ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

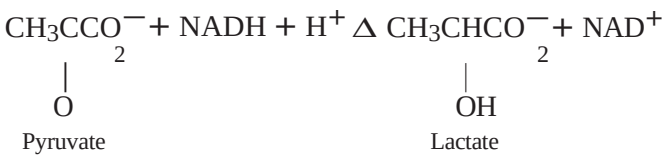
You only have to look at the ATP yield from the TCA cycle, 12 of them per molecule of acetyl-CoA, to know that oxidative phosphorylation must be important. That's where all the electrons from NADH and $FADH_2$ go after they're made by the TCA cycle.

OXIDATION AND REDUCTION

Oxidation is the loss of electrons.
NADH is oxidized to NAD^+.
Reduction is the gain of electrons.
O_2 is reduced to H_2O.

Electrons usually aren't floating around in space; they're stuck on some atom or other. The simple consequence of this is that when one

thing loses electrons, something else must gain them. Every oxidation of something must be coupled to the reduction of something else. The molecule or atom that loses the electrons has been oxidized; the one that gains them has been reduced. Oxidants, or oxidizing agents, are compounds that oxidize other compounds—they are reduced in the process. Reductants, or reducing agents, are compounds that reduce other compounds—they are oxidized in the process.



Pyruvate is reduced to lactate. Lactate is oxidized to pyruvate. NADH is oxidized to NAD^+. NAD^+ is reduced to NADH. Pyruvate and NAD^+ are oxidizing agents. Lactate and NADH are reducing agents.

As fuel molecules are oxidized, the electrons they have lost are used to make NADH and $FADH_2$. The function of the electron transport chain and oxidative phosphorylation is to take electrons from these molecules and transfer them to oxygen, making ATP in the process.

THE ELECTRON TRANSPORT CHAIN

Two electrons flowing down the chain make
- 3 ATP/NADH
- 2 ATP/$FADH_2$

As electrons move down the electron transport chain, the carriers become reduced (Fig. 1). The next carrier oxidizes the previous carrier, taking its electrons and transferring them on to the next carrier. Finally the electrons end up reducing oxygen to water. The cytochromes are named with letters in no particular order, making them tough to memorize, but you probably should learn them, at least right before the exam—after that you can look them up if you ever need to.

The energetics of the electron transport steps makes the process work. Overall there's a lot of free energy lost in the tranfer of electrons from NADH to oxygen—the overall reaction is very favorable, with an equilibrium constant that's overwhelmingly large. At the three sites where ATPs are made (labeled I, II, and III), the reaction is the most downhill.

