(with embedded Q&A)

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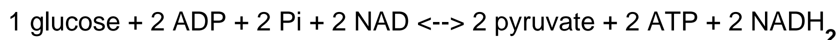
Note: You are not responsible for material not covered in lecture that is included here in green italics.

anaerobiosis
fermentation
energy yield
Krebs Cycle
FAD
GTP
electron transport chain
mitochondria
chemiosmotic theory
proton pump
ATP synthetase
oxidative phosphorylation
substrate level phosphorylation
alternative energy sources
transamination, deamination
biosynthetic pathways

Links to movies:

Rotation of actin filament arm
Animation 1
Animation 2
Animation 3

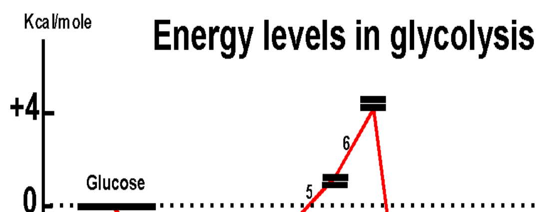
So after the 10 steps of glycolysis we have 4 ATPs produced and 2 invested, for a net gain of 2 ATPs produced from ADP. So glycolysis does produce energy in the form of ATP. And the overall reaction will run far to the right, as the Delta G^o from glucose to 2 pyruvates is -18 kcal/mole (even taking coupled reactions producing the 2 net ATP into account). That is,


1 glucose + 2 ADP + 2 Pi + 2 NAD <--> 2 pyruvate + 2 ATP + 2 $NADH_2$

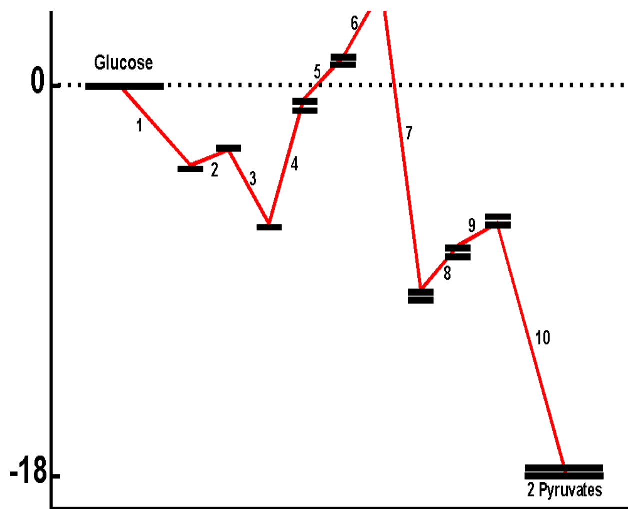
Delta Go = -18 kcal/mole

The diagram below shows Delta G^o's for the individual steps in glycolysis.

(For a different way of looking at it, see the chart of Delta G's drawn by Purves [Purves picture])



Although the pathway overall is quite favorable, some individual reactions are quite unfavorable, the most extreme being reaction #4. The energy-requiring reactions in this case are being pushed by the build-up of reactants by the more favorable reactions before them, and they are being pulled by



the withdrawal of products by the more exergonic reactions further downstream. The actual Delta G's will be influenced by the drain of products, so that the second term in the equation for Delta G is being brought into play here. {Q&A}

Delta G = Delta G^o + RTln([products]/[reactants]), where RT~0.6 (in **k**cal/mole)

For instance, if the products are drained such that the ratio of P/R reaches down to 0.00001, this produces ~ 7 kcal/mole of negative Delta G , enough to balance out the unfavorable Delta G$_o$ of +6.8 for rxn. 4.

This indirect affect on the Delta G is the second method the cell utilizes to carry out an individual reaction that has an unfavorable Delta G^o.

So we have 2 methods:

1.) DIRECT COUPLING of the unfavorable reaction to an energetically favorable one to produce a new coupled reaction with a net negative (favorable) Delta G^o (as in the hexokinase reaction #1).

2.) INDIRECTLY, via the WITHDRAWAL OF PRODUCTS or buildup of substrates {Q&A}

SO, we have our overall negative Delta G^o , and we have generated net ATP, so we should feel pretty good, except for one thing: we have an important loose end to tie up. We borrowed an NAD to oxidize Gal-3-PO_4. That was a key reaction in the path, as oxidations are usually accompanied by large changes in free energy. We were able to get a PO_4 added to our 3-carbon compound, and this phosphate was the one that was used to phosphorylate ADP in the very next step (#7). We used NAD, and it became reduced to NADH2. So now we must consider how we are going to repay that debt. Otherwise the small stores of NAD in the cell would very soon all be converted to $NADH_2$ and glycolysis would quickly grind to a halt.

We need an oxidizing agent to oxidize $NADH_2$. A great one abounds: O_2.

It readily takes up electrons, for instance from Fe++ to make it Fe+++ as steel goes to rust.

(Anaerobiosis)
Does E. coli have access to oxygen? In the lab, yes. We vigorously and constantly shake the E. coli cultures on mechanical shakers to get air dissolved in the culture medium to provide a constant source of oxygen, called an AEROBIC state. In the gut, sometimes yes, sometimes no (crowded). Do our own cells have access to oxygen? Sure, through the lungs via the blood vessels to all tissues. But when you are running across campus to class so as not to miss the first golden words here, your muscles may need ATP faster than you can deliver oxygen to them for $NADH_2$ oxidation, your muscles will be in an AN-AEROBIC state. And are many organisms that live in naturally anaerobic environments, in mud at the bottom of rivers, e.g. So let's first consider the anaerobic case when no oxygen is available for the oxidation of $NADH_2$. Under these circumstances the cell must make do with what it has available, which is mainly: 2 pyruvates. Fortunately, pyruvate itself is able to act as an oxidizing agent, as seen in rxn #11, where it accepts electrons into its C=O bond, adding two hydrogens as well, to form lactate, or **LACTIC ACID**. It gets the electrons from our $NADH_2$ molecule, which is really NADH and H+, so it is recapturing one proton (an H+ ion) from the aqueous pool. Even the Delta Go is favorable, and we get our NAD regenerated from $NADH_2$. The NAD shuttles back and forth then, getting reduced in rxn 6 and reoxidized in rxn 11 over and over and over again, while glucose runs down to lactate, and ADP's are converted to ATPs to power cell division for E. coli or running up stairs for humans even in the case of insufficient oxygen.

It is thought that it's the build-up of acidity from lactic acid that makes your muscles hurt if they are doing anaerobic glycolysis too long (acidity sensed as pain by neurons in the muscle).

