

Oxygen Metabolism and Oxygen Toxicity

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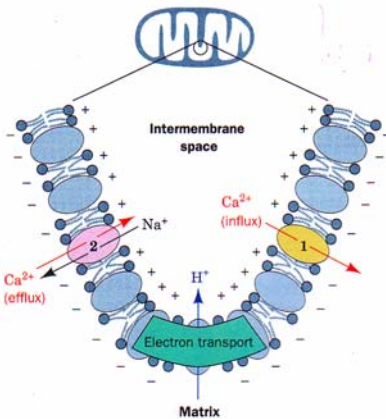
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Chemiosmotic Theory

Chemiosmotic Theory states that the free energy of electron transport is coupled to the pumping of protons from the matrix to the intermembrane space to create a pH gradient. This potential energy stored in this pH gradient is used to drive the synthesis of ATP. This process requires:

1. An intact inner mitochondrial membrane that is impermeable to protons,
2. The inner mitochondrial membrane must also be impermeable to ions such as OH^- , K^+ , Na^+ and Cl^- because free diffusion of these ions would discharge the transmembrane electrical potential which is a key component of the proton motive force.
3. Compounds that increase the permeability of the membrane to protons dissipate the electrochemical gradient and uncouple electron transport from oxidative phosphorylation.

Ca^{2+} transport in the mitochondria



The transport of calcium ions into and out of the matrix of the mitochondria is really interesting. There are two separate transport proteins for calcium ions. One integral membrane protein transports calcium ions into the matrix using the transmembrane electrical potential. The rate of Ca^{2+} influx depends on the cytosol concentration of calcium because the K_m for Ca^{2+} for this transport protein is greater than physiological cytosolic concentrations of Ca^{2+} . A separate protein transports Calcium ions out of the matrix antiporting in a sodium ion. This transporter protein always operates at its maximal velocity.

Thus an increase in cytosolic Ca^{2+} increases the rate of influx while the rate of efflux remains unchanged. This produces a net increase in the concentration of Ca^{2+} in the matrix. A drop in cytosolic Ca^{2+} cause a decrease in the rate of influx while the rate of efflux

remains unchanged thus producing a net drop in the concentration of Ca^{2+} in the matrix. Cool.

Regulation of ATP Producing Pathways

We have seen over and over again how the irreversible steps in metabolic pathways control the flux of metabolites through the pathway and are the sites of allosteric regulation. The electron transport chain functions near equilibrium from NADH to cytochrome *c*. There is only one irreversible step in electron transport-oxidative phosphorylation. That is the reduction of oxygen by cytochrome *c* oxidase, Complex IV. Cytochrome *c* oxidase is regulated primarily by the concentration of reduced cytochrome *c*. The concentration of reduced cytochrome *c* is in equilibrium with the rest of the electron transport pathway. Thus a high NADH/NAD⁺ ratio and a low ATP/ADP ratio increase the concentration of reduced Cyt *c*. Increased concentration of reduced Cyt *c* results in an increase in the rate of oxygen reduction.

Oxygen Metabolism.

Molecular oxygen has two unpaired electrons which have parallel spin states. The parallel spin states prevent carbon based organisms such as our selves from spontaneously igniting in our oxygen atmosphere. The parallel electron spins prevent oxidation by 2 electron transfers. Oxidation by molecular oxygen can only occur by the transfer of single electrons.

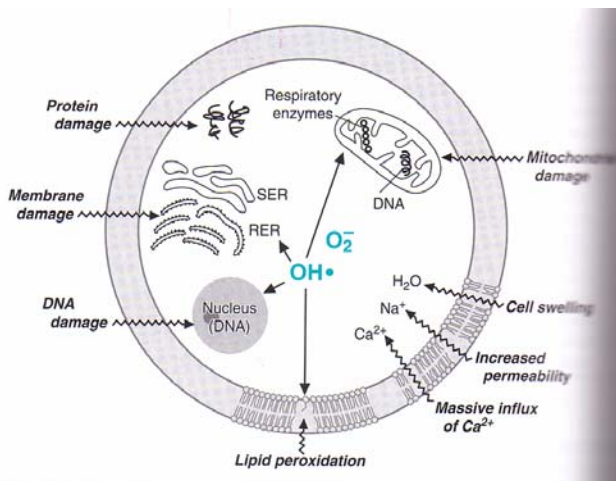
Organic molecules that serve as substrates for oxidation do not contain unpaired electrons. Their bonds are in the stable form of two electrons with antiparallel spins. For O_2 to accept a pair of electrons from an organic substrate, one of the electrons of oxygen or one of the electrons from the donating substrate has to invert its spin. There is a large thermodynamic barrier to such spin inversions. As a result two electron oxidations by molecular oxygen have to occur stepwise by two single electron transfers. The large barrier to spin inversions keeps us from spontaneously combusting in our oxygen atmosphere.

