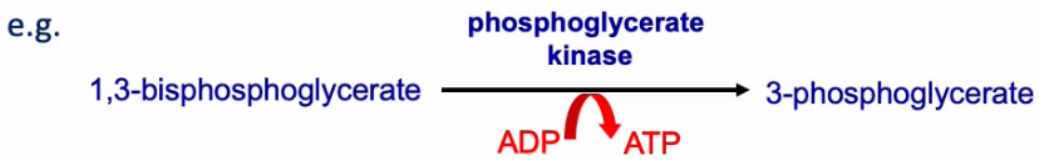
2.7 Cell Integrity

1-POM-1-2: Cell integrity: Explain how metabolism generates utilisable energy, and why this is important for maintaining cell integrity.

Oxidative Phosphorylation

▼ How does substrate level phosphorylation differ from oxidative phosphorylation?

- Substrate level phosphorylation is the production of ATP by the **direct transfer of a high energy phosphate group** from an intermediate substrate to ADP e.g. kinases



- Oxidative phosphorylation utilises an **electron transport chain** to drive ATP production

▼ Where does OxPhos take place?

inner mitochondrial membrane

▼ How do the cristae of the mitochondria facilitate ATP production by oxidative phosphorylation?

they provide a greater surface area for the membrane-bound components of the electron transport chain (increase SA for ox phos to take place)

▼ What is a common cause of failure of oxidative phosphorylation?

lack of oxygen (oxygen dependant)

▼ What is the difference between hypoxia and anoxia?

hypoxia - reduced oxygen supply

anoxia - total oxygen loss

▼ Why is it aerobic?

as oxygen is the final electron acceptor

▼ What happens to the co-enzymes in OxPhos and why is this important?

they are re-oxidised so the energy released generates phosphoanhydride bonds, subsequently making ATP from ADP.

▼ What are the overall reactions for the oxidation of NADH and FADH2 by oxygen in the mitochondria?

delta G for ATP hydrolysis is -31, so multiple ATPs can be produced from this

Within the mitochondria, the co-enzymes NADH and $FADH_2$ are re-oxidised by molecular oxygen in the reactions:

$NADH + H^+ + ½\ O_2$	$\longrightarrow$ $NAD^+ + H_20$	ΔG = **-220** kJ/mol
$FADH_2 + ½\ O_2$	$\longrightarrow$ $FAD + H_20$	ΔG = **-167** kJ/mol

Electron Transport Chain

▼ What does the (ETC) consist of? (6)

4 membrane proteins:

- Complex I (NADH dehydrogenase)
- Complex II (Succinate dehydrogenase)
- Complex III (Q-cytochrome C oxidoreductase)
- Complex IV (cytochrome c oxidase)

2 mobile electron carriers:

- Co-enzyme Q (ubiquinone)
- Cytochrome C

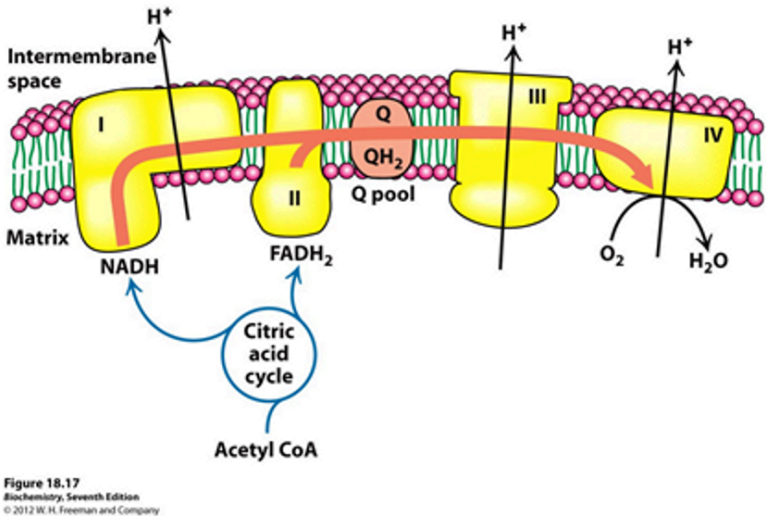


Figure 18.17
Biochemistry, Seventh Edition

▼ How and why are electrons passed along the ETC?

