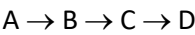


Intermediary Metabolism: An Introduction

Fundamentally, metabolism is the study of inter- and intra-cellular activities that keep cells functioning. The cells keep tissues functioning. The tissues keep the organ functioning and the organ keeps the organism functioning.

Metabolism is a systematic and sequential approach to these activities. In some cases, metabolic end products or enzymes may be used clinically e.g., creatine phosphate kinase’s (CK) heart fraction (MB) – together referred to as CK-MB, to understand just how large an infarcted area in the heart really is. A myocardial infarction (MI) is death of heart muscle and is colloquially known as a heart attack. The higher the CK-MB in a patient’s blood, the bigger the area in the heart that is dying and, hence, the worse the infarct is.

When metabolism is studied, it is quite detailed and consists of a series of metabolic pathways. Pathways, biochemically speaking, are biochemical reactions that occur in sequence, either anabolically or catabolically or amphibolically. In general, one can think of a pathway as follows:



Where “A” is the initial substrate and “D” is the final product of the reaction sequence. “C” and “D” are referred to as intermediates. In a biochemical pathway, co-factors necessary for the reaction to go are “arrowed in” with the reaction direction arrow. There are those who use strict “arrowing”, e.g., only arrow in and out on top of the reaction arrow and there are those of us who are a little less “up tight” and arrow in and out from top and/or bottom, as shown in the figure below:

In addition, the name of the enzyme catalyzing the reaction is written either over or beneath the reaction arrow for the specific step[s].

A reaction may be bidirectional (amphibolic) depending on the conditions in the cell, e.g.,



For the purposes of this monograph/course, we’re only going to focus on the unidirectional reactions. If you desire more knowledge about additional reactions, a one semester course in

General Biochemistry or in something like Metabolic Regulation will provide you with a great deal of satisfaction.

There is some good news as we move into intermediary metabolism: you’ve already studied and learned some of the fundamental amino acids, structures of proteins (primary, secondary, tertiary and quaternary structures), as well as how they’re joined by the peptide bond, a brief introduction to enzymes (proteases, saccharidases, lipases and D(R)NA’ases (nucleases), nucleotidases, nucleosidases) carbohydrates, lipids and nucleic acids (anabolic and catabolic reactions). This topic builds upon those previously learned lessons: if you’ve got your structures of amino acids, mono-, di- and poly-saccharides, saturated and unsaturated fatty acids, triglycerides, phospholipids, steroids, purines and pyrimidines down cold, you’ll save yourself about 35% in your study time – if not, your work just became more challenging as you’ll have to learn the basics along with the advanced coursework – never a pretty picture.

Do keep in mind that what information you’re getting is minimal and will get you through Anatomy and Physiology and Microbiology and into Nursing. Each topic can be taught as a one-semester course and is in graduate school. You’re getting about 3 weeks of metabolism versus 4-6 semesters depending on one’s grad school focus.

Biochemists are famous for using acronyms, e.g., NAD+ for nicotinamide adenine dinucleotide. Most are standard, however, each biochemist has his or her favorites. Your professor is no different, hence, a sort of mini-glossary is below to get you started thinking about some of these acronyms:

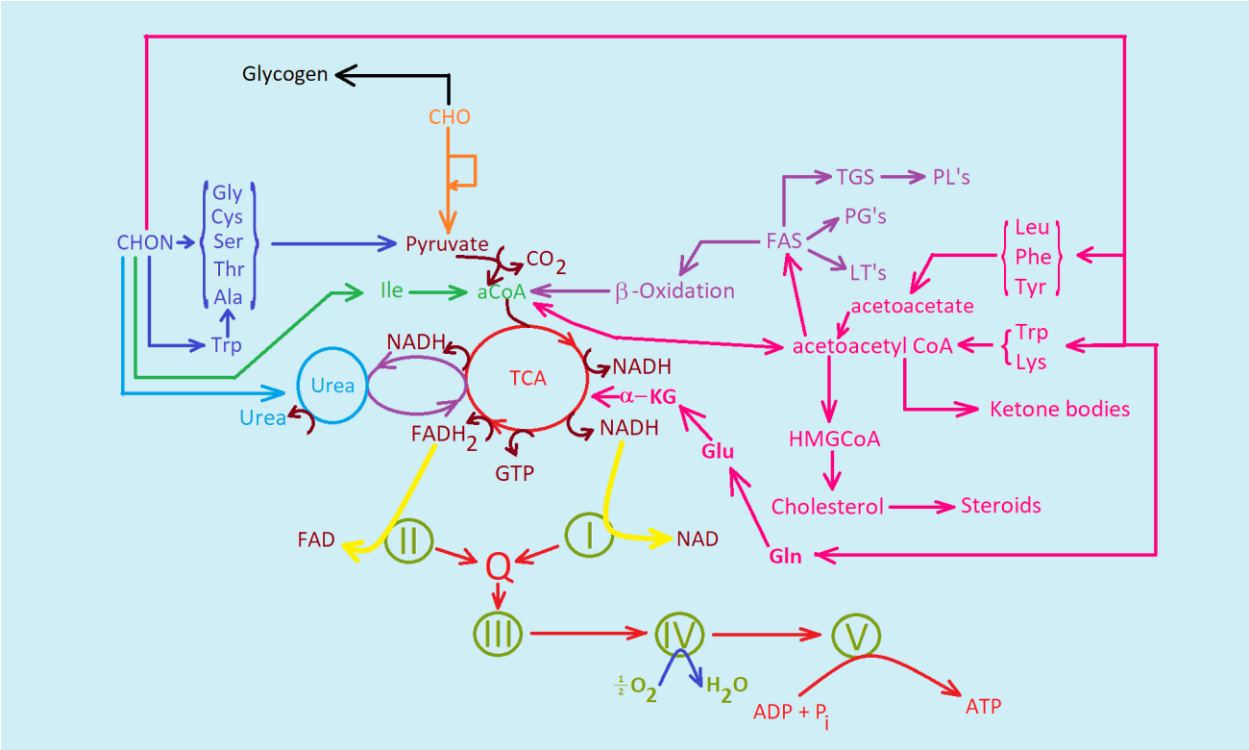
Acronym	“Real” Name	Acronym	“Real” Name
F	Fructose	F-1,6-bP	Fructose-1,6-bisphosphate
G	Glucose	Ga	Galactose
G-6-P	Glucose-6-phosphate	Ga-1-P	Galactose-1-phosphate

G-3-P	Glyceraldehyde-3-phosphate	DHAP	Dihydroxyacetone phosphate
G-3-PDH	Glyceraldehyde-3-phosphate dehydrogenase	aCoA	Acetyl CoEnzyme A
HMGCoA Reductase	β -hydroxy- β -methyl-glutaryl Coenzyme A reductase	LDH	Lactate dehydrogenase

There are many more acronyms that you’ll be learning as we go along. While it makes the pathways a little easier, it still requires learning the acronyms and what it is that they stand for.

An Integrated Overview of Metabolism

The image below succinctly summarizes a great deal of the metabolism that we’ll be going over in this monograph:



Note all of the acronyms. As we go over this in lecture, they’ll be pointed out and you’ll be expected to write them down as we go along.

The image above ties together the metabolism of protein (CHON), carbohydrate (CHO) and lipids (fats like fatty acids, prostaglandins [PG’s], leukotrienes [LT], cholesterol) through the Krebs’ cycle (TCA), urea cycle and electron transport and oxidative phosphorylation (ET-OP) at the bottom of the image. We’ll go through all of this in greater detail and, then, tie it back together at the end of the overall lecture on metabolism.

Metabolism

Historically, carbohydrate metabolism was taught before any other sort of metabolism. Carbohydrate metabolism seems to get most of the time and attention throughout all of metabolism. This was most likely because it was studied first and because it typically involves only molecules with carbon, hydrogen and oxygen (CHO). After that, it was a toss up which metabolism topic came next. In this monograph, CHO metabolism is studied, first, followed by amino acid (AA) metabolism, then lipid metabolism and, lastly, by nucleic acid metabolism.

Carbohydrate Metabolism

There are some “general rules” that encompass all of metabolism, not just CHO’s.

As a general rule, whenever you hear the word "kinase", think ATP and magnesium ions. We’re not saying “making” or “breaking” ATP and magnesium ions, we’re just saying to automatically think of those two co-factors any time you hear an enzyme called a kinase.

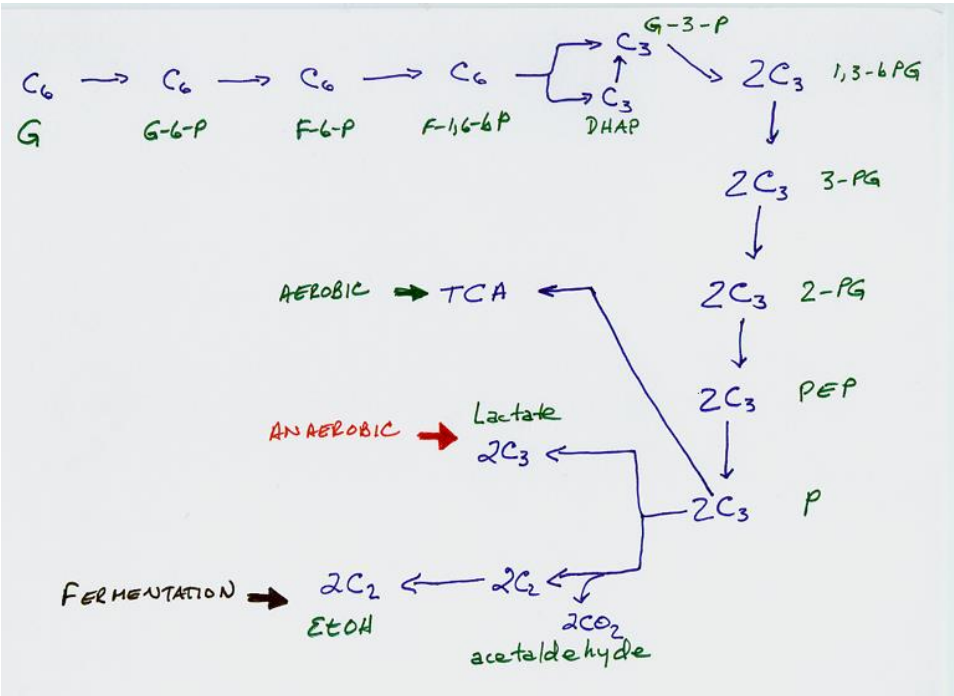
Hexokinase is a generic enzyme, capable of "working" on most hexoses. Glucokinase is specifically for the phosphorylation of glucose ... and only glucose. Typically this is after a load of glucose that’s been ingested and needs rapid removal from the blood and to be stored in some form in a cell.

PFK is one of the major regulatory enzymes in the EMP pathway. PFK is phosphofructokinase. It’s the main enzyme of regulation in the glycolysis (Embden-Myerhof-Parnas [EMP]) pathway. High amounts of

ATP and high levels of citrate inhibit the enzyme in a reversible, allosteric (feedback) manner. High amounts of ADP, AMP and low levels of citrate turn PFK on to re-initiate EMP. EMP is a catabolic pathway that **catabolizes glucose to form pyruvate (aerobic glycolysis), lactate (anaerobic glycolysis) or, in some organisms like *Saccharomyces cerevisiae*, ethanol (fermentation).**

This pathway (EMP) begins with a six-carbon sugar, glucose, and ends with 2 three-carbon intermediates (pyruvate) as glucose is oxidized. Below is a quick overview of EMP using only acronyms and counting carbon atoms to familiarize you with this pathway:

Note that glucose (G) starts the pathway and it has six carbon atoms. Those 6 carbon atoms remain even though glucose is rapidly phosphorylated (G-6-P) and isomerized to fructose-1,6-bisphosphate (F-1,6-bP). At F-1,6-bP, though, an enzyme called aldolase symmetrically “splits” F-1,6-bP into 2 three carbon compounds: G-3-P and DHAP. Note that

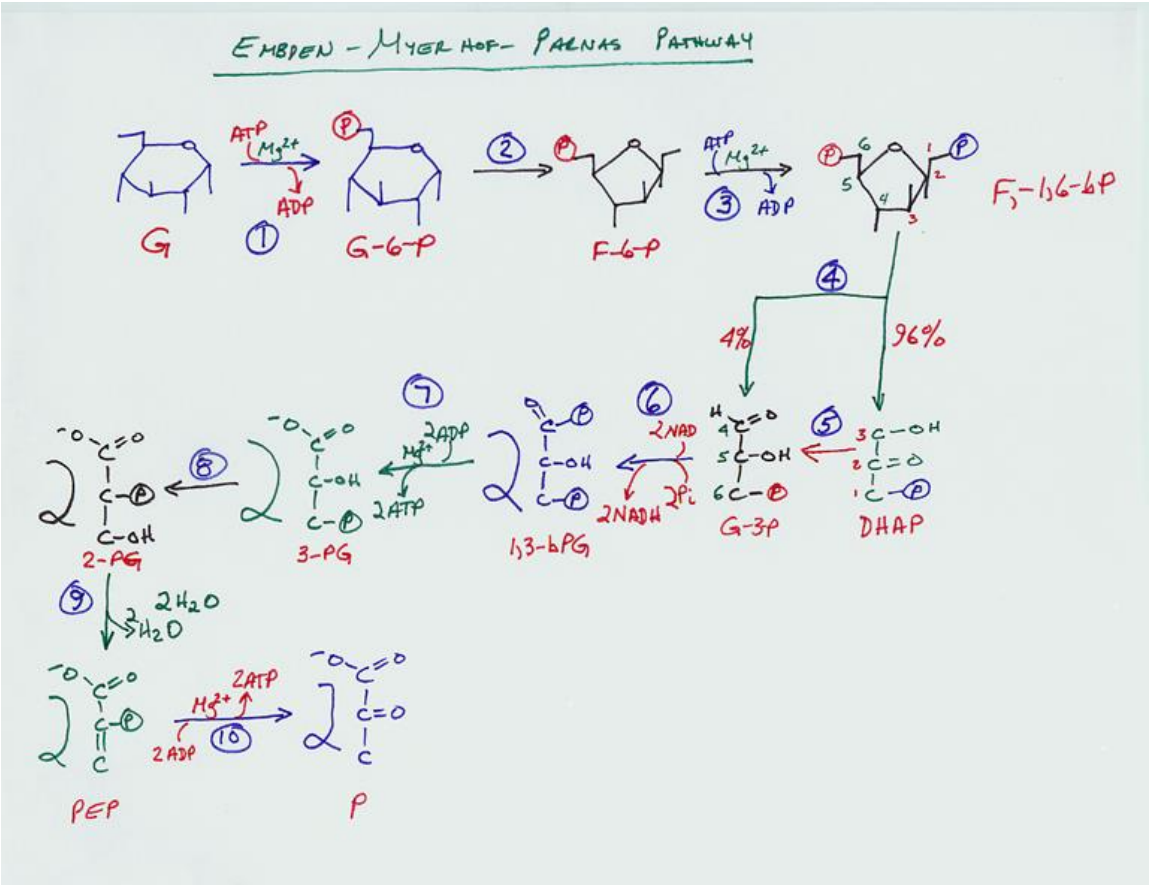


there are still 6 carbon atoms – they’re just between two compounds ($2 \times 3 = 6$, right?) – at this stage.

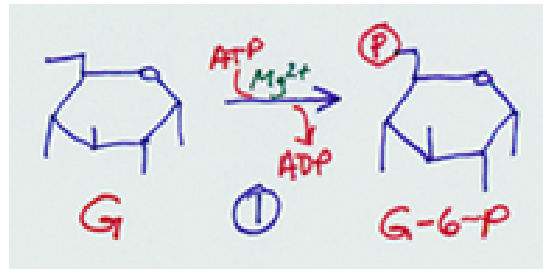
At issue is what happens to the molecules of G-3-P and DHAP. 96% of the time, the cell makes DHAP. This is a problem because DHAP won’t traverse EMP. An enzyme called triose phosphate isomerase (TPI) isomerizes the DHAP to G-3-P, which WILL traverse EMP and we now have, stoichiometrically, 2 three-carbon molecules of G-3-P. The significance of this is that as we complete EMP, any energy (ATP or equivalents) we generate is doubled in this portion of EMP. More details coming shortly.

Also note the three possible end products of EMP, depending on the cell conditions/environment.

The image, below, is that of EMP with more detail, i.e., not just acronyms and carbon counts, rather also with structures, enzymes and cofactors:

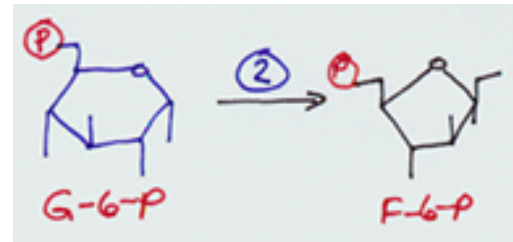


Note that each reaction step has its own number. That number corresponds to a specific enzyme name. Reaction 1, shown at right, is catalyzed by the enzyme, hexokinase (HK). The purpose of phosphorylating the glucose to glucose-6-phosphate is to trap the glucose in the cell. EMP occurs in the cell once the glucose has been transported into the cell.

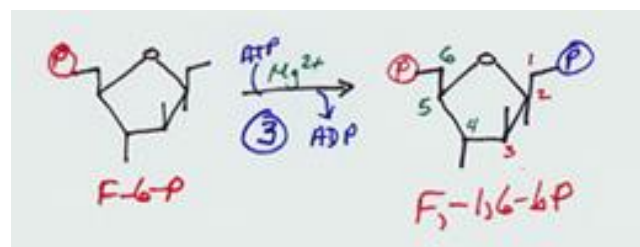


Note the hydrolysis of the ATP to ADP when G-6-P is formed. The divalent cationic magnesium ion is required to form a stable intermediate between the enzyme, the ATP and the substrate (glucose) so that the inorganic phosphate (P_i) can bind on/to the 6th carbon of the glucose molecule. Once G-6-P is formed, only the liver has the enzyme glucose-6-phosphatase to remove the #6 phosphate to release the glucose into the blood for transport to cells that need the glucose.

