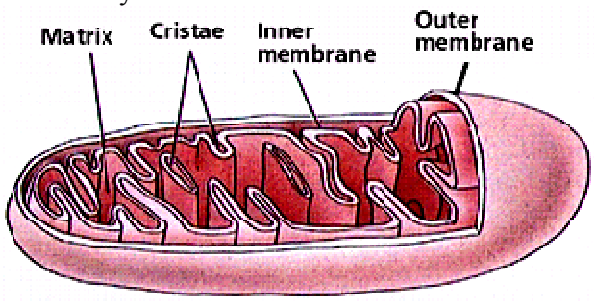
Mitochondrial Physiology

Mitochondria contain their own DNA (termed mDNA) and are thought to represent bacteria-like organisms incorporated into eukaryotic cells over 700 million years ago (perhaps even as far back as 1.5 billion years ago). They function as the **sites of energy release** (following **glycolysis** in the cytoplasm) and ATP formation (by **chemiosmosis**). The mitochondrion has been termed the powerhouse of the cell. Mitochondria are bounded by two membranes.

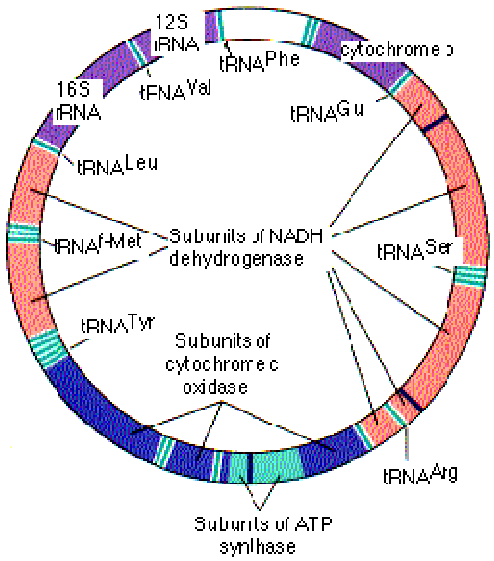


Structure of a mitochondrion

Mitochondria are membrane-enclosed organelles distributed through the cytosol of most eukaryotic cells. Their main function is the conversion of the potential energy of food molecules into ATP. Mitochondria have an **outer membrane** that encloses the entire structure and an **inner membrane** that encloses a fluid-filled **matrix** between the two is the **intermembrane space**. The inner membrane is elaborately folded with shelflike **cristae** projecting into the matrix and a small number (some 5–10) circular molecules of **DNA.** The outer membrane contains many complexes of integral membrane proteins that form channels through which a variety of molecules and ions move in and out of the mitochondrion. The inner membrane contains 5 complexes of integral membrane proteins **NADH dehydrogenase,** succinate dehydrogenase, **cytochrome c reductase**(also known as the cytochrome b-c_1 complex), **cytochrome c oxidase** and **ATP synthase.** The matrix contains a complex mixture of soluble enzymes that catalyze the respiration of pyruvic acid and other small organic molecules. Here pyruvic acid is oxidized by NAD^+ producing NADH + H^+ and decarboxylated producing a molecule of carbon dioxide (CO_2) and a 2-carbon fragment of acetate bound to coenzyme A forming acetyl-CoA

Mitochondrial DNA (mtDNA)

The human mitochondrion contains 5–10 identical, **circular molecules** of DNA. Each consists of 16,569 base pairs carrying the information for **37 genes** which encode 2 different molecules of **ribosomal RNA** (**rRNA**), 22 different molecules of **transfer RNA** (**tRNA**) (at least one for each amino acid) and **13 polypeptides.** The **rRNA** and **tRNA** molecules are used in the machinery that synthesizes the 13 polypeptides.



Structure of mitochondrial DNA (mDNA)

The 13 polypeptides are subunits of the protein complexes in the inner mitochondrial membrane, including subunits of **NADH dehydrogenase**, **cytochrome c oxidase**, and **ATP synthase**. However, each of these protein complexes also requires subunits that are encoded by nuclear genes, synthesized in the cytosol, and imported from the cytosol into the mitochondrion.

