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Protein Metabolism

Much of the body is made of protein, and these proteins take on a myriad of forms. They represent cell signaling receptors, signaling molecules, structural members, enzymes, intracellular trafficking components, extracellular matrix scaffolds, ion pumps, ion channels, oxygen and CO_2 transporters (hemoglobin). That is not even the complete list! There is protein in bones (collagen), muscles, and tendons; the hemoglobin that transports oxygen; and enzymes that catalyze all biochemical reactions. Protein is also used for growth and repair. Amid all these necessary functions, proteins also hold the potential to serve as a metabolic fuel source. Proteins are not stored for later use, so excess proteins must be converted into glucose or triglycerides, and used to supply energy or build energy reserves. Although the body can synthesize proteins from amino acids, food is an important source of those amino acids, especially because humans cannot synthesize all of the 20 amino acids used to build proteins.

The digestion of proteins begins in the stomach. When protein-rich foods enter the stomach, they are greeted by a mixture of the enzyme pepsin and hydrochloric acid (HCl; 0.5 percent). The latter produces an environmental pH of 1.5–3.5 that denatures proteins within food. Pepsin cuts proteins into smaller polypeptides and their constituent amino acids. When the food-gastric juice mixture (chyme) enters the small intestine, the pancreas releases sodium bicarbonate to neutralize the HCl. This helps to protect the lining of the intestine. The small intestine also releases digestive hormones, including secretin and CCK, which stimulate digestive processes to break down the proteins further. Secretin also stimulates the pancreas to release sodium bicarbonate. The pancreas

1

releases most of the digestive enzymes, including the proteases trypsin, chymotrypsin, and elastase, which aid protein digestion. Together, all of these enzymes break complex proteins into smaller individual amino acids ,which are then transported across the intestinal mucosa to be used to create new proteins, or to be converted into fats or acetyl CoA and used in the Krebs cycle.

Fate of Ammonia

1. **Amination of keto acids ♣**

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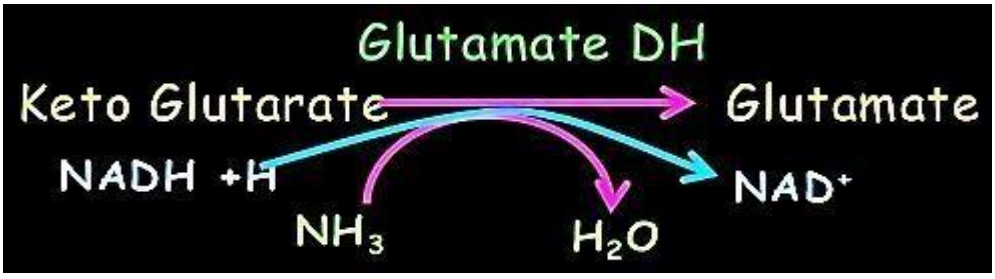

3. Amination of glutamate to Glutamine ♣

One more molecule of Ammonia added on to Glutamate to form Glutamine . Glutamate and glutamine are the major buffering molecule for Ammonia.

4. Detoxification of ammonia as UREA ♣

Most of the ammonia produce in blood is taken to the liver for the conversion of UREA. ♣ In liver CO_2 and NH_3 forms carbamoyl-phosphate which then enter Urea cycle .

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Ammonia

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Urea cycle

The Urea cycle (also known as the ornithine cycle) is a cycle of biochemical reactions occurring in many animals that produces urea ($(NH_2)_2CO$) from ammonia (NH_3). This cycle was the first metabolic cycle discovered (Hans Krebs and Kurt Henseleit, 1932), five years before the discovery of the TCA cycle. In mammals, the urea cycle takes place primarily in the liver, and to a lesser extent in the kidney

The urea cycle consists of five reactions: two mitochondrial and three cytosolic . The cycle converts two amino groups, one from NH_4^+ and one from Asp, and a carbon atom from HCO_3^- to the relatively nontoxic excretion product urea at the cost of four "high-energy" phosphate bonds (3 ATP hydrolyzed to 2 ADP and one AMP). Ornithine is the carrier of these carbon and nitrogen atoms. Urea cycle is discussed in detail as follows:

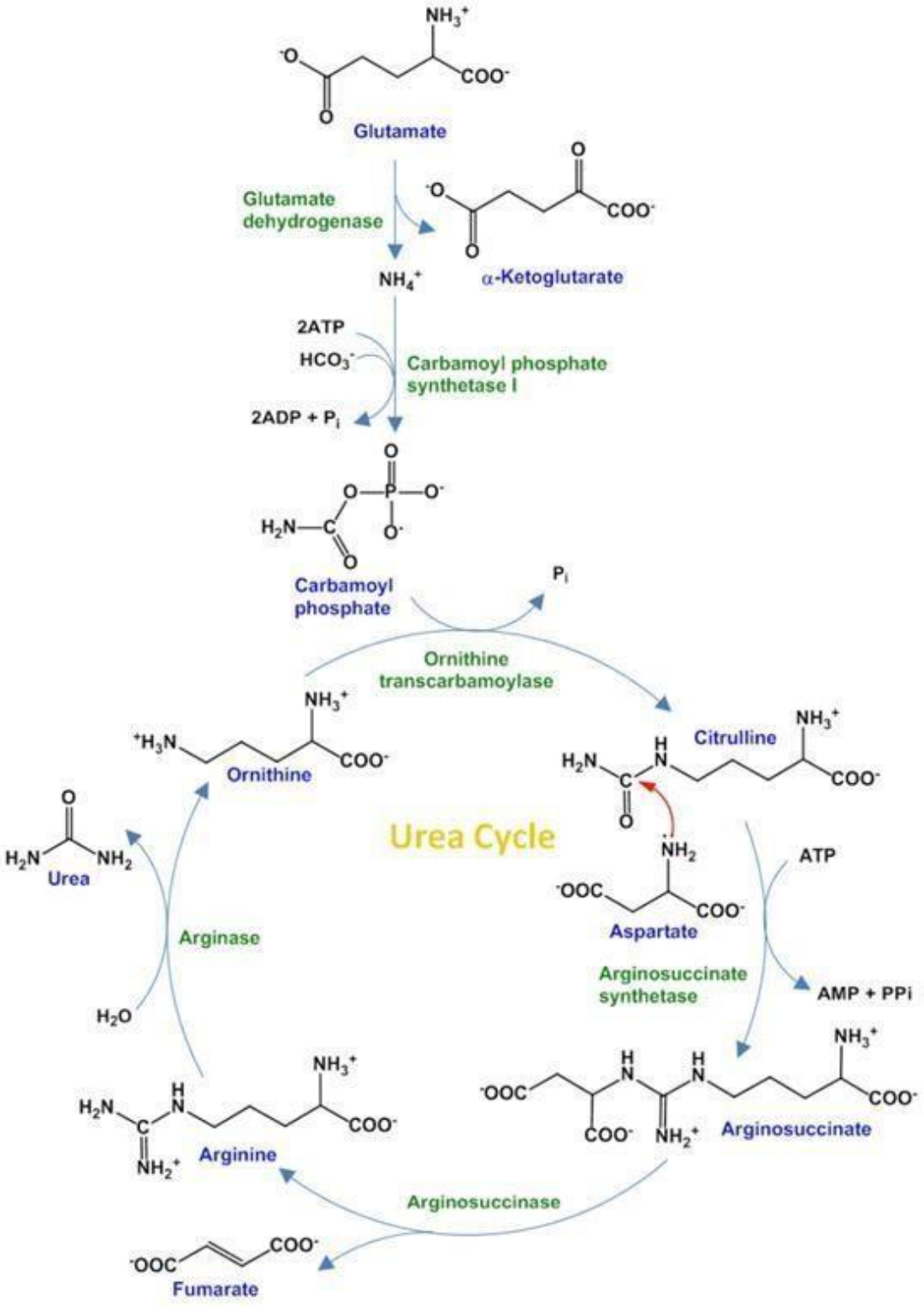
- **Synthesis of carbamoyl phosphate–** This step takes place in the mitochondria of the liver cells. Here the ammonium ions react with carbon dioxide (product of mitochondrial respiration) to form carbamoyl phosphate catalyzed by the enzyme carbamoyl phosphate synthetase I. This is an irreversible, rate-limiting, ATP-dependent reaction and consumes 2 ATP. Carbamoyl phosphate synthetase I (in mitochondria) is different from carbamoyl phosphate synthetase II (in cytosol) as the latter one has a different role to play and is involved in pyrimidine synthesis.

- **Synthesis of citrulline**– Carbamoyl phosphate produced in the first step reacts with ornithine in the presence of ornithine transcarbamoylase to synthesize citrulline. Just like oxaloacetate in Kreb's cycle, ornithine plays a similar role acting as accepting substrate at each turn of the cycle. Via a transporter system this citrulline is now transferred to the cytosol of the liver cells.

