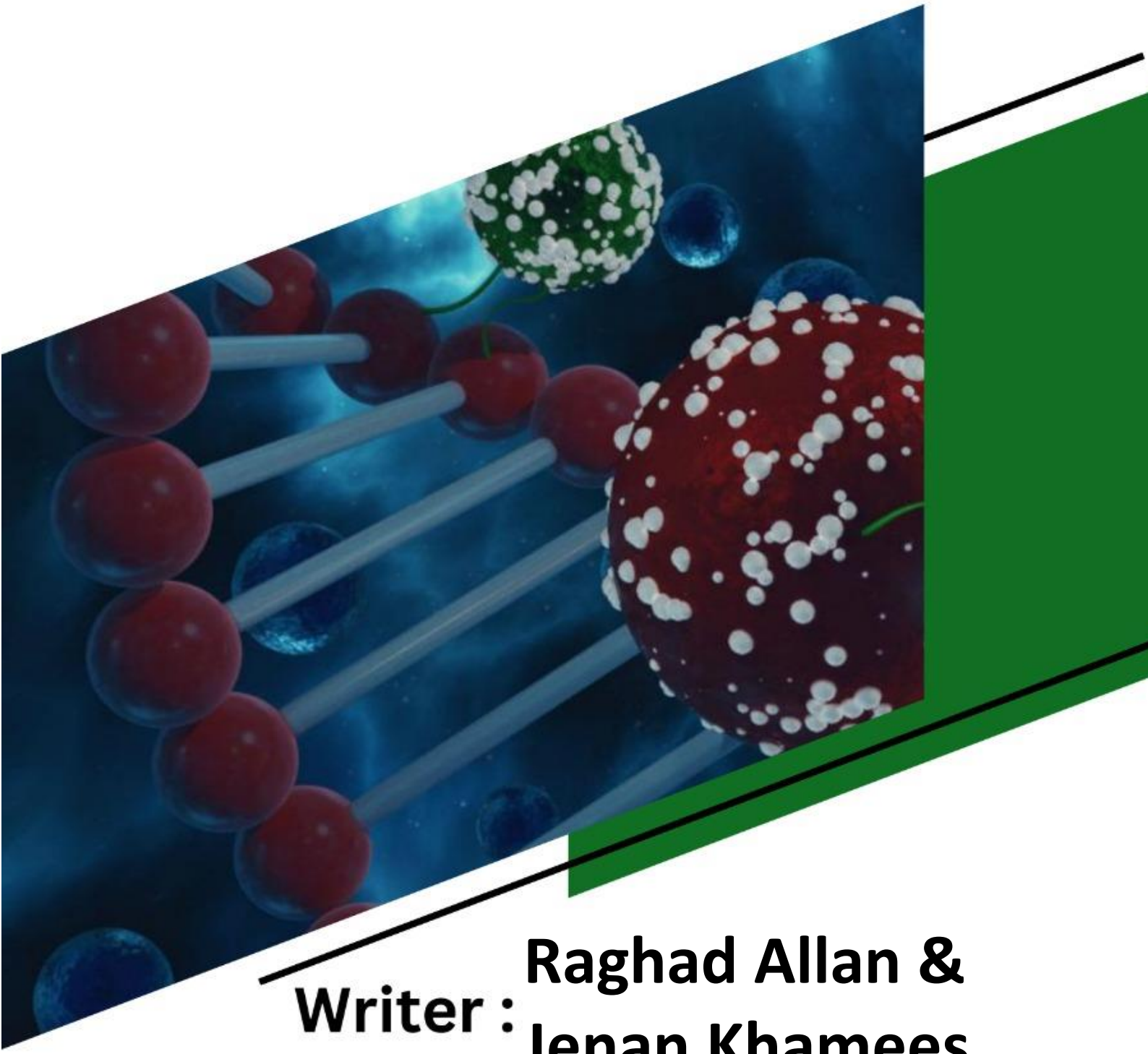
Doctor 021

METABOLISM

Sheet no. 8



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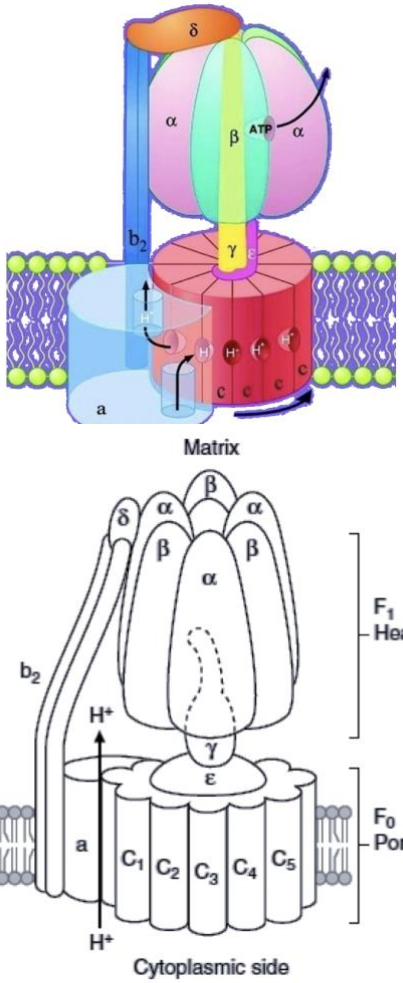
ATP SYNTHASE

- ✓ **F1:**
  - ➢ **"γ" subunit: rotates**
  - ➢ **"β" subunit: binds**
  - ➢ **"α" subunit: structural**
  - ➢ **3 conformations: tight (T), loose (L), open (O)**
- ✓ **F0:**
  - ➢ **"a" subunit: point of entry & exit**
  - ➢ **"c" subunit rotates**
  - ➢ **4H+/ATP**
- ✓ **Can run backwards**



Why does pumping of protons occur?

To generate ATP.

Common Exam Question: What is the driving force for ATP synthesis?

ATP synthesis occurs due to **proton motive force**, which is the flow of protons from outside to inside (from inter-membrane space into the matrix of the mitochondria).

Those protons will come inside by a big complex called **ATP Synthase** which is also called **Complex V** (because it comes after **Complex IV**).