(Fermentation)
So pyruvate is a crossroads: If no oxygen, then if you are E. coli or humans, you carry out a lactic acid fermentation... [Purves picture], [another Purves picture]

If you are yeast, there is a variation on this theme, you break down pyruvate first to acetaldehyde and CO_2, which is not an oxidation, but then use the acetaldehyde as an oxidizing agent for $NADH_2$, with the product here being the 2 carbon alcohol, ethanol (rxns 12 and 13). Beer drinkers appreciate this variation, as lactic acid beer would be pretty bad, and probably not even produce those psycho-pharmacological effects for which ethanol is famous. {Q&A}

Just as in the case of ATP, the NAD - $NADH_2$ case is one of re-generation, not generation. Once a little NAD is made, it can shuttle back and forth millions of times getting alternately reduced and oxidized.

Let's consider for a change an alternative source of carbon and energy for E. coli, glycerol. Remember glycerol is the tri-alcohol that serves as the backbone for triglycerides (fat) and phospholipids.

See glycolysis handout. From the PowerPoint graphic, it can be seen that glycerol is metabolized as follows:

glycerol + ATP --> glycerol-1-P

glycerol-1-P + NAD --> DHAP (= dihydroxyacetone phosphate) + $NADH_2$

DHAP --> continues in glycolysis (rxn 5). See recitation problems #3, problem 2.

Under aerobic conditions, no problem, the $NADH_2$'s produced will get re-oxidized by oxygen, as we will see. And so E. coli grow just fine in a glycerol minimal medium using glycerol instead of glucose.

How about under ANAEROBIC conditions?

We used *two* NADs to get down to pyruvate from glycerol, but we get back only *one* in going to lactate. If we try to run the lactate fermentation using glycerol as our only carbon and energy source, we will grind to a halt as all our NAD ends up as $NADH_2$. (You will reach a state where all the NAD is in the form of $NADH_2$, and there is no pyruvate left, just DHAP waiting for an NAD that is not there.) So, although E. coli will grow just fine on glycerol in the presence of air (oxygen), it will NOT, in fact, grow on glycerol in the absence of air (anaerobically). So: these loose-end debts are real, there is no magic, we must play by the rules.

See Problem 4-15 - why was he fired?]

Consider the **efficiency** of glucose fermentation:

For: glucose--> 2 lactates, without considering the couplings for the formation of ATP's (no energy harnessing): Delta G^o = -45 kcal/mole. {Q&A}.

Out of this comes 2 ATPs, worth 14 kcal/mol. So the efficiency is about 14/45 = 30%, which is not bad, about what a gasoline engine can do.

Where did the other 31 kcal get to? They are released as heat, which is why, after you've run fast to class, in addition to the lactic acid PAIN in your legs, you are also SWEATING.

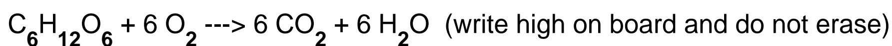
Now taking the ATPs into account, the overall Delta Gº is about -45 + 14 = -31 kcal/mole, so the lactic acid fermentation runs essentially completely to the right (or clockwise) as written.

(Energy yield)
The efficiency is pretty good, but on the other hand the **YIELD** is poor. What do I mean by YIELD? Well, glucose has a lot more chemical energy in it than we are tapping here. For example, if we BURN glucose (i.e., react it with oxygen) and measure the calories of heat given off, we get no less than 686 kcal/mole. Compare that to the measly 45 kcal/mole we got from converting glucose to two lactates. The lactic acid that we are throwing away at the end could be used for more energy, but in the absence of oxygen there is no way to use it.

With oxygen, it's another story ....and our next chapter.

[To review glycolysis and fermentation, try Problems 4-11 and then 4-13.]

(Krebs Cycle (TCA cycle, citric acid cycle))
So now let us turn to the case when oxygen **is** present. In this case we are headed to the complete oxidation of glucose to 6 molecules of CO_2 and 6 molecules of water:



$C_6H_{12}O_6 + 6\ O_2 \text{ ---> } 6\ CO_2 + 6\ H_2O$ (write high on board and do not erase)

The Delta G^o for this reaction is -686 kcal/mole, and we are going to hope to get a lot more ADP --> ATP conversions out of this.

In the presence of oxygen, glycolysis down to pyruvate is the same. But the fate of pyruvate is now different.

THE KREBS CYCLE

Rather than heading toward lactate or ethanol, as shown at the bottom of the glycolysis handout, the pyruvate jumps to another handout: the KREBS CYCLE. Keep in mind that by forgoing the reduction of pyruvate, we have not satisfied our loose end of $NADH_2$ accumulation from the oxidation step (step 6) in glycolysis.

The fate of pyruvate is now different, it will enter a series of reactions known as the Krebs Cycle, (also TCA cycle, or citric acid cycle) in which all of its carbons will indeed end up as CO_2. However, as we are about to see, its H's will not be converted to water here, and very little ATP will be produced here. [Purves picture], [another Purves picture]

Please follow along in the Krebs Cycle diagram as the reactions are discussed. At the top of the Krebs Cycle diagram, we see our pyruvate entering from the top left, in a reaction that is not part of the cycle of reactions seen below (it is sometimes called the entrance reaction). Before entering the cycle, pyruvate undergoes a relatively complicated reaction involving an oxidation, once again using NAD as the oxidizing agent, as well as a **DECARBOXYLATION**, the splitting off of the carboxyl carbon as CO_2 (as when ethanol was made in yeast) and leaving a 2-carbon acetate group. So here is some CO_2 produced, which is what we expected from the oxidation of glucose. In addition, a new co-factor makes an appearance: Coenzyme A (abbreviated CoA), a sulfur-containing small molecule [pADP-pantothenate-SH] which becomes bound to the acetate in a thioester linkage. A thioester is analogous to an ester except a sulfhydryl is one of the reactants instead of an alcohol, so a sulfur atom takes the place of an oxygen. A thioester contains a high energy bond, and so should be a SQUIGGLE (see Becker 6th Ed.

Fig. 10-7 for the exact structures, if you wish). It is **acetyl-CoA**, the product of this dehydrogenation of pyruvate, that is the compound that now enters the Krebs Cycle proper. [Purves 7.9]

Acetyl-CoA condenses with a molecule of **oxaloacetate**, a 4-carbon dicarboxylic acid, to produce citrate, a 6-carbon tricarboxylic acid (thus the name tricarboxylic acid cycle or TCA cycle as a synonym for the Krebs Cycle). CoA is split off in the course of this reaction. The high energy bond to CoA is utilized to help drive this otherwise endergonic synthesis of a 6C molecule. The free CoA is regenerated and so is not consumed.