- **Formation of arginosuccinate**– In this ATP dependent step, the carbonyl carbon of citrulline is attacked by the lone pair of the amine in aspartate to produce arginosuccinate in presence of arginiosuccinate synthetase. In this step, the second nitrogen of urea is incorporated by condensation. ATP is broken down into AMP and pyrophosphate.

- **Breakdown of arginosuccinate–** Arginosuccinase promotes the cleavage of arginosuccinate to give arginine and fumarate in a reversible manner. Fumarate formed here joins the citric acid cycle forming a link between urea and citric acid cycle.

- **Formation of urea**– Arginine produced in the earlier step is broken down by arginase to give urea and ornithine. Ornithine is recycled back to the mitochondria for the next cycle.



As we discussed, five enzymes took part in the formation of urea. Out of these the first four are found in all cells. But the last enzyme arginase is found only in the liver cells thus assuring the formation of final product urea only in the liver despite the formation of arginine in other tissues.

Energetics of Urea Cycle

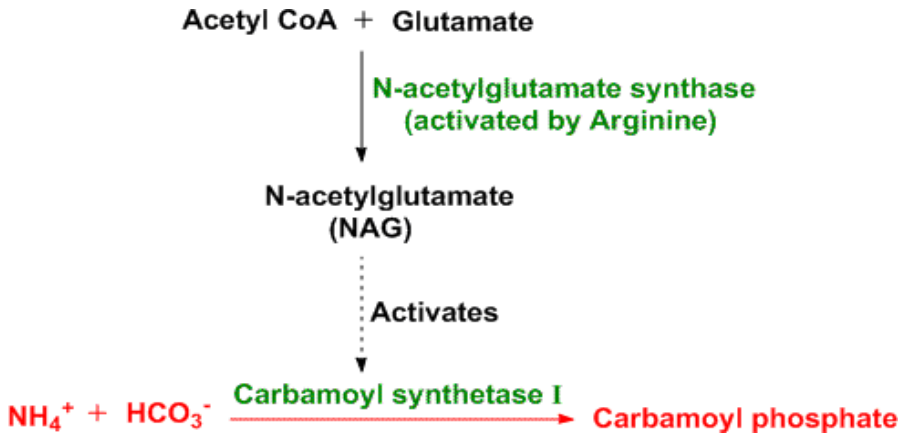
Let us take a look at how much energy is consumed during one turn of the urea cycle. On considering only the urea cycle, and not considering the other biopathways linked, to produce one urea molecule 4 ATP molecules are used up as shown below:

NH_4^+ ions to Carbamoyl phosphate- utilisation of 2ATP
Citrulline to arginosuccintae- breakdown of 1 ATP to AMP + PPi which is equivalent to 2 Pi

Therefore the entire reaction can be summarised as follows:

$2NH_4^+ + HCO_3^- + H_2O + 3ATP + Aspartate \rightarrow Urea + 2ADP + 4Pi + AMP + 2H^+ + Fumarate$

Rate Limiting Steps of Urea cycle



Rate limiting step of Urea cycle

The conversion of ammonium ions to carbamoyl phosphate catalysed by carbamoyl synthetase I is a rate limiting step. This enzyme (carbamoyl synthetase I) gets activated by N-acetylglutamate (NAG) which is formed by reaction between acetyl CoA and glutamate catalysed by the enzyme

N-acetylglutamate synthase (activated by arginine).Thus concentrations of glutamate and acetyl CoA as well as levels of arginine determine the steady state levels of N-acetylglutamate (NAG) which in turn regulates the concentration of urea. When a high protein diet is is consumed, levels of NAG increases and in turn urea levels increase. Also during starvation, when muscle proteins start breaking down to source out energy, urea levels increase in response. The rest all enzymes participating in the urea cycle are mostly regulated by the concentrations of their respective substrates.

Amino acids as buffers

Amino acids are compounds that have both an amine group and a carboxylic acid group.

An amino acid can act as a buffer **because** it can react with added acids and bases to keep the pH nearly constant.

A good example of this would be the protein hemoglobin. It can bind to small amounts of acid in the blood, helping to remove that acid before it changes the blood's pH thus making it an excellent buffer.

The general formula of an amino acid is $H_2NCHRCOOH$, where R is a side chain characteristic of each amino acid.

Because an amino acid has both an acidic group (COOH) and a basic group (NH_2), it can act as both an acid and a base.

In very acidic media, the NH_2 group is in the protonated form, and in very basic media, the COOH group is in the deprotonated form.

At an intermediate pH (the **isoelectric point, pI**), both ends are in their ionic form.