- Electrons are transferred from one complex to another due to the oxidation of NADH and FADH2 which is passed through each of the complexes.
- The electrons lose energy along the chain which is used to pump H+ ions from the mitochondrial matrix to the intermembrane space

Transfer of electrons is energetically favourable

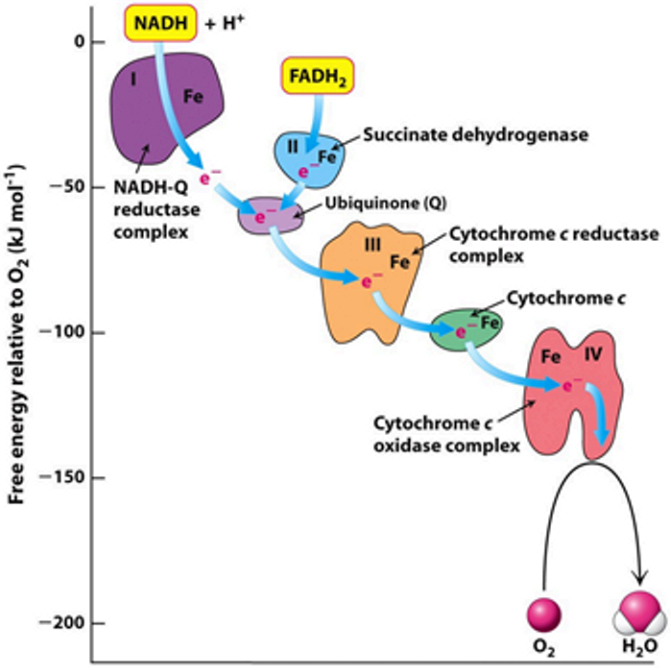


Figure 18.6
Biochemistry, Seventh Edition

▼ Which complexes in the ETC use NADH as a co-factor?

Complexes I, III and IV (accept electrons and allows acceptance of protons from aq. Solution, as electrons pass through complexes, protons pumped to inter-membrane space)

▼ What is the role of Complex II in the ETC?

- electrons are passed from FADH2 to co-enzyme Q which also picks up 2 protons from the matrix to form QH2 and regenerate FAD+
- Complex II doesn't pump electrons unlike the others

(complex II, succinate dehydrogenase also enzyme of TCA)

(as you bypass complex I fewer protons into intermembrane space compared to NADH so fewer ATP)

▼ At which complex in the ETC is water produced?

Complex IV (oxygen is final electron acceptor)

▼ What is the order in which electrons flow from one member of the chain to another?

Complex II / *I*, Co enzyme Q, Complex III, Cytochrome C, Complex IV

▼ Why does FADH2 form 2 ATP molecules compared with NADH which forms 3? (Succinate dehydrogenase)

- FADH2 bypasses complex I when being oxidised (oxidised at complex II)
- 1 fewer proton is pumped into the intermembrane space compared with NADH which is oxidised at complex I
- high conc. of protons in intermembrane space and low con. in matrix
- protons flowing back into the matrix down the gradient via ATP synthase are used to generate ATP
- so fewer ATP molecules are generated by the reoxidation of FADH2 than NADH

Redox

▼ What is a redox couple?

A molecule that can exist in both an oxidised and reduced form e.g NAD+/NADH, FAD/FADH2, O2,H20

▼ What is a redox potential?

the ability of a redox couple to accept or donate its electrons

▼ What does a standard negative redox potential imply (measured by hydrogen electrode)?

The redox couple has a tendency to **donate** electrons so it has more reducing power than hydrogen.

e.g. NAD+/NADH $E^0 = -0.32$ V

▼ What does a standard positive redox potential imply?

