RESPIRATION

Competency: The learner appreciates how living organisms generate cellular energy, by analysing respiratory processes and the chemical breakdown of food within cells, to make informed decisions that promote good health and wellbeing.

examine the relationship between the structure of the mitochondrion and the stages of cellular respiration in living organisms.

- Ultrastructure of a mitochondrion.
- the structural relationship of the mitochondrion to its functions and hydrolysis of Adenosine triphosphate (ATP) to release energy.
- the detailed structure and energy release from ATP.

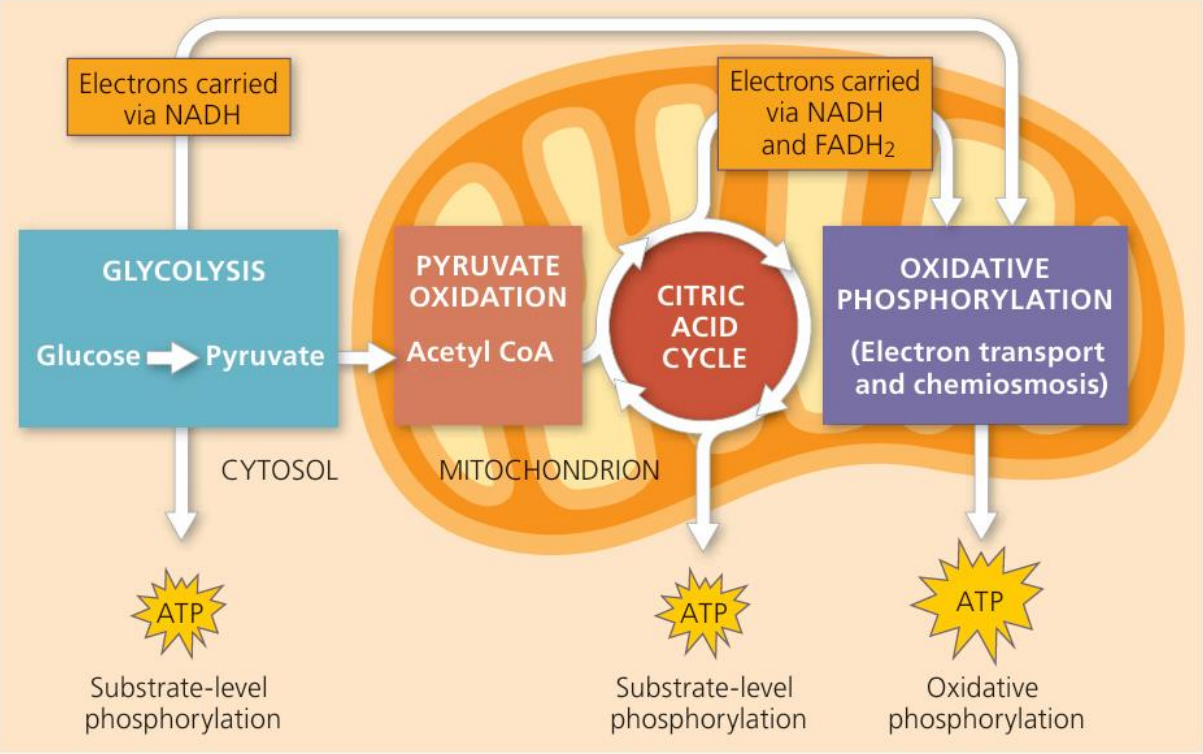


Figure 1 showing the processes involved in cellular respiration in the mitochondria

RESPIRATION

This is a process by which organic food materials are broken down in a cell to release energy in the form of ATP.

The process involves a series of enzyme controlled reactions in which an organic molecule is broken down through a series of redox reactions. The organic food materials are oxidized to carbon dioxide.

The energy used to make ATP (Adenosine triphosphate) is unlocked from the chemical energy in the organic molecules during oxidation. The chemical energy is in each of the C-H bonds of the organic molecules, thus the more the number of C-H bonds, the more the energy that can be obtained from an organic molecule. (Size of organic molecule).

ULTRASTRUCTURE OF A MITOCHONDRION AND ITS STRUCTURAL RELATIONSHIP TO ITS FUNCTIONS

Mitochondria are the **powerhouses of the cell**. They are unique organelles present in almost all eukaryotic cells that are responsible for generating the cell's supply of adenosine triphosphate (ATP), the energy currency of the cell.

Carl Benda coined the word "mitochondrion" in 1898.The term "mitochondrion" comes from the Greek words "**mitos**," meaning **thread**, and "**chondrion**," meaning **granule**. This term reflects their thread-like or granular appearance under the microscope. The plural of "mitochondrion" is "mitochondria."

Structure of a Mitochondrion

Mitochondria have a unique structure which is key to their function:

- **Outer Membrane**: This smooth membrane encloses the entire organelle and is permeable to ions and small molecules. It is similar in composition to the cell's plasma membrane. Integral membrane proteins called porins allow for transport between the mitochondrion and the cell's cytosol.
- **Inner Membrane**: The inner membrane is highly convoluted, forming folds known as cristae. This membrane contains proteins involved in the electron transport chain and ATP synthesis. The cristae greatly increase the surface area of the inner membrane, allowing for more ATP production.

