others do not agree. Carbon dioxide is involved in many biochemical reactions (**carboxylation**); **biotin** serves as a carrier of CO_2 in these reactions. For instance, conversion of pyruvate to oxaloacetate in gluconeogenesis.

Metabolic Diseases of Amino Acid Catabolism

Defective synthesis or decreased activity of enzymes involved in amino acid catabolism is associated with many metabolic diseases.

Metabolic defects in transaminases, which fulfill central metabolic functions, may be incompatible with life. Consequently, no known metabolic defect is associated with this short catabolic pathway.

Proline

There are two metabolic disorders of proline catabolism.

The metabolic block in **type I hyperprolinemia** is at **proline dehydrogenase**.

The metabolic block in **type II hyperprolinemia** is at **Δ^1-pyrroline-5-carboxylate dehydrogenase**, which also participates in the catabolism of arginine, ornithine, and hydroxyproline. Since proline and hydroxyproline catabolism are affected, both *Δ^1-pyrroline-5-carboxylate* and *Δ^1-pyrroline-3-hydroxy-5-carboxylate* are excreted.

Hyperpolinemia is associated with seizures, intellectual disability, or other neurological problems.

Arginine and Ornithine

Mutations in **ornithine transaminase** elevate **plasma** and **urinary ornithine** and are associated with **gyrate atrophy of the choroid and retina.** Treatment involves restricting dietary arginine.

Note:

Defective arginase is associated with hyperargininemia

In the **hyperornithinemia–hyperhomocitrulinuria-hyperammonemia syndrome,** a defective mitochondrial **ornithine permease** impairs transport of ornithine into mitochondria where it participates as an intermediate in urea synthesis.

Histidine

Benign disorders of histidine catabolism include **histidinemia** and **urocanic aciduria** associated with impaired **histidase and urocanase,** respectively.

In **folic acid deficiency,** transfer of the formimino group is impaired, and formiminoglutamate (Figlu) is excreted.

Note:

Excretion of Figlu following a dose of histidine thus can be used to detect folic acid deficiency.

Glycine

Nonketotic hyperglycinemia or **Glycine encephalopathy,** is a rare inborn error of glycine degradation, due to defective **glycine cleavage complex**. Glycine accumulates in all body tissues including the central nervous system.

The defect in **primary hyperoxaluria** is the failure to catabolize glyoxylate formed by the deamination of glycine. Subsequent oxidation of glyoxylate to oxalate results in urolithiasis, nephrocalcinosis, and early mortality from renal failure or hypertension.

Cystine and Cysteine

Homocystinurias (vitamin B_6-responsive or vitamin B_6-unresponsive) result from a deficiency in the reaction catalyzed by **cystathionine β-synthase**:

Consequences include osteoporosis and mental retardation.

Note:

Epidemiologic and other data link plasma homocysteine levels to cardiovascular diseases risk, but the role of homocysteine as a causal cardiovascular risk factor remains controversial.

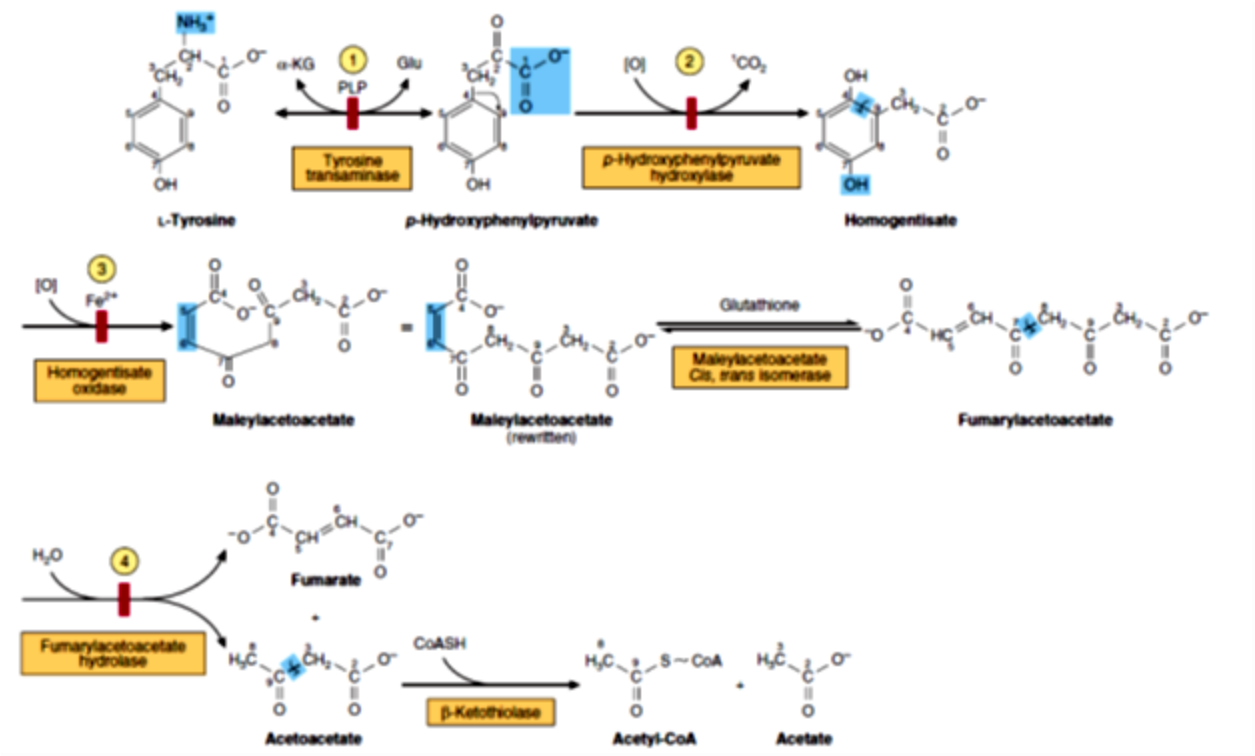
Tyrosine

Several metabolic disorders are associated with the tyrosine catabolic pathway:

- ✓ **Type I tyrosinemia (tyrosinosis)** results from defective **fumarylacetoacetate hydrolase**.
- ✓ **Type II tyrosinemia (Richner-Hanhart syndrome),** a defect in **tyrosine aminotransferase**.
- ✓ **Neonatal tyrosinemia,** due to lowered activity of ***p*-hydroxyphenylpyruvate hydroxylase**.
- ✓ **Alkaptonuria** is a defective **homogentisate oxidase**. The urine darkens on exposure to air due to oxidation of excreted homogentisate. Late in the disease, there is arthritis and connective tissue pigmentation (ochronosis) due to oxidation of homogentisate to benzoquinone acetate, which polymerizes and binds to connective tissue.

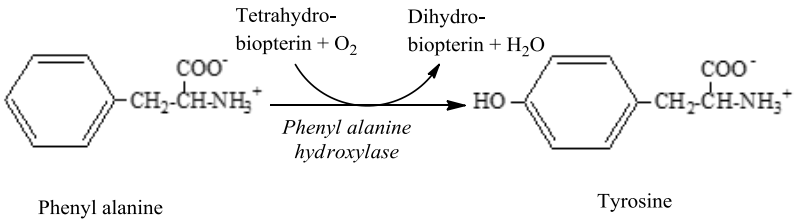
Except for alkaptonuria, these disorders result in elevated blood tyrosine levels.

Hypertyrosinemia results in liver disease or neurologic abnormalities, such as seizures or developmental delay. Therapy employs a diet low in tyrosine and phenylalanine.

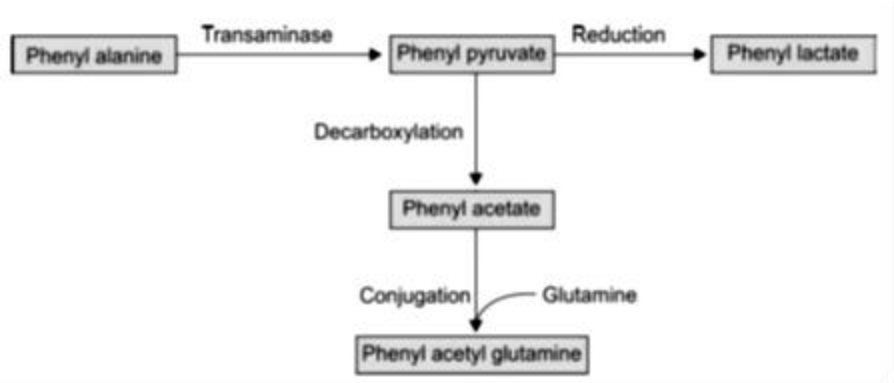


Phenylalanine

Hyperphenylalaninemias arise from defects in **phenylalanine hydroxylase** (**type I, classic phenylketonuria (PKU)**), in dihydrobiopterin reductase **(types II and III),** or in dihydrobiopterin biosynthesis **(types IV and V)**.



There are two routes by which the excess phenylalanine can be metabolized: oxidation to tyrosine (the normal and main route for degradation of phenylalanine, and the normal route for biosynthesis of tyrosine), and transamination to phenylpyruvate and subsequent further metabolism to phenyl lactate, phenylacetate, and phenylacetyl glutamine (a minor route, which comes to the fore when the main route is blocked).



- ✓ A diet low in phenylalanine can prevent the mental retardation of PKU.
- ✓ Elevated blood phenylalanine may not be detectable until 3 to 4 days postpartum.
- ✓ False-positives in premature infants may reflect delayed maturation of enzymes of phenylalanine catabolism.

Branched chain amino acids

Defect in the **α-keto acid dehydrogenase complex** results in **maple syrup urine disease (MSUD)** or **branched-chain ketonuria**. As the name implies, the odor of urine in suggests maple syrup, or burnt sugar.

