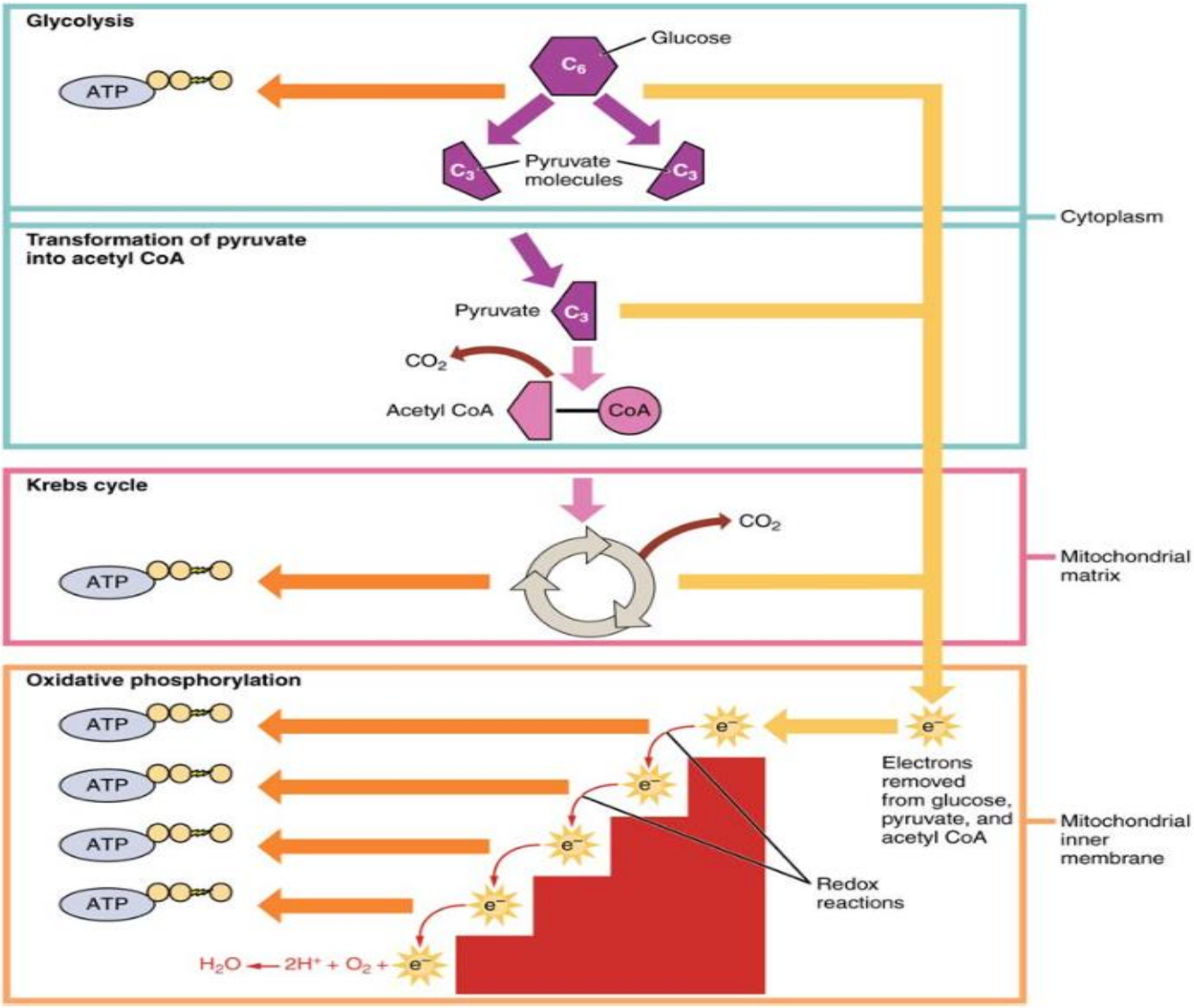
3.1 CARBOHYDRATE METABOLISM

Carbohydrates are organic molecules composed of carbon, hydrogen, and oxygen atoms. The family of carbohydrates includes both simple and complex sugars. Glucose and fructose are examples of simple sugars, and starch, glycogen, and cellulose are all examples of complex sugars. The complex sugars are also called **polysaccharides** and are made of multiple **monosaccharide** molecules. Polysaccharides serve as energy storage (e.g., starch and glycogen) and as structural components (e.g., chitin in insects and cellulose in plants).

During digestion, carbohydrates are broken down into simple, soluble sugars that can be transported across the intestinal wall into the circulatory system to be transported throughout the body. The main function of carbohydrates is to provide energy in the form of ATP. This production of ATP from carbohydrates or glucose is done in various steps and cycles Like Glycolysis, krebs cycle and electron transport chain as shown in the diagram below.



GLYCOLYSIS

Glycolysis is derived from the Greek words (glykys = sweet and lysis = splitting). It is a universal catabolic pathway in the living cells. Glycolysis can be defined as the sequence of reactions for the breakdown of Glucose (6-carbon molecule) to two molecules of pyruvic acid (3-carbon molecule) under aerobic conditions; or lactate

under anaerobic conditions along with the production of small amount of energy. This pathway was described by Embden, Meyerhof and Parnas. Hence, it is also called as Embden-Meyerhof pathway (EM pathway).

Site of Glycolysis

Glycolysis occurs in the cytoplasm of virtually all the cells of the body.

Types of Glycolysis

There are two types of glycolysis.

- **Aerobic Glycolysis**: It occurs when oxygen is plentiful. Final product is **pyruvate** along with the production of Eight ATP molecules.
- **Anaerobic Glycolysis**: It occurs when oxygen is scarce. Final product is **lactate** along with the production of two ATP molecules.

Steps of Glycolysis

Glycolysis is an extramitochondrial pathway and is carried by a group of eleven enzymes. Glucose is converted to pyruvate in 10 steps by glycolysis. The glycolytic patway can be divided into two phases:

Preparatory Phase :

This phase is also called **glucose activation phase**. In the preparatory phase of glycolysis, two molecules of ATP are invested and the hexose chain is cleaved into two triose phosphates. During this, phosphorylation of glucose and it's conversion to glyceraldehyde-3-phosphate take place. The steps 1, 2, 3, 4 and 5 together are called as the preparatory phase.

Payoff Phase :

This phase is also called **energy extraction phase**. During this phase, conversion of glyceraldehyde-3-phophate to pyruvate and the coupled formation of ATP take place.
Because Glucose is split to yield two molecules of D-Glyceraldehyde-3-phosphate, each step in the payoff phase occurs twice per molecule of glucose. The steps after 5 constitute payoff phase

Step 1 : Uptake and Phosphorylation of Glucose

- Glucose is phosphorylated to form glucose-6-phosphate.
- The reaction is catalysed by the specific enzyme **glucokinase** in liver cells and by non specific enzyme **hexokinase** in liver and extrahepatic tissue. The enzyme splits the ATP into ADP, and the Pi is added onto the glucose.
- Hexokinase is a **key glycolytic enzyme**. Hexokinase catalyses a regulatory step in glycolysis that is irreversible.
- Hexokinase, like many other kinases, requires Mg2+ for its activity.

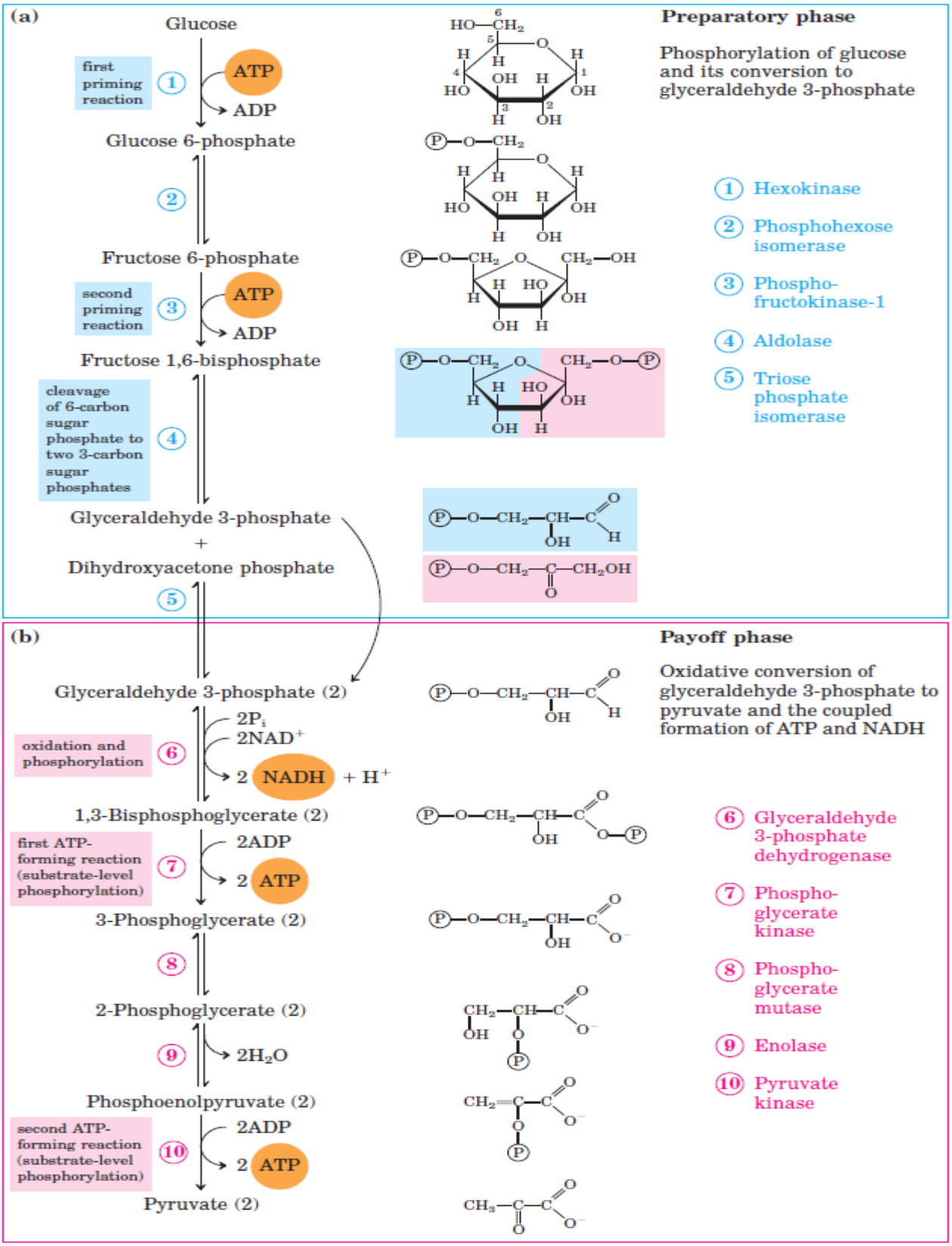
Step 2 : Isomerization of Glucose-6-Phsphate to Fructose-6-Phosphate

- Glucose-6-phosphate is isomerised to fructose-6-phosphate by **phosphohexose isomerase**.
- This reaction involves an aldose-ketose isomerisastion catalysed by phosphohexose isomerase. There is opening of the glucopyranose ring of glucose-6-phosphate to a linear structure which then changes to the furanose ring structure of fructose-6-phosphate.