- **Intermembrane Space:** The intermembrane space is the region between the inner and outer membranes. Its fluid composition resembles the cell's cytosol in terms of ions and sugars. However, cytochrome c and certain other large proteins also occur here.
- **Matrix**: The matrix is innermost compartment, which contains enzymes, mitochondrial DNA, and ribosomes. Essentially, it is the mitochondrial analog to the cell's cytoplasm. Enzymes in the matrix play roles in the citric acid cycle and oxidation of fatty acids and pyruvate.
- **Mitochondrial Ribosomes:** Mitochondrial ribosomes are smaller than their cytoplasmic counterparts are usually 55S to 70S, depending on the species. The 'S' here stands for Svedberg units, a measure of sedimentation rate during ultracentrifugation. Although both types of ribosomes perform protein synthesis, mitochondrial ribosomes specialize in synthesizing proteins for use within the mitochondria. These proteins are often integral to mitochondrial functions, such as oxidative phosphorylation.

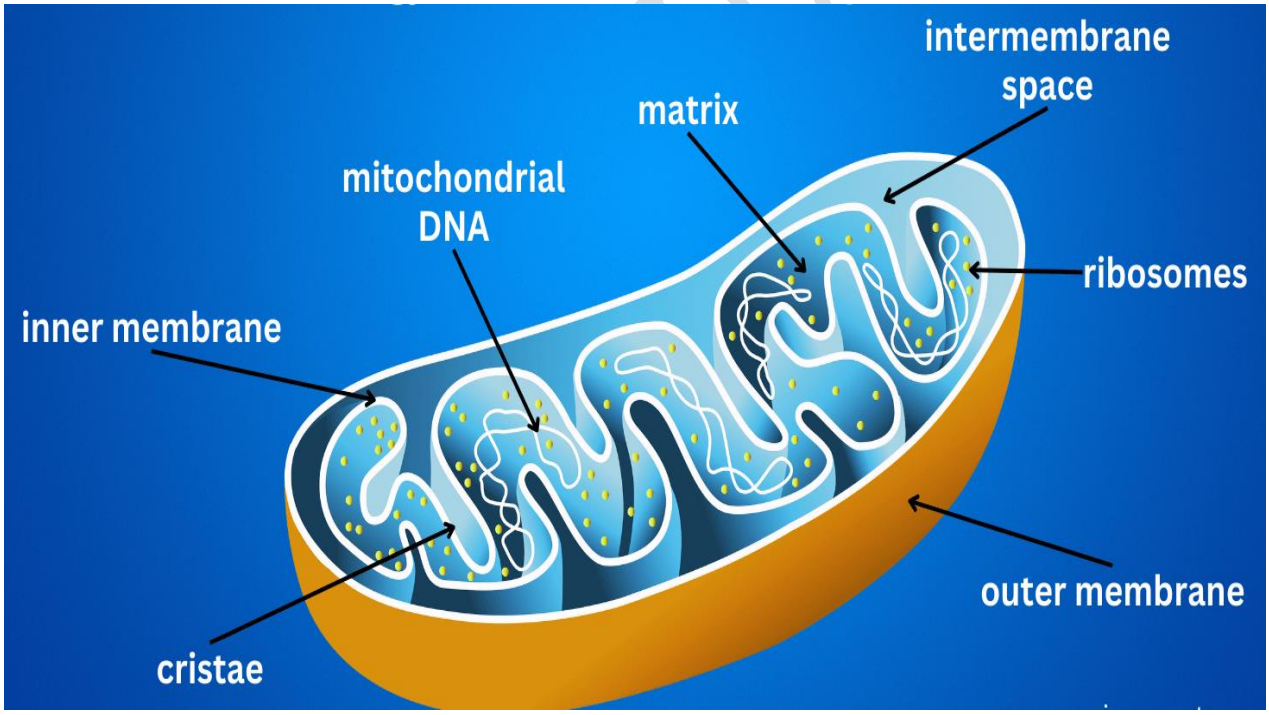


Figure 2 showing the ultra-structure of the mitochondria

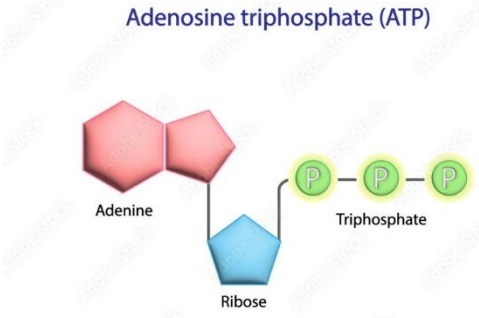
The structure of mitochondria makes them well **adapted to their function:**

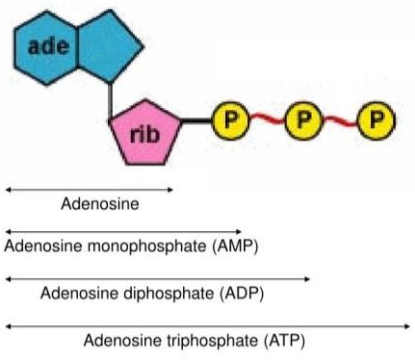
- They have a **double membrane** and a **small volume of intermembrane space**; this means that this space can be used for the concentration buildup of hydrogen ions required for respiration reactions.
- They have a **large surface area** due to the presence of **cristae** (inner folds) which enables the membrane to hold many electron transport chain proteins and ATP synthase enzymes
- More active cell types can have larger mitochondria with longer and more tightly packed cristae to enable the **synthesis of more ATP** because they have a **larger surface area.**
- The **number** of mitochondria in each cell can vary depending on cell activity
- Muscle cells are more active and have more mitochondria per cell than fat cells
- **Compartmentalization of enzymes and substrates** using the matrix ensures that respiration reactions, like the Krebs cycle, can happen more efficiently

THE DETAILED STRUCTURE AND ENERGY RELEASE FROM ATP

Structure of ATP

ATP is a molecule made up of a nitrogen base; **adenine**, a **ribose sugar** and three **phosphate groups**. Adenine is attached to carbon 1 of ribose sugar while the chain of phosphate groups is attached to carbon 5 of ribose.