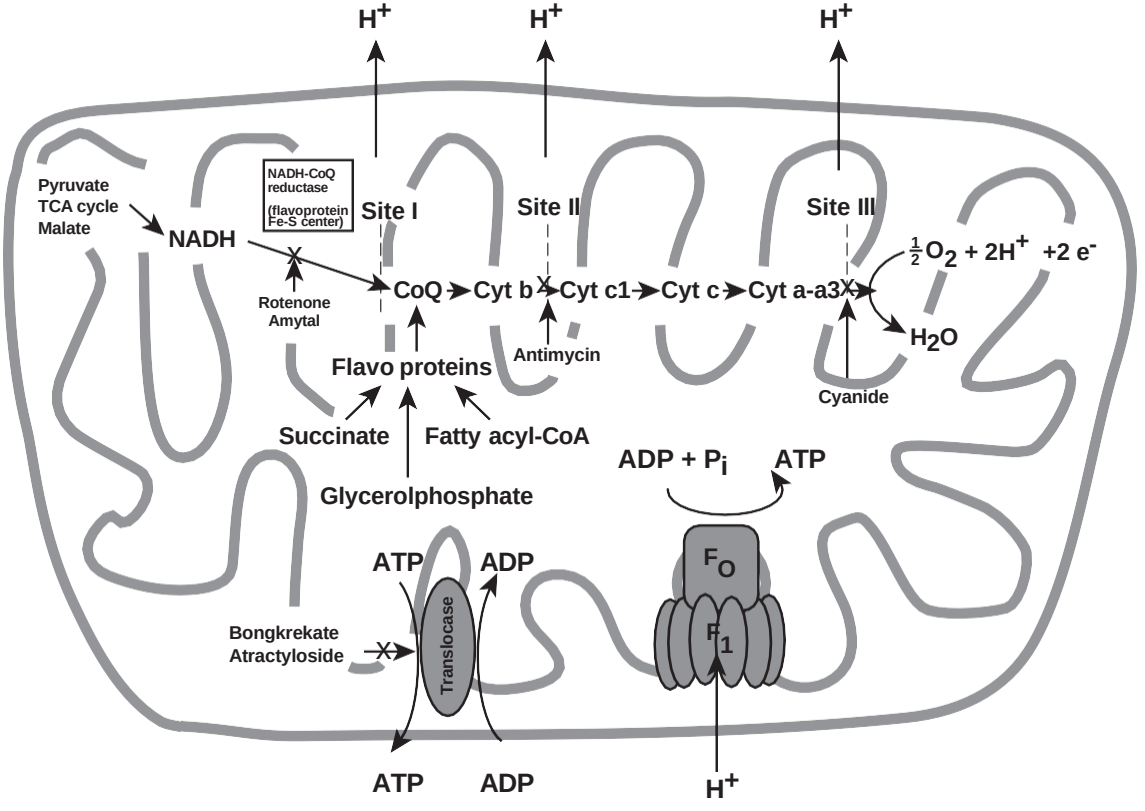


Figure 1 The Electron Transport Chain

During the electron tranfers at the three classic sites of phosphorylation (marked I, II, and III), protons are pumped out of the mitochondria into the cytoplasm. The exact number of protons pumped at each site is somewhat controversial; however, this proton pumping makes the interior of the mitochondria alkaline.

ATP is made by the F_1F_0 ATPase. This enzyme allows the protons back into the mitochondria. Since the interior is alkaline, the reaction is favorable—favorable enough to drive the synthesis of ATP by letting protons back into the mitochondria. Exactly how the F_1F_0 ATPase couples the flow of protons down their concentration gradient to the formation of ATP is not known in molecular detail. The proton flow through the F_1F_0 ATPase is required to release ATP from the active site where it was synthesized from ADP and P_i. The ATP is made in the interior of the mitochondria and must be exchanged for ADP outside the mitochondria to keep the cytosol supplied with ATP. The exchange of mitochondrial ATP for cytoplasmic ADP is catalyzed by the ATP/ADP translocase.

The complete transfer of 2 electrons from NADH through the entire electron transport chain to oxygen generates 3 ATPs.[1] $FADH_2$ feeds electrons into coenzyme Q (a quinone) after the first ATP-generating step. Flavin-linked substrates (those that make $FADH_2$) generate only 2 ATPs per 2 electrons transferred down the chain. Flavin-linked substrates generate less ATP, not only because they feed in after the first ATP has already been made; they make 2 ATPs because $FADH_2$ is not as strong a reducing agent as NADH. There is not enough energy in the oxidation of $FADH_2$ to generate 3 ATPs.

CONNECTIONS

NADH and $FADH_2$ from the TCA Cycle.

Electrons from NADH outside the mitochondria are transported into the mitochondria by the malate-aspartate shuttle or the α-glycerol phosphate shuttle.

O_2 is a gas supplied by the blood.

ADP outside the mitochondria is swapped for ATP inside the mitochondria by a specific translocase.

F_1F_0 ATPase couples H^+ gradient to ATP synthesis.

[1] The current estimate for the number of ATPs made per 2 electrons is actually about 2.5. This is because of the uncertainties in the number of protons pumped out at each electron transfer step. This affects ATP yields from glucose (30 instead of 36), so be sure you ask your professor which to use.

The electron transport chain gets its substrates from the NADH and $FADH_2$ supplied by the TCA cycle. Since the TCA cycle and electron transport are both mitochondrial, the NADH generated by the TCA cycle can feed directly into oxidative phosphorylation. NADH that is generated outside the mitochondria (for example, in aerobic glycolysis) is not transported directly into the mitochondria and oxidized—that would be too easy.