The two unpaired electrons of molecular oxygen have a biradical nature which has a tendency to form highly reactive oxygen species (ROS) which initiate free radical reactions. Free radicals react indiscriminately with any molecule they come in contact with. Free radical reactions are a chain of single electron transfer reactions which damage cellular components.

Reactive Oxygen Species (ROS)

$\cdot OH$	Hydroxyl radical
$\cdot O_2^-$	Superoxide
H_2O_2	Hydrogen peroxide

Reactive oxygen species can be lethal to cells. Proteins, membrane lipids, carbohydrates and nucleic acids are subject to cellular damage by oxygen radicals. Free radical damage contributes to complications of many chronic diseases. In some cases oxygen free radicals are the direct cause of the disease state. In other cases such as rheumatoid arthritis, radical oxygen species perpetuate cellular damage caused by a different process. Macrophages, neutrophils use ROS to destroy foreign organisms during phagocytosis.



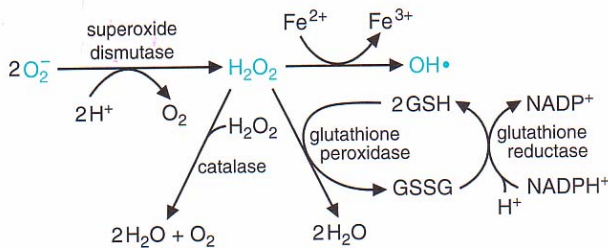
Free radical mediated cellular injury.

Superoxide and hydroxyl free radicals initiate peroxidation in the cellular, mitochondrial, nuclear, and endoplasmic reticulum membranes. This increases the cellular permeability for Ca^{2+} . Increased cellular concentrations of calcium ions damage the mitochondria. Amino acids are oxidized and degraded. Nuclear and mitochondrial DNA is oxidized, resulting in strand breaks and other types of DNA damage.

Coenzyme Q generates superoxide.

One of the major sites of superoxide generation is the electron transport chain which leaks free radicals in the form of semiquinone radicals of coenzyme Q. The one electron form of CoQ occasionally leaks into the inner mitochondrial membrane. The nonspecific interaction of a $CoQH\cdot$ with molecular oxygen results in the formation of a superoxide radical which abstracts an electron from some other molecule and initiates a free radical chain reaction

Cellular Defences Against ROS



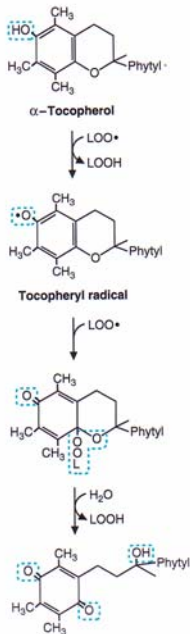
Cells protect themselves by compartmentalizing processes which generate highly reactive oxygen species. Oxidative stress occurs when the rate of generation of ROS exceeds the capacity of the cell to remove them.

Aerobic cells have to protect themselves from damage by the naturally occurring, continuous generation of ROS. Superoxide dismutase (SOD) removes the

superoxide free radical. Superoxide dismutase is one of the primary defenses against oxidative stress because the superoxide radical is a strong free radical initiator.

Catalase and glutathione peroxidase removes hydrogen peroxide and lipid peroxides. Hydrogen peroxide is a source of hydroxyl free radicals. Catalase reduces hydrogen peroxide into water. Catalase is found in the peroxisomes. Glutathione peroxidase also protects the cell from oxidative injury by reducing hydrogen peroxide into water and lipid peroxides into acids.