The structure of ATP

ATP is a temporary energy store compound which releases energy when the bond between the phosphate groups is broken during hydrolysis. Due to its synthesis in all living organisms, ATP is referred to as a **universal energy currency.**

Energy Release from ATP

Energy is released from ATP by breaking the bond between the second and third phosphate groups. This process is called **hydrolysis**.

The reaction is: $ATP + H_2O \rightarrow ADP + P_i + Energy$

Where:

- ADP = Adenosine Diphosphate
- P_i = Inorganic phosphate
- About 30.5 kJ/mol of energy is released.

This energy is used immediately by cells for biological work.

More energy is obtained from the hydrolysis of ATP to ADP and ADP to AMP than from hydrolysis of AMP. This explains why hydrolysis of AMP to release energy isn't feasible.

ATP is formed by a process called **phosphorylation** in which a phosphate is added to ADP.

ATP is preferred to other high energy compounds to provide energy for cell metabolism because;

- ✓ Provides the right amount of energy for cellular needs when hydrolyzed
- ✓ ATP can be moved to any place when need arises
- ✓ Is easily hydrolyzed to provide energy at the right time.

Note:

- ATP can't be stored for long and is thus continually hydrolyzed and regenerated.
- Brain cells only have a few minutes supply of ATP and thus must be continuously supplied with oxygen to regenerate it.
- Muscle cells however store creatine phosphate which is a source of phosphate for rapid regeneration of ATP.

Assignment one: research and write down the importances of energy released from ATP.

There are three types of phosphorylation;

i. ***Substrate level phosphorylation:*** this involves the transfer of phosphate group directly from a high energy compound to ADP to form ATP. Examples of high energy compounds include 1, 3bisphosphoglycerate, creatine-phosphate.

ii. ***Oxidative phosphorylation:*** This the process of ATP synthesis using energy from oxidation of compounds such as NADH and $FADH_2$

iii. ***Photophosphorylation:*** this is the process by which ATP synthesis takes place in a cell using energy from light. E.g. during photosynthesis.

Analyse the biochemical processes leading to ATP production in living organisms, and how these processes are affected by physical activities and respiratory poisons (cyanide).

- ✓ The process of glycolysis, focusing on its stages, key molecules involved, and its significance in cellular respiration.
- ✓ The role of acetyl coenzyme A (Acetyl-CoA) in the metabolism of carbohydrates, lipids, and proteins.
- ✓ The citric acid cycle, focusing on key steps (substrate-level phosphorylation, decarboxylation, and the production of NADH and $FADH_2$).
- ✓ The fate of Acetyl-CoA, NAD, and FAD.
- ✓ Cyanide effect on the electron transport system
- ✓ The exercise intensity on ATP production.

The process of glycolysis and its significance in cellular respiration

This is a series of enzyme controlled reactions that involve the splitting of a single glucose molecule to form two molecules of pyruvate with release of 2ATP molecules. The pyruvate formed is a three carbon sugar compound.

Or

Glycolysis is the **breakdown of glucose** to form energy for cellular processes.

Glycolysis occurs in the **cytoplasm**.

Red blood cells and corneal cells completely **lack mitochondria**; other cells, such as retinal, kidney medulla, leukocytes, and testicular cells, have few. Therefore, glycolysis is the only way to produce **ATP** in these tissues.

Sources of glucose

Glucose is required for the process of glycolysis and can be sourced from three places:

1**.** Diet: absorbed via the gut and delivered through the portal vein to the liver

2**.** Glycogenolysis**:** glycogen is essentially a storage form of glucose, and can be broken down to release glucose.

3**.** Gluconeogenesis: the liver (and kidneys) can make glucose from non-carbohydrate sources like amino acids, glycerol, and lactate.

DESCRIPTION OF GLYCOLYSIS

Glycolysis can be divided into two phases: the energy investment phase and the energy payoff phase. During the energy investment phase, the cell actually spends ATP. This investment is repaid with interest during the energy payoff phase, when ATP is produced by substrate-level phosphorylation and NAD+ is reduced to NADH by electrons released from the oxidation of glucose.

- Glucose is phosphorylated to form glucose-6-phosphate **to raise its energy level** and **prevent it from leaving the cell** since large ions can't move across the cell membrane. Reaction is catalyzed by the enzyme hexokinase.
- The glucose-6-phosphate is isomerized to form fructose-6-phosphate, the reaction catalyzed by ***phosphoglucoisomerase enzyme*****.**
- Fructose-6-phosphate is phosphorylated to form fructose-1, 6bisphophate. Reaction catalyzed by enzyme ***phosphofructokinase*****.**
- The fructose-1, 6-bisphosphate is unstable and split to form two 3 carbon compounds which are isomers; **dihydroxyacetone phosphate** and **glyceraldehyde-3-phosphate**. Reaction is catalyzed by enzyme ***aldolase***.

