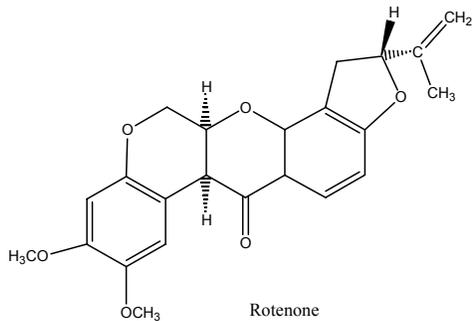


# Inhibitors & Uncouplers

February 24, 2003

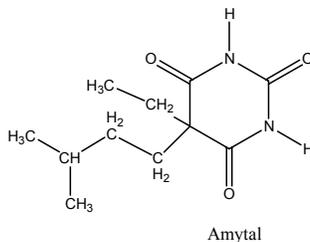
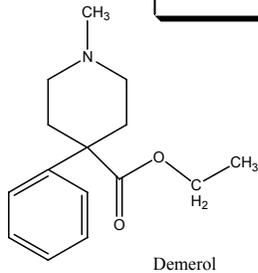
Bryant Miles

The electron transport chain was determined by studying the effects of particular inhibitors.



Rotenone is a common insecticide that strongly inhibits the electron transport of complex I. Rotenone is a natural product obtained from the roots of several species of plants. Tribes in certain parts of the world beat the roots of trees along riverbanks to release rotenone into the water which paralyzes fish and makes them easy prey.

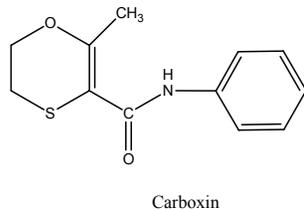
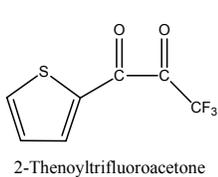
## Inhibitors of Complex I



Amytal is a barbiturate that inhibits the electron transport of complex I.

Demerol is painkiller that also inhibits complex I.

All three of these complex I inhibitors block the oxidation of the Fe-S clusters of complex I.

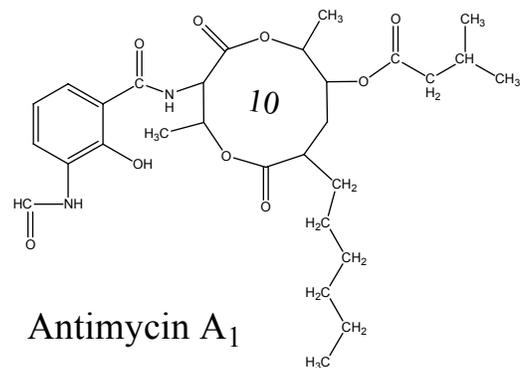


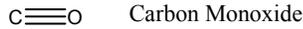
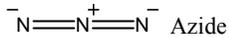
2-Thienyltrifluoroacetone and carboxin specifically block electron transport in Complex II.

## Inhibitors of Complex II

Antimycin A<sub>1</sub> is an antibiotic that inhibits electron transfer in complex III by blocking the transfer of electrons between Cyt *b<sub>H</sub>* and coenzyme Q bound at the Q<sub>N</sub> site.

## Inhibitor of Complex III





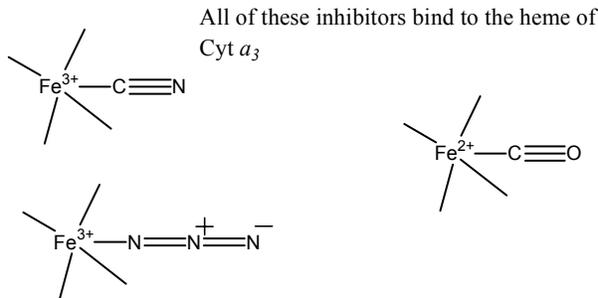
## Inhibitors of Complex IV

Cyanide, azide and carbon monoxide all inhibit electron transport in Complex IV. They all inhibit electron transfer by binding tightly with the iron coordinated in Cyt  $a_3$ .