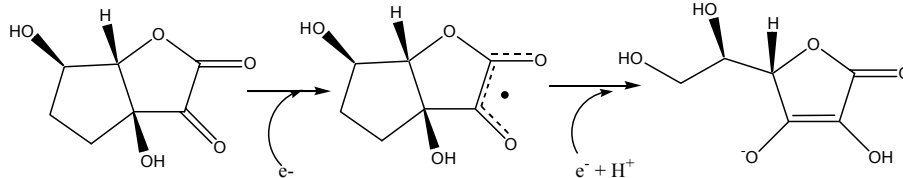
The mitochondria is a major source of ROS. The mitochondria have a high concentration of SOD and glutathione peroxidase to prevent oxidative stress.



Vitamins E and C act as antioxidants.

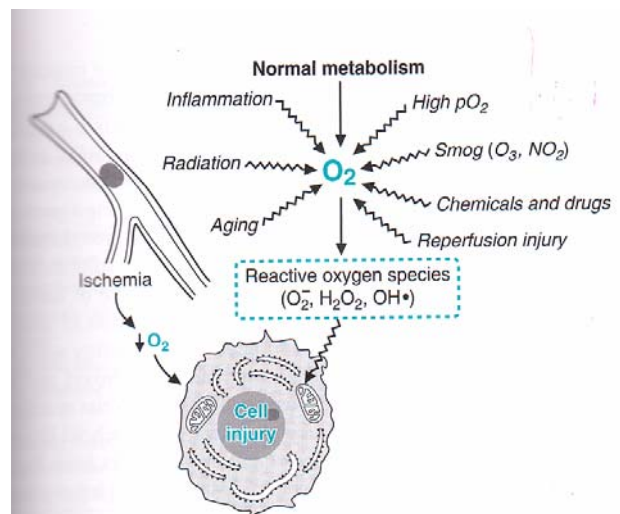
Vitamin E (tocopherol) is the most abundant antioxidant in nature. It is a lipophilic free radical scavenger to protect lipids from peroxidation in the membranes. Its sole biological purpose is to quench free radical reactions in membranes.

Vitamin C, ascorbic acid is a water soluble free radical scavenger. It accepts electrons from superoxide, hydrogen peroxide, hypochlorite, hydroxyl radicals and peroxy radicals. It also quenches ozone and nitrous oxide.



The role of oxygen in cell injury.

O_2 is necessary for the generation of ATP by oxidative phosphorylation. But oxygen is also toxic. Normal oxygen metabolism generates reactive oxygen species which can cause cell injury. Protective enzymes and antioxidants remove ROS. Various stimuli, such as radiation, inflammation, aging and high concentrations of oxygen greatly increase the rate of formation of ROS. Ozone and nitrous oxide are air pollutants that form free radicals in the cells of the lungs resulting in pulmonary



emphysema and pulmonary fibrosis. The lack of oxygen due to decreased blood flow (ischemia) also causes cell injury. The reintroduction of oxygen (reperfusion) enhances cellular injury due to ROS.

Hypoxic Injury

Hypoxia- deficiency of oxygen.

Hypoxic injury-injury caused by a deficiency of oxygen.

Acute hypoxic tissue injury has been extensively studied. The occlusion of a major coronary artery produces an array of biochemical and physiological complications. When a tissue is deprived of oxygen, the mitochondrial electron transport-oxidative phosphorylation is inhibited, resulting in a severe decline in cellular ATP levels. Anaerobic glycolysis is activated in an attempt to restore cellular ATP levels. Glycogen stores are rapidly depleted, and lactic acid concentrations increase, lowering the cellular pH (lactic acidosis). Hypoxic cells begin to swell as they can no longer maintain their normal electrolyte concentrations. The mitochondria begin to swell and accumulate calcium which precipitates as calcium phosphate. As the membranes swell they become permeable leading to the leakage of enzymes, coenzymes and other cellular constituents. As the pH continues to fall, the lysosomal membranes release hydrolytic proteases, lipases, glucosides and phosphatases that digest the cell.

Amazingly cells that have been exposed to short periods of hypoxia can recover without irreversible damage upon reperfusion with oxygen containing medium.

Enzymes that use oxygen as a substrate.

