Electron Transport and Oxidative Phosphorylation

Objectives:
  I. Describe the structure of the Mitochondrion.
     A. Compare the structure of the outer membrane to the structure of the inner membrane.
     B. What metabolic pathways are localized to the mitochondrion?
        1. Where in the mitochondrion are they located?
  II. What is(are) the overall chemical reaction(s) of electron transport and oxidative phosphorylation?
  III. Describe the overall makeup of the Electron Transport / Oxidative Phosphorylation pathway.
     A. Number of complexes present in the system.
        1. Which complexes carry electrons?
     B. Describe the electron carriers present in each of the complexes.
     C. Describe the electron carriers that are part of the pathway but are not part of the complexes.
     D. Describe the “flow” of electrons “down” the Electron Transport Chain.
     E. Describe the use of Respiratory Inhibitors in elucidating the sequence of electron transport.
  IV. Describe how the energy released by the Electron Transport Chain is used.
     A. How does the nature of the complexes facilitate establishment of the proton gradient?
     B. How does the nature of the inner mitochondrial membrane function to maintain the proton gradient?
  V. Describe ATP synthesis during Electron Transport / Oxidative Phosphorylation pathway.
     A. Complex involved.
     B. How is the proton gradient utilized to synthesize ATP?
        1. Describe the use of Uncouplers in elucidating the mechanism of ATP synthesis.
     C. Describe the mechanism of ATP synthesis.
     D. Distinguish substrate level phosphorylation from oxidative phosphorylation.
  VI. ATP Yield
     A. Yield of ATP (moles) per mole of NADH/H+ oxidized by the electron transport chain.
        1. Theoretical versus Actual
     B. Yield of ATP (moles) per mole of FADH2 oxidized by the electron transport chain.
        1. Theoretical versus Actual
     C. What other process utilizes the proton gradient?
        1. How?
  VII. Calculate the overall yield of ATP from glucose metabolized under aerobic conditions.
     A. Compare and contrast the shuttles that move NADH from the cytoplasm into the mitochondrion.
        1. Discuss why the shuttles result in different ATP yields per NADH transported from the cytoplasm.
  VIII. Ask yourself “What If Questions”; e.g., What happens if complex III is inhibited?

Background

During the TCA cycle the carbons of an acetate ion were completely oxidized to two CO₂. Some of the energy released during the oxidative process was stored as electrons / hydride ions on 3 molecules of NADH, some was stored as electrons / hydrogens atoms on a FADH₂, and some was stored as GTP / ATP by substrate level phosphorylation. The remaining energy was lost as heat.
The NADH and FADH₂ must be oxidized (back) to NAD⁺ and FAD in order for the TCA cycle to continue operating. During this oxidation of NADH and FADH₂ the energy released is used to generate high energy phosphate compounds, i.e., ATP. The Electron Transport / Oxidative Phosphorylation Pathway (ET/OxPhos) couples the oxidation of NADH and FADH₂ with the generation of ATP.

The Electron Transport / Oxidative Phosphorylation Pathway (ET/OxPhos) occurs in the mitochondria. The Mitochondrion is a subcellular organelle enclosed by two membranes. The Outer Membrane is porous. It contains a significant amount of Porin, a transmembrane protein that forms large pores through the outer membrane. Substances with a molecular mass of 5000 g/mol (Daltons) or less can freely pass through these pores. The Inner Membrane is impervious to all compounds, save for N₂, O₂, CO₂, and H₂O. Molecules cross the inner membrane only with the aid of specific transport proteins. The space between the membranes is the Intermembrane Space. The inner membrane encloses the mitochondrial Matrix. The reactions catalyzed by the pyruvate dehydrogenase complex and pyruvate carboxylase occur in the matrix of the mitochondrion. Seven of the eight enzymes of the TCA Cycle are in the matrix of the mitochondria organized into a Metabolon that is bound to the inner mitochondrial membrane by protein-protein interactions with the eighth enzyme of the TCA Cycle. This 8th TCA Cycle enzyme is an intrinsic membrane protein embedded in the inner membrane. The Electron Transport / Oxidative Phosphorylation Pathway is composed of five large protein complexes imbedded in the inner mitochondrial membrane.