Step 3 : Phosphorylation of F-6-P to Fructose 1,6-Biphosphate

- Fructose-6-phosphate is further phosphorylated to fructose 1,6-bisphosphate.

- The enzyme is **phosphofructokinase-1**. It catalyses the transfer of a phosphate group from ATP to fructose-6-phosphate.
- The reaction is irreversible.
- One ATP is utilised for phosphorylation.
- Phosphofructokinase-1 is the **key enzyme** in glycolysis which regulates breakdown of glucose.



Step 4 : Cleavage of Fructose 1,6-Biphosphate

- The 6 carbon fructose-1,6-bisphosphate is cleaved into two 3 carbon units; one glyceraldehyde-3-phosphate (GAP) and another molecule of dihydroxy acetone phosphate (DHAP).
- The enzyme which catalyses the reaction is **aldolase**. Since the backward reaction is an aldol condensation, the enzyme is called aldolase.
- The reaction is reversible.

Step 5 : Interconversion of the Triose Phosphates

- GAP is on the direct pathway of glycolysis, whereas DHAP is not. Hence **Triose-phosphate isomerase** converts DHAP into GAP useful for generating ATP. Thus net result
- is that glucose is now cleaved into 2 molecules of glyceraldehyde-3-phosphate.
- This reaction is rapid and reversible.

Step 6 : Oxidative phosphorylation of GAP to 1,3-Bisphosphoglycerate

- The first step in the payoff phase is the oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.
- This reaction is catalyzed by **glyceraldehyde 3-phosphate dehydrogenase**.
- It is the energy-yielding reaction. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy. During this reaction, NAD+ is reduced to NADH.
- This is a reversible reaction.

Step 7 : Conversion of 1,3-Biphosphoglycerate to 3-Phosphoglycerate

- The enzyme **phosphoglycerate kinase** transfers the high-energy phosphoryl group from the carboxyl group of 1,3-bisphosphoglycerate to ADP, forming ATP and 3-phosphoglycerate.
- This is a unique example where ATP can be produced at substrate level without participating in electron transport chain. This type of reaction where ATP is formed at substrate level is called as Substrate level phosphorylation.

Step 8 : Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate

- 3-phospho glycerate is isomerized to 2-phospho glycerate by shifting the phosphate group from 3rd to 2nd carbon atom.
- The enzyme is **phosphogluco mutase**.
- This is a readily reversible reaction.
- Mg2+ is essential for this reaction.

Step 9 : Dehydration of 2-Phosphoglycerate to Phosphoenolpyruvate

- 2-phosphoglycerate is converted to phosphoenol pyruvate by the enzyme **enolase**.
- One water molecule is removed.
- A high energy phosphate bond is produced. The reaction is reversible.
- Enolase requires Mg++.

Step 10 : Conversion of Phosphoenol Pyruvate to Pyruvate

- Phosphoenol pyruvate (PEP) is dephosphorylated to pyruvate, by **pyruvate kinase**.
- One mole of ATP is generated during this reaction. This is again an example of substrate level phosphorylation.
- The pyruvate kinase is a **key glycolytic enzyme**. This step is irreversible

Glycolysis can be expressed as the following equation:

Glucose + 2ATP + 2NAD$^+$ + 4ADP + 2Pi -------- 2Pyruvate + 4ATP + 2NADH + 2H$^+$

This equation states that glucose, in combination with ATP (the energy source), NAD+ (a coenzyme that serves as an electron acceptor), and inorganic phosphate, breaks down into two pyruvate molecules, generating four ATP molecules—for a net yield of two ATP—and two energy-containing NADH coenzymes. The NADH that is produced in this process will be used later to produce ATP in the mitochondria. Importantly, by the end of this process, one glucose molecule generates two pyruvate molecules, two high-energy ATP molecules, and two electron-carrying NADH molecules.

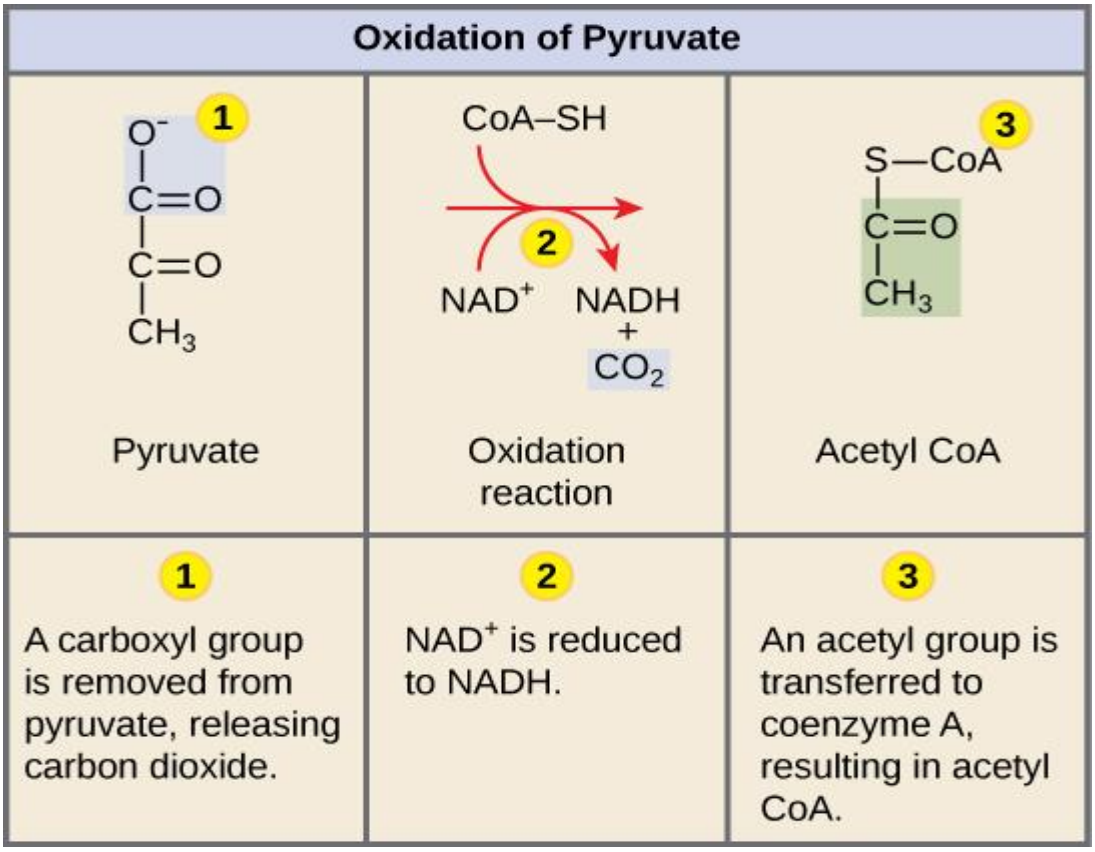
In Case of aerobic metabolism of glucose pyruvate enters the mitochondria and undergoes following processes.

OXIDATIVE DECARBOXYLATION OF PYRUVATE

In this process pyruvic acid undergoes oxidation (dehydrogenation) and decarboxylation (loss of CO_2) in the presence of an H-acceptor (NAD^+), Pyruvic acid dehydrogenase complex and Coenzyme-A (Co-A-SH) to produce Acetyl Co-A and reduced Coenzyme

Pyruvate dehydrogenase complex is formed of 3 different enzymes (E1-Pyruvate dehydrogenase, E2-Dihydrolipoyl Transacetylase, E3- Dihydrolipoyl Dehydrogenase) and 5 coenzymes (TPP-Thiamine Pyrophosphatase), Coenzyme-A, FAD and NAD^+)

This process occurs in mitochondrial matrix.



Then the acetyl-Co-A enters into a cycle of reactions called krebs cycle, which is described as.

KREBS CYCLE

The pyruvate molecules generated during glycolysis are transported across the mitochondrial membrane into the inner mitochondrial matrix, where they are metabolized by enzymes in a pathway called the Krebs cycle. The Krebs cycle is also commonly called the citric acid cycle or the tricarboxylic acid (TCA) cycle. During the Krebs cycle, high-energy molecules, including ATP, NADH, and FADH2, are created. NADH and FADH2 then pass electrons through the electron transport chain in the mitochondria to generate more ATP molecules.

In eukaryotes, the citric acid cycle takes place in the matrix of the mitochondria, just like the conversion of pyruvate to acetyl Co-A. In prokaryotes, these steps both take place in the cytoplasm. The citric acid cycle is a closed loop; the last part of the pathway reforms the molecule used in the first step. The cycle includes eight major steps.

In this cycle the Acetyl Co-A formed from pyruvate combine with the oxaloacetate and produce NADH, FADH2 and GTP through a cycle of enzyme controlled reactions where oxaloacetate is regenerated and and the cycle starts again.

Steps in the Citric Acid Cycle

Step 1 (Condensation). The first step is a condensation step, combining the two-carbon acetyl group (from acetyl CoA) with a four-carbon oxaloacetate molecule to form a six-carbon molecule of citrate. This step is irreversible because it is highly exergonic. The rate of this reaction is controlled by negative feedback and the amount of ATP available. If ATP levels increase, the rate of this reaction decreases. If ATP is in short supply, the rate increases.

This step is catalysed by enzyme citric acid synthetase

Step 2 (Isomerisation). Citrate loses one water molecule and gains another as citrate is converted into its isomer, isocitrate.

This reaction is catalysed by enzyme aconitase

Steps 3 and 4 (Dehydrogenation and Decarboxylation). In step three, isocitrate is oxidized, producing a five-carbon molecule, α-ketoglutarate, together with a molecule of CO_2 and two electrons, which reduce NAD+ to NADH. This step is also regulated by negative feedback from ATP and NADH and by a positive effect of ADP. Steps three and four are both oxidation and decarboxylation steps, which release electrons that reduce NAD^+ to NADH and release carboxyl groups that form CO_2 molecules. α-Ketoglutarate is the product of step three, and a succinyl group is the product of step four. CoA binds the succinyl group to form succinyl CoA. The enzyme that catalyzes step four is regulated by feedback inhibition of ATP, succinyl CoA, and NADH.