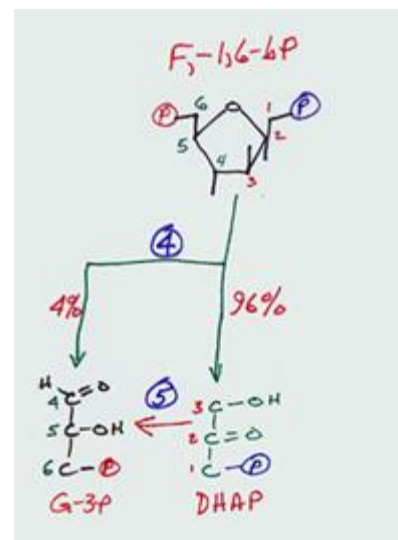
Reaction 2, shown at right, is catalyzed by the enzyme, phosphoglucosomerase (PGI). This enzyme isomerizes G-6-P to F-6-P. While this step seems a sort of waste, Ma Nature had it all figured out and it's actually quite important in 2 more reactions. This is not uncommon in metabolic pathways: to find something that seems, intuitively, weird, only to see that 2-5 steps later, it makes perfect sense. Do you also observe that we're using Haworth projections in this pathway?



Reaction 3, shown at right, is catalyzed by the enzyme phosphofructokinase (PFK) and phosphorylates F-6-P at the expense of another ATP molecule to form fructose-1,6-bisphosphate (F-1,6-bP). PFK is THE major step of regulation in EMP and is turned off by high levels of ATP and Citrate (a state of high energy). PFK is turned on by low levels of citrate and high levels of ADP and AMP (a state of low energy).



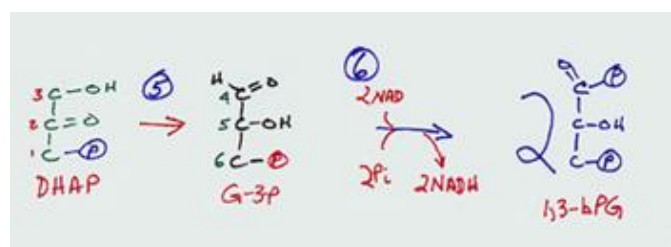
It's reaction 4, shown at right, that makes reaction 2, above, make sense: aldolase symmetrically splits F-1,6-bP into a molecule of glyceraldehyde-3-phosphate (G-3-P) and a molecule of dihydroxyacetone phosphate (DHAP). Note the numbering in the F-1,6-bP molecule and the corresponding numbering in the G-3-P and DHAP. Those numbers in the latter two molecules correspond to the carbon atoms indicated in the F-1,6-bP molecule.



As mentioned previously, 96% of the time aldolase produces DHAP. This is not a good thing as the DHAP won't go through EMP. Because G-3-P will go through EMP, triose phosphate isomerase (TPI) catalyzes the isomerization of DHAP to G-3-P at reaction 5. This, in effect, means that our cells produce two molecules of G-3-P and we have to take that into account from this point forward in EMP.

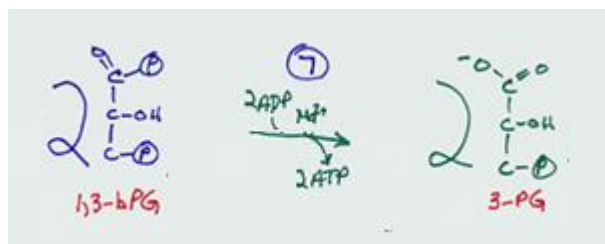
Additionally, to this point in EMP, our cells have used up energy to get this far. The remainder of EMP is to produce energy and each step will produce twice as much energy as was used due to the stoichiometry, i.e., one glucose, one ATP is spent vs one phosphoenolpyruvate (PEP), two ATP are produced when pyruvate (P) is formed at the end of aerobic glycolysis proper.

Reaction 6, shown at right, is catalyzed by glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH). This enzyme requires NAD and inorganic phosphate to phosphorylate the first carbon to form 1,3-bisphosphoglycerate (1,3-bPG). Note the stoichiometry, now: there are two each of the 1,3-bPG, NAD, NADH and P_i – just as previously indicated.



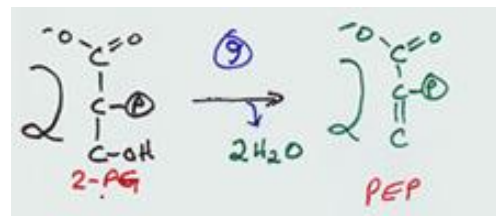
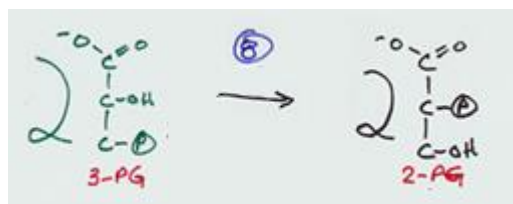
Additionally, each NADH molecule is worth "about" 3 ATP molecules (lots more on this coming up), hence, at reaction 6, there have been the equivalent of 6 ATP molecules formed.

Reaction 7, shown at the top of the following page, is catalyzed by phosphoglycerate kinase (PGK) and produces 2 molecules of ATP, along with 2 molecules of 3-phosphoglycerate (3-PG). Again, twice as much energy is produced at reaction 7 due to the stoichiometry.



Reaction 8, shown at right, is another reaction that doesn't seem to make sense, once again. In reaction 8, 3-PG is isomerized to 2-PG and retains the stoichiometry.

The purpose of this isomerization will become clearer in reaction 9, **below**. Reaction 9 is catalyzed by an enzyme called enolase. Water is removed between the H on carbon #2 and the OH on carbon #3.



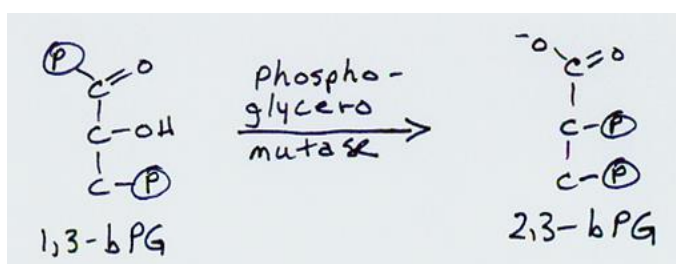
This places a double bond between carbon 2 and carbon 3, where carbon 2 has a phosphate on it. This places a tremendous amount of strain on the double bond that releases a great

deal of energy in reaction 10, when pyruvate (P) and 2 molecules of ATP are formed under the catalysis of pyruvate kinase (PK), image at right. Reaction 10 ends aerobic glycolysis (EMP) proper.



Before we go into two forms of anaerobic glycolysis (lactate synthesis and alcohol fermentation), there is a molecule that is related to 1,3-bPG about which we need to expand. This molecule is 2,3-bPG and is formed by the catalysis with phosphoglyceromutase (PGM), image at right.

2,3-bPG is an important molecule in short term adaptation to higher altitudes so that molecular oxygen can be more easily released from oxyhemoglobin ($\text{Hb} \cdot \text{O}_2$) and transported to the cells. 2,3-bPG reduces the affinity of Hb for O_2 .

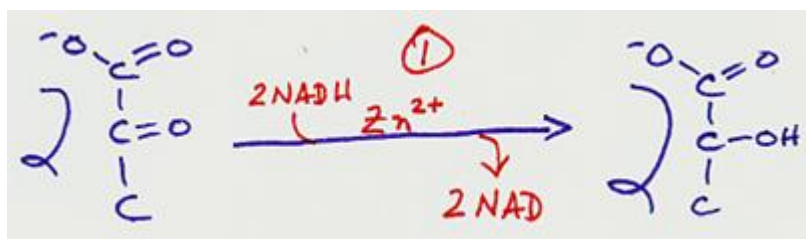


The synthesis of 2,3-bPG is a side-rxn of EMP. If one lives in San Francisco, to adapt (short term) to life at Lake Tahoe, the body's cells increase the levels of 2,3-bPG so more oxygen is released to the cells in the body. Overall, the basic effect is as follows:



Thus closes out EMP (aerobic glycolysis, proper). The graphic on the bottom of page 3, again, summarizes EMP in one graphic.

There are two remaining extensions to EMP that are anaerobic: lactate synthesis and ethanol fermentation. The former humans perform; the latter is performed by other organisms as previously stated. Lactate synthesis (a new reaction 1 – keep a close eye on the reaction numbers as we go along) is shown at right. This reaction is catalyzed by lactate dehydrogenase (LDH or LD). The source of the NADH to reduce the pyruvate to lactate is reaction 6 in EMP.

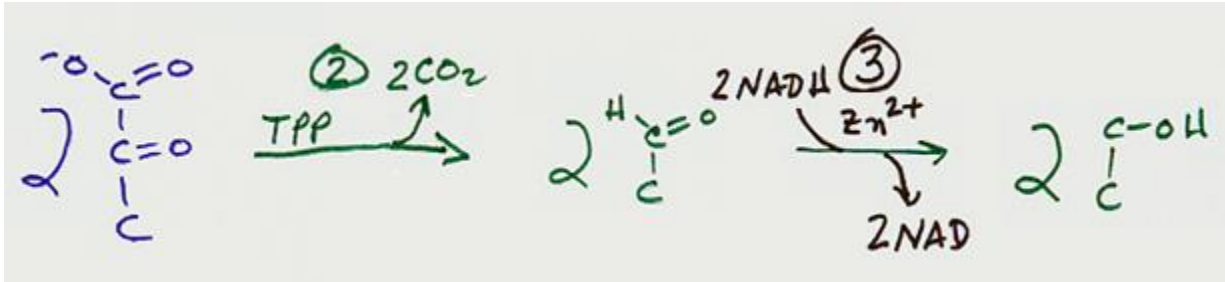


Remember that NADH is equivalent to 3 ATP molecules, hence, when the anaerobic pathway is activated to form lactate, the 6 ATP equivalents are lost to lactate formation. The stoichiometry – **aerobic AND anaerobic** -- is that per molecule of glucose (1 six-carbon sugar), TWO molecules of three-carbon sugars are formed, i.e., one times six is six, as are two times three.

Man has been brewing alcoholic beverages for thousands of years (longer than man has been making soap, believe it or not!). Well known liquors, e.g., wine from grapes, vodka from potatoes, whiskey from

corn, come from foods with a source of carbohydrate which, while enjoying anaerobic conditions (what are these conditions?), will be metabolized to ethyl alcohol and carbon dioxide.

While this monograph is focusing on fermentation starting from glucose, the source of carbohydrate for fermentation from plants is, as you recall, starch. As grains begin to sprout (potatoes, as well), enzymes are activated which catabolize (what does this mean?) the starch to glucose. If the process is stopped, e.g., by heating until the “sprouts” are dried, the glucose will not be further catabolized. If the dried residue is rehydrated, has its pH and temperature adjusted to optimal conditions, has yeast added and then the resulting mix isolated from an aerobic atmosphere, then anaerobic fermentation will proceed. Fermentation is shown below.



Pyruvate is decarboxylated by pyruvate decarboxylase (PDC) at reaction 2. It’s the carbon dioxide that forms that fills balloons in home brew systems that are very small scale. PDC requires an active form of a water soluble vitamin, thiamine, to drive the reaction forward. The form of the vitamin is thiamine pyrophosphate (TPP). The product of this reaction is acetaldehyde (common name) or ethanal (IUPAC name). Ethanal is reduced to ethanol at step 3. Reaction 3 is catalyzed by alcohol dehydrogenase (ADH). Note that ADH and LDH both require the divalent cation, zinc, as a co-factor. In addition, did you note the presence of the NADH in reaction 3, above? Good! That, too comes from reaction 6 in EMP, making fermentation a non-energetically helpful reaction ... other than to generate ethanol (EtOH).

When considering the regulation of EMP, keep in mind that regulation is allosteric and the regulators act on specific enzymes as tabulated below.

Stimulators (“Activators”)	Inhibitors
PFK: ADP and AMP	PFK: Citrate and ATP
	G-3-PDH: AsO_4^{3-} – arsenate inhibits because it “looks like” phosphate
	Enolase: fluoride ion (F ⁻)
	Pyruvate kinase: ATP
Low energy turns on EMP	High energy turns off EMP
As a general rule, wherever ATP is generated or expended, the enzyme is regulated in a feedback manner	

Biochemists are notorious “bean counters”: we want to know how much energy it takes to beget energy. In the case of aerobic glycolysis, those attributes are tabulated below:

ATP Used	ATP Gained
Hexokinase: -1	G-3-PDH: +6 (⇌)
PFK: -1	Phosphoglycerate kinase: +2
	Pyruvate kinase: +2
Total USED = 2 ATP	Total GAINED: 10 ATP

Overall: **8 ATP are produced** in EMP.

Likewise, in the case of anaerobic (or fermentative) glycolysis, we want to know how much energy it takes to beget energy, tabulated below:

ATP Used	ATP Gained
Hexokinase: -1	G-3-PDH: +6 (⇌)
PFK: -1	Phosphoglycerate kinase: +2
LDH (or ADH): -6 (⇌)	Pyruvate kinase: +2
Total USED = 8 ATP	Total GAINED: 10 ATP

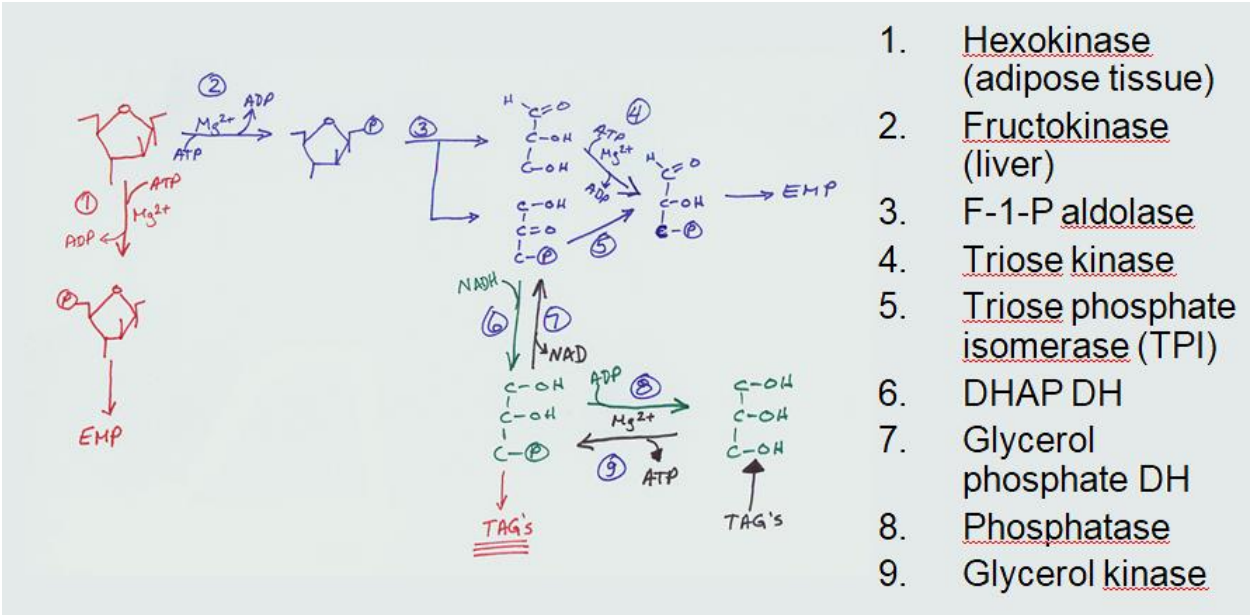
In anaerobic glycolysis (fermentation), the number of ATP go down from 8 to 2 because the NADH and NAD cycle, as previously pointed out.

Fructose Metabolism

Every now and again, a diabetic patient's parent, sibling or other relative reads about how diabetes is a disease of glucose metabolism. On occasion, they read a little more and discover that fructose is a carbohydrate, but not glucose.

They then come in to see you as the health care person who knows something about diabetes and ask you, "Since fructose isn't glucose, can I substitute all of my relative's carbohydrate needs with fructose?"

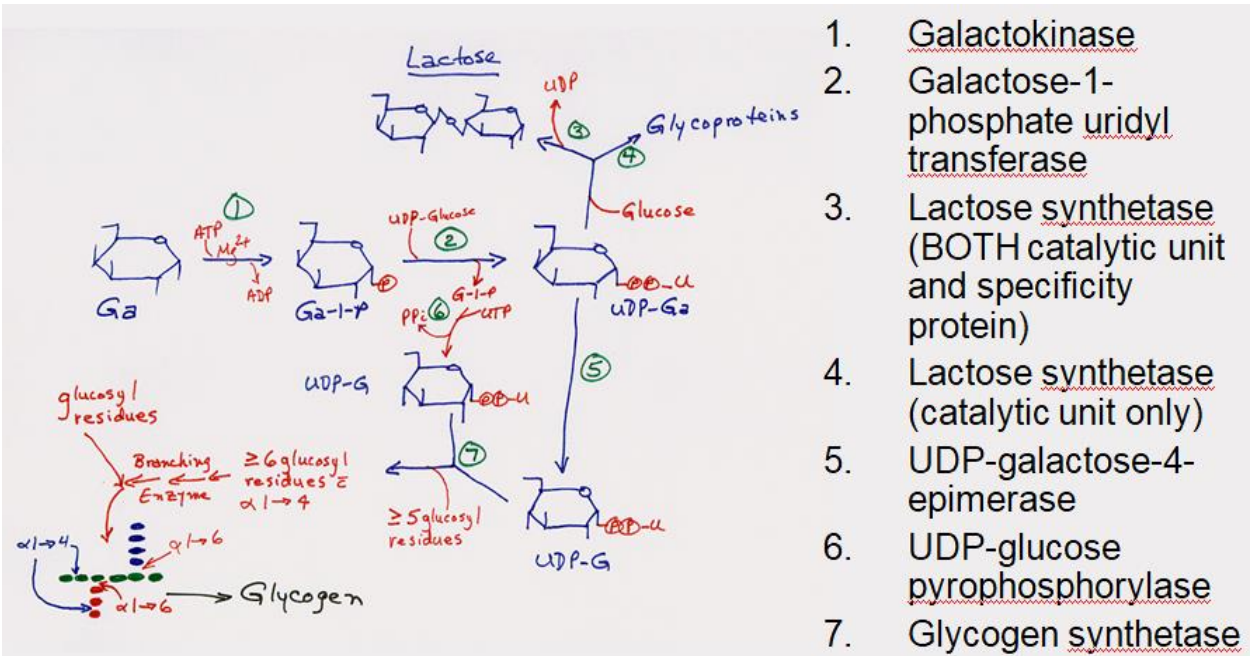
Your answer is, of course, no. So, why is it that your answer is no? The catabolism of fructose and how it intertwines with triglyceride (TGS) synthesis is the reason you said "no". The metabolic fate of fructose is illustrated below:



While we're not going to go into this pathway in the detail we did in EMP, note that reaction 1 drives fructose into EMP in adipose tissue. Note that reaction 2 occurs on the liver tissue. Note that TGS' may be synthesized, *de novo*, from reactions 3 and 6, while, if the fructose is not used in TGS biosynthesis, it will continue on into EMP in adipose tissue via reactions 4 and 5. In addition, TGS may contribute to more TGS synthesis via reaction 9.

