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T.Y.B.SC.(MICROBIOLOGY)(CBCS)

NEW PROPOSED SYLLABUS -JUNE 2021

MB-502 BACTERIAL METABOLISM (THEORY)

UNIT :2 HETEROTROPHIC MODE OF METABOLISM

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CONTENT

- Glycolysis
- Glycolysis and its regulation
- The pentose phosphate pathway and its regulation
- The Enters -Doudroff pathway
- The citric acid cycle and its regulation
- The glyoxylate cycle
- Protein metabolism
- General reaction of amino acid catabolism
- Strickland reaction
- Lipid catabolism
- Oxidation of fatty acids
- Beta -oxidation of fatty acid

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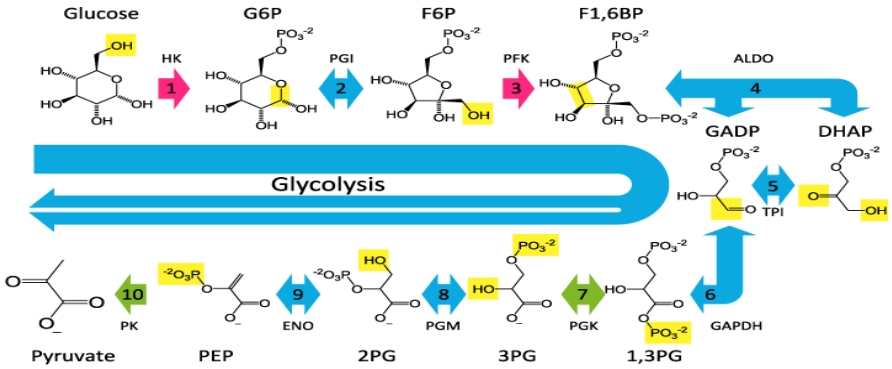
Glycolysis

Glycolysis is the metabolic process that converts glucose into pyruvic acid."

What is Glycolysis?

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water. The process takes place in the cytoplasm of a cell and does not require oxygen. It occurs in both aerobic and anaerobic organisms.



Glycolysis is the primary step of cellular respiration, which occurs in all organisms. Glycolysis is followed by the Krebs cycle during aerobic respiration. In the absence of oxygen, the cells make small amounts of ATP as glycolysis is followed by fermentation.

This metabolic pathway was discovered by three German biochemists- Gustav Embden, Otto Meyerhof, and Jakub Karol Parnas in the early 19th century and is known as the EMP pathway (Embden–Meyerhof–Parnas).

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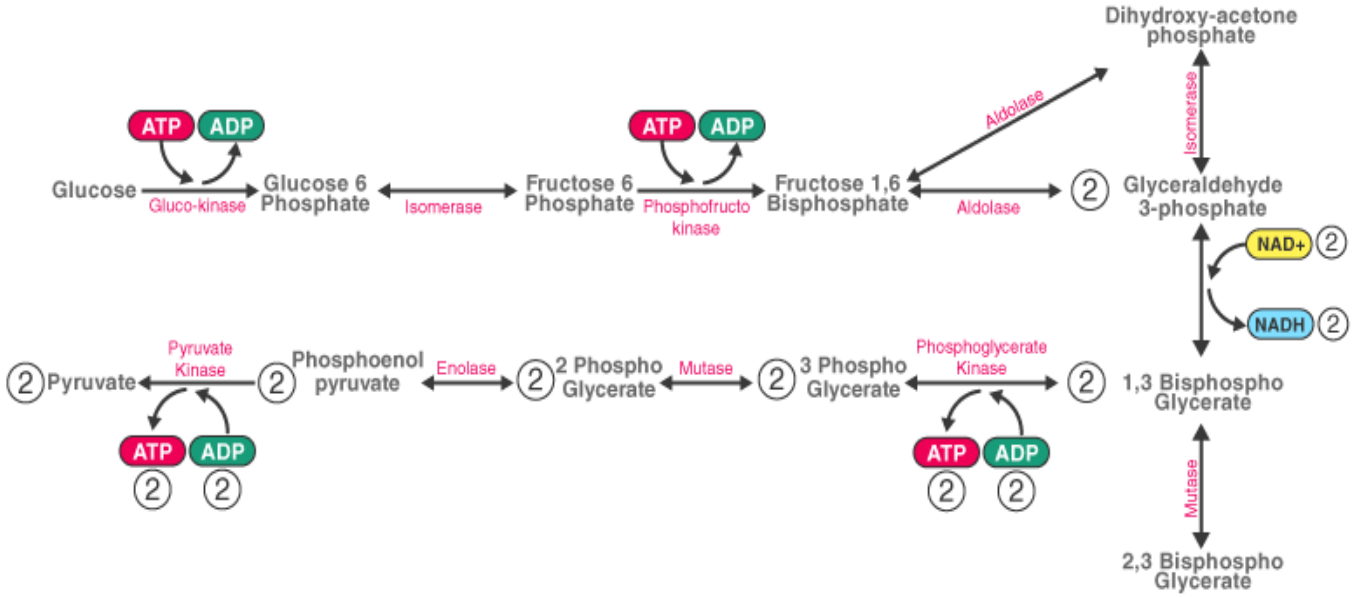
Glycolysis Pathway