Azide and cyanide bind to the iron when the iron is in the ferric state.

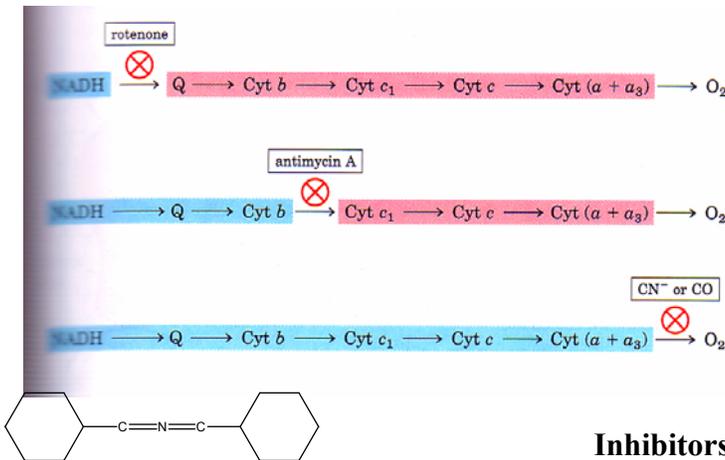
Carbon Monoxide binds to the iron when it is in the ferrous state.

Cyanide and azide are potent inhibitors at this site which accounts for their acute toxicity.

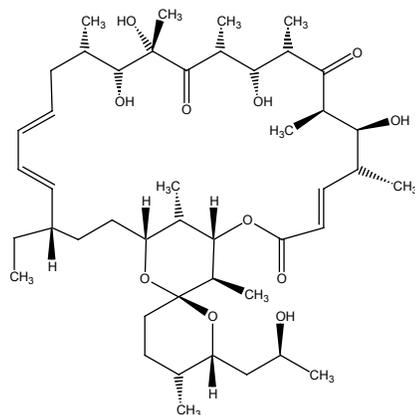


Carbon monoxide is toxic due to its affinity for the heme iron of hemoglobin. Animals carry many molecules of hemoglobin, therefore it takes a large quantity of carbon monoxide to die from carbon monoxide poisoning. Animals have relatively few molecules of Cyt  $a_3$ . Consequently an exposure to a small quantity of azide or cyanide can be lethal. The toxicity of cyanide is solely from its ability to arrest electron transport.

To the left is the effect of inhibitors of electron transport in the presence of  $\text{O}_2$  and an electron donor such as NADH. The electron carriers before the inhibited step become fully reduced (blue). The electron carriers after the block are completely oxidized (red).



Dicyclohexylcarbodiimide  
DCCD



Oligomycin A

Inhibitors of ATP Synthase

## Inhibitors of ATP Synthase

DCCD, shown to the left, forms covalent bonds to a glutamate residue of the c subunit of  $F_0$ . When DCCD is covalently attached it blocks the proton channel, which causes the rotation and ATP synthesis to cease.

Oligomycin binds directly to ATP synthase  $F_0$  subunit and blocks the flow of protons through the channel.

## P/O Ratios

The P/O ratio is the number of ATP molecules formed per 2 electrons transferred to oxygen.

Beginning with NADH, two electrons are transferred via the electron transport chain to oxygen resulting in 10 protons being pumped across the mitochondrial inner membrane. 4 protons are channeled back the matrix per ATP synthesized.

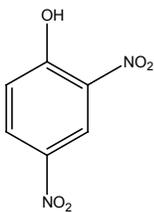
The P/O ratio is  $10/4 = 2.5$ .

$$\frac{P}{O} = \left( \frac{1 \text{ ATP}}{4 \text{ H}^+} \right) \left( \frac{10 \text{ H}^+}{[2e^- \text{ NADH} \rightarrow 1/2 \text{ O}_2]} \right) = \frac{10}{4} = 2.5$$

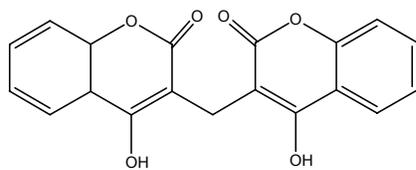
Beginning with FADH<sub>2</sub>, 6 protons pumped per 2 electrons transferred. Still 4 protons/ATP.