Plasma and urinary levels of leucine, isoleucine, valine, and their α-keto acids and α-hydroxy acids (reduced α-keto acids) are elevated, but the urinary keto acids derive principally from leucine.

Signs and symptoms of MSUD include often, fatal ketoacidosis, neurological derangements, mental retardation, and a maple syrup odor of urine.

In **intermittent branched-chain ketonuria,** the α-keto acid dehydrogenase retains some activity, and symptoms occur later in life.

In **isovaleric acidemia,** ingestion of protein-rich foods elevates isovalerate, the deacylation product of isovaleryl-CoA. The impaired enzyme in **isovaleric acidemia** is **isovaleryl-CoA dehydrogenase**.

Signs include vomiting, acidosis, and coma follow ingestion of excess protein.

CLINICAL ENZYMOLOGY

In general, most enzymes are present in cells at much higher concentrations than in plasma. Measurement of their levels in plasma indicates whether their tissue of origin is damaged leading to the release of intracellular components into the blood. This forms the basis of clinical enzymology. Thus, **clinical enzymology** refers to measurement of enzyme activity for the diagnosis and treatment of diseases.

Since the tight control of enzyme activity is essential for homeostasis, any malfunction of a single critical enzyme (mutation, overproduction, underproduction or deletion) can lead to a genetic disease - commonly called **inborn errors of metabolism**. One example is the most common type of phenylketonuria caused by a mutation of a single amino acid in the enzyme phenylalanine hydroxylase, which catalyzes the first step in the degradation of phenylalanine. The deficiency results in build-up of phenylalanine and related un-physiological by-products. This can lead to mental retardation if the disease is untreated early.

Detection of the plasma level of an enzyme immunologically (for its protein amount) or colorimetrically (for its activity, preferred) have the following applications:

Diagnosis: detect and localize tissue cell damage or proliferation; e.g., high serum creatine phosphokinase (CK) on the day of a suspected case of myocardial infarction strengthen the diagnosis if ECG changes are doubtful.

Differential diagnosis: e.g., chest pain associates myocardial infarction and pulmonary embolism. Elevated serum aspartate transaminase (AST) and lactate dehydrogenase (LDH) characterizes myocardial infarction, whereas, elevated serum LDH only characterizes pulmonary embolism.

Therapeutic follow up and/or early detection of a disease:

Chronic administration of several therapeutics - e.g., anti-depressant and anticancer chemotherapies - elevates serum isocitrate dehydrogenase or ornithine carbamoyl-transferase level when they induce minimal hepatotoxicity.

Serum alanine transaminase (ALT) level elevates in sub-clinical early viral hepatitis.

PLASMA ENZYMES:

Enzymes present in plasma can be classified into **2 types**, they are: Functional Plasma enzymes and Non-functional plasma enzymes.

Plasma-derived enzymes (functional plasma enzymes):

- ✓ They are normally occurring functional plasma enzymes.
- ✓ Mostly synthesized by the liver.
- ✓ Usually decreased in disease conditions.
- ✓ Their field of activity is plasma components and their activity is higher in plasma than in cells, e.g., coagulation and lipoprotein-metabolizing enzymes.
- ✓ Their clinical importance is limited to diseases related to their own synthesis and function; i.e., abnormalities of metabolism of plasma lipoproteins and blood clotting, and the organ function of their synthesizing tissues, e.g., thromboplastin as a liver function test.

Cell-Derived enzymes (**Non-functional plasma enzymes**):

- ✓ Normally they locate to intracellular compartments; i.e., they are non-functional plasma enzymes.
- ✓ A very low plasma level normally exists due to normal wear and tear and diffusion through undamaged cell membranes.
- ✓ Gross damage to the cells or abnormal membrane permeability, overproduction of the enzymes or abnormal high cellular proliferation may allow their leakage in abnormally high amount into plasma and other body fluids.
- ✓ The amount and nature of the plasma enzyme(s) reflects the extent and nature of the damaged tissue.
- ✓ Measurement of these enzymes in plasma can be used to assess cell damage and proliferation i.e. diagnosis of disease.
- ✓ **They are further subdivided** into; secretory and metabolic non-functional plasma enzymes:

Secretory: They are synthesized and secreted by specialized glands into body lumens mainly for digestion. Their retrograde escape into blood reflects damage in the tissue of their origin, e.g., pancreatic amylase and lipase in pancreatitis.
Metabolic: They are intracellular metabolic enzymes and their appearance in the plasma is mainly due to cellular damage among other factors.

The cell derived enzyme activity in plasma may be:
Higher than normal, due to the proliferation of cells, an increase in the rate of cell turnover or damage or in enzyme synthesis (induction), or to reduced clearance from plasma (As the case for other plasma proteins, enzymes have specific plasma half-life after which they are disposed by cellular reuptake, degradation and/or excretion in bile or urine).
Lower than normal, due to reduced synthesis, congenital deficiency or the presence of inherited variants of relatively low biological activity – examples of the latter are the cholinesterase variants.

ASSESSMENT OF CELL DAMAGE AND PROLIFERATION

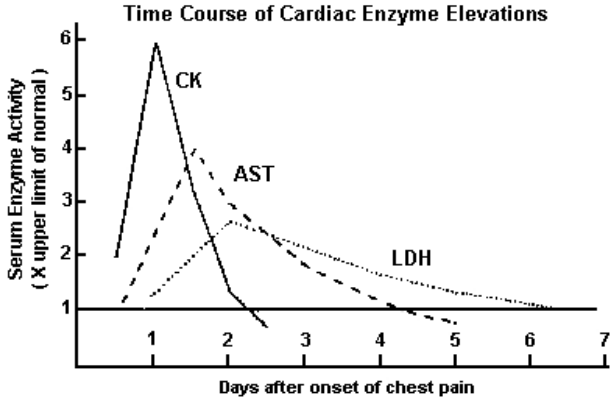
Plasma enzyme levels depend on the extent of cell damage and the rate of release from damaged cells, which, in turn, depends on the rate at which damage is occurring. In the absence of cell damage, the rate of release depends on the degree of induction of enzyme synthesis and the rate of cell proliferation. These factors are balanced by the rate of enzyme clearance from the circulation.

Acute cell damage, for example in viral hepatitis, may cause very high plasma aminotransferase activities that reduce as the condition resolves. By contrast, the liver may be much more extensively involved in advanced cirrhosis but the *rate* of cell damage is often low, and consequently plasma enzyme activities may be only slightly raised or within the reference range. In very severe liver disease, plasma enzyme activities may even fall terminally when the number of hepatocytes is grossly reduced.

Relatively small enzymes, such as amylase, can be cleared by the kidneys. Thus, plasma amylase activity may be high as a result of renal glomerular impairment rather than pancreatic damage. However, most enzymes are large proteins and may be catabolized by plasma proteases before being taken up by the reticuloendothelial system.

In healthy individuals, each enzyme has a fairly constant and characteristic biological half-life, a fact that may be used to assess the time since the onset of an acute

illness. After a myocardial infarction, for example, plasma levels of CK and aspartate aminotransferase (AST) fall to normal before those of LDH, which has a longer half-life.



Localization of damage

Most of the enzymes commonly measured to assess tissue damage are present in nearly all body cells, although their relative concentrations in certain tissues may differ. The following table lists several enzymes of value in clinical diagnosis.

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson disease)
Creatine kinase	Muscle disorders and myocardial infarction
γ-Glutamyl transferase	Various liver diseases
Lactate dehydrogenase isozyme 5	Liver diseases
Lipase	Acute pancreatitis
β-Glucoscerebrosidase	Gaucher disease
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

Note: Many of the above enzymes are not specific to the disease listed

Measurement of the plasma activity of an enzyme known to be in high concentration within cells of a particular tissue may indicate an abnormality of those cells, but the results will rarely enable a specific diagnosis to be made. For example, if there is circulatory failure after a cardiac arrest, very high plasma concentrations of enzymes originating from many tissues may occur because of hypoxic damage to cells and reduced rates of clearance.

The distribution of enzymes within cells may differ. Alanine aminotransferase (ALT) and LDH are predominantly located in cytoplasm, and glutamate dehydrogenase (although this is not usually measured clinically) in mitochondria, whereas AST occurs in both these cellular compartments. Different disease processes in the same tissue may affect the cell in different ways, causing alteration in the relative plasma enzyme activities.

The diagnostic precision of plasma enzyme analysis may be improved by the following:

Serial enzyme estimations. The rate of change of plasma enzyme activity is related to a balance between the rate of entry and the rate of removal from the circulation. A persistently raised plasma enzyme activity is suggestive of a chronic disorder or, occasionally, impaired clearance.

Isoenzyme determination. Some enzymes exist in more than one form; these isoenzymes may be separated by their different physical or chemical properties. If they originate in different tissues, such identification will give more information than the measurement of plasma total enzyme activity; for example, CK may be derived from skeletal or cardiac muscle, but one of its isoenzymes is found predominantly in the myocardium.

Estimation of more than one enzyme. Many enzymes are widely distributed, but their relative concentrations may vary in different tissues. For example, although both ALT and AST are abundant in the liver, the concentration of AST is much greater than that of ALT in heart muscle.

Non-specific causes of raised plasma enzyme activities

Before attributing a change in plasma enzyme activity to a specific disease process, it is important to exclude the presence of factitious or non-specific causes. Slight rises in plasma ALT and AST activities are common, non-specific findings in many illnesses. Moderate exercise, or a large intramuscular injection, may lead to a rise in plasma CK activity; isoenzyme determination may identify skeletal muscle as the tissue of origin. Some drugs, such as the anticonvulsants phenytoin and phenobarbital, may induce the synthesis of the microsomal enzyme γ-glutamyl transferase (GGT), and so increase its plasma activity in the absence of disease.