Reactions of The ET/OxPhos Pathway

1. \[ 2 \text{NADH} + 2 \text{H}^+ + \text{O}_2 \rightarrow 2 \text{NAD}^+ + \text{H}_2\text{O} \]
2. \[ 2 \text{FADH}_2 + \text{O}_2 \rightarrow 2 \text{FAD} + 2 \text{H}_2\text{O} \]
3. \[ \text{ADP} + \text{P}_{\text{O}_4}^{-3} \rightarrow \text{ATP} \]
Reaction (1) & (2) show that NADH and FADH$_2$ reduce O$_2$ to H$_2$O as they are oxidized (back) to NAD$^+$ and FAD, respectively. NADH and FADH$_2$ are high energy (energy rich) compounds and H$_2$O is a very low energy compound. When NADH and FADH$_2$ are oxidized to NAD and FAD a large amount of energy is released. Because reactions (1) & (2) do not share a common intermediate with reaction (3), the oxidation of NADH and FADH$_2$ must indirectly drive the synthesis of ATP. Some cellular process links these three reactions. The energy carried by NADH and FADH$_2$ is not released all in one step. Rather, the high energy electrons on NADH and FADH$_2$ are passed to O$_2$ by a series of oxidation-reduction reactions using a series of electron carrier molecules. A small amount of the total energy is released at each of the redox reactions and this energy is trapped for subsequent use by the ATP synthesizing apparatus.

System Components

Before the specific steps of the ELECTRON TRANSPORT / OXIDATIVE PHOSPHORYLATION PATHWAY are examined, the organization of the pathway in the inner membrane of the mitochondrion and the components that comprise the pathway must be described. As mentioned above, ET/OXPHOS is composed of five large protein complexes, Complex I, II, III, IV, and V. All five complexes are intrinsic membrane proteins embedded in the inner mitochondrial membrane. The relative ratios of complexes I, II, III, IV, and V have been estimated to be 1:2:3:6:6. Complex I, II, III and IV collectively contain a series of molecules that undergo reversible oxidation/reduction reactions. These electron carriers pass electrons down an energy gradient starting with high energy electrons carried by NADH or FADH$_2$ and ending when four electrons are passed to a molecule of oxygen (O$_2$) to form two molecules of low energy H$_2$O. Complexes I, II, III, and IV contain the enzymes necessary to catalyze the electron transfer reactions and the substrate molecules that undergo reversible oxidation and reduction. Complex I, III, and IV are also proton translocators / hydrogen ion transport proteins / proton pumps. As electrons move down the energy gradient through complexes I, III, and IV, the energy liberated is used to translocate (pump) protons (H$^+$) across the inner mitochondrial membrane. Protons are pumped from the matrix to the intermembrane space. These complexes are asymmetrically oriented in the membrane to accomplish this task.

Complex V, also called the F$_0$/F$_1$ Complex or ATP Synthase, uses the energy stored in the proton gradient established by electron transport to generate ATP from ADP and PO$_4^{-3}$.

Small Molecules Present in the Supramolecular Complexes - I, II, III, or IV

Included among the macromolecules that make up the supramolecular complexes of the ET/OXPHOS PATHWAY are the IRON-SULFUR PROTEINS or IRON-SULFUR CENTERS. The Iron-Sulfur Proteins are small proteins that contain iron ions in coordinate covalent linkage to sulfur. The sulfur is donated by cysteine residues of the protein and by inorganic sulfide ions. Iron-sulfur centers can contain two iron and six sulfur
centers (four cysteines & two sulfide ions) or four iron and eight sulfur centers (four cysteines & four sulfide ions). The individual iron ions in these proteins undergo reversible one electron oxidations and reductions (Fe$^{2+}$ $\rightleftharpoons$ Fe$^{3+}$). The two iron/six sulfur centers can carry a total of two electrons and the four iron/eight sulfur centers can carry four.

The CYTOCHROMES (CELL COLORS) are small proteins containing a HEME PROSTHETIC GROUP similar to the heme group of hemoglobin. Unlike the heme iron of hemoglobin, the heme iron of the cytochromes undergo reversible one electron redox reactions (Fe$^{2+}$ $\rightleftharpoons$ Fe$^{3+}$). The cytochromes are colored compounds, grouped according to the heme group they contain which gives them their distinctive and characteristic color. Cytochromes of the ‘a’, ‘b’, and ‘c’ type take part in the electron transport chain. One of the ‘c’ type cytochromes, cytochrome c, is not part of any of the large protein complexes. This molecule is an independent part of ET/OXPHOS. It is an extrinsic protein loosely bound to the outer leaflet of the inner mitochondrial membrane.

