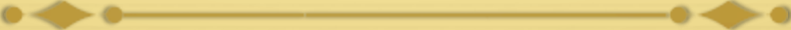




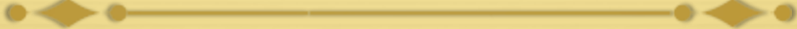
- 
- ✦ The cells convert physical and chemical energy to biological energy by a special system which is transported as ATP.
  - ✦ ATP is used to produce chemical, osmotic and mechanical work.

# Metabolism



- ✦ Total chemical reactions in organism
- ✦ ATP and ADP are known as high energy phosphates as the cleavage of phosphate bond in them yield energy and inorganic phosphate.
- ✦ This energy is utilized for the anabolic and catabolic processes.
- ✦ Anabolism: Consumes ATP
- ✦ Catabolism: Produces ATP

# ATP: The basic transporter of cellular energy !!!



✦ 1 molecule ATP energy: -7.3 kcal/mol

## ✦ Functions of ATP

-Mobility

-Membrane transport

-Signal transduction

--Synthesis of nucleotides

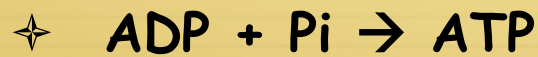
# Redox Reactions



- Every oxidation is always accompanied by a reduction process.
- All such reactions are termed as "oxidation-reduction" reactions and shortly referred as "redox".
- These redox reactions are associated with movements of electron.
- The electron donor is called as **reductant** or reducing agent and
- The electron acceptor, the **oxidant** or oxidizing agent.
- The system which transfer its electron is changed into oxidant form while the system which accepts electrons gets converted to the reductant form.

- The oxidoreduction reactions occur in living organisms are known as **biological oxidation**.

# The Principles of Redox Reactions



+ 7.3 kcal/mol

2 e<sup>-</sup> transport from

NADH' to O<sub>2</sub> : 2.5 ATP,

FADH<sub>2</sub> to O<sub>2</sub>: 1.5 ATP

# Which Enzymes Display Roles in Oxidoreduction Reactions



- ✦ Oxidases: Utilize oxygen as electron acceptor
- ✦ Dehydrogenases: can't utilize oxygen as electron acceptor (Uses nicotinamides and riboflavins as coenzymes)
- ✦ Hydroperoxidases: uses  $H_2O_2$  as substrate
- ✦ Oxygenases: Catalyses the direct transport of  $O_2$  to the substrate



# Oxidative Phosphorylation

Oxidative phosphorylation is defined as "ATP synthesis by the transport of electron to the molecular oxygen".

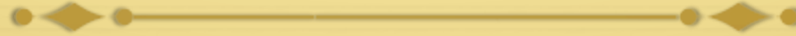
The oxidative phosphorylation enables the aerobic living organisms to capture a far greater proportion of available free energy of the oxidizing substrates in the form of ATP !!!

The background features a light yellow-to-orange gradient. At the top, there are several faint, overlapping circular stamps in red and black ink, some containing text like 'POSTAGE' and 'AIR MAIL'. A faint globe is also visible in the upper center.

**Where does oxidative phosphorylation occur???**

# Mitochondria

# The Structure of Mitochondria



# Components of Mitochondria



## ✦ Outer membrane

Outer membrane is freely permeable to most of the small ions and contains monoamine oxidase and Acetyl CoA synthase.

# Components of Mitochondria

## ✦ Inner Membrane

✦ The folding of the inner membrane produces a number of partitions called cristae that extend into the matrix

✦ Very selective in its permeability

✦ Contains;

-Electron transport chains (complex I-IV)

-ATP synthesizing enzymes

-Many membrane transport systems

-Pyruvate and fatty acid transporter proteins

-Other transporter systems

! It's not permeable to small molecules such as ATP, ADP, pyruvate and ions such as  $H^+$ ,  $Na^+$ ,  $K^+$ . (Needs special transporters)

# Components of Mitochondria

## ✦ Matrix contains

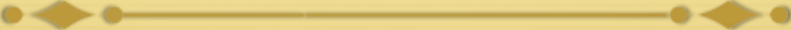
- Most of citric acid cycle enzymes
- Pyruvate dh system
- Fatty acid-ox enzymes
- Aminoacide ox enzymes
- DNA, ribosomes
- ATP, ADP, Pi, Mg<sup>+2</sup>, Ca<sup>+2</sup>, K<sup>+</sup>
- NAD<sup>+</sup>, FAD
- Solubl metabolytes

