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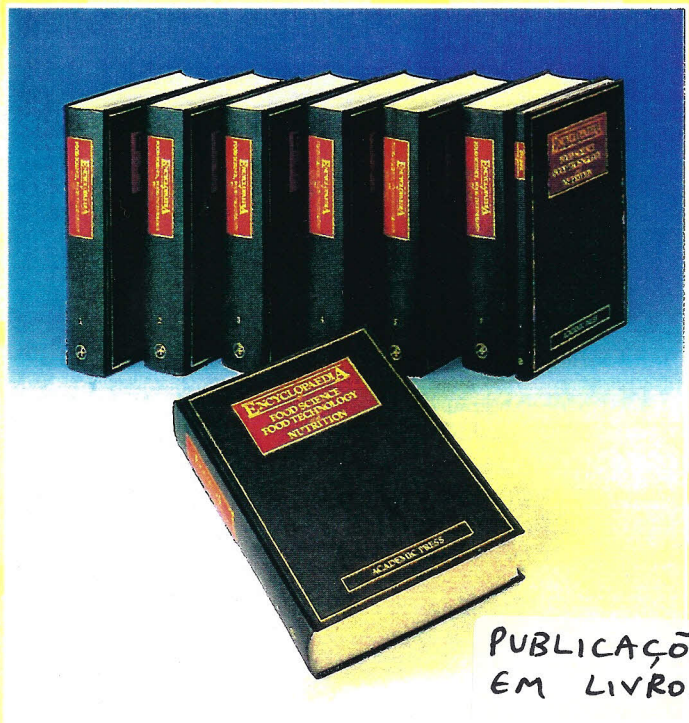

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tion with OPA. Alternatively an RPC method using a chiral stationary phase, in which the Cu–Pro or Cu–hydroxyproline complex is bound to a silica stationary phase, can be used.

D- and L-amino acids can also be determined by GLC with the introduction of a second, optically pure, asymmetric centre into the molecule to make diastereoisomers which can be separated on conventional packed columns. The use of (+)-butan-2-ol to form (+)-2-butyl esters appears to be the best method. Alternatively the enantiomers, converted to normal derivatives, e.g. TFA isopropyl esters, can be separated on capillary columns coated with chiral stationary phases, e.g. *N*-TFA-L-valyl-L-valine cyclohexyl ester.

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Metabolism

Naturally occurring amino acids may be conveniently grouped into three categories: protein amino acids (sometimes known as 'standard', 'primary' and 'normal'), uncommon amino acids, and nonprotein amino acids. Protein amino acids are those that are coded for in the genes and incorporated directly into proteins. For some time it seemed well established that all proteins, whatever their origin, were constructed from the same set of 20 amino acids. Recent studies, however, have shaken the foundation of this classical dogma. It now seems that the genetic code may dictate the incorporation of more than 20 amino acids. Thus, for example, selenocysteine and phosphoserine, previously considered to be uncommon amino acids, can be directly incorporated into the polypeptide chain. All protein amino acids are α,L-amino acids. It is not clear why amino acids incorporated by organisms into proteins are of the L form, since L-amino acids have no obvious inherent superiority over their D isomers for biological function. Thus, in this article, unless otherwise stated, an L configuration is assumed.

The 20 classical protein amino acids may be grouped into several classes reflecting important characteristics of their side-chains: straight aliphatic amino acids (glycine, alanine), branched-chain amino acids (valine, leucine, isoleucine), hydroxy amino acids (serine, threonine), sulphur-containing amino acids (cysteine, methionine), aromatic amino acids (phenylalanine, tyrosine), heterocyclic amino acids (tryptophan, histidine), basic amino acids (lysine, arginine), acidic amino acids and their amides (aspartate, glutamate, asparagine, glutamine) and imino acid (proline). Amino acids can also be classified on the basis of the polarity of their side-chains. *See* Protein, Chemistry

Analyses of proteins have revealed that they contain well over 100 different amino acids. The occurrence of uncommon amino acids in proteins is the result of post-translational, covalent modification of protein amino acids. Cystine, for example, is formed by the post-translational cross-linking of two cysteine residues. Citrulline, *N*-formylmethionine, *O*-galactosylserine, and *N*-acetylthreonine constitute other examples of amino acids found in proteins.

The amino acids found in proteins are by no means the only ones to occur in living organisms. Thus the term 'nonprotein amino acids' is used to include those naturally occurring amino acids which are present in free or combined forms but not in proteins. Over 200 nonprotein amino acids are known, most of them occurring in plants and frequently limited, in each case, to certain taxonomic groups. Some, such as cystathionine and saccharopine, fulfil important roles in the primary metabolic pathways. However, the great majority of these compounds have obscure functions and are generally regarded as secondary products. Many of the nonprotein amino acids from plants are known to be toxic to animals, plants and microorganisms. Some accumulate to exceptionally high levels, as in the case of 5-hydroxytryptophan, canavanine or 3,4-dihydroxyphenylalanine, which may constitute up to 14% of the seed weight in some Leguminosae species. Storage and protection against predation are probably two of the many possible roles that these amino acids play in plants.

