Amino Acid Oxidation, the Production of Urea, and Amino Acid Biosynthesis

Objectives:
I. Describe the digestion of proteins and absorption of amino acids.
   A. What enzymes are involved?
      1. Where are they synthesized.
      2. How are they activated.
II. Describe the usual route(s) by which NH₄⁺ is removed from an amino acid.
    A. Describe transamination (aminotransferase reaction).
       1. What coenzyme / vitamin is required for the transamination reaction?
       2. Given an α-amino acid and α-keto acid, be able to predict the products of the transamination reaction.
    B. Describe oxidative deamination.
       1. Which amino acids undergo oxidative deamination.
III. Describe how NH₄⁺ is transported from the tissues to the liver.
    A. Describe what happens to the amino acids sent to the liver by the tissues.
       1. How is the amino group processed and released?
       2. How is the carbon skeleton usually sent back to the tissues?
IV. Describe how NH₄⁺ from excess amino acids is metabolized in the liver.
    A. How is the amino group processed and released?
V. Understand the importance of the urea cycle and describe its essential steps.
    A. State the overall result of the urea cycle.
    B. How is the urea cycle related to the TCA cycle?
VI. Describe the conversion of amino acids into molecules that can enter the citric acid cycle.
    A. Define the terms glucogenic amino acid and ketogenic amino acid.
    B. Which of the amino acids are glucogenic, which are ketogenic, and which are both?
    C. Describe the entry points of the amino acid carbon skeletons.
       1. The compounds that enter intermediary metabolism and where they enter.
    D. Describe the reactions common to the metabolism of the branched chain amino acids (Ile, Met, Val, Thr), the odd chain length fatty acids, and the branched chain fatty acids.
VII. Serine, Glycine, Tetrahydrofolate and One carbon Metabolism
    A. Describe the catabolic pathway for Serine and Glycine most often used by cells.
       1. For what reason is Serine and Glycine metabolized in this manner?
       2. What are the activated one carbon groups used for?
       3. Describe the interrelationships between S-Adenosylmethionine (SAM) and Tetrahydrofolate
       4. What is the function of SAM in the cell?
    B. Describe the anabolic pathway for Serine and Glycine synthesis.
VIII. Distinguish between essential and nonessential amino acids.
    A. What are the essential amino acids
    B. What are the nonessential amino acids.
       1. Describe the precursors for the nonessential amino acids
IX. Other biomolecules synthesized from amino acids.
X. Integrate Amino Acid Metabolism with Carbohydrate Metabolism & Lipid Metabolism.
XI. Ask yourself “What If Questions”; e.g. Does a lack of serine in the diet effect one carbon
metabolism?; Does a lack of folate in the diet effect one carbon metabolism?; Does a lack of methionine in the diet effect one carbon metabolism?; Does a lack of methionine in the diet effect the metabolism of other elements or amino acids?

Background

Amino acids function primarily as building blocks for proteins and other nitrogen containing compounds in the cell. During times of extreme need (starvation) amino acids are utilized directly as an energy source and as a carbon source for gluconeogenesis. When present in excess amino acids can be converted to glucose to meet the glucose needs of the organism, converted to fatty acids and stored as triacylglycerols, or used for energy generation. When amino acids are utilized for energy metabolism (generation or storage) the cell/organism has two parts to deal with; the nitrogen containing amino group and the carbon skeleton (the carbon, oxygen, hydrogen containing part). Ammonia (NH$_4^+$) is very toxic to cells so the amino part must be dealt with carefully or else the cell would kill itself. The present discussions will examine the route for safe amino group removal, detoxification, and excretion, as well as some of the pathways necessary for the metabolism of the amino acid carbon skeletons to CO$_2$ and H$_2$O with the concomitant generation of cellular energy, their conversion into glucose, and/or their conversion into fatty acids. Selected pathways for the synthesis of amino acids will be explored. Finally, the utilization of amino acids for non protein compounds will be touched upon briefly.

