Session 4
Lecture 1
Oxidative Phosphorylation

Oxidative Phosphorylation (Stage 4 of Metabolism)

- **The complete oxidation of glucose can be represented by the following equation:**

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

$$\Delta Go = -\ 2{,}870\ kJ/mole$$

- **Till now only 2ATP and 2GTP are formed from oxidation of glucose which equal to 124 kJ/mole**
- **The question is Where has all the energy gone?**
- **The answer is that energy is still carried by the carriers of the reducing equivalents (NADH & FADH2)**

Consider the energy liberated from oxidation of these carriers:

- NADH + H^+ (FAD2H) → NAD^+ (FAD)

oxidised carrier → reduced carrier

H_2O → O

$$NADH + H^+ + O \rightarrow NAD^+ + H_2O \qquad \Delta G^\circ = -220 \text{ kJ/mole}$$

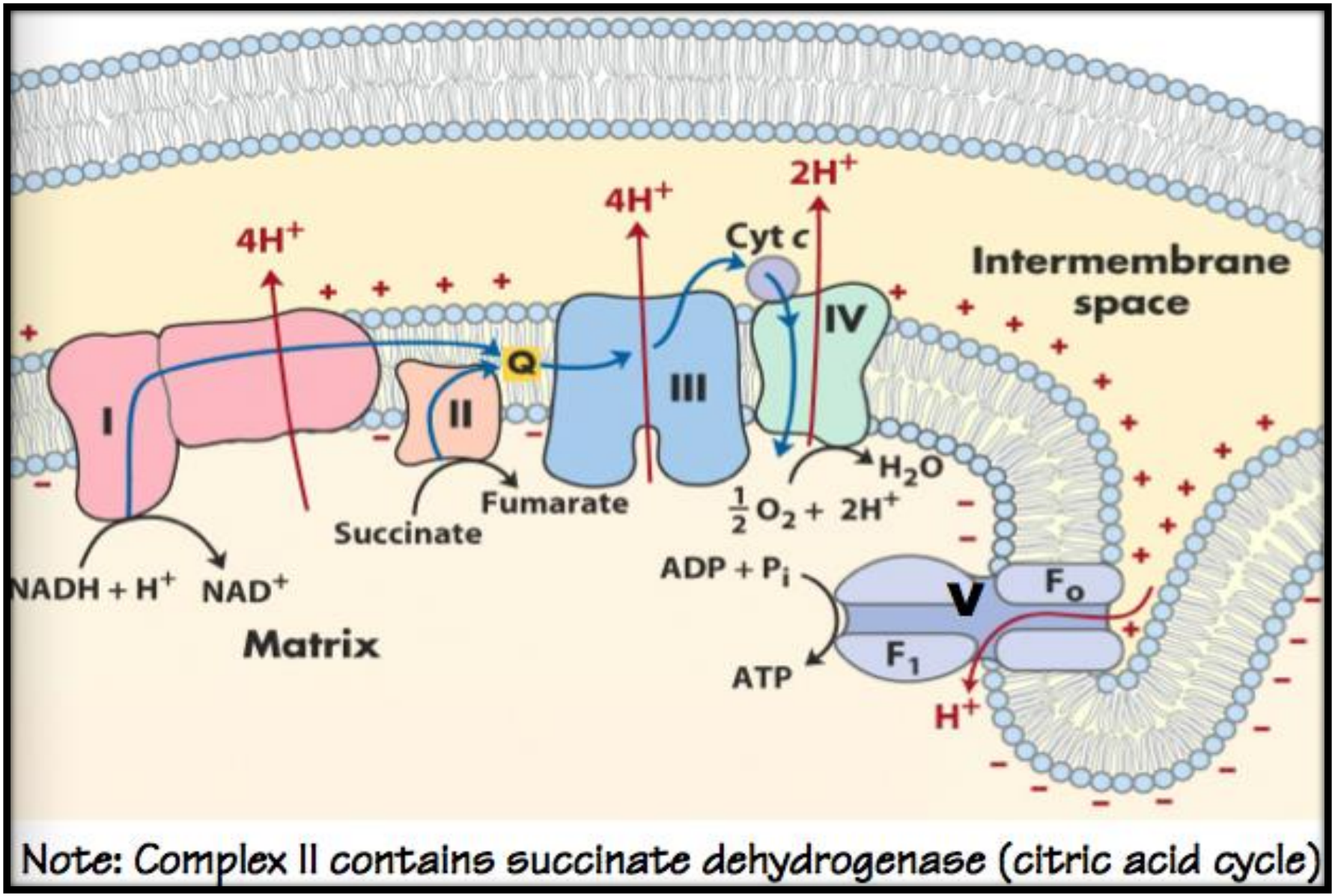
$$FAD2H + O \rightarrow FAD + H_2O \qquad \Delta G^\circ = -152 \text{ kJ/mole}$$