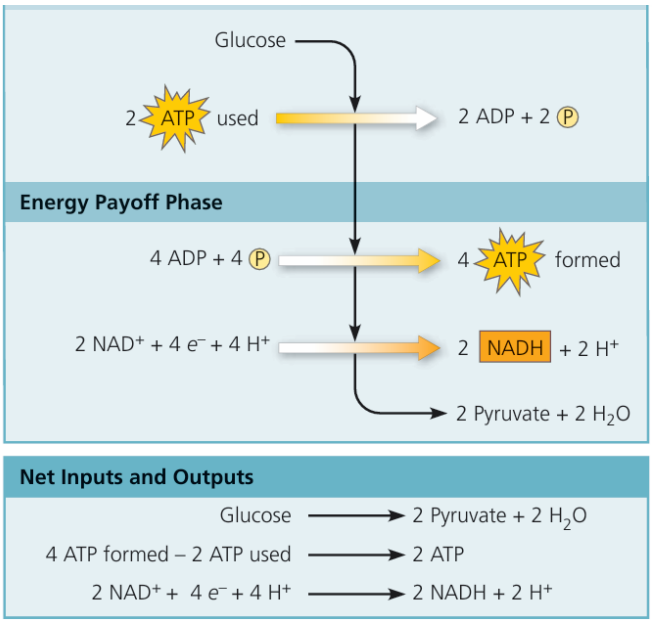
- Dihydroxyacetone phosphate is isomerized to glyceraldehyde-3phosphate. The reaction is catalyzed by enzyme ***triose phosphate isomerase***.

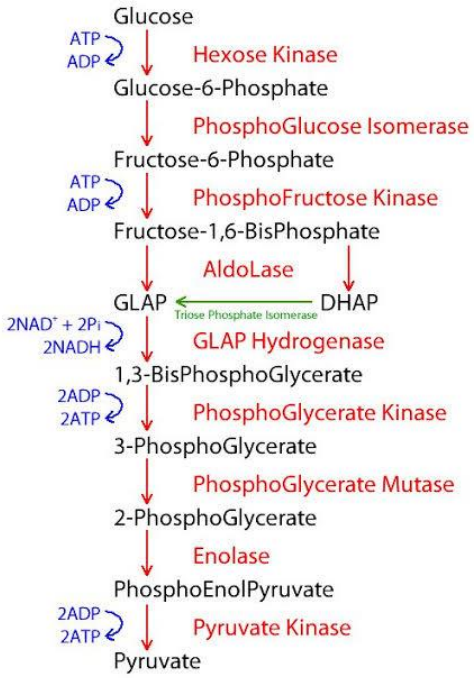
Thus, each glucose molecule is cleaved to form 2 molecules of three carbon compound glyceraldehyde-3-phosphate.

- Each of the glyceraldehyde-3-phosphate is oxidized by oxidized Nicotinamide adenine dinucleotide (NAD^+) and then phosphorylated to increase its potential energy to form **1,3-bisphosphoglycerate** and the reaction is catalyzed by ***glyceraldehyde-3-phospahte dehydrogenase.*** Two molecules of reduced nicotinamide adenine dinucleotide (**NADH)** are formed.
- Each molecule of 1, 3-bisphosphoglycerate is used to from an ATP molecule during **substrate level phosphorylation** of ADP. Two molecules of 3-phosphoglycerate (PGA) are formed. Reaction is catalyzed by ***phosphoglycerokinase*** enzyme.
- Each of the PGA molecules isomerizes to form **2-phosphoglycerate.** The molecules now lose a water molecule under catalysis of enzyme enolase to form 2 molecules of phosphoenolpyruvate (PEP).
- A phosphate group is transferred from each of the PEP molecules to ADP, forming 2 molecules of ATP, during substrate level phosphorylation.
- Two molecules of pyruvate are formed. Reaction is catalyzed by pyruvate kinase enzyme.
- Thus from each molecule of glucose, 2ATP molecules are invested and 4 molecules of ATP are formed during substrate level phosphorylation and 2 molecules of (NADH)
- The net gain in ATP during glycolysis for one glucose molecule is thus 2ATPs.

All of the carbon originally present in glucose is accounted for in the two molecules of pyruvate, no carbon is released as CO_2 during glycolysis.

Glycolysis occurs **whether or not oxygen** is present. However, if oxygen is present, the chemical energy stored in pyruvate and NADH can be extracted by pyruvate oxidation, the citric acid cycle, and oxidative phosphorylation.





SUMMARY OF GLYCOLYSIS

SIGNIFICANCE OF GLYCOLYSIS

- ✓ Formation of ATP used to power cell activities
- ✓ Formation of NADH from which more energy is extracted during the electron transport system
- ✓ Formation of pyruvate from which more energy can be extracted either in aerobic conditions or anaerobic conditions during Krebs cycle.

In summary, for one molecule of glucose, glycolysis produces:

1. **Pyruvate**: two pyruvate molecules, which are utilized in either aerobic or anaerobic conditions

2. **ATP**: glycolysis **uses two ATP** and **produces four ATP**, resulting in a **net gain of two ATP** per glucose molecule

3. **NADH**: one NADH is formed from NAD^+. This high-energy electron carrier can later be utilized by the ETC for further ATP generation.

Fate of pyruvate

Glycolysis produces **two pyruvate molecules** per glucose molecule. The fate of pyruvate is dependent on the availability of **oxygen**:

Aerobic conditions: pyruvate enters the mitochondria and is utilized within the citric acid cycle to produce NADH, which then enters the electron transport chain to produce the maximum ATP

Anaerobic conditions: pyruvate is converted to **lactate**, which enters the blood, and is used in the **Cori cycle** (lactate shuttle) within the liver to produce a small amount of ATP.

Glycolysis releases less than a quarter of the chemical energy in glucose that can be harvested by cells most of the energy remains stockpiled in the two molecules of pyruvate. When Oxygen is present, the pyruvate in eukaryotic cells enters a mitochondrion, where the oxidation of glucose is completed.

LINK REACTION

Mitochondrial matrix and it only occurs only under aerobic conditions (oxygen must be available).

Upon entering the mitochondrion via active transport, the **pyruvate** is *decarboxylated* and then *oxidized* to form **acetate**. This step, linking glycolysis and the citric acid cycle, is carried out by a multi-enzyme complex that catalyzes three reactions.