Essential and Nonessential Amino Acids

Organisms differ greatly in their abilities to synthesize the amino acids required for protein synthesis. Many

microorganisms and plants are entirely self-sufficient in that they can synthesize the entire basic set of protein amino acids. However, the bacterium *Leuconostoc mesenteroides* can synthesize only four of the protein amino acids, whereas *Lactobacillus*, which flourishes in milk, must be provided with all amino acids required for protein synthesis. Mammals are intermediate, being able to synthesize about half of the protein amino acids. Amino acids which cannot be synthesized by an organism in adequate amounts are called essential or indispensable because they must be supplied by the diet. Those which can be synthesized by an organism from readily available precursors in sufficient amounts to meet its needs are not required in the diet and are referred to as nonessential or dispensable amino acids. A consensus of current nutritional opinion indicates that the L-isomers of 10 amino acids – arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine – are considered to be essential for mammals, including humans.

The designations of essential and nonessential amino acids refer to the needs of an organism under a particular set of conditions. Thus essential amino acids are often species specific, i.e. the set of amino acids that are essential for a particular organism is not necessarily the same for other organisms. The essential amino acids requirements depend on a variety of factors including age, sex, physiological conditions and diet. Arginine (see *Urea Cycle*, below) and histidine are synthesized by humans in quantities sufficient to meet the needs of an adult but not those of a growing child. These amino acids have been termed semi- or half-essential. Adults have proportionally lower demands for essential amino acids than infants and children because adults are able to efficiently recycle such amino acids, whereas infants need them for tissue growth. When the ratio of total essential amino acids required to total protein required is considered, it is 0·37 for infants and 0·15 for adults. Tyrosine and cysteine are considered nonessential amino acids for mammals only as long as the diet contains adequate amounts of, respectively, phenylalanine and methionine; this is because, in mammals, tyrosine is formed in one step directly from phenylalanine, and cysteine derives its sulphur uniquely from dietary methionine (see *Synthesis of Amino Acids* below). Hence the apparent quantitative phenylalanine requirement is actually a requirement for phenylalanine plus tyrosine, whereas that of methionine is for methionine plus cysteine.

The essential amino acids include those with complex structures, which are formed by complex routes, whereas the nonessential are those whose syntheses are the simplest and whose intermediate precursors are always present in all organisms. Indeed, 59 enzymes are required by prokaryotic cells to synthesize the essential amino acids for humans, but only 15 are required for the non-essential. Essential amino acids other than lysine and threonine (the only amino acids that, in mammals, do not participate in transamination reactions; see *Transamination and Deamination*, below) can be replaced by their α-keto analogues in the diet. This indicates that the carbon skeleton of the essential amino acid is the fundamental part of the amino acid molecule.

A deficiency of even one essential amino acid in the diet of an organism results promptly in a negative nitrogen balance, i.e. total nitrogen excretion exceeding total nitrogen intake, indicating that tissue protein is being degraded and used to supply the missing amino acid for those 'high priority' proteins that need to be continually synthesized. The remaining amino acids then accumulate and are shunted into catabolic pathways – hence the loss of nitrogen. Under these conditions protein synthesis is severely inhibited because the ribosome – mRNA (messenger ribonucleic acid) – nascent polypeptide complex must suspend its operation at the point where the missing amino acid should be incorporated. Thus the degree of negative nitrogen balance is similar whether only one, several or all of the essential amino acids are missing. This is logical because nearly all body proteins contain all the essential amino acids.

Regardless of the organism or of the essential amino acids considered, the net result of its deficiency involves inevitably a decreased growth rate, increased susceptibility to disease and biochemical dysfunctions along with ultimate death. However, deficiencies of a specific essential amino acid may also result in disturbances characteristic of that particular amino acid. This is the case for tryptophan in nicotinic acid formation, and lysine in the formation of hydroxylysine in the biosynthesis of collagen. *See* Niacin, Physiology

Amino Acid Biosynthesis

Amino acid metabolism involves the dynamic occurrence of anabolic and catabolic pathways. It is sometimes difficult to distinguish between catabolic and anabolic reactions because the catabolism of one amino acid may be involved in the biosynthesis of another. Because of the complexity and multiplicity of these pathways only a simplified version of the major routes will be considered.

Nitrogen Assimilation

Inorganic nitrogen is incorporated into organic nitrogen compounds as ammonium. This process, called ammonium assimilation, leads to the formation of glutamate, glutamine and carbamoyl phosphate. Utilization of the nitrogen of carbamoyl phosphate is limited to the

biosynthesis of arginine (see *Urea Cycle*, below) and pyrimidine nucleotides. Essentially, all other nitrogen atoms of amino acids and other nitrogenous compounds are derived directly or indirectly from glutamate or glutamine.