These steps are controlled by enzymes Isocitrate dehydrogenase and oxalsuccinate decarboxylase respectively

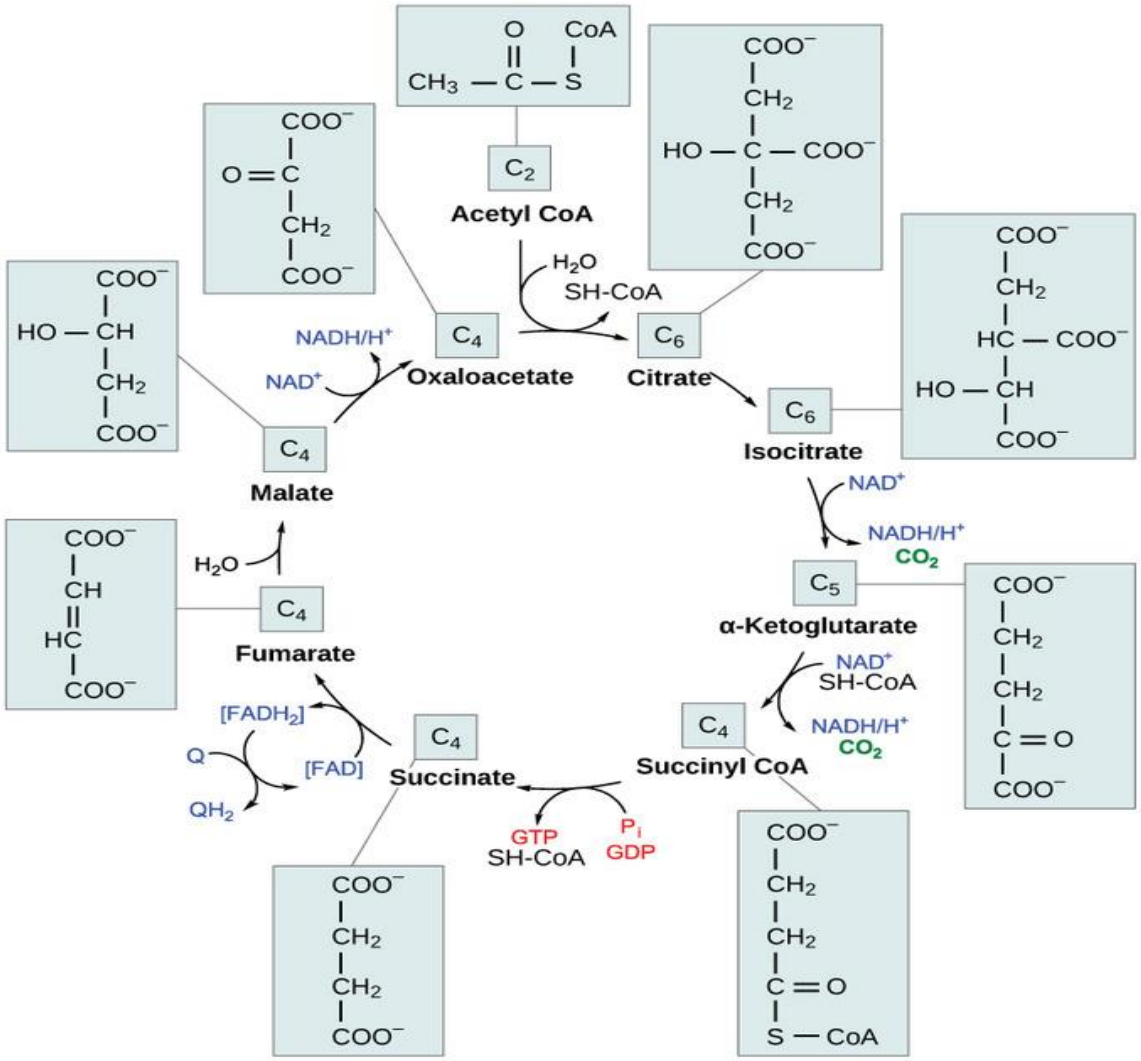
Step 5 (Oxidative Decarboxylation). A phosphate group is substituted for coenzyme A, and a high- energy bond is formed. This energy is used in substrate-level phosphorylation (during the conversion of the succinyl group to succinate) to form either guanine triphosphate (GTP) or ATP. There are two forms of the enzyme, called isoenzymes, for this step, depending upon the type of animal tissue in which they are found. One form is found in tissues that use large amounts of ATP, such as heart and skeletal muscle. This form produces ATP. The second form of the enzyme is found in tissues that have a high number of anabolic pathways, such as liver. This form produces GTP. GTP is energetically equivalent to ATP; however, its use is more restricted. In particular, protein synthesis primarily uses GTP.

Step 6 (Dehydrogenation). Step six is a dehydration process that converts succinate into fumarate. Two hydrogen atoms are transferred to FAD, producing $FADH_2$. The energy contained in the electrons of these atoms is insufficient to reduce NAD^+ but adequate to reduce FAD. Unlike NADH, this carrier remains attached to the enzyme and transfers the electrons to the electron transport chain directly. This process is made possible by the localization of the enzyme catalyzing this step inside the inner membrane of the mitochondrion.

This reaction is catalysed by succinate dehydrogenase.

Step 7 (Hydration). Water is added to fumarate during step seven, and malate is produced in presence of enzyme fumarase

Step 8 (Dehydrogenation). The last step in the citric acid cycle regenerates oxaloacetate by oxidizing malate. Another molecule of NADH is produced. Enzyme required is malate dehydrogenase.



In the citric acid cycle, the acetyl group from acetyl CoA is attached to a four-carbon oxaloacetate molecule to form a six-carbon citrate molecule. Through a series of steps, citrate is oxidized, releasing two carbon dioxide molecules for each acetyl group fed into the cycle. In the process, three NAD+ molecules are reduced to NADH, one FAD molecule is reduced to FADH2, and one ATP or GTP (depending on the cell type) is produced (by substrate-level phosphorylation). Because the final product of the citric acid cycle is also the first reactant, the cycle runs continuously in the presence of sufficient reactants

The krebs cycle is a common pathway of oxidative breakdown of carbohydrate, fatty acids and amino acids.

The FADH2 and NADH then enter the Electron Transport Chain to undergo oxidative phosphorylation and produce ATP.

ELECTRON TRANSPORT CHAIN (ETC)-Oxidative Phosphorylation

The electron transport chain is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called chemiosmosis. Together, the electron transport chain and chemiosmosis make up oxidative phosphorylation

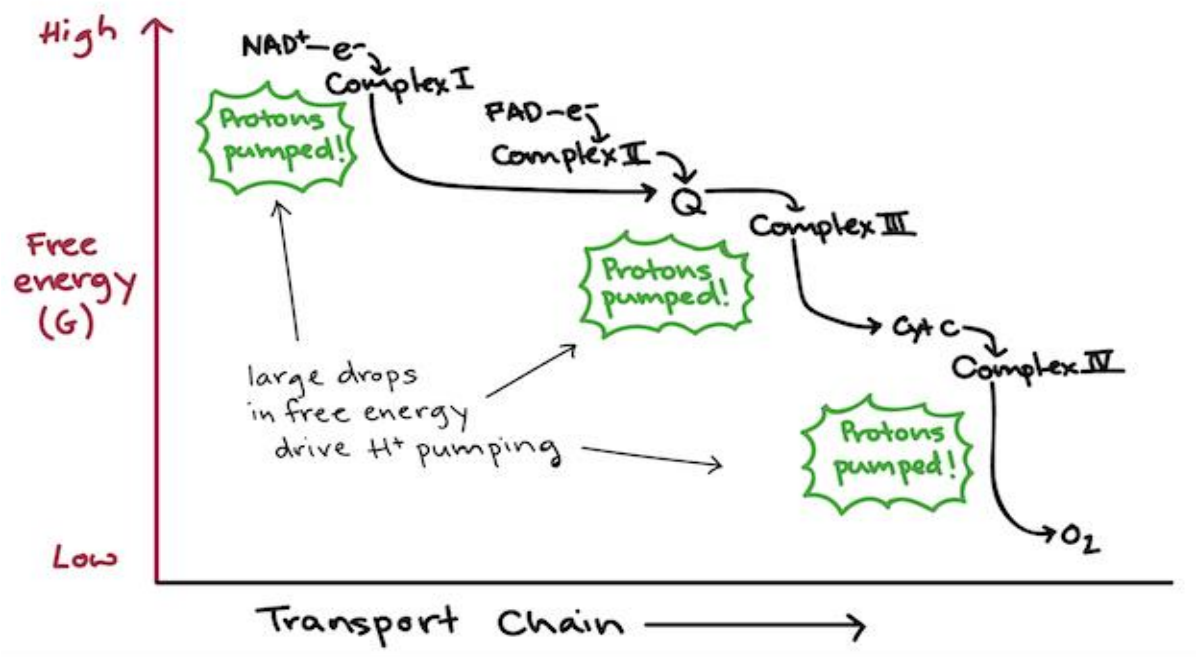
The key steps of this process, shown in simplified form in the diagram above, include:

- **Delivery of electrons by NADH and FADH2.** Reduced electron carriers (NADH and FADH2) from other steps of cellular respiration transfer their electrons to molecules near the beginning of the transport chain. In the process, they turn back into NAD+ and FAD, which can be reused in other steps of cellular respiration.
- **Electron transfer and proton pumping.** As electrons are passed down the chain, they move from a higher to a lower energy level, releasing energy. Some of the energy is used to pump H+ ions, moving them out of the matrix and into the intermembrane space. This pumping establishes an electrochemical gradient.
- **Splitting of oxygen to form water.** At the end of the electron transport chain, electrons are transferred to molecular oxygen, which splits in half and takes up H+ to form water.
- **Gradient-driven synthesis of ATP.** As H+ ions flow down their gradient and back into the matrix, they pass through an enzyme called ATP synthase, which harnesses the flow of protons to synthesize ATP.

The electron transport chain

The **electron transport chain** is a collection of membrane-embedded proteins and organic molecules, most of them organized into four large complexes labeled I to IV. In eukaryotes, many copies of these molecules are found in the inner mitochondrial membrane. In prokaryotes, the electron transport chain components are found in the plasma membrane.

As the electrons travel through the chain, they go from a higher to a lower energy level, moving from less electron-hungry to more electron-hungry molecules. Energy is released in these "downhill" electron transfers, and several of the protein complexes use the released energy to pump protons from the mitochondrial matrix to the intermembrane space, forming a proton gradient.