Galactose Metabolism

Galactose is an important constituent of lactose, as you have learned previously. Galactose may be used in the synthesis of glycoproteins (cell identifiers, remember??). Galactose may be used in the synthesis of glycogen, pathways illustrated below:



In the synthesis of lactose, the reaction sequence is reactions 1, 2 and 3. These reactions are under the direct regulation by two hormones: oxytocin and prolactin. Oxytocin (OT) is known as the “milk let-down hormone” and performs two functions in regards to breast milk synthesis: 1) it causes the release of prolactin and 2) it causes myoepithelial cells around the milk lobules in their breasts to contract (that’s the “tingle” women feel in their breasts when they prepare to breast feed their babies) for milk let-down. Prolactin (pro = in favor of; lact = milk; in = protein) drives lactose synthesis (via these three reactions) and milk synthesis.

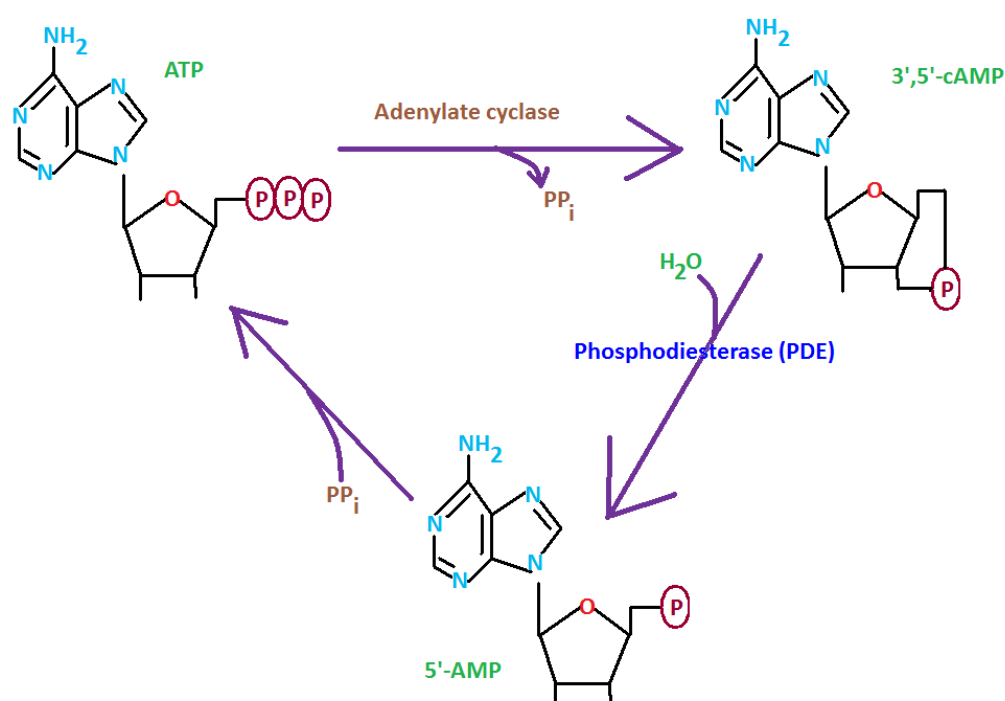
In the case of the synthesis of cell identifiers, the reaction sequence is reactions 1, 2 and 4.

The synthesis of glycogen is more complex inasmuch as that requires reactions 1, 2, 5, 6, 7 along with the un-numbered reactions in the lower left of the pathway, above. Note that this is energy demanding as PP_i is released at reaction 6, which is the equivalent of two inorganic phosphates (or the hydrolysis of two ATP molecules).

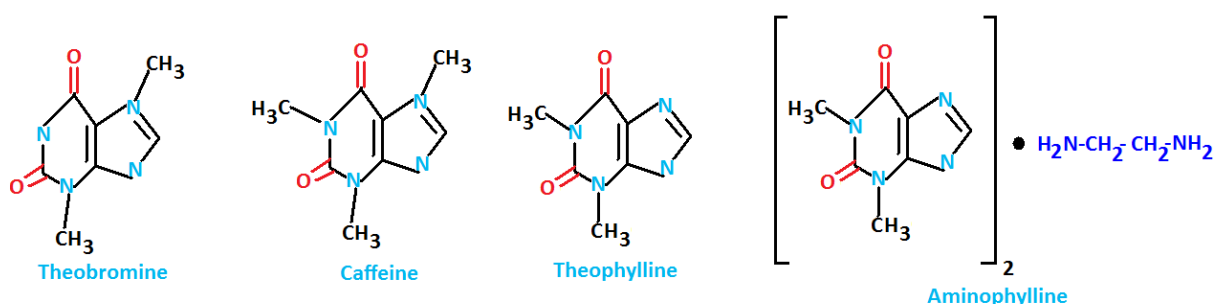
The branching enzyme adds α 1→6 glycoside bonds to increase the efficiency of packing of the molecule so that a lot of energy may be stored in a small space.

Glycogen Metabolism Regulated by cAMP

Elevated levels of cAMP drive protein synthesis, enzyme cascades and changes in membrane permeability:



Phosphodiesterase (PDE) is inhibited by Theophylline, Caffeine and Theobromine. These compounds are called xanthines, illustrated below:



When PDE is inactivated, cAMP levels build up, making it easier for patients to breathe (this is still a controversial statement as there is literature that supports and debunks this hypothesis – it’s possible that the effect on breathing is actually through an adenosinergic receptor).

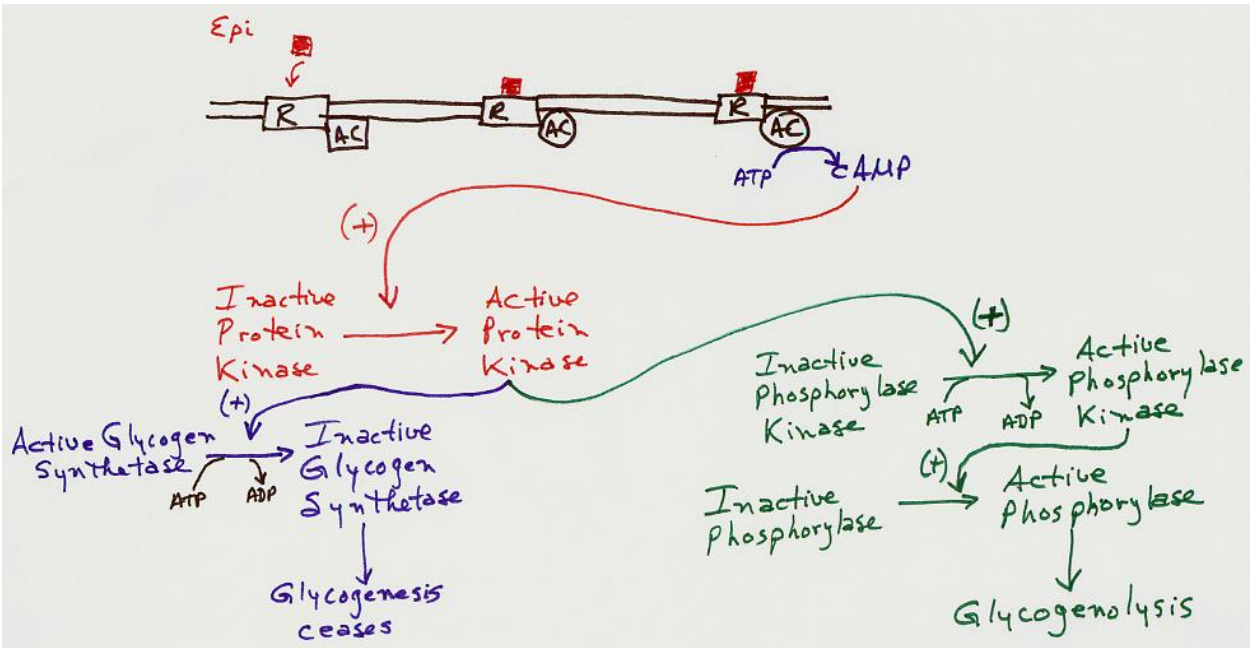
In General: Glycogen – olysis and genesis

Glycogenolysis (destruction of glycogen)

Glycogenesis (production of glycogen)

As the body doesn't like to be confused during times of stress, cAMP inhibits glycogenesis and activates glycogenolysis. One example of this occurs when epinephrine binds with the appropriate receptor on the cell membrane of a target cell in, e.g., flight, fight or freeze.

In a little more detail, glycogenesis and glycogenolysis under the influence of epinephrine are illustrated below:



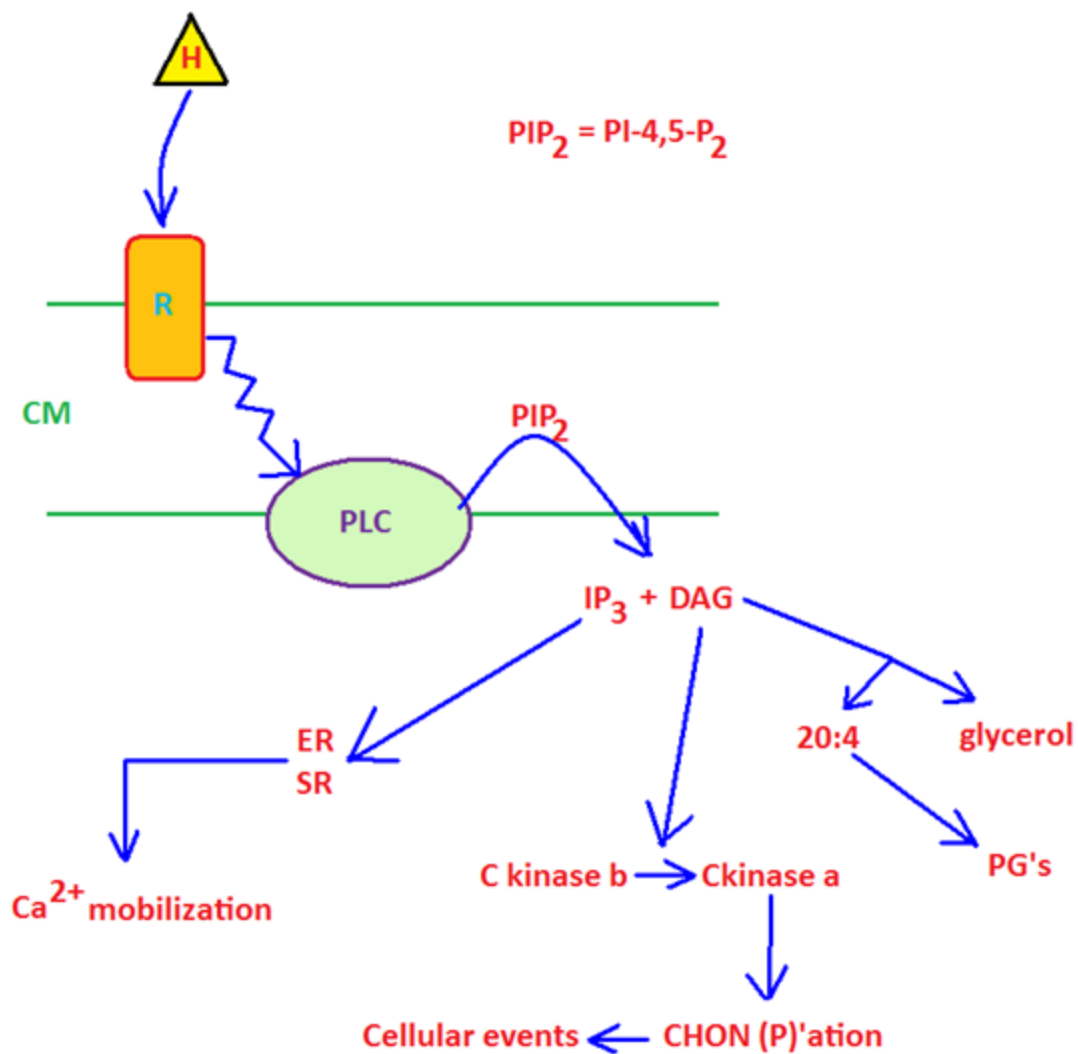
In the graphic above, Epi = epinephrine; R = receptor; AC = adenylate cyclase; (+) = activates or “turns on”; the two black parallel lines at the top of the image represent the lipid bilayer cell membrane. cAMP activates inactive protein kinase to initiate the cascade to shut down glycogenesis (lower left of image) or turn on glycogenolysis (lower right of image).

While the graphic seems simple, there are some details that one needs to be aware of with this pathway: between the receptor (R) and adenylate cyclase (AC), above, there is a switch of sorts called a G protein. For the purposes of this monograph, there are three G proteins that students need to know cold, tabulated, below:

G _s	G _i	G _p
This is a “G stimulating” protein – it turns on cAMP synthesis.	This is a “G inhibiting” protein – it turns off cAMP synthesis.	This is a “G phosphatide” protein – it turns on the release of IP ₃ from phospholipids in the cell membrane. IP ₃ is 1,4,5-inositol trisphosphate. Any time one sees “IP ₃ ”, one needs to think about calcium ion movement; it may also involve IP ₄ .
In all three images, the “cactus” in its “pot” represents the ligand (cactus) bound to its receptor (pot); in the left and center image, the purple pentagon is adenylate cyclase; in the right image, the purple pentagon is phospholipase C. The red “X” in the center panel indicates inhibition of adenylate cyclase.		

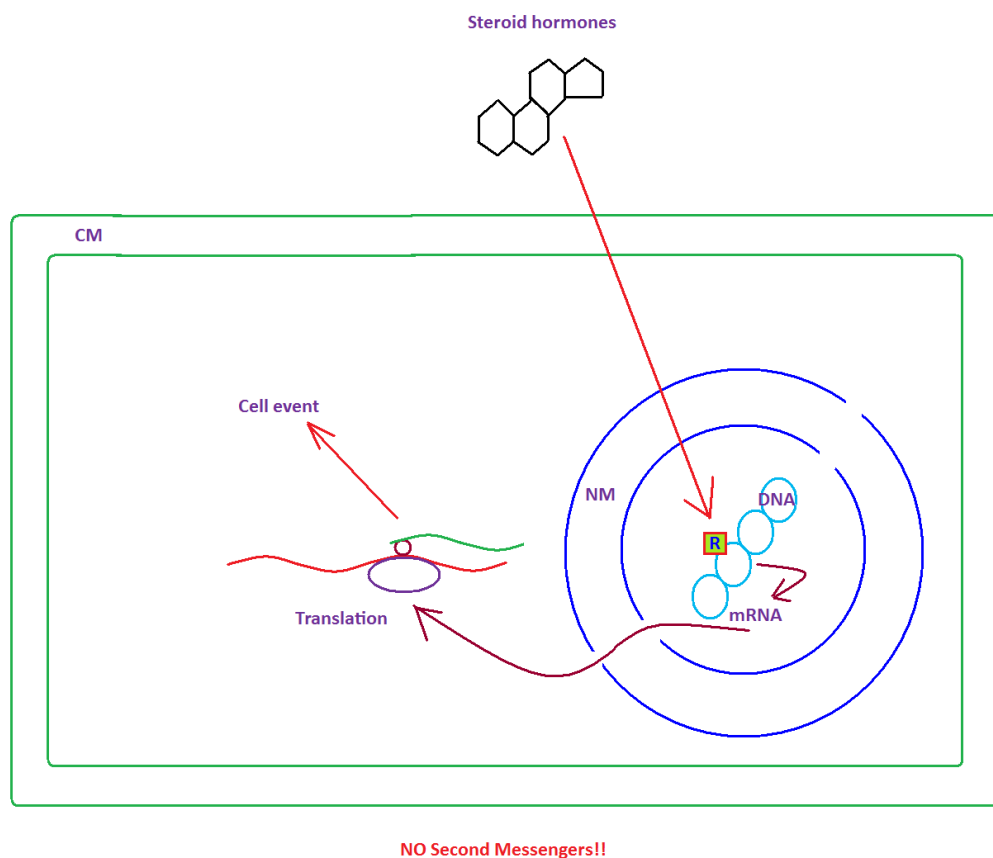
IP₃ drives changes in: Ca²⁺ concentration and Ca²⁺ mobilization. In addition, GABA (γ-aminobutyric acid), AVP (arginine vasopressin), ANG (angiotensin II), TSH (thyroid stimulating hormone) utilize IP₃ as a second messenger.