# Components of Mitochondria



- ✦ The space between the inner and the outer membrane is called as Inter membrane space which is surrounded by matrix.
- ✦ The mitochondria contains its own circular DNA and ribosomes.
- ✦ Some mitochondrial proteins are thus coded for and produced by the mitochondria itself.
- ✦ Other mitochondrial proteins are coded by nuclear DNA, synthesized by cytosolic ribosomes, and subsequently transported to the mitochondria.

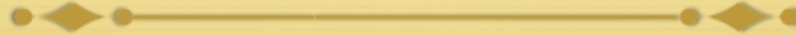


- 
- ✦ Oxidative phosphorylation begins with the entrance of electrons to the , electron transport chain !!!

# Members of the electron transport chain

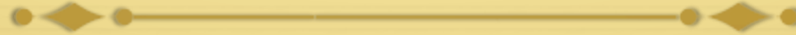
- ✦ The electron transport chain is initiated by the reaction of an organic metabolite (intermediate in metabolic reactions) with the coenzyme NAD<sup>+</sup> (nicotinamide adenine dinucleotide is a coenzyme containing the B-vitamin, nicotinamide).
- ✦ This is an oxidation reaction where 2 hydrogen atoms (or 2 hydrogen ions and 2 electrons) are removed from the organic metabolite.
- ✦ (The organic metabolites are usually from the citric acid cycle and the oxidation of fatty acids—details in following pages).
- ✦ The reaction can be represented simply where M = any metabolite.
- ✦  $MH_2 + NAD^+ \rightarrow NADH + H^+ + M: + \text{energy}$

# Members of the electron transport chain



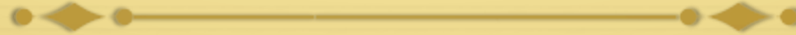
- ✦ **Complex I** - NADH dehydrogenase, also called NADH coenzyme Q reductase located in the inner mitochondrial membrane and also contains non heme iron atoms.
- ✦ These dehydrogenase enzyme does not react with oxygen instead an electron carrier is interposed between the metabolite and next member in the chain.
- ✦ These enzymes consist of a protein part and a non protein part which is a coenzyme. The co enzyme  $\text{NAD}^+$  or  $\text{NADP}^+$  are utilized as the prime carriers of hydrogen.

# Members of the electron transport chain



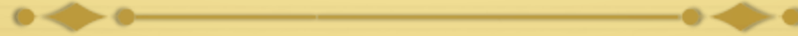
- ✦ **Complex II** - Coenzyme Q (Q for Quinone) or cytochrome c reductase is a Ubiquinone.
- ✦ It is in the inner membrane in the free form or protein bound form.
- ✦ Coenzyme Q occupies the position between metalloflavoproteins and cytochrome in the chain.
- ✦ At the point of coenzyme the  $H^+$  ion dissociate and go into solution, leaving the electrons to the cytochromes .

# Members of the electron transport chain



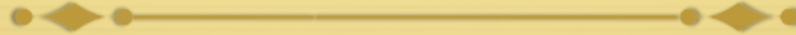
- ✦ **Complex III** -Cytochrome c oxidase.
- ✦ Cytochromes are very similar to the structure of myoglobin or hemoglobin.
- ✦ The significant feature is the heme structure containing the iron (Fe) ions, initially in the +3 state and changed to the +2 state by the addition of an electron.

# Members of the electron transport chain



- ✦ Cytochrome molecules accept only the electron from each hydrogen, not the entire atom. The several types of cytochromes hold electrons at slightly different energy levels.
- ✦ Electrons are passed along from one cytochrome to the next in the chain, losing energy as they go.
- ✦ Finally, the last cytochrome in the chain, cytochrome a<sub>3</sub>, passes two electrons to molecular oxygen. .