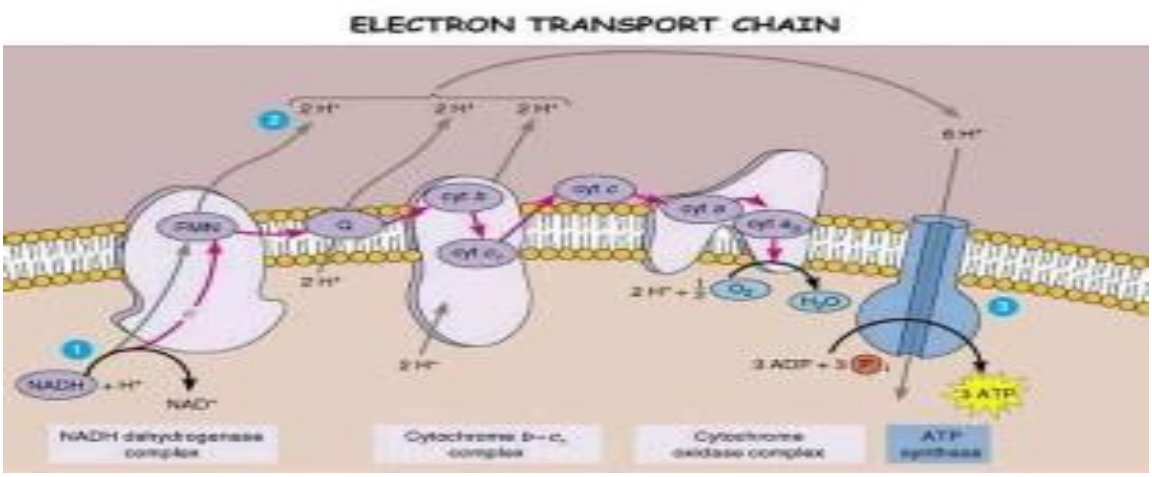


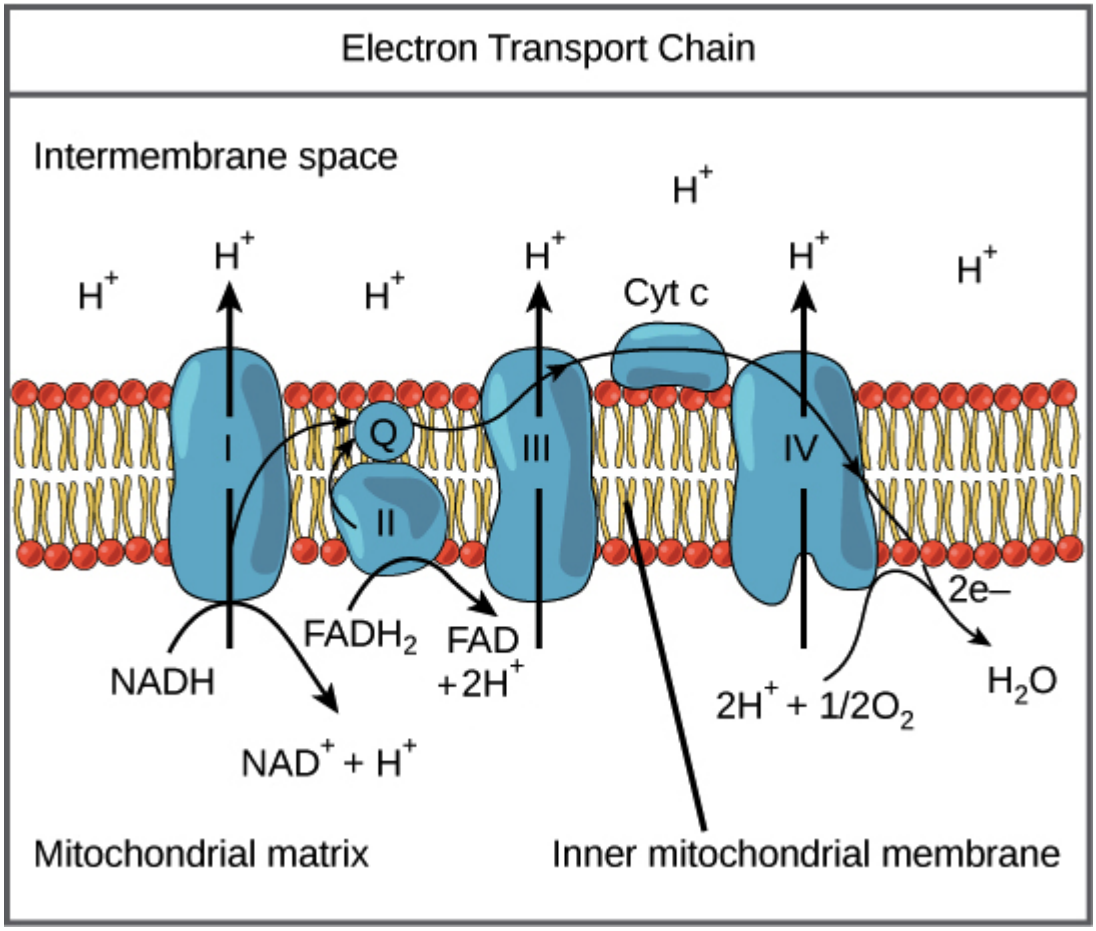
Complex 1. It is formed **of NADH-Ubiquinone oxidoreductase** which transfers two electrons from NADH2 to lipid soluble carrier Coenzyme Q through FMN

Complex 2. It is formed of succinate dehydrogenase enzyme which transfers two electrons from succinate via FAD to coenzyme Q.

Complex 3. It is also called cytochrome bc1 complex. It removes two electrons from coenzyme Q and transfers them to two molecules of cytochrome –c.

Complex 4. It is also called cytochrome-c oxidase it removes 4 electrons from four molecules of cytochrome-c and then transfer them to oxygen to produce 2 molecules of water





All the components of the chain are embedded in or attached to the inner mitochondrial membrane. In the matrix, NADH deposits electrons at Complex I, turning into NAD+ and releasing a proton into the matrix. FADH2 in the matrix deposits electrons at Complex II, turning into FAD and releasing 2 H+. The electrons from Complexes I and II are passed to the small mobile carrier Q. Q transports the electrons to Complex III, which then passes them to Cytochrome C. Cytochrome C passes the electrons to Complex IV, which then passes them to oxygen in the matrix, forming water. It takes two electrons, 1/2 O2, and and 2 H+ to form one water molecule. Complexes I, III, and IV use energy released as electrons move from a higher to a lower energy level to pump protons out of the matrix and into the intermembrane space, generating a proton gradient.

All of the electrons that enter the transport chain come from NADH and FADH2 molecules produced during earlier stages of cellular respiration: glycolysis, pyruvate oxidation, and the citric acid cycle.

- **NADH** is very good at donating electrons in redox reactions (that is, its electrons are at a high energy level), so it can transfer its electrons directly to complex I, turning back into NAD+. As electrons move through complex I in a series of redox reactions, energy is released, and the complex uses this energy to pump protons from the matrix into the intermembrane space.
- **FADH**2is not as good at donating electrons as NADH (that is, its electrons are at a lower energy level), so it cannot transfer its electrons to complex I. Instead, it feeds them into the transport chain through complex II, which does not pump protons across the membrane.

Because of this "bypass," each FADH2 molecule causes fewer protons to be pumped (and contributes less to the proton gradient) than an NADH.

The simplified pathway for electron flow in electron transport chain is as

NADH---------FMN---------Co-Q----------Cyt.b----------Cyt.c----------Cyt.c1----------Cyt.a----------Cyt.a3

FADH2 ↗

Beyond the first two complexes, electrons from NADH and FADH2 travel exactly the same route. Both complex I and complex II pass their electrons to a small, mobile electron carrier called **ubiquinone** (**Q**), which is reduced to form QH2 and travels through the membrane, delivering the electrons to complex III. As electrons move through complex III, more H+ ions are pumped across the membrane, and the electrons are ultimately delivered to another mobile carrier called **cytochrome C** (**cyt C**). Cyt C carries the electrons to complex IV, where a final batch of H+ ions is pumped across the membrane. Complex IV passes the electrons to O2, which splits into two oxygen atoms and accepts protons from the matrix to form water. Four electrons are required to reduce each molecule of O2, and two water molecules are formed in the process.

Overall, what does the electron transport chain do for the cell? It has two important functions:

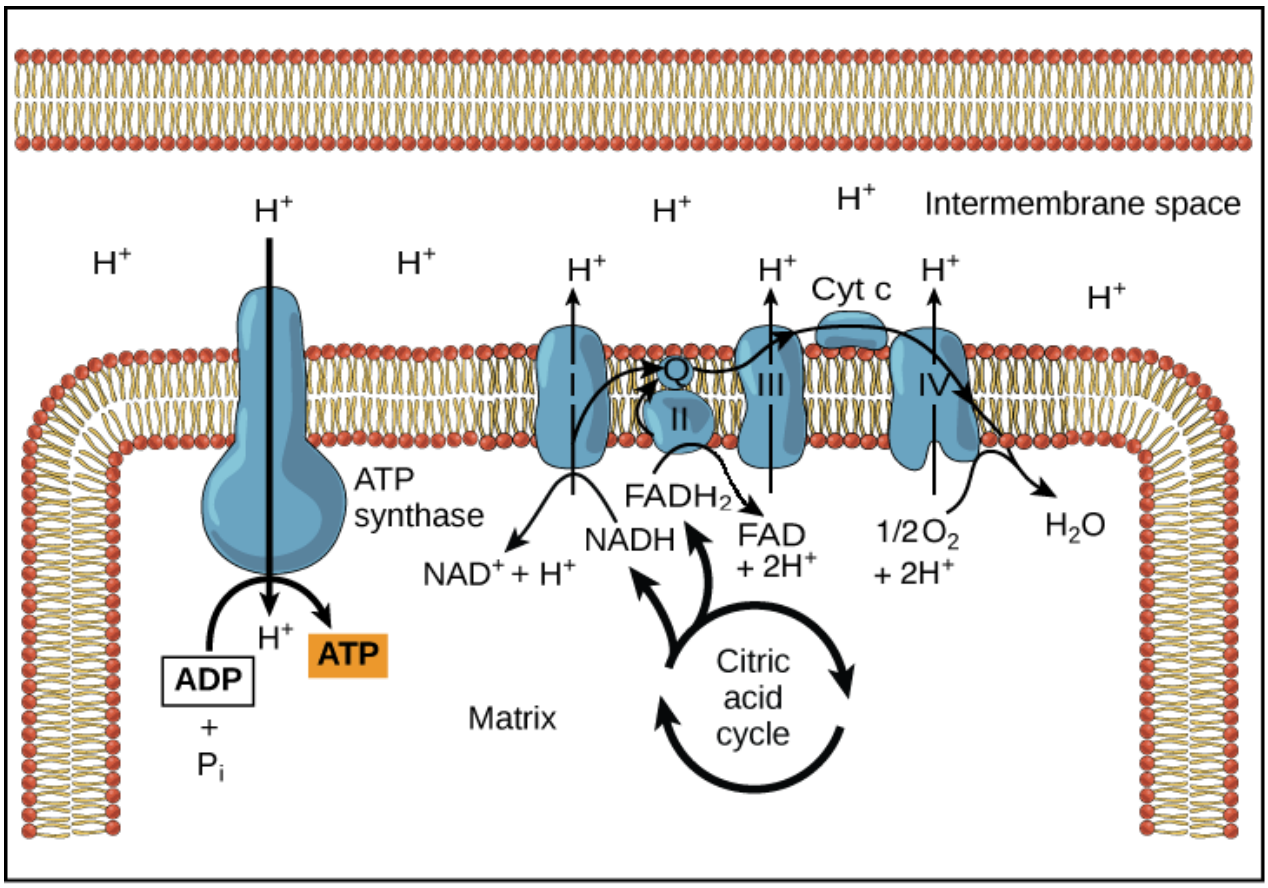
- **Regenerates electron carriers.** NADH and FADH2 pass their electrons to the electron transport chain, turning back into NAD+and FAD. This is important because the oxidized forms of these electron carriers are used in glycolysis and the citric acid cycle and must be available to keep these processes running.
- **Makes a proton gradient.** The transport chain builds a proton gradient across the inner mitochondrial membrane, with a higher concentration of H+ in the intermembrane space and a lower concentration in the matrix. This gradient represents a stored form of energy, and, as we'll see, it can be used to make ATP.

Chemiosmosis

Complexes I, III, and IV of the electron transport chain are proton pumps. As electrons move energetically downhill, the complexes capture the released energy and use it to pump H+ ions from the matrix to the intermembrane space. This pumping forms an electrochemical gradient across the inner mitochondrial membrane. The gradient is sometimes called the **proton-motive force**, and you can think of it as a form of stored energy, kind of like a battery.