P/O ratio =  $6/4 = 1.5$

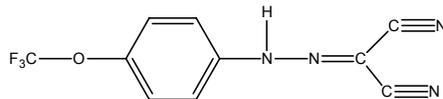
### Uncouplers



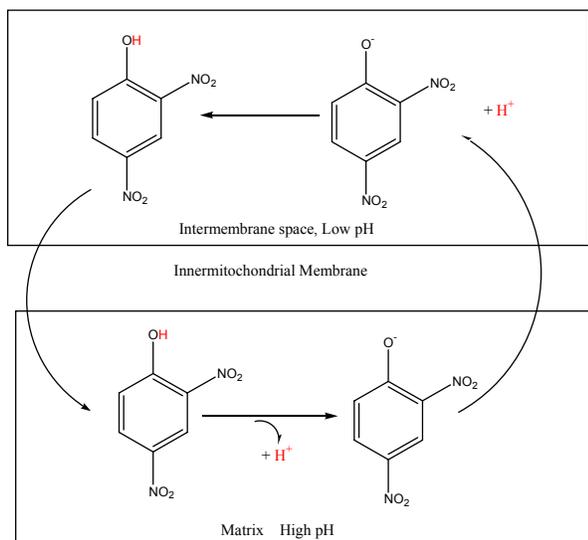
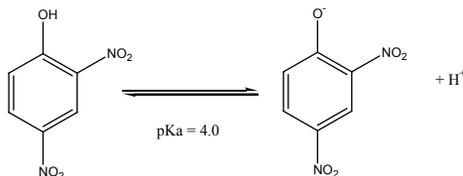
Dinitrophenol



Dicumarol



Carbonyl cyanide-p-trifluoromethoxyphenyl hydrazone  
FCCP



The coupling between electron transport and oxidative phosphorylation depends on the impermeability of the inner mitochondrial membrane to H<sup>+</sup> translocation. The only way for protons to go from the intermembrane space to the matrix is through ATP synthase.

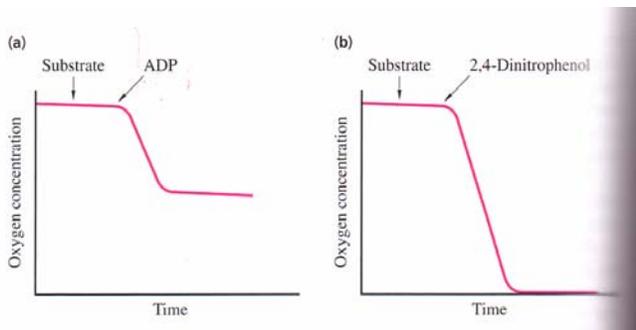
Uncouplers uncouple electron transport from oxidative phosphorylation. They collapse the chemiosmotic gradient by dissipating protons across the inner mitochondrial membrane.

All of the uncouplers shown to the left, collapse the pH gradient by binding a proton on the acidic side of the membrane, diffusing through the inner mitochondrial membrane and releasing the proton on the membranes alkaline side.

2,4-Dinitrophenol, dicumarol and carbonyl cyanide-p-trifluorocarbonyl-cyanide methoxyphenyl hydrazone (FCCP) all have hydrophobic character making them soluble in the bilipid membrane. All of these decouplers also have dissociable protons allowing them to carry protons from the intermembrane space to the matrix which collapses the pH gradient.