Key Points of Glycolysis

- It is the process in which a glucose molecule is broken down into two molecules of pyruvate.
- The process takes place in the cytoplasm of plant and animal cells.
- Six enzymes are involved in the process.
- The end products of the reaction include 2 pyruvate, 2 ATP and 2 NADH molecules.

Stage 1

- A phosphate group is added to glucose in the cell cytoplasm, by the action of enzyme hexokinase.
- In this, a phosphate group is transferred from ATP to glucose forming glucose,6-phosphate.

Stage 2

Glucose-6-phosphate is isomerised into fructose,6-phosphate by the enzyme phosphoglucomutase.

Stage 3

The other ATP molecule transfers a phosphate group to fructose 6-phosphate and converts it into fructose 1,6-bisphosphate by the action of the enzyme phosphofructokinase.

Stage 4

The enzyme aldolase converts fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which are isomers of each other.

Step 5

Triose-phosphate isomerase converts dihydroxyacetone phosphate into glyceraldehyde 3-phosphate which is the substrate in the successive step of glycolysis.

Step 6

This step undergoes two reactions:

- The enzyme glyceraldehyde 3-phosphate dehydrogenase transfers 1 hydrogen molecule from glyceraldehyde phosphate to nicotinamide adenine dinucleotide to form NADH + H^+.
- Glyceraldehyde 3-phosphate dehydrogenase adds a phosphate to the oxidised glyceraldehyde phosphate to form 1,3-bisphosphoglycerate.

Step 7

Phosphate is transferred from 1,3-bisphosphoglycerate to ADP to form ATP with the help of phosphoglycerokinase. Thus, two molecules of phosphoglycerate and ATP are obtained at the end of this reaction.

Step 8

The phosphate of both the phosphoglycerate molecules is relocated from the third to the second carbon to yield two molecules of 2-phosphoglycerate by the enzyme phosphoglyceromutase.

Step 9

The enzyme enolase removes a water molecule from 2-phosphoglycerate to form phosphoenolpyruvate.

Step 10

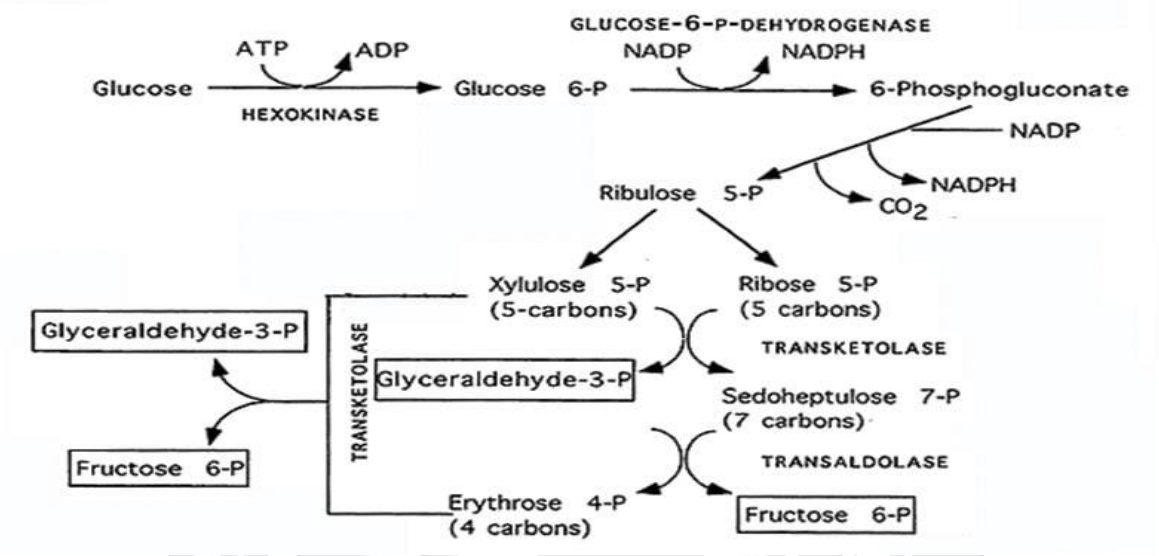
A phosphate from phosphoenolpyruvate is transferred to ADP to form pyruvate and ATP by the action of pyruvate kinase. Two molecules of pyruvate and ATP are obtained as the end products.