UBIQUINONE or COENZYME Q (CoQ) is a lipid soluble QUINONE that can undergo reversible one or two electron oxidation/reduction reactions. The molecule is an ISOPRENE. CoQ is dissolved in the inner membrane; it is one of the lipids that make up the inner mitochondrial membrane. It is not part of any of the complexes, rather it serves to shuttle electrons from Complex I or Complex II to Complex III.

The Electron Transport Pathway

The sequence of electron carriers in the Electron Transport Chain was determined by a combination of calculating the redox potentials of the isolated components of the pathway and by adding inhibitors to the electron transport system (intact mitochondria and/or isolated complex) and then determining which electron carrier molecules were completely reduced and which were completely oxidized. Some of the inhibitors that were employed include:

1. The insecticide ROTENONE, the barbiturates AMYTAL, & PIERICIDIN A, and the painkiller DEMEROL. These molecules inhibit the flow of electrons within Complex I and from Complex I to CoQ.
2. The antibiotic ANTIMYCIN A inhibits the flow of electrons from Complex III to cytochrome c.
3. AZIDE (N$_3^-$), CYANIDE (CN$^-$), and CARBON MONOXIDE (CO) inhibit electron flow within Complex IV and from Complex IV to O$_2$.
**Complex I** is also called NADH:UBIQUINONE OXIDOREDUCTASE or NADH:COENZYME Q REDUCTASE or NADH DEHYDROGENASE. This complex accepts electrons from NADH and passes them to FMN oxidizing NADH to NAD and reducing FMN to FMNH$_2$. The electrons are passed from FMNH$_2$ to oxidized (Fe$^{+3}$) iron sulfur centers oxidizing FMNH$_2$ to FMN and reducing the iron in the iron sulfur centers to Fe$^{+2}$. Ultimately, the electrons are passed from the reduced iron sulfur centers (Fe$^{+2}$) in Complex I to Coenzyme Q in the inner membrane oxidizing the iron sulfur centers (Fe$^{+3}$) and reducing CoQ to CoQH$_2$. As electrons are passed between these components, the energy liberated by the redox reactions is used to translocate (pump) approximately four protons (H$^+$) from the matrix of the mitochondria to the intermembrane space.

**Complex II** is the Succinate Dehydrogenase Complex of the TCA CYCLE. Seven of the eight enzymes of the TCA cycle are soluble enzymes that form a metabolon in the mitochondrial matrix. The eighth enzyme, the Succinate Dehydrogenase Complex, is an intrinsic membrane protein embedded in the inner mitochondrial membrane. (The other seven enzymes form the metabolon in complex with this membrane bound enzyme.) Succinate is oxidized to fumarate and the electrons are initially passed to FAD reducing it to FADH$_2$. These electrons are passed from FADH$_2$ to iron sulfur centers and then ultimately to the Coenzyme Q that is part of the inner membrane reducing it to CoQH$_2$. No protons are pumped by this complex.

**Complex III** is also called UBIQUINONE:CYTOCHROME c OXIDOREDUCTASE or CYTOCHROME bc$_1$ COMPLEX. CoQH$_2$ undergoes a two electron reduction when it accepts electrons from Complex I or Complex II. The individual cytochromes of Complex III and Complex IV are capable of only one electron redox reactions, the iron ion on the heme can only accept or donate a single electron. Complex III accepts electrons from reduced CoQ (CoQH$_2$) and by a circuitous route involving two ‘b’ type cytochromes, cytochrome c$_1$, and iron sulfur centers ultimately passes the electrons one at a time to two molecules of cytochrome c. This complex route is required to funnel electrons from the two electron carrier, CoQH$_2$, to the one electron carrying cytochromes present in complex III and complex IV. The postulated pathway for
electron flow in this complex is diagramed below. A large pool of oxidized Coenzyme Q (CoQ) and reduced Coenzyme Q (CoQH₂) is present as part of the inner mitochondrial membrane. Reduced Coenzyme Q is oxidized by Complex III in two steps. The two steps are called the Q CYCLE.