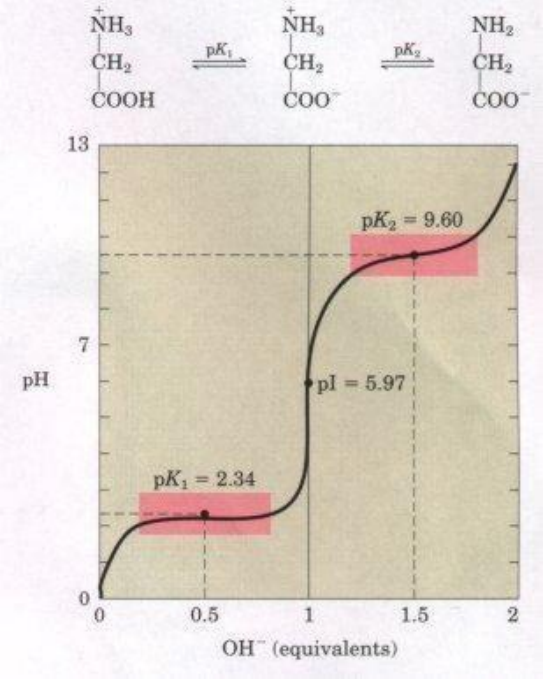
In glycine, the COOH group has pKa_1=2.34, and the H_3N^+ group has Ka_2=9.60.

At the isoelectric point, $pI = \frac{pK_{a1} + pK_{a2}}{2} = 5.97$

$\underbrace{H_3\overset{+}{N}CHRCOOH}_{\text{At low pH}} \rightleftharpoons \underbrace{H_3\overset{+}{N}CHRCOO^-}_{\text{At pI}} \rightleftharpoons \underbrace{H_2NCHRCOO^-}_{\text{At high pH}}$

Amino acids have characteristic titration curves.

For example, the titration curve for glycine looks like this:



At pH 2.34, we have equal amounts of the weak base RNH2 and its conjugate acid RNH+3.

A pH 9.60, we have equal amounts of the weak acid RCOOH and its conjugate base $RCOO^-$.

A mixture of a weak acid and its conjugate base is a **buffer**

In both regions, we can add small amounts of acid or base, and the pH will not change much.

Thus, an amino acid has **two** regions in which it can act as a buffer.

The buffer regions for glycine are pH 1.3 to pH 3.3 and from pH 8.6 to pH 10.6

Serum Protein Components

Serum proteins (also blood or plasma proteins) are proteins present in blood that serve many different functions, including transport of lipids, hormones, vitamins and minerals in the circulatory system and the regulation of acellular activity and functioning of the immune system. Other blood proteins act as enzymes, complement components, protease inhibitors or kinin precursors. Although serum proteins have very high concentration, they exhibit an uneven distribution in terms of composition. That is, only about 22 proteins account for 99% of all the serum proteins. These include serum albumin, globulins and fibrinogen. The remainder 1% of blood proteins is composed of low abundance circulatory proteins as well as proteins secreted by live, apoptotic and necrotic cells. Most of blood proteins are secreted by the liver and intestines except for the gamma globulins, synthesized by the immune system.

Fraction	Protein Type	Function	Abundance
Serum and Plasma	Albumin	• Prevention of blood vessel leakiness. • Blood carrier (transporter) of insoluble molecules. • Tissue growth and healing.	55%
Serum and Plasma	Alpha-1 globulin Fraction	• Contains high-density lipoprotein (HDL) known as "good" cholesterol.	38%
	Alpha-2 globulin Fraction	• Contains haptoglobin that binds hemoglobin and prevents loss of iron.	
	Beta globulin Fraction	• Carry substances, such as iron, through the bloodstream and help fight infection.	
	Gamma globulin Fraction	• Antibodies. Prevent and fight infection.	
Plasma	Fibrinogen	• Blood coagulation.	7%
Plasma	Clotting Factors	• Conversion of fibrinogen into fibrin	<1%
Serum and Plasma	Regulatory proteins	• Regulation of gene expression and other functions.	<1%

