BIOLOGICAL OXIDATION

Biochemistry-1 (PHL-284)

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- **Metabolic fuels.**
- **Stages in the extraction of energy from fuels.**
- **ATP .**
- **Oxidation of pyruvate to acetyl CoA.**
- **The citric acid cycle.**
- **Oxidative phosphorylation.**

METABOLIC FUELS

1. Fuels:

The carbohydrates, fats, and proteins that serve as the major fuels of the human are obtained from the diet. After digestion and absorption, these fuels may be oxidized for energy.
When fuels are oxidized to CO_2 and H_2O:

- Carbohydrates provide 4 kcal/g (partly reduced)
- Proteins provide 4 kcal/g and (partly reduced)
- Fats provide 9 kcal/g (highly reduced)

$$C_6H_{12}O_6 + 6O_2 \xrightarrow[\text{oxidation}]{\text{Biological}} 6CO_2 + 6H_2O + \text{E}\mathbf{nergy}$$

When fuels are oxidized in the body:

- Heat is generated to maintain body temperature.
- ATP is synthesized and is used for driving biochemical reactions, muscle contraction and other energy-requiring processes in the body.

$$ADP + P_i + \mathbf{Energy} \longrightarrow ATP$$

2. The composition of body fuel stores:

The average 70 kg man has fuel stores consisting of:

- 15 kg of triglyceride (lipids)
- 6 kg of protein
- 0.2 kg of glycogen (carbohydrates)

STAGES IN THE EXTRACTION OF ENERGY FROM FUEL MOLECULES

A. Stage 1:

1. The complex molecules of carbohydrates, proteins, and triacyglycerols are broken into smaller units such as
   - monosacharides,
   - amino acids,
   - glycerol,and fatty acids.
2. During this stage, **no** free energy is trapped.

B. Stage 2:

1. The simple molecules of different kinds are catabolized to a few molecules (mainly acetyl CoA).
2. In this stage, **some** free energy is trapped as ATP.

C. Stage 3:

1. Stage 3 consists of:
   a) The tricarboxylic acid (TCA) cycle.
   b) The Oxidative phosphorylation.
2. Together, these processes oxidize acetyl CoA to CO_2 and water along a common pathway.
3. In this stage, **most** free energy is trapped as ATP.

ATP (adenosine triphosphate)

1.Chemistry of ATP:

Adenine-ribose - P ~ P ~ P

- ATP contains the base **adenine**, the sugar **ribose**, and **three phosphate** groups joined to each other via two anhydride bonds
- ATP may be hydrolyzed to ADP and P_i

ATP ⟶ ADP + Pi $\quad \Delta G^{o'} = -7.3$ kcal/mol

or to AMP and PP_i (pyrophosphate)

ATP ⟶ AMP + PPi $\quad \Delta G^{o'} = -7.3$ kcal/mol

- The anhydride bonds of ATP are often called "**high-energy bonds**". Any bond whose breakdown is accompanied by large decrease in free energy (> 5 kcal/mol) is termed a high-energy bond.

2. Formation of ATP:

a) Oxidative phosphorylation (major pathway)

NADH ⟶ 3 ATP
$FADH_2$ ⟶ 2 ATP

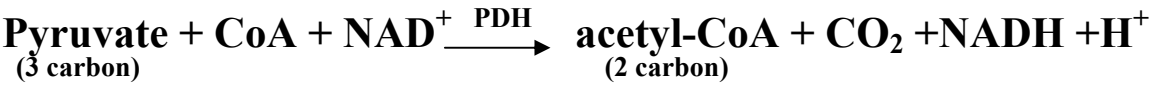
b) Substrate-level phosphorylation.

ADP can accept phosphate groups from compounds such as phosphoenolpyruvate

phosphoenolpyruvate + ADP ⟶ pyruvate + ATP

OXIDATION OF PYRUVATE TO ACETYL-CoA

(Oxidative decarboxylation of pyruvic acid)



This reaction (see figure) is catalyzed by a multienzyme complex known as **pyruvate dehydrogenase complex** (**PDH complex**).

This complex is formed of **three** enzymes
This reaction requires **five coenzymes.** These are:

- TPP (thiamine pyrophosphate)
- Lipoic acid.
- CoA.
- FAD.
- NAD^+.

Control of the PDH complex:

1.Product inhibition:

Both acetyl CoA and NADH inhibit the PDH complex.

2. Phosphorylation dephosphorylation:

Enzyme becomes **inactive** when it is phosphorylated.
Enzyme becomes **active** when it is dephosphorylated.
-Phosphorylation reaction is inhibited by pyruvate, NAD^+ and CoA (substrates of PDH).
-Phosphorylation is stimulated by excess acetyl CoA and NADH (products of PDH).

THE CITRTIC ACID CYCLE
(Krebs cycle or Tricarboxylic acid cycle)

TCA cycle oxidises acetyl CoA to two CO_2 molecules with the generation of 3 NADH, 1 $FADH_2$ and 1 GTP.