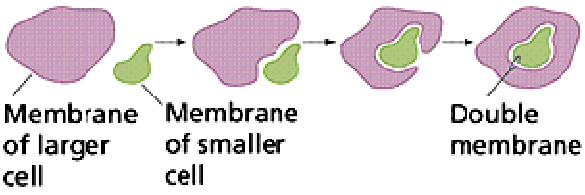
A number of human diseases are caused by mutations in genes in our mitochondria: **cytochrome b**, **12S rRNA, ATP synthase, subunits of NADH dehydrogenase** and several **tRNA** genes. Although many different organs may be affected, disorders of the brain and muscles are the most common. Perhaps this reflects the great demand for energy of both these organs. Some of these disorders are inherited in the germ line. In every case, the mutant gene is received from the mother because none of the mitochondria in sperm survives in the fertilized egg. Other disorders are somatic; that is, the mutation occurs in the somatic tissues of the individual. A number of humans who suffer from easily-fatigued muscles turn out to have a mutations in their **cytochrome b** gene. Curiously, only the mitochondria in their muscles have the mutation; the mtDNA of their other tissues is normal. Presumably, very early in their embryonic development, a mutation occurred in the cytochrome b gene in a cell destined to produce their muscles.

Many of the features of the mitochondrial genetic system resemble those found in **prokaryotes** like bacteria. This has strengthened the theory that mitochondria are the evolutionary descendants of a prokaryote that established an **endosymbiotic** relationship with the ancestors of eukaryotic cells early in the history of life on earth. However, many of the genes needed for mitochondrial function have since moved to the nuclear genome. The recent sequencing of the complete genome of **Rickettsia prowazekii** has revealed a number of genes closely related to those found in mitochondria. Perhaps rickettsias are the closest living descendants of the endosymbionts that became the mitochondria of eukaryotes.

Endosymbiosis and the Origin of Eukaryotes

The endosymbiosis theory postulates that the **mitochondria** of eukaryotes evolved from aerobic bacteria (probably related to the **rickettsias**) living within their host cell. The **chloroplasts** of eukaryotes evolved from endosymbiotic **cyanobacteria** (autotrophic

prokaryotes). Eukaryotic **cilia** and flagella may have arisen from endosymbiotic spirochetes. The basal bodies from which eukaryotic cilia and flagella develop would have been able to create the mitotic spindle and thus made mitosis possible.



The basic events in endosymbiosis

There are several evidences supporting the theory of endosymboisis for both mitochondria and chloroplasts.. Both mitochondria and chloroplasts can arise only from preexisting mitochondria and chloroplasts. They cannot be formed in a cell that lacks them because nuclear genes encode only some of the proteins of which they are made. Both mitochondria and chloroplasts have their own **genome** and it resembles that of prokaryotes not that of the nuclear genome. Both genomes consist of a **single circular molecule of DNA**. There are **no histones** associated with the DNA. Both mitochondria and chloroplasts have their own protein-synthesizing machinery, and it more closely resembles that of prokaryotes than that found in the cytoplasm of eukaryotes. The first amino acid of their transcripts is always **fMet** as it is in bacteria (not **methionine** [Met] that is the first amino acid in eukaryotic proteins). A number of antibiotics (e.g., **streptomycin**) that act by blocking protein synthesis in bacteria also block protein synthesis within mitochondria and chloroplasts. They do not interfere with protein synthesis in the cytoplasm of the eukaryotes. Conversely, inhibitors (e.g., **diphtheria toxin**) of protein synthesis by eukaryotic ribosomes do not have any effect on bacterial protein synthesis nor on protein synthesis within mitochondria and chloroplasts. The antibiotic **rifampicin**, which inhibits the RNA polymerase of bacteria, also inhibits the RNA polymerase within mitochondria. It has no such effect on the RNA polymerase within the eukaryotic nucleus.

Metabolism

The **metabolism** of a person is the sum of all chemical reactions in which energy is made available and consumed in the body. The bindings between hydrogen and carbon in nutrients are a source of energy for animals. Such substances are changed into **metabolic end products** (eliminated as bilirubin, urobilin, urea, uric acid, creatinine etc.) and to **metabolic intermediary products** (i.e., products that participate in other chemical reactions). The **net metabolism** is the sum stochiometry of the single net reactions in the body.

The **oxidation** of fuel (carbohydrates, glycerol, fatty acids) to CO_2 and water is the primary pathway for generation of energy and subsequent heat energy liberation. Protein can also serve as an important energy source during prolonged exercise, but it must first be broken down to amino acids, which are then partially oxidised (to CO_2, water, NH_4^+ etc). During forceful exercise, energy is obtained primarily from **non-oxidative sources** (glycolysis). There is, therefore, a net formation of **lactic acid** from glycogen. Following anaerobic exercise the **lactate elimination** accounts for an extra O_2 consumption called **oxygen debt**.