The pathway on top of the following page provides you with a rapid overview of the synthesis and functions of “IP₃”:



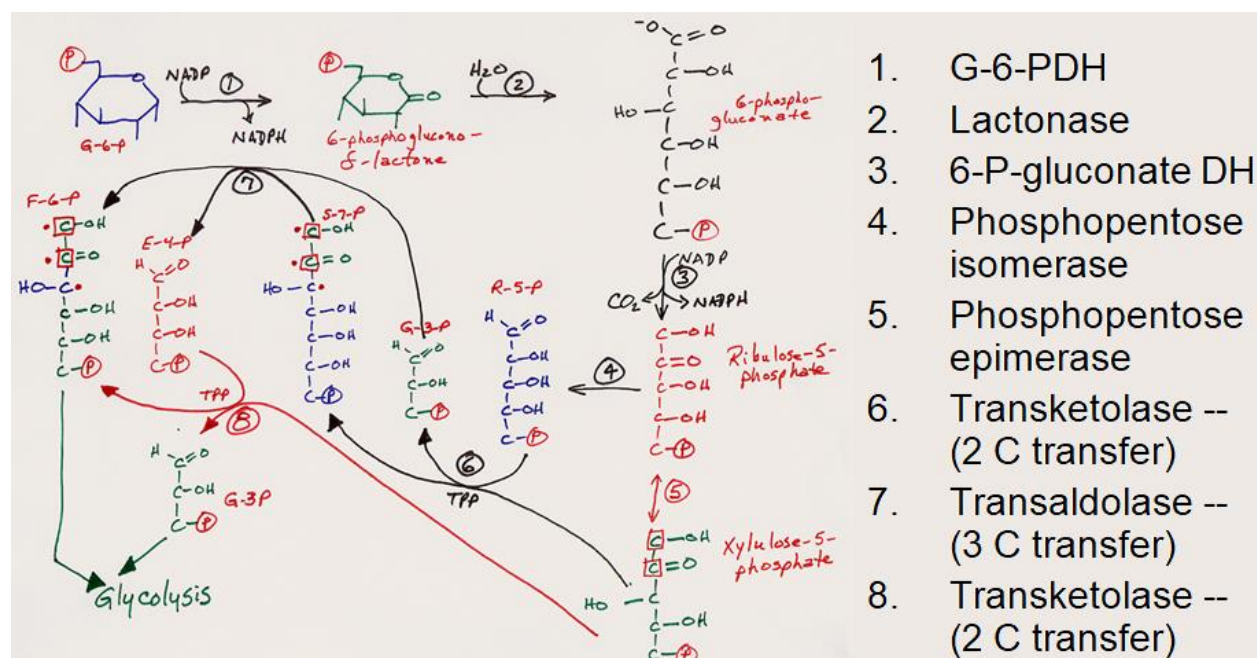
“H” represents the hormone or ligand that will bind to the receptor “R”; PLC is phospholipase C; PIP₂ is phosphatidyl inositol biphosphate; DAG – diglycerides; ER = endoplasmic reticulum; SR = sarcoplasmic reticulum; CM = cell membrane.

Steroid Hormones require NO second messenger as they are lipid soluble:



Steroids drive translation in such a manner that functionally: increase activity in the cell and structurally: they drive bulking up (think anabolic steroids). Steroids interact directly with DNA-bound receptors to turn on transcription and, hence, translation for increased protein synthesis.

Another pathway of great clinical importance that's a shunt away from and back to EMP is the hexose monophosphate shunt (or pentose phosphate pathway; HMP). A shunt is the process of moving to an alternate metabolic pathway. The HMP starts with/from G-6-P in EMP and returns F-6-P and G-3-P to the EMP while generating two very important substances: NADPH and ribose. We'll return to these after we've gone over the HMP, shown below:



The initial substrate is G-6-P. It's acted on by glucose-6-phosphate dehydrogenase (G6PDH) at reaction 1, along with NADP. NADP is a bit different from NAD, in that it has an additional phosphate (hence the "P") and NADP is "usually" (there are exceptions) found in anabolic reactions (NAD is "usually" found in catabolic reactions). NADPH is formed, as is 6-phosphoglucono-δ-lactone (6-PGL).

Lactonase at reaction 2 hydrolyzes 6-PGL and opens up the Haworth projection into the Fisher projection (stick structures) as 6-phosphogluconate (6-PG) is formed. 6-PG is then oxidized at reaction 3 by 6-PGDH which forms ribulose-5-phosphate and another molecule of NADPH.

The NADPH formed at reactions 1 and 3 is the first of the clinically significant compounds from the HMP.

From ribulose-5-phosphate, things get very exciting, very quickly, as reaction 5, catalyzed by phosphopentose epimerase, forms xylulose-5-phosphate.

Before we get into reactions 6, 7 and 8, note the red squares around the top two carbon atoms in the xylulose-5-phosphate. Those red squared carbons will be transported shortly to another molecule. Note, as well, the three red dots next to the top three carbons in sedoheptulose-7-phosphate (S-7-P in the pathway). Those three carbons, along with the carbons with the two red squares next to the red dots, will also be transported to another compound.

Reaction 6 is catalyzed by an enzyme called transketolase. This enzyme requires TPP to properly function. Transketolase drives a two carbon transfer from xylulose-5-phosphate on top of ribose-5-phosphate (do NOT confuse the ribose with the ribulose!) that is formed at reaction 4 by phosphopentose epimerase. The new products are G-3-P and S-7-P. Do you see the red squared carbons and how they moved? Note also the color coding between molecules to help you follow along.

The ribose is the second product of significance from the HMP.

As soon as the S-7-P and G-3-P are formed, the top three carbons from S-7-P are transferred to the top of the G-3-P by an enzyme called transaldolase at reaction 7. Transaldolase drives a three carbon transfer, hence the three red dots to help you follow that transfer. The new products are erythrose-4-phosphate (E-4-P) and our old friend F-6-P. The F-6-P feeds right back into EMP.

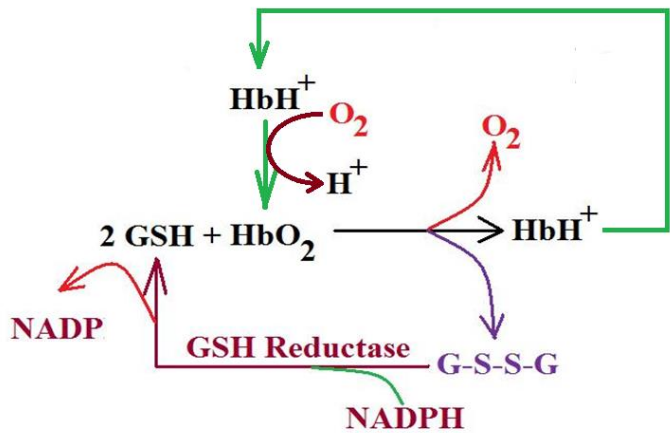
At reaction 8, the E-4-P receives the top two carbons from the xylulose-5-phosphate by the activity of transketolase, again. In addition, another molecule of F-6-P is formed along with a molecule of G-3-P, both of which dump right back into EMP (see bottom of following page).

Hexose Monophosphate Shunt (Pentose Phosphate Pathway): Clinical/Significance

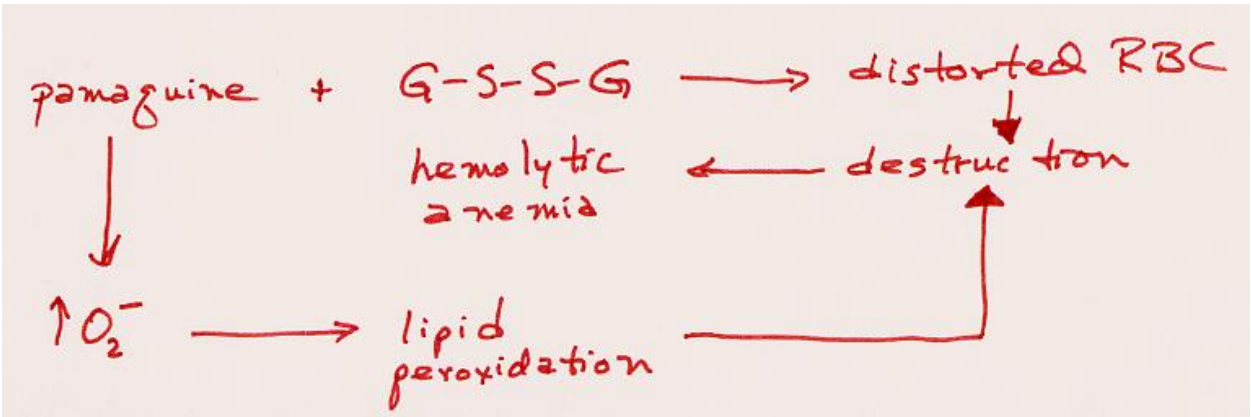
The HMP produces 2 NADPH for reductive power and Ribose for nucleoside/nucleotide synthesis. People with G-6-PDH deficiency don't make enough NADPH to reduce G-S-S-G and causes health problems. GSSG

is dimeric glutathione. It's synthesized from two molecules of GSH (monomeric form; SH is the active portion and is a thiol).

The function of the NADPH is summarized in the following graphic at right. When de-oxyhemoglobin (HbH^+ in the graphic) binds with oxygen and releases its proton, 2 molecules of GSH reduce the oxy-hemoglobin (HbO_2 in the image) to release its oxygen to the cells, leaving HbH^+ , again. GSSG is formed concurrently (the two SH groups in the 2 GSH molecules donate their hydrogen/proton to the HbO_2 and are oxidized as the oxyhemoglobin is reduced (classical redox reaction). In order for the GSSG to be re-reduced to keep the cycle going, the NADPH from the HMP must be oxidized. The NADP will be reduced at reactions 1 and 3 in HMP to keep this cycle going.

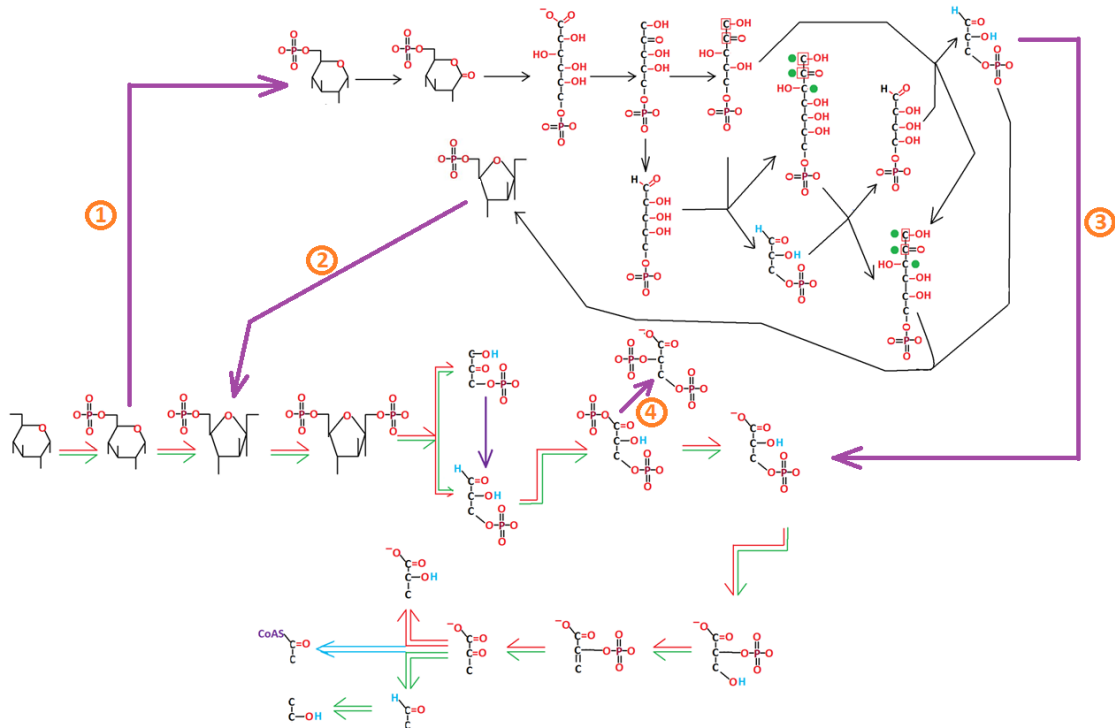


In addition, patients who have a G6PDH deficiency may develop a reversible anemia if they take a drug like primaquine:



Primaquine is an anti-malarial medication that was used a great deal during the Korean War. It caused problems of a health-related nature in folks from middle-eastern descent. It turned out that many of those folks have a natural G6PDH deficiency that isn't noticeable until it's brought out iatrogenically. The end result is a reversible hemolytic anemia. It's not an issue, any more, with this specific medication as it's no longer "out there". It was replaced with a medication called chloroquine that doesn't cause the anemia.

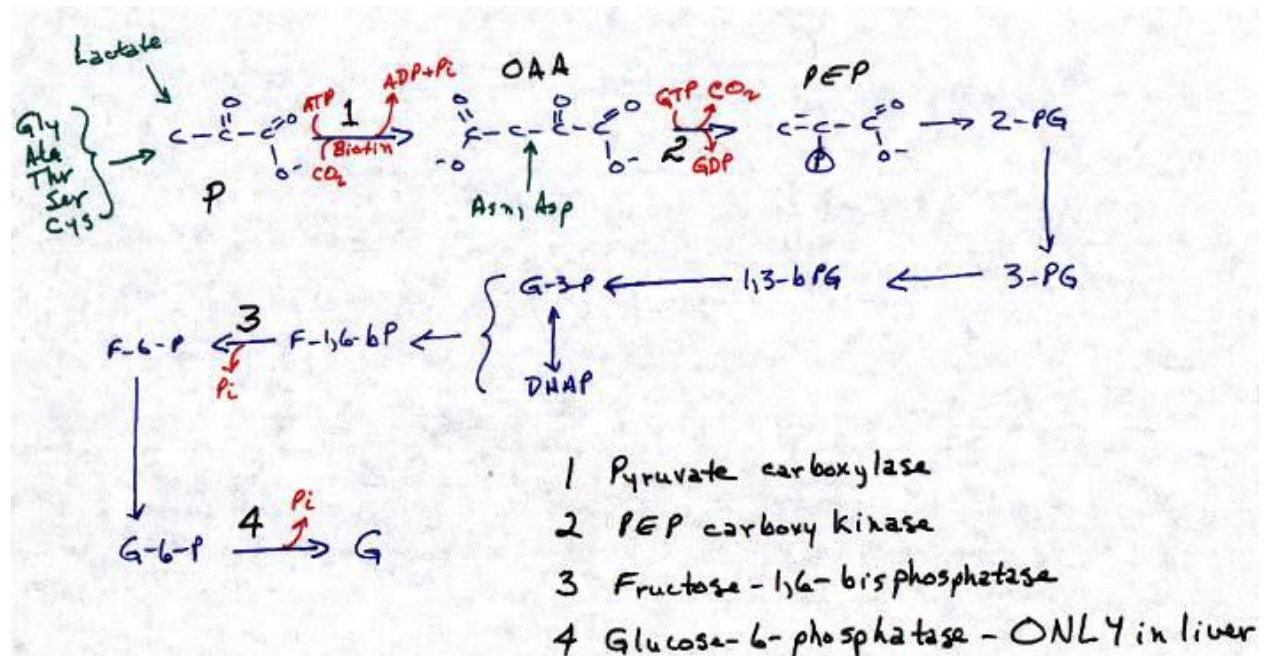
The image below combines [aerobic, anaerobic and fermentative] EMP, HMP and 2,3-bPG biosynthesis in one graphic:



Gluconeogenesis

Gluconeogenesis is a different carbohydrate-oriented pathway. It's anabolic (unlike EMP) and means "production of new glucose".

Gluconeogenesis is NOT the absolute reverse of glycolysis. Some enzymes are the same -- 4 are NOT. When the body produces new glucose, it utilizes various substrates as necessary. These include the carbon skeletons of amino acids and anaerobic end-products of catabolism. The carbon skeletons of Gly, Ala, Thr, Ser and Cys feed into gluconeogenesis via pyruvate, as does lactate. The carbon skeletons of Asn and Asp feed in to OAA (oxaloacetic acid) synthesis. Gluconeogenesis is illustrated below:



The four enzymes that differ between EMP are pyruvate carboxylase (enzyme 1), PEP carboxykinase (enzyme 2), fructose-1,6-bisphosphatase (enzyme 3) and glucose-6-phosphatase (enzyme 4). Enzyme 1 catalyzes the reaction between pyruvate and carbon dioxide to form OAA. This is an energy demanding reaction as ATP is hydrolyzed to drive the reaction forward. Note, too, that biotin is a cofactor for the reaction. Biotin is a carbon dioxide fixing vitamin, i.e., it binds carbon dioxide from biochemical reactions in such a manner that the carbon dioxide is added onto a molecule in the form of the carboxylate ion and extends the chain by one carbon.

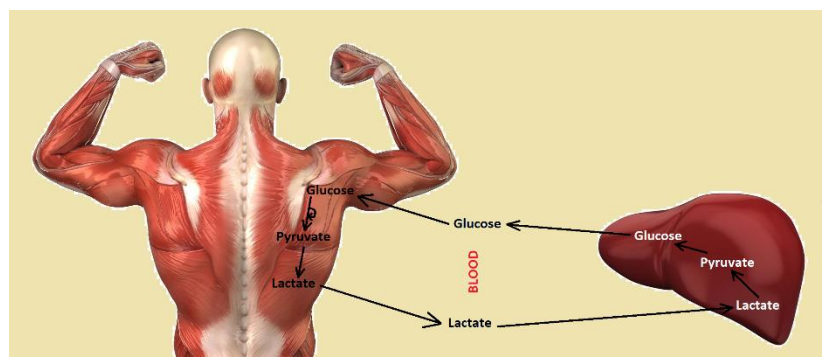
Enzyme 2 utilizes GTP hydrolysis to a) add a phosphate onto the b) now decarboxylated OAA forming PEP. From this point, gluconeogenesis uses the enzymes of EMP until the pathway reaches F-1,6-P.

At F-1,6-P, fructose-1,6-bisphosphatase removes the phosphate from carbon #1 to leave F-6-P, which is isomerized to G-6-P.

Remember that the purpose of phosphorylation of glucose in the first place is to trap it in the cell as G-6-P. Since that is a trapping mechanism, there has to be a way to remove the phosphate so that the newly formed glucose can get "dumped" into the blood. ONLY the liver cells contain G-6-phosphatase (enzyme 4) that cleaves off the phosphate so that the glucose may be routed through the body for use as needed by its cells. Which tissue does NOT require insulin for glucose uptake?

These pathways are all fine and dandy, but of what significance are anaerobic glycolysis and gluconeogenesis? The significance is the Cori cycle, named after Dr. and Dr. Cori who discovered it. As muscle tissue anaerobically catabolizes glucose for whatever energy needs, lactate is produced.

Lactate is small enough to freely diffuse across the muscle cell membranes into the blood. Once in the blood, it travels to the liver where it diffuses into liver cells. The lactate is used in gluconeogenesis



to synthesize more glucose in the liver, which is then sent back to the muscles for utilization until aerobic catabolism catches up or until the muscle needs no more glucose. The Cori cycle buys time and changes the metabolic burden to the liver. The Cori Cycle is illustrated above.