The pentose phosphate pathway

The hexose monophosphate (HMP) shunt, also known as the pentose phosphate pathway or phosphogluconate pathway, is a metabolic pathway that runs parallel to glycolysis.
This pathway produces NADPH and intermediates required for the synthesis of nucleic acids and amino acids.

- It is an anabolic pathway that takes place in the cytosol for most organisms. However, in plants, it takes place in plastids.
- The pathway takes place in two distinct phases: oxidative and non-oxidative phases.
- The reactions of this pathway are enzyme catalysed.

- The pentose phosphate pathway is also called as the phosphogluconate pathway or hexose monophosphate shunt.
- While it involves oxidation of glucose, its primary role is anabolic rather than catabolic.
- It is an important pathway that generates precursors for nucleotide synthesis and important in red blood cells (erythrocytes).



Overall reaction of the pentose phosphate pathway

3 Glucose-6-P + 6 $NADP^+ \rightarrow$ 3 ribulose-5-P + 3 CO_2 + 6 NADPH

3 Ribulose-5-P → 2 xylulose-5-P + Ribose-5-P

2 Xylulose-5-P + Ribose-5-P → 2 fructose-6-P + Glyceraldehyde-3-P

1. The Oxidative Reactions

- Glucose-6-phosphate is converted to 6-phosphogluconolactone, and $NADP^+$ is reduced to NADPH + H^+.
  - Enzyme: glucose-6-phosphate dehydrogenase
- 6-Phosphogluconolactone is hydrolyzed to 6-phosphogluconate.
  - Enzyme: Gluconolactonase
- 6-Phosphogluconate undergoes an oxidation, followed by a decarboxylation. CO2 is released, and a second NADPH + H^+ is generated from $NADP^+$. The remaining carbons form ribulose-5-phosphate.
  - Enzyme: 6-phosphogluconate dehydrogenase

2. The Non-oxidative Reactions

- Ribulose-5-phosphate is isomerized to ribose-5-phosphate or epimerized to xylulose-5-phosphate.
- Ribose-5-phosphate and xylulose-5-phosphate undergo reactions, catalyzed by transketolase and transaldolase, that transfer carbon units, ultimately forming fructose 6-phosphate and glyceraldehyde-3-phosphate.
  - Transketolase, which requires thiamine pyrophosphate, transfers two-carbon units.
  - Transaldolase transfers three-carbon units.

Result of Pentose Phosphate Pathway

- **Oxidative portion:** Irreversible.

Generates two NADPH, which can then be used in fatty acid synthesis and cholesterol synthesis and for maintaining reduced glutathione inside RBCs.

- **Nonoxidative portion:** Reversible.

Generates intermediate molecules (ribose-5-phosphate; glyceraldehyde-3-phosphate; fructose-6-phosphate) for nucleotide synthesis and glycolysis.

The Entner–Doudoroff pathway

describes an alternate series of reactions that catabolize glucose to pyruvate using a set of enzymes different from those used in either glycolysis or the pentose phosphate pathway. This pathway was first reported in 1952 by Michael Doudoroff and Nathan Entner.

- The Entner–Doudoroff pathway has a net yield of 1 ATP for every glucose molecule processed, as well as 1 NADH and 1 NADPH. By comparison, glycolysis has a net yield of 2 ATP and 2 NADH for every one glucose molecule processed.
- This pathway used two specific enzymes ie. 6-phosphogluconate dehydratase and KDPG aldolase.
- The Entner-Doudoroff pathway is generally found in Pseudomonas, Rhizobium, Azotobacter, Agrobacterium, and a few other gram-negative genera. Very few Gram-positive bacteria have this pathway, with Enterococcus faecalis being a rare exception

Steps: 1. At first glucose is phosphorylated to glucose -6-phosphate by the enzyme hexokinase.