ATP Synthase complex is described as a lollipop, it has a part embedded in the inner mitochondrial membrane, and a head piece projecting towards the matrix of the mitochondria.

-The part embedded in the membrane is called F0 portion.

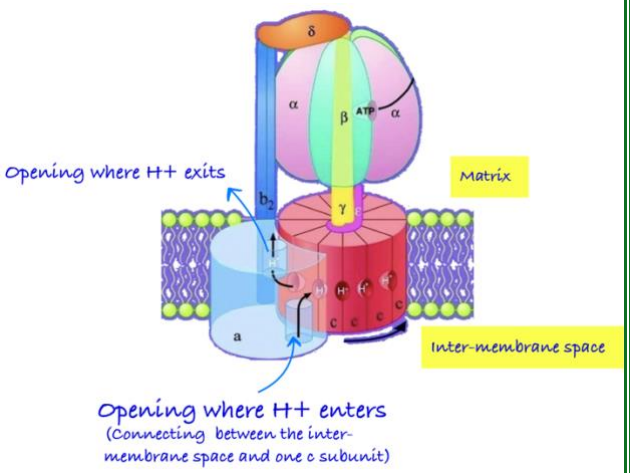
-The part projecting towards the matrix is called F1 Head piece.

F0 portion contains 12 **c subunits** forming a cylinder shape, this cylinder is covered from one side by another subunit that is called **a subunit.**

"a subunit" has two openings, one opening is projecting to the matrix, the other is projecting toward the inter-membrane space (the two openings are not connected to each other).

H+(protons) existing in the inter-membrane space enter through the first opening facing one of the **C subunits.**

Remember: any subunit is a polypeptide chain, which is a sequence of amino acids.



The amino acid that is facing the opening directly is **Glutamate**, the negative form of Glutamic Acid.