There are two shuttles involved in getting the electrons from NADH into mitochondria. The α-glycerol phosphate shuttle works most simply. In this shuttle, NADH in the cytoplasm is used to reduce dihydroxyacetone phosphate (DHAP) to α-glycerol phosphate. The α-glycerol phosphate is actually the molecule transported into the mitochondrion, where it is oxidized back to DHAP, giving mitochondrial $FADH_2$. The DHAP then leaves the mitochondrion to complete the shuttle. With this shuttle in operation, there's a cost. Normally, the oxidation of mitochondrial NADH gives 3 ATPs. However, the mitochondrial enzyme that oxidizes α-glycerol phosphate uses FAD as the oxidizing agent. The $FADH_2$ that results gives only 2 ATP equivalents. Using this shuttle, the cytoplasmic NADH yields only 2 ATPs.

The other shuttle is the malate-aspartate shuttle. The advantage of this shuttle is that it gives you 3 ATPs for the oxidation of each cytoplasmic NADH. In red muscle, heart, and brain tissues the malate-aspartate shuttle is the major pathway for shuttling electrons into mitochondria. In white muscle, the α-glycerol phosphate shuttle predominates (Fig. 2).

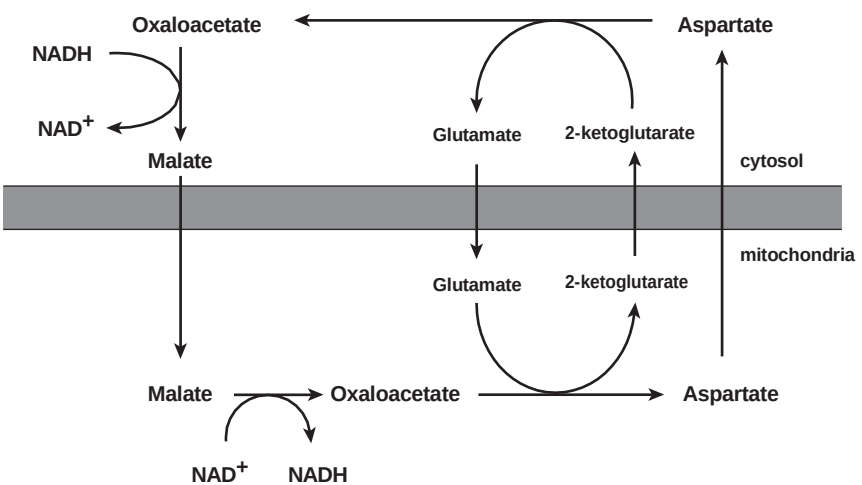


Figure 2
The **MALATE-ASPARTATE SHUTTLE** gets reducing equivalents (electrons) from cytosolic NADH into the mitochondria so that 3 ATPs can be made.

REGULATION

The rate of oxidative phosphorylation is controlled by the supply of ADP and phosphate.

Assuming that oxygen is available and that there is a supply of NADH- or $FADH_2$-generating substrates, the activity of oxidative phosphorylation is determined by the availability of ADP. If ADP is available and there is enough phosphate around (there usually is), the ADP and P_i are converted to ATP. If not, not.

P/O RATIOS

These are the numbers of ATP equivalents made per 2 electrons passed down the electron transport chain.

NADPH: P/O = 3
$FADH_2$: P/O = 2
Succinate (with rotenone present): P/O = 2
Acetate: P/O = 2.5

The P/O ratio is the number of ATPs made for each O atom consumed by mitochondrial respiration. The P stands for high-energy phosphate equivalents, and the O actually stands for the number of ¿ O_2's that are consumed by the electron transport chain. The full reduction of O_2 to 2 H_2O takes 4 electrons. Therefore, 2 electrons reduce ¿ of an O_2. The oxidation of NADH to NAD and the oxidation of $FADH_2$ to FAD are both 2-electron oxidations. O can be read as the transfer of 2 electrons. It's not quite as obscure as it sounds.[2]

To figure out a P/O ratio[3] you have to figure out two things—the P and the O. The P is easy if you've learned to count ATPs made by various metabolic pathways. The P is the net number of ATPs made by the metabolism of the substance you're dealing with. The O is a little harder. Here you must figure out how many times 2 electrons have been passed down the electron transport chain. For each NADH or each $FADH_2$ made during the metabolism of your substance, 1 O is consumed as 2 electrons are passed down the chain.

