ELECTRON TRANSPORT CHAIN, OXIDATIVE PHOSPHORYLATION, SUPEROXIDES

University of Papua New Guinea
School of Medicine & Health Sciences,
Division of Basic Medical Sciences,
Discipline of Biochemistry & Molecular Biology,
BMLS II, Bpharm II, BDS II

VJ Temple

What is metabolism?

- **Metabolism**: It is sum total of all chemical reactions involved in maintaining the living state of all cells;
- **Categories of Metabolism**:
  - Anabolism, Catabolism and Amphibolism;
- **Anabolism** (Biosynthesis) of compounds in the cells;
  - Examples: biosynthesis of DNA, RNA, or Proteins;
- **Catabolism** (break down) of compounds to obtain energy in the cells;
  - Examples: break down of Glucose to obtain energy,

- **Amphibolism**: Link of Anabolism and Catabolism,
  - TCA (Krebs Cycle) is the major Amphibolic pathway because it links Anabolic and Catabolic pathways;

- **Bioenergetics** describe the biochemical or metabolic pathways by which cells obtain energy;

How is energy used in cells?

- Catabolism provides the energy needed for useful work,
- Energy is used mainly as Adenosine Tri-phosphate (**ATP**),
- ATP links Exothermic and Endothermic Reactions,
- **ATP**: Adenosine and Ribose bonded to 3-Phosphate groups via Phosphate Ester bonds,
- Two bonds in ATP are High-energy bonds
  - Bond energy = 7 kcal/mole,
- **ADP** contains 2-Phosphate groups:
  - One of them is high energy bond,
- **AMP** contains 1-Phosphate group, with no high energy bond;

- Hydrolysis of ATP:

ATP + H_2O =====➔ ADP + P + Energy

- Under certain conditions ATP may be hydrolyzed to AMP

ATP + H_2O ====➔ AMP + PP + Energy

- Formation of ATP :

ADP + P + Energy =====➔ ATP + H_2O

- Other High energy Phosphates molecules are:
  - Guanosine Tri-phosphate (**GTP**),
  - Creatine Phosphate (**$CrPO_3$**),
  - Phosphoenolpyruvate (**PEP**),
  - 1,3-Bisphosphoglycerate (**1,3BPG**),
  - Succinyl-CoA, etc.

What are Coupled reactions, give examples?

- Some reactions produce energy (Exothermic reactions),
- Others reactions require energy (Endothermic reactions),
- Both processes occur efficiently when they are "Coupled"
- Couple reaction means:
  - Two reactions occurring to support each other,
  - The Fists reaction **must** be **Exothermic,**
  - The Second reaction which is **Endothermic**, picks up the energy produce by Exothermic reaction,

- Couple reaction requires ATP or other high-energy compound

Two examples of Coupled Reactions

- (**1**) Hydrolysis of ATP and Contraction of muscle tissue:
  - Energy releases from ATP is used for muscles to contract,

$$\mathbf{ATP + H_2O ====\rightarrow ADP + P + Energy}$$

Relaxed muscle + Energy ====➔ Contracted muscle

- (**2**) Hydrolysis of $CrPO_3$ and formation of ATP:

$$\mathbf{CrPO_3 + H_2O ===\rightarrow Creatine + HPO_4^{-3} + Energy}$$

$$\mathbf{ADP + HPO_4^{-3} + Energy =====\rightarrow ATP + H_2O}$$

- During periods of rest the muscular activity is low, thus the reactions are reversed to replenish ATP and $CrPO_3$

$$\mathbf{ATP + Creatine ======\rightarrow CrPO_3 + ADP}$$

How is ATP produced in mitochondria?

- Mitochondria is the power house of the cell,
- Cells use Proton-Pumping System made up of proteins inside Mitochondria to generate ATP;
- Production of ATP is coupled with Oxidation of Reducing Equivalent (NADH) and reduction of Oxygen in Electron Transport Chain (ETC),
- Process is known as Oxidative Phosphorylation;

- Process involved 3 key steps:
  - Transfer of electrons from NADH via Electron carriers to Oxygen,
  - Transfer of electrons by carriers generates Proton (H^+) Gradient across Inner Mitochondrial membrane;
  - ATP is produced when **H^+** spontaneously diffuses back across the Inner Mitochondrial membrane;

- **ATP Synthetase** converts the Free Energy of the Proton Gradient to Chemical Energy in the form of ATP;

What is the Electron Transport Chain (ETC)?