2. Glucose-6-phosphate is then oxidized to 6- phosphogluconolactone releasing a molecule of NADPH. This reaction is catalyzed by the enzyme glucose-6- phosphate dehydrogenase.

3. Hydrolase enzyme converts 6- phopshogluconolactone to 6- phosphogluconate.

4. 6-phosphogluconate undergoes dehydration reaction catalyzed by 6-phosphogluconate dehydratase to form 2-keto 3-deoxy 6- Phosphogluconate (KDPG)
5.KDPG splits to form pyruvate and glceraldehyde-3-phosphate. It is catalyzed by KDPG aldolase enzyme
6. Glyceraldehyde-3-phosphate is then metabolized by glycolysis to form pyruvate

The Krebs cycle or TCA cycle (tricarboxylic acid cycle)

The Krebs cycle or TCA cycle (tricarboxylic acid cycle) or Citric acid cycle is a series of enzyme catalysed reactions occurring in the mitochondrial matrix, where acetyl-CoA is oxidised to form carbon dioxide and coenzymes are reduced, which generate ATP in the electron transport chain.

Krebs cycle was named after Hans Krebs, who postulated the detailed cycle. He was awarded the Nobel prize in 1953 for his contribution.

It is a series of eight-step processes, where the acetyl group of acetyl-CoA is oxidised to form two molecules of CO_2 and in the process, one ATP is produced. Reduced high energy compounds, NADH and $FADH_2$ are also produced.

Two molecules of acetyl-CoA are produced from each glucose molecule so two turns of the Krebs cycle are required which yields four CO_2, six NADH, two $FADH_2$ and two ATPs.

Krebs Cycle is a part of Cellular Respiration

Cellular respiration is a catabolic reaction taking place in the cells. It is a biochemical process by which nutrients are broken down to release energy, which gets stored in the form of ATP and waste products are released. In aerobic respiration, oxygen is required

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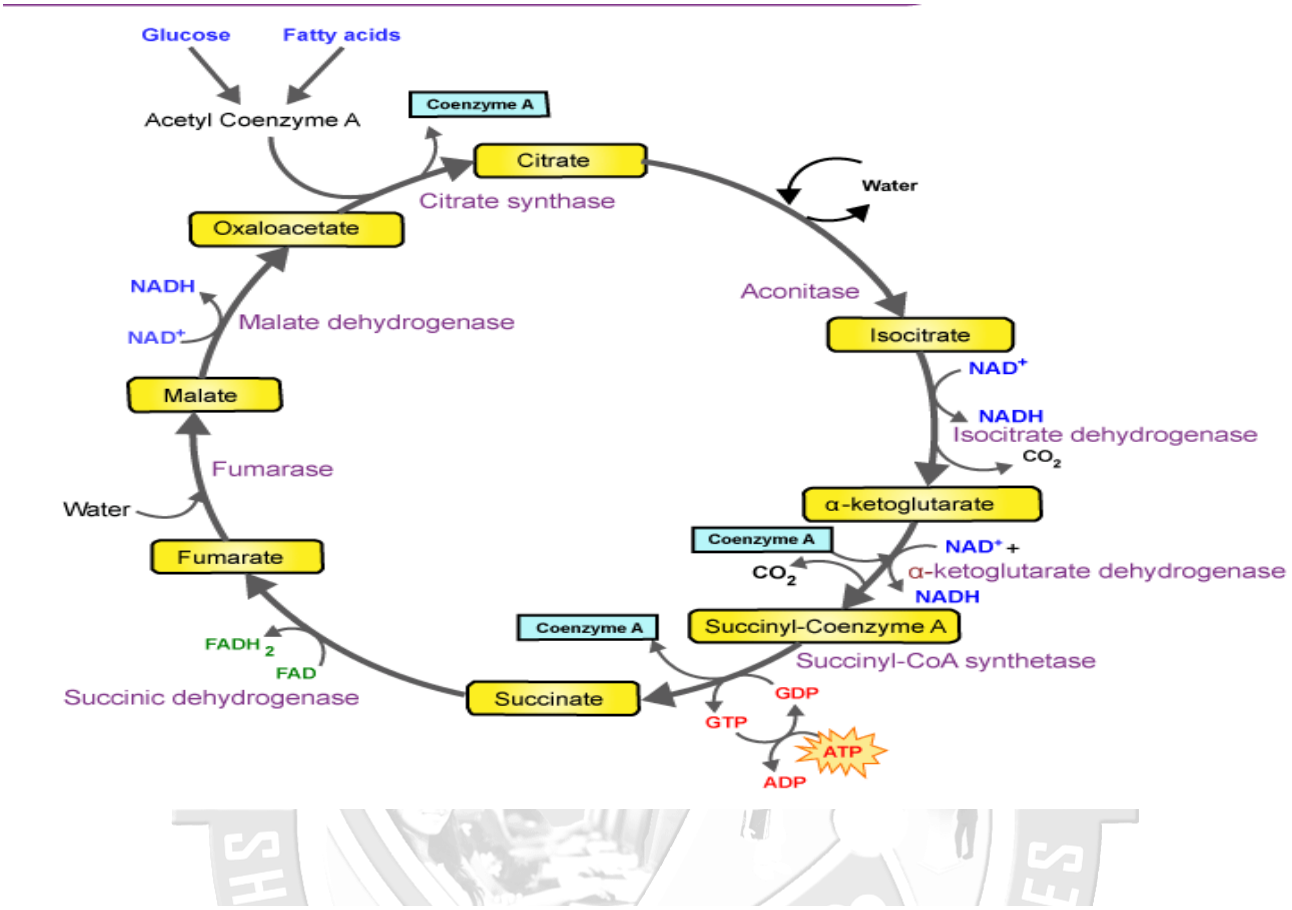