Aerobic Energy Sources from Intermediary Metabolism

There are three systems that provide energy to cells: System 1: Phosphagen System; System 2: Creatine Phosphate System; System 3: The Krebs' Cycle (TCA; Citric Acid Cycle).

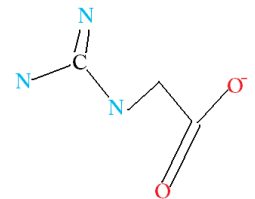
The first system is the phosphagen system. In this system, the source of the energy is ATP. During muscular contraction, ATP is hydrolyzed to ADP, P_i and energy. When this happens, there is only enough energy for 5-6 seconds. So how do our cells get additional energy?

Our cells get it via a compound called phosphocreatine (PCr; System 2). The concentration of PCr is about 2-3 times greater than the concentration of ATP. When PCr is available, it is hydrolyzed to Cr and P_i and energy. The P_i is used to re-phosphorylate ADP to make more ATP. This gives us about 15 seconds of maximal contractions and is used for short bursts.

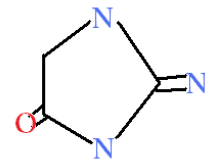
As long as the system (cell and/or tissues and/or body) remains in an aerobic state and fuel is present, the TCA (System 3) will continue to provide energy to the cells.

Aerobic Energy System 2

Creatine (at right) is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to muscle and nerve cells. In humans and animals, approximately half of stored creatine originates from food (mainly from fresh meat). Ninety-five percent of creatine is later stored in the skeletal muscles.



Creatinine (at right) is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatinine is chiefly filtered by the kidney, though a small amount is actively secreted. There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, blood levels rise. Men tend to have higher levels of creatinine because they have more skeletal muscle than women. Vegetarians tend to have lower creatinine levels, because vegetables contain no creatine.

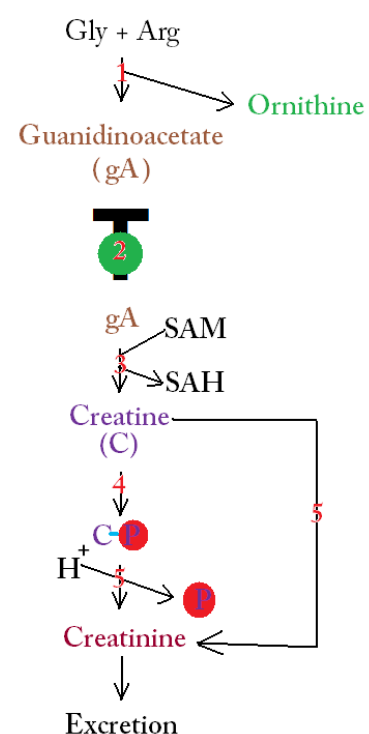


The biosynthesis of creatinine from creatine is illustrated at right. The enzymes that run this pathway are as enumerated:

1. Mitochondrial Arginine-glycine: amidinotransferase (E.C. 2.1.4.1);
2. gA Transporter – (from mito to cytosol);
3. Cytosolic S-adenosylmethionine :guanidinoacetate-N-methyltransferase (SAM:gA NMT);
4. Cytosolic Creatine kinase;
5. Cytosolic non-enzymatic cyclization.

Note the use of the EC nomenclature as a reminder not to forget that system and as an example of just how detailed the EC system can actually get. Glycine and arginine are condensed with the release of ornithine (a urea cycle intermediate) by E.C. 2.1.4.1 to form guanidinoacetate. The gA is then transferred via its specific transporter from the mitochondrion to the cytosol where it's acted on by SAM:gA NMT to form creatine. This reaction involves a one carbon transfer from SAM to form SAH, as well. SAM as you recall is S-adenosylmethionine.

The creatine then follows one of two steps: 1) creatine kinase phosphorylates it to form creatine phosphate (PCr or CrP). Which also follows the second pathway, 2) that creatine undergoes to form creatinine in a non-enzymatic cyclization process. Creatinine is then excreted.



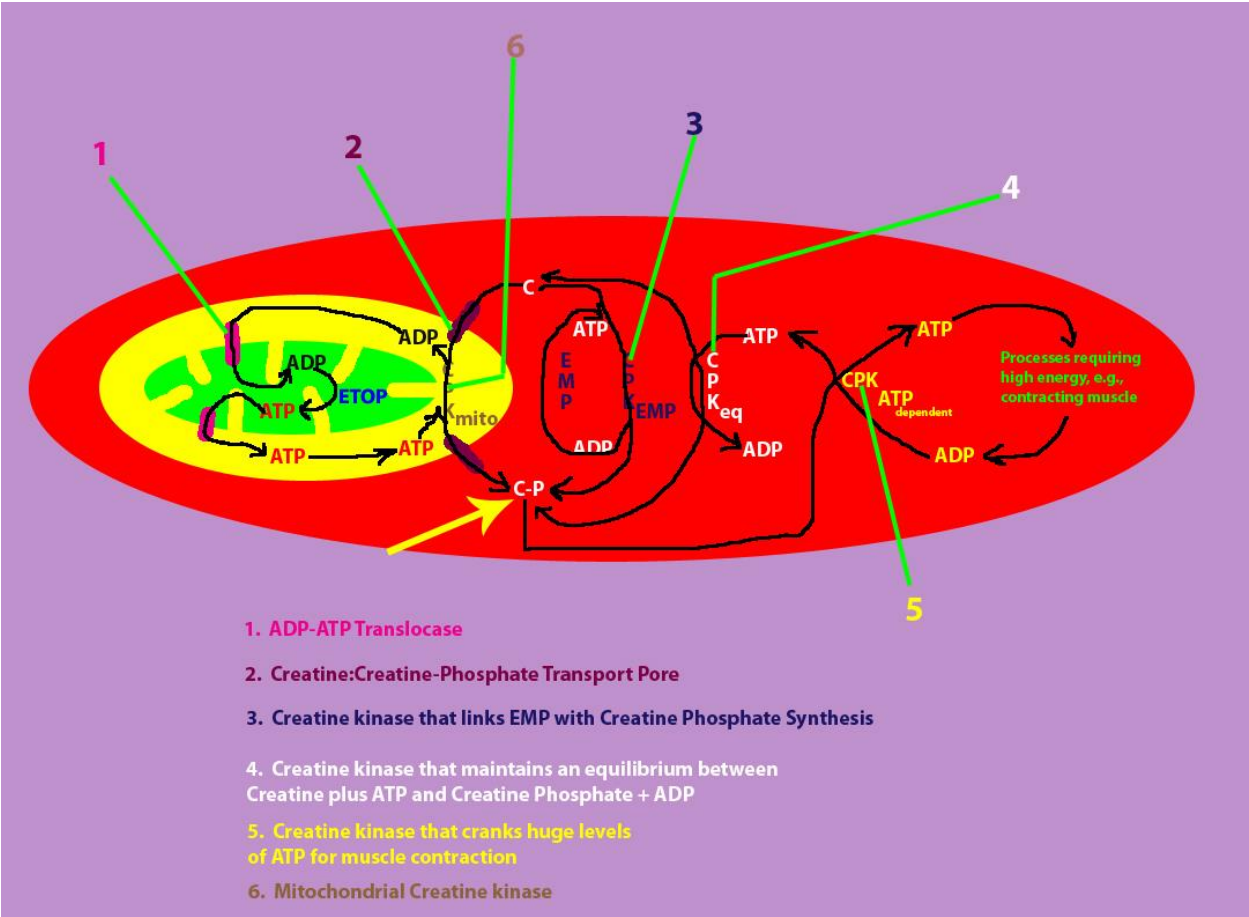
As previously indicated, creatine plays an important role in aerobic energy production.

PCr Shuttle – Quick View

To give you an idea of what is involved in PCr utilization and synthesis, let's examine the PCr shuttle in cardiac and skeletal muscle (pathway image on top of following page). This shuttle increases incredibly the movement/transport of high-energy phosphate (ATP) from the matrix of the mitochondrion to the cytosol of the cell.

In the first step (1), an ADP-ATP translocase re-phosphorylates ADP to form ATP in the mitochondrial matrix. This occurs via electron transport/oxidative phosphorylation (the "ETOP" in the graphic). When the ATP is "dumped" into the intermembrane space, it is reacted with creatine via a mitochondrial creatine

kinase (6) to form PCr. The PCr is then transported via a creatine-creatine phosphate (C:PCr) transport pore (2) into the cytosol to "dump" into a cytosolic PCr store.

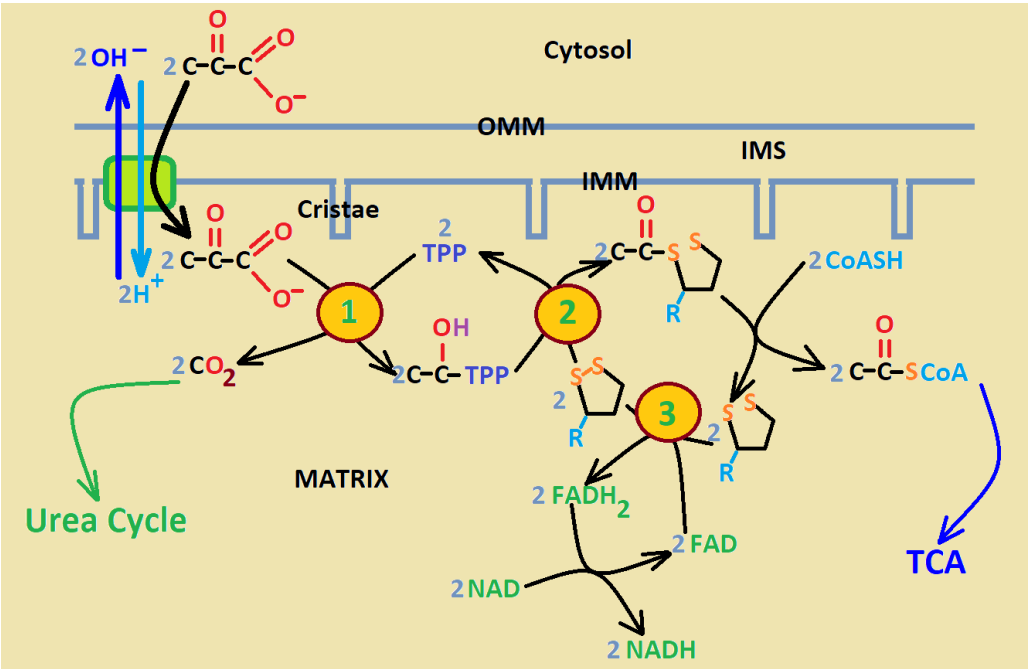


The cytosolic PCr store is derived from an EMP-linked (glycolysis-linked) creatine kinase (CK) (3) and another CK that maintains the equilibrium between C and PCr (or ATP and ADP, if you prefer)(4). The utilization of the PCr from the cytosolic store occurs via an ATP dependent CK that cranks out hugely elevated levels of ATP (5). The ATP is used by processes requiring high energy, e.g., contracting muscle.

There’s more to the cell and metabolic pathways than this brief information would suggest.

We need to get the pyruvate from EMP out of the cytosol and into the matrix of the mitochondrion of the cells. NOTE: there is an hypothesis that suggests that each cell has only one mitochondrion per cell and the reason we think that there appear to be more than one mito is because some cells have a larger mito and some have a smaller mito and when the cell is sliced for microscopic study, it appears that there are numerous (or fewer) mito in each cell.

Pyruvate freely diffuses through pores (proteins called porins) in the outer mitochondrial membrane and, then, is transported in symport with a proton (making this an energy requiring, active transport mechanism) and in anti-port with a hydroxide ion, pyruvate translocase, from the intermembrane space into the matrix of the mitochondrion. This, in addition to the pyruvate dehydrogenase complex, are illustrated below:



The pyruvate translocase is at the upper left in the graphic at the bottom of the previous page and is represented by the darker green outlined rounded rectangle (filled with lime green). Note the symport transport with the pyruvate and proton and the antiport transport between the pyruvate and the hydroxide ion across the inner mitochondrial membrane (through the pyruvate translocase) and the relatively free diffusion across the outer mitochondrial membrane.

Once the pyruvate is in the matrix, pyruvate is first decarboxylated by PDH in the presence of thiamine pyrophosphate (TPP) to form carbon dioxide (2 moles, remember) and an acetol-TPP intermediate at step 1, catalyzed by pyruvate dehydrogenase (PDH).

The acetol is transferred from TPP to lipoic acid at step 2 by dihydrolipoyl transacetylase (DHLTA) and, then thiolated with the addition of HSCoA (Coenzyme A) and release of reduced lipoic acid and acetyl CoA (aCoA).

Lastly, the reduced lipoic acid is oxidized at step 3 by dihydrolipoyl dehydrogenase. It's this step where additional ATP equivalents are formed. The 2 FADH₂ molecules are very rapidly oxidized to FAD at the expense of 2 NAD molecules to produce 2 molecules of NADH. This step, then, produces the equivalent of 6 ATP molecules rather than 4 ATP molecules as could have occurred without the FADH₂ oxidation step by NAD.

The carbon dioxide generated by the PDH complex is used in the urea cycle and the acetyl CoA (aCoA) is used to synthesize the first product in the Krebs' cycle (TCA or citric acid cycle).

Aerobic Energy System 3: The Krebs' Cycle – TCA

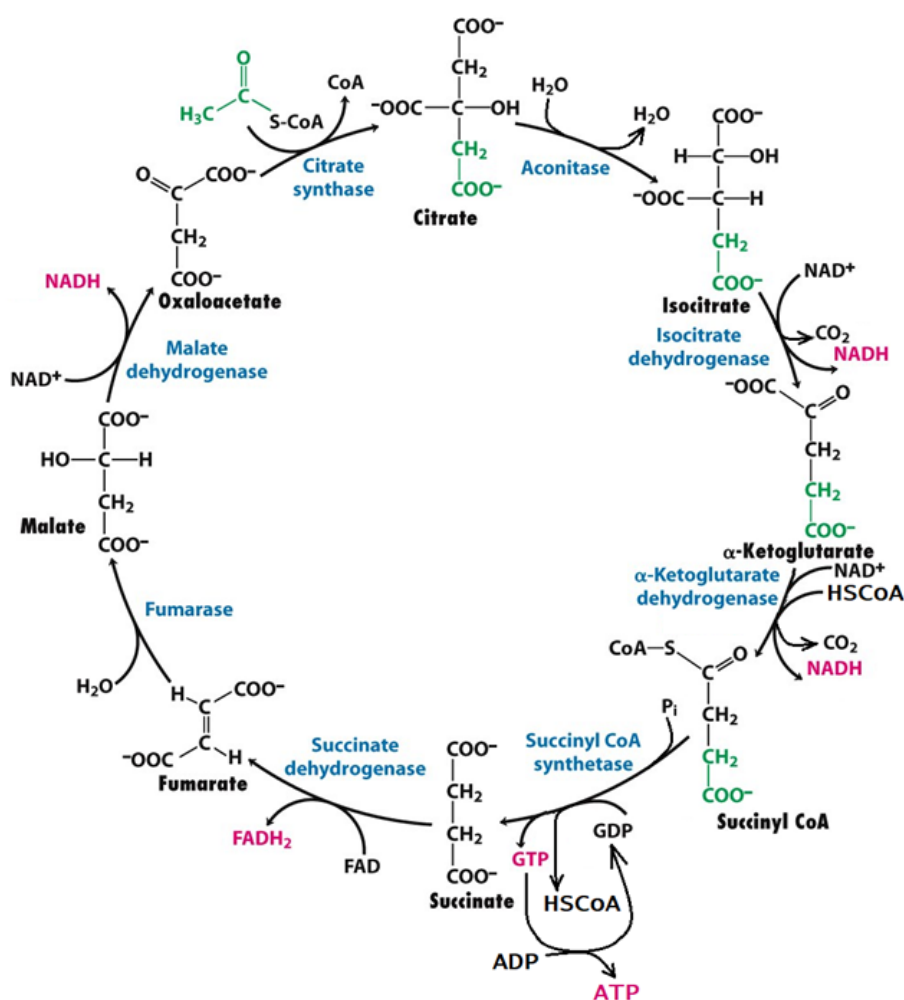
The Krebs' cycle is known by a number of different names and acronyms: the Tricarboxylic Acid Cycle (TCA – after the first product of the TCA, which is citrate, a tricarboxylic acid), the citric acid cycle (after citrate) and the Krebs' cycle, after Hans Krebs who discovered this cycle (he also discovered the urea cycle).

The TCA is illustrated at right. Acetyl CoA (aCoA – green molecule at upper left of TCA) from the PDH complex condenses with OAA to form citrate, a tricarboxylic acid, with citrate synthetase.

Citrate is isomerized via aconitase to aconitic acid, then to isocitrate, also by aconitase. This involves the movement of the –OH group on the third carbon to the second carbon. Aconitase is a reaction that both uses and releases water.

Isocitrate is acted on by isocitrate dehydrogenase, that requires NAD to form NADH and to decarboxylate isocitrate to form α-ketoglutarate with the loss of carbon dioxide. This is the first reaction step in the TCA to produce energy (in the form of NADH).

α-ketoglutarate is decarboxylated, oxidized and thiolated by α-ketoglutarate dehydrogenase, which adds NAD, CoASH (Coenzyme A) and removes carbon dioxide and releases NADH, the second reaction in the TCA to release energy (note that the stoichiometry is only half that of reality in the graphic, hence, in the two steps, 4 NADH molecules or 12 ATP equivalents have really been formed/produced at this point). The product of this reaction is succinyl CoA.



Succinyl CoA synthetase adds GDP and inorganic phosphate as co-factors to remove HSCoA and form GTP in the production of succinate. Once GTP is formed, it automatically phosphorylates ADP to form ATP.

FAD is reduced to FADH₂ when reacting with succinate in the presence of succinate dehydrogenase. The product of this reaction is fumarate. Remember that each FADH₂ is equivalent to 2 ATP molecules, hence 4 ATP equivalents are produced here. Between this and the immediately previous reactions, the cell has now produced 18 ATP molecules.