Plasma enzyme activities may be raised if the rate of clearance from the circulation is reduced. In the absence of hepatic or renal disease, this may occur if, for example, the plasma enzyme forms complexes with immunoglobulins, known as a macroenzyme. Various enzymes can form clinically significant macroenzymes including amylase, LDH, ALP and CK.

FACTORS AFFECTING RESULTS OF PLASMA ENZYME ASSAYS

Analytical factors

The total concentration of all plasma enzyme proteins is less than 1 g/L. The results of enzyme assays are not usually expressed as concentrations, but as activities.

These activities are usually measured in terms of the activity unit (**U**) which is defined as the amount which will catalyse the transformation of 1 micromole of the substrate per minute.

Another unit of enzyme activity has been used. This is the **katal** (**kat**) which is defined as the amount which will catalyse the transformation of one mole of substrate per second. It is an impracticable unit and has not received widespread acceptance.

Changes in concentration may give rise to proportional changes in catalytic activity, but the results of such measurements depend on many analytical factors, including:

substrate concentration, product concentration, enzyme concentration, reaction temperature, reaction pH, and presence of activators or inhibitors.

The definition of ‘international units’ does not take these factors into account, and the results from different laboratories, which are apparently expressed in the same units, may not be directly comparable.

Non-disease factors

Examples of non-disease factors affecting enzyme activities include the following.

Age

Plasma AST activity is moderately higher during the neonatal period than in adults. Plasma ALP activity of bony origin is higher in children than in adults and peaks during the pubertal bone growth spurt before falling to adult levels. A second peak occurs in the elderly.

Sex

Plasma GGT activity is higher in men than in women. Plasma CK activity is also higher in males, probably in part due to their increased muscle bulk.

Race/ethnicity

Plasma CK activity is higher in black people and Afro-Caribbeans than in white people.

Physiological conditions

Plasma ALP activity rises during the last trimester of pregnancy because of the presence of the placental isoenzyme. Several enzymes, such as AST and CK, rise moderately in plasma during and immediately after labour or strenuous exercise.

Plasma enzyme activities should therefore be interpreted in relation to the sex-, race-ethnicity- and age-matched reference ranges of the issuing laboratory.

Isoenzymes and their Clinical Significance in Clinical Enzymology

Isoenzymes (also known as **isozymes**) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. Isoenzymes are produced by different genes and are not redundant despite their similar functions. They occur in many tissues throughout the body and are important for different developmental and metabolic processes.

Isoenzymes are useful biochemical markers and can be measured in the bloodstream to diagnose medical conditions. Isoenzymes can be differentiated from one another using gel electrophoresis. In gel electrophoresis, isoenzyme fragments are drawn through a thick gel by an electric charge. Each isoenzyme has a distinct charge of its own because of its unique amino acid sequence. This enables gel electrophoresis to separate the fragments into bands for identification. Some clinically important isoenzymes are as follows:

1) Creatine Kinase (CK)

It is an enzyme found primarily in the heart and skeletal muscles, and to a lesser extent in the brain. Small amounts are also found in lungs, liver, thyroid and adrenal glands. Significant injury to any of these structures will lead to a measurable increase in CK levels. It is not found in red blood cells and its level is not affected by hemolysis.

CK Isoenzymes

There are three Isoenzymes. Measuring them is of value in the presence of elevated levels of CK to determine the source of the elevation. Each isoenzyme is a dimer composed of two protomers ‘M’ (for muscles) and ‘B’ (for Brain).

The three possible isoenzymes are;

Isoenzyme	Electrophoretic mobility	Tissue of origin	Mean percentage in blood
MM(CK3)	Least	Skeletal muscle	97-100%
MB(CK2)	Intermediate	Heart muscle	0-3%
BB(CK1)	Maximum	Brain	0%

Following an MI attack of acute myocardial infarction, this isoenzyme appears within 4 hours following onset of chest pain, reaches a peak of activity at approximately 24 hours and falls rapidly.

Atypical Isoenzymes: In addition to the above three isoenzymes two atypical iso enzymes of CK have been reported. They are; Macro CK(CK-Macro) and Mitochondrial CK(CK-Mi).

- *Macro CK(CK-Macro)*: It is formed by the aggregation of CK with immunoglobulins usually with IgG but sometimes IgA. It may also be formed by complexing CK with lipoproteins. No specific disease has been found to be associated with this isoenzyme.
- *Mitochondrial CK(CK-Mi)*: It presents bound to the inner mitochondrial membrane of muscle, liver and brain. It can exist in dimeric form or as oligomeric aggregates having molecular weight of approximately 35,000. It presents in serum only when there is extensive tissue damage causing breakdown of mitochondrial membrane and cell membrane. Thus, its presence in serum indicates severe illness and cellular damage. It is not related with any specific disease states but it has been detected in certain cases of malignant tumors.

2) Lactate dehydrogenase (LDH)

Lactate dehydrogenase catalyzes the reversible conversion of lactate and pyruvate. LDH is essential for anaerobic respiration. When oxygen levels are low, LDH converts pyruvate to lactate, providing a source of muscular energy.

LDH level is 100 times more inside the RBCs than in plasma, and therefore minor amount of hemolysis results in false positive result.

In Acute MI-The serum activity rises within 12 to 24 hours, attains a peak at 48-72 hours, and then returns gradually to normal from 7^{th} to 12^{th} day. The magnitude of rise is proportional to the extent of myocardial infarction.

Other diseases: The increase in serum activity of LDH is also seen in hemolytic anemia, hepatocellular damage, muscular dystrophies, carcinoma, leukemias, and any condition which causes necrosis of the body cells. Since the total LDH is increased in many diseases, so the study of Isoenzymes of LDH is of more significance.

Isoenzymes of LDH

LDH enzyme is tetramer with 4 subunits. The subunit may be either H(Heart) or M(Muscle) polypeptide chains. These two chains are the product of 2 different genes. Although both of them have the same molecular weight, there are minor amino acid variations. There can be 5 possible combinations; H4, H3M1, H2M2, H1M3, and M4. Thus, there are 5 different types of isoenzymes seen in all individuals.

No. of Isoenzyme	Subunit make up of isoenzyme	Electrophoretic mobility	Tissue origin	Percentage in human serum (Mean)
LDH-1	H4	Fastest	Heart muscle	30%
LDH-2	H3M1	Faster	RBC	35%
LDH-3	H2M2	Fast	Brain	20%
LDH-4	H1M3	Slow	Liver	10%
LDH-5	M4	Slowest	Skeletal Muscles	5%

Normally LDH-2 (H3M1) level in blood is greater than LDH-1, but this pattern is reversed in myocardial infarction, this is called **'flipped pattern'**.

3) Alkaline phosphatase (ALP)

It is an enzyme that removes phosphate groups from organic or inorganic compounds in the body. It is present in a number of tissues including liver, bone, intestine, and placenta. The activity of ALP found in serum is a composite of isoenzymes from those sites. A placental-like, so-called 'Regan' and 'Nago' isoenzymes may occasionally be identified in plasma in patients with malignant disease.

The optimum pH for enzyme action varies between 9-10. It is a zinc containing metalloenzyme and is localized in the cell membranes (ectoenzyme). It is associated with transport mechanism in the liver, kidney and intestinal mucosa.

In children, the upper level of normal value may be more, because of increased osteoblastic activity.

ALP Isoenzymes

ALP has many isoenzymes (the following order represents their speed and position on the electrophoretic graph).

i. **Hepatic ALP isoenzyme:** It is associated with biliary epithelium and is elevated in cholestatic processes. Various liver diseases increase the liver isoenzyme but extrahepatic biliary obstruction is associated with the higher levels.
ii. **Bone isoenzyme:** Osteoblastic bone tumors and hyperactivity of osteoblasts involved in bone remodeling (eg, Paget's disease) increase the bone isoenzyme. Paget's disease leads to a striking, solitary elevation of bone ALP.
iii. **Placental isoenzyme:** It increases during the third trimester of pregnancy.
iv. **The intestinal isoenzyme:** It may be increased in patients with cirrhosis and in patients undergoing hemodialysis due to decrease clearance. Individuals with blood types O and B can have elevated levels of intestinal ALP. The levels of serum ALP increase after eating a fatty meal due to the influx of intestinal alkaline phosphatase into the blood.

Atypical ALP isoenzymes (Oncogenic markers)-In addition to 4 major isoenzymes, 2 more abnormal fractions are seen associated with tumors. These are Regan and Nagao isoenzymes. They are also called "**Carcino placental ALP",** as they resemble placental isoenzymes.

Other Clinical Applications of Enzymes

1. Enzyme-Linked Immunoassays

The sensitivity of enzyme assays can be exploited to detect proteins that lack catalytic activity. **Enzyme-linked immunosorbent assays** (ELISAs) use antibodies covalently linked to a "reporter enzyme" such as alkaline phosphatase or horseradish peroxidase whose products are readily detected, generally by the absorbance of light or by fluorescence. Serum or other biologic samples to be tested are placed in plastic, multi-well microtiter plates, where the proteins (the antigen) immobilized on the plastic surface either non-specifically (via adsorption) or specifically (via capture by another antibody specific to the same antigen). Any exposed plastic (or fixed antibodies) that remains is subsequently "blocked" by adding a nonantigenic protein such as bovine serum albumin. A solution of antibody covalently linked to a reporter enzyme (detection antibodies) is then added. The antibodies adhere to the immobilized antigen and are themselves immobilized. Excess free detection antibody molecules are then removed by washing. The presence and quantity of bound antibody is then determined by adding the substrate for the reporter enzyme.