Now we have a new loose end, however: **OA** (oxaloacetate). We have introduced, borrowed, this molecule, much as we borrowed an NAD, so it's now OA plus 2 NADs; no, really 4 NADs and 2 OA's (per glucose molecule). We must pay back these debts by the end of our path (which remember now is going to be 1 molecule of glucose going to 6 molecules of CO_2).

We have labeled the carbons of acetyl-CoA with an asterisk and a dot so that we can follow them as they go through this set of reactions. In the laboratory, it is also possible to use organic molecules labeled in this way, by using molecules in which particular carbons or hydrogens have been replaced with their radioactive counterparts, or isotopes, for instance ^{14}C instead of the usual ^{12}C or ^{3}H instead of ^{1}H. Radioactivity counters can then be used to track and measure the appearance of the isotopes in various purified intermediate compounds.

The citrate is isomerized to isocitrate; note the movement of the hydroxyl group from the middle to one end. The isocitrate is oxidized once again using NAD and it's simultaneously decarboxylated to produce alpha-ketoglutarate and now our second molecule of CO_2. We need to get 3 from our 3-carbon pyruvate molecule. So this is 2. Except the actual carbon is not from our acetyl-CoA, i.e., from our pyruvate, but rather from the OA that we borrowed. Looks like it may be a bad debt: here we've borrowed an OA and now we've blown it into CO_2.......How are we ever going to pay it back? Oh well, let's go on.

Next we again have an oxidative decarboxylation, from the 5-carbon alpha-ketoglutarate to the 4-carbon succinate. This is actually a set of two reactions, as can be seen in the Becker text. Once again the CO_2 comes not from the acetyl-CoA carbons, but from a carbon atom originally in OA. But here we have a new and welcome feature, the production of GTP from GDP. The free energy from this oxidation is coupled to the phosphorylation of GDP with Pi. GDP is a compound, a nucleotide, related to ADP, the structure being the same except for the substitution of the guanine ring for the adenine ring. The production of GTP is energetically equivalent to producing ATP, since:

GTP + ADP --> ATP + GDP, Delta G^{o} = 0

So we finally get some ATP here, 2 moles per mole of glucose, equal to what we netted in glycolysis. {Q&A}

The mechanism of this coupling is less obvious than those we saw in glycolysis (where the substrates were phosphorylated). Here the inorganic phosphate and GDP are both bound by the enzyme as part of the overall reaction.

[not responsible for this mechanistic detail:

aKG + NAD + CoA --> CO_2 + $NADH_2$ + Succ~CoA;

Succ~CoA + Pi + E --> CoA + E-succ~P --> E~P + Succinate,

E~P + GDP --> E + GTP]

(FAD)

Next the succinate is dehydrogenated across its central 2 C's, producing fumaric acid This is the reaction we discussed earlier illustrating enzyme specificity. Here the oxidizing agent is FLAVIN ADENINE DINUCLEOTIDE

(**FAD**), rather than NAD. FAD is a better oxidizing agent (it is more easily reduced) than NAD; the Delta G^o for this reaction using NAD would be highly unfavorable, whereas with FAD it is much more favorable, about a 10 kcal/mole difference. So now we have to add 2 $FADH_2$'s to our debt LIST. Our debt list now includes: NAD's, FAD's, and OA.

Continuing, we add water across fumarate's C=C double bond to get malate, and then once again dehydrogenate, i.e., oxidize, using NAD to get the 4-carbon dicarboxylic keto acid OA.

So we can pause here, our OA has been regenerated, and is ready to take on another acetyl-CoA. We have utilized one pyruvate and have released 1, 2, 3 CO_2 molecules. We have carried out 5 oxidations per pyruvate, 4 with NAD and 1 with FAD, 4 oxidations in the cycle proper and one oxidation getting into the cycle. We produced one ATP equivalent per pyruvate. And we have accumulated 4 $NADH_2$'s and 1 $FADH_2$ per pyruvate (double all these for a per glucose accounting). But we have paid back our OA debt, not with the original OA molecule, but a "refurbished" molecule. No one will ever know the difference (except perhaps some nosy biochemist with radioactive isotopes).

In Krebs Cycle	per pyruvate	per glucose
CO2 released	3	6
oxidations:	5	10
$NADH_2$ produced	4	8
$FADH_2$ produced	1	2
ATP produced (as GTP)	1	2
O_2 consumed	0	0

Our labeling showed that the CO_2 molecules produced in this turn of the cycle were not derived from the acetyl-CoA.

The acetyl carbons have ended up on either the top or the bottom of the OA that was regenerated. We don't know which end because fumarate is a symmetric molecule, and the water addition forming malate could have produced a hydroxyl on either the labeled or the unlabeled end.

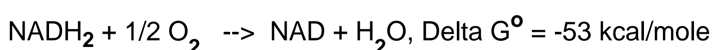
So far we've gotten precious little energy out in the form of ATP, and we still have the NAD and FAD to pay back. And oxygen has not been involved yet. The electrons of glucose (entering here as pyruvate) have not been delivered to O_2, but are still on the way-station of $NADH_2$ and $FADH_2$.

To review the Krebs Cycle, try problem 4-17 (4-12 in 16th ed.)

(electron transport chain)

In the next part of the story, $NADH_2$ and $FADH_2$ will pass their electrons on to oxygen AND we will get a lot of ATP from this passage of electrons. This oxidation of $NADH_2$ and $FADH_2$ will return them for further action as NAD and FAD.

So let' s pass them on to oxygen:



53 kcal/mole released, TOO high. Too much energy released: if it were used in one fell swoop of the usual coupled reaction, we would get only a single ATP's worth, 7 kcal/mole, from this 53 kcal/mole, and we'd release a LOT of heat besides. It would be better if we could break up this -53 kcal/mole into smaller bits to use. The scheme for breaking up the free energy change involved in the reduction of oxygen involves passing the electrons from $NADH_2$ and $FADH_2$ not directly to oxygen but rather through a chain of intermediate transfer steps. This chain of steps is called the **ELECTRON TRANSPORT CHAIN** (E.T.C.).

Let's look at View #2, on the ETC handout: The electrons from $NADH_2$ are seen to be passed to various ELECTRON CARRIERS of the electron transport chain, in a precise sequence of transfers (here simplified, see Becker).

Some of the participants in this chain are:

- Proteins = Iron-sulfur protein (in which iron as Fe+++ (ferric) accepts the electrons).

- Coenzyme Q (CoQ) , a small molecule, hydrophobic, lipid soluble (also called ubiquinone).

