Nitrogen as a key component of amino acids

The atmosphere is rich in nitrogen gas (N₂), a very unreactive molecule. Certain organisms such as bacteria that live in the root nodules of bird’s foot trefoil (photo above) can convert nitrogen gas into ammonia. Ammonia can then be used to synthesize first glutamate and then other amino acids.

[Courtesy: (Upper) Vu/Cabisco]
surrounding environment. In the first case, where there is de novo synthesis of the compounds from simple substances, they are often described as being of endogenous origin. The second case, where the compounds are obtained preformed from the environment, would be termed an exogenous source. Respectively, these two terms literally mean ‘formed within’ and ‘formed without’.

Amino acids rely upon the diet for the provision of some amino acids. Indeed, it might be questioned why animals synthesize any of the amino acids, since all 20 are obtained from the food. However, we know that some of the amino acids have important metabolic roles, which in themselves involve a constant synthesis and degradation of the compounds in quantities much higher than those needed for protein synthesis.

It is of interest to note that the catabolic breakdown of amino acids produces intermediates of citric acid cycle whereas the anabolic formation of amino acids utilizes citric acid cycle intermediates as precursors. Fig 27-2 (on the next page) highlights the relationship between amino acid metabolism and the citric acid cycle intermediates.

**Essential and Nonessential Amino Acids**

The organisms differ markedly in terms of their ability to carry out the de novo synthesis of the protein amino acids from inorganic substances. Most microorganisms and plants are competent in all such syntheses, but most animals lack about half of these synthetic capacities. For the latter organisms, amino acids may then be classified as ‘essential’ or ‘nonessential’. On the basis of the most frequently employed experimental criteria, an amino acid is considered essential if it is included in the diet for:

(a) optimal growth, or

(b) the maintenance of nitrogen balance.

An animal is said to be in nitrogen balance, if its daily intake of nitrogen is just balanced by its daily excretion of nitrogen.

The ‘essentiality’ of an amino acid is not only a function of the criteria employed for its determination but is a function of many other variables, including the presence of metabolically related substances in the diet. Besides, the amino acid requirement may also vary with the physiological state of the animal (i.e., in pregnancy, lactation and disease), with age, and probably with the nature of the intestinal flora. It is also noteworthy that the ‘essential’ feature of the essential amino acids is their carbon skeleton; most of these may be derived from the corresponding keto acids through transamination:

\[
\begin{align*}
\text{COO}^- & \quad \text{COO}^- \\
\text{C} = \text{O} + \text{H}_2\text{N}^- & \quad \text{C} = \text{H} \\
\text{R}_E & \quad \text{R}_N
\end{align*}
\]

Transamination

\[
\begin{align*}
\text{C} = \text{O} + \text{H}_2\text{N}^- & \quad \text{C} = \text{H} \\
\text{R}_E & \quad \text{R}_N
\end{align*}
\]

Aminotransferase

\[
\begin{align*}
\text{α–keto acid} & \quad \text{A second amino acid} \\
\text{skeleton of essential amino acid} & \quad \text{Essential amino acid}
\end{align*}
\]

\[
\begin{align*}
\text{α–keto acid} & \quad \text{skeleton of the second amino acid} \\
\text{R}_E & \quad \text{R}_N
\end{align*}
\]

[\text{R}_E and \text{R}_N represent R groups of the essential and nonessential amino acids, respectively.]

In fact, all the 20 amino acids are essential to the organism in the sense that all must be present in order for protein synthesis and, therefore, life to occur. As already stated, some life forms (e.g., bacteria and plants) can form all the 20 amino acids from amphibolic intermediates, whereas other forms including human and other animals, can biosynthesize only half of those required. These amino acids are termed nutritionally-nonessential amino acids (refer Table 27–1). The remaining
Fig. 27–2. Interrelation between amino acid metabolism and the citric acid cycle.
Fundamentals of Biochemistry

Table 27–1. Essential and nonessential amino acids and the number of enzymes required for their synthesis

<table>
<thead>
<tr>
<th>Nutritionally essential amino acids</th>
<th>Nutritionally nonessential amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine*, a 7</td>
<td>Alanine</td>
</tr>
<tr>
<td>Histidine* 6</td>
<td>Asparagine b 1</td>
</tr>
<tr>
<td>Isoleucine 8 (6 shared)</td>
<td>Aspartic acid 1</td>
</tr>
<tr>
<td>Leucine 3 (7 shared)</td>
<td>Cysteine d 2</td>
</tr>
<tr>
<td>Lysine 8</td>
<td>Glutamic acid 1</td>
</tr>
<tr>
<td>Methionine 5 (4 shared)</td>
<td>Glutamine a 1</td>
</tr>
<tr>
<td>Phenylalanine 1 (9 shared)</td>
<td>Glycine c 1</td>
</tr>
<tr>
<td>Threonine 6</td>
<td>Proline a 3</td>
</tr>
<tr>
<td>Tryptophan 5 (8 shared)</td>
<td>Serine 3</td>
</tr>
<tr>
<td>Valine +1 (7 shared)</td>
<td>Tyrosine +10</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

* Arginine and histidine, though belonging to the nutritionally essential category, are sometimes classified as nutritionally semiessential amino acids because they may be synthesized in tissues at rates inadequate to support growth of children.

**From glutamic acid**  
**From aspartic acid**  
**From serine**  
**From serine plus S⁻²**

Amino acids, which must therefore be supplied by the diet, are termed nutritionally essential amino acids. It may be emphasized that a given amino acid may be nutritionally essential for one form of life but nutritionally nonessential for another. These differences reflect their different genetic heritages. Confusion may arise because nutritionists frequently refer to the nutritionally essential amino acids as ‘essential’ or ‘indispensable’ amino acids and the nutritionally nonessential amino acids as ‘nonessential’ or ‘dispensable’ amino acids. Although in a nutritional context these terms are correct, it is unfortunate that they obscure the biologically essential nature of all the 20 amino acids.

A perusal of the Table 27–1 indicates that 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.

Metabolic Precursors of Amino Acids

The pathways for the biosynthesis of amino acids are diverse. However, they have an important common feature: their carbon skeletons come from intermediates in glycolysis, tricarboxylic acid cycle or pentose phosphate pathway (Fig. 27–3). The nonessential amino acids are synthesized by quite simple reactions, whereas the pathways for the formation of essential amino acids are quite complex. Ten of the amino acids are only one or a few enzymatic steps removed from their precursors. The pathways for others, such as the aromatic amino acids, are more complex. A useful way to organize the amino acid biosynthetic pathways is to group them into families corresponding to the metabolic precursor of each amino acid (Table 27-2).
Table 27–2. Six biosynthetic families of amino acids, based on different metabolic precursors (shown in boldface)

<table>
<thead>
<tr>
<th>α-ketoglutarate</th>
<th>Oxaloacetate</th>
<th>Phosphoenolpyruvate and erythrose-4-phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>Aspartate</td>
<td>Phenylalanine*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Asparagine</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Proline</td>
<td>Methionine*</td>
<td>Tryptophan*</td>
</tr>
<tr>
<td>Arginine†</td>
<td>Threonine*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isoleucine*</td>
<td></td>
</tr>
<tr>
<td>3-phosphoglycerate</td>
<td>Glycine</td>
<td>Ribose-5-phosphate</td>
</tr>
<tr>
<td>Serine</td>
<td>Alanine*</td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>Valine*</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>Leucine*</td>
<td></td>
</tr>
</tbody>
</table>

* Essential amino acids; rest are nonessential.
† Although essential in young, growing animals but not in adults, the status of arginine is rather ambiguous since the entire arginine molecule can be synthesized by human and other animals.
In addition to these, there is a notable intermediate that recurs in several pathways: phosphoribosyl pyrophosphate (PRPP). PRPP is synthesized from ribose-5-phosphate derived from the pentose phosphate pathway, in a reaction catalyzed by ribose phosphate pyrophosphokinase:

\[ \text{Ribose-5-phosphate} + \text{ATP} \rightleftharpoons 5\text{-phosphoribosyl-1-pyrophosphate} + \text{AMP} \]

Ribose phosphate pyrophosphokinase is allosterically regulated by many of the biomolecules for which PRPP is a precursor. PRPP is an intermediate in tryptophan and histidine biosyntheses, with the ribose ring contributing many of its carbons to the final structure of these amino acids. It is also of fundamental importance in the biosynthesis of nucleotides.