H^+ (one proton) will then bind to the Glutamate, removing its negative charge (neutralising it), causing a conformational change in the **c subunit** facing the opening.

This conformational change causes a movement of that c subunit, exposing another c subunit to the opening, after that, this proton will leave through the other opening to the matrix, then another binding of H+ happens, and consequently another conformational change happens and so on.

Note: Binding of each H^+ causes the c subunit to conformationally change, and releasing it causes the c subunit to return to its original form (binding and releasing of each proton depends on the different pH values)

This movement is what causes the cylinder to keep rotating, and this rotation is proven experimentally.

-Since we have 12 **c subunits**, we need 12 H^+ to fully rotate the cylinder.

H^+ is moved from outside to inside(matrix), releasing energy expressed as a rotation (conformational changes).

-Did we produce ATP?

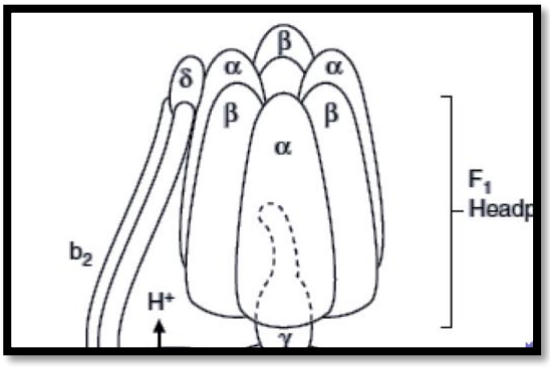
Not yet!

-How will this rotation produce ATP eventually?

Let's first move to the other part of ATP synthase complex, F1 head piece.

F1 head piece consist of:

- **3α subunits**: For structural reasons (No catalytic activity).
- **3β subunits**: for catalytic activity (producing ATP).



Alpha and Beta subunits goes in sequential manner (α, β, α, β,…)

- And **one γ subunit**

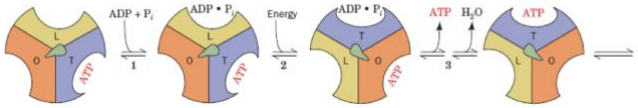
Gamma subunit **γ** is in the middle of the cylinder, it rotates with the rotation of the c subunits (cylinder).Due to its angled tip, it will keep hitting the Beta subunits, causing changes in their conformation.

Each Beta subunit goes through 3 conformations:

-Loose (L)

-Open (O)

-Tight (T)



Inorganic phosphate

Switching between them produces ATP out of (ADP and Pi)

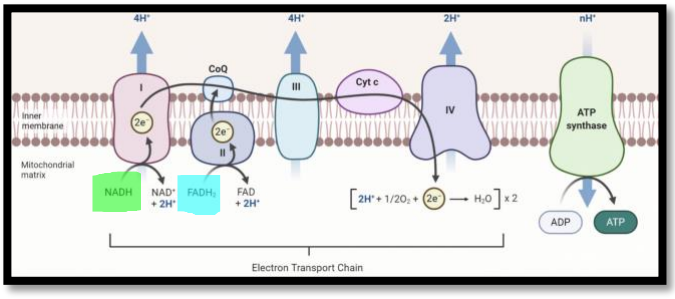
You are not required to know the function of each conformation

-For a full rotation, 12 H^+ are needed, releasing 3 ATP molecules.

So, to produce 1 ATP molecule, we need 4H^+

That's what explains the **2.5 ATP** molecules produced by the 2 electrons held by NADH. (Pumping 10 H^+ will produce 10/4= 2.5 ATP molecules).

And the **1.5 ATP** molecules produced by the 2 electrons of FADH2. (Pumping 6 H^+ will produce 6/4= 1.5 ATP molecules).



ATP Synthase can run backwards by reversing the electrochemical gradient. (If protons inside are higher than outside).

This will cause protons to enter from the other opening, rotating the cylinder and γ subunit the other side,

Then the enzyme will be called **ATPase** which breaks down ATP producing ADP and Pi.

-This occurs when the proton motive force is not strong enough to generate ATP.