# Members of the electron transport chain



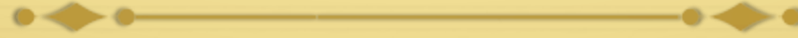
- ✦ These cytochromes are proteins that carry a prosthetic group that has an embedded metal atom.
- ✦ The protein 'steals' the ability of the metal atom to accept and release electrons.
- ✦ The electrons are transferred from Complex III to cytochrome c

## Complex IV (cytochrome oxidase)

- ✧ **Complex IV**-(cytochrome oxidase), catalyses the transport of electrons from cytochrome c to molecular  $O_2$  and thus the reduction of  $O_2$  to  $H_2O$ .
- ✧ It contains cytochrome a and a.3

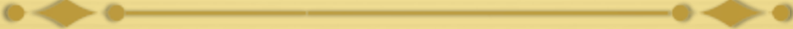


# Members of the electron transport chain



- ✦ **Complex V** - ATP synthase, also known as the F<sub>0</sub> F<sub>1</sub> particle has two components F<sub>0</sub> and F<sub>1</sub>
- ✦ (F - indicates the factor). F<sub>1</sub> protruding into matrix from the inner membrane and F<sub>0</sub> embedded and extend across the inner membrane.
- ✦ The protruding F<sub>1</sub> is essential for the energy coupling to ATP molecule.
- ✦ Careful removal of this component (experimentally) leads to impairment in ATP production though the intact respiratory chain is present.

# Oxidative Phosphorylation



- ✦ Hydrogens or their electrons, pass down the electron transport chain in a series of redox reaction. The electrons entering the electron transport system have relatively high energy content.
- ✦ As they pass along the chain of electron acceptors, they lose much of their energy, some of which is used to pump the protons across the inner mitochondrial membrane.

# Chemiosmotic Theory



- ✦ The chemiosmotic theory claims that oxidation of components in respiratory chain generates hydrogen ion and ejected across the inner membrane.
- ✦ The electrochemical potential difference resulting from the asymmetric distribution of the hydrogen ion is used as the driving force (potential energy).
- ✦ This consist of a chemical concentration gradient of protons across the membrane (pH gradient) also provides a charge gradient.

# Chemiosmotic Theory



- ✦ The inner mitochondrial membrane is impermeable to the passage of protons, which can flow back into the matrix of the mitochondrion only through special channels in the inner mitochondrial membrane.
- ✦ In these channels, the enzyme *ATP synthetase* is present. As the protons move down the energy gradient (proton motive force = chemiosmotic energy), the energy released is used by *ATP synthetase* to produce *ATP*.

# Chemiosmotic Theory



- ✦ The chemiosmotic model explains that this electrochemical potential difference across the membrane is used to drive a membrane located ATP synthetase which couple the energy to ADP, to form ATP.
- ✦ Protons are pumped across the inner mitochondrial membrane by four electron transfer complexes, each associated with particular steps in the electron transport system.
- ✦ As electrons are transferred along the acceptors in the electron transport chain, sufficient energy is released at three points to convey protons across the inner mitochondrial membrane and ultimately to synthesize ATP.

# Inhibitors of Oxidative Phosphorylation



- ✦ The use of inhibitors gives much information about the electron transport chain.
- ✦ They are classified as
  - A. Inhibitors of respiratory chain,
  - B. Inhibitors of oxidative phosphorylation,
  - C. Uncouplers of phosphorylation.

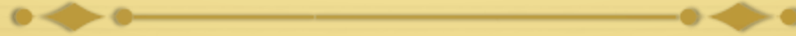
# Inhibitors of Oxidative Phosphorylation



## A. Inhibitors of Respiratory Chain

- ✓ Inhibitors that arrest respiration are barbiturates like **amobarbital**, antibiotic like **piERICIDIN A**, **antimycin A** and fish poison **retinone**.
- ✓ The **carbon monoxide (CO)** and **cyanide (CN)** inhibit cytochrome oxidase so that it cannot transport electrons to oxygen.
- ✓ This blocks the further passage of electrons through the chain, halting ATP production and life.

# Inhibitors of Oxidative Phosphorylation



## B. Inhibitors of oxidative phosphorylation

- ✓ Inhibitors of oxidative phosphorylation are oligomycin and atrcctyloside.
- ✓ Inhibitors of ATP synthase
  - Oligomycin
  - VenturicidinInhibition of Fo and CFo units
- ✓ Dicyclohexylcarbodiimide (DCCD) : Inhibition of proton efflux from Fo and Cfo



# Inhibitors of Oxidative Phosphorylation



## C. Uncouplers of phosphorylation

- ✦ Uncouplers dissolve in the membrane, and function as **carriers for  $H^+$**  or it can be an **ionophores**.
- ✦ Uncouplers block oxidative phosphorylation by dissipating the  $H^+$  electrochemical gradient by uncoupling the essential linkage between electron transport and ATP synthesis.
- ✦ Uncouplers are **2,4 dinitro phenol, dinitrocresol, pentachlorophenol**.