Cells maintain a relatively fixed concentration (a pool) of amino acids within them. The cell uses amino acids from this pool for protein biosynthesis, for the synthesis of other nitrogen containing compounds, (e.g., the nucleotides) and, as a source of energy. The amino acid pool is replenished by the amino acids obtained from dietary proteins and by recycling the amino acids from cellular proteins upon their degradation. In the fed state, when an individual is in nitrogen balance, the quantity of amino acids leaving
the pool is exactly balanced by the quantity that enters the pool.

Digestion of Proteins

Protein digestion begins in the stomach. The stomach secretes HCl which lowers the pH of the stomach contents to between 1 and 2. The low pH serves to denature the dietary proteins. Denatured proteins are more easily attacked by the digestive proteases (protein hydrolases). Stomach mucosal cells secrete a proteolytic zymogen - Pepsinogen. The zymogen Pepsinogen is partially activated to Pepsin by the low pH of the stomach. Complete activation occurs when the partially active enzyme cleaves an activation peptide from other partially activated Pepsin(ogen) molecules. Pepsin is an endoprotease, it begins the digestion of proteins by hydrolyzing peptide bonds within the protein releasing peptides of various lengths.

As the food moves from stomach to small intestine the pancreas is stimulated to secrete several substances important for continued protein digestion. It secretes bicarbonate (HCO₃⁻) that neutralizes the stomach HCl and raises the pH of the chyme back to neutrality or slightly basic. The increase in pH inhibits the action of Pepsin. The pancreas also secretes numerous zymogens, including Trypsinogen, Chymotrypsinogen, Procarboxypeptidases, Proaminopeptidases, Procollagenase, and Proelastase. A very specific protease called Enteropeptidase is secreted by the mucosal cells lining the small intestine. Enteropeptidase activates Trypsinogen to Trypsin by the proteolytic removal of a small activation peptide. The active protease Trypsin activates Chymotrypsinogen to Chymotrypsin, the Procarboxypeptidases to the Carboxypeptidases, the Proaminopeptidases to the Aminopeptidases, Procollagenase to Collagenase, and Proelastase to Elastase by cleaving one or more small activation peptides from each of the zymogens. Trypsin, Chymotrypsin, Collagenase, and Elastase are endoproteases, they cleave proteins at specific sites within the molecule. The Aminopeptidases and Carboxypeptidases cleave one amino acid at a time starting at the amino terminus or carboxy terminus, respectively. In addition, the mucosal cells lining the small intestine secrete or have proteolytic enzymes bound to their surface that hydrolyze dietary proteins and peptides. The products are a mixture of small peptides (≤5 AA) and amino acids. Mucosal cells lining the small intestine absorb these products by secondary active transport. Once in the cell the small peptides are hydrolyzed to amino acids, the amino acids are passed into the blood stream by a passive transport mechanism, and the blood distributes them to the cells of the body.

Transport of Ammonia

Amino acids are metabolized for one of three reasons; to generate energy, to generate precursors for gluconeogenesis, or to be converted to fatty acids. The energy and precursors are generated during fasting or starvation, whereas fatty acids are made during times of plenty when the body has more amino acids than it needs. During energy poor times and/or starvation, amino acids are metabolized by all tissues, except the four glucose dependent tissues. When amino acids are utilized for gluconeogenesis or excess amino acids converted to fat the process occurs primarily in the liver.

When amino acids are metabolized by the tissues to generate energy, the cells have two tasks to perform. First, the cell must remove the amino group and prepare the “ammonia” part of the amino acid for safe transport to the liver. Ammonia is very toxic to animal tissues, especially the brain. Ammonia is a weak base and its primary toxic effect on the brain involves an increase in cytoplasmic pH. Excess ammonia also causes the depletion TCA cycle intermediates because the cells, especially the brain, use TCA cycle
intermediates (oxaloacetate & α-ketoglutarate) to detoxify excess free ammonia. Second, the cell must convert the resulting carbon skeletons into intermediates that can enter “mainstream” metabolism, i.e., the TCA Cycle, and ET/OxPhos. When amino acids are utilized for gluconeogenesis or an excess converted into fat for storage, the liver removes the “ammonia” part of the molecule. The “ammonia” that the liver generates along with the “ammonia” collected from the tissues is convert it into the less toxic compound urea. Each urea molecule contains two ammonia molecules and one carbon dioxide molecule for excretion. The liver is the only site of urea production, the kidney is the site of urea excretion.