ENERGY YEILD FROM THE ETC

- **NADH, -53 kcal, ATP?**
- **FADH2, -41 kcal, ATP?**
- **ΔGo' is so negative, never reversible**
- **ATP machine efficiency, (anions, Ca+2, heat, phosphate, substrates)**
- **Electron transport chain is our major source of heat**

Is producing ATP this way efficient?

No!

Efficiency of ATP synthesis is calculated by dividing the actual energy produced over the theoretical one.

For NADH: (giving its electrons to O_2)

Theoretically: 53 kilo calories

Actually: 2.5 ATP molecules, 2.5*7.3=19 kilocalories

19/53= Nearly 34%

For FADH2 : (as an electron donor)

Theoretically: 41 kilo calories

Actually: 1.5 ATP molecules, 1.5*7.3=11 kilocalories

11/41= Nearly 25%

Remember: 7.3 kcal/mol is the energy produced from ATP hydrolysis.

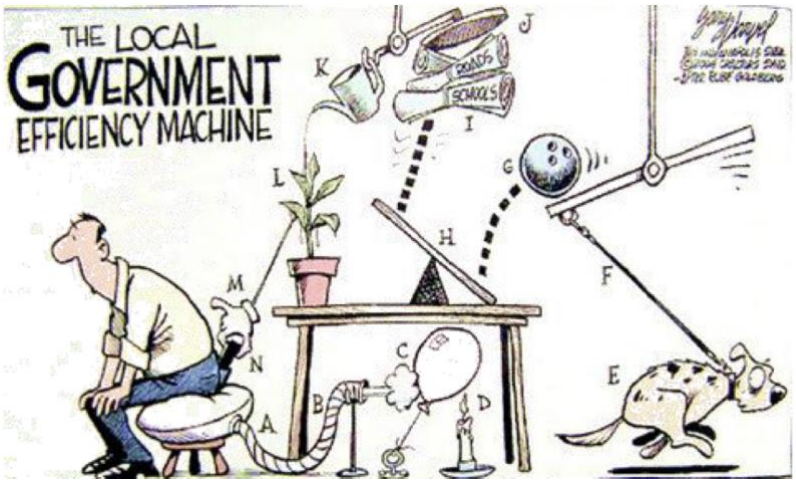
Where is the wasted energy?

1-Heat.

Presented by the non-shivering thermogenesis (Adaptive thermogenesis) and will be explained later in this sheet.

2-To move molecules across the inner mitochondrial membrane.

Remember that the inner mitochondrial membrane is impermeable, so for any molecule to pass this membrane (to inside or outside), it needs a carrier(transporter) which might need that energy.



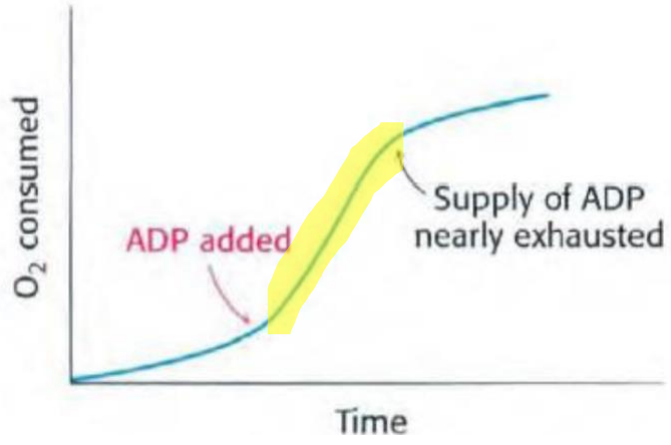
REGULATION:

- **What OxPhos needs? (NADH, O2, ADP, and Pi)**
- **In skeletal muscles, 20% drop in ATP concentration.**
- **In the heart, Ca^{+2} activates TCA enzymes for extra push (NADH, ATP), no drop.**
- **ET is tightly coupled to phosphorylation (simultaneously)**
- **ADP is the most important factor in determining the rate.**
- **The regulation of the rate of oxidative phosphorylation by the ADP level is called respiratory control.**

-The main physiological regulator of the Electron Transport Chain is ADP, it is the main regulator in Citric Acid Cycle too. (ADP allosterically activates Isocitrate Dehydrogense enzyme).