Like many other ions, protons can't pass directly through the phospholipid bilayer of the membrane because its core is too hydrophobic. Instead, H+ ions can move down their concentration gradient only with the help of channel proteins that form hydrophilic tunnels across the membrane.

In the inner mitochondrial membrane, H+ ions have just one channel available: a membrane-spanning protein known as **ATP synthase**. Conceptually, ATP synthase is a lot like a turbine in a hydroelectric power plant. Instead of being turned by water, it's turned by the flow of H+ ions moving down their electrochemical gradient. As ATP synthase turns, it catalyzes the addition of a phosphate to ADP, capturing energy from the proton gradient as ATP.



The electron transport chain and ATP synthase are embedded in the inner mitochondrial membrane. NADH and FADH2 made in the citric acid cycle (in the mitochondrial matrix) deposit their electrons into the electron transport chain at complexes I and II, respectively. This step regenerates NAD+ and FAD (the oxidized carriers) for use in the citric acid cycle. The electrons flow through the electron transport chain, causing protons to be pumped from the matrix to the intermembrane space. Eventually, the electrons are passed to oxygen, which combines with protons to form water. The proton gradient generated by proton pumping during the electron transport chain is a stored form of energy. When protons flow back down their concentration gradient (from the intermembrane space to the matrix), their only route is through ATP synthase, an enzyme embedded in the inner mitochondrial membrane. When protons flow through ATP synthase, they cause it to turn (much as water turns a water wheel), and its motion catalyzes the conversion of ADP and Pi to ATP

This process, in which energy from a proton gradient is used to make ATP, is called **chemiosmosis**. More broadly, chemiosmosis can refer to any process in which energy stored in a proton gradient is used to do work. Although chemiosmosis accounts for over 80% of ATP made during glucose breakdown in cellular respiration, it's not unique to cellular respiration. For instance, chemiosmosis is also involved in the light reactions of photosynthesis

ATP yield

How many ATP do we get per glucose in cellular respiration? If you look in different books, or ask different professors, you'll probably get slightly different answers. However, most current sources estimate that the maximum ATP yield for a molecule of glucose is around 30-32 ATP.

Stage	Direct products (net)	Ultimate ATP yield (net)
Glycolysis	2 ATP	2 ATP
	2 NADH	3-5 ATP
Pyruvate oxidation	2 NADH	5 ATP
Citric acid cycle	2 ATP/GTP	2 ATP
	6 NADH	15 ATP
	2 FADH_22start subscript, 2, end subscript	3 ATP
Total		**30-32 ATP**

3.2 FERMENTATION

Fermentation is a form of anaerobic (non-oxygen-requiring) pathway for breaking down glucose, one that's performed by many types of organisms and cells. In fermentation, the only energy extraction pathway is glycolysis, with one or two extra reactions tacked on at the end.

Fermentation and cellular respiration begin the same way, with glycolysis. In fermentation, however, the pyruvate made in glycolysis does not continue through oxidation and the citric acid cycle, and the electron transport chain does not run. Because the electron transport chain isn't functional, the NADH made in glycolysis cannot drop its electrons off there to turn back into NAD+

The purpose of the extra reactions in fermentation, then, is to regenerate the electron carrier NAD+ from NADH produced in glycolysis. The extra reactions accomplish this by letting NADH drop its electrons off with an organic molecule (such as pyruvate, the end product of glycolysis). This drop-off allows glycolysis to keep running by ensuring a steady supply of NAD+.

In simple terms fermentation is actually, chemical process by which glucose IS broken down anaerobically (In the absence of Oxygen) to produce products like organic acids, gases, or alcohol.

Fermentation is found in some bacteria, yeast, intestinal endoparasitic helminthes and oxygen starved muscle cells.

Fermenting bacteria are also called as **obligatory anaerobes** as they are unable to live in presence of oxygen

However yeast and muscle cells are **facultative anaerobes** as they can switch between fermentation and aerobic respiration depending upon the absence and presence of oxygen respectively.

In case of fermentation the pyruvic acid formed from glycolysis does not undergo oxidation or enter krebs cycle however acts as hydrogen acceptor and forms different products.

Mainly two types of end products are formed in fermentation, lactic acid or alcohol. So depending upon the end products fermentation is mainly of two types.

1. Alcohol Fermentation

Alcohol fermentation by yeast produces the ethanol found in alcoholic drinks like beer and wine. It is done by brewing yeast *Sachromyces cerevisiae* . However, alcohol is toxic to yeasts in large quantities (just as it is to humans), which puts an upper limit on the percentage alcohol in these drinks. Ethanol tolerance of yeast ranges from about 555 percent to 212121 percent, depending on the yeast strain and environmental conditions This type of fermentation involves following steps.

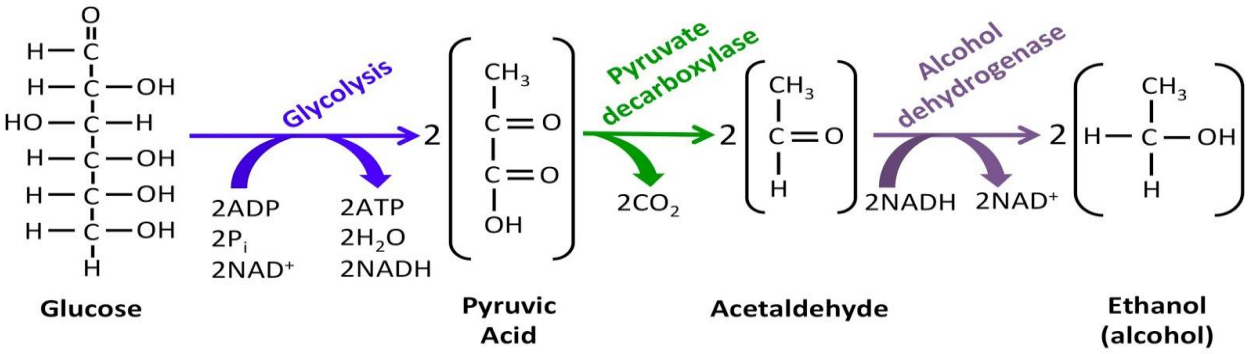
Step 1. Decarbpxylation of pyruvic acid in the presence of enzyme pyruvate decarboxylase and co-enzyme Thiamine Pyrophosphate (TPP) aand Zn^{2+} as cofactor. This produces acetaldehyde.

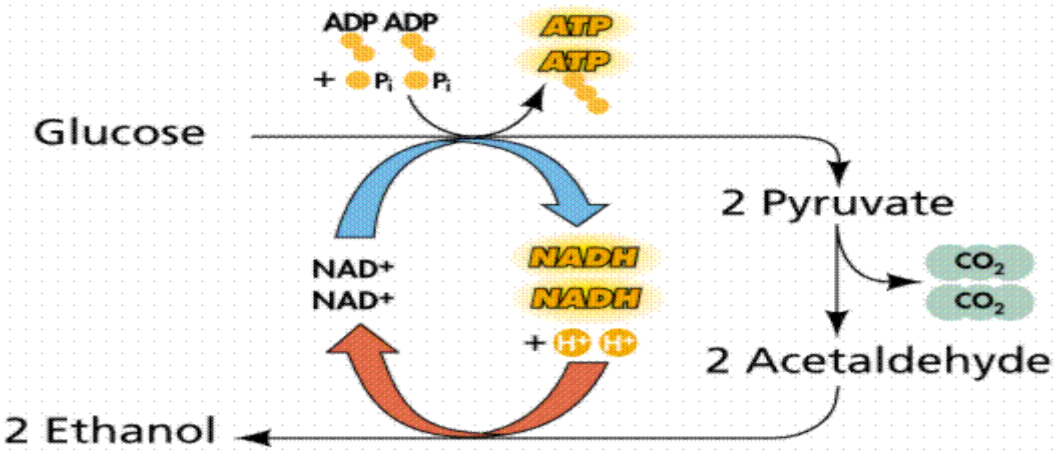
Step2. Acetaldehyde accepts 2 hydrogen atoms from Nadh2, in the presence of enzyme ethanol dehydrogenase and reduces to ethanol.

So overall reaction of alcoholic fermentation is

Glucose + 2Pi + 2ADP---------- 2 Ethanol + $2CO_2$ + 2ATP + $2H_2O$

So both glycolysis and alcoholic fermentation produces 2 molecule of ATP



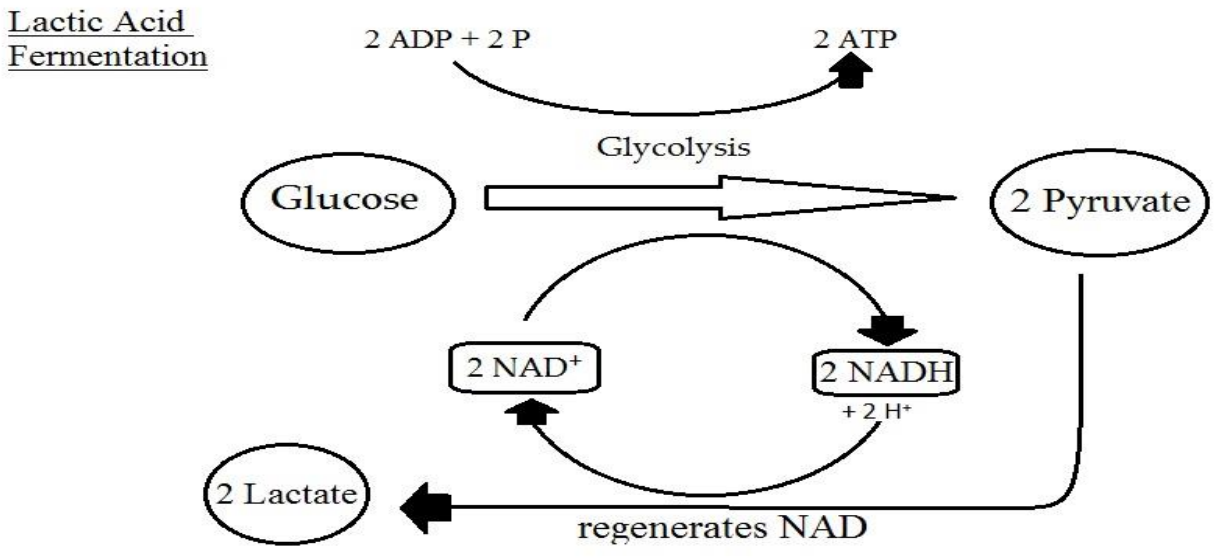


2. Lactic acid fermentation.

In lactic acid fermentation, NADH transfers its electrons directly to pyruvate, generating lactate as a byproduct. Lactate, which is just the deprotonated form of lactic acid, gives the process its name. The bacteria that make yogurt carry out lactic acid fermentation, as do the red blood cells in your body, which don't have mitochondria and thus can't perform cellular respiration.

Muscle cells also carry out lactic acid fermentation, though only when they have too little oxygen for aerobic respiration to continue—for instance, when you've been exercising very hard. It was once thought that the accumulation of lactate in muscles was responsible for soreness caused by exercise, but recent research suggests this is probably not the case.