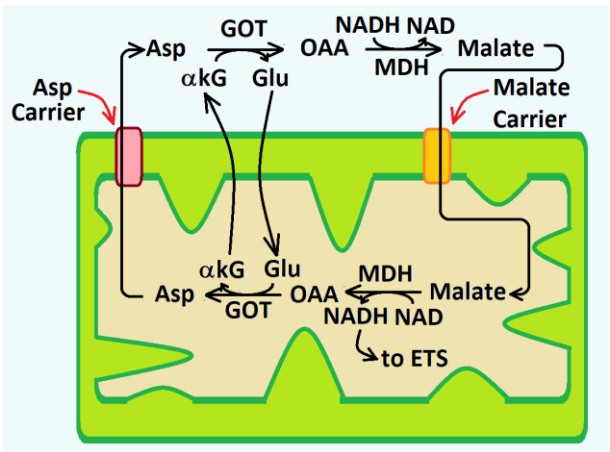
Fumarate is hydrated by fumarase to form malate. Water is added across the double bond to open the chain up to form malate so that malate dehydrogenase can oxidize the secondary alcohol, at the expense of NAD, to a ketone to form OAA and NADH. 6 more ATP equivalents are formed here. The ATP molecules synthesized in TCA are as follow, at right. At this point, 24 ATP's are formed in TCA, 6 in PDH and 8 in EMP for a grand total of 38 ATP's from one molecule of glucose!

TCA: ATP Summary – All MADE	
PDH:	+6 (⇔)
iCDH:	+6 (⇔)
αKGDH:	+6 (⇔)
sCoA synthetase:	+2
SDH:	+4 (⇔)
MDH:	+6 (⇔)

ATP Synthesis by Tissue: Clarification

Not all tissues actually produce 38 ATP per molecule of glucose. How does this all work itself out?

NAD/NADH will NOT cross the mitochondrial membranes – how does it “get in” the mitochondria? It gets in via the Aspartate-Malate Shuttle, which is illustrated at right! This shuttle is found in the HEART and LIVER tissues; these tissues DO produce 38 ATP from aerobic glycolysis and TCA. Note: OAA won’t cross the mitochondrial membranes, either.

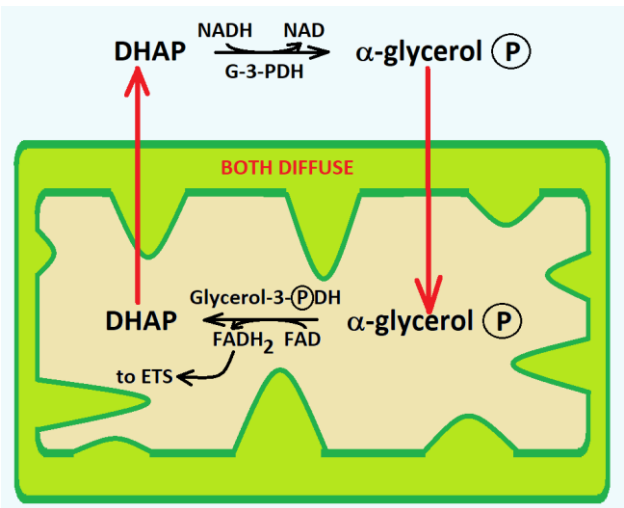


Note the paucity of biochemical structures: you’ve already learned them, so they’re not needed here. Both aspartate and malate will cross the mitochondrial membranes via specific carrier proteins as shown in the image. In the cytosol (in the blue area), asp is acted on by GOT (glutamate-oxaloacetate transaminase) which swaps the α-amine from asp for the ketone on αKG to produce OAA and glu.

OAA is acted on by a cytosolic malate dehydrogenase (MDH) at the expense of cytosolic NADH to reduce the OAA to malate. The cytosolic malate is transported from the cytosol into the matrix, where it’s immediately oxidized to OAA by a mitochondrial MDH and NAD is reduced to NADH for transport to the electron transport system (ETS) for ATP biosynthesis.

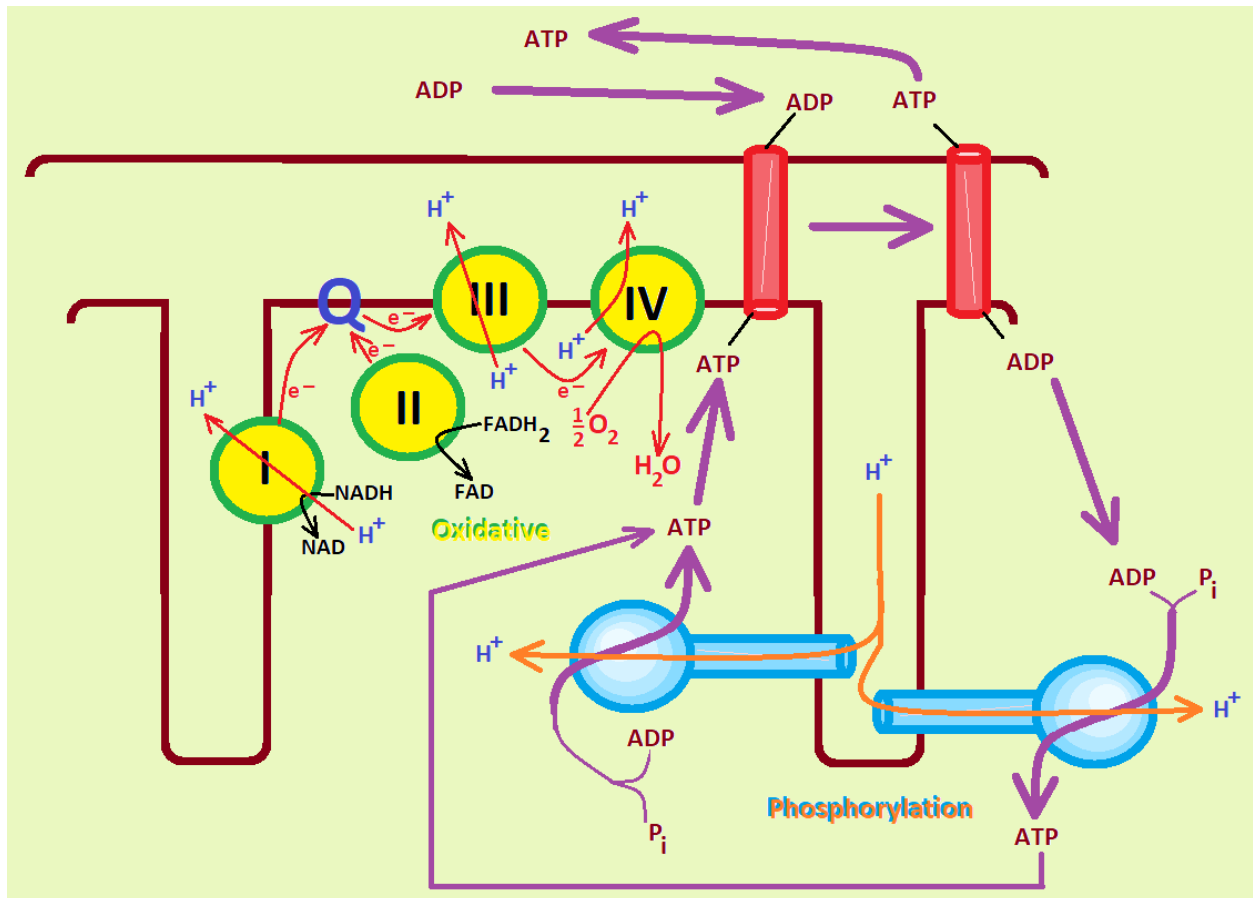
Mitochondrially formed OAA is then transaminated by GOT with glu to form asp and αKG. Asp is then transported into the cytosol to propagate this shuttle.

This doesn’t fully answer our inquiry regarding the production of ATP in a variety of cells. Is there any other way? YES!!!! The Glycerol Phosphate Shuttle in SKELETAL MUSCLE, at right! This shuttle has an effect on ATP production because NADH is equivalent to 3 ATP and FADH₂ is equivalent to 2 ATP. Why is FADH₂ being brought up here? The α-glycerol phosphate shuttle is illustrated below to assist in the response to this inquiry.



Both DHAP and α-glycerol phosphate freely diffuse across the mitochondrial membranes between the matrix and cytosol. NADH is oxidized to NAD in the cytosol (blue region), which reduces DHAP to α-glycerol phosphate. Once the α-glycerol phosphate is in the matrix, though, a different enzyme (glycerol-3-phosphate dehydrogenase, which requires FAD, oxidizes the α-glycerol phosphate to DHAP and forms FADH₂. Because each FADH₂ produces one less ATP than each NADH molecule, there are, hence, 36 ATP produced in aerobic glycolysis and TCA in MUSCLE.

We've reached a point in the metabolism discussion where we need to take the NADH and FADH₂ and use it to generate energy in the form of ATP. This is done in electron transport and oxidative phosphorylation (ET-OP). ET-OP is illustrated below:



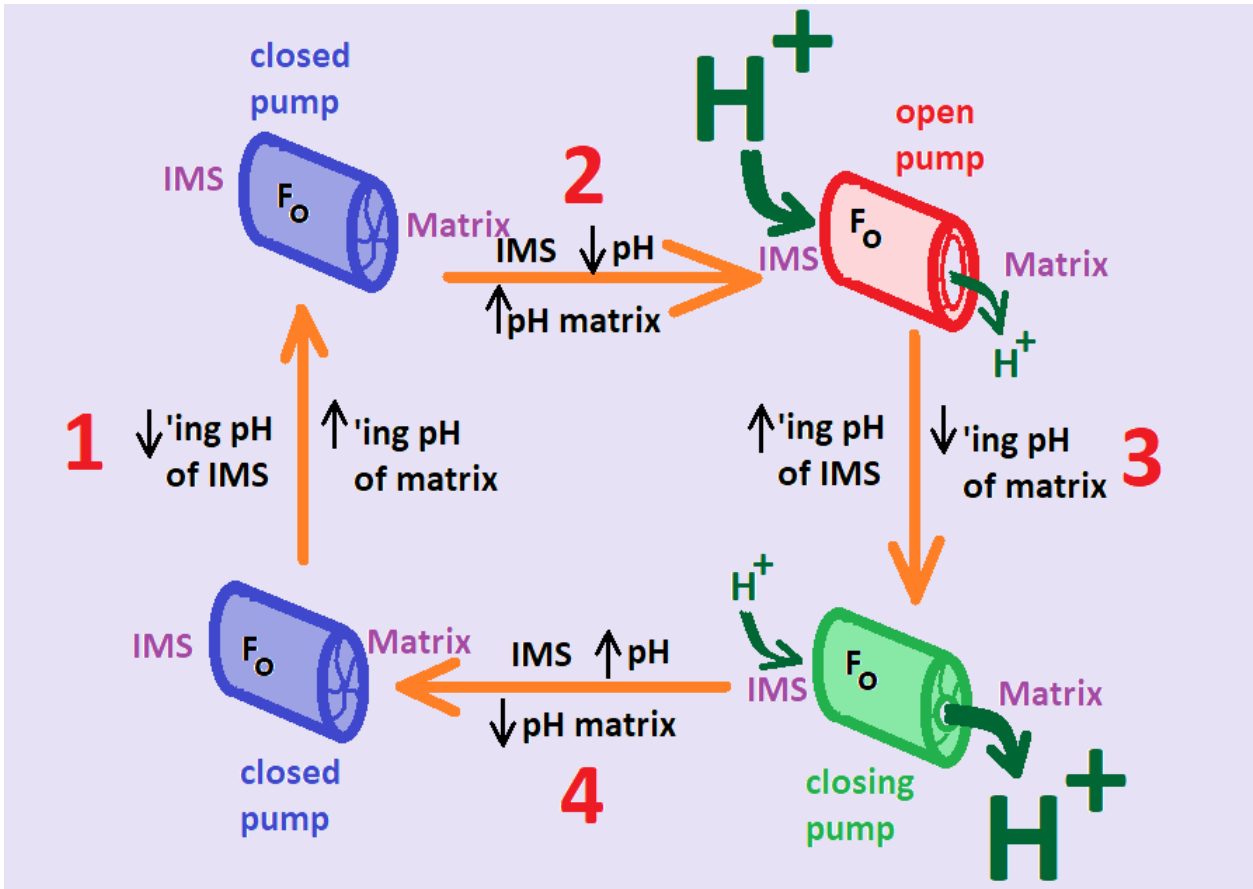
In order to fully understand why one mole of NADH is worth 3 ATP molecules and why one mole of FADH₂ is worth 2 molecules, we need to discuss how each is oxidized by electron transport (ET) to form ATP through oxidative phosphorylation (OP; the combination of the two is called ET-OP).

The primary purpose of ET-OP is to generate ATP. Neither ADP nor ATP easily cross the mitochondrial membranes from cytosol to matrix or vice versa. In order for the ADP and ATP to be interchanged, as it were, NADH and FADH₂ must undergo oxidation in the matrix. The oxidation of these two high energy compounds is performed by a series of four (or five, depending on how you look at it) high energy protein complexes that, with the exception of Complex II, are bound to the inner mitochondrial membrane. The complexes are generally named Complex I, Complex II, Complex III, Complex IV and Complex V. Complex I is also known as NADH:NAD oxidoreductase; Complex II is also known as FADH₂:FAD oxidoreductase; Complex V is also known as the F₁F₀ATPase. We'll stick with "Complex X" for the most part – there will be exceptions eventually.

NADH is oxidized to NAD by Complex I. Simultaneously, a proton is transported from the matrix into the IMS across Complex I. An electron is passed from Complex I to CoQ concurrently. CoQ is an electron acceptor that's also inner mitochondrial membrane-bound (you may have seen commercials for CoQ₁₀ or seen it in the supplements section of grocery stores – for some folks it does help with cardiac function, although not for all). CoQ passes the electron onto Complex III and another proton is transported from the matrix to the IMS. An electron continues to Complex IV. Where a half a molecule of oxygen is reduced to water and a 3d proton is shunted across Complex IV from the matrix to the IMS. Didja notice the three (3) protons that were transported into the IMS with the oxidation of NADH?

FADH₂ is oxidized to FAD by Complex II in the matrix. No proton is passed into the IMS from this complex. An electron, however, is passed to CoQ, and the electron transport chain continues as with the NADH oxidation. Didja notice that only two (2) protons are transported into the IMS from the matrix with FADH₂ oxidation?

It's the 3 protons from NADH and the 2 protons from FADH₂ that drive the respective synthesis of 3 and 2 ATP's via Complex V. Complex V's alternative name reflects its electron microscopic anatomy: it looks like a lollipop, with the stick called the F₀ fraction and the lollipop portion called the F₁ fraction. The former is a proton pump and the latter the ATP synthetase activity. It's the proton pump that responds to the protons shunted from the matrix across the Complexes into the IMS that turns on the ATP synthetase activity.

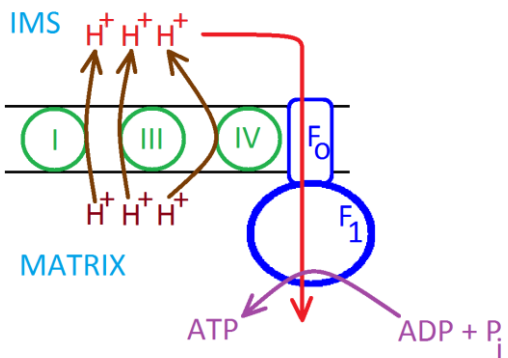


The proton pump elementary mechanism is illustrated below:

During step 1, H^+ are moving out of the matrix at Complexes I, III, IV. The pH of the IMS drops and the pH of the matrix rises. In step 2, the pH gradient established – F_o opens and H^+ run through to drive ATP’ase. The pH of the IMS continues to drop and the pH of the matrix continues to rise.

The proton pump (F_o fractrion) works sort of like a twisted up paper towel roller: the protons on the IMS side act to unwind the proton pump, much like when one unwinds the cardboard paper towel roller. Eventually, the proton pump is fully unwound/untwisted and the protons rush through the proton pump activating the ATP synthetase.

In step 3, the pH of the IMS begins to increase due to the paucity of protons in that space, whilst the pH of the matrix is dropping in response to the rapid influx of protons. With the reduction of protons, the proton pump begins to re-wind/re-twist itself closed and the IMS pH rises more, while the pH of the matrix continues to drop in step 4, with a closed proton pump awaiting more IMS protons to re-open itself.

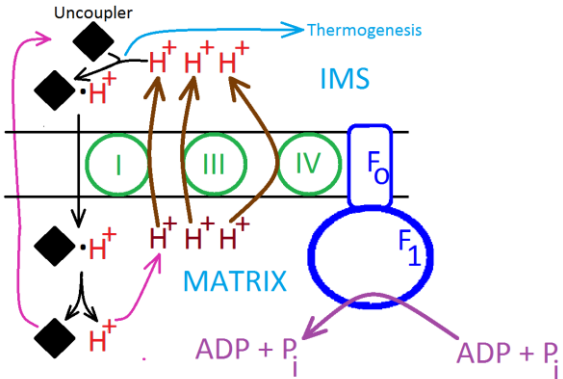


Application of Proton Pump in ETOP – White Adipose Tissue (WAT)

White adipose tissue is so called because it contains only a few mitochondria. White adipose tissue runs through normal ET-OP, image at right, producing ATP is it’s supposed to.

Application of Proton Pump in ETOP – Brown Adipose Tissue (BAT)

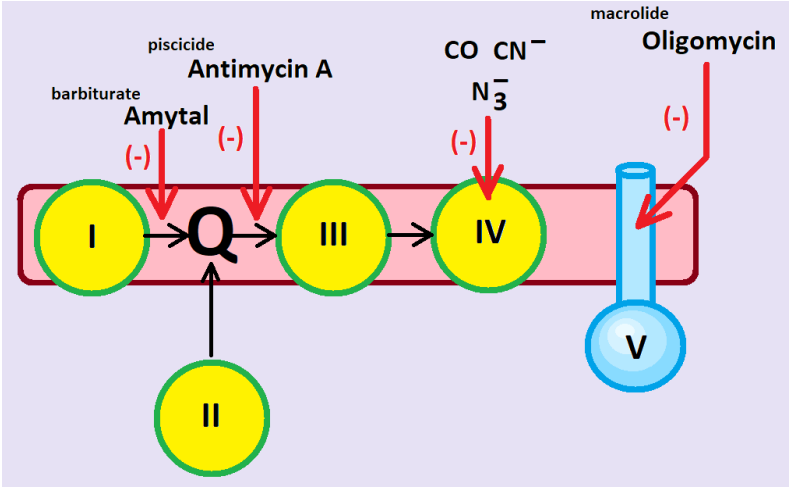
In contrast, BAT contains a great deal of mitochondria (that makes the tissue look “brown”). The primary duty of BAT is thermogenesis, the production of heat. This heat production is regulated by two thyroid hormones: T_4 and T_3 . Fatty Acids act as uncouplers of ET from OP; norepinephrine regulates FA release in BAT. The purpose of uncoupling the two functions is to generate heat only, and no ATP, per the BAT image at right. The fatty acid uncouplers are transported to the matrix with the protons to drive the process all over,



again. When we're very young, we have a great deal of BAT: it helps us stay warm. Eventually, though, over time, we lose BAT and we begin to "catch a chill" where BAT used to be.