**AN OVERVIEW OF NITROGEN METABOLISM**

The pathways leading to the biosynthesis of amino acids and nucleotides both share a requirement for nitrogen. But as soluble and biologically-useful nitrogen compounds are scarce in natural environment, therefore ammonia, amino acids and nucleotides are used economically by most organisms. Nitrogen from the environment is introduced into biological systems by many pathways which are described below.

**Nitrogen Cycle**

The atmospheric air contains 79% (i.e., about four-fifths) molecular nitrogen (N\(_2\)). However, only a relatively few species can convert atmospheric nitrogen into forms useful to living organisms. Therefore, different organisms display an interdependent manner to salvage and reuse biologically available nitrogen in a vast nitrogen cycle (Fig. 27–4).

![Fig. 27–4. The nitrogen cycle](image-url)

The total amount of nitrogen fixed annually in the biosphere exceeds 10\(^{11}\) kg.

The first step in nitrogen cycle is nitrogen fixation, i.e., reduction of N\(_2\) to ammonia (NH\(_3\) or NH\(_4^+\)) which is accomplished only by nitrogen-fixing bacteria. Plants cannot reduce but live symbiotically with the bacteria (usually Rhizobium) and thereby enrich the nitrogen content of the soil. Thus, the symbiotic and photosynthetic N\(_2\)-fixing systems fix a major amount of the total of
approximately $10^8$ tons of $N_2$ fixed annually. On the contrary, the free-living and nonphotosynthetic bacteria (Azotobacter, Klebsiella, Clostridium) contribute relatively little to the total.

Although ammonia can be used by most living organisms, the soil bacteria, which are so abundant and active, oxidize soil ammonia first to $NO_2^-$ and then to $NO_3^-$, a process called nitrification. Plants and many bacteria can easily reduce $NO_3^-$ to ammonia by the action of nitrate reductases. Ammonia so formed can be synthesized into amino acids by plants which are then used up by animals as source of amino acids, both essential and nonessential, to built animal proteins. When organisms die, the microbes degrade their body proteins to ammonia in the soil, where nitrifying bacteria convert it into nitrite and nitrate again.

Certain denitrifying bacteria, however, convert nitrate to $N_2$ under anaerobic conditions, so that a balance is maintained between fixed nitrogen and atmospheric nitrogen. In this process of denitrification, these soil bacteria utilize $NO_3^-$, rather than $O_2$, as the ultimate electron acceptor.

Fig. 27–5. The 3-dimensional structure of nitrogenase complex
(a) Ribbon diagram. Nitrogen is an essential component of many biochemical building blocks. This enzyme complex, shown here, converts nitrogen gas, an abundant but inert compound, into a form that can be used for synthesizing amino acids, nucleotides, and other biochemicals. The dinitrogenase subunits are shown in grey and pink, the dinitrogenase reductase subunits in blue and green. Bound ADP is shown in red. Note the 4Fe–4S complex (Fe and S atoms orange and yellow, respectively), and the Fe-Mo cofactor (Mo Black, homocitrate (light grey). The P clusters (bridged pairs of the 4Fe–4S complexes) are also shown.
(b) Schematic diagram. The dinitrogenase reductase component dissociates from the nitrogenase component before $N_2$ is converted into $NH_4^+$. 
Nitrogenase Complex

The conversion of nitrogen to ammonia is a reduction reaction which is exergonic in nature:

\[ \text{N}_2 + 3\text{H}_2 \rightarrow 2\text{NH}_3 \quad \Delta G^\circ = -33.5 \text{ kJ/mol} \]

The N≡N triple bond, which has a bond energy of 942 kJ/mol, is highly resistant to chemical attack. Indeed, Lavoisier named it “azote”, meaning “without life”, because it is quite unreactive. The industrial process for nitrogen fixation, devised by Fritz Haber in 1910 and currently used in fertilizer factories, is typically carried out over an iron catalyst at about 500°C and a pressure of 300 atm of N\(_2\) and H\(_2\) to provide the necessary activation energy. Biological nitrogen fixation must occur at 0.8 atm of N\(_2\), and the high activation barrier is partly overcome by the binding and hydrolysis of ATP. The stoichiometry of the overall process of nitrogen fixation can be written as:

\[ \text{N}_2 + 10\text{H}^+ + 8\text{e}^- + 16\text{ATP} \rightarrow 2\text{NH}_4^+ + 16\text{ADP} + 16\text{Pi} + \text{H}_2 \]

Biological fixation of nitrogen is carried out by a highly conserved complex of proteins called nitrogenase complex (Fig. 27–5.). The nitrogenase complex comprises 2 protein components: component I or dinitrogenase and component II or dinitrogenase reductase; neither of the two components is active in the absence of the other and their sizes vary with the microbial source. Dinitrogenase or Mo-Fe protein (MW ≈ 2,10,000 to 2,40,000) is a tetramer with 4 identical peptide chains, each with an Mo-Fe coenzyme of unknown structure and an Fe\(_4\) – S\(_4\) group. Its redox centres have a total of 2 Mo, 32 Fe and 30 S per tetramer. Dinitrogenase reductase or Fe protein (MW = 55,000 to 60,000) is a dimer with 2 identical chains, each having an Fe\(_4\) – S\(_4\) group and can be oxidized and reduced by one electron. It also has two binding sites for ATP. The nitrogenase complex has one or two units of component II for each component I. The reductase component provides electrons with high reducing power and the nitrogenase component uses these electrons to reduce N\(_2\) to NH\(_4^+\).

Nitrogen fixation is carried out by a highly reduced form of dinitrogenase, and it needs 8 electrons: 6 for the reduction of N\(_2\), and 2 to produce one mole of H\(_2\) as an obligate part of the reaction mechanism (Fig. 27–6). Dinitrogenase is reduced by the transfer of electrons from dinitrogenase reductase. Dinitrogenase has 2 binding sites for the reductase and the required 8 electrons are transferred to dinitrogenase one at a time, with the reduced reductase binding and the oxidized reductase dissociating from dinitrogenase in a cycle. This cycle requires the hydrolysis of ATP by the reductase. The immediate source of electrons to reduce dinitrogenase reductase varies, although in at least one instance, the ultimate source of electrons is pyruvate, as shown in the figure.

Two important characteristics of the nitrogenase complex are:

(a) The ATP seems to play a catalytic role, rather than thermodynamic. It may be recalled that ATP, besides contributing chemical energy through the hydrolysis of one or more of its phosphodiester bonds, can also contribute binding energy through noncovalent interactions that can be used to lower the activation energy. In the reaction carried out by dinitrogenase reductase, both ATP binding and ATP hydrolysis bring about protein conformational changes that help overcome the high activation energy of nitrogen fixation.

(b) Another characteristic of nitrogenase complex is that it is extremely labile when oxygen is present. The reductase is inactivated in air, with a half-life of 30 s. The dinitrogenase has a half-life of 10 min in air. Free-living bacteria, that fix nitrogen, avoid or solve this problem by diverse means.
Electrons are transferred from pyruvate to dinitrogenase via ferredoxin (or flavodoxin) and dinitrogenase reductase. Dinitrogenase is reduced one electron at a time by dinitrogenase reductase, and must be reduced by at least 6 electrons to fix one mole of $N_2$. An additional 2 electrons (thus a total of 8) are used to reduce $2H^+$ to $H_2$ in a process that accompanies nitrogen fixation in anaerobes.

Both components of the nitrogenase complex are irreversibly poisoned by oxygen. The apparent function of the leghemoglobins (= legume hemoglobins) supplied to the nodules of *Rhizobium* in the legumes, is to bind $O_2$ and maintain $pO_2$ below 0.001 mm Hg so that it cannot interfere with nitrogen fixation. In the cyanophycean members (blue-green algae), the walls of the heterocysts separate the nitrogenase from the oxygen produced by photosynthesis.

The activity of nitrogenase complex is controlled in 2 ways: one is a *coarse control*, in which enzyme synthesis is repressed by excess of ammonia; the other is a *fine control*, in which the activity of the nitrogenase is regulated by ADP. Should the amount of ADP increase to about twice that of ATP, further utilization of ATP by nitrogenase is completely inhibited.

It may, thus, be inferred from the foregoing discussion that the **basic features** of biological nitrogen fixation are (Fig. 27–7):
1. nitrogenase, the cardinal enzyme complex in the process,
2. a strong reductant, such as reduced ferredoxin or flavodoxin,
3. ATP,
4. a system for regulation of the rate of ammonia production and one for assimilation, since biosynthesis of the nitrogenase complex ceases when ammonia accumulates, and
5. protection of the nitrogen fixation system from molecular oxygen, which inactivates nitrogenase and competes for reductant (in aerobic bacteria).