- Electron Transport (Respiration) Chain (ETC) is the Final Common Pathway in Aerobic cells,
- In ETC electrons derived from various substrates are transferred to Oxygen;
- ETC is composed of a series of highly organized **Oxidation-Reduction Enzymes** whose reactions can be represented by:

Reduced A + Oxidized B ⟵⟶ Oxidized A + Reduced B

Where is ETC located in the cell?

- ETC is located in the Inner membrane in the Mitochondria,

- Enzymes of the ETC are embedded in the inner membrane in association with the enzymes of Oxidative Phosphorylation;

What are Reducing Equivalents?

- Reducing Equivalents are sources of electrons for ETC,
- Two major Reducing Equivalents:
- **$NADH+H^+$** : Reduced Nicotinaminde-Adenine Dinucleotide
  - It produces **3 molecules of ATP in ETC**;
- **$FADH_2$** : Reduced Flavin-Adenine Dinucleotide,
  - It produces **2 molecules of ATP in ETC**;
- Other reducing equivalents are:
  - **$NADPH + H^+$;**
  - **$FMNH_2$**;

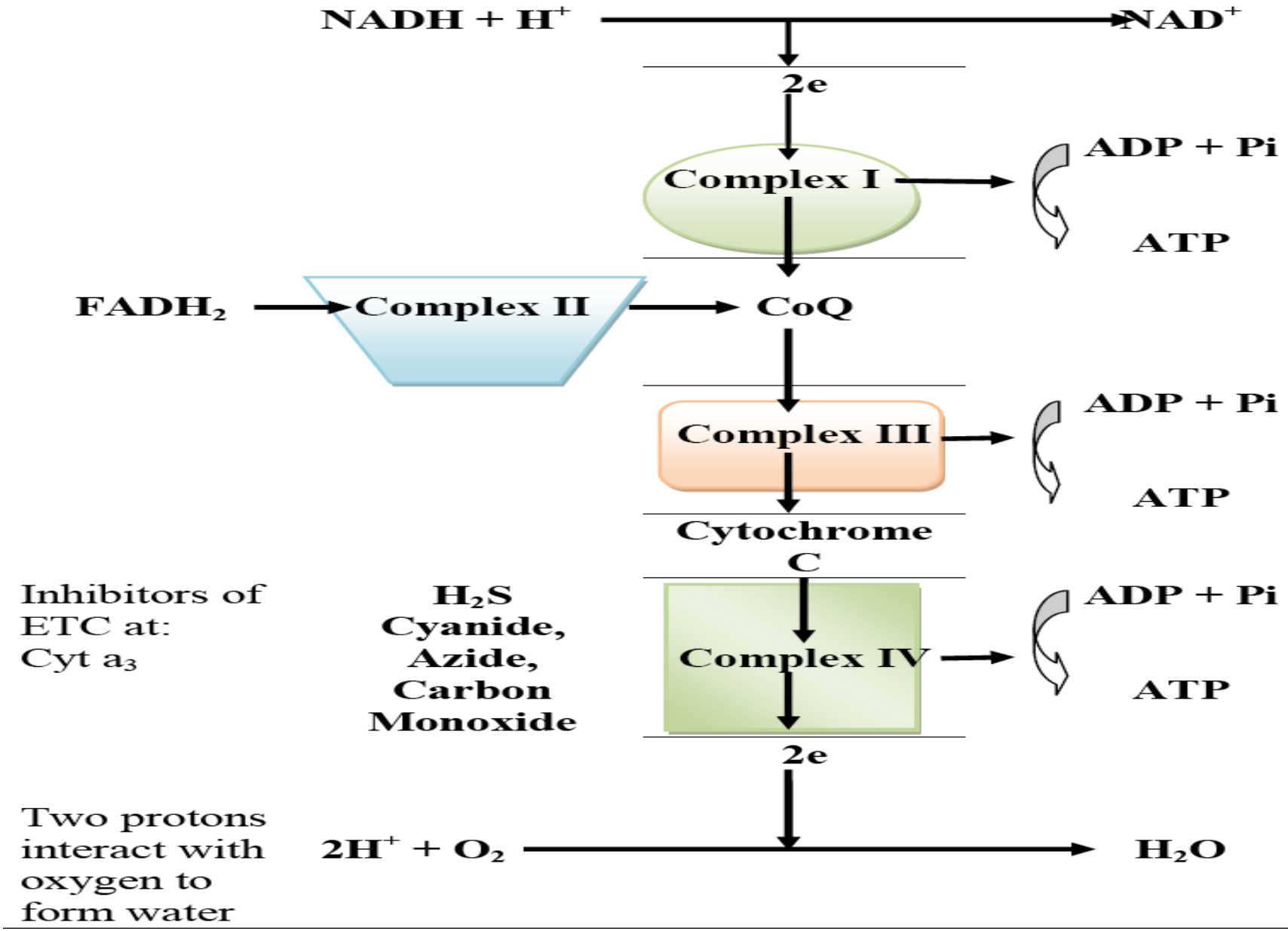
What are the major components of the ETC?