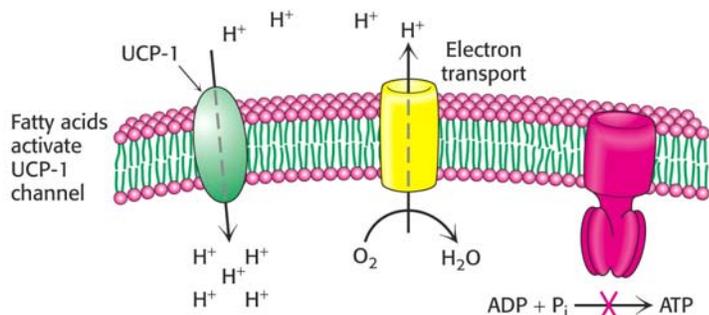
The potential energy of the proton gradient is lost as heat.

The link between electron transport and ATP synthesis is below. (a) In the presence of excess phosphate and substrate and intact mitochondria, oxygen is consumed only when ADP is added. When all of the added ADP has been converted into ATP, electron transport stops and oxygen consumption ceases. (b) The addition of 2,4-dinitrophenol uncouples electron transfer from ATP synthesis. The oxygen is completely consumed in the absence of ADP.



### Endogenous Uncouplers Enable Organisms to Generate Heat.

The uncoupling of oxidative phosphorylation from electron transport generates heat. Hibernating animals and newborn animals (including human beings) contain brown adipose tissue. The adipose tissue is brown due to the high mitochondria content of the tissue. An endogenous protein called thermogenin uncouples ATP synthesis from electron transport by opening up a passive proton channel (UCP-1) through the inner mitochondrial membrane. The collapse of the pH gradient generates heat.

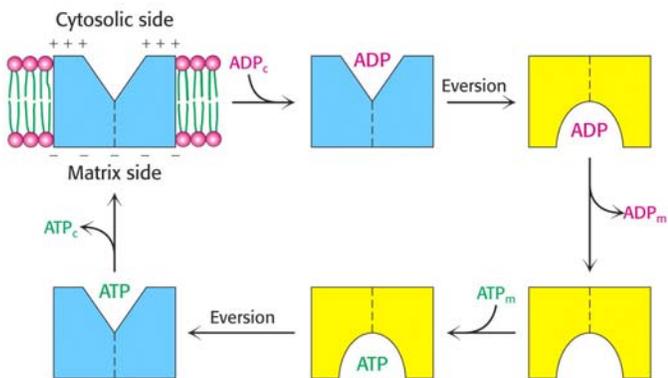


### Proton Motive Force Drives Transport

The primary purpose of the proton gradient is to generate ATP by oxidative phosphorylation. The potential energy of the gradient can also be used for active transport. The inner mitochondrial membrane is impermeable to charged molecules.

### ATP-ADP Translocase

There are two specific systems to transport ADP and Pi into the mitochondrial matrix. A specific transport protein **ATP-ADP translocase** enables ATP and ADP to transverse the inner mitochondrial membrane. The transport of ADP in and ATP out are coupled. ADP only enters the matrix if ATP exits or vice versa.



ATP-ADP translocase has a single nucleotide binding site which binds ADP and ATP with equal affinity. Due to the negative electrostatic charge of the matrix, ATP<sup>4-</sup> is bound on the N phase of the membrane because it has greater negative charge than ADP<sup>3-</sup>. Hence ATP is transported from the matrix to the intermembrane space 30 times more rapidly than ADP is transported.

Once an ATP has been exported into the

intermembrane space, a new molecule of ADP must be bound to the transport protein. This precisely couples the exchange of ATP for ADP in the matrix. The net result of the translocation is the decrease of the proton motive force by the net exchange of one negative charge out the matrix. The ATP-ADP translocase can be specifically inhibited by a low concentration of atractyloside which is a plant glycoside. Atractyloside binds to the translocase when its nucleotide binding site is facing the intermembrane space. This inhibitor completely blocks ATP-ADP translocation and stops oxidative phosphorylation.