Krebs Cycle Steps

It is an eight-step process. Krebs cycle or TCA cycle takes place in the matrix of mitochondria under aerobic condition.

Step 1: The first step is the condensation of acetyl CoA with 4-carbon compound oxaloacetate to form 6C citrate, coenzyme A is released. The reaction is catalysed by *citrate synthase*.

Step 2: Citrate is converted to its isomer, isocitrate. The enzyme *aconitase* catalyses this reaction.

Step 3: Isocitrate undergoes dehydrogenation and decarboxylation to form 5C **α**-ketoglutarate. A molecular form of CO_2 is released. *Isocitrate dehydrogenase* catalyses the reaction. It is an NAD^+ dependent enzyme. NAD^+ is converted to NADH.

Step 4: **α**-ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by the ***α-ketoglutarate dehydrogenase*** enzyme complex. One molecule of CO_2 is released and NAD^+ is converted to NADH.

Step 5: Succinyl CoA forms succinate. The enzyme *succinyl CoA synthetase* catalyses the reaction. This is coupled with substrate-level phosphorylation of GDP to get GTP. GTP transfers its phosphate to ADP forming ATP.

Step 6: Succinate is oxidised by the enzyme *succinate dehydrogenase* to fumarate. In the process, FAD is converted to $FADH_2$.

Step 7: Fumarate gets converted to malate by the addition of one H_2O. The enzyme catalysing this reaction is *fumarase*.

Step 8: Malate is dehydrogenated to form oxaloacetate, which combines with another molecule of acetyl CoA and starts the new cycle. Hydrogens removed, get transferred to NAD^+ forming NADH. *Malate dehydrogenase* catalyses the reaction.

The glyoxylate cycle
The glyoxylate cycle centers on the conversion of acetyl-CoA to succinate for the synthesis of carbohydrates.
In microorganisms, the glyoxylate cycle allows cells to use two carbons (C2 compounds), such as acetate, to satisfy cellular carbon requirements when simple sugars such as glucose or fructose are not available.