The first half of the Q cycle starts when reduced CoQ binds to the oxidizing face of Complex III. One electron is transferred from CoQH₂ to the iron sulfur centers reducing the iron (Fe³⁺ to Fe²⁺) and half oxidizing CoQ to the one electron semiquinone, CoQH•. The electron on the iron sulfur center is passed to cytochrome c₁ and from cytochrome c₁ it is passed to cytochrome c. The semiquinone form of CoQ,
CoQH•, passes the second electron to cytochrome bL, reducing the cytochrome and oxidizing CoQH• to CoQ. The electron on cytochrome bL is passed to cytochrome bH and then it is passed to a CoQ molecule bound to the opposite face, the reducing face, of Complex III. The CoQ bound to the reducing face is reduced it to the semiquinone, CoQH•. This completes the first half of the Q cycle.

The second half of the cycle is similar to the first half, a second CoQH2 binds to the oxidizing face and is oxidized to CoQ with one electron being passed to cytochrome c and the other going to cytochrome bH. In the second half of the Q cycle the electron on cytochrome bH is passed to the CoQ semiquinone (CoQH•) formed by the first half of the cycle, reducing it to CoQH2. The CoQH2 formed on Complex III leaves the complex and re-enters the pool of reduced CoQ. This round about pathway results in two electrons (net) entering Complex III and leaving the complex one at a time. As two electrons flow through Complex III, approximately four protons (H+) are pumped from the matrix of the mitochondrion to the intermembrane space.

There is an oxidized pool of cytochrome c that excepts electrons from Complex III. The resulting pool of reduced cytochrome c serves to shuttle electrons to Complex IV.

**COMPLEX IV** is also called **CYTOCHROME c OXIDASE**. The flow of electrons in Complex IV is cytochrome c → CuA → cytochrome a → CuB → cytochrome a3 → O2. CuA is a pair of copper ions that are adjacent to a part of cytochrome a. They are within 1.5 nm of the heme iron on cytochrome a. CuB is a single copper ion that is part of cytochrome a3 and equally close to the heme iron on cytochrome a3.

It should be noted that oxygen (O2) and the intermediates formed during its reduction remain tightly bound to the complex until completely converted to water, thereby limiting the generation of highly reactive toxic oxygen intermediates (O2•, O2–, OH•, H2O2). The precise mechanism is more complex than described here, in essence Complex IV accepts two electrons from a pair of cytochrome c and passes these two electrons to each of the CuA’s. Electrons are passed one at a time through cytochrome a and they are collected again on the cytochrome a3-CuB complex. These two electrons are passed to O2 reducing it to O2–2. A third electron is passed from cytochrome c down the chain to cytochrome a3. This electron along with a second electron from the iron of cytochrome a3 is passed to the tightly bound O2–2, simultaneously 4 H+ react forming 2 H2O. This oxidizes the iron of cytochrome a3 to the Fe+4 state. A fourth electron is passed down the chain from cytochrome c to reduce cytochrome a3 from Fe+4 to Fe+3. Two protons are used by Complex IV during the formation of one water molecule and it pumps approximately two (H+) protons across the inner mitochondrial membrane for every pair of electrons that are passed to an oxygen atom. Four protons are removed from the matrix for every pair of electrons transported through Complex IV and eight are removed.
by Complex IV for every O$_2$ reduced to 2 H$_2$O.

Remember the complexes contain not only the electron carriers, they also contain the enzymes that catalyze the reactions that transfer electrons from one electron carrier to the next and the proton translocases, the proton pumps, that move protons (H$^+$, H$_3$O$^+$) across the inner mitochondrial membrane.

**ATP Generation**

Prior to 1961 investigators working to decipher the **ELECTRON TRANSPORT / OXIDATIVE PHOSPHORYLATION PATHWAY** searched exhaustively for high energy intermediates formed by or during electron transport. It was initially postulated that a high energy phosphorylated intermediate (e.g., phosphoenolpyruvate) was generated during electron transport and subsequently used to transfer phosphoryl groups to ADP forming ATP at the substrate level. When this hypothesis didn't pan out, investigators postulated that the flow of electrons down the electron transport chain caused conformational changes in proteins raising them to an “energized conformation” and ATP was synthesized as these proteins fell back to their ground state. This hypothesis was likewise discarded.