The first step is the one in which carbondioxide is released during decarboxylation.

the remaining two-carbon fragments are oxidized and the electrons transferred to NAD+, storing energy in the form of NADH.

Finally, coenzyme A (CoA), a sulfur-containing compound derived from a B vitamin, is attached via its sulfur atom to the two-carbon intermediate, forming acetyl CoA.

Acetyl CoA has a high potential energy, which is used to transfer the acetyl group to a molecule in the citric acid cycle, a reaction that is therefore highly exergonic.

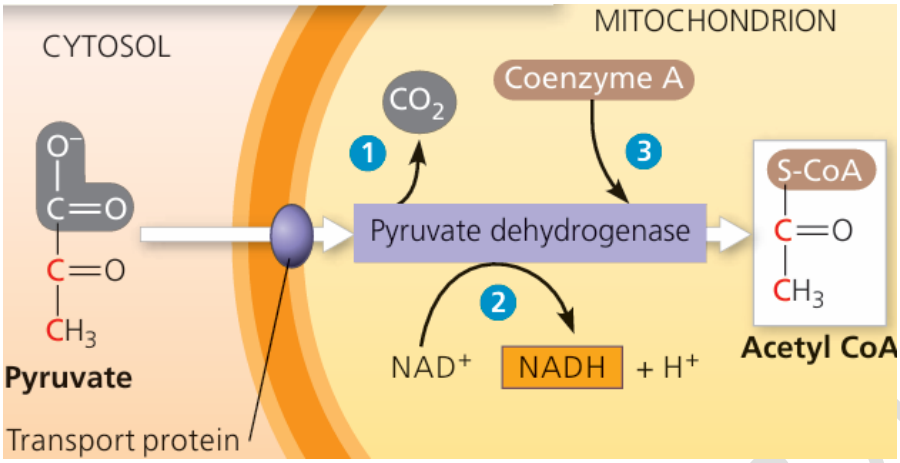


Figure 3 showing a summary of Link's reaction

THE ROLE OF ACETYL COENZYME A (ACETYL-COA) IN THE METABOLISM OF CARBOHYDRATES, LIPIDS, AND PROTEINS

Acetyl-CoA (acetyl coenzyme A) is a central molecule in cellular metabolism.

It's the starting point for the Krebs cycle (also called the citric acid cycle or TCA cycle).

Role of coenzyme A

- Activates acetate so that more energy can be obtained from it
- Transfers the acetyl group formed from pyruvate to combine with 4C compound oxaloacetate, forming 6C compound citrate. This reaction is catalysed by the enzyme citrate synthatase.
- Provides a pathway by which fatty acids and proteins can be used as respiratory substrates via a central link of acetyl coenzyme A.

The fatty acids

Fatty acids are broken down in the mitochondria through **beta-oxidation**, producing multiple **acetyl CoA** molecules. These acetyl CoA units enter the **citric acid cycle** to generate ATP, especially during fasting or low-carb states. This process is highly efficient and that's why fats yield more energy per gram than carbohydrates.

Lipogenesis: When energy is abundant, **excess acetyl CoA** (from carbs or proteins) is used to build **fatty acids**. In the cytoplasm, acetyl CoA is converted to **malonyl CoA** by **acetyl CoA**

carboxylase, then elongated into fatty acid chains. These fatty acids are stored as **triglycerides** in adipose tissue.

KREB'S CYCLE

The citric acid cycle functions as a metabolic furnace that further oxidizes organic fuel derived from pyruvate.

Pyruvate is broken down to three CO_2 molecules. The cycle generates 1 ATP per turn by **substrate-level phosphorylation**, but most of the chemical energy is transferred to NAD+ and FAD during the redox reactions. The reduced coenzymes, NADH and FADH2, shuttle their cargo of high-energy electrons into the electron transport chain. The citric acid cycle is also called the **tricarboxylic acid cycle** due to three carboxyl groups on its first two intermediates, or the **Krebs cycle**, the latter honoring Hans Krebs. Krebs was the German-British scientist largely responsible for working out the pathway in the 1930s. In eukaryotic cells, it occurs in the **matrix** of the mitochondrion as it happens in the conversion of pyruvate to acetyl CoA. In prokaryotes, it happens in the cytoplasm. Both processes occur in the presence of oxygen.

The process oxidizes glucose derivatives, fatty acids and amino acids to carbon dioxide (CO2) through a series of enzyme controlled steps. The purpose of the Krebs Cycle is to collect (eight) high-energy electrons from these fuels by oxidizing them, which are transported by activated carriers NADH and FADH2 to the electron transport chain. The Krebs Cycle is also the source for the precursors of many other molecules, and is therefore an amphibolic pathway (meaning it is both anabolic and catabolic).

- In the Krebs cycle, acetyl CoA reacts with oxaloacetate a 4C compound to form **citrate**, a 6C compound.
- Coenzyme A is reformed and may be used to combine with another acetate molecule from pyruvate.
- Citrate isomerises to form **isocitrate**, a more reactive molecule by addition and removal of a water molecule.

- The isocitrate is oxidized by NAD^+ to NADH and then decarboxylated by loss of carbon dioxide to form **α-ketoglutarate**.
- The α-ketoglutarate loses a carbon dioxide molecule and oxidized by (NAD^+), the remaining product reacts with coenzyme A to form a 4C compound, **succinyl CoA**, which is unstable. The CoA is displaced, ATP is formed and a more stable 4C compound, **succinate** forms.
- The succinate is oxidized by removal of two hydrogen atoms by (Flavine adenine dinucleotide) FAD, to form $FADH_2$. **Fumarate** is formed.
- Addition of a water molecule to the fumarate results into formation of **malate**, a 4C compound.
- Malate is oxidized by NAD^+ to regenerate **oxaloacetate**. (NADH) is also formed.