Detection of Troponins by ELISA

Troponin, or the troponin complex, is a complex of three regulatory proteins (troponin C, troponin I, and troponin T) that is integral to muscle contraction in *skeletal muscle* and *cardiac muscle*, but **not in smooth muscle**. Immunological measurement of plasma levels of **cardiac troponins** I and T provide sensitive and specific indicators of damage to heart muscle. Cardiac troponin levels rise for 2 to 6 hours after an MI and remain elevated for 4 to 10 days. In addition to MI, other heart muscle damage also elevates serum troponin levels. Cardiac troponins thus serve as a marker of all heart muscle damage.

Note: *The genes that encode for the skeletal and cardiac isoforms of troponin C are identical; thus, no structural difference exists between them. However, the skeletal and cardiac subforms for troponin I and troponin T are distinct, and immunoassays have been designed to differentiate between them.*

2. Enzymes also can be employed in the clinical laboratory as tools for determining the concentration of critical metabolites.

For example, glucose oxidase is frequently utilized to measure plasma glucose concentration.

3. Enzymes facilitate diagnosis of genetic diseases.

Many diagnostic techniques take advantage of the specificity and efficiency of the enzymes that act on oligonucleotides such as DNA. Enzymes known as **restriction endonucleases,** for example, cleave double-stranded DNA at sites specified by a sequence of four, six, or more base pairs called **restriction sites.** Cleavage of a sample of DNA with a restriction enzyme produces a characteristic set of smaller DNA fragments. Deviations in the normal product pattern, called **restriction fragment length polymorphisms (RFLPs)**, occur if a mutation renders a restriction site unrecognizable to its cognate restriction endonuclease or, alternatively, generates a new

recognition site. RFLPs are currently utilized to facilitate prenatal detection of a number of hereditary disorders, including sickle cell trait, β-thalassemia, infant phenylketonuria, and Huntington disease.

4. Medical applications of the Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) employs a thermostable DNA polymerase and appropriate oligonucleotide primers to produce thousands of copies of a defined segment of DNA from a minute quantity of starting material. PCR enables medical, biological, and forensic scientists to *detect and characterize DNA present initially at levels too low for direct detection*. In addition to *screening for genetic mutations*, PCR can be used to detect and *identify pathogens and parasites*, through the selective amplification of their DNA.

5. Enzymes are employed with increasing frequency as tools for the treatment of injury and disease.

Tissue plasminogen activator (tPA) or streptokinase is used in the treatment of acute MI, while trypsin has been used in the treatment of cystic fibrosis. Intravenous infusion of recombinantly produced enzymes has been approved for the treatment of several lysosomal storage diseases (are a group of approximately 50 rare inherited metabolic disorders that result from defects in lysosomal function).

Conversion of Amino Acids to Specialized Products

Certain proteins contain amino acids that have been posttranslationally modified to permit them to perform specific functions. One example is the hydroxylation of lysine to 5-hydroxylysine, whose subsequent modification and cross-linking stabilizes maturing collagen fibers.

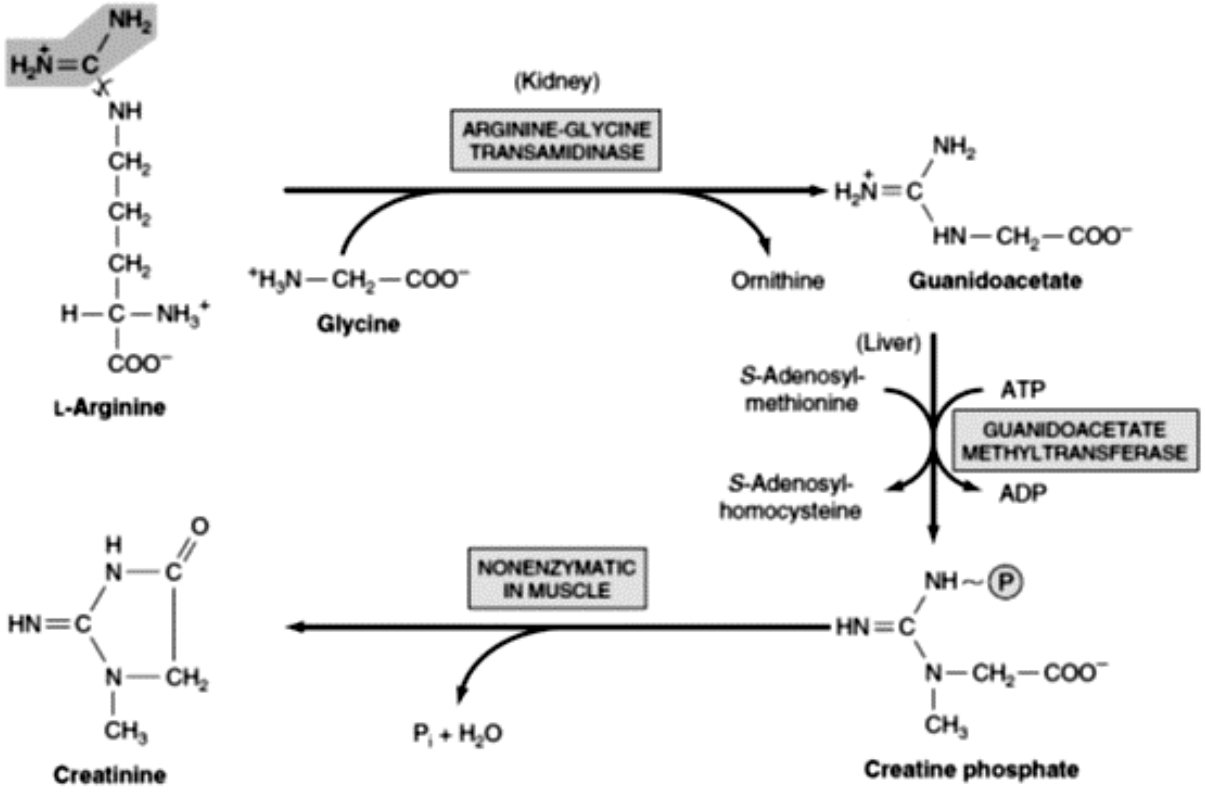
In addition to serving as the building blocks for protein synthesis, amino acids serve as precursors of diverse biologic materials.

Some important biological molecules derived from amino acids

Creatine and Creatinine:

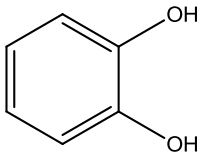
Glycine, **arginine**, and **methionine** all participate in creatine biosynthesis. The first step occurs in kidney which involve transfer of amidine group from arginine to glycine to form guanidinoacetic acid; this step is catalyzed by **arginine-glycine transamidinase**. The second step occur in the liver, which involve the transfer of a methyl group from S-adenosylmethionine to form creatine; catalyzed by **guanidinoacetate methyltransferas**. Creatine is phosphorylated by **creatine kinase** into creatine phosphate (or phospho creatine), which is stored in the muscle and acts as energy store.

Creatinine is formed in muscle from creatine phosphate by **irreversible, nonenzymatic dehydration**, and loss of phosphate (used for muscle contraction). The amount of creatinine produced is constant from day to day and depend on muscle mass. Creatinine is excreted in urine.



Catecholamines:

The name catechol refers to the **dihydroxylated phenyl ring**. The amine derivatives of catechol are called catecholamines.



Catechol

Tyrosine is the precursor for the synthesis of catecholamines, namely **dopamine**, **norepinephrine** (noradrenaline) and **epinephrine** (adrenaline).

The conversion of tyrosine to catecholamines occurs in adrenal medulla and central nervous system involving the following reactions:

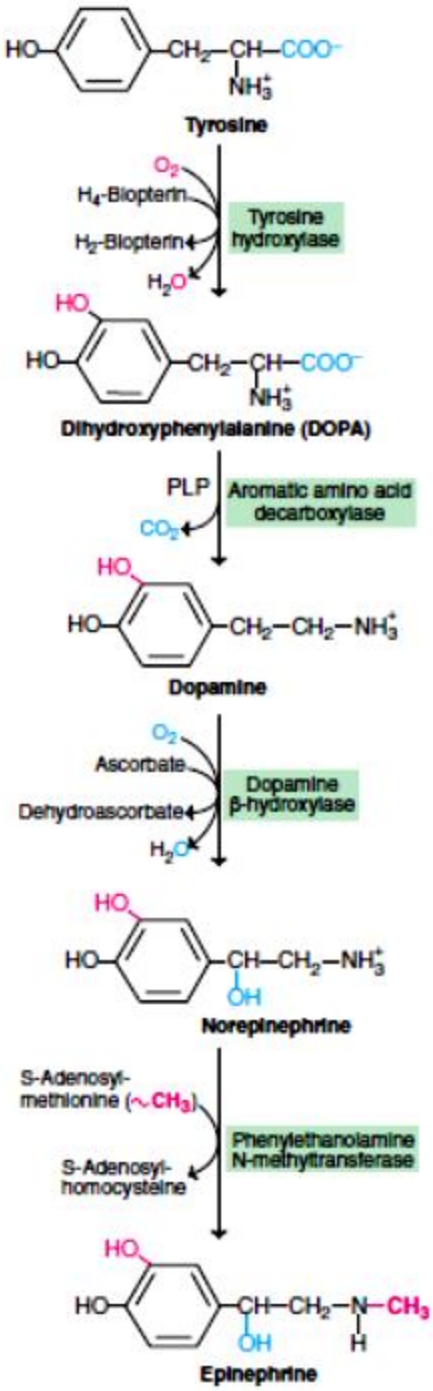
Tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) by **tyrosine hydroxylase**. This enzyme catalyses the **rate limiting** reaction and requires tetrahydrobiopterin as coenzyme.