Most of the chemical reactions in our body are **degradativ***e* or **catabolic** - they break a molecule down to smaller units. These reactions are often also **exothermic** (heat releasing) and **exergoni***c* The **synthetic** or **anabolic** reactions (the formation of protein from amino

acids) are obviously coupled to these degradative reactions. Synthetic reactions are most often also **endothermic** and **endergonic.**

Energy exchange

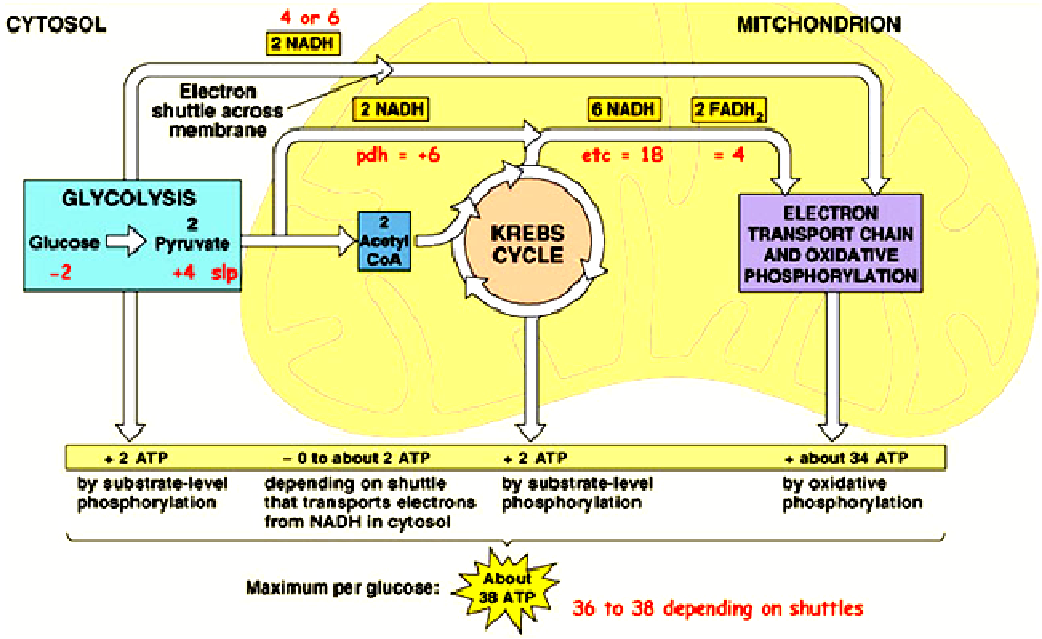
The **first law of thermodynamics** states that energy can neither be created nor destroyed but is **converted** from one form to another or from one place to another. Life is thermodynamically the maintenance of an infinite row of non-equilibrium reactions in such a way that appear to be in a stationary condition, a **steady state***.* Real life is **chaos***,* a steady state only maintained as long as we derive chemical energy from food. Only part of the dietary energy is available for ATP formation in humans. Cellulose, for example, passes the digestive tract without being absorbed. The absorbable chemical energy passes through the intestinal mucosa, and is in the body transformed to energy rich phosphate bindings in **ATP**. ATP is broken down to ADP during muscular contractions. Muscular contractions stimulate the oxidation of fatty acids and carbohydrates in the muscle cells which liberate more energy for rephosphorylation of ADP to ATP. The energy is used for the maintenance of chemical syntheses, electrochemical potentials and for the net-transport of substances across membranes.

The **Gibbs energy** is the free chemical energy available in food. However, 75% is lost as heat energy, and the **mechanical efficiency** of exercise is therefore only 25%. The ratio between **external work (W')** and the total energy used during work ($-\Delta U$) is called the **mechanical efficiency**. In this case ΔU equals ΔG. The **mechanical efficiency** is always less than one and often only 0.25 as stated above. The energy, which is not transferred to external work, is released as heat energy ($-Q$) or is accumulated in the body as heat. At the onset of exercise 50% of the total energy from hydrolysis of ATP is converted into mechanical energy in the myofibrils. The remaining 50% are lost as **initial heat**. This is because energy recapturing recovery processes (oxidative regeneration of ATP etc) occur outside the myofibrils. Hereby, half of the energy is dissipated as so-called **recovery heat.**

Energy sources

The predominant source of energy is **oxidation** of fuel in the mitochondrion. Hereby, high-energy compounds such as **creatine phosphate** and **ATP** are formed. Glucose is oxidised by nicotinamide-adenine-dinucleotide (**NAD^+),** so by **glycolysis** resulting in two **pyruvate** molecules formed in the cytosol, transported to the mitochondrion, and transformed to a **Co-enzyme-A** derivative (acetyl-CoA), which then is involved in theTri-Carboxylic Acid (TCA) or **Krebs cycle**.