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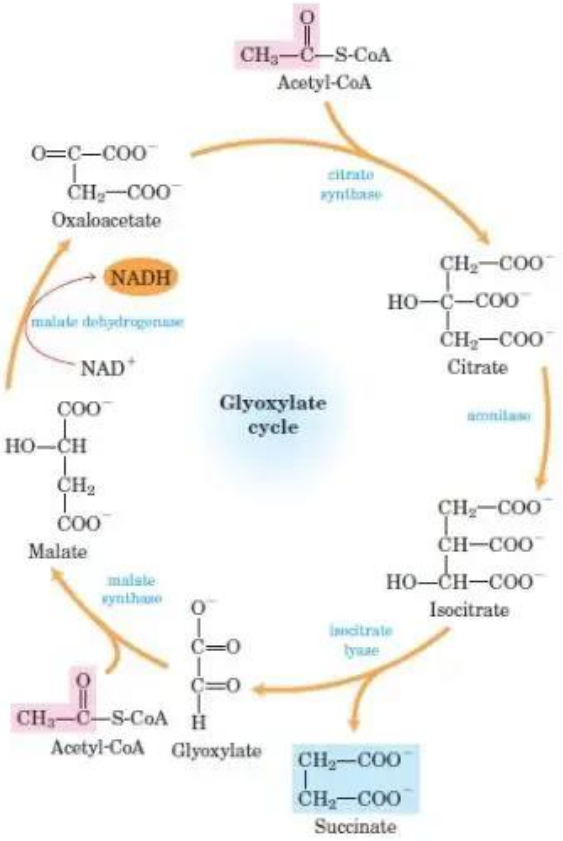


Protein catabolism

Protein catabolism is the breakdown of proteins into absorbable monomers for further degradation or reassembly. Protein catabolism in the intestinal lumen is important for several reasons, one of which is mobilizing essential amino acids for absorption.

In molecular biology, **protein catabolism** is the breakdown of proteins into smaller peptides and ultimately into amino acids. Protein catabolism is a key function of digestion process. Protein catabolism often begins with pepsin, which converts proteins into polypeptides. These polypeptides are then further degraded.
In humans, the pancreatic proteases include trypsin, chymotrypsin, and other enzymes.

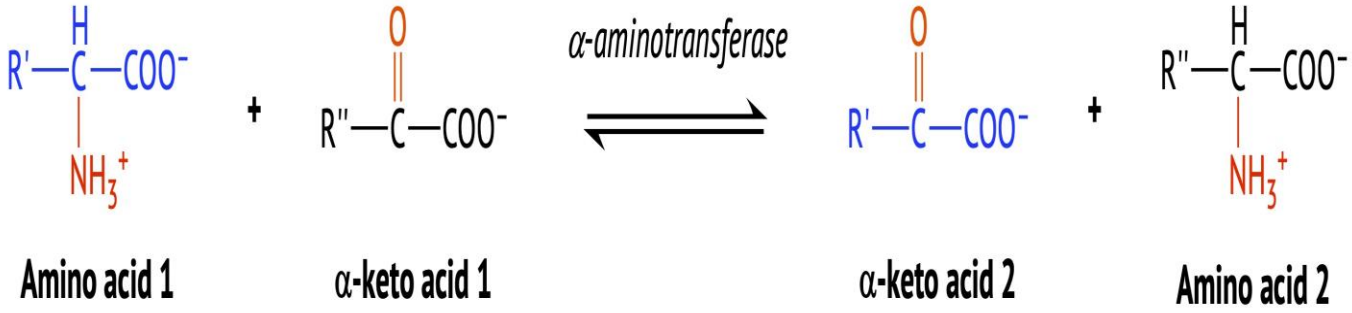
In the intestine, the small peptides are broken down into amino acids that can be absorbed into the bloodstream. These absorbed amino acids can then undergo amino acid catabolism, where they are utilized as an energy source or as precursors to new proteins.[1]

The amino acids produced by catabolism may be directly recycled to form new proteins, converted into different amino acids, or can undergo amino acid catabolism to be converted to other compounds via the Krebs cycle.[