- ETC is made up of Four Major Complexes:
- **Complex I**:
  - NADH, Coenzyme Q Reductase,
  - Point of entry into ETC for electrons from NADH
- **Complex II**:
  - Succinate, Coenzyme Q Reductase,
  - Point of entry into ETC for electrons from Succinate;

- **Complex III**:
  - Coenzyme Q, Cytochrome C Reductase,
  - Electron acceptor for Coenzyme Q;
- **Complex IV**:
  - Cytochrome C Oxidase,
  - Electron acceptor for Cytochrome C
  - Cytochrome **a** $\mathbf{a_3}$

Fig 1: Simplified schematic diagram of ETC,

Fig. 1: Schematic diagram of ETC: showing the complexes, points of formation of ATP and point of action of Inhibitors of ETC



What do you understand by Oxidative Phosphorylation?

- It is main source of energy in Aerobic metabolism
- Process by which Free Energy released when electrons are transferred along the ETC is coupled to the formation of ATP from ADP and Pi

ADP + Pi + Energy ==========➔ ATP

- Two possibilities must be considered:
- **Intact Mitochondria**:
  - Transport of Electrons and Oxidative Phosphorylation of ADP are tightly Coupled reactions,
  - Free Energy released is stored as ATP,

- **Damaged Mitochondria**:
  - Electron transport may occur without Oxidative Phosphorylation,
  - Free Energy released as Electrons are transported will not be stored as ATP but will instead be lost as heat,

What are some effects of prolonged Anaerobic Glycolysis?

- Anaerobic Glycolysis leads to production of:
  - Two molecules of Lactic Acid (Lactate);
  - Total of 4 ATP,
  - Net of 2 ATP per molecule of Glucose,
- Summary of equation for Anaerobic Glycolysis:
- (All enzymes are present in Cytosol)

Glucose + 2ADP + 2P ===➔ 2 Lactate + 2 ATP + $2H^+$

- End product of Anaerobic Glycolysis is Lactate;

- Prolonged Anaerobic Glycolysis causes Lactic Acidosis;
- Muscles become Tired and Sore;
- Lungs respond by Hyperventilation, blowing out CO_2, which helps to reduce accumulation of acid in the cells and restore Acid – Base balance;
- Lactic acid is removed via Cori Cycle in the Liver;

What is Superoxide and where is it formed?

- Partial reduction of Oxygen gives a highly reactive, highly unstable molecule called Superoxide (O_2^-),
- Superoxide is an anion free radical that can react with and damage DNA, Proteins and Cell membranes in general;

Superoxide is usually formed in:

- Mitochondria by reactions of O_2 with $FADH_2$ and reduces Cytochrome Q,
- Reactions involving molecular Oxygen in the cells,
- Red Blood Cells, because Hemoglobin contains Ferrous ions that can be converted to Ferric ions;

How can Superoxide be removed from cells?

- They are removed by enzymatic reactions;
- Two step reactions for removal of Superoxide:

First Step:

- **Superoxide Dismutase**: Metallo-enzyme that catalyzes removal of Superoxide from cells:

$$2\ O_2^- + 2\ H^+ \xrightarrow{\text{=====}} H_2O_2 + O_2$$

Second Step:

- **Hydrogen Peroxidase:** catalyzed break down of Hydrogen Peroxide formed:

$$2\ H_2O_2 \xrightarrow{\text{======}} 2H_2O + O_2$$

STUDY QUESTIONS

- What is a Superoxide?
- How can Superoxide be removed from cells?
- What is the Electron Transport Chain (ETC)?
- Where is the ETC located in the cell?
- How is energy used in the cells?
- What are coupled reactions (give one example)?
- What are the major components of the ETC?
- How many molecules of ATP are produced by NADH, and FADH2

REFERENCES

- Textbook of Biochemistry, with clinical correlations, Ed. By T. M. Devlin, 4th Ed.
- Harper's Illustrated Biochemistry 26th Edition; 2003; Ed. By R. K. Murray et. al.
- Biochemistry, By V. L. Davidson & D. B. Sittman. 3rd Edition.
- Hames BD, Hooper NM, JD Houghton; Instant Notes in Biochemistry, Bios Scientific Pub, Springer; UK.
- VJ Temple Biochemistry 1001: Review and Viva Voce Questions and Answers Approach; Sterling Publishers Private Limited, 2012, New Delhi-110 – 020.