Reactions in the Tissues to Remove and Transport Ammonia (NH4+)

During a fast or starvation all tissues except the brain, erythrocytes, adrenal medulla, and testes can use amino acid carbon skeletons for energy generation. Animals need a safe method for transporting the ammonia from the tissues to the liver. The following sequence of reactions accomplishes this task.

The initial step of this sequence is a TRANSAMINATION of the amino acid(s) to be metabolized. These reactions are catalyzed by the Aminotransferases or Transaminases. PYRIDOXAL PHOSPHATE is a necessary coenzyme for this reaction. During TRANSAMINATION the amino group of the amino acid is removed and passed to an α-ketoacid. The products of this reaction are an α-ketoacid with the carbon skeleton of the amino group donor and a new amino acid with the carbon skeleton of the α-ketoacid acceptor.

One might think that TRANSAMINATION as depicted above is less than useless since nothing has really happened. The substrates were an amino acid & an α-ketoacid and the products were an amino acid & an α-ketoacid. The usefulness of transamination reactions comes about by limiting the number of acceptor α-ketoacids. In most tissues, α-ketoglutarate is the usual α-ketoacid acceptor and glutamate is the product.
In skeletal muscle, pyruvate generated during glycolysis in the actively contracting tissue is the usual amino group acceptor and alanine is the resulting amino acid.

In human cells eighteen of the twenty amino acids can be transaminated. There is no aminotransferase for the amino groups of serine and/or threonine.

Threonine and serine are oxidatively deaminated by the action of Serine-Threonine Dehydratase. When serine is the substrate the products are $\text{H}_2\text{O}$, $\text{NH}_4^+$ and pyruvate. Oxidative deamination of threonine produces $\text{H}_2\text{O}$, $\text{NH}_4^+$ and $\alpha$-ketobutyrate, which is oxidized to propionyl-CoA by $\alpha$-Keto Acid Dehydrogenase. The propionyl-CoA is converted to succinyl-CoA by the enzymes of odd chain fatty acid metabolism.

Lysine is a special case. The human animal does not have a transaminase that uses the $\alpha$-amino group nor the $\varepsilon$-amino group (side chain) of lysine as a substrate. The $\varepsilon$-amino group of lysine (side chain amino group) is transfered to $\alpha$-ketoglutarate in two enzymatic steps to form glutamate and $\alpha$-amino adipate. The $\alpha$-amino group of $\alpha$-amino adipate is then passed to $\alpha$-ketoglutarate by a transamination reaction to form glutamate and $\alpha$-keto adipate. While “lysine” does not directly take part in transamination reactions, intermediates of its catabolic pathway do undergo transamination and both amino groups of lysine ultimately end up on glutamate.

Aminotransferase reactions serve an important purpose. Ammonia is removed from 18 of the amino acids and “concentrated” on two; alanine and glutamate. The alanine formed by the transaminase reaction is
transported from the tissues and carried by the blood to the liver for processing. The glutamate usually undergoes several additional reactions in the tissues before the final product is released into the blood.

The glutamate formed in the tissues by transamination is OXIDATIVELY DEAMINATED by the enzyme Glutamate Dehydrogenase. Glutamate Dehydrogenase is the only amino acid dehydrogenase present in tissues. It is a metalloenzyme, it requires Zn$^{2+}$ for activity. The products of this reaction are $\alpha$-ketoglutarate and NH$_4^+$. Either NAD$^+$ (mitochondrial) or NADP$^+$ (cytoplasmic) can accept the electrons released during this reaction. This reaction is reversible and when run in the reverse direction it is used for the synthesis of glutamate from $\alpha$-ketoglutarate and free NH$_4^+$. Under conditions of hyperammoniaemia, the brain uses this enzyme in the reverse direction to detoxify ammonia resulting in the depletion of TCA cycle intermediates.

Under usual conditions the $\alpha$-ketoglutarate formed by the Glutamate Dehydrogenase reaction enters the TCA cycle or it acts as the substrate for the aminotransferase reactions accepting additional ammonia from other amino acids. The ammonia formed by this reaction immediately takes part in the next step of the sequence.