-It is called **Respiratory Control** since it controls the process of aerobic respiration.

-We check the level of O_2 consumption to determine whether ADP controls the process or not.



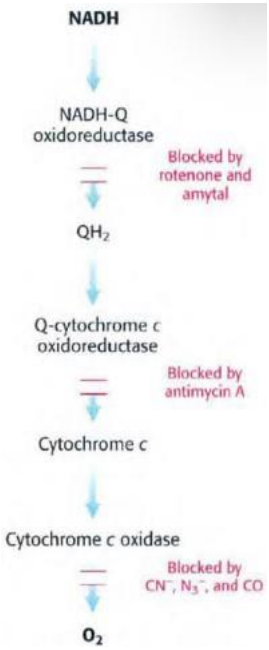
-In the absence of ADP, the process is going slowly according to time.

-Once we add ADP which acts as a stimulator for the process, there is a sharp increase in O_2 consumption.

-The process is back to the way it was once ADP supply is over.

INHIBITION OF ETC:

- Can occur at any stage.
- **Specific inhibitors:**
  - ✓ **Cyanoglycosides are present in edible plant pits: (e.g.amygdalin)**
  - ✓ **Oligomycin prevents the influx of H^+ through ATP synthase.**



Specific inhibitor	Target
Rotenone (insecticide) & Amytal (sedative)	Complex I
Antimycin A (antibiotic)	Complex III
Cyanide (CN-), Azide (N_3-), & (CO)	Complex IV
Oligomycin (antibiotic)	* Complex V
Atractyloside	Translocase

***ATP synthase complex**

-Those are molecules which inhibit ETC.

-If we inhibit any of the complexes mentioned above, CoQ or cytochrome c, the flow of electrons will stop. Pumping of protons will stop as well resulting in no ATP production.

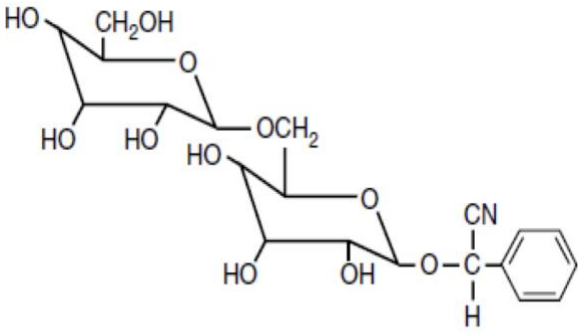
-Inhibitors of the electron transport chain are fatal at appropriate concentrations.

-Cyanide is fatal because it binds (where Oxygen binds) on the heme in **complex IV**, preventing the reduction of O_2 ›› no flow of electrons ›› pumping of protons stops.

*Note: **f0** portion of ATP synthase is the part inhibited by **Oligomycin**, this explains the fragment's name.(**Oligomyocin** inhibits the flow of protons into the matrix by the **a subunit** of **f0** fragment).

-Cyanide is present in **Amygdalin, a cyanoglycoside** (cyanide connected to sugars).

-Amygdalin is found in edible plants' pits (peaches, apricots, almonds, apples). Having Beta Bond within its structure.



Amygdalin, a cyanoglycoside

Beta glucosidases (which break down the beta bond) are found in some plants and bacteria.

Bacterial enzymes in human intestines can break it off releasing the Cyanide in the body. However, large amount is needed to cause toxicity.