The reductive amination of 2-oxoglutarate by ammonium ions (NH_4^+), catalysed by glutamate dehydrogenase, is the simplest route to the formation of α-amino groups:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADPH} + H^+ \longrightarrow \text{glutamate} + H_2O + NADP^+$$

(NADPH, $NADP^+$ represent the reduced and oxidized forms of the nicotinamide adenine dinucleotides.) This reaction occurs in plants and bacteria only under situations of high NH_4^+ concentration, which is toxic to cells and does not happen frequently under natural conditions, implying that this enzyme does not play a significant role in primary ammonium assimilation. Under natural conditions, the glutamate synthase cycle constitutes the major pathway by which plants and microorganisms assimilate NH_4^+. This cycle involves the sequential action of two enzymes: glutamine synthetase, which catalyses the adenosine triphosphate (ATP)-dependent amidation of glutamate to produce glutamine, and glutamate synthase, which catalyses the reductive transfer of the δ-amino group of glutamine to 2-oxoglutarate, to produce two molecules of glutamate. The sum of these reactions is as follows:

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NADPH} + \text{ATP} \longrightarrow \text{glutamate} + NADP^+ + \text{ADP} + P_i$$

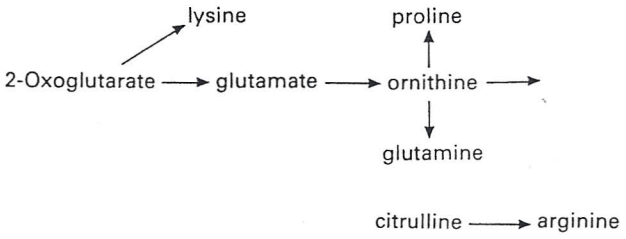
(or reduced ferredoxin; or oxidized ferredoxin; (inorganic phosphate))

Synthesis of Amino Acids

The biosyntheses of protein amino acids arise as branching pathways from a few key intermediates in the central metabolic routes that are common to all cells, namely glycolysis, the pentose phosphate pathway, and the tricarboxylic acid (TCA) cycle. It is convenient to divide the 20 classical protein amino acids into six biosynthetic families according to the central metabolites that serve as starting points for their syntheses. ***See*** Glucose, Function and Metabolism

The Glutamate Family

2-Oxoglutarate, a TCA cycle intermediate, serves as the starting point in the formation of glutamate and the other members of the glutamate family, glutamine, proline, arginine and, in the fungi and *Euglena*, lysine:



The Serine Family

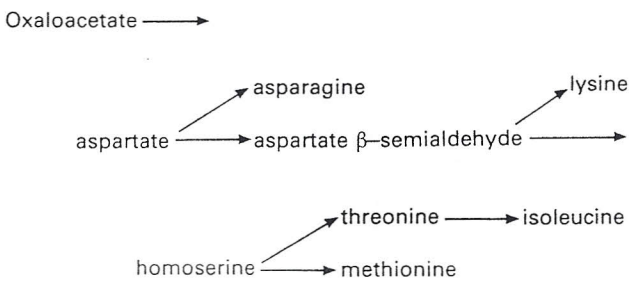
3-Phosphoglycerate, an intermediate of the glycolytic pathway, serves as a precursor for the serine family of amino acids, comprising serine and its derivative amino acids, glycine and cysteine:

In common with carbon and nitrogen, environmental sulphur is available to organisms in the form of inorganic compounds. Sulphur assimilation is largely confined to plants and microorganisms since higher animals, unable to assimilate inorganic sulphur, must rely on ingested methionine and cysteine. Thus, whilst some microorganisms can reduce sulphate, thiosulphate or elemental sulphur, higher plants use sulphate for amino acids synthesis. Reductive assimilation of sulphate, i.e. incorporation of sulphate sulphur into thiol groups of amino acids and other organic compounds, requires the reduction of sulphate to sulphite and, subsequently, of sulphite to sulphide.

Two major pathways exist for the biosynthesis of cysteine in living organisms. Plants and microorganisms, which utilize H_2S as the source of sulphur, synthesize cysteine by the direct sulphhydrylation pathway. However, in mammals, which synthesize cysteine by the transsulphuration pathway, cysteine derives its carbon skeleton from serine but its sulphur atom is obtained uniquely from methionine.

The Aspartate Family

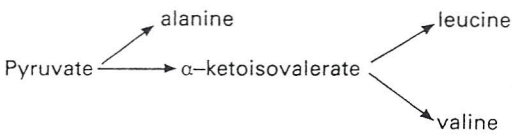
Oxaloacetate, an intermediate of the TCA cycle, provides the carbon skeleton for the synthesis of six different amino acids: aspartate, asparagine, lysine (in bacteria and plants but not in fungi), methionine, threonine and isoleucine, which constitute the aspartate family of amino acids:



However, isoleucine is frequently included in the pyruvate family since four of its five biosynthetic enzymes are common to the valine pathway. Methionine derives its sulphur atom from cysteine.