Fig.  27–7. Overall representation of nitrogen fixation

**Reduction of Nitrate and Nitrite**

Whereas nitrogen-fixing plants can obtain their nitrogen requirements from N\textsubscript{2}, nonfixing plants acquire most of their nitrogen from nitrate (NO\textsubscript{3}\textsuperscript{−}), the most abundant form of nitrogen. Most anaerobic and facultative aerobes (those that can also grow anaerobically) and the algae are capable of converting NO\textsubscript{3}\textsuperscript{−} into biological nitrogen. This utilization of NO\textsubscript{3}\textsuperscript{−} entails its reduction to ammonia, which is then incorporated into nitrogenous biomolecules. Reduction of nitrate to ammonia in diverse microorganisms and most higher plants is accomplished by the catalytic action of two enzymes, *nitrate reductase* and *nitrite reductase*.

**Nitrate reductase.** This enzyme catalyzes the two-electron reduction of nitrate to nitrite, with NADH (bacterial and plant cells) or NADPH (fungal cells) as electron donor. Nitrate reductase (MW = 8,00,000) is a tetrameric aggregation of 4 dimers, each possessing one protomer of MW $\approx 1,50,000$ and another of MW $\approx 50,000$. The mechanism of nitrate reduction is analogous to that of nitrogen fixation in that nitrate reductase also contains an electron pathway with one FAD, one Mo atom and many Fe\textsubscript{4}−S\textsubscript{4} centres per basic dimer, serving as electron carriers. The flow of electrons derived from NADH in plants, that results in reduction of NO\textsubscript{3}\textsuperscript{−} to NO\textsubscript{2}\textsuperscript{−}, appears to proceed as follows:

\[
\text{NADH} \xrightarrow{2e^-} \text{FAD} \xrightarrow{e^-} \text{Fe}_4^-\text{S}_4 \xrightarrow{e^-} \text{Mo} \xrightarrow{e^-} \text{NO}_3^- \xrightarrow{e^-} \text{NO}_2^- 
\]

The overall reaction catalyzed is:

\[
\text{NO}_3^- + \text{NADH} + \text{H}^+ \rightarrow \text{NO}_2^- + \text{NAD}^+ + \text{H}_2\text{O}
\]

Molybdenum undergoes cyclic changes from Mo\textsuperscript{v+} to Mo\textsuperscript{v+} during reduction of nitrate. The nitrate reductase of plants is induced by nitrate and is believed to be repressed by ammonia.

**Nitrite reductase.** This enzyme catalyzes the six-electron reduction of nitrite to ammonia, with NADH (bacterial and plant cells) or NADPH (fungal cells) as reductant. The nitrite reductase of plants has an Fe\textsubscript{2}−S\textsubscript{2} centre and siroheme, the immediate reductant of nitrogen, as carriers of the electrons furnished to the enzyme by reduced ferredoxin. The siroheme iron is the binding site for NO\textsubscript{2}\textsuperscript{−}; no intermediates dissociate NH\textsubscript{3}\textsuperscript{+} appears. Some fungal nitrite reductases also possess an FAD or FMN as an electron carrier. Presumably, the electron pathway is similar to that in sulfite reductase. The overall reaction for the reduction of NO\textsubscript{2}\textsuperscript{−} to NH\textsubscript{4}\textsuperscript{+} is:

\[
\text{NO}_2^- + 6e^- + 8H^+ \rightarrow \text{NH}_4^+ + 2\text{H}_2\text{O}
\]

Siroheme is an iron tetrahydroporphyrin of the isobacteriochlorin type, viz., two adjoining pyrrole rings are fully reduced, and each pyrrole bears one propionic and one acidic side chain. Hence, it is a derivative of uroporphyrin.
FIXATION OF AMMONIA INTO AMINO ACIDS

Ammonia, of whatever origin, can be combined into organic linkage by 3 major reactions that occur in all organisms. These reactions result in the formation of glutamate, glutamine and carbamoyl phosphate. Utilization of the nitrogen of carbamoyl phosphate is limited to two pathways, one contributing a single nitrogen atom in the synthesis of pyrimidines and the other donating one nitrogen atom to the synthesis of arginine. *Essentially all other nitrogen atoms of amino acids (or of other organic compounds) are derived directly or indirectly from glutamate or the amide group of glutamine.* Although NH₃ can be utilized in place of glutamine in some enzymic reactions, glutamine is preferred in most cases. The α amino group of most amino acids is derived from the α amino group of glutamate by a process called transamination. Glutamine, the other major nitrogen donor, contributes its side-chain nitrogen in the biosynthesis of a wide range of compounds.

Glutamate is synthesized from NH₄⁺ and α-ketoglutarate, a tricarboxylic acid cycle intermediate, by the action of *L-glutamate dehydrogenase* which is present in all organisms. This reaction, which is termed reductive amination, has already been dealt with in the catabolism of amino acids. NADPH acts as a reductant in glutamate biosynthesis, whereas acts as an oxidant in its degradation.

\[
\alpha\text{-ketoglutarate} + \text{NH}_4^+ + \text{NADPH} \rightleftharpoons \text{L-glutamate} + \text{NADP}^+ + \text{H}_2\text{O}
\]

*Glutamate dehydrogenase* from *Escherichia coli* (MW = 50,000) is a hexamer of 6 identical subunits. In eukaryotic cells, L-glutamate dehydrogenase is located in the mitochondrial matrix. The equilibrium for the reaction favours reactants and the *Kₘ* for NH₄⁺ (~ 1 mM) is so high that a modest NH₄⁺ assimilation takes place. Soil bacteria and plants rarely have sufficiently high concentrations of NH₄⁺ and as such not enough glutamate is formed and these organisms have to generally rely on the two-enzyme pathway, described below.

The pathway for the conversion (or assimilation) of NH₄⁺ into glutamate requires 2 reactions. First, glutamate and NH₄⁺ react to produce glutamine by the action of *glutamine synthetase*, which is found in all organisms.

\[
\text{Glutamate} + \text{NH}_4^+ + \text{ATP} \rightleftharpoons \text{Glutamine} + \text{ADP} + \text{P}_i + \text{H}^+
\]

In fact, this is a two-step reaction, with enzyme-bound γ-glutamyl phosphate as an intermediate.

\[
\text{Glutamate} + \text{ATP} \rightleftharpoons \gamma\text{-glutamyl phosphate} + \text{ADP}
\]

\[
\gamma\text{-glutamyl phosphate} + \text{NH}_4^+ \rightleftharpoons \text{Glutamine} + \text{P}_i + \text{H}^+
\]

Besides its importance for NH₄⁺ assimilation in bacteria, this is a central reaction in amino acid metabolism. It is the main pathway for converting toxic free ammonia into the nontoxic glutamine for transport in the blood.

*Glutamine synthetase* (Fig 27–8), (MW from that of *E. coli* = 6,00,000), consists of 12 identical subunits and is inactivated by the transfer from ATP of a 5′-adenylyl group to form a phosphodiester linkage with the phenolic hydroxyl group of a specific tyrosine residue of each subunit. At least 6 end products of glutamine metabolism plus alanine and glycine are allosteric inhibitors of the enzyme (Fig. 27–9) and each subunit (MW = 50,000) has binding sites for all 8 inhibitors as well as an active site for catalysis. Each inhibitor alone gives only partial inhibition. The effects of the different inhibitors, however, are more than additive, and all 8 together virtually shut down the enzyme. Thus, *glutamine synthetase is a primary regulatory point in nitrogen metabolism.*
Fig. 27–8. The structure of glutamine synthetase from *Salmonella typhimurium*

The enzyme consists of twelve identical subunits arranged in two rings of six subunits, here represented by their C$_\alpha$ backbones, arranged with D$_6$ symmetry (the symmetry of a hexagonal prism).

(a) Top view of the enzyme down the sixfold axis of symmetry. The top ring of monomers are alternately colored light and dark blue and the bottom ring of monomers light and dark red. The subunits or monomers of the bottom ring are roughly directly below those of the top ring. The protein, including its side chains (not shown), has a diameter of 143 Å. The active sites of each monomer shown are marked by pairs of Mn$^{2+}$ ions (white spheres).

(b) Side view of the enzyme along one of the twofold axes of symmetry showing only the six nearest subunits. The molecule extends 103 Å along the sixfold axis which is vertical in this view.