The redox couple has a tendency to **accept** electrons so it has more oxidising power than hydrogen

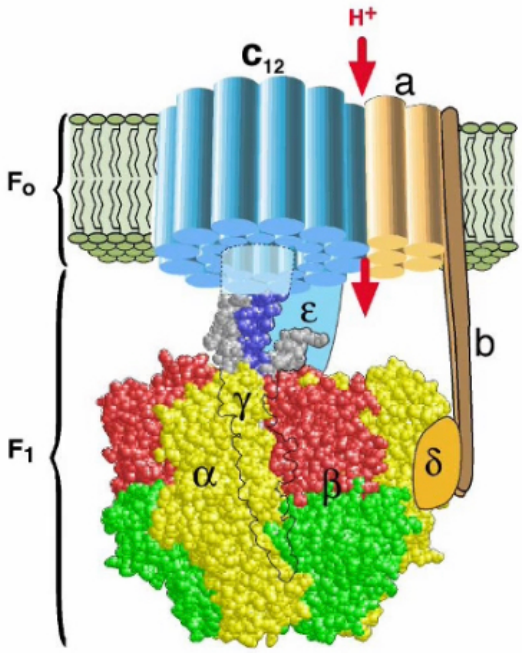
e.g.	Fe^{3+} / Fe^{2+}	$E^0 = +0.77\ V$
	$½\ O_2 + 2\ H^+ / H_2O$	$E^0 = +0.82\ V$

ATP synthase

▼ Which two components make up ATP synthase and how many subunits are there in each?

ATP synthase is a mol ulnar turbine powered by protons

- **F0 (membrane-bound)** - has a,b and c subunits
  - c subunit rotates
- **F1 (projecting into matrix)** - has alpha, beta and gamma subunits (these have altering affinities for ADP, ATP, Pi)



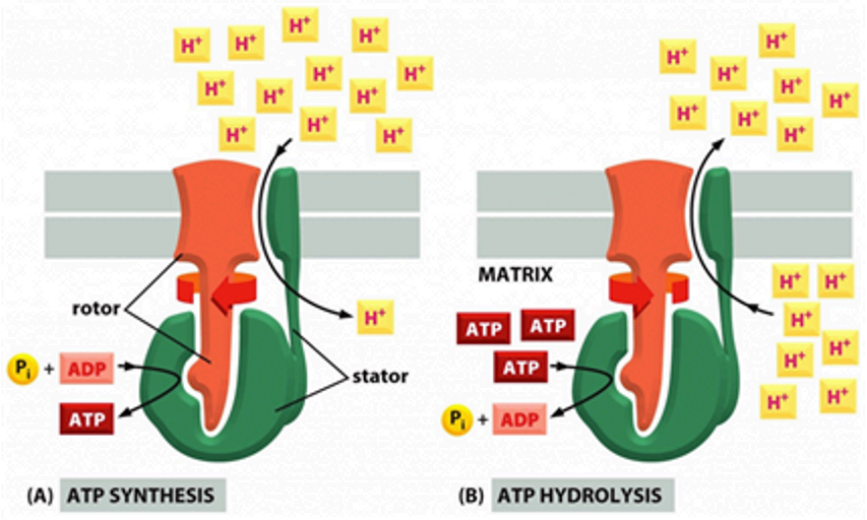
▼ How does ATP synthase work?

1. protons flow into the matrix → the F0 component of ATP rotates → conformational change in F1 subunit = Pi combines with ADP and form ATP (confirmation energy translated into chemical energy in phosphoanhydride bonds of ATP)

Direction of electron flow dictates whether enzyme makes or consumes ATP

Into matrix - synthesis

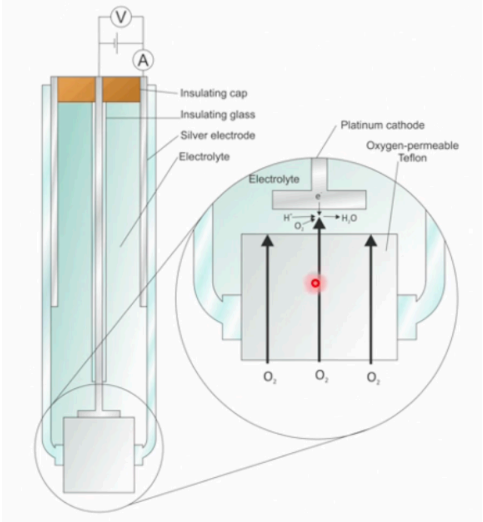
Out of matrix - hydrolysis



Oxygen electrode

▼ What is the oxygen electrode made of?

small chamber with a base made of a teflon membrane permeable to oxygen, underneath which is a compartment with a platinum cathode and silver anode



▼ What is it used for?

measure OxPhos in a lab (by measuring consumption of oxygen)

▼ How does it work?