- CYTOCHROMES: cytochromes b, an Fe/S protein, and cytochromes c1, c, a, and a3 (with prosthetic groups containing Fe or Cu)]

- then to O_2 (forming water)

Follow the electrons in this diagram (ETC handout). The electrons from an $NADH_2$ are transferred to FMN (a simpler form of FAD) and then CoQ, reducing it to $CoQH_2$. Subsequently, the electrons are passed from $CoQH_2$: that is, $CoQH_2$ gets oxidized by passing the electrons to cytochrome b. The cytochromes contain heme as a prosthetic group, in which an iron oxidation-reduction occurs: Fe+++, +1 electron ---> Fe++. So now cytochrome B has the electrons. Cytochrome B gets oxidized by an iron-sulfur protein and that in turn gets oxidized by a heme in cytochrome C1. Its iron returns to ferric (Fe+++), while cytochrome C's heme group get reduced from the Fe+++ to the Fe++ state. Similar transfers occur between cytochromes C1, C, A, and A3. Finally, cytochrome A3 passes the electrons to molecular oxygen, which also picks up 2 protons to go with two electrons to form hydrogen atoms, so that the product of this reduction is H_2O, which is the final resting place for these travel-weary electrons.

Note that the electrons become separated from the hydrogen ions early in this process (after FMN); after that we follow the electrons and let the protons join the general proton pool, for the moment.

An energy level handout shows the free energy changes associated with some of these electron transfers. Each transfer is energetically favorable, with some of the changes releasing much more free energy than others. It can be seen here that the 53 kcal per mole for the reaction between $NADH_2$ and oxygen has been broken up into smaller packets of free energy changes. The free energy changes marked with an asterisk are those that have been found capable of generating a molecule of ATP from ADP. We will get to the mechanism of that generation a little later. You can also see that whereas $NADH_2$ can generate 3 ATPs, $FADH_2$ can only produce 2.

Thus CoQ and the cytochromes C, and NAD and FAD, are constantly shuttling electrons, picking them up originally from glucose-derived molecules and then delivering them elsewhere and then returning to pick up another load.

In the end, O_2 receives the electrons. All the reduced forms of the oxidative cofactors ($NADH_2$, $FADH_2$) return to the oxidized state (NAD, FAD), having gotten rid of these electrons..

So: ..... we have no NAD or FAD loose-end debt any longer.

All debts have been paid. All the glucose carbon atoms have been converted to CO_2, and the electrons from glucose have now all been delivered to O_2 to form water.

To review electron transport try problem 5-9 and then 5-10.

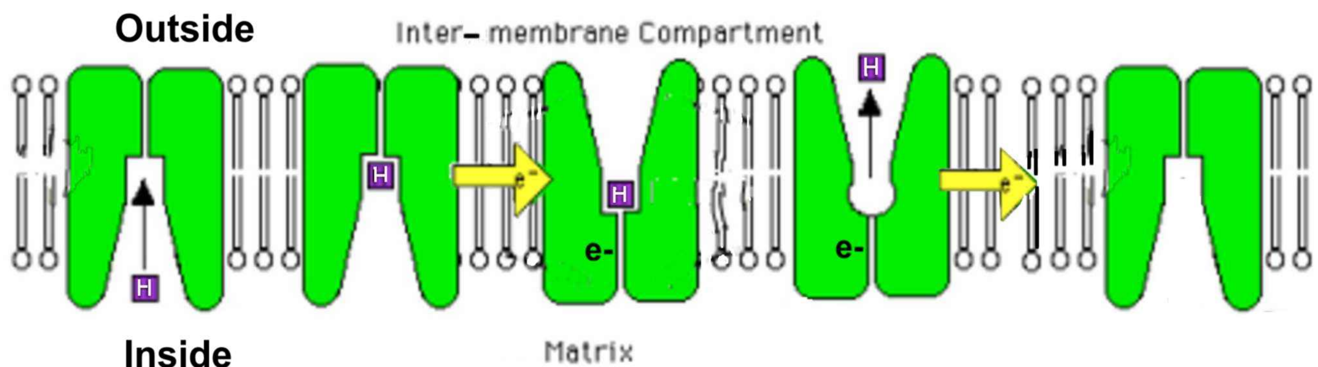
(electron transport chain, continued)
The **ETC** proteins complexes are held within the **INNER MEMBRANE** (within the cristae).

The mitochondrion handout shows that these electron transport proteins of the ETC are organized into 3 groups; these protein complexes, called respiratory complexes are geographically fixed next to each other within a membrane in the cell, as we shall soon see. That is, these are membrane-bound complexes of proteins. [Purves picture].

But where is the energy? the ATP?

(chemiosmotic theory)
The answer lies in the **Chemiosmotic Theory** first proposed by Peter Mitchell in 1961.

(proton pump)
The energy released at each of the electron transfers is stored in an electro-chemical gradient, established across the mitochondrial inner membrane. Concomitant with electron flow, H^+'s are being pumped out of the matrix into the inter-membrane space. These hydrogen ions are not clearly from $NADH_2$ *per se*, as H^+'s get pumped out even in later steps in the ETC where no protons are directly involved (just electrons on Fe++ atoms) [Purves picture]. So this pumping out of H+ ions must be coupled the binding and release of electrons by the proteins involved, or schematically:



The immediate effect of this H+ pumping is a higher concentration of H+ ions outside the mitochondrion's inner membrane and a lower concentration of H+ ions inside the mitochondrion. Now we allow the pumped-out H^+'s to flow back. By mass action, the protons will flow from a region of high concentration to a region of lower concentration. A steady-state increment (between the outside and the inside of the inner membrane is thus maintained, resulting in the matrix (inside) being about 1 pH unit higher than the outside). A constant kicking-out, and flowing-back.

The H^+'s may not get that far, but the outer membrane is no barrier to H^+ ions, so how far they get is not an important factor.

To review the movement of protons and electrons see problem 5-13 part A (part C-1 in 16th ed.)

(oxidative phosphorylation and substrate level phosphorylation)
This process of forming ATP by a proton motive force is called **OXIDATIVE PHOSPHORYLATION** (OXPHOS). {Q&A}. And so there are two methods of producing ATP from glucose metabolism: Oxidative phosphorylation and the "regular", direct phosphorylation of ADP from phosphorylated intermediates that was seen during glycolysis, or in

the GTP-forming step in the Krebs Cycle. This direct phosphorylation is called **SUBSTRATE-LEVEL PHOSPHORYLATION** (SLP)**,** to distinguish it from OXPHOS.

Some evidence for the validity of the chemiosmotic theory of oxidative phosphorylation is:

1) Adding H^+ ions (adding acid, in moderation) to closed vesicles (or membrane-bound spheres) that have been formed from membranes containing the F1-Fo protein complexes generates ATP from ADP + Pi in the test tube.

2) Isolated ETC complexes I, III, or IV inserted into artificial membranes are able to pump H+ ions in the predicted directions when provided with the appropriate substrates (reduced electron carriers like $NADH_2$).