(Courtesy: Michael Pique/Scripps Research Institute)

Fig. 27–9. Cumulative allosteric inhibition of glutamine synthetase by 6 end products of glutamine metabolism

Alanine and glycine probably serve as indicators of the general status of cellular amino acid metabolism.
In bacteria, an enzyme glutamate synthase catalyzes the reductive amination of α-ketoglutarate, using glutamine as nitrogen donor. Thus, two moles of glutamate are produced.

\[
\alpha\text{-ketoglutarate} + \text{Glutamine} + \text{NADPH} + H^+ \rightarrow 2 \text{Glutamate} + \text{NADP}^+
\]

Glutamate synthase obtained from \emph{E. coli} (MW = 8,00,000) has two types of subunits: one contains nonheme iron, FAD and FMN; the other subunit binds NADPH.

When \(
\text{NH}_4^+
\) is limiting, most of the glutamate is made by the sequential action of glutamine synthetase and glutamate synthase. The net reaction of these two enzymes in bacteria is:

\[
\alpha\text{-ketoglutarate} + \text{NH}_4^+ + \text{NADPH} + \text{ATP} \rightarrow \text{L-glutamate} + \text{NADP}^+ + \text{ADP} + \text{Pi}
\]

It may, however, be noted that this stoichiometry differs from that of the glutamate dehydrogenase reaction in that here an ATP is hydrolyzed. At this stage, a query may arise as to why this more expensive pathway sometimes is used by \emph{E. coli}? The answer lies in the fact that the \(K_m\) of glutamate dehydrogenase for \(\text{NH}_4^+\) is high (~ 1 mM), and so this enzyme is not saturated when \(\text{NH}_4^+\) is limiting. In contrast, glutamine synthetase has very high affinity for \(\text{NH}_4^+\).

**BIOSYNTHESIS OF AMINO ACIDS**

All the 20 protein amino acids (Fig. 27–10) are derived from intermediates in glycolysis, citric acid cycle or the pentose phosphate pathway (refer Fig. 27–3). Nitrogen enters these pathways by way of glutamate and glutamine. The pathways for 10 amino acids are simple and are only one or a few enzymatic steps removed from their precursors, whereas the pathways for others (such as aromatic amino acids) are more complex. Different organisms have varied capacity to synthesize these 20 amino acids. \emph{Whereas most bacteria and plants can synthesize all the 20 amino acids, mammals including man can synthesize only about half of them.} These are termed as nonessential amino acids and the remaining ones, which must be obtained from food, as the essential amino acids (refer Table 27–1).

\emph{Most pathways are essentially irreversible (i.e.,} they proceed with a substantial loss of free energy) and as such a continuous supply of all the amino acids is ensured. This is accomplished, in general, by reactions in which ATP is utilized and in effect hydrolyzed to ADP + Pi. The cases, where ATP splits into AMP + PPi, are more effective because the pyrophosphate is irreversibly hydrolyzed (PPi \(\rightarrow\) 2 Pi). In other instances, synthesis is ensured by a reductive amination, usually employing a pyridine nucleotide, in which equilibrium strongly favours such reduction. Most of the pathways for amino acid syntheses have been established mainly for bacteria, yeast and other moulds. It is believed that the pathways in higher plants and animals are similar, but comparatively less is known of the enzymes involved.

\**A. Syntheses of Amino Acids of a-ketoglutarate Precursor Family**

The biosynthesis of glutamate and glutamine has already been discussed earlier in this chapter. The synthesis of proline, a cyclized derivative of glutamate is depicted in Fig. 27–11. In the first reaction, the \(\gamma\)-carboxyl group of glutamate is phosphorylated using ATP to form an acyl phosphate, which is then reduced by NADPH to form glutamate \(\gamma\)-semialdehyde. This intermediate ultimately undergoes cyclization and further reduction to form proline.

\emph{Arginine} is synthesized from glutamate via ornithine and the urea cycle (refer page 651). Ornithine could also be synthesized from glutamate \(\gamma\)-semialdehyde by transamination but cyclization of the semialdehyde in the proline pathway is a rapid spontaneous reaction so that only a little
Alanine  
Cysteine  
Histidine  
Methionine  
Threonine  
Tryptophan  
Phenylalanine  
Isoleucine  
Glutamic acid  
Arginine

Fig. 27–10. (Cont’d.)
The structure of the 20 protein amino acids

(For each amino acid, diagram on the left represents the ball-and-stick model and the diagram on the right represents the space-filling model)

The various proteins found in the living beings are made of these 20 amino acids which are synthesized by
discrete pathways.
Fig. 27–11. Biosynthesis of proline from glutamate

Note that all 5 carbon atoms of proline arise from glutamate. The nonenzymatic cyclization of glutamate \( \gamma \)-semialdehyde is so rapid that the \( \gamma \)-semialdehyde cannot give rise to ornithine via transamination. The amount of this intermediate is left for ornithine synthesis. The biosynthetic pathway for ornithine therefore parallels some steps of the proline pathway but includes 2 additional steps to chemically block the amino group of glutamate \( \gamma \)-semialdehyde and prevent cyclization (Fig. 27–12). To begin with, the \( \alpha \)-amino group of glutamate is blocked by acetylation using
Fig. 27.12. Biosynthesis of arginine from glutamate

Note that, in contrast to proline pathway, cyclization is averted in ornithine/arginine pathway by acetylating the α-amino group of glutamate in the first step and removing the acetyl group after the transamination. Arginine is synthesized from ornithine via the urea cycle, as shown in Fig. 26–10.
acetyl-CoA, and after the transamination step the acetyl group is removed to yield ornithine. Most of the arginine formed in mammals is cleaved to form urea so that the available arginine is depleted. This makes arginine an essential amino acid in young animals that need higher amounts of amino acids for growth.

**B. Syntheses of Amino Acids of 3-phosphoglycerate Precursor Family**

Fig. 27–13 outlines the serine pathway. In the first step, the hydroxyl group of 3-phosphoglycerate is oxidized by NAD⁺ to produce an 3-phosphohydroxypyruvate. This is the

\[ \text{3-phosphoglycerate} \rightarrow \text{3-phosphohydroxypyruvate} \]

**Fig. 27–13.** Biosynthesis of serine from 3-phosphoglycerate and the subsequent conversion of serine into glycine
committed step in the serine biosynthetic pathway and is catalyzed by the enzyme 3-phosphoglycerate dehydrogenase (Fig. 27-14). Transamination of 3-phosphohydroxypyruvate (an α-keto acid) from glutamate produces 3-phosphoserine, which upon hydrolysis by phosphoserine phosphatase yields free serine.

Serine is the precursor of glycine and cysteine. The 2-carbon glycine is produced from its precursor 3-carbon amino acid serine through removal of its side chain β carbon atom by serine hydroxymethyl transferase (= serine transhydroxymethylase), a PLP enzyme (Fig. 27–15). The bond between the α and β carbon atoms of serine is labilized by the formation of a Schiff base between serine and PLP. The β carbon atom of serine is then transferred to tetrahydrofolate (= tetrahydropteroylglutamate), a highly versatile carrier of activated one-carbon units.

In the vertebrate liver, glycine is also produced from CO₂ and NH₄⁺ by the action of glycine synthase:

\[
\text{CO}_2 + \text{NH}_4^+ + \text{NADH} + \text{H}^+ + \text{N}^5\text{N}^{10}\text{-methylene-tetrahydrofolate} \rightleftharpoons \text{Glycine} + \text{NAD}^+ + \text{tetrahydrofolate}
\]

In mammals, cysteine is produced from two other amino acids, namely Met which provides the sulfur atom and Ser which furnishes the carbon atom (Fig. 27–15).

\[
\text{OOC—CH—CH}_2—\text{CH}_2—\text{SH} + \text{HOCH}_2—\text{CH—COO}^- 
\]

Homocysteine

\[
\text{Cystathionine-β-synthase} \rightarrow \text{H}_2\text{O}
\]

Cystathionine

\[
\text{OOC—CH—CH}_2—\text{CH}_2—\text{S—CH}_2—\text{CH—COO}^- 
\]

Cystathionine-γ-lyase

\[
\text{OOC—C—CH}_2—\text{CH} + \text{HS—CH}_2—\text{CH—COO}^- 
\]

α-ketobutyrate

Fig. 27–14. Structure of 3-phosphoglycerate dehydrogenase

This enzyme, which catalyzes the committed step in the serine biosynthetic pathway, includes a serine-binding regulatory domain. Serine binding to this domain reduces the activity of the enzyme.