The Pyruvate Family

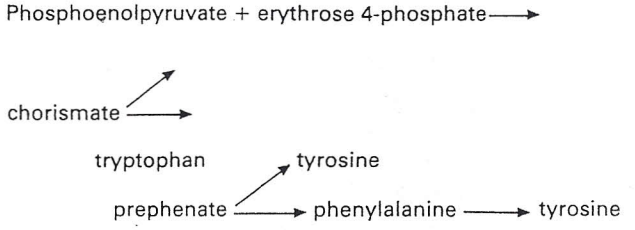
The pyruvate family of amino acids includes alanine, valine and leucine:



Pyruvate, a glycolytic intermediate, gives rise to the carbon skeletons of alanine and valine, and to 4 of the 6 carbons of leucine. In addition, pyruvate also donates 2 carbon atoms to the synthesis of isoleucine and, on average, 2·5 carbons to the synthesis of lysine in bacteria and plants. As mentioned earlier, isoleucine, a member of the aspartate family, is most conveniently considered along with valine, since the biosynthesis of both involves a common set of enzymes.

The Aromatic Family

Phenylalanine, tyrosine and tryptophan, which comprise the aromatic family of amino acids, are synthesized from phosphoenolpyruvate and erythrose 4-phosphate, intermediates of glycolysis and the pentose phosphate pathway, respectively:



These amino acids are synthesized by a branched pathway in which chorismate is the major branch-point metabolite. Chorismate is synthesized by a seven-step pathway, often referred to as the shikimate or common aromatic pathway, to build the benzene ring.

In some organisms, including humans, tyrosine can be synthesized by hydroxylation of phenylalanine in a reaction catalysed by phenylalanine hydroxylase. This reaction, the only known reaction of aromatic amino acid biosynthesis in animals, accounts for the nonessentiality of tyrosine in mammals, and is not reversible, which explains why tyrosine cannot replace the nutritional requirement for phenylalanine.

The Histidine Family

Histidine is synthesized from ribose 5-phosphate, a pentose phosphate pathway intermediate, by a pathway unrelated to those of the other amino acids.

Uncommon amino acids are synthesized by post-translational, covalent modification of protein amino acids. These modifications, which may either be enzyme-catalysed or occur spontaneously, involve a variety of chemical processes including glycosylation, phosphorylation, hydroxylation, methylation, acetylation and amidation. Hydroxyproline and hydroxylysine, for example, are two uncommon amino acids almost exclusively associated with collagen. The preformed amino acids, as they may occur in ingested food protein, are not incorporated into collagen since there are no tRNAs (transfer RNAs) capable of recognizing and inserting them into a nascent polypeptide chain. Rather, these amino acids are synthesized by hydroxylation of prolyl and lysyl residues, in reactions catalysed by prolyl hydroxylase and lysyl hydroxylase, respectively. 3-*N*-Methylhistidine constitutes another example of an uncommon amino acid. This amino acid, found in actin and myosin, is synthesized by methylation of an histidyl residue in an enzymatic reaction that utilizes *S*-adenosylmethionine as the methyl group donor. This process is clearly highly specific because only one histidine out of the 35 found in the heavy chain of myosin is methylated. Furthermore, the extent of methylation varies with a number of factors including age and diet and is generally not complete in that specific residues are found to be methylated in only a fraction of the myosin molecules.

Relatively little work has been done on the biosynthesis of non-protein amino acids. There are four different ways by which these amino acids may be formed: (1) as intermediates in protein amino acid synthesis; (2) modification of protein amino acids; (3) modification of pathways to protein amino acids; (4) novel pathways.

Regulation of Amino Acid Biosynthesis

Living cells contain a small pool of free protein amino acids resulting from a precise and coordinated control of

the rates at which each amino acid is synthesized and degraded. The mechanisms that control amino acid synthesis vary widely in the various pathways and, for the same pathway, in different organisms. Most studies have been performed with microorganisms, in particular with *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhimurium*. The regulation of amino acid biosynthesis occurs at two levels: regulation of enzyme activity or metabolite flow over a pathway and regulation of enzyme amount. *See* Enzymes, Functions and Characteristics

Control of Enzyme Activity

The control over the flow of metabolites into an amino acid biosynthetic pathway can be efficiently achieved by blocking the first, usually irreversible step which is specific for that amino acid. The inhibition of the committed step by the end product, i.e. the amino acid itself, constitutes the simplest kind of feedback inhibition. Some examples include the regulation of the biosynthesis of proline, arginine, histidine and of the branched-chain amino acids. Alanine, aspartate, glutamate and glycine are four amino acids for which no form of feedback inhibition is known. However, these amino acids are usually in equilibrium, by means of reversible reactions, with compounds that are key intermediates in the central metabolic routes. Metabolite flow into the biosynthetic pathways of the remaining 16 protein amino acids is controlled by several types of feedback inhibition.

Sequential feedback inhibition regulates the synthesis of aromatic amino acids in *B. subtilis*. The first divergent steps in the synthesis of these amino acids are inhibited by their final products. If all three are present in excess, the branch-point intermediates chorismate and prephenate will accumulate, inhibiting the first common enzyme in the overall pathway, i.e. the first reaction of the shikimate pathway.