[2] It probably *is* as obscure as it sounds. Remember O = transfer of 2 electrons down the entire chain.

[3] Pronounced "pee to oh," "pee over oh," or "pee oh"—all are used.

In the presence of the inhibitor rotenone (to prevent the oxidation of NADH by the electron transport chain), succinate can be metabolized only to fumarate, producing an $FADH_2$ in the process.

$$\text{Succinate} + \text{FAD} \rightarrow \text{fumarate} + FADH_2$$

The oxidation of the $FADH_2$ makes 2 ATPs and consumes 1 O. The P/O for succinate is 2.

In the absence of rotenone, the NADH that is made from the conversion of succinate to oxaloacetate can be oxidized by the electron transport chain. The metabolism of succinate then becomes

$$\text{Succinate} + \text{FAD} \rightarrow \text{fumarate} + FADH_2$$

$$\text{Fumarate} + H_2O \rightarrow \text{malate}$$
$$\text{Malate} + NAD^+ \rightarrow \text{oxaloacetate} + \text{NADH} + H^+$$

Net: $\text{Succinate} + \text{FAD} + H_2O + NAD^+ \rightarrow \text{oxaloacetate} + FADH_2 + \text{NADH} + H^+$

In this case, succinate metabolism to oxaloacetate produces 5 ATPs from the oxidation of the NADH and $FADH_2$—2 from the $FADH_2$ and 3 from the NADH. Two O's are consumed, 1 for each of the NADH and $FADH_2$ molecules. The P/O is then 5/2 = 2.5. Thus the P/O can be a noninte-gral number.

Just one more. Let's do acetate. Before it can be metabolized, acetate must be activated in a reaction that uses 2 ATP equivalents.[4]

$$\text{Acetate} + \text{CoA} + \text{ATP} \rightarrow \text{acetyl-CoA} + \text{ADP} + PP_i$$

Acetyl-CoA metabolized through the TCA cycle yields 3 NADH, 1 $FADH_2$, and 1 GTP—a total of 12 ATP equivalents (3 from each NADH, 2 from each $FADH_2$, and 1 GTP—12 in all). Four O's are used, 1 for each NADH and $FADH_2$. The P in this case is 10 (12 — 2 for the activation of acetate to acetyl-CoA). The P/O = 10/4 = 2.5. P/O ratios for anything else are calculated in the same way.

UNCOUPLERS

Allow protons back into the mitochondria without making any ATP
Stimulate oxygen consumption

[4] For reactions that make PP_i (pyrophosphate), the PP_i is rapidly hydrolyzed to 2 P_i in the cell, so we'll consider the formation of PP_i to use 2 high-energy-phosphate bonds.

Mitochondria do three things: oxidize substrates, consume oxygen, and make ATP. Uncouplers prevent the synthesis of ATP but do not inhibit oxygen consumption or substrate oxidation. Uncouplers work by destroying the pH gradient. The classic uncoupler is dinitrophenol (DNP). This phenol is a relatively strong acid and exists as the phenol and the phenolate anion.

$$\text{2,4-DNP—OH} + H_2O \;\Delta\; \text{2,4-DNP—}O^- + H_3O^+$$

Because both the anion and the acid are lipophilic (greasy) enough to cross the mitochondrial membrane, DNP can transport protons across the membrane and destroy the pH gradient. The 2,4-DNP–OH crosses from the cytosol into the mitochondrion, carrying its proton with it. In the more alkaline environment of the mitochondrion, the DNP–OH loses its proton and the pH falls. The 2,4-DNP–O^- then leaves the mitochondrion and repeats the cycle again until the pH inside is the same as the pH outside. With no pH gradient, there is no ATP synthesis. However, there is still oxidation of substrates and consumption of oxygen. With no ATP synthesis, the ADP concentration is high and the electron transport chain keeps trying to pump out protons. In fact, uncouplers usually stimulate oxygen and substrate consumption. Long-chain fatty acids can uncouple mitochondria by the same mechanism. There are other ways to collapse the pH gradient. Valinomycin is a potassium ionophore[5] and collapses the electrochemical gradient[6] of the mitochondria. Collapsing the electrochemical gradient also prevents ATP synthesis.