1. Site:

Mitochondrial matrix : 7 Enzymes .
Inner mitochondrial membrane : Succinate dehydrogenase

2. The Reactions of the TCA Cycle:

1. Acetyl CoA condenses with oxaloacetate (a 4-carbon keto-acid), forming citrate (a 6-carbon, ß-hydroxy tricarboxylic acid).

2. Citrate is isomerized to isocitrate, by a rearrangement of the molecule.

3. The first oxidative decarboxylation occurs when isocitrate is oxidized to α -ketoglutarate (a 5-carbon, α -keto dicarboxylic acid) CO_2 is produced, and the electrons are passed to NAD^+ to form NADH + H^+.

4. α-Ketoglutarate is converted to succinyl CoA in a second oxidative decarboxylation reaction, CO_2 is released, and the keto group of α -ketoglutarate is oxidized to an acid which combines with CoASH to form succinyl CoA. NADH + H^+ are produced.

5. The high-energy, thioester bond of succinyl CoA is cleaved, providing energy for the synthesis of GTP from

GDP and inorganic phosphate (a **substrate level phosphorylation**). Succinate (a 4-carbon, dicarboxylic acid) is formed.

6. Succinate is oxidized. Two hydrogens are removed together with their electrons, and FAD is converted to $FADH_2$. Fumarate (a 4-carbon, dicarboxylic acid with a double bond between carbons 2 and 3) is generated.

7. Water adds across the double bond of fumarate, generating malate (a 4-carbon, α -hydroxy dicarboxylic acid).

8. Malate is oxidized. Two hydrogens along with their electrons are removed from the α -carbon and its hydroxyl group. They are passed to NAD^+, producing $NADH + H^+$. Oxaloacetate is regenerated and, thus, the cycle is complete.

3. Energy Production by the TCA Cycle:

The NADH and $FADH_2$, produced by the cycle, donate electrons to the electron transport chain. From each NADH, **3 ATP** are generated, and from each $FADH_2$, **2 ATP** are generated.

- From 3 NADH, produced	9 ATP are generated
- From 1 $FADH_2$, produced	2 ATP are generated
- From 1 GTP, produced	1 ATP is generated
	12 ATP

4. Inhibitors of the TCA cycle:

1. **Fluoroacetate** inhibits **aconitase**.

2. **Arsenite** inhibits **α -ketoglutarate dehydrogenase.**

3. **Malonate** inhibits **succinate dehydrogenase**.

5. Regulation of the Cycle:

High ATP (or low ADP) will inhibit the cycle
Low ATP (or high ADP) will stimulate the cycle.
They are 3 possible regulatory enzymes which catalyze irreversible reactions:

1. Citrate synthetase:
- Allosterically inhibited by ATP.
- Competitively inhibited by succinyl CoA and citrate.

2. Isocitrate dehydrogenase:
- Allosteric activation by ADP.
- Inhibition of NADH and excess ATP.

3. α-ketoglutarate dehydrogenase:
- Inhibited by succinyl CoA, NADH and ATP.

6. Synthetic Functions of the TCA Cycle:

1. The Synthesis of Glucose:

The synthesis of glucose occurs by the pathway of gluconeogenesis, which involves intermediates of the TCA cycle.

2. The Synthesis of Fatty Acids:

The pathway for fatty acid synthesis from glucose includes reactions of the TCA cycle.

3. The Synthesis of Amino Acids:

The synthesis of amino acids from glucose involves intermediates of the TCA cycle.

4. The Synthesis of porphyrin from succinyl CoA.

Anaplerotic Reactions:

As intermediates are removed from the cycle for the synthesis of glucose, fatty acids, amino acids, or other compounds, the intermediates of the cycle must be replenished by anaplerotic reactions.

- A key **anaplerotic** reaction is catalyzed by **pyruvate carboxylase**, which carboxylates pyruvate, forming oxaloacetate.
- **Amino acids** may produce intermediates of the TCA cycle through anaplerotic reactions. e.g. glutamate to α-ketoglutarate and aspartate to oxaloacetate

OXIDATIVE PHOSPHORYLATION

1. Site: inner mitochondrial membrane

2. Mechanism of oxidative phosphorylation:
(Chemiosmotic hypothesis or Michell hypothesis)

Oxidation: Energy derived from the oxidation of NADH and $FADH_2$ is used to pump protons across the inner mitochondrial membrane from the matrix to the space between the inner and outer membrane. An electrochemical gradient is generated, consisting of a proton gradient and a membrane potential.

Phosphorylation: Protons move back into the matrix through the ATP synthase complex, causing ATP to be produced from ADP and inorganic phosphate.

Oxidation and phosphorylation are tightly coupled.
NADH and $FADH_2$ are oxidized only if ADP is available for conversion to ATP (that is, if ATP is being utilized and converted to ADP).

The oxidation of one NADH generates 3 ATP,
The oxidation of one $FADH_2$ generates 2 ATP.