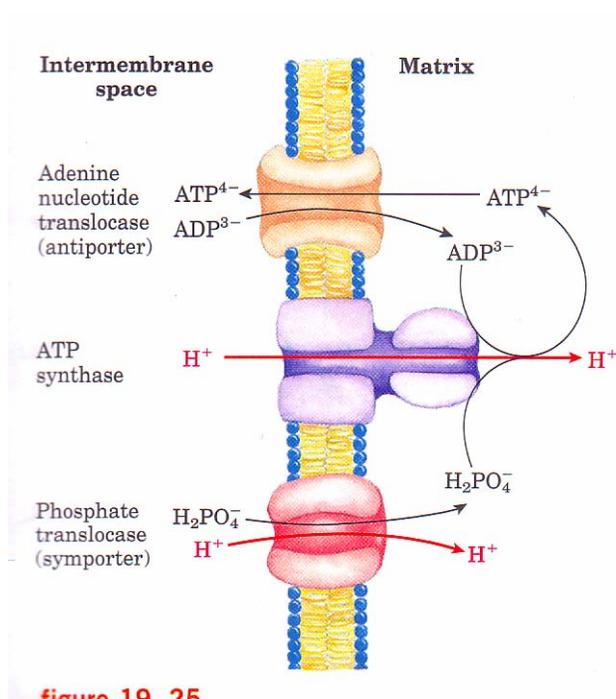
### Other Mitochondrial Transporters.

Recall from 410 symporters and antiporters.

ATP-ADP translocase is an example of an antiporter.

### Phosphate Carrier Protein.

A example of a symport carrier is the electroneutral symport of inorganic phosphate with a proton by the **phosphate carrier protein**. The combined activity of ATP-ADP translocase and the phosphate carrier is the exchange of ADP + Pi for ATP at the cost of one proton transported from the P-phase to the N-phase. **Secondary active transport.**



### Dicarboxylate Carrier Protein

This protein enables malate, succinate and fumarate to be transported from the matrix of the mitochondria in exchange for phosphate or vice versa. This is an example of antiport.

### Tricarboxylate Carrier Protein

This protein antiports citrate and H<sup>+</sup> in exchange for malate.

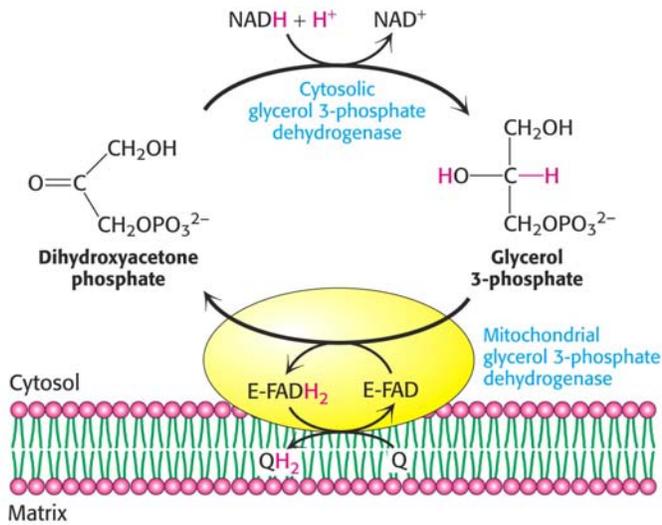
### Pyruvate Carrier Protein

Symports pyruvate and a proton from the intermembrane to the matrix.

## Electron Shuttle Systems

Most of the NADH used in electron transport is produced in the mitochondrial matrix. The binding site of Complex I for NADH is on the matrix side of the inner membrane. The inner mitochondrial membrane is impermeable to NAD<sup>+</sup> and NADH. NADH formed in the cytosol of cells needs to be reoxidized into NAD<sup>+</sup> to keep glycolysis going. Under anaerobic conditions, NAD<sup>+</sup> is regenerated by lactate dehydrogenase. Under aerobic conditions, the electrons of NADH need to be shuttled into the matrix of the mitochondria.