# Inhibitors of Oxidative Phosphorylation



## C. Uncouplers of phosphorylation

- ✓ Ionophores (ion carriers) are lipid soluble substances capable of carrying specific ions through the membrane.
- ✓ They slightly differ in their action from the uncouplers as they also transport cations other than  $H^+$  through the membrane.
- ✓ **Valiomycin** forms a lipid complex through which the  $K^+$  ion readily pass through.
- ✓ The ionophore **gramicidin** induces penetration to  $H^+$ ,  $K^+$  or  $Na^+$  and uncouples the oxidative phosphorylation.

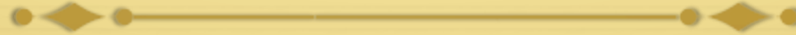
## ✦ Uncoupled Mitochondria in Brown Fat Produce Heat

✦ There is a remarkable and instructive exception to the general rule that respiration slows when the ATP supply is adequate.

✦ Most newborn mammals, including humans, have a type of adipose tissue called brown fat in which fuel oxidation serves not to produce ATP but to generate heat to keep the newborn warm.

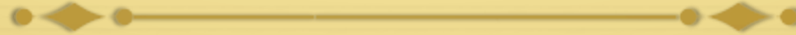
✦ This specialized adipose tissue is brown because of the presence of large numbers of mitochondria and thus large amounts of cytochromes, whose heme groups are strong absorbers of visible light.

## ✦ **Uncoupled Mitochondria in Brown Fat Produce Heat**



- ✦ The mitochondria of brown fat are like those of other mammalian cells in all respects, except that they have a unique protein in their inner membrane.
- ✦ Thermogenin, also called the uncoupling protein provides a path for protons to return to the matrix without passing through the Fo F1 complex.

# Shuttle Systems



- ✦ Shuttle Systems Indirectly Convey Cytosolic NADH into Mitochondria for Oxidation

# Shuttle Systems

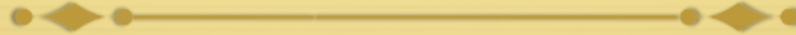


✦ The NADH dehydrogenase of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix.

✦ Given that the inner membrane is not permeable to NADH,

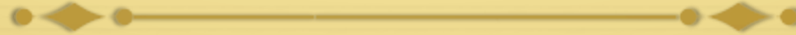
**!!! How can the NADH generated by glycolysis in the cytosol be reoxidized to NAD by  $O_2$  via the respiratory chain?**

# Malate-Aspartate Shuttle



- ✦ The most active NADH shuttle
- ✦ It functions in liver, kidney, and heart mitochondria
- ✦ The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate, catalyzed by cytosolic malate dehydrogenase.
- ✦ The malate thus formed passes through the inner membrane via the malate- $\alpha$ -ketoglutarate transporter.
- ✦ Within the matrix the reducing equivalents are passed to NAD by the action of matrix malate dehydrogenase, forming NADH; this NADH can pass electrons directly to the respiratory chain.
- ✦ About 2.5 molecules of ATP are generated as this pair of electrons passes to  $O_2$ . Cytosolic oxaloacetate must be regenerated by transamination reactions and the activity of membrane transporters to start another cycle of the shuttle.

# Glycerol-3-Phosphate Shuttle



- ✦ Skeletal muscle and brain use this shuttle.
- ✦ It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH to ubiquinone and thus into Complex III, not Complex I, providing only enough energy to synthesize 1.5 ATP molecules per pair of electrons.
- ✦ The mitochondria of plants have an externally oriented NADH dehydrogenase that can transfer electrons directly from cytosolic NADH into the respiratory chain at the level of ubiquinone. Because this pathway bypasses the NADH dehydrogenase of Complex I and the associated proton movement, the yield of ATP from cytosolic NADH is less than that from NADH generated in the matrix