DOXORUBICIN

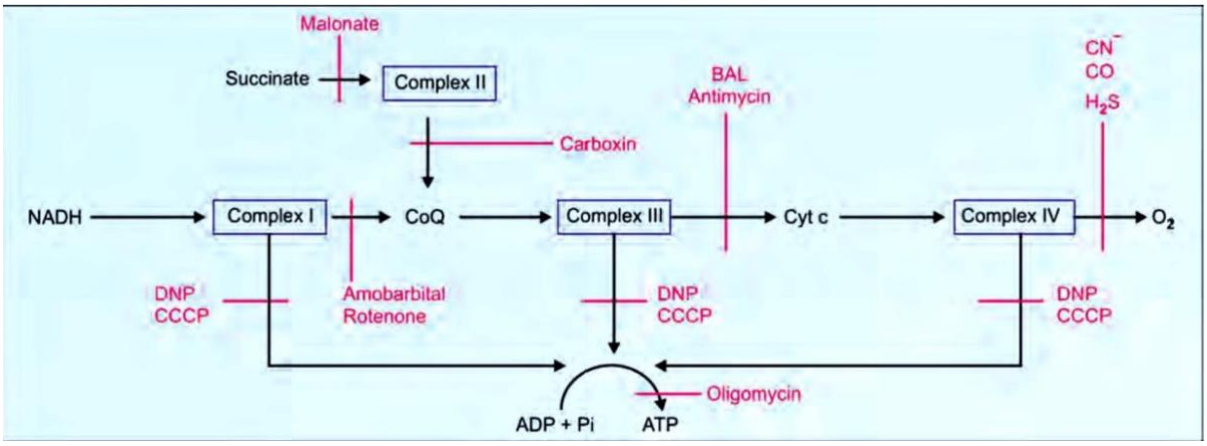
- **MECHANISM**
  - ✓ **Binds to cardiolipin (in the inner mitochondrial membrane)**
  - ✓ **Inhibits succinate oxidation**
  - ✓ **Inactivates cytochrome oxidase**
  - ✓ **Interacts with CoQ**
  - ✓ **Affects ion pumps**
  - ✓ **Inhibits ATP synthase**

- **EFFECT**
  - ✓ **Decreased ATP levels**
  - ✓ **Swollen mitochondria**
  - ✓ **Decreased mitochondrial ability to sequester calcium ions**
  - ✓ **increased free radicals leading to mitochondrial membrane damage**

-Doxorubicin is famously used as an anticancer drug.

-It effects cancerous cells by increasing the number of free radicals ending up with a swollen mitochondria that bursts, leaving the cell with no source of energy. Therefore, cells die.

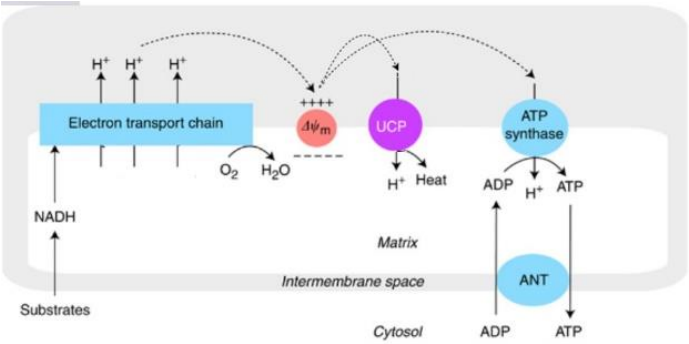
-It also affects normal cells the same way, which makes this drug unspecific to cancer cells.



Note that Malonate inhibits the conversion of succinate into fumarate at the level of complex II.

UNCOUPLING

- **Short-circuiting ATP synthase.**
- **UCP1 (thermogenin):**
  - ✓ **Brown adipose tissue, non-shivering thermogenesis.**
  - ✓ **Infants: neck, breast, around kidneys.**
  - ✓ **Fatty acids directly activate UCP1.**



- **UCP2 (most cells); UCP3 (skeletal muscle); {UCP4, UCP5} (brain)**
- **Obesity tendency in some populations.**

-If there is a flow of electrons, there should be pumping of protons then ATP generation. The oxidative part is coupled to the phosphorylation's part. Can they be uncoupled?

-This process can be **uncoupled** by the presence of **UCP (Uncoupling proteins)**.

-Those proteins enables H+ to be leaked back to the matrix before reaching ATP synthase, this will result in losing electrons' energy in the form of heat. (Protons here are moving with their gradient, so they will lose energy).