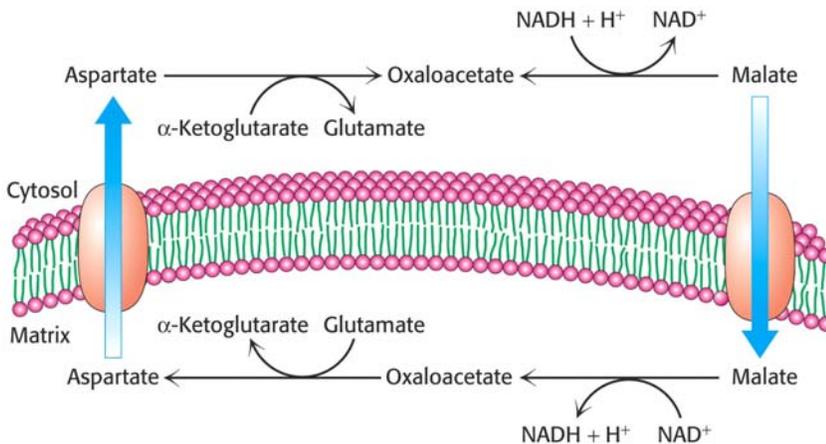
## The Glycerophosphate Shuttle



electrons of NADH are transferred to FAD to form FADH<sub>2</sub>. The two electrons bound by the FADH<sub>2</sub> are transferred directly to coenzyme Q forming QH<sub>2</sub>. QH<sub>2</sub> carries the electrons to complex III. The result of this shuttle is 1.5 ATP/NADH.

Note that this shuttle is essentially irreversible.

## The Malate-Aspartate Shuttle.



In the cytosol, oxaloacetate is reduced to malate by malate dehydrogenase which uses NADH as the reductant. Malate is transported across the inner mitochondrial membrane by the dicarboxylic acid or tricarboxylic acid carrier. Now in the matrix, the malate is reoxidized by malate dehydrogenase to generate oxaloacetate and NADH which can now transfer its electrons to Complex I. The oxaloacetate is transaminated by glutamine to form aspartate and  $\alpha$ -ketoglutarate. Aspartate can be transported across the inner mitochondrial membrane by the dicarboxylic acid carrier. In the cytosol aspartate transaminates  $\alpha$ -ketoglutarate to reform oxaloacetate completing the cycle.

This shuttle system generates 2.5 ATP/NADH and is completely reversible.

The net yield of ATP by glucose oxidation depends on the shuttle used.

Glycolysis Glucose → 2 pyruvate		2ATP
	2NADH	
<b>Glycerophosphate shuttle</b>		
2NADH → 2FADH <sub>2</sub>		
1.5 ATP/FADH <sub>2</sub>		3 ATP
Conversion of 2pyruvate into 2acetyl CoA in the mitochondria	2NADH	
2.5 ATP/NADH		5 ATP
Citric Acid cycle oxidation of 2 acetyl CoA	2GTP = 2FADH <sub>2</sub> +6NADH	2 ATP
1.5 ATP/FADH <sub>2</sub>		3 ATP
2.5 ATP/NADH		15 ATP

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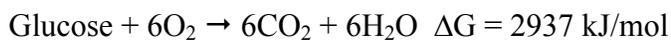
**30 ATP**

Glycolysis Glucose → 2 pyruvate		2ATP
	2NADH	
<b>Malate-Aspartate shuttle</b>		
2.5 ATP/NADH		5 ATP
Conversion of 2pyruvate into 2acetyl CoA in the mitochondria	2NADH	
2.5 ATP/NADH		5 ATP
Citric Acid cycle oxidation of 2 acetyl CoA	2GTP = 2FADH <sub>2</sub> +6NADH	2 ATP
1.5 ATP/FADH <sub>2</sub>		3 ATP
2.5 ATP/NADH		15 ATP

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**32 ATP**

Back to the very beginning.



Under cellular conditions,  $\Delta G$  for ATP hydrolysis  $\approx 50 \text{ kJ/mol}$   
 $32 \text{ ATP /glucose} = 1,600 \text{ kJ/mol}$

Efficiency  $1600/2937 \times 100 = 54\%$

3.5 billion years of evolution has resulted in 54 % efficiency.