- silver anode and platinum cathode suspended in electrolyte (KCl)
- oxygen dissolved in electrolyte

- small voltage of 0.6 volts is applied between the electrodes
- oxygen is reduced to water at the Pt cathode (- Ve)

$$O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O$$

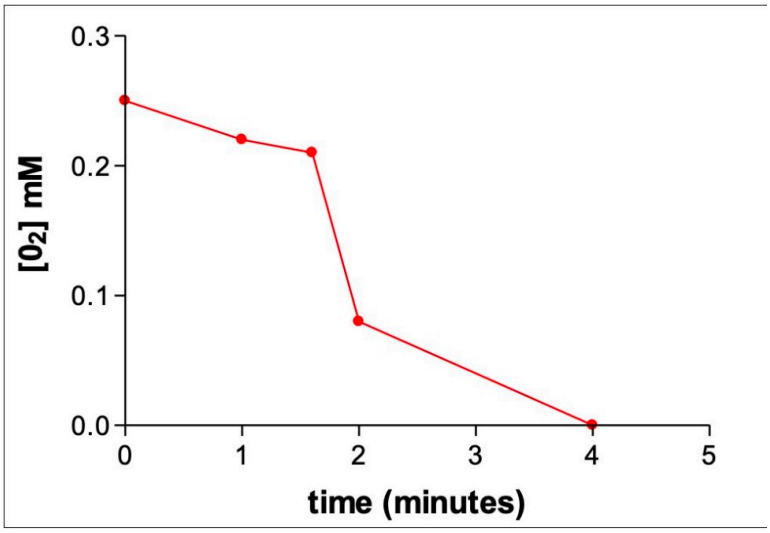
- silver is slowly oxidised to AgCl at the silver anode due to KCl electrolyte

$$4\,Ag^0 + 4Cl^- \rightarrow AgCl + 4e^-$$

- the resulting current is proportional to the O2 conc in the sample chamber

▼ How can we use it to measure OxPhos?

1. prepare a suspension of mitochondria
2. place suspension into chamber of oxygen electrode
3. monitor O2 consumption in a given time



▼ What is this graph showing between 0 and 1.7 mins?

no additives, oxygen consumed by mitochondria, gentle decline (**basal respiration**)

▼ What is this graph showing between 1.7 and 2 mins?