In 1961 **PETER MITCHELL** noted that an intact inner mitochondrial membrane and a proton gradient was necessary for ATP generation. He demonstrated that if the proton gradient was destroyed, electrons would flow down the energy gradient of the electron transport chain but no ATP would be generated. When the proton gradient was reestablished, ATP generation would begin again. Mitchell destroyed the proton gradient by either physically disrupting the inner mitochondrial membrane (irreversible damage) or by adding **UNCOUPLERS** to intact mitochondria (reversible damage). An uncoupler is a lipid soluble weak acid that transports protons from the intermembrane space back into the matrix destroying the electrochemical proton gradient. 2,4-DINITROPHENOL is the classic example of an uncoupler. Mitchell’s hypothesis became known as the **CHEMIOSMOTIC THEORY** of ATP generation. In the 1990’s Mitchell’s hypothesis was broadened to include a **CONFORMATIONAL COMPONENT** into the **CHEMIOSMOTIC THEORY**. To put it in simple terms, the movement of protons down the concentration gradient from the intermembrane space to the matrix causes conformational changes in Complex V and these changes in conformation drive the synthesis of ATP from ADP and phosphate.

ATP is generated by **COMPLEX V**, or ATP SYNTHASE, using the
energy stored in the electrochemical proton gradient. Complex V is a large complex of proteins. It is also called the F₀/F₁ COMPLEX.

The F₀ part in mammals consists of, 1 ‘a’, 2 ‘b’, & 12 ‘c’ subunits. F₁ is composed of 3 α, 3 β, 1 γ, 1 δ, and 1 ε subunits. The 12 ‘c’ subunits of the F₀ part of the complex each contain two transmembrane helical segments and these 24 helices of the 12 ‘c’ subunits form a disc that is embedded in the inner mitochondrial membrane. The ‘a’ subunit is a voltage gate controlling the flow of protons from the intermembrane space to the matrix. The 2 ‘b’ subunits are tightly bound to the ‘a’ subunit of F₀ and the δ subunit of F₁. The F₀ part of the complex is a gated proton channel.

One α subunit and one β subunit forms a functional unit of the F₁ part of the complex, the αβ pair perform the ATP synthesis reaction. As was mentioned above, the δ subunit is bound to the 2 ‘b’ subunits of F₀ and it is tightly bound to one of the α subunits of F₁. The γ and ε subunits of F₁ are fixed to the center of the disc of 12 ‘c’ subunits in the F₀ part of the complex. When the concentration of protons in the intermembrane space is sufficiently high the gate on the ‘a’ subunit of F₀ is opened and protons flow from the intermembrane space to the mitochondrial matrix. The protons cannot move in a straight line from the intermembrane space to the matrix. The arrangement of subunits in F₀ is such that the protons must interact with the ‘c’ subunits to get from one side of the inner membrane to the other. As the protons enter through the ‘a’ subunit the protons bind to an essential aspartate on a ‘c’ subunit. Protonation of the Asp causes the helix to swivel and this movement causes the disc of the 12 ‘c’ subunits to rotate a distance of one ‘c’ subunit. Since the γ subunit of F₁ is very tightly bound to the center of the 12 ‘c’ subunits it also rotates. The α and β subunits of the F₁ complex, however, cannot rotate because they are fixed in a stationary position by the interactions between the δ subunit and the 2 ‘b’ subunits of F₀, which is anchored to the ‘a’ subunit in the membrane. The γ subunit is triangular in cross section and it functions as an asymmetrical axle (eccentric cam). As the γ subunit rotates, the β subunits are required, physically forced, to undergo conformational changes. The γ subunit rotates at 1000 rps.
As the eccentric cam of the γ subunit rotates it forces the β subunit to adopt one of three unique conformations. These conformations are termed OPEN or EMPTY (β₀), LOOSE or ADP (β₁), and TIGHT or ATP (β₂). The β₀ conformation has no affinity for the nucleotides. The β₁ conformation binds ADP and PO₄⁻³, brings these two components together, and aligns them properly for phosphoanhydride bond formation. The β₂ conformation joins the two substrates forming ATP. The β₂ conformation then reverts back to the β₀ form and ATP leaves the active site. At any given time one β subunit is in each of the three conformations, i.e., three ATP are synthesized per rotation of the γ subunit. In summary, the flow of protons through the F₀/F₁ Complex causes conformational changes in the β subunit of F₁ that drives the formation of ATP. The F₀/F₁ Complex is called a molecular motor because it moves/rotates like a motor or a generator.