An easy way to memorize the names of the compounds taking part in the Krebs cycle is through these sentences: (Citrate Is Krebs Starting Substrate For Making Oxaloacetate)

Thus one turn of the cycle produces:

- CO_2
- 3 NADH
- $FADH_2$
- GTP

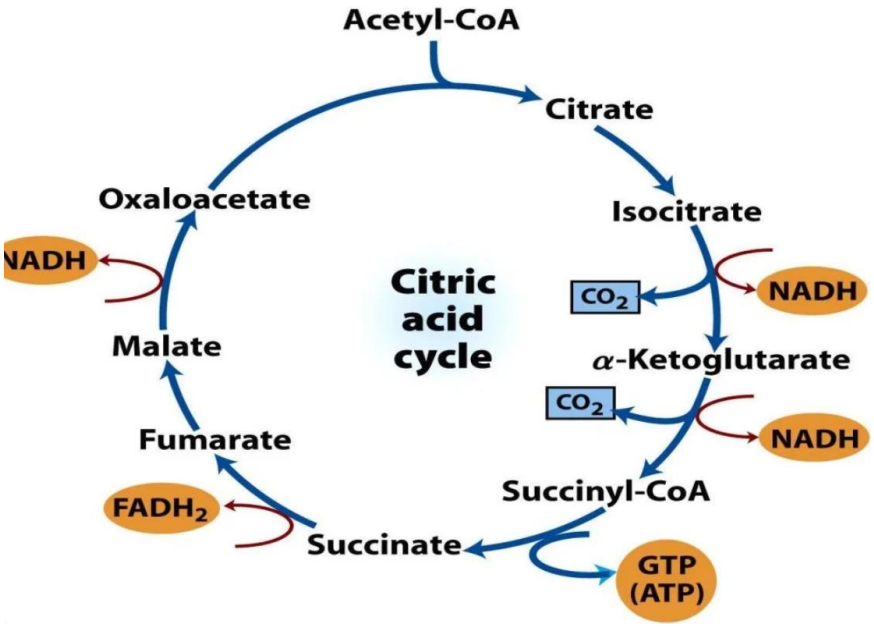


Figure 4 summary of the Krebs cycle

THE PATHWAY OF ELECTRON TRANSPORT/ ELECTRON TRANSPORT CHAIN

American biochemist, Albert Lehninger, discovered the electron-transport chain in 1961. The complete ETC was found to have four membrane-bound complexes named complex I, II, III, and IV and two mobile electron carriers, namely coenzyme Q and cytochrome c.

The electron transport chain has two essential functions in the cell:

- Regeneration of electron carriers: Reduced electron carriers NADH and $FADH_2$ pass their electrons to the chain, turning them back into NAD^+ and FAD. This function is vital because the oxidized forms are reused in glycolysis and the citric acid cycle (Krebs cycle) during cellular respiration.
- Generating proton gradient: The transport of electron through the chain results in a gradient of a proton across the inner membrane of mitochondria, later used in ATP synthesis.

The electron transport chain is a collection of molecules embedded in the inner membrane of the mitochondrion in eukaryotic cells. The folding of the inner membrane to form cristae increases its surface area, providing space for thousands of copies of each component of the

electron transport chain in a mitochondrion. The in-folded membrane with its concentration of electron carrier molecules is well-suited for the series of sequential redox reactions that take place along the electron transport chain. Most components of the chain are proteins, which exist in multi-protein complexes numbered I through IV. Tightly bound to these proteins are prosthetic groups, non-protein components such as cofactors and coenzymes essential for the catalytic functions of certain enzymes.

How does the Electron transport system work?

- Electrons removed from glucose during glycolysis and from Krebs cycle by coenzyme (NAD^+) are transported by NADH to complex one, at a higher energy level. The electron carrier is thus reduced.
- The electrons are passed onto ubiquinone (cytochrome Q) which is not a protein, reducing it.
- Ubiquinone becomes oxidized when it passes on its electrons to complex III which is itself oxidized.
- Electrons are passed on successively down a series of cytochromes during a series of redox reactions, from cytochrome C_1, cytochrome C, cytochrome a, with the last one in protein complex IV being cytochrome a_3.
- The final electron acceptor, which more electronegative than all the electron carriers is oxygen and it receives the electron and combines with hydrogen to form water.
- Another source of electrons from the Krebs cycle is $FADH_2$, However, since it joins the electron transport chain at a lower energy level than NADH i.e. at protein complex II, it yields a third less energy than NADH