DOPA undergoes PLP-dependent decarboxylation, catalyzed by **aromatic amino acid decarboxylase**, to give dopamine. In turn, dopamine is hydroxylated by **dopamine β-hydroxylase** to produce norepinephrine. Methylation of norepinephrine by S-adenosylmethionine, catalyzed by **phenylethanolamine N-methytransferase** gives epinephrine.

Norepinephrine and epinephrine regulate carbohydrate and lipid metabolisms. They stimulate the degradation of triacylglycerol and glycogen. They cause an increase in the blood pressure. Dopamine and norepinephrine serve as neurotransmitters in the brain and autonomous nervous system.

NOTE:

In contrast to this enzyme, **tyrosinase** present in **melanocytes** converts tyrosine to DOPA. ***Hence, two different enzyme systems exist to convert tyrosine to DOPA.***



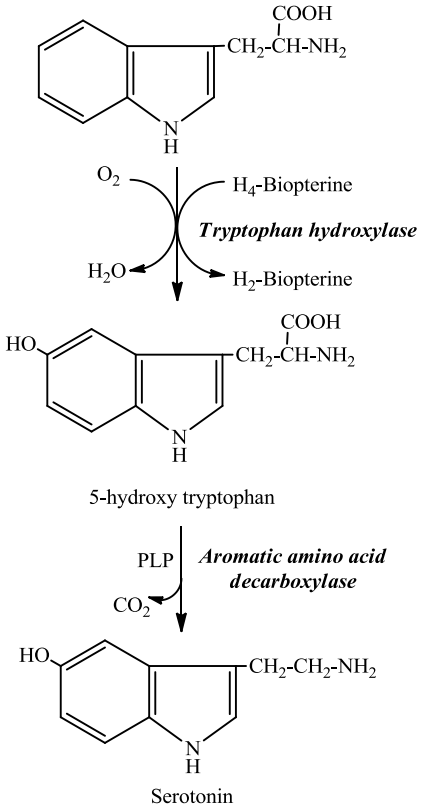
In melanocytes, **tyrosinase** hydroxylates tyrosine to form DOPA. DOPA in turn is converted to dopaquinone, followed by couple of spontaneous reactions occur, forming leucodopachrome then 5,6-dihydroxyindole. Oxidation of 5,6-dihydroxyindole yeilds indole 5,6-quinone.

Melanochromes are formed from indole quinone, which on **polymerization** are converted to **black melanin**.

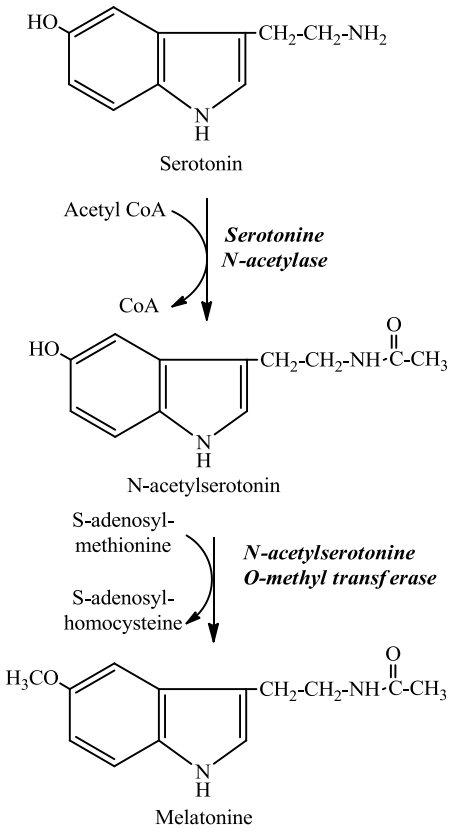
Deficiency of tyrosinase results in **albinism**.

Serotonin and Melatonin:

Serotonin or **5-hydroxytryptamine** (5HT) is a neurotransmitter, synthesized from tryptophan. Tryptophan is first hydroxylated at 5th carbon by **tryptophan hydroxylase**. This enzyme requires tetrahydrobiopterin as a coenzyme. 5-Hydroxytryptophan is decarboxylated by **aromatic amino acid decarboxylase** (PLP dependent) to give serotonin.



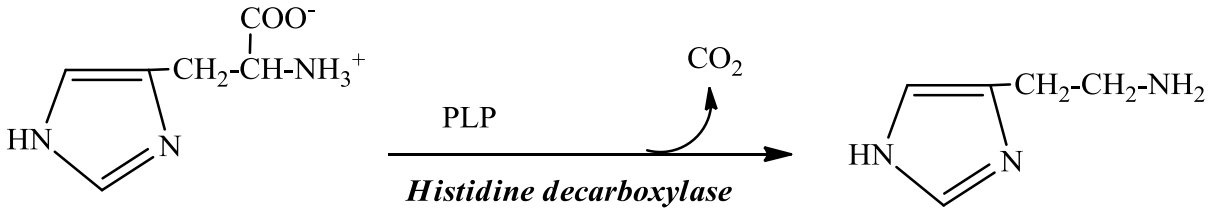
Melatonin or N-acetyl 5-methoxyserotonin is a hormone, mostly synthesized by the **pineal gland.** Serotonin (produced from tryptophan) is acted upon by **serotonin N-acetylase** (the rate limiting reaction), to give N-acetylserotonin. The latter undergoes O-methylation catalyzed by **N-acetylserotonine O-methyltrransferase**, S-adenosylmethionine being the methyl group donor to produce N-acetyl,5-methoxyserotonin (melatonin). The synthesis and secretion of melatonin from pineal gland is controlled by light.



Histamine:

A biogenic amine that functions in allergic reactions and gastric acid secretion, histamine is present in all tissues.

Histamine is formed by **decarboxylation of histidine**; the reaction is catalyzed by the pyridoxal phosphate-dependent enzyme **histidine decarboxylase**.

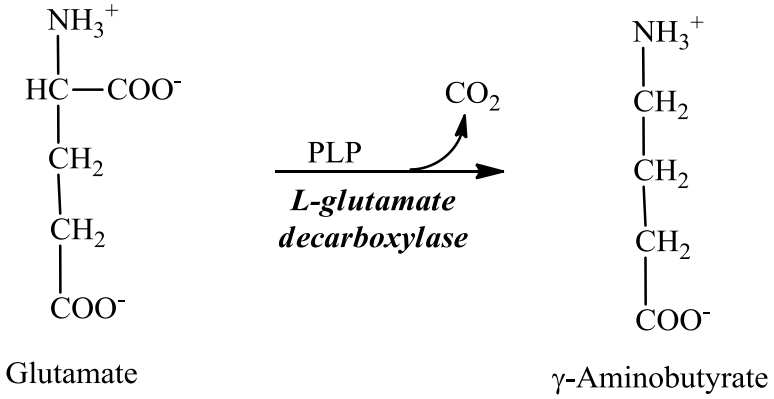


Histidine

Histamine

γ-aminobutyric acid (GABA):

γ-aminobutyrate (GABA) functions in brain tissue as an inhibitory neurotransmitter. GABA is formed by **decarboxylation** of glutamate by **L-glutamate decarboxylase** (PLP dependent).

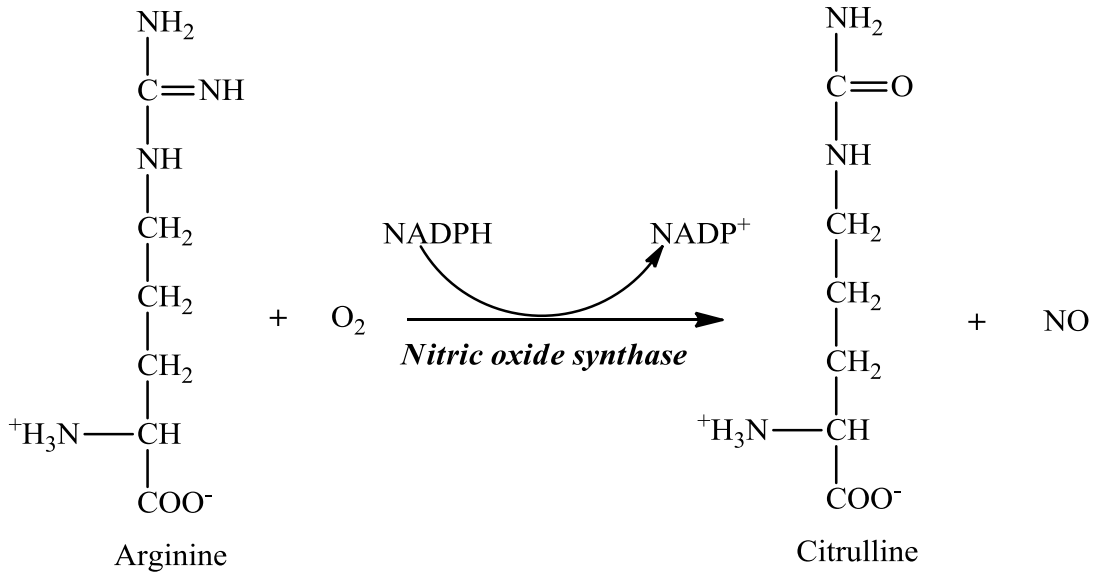


Glutamate γ-Aminobutyrate

Nitric oxide (NO):

In addition to serving as a carrier of nitrogen atoms in urea and creatine biosynthesis, arginine is also the source of nitric oxide (NO). NO is an intercellular signaling molecule that serves as a neurotransmitter, smooth muscle relaxant, vasodilator, and prevents platelet aggregation.

The biosynthetic reaction of NO is catalyzed by **NO synthase** (NOS), which converts one nitrogen of the guanidine group of arginine to NO.