3) DINITROPHENOL, a small partially hydrophobic molecule, can return H^+'s to the inside of the mitochondria via a short circuit; it ferries them across the inner membrane; the H^+'s thus avoid the Fo channel. This compound uncouples H^+ transport from ATP generation, so you get electron transport, but no OX-PHOS (since there is no longer a build-up of a proton gradient across the membrane). What about E. coli, they have no mitochondria (in fact a mitochondria is about the size of an E. coli cell). Bacteria simply use their own plasma (cell) membrane, and kick the H^+'s out into the medium (or in the immediate vicinity of the periplasmic space between the cell membrane and the cell wall).

To review all of oxidative phosphorylation try problems 5-11 and 5-12.

OK, so how much ATP do we get after all this?

1 ATP per pair of electrons transferred through EACH of the 3 enzyme complexes (**I**, **III**, and IV). The number of protons transferred per pair of electrons is not really known precisely (it's been estimated at 10-12, so 3-4 protons flowing back can produce an ATP).

So 3 ATPs per pair of electrons passing through the full ETC.

So 3 ATPs per 1/2 O_2

So 3 ATPs per $NADH_2$

But only 2 ATPs per $FADH_2$, which skips complex **I**, and delivers its electrons to CoQ via complex **II**, with little free energy released at that first step.

Overall ATP tally of **RESPIRATION** (as this overall oxidative metabolism of glucose is called) (see also OUTLINE of energy metabolism handout):

*ATP from substrate-level phosphorylation, **SLP** (per glucose)*:

Glycolysis: -2 that have to be invested, then +4 for a net of +2

Krebs Cycle: 2 (as GTP)

Total SLP = 4

ATP from OxPhos (per glucose)

(first calculating per Glyceradhyde-3-P, where the first oxidation takes place:

1 $NADH_2$ from glycolysis and 1 from entry into the KC and 3 from the KC proper)

So 5 $NADH_2$ @ 3 ATP/$NADH_2$ = 15

$FADH_2$: 1 from the KC @ 2 ATP/$FADH_2$ = 2

Total OXPHOS per molecule of glyceraldehyde-3-phosphate = 17

Per glucose molecule, multiply by 2:

17 X 2 = 34

Grand total = 4 + 34 = 38 ATPs per glucose (in E.coli and other prokaryotes)

Now, if we are considering eukaryotic cells, we need to subtract 2 ATPs from this total, as 2 ATPs are used to get electrons from the 2 cytoplasmic $NADH_2$'s from glycolysis into the mitochondria (by an indirect mechanism), so the net for eukaryotes is 36.

Efficiency: 36 ATPs X 7 kcal/mole ATP hydrolysis = 252 kcal/mole harnessed as ATP.

252/686 available from glucose combustion = 37% efficiency.

Once again, better than most engines (20-25%).

And compare the yield: 36 ATPs per glucose from *respiration* to 2 per glucose for *fermentation* . {Q&A}

So with or without air, ATP is no problem............

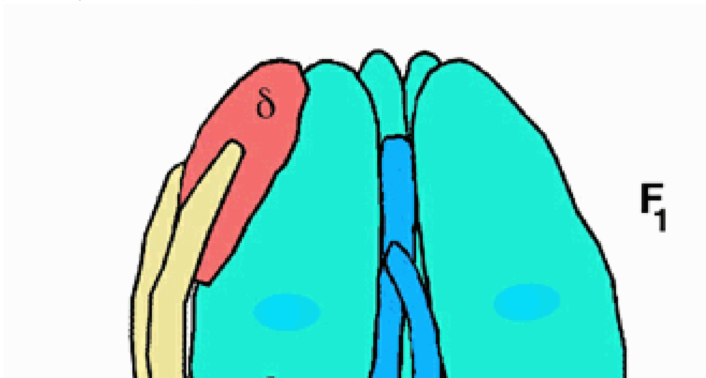
To review overall energy metabolism and compare fermentation and respiration, try problems 5-1, 5-3, 5-5.

To review energy (ATP) yields +/- oxygen, try problems 5-2 and 5-4.

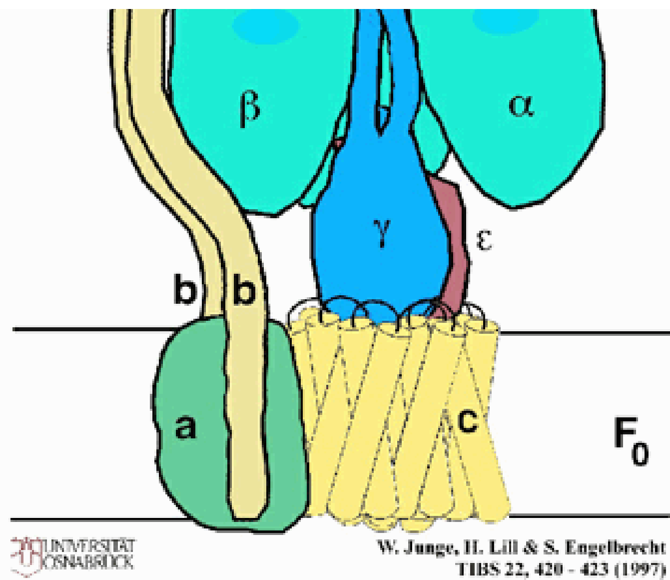
[ATP-synthetase 3-D animation (.mov)]

Mechanism of ATP synthesis in mitochondria

(ATP synthetase)

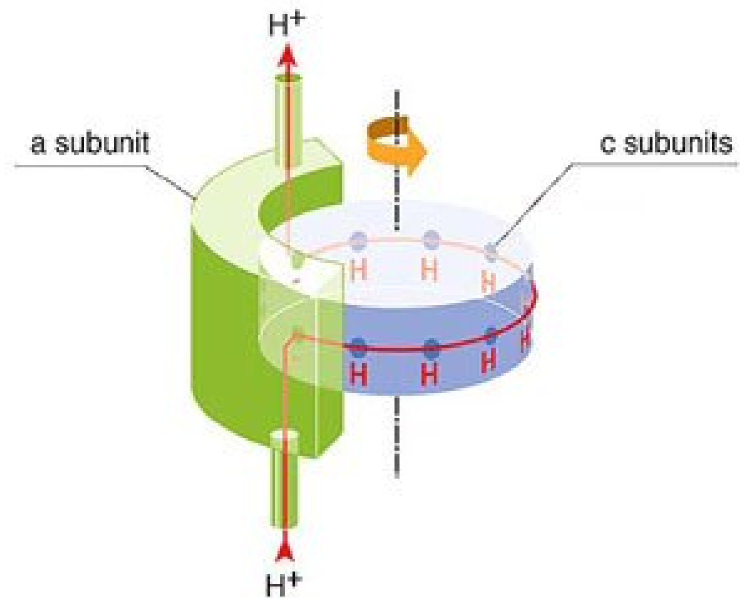


The flow-back is through lollipop-like structures that populate the inner surface of the inner membrane. See handout on ATP-synthetase for the structure of this multi-subunit protein complex. Each lollipop is a complex of proteins; the stem is called **F_o** and forms a channel through the membrane. The sphere is called **F_1** and contains the **ATP SYNTHETASE** activity; that is, it is in the spheres that the generation of ATP from