Oxidation of NADH and FADH2

- It is carried out in a pathway called oxidative phosphorylation (OP)
- OP represents 2 processes, the first is the oxidation of NADH and FADH2 in the **electron transport chain** and the second **phosphorylation** of ADP to produce ATP
- **The energy liberated from the oxidation of the carriers of the reducing equivalents was used for phosphorylation of ADP to produce ATP**

Electron Transport Chain (ET)

- **complex I** (NADH-Q oxidoreductase): oxidizes NADH and transfers electrons to coenzyme Q
- **coenzyme Q** (Ubiquinone)
- **complex П** (Succinate-Q reductase): oxidizes $FADH_2$ and transfers electrons to coenzyme Q
- **complex IП** (Q-cytochrome c oxidoreductase): passes the electrons to cytochrome c
- **complex IV** (Cytochrome c oxidase): passes the electrons to O_2
- **Comlex V** (ATP synthase): production of ATP.



Note: Complex II contains succinate dehydrogenase (citric acid cycle)

- Three of the complexes (I, IΠ and IV) in addition to transferring electrons, also act as **proton translocating complexes**.
- They use the **free energy** to move protons from the **inside** to the **outside** of the inner mitochondrial membrane. The membrane itself is impermeable to protons
- The proton translocating complexes transform the chemical bond energy of the electrons into an electro-chemical potential difference of protons. **This is known as the proton motive force (p.m.f).**

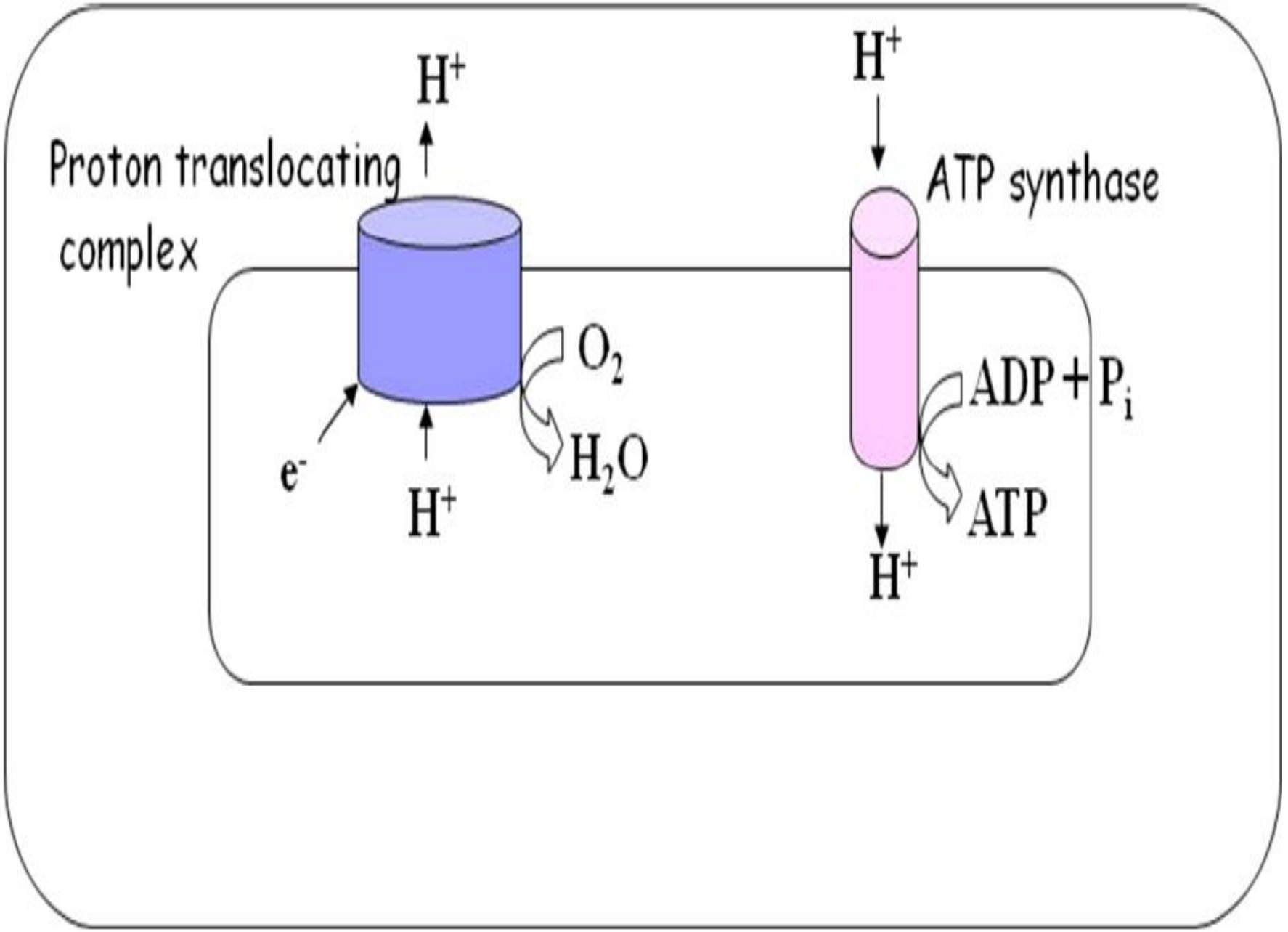
How does ATP synthesize after oxidation of NADH and FADH2?