Provided a certain oxygen flux from the lungs to the mitochondria is present, the electron **transport chain** (the glycero-phosphate shuttle) will reoxidize ($NADH+H^+$) and $FADH_2$ to NAD^+ and FAD



Pathways of cellular respiration

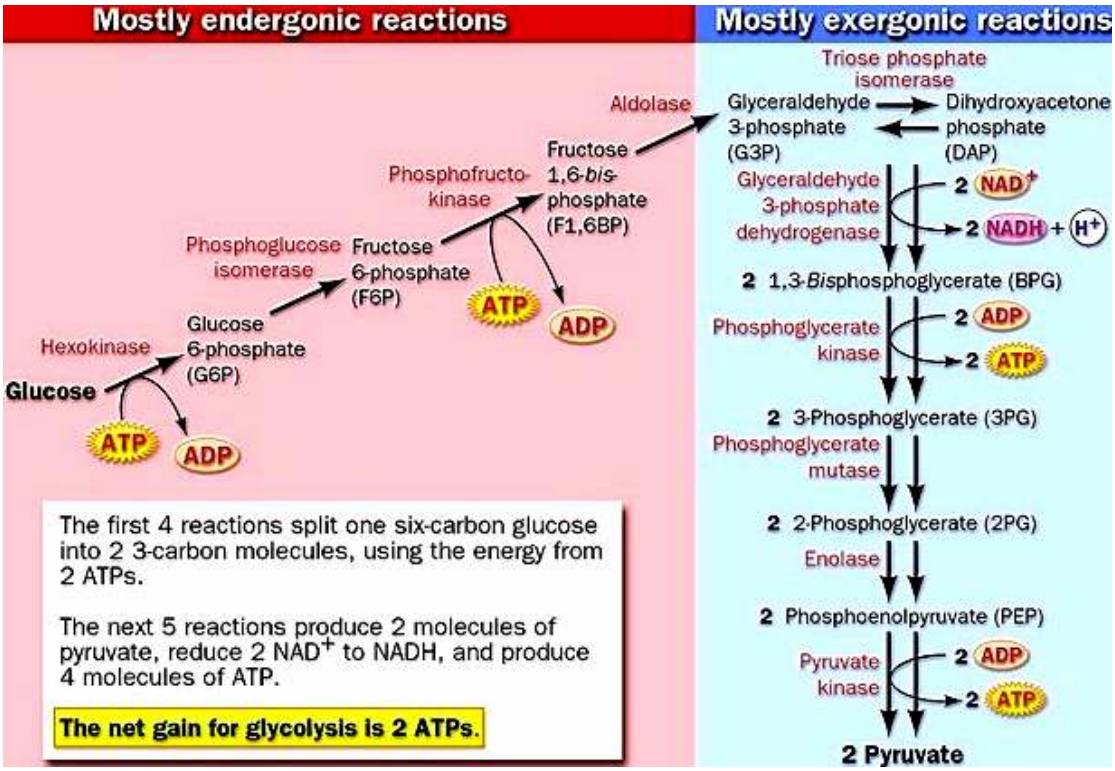
Cellular Respiration

Glycolysis, the Universal Process

Nine reactions, each catalyzed by a specific enzyme, makeup the process we call **glycolysis**. All organisms have glycolysis occurring in their **cytoplasm**.

At steps 1 and 3 ATP is converted into ADP, inputting energy into the reaction as well as attaching a phosphate to the glucose. At steps 6 and 9 ADP is converted into the higher energy ATP. At step 5 NAD^+ is converted into NADH + H^+.

The process works on glucose, a 6-C, until step 4 splits the 6-C into two 3-C compounds. Glyceraldehyde phosphate (GAP, also known as phosphoglyceraldehyde, PGAL) is the more readily used of the two. Dihydroxyacetone phosphate can be converted into GAP by the enzyme Isomerase. The end of the glycolysis process yields two pyruvic acid (3-C) molecules, and a net gain of 2 ATP and two NADH per glucose.