Enzyme multiplicity regulates the synthesis of aromatic amino acids in *E. coli*, *S. typhimurium* and *Neurospora crassa* and the synthesis of the aspartate family of amino acids in *E. coli*. In the former, those organisms possess three isoenzymes which catalyse the first reaction of the shikimate pathway – one inhibited by phenylalanine, one by tyrosine, and one by tryptophan. In the latter, three forms of the enzyme catalysing the first reaction of the pathway leading from aspartate to aspartate β-semialdehyde exist – one inhibited by methionine, one by threonine, and one by lysine.

Bacillus polymyxa and *Rhodopseudomonas capsulata* possess a single enzyme catalysing the first reaction of the pathway leading from aspartate to aspartate β-semialdehyde, and its regulation is achieved by concerted feedback inhibition. Lysine and threonine alone are only weak inhibitors, but when both present, a strong synergistic inhibition occurs.

The regulation of *E. coli* glutamine synthetase, a key enzyme in the flow of inorganic nitrogen to organic compounds, is an example of cumulative feedback inhibition. Eight inhibitors are either metabolic end products of glutamine (tryptophan, histidine, carbamoyl phosphate, glucosamine 6-phosphate, cytidine triphosphate and adenosine monophosphate, or AMP) or in some other way indicators of the general status of amino acid metabolism (alanine and glycine). Each of the eight compounds alone gives only partial inhibition, but in combination, with each acting independently of the others, the degree of inhibition is increased until the activity is almost completely switched off when all eight compounds are simultaneously present.

Other ways of controlling enzyme activity include the following: (1) activation of enzyme activity by metabolites; (2) modification of enzymes (e.g. adenylation of certain enzymes may render them more susceptible to feedback inhibition); (3) protein–protein interactions (e.g. activity of multienzyme complexes may change with the amounts of its components present).

Control of Enzyme Amount

The amount of an enzyme may be controlled by a number of different mechanisms: (1) end-product repression of enzyme synthesis (e.g. the coordinate repression of the synthesis of all the enzymes involved in histidine biosynthesis in *E. coli* by histidine); (2) substrate induction of enzyme synthesis (e.g. the induction of the synthesis of the first enzyme involved in cysteine biosynthesis in *E. coli* by the product of its reaction); (3) metabolite depression of enzyme synthesis (e.g. the synthesis of all amino acid biosynthetic enzymes is strongly reduced when *E. coli* is grown in a rich medium); (4) regulation of enzyme degradation. Very little is known on this last topic. Nevertheless, the protection of a given enzyme against proteolysis is probably an important regulatory process.

Amino Acid Catabolism

All living cells undergo intracellular protein degradation, with the resulting amino acids being recycled into proteins or degraded oxidatively to yield energy. In microorganisms and plants amino acids are not generally present in excessive amounts. In higher animals, however, where amino acids intake may largely exceed the metabolic needs, amino acids present in excess are not stored or excreted as such. Instead, they are used for energy production. It is estimated that amino acids supply about 15% of the total energy required by an average human adult. This value may be increased under conditions of energy insufficiency or nutritional

pathologies. Amino acids can also constitute an important energy source in plants, during the germination of protein-storing seeds, and in microorganisms, when carbohydrates or fatty acids are not available. This is the case in many bacteria that can grow in media containing amino acids as the source of energy, carbon and nitrogen. These organisms utilize amino acids catabolic pathways analogous to those of higher animals.

The catabolic metabolism of amino acids is mainly concerned with the separation of the amino groups from the carbon skeletons and the subsequent fate of both the amino groups and the carbon chains. *See* Energy, Measurement of Food Energy

Transamination and Deamination

In general, one of the first steps in the degradation of amino acids involves the removal of the α-amino group to give the corresponding 2-oxo acid. Two distinct types of reactions are known to accomplish this task: transamination and deamination.

Transamination, the most common mechanism for deamination of amino acids, involves the transfer of an amino group from a donor amino acid to an acceptor 2-oxo acid, with the formation of a new amino acid and a new oxo acid. Transamination reactions are catalysed by pyridoxal phosphate-dependent enzymes termed transaminases or, more properly, aminotransferases. These enzymes have a twofold specificity in that they are specific for the acceptor 2-oxo acid but nonspecific for the donor amino acid. Most aminotransferases are specific for 2-oxoglutarate as the acceptor 2-oxo acid, although some may use either pyruvate or oxaloacetate. Accordingly, there are three classes of aminotransferases, which form glutamate, alanine and aspartate, respectively. More than 50 aminotransferases have been identified. With the exception of lysine and threonine, the α-amino groups of all the amino acids found in proteins can be removed by transamination. Moreover, transamination is not restricted to α-amino groups since, for example, the δ-amino group of ornithine is readily transaminated. Transaminases fulfil central catabolic as well as anabolic functions in the metabolism of several amino acids because they catalyse freely reversible reactions, having equilibrium constants close to unity.

Transamination does not result in a net removal of nitrogen from amino acids. It does, however, allow for the collection of amino groups in glutamate. Oxidative deamination of glutamate by glutamate dehydrogenase results in the liberation of ammonium. The 2-oxoglutarate thus produced can either be used as the acceptor 2-oxo acid in further transamination reactions or enter the TCA cycle. Glutamate is the only amino acid for which a specific and highly active dehydrogenase exists. This pathway, i.e. the concerted action of the aminotransferases and glutamate dehydrogenase, is responsible for most of the ammonium produced by the catabolism of amino acids.