In the tissues as fast as NH$_4^+$ is formed by oxidative deamination of glutamate and by the Serine-Threonine

©Kevin R. Siebenlist, 2018
Dehydratase reaction, it reacts with (a different) glutamate to form glutamine by the action of the enzyme Glutamine Synthetase. Glutamine synthetase has a very very low $K_m$ (very high affinity) for ammonia. The ammonia produced in the tissues is quantitatively incorporated into glutamine by Glutamine Synthetase. The glutamine synthesized by this series of reaction in the tissues is released into blood stream and transported to the liver.

Glutamine synthetase is an allosteric enzyme. Glutamine Synthetase is allosterically inhibited by Glycine, Serine, Alanine, CTP, AMP, Histidine, Tryptophan, Carbamyl phosphate, and Glucosamine-6-phosphate. As will be apparent shortly, glutamine is an amine group donor in the biosynthetic reactions of these molecules.
Reactions in the Liver Using the Glutamine and Alanine from the Tissues

**Glutamine** that arrives in the liver from the tissues is transported into mitochondrial matrix where it is converted to glutamate and NH$_4^+$ by the action of Glutaminase. Ammonia enters the urea cycle. The glutamate formed by the Glutaminase reaction has several possible fates.

1. It can be released from the liver and transported back to the tissues to shuttle additional NH$_4^+$ (least likely option).

2. It can be transaminated using oxaloacetate as the acceptor ketoacid to form aspartate and $\alpha$-ketoglutarate. The aspartate is transported to the cytoplasm by the Malate-Aspartate Shuttle and it takes part in the cytoplasmic reactions of the urea cycle. The $\alpha$-ketoglutarate that results enters the TCA Cycle followed by gluconeogenesis with the resulting glucose being sent back to the tissues (good possibility).

3. It can be oxidatively deaminated by Glutamate Dehydrogenase. The NH$_4^+$ released enters the urea cycle and the $\alpha$-ketoglutarate that results enters the TCA Cycle followed by gluconeogenesis with the resulting glucose being sent back to the tissues (very likely possibility).

**Alanine** from the tissues is transported into the liver mitochondrial matrix where it is transaminated with $\alpha$-ketoglutarate as the ammonia acceptor to form glutamate and pyruvate. The pyruvate that is formed enters gluconeogenesis and the glucose is sent back to the tissues.

**Glutamate** formed by the transamination of alanine in the liver is Oxidatively Deaminated by the action of Glutamate Dehydrogenase in the mitochondria. The $\alpha$-ketoglutarate recycles to accept additional NH$_4^+$ by transamination.

**Glutamate Dehydrogenase** is an allosteric enzyme. ADP, GDP, and some amino acids act as positive modulators, and ATP, GTP, and NADH are negative allosteric effectors.

Reactions in the Liver Using Amino Acids
Preparation for Gluconeogenesis or Fatty Acid Biosynthesis

When amino acids are used as precursors for gluconeogenesis (glucose poor state) or when they are present in excess and are going to be converted into fatty acids (energy rich state) the liver is the primary site of amino acids metabolism. The liver uses $\alpha$-ketoglutarate or oxaloacetate as the acceptor molecule for transamination. The resulting glutamate, as well as any serine or threonine is then oxidatively deaminated to release the ammonia within the mitochondrial matrix. The ammonia is detoxified by the urea cycle, and the $\alpha$-ketoglutarate is used to accept additional amino groups or it re-enters the TCA cycle. The aspartate formed by transamination takes an active part in the urea cycle and/or the malate-aspartate shuttle.
The Urea Cycle

Before the \( \text{NH}_4^+ \) can enter the Urea Cycle it must be activated. Ammonia is activated by the reaction catalyzed by Carbamoylphosphate Synthetase I. This reaction occurs in the matrix of liver mitochondria, it utilizes \( \text{NH}_4^+ \), \( \text{CO}_2 \), and 2 ATP as substrates, and it forms carbamoylphosphate.