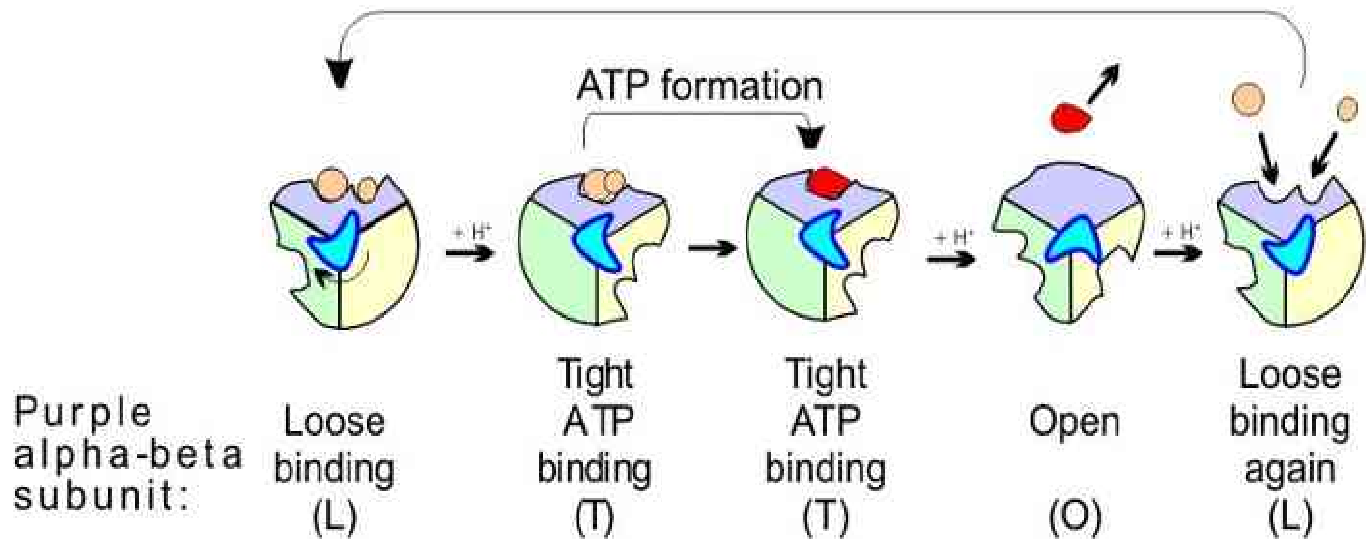


ADP + Pi takes place. It is the flow-back of H^+'s through the F1 spheres that generates the phosphorylation of ADP by P_i to form ATP. An analogy would be to use one source of energy to pump water up to a high level behind a dam (the pumping of protons tied to the free energy released in the oxidations of the electron transport chain components) and then letting the water drive turbines to generate electricity as it falls from the high level behind the dam (the generation of ATP)

This is one place where reading of both texts can help in the understanding of this very indirect mechanism. Indeed, this theory was doubted for many years after its proposal by Mitchell (it is also known as the Mitchell Hypothesis; Mitchell was known to do experiments in the basement of his mansion, like in the movies).



The mechanism of this reaction, the ATP synthetase, has only become clearer in the last few years. The Fo channel includes 10 c-subunits surrounding a central stalk, the gamma subunit. A proton outside the mitochondrial membrane flows back by entering the F_o stem channel where it bind to an amino acids on one of 10 c subunits comprising the cylindrical channel. This binding produces a shift in the cylinder by 1/10 turn (see right). As the c-cylinder turn so does its attached gamma stalk. The top the gamma subunit reaches into the center of the F1 head structure, and its shape there is in the form of an asymmetric cam. The F_1 head is

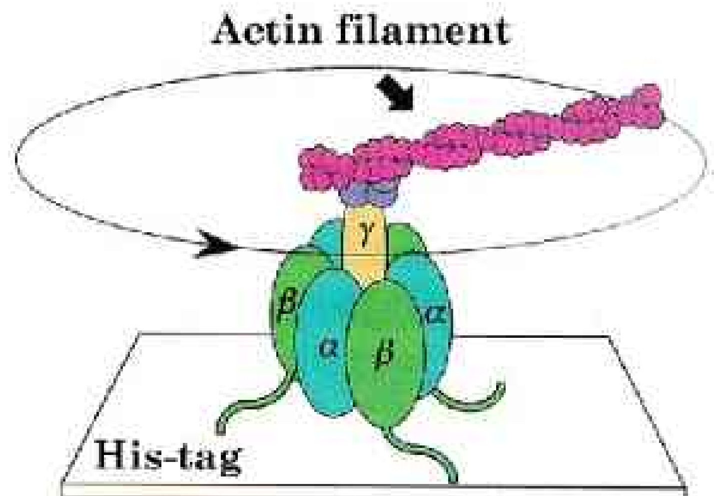


organized like a pie divided into 3 identical wedges [Purves picture]. The wedges can be configured in space to provide a binding site for ADP and Pi or for ATP. As the cam rotates amidst the alpha-beta subunits of the F1 head structure, it distorts their structure. Three distinct distortions are produced and each has an effect on the ability of the alpha-beta subunits to bind ATP and ADP and Pi on their face that is exposed to the inside of the mitochondrion. See the ATP synthetase handout to follow this process.

One distortion forces the ADP and Pi together in one wedge, while the ATP that just had been formed on another wedge is distorted in the opposite way to release the ATP. The sequence of these 3 events is thus 1) the binding of ADP and Pi (L), 2) a kind of mechanical force pushing them together (T), followed by 3) a quick release of the ATP (O). The formation of these 3 conformations is driven by protons binding to specific amino acids in the Fo channel. Thus as the protons flow back into the mitochondrion, the F_o shaft with its cam is spinning.

See a pretty animation of ATP synthesis from the Website of W. Junge.