ET-OP Inhibition

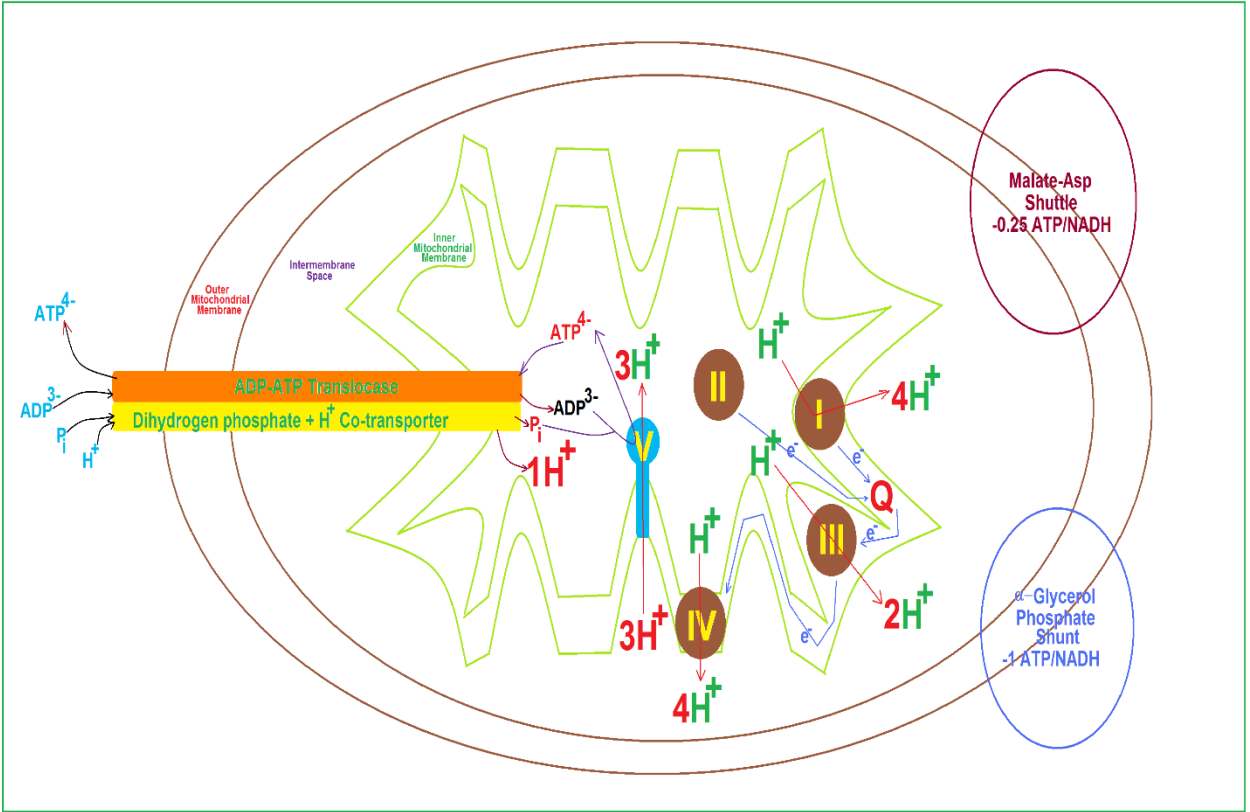
ET-OP can be inhibited both in the lab and in "real life", see image at right. The flow of electrons from I to Q may be inhibited by a barbiturate called amytal. The electron flow from Q to III may be inhibited by a poscocide called Antimycin A. Carbon monoxide, cyanide and azide all inhibit IV and block the reduction of the half mole of oxygen to water. These three inhibitions simply shut ET-OP down.



Oligomycin, a macrolide of the same group as Zithromax, inhibits the proton pump in V, shutting off ATP synthesis.

ATP Fine Tune

We've previously discussed the ATP equivalency from NADH (3 ATP's) and FADH₂ (2 ATP's). While this is a good rule of thumb, it's not quite 100% the whole story. The graphic below, illustrates what's really going on with ATP synthesis from NADH and FADH₂ oxidation in ET-OP:



NADH: through ETS

ADP-ATP Translocase is coupled with the Dihydrogen Phosphate/Proton Co-Transporter. Complex 1 requires 4 protons to pass on electron to Complex III. Complex III requires 2 protons to pass on electron to Complex IV. Complex IV requires 4 protons to reduce molecular oxygen to water. Hence, 10 protons are exported into the IMS per NADH to make an ATP.

FADH₂: through ETS

ADP-ATP Translocase is coupled with the Dihydrogen Phosphate/Proton Co-Transporter. Complex III requires 2 protons to pass on electron to Complex IV. Complex IV requires 4 protons to reduce molecular oxygen to water. Hence, 6 protons are exported into the IMS per FADH₂ to make an ATP.

Complex V and Co-Transporter

3 protons are required to turn on F_o. 1 proton is co-transported with P_i and 4 protons are imported from the IMS into the matrix to synthesize 1 ATP molecule.

For the production of ATP from NADH, the currently accepted stoichiometry is in the graphic at lower left.

$$\frac{\# \text{ ATP}}{1 \text{ NADH}} = \frac{\frac{10 \text{ protons}}{\text{NADH}}}{\frac{4 \text{ protons}}{\text{ATP}}} = 2.5 \frac{\text{ATP}}{\text{NADH}}$$

$$\frac{\# \text{ ATP}}{1 \text{ FADH}_2} = \frac{\frac{6 \text{ protons}}{\text{FADH}_2}}{\frac{4 \text{ protons}}{\text{ATP}}} = 1.5 \frac{\text{ATP}}{\text{FADH}_2}$$

For the production of ATP from FADH₂, the currently accepted stoichiometry is in the graphic at upper right.

As you can see, we’re pretty close with 3 ATP per NADH and 2 ATP per FADH₂. In spite of the details, above, we’ll stick with the 3 ATP per NADH and 2 ATP per FADH₂ for this course.

Below is information tabulated on two types of muscle fibers, including metabolic differences and similarities related to CMB and A&P.

Type I Muscle Fibers	Type II Muscle Fibers
• Red Fibers • Slow Twitch • For Endurance • Small Diameter • Aerobic • Lots of Mitochondria • Malate-Aspartate Shuttle • Liver, Heart, Kidney • Back Muscles, too	• White Fibers • Fast Twitch • For Explosive Bursts of Power • Large Diameter • Anaerobic • Fewer Mitochondria • α-Glycerol Phosphate Shunt • Skeletal Muscle, Brain • Digits and Extraocular Muscles • Store Glycogen

Q&D Energy Systems’ Summary and New Terminology

Chemiosmosis is the diffusion of hydrogen ions (protons) across the biological membrane via the ATP synthase (a transport protein) due to a proton gradient that forms on the other side of the membrane. The proton gradient forms when the hydrogen ions accumulate as they are forcibly moved to the other side of the membrane by carrier proteins while the electrons pass through the electron transport chain in the membrane. Since more hydrogen ions are on the other side they tend to move back across the membrane via the ATP synthase. As they flow through energy is released which is then used to convert ADP to ATP (by a process called phosphorylation). Chemiosmosis is one of the processes by which ATP is synthesized. In eukaryotes, it takes place in the mitochondria during cellular respiration and in the chloroplasts during photosynthesis. In prokaryotes, it occurs in the cell membrane.

This process is called **chemiosmosis** because the chemical ions move from an area of higher concentration to an area of lower concentration across a semipermeable membrane, similar to the movement of water molecules by osmosis.

Source accessed 07/16/2017, 0647 PDT: <http://www.biology-online.org/dictionary/Chemiosmosis>

Cellular respiration is a series of metabolic processes that take place within a cell in which biochemical energy is harvested from organic substance (e.g. glucose) and stored as energy carriers (ATP) for use in energy-requiring activities of the cell.

Simplified Reaction: C₆H₁₂O₆ + 6O₂ → 6CO₂ + 6H₂O

It consists of:

- (1) Glycolysis
- (2) Citric Acid Cycle, TCA or Krebs Cycle
- (3) Oxidative phosphorylation

The cell seems to “respire” in a way that it takes in molecular oxygen (as an electron acceptor) and releases carbon dioxide (as an end product), hence, the process is described to be aerobic. There are organisms that use other organic molecules as electron acceptors instead of oxygen. This type of respiration in which oxygen is not used as a final electron acceptor is referred to as anaerobic.

In anaerobic respiration (respiration in absence of oxygen), pyruvate is not metabolized by cellular respiration but undergoes a process of fermentation. The pyruvate is not transported into the mitochondrion, but remains in the cytoplasm, where it is converted to lactate in humans or ethanol in specific micro-organisms that may be removed from the cell.

Cellular respiration is essential to both eukaryotic and prokaryotic cells since biochemical energy is produced to fuel many metabolic processes, such as biosynthesis, locomotion, and/or transportation of molecules across membranes.

The entire process occurs in the cytoplasm of prokaryotes. In eukaryotes, glycolysis occurs in the cytoplasm whereas the Krebs Cycle and oxidative phosphorylation occur in the mitochondrion.

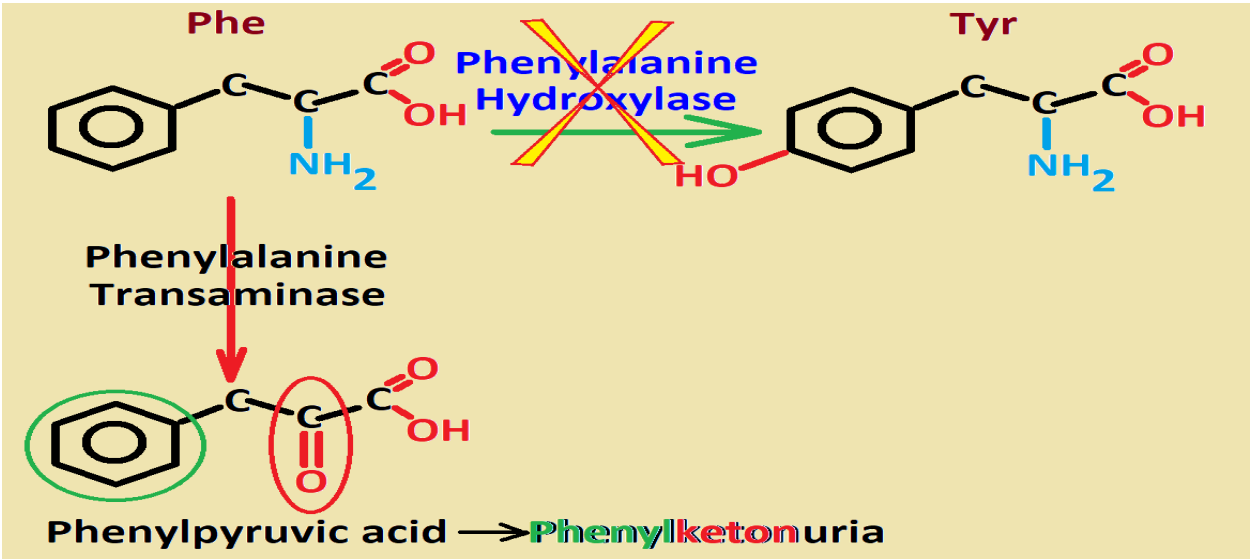
Source accessed 07/16/2017, 0647 hours PDT: http://www.biology-online.org/dictionary/Cellular_respiration

Amino Acid Metabolism

This section of metabolism focuses on a very narrow view of [semi-]essential amino acid metabolism. In previous coursework and readings, you learned the mnemonic for those amino acids: PVT TIM HALL. In this monograph, we'll examine amino acid metabolism as groups of amino acids with similar properties where possible, i.e., Positively charged Amino Acids: Histidine (his), Arginine (arg), Lysine (lys); Aromatic Amino Acids: Phenylalanine (phe), Tryptophane (trp); BCAA: Valine (val), Isoleucine (ile or ileu), Leucine (leu); Neutral Amino Acids: Methionine (met), Threonine (thr).

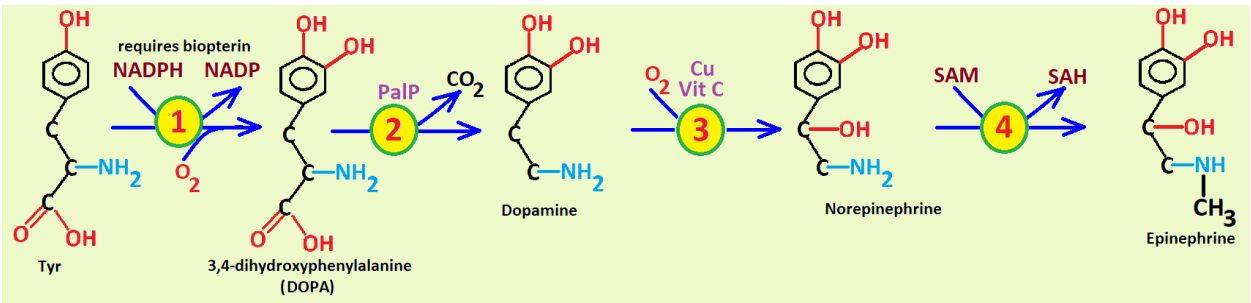
Aromatic Amino Acids: Phenylalanine (phe), Tryptophane (trp)

Phenylalanine is the only aromatic amino acid we'll discuss here. An inborn error of metabolism called PKU (phenylketonuria) is the focus of this discussion:



PKU is a cause of mental retardation. People who have PKU tend to be blonde, blue-eyed and fair skinned. WHY??? PIGMENT! In PKU, the patients are unable to biosynthesize tyrosine, which negates pigment synthesis. Which amino acid becomes essential, then?

Phenylalanine is also important in the biosynthesis of compounds called catecholamines: DOPA, dopamine, norepinephrine (NE) and epinephrine (E):



While only a small amount of the total tyrosine metabolism, catecholamine synthesis is rather important. First off, you'll recall phe is hydroxylated to tyr by phenylalanine hydroxylase.

At step 1, tyr is, again, hydroxylated by tyr hydroxylase. This enzyme requires molecular oxygen and NADPH, as well as a molecule of biotin, to generate the DOPA and NADP.

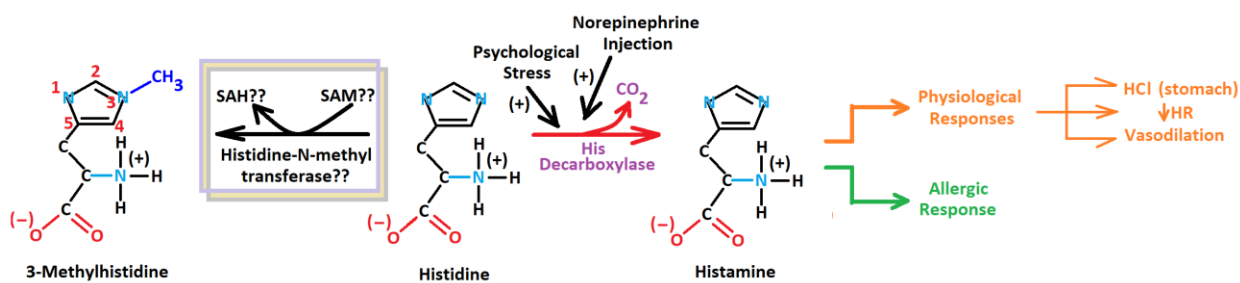
At step 2, DOPA is decarboxylated by DOPA decarboxylase, which requires PalP, to form dopamine and release carbon dioxide.

Dopamine-β-hydroxylase reduces oxygen and uses copper (redox cyclers) and vitamin C (more redox cycling) to add an alcohol onto the second carbon of the aliphatic chain to form norepinephrine (NE).

NE is acted upon by phenylethanolamine-N-methyl transferase (PNMT) with SAM to generate epinephrine (E) and S-adenosyl homocysteine (SAH) at step 4.

E drives bronchodilation, elevated heart rate, superficial vasoconstriction, deep vasodilation and is the chemical responsible for “Flight, Fight or Freeze”. NE is the neurotransmitter of rage. DOPA has had some limited success treating folks with Parkinson’s. Dopamine has been used to elevate the blood pressure in shocky patients who aren’t in any sort of renal injury/failure/damage.