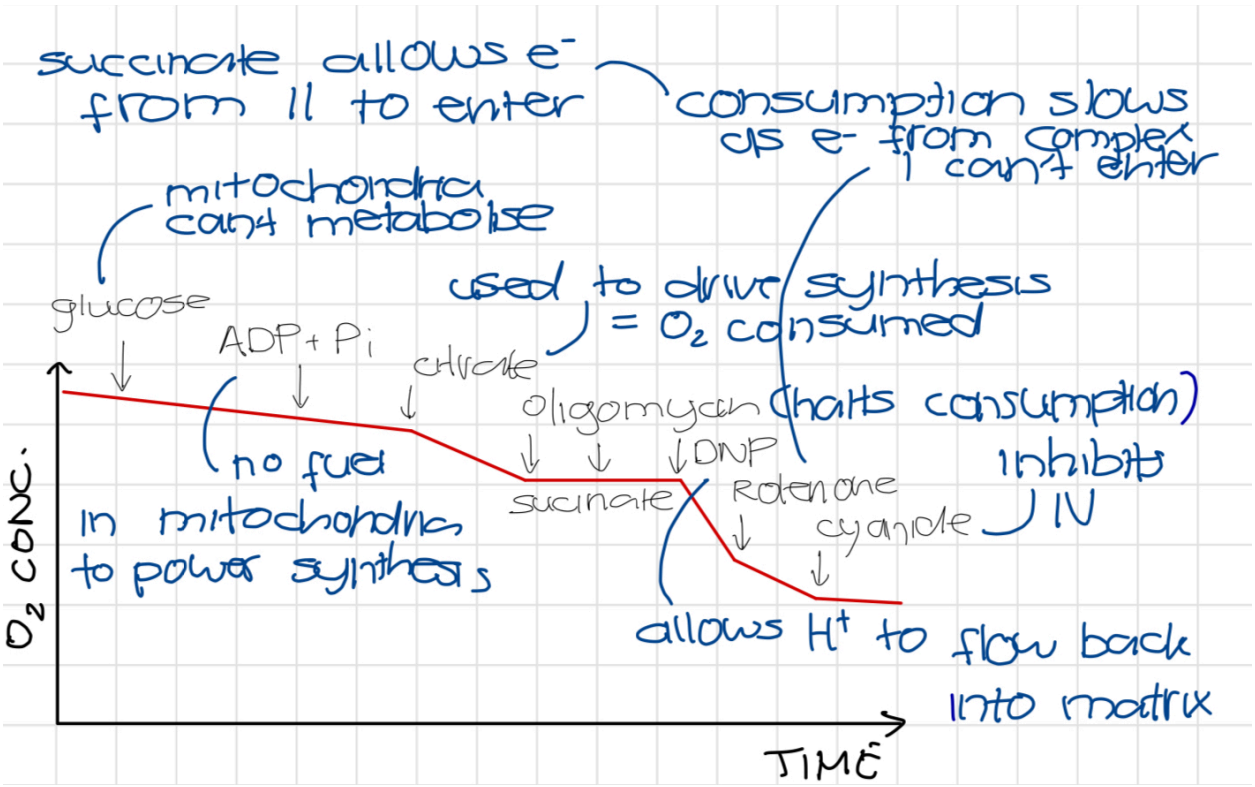
- ADP added at 1.7 mins
- rapid consumption of oxygen by **Oxidative Phosphorylation**
- This is **respiratory control** which allows the body to adapt oxygen consumption to the actual energy requirements (amount of ADP and Pi there)
- oxygen reduced to water by electrons along etc
- ADP consumed at 2 mins

▼ What is this graph showing between 2 and 4 mins?

oxygen gradually used up until there is none left **(O2 exhausted)**

Metabolic poisons

(all decrease or stop oxygen consumption except rotenone has no effect)



▼ Give two ways in which metabolic poisons interrupt ATP synthesis.

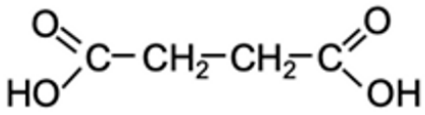
1. interfere with flow of electrons along ETC
2. interfere with flow of protons through ATP synthase

▼ How does **rotenone** act as a metabolic poison?

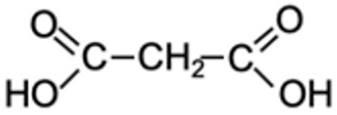
- inhibits transfer of electrons from complex I to co-enzyme Q (ubiquinone)
- less NADH oxidised
- smaller H+ gradient
- less ATP produced
- (Metabolism of succinate bypasses complex 1 thus rotenone has no effect on oxygen consumption)

▼ How does **malonate** act as a metabolic poison?

- resembles succinate
- acts as a competitive inhibitor of complex II (succinate dehydrogenase)
- slows down flow of electrons from succinate to co-enzyme Q (ubiquinone) (from FAD) by inhibiting the oxidation of succinate to fumarate



succinate



malonate

▼ How does **cyanide/azide** act as a metabolic poison?

- binds to Fe3+ in complex IV
- blocks the flow of electrons through the ETC

▼ How does **oligomycin** act as a metabolic poison?

- antibiotic that binds to stalks on ATP synthase
- blocks H+ channel
- creates a high conc. of H+ in intermembrane space

- prevents flow of electrons down ETC as the energy they lose can’t be used to pump H+ into intermembrane space due to steep gradient
- no ATP

▼ How does **dinitrophenol** (DNP) act as a metabolic poison? (initially used to lose weight, but toxic)

- transports H+ from intermembrane space into mitochondrial matrix
- bypasses ATP synthase
- no ATP is produced
- increases metabolic rate and body temp as energy is lost as heat as more protons have to be pumped more fuel metabolised to make ATP

▼ What is **non-shivering thermogenesis**?