<u>Uncoupling agents</u>:
They allow protons to cross the inner mitochondrial membrane and reenter the matrix without going through the pore in the ATP synthase complex. ATP production will not occur i.e. oxidation without phosphorylation.
Heat is generated.
Examples: **dinitrophenol** and **aspirin** in high dose

3. Electron Carriers:

A. NAD^+ and FAD

As food is oxidized to CO_2 and H_2O, electrons are transferred mainly to NAD^+ and FAD.

1. **NAD^+** accepts a hydrogen atom plus an additional electron (the equivalent of a hydride ion), which react with its nicotinamide ring. NAD^+ is reduced, the substrate (RH_2) is oxidized, and a proton is released.

$$NAD^+ + RH_2 \longrightarrow NADH + H^+ + R$$

The nicotinamide ring of NAD^+ is derived from the vitamin (nicotinic acid). It is also produced to a limited extent from the amino acid tryptophan.

2. **FAD** accepts two hydrogen atoms together with their electrons that react with its isoalloxazine ring. FAD is reduced and the substrate is oxidized.

$$FAD + RH_2 \longrightarrow FADH_2 + R$$

FAD is derived from a vitamin riboflavin.

B. Coenzyme Q (Ubiquinone).

Coenzyme Q may be synthesized in the body.It is not derived from a vitamin.

C. Heme

Heme is synthesized from glycine and succinyl CoA. It is not derived from a vitamin. The iron of the heme group is reduced when the cytochrome accepts an electron.

$$Fe^{3+} + e \longrightarrow Fe^{2+}$$

4. Electron Transport

A.Transfer of Electrons from NADH to O_2:

The transfer of electrons from NADH to oxygen occurs in **three** stages.

1.The transfer of electrons from NADH to Coenzyme Q:
Electrons are passed by the **NADH dehydrogenase** complex from NADH to flavin mononucleotide (FMN). FMN passes the electrons through a series of iron sulfur complexes to ubiquinone.

2.The Transfer of Electrons from Coenzyme Q to Cytochrome c:
Electrons are transferred from ubiquinone through Fe-S centers to cytochromes b and c1, which transfer the electrons to cytochrome c. The protein complex involved in these transfers is called **cytochrome c reductase**.

3.The Transfer of Electrons from Cytochrome c to Oxygen:
Cytochrome c transfers electrons to the cytochrome aa3 complex, which transfers the electrons to molecular oxygen, which is reduced to water. **Cytochrome c oxidase** catalyzes this transfer of electrons.

B.The Transfer of Electrons from $FADH_2$ to Oxygen

The transfer of electrons from $FADH_2$ occurs in **two** stages:

1. $FADH_2$ passes electrons to an iron sulfur center, which transfers them to Coenzyme Q.
2. The electrons are then transferred to cytochrome c **(cytochrome c reductase**) and from cytochrome c to oxygen **(cytochrome c oxidase**).

5. Inhibitors of Electron Transport and Oxidative Phosphorylation:

Inhibitor	Site of inhibition
Rotenone	NADH dehydrogenase
Antimycins	Cytochrome c reductase
Cyanide and carbon monoxide	Cytochrome c oxidase
Oligomycin	ATP synthase
Atractyloside	ADP-ATP antiport

6. Selected Medical Problems:

A. Cyanide Poisoning:

- Cyanide binds to Fe^{3+} in cytochrome c oxidase.
- As a result, O_2 cannot recieve electrons
- Oxidative phosphorylation is inhibited and death occurs within a very short time.

B. Acute Myocardial Infarction:

- Coronary occlusions may occur and regions of heart muscle may be deprived of blood flow.
- Lack of oxygen causes inhibition of the processes of electron transport and oxidative phosphorylation, which results in a decreased production of ATP.
- The heart muscle becomes damaged.
- If the damage is relatively mild, the person may recover. If the damage is severe, death may result.

7. Transport of NADH from cytosol to mitochondria:

NADH cannot directly cross the mitochondrial membrane. Therefore, the **electrons** are passed to the mitochondrial electron transport chain by two **shuttle** systems.

a) The Glycerol Phosphate Shuttle:
Cytosolic dihydroxyacetone phosphate(DHAP) is reduced to glycerol 3-phosphate by NADH. Glycerol 3-phosphate reacts with an FAD-linked dehydrogenase in the inner mitochondrial membrane. DHAP is regenerated and reenters the cytosol.
Each mole of **$FADH_2$** that is produced generates **2 moles of ATP** via oxidative phosphorylation.

b) The Malate Aspartate Shuttle:
Oxaloacetate is reduced to malate by NADH in the cytosol.The reaction is catalyzed by cytosolic malate dehydrogenase.The malate enters the mitochondrion and is reoxidized to oxaloacetate by the mitochondrial malate dehydrogenase that generates NADH in the matrix. Oxaloacetate cannot cross the mitochondrial membrane.In order to return carbon to the cytosol, oxaloacetate is transaminated to aspartate, which can be transported into the cytosol and reconverted to oxaloacetate by another transamination reaction.

Each mole of **NADH** generates **3 moles of ATP** via oxadative phosphorylation.