Lactic acid produced in muscle cells is transported through the bloodstream to the liver, where it's converted back to pyruvate and processed normally in the remaining reactions of cellular respiration.



Lactic acid fermentation is of two types.

A . Homolactic fermentation.

It is found in homofermentative strains of bacteria like *Streptococcus , Lactobacillus etc* . It is also found in the muscle cells of animals. In this type of fermentation the main end product from metabolism of monosaccharides and disaccharides is lactic acid with traces of other end products of fermentation.

B . Heterolactic fermentation

It is found in heterofermentative strains of bacteria like *Leuconostoc, Eubacterium etc.* In this type in addition to small amounts of lactic acid some other end products are also formed such as acetate, ethanol, glycerol etc.

Disadvantages of fermentation

- It is low energy producing process.
- There is considerable loss of energy in end products.
- Lactic acid causes muscle fatigue.

Advantages of fermentation

- It supplements the aerobic respiration
- It has important role in brewing baking and milk industry
- It regenerates NAD+ to trap more H-atoms. If this does not happen glycolysis will stop, resulting in the death of the organism.

3.3 DEAMINATION, TRANSAMINATION AND ORNITHINE CYCLE.

As there is no store for excess amino acids, and as proteins are constantly being turned over, amino acids have to be continually degraded. The α-amino group is removed first and the resulting carbon skeleton is converted into one or more major metabolic intermediates and used as metabolic fuel. The carbon skeletons of the 20 standard amino acids are funneled into only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate . Amino acids that are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate and oxaloacetate are termed glucogenic as they can give rise to the net synthesis of glucose. This is because the citric acid cycle intermediates and pyruvate can be converted into phosphoenolpyruvate and then into glucose via gluconeogenesis. In contrast, amino acids that are degraded to acetyl CoA or acetoacetyl CoA are termed ketogenic because they give rise to ketone bodies, the acetyl CoA or acetoacetyl CoA can also be used to synthesize lipids. Of the standard set of 20 amino acids, only Leu and Lys are solely ketogenic. Ile, Phe, Trp and Tyr are both ketogenic and glucogenic as some of their carbon atoms end up in acetyl CoA or acetoacetyl CoA, whereas others end up in precursors of glucose. The remaining 14 amino acids are classified as solely glucogenic.

TRANSAMINATION

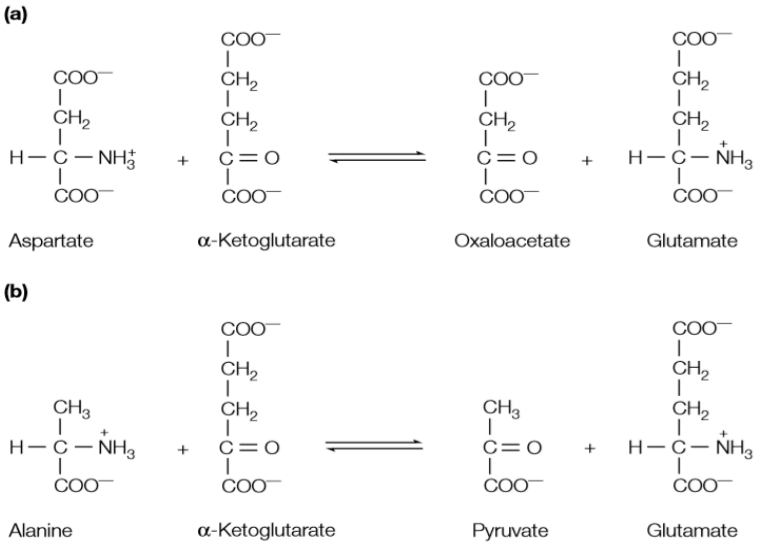
Transamination is a process of transfer of amino group from an amino acd to a keto acidwhich results into the formation of new α-keto acid and new amino acid.

Prior to the metabolism of their carbon skeletons into a major metabolic intermediate, the α-amino group of the amino acid has first to be removed by a process known as transamination. In this process the α-amino group of most amino acids is transferred to α -ketoglutarate to form glutamate and the corresponding α-keto acid

α-amino acid + α-ketoglutarate ⇌ α-keto acid + glutamate

The enzymes that catalyze these reactions are called transaminases (aminotransferases) and in mammals are found predominantly in the liver.

For example, aspartate transaminase catalyzes the transfer of the amino group of aspartate to α-ketoglutarate , while alanine transaminase catalyzes the transfer of the amino group of alanine to α-ketoglutarate . The coenzyme (or prosthetic group) of all transaminases is pyridoxal phosphate, which is derived from pyridoxine (vitamin B6), and which is transiently converted into pyridoxamine phosphate during transamination.



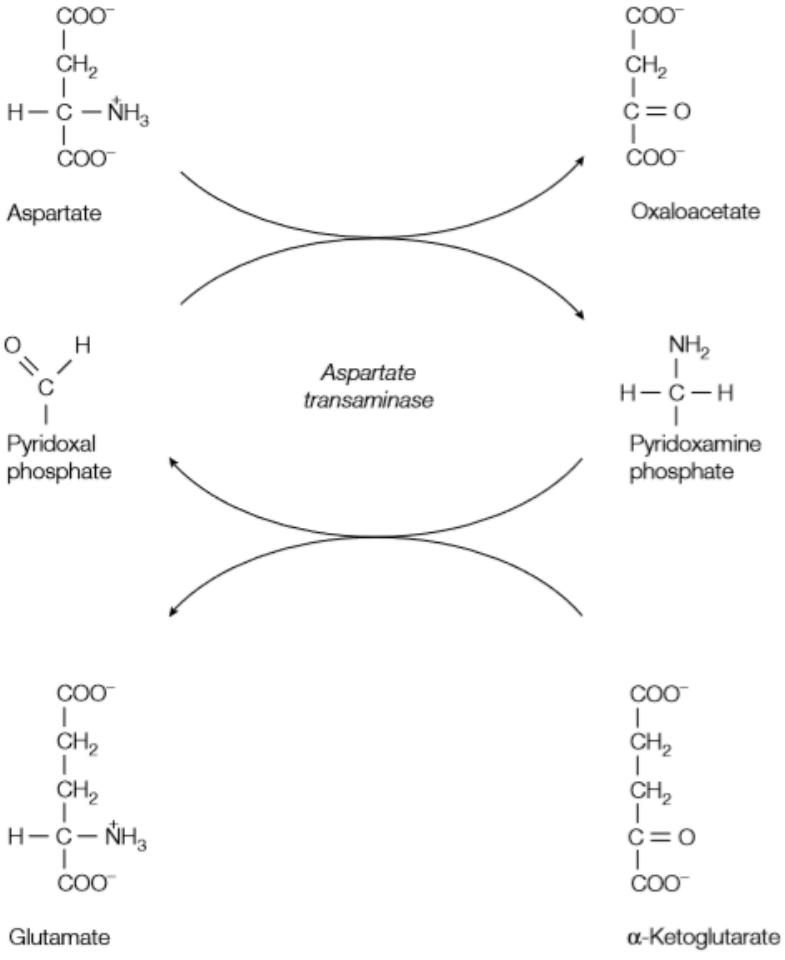
. *Reactions catalyzed by (a) aspartate transaminase and (b) alanine transaminase.*

Mechanism of transamination

Pyridoxal phosphate The coenzyme (or prosthetic group) of all transaminases is pyridoxal phosphate, which is derived from pyridoxine (vitamin B6), and which is transiently converted into pyridoxamine phosphate during transamination . In the absence of substrate, the aldehyde group of pyridoxal phosphate forms a covalent Schiff base linkage (imine bond) with the amino group in the sidechain of a specific lysine residue in the active site of the enzyme.

On addition of substrate, the α-amino group of the incoming amino acid displaces the amino group of the active site lysine and a new Schiff base linkage is formed with the amino acid substrate. The resulting amino acid–pyridoxal phosphate–Schiff base that is formed remains tightly bound to the enzyme by multiple noncovalent interactions.

The amino acid is then hydrolyzed to form an α-keto acid and pyridoxamine phosphate, the α-amino group having been temporarily transferred from the amino acid substrate on to pyridoxal phosphate . These steps constitute one half of the overall transamination reaction. The second half occurs by a reversal of the above reactions with a second α-keto acid reacting with the pyridoxamine phosphate to yield a second amino acid and regenerate the enzyme–pyridoxal phosphate complex



The overall reaction of transamination.

Significance

Transamination is main metabolic pathway to form amino acids.

The glutamate formed in transamination gets deaminated to produce ammonia, which is converted into urea through urea cycle.

DEAMINATION

The α-amino groups that have been funneled into glutamate from the other amino acids are then converted into ammonia by the action of glutamate dehydrogenase

(i) Definition. It is the catabolic pathway by which amino group of the amino acids is removed in the form of ammonia and amino acid changes into α-keto acid.

(ii) Site. Though most of the deamination occurs in liver but kidneys and several other tissues contain the D- or L-amino acid oxidases involved in the deamination of D- or L-amino acids.

(iii) Mechanism and examples. The oxidative deamination of specific amino acids is catalysed by specific enzymes; most commonly involved enzymes are **oxidases** (e.g. **glycine** amino acid is acted upon by **Glycine oxidase**) which remove two hydrogen atoms **(dehydrogenation)** from amino acid in the presence of an oxidised form of coenzyme, FAD or FMN. The amino acid changes into imino acid which undergoes hydrolysis to form α-keto acid and ammonia (**Fig. 2**). Reduced coenzyme ($FADH_2$ or $FMNH_2$) is reoxidised by moleculer oxygen to form hydrogen peroxide which is then decomposed by a catalase enzyme. It is an irreversible process.

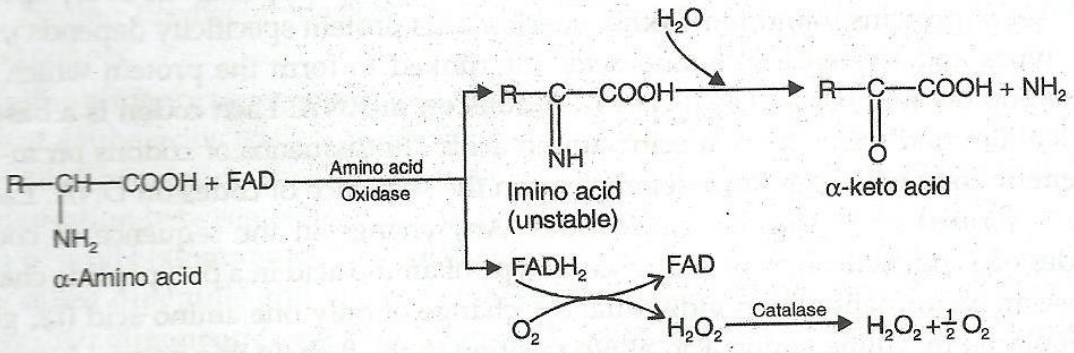


Fig. 2. Oxidative deamination of amino acids.

Amino acid oxidases are of 2 types on the basis of their **prosthetic group :**

(a) L-amino acid oxidases. These cause the oxidative deamination of most of the L-amino acid and have FMN as their prosthetic group.