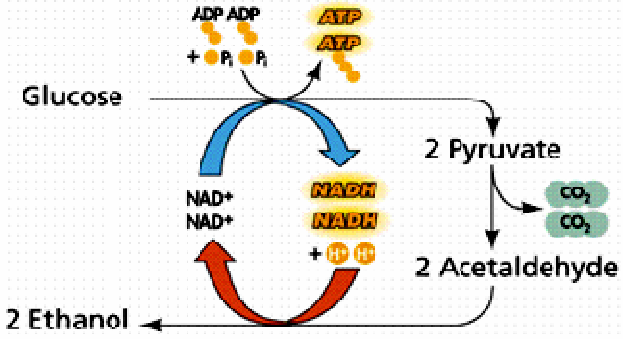


Graphic summary of the glycolysis process

Anaerobic Pathways

Under **anaerobic** conditions i.e., the absence of oxygen, **pyruvic acid** can be routed by the organism into one of three pathways: **lactic acid fermentation, alcohol fermentation,** or **cellular (anaerobic) respiration**. Humans cannot ferment alcohol in their own bodies, we lack the genetic information to do so. These biochemical pathways, with their myriad reactions catalyzed by reaction-specific enzymes all under genetic control, are extremely complex.

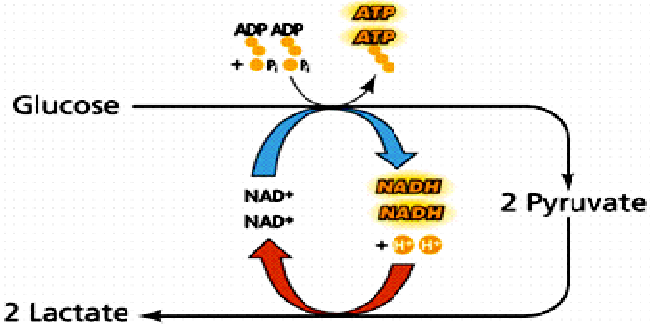
Alcohol fermentation is the formation of **alcohol** from **sugar.** Yeast, when under anaerobic conditions, convert glucose to **pyruvic acid** via the glycolysis pathways, then go one step farther, converting pyruvic acid into **ethano**l, a C-2 compound.



Fermentation of ethanol

Many organisms will also ferment pyruvic acid into, other chemicals, such as **lactic acid.** Humans ferment lactic acid in **muscles** where oxygen becomes depleted, resulting in localized anaerobic conditions. This lactic acid causes the muscle stiffness after beginning exercise programs. The stiffness goes away afterwards since the

cessation of strenuous activity allows aerobic conditions to return to the muscle, and the lactic acid can be converted into ATP via the normal aerobic respiration pathways.



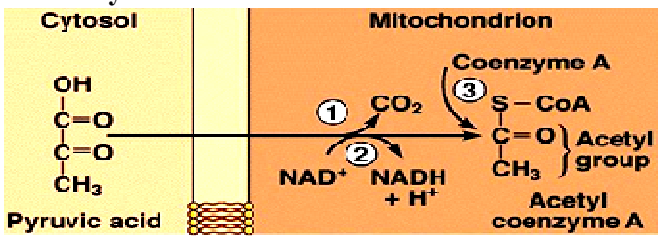
Fermentation of lactate (lactic acid)

Aerobic Respiration

When oxygen is present (aerobic conditions), most organisms will undergo two more steps, **Kreb's Cycle**, and **Electron Transport**, to produce their ATP. In eukaryotes, these processes occur in the mitochondria, while in prokaryotes they occur in the cytoplasm.

Acetyl Co-A: The Transition Reaction

Pyruvic acid is first altered in the **transition reaction** by removal of a carbon and two oxygens (which form carbon dioxide). When the **carbon dioxide** is removed, energy is given off, and **NAD^+** is converted into the higher energy form **NADH**. **Coenzyme A** attaches to the remaining 2-C (acetyl) unit, forming **acetyl Co-A**. This process is a prelude to the **Kreb's Cycle**.