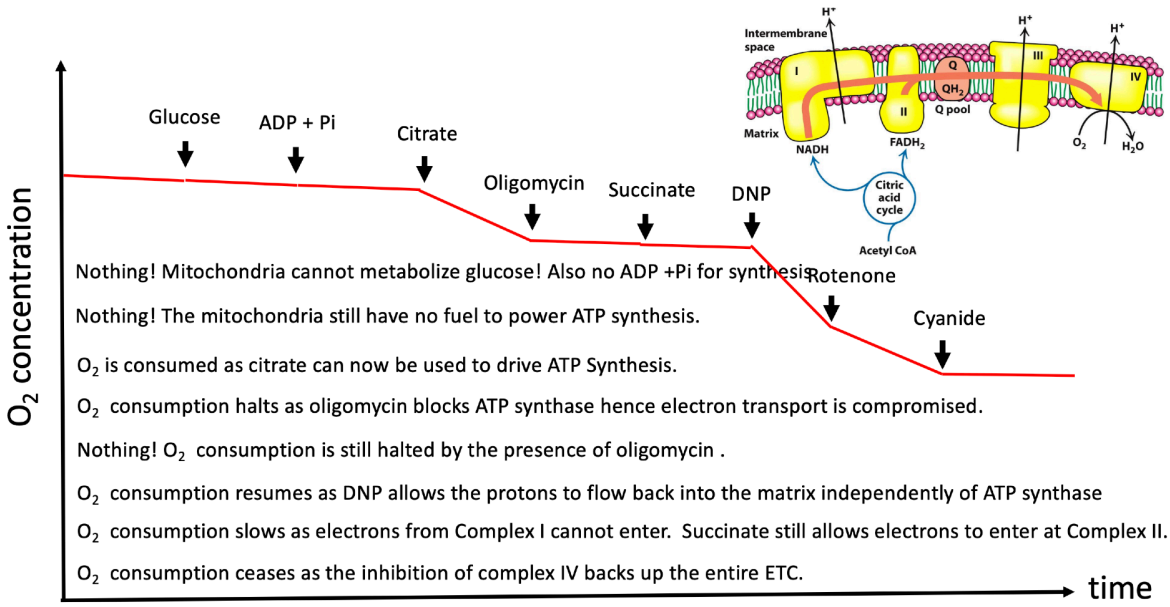
increase in metabolic heat production by uncoupling of OxPhos

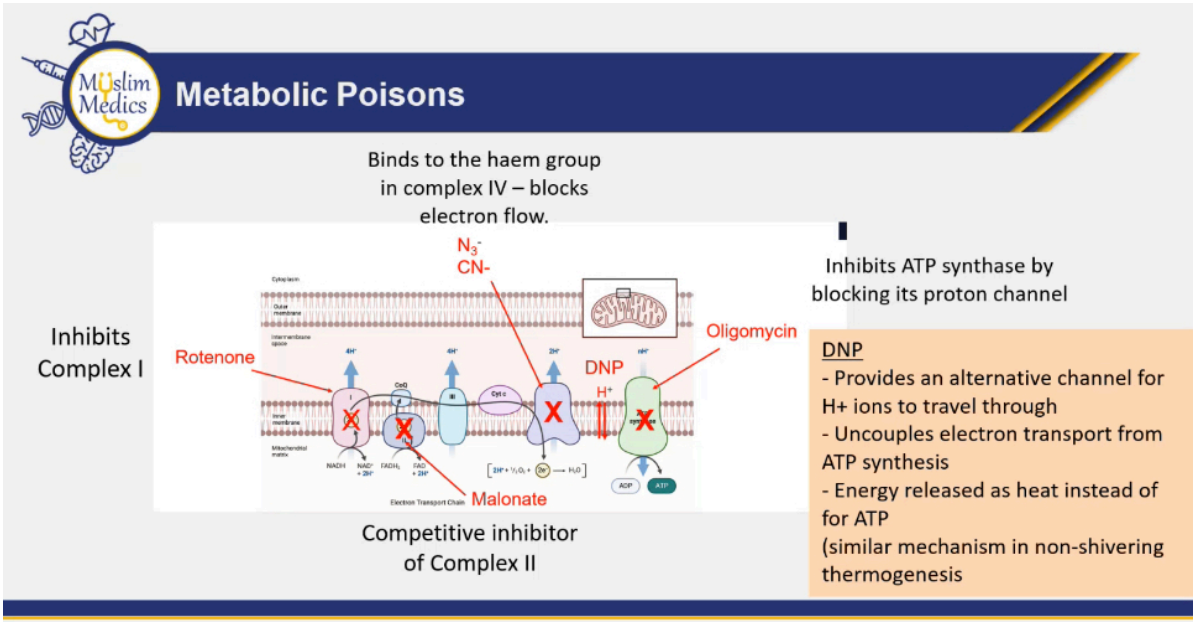
▼ What is it caused by?