(b) D-amino acid oxidases. These cause oxidative deamination of D-amino acids and have FAD as their prosthetic group.

In eukaryotes, these oxidases are present in the microbodies which also have catalase enzyme. So these microbodies are called **peroxisomes.**

But there are certain exceptions from this basic mechanism of oxidative deamination e.g.

(a) Oxidative deamination of L-Glutamic acid (Fig. 3). It is characterised by :

(1) It is deaminated by enzyme **L-Glutamic acid dehydrogenase.** It is widely distributed in mammalian tissues.

(2) Coenzyme required is **NAD^+** or **$NADP^+$.**

(3) It is a **reversible process** so the enzyme can participate in both the amino acid catabolism and biosynthesis.

(4) L-Glutamic acid changes into **α-Ketoglutaric acid** and vice versa. As α-Ketoglutaric acid is one of the compounds of Kreb's cycle so it constitutes the link between the carbohydrate and protein metabolism.

(5) The activity of enzyme glutamate dehydrogenase is inhibited by allosteric modifiers such as ATP, GTP and NADH, while ADP activates the enzyme.

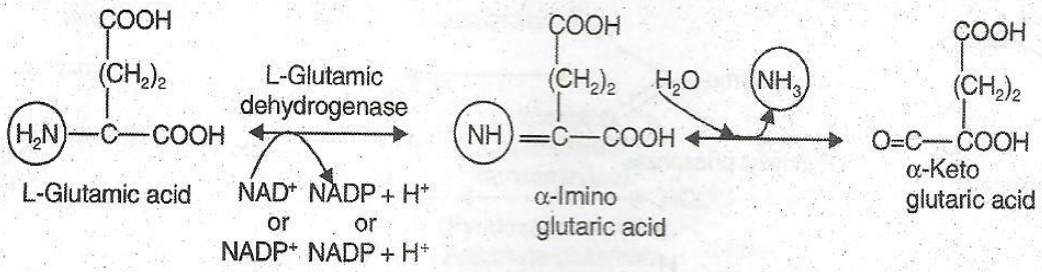


Fig. 3. Oxidative deamination of L-glutamic acid.

(b) Deamination of Serine (Fig. 4). It is characterized by :

(1) It is catalyzed by enzyme **serine dehydrase** which deaminates the amino acids having –OH molecule in their structure.

(2) Amino acids are deaminated **non-oxidatively.**

(3) Serine changes into **pyruvic acid** which is also one of the products of glucose catabolism. So it also shows the link between carbohydrate and protein metabolism.

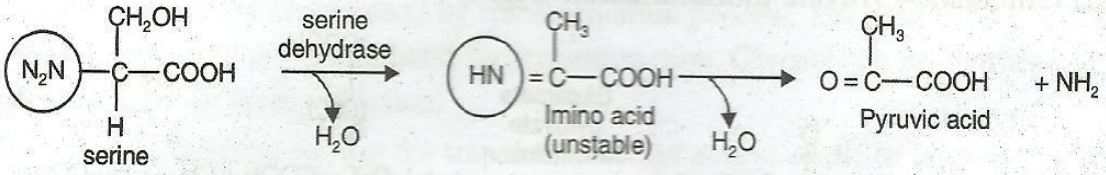


Fig. 4. Deamination of Serine.

ORNITHINE CYCLE (Urea Cycle)

Excess nitrogen is excreted as ammonia. Ammonotelic organisms excrete ammonia directly, uricotelic organisms excrete it as uric acid, and ureotelic organisms excrete it as urea.

In the urea cycle ammonia is first combined with CO2 to form carbamoyl phosphate. This then combines with ornithine to form citrulline. Citrulline then condenses with aspartate, the source of the second nitrogen atom in urea, to form argininosuccinate. This compound is in turn split to arginine and fumarate, and the arginine then splits to form urea and regenerate ornithine The first two reactions take place in the mitochondria of liver cells, the remaining three in the cytosol.

The fumarate produced in the urea cycle can enter directly into the citric acid cycle and be converted into oxaloacetate. Oxaloacetate can then be either transaminated to aspartate which feeds back into the urea cycle, or be converted into citrate, pyruvate or glucose.

Mechanism of urea formation

Urea is synthesized in the liver by the urea cycle. It is then secreted into the bloodstream and taken up by the kidneys for excretion in the urine. The urea cycle was the first cyclic metabolic pathway to be discovered by Hans Krebs and Kurt Henseleit in 1932, 5 years before Krebs discovered the citric acid cycle.

The overall reaction of the pathway is:

$$NH4^+ + HCO_3 + H_2O + 3\ ATP + aspartate \rightarrow urea + 2\ ADP + AMP + 2\ Pi + PPi + fumarate$$

One of the nitrogen atoms of urea comes from ammonia, the other is transferred from the amino acid aspartate, while the carbon atom comes from CO2. Ornithine, an amino acid that is not in the standard set of 20 amino acids and is not

found in proteins, is the carrier of these nitrogen and carbon atoms. Five enzymatic reactions are involved in the urea cycle, the first two of which take place in mitochondria, the other three in the cytosol:

1. Carbamoyl phosphate synthetase, which is technically not a member of the urea cycle, catalyzes the condensation and activation of ammonia (from the oxidative deamination of glutamate by glutamate dehydrogenase) and CO2(in the form of bicarbonate, HCO3–) to form carbamoyl phosphate. The hydrolysis of two ATP molecules makes this reaction essentially irreversible.

2. The second reaction also occurs in the mitochondria and involves the transfer of the carbamoyl group from carbamoyl phosphate to ornithine by ornithine transcarbamoylase. This reaction forms another nonstandard amino acid citrulline which then has to be transported out of the mitochondrion into the cytosol where the remaining reactions of the cycle take place.

3. The citrulline is then condensed with aspartate, the source of the second nitrogen atom in urea, by the enzyme argininosuccinate synthetase to form argininosuccinate. This reaction is driven by the hydrolysis of ATP to AMP and PPi, with subsequent hydrolysis of the pyrophosphate. Thus both of the high-energy bonds in ATP are ultimately cleaved.

4. Argininosuccinase then removes the carbon skeleton of aspartate from argininosuccinate in the form of fumarate, leaving the nitrogen atom on the other product arginine. As the urea cycle also produces arginine, this amino acid is classified as nonessential in ureotelic organisms. Arginine is the immediate precursor of urea.

5. The urea is then formed from arginine by the action of arginase with the regeneration of ornithine. The ornithine is then transported back into the mitochondrion ready to be combined with another molecule of carbamoyl phosphate.

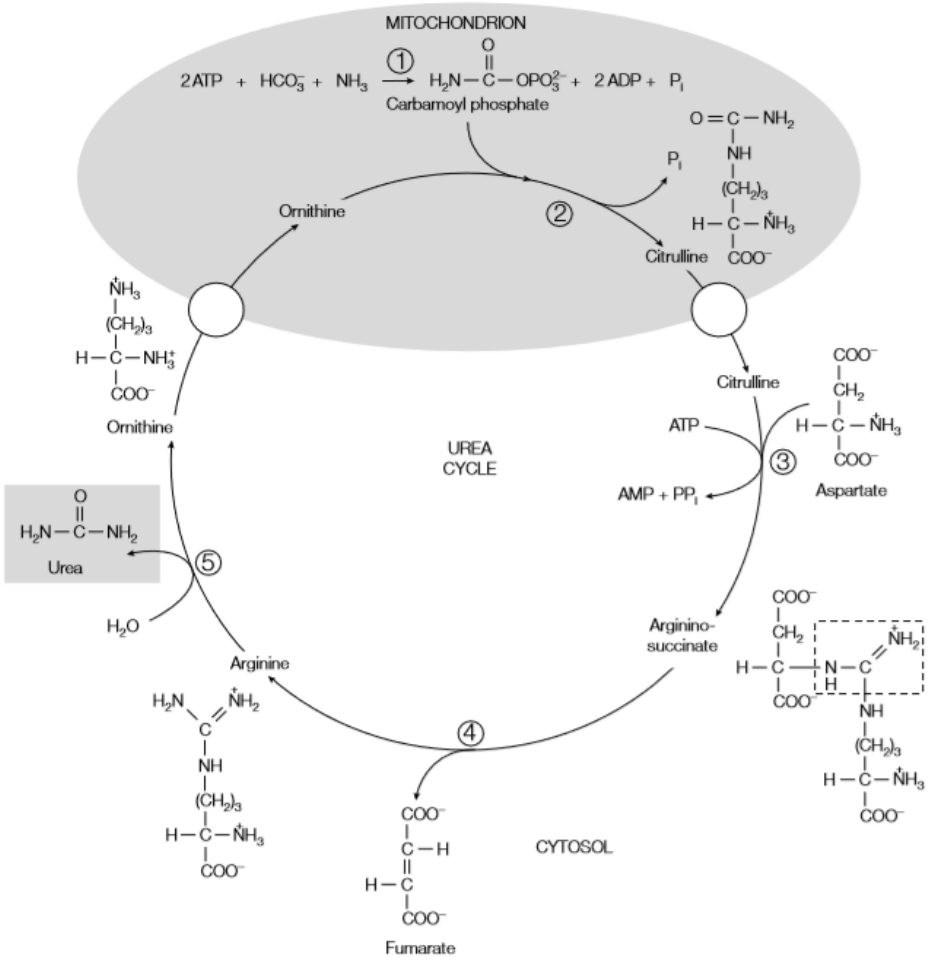


Fig. 1. The urea cycle. The enzymes involved in this cycle are: (1) carbamoyl phosphate synthetase; (2) ornithine transcarbamoylase; (3) argininosuccinate synthetase; (4) arginosuccinase; and (5) arginase.

Link to the citric acid cycle

The synthesis of fumarate by argininosuccinase links the urea cycle to the citric acid cycle. Fumarate is an intermediate of this latter cycle which is then hydrated to malate, which in turn is oxidized to oxaloacetate.

Oxaloacetate has several possible fates:

● transamination to aspartate which can then feed back into the urea cycle;

● condensation with acetyl CoA to form citrate which then continues on round the citric acid cycle;

● conversion into glucose via gluconeogenesis

● conversion into pyruvate.

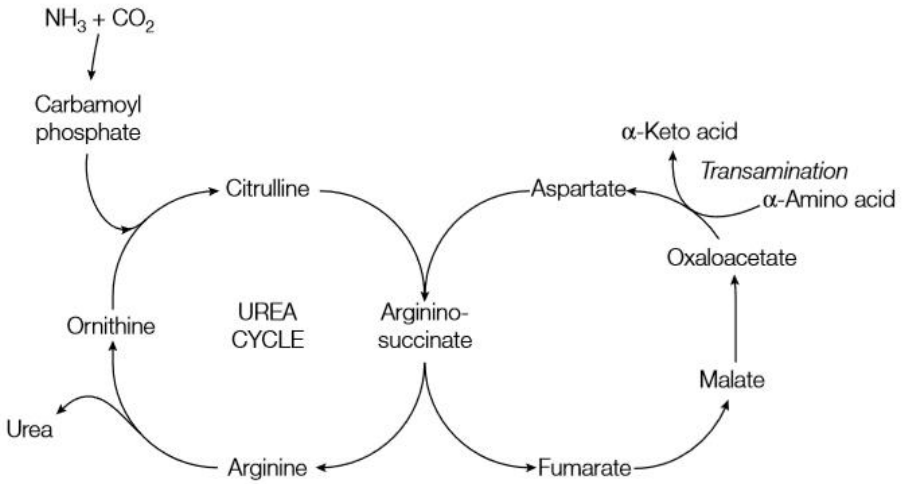


Fig. 2. The urea cycle and the citric acid cycle are linked by fumarate and the transamination of oxaloacetate to aspartate.