Additional minor routes for the deamination of amino acids are provided by amino acid oxidases, capable of oxidizing most naturally occurring amino acids, and by dehydratases, capable of removing non-oxidatively the amino groups of some amino acids.

Urea Cycle

Plants and microorganisms commonly excrete very little nitrogen. Growth of these organisms is often restricted by a limited availability of nitrogen so that nitrogen liberated by catabolic pathways is usually reassimilated. However, because high concentrations of NH_4^+ are extremely toxic to cells, animals must get rid of the excess ammonium produced by the catabolism of amino acids, either by direct excretion or, when removal of NH_4^+ by simple diffusion is difficult, by conversion to less toxic excretory products. Most terrestrial vertebrates, including mammals, excrete ammonia in the form of urea. Urea is highly soluble in water but non-toxic to cells.

Urea is synthesized by the urea cycle, which is carried out almost exclusively in liver cells. This cycle, discovered by Hans Krebs and Kurt Henseleit in 1932, consists of five sequential enzymatic reactions:

1. $NH_4^+ + HCO_3^- + 2ATP \longrightarrow$ carbamoyl phosphate + $2ADP + P_i + H^+$
2. Carbamoyl phosphate + ornithine $\longrightarrow$ citrulline + P_i
3. Citrulline + aspartate + ATP $\longrightarrow$ argininosuccinate + $AMP + PP_i$
4. Argininosuccinate $\longrightarrow$ arginine + fumarate
5. Arginine + $H_2O \longrightarrow$ ornithine + urea

The sum of these reactions is as follows:

$$NH_4^+ + HCO_3^- + 3ATP + H_2O + \text{aspartate} \longrightarrow \text{urea} + 2ADP + AMP + 2P_i + PP_i + H^+ + \text{fumarate}$$

Virtually all organisms synthesize arginine from ornithine by reactions 2–4. However, only ureotelic organisms are capable of catalysing the hydrolysis of arginine (reaction 5), the reaction responsible for the cyclic nature of the urea cycle. The synthesis of urea is energetically expensive, requiring the hydrolysis of 4 molecules of ATP per turn of the cycle (2 molecules of ATP are needed to convert AMP to ATP). The fumarate produced is hydrated to malate and oxidized to oxaloacetate by TCA cycle enzymes. Aspartate is then

Table 1. Metabolic fates of the carbon skeletons of amino acids

Amino acid	Product(s) of catabolism	Metabolic fate
Alanine	Pyruvate	Glycogenic
Arginine→glutamate	2-Oxoglutarate	Glycogenic
Asparagine→aspartate	Oxaloacetate	Glycogenic
Aspartate	Oxaloacetate, fumarate[a]	Glycogenic
Cysteine	Pyruvate	Glycogenic
Glutamate	2-Oxoglutarate	Glycogenic
Glutamine→glutamate	2-Oxoglutarate	Glycogenic
Glycine→serine	Pyruvate	Glycogenic
Histidine→glutamate	2-Oxoglutarate	Glycogenic
Methionine	Succinyl-CoA	Glycogenic
Proline→glutamate	2-Oxoglutarate	Glycogenic
Serine	Pyruvate	Glycogenic
Threonine	Pyruvate	Glycogenic
Valine	Succinyl-CoA	Glycogenic
Isoleucine	Succinyl-CoA, acetyl-CoA	Glycogenic and ketogenic
Phenylalanine→tyrosine	Fumarate, acetoacetate	Glycogenic and ketogenic
Tryptophan	Pyruvate, acetyl-CoA, acetoacetate	Glycogenic and ketogenic
Tyrosine	Fumarate, acetoacetate	Glycogenic and ketogenic
Leucine	Acetyl-CoA, acetoacetate	Ketogenic
Lysine	Acetoacetate	Ketogenic

[a] See text (*Urea Cycle*).

regenerated from oxaloacetate by transamination. Thus both amino groups of urea originate from amino acids: one is derived from ammonium produced by deamination (reaction 1) and the other is provided by aspartate (reaction 3). Bicarbonate (reaction 1) furnishes the carbon atom of urea. In this respect it is interesting to note that not all the urea produced in the human liver is excreted in the urine, a considerable fraction being hydrolysed in the colon by bacterial ureases. The mucosa of the human colon is relatively permeable to urea. However, the great majority of the urea molecules is rapidly hydrolysed within the lumen of the colon with a large proportion of the resulting ammonia nitrogen being absorbed into the portal system or metabolised by the intestinal flora. The ammonium absorbed from the colon may be available for transamination into amino acids in the liver or resynthesized to urea also in the liver, with some of this urea distributed back to the gastrointestinal tract for degradation to ammonia and consequent recycling.