Fig. 27–15. Biosynthesis of cysteine from homocysteine and serine
Fig. 27-16. (Cont’d)
Fig. 27–16. Biosynthesis of lysine, methionine and threonine

Note that L-L-, ε-diaminopimelate, the product of Step 7, is symmetric. The carbons derived from pyruvate (and amino group derived from glutamate) cannot be traced beyond this point because subsequent reactions may place them at either end of the lysine molecule. The enzymes involved in the numbered reactions are: 1 aspartokinase, 2 aspartate-β-semialdehyde dehydrogenase, 3 dihydropicolinate synthase, 4 Δ-piperidine-2, 6-dicarboxylate dehydrogenase, 5 N-succinyl-2-amino-6-ketopimelate synthase, 6 succinyl diaminopimelate aminotransferase (a PLP enzyme), 7 succinyl diaminopimelate desuccinylase, 8 diaminopimelate epimerase, 9 diaminopimelate decarboxylase, 10 homoserine dehydrogenase, 11 homoserine acyltransferase, 12 cystathionine-γ-synthase, 13 cystathionine-β-lyase, 14 methionine synthase, 15 homoserine kinase and 16 threonine synthase (a PLP enzyme).
Note that pyruvate is the metabolic precursor for both isoleucine and valine pathways. The pathway enzymes for the numbered reactions are: 1 and 2, acetoacetate synthase (a TPP enzyme), 3, acetohydroxy acid isomeroreductase, 4, dihydroxy acid dehydratase, and 5, valine aminotransferase (a PLP enzyme).
In a multistep reaction, the –OH group of serine is replaced by an –SH group derived from methionine to form cysteine. In the first step, methionine is converted into S-adenosylmethionine. After the enzymatic transfer of the methyl group to any of a number of different acceptors, S-adenosylhomocysteine, the demethylated product, is hydrolyzed to free homocysteine. Homocysteine

\[
\text{CH}_3 \text{ COO}^- \quad \text{CH}_3 \text{ COO}^- \quad \text{CH}_3 \text{ COO}^- \\
\text{iso-propylmalate} \quad \text{isopropylmalate} \quad \text{ketoisocaproate}
\]

Note that α-ketoisovalerate, an intermediate in valine pathway, is the starting point for leucine synthesis. The enzymes involved in the numbered reactions are: 1 α-isopropylmalate synthase, 2 isopropylmalate β-isopropylmalate isomerase, 3 dehydrogenase, and 4 leucine aminotransferase (a PLP enzyme).
then condenses with serine to yield cystathionine; the reaction being catalyzed by cystathionine-β-synthase, a PLP enzyme. In the last step, cystathionine is then deaminated and cleaved to cysteine plus α-ketobutyrate, by cystathionine-γ-lyase (= cystathioninase), another PLP enzyme. Note that the sulfur atom of cysteine is derived from homocysteine, whereas the carbon skeleton comes from serine.

C. Syntheses of Amino Acids of Oxaloacetate and Pyruvate Precursor Families

Alanine and aspartate are synthesized from pyruvate and oxaloacetate respectively, by transamination from glutamate. Asparagine is then synthesized by amidation of aspartate; the NH₄⁺ being donated by glutamine. These are all nonessential amino acids and their simple biosynthesis occurs in all organisms. The biosynthetic pathways for the 6 of the essential amino acids, viz., methionine, threonine, lysine, isoleucine, valine and leucine, are complex and interlinked. In some cases, there are marked differences in the pathways found in bacteria, fungi and higher plants. The bacterial pathways have been outlined in Figs. 27–16, 27–17 and 27–18.

Aspartate gives rise to lysine, methionine and threonine. Branch points occur at aspartate β-semialdehyde, an intermediate in all three pathways and at homoserine, a precursor of methionine and threonine. Threonine, in turn, is one of the precursors of isoleucine.

The isoleucine and valine pathways (Fig. 27–17) have 4 common enzymes. These two pathways begin with the condensation of 2 carbons of pyruvate with either another mole of pyruvate (valine path) or with α-ketobutyrate (isoleucine path). The α-ketobutyrate is derived from threonine in a reaction that requires pyridoxal phosphate (PLP).

The α-ketoisovalerate, an intermediate in the valine pathway, is the starting point for a 4-step branch pathway which leads to the production of leucine (Fig. 27–18).

D. Synthesis of Amino Acids of Phosphoenolpyruvate–erythrose-4-phosphate Precursor Family

The synthesis of tryptophan, tyrosine and phenylalanine takes place by pathways that share a number of early steps. The first 4 steps lead to the production of shikimate whose 7 carbon atoms are derived from phosphoenolpyruvate and erythrose-4-phosphate. Shikimate is then converted to chorismate through 3 more steps that include the addition of 3
more carbon atoms from another molecule of phosphoenolpyruvate (Fig. 27–20). Chorismate is the first branch point, with one branch leading to tryptophan and the other to tyrosine and phenylalanine through prephenate, the other branch point.

Fig. 27–20. Synthesis of chorismate, a key intermediate in the synthesis of the aromatic amino acids

Note that all carbons are derived from either erythrose-4-phosphate or phosphoenolpyruvate.

The pathway enzymes for the numbered reactions are: 1 2-keto-3-deoxy-D-arabinohexulose-7-phosphate synthase, 2 dehydroquinate synthase, 3 dehydroquinate dehydrogenase, 4 shikimate dehydrogenase, 5 shikimate kinase, 6 3-enolpyruvylshikimate-5-phosphate synthase, and 7 chorismate synthase. Note that Step 2 requires NAD⁺ as a cofactor, and NADH is released unchanged. It may be transiently reduced to NADH during the reaction to produce an oxidized reaction intermediate.

Tryptophan is synthesized from chorismate in a 5-step process (Fig. 27–21). Chorismate acquires an amino group from the side chain of glutamine and releases pyruvate to form anthranilate.
Fig. 27–21. **Biosynthesis of tryptophan from chorismate**

The pathway enzymes for the numbered reactions are: 1. anthranilate synthase, 2. anthranilate phosphoribosyl transferase, 3. N-(5'-phosphoribosyl)-anthranilate isomerase, 4. indole-3-glycerol phosphate synthase, and 5. tryptophan synthase. In *Escherichia coli*, the two enzymes, anthranilate synthetase and anthranilate phosphoribosyl transferase, are subunits of a single complex called **anthranilate synthase**.
In fact, glutamine serves as an amino donor in many biosynthetic reactions. Anthranilate then undergoes condensation with phosphoribosyl pyrophosphate (PRPP), an activated form of ribose phosphate. PRPP is also a key intermediate in the synthesis of histidine, pyrimidine nucleotides and purine nucleotides. The C-1 atom of ribose 5-phosphate becomes bonded to the nitrogen atom of anthranilate in a reaction that is driven by the hydrolysis of pyrophosphate. The ribose moiety of ribosylanthranilate undergoes rearrangement to yield enol-1-o-carboxylphenylamino-1-deoxysibulose-5-phosphate. This intermediate is dehydrated and then decarboxylated to indole-3-glycerol phosphate, which reacts with serine to form tryptophan. In this reaction, the glyceral phosphate side chain of indole-3-glycerol phosphate is replaced by the carbon skeleton and amino group of serine. This final reaction in the process is catalyzed by tryptophan synthase or tryptophan synthetase (Fig 27–19), an enzyme with an $\alpha_2\beta_2$ subunit structure. The enzyme can be dissociated into two $\alpha$ subunits and a $\beta_2$ subunit. The $\alpha$ subunit catalyzes the formation of indole from indole-
3-glycerol phosphate, whereas the \( \beta_2 \) subunit catalyzes the condensation of indole and serine to form tryptophan.

\[
\text{Indole-3-glycerol phosphate} \xrightarrow{\alpha\text{-subunit}} \text{Indole + Glyceraldehyde-3-phosphate} \\
\text{Indole + Serine} \xrightarrow{\beta_2\text{-subunit}} \text{Tryptophan + Water}
\]

The second reaction requires a pyridoxal phosphate as cofactor. Indole is rapidly channeled from the \( \alpha \)-subunit active site to the \( \beta \)-subunit active site, where it undergoes condensation with a Schiff base intermediate, derived from serine and PALP.

Tyrosine and phenylalanine are synthesized from chorismate in plants and microorganisms via simpler pathways. A mutase converts chorismate into prephenate, the immediate precursor of the aromatic ring of tyrosine and phenylalanine (Fig. 27–22). Prephenate is oxidatively decarboxylated to \( p \)-hydroxyphenylpyruvate. Alternatively, dehydration followed by decarboxylation yields phenylpyruvate. These \( \alpha \)-keto acids are then transaminated, with glutamate as amino group donor, to form tyrosine and phenylalanine, respectively.