Control of Electron Transport / Oxidative Phosphorylation

At Complex IV the pathway is controlled by the cellular demand for ATP. When ADP, PO₄⁻³, and NADH levels are high within the matrix of the mitochondria, the rate of the pathway increases. When ATP levels are high the pathway is slowed. There are ATP binding regulatory sites on cytochrome c and complex IV. When ATP concentrations are high, ATP binds to the sites and acts as an allosteric inhibitor decreasing electron transport activity.

Energy Yield

How many ATP molecules are generated per pair of electrons passed down the electron transport / oxidative pathway?

The current consensus opinion is that about four protons must be translocated from the matrix to the intermembrane space for every ATP generated. As a pair of electrons move from NADH to oxygen 4 protons are translocated at Complex I, 4 are pumped at Complex III, and 2 are pumped by Complex IV. From the number of protons translocated, in theory, 2.5 ATP should be generated per NADH oxidized. The electrons from one FADH₂ (succinate) translocates six protons as they flow through Complex III and Complex IV and therefore, in theory, 1.5 ATP should be generated per FADH₂ (succinate) oxidized. The P/O ratio is the number of ATP molecules generated per oxygen atom reduced to H₂O which is equivalent to the number of ATP molecules generated per pair of electrons passed down the chain. Early experiments with isolated mitochondria yielded P/O ratios between 2.4 and 2.8 when NADH was the electron donor and P/O ratios between 1.3 and 1.7 were obtained when FADH₂ was the electron donor. These values were rounded to whole numbers; 3 ATP per NADH and 2 ATP per FADH₂. More carefully controlled experiments have shown that the electrons from NADH generate about 2.5 ATPs, and about 1.5 ATPs are generated per FADH₂ (succinate) that enters ET/OxPhos. This fits the theory.

For electrons to flow down the electron transport chain ADP and PO₄⁻³ must be present in the mitochondrial matrix. ADP is transported into the mitochondrial matrix in exchange for ATP by an antipporter. This movement is facilitated by the charge difference across the inner mitochondrial membrane. The matrix becomes more negative as protons are moved across the inner membrane, and it is energetically favorable to move the ATP with 4 negative charges out and ADP with 3 negative charges in. We speak about phosphate (PO₄⁻³), in reality the cell contains a mixture of dihydrogenphosphate (H₂PO₄⁻), monohydrogenphosphate (HPO₄⁻²), and phosphate (PO₄⁻³). The transporter that brings “phosphate” into the mitochondrial matrix is specific for H₂PO₄⁻ and it is a symport bringing one H₂PO₄⁻ in along with one H⁺. One of the four protons
needed to synthesize ATP is used to transport the $H_2PO_4^-$ into the mitochondrial matrix. It must be noted that some of the energy of the gradient is lost by non specific leakage of protons across the outer or inner mitochondrial membrane and by compounds in the intermembrane space that can buffer the pH gradient.

**Cellular Metabolism**

ATP $\rightarrow$ ADP + phosphate

**Energy Yield per Glucose Molecule**

*How many ATP molecules are generated by the complete oxidation of a single glucose molecule?*

Starting at the beginning -

Glycolysis produces 2 ATP, 2 NADH, and 2 pyruvate in the cytoplasm of the cell.

There is no transporter in the inner mitochondrial membrane that can directly transport NAD or NADH into or out of the mitochondrial matrix. So for the moment the 2 NADH generated by glycolysis are trapped in the cytoplasm. How it ultimately enters the mitochondria will be discussed ere long.

The 2 pyruvate from glycolysis are transported into the mitochondrial matrix where the Pyruvate Dehydrogenase Complex (PDHC) produces 2 NADH, 2 acetyl-CoA, and 2 $CO_2$. 
The 2 acetate fragments carried by CoA enter the TCA Cycle. For every acetate fragment that enters the TCA Cycle, 3 NADH, 1 FADH₂, 1 GTP (1 ATP) and 2 CO₂ are produced. Therefore, the two acetate fragments from glucose produces 6 NADH, 2 FADH₂, 2 GTP (2 ATP) and 4 CO₂.