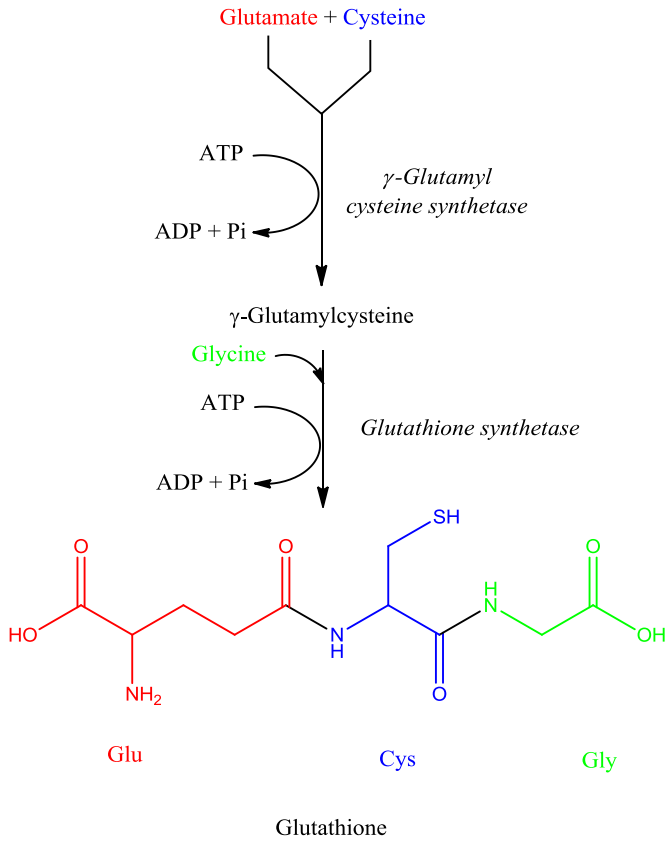


Arginine Citrulline

Glutathione (GSH):

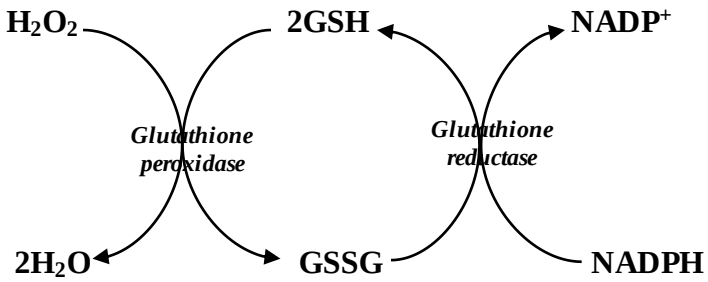
Glutatione is γ-Glutamylcysteinylglycine. γ-glutamate is attached via the γ-carbon instead of the α-carbon. The active part is the -SH group of cysteine (sulfhydryl group).

Biosynthetic reactions of GSH are catalyzed by **γ-glutamyl cysteine synthetase** and **GSH synthetase**.



Glutathione

GSH in the reduced form has free -SH group. Two molecules can be bridged by a disulfide bond; which produces the oxidized form (GS-SG). Conversion reactions between the reduced and the oxidized forms are catalyzed by **glutathione peroxidase** and **glutathione reductase.**
By this mechanism GSH peroxidase and GSH reductase **scavenge peroxide free radicals.**



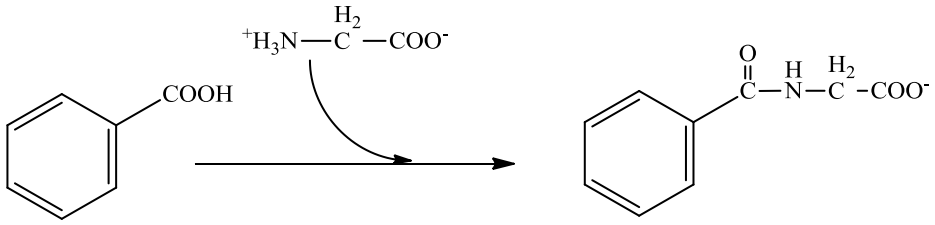
Other functions of GSH include; **conjugation** of lipophilic drugs which converts them to hydrophilic molecules for excretion, and **transport of amino acids** especially in the renal epithelium.

NOTE:

In addition to the role of glutathione in conjugation reactions, glycine also performs two important functions in this regard:

(a) The bile acids, cholic acid and chenodeoxy cholic acid are conjugated with glycine, forming glycocholic acid and glycochenodeoxy cholic acid respectively

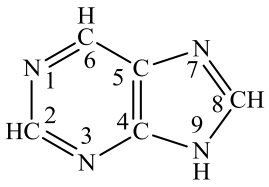
(b) Glycine is important for **detoxification** of benzoic acid (commonly used as a food preservative) to hippuric acid.



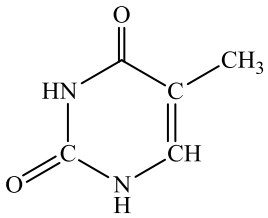
Benzoic acid Hippuric acid

Formation of purine and pyrimidine rings:

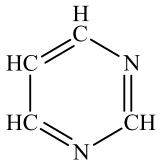
Glycine and serine are involved in purine and pyrimidine rings biosynthesis. The entire molecule of glycine is utilized for the formation of carbons 4 and 5 and nitrogen at position 7 of purines. Serine provides carbons 2 and 8 of purines and the methyl group of thymine.



Purine rings



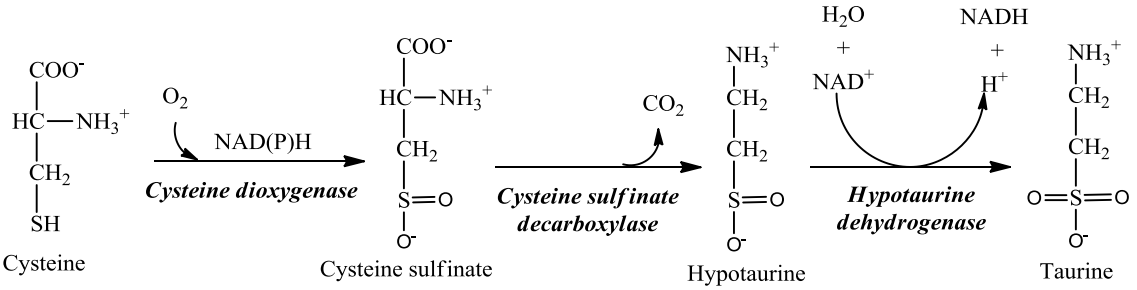
Thymine



Pyrimidine ring

Taurine:

Three enzyme catalyze reactions that convert cysteine to taurine, which can displace the coenzyme A moiety of cholyl-CoA to form the bile acid taurocholic acid. The conversion of cysteine to taurine is initiated by its *oxidation* to cysteine sulfinate, catalyzed by the enzyme **cysteine dioxygenase**. *Decarboxylation* of cysteine sulfinate by **cysteine sulfinate decarboxylase**, forms hypotaurine, whose *oxidation* by **hypotaurine dehydrogenase** forms taurine.

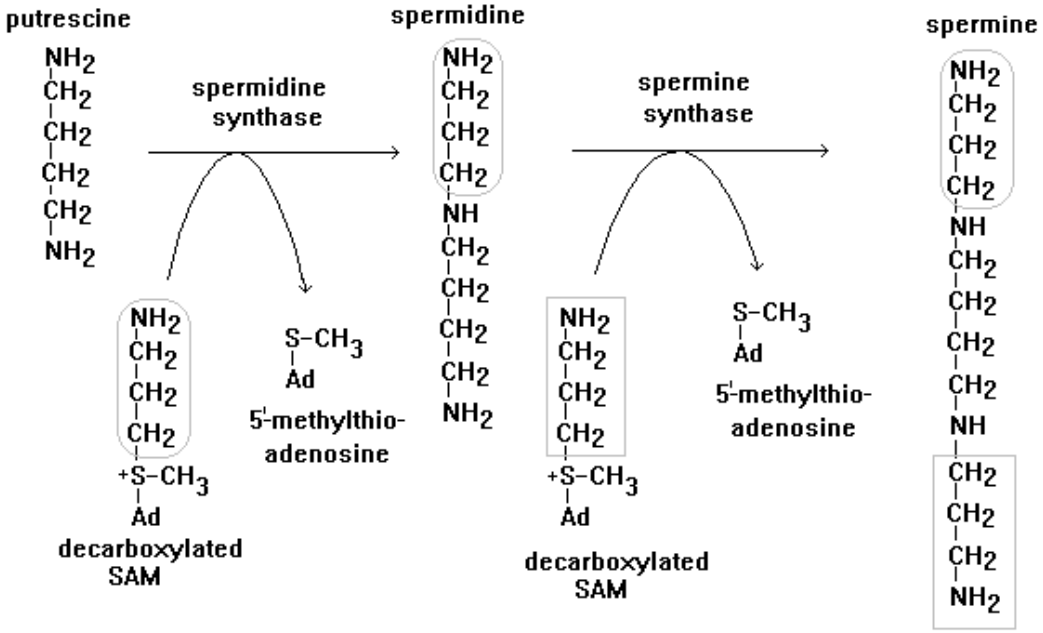


Spermine and spermidine:

These polyamines function in cell proliferation and growth. Following decarboxylation of *S*-adenosylmethionine by **S-adenosylmethionine decarboxylase**, three carbons and the α-amino group of methionine contribute to the biosynthesis of spermine and spermidine.