Catabolic Pathways

Once the amino groups of amino acids have been removed, the remaining carbon skeletons are funnelled into seven major metabolic intermediates, namely pyruvate, acetyl coenzyme A (acetyl-CoA), acetoacetate, 2-oxoglutarate, succinyl-CoA, fumarate, and oxaloacetate (Table 1), which may be either directly oxidized into carbon dioxide and water by the TCA cycle or reincorporated into glucose or fatty acids. Glycogenic amino acids are those possessing carbon skeletons which generate pyruvate or TCA cycle intermediates and can, therefore, be converted to glucose via gluconeogenesis. In contrast, amino acids possessing carbon skeletons which are metabolized to acetyl-CoA or acetoacetate, precursors of fatty acids and ketone bodies, are termed ketogenic. Recall that, with the exception of some species of plants and microorganisms which possess the glyoxylate cycle, all other organisms lack a pathway for the net synthesis of glucose from acetyl-CoA or acetoacetate. A few amino acids are both glycogenic and ketogenic since portions of their carbon skeletons are converted into carbohydrate derivatives whereas other portions are converted into ketone bodies. Note that the classification presented in Table 1 is not universally accepted because several amino acids are glycogenic under some conditions but ketogenic under others.

Regulation of Amino Acid Catabolism

Microorganisms regulate the level of their amino acid degradative enzymes in different ways. (1) The enzymes are subjected to catabolite repression, i.e. repression of the amino acid catabolic pathway by a carbon and energy source, even in the simultaneous presence of that amino acid as the only source of nitrogen. Thus these enzymes are induced only when carbon and energy limit growth (e.g. the induction of tryptophanase – the enzyme which cleaves tryptophan to yield ammonium,

pyruvate and indole – by tryptophan in *E. coli*). (2) The enzymes are induced when nitrogen limits growth (e.g. the induction of proline oxidase – the enzyme which catalyses the first step of proline degradation – by proline in *E. coli*, even in the presence of ample carbon supply). (3) The enzymes are induced independently of carbon and energy or nitrogen supply (e.g. the induction of threonine dehydrogenase – an enzyme involved in threonine catabolism – in *E. coli* by growth in leucine, even in the presence of other carbon and nitrogen sources). In some microorganisms, catabolite repression can be bypassed by a nitrogen limitation signal that allows the induction of a particular amino acid catabolic pathway. This nitrogen limitation signal is probably related to the complex regulation mechanisms of glutamine synthetase (see *Regulation of Amino Acid Biosynthesis*, above).

In animal cells, amino acid catabolism is also subjected to control mechanisms. Thus, for example, removal of amino groups from amino acids is regulated mainly by control of glutamate dehydrogenase. This enzyme is allosterically inhibited by ATP and guanosine triphosphate (GTP) and stimulated by ADP and GDP. Hence, when cellular energy charge is low the rate of amino acid oxidation increases. On the other hand, the urea cycle is controlled by *N*-acetylglutamate. This compound is a positive allosteric effector of carbamoyl phosphate synthetase, which catalyses the first and rate-limiting step in the pathway. *N*-acetylglutamate is also a precursor of arginine and its synthesis is inhibited by arginine. However, the amino acid catabolic enzymes of animal cells are much more often subjected to a hormonal control than are the microbial enzymes. Thus, for example, the synthesis of tryptophan oxygenase, regulated by adrenal activity, is developmentally controlled so that the enzyme is formed only in certain tissues and at certain times during development. A high-protein diet is also a factor that is known to stimulate the formation of a number of amino acid degradative enzymes in liver, namely urea cycle enzymes and tryptophan oxygenase.

Synthesis of Biologically Important Compounds

In addition to their role in protein synthesis, energy production, and gluconeogenesis, many amino acids serve as precursors for the synthesis of other amino acids and other biologically important compounds.

Many oligopeptides containing up to 20 residues, including hormones, antibiotics and antitumour agents, are synthesized in living organisms by mechanisms different from the usual ribosome-dependent processes of protein synthesis. The dipeptides carnosine (β-alanylhistidine) and anserine (β-alanyl-1-*N*-methylhistidine) are synthesized enzymatically from β-alanine and histidine, and from carnosine and *S*-adenosylmethionine, respectively.

Glutathione (γ-glutamylcysteinylglycine) plays a variety of roles in living organisms. This tripeptide is synthesized by a two-step enzymatic pathway: (1) the formation of a peptide linkage between the γ-carboxyl group of glutamate and the amino group of cysteine, to produce γ-glutamylcysteine; (2) the condensation of this dipeptide with glycine, to form glutathione. Thus the order of the amino acids in glutathione is specifically determined by the enzymes catalysing the formation of each peptide bond.

At least 90 different peptide antibiotics are produced by strains of *Bacillus subtilis* and *B. brevis*. Gramicidin S, for example, is a cyclic decapeptide composed of two identical pentapeptides (D-phenylalanine-L-proline-L-valine-L-ornithine-L-leucine). Gramicidin S is synthesized by a multienzyme complex, gramicidin synthetase, composed of two enzymes, one of which, serving as a template, specifies the amino acid sequence in the antibiotic.