The 8 NADH (2 from PDHC + 6 from TCA) and the 2 FADH₂ (TCA) enter the Electron Transport / Oxidative Phosphorylation Pathway and the high energy electrons are passed to oxygen to form water, the electrochemical gradient is established, and the F₀/F₁ Complex generates ATP.

\[
\begin{align*}
8 \text{NADH} &\times 2.5 \text{ATP/NADH} = 20 \text{ATP} \\
2 \text{FADH}_2 &\times 1.5 \text{ATP/FADH}_2 = 3 \text{ATP}
\end{align*}
\]

The total ATP to this point is 23 ATP (ET/OxPhos) + 2 ATP (TCA) + 2 ATP (glycolysis) = 27 ATP.

Shuttle Systems

The 2 NADH generated in the cytoplasm during glycolysis have not been accounted for. This NADH must be oxidized to NAD⁺ in order for glycolysis to continue as well as to produce ATP from the high energy electrons. As was noted above, the NADH can not be directly transported into the mitochondria for oxidation by ET/OxPHOS. The cytoplasmic pool of NADH/NAD⁺ is kept separate from the mitochondrial
pool of NADH/NAD⁺. Shuttle pathways transport the electrons from cytoplasmic NADH to the ET/OxPhos pathway in the mitochondria.

There are two shuttle pathways present in cells. The simpler of the two shuttles is the GLYCEROL SHUTTLE (above). In this shuttle Cytosolic Glycerol-3-phosphate Dehydrogenase uses the NADH generated by glycolysis to reduce dihydroxyacetone phosphate to glycerol-3-phosphate. The NAD formed returns to glycolysis. Glycerol-3-phosphate enters the mitochondria via the porin channels where it encounters Mitochondrial Glycerol-3-phosphate Dehydrogenase located on the outer surface of the inner mitochondrial membrane. This isoenzyme oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate reducing covalently bound FAD to FADH₂. Dihydroxyacetone phosphate leaves the mitochondria to continue the shuttle process or to (re)enter glycolysis. The Mitochondrial Glycerol-3-phosphate Dehydrogenase passes the electrons from FADH₂ to Coenzyme Q, to Complex III, to Complex IV, and ultimately to O₂. 1.5 ATPs are generated per pair of electrons that enter ET/OxPhos by this shuttle. Therefore, 3 ATPs are generated from the 2 glycolytic NADH, for a grand total of 30 ATP. This shuttle system is very active in mammalian muscle and brain.

The other shuttle system, found in kidney, liver, and heart, is the MALATE-ASPARTATE SHUTTLE. In this shuttle Cytosolic Malate Dehydrogenase uses the NADH generated by glycolysis to reduce oxaloacetate to malate. The NAD formed returns to glycolysis. Malate enters the mitochondrial matrix by the Malate / α-Ketoglutarate Antiport. Malate is oxidized to oxaloacetate in the mitochondria and the NADH formed returns to glycolysis.
Ketoglutarate Antiport; for every malate that enters the matrix an α-ketoglutarate leaves. Malate in the matrix is oxidized to oxaloacetate and NAD is reduced to NADH by the Mitochondrial Malate Dehydrogenase. The NADH enters ET/OxPhos at Complex I. To keep the shuttle running the oxaloacetate must be transported back to the cytoplasm. It is not transported as oxaloacetate, since there is no transporter for oxaloacetate in the inner mitochondrial membrane, rather oxaloacetate is transaminated by Aspartate Aminotransferase. Glutamate donates the amino group to oxaloacetate forming aspartate and α-ketoglutarate. α-Ketoglutarate leaves the matrix by the Malate / α-Ketoglutarate Antiport and aspartate leaves via the Glutamate / Aspartate Antiport. Once in the cytoplasm Aspartate Aminotransferase converts aspartate and α-ketoglutarate back to oxaloacetate and glutamate. The oxaloacetate takes part in another round of shuttle operation and the glutamate is returned to the mitochondrial matrix by the Glutamate / Aspartate Antiport. Since the NADH generated by this shuttle enters at Complex I, 2.5 ATPs are generated for each of the cytoplasmic NADH for a grand total of 32 ATP per glucose molecule. How much ATP is generated by a resting adult per day?