- ATP hydrolysis results in the release of energy:

$$ATP + H_2O \rightarrow ADP + Pi \quad \Delta Go = -31 \text{ kJ/mole}$$

- This means that ATP synthesis from ADP and Pi requires +31 kJ/mole to drive the reaction
- This amount is derived from the **p.m.f** that has been produced across the inner mitochondrial membrane .
- Protons can normally only re-enter the mitochondrial matrix via the ATP synthase complex, driving the synthesis of ATP from ADP and Pi as in the following:

$$2H^+_{outside} + ADP + Pi \rightarrow 2\ H^+_{inside} + ATP$$


H+
Proton translocating complex
H+
ATP synthase
O2
ADP + Pi
e-
H2O
H+
ATP
H+

Coupling between electron transport (ET) and ATP synthesis

Normally ET and ATP synthesis are tightly coupled.

The mitochondrial concentration of ATP regulates both processes. **When [ATP] is high**:

- The [ADP] is **low** and the ATP synthase stops (lack of substrate).
- This prevents the transport of protons back into the mitochondria.
- The [H^+] outside increases to a level that prevents more protons being pumped.
- In the absence of proton pumping, the electron transport stops.
- The reverse occurs **when [ATP] is low.**

Inhibitors of OP

- **Uncouplers**
- **Inhibitors of electron transport**
- **Inhibitors of ATP synthase**