Carbamoylphosphate once formed enters the urea cycle. In the first step of the cycle ornithine reacts with carbamoylphosphate under the action of Ornithine Transcarbamoylase to form citrulline. This reaction
occurs in the matrix of the mitochondria. An antiport moves citrulline from the matrix to the cytoplasm in exchange for ornithine. The remaining steps of the urea cycle occur in the cytoplasm.

Citrulline reacts with aspartate to form argininosuccinate in a reaction catalyzed by Argininosuccinate Synthetase. Both high energy phosphate bonds of ATP are required as energy for the formation of the new chemical bond.
Argininosuccinate is cleaved into arginine and fumarate by the action of Argininosuccinate Lyase.

In the final step of the urea cycle, arginine is hydrolyzed into urea and ornithine by the action of the enzyme Arginase. Ornithine reenters the mitochondria by the antiport, in exchange for citrulline, to start the next cycle and the urea is excreted.

The fumarate that is formed in the cytosol during the urea cycle is converted to malate by the cytosolic isoenzyme of Fumarase. This malate can be transported into the matrix of the mitochondria, converted to oxaloacetate, and used by the TCA cycle. The oxaloacetate can be used as an acceptor by the Aminotransferases of the liver and the resulting aspartate used for urea synthesis. Alternatively, the malate formed in the cytosol can be converted to oxaloacetate and used in gluconeogenesis.

The Urea Cycle was elucidated by Hans Krebs. The aspartate to fumarate back to aspartate sequence of reactions connects (bridges) the urea cycle and TCA cycle.

Fates of the Carbon Skeletons

Once the NH₄⁺ has been removed, the remaining amino acid carbon skeletons are converted into intermediates of Glycolysis or the TCA Cycle; intermediates of “mainstream” metabolism. Depending upon the conditions within the organism, these intermediates can be oxidized for energy, they can serve as precursors for gluconeogenesis, they can serve as precursors for ketone body biosynthesis, and/or they can serve as precursors for fatty acid biosynthesis. Ketone body synthesis from amino acid carbon skeletons occurs rarely, and only during times of starvation.

Each of the 20 amino acids has a unique pathway for the conversion of its carbon skeleton into intermediates that can enter metabolism. Many of these pathways share common steps and enzymes. Some of the pathways are as simple as a single step, e.g., Glutamate Dehydrogenase, and some are as complex as 10 or more steps. A great deal of “pretty” biochemistry occurs during the conversion of amino acid carbon...
skeletons into intermediates of mainstream metabolism and a large number of the more common inborn errors in metabolism occur in these pathways. Parts of some of the pathways will be examined subsequently. The carbon skeletons of the 20 amino acids are converted into seven intermediates of “mainstream” metabolism. The final fates of the carbon skeletons are as follows:

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Intermediates</th>
<th>Fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>Oxaloacetate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Oxaloacetate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Glutamate</td>
<td>α-Ketoglutarate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Glutamine</td>
<td>α-Ketoglutarate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Arginine</td>
<td>α-Ketoglutarate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Proline</td>
<td>α-Ketoglutarate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Histidine</td>
<td>α-Ketoglutarate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Alanine</td>
<td>Pyruvate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Pyruvate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Serine</td>
<td>Pyruvate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Glycine</td>
<td>Pyruvate</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Methionine</td>
<td>Succinyl-CoA</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Threonine</td>
<td>Succinyl-CoA</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Valine</td>
<td>Succinyl-CoA</td>
<td>Glucogenic</td>
</tr>
<tr>
<td>Lysine</td>
<td>Acetoacetyl-CoA</td>
<td>Ketogenic</td>
</tr>
<tr>
<td>Leucine</td>
<td>Acetoacetyl-CoA &amp; Acetyl-CoA</td>
<td>Ketogenic</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Fumarate &amp; Acetoacetyl-CoA</td>
<td>Both</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>Fumarate &amp; Acetoacetyl-CoA</td>
<td>Both</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>Succinyl-CoA &amp; Acetyl-CoA</td>
<td>Both</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>Pyruvate, Acetoacetyl-CoA, &amp; Acetyl-CoA</td>
<td>Both</td>
</tr>
</tbody>
</table>

The carbon skeletons of the amino acids labeled GLUCOGENIC can be used as precursors for gluconeogenesis if glucose is needed by the organism.