- UCP-1 is activated in response to a drop in core body temp
- acts as a channel for H+ to move into matrix and bypass ATP synthase
- energy in H+ gradient is lost as heat

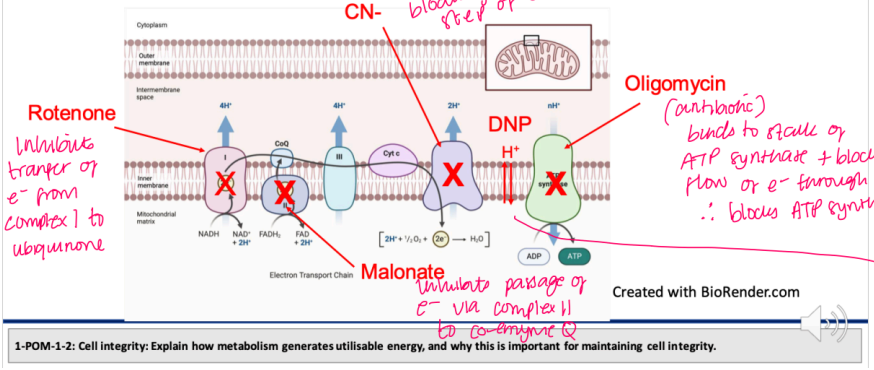
▼ **Summary of 5 metabolic poisons and where they act**

rotenone, malonate, cyanide/azide, dinitrophenol, oligomycin


O_2 concentration
Glucose
ADP + Pi
Citrate
Oligomycin
Succinate
DNP
Rotenone
Cyanide
time
Intermembrane space
Matrix
NADH
$FADH_2$
Q
QH_2
Q pool
Citric acid cycle
Acetyl CoA
O_2
H_2O
Nothing! Mitochondria cannot metabolize glucose! Also no ADP +Pi for synthesis
Nothing! The mitochondria still have no fuel to power ATP synthesis.
O_2 is consumed as citrate can now be used to drive ATP Synthesis.
O_2 consumption halts as oligomycin blocks ATP synthase hence electron transport is compromised.
Nothing! O_2 consumption is still halted by the presence of oligomycin .
O_2 consumption resumes as DNP allows the protons to flow back into the matrix independently of ATP synthase
O_2 consumption slows as electrons from Complex I cannot enter. Succinate still allows electrons to enter at Complex II.
O_2 consumption ceases as the inhibition of complex IV backs up the entire ETC.


Muslim Medics
Metabolic Poisons
Binds to the haem group in complex IV – blocks electron flow.
N_3^-
CN-
Inhibits ATP synthase by blocking its proton channel
Inhibits Complex I
Rotenone
Oligomycin
DNP
H^+
Malonate
Competitive inhibitor of Complex II
DNP
- Provides an alternative channel for H+ ions to travel through
- Uncouples electron transport from ATP synthesis
- Energy released as heat instead of for ATP
(similar mechanism in non-shivering thermogenesis

Metabolic poisons – Summary



1-POM-1-2: Cell integrity: Explain how metabolism generates utilisable energy, and why this is important for maintaining cell integrity.