INHIBITORS

Inhibitors block the flow of electrons at a specific site and inhibit electron flow and ATP synthesis.

Inhibitors inhibit oxygen consumption and ATP synthesis.

[5] An *ionophore* is a compound that is capable of selectively carrying ions across a membrane. The ion fits into a specific binding site in a molecule that is hydrophobic enough to cross the membrane. There are calcium-specific ionophores, proton ionophores, sodium ionophores, etc.

[6] *Electrochemical gradient* is the name given to the gradient of charge and ions that exists across the inside and the outside of a cellular membrane. The outside of the mitochondrion is more positively charged than the inside, and the concentration of potassium ions is higher outside than inside. When an ion falls through (an expression that gives a nice image, but *moves* would be just as good) the electrochemical gradient, it is driven by both its concentration gradient and the charge difference between the inside and the outside of the membrane. Ions tend to move from areas of high concentration to areas of low concentration. Positive ions tend to move toward the more negative compartment. The bigger the charge difference between the inside and the outside, the bigger the free-energy difference that drives the ion movement (see Chapter 3).

Inhibitors actually block one of the steps of oxidative phosphorylation. *Cyanide* blocks the last step of electron transfer by combining with and inhibiting cytochrome oxidase. The effect is similar to oxygen deprivation. The less obvious effect is that all the electron carriers become more reduced than they would be without the inhibitor. The reason is that the substrates are still pushing reducing equivalents (electrons) down the electron transport chain. But it's blocked at the end. The result is that all the carriers before the block become reduced. For the same reason, inhibiting electron transport also tends to keep the NADH and $FADH_2$ reduced (depending on where the inhibitor acts). Carriers after the block become more oxidized. Carriers after the block can still transfer their electrons to oxygen. Once they've done this, though, there are no more reducing equivalents available because of the block, and they are left in the oxidized state.

Different inhibitors block at different points of the chain. The general rule is that all electron carriers that occur before the block become reduced and all that occur after the block become oxidized.

INHIBITOR	SITE	EFFECT
Cyanide	Cytochrome oxidase	Blocks transfer of electrons to O_2. Blocks at site III.
Antimycin	Electron transfer from cyt b to cyt c_1	All intermediates before and including cyt a will be in the reduced state; all intermediates after and including cyt c_1 will be in the oxidized state. Blocks at site II.
Rotenone	NADH-CoQ reductase	Blocks oxidation of NADH (site I). NADH will become reduced; Substrates such as succinate that enter via FADH will still be oxidized and make 2 ATPs/mol.
Oligomycin	ADP phosphorylation	Blocks phosphorylation of ADP. Does not inhibit uncoupled oxidations.
Atractyloside and bongkrekate	ADP-ATP transporter	Inhibits entry of ADP into mitochondria and ATP export. Stops electron transport because of lack of ADP. Inside, all ADP is converted to ATP.

Rotenone inhibits the transfer of electrons from NADH into the electron transport chain. The oxidation of substrates that generate NADH is, therefore, blocked. However, substrates that are oxidized to generate $FADH_2$ (such as succinate or α-glycerol phosphate) can still be oxidized and still generate ATP. Because NADH oxidation is blocked, the NADH pool becomes more reduced in the presence of rotenone since there's nowhere to transfer the electrons.

Atractyloside and bongkrekate inhibit the entry of ADP into the mitochondria. After all the ADP in the mitochondria has been converted to ATP, oxidative phosphorylation stops, since the ATP that's made can't get out and new ADP can't get in from the outside.