The carbon skeletons of the amino acids marked KETOGENIC are converted directly into one of the precursors of ketone body or fatty acid biosynthesis. The carbon skeletons of leucine and lysine can only be used for energy generation, ketone body synthesis, and/or fatty acid biosynthesis. They cannot be used for glucose synthesis.

Four amino acids are indicated as BOTH. These amino acids are BOTH GLUCOGENIC and KETOGENIC. Parts of their carbon skeletons can be used for glucose synthesis, if glucose is needed, whereas the remaining parts can not be used in gluconeogenesis. They can be used for energy generation, for the synthesis of...
ketone bodies, and/or for fatty acid biosynthesis.

Just because an amino acid is GLUCOGENIC does not mean that the cell will only use it to synthesize glucose. Under conditions of low glucose and low energy (fasting or starvation conditions) the tissues completely oxidize the carbon skeletons from all 20 amino acids to CO₂ and H₂O with the concomitant generation of ATP. Under these low glucose and low energy conditions the liver and kidney will oxidize some of the carbon skeletons for energy and use others for gluconeogenesis to feed the glucose dependent tissues. The liver will also produce and release ketone bodies from the amino acid carbon skeletons and from fatty acids. Conversely, under conditions of plenty, the carbon skeletons from all of the “excess” amino acids will be converted to fatty acids and triacylglycerols.

The carbon skeleton of leucine and lysine enter “mainstream” metabolism as acetoacetyl-CoA and/or acetyl-CoA. These compounds can very easily enter the TCA cycle and be completely oxidized to CO₂ and H₂O. They can just as easily enter ketogenesis and/or fatty acid biosynthesis.

The amino acids that become TCA Cycle intermediates follow a less direct pathway. Remember, the TCA cycle intermediates not oxidized as they travel around the pathway. Oxaloacetate accepts the two carbon acetate fragment and the intermediates carry the acetate fragment around the cycle as it is oxidized to CO₂. If the carbon skeletons of the amino acids entered TCA cycle and stayed there, all that would result is a net increase in the concentration of TCA cycle intermediates. There would be very little ATP generation from these carbon skeletons and no glucose or fatty acid biosynthesis. The excess TCA Cycle intermediates must be “drained off” if they are to be used for gluconeogenesis, energy generation and/or fatty acid biosynthesis. This illustrates the AMPHIBOLIC nature of the TCA cycle.

The amino acid carbon skeletons that are converted to TCA Cycle intermediates enter the TCA Cycle at various points, travel around the cycle until they are converted to malate, and are then transported out of the mitochondria. Once in the cytoplasm they can travel one of several routes.

If the body needs glucose, the **malate** is converted to **oxaloacetate** by cytoplasmic Malate Dehydrogenase and the **oxaloacetate** enters Gluconeogenesis by the action of Phosphoenolpyruvate Carboxykinase.

If the cell needs to oxidize them for energy the **malate** is converted to **pyruvate** by Malic Enzyme. **Pyruvate** reenters the mitochondria where it is converted to **acetyl-CoA** by the **Pyruvate Dehydrogenase Complex**, the **acetyl-CoA** combines with **oxaloacetate** to form **citrate**, and the TCA Cycle proceeds to oxidize the acetate fragment to CO₂.

If the cell is energy rich these carbon skeletons are to be converted to fatty acids and stored as triacylglycerols. The **malate** is converted to **pyruvate** by Malic Enzyme and the **pyruvate** reenters the mitochondria where it is converted to **acetyl-CoA** by the **Pyruvate Dehydrogenase Complex**. The **acetyl-CoA** combines with **oxaloacetate** to form **citrate** and the citrate is transported into the cytoplasm for lipid biosynthesis.
Branched Chain Amino Acid Catabolism

The amino acids methionine, valine, isoleucine, and threonine are termed the Branched Chain Amino Acids and their metabolism requires enzymes from the pathway for the metabolism of odd chain length fatty acids. Six enzymatic steps convert methionine to propionyl-CoA, valine is converted in seven steps, threonine requires two, and isoleucine needs six. The propionyl-CoA is converted to D-methylmalonyl-CoA by Propionyl-CoA Carboxylase; the D-methylmalonyl-CoA is converted to L-methylmalonyl-CoA by Methylmalonyl-CoA Epimerase; and finally the L-methylmalonyl-CoA is converted to succinyl-CoA by Methylmalonyl-CoA Mutase (Vitamin B₁₂).