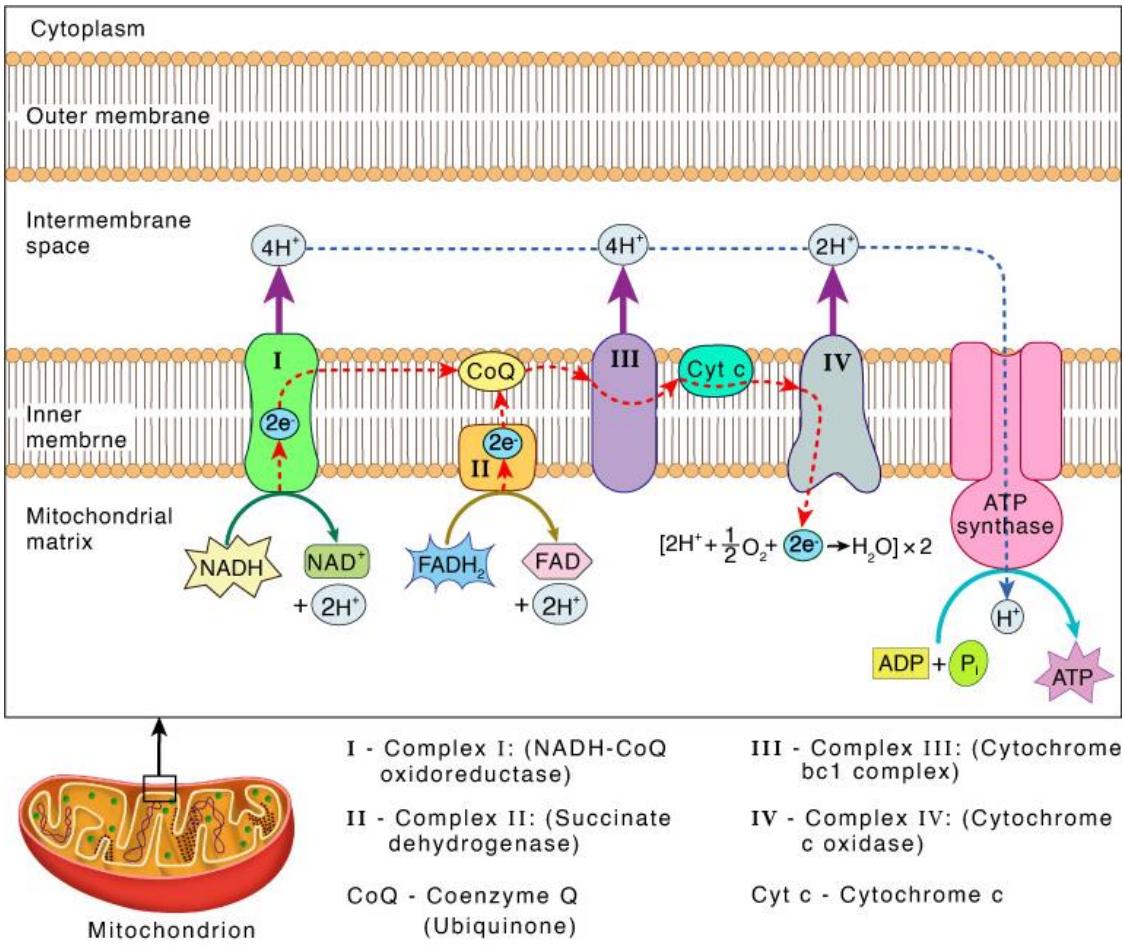


Figure 5 the electron transport system

CHEMIOSMOSIS

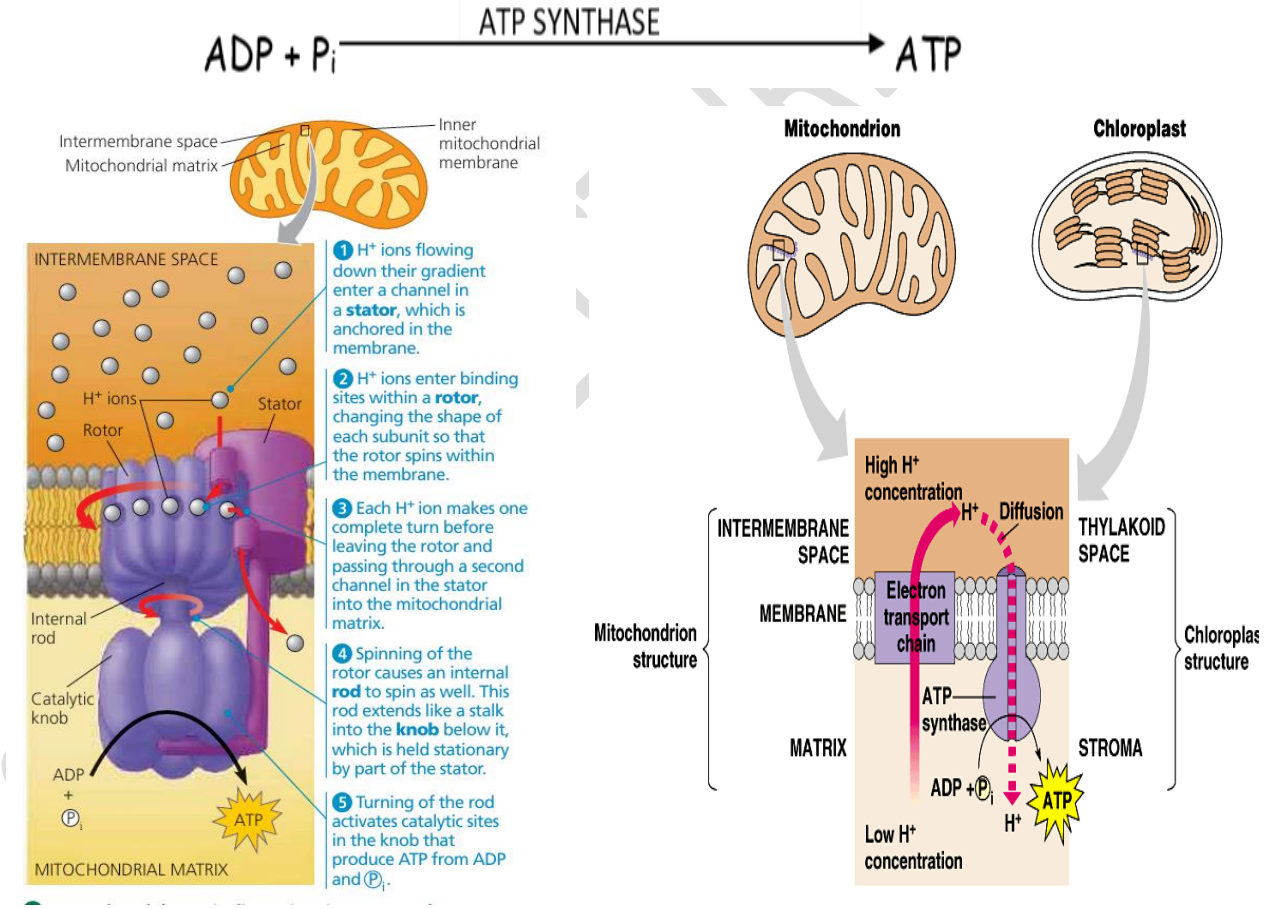
This is the process by which a hydrogen ion gradient generated across the inner mitochondrial membrane is used to provide energy for ATP synthesis.