Insulin

Function of Insulin

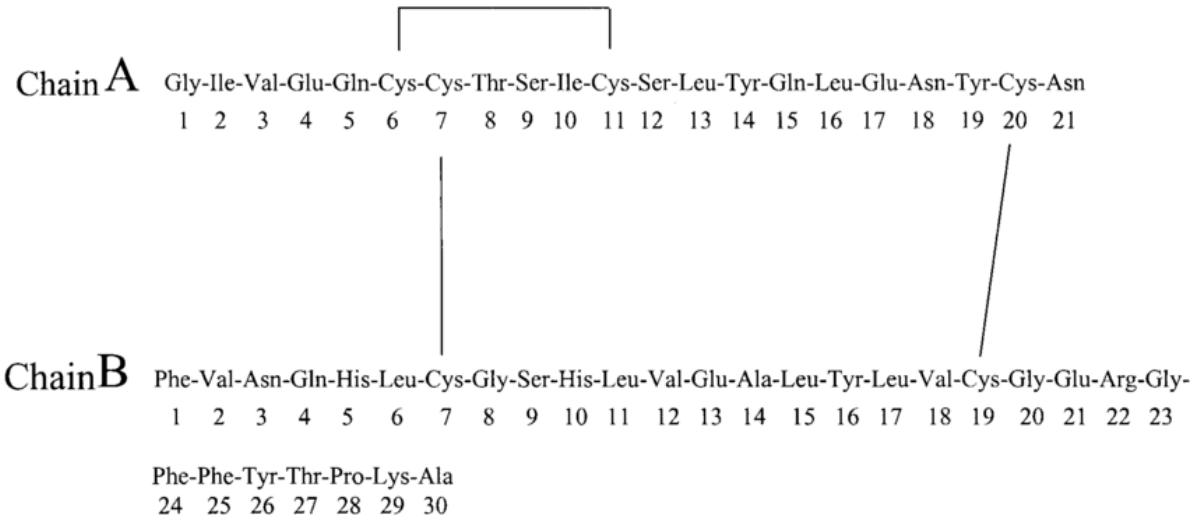
Insulin is made by the pancreatic islet beta cells in response to elevated blood glucose levels. Insulin signals cells that the body is in the "fed" state, and that it should take up glucose from the blood and make other appropriate response. For example, in the liver glycogen synthesis is turned on, which provides a supply of glucose when the blood glucose levels fall under fasting conditions. Insulin also increases fat synthesis in adipocytes. In type 1 diabetes, the pancreatic cells do not release insulin, resulting in high blood sugar levels and increased fat metabolism.

Consequently, there is "spillover" of glucose into the urine, and weight loss due to the loss of body fat stores.

Structure of Insulin

Insulin is composed of two different types of peptide chains. Chain A has 21 amino acids and Chain B has 30 amino acids. Both chains contain alpha helices but no beta strands. There are 3 conserved disulfide bridges which help keep the two chains together. Insulin can also form dimers in solution due to the hydrogen bonding between the B chains (shown as white lines). The dimers can further interact to form hexamers due to interaction between hydrophobic surfaces. This scene highlights the hydrophobic (gray) and polar (purple) parts of an insulin monomer at a pH of 7.

A number of insulin variants have been made to favor either the monomeric or hexameric form. Deletion of the five C terminal residues of the B chain creates a monomer only form. This portion of the B chain is involved in hydrogen bonds between the B chain of one monomer and the A (marked C) and B (marked D) chain of another monomer.



Inborn errors of amino acid metabolism

Inborn errors of amino acid metabolism are commonly caused by mutant genes that generally result in abnormal proteins, most often enzymes.

1. **Phenylketonuria (PKU)** is an important disease of amino acid metabolism because it is relatively common and responds to dietary treatment. And is caused by a **deficiency** of **phenylalanine hydroxylase** (**PAH**), the enzyme that converts phenylalanine to tyrosine.

Untreated patients with PKU suffer from severe intellectual disability, developmental delay, microcephaly, seizures and a characteristic mousey smell of the urine. Treatment involves controlling dietary phenylalanine. Tyrosine becomes an essential dietary component for people with PKU.

2. **Hyperphenylalaninemia** may also be caused by deficiencies in the enzymes that synthesize or regenerate the coenzyme for PAH, **tetrahydrobiopterin**.

3. **Maple syrup urine disease** is caused by a partial or complete **deficiency** in **branched-chain α-keto acid dehydrogenase**, the enzyme that decarboxylates **leucine**, **isoleucine**, and **valine**. Symptoms include feeding problems, vomiting, ketoacidosis, changes in muscle tone, and a characteristic sweet smell of the urine. If untreated, the disease leads to neurologic problems that result in death. Treatment involves controlling dietary leucine, isoleucine, and valine.

4. Other important genetic diseases associated with amino acid metabolism include **albinism**, **homocystinuria**, **methylmalonyl CoA mutase deficiency**, **alkaptonuria**, **histidinemia**, **tyrosinemia**, and **cystathioninuria**.