3.4 OXIDATION OF FATTY ACIDS

Fatty acid breakdown (also called β-oxidation) brings about the oxidation of long-chain fatty acids with the production of energy in the form of ATP. The fatty acids are converted into their acyl CoA derivatives and then metabolized by the removal of two-carbon acetyl CoA units from the end of the acyl chain.

Fatty acid breakdown occurs in the cytosol of prokaryotes and in the mitochondrial matrix of eukaryotes. The fatty acid is activated by forming a thioester link with CoA before entering the mitochondria.

The inner mitochondrial membrane is not permeable to long-chain acyl CoA derivatives and so these are transported into the mitochondria

β-oxidation

Fatty acid breakdown occurs in the cytosol of prokaryotes, in peroxisomes in plants and in the mitochondrial matrix of all other eukaryotes. Before entering the mitochondrial matrix, the fatty acid is activated by forming a thioester link with CoA (Fig. 1). This reaction is catalyzed by acyl CoA synthase (also called fatty acid thiokinase) which is present on the outer mitochondrial membrane, and uses a molecule of ATP. The overall reaction is irreversible due to the subsequent hydrolysis of PPi to two molecules of Pi.

Small- and medium-chain acyl CoA molecules (up to 10 carbon atoms) are readily able to cross the inner mitochondrial membrane by diffusion. However, longer chain acyl CoAs do not readily cross the inner mitochondrial membrane, and require a specific transport mechanism. To achieve this, the longer chain acyl CoAs are conjugated to the polar carnitine molecule which is found in both plants and animals. This reaction, catalyzed by an enzyme on the outer face of the inner mitochondrial membrane (carnitine acyltransferase I), removes the CoA group and substitutes it with a carnitine molecule (Fig. 2). The acylcarnitine is then transported across the inner mitochondrial membrane by a carnitine/acylcarnitine translocase. This integral membrane transport protein transports acylcarnitine molecules into the mitochondrial matrix and free carnitine molecules out. Once inside the mitochondrial matrix the acyl group is transferred back on to CoA, releasing free carnitine, by the enzyme carnitine acyltransferase II which is located on the matrix side of the inner mitochondrial membrane (Fig. 2).

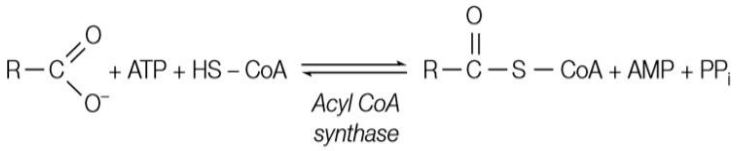


Fig. 1. Activation of a fatty acid.

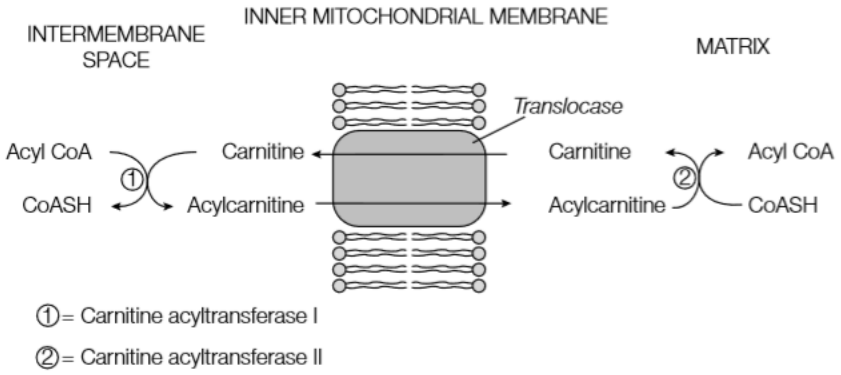


Fig. 2. Transport of fatty acids across the inner mitochondrial membrane.

The individual reactions involved in the degradation of fatty acids by βoxidation are as follows (see Fig. 3):

1. Oxidation of the fatty acyl CoA to enoyl CoA forming a trans Δ2-double bond on the fatty acyl chain and producing FADH2 (catalyzed by acyl CoA dehydrogenase).

2. Hydration of the trans Δ2-enoyl CoA to form 3-hydroxyacyl CoA (catalyzed by enoyl CoA hydratase).

3. Oxidation of 3-hydroxyacyl CoA to 3-ketoacyl CoA producing NADH (catalyzed by hydroxyacyl CoA dehydrogenase).

4. Cleavage, or thiolysis, of 3-ketoacyl CoA by a second CoA molecule, giving acetyl CoA and an acyl CoA shortened by two carbon atoms (catalyzed by βketothiolase).

Thus, the breakdown of individual fatty acids occurs as a repeating sequence of four reactions: oxidation (by FAD), hydration, oxidation (by NAD^+) and thiolysis. These four reactions form one 'round' of fatty acid degradation (Fig. 3) and their overall effect is to remove two-carbon units sequentially in the form of acetyl CoA from the fatty acid chain. The cleavage of the Δ2 (or β) bond of the fatty acyl chain (see Fig. 3, top structure, for nomenclature) gives fatty acid breakdown its alternative name, β-oxidation. The shortened acyl CoA then undergoes further cycles of β-oxidation until the last cycle, when the acyl CoA with four carbon atoms is split into two molecules of acetyl CoA. Thus a C16 saturated acyl CoA, such as palmitoyl CoA (of palmitic acid), would be completely degraded into eight molecules of acetyl CoA by seven rounds of degradation, leading to the overall equation:

Palmitoyl CoA + 7 FAD + 7 NAD^+ + 7 CoA + 7 H2O → 8 acetyl CoA + 7 FADH2 + 7 NADH + 7 H+

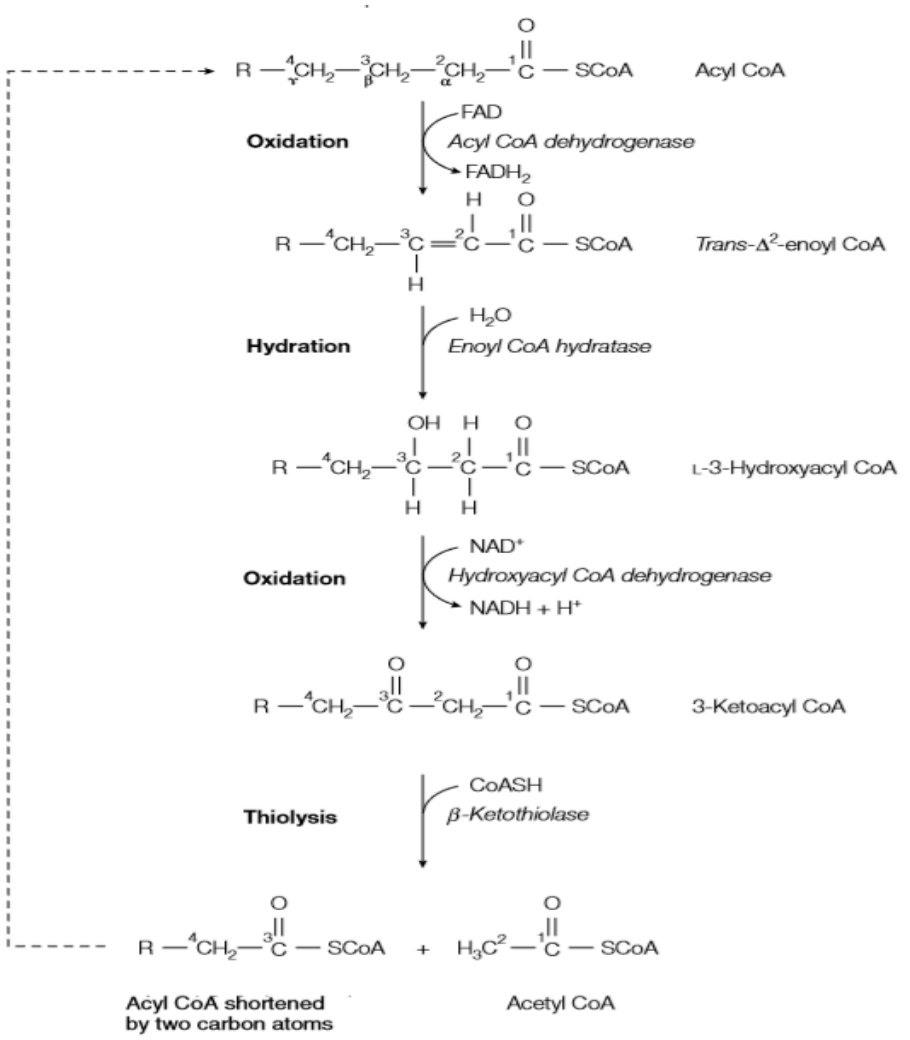


Fig. 3. Summary of the reactions involved in the degradation of fatty acids.

Mitochondria contain three acyl CoA dehydrogenases which act on short-, medium- and long-chain acyl CoAs, respectively. In contrast, there is just one each of the enzymes enoyl CoA hydratase, hydroxyacyl CoA dehydrogenase and β-ketothiolase which all have a broad specificity with respect to the length of the acyl chain.

In animals the acetyl CoA produced from fatty acid degradation cannot be converted into pyruvate or oxaloacetate. Although the two carbon atoms from acetyl CoA enter the citric acid cycle, they are both oxidized to CO2 in the reactions catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase . Thus, animals cannot convert fatty acids into glucose. In contrast, plants have two additional enzymes, isocitrate lyase and malate synthase, that enable them to convert the carbon atoms of acetyl CoA into oxaloacetate. This is accomplished via the glyoxylate pathway, a route involving enzymes of both the mitochondrion and the glyoxysome, a specialized membranous plant organelle.

α -Oxidation

Defined as the oxidation of fatty acids with the removal of one carbon unit adjacent to the alpha carbon from the carboxylic end in the form of CO_2.

Alpha oxidation occurs in those fatty acids which have methyl group at the beta carbon thereby preventing beta oxidation

This type of oxidation occurs in peroxisomes.

Fatty acid present in milk called Phytanic acid undergoes alpha oxidation

Mechanism

1. Phytanic acid is first attached to CoA to form phytanoyl-CoA.

2. Phytanoyl-CoA is oxidized by phytanoyl-CoA dioxygenase, in a process using Fe2+ and O2, to yield 2-hydroxyphytanoyl-CoA.

3. 2-hydroxyphytanoyl-CoA is cleaved by 2-hydroxyphytanoyl-CoA lyase in a TPP-dependent reaction to form pristanal and formyl-CoA (in turn later broken down into formate and eventually CO2).

4. Pristanal is oxidized by aldehyde dehydrogenase to form pristanic acid (which can then undergo beta-oxidation)