ATP synthesis during the electron transport chain is by oxidative phosphorylation since the hydrogen ion gradient across the membrane couples oxidation in the ETS to ATP synthesis.

DESCRIPTION OF CHEMIOSMOSIS

- As electrons flow from one electron carrier to the next, energy is generated which is used to pump protons from the matrix of the mitochondrion into the intermembrane space.

- Protons accumulate such that a steep proton gradient develops between the intermembrane space and the matrix.
- The cristae is generally impermeable to protons.
- protons can only move down their electrochemical gradient through stalked granules embedded in the membrane which are basically enzymes i.e ATP synthase
- The energy generated due to flow of protons down their electro-chemical gradient is used by the enzyme at its bulbous end to synthesize ATP from ADP and inorganic phosphate.



NUMBER OF ATP MOLECULES GENERATED FROM ONE GLUCOSE MOLECULE DURING CHEMIOSMOSIS

- One ATP molecule is generated from each proton activated in the electron transport chain.
- Electrons from NADH activate 3 proton channels, 3ATP molecules are synthesized.
- Electrons from $FADH_2$ activate 2 proton channels thus only 2ATPs are synthesized.

An account of ATPs from ETS per glucose molecule

SOURCE OF ELECTRONS	NUMBER OF ELECTRON CARRIERS	NUMBER OF ATPS FORMED
GLYCOLYSIS	2 NADH	6
KREBS CYCLE	2 NADH	4
	6 NADH	18
PYRUVATE DECARBOXYLASE	2 NADH	6
TOTAL		34

Assignment 2: From your knowledge of the above processes describe the fate of Acetyl-CoA, NAD, and FAD.

CYANIDE EFFECT ON THE ELECTRON TRANSPORT SYSTEM

Cyanide is a highly toxic substance that can cause severe harm to the human body. It is a potent inhibitor of cellular respiration, which is the process by which cells produce energy in the form of adenosine triphosphate (ATP). Cyanide binds to the enzyme **cytochrome c oxidase**, which is a crucial component of the electron transport chain in mitochondria. This binding prevents the transfer of electrons to oxygen, the final electron acceptor, and thereby blocks the production of ATP.

When cyanide enters the body, it rapidly diffuses across cell membranes and enters the mitochondria of cells. Once inside the mitochondria, cyanide binds to cytochrome c oxidase and inhibits its activity. This results in a decrease in the production of ATP, which is essential for the normal functioning of cells. As a consequence, the affected cells begin to switch to anaerobic respiration, a process that does not require oxygen, but is much less efficient in producing ATP.

- Cyanide, a highly toxic chemical compound, interferes with the electron transport chain, a crucial process in cellular respiration. Specifically, cyanide inhibits the activity of cytochrome c oxidase, a key enzyme in the electron transport chain that facilitates the transfer of electrons from cytochrome c to molecular oxygen by binding to the Fe^{3+} inhibiting oxidative phosphorylation. This process is essential for the production of ATP, which is the main source of energy for cellular activities.

- The inhibition of cytochrome c oxidase by cyanide leads to a halt in the flow of electrons in the electron transport chain, resulting in the accumulation of electrons and the depletion of oxygen in the cell. As a consequence, the cell is unable to produce ATP through oxidative phosphorylation and instead, switches to anaerobic respiration, which produces lactic acid as a byproduct.

- Furthermore, the accumulation of electrons in the electron transport chain can lead to the formation of reactive oxygen species (ROS), which can cause oxidative damage to cellular components and further impair cellular function.

Cyanide disrupts the flow of electrons in cellular respiration by inhibiting cytochrome c oxidase, leading to cellular hypoxia, lactic acidosis, and ROS formation.

Natural Sources of Cyanide

Several plants are known to produce cyanogenic compounds that can release cyanide when ingested or processed improperly. Some of these include:

Bitter Almonds: These contain amygdalin, which converts to cyanide when metabolized.

Cassava: A staple food in many tropical regions, cassava must be properly processed to reduce its cyanogenic content.

Cherry Pits: The seeds of cherries also contain amygdalin.

These natural sources illustrate how easily cyanide can enter the food chain if not handled with care. Ingesting large amounts of these foods without proper preparation can lead to poisoning.

Cyanide production in industrial settings

In industry, cyanide is produced through various chemical reactions. One common method involves the reaction of sodium carbonate with ammonia and methane at high temperatures. This process yields sodium cyanide (NaCN), which is then used in several applications:

Gold Mining: Cyanide leaching is a widely used method for extracting gold from ore.

Electroplating: It serves as an important component in electroplating processes for metals.

Chemical Synthesis: Cyanides are precursors for producing numerous chemicals.

The production methods are tightly regulated due to the toxicity of the compound involved. Industries must adhere to strict guidelines to minimize environmental contamination and ensure worker safety.

Symptoms of cyanide poisoning

- ✓ Headache
- ✓ Dizziness
- ✓ Shortness of breath
- ✓ Confusion
- ✓ Loss of consciousness