Serine, Glycine, and Tetrahydrofolate (TH₄)

The coenzyme Tetrahydrofolate (TH₄) carries and donates “activated methyl groups”, activated one carbon fragments, for biosynthetic reactions. These “activated methyl groups” are used extensively during
nucleotide biosynthesis. If Tetrahydrofolate carries and donates “activated methyl groups”, it must obtain these methyl groups from some biological source. The major source of methyl groups for biosynthetic reactions is the amino acid serine, and to a lesser extent the amino acid glycine. The conversion of serine and glycine to pyruvate is a minor pathway for the metabolism of these two amino acids. The majority of serine and glycine not used for protein biosynthesis is used as a source of “activated methyl groups”.

When serine acts as a source of methyl groups the hydroxymethyl side chain of serine is transferred to Tetrahydrofolate (TH$_4$) by the enzyme Serine Hydroxymethyl Transferase. The products are glycine and N$_5$N$_{10}$-methylene-tetrahydrofolate. A second methyl group from glycine can be transferred to TH$_4$ by the enzyme Glycine Synthase. This enzyme requires NADH as a cosubstrate. It produces N$_5$N$_{10}$-methylene-tetrahydrofolate, NH$_4^+$, and HCO$_3^-$.

Since all of these reactions are reversible, the cell can and does use these enzymes for the biosynthesis of serine and glycine. However, the reverse of these reactions is a very minor pathway for their biosynthesis.
Methyl groups carried by TH₄ are absolutely essential for the synthesis of nucleotides. What happens if the diet is completely lacking in serine and glycine? Does nucleotide biosynthesis stop? Does the cell die? Does the organism die? Conversely, if the cell is deficient in Tetrahydrofolate does it lose its ability to synthesize serine and glycine.

The main pathway for synthesizing serine starts with 3-phosphoglycerate obtained from Glycolysis. The 3-phosphoglycerate is first oxidized to 3-phosphohydroxypyruvate by the enzyme Phosphoglycerate Dehydrogenase. NAD accepts the electrons released during this oxidation. An amino group is then transferred from glutamate to 3-phosphohydroxypyruvate to form 3-phosphoserine and α-ketoglutarate. This is an aminotransferase reaction. The enzyme Phosphoserine Aminotransferase catalyzes this reaction. Finally, the phosphate group is removed from 3-phosphoserine to form serine by the enzyme Phosphoserine Phosphatase. Glycine is synthesized from serine by the enzyme Serine Hydroxymethyl Transferase.

©Kevin R. Siebenlist, 2018
**N5,N10-methylene-tetrahydrofolate** is the other product of this reaction.

In addition to tetrahydrofolate, the cell uses another compound as a source for methyl groups during biosynthetic reactions. The other compound that donates methyl groups is S-ADENOSYL METHIONINE or SAM. The methyl group donated by SAM is the methyl group that is in thioether linkage on methionine.

Methionine is an essential amino acid, it must be obtained from some outside sources, i.e., the diet. Humans cannot synthesize it. To keep the biosynthetic processes that use SAM functioning, the methionine and SAM must be regenerated. It is regenerated by a series of reactions employing N5-methyl-tetrahydrofolate.
The “SAM Cycle” starts with **Methionine** reacting with ATP to form **S-adenosylmethionine (SAM)** and three phosphate groups in a reaction catalyzed by **Methionine Adenosyl Transferase**. SAM donates its methyl group to a “methyl group acceptor” to form a “methylated product” and S-adenosylhomocysteine. {For example the cell needs to synthesize choline from ethanolamine. Choline is synthesized from ethanolamine by the transfer of three methyl groups from three SAM molecules.} The **S-adenosylhomocysteine** is hydrolyzed to **adenosine** and **homocysteine**. The adenosine by a series of enzymatic steps is converted to ATP. **Homocysteine** is converted back to **methionine** by **Methionine Synthase**, homocysteine accepts a methyl group from **N5-methyl-tetrahydrofolate**. **N5-methyl-tetrahydrofolate** is regenerated when tetrahydrofolate accepts a methyl group from **serine** or **glycine** as described above.