Tyrosine can also be made by animals directly from phenylalanine via hydroxylation at C-4 of the phenyl group by phenyl hydroxylase, which also participates in the degradation of phenylalanine. Tyrosine is considered a nonessential amino acid only because it can be synthesized from the essential amino acid phenylalanine.

### E. Synthesis of Amino Acid of Ribose-5-phosphate Precursor Family

The biosynthetic pathway for histidine in all plants and bacteria is unique in many respects:

1. Histidine is derived from 3 precursors (Fig. 27–23):
   - \( (a) \) PRPP contributes 5 carbon atoms
   - \( (b) \) the purine ring of ATP contributes a nitrogen and a carbon
   - \( (c) \) glutamine contributes the second ring nitrogen.

2. The key steps of histidine biosynthesis are:
   - \( (a) \) the condensation of ATP and PRPP (step \( \oplus \) )
   - \( (b) \) purine ring opening that ultimately leaves N-1 and C-2 linked to the ribose (Step \( \ominus \) )
   - \( (c) \) formation of the imidazole ring in a reaction during which glutamine donates a nitrogen (Step \( \ominus \) ).

3. The use of ATP as a metabolite rather than a high-energy cofactor is unusual, but not wasteful because it dovetails with the purine biosynthetic pathway.

4. The remnant of ATP that is released after the transfer of N-1 and C-2 is 5-aminoimidazole-4-carboxamide ribonucleotide, an intermediate in the biosynthesis of purines that can rapidly be recycled to ATP.

### REGULATION OF AMINO ACID BIOSYNTHESIS

The amino acid biosynthesis is regulated by feedback inhibition. The rate of synthesis depends mainly on the amount and activity of the biosynthetic enzymes.

The first irreversible reaction in a biosynthetic pathway, called the committed step, is usually an important regulatory site. In a linear (i.e., unbranched) biosynthetic pathway, the final product (Z) often inhibits the enzyme that catalyzes the committed step (A \( \longrightarrow \) B). This kind of control is essential for the conservation of building blocks and metabolic energy.
The image depicts a biochemical pathway involving the biosynthesis of amino acids. The pathway begins with the reaction of 5-phosphoribosyl-\(\alpha\)-pyrophosphate (PRPP) with ATP to form \(N^\prime-5\prime\)-phosphoribosyl-ATP. This compound then undergoes further transformations, such as the condensation with glutamine to form glutamate, which can be used in the synthesis of purine and pyrimidine bases.

Key steps in the pathway include:

1. Formation of \(N^\prime-5\prime\)-phosphoribosyl-ATP from PRPP and ATP.
2. Conversion of glutamine to glutamate.
3. Formation of \(N^\prime-5\prime\)-phosphoribosylformiminoo-5-aminoimidazole-4-carboxamide ribonucleotide from \(N^\prime-5\prime\)-phosphoribosyl-ATP.
4. Further conversions leading to the synthesis of purine bases.

The diagram illustrates these reactions with specific chemical structures and labels, showing the flow of the biosynthetic process.

**Fig. 27–23 (Cont’d)**
Fig. 27–23. **Biosynthesis of histidine**

Atoms derived from PRPP and ATP are shaded red and blue, respectively. Two of the histidine nitrogens are derived from glutamine and glutamate (green). The pathway enzymes for the numbered reactions are: ① ATP phosphoribosyl transferase, ② pyrophosphohydrolase, ③ phosphoribosyl-AMP cyclohydrolase, ④ phosphoribosyl formimino-5- aminimidazole-4-carboxamide-ribonucleotide isomerase, ⑤ glutamine amidotransferase, ⑥ imidazole glycerol-3-phosphate dehydratase, ⑦ L-histidinol phosphate aminotransferase, ⑧ histidinol phosphate phosphatase, and ⑨ histidinol dehydrogenase. Note that the derivative of ATP
The first example of this unbranched pathway was observed in the biosynthesis of isoleucine in *E. Coli*. The dehydration and deamination of threonine to α-ketobutyrate is the committed step. *Threonine deaminase*, the PLP enzyme that catalyzes this reaction, is allosterically inhibited by isoleucine:

\[
\text{CH}_3\text{-CH-CH-COO}^- \xrightarrow{\text{OH, NH}_3^+} \text{CH}_3\text{-CH}_2\text{-C-COO}^- \xrightarrow{\text{O}} \text{CH}_3\text{-CH}_2\text{-CH-CH-COO}^- \xrightarrow{\text{CH}_3, \text{NH}_3^+} \text{Isoleucine} \]

Likewise, tryptophan inhibits the enzyme complex that catalyzes the first two steps in the conversion of chorismate into tryptophan (see page 700).

Take a hypothetical example of a branched biosynthetic pathway, in which *Y* and *Z* are the final products:

\[
\text{A} \rightarrow \text{B} \rightarrow \text{C} \rightarrow \text{D} \rightarrow \text{E} \rightarrow \text{Y} \]

\[
\text{A} \rightarrow \text{B} \rightarrow \text{C} \rightarrow \text{F} \rightarrow \text{G} \rightarrow \text{Z} \]

Suppose that high levels of *Y* or *Z* completely inhibit the first common step (A → B). Then, high levels of *Y* would prevent the synthesis of *Z* even if there were a deficiency of *Z*. In fact, several complex control mechanisms operate in branched biosynthetic pathways:

1. **Sequential feedback control.** In this mechanism, the first common step (A → B) is not inhibited directly by *Y* or *Z*. Rather, these final products inhibit the reactions after the branch point: *Y* inhibits the C → D step, and *Z* inhibits the C → F step. In turn, high levels of *C* inhibit the A → B step. Thus, the first common reaction (A → B) is blocked only if both *Y* and *Z* are present in excess.

This mechanism operates in the synthesis of aromatic amino acids in *Bacillus subtilis*. The first divergent steps in the synthesis of phenylalanine, tyrosine and tryptophan are inhibited by their final products. If all 3 are present in excess, chorismate and prephenate accumulate. These branch-point intermediates, in turn, inhibit the first common step in the overall pathway, *i.e.*, the condensation of phosphoenolpyruvate and erythrose-4-phosphate.
2. **Differential multiple enzyme control (Enzyme multiplicity).** Here, the first common step (A → B) is catalyzed by either of 2 different enzymes. One of them is directly inhibited by Y, and the other by Z. A high level of either Y or Z partially blocks the first step. In fact, both Y and Z must be present in sufficient quantities to prevent the conversion of A into B completely. In the rest of this control mechanism, like in sequential feedback control, Y inhibits the C → D step and Z inhibits the C → F step.

![Diagram of Enzyme Multiplicity](image)

Enzyme multiplicity mechanism operates in a variety of biosynthetic pathways in microbes. In *E. coli*, the condensation of phosphoenolpyruvate and erythrose-4-phosphate is catalyzed by 3 different enzymes. One is inhibited by phenylalanine, another by tyrosine, and the third by tryptophan. Furthermore, there are 2 different mutases that convert chorismate into prephenate.

One of them is inhibited by phenylalanine, and the other by tyrosine.

3. **Concerted feedback control.** Here, the first common step (A → B) is inhibited if high quantities of both Y and Z are simultaneously present. In contrast with enzyme multiplicity mechanism, a high level of either Y or Z alone does not appreciably inhibit the A → B step. Like the two preceding control schemes, here also Y inhibits the C → D step and Z inhibits C → F step.

![Diagram of Concerted Feedback Control](image)

An example of the concerted feedback control is the inhibition of bacterial aspartyl kinase by threonine and lysine, the two final products.

4. **Cumulative feedback control.** In this mechanism, the first common step (A → B) is partially inhibited by each of the final products, which act independently of the other. Suppose that a high level of Y decreased the rate of the A → B step from 100 to 70 s⁻¹ and that Z alone decreased the rate from 100 to 30 s⁻¹. Then, the rate of the A → B step in the presence of high levels of Y and Z would be \((0.7 \times 0.3 \times 100 \text{ s}^{-1}) = 21 \text{ s}^{-1}\).