This spinning turbine-type mechanism has received dramatic support from an experiment in which the ATP synthetase is constructed to run in reverse in the laboratory. In this case, ATP is added to a pieces of the synthetase (alpha, beta and gamma subunits); a long polymer of the protein actin has been attached to the free end of the gamma subunit. The actin has been labeled by attaching fluorescent groups to it, so it can be visualized in a fluorescent light microscope. The molecule is mount head down on a glass slide, with its gamma subunit pointing up, and with the attached actin polymer as a sort of flag. When ATP is added and the molecule is observed under the microscope, the actin polymer can be seen to be turning as the ATP is hydrolyzed and the synthetase revolves in reverse. See the PowerPoint graphics for pictures, which are necessary to understand this experiment.



C

W. Junge, H. Lill & S. Engelbrecht
TIBS 22, 420 - 423 (1997)

To review the action of ATP synthetase see problem 5-13.

(oxidative phosphorylation and substrate level phosphorylation)

From glucose, at least …... But we do not live by cake alone ….

How about a carbon and energy source OTHER THAN GLUCOSE?

Not in live lecture - not responsible for italicized text:

Where do we get this glucose? Ultimately from plants, who are able to synthesize it using an energy source obviously other than glucose, solar energy. I want to now rather briefly turn to photosynthesis, to summarize what is involved. Just sit back and listen, because photosynthesis will not be on the exam.

Photo - synthesis, the word and the problem consists of two parts.

1. Synthesis of glucose from simple CO_2 in the air.

2. Photo: the source of the energy, in the form of ATP, from sunlight, to carry out this synthesis.

First, the synthesis. Overall:

$6\ CO_2 + 6\ H_2O \text{ --> } C_6H_{12}O_6 + 6\ O_2$

CO_2 is highly oxidized carbon. Need to add H's from a reducing agent. The ultimate source of these hydrogen atoms is going to be water, but water is a poor reducing agent (O_2 holds its electrons tightly). So for photosynthesis, we need a source of reducing power as well as a way to make ATP from ADP.

Overall with ATP:

$6\ CO_2 + 12\ NADPH_2 + 18\ ATP \text{ --> } 18\ ADP + 12\ NADP + 1\ \text{glucose.}$

So we need NADPH2 and ATP. Get them both from the light reactions.

Another piece of cellular machinery: the chloroplasts, like mitochondria, a membrane delimited cytoplasmic organelle. But they also contain light-absorbing pigments, including the chlorophylls. When light energy (one photon) is absorbed by these pigments (each at a characteristic wavelength), the energy is used to excite a water electron to a higher energy state.

The excited e- goes down a (different) E.T.C. ending up at NADP, where a pair of e-'s are accepted to produce $NADPH_2$. This is the $NADPH_2$ we needed to make glucose.

$12\ H_2O + 12\ NADP + 18\ ADP + 18\ Pi + 60\ \text{photons -->}$

$6\ O_2 + 12\ NADPH_2 + 18\ ATP + 18\ H_2O\ \text{(from ADP+Pi)}$

The ATP is made via proton pumping to establish a hydrogen ion gradient as the electron travels down an electron

transport chain, as in mitochondria.

Efficiency 27%, once e-'s have been excited. But much less from light overall, as many e- fall back from excited state.

In plants these reaction occur only in chlorophyll-containing tissues (leaves, mainly); other plant tissues use glucose via respiration. And so do the leaves at night. Glucose is stored, transported, and used for energy.

So plants: CO_2 --> glucose + O_2

and we (and plants): glucose + O_2 --> CO_2

The carbon gets shuttles back and forth. In the planet as a whole, the CO_2 is also in equilibrium with vast stores of inorganic CO_2 in the oceans and in the form of carbonates ($CaCO_2$), limestone.

We discussed glucose. But one does not live by refined sugar alone. Most parts of a big Mac dinner will provide us with energy. These pathways of degradation of compounds is called CATABOLISM.

The roll:

1. POLYSACCHARIDES:

STARCH = Gluc-gluc-gluc-gluc-....Hydrolyzed to glucose-1-P, then PhosphoGlucoMutase catalyzes its isomerization to G6P = a glycolytic intermediate . Glycolysis takes it from there.

See problem 4-13 (4-11 in 16th ed.) Ask yourself, "What steps and enzymes are needed to carry out the reaction shown in the problem: Glucose-P + ATP --> 2 PGAlde?"

Milk shake:

More complex sugars: LACTOSE = glucose-galactose --> glucose + galactose; galactose then --> --> glucose.

Lettuce:

CELLULOSE --> nowhere (excreted). We can't handle it (no catabolic enzyme, no cellulase).

The French fry:

2. LIPIDS: fats = triglycerides (structures on board, refer to handout) = more interesting:

Triglyceride hydrolysis --> glycerol + 3 free Fatty Acids

For the main Fatty Acid (FA) part of the triglyceride (see handout):

FA + CoA (ATP [--> AMP]) to get started --> FA-CoA --> via FAD (2,3-ene C=C double bond), then HOH addition (OH added distally), --> via NAD --> C=O

(3-keto, or beta-keto acid, so called "beta-oxidation", in mitochondria)

+ CoA again (no ATP needed here) --> FA-CoA (smaller by 2 carbons now), + CH_3-CO-CoA = acetyl-CoA = the KC entry compound --> continues around the Krebs Cycle.

etc. etc. in blocks of 2 carbons. See problem 5-14.

About twice the energy as from glucose, pound for pound.

How about the glycerol part?

See glycolysis handout. On PowerPoint graphic,

glycerol + ATP --> glycerol-1-P

glycerol-1-P + NAD --> DHAP (= dihydroxyacetone phosphate) + $NADH_2$

DHAP --> continues in glycolysis (rxn 5). See recitation problems #3, problem 2.

Under aerobic conditions, no problem, the $NADH_2$'s produced will get re-oxidized in the E.T. chain.

Under ANAEROBIC conditions cannot be fermented (see last lecture).

The burger,

3. = PROTEIN:

Common step for all proteins: hydrolysis of the peptide bonds by several enzymes (pepsin, trypsin), stomach acid (pH1) denatures the protein, making it accessible to proteolytic attack --> Amino Acids.
Subsequent metabolism depends on which of the 20 aa's we're talking about.

A central reaction is:

Glutamate (H_2N-CH(COOH)-CH_2-CH_2-COOH) + NAD + H_2O --> alpha-ketoglutarate (--> Krebs Cycle continuation) + NH_3 + $NADH_2$
This enzyme is glutamic dehydrogenase (GDH).