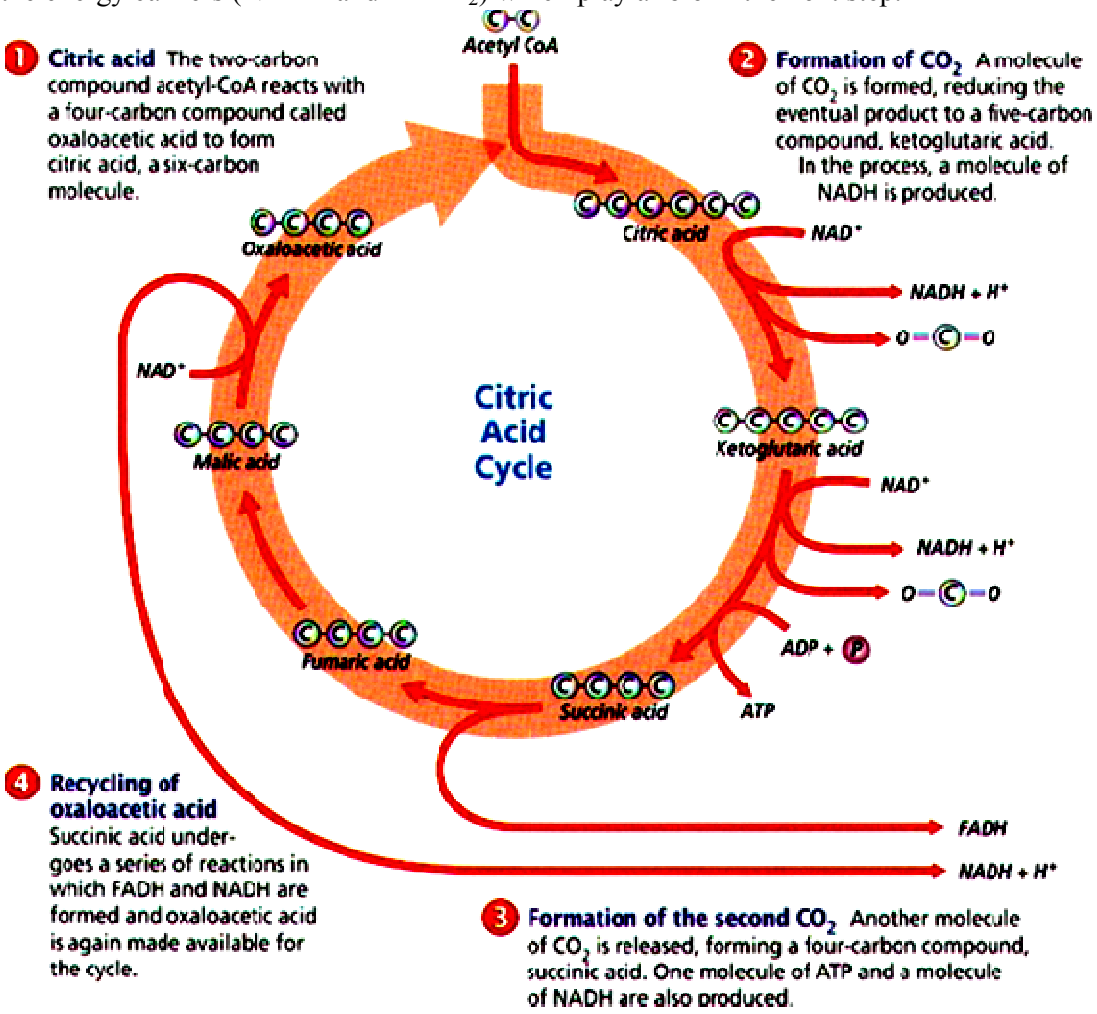


The transition reaction

Kreb's Cycle (Citric Acid Cycle)

The Acetyl Co-A (2-C) is attached to a 4-C chemical (**oxaloacetic acid**). The Co-A is released and returns to await another pyruvic acid. The 2-C and 4-C make another chemical known as **Citric acid**, a 6-C. Kreb's Cycle is also known as the Citric Acid Cycle. The process after Citric Acid is essentially removing carbon dioxide, getting out energy in the form of ATP, GTP, NADH and $FADH_2$, and lastly regenerating the cycle. Between **Isocitric Acid** and **α-Ketoglutaric Acid**, carbon dioxide is given off and NAD+ is converted into **NADH**. Between α-Ketoglutaric Acid and **Succinic Acid** the release of carbon dioxide and reduction of NAD^+ into NADH happens again, resulting in a 4-C chemical, succinic acid. GTP (**Guanine Triphosphate**, which transfers its energy to **ATP**) is also formed here (GTP is formed by attaching a phosphate to GDP). The remaining energy carrier-generating steps involve the shifting of atomic arrangements within the 4-C molecules. Between Succinic Acid and **Fumaric Acid,** the molecular shifting releases not enough energy to make ATP or NADH outright, but instead this energy is captured by a new energy carrier, **Flavin adenine dinucleotide** (FAD). FAD is reduced by the addition of two H's to become **$FADH_2$.** $FADH_2$ is not as rich an energy carrier as NADH, yielding less ATP than the latter.The last step, between **Malic Acid** and **Oxaloacetic Acid** reforms OA to