General reaction of amino acid catabolism

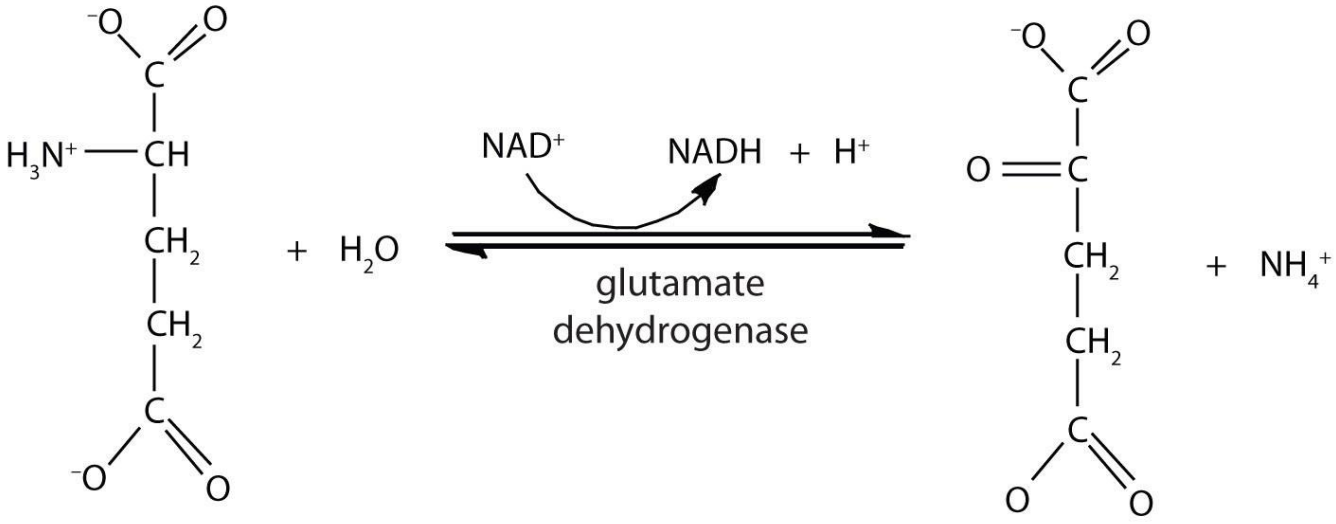
Transamination

Transamination is an exchange of functional groups between any amino acid (except lysine, proline, and threonine) and an α-keto acid. The amino group is usually transferred to the keto carbon atom of pyruvate, oxaloacetate, or α-ketoglutarate, converting the alpha***h*-keto acid to alanine, aspartate, or glutamate, respectively. Transamination reactions are catalyzed by specific transaminases (also called aminotransferases), which require pyridoxal phosphate as a coenzyme.



Oxidative Deamination

In the breakdown of amino acids for energy, the final acceptor of the alpha***h*-amino group is alpha***h*-ketoglutarate, forming glutamate. Glutamate can then undergo oxidative deamination, in which it loses its amino group as an ammonium (NH_4^+) ion and is oxidized back to alpha***h*-ketoglutarate (ready to accept another amino group):



This reaction occurs primarily in liver mitochondria. Most of the NH_4^+ ion formed by oxidative deamination of glutamate is converted to urea and excreted in the urine in a series of reactions known as the **urea cycle**.

The synthesis of glutamate occurs in animal cells by reversing the reaction catalyzed by glutamate dehydrogenase.

For this reaction nicotinamide adenine dinucleotide phosphate (NADPH) acts as the reducing agent. The synthesis of glutamate is significant because it is one of the few reactions in animals that can incorporate inorganic nitrogen (NH_4^+) into an α-keto acid to form an amino acid.

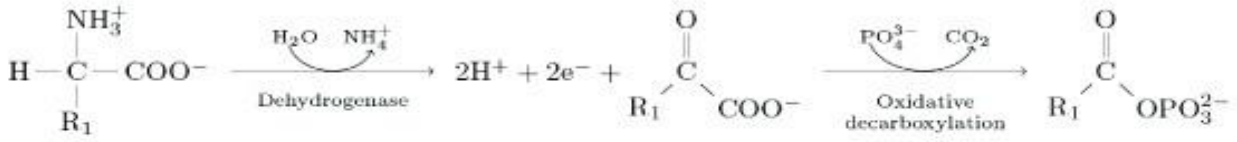
The amino group can then be passed on through transamination reactions, to produce other amino acids from the appropriate α-keto acids.

The Stricklands reaction

usually involves one amino acid that acts as an electron donor (the product is shorter by one carbon atom than the original amino acid), while another acts as an electron acceptor (the product has the same number of carbon atoms as the original amino acid) (Strickland 1934; Nisman 1954).

For example, alanine with a three carbon chain is converted to acetate with two carbons. The electron acceptor amino acid is reduced to a volatile carboxylic acid the same length as the original amino acid. For example, glycine with two carbons is converted to acetate.