An important example of cumulative feedback control is in the bacterial synthesis of glutamine from glutamate, NH₄⁺ and ATP which is regulated by glutamine synthetase. Earl Stadtman showed that this enzyme controls the flow of nitrogen. The amide group of glutamine is a source of nitrogen in the biosynthesis of a variety of compounds such as tryptophan, histidine, glucose-6-phosphate, CTP and ATP. Glutamine synthetase is cumulatively inhibited by each of these final products of glutamine metabolism, as well as by alanine and glycine. The enzymic activity of glutamine synthetase is switched off almost completely when all final products are bound to the enzyme.
The activity of glutamine synthetase is also regulated by reversible covalent modification — the attachment of an AMP unit by a phosphodiester bond to the hydroxyl group of a specific tyrosine residue in each subunit. There are two forms of glutamine synthetase: the more active deadenylation and the less active adenylated form. The adenylated form is more susceptible to cumulative feedback inhibition than the deadenyalted form. The covalently-attached AMP unit can be removed from the adenylated enzyme by phosphorolysis. Adenylation and deadenylation both are catalyzed by the same enzyme, adenyl transferase (AT). It has been observed that the specificity of adenyl transferase is controlled by a regulatory protein (designated $P$), which can exist in 2 forms, $P_A$ and $P_D$ and (Fig. 27–24). Adenylation is catalyzed when the enzyme AT forms a complex with one form ($P_A$) of the regulatory protein. The same enzyme (AT) catalyzes deadenylation when it is complexed with the other form ($P_D$) of the regulatory protein.

### MOLECULES DERIVED FROM AMINO ACIDS

Amino acids are the building blocks of peptides and proteins. They also serve as precursors of specialized biomolecules, such as hormones, coenzymes, nucleotides, porphyrins, alkaloids, antibiotics, pigments, neurotransmitters etc. Some of the leading biosynthetic pathways are discussed below.

1. **Biosynthesis of Porphyrins**

Glycine acts as a major precursor of porphyrins, which are constituents of hemoglobin, the cytochromes, and chlorophyll. The porphyrins are formed from four moles of the monopyrrole derivative, porphobilinogen (Fig. 27–25). In the first reaction, glycine reacts with succinyl-CoA to yield $\alpha$-amino-$\beta$-ketoadipate, which is then decarboxylated to produce $\delta$-aminolevulinate. This reaction is catalyzed by $\delta$-aminolevulinate synthase, a PLP enzyme in mitochondria. Two moles of $\delta$-aminolevulinate condense to form porphobilinogen, the next intermediate. This dehydration reaction is catalyzed by $\alpha$-aminolevulinate dehydrase. Four moles of porphobilinogen come together to form protoporphyrin, through a series of complex enzymatic reactions. The iron atom is incorporated after the protoporphyrin has been assembled.

In humans, genetic defects of certain enzymes of this pathway lead to the accumulation of specific porphyrin precursors in body fluids and in the liver. These genetic diseases are known as porphyrias. In congenital erythropoietic porphyria, which affects mainly erythrocytes, there is an

**Porphyrias** are inherited as acquired disorders caused by a deficiency of an enzyme in the heme biosynthetic pathway. About 15% of heme synthesis occurs in the liver and nearly all of the remainder occurs in erythrocyte precursors. Hence, the porphyrias are separated into hepatic or erythropoietic types. All types of porphyrias, except a few hepatic ones, are inherited. Those in which biochemical defect appears to be known include erythropoietic porphyria, acute intermittent porphyria, hereditary porphyria, porphyria cutanea tarda and lead poisoning.
accumulation of uroporphyrinogen I, the useless symmetric isomer of a precursor of protoporphyrin. It stains the urine red and causes the teeth to fluoresce strongly in UV light and the skin to be abnormally sensitive to light. Because insufficient heme is synthesized, patients with this disease are anemic, shy away from sunlight and have a propensity to drink blood. This condition may have given rise to the vampire myths in medieval folk legend. The disease is inherited as an autosomal dominant.

![Molecular diagram](image)

**Fig. 27–25.** Biosynthesis of protoporphyrin IX, the porphyrin of hemoglobin and myoglobin

The atoms furnished by glycine are shown in boldface. The remaining carbon atoms are derived from the succinyl group of succinyl-CoA. The enzymes involved in the numbered reactions are: 1 δ-aminolevulinate synthase, 2 porphobilinogen synthase, 3 uroporphyrinogen synthase, 4 uroporphyrinogen III cosynthase 5 uroporphyrinogen decarboxylase, and 6 coproporphyrinogen oxidase.
Acute intermittent porphyria is a quiet different disease. The liver, rather than the red cells, is affected and the skin is not typically photosensitive. Symptoms rarely occur before puberty; they include in order of frequency intermittent abdominal pain, vomiting, constipation, paralysis and psychological symptoms. The urine of the afflicted may have a port wine colour from photooxidation of the porphobilinogen excreted, together with 5-aminolevulinate, in large amounts. As its name implies, the disease is episodic in its clinical expression. The disease is inherited as an autosomal dominant.

2. Biosynthesis of Bile Pigments
The normal human erythrocyte has a life span of about 120 days. Old cells are removed from the circulation and degraded by the spleen. The apoprotein of hemoglobin is hydrolyzed to its constituent amino acids. The first step in the degradation of the heme group to bilirubin (Fig. 27–26) is the cleavage of its α-methene bridge to form biliverdin, a linear tetrapyrrole. This reaction is catalyzed by heme oxygenase. The central methene group of biliverdin is then reduced by biliverdin reductase to form bilirubin. Bilirubin binds to serum albumin and is transported to the liver, where it is transformed into the bile pigment bilirubin glucuronide. Bilirubin glucuronide is sufficiently water-soluble to be secreted with other components of bile into the small intestine. Impaired liver function or blocked bile function causes bilirubin to leak into the blood. This results in a yellowing of the skin and white of the eye, a general condition called jaundice.

![Biosynthesis of bilirubin from heme group of porphyrin](image)

3. Biosynthesis of Creatine and Glutathione
Phosphocreatine (PC) is derived from creatine (Cr) and acts as an important energy reservoir in skeletal muscle. Creatine is derived from glycine and arginine, and methionine (as S-adenosylmethionine) plays an important role as donor of a methyl group (Fig. 27–27).
Fig. 27–27. Biosynthesis of creatine and phosphocreatine

Note that creatine is made from 3 amino acids: glycine, arginine, and methionine. This pathway shows the versatility of amino acids as precursors in the biosynthesis of other nitrogenous biomolecules.
Glutathione (GSH) is a tripeptide containing glycine, glutamate and cysteine (Fig. 27–28). The first step in its synthesis is the formation of a peptide linkage between the γ-carboxyl group of glutamate and the α-amino group of cysteine, in a reaction catalyzed by γ-glutamylcysteine synthase. Formation of this peptide bond requires activation of the γ-carboxyl group, which is achieved by ATP. The resulting acyl phosphate intermediate is then attacked by cysteine amino group. This reaction is feedback-inhibited by glutathione. In the second step, which is catalyzed by glutathione synthetase, ATP activates the carboxyl group of cysteine to enable it to condense with the amino group of glycine. Glutathione is a highly distinctive amino acid derivative with several important functions. Glutathione is present in virtually all cells, often at high levels (~5 mM), and serves as a sulfhydryl buffer. It cycles between a reduced thiol form (GSSG) in which 2 tripeptides are linked by a disulfide bond. GSSG is reduced to GSH by glutathione reductase. The ratio of GSH to GSSG in most cells is more than 500. Glutathione also plays a key role in detoxification by reacting with H2O2 and organic peroxides, the harmful byproducts of organic life.

\[
2\text{GSH} + \text{R—O—OH} \xrightarrow{\text{Glutathione peroxidase}} \text{GSSG} + \text{H}_2\text{O} + \text{R—OH}
\]

*Glutathione peroxidase*, the enzyme catalyzing this reaction, is unique in possessing a covalently-attached selenium (Se) atom.

**Fig. 27–28. Biosynthesis and structure of glutathione**

Also shown is the oxidized form of glutathione (GSSG), which contains 2 moles of glutathione linked by a disulfide bond.

4. Biosynthesis of Neurotransmitters

The small diffusible molecules which communicate nerve impulses across most synapses are called neurotransmitters. Many important neurotransmitters are primary or secondary amines, derived from amino acids in simple pathways. Besides, certain polyamines, that are conjugated with DNA, are derived from the nonprotein amino acid, ornithine.