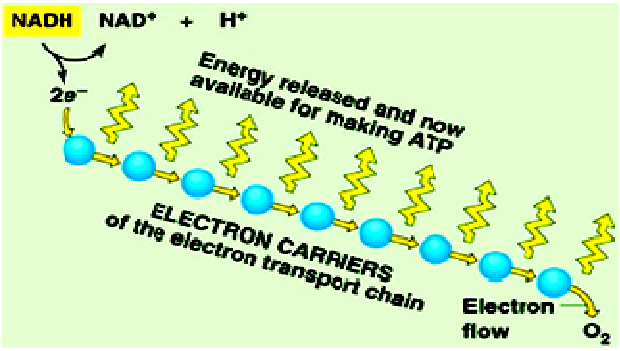
complete the cycle. Energy is given off and trapped by the reduction of NAD^+ to NADH. The **carbon dioxide** released by cells is generated by the Kreb's Cycle, as are the energy carriers (NADH and $FADH_2$) which play a role in the next step.



Summary of the Krebs' (or citric acid) cycle

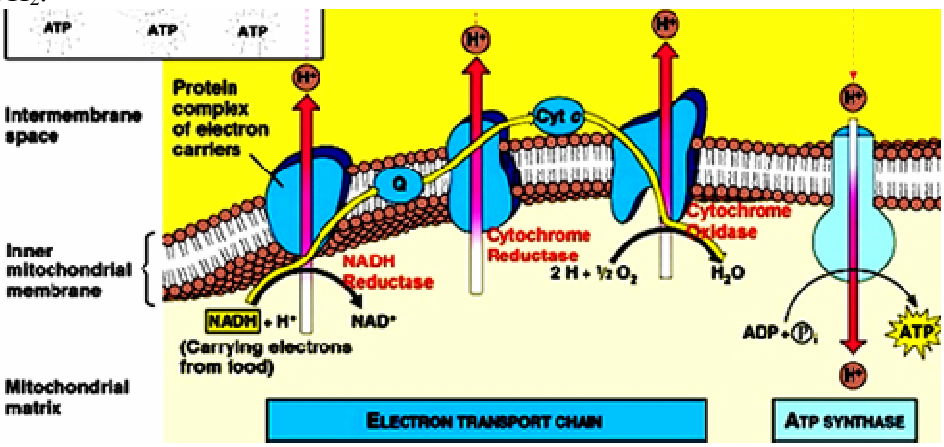
Electron Transport Phosphorylation

Whereas Kreb's Cycle occurs in the **matrix** of the mitochondrion, the **Electron Transport System** (ETS) chemicals are embedded in the **membranes** known as the **cristae**. Kreb's cycle completely oxidized the carbons in the pyruvic acids, producing a small amount of ATP, and reducing NAD and FAD into higher energy forms. In the ETS those higher energy forms are cashed in, producing ATP. **Cytochromes** are molecules that pass the electrons along the ETS chain. Energy released by the "downhill" passage of electrons is captured as ATP by ADP molecules. The ADP is reduced by the **gain of electrons**. ATP formed in this way is made by the process of **oxidative phosphorylation**. The mechanism for the oxidative phosphorylation process is the **gradient of H^+ ions** discovered across the inner mitochondrial membrane. This mechanism is known as **chemiosmotic coupling**.



Chemiosmotic coupling

This involves both chemical and transport processes. Drops in the potential energy of electrons moving down the ETS chain occur at three points. These points turn out to be where ADP + P are converted into ATP. Potential energy is captured by ADP and stored in the pyrophosphate bond. NADH enters the ETS chain at the beginning, yielding 3 ATP per NADH. $FADH_2$ enters at Co-Q, producing only 2 ATP per $FADH_2$.



Electron transport system.