-This is how we produce heat in our bodies to maintain a body temperature of 37^{o} .

-This is called **adaptive thermogenesis**: The amount of energy we use out of food (originally) to produce heat .

And it differs from one person to another.

-Any mutation in UCP proteins results in less heat production and more ATP synthesis than needed in the body.

Remember: ATP produced is used to build up many macromolecules in the body(Carbs, proteins,..) but **mainly fats,** so the more ATP you have, the fatter you get.

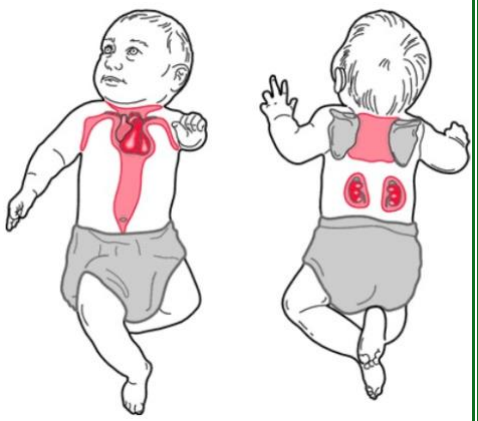
That explains the link between mutations in UCPs and obesity.(A study was formed on a population with obesity, showed mutations in their UCPs).

-There are many types of uncoupling proteins including **uncoupling protein 1** which is called **Thermogenin**.

-**Thermogenin** produces heat so the more you have of it ›› the more protons will come back ›› the more heat generated.

-It is highly abundant in brown adipose tissue that babies have in more quantities (than adults), since they can't cover themselves when they get cold, so they need to generate more heat.

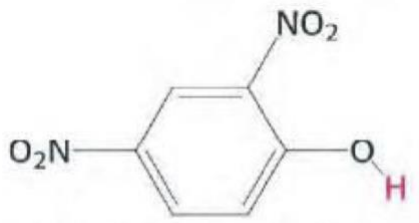
Note that brown and white adipose tissues differ in the **Thermogenin** quantity, which is higher is the brown adipose tissue, meaning more heat formation.



UNREGULATED – CHEMICAL UNCOUPLERS

- What is uncoupling?
- How does it occur? Dissipation of PMF
- What is the result?
- Is it physiological or not?
- 2,4-dinitrophenol (DNP) & other acidic aromatic compounds
- What changes happen? ↑O2 consumption, ↑NADH oxidation
- Soviet soldiers were given DNP, FDA banned DNP (1938)

-Pharmaceutical companies came up with a drug called **Dinitrophenol** that can pass through the inner mitochondrial membrane to transfer protons from outside to inside, giving a solution to obesity.

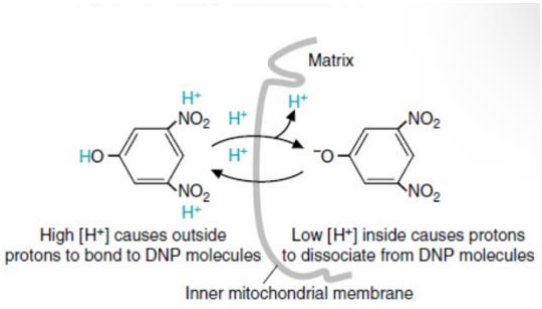


2,4-Dinitrophenol (DNP)

-Dinitrophenol consists of one benzene ring (lipid soluble), 2 nitrous groups and OH.

-When it reaches the outer surface of the inner mitochondrial membrane (where there's high content of protons), it binds to H^+.

-It is a benzene ring so it can swim freely in the membrane. When it reaches the inner side of the membrane (where there's low content of protons) it releases the H^+.



-The drug worked for some time but then unexpected cases of bleeding in the eyes, blindness and death started to occur due to **malignant hyperthermia** (very high amount of heat produced).

-Side effects weren't dose-dependent.

-The drug was banned in the USA in 1939 but it was used again in WW2 by the Soviet Union on soldiers to tolerate the cold.

-GOOD LUCK-