The synthesis of some neurotransmitters is illustrated in Fig. 27–29. Tyrosine gives rise to a family of catecholamines that comprises dopamine, norepinephrine and epinephrine. Levels of catecholamines are correlated

The word catecholamine is derived from catechol, which is a fairly recent shortening of the trivial name pyrocatechol or 1,2-dihydroxybenzene (catechol was a more complex aromatic compound from which pyrocatechol was obtained by heating). The nomenclature is unfortunate, but it is in common usage.
with changes in blood pressure in animals. Parkinson’s disease, a neurological disorder, is associated with an underproduction of dopamine. The disease is cured by administering L-dopa. An overproduction of dopamine in the brain leads to psychological disorders such as schizophrenia. An inhibitory neurotransmitter called γ-aminobutyrate (GABA) is produced from glutamate on decarboxylation. An underproduction of GABA leads to epilepsy. GABA is used in the treatment of epileptic seizures and hypertension. Tryptophan, in a 2-step pathway, produces serotonin (= 5-hydroxytryptamine), another important neurotransmitter.

![Chemical structures and pathways involving neurotransmitters and related enzymes.](image-url)
BIOSYNTHESIS OF AMINO ACIDS

Fig. 27–29. Biosynthesis of some neurotransmitters derived from amino acids

Note that the key biosynthetic step is the same in each case: a PLP-dependent decarboxylation (shaded).

Histidine, upon decarboxylation, produces histamine which is a powerful vasodilator found in animal tissues. In allergic responses, histamine is produced in large amounts. It also stimulates acid secretion in the stomach.

5. Formation of Nitric Oxide from Arginine

Nitric Oxide (NO), a short-lived signal molecule, is produced endogenously from arginine in a complex reaction that is catalyzed by nitric oxide synthase (NOS); citrulline is the other product.

Nitric oxide, a free-radical gas, is an important messenger in many vertebrate signal transduction processes. It diffuses freely across membranes but has a short life span, less than a few seconds,
because it is highly reactive. Hence, nitric oxide is well suited to serving as a transient signal molecule within cells and between adjacent cells.

6. Biosynthesis of Lignin, Tannin and Auxin

The aromatic amino acids, phenylalanine, tyrosine and tryptophan are precursors of many plant substances. **Lignin**, a complex rigid polymer, is derived from phenylalanine and tyrosine. *It is second only to cellulose in abundance in plant tissues.* Phenylalanine and tyrosine also give rise to many natural products such as **tannins** that inhibit oxidation in wines.

Tryptophan gives rise to plant growth hormone, **indole-3-acetate** (IAA) or **auxin** (Fig. 27–30). This molecule regulates some important physiological processes.

![Fig. 27–30. Biosynthesis of indole-3-acetate](image)

7. Biosynthesis of Papaverine from Tyrosine

The term ‘**alkaloid**’ (which means alkali-like), first proposed by a pharmacist, C.F.W. Meissner in 1819, was originally applied to all organic bases isolated from plants. As this definition included unnecessarily an extraordinary wide variety of compounds, so the definition changed. Later, Landenburg defined alkaloids as natural plant compounds having a basic character and containing at least one nitrogen atom in a heterocyclic ring. His definition noticeably excluded any synthetic compounds and any compounds obtained from animal sources. Some authors specify those alkaloids obtained from plants as **plant alkaloids** or **vegetable alkaloids**. They are very poisonous, but are used medicinally in very small quantities. Thus, plant alkaloids are characterized by their basic properties, (usually) complex structures, specific physiological actions and their plant origin. In fact, they represent another group of secondary metabolites, parallel to terpenes or terpenoids. About 4,500 alkaloids have so far been isolated from more than 4,000 species of plants, mostly from dicot families such as Papaveraceae, Apocynaceae, Papilionaceae, Ranunculaceae, Rubiaceae, Rutaceae and Solanaceae: the first one being unusual in that almost all its members contain alkaloids. Among monocot families, they are found in Liliaceae and Gramineae (=Poaceae). Alkaloids are generally found in the roots, bark, stem, leaves and seeds of these plants and are stored in the vacuoles (hence, they do not appear in young cells until they are vacuolated) as salts of various plant organic acids, such as acetic, citric, oxalic, tartaric etc.

The alkaloids are usually colourless, crystalline, nonvolatile solids, which are insoluble in water but are soluble in ethanol, ether, chloroform etc. Some alkaloids are liquid which are soluble in water (e.g., conine and nicotine) and a few are coloured (e.g., berberine is yellow). Most alkaloids have a bitter taste and are optically active (levorotatory). Alkaloids form insoluble precipitates with solutions of phosphotungstic acid, phosphomolybdic acid, picric acid etc; many of these have definite crystalline shapes, hence may be used to help in their identification. The alkaloids are stored in sites other than the site of their synthesis. For example, the alkaloid **nicotine** is synthesized in tobacco roots but is transported and stored in the leaves. Some alkaloids are even modified at the storage sites. Basically, the alkaloids are synthesized from various amino acids (ornithine, lysine, tyrosine, phenylalanine, asparagine, tryptophan, proline, glutamic acid) or their derivatives. Because of their physiological effects on human systems, alkaloids have been used in pharmacy since time immemorial. Conine is principle alkaloid of hemlock (Conium masculatum) which was responsible for the death of the great Greek philosopher Socrates in 399 B.C. when he drank the cup of poison–hemlock oil. They act as nitrogen excretory products. They also help in maintaining ionic balance. Besides, they act as growth regulators, most probably germination inhibitors because they have chelating power.
reveals their close relationship to amino acids such as phenylalanine, tyrosine, tryptophan, lysine or proline. As an example, the structure of papaverine, one of the alkaloids of opium, is given in Fig. 27–31. Note that this compound could be formed rather simply from two molecules of dihydroxyphenylalanine with the elimination of both carboxyls and one amino group. The actual pathway is not known.

Fig. 27–31. Chemical structure of papaverine

REFERENCES


**PROBLEMS**

1. Write a balanced equation for the synthesis of alanine from glucose.

2. Isovaleric acidemia is an inherited disorder of leucine metabolism caused by a deficiency of isovaleryl CoA dehydrogenase. Many infants having this disease die in the first month of life. The administration of large amounts of glycine sometimes leads to marked clinical improvement. Propose a mechanism for the therapeutic action of glycine.

3. Most cytosolic proteins lack disulfide bonds, whereas extracellular proteins usually contain them. Why?

4. The synthesis of δ-aminolevulinate takes place in the mitochondrial matrix, whereas the formation of porphobilinogen takes place in the cytosol. Propose a reason for the mitochondrial location of the first step in heme synthesis.

5. Which of the 20 amino acids can be synthesized directly from a common metabolic intermediate by a transamination reaction?

6. For the following example of a branched pathway, propose a feedback inhibition scheme that would result in the production of equal amounts of Y and Z.
7. The first common step \((A \rightarrow B)\) is partly inhibited by both of the final products, each acting independently of the other. Suppose that a high level of \(Y\) alone decreased the rate of the \(A \rightarrow B\) step from 100 to 60 s\(^{-1}\) and that a high level of \(Z\) alone decreased the rate from 100 to 40 s\(^{-1}\). What would the rate be in the presence of high levels of both \(Y\) and \(Z\)？

8. How might increased synthesis of aspartate and glutamate affect energy production in a cell? How would the cell respond to such an effect?

9. The mitochondrial form of carbamoyl phosphate synthetase is allosterically activated by \(N\)-acetylglutamate. Briefly describe a rationale for this effect.

10. Most bacterial mutants that require isoleucine for growth also require valine. Why? Which enzyme or reaction would be defective in a mutant requiring only isoleucine (not valine) for growth?

11. Describe a series of allosteric interactions that could adequately control the biosynthesis of valine, leucine, and isoleucine.

12. Identify carbon atoms, by number, that are incorporated from this structure into the compounds listed in \((a)\) to \((d)\).

\[
\begin{align*}
5\text{CH}_3 & \quad \text{S} & \quad \text{CH}_2 \\
4\text{CH}_2 & \quad \text{O} & \quad \text{Adenine} \\
3\text{CH}_2 & \quad \text{OH} & \quad \text{OH} \\
2\text{HC} & \quad \text{NH}_3 & \\
1\text{COO} & \\
\end{align*}
\]

\((a)\) Creatine phosphate .................

\((b)\) Ethylene ......................

\((c)\) Glycine betaine ...................

\((d)\) Epinephrine ......................

13. Why is phenylketonuria resulting from dihydropteridine reductase deficiency a more serious disorder than PKU resulting from phenylalanine hydroxylase deficiency?

14. Tyrosine is normally a nonessential amino acid, but individuals with a genetic defect in phenylalanine hydroxylase require tyrosine in their diet for normal growth. Explain.

15. Write the net equation for the synthesis of the nonessential amino acid aspartate from glucose, carbon dioxide, and ammonia.