The electron transport system of the mitochondria accounts for 90% of the oxygen consumption of a cell. The remainder 10 % of the oxygen is consumed in oxygen requiring reactions in the body by oxidases or oxygenases. An oxidase is an enzyme that reduces molecular oxygen to water or hydrogen peroxide. These oxidases play specific roles in metabolism. An oxygenase directly incorporates oxygen into the molecules being oxidized

Oxidases

Oxidases reduce oxygen to either water or hydrogen peroxide.

Cytochrome c oxidase (Complex IV) is an example of an oxidase. This enzyme complex catalyzes the four electron reduction of O_2 to $2H_2O$. The electrons are transferred to oxygen one at a time to overcome the spin restrictions. The mechanism of this enzyme allows the peroxy intermediates to be formed in a controlled fashion without interaction between oxygen free radicals and other mitochondrial components. Other oxidases reduce oxygen to peroxide instead of water. These enzymes are compartmentalized into peroxisomes and lysosomes where catalase and glutathione peroxidase remove the hydrogen peroxides.

Oxygenases

Oxygenases incorporate oxygen into the substrate.

Monoxygenases incorporate one of the atoms of oxygen into the substrate while reducing the other atom of oxygen into water. The common name for monoxygenases is hydroxylases (ie phenylalanine hydroxylase which adds a hydroxyl group to phenylalanine to form tyrosine.) Monoxygenases require an electron donor such as NADPH, a coenzyme capable of mediating between a two electron donor and a one electron acceptor (FAD) and a metal cofactor to form a stable reactive oxygen complex.

Cytochrome P450 enzymes are a superfamily of structurally related monoxygenases which hydroxylate many compounds such as steroids, fatty acids, drugs, carcinogens, ect. There are over 100 isozymes of P450 enzymes in humans, each with a different but overlapping specificity.

Dioxygenases incorporate both atoms of oxygen into the substrate.

Pyruvate Dehydrogenase Deficiency

A variety of pyruvate metabolism disorders have been detected in children. Some involve deficiencies in the regulatory subunits of pyruvate dehydrogenase other disorders involve the catalytic subunits. Children with deficiencies have high serum concentrations of lactate, pyruvate and alanine which produce a chronic lactic acidosis.

In addition there are severe neurological defects that in most cases result in death.

Patients in shock suffer from lactic acidosis because of the decreased delivery of oxygen increases anaerobic glycolysis. Decreased concentrations of oxygen increase mitochondrial concentrations of NADH which activates pyruvate dehydrogenase kinase which inhibits pyruvate dehydrogenase.

Patients in shock are treated with dichloroacetate which is an inhibitor of pyruvate dehydrogenase kinase and therefore an activator of pyruvate dehydrogenase.

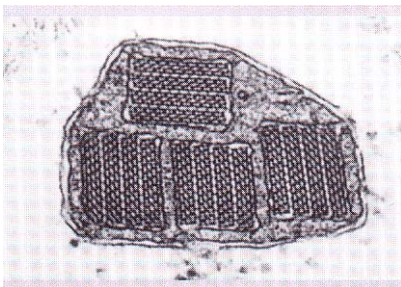
Fumarase Deficiency

Deficiencies in the enzymes of the citric acid cycle are rare because these enzymes are so crucial for life. Several cases are now on record in which there is a severe deficiency in fumarase in both the mitochondria and the cytosol of tissues. The condition is characterized by encephalopathy (a disease of the brain involving alteration of brain structure.), severe impairment of neurological function and dystonia (disordered tonicity of muscles). These conditions develop immediately after birth. The urine contains abnormally high concentrations of fumarate, succinate, citrate and malate.

Mitochondrial Myopathies

Mitochondrial myopathies are mitochondrial diseases that effect the muscles. Clinically patients with mitochondrial myopathies complain of weakness and severe cramping of the affected muscles. Infants have difficulty feeding and crawling. Continued severe fatigue results in minimal exertion and muscle wasting. Examining the mitochondria of these myopathies have discovered primary reasons for mitochondrial malfunction.

- 1.) Deficiencies in mitochondrial transport.
- 2.) Deficiencies in electron transport in either Complex I, Complex II, Complex III, Complex IV and ATP synthase have been described.



- 3.) Crystalline inclusions within the mitochondrial matrix. Under these conditions electron transport and oxidative phosphorylation are only loosely coupled.

←Example of paracrystalline inclusions in the mitochondria from muscles of ocular myopathic patients.