Decarboxylated ornithine (by **ornithine decarboxylase**) which is called *putrescine*, react with decarboxylated SAM to form spermidine. This reaction is catalyzed by **spermidine synthase.**

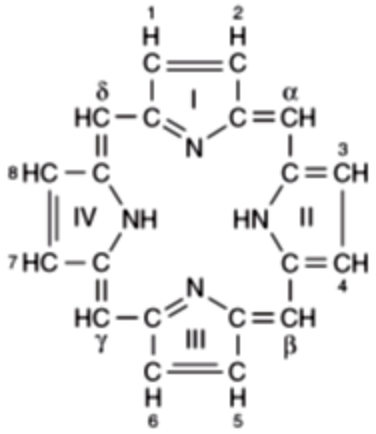
Another molecule of decarboxylated SAM react with spermidine to form spermine, this reaction is catalyzed by **spermine synthase**.



Porphyrins and Bile Pigments

The biochemistry of the porphyrins and of the bile pigments are closely related topics. Heme is synthesized from porphyrins and iron, and the products of degradation of heme are the bile pigments and iron.

Porphyrins are cyclic compounds formed by the linkage of four **pyrrole rings** through **methyne** (=HC–) bridges (also called **methenyl** bridges). In the naturally occurring porphyrins, various side chains replace the eight numbered hydrogen atoms of the pyrroles.



Porphyrin
($C_{20}H_{14}N_4$)

- *Roman numerals designate the pyrrole rings.*
- *Arabic numbers indicate positions at which substituents may be attached (1 through 8).*
- *Greek letters denote methyne bridges (=HC–).*

The common substituents are often abbreviated as follows:

A = acetic acid (–CH_2–COOH)
P = propionic acid (–CH_2–CH_2–COOH)
M = methyl (–CH_3)
V = vinyl (–CH=CH_2)

Names of Porphyrins:

The names of the porphyrins of interest consist of a **word** and a **number**, e.g., uroporphyrin III. The word denotes the kinds of substituents found on the ring, and the number denotes how they are arranged.

There are three important **words**:

- ✓ **Uroporphyrin** contains A and P only
- ✓ **Coproporphyrin** contains M and P only (A has been changed to M)
- ✓ **Protoporphyrin** contains M and P and V (some P has been changed to V)

There are two important **numbered series**, **I** and **III**.
(Series II and IV do not occur in natural systems).

- ✓ In series I the substituents repeat in a regular manner, e.g., APAPAPAP (starting with ring I).
- ✓ In series III the order of substituents in ring IV is reversed: APAPAP**PA**.

If three kinds of groups are present, as in the protoporphyrins, its immediate precursor is variously referred to as protoporphyrin III or protoporphyrin IX (being the 9^{th} isomer to be discovered).

Water Solubility of Porphyrins:
Depends on number of carboxylate groups, $-COO^-$

- ✓ Uroporphyrins, 8 carboxylates (more soluble).
- ✓ Coproporphyrins, 4 carboxylates.
- ✓ Protoporphyrins, 2 carboxylates (less soluble).

This determines routes of excretion. Water soluble compounds are excreted in urine via kidney. While, water insoluble compounds are excreted in feces via GIT.

NOTE:
Porphyrins form complexes with metal ions that bind to the nitrogen atom of each of the four pyrrole rings.
Examples:

- ✓ **Iron porphyrins** such as the **heme** of hemoglobin.
- ✓ **Magnesium porphyrin** of **chlorophyll,** the photosynthetic pigment of plants.

Functions of Heme:

Heme proteins serve diverse functions including (but not limited to):

Protein	**Protein Function**
Hemoglobin	Transport of oxygen in blood
Myoglobin	Storage of oxygen in muscle
Cytochrome *c*	Involvement in the electron transport chain
Cytochrome P_{450}	Hydroxylation of xenobiotics
Catalase	Degradation of hydrogen peroxide
Tryptophan pyrrolase	Oxidation of tryptophan

Biosynthesis of Heme:

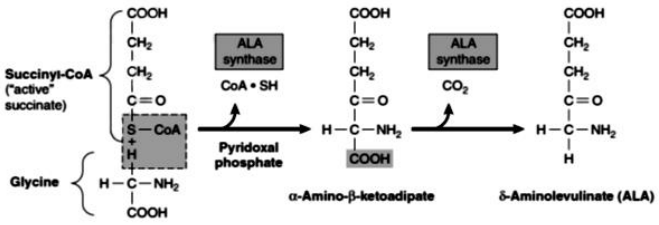
Site of synthesis

- ✓ Heme biosynthesis occurs in most mammalian cells except mature erythrocytes, which lack mitochondria. Approximately 85% of heme synthesis occurs in **bone marrow**, and the majority of the remainder in **hepatocytes.**
- ✓ Within the cells, part of the biosynthesis reactions occurs in the **mitochondria** and part in the **cytoplasm**.

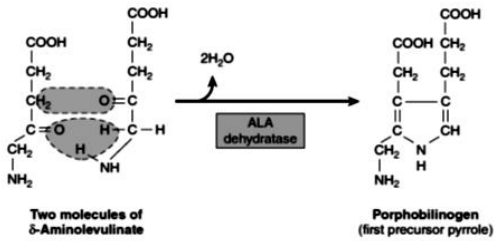
Reactions 1, 6, 7, and 8 take place in mitochondria.
Reactions 2, 3, 4, and 5 take place in cytoplasm.

Reaction of Heme Biosynthesis

1. Heme biosynthesis is initiated by the formation of δ-aminolevulinic acid (ALA) from glycine and succinyl-CoA, catalyzed by the *mitochondrial* **ALA synthase**. This reaction is the rate-limiting reaction of heme synthesis in all tissues, and it is therefore tightly regulated. The reaction is pyridoxal phosphate-dependent.

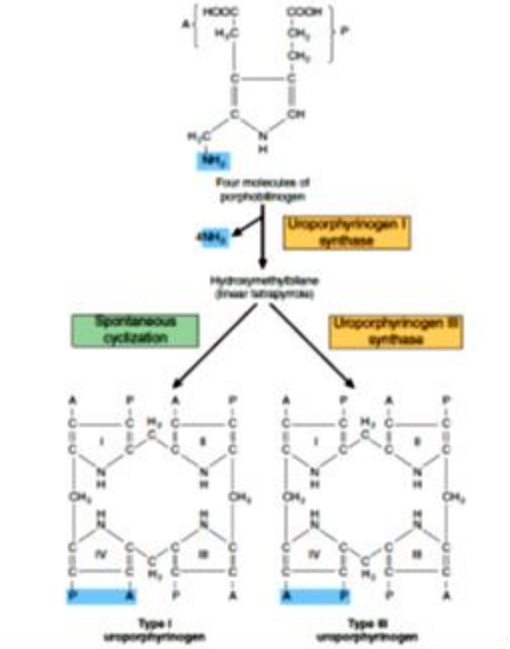


2. Two ALA molecules are joined together to form porphobilinogen (the first pyrrole) by the *cytoplasmic* enzyme, **ALA dehydratase**. ALA dehydratase is sensitive to inhibition by **lead,** as can occur in lead poisoning.

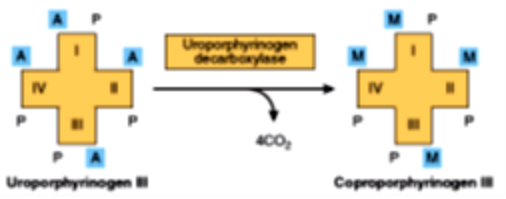


3. Condensation of four molecules of porphobilinogen catalyzed by the *cytoplasmic* **hydroxymethylbilane synthase** (uroporphyrinogen I synthase) forms **hydroxymethylbilane.** The four pyrrole rings in porphyrin are interconnected by methylene (-CH_2-) bridges derived from α-carbon of glycine.

4. Hydroxymethylbilane undergo cyclization reaction which is catalyzed by **uroporphyrinogen III synthase** to form uroporphyrinogen III. Hydroxymethylbilane can also cyclize spontaneously to form **uroporphyrinogen I**, but under normal conditions the uroporphyrinogen formed is almost exclusively the type III isomer. The type-I isomers of porphyrinogens are, however, formed in excess in certain porphyrias.



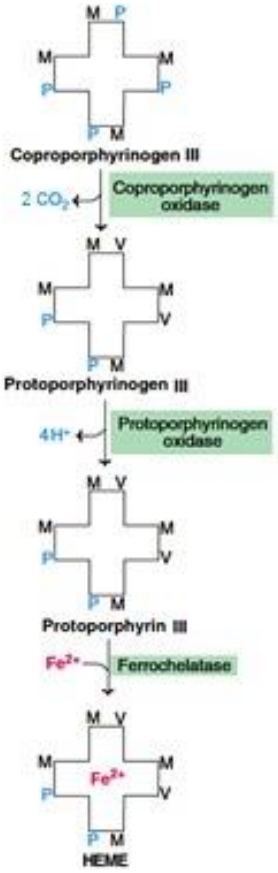
5. the *cytoplasmic* enzyme, **uroporphyrinogen decarboxylase** decarboxylates all the four acetate (A) side chains to form methyl groups (M), to form coproporphyrinogen III.
   Uroporphyrinogen decarboxylase can also convert uroporphyrinogen I, if present, to coproporphyrinogen I.



6. The coproporphyrinogen III formed is acted on by the enzyme, **coproporphyrinogen oxidase**, which by oxidative decarboxylation converts two of the propionate side chains (P) to vinyl groups (V) and results in the formation of protoporphyrinogen III.
   Coproporphyrinogen oxidase *is specific for type III coproporphyrinogen, so type I protoporphyrins generally do not occur in humans.*

7. Protoporphyrinogen III is next oxidized to **protoporphyrin III (also called IX)** in a reaction catalyzed by **protoporphyrinogen oxidase**.
   Protoporphyrinogen oxidase oxidizes methylene groups (-CH_2-) interconnecting pyrrole rings to methenyl groups (-CH=).