Also:

Aspartate: (H_2N-CH(COOH)-CH_2-COOH) --> -NH_3, --> HOOC-CH=CH-COOH (that's fumarate = a Krebs Cycle intermediate)
Aspartate deaminase (E. coli but not humans)

The rest of the amino acids cannot lose their amino group so directly.
So a general problem for aa's: to get rid of the NH_2 group: General solution: Transamination:

aspartate + alpha-KG --> oxaloacetate (OA) + glutamate (=transamination)

Pass the NH_2 to alpha-KG to form glu, then use GDH to make alpha-KG + NH_3 from glu.

Asp becomes OA, another old friend.

Another simple example: ala + a-KG --> glutamate + pyruvate (--> KC)

What happens to this ammonia (NH3), sounds bad. High conc. of NH_3 are toxic for many cells (OK for E. coli, excretes directly and quickly), so combine with CO_2 to form urea for excretion (low urea conc. here, so OK, no denaturation of proteins).

Other catabolic paths are more complex, but the principle is the same, they get converted to intermediates of the Krebs Cycle or of glycolysis..

His --> --> --> --> glutamate in humans, but not in E. coli. So we can do something E. coli can't, for a change (my graduate work involved this pathway)

So we get energy from protein. Fast for a day or two, and you start breaking down your own muscle protein to get energy to keep going. See Problems 5R-4 & 5R-5 for an example of amino acid metabolism.

4. Nucleic acids (in a burger, though not much)

Enough is enough: again, --> KC + glycolytic pathway intermediates.

*Now we are going to turn from the breakdown of chemicals to the BIOSYNTHESIS of new ones. The process of building up new compounds from simpler ones is called **ANABOLISM**.*

*1. **POLYSACCHARIDES***

Glucose: run glycolysis in reverse, but often with different enzymes for a particular step.

e.g., a phosphatase, uncoupled to ATP for F-1,6-diP to F6P.

UTP (similar to ATP) + glucose-1-P --> UDP-gluc + PPi, + $glycogen_{(n)}$ --> UDP + $glycogen_{(n+1)}$

UDP + ATP --> ADP + UTP

So 2 ATPs are used to put each glucose residue in glycogen (one to make the G-1-P plus one to regenerate the UTP)).

Also: glucose --> --> gal --> +glucose --> lactose

All enzymatic, and 1 or a few enzymes for each polysaccharide, then a repeating structure, so the same few enzymes are used again and again.

Hyaluronic acid = 2 modified sugars, repeating:

N-acetyl-glucosamine + glucuronic acid: NAcGA-Glucu-NAcGA-Glucu-NAcGA-Glucu-NAcGA-Glucu - - - -

(see handout) : glucose --> N-acetyl-glucosamine; glucose --> glucuronic acid

N-acetylglucosamine + UTP --> UDP-N-acetyl-glucosamine; + growing chain --> growing chain$_{+1}$ + UDP

glucuronic acid + UTP --> UDP-glucuronic acid; + growing chain --> growing chain$_{+1}$ + UDP

Two alternating enzymes build the polymer from UDP-sugar monomers

*2. **LIPIDS**, **phospholipids**: biosynthesis is like degradation, but reverse, and different enzymes (and not in mitochondria). See handout.*

glucose --> DHAP, (+NADH) --> glycerol-1-P,

+ 2 FAs --> phospholipid; + 3 FA = triglyceride

Biosynthesis of FATTY ACIDS (see handout)

1) Acetyl-CoA + $NADPH_{2;}$ 2) -HOH step ; 3) $NADPH_2$ again --> repeat in blocks of 2, --> Fatty Acid

NADP is like NAD but with an additional phosphate group. NADPH is usually used as a reducing agent for biosynthetic reactions, while NAD is used as an oxidizing agent in catabolic reactions.

3. NUCLEIC ACIDS

Defer, but how about ATP, a NA monomer

glucose --> --> glyceraldehyde-3-P --> ser --> gly

G6P--> --> ribose-P

ser + gly + ribose-P + NH_3 --> + about 10 steps -->ATP.

(Then ATP <--> ADP many times, for plenty of energy)

4. Proteins:

Monomers = aa's:

Serine from glycolysis: see handout

Krebs Cycle --> alpha-keto-glutarate, +NH_3 + $NADH_2$ --> glutamate,

Krebs Cycle --> OA --> transam (via glu) --> aspartate

Pyruvate, via transamination --> alanine

Isoleucine (ile):

Glucose --> Oxaloacetate --> asp --> asp-P --> --> --> thr --> --> --> --> --> ile
Added in this path: pyruvate, 2 $NADPH_2$'s, 1 $NADH_2$, 2 ATP, transaminations

Made other aa's along the way: asp, thr

Typical biosynthetic pathway: branches, requires reducing power ($NADH_2$, or $NADPH_2$), ATP, subject to Feedback Inhibition.

Summary:

YOU ARE WHAT YOU EAT (simplified)(handout). Yes and No. Same atoms, but different compounds. All paths funnel into (catabolism) or fan out from (anabolism) the central energy metabolism pathways of glycolysis and the Krebs cycle. Due to coupled reactions or an overall favorable free energy change, these pathways have Delta Go's that are large and negative.

So that finishes a long section that started with one important function of proteins, being enzymes. But we included a discussion of the directionality of the enzymatic reactions, the need for energy, the metabolic pathways that produce the needed energy, and then metabolic pathways leading to the needed small molecules in general.

Now let's return to proteins as macromolecules, and finish off our consideration by seeing how they are synthesized, how the monomer amino acids are polymerized into polypeptides.

We saw how polysaccharides are made by the action of a few biosynthetic enzymes, which had as substrates the growing end of a polymer chain and the free monomer that is being attached to it. So a handful of enzymes can take care of polysaccharide synthesis. Similarly, a handful of enzymes can handle the biosynthesis of fatty acids and fats and phospholipids.

So now let's consider the biosynthesis of proteins in the same way. Let's start with the synthesis of hexokinase, the first enzyme of glucose metabolism. Suppose the first few amino acid were met-val-his-leu-gly. In this case, the growing end is different for each step, so we would need one enzyme to add val to met, and then another to add his to met-val, and a third to add leu to met-val-his, and so on. So if hexokinase has 500 amino acids, we would need about 500 enzymes to put it together. If there are 3000 enzyme proteins that have to be made this way, then that's 3000 x 500 = 1,500,000 enzymes to do the job. That's a lot of enzymes. And wait a minute: each of these 1,500,000 enzymes is a unique protein itself, and has to be synthesized! So we quickly see the impossibility of making proteins by a series of simple enzymatic condensation reactions. We need a new principle, a way of using some common synthetic apparatus that somehow knows what amino acid comes after what amino acid in every protein. A mystery until the 1960's. The discovery of how this information (what a protein's primary sequence should be) is realized in a growing cell is one of the greatest achievements in the history of experimental biology. The answer of course is written in the nucleic acids.

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