S-Adenosylmethionine (SAM), the metabolically activated form of methionine, functions as an important source of methyl and propylamino groups for a wide variety of compounds, including alkaloids, choline, creatine, adrenaline, *N*-methylated amino acids, nucleotides, and polyamines, as well as for phospholipids, proteins, polysaccharides, and nucleic acids. It is synthesized from methionine and ATP, in a reaction catalysed by SAM synthase.

A wide variety of amines occurring in bacteria, plants and animals are derived directly or indirectly from amino acids by decarboxylation; these include ethylamine (from alanine), agmatine (from arginine), γ-aminobutyric acid (from glutamate), methylamine (from glycine), histamine (from histidine), cadaverine (from lysine), putrescine (from ornithine), phenylethylamine (from phenylalanine), ethanolamine (from serine), tryptamine and 5-hydroxytryptamine (from tryptophan), and tyramine and dopamine (from tyrosine). These amines and their derivatives often play a variety of physiologically important roles. For example, γ-aminobutyric acid, phenylethylamine, tryptamine, 5-hydroxytryptamine, or serotonin, tyramine, dopamine, noradrenaline, and adrenaline are all neurologically active compounds, whereas histamine, a powerful vasodilator, is involved in allergic reactions. ***See*** Amines

Tyrosine plays several important roles in animal metabolism as a precursor to melanins, thyroid hormones (thyroxine and triiodothyronine), and catecholamines (dopamine, noradrenaline and adrenaline). In the synthesis of melanins, tyrosinase catalyses first the hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (dopa), followed by the oxidation of dopa to phenylalanine-3,4-quinone (dopaquinone). Dopaqui-

none undergoes a sequence of reactions, including polymerization, to yield both red and black melanins. Another enzyme forming dopa is tyrosine hydroxylase, which catalyses the first reaction in the sequential enzymatic pathway leading to the biosynthesis of catecholamines. Dopa is then decarboxylated to yield 3,4-dihydroxyphenylethylamine (dopamine). Dopamine is hydroxylated to norepinephrine (noradrenaline), which in turn is methylated by SAM to give epinephrine (adrenaline). ***See*** Hormones, Adrenal Hormones; Hormones, Thyroid Hormones

Putrescine, or 1,4-diaminebutane, is synthesized by decarboxylation of ornithine, in a reaction catalysed by ornithine decarboxylase, a highly regulated enzyme. Another route to putrescine formation involves the conversion of arginine to agmatine by arginine decarboxylase, followed by cleavage of agmatine to putrescine and urea by agmatine ureohydrolase. Putrescine is an intermediate in the biosynthesis of two important polyamines, spermidine and spermine. Spermidine is synthesized enzymatically by the SAM-mediated transfer of a propylamino group to putrescine. The enzymatic transfer of an additional propylamino group from SAM to spermidine produces spermine. These polycations play multiple roles in stabilizing negatively charged intracellular components such as nucleic acids and membranes.

Creatine phosphate, which serves as a source of high-energy phosphate in mammalian muscle and brain, is synthesized in three steps from arginine, glycine, and methionine.

There are four classes of tetrapyrrole compounds, haems, chlorophylls, phicobilins and cobalamins, all of which are synthesized from a common precursor, δ-aminolevulinic acid (ALA). In bacteria and animals, ALA is synthesized by the condensation of glycine and succinyl-CoA, with loss of carbon dioxide, in a reaction catalysed by ALA synthase. In plants, however, ALA is formed from glutamate by a three-step pathway.

An enormous amount of carbon in the biosphere passes through the pathway leading to lignin biosynthesis, the major constituent of woody tissue. In the first reaction, phenylalanine ammonia lyase catalyses the cleavage of phenylalanine to *trans*-cinnamic acid and NH_4^+. Cinnamic acid is a precursor for the synthesis of a huge number of plant substances, including lignin, tannins, flavonoids, pigments, many of the flavour components of spices, and various alkaloids, such as morphine and colchicine. ***See*** Lignin

In addition, the synthesis of a variety of other important molecules utilizes various amino acids as precursors. Thus β-alanine is a component of CoA asparagine is a major form of transport of organi nitrogenous compounds in plants, and aspartate i involved in purine and pyrimidine biosynthesis. Gluta mate is a precursor of folic acid; glutamine contribute to the synthesis of a variety of substances, includin purines, pyrimidines, ATP, cytidine triphosphat (CTP), NAD, amino sugars, and glycoproteins; cystein is a precursor of taurine, isethionic acid, CoA, vasopres sin, various types of pigments, including phaeomalin and trichochromes, and other sulphur-containing com pounds. Glycine also plays multiple roles, includin contributions to the one-carbon pool and as a precurso of purines, glyoxylate, and various conjugates such a hippurate and glycocholate. Histidine is involved i ergothionine and homocarnosine biosynthesis, an methionine, via SAM, is the precursor of the plan hormone ethylene, which influences plant growth an development and induces the ripening of fruits. Serine i involved in the biosynthesis of phospholipids, an tryptophan is the precursor of several important physic logical substances, including NAD, NADP, and th plant hormone indole 3-acetic acid. ***See*** Niacin, Physic logy; Ripening of Fruit

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