8. The final step of heme biosynthesis, catalyzed by the enzyme **ferrochelatase (heme synthase)**, is the insertion of ferrous iron into protoporphyrin III to produce heme. Ferrochelatase is inhibited by **lead**.



Regulation of heme synthesis:

- ✓ **Feedback regulation**: heme is a feedback inhibitor of ALA synthase. It represses the synthesis and diminishes the transport of ALA synthase from cytoplasm to mitochondria.
- ✓ **Substrate availability**: Fe^{2+} must be available for ferrochelatase.
- ✓ **Effects of drugs**: Many drugs whose metabolism requires the hemoprotein cytochrome P_{450} increase cytochrome P_{450} biosynthesis. The resulting depletion of the intracellular heme pool induces synthesis of ALA synthase

Porphyrinogens differ from porphyrins:

- ✓ Porphyrinogens have more hydrogen atoms and less double bonds than porphyrins.
- ✓ Porphyrinogens are color less, However, they are easily auto-oxidized to the **colored porphyrins**.
- ✓ Porphyrinogens are not fluorescent while porphyrins are illuminated by ultraviolet light and emit a strong red **fluorescence.**

Disorders of heme synthesis (porphyrias)

Porphyria can be classified depending on the etiology into:

- **Genetic** (defective enzyme).
- **Acquired** (e.g., lead poisoning).

Porphyrias may be divided into **two** major types based on the organs most affected:

- ✓ **Erythropoietic porphyria** is a defect of porphyrin synthesis of bone marrow.
- ✓ **Hepatic porphyria** is a defect in porphyrin synthesis of the liver.

Example of acquired porphyrias

Lead toxicity:

- ✓ Cause: inhibition of **ALA dehydratase** and **Ferrochelatase** by lead.
- ✓ Results: elevated levels of protoporphyrin in erythrocytes and elevated urinary levels of ALA and coproporphyrin.

Examples of hereditary porphyria

Congenital erythropoietic porphyria:

- ✓ Cause: Deficiency of uroporphyrinogen III synthase.
- ✓ Results: increase in type I porphyrin.

Acute intermittent porphyria:

- ✓ Cause: Deficiency of hepatic uroporphyrinogen I synthase.
- ✓ Result: increase in ALA and porphobilinogen.

NOTE:

Certain drugs (enzyme inducers e.g, barbiturates, griseofulvin) induce the production of cytochrome P_{450}. In patients with porphyria, this can precipitate an attack of porphyria (***drug-induced porphyria***) by depleting heme (the negative regulator of ALA synthase) levels. Thus, the rate of synthesis of ALA synthase is increased with the subsequent increase in levels of potentially harmful heme precursors.

Catabolism of Heme

Most of the heme which is degraded comes from **hemoglobin** in red blood cells, which have a life span of about 120 days. Human adults normally destroy about 200 billion erythrocytes per day. Thus, a 70-kg human turns over approximately **6 g of hemoglobin** daily. Since 1 g of hemoglobin yields about 35 mg of bilirubin, human adults form **250 to 350 mg of bilirubin per day**. The **globin** is degraded to its constituent amino acids, the released **iron** enters the iron pool, and all products are reused. The iron-free **porphyrin** portion of heme is also degraded.

1. Conversion of heme to bilirubin:

- ✓ **Site:**
- − Mainly in the reticuloendothelial cells of the **liver**, **spleen**, and **bone marrow**.
- ✓ **Enzymes:**
- − ***Heme oxygenase*** cleaves heme ring between the I and II pyrrole rings producing biliverdin (green) and carbon is released as carbon monoxide (CO).
- − ***Biliverdin reductase*** reduces the central (-CH=) bridge of biliverdin to ($-CH_2-$), producing bilirubin (red).

2. Transport of bilirubin in blood to the liver:

Bilirubin is only sparingly soluble in water. It must be transported in the blood by a **carrier**. The physiological carrier is serum **albumin**.

3. Uptake of bilirubin by hepatocytes:

- ✓ Hepatocytes take up bilirubin from albumin by a **large capacity**, saturable facilitated transport system. Thus, transport does not appear to be rate-limiting for the metabolism of bilirubin.
- ✓ The net uptake of bilirubin depends upon its removal by subsequent metabolism.
- ✓ Once internalized, bilirubin binds to cytosolic proteins such as glutathione *S*-transferase, to prevent bilirubin from reentering the blood stream.

4. Conversion of bilirubin to bilirubin diglucuronide (Conjugation):

- ✓ Bilirubin is **nonpolar,** and would persist in cells (e.g., bound to lipids) if not converted to a more water-soluble form.
- ✓ Bilirubin is converted to a more **polar** molecule by conjugation with glucuronic acid.
- ✓ A bilirubin-specific **UDP-glucuronyl transferase** catalyzes transfer of **two** glucuronyl moieties from UDP-glucuronate to bilirubin to form **bilirubin diglucuronide** in **two steps**.

5. Secretion of bilirubin diglucuronide into bile:

- ✓ It is an **active transport** mechanism.
- ✓ Probably is rate-limiting for the entire process of **hepatic bilirubin metabolism**.
- ✓ The protein involved is a **multispecific organic anion transporter** (**MOAT)** located in the plasma membrane of the bile canaliculi.

6. Processing of bilirubin diglucuronide by intestinal bacteria:

- ✓ In the intestine, the glucuronyl moieties of the conjugated bilirubin are removed by specific bacterial β-**glucuronidases** to reform bilirubin.
- ✓ Bilirubin in the intestine is converted to urobilinogens compounds most of them are excreted into the stool as **stercobilinogen** (cause of brown color of stools) after oxidation.
- ✓ A lesser amount (of urobilinogens) is recycled to the liver and either returned to bile or excreted in urine as urobilin (cause of yellow color of urine) after oxidation.
- ✓ Bilirubin and its catabolic products are collectively known as the **bile pigments**, which provide the yellow tinge in normal serum, the yellow-green hue in bile, the brown in stools, and the yellow in urine.

Hyperbilirubinemia causes Jaundice

- − **Hyperbilirubinemia**, a blood level that exceeds 1 mg of bilirubin per dL (17 μmol/L), may result from:
- ✓ **Production** of more bilirubin than the normal liver can conjugate, or
- ✓ Failure of a damaged liver to **conjugate** normal amounts of bilirubin, or
- ✓ **Obstruction** of the excretory ducts of the liver prevents the excretion of bilirubin.

- − When the blood concentration reaches 2 to 2.5 mg of bilirubin per dL, it diffuses into the tissues, which turn yellow, a condition termed **jaundice (**bilirubin deposits in the skin, mucous membranes, and eyes), or **icterus** (bilirubin deposits in the blood).

NOTE:

- − Hyperbilirubinemia may be classified depending on the type of bilirubin present in plasma, as:
- ✓ **Retention hyperbilirubinemia** due to overproduction of bilirubin (unconjugated).
- ✓ **Regurgitation hyperbilirubinemia,** due to reflux into the bloodstream because of biliary obstruction (conjugated).
- − Only ***unconjugated*** bilirubin (due to its **hydrophobicity**), can cross the blood-brain barrier into the central nervous system causing **kernicterus** (an encephalopathy due to hyperbilirubinemia), only in retention hyperbilirubinemia.
- − Only ***conjugated*** bilirubin (due to its **hydrophilicity)**, can appear in urine. Accordingly:
- ✓ **Choluric jaundice** (choluria is the presence of bile pigments in the urine) occurs only in regurgitation hyperbilirubinemia.
- ✓ **Acholuric jaundice** occurs only in the presence of an excess of unconjugated bilirubin.

Causes of Hyperbilirubinemia (jaundice)

1. Pre-hepatic (hemolytic jaundice):

- ✓ Hemolysis results in increased production of bilirubin.
- ✓ More bilirubin is conjugated and excreted than normally, but the conjugation mechanism is overwhelmed, and an abnormally large amount of **unconjugated** bilirubin is found in the blood.

2. Hepatic:

Toxic Hyperbilirubinemia

- ✓ Can result from **toxin induced liver dysfunction** caused by chloroform, carbon tetrachloride, acetaminophen, hepatitis virus, or cirrhosis.
- ✓ These acquired disorders involve hepatic parenchymal cell damage, which impairs bilirubin conjugation, thus **unconjugated** bilirubin is retained in blood.

Gilbert's disease

- ✓ A hereditary disorder in which there is decreased bilirubin **uptake** into the hepatocytes
- ✓ As a result, **unconjugated** bilirubin accumulates.

Physiological jaundice and Crigler-Najjar syndrome

- − Physiological jaundice results from inability of the immature liver of the newborn to produce UDPG-transferase.
- − Crigler-Najjar syndrome a congenital disease results from complete absence (type I) or relative decrease (type II) in hepatic UDPG-transferase.
- ✓ **Conjugation** is impaired in these conditions thus **unconjugated** bilirubin is retained in the body.

Dubin-Johnson syndrome

- ✓ Caused by mutations in the gene encoding the protein involved in the **secretion of** conjugated bilirubin after it has been formed.
- ✓ **Conjugated** bilirubin returns to the blood.

3. Post-hepatic (biliary obstruction)

- ✓ Results from blockage of the hepatic or common bile ducts, most often due to a **gallstone** or to **cancer of the head of the pancreas.**
- ✓ Bilirubin diglucuronide that cannot be excreted regurgitates into blood.
- ✓ **Conjugated** bilirubin appears in the ***blood*** and ***urine*** **(choluric jaundice)**, and the stools typically are a pale color.