Uncouplers

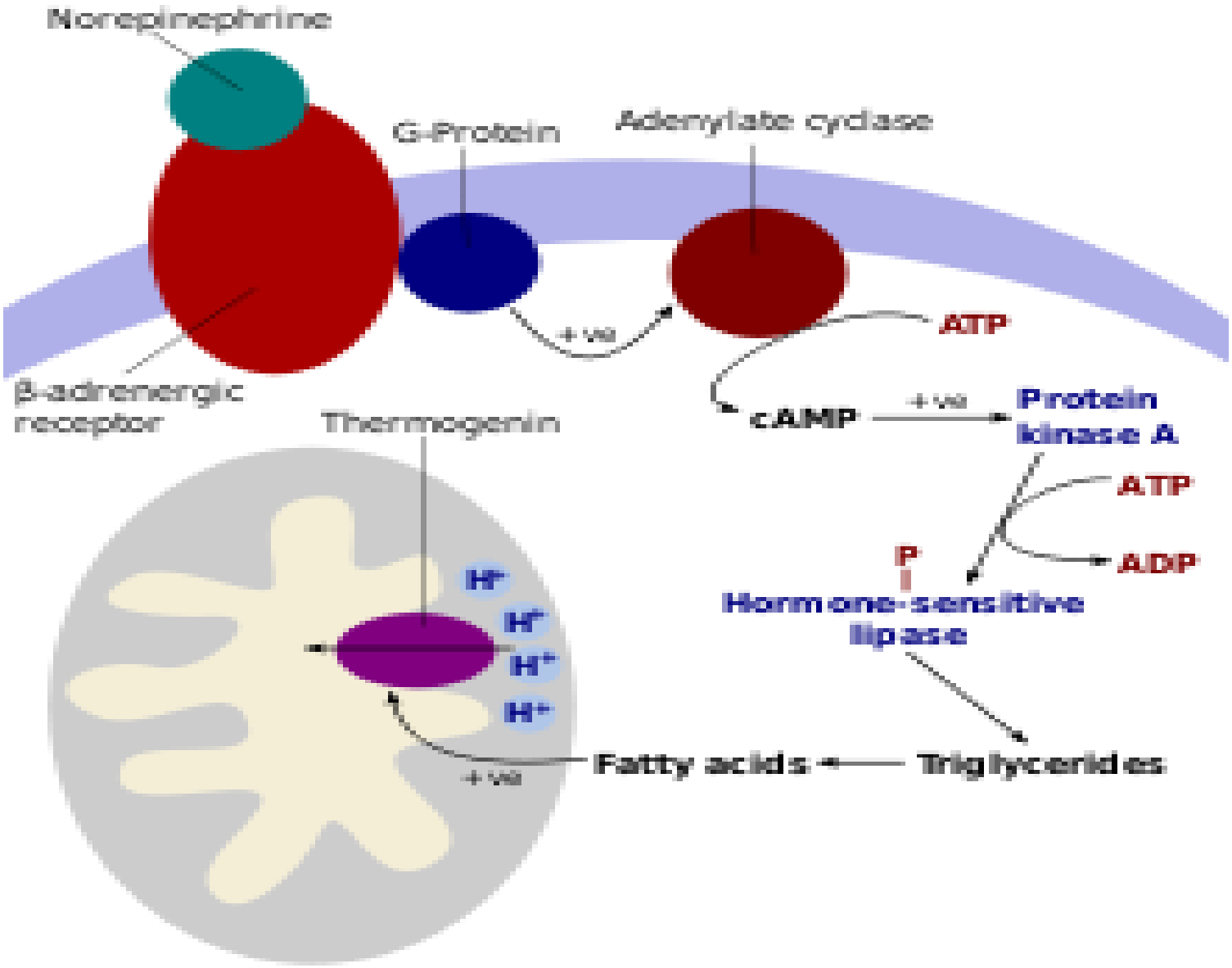
- Some synthetic substances like **dinitrophenol** and **dinitrocresol** increase the permeability of the inner mitochondrial membrane to protons
- ET continues, ATP synthesis stops and energy is dissipated as heat (**thermogenesis**).
- **Uncoupling proteins** (UCP1 to UCP5) are identified in tissues to produce heat.

Uncoupling proteins

- UCP1-3 are important to induce a leak of protons across the membrane, reducing the **p.m.f** and inhibiting **ATP** synthesis.
- Proton leak is physiologically important and accounts for 20-25% of the **basal metabolic rate**.
- UCP1 (previously known as thermogenin) is expressed in brown adipose tissue and is involved in non-shivering thermogenesis, which enables mammals to survive in cold environments.

Uncoupler Proteins

- In response to cold, noradrenaline is released and stimulates lipolysis releasing fatty acids to provide fuel for oxidation in brown adipose tissue
- Fatty acids breakdown produce FADH2 and NADH, driving ET and increasing the **p.m.f.** Noradrenaline also activates UCP1 to inhibit ATP synthesis, which dissipates the **p.m.f** as heat.
- UCP3 has been found in skeletal muscle, brown adipose tissue and the heart and it appears to be involved in modifying fatty acid metabolism


Norepinephrine
G-Protein
Adenylate cyclase
β-adrenergic receptor
+ Mg
ATP
cAMP
+ Mg
Protein kinase A
ATP
ADP
P
Hormone-sensitive lipase
Thermogenin
H+
H+
H+
H+
+ Mg
Fatty acids
Triglycerides

Inhibitors of Electron Transport

- **Carbon monoxide, cyanide, rotenone and antimycin are inhibitors for components of ET.**
- **Without the p.m.f, ATP cannot be synthesized and no heat is generated. Irreversible cell damage rapidly occurs**

Oxidative stress

- During oxidative phosphorylation, reactive oxygen species are produced as a by-product.
- Mitochondria produce superoxide radicals ($O2^{-}\bullet$) which may be converted to hydrogen peroxide (H_2O_2) .
- Some superoxide radicals react with nitric oxide to form peroxynitrite (ONOO-) and hydroxyl radicals (•OH).
- These highly reactive agents can cause damage to cells, particularly to membranes.
- Some cells, such as neutrophils release large amounts of ROS to destroy bacteria