---

**Biosynthesis of the Amino Acids**

Plants and bacteria can synthesize all 20 amino acids. Current data indicates that humans can synthesize ten of them in adequate amounts for growth and maintenance. The 10 amino acids that humans can synthesize are the **NONSESENTIAL AMINO ACIDS**. It is nonessential that they be obtained from some outside source, i.e., the diet. The other 10 amino acids are the **ESSENTIAL AMINO ACIDS**. The human animal can not synthesize the Essential Amino Acids, these amino acids must be obtained in adequate amounts from some outside source, i.e., the diet. The 10 essential amino acids are **PVT TIM HALL**

- Phenylalanine
- Valine
- Threonine
- Tryptophan
- Isoleucine
- Methionine
- Histidine
- Arginine*
- Leucine
- Lysine

**Arginine** can be considered a nonessential amino acid under certain conditions. Non-stressed adults contain the enzymes necessary for the synthesis of arginine (urea cycle) and it can be synthesized in adequate amounts to support bodily needs. Infants, growing children, and adults under stress (e.g., disease states, post surgery, strenuous conditioning, etc.) still contain the enzymes necessary for the synthesis of arginine, but the capacity (rate) of the enzymes cannot meet the needs of these individuals. For these individuals arginine is considered an essential amino acid.

The ten amino acids that humans can synthesis use glycolytic or TCA cycle intermediates as precursors.

- **Pyruvate** is the precursor for **alanine**. A single aminotransferase reaction converts one to the other.
- **Oxaloacetate** is the precursor for **aspartate** and **asparagine**.
**α-Ketoglutarate** is the precursor for glutamate, glutamine, proline, as well as arginine in adults.

**3-Phosphoglycerate** is the precursor for serine, glycine, and cysteine.

**Phenylalanine** is the precursor for tyrosine. However, phenylalanine is an essential amino acid. There must be enough phenylalanine in the diet to cover the needs for both phenylalanine and tyrosine.

A similar relationship exists between the essential amino acid **methionine** and the nonessential amino acid **cysteine**. The sulfur for cysteine biosynthesis comes from methionine. Therefore, there must be sufficient methionine if cysteine is to be synthesized.

**Other Biomolecules Derived from Amino Acids**

A wide variety of important biomolecules are derived from amino acids.

**Aspartate, Glycine, and Glutamine** are necessary for the synthesis of Adenine and Guanine, the heterocyclic purine base (the ring) of ATP and GTP respectively.

**Aspartate** and **Glutamine** are necessary for the synthesis of Uracil, Thymine and Cytosine the pyrimidine bases on UTP, dTTP and CTP.

**Heme** is synthesized from glycine and the TCA cycle intermediate succinyl-CoA. Heme is a necessary prosthetic group for hemoglobin and the cytochromes.

**Nitric Oxide (NO)** has a variety of biological functions in the body. It is involved in bacterial killing by macrophages, it relaxes smooth muscle surrounding blood vessels, and it is a second or third messenger in brain tissues. In the brain, the release of NO causes an increase in cellular concentrations of cAMP and cGMP. The enzyme **Nitric Oxide Synthase**, when activated by some stimulus, converts arginine into NO and citrulline.

![Chemical structures](image)

**Ornithine** is synthesized from proline by the action of **Ornithine δ-aminotransferase**.

**Creatine** is derived from glycine and arginine.

Several Neurotransmitters and Hormones are synthesized from amino acids.
1. A decarboxylation reaction is used to form $\alpha$-aminobutyrate from glutamate.
2. Likewise, the decarboxylation of histidine results in histamine.
3. The decarboxylation and hydroxylation of tryptophan results in serotonin (5-hydroxytryptamine).
4. Dopamine, Norepinephrine, Epinephrine, and the Thyroid Hormones are all derived from tyrosine.