(A)



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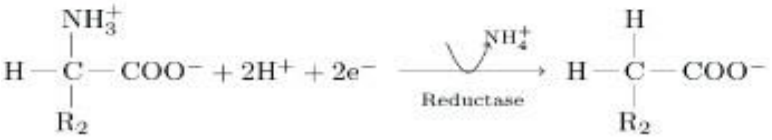

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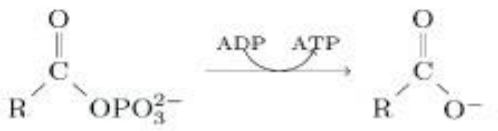

Lipid catabolism

comprises two major spatially and temporarily separated steps, namely lipolysis, which releases fatty acids and head groups and is catalyzed by lipases at membranes or lipid droplets, and degradation of fatty acids to acetyl-CoA, which occurs in peroxisomes through the β-oxidation pathway in green .

Types of Lipids- simple lipid; compound lipid and derive lipid The four main groups of lipids include: 1. Fatty acids (saturated and unsaturated) 2. Glycerides (glycerol-containing lipids) 3. Nonglyceride lipids (sphingolipids, steroids, waxes) 4. Complex lipids (lipoproteins, glycolipids)

Oxidation of fatty acid removal of successive two carbon atom unit fatty acid oxidation to form carbon dioxide

Fatty acid oxidation takes place in four stages - dehydrogenation, hydration, oxidation, and thiolysis.
These four stages keep repeating until the whole molecule is oxidized.
Each of these four stages is catalyzed by a different enzyme.

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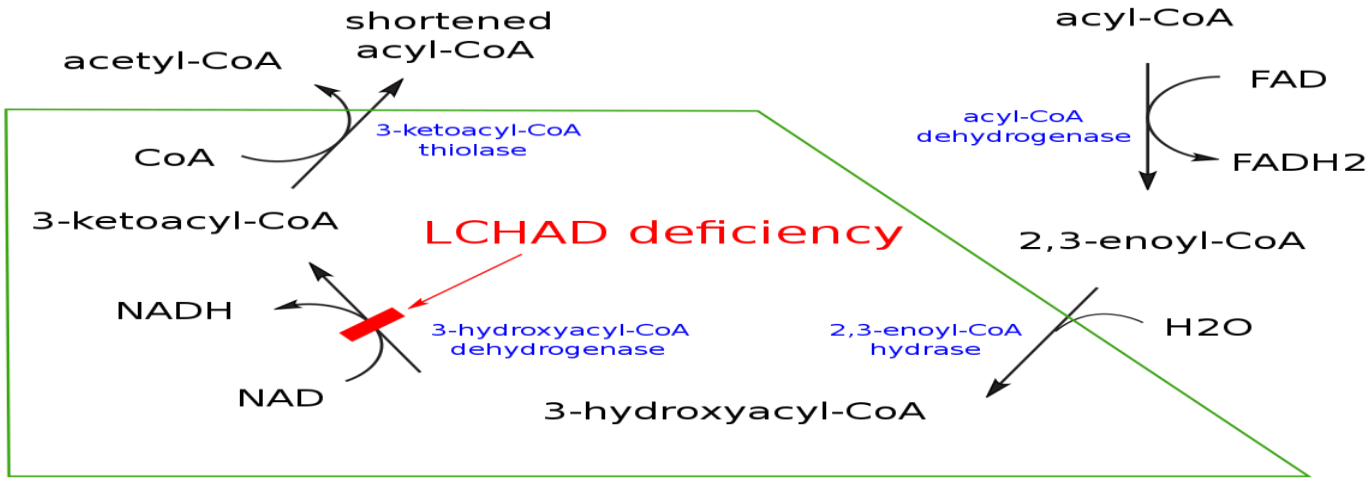
Oxidation of fatty acid

Oxidation of fatty acids occurs in multiple regions of the cell within the human body; the mitochondria, in which only Beta-oxidation occurs; the peroxisome, where alpha- and beta-oxidation occur; and omega-oxidation, which occurs in the endoplasmic reticulum

Beta oxidation occurs in the mitochondria of eukaryotic cells and in the cytosol of prokaryotic cells. However, before this happens, fatty acids must first enter the cell and, in the case of eukaryotic cells, the mitochondria. In cases where fatty acid chains are too long to enter the mitochondria, beta oxidation can also take place in peroxisomes.

First, fatty acid protein transporters allow fatty acids to cross the cell membrane and enter the cytosol, since the negatively charged fatty acid chains cannot cross it otherwise. Then, the enzyme fatty acyl-CoA synthase (or FACS) adds a CoA group to the fatty acid chain, converting it to acyl-CoA.



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Beta -oxidation of fatty acids