Positively Charged Amino Acids: Histidine (his), Arginine (arg), Lysine (lys) (Remember: His and Arg are semi-essential)

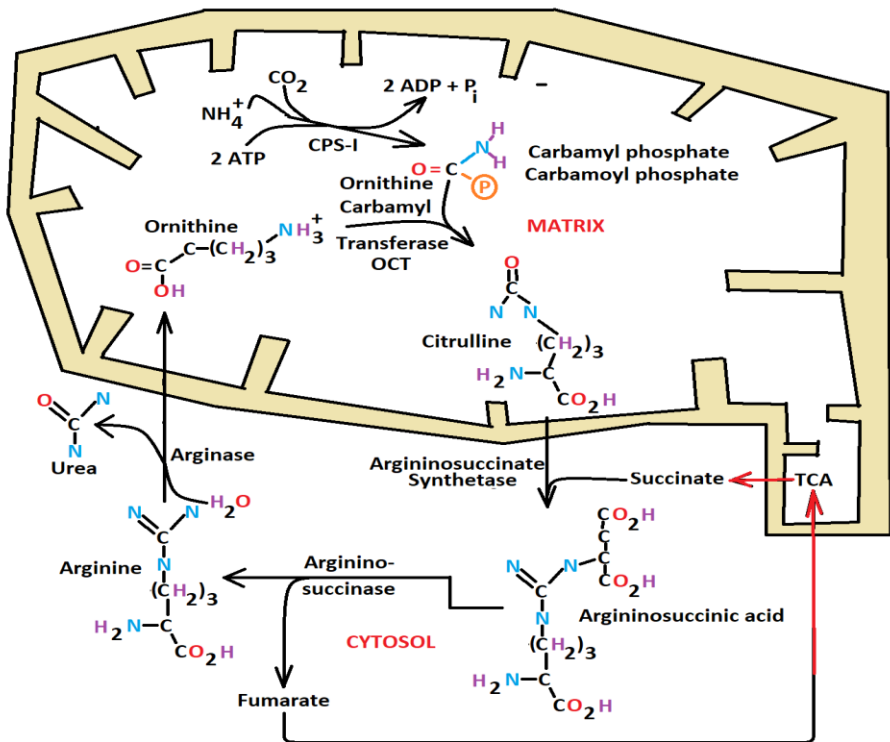


The first amino acid in this category that we’ll look at is histidine. For all practical purposes, there are really two metabolically important reactions: decarboxylation to form histamine and 3-methylation to form 3-methylhistidine:

3-methylhistidine is the first metabolic end product to examine. It’s a catabolite of muscle contraction found in actin and myosin (muscle contractile proteins). It also seems to be a product of peptide bond synthesis and is NOT further metabolized but excreted in the urine unchanged following muscle contraction. It’s useful in determining muscle protein turnover ACCURATELY (much more accurate than the old 24 hour urine creatinine’s that used to be used). It appears to be a post-translational modification. There’s not much information on its synthesis, however, given its similarity to epinephrine, it’s very likely that a sort of “histidine-N-methyl transferase” (“HNMT”) that uses SAM as one carbon donor is the “point of conversion”.

Histamine synthesis is driven by the effects of psychological stress or by norepinephrine injection on histidine decarboxylase. We’ll go over more of this in BIOL 223 in the nervous system. Suffice it to say that histamine’s allergic effects are very well known in NV.

While not the last positively charged amino acid to discuss, lysine is of considerable importance from a biological point of view. Lysine provides crosslinking in collagen; it provides crosslinking in elastin. When crosslinking is inhibited in severe Cu deficiency or after ingesting sweet pea toxin [β-aminopropionitrile], it causes lathyrism. The lathyrism described here is sometimes referred to as osteolathyrism. There is increased solubility of the collagen, as well as increased rigidity of



elastin. Both cause death – generally due to aortic rupture from a lack of elasticity at the aortic root – an “aortic blow out” or aneurism.

The other form of lathyrism is referred to as neurolathyrism and causes paralysis.

The last positively charged amino acid is arginine. While arg is a semi-essential amino acid, hypo-argininemia can lead to developmental disorders in the young. As humans age, their diet changes and more arg is taken up and the deficiency isn’t an issue. In addition, arg is a necessary participant on the urea cycle (bottom left on previous page).

The urea cycle is how the cells get rid of toxic ammonia. To some extent it’s also how the cells get rid of a little carbon dioxide. The first reaction condenses ammonia in the form of the ammonium ion with carbon dioxide at the expense of two ATP molecules to form carbamoyl phosphate. This reaction is catalyzed by carbamoyl phosphate synthetase I (CPS-I). There is a CPS-II, however, it’s involved in nucleic acid synthesis and we won’t go into that in this course.

The second reaction condenses carbamoyl phosphate with ornithine to form citrulline. This reaction is catalyzed by ornithine carbamoyl transferase (OCT). The citrulline is then transported across the mitochondrial membranes via an Ornithine-Citrulline AntiPort mechanism (OCAP mechanism).

Once the Citrulline is in the cytosol of the cell, it reacts with succinate from the TCA to form argininosuccinate. This reaction is catalyzed by argininosuccinate synthetase (ASS).

The argininosuccinate is hydrolyzed by argininosuccinase (AS) to arginine and fumarate (which is transported back to the mitochondrion and, hence, the TCA).

Arginine is then hydrolyzed by arginase to form urea (for excretion) and ornithine, which is transported back to the matrix of the mitochondrion via the OCAP Mechanism, and the cycle perpetuates.

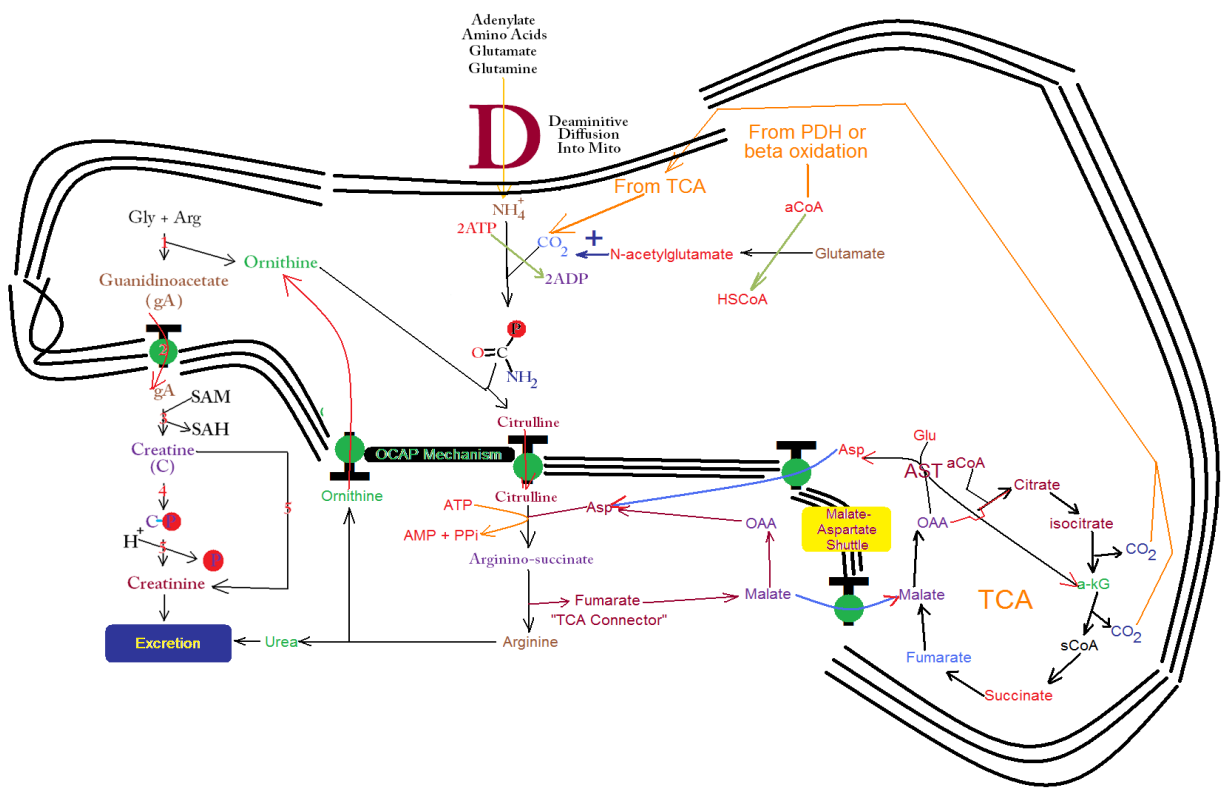
Did you notice that the first two reactions of the urea cycle are in the matrix? And that the remaining three reactions occur in the cytosol of the cell? In addition to the TCA, Hans Krebs also discovered the urea cycle and its link to the TCA.

BCAA: Valine (val), Isoleucine (ile or ileu), Leucine (leu)

BCAA’s aren’t going to be covered in this monograph, other than to remind you of leucine’s importance in ketone synthesis, as well as in cholesterol biosynthesis, albeit in a minor role, coming up shortly in the section on lipid metabolism.

Neutral Amino Acids: Methionine (met), Threonine (thr)

Methionine is the only amino acid in this group that we’ll briefly discuss. Its role is that of a one carbon donor to reactions that require methylation. PNMT (aromatic’s, above) and HNMT (positively charged amino acids, above) basically cover the metabolism of methionine. Further discussion on methionine involving homocysteine is reserved for BIOL 224.



The graphic emphasizes adipokinesis (fat movement) from BAT (and is why the bottom graphic is colored the way it is).

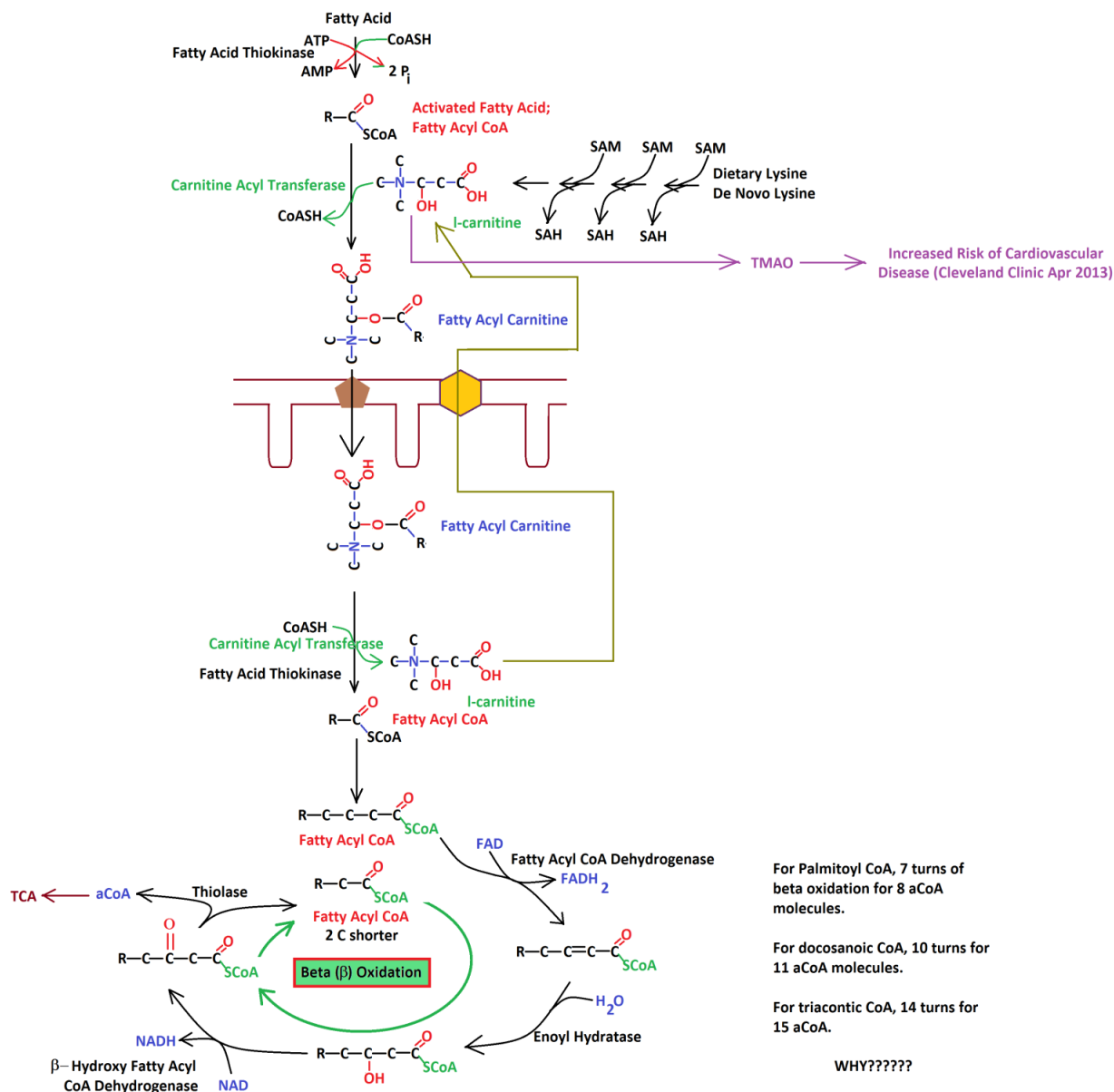
In order for triglycerides (TGS' or TAG's) to be mobilized for oxidation, a receptor in the cell membrane in BAT cells binds norepinephrine (NE), ACTH (adrenocorticotrophic hormone) or epinephrine (E). Note that the graphic indicates that glucagon also activates the cascade for TGS oxidation. It's not so much the binding of the glucagon to the receptor as much as it's the lack of binding of insulin in humans that drives the process. The process is inhibited by 10 μ M insulin.

Once the receptor is bound by an activator, adenylate cyclase undergoes a conformational change to dephosphorylate and cyclize ATP to form cAMP. cAMP turns on an inactive protein kinase to its active form that phosphorylates a hormone sensitive lipase to its active form.

The activated hormone sensitive lipase hydrolyzes the fatty acid off of the #1 carbon in the triglyceride to a diglyceride (DG or DAG) and a free fatty acid (FFA). Once this hydrolysis is complete, a hormone INsensitive lipases clip off the remaining two fatty acids from the DAG, leaving glycerol (to feed into EMP) and two additional FFA's which will diffuse into the blood, where they're picked up by albumin for transport to target organs for cellular uptake.

Note that the hormone sensitive lipase, once activated, is the rate limiting step in the TAG hydrolysis; the hormone INsensitive lipases are remarkably rapid in their activity.

Once the fatty acids are in the cells that need them, what happens to those FFA's? Given that we're looking at cold-, exercise- or Irisin-induced activities, they'll be oxidized by a process known as beta-oxidation (β -oxidation). Cellular uptake and β -oxidation are illustrated in the graphic below:



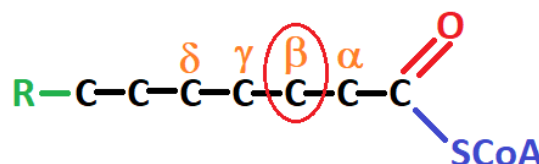
Once the fatty acid has diffused into the cell, it's immediately thiolated with the addition of CoASH at the expense of the equivalent of two ATP molecules (ATP hydrolyzed to AMP and PP_i), catalyzed by fatty acid thiokinase. Keep in mind that fatty acid thiokinase is a generic enzyme (and name): if it were stearic acid that was thiolated, the enzyme is stearic acid (or steroyl) thiokinase. The fatty acyl CoA formed is being prepped for mitochondrial uptake (again, if it were stearic acid, its name would be steroyl CoA).

In order for the fatty acyl CoA to be transported across the mitochondrial membranes, it must condense with carnitine at the expense of losing the CoASH to form the fatty acyl carnitine. This is all, so far, occurring in the cytosol.

ASIDE: Carnitine can also be synthesized from lysine and SAM. Most of the carnitine comes from the diet of those who eat meat. Levels of carnitine are lower in non-meat eaters. Carnitine is inactivated by an enzyme called trimethylamine oxidase (TMAO). Carnitine also has a bit of a shady history: in Spring 2013, both the Mayo Clinic and the Cleveland Clinic published three articles in a three week period that served only to confuse and confound those of us who read them, i.e., carnitine was, at once, good and bad regarding heart disease. Since then, there hasn't been much noise published about carnitine. It's something to keep an eye on, though, in the literature. End of ASIDE

The fatty acyl carnitine is transported across a transport protein in the mitochondrial membranes and carnitine acyl transferase then removes the carnitine and replaces it with CoASH to re-form the fatty acyl CoA in the matrix.

Before we get into β -oxidation, let's recall the nomenclature of the carbon atoms in a hydrocarbon chain bonded to a carbonyl group, image at right. The beta carbon (β -carbon) is circled in red. Eventually that's the carbon we're going to oxidize in this catabolic process, and the carbon to keep an eye on.



The fatty acyl CoA is oxidized by fatty acyl CoA dehydrogenase, that requires FAD to form FADH₂ and the intermediate fatty enoyl CoA. Again, if this was from stearic acid, it'd be stearenoyl CoA. Note that a hydrogen atom and a proton were removed from the α and the β carbons to form the double bond in their places.

Enoyl hydratase adds water across the new double bond adding the -OH to the β -carbon and the H⁺ to the α -carbon. This is the first " β -carbon oxidation", for all practical purposes, and leaves us with a β -hydroxy fatty acyl CoA.

β -hydroxy fatty acyl CoA dehydrogenase oxidizes the alcohol to a ketone at the expense of NAD and forms β -keto fatty acyl CoA. Do these three reaction steps remind you of any other reaction sequences that are similar?

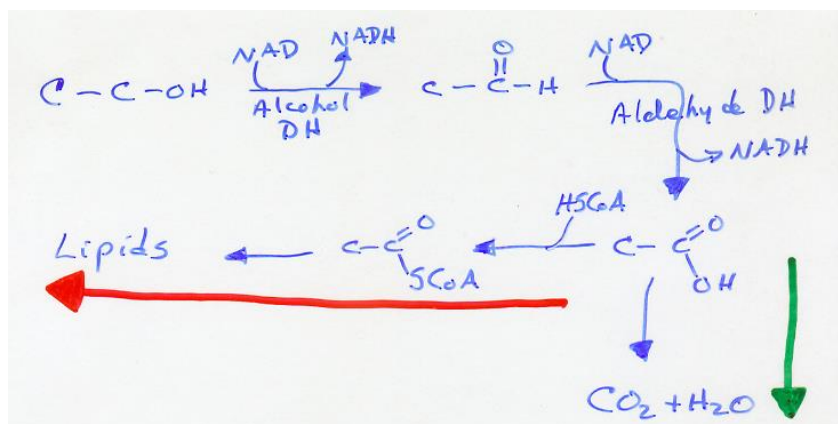
Thiolase clips between the α and the β carbons, leaving an aCoA and a free carbonyl end that binds another molecule of CoASH to form a **new fatty acyl CoA that is two carbons shorter than the original fatty acyl CoA**. The fatty acyl CoA thus formed runs through β -oxidation releasing two carbon fragments (aCoA) until there are four carbons left as a β -keto fatty acyl CoA. Once this happens, thiolase still clips between the α and the β carbons, leaving an aCoA and a free carbonyl end that binds another molecule of CoASH, however, this is the end of β -oxidation for this intermediate as **thiolase releases two molecules of aCoA!**

The point here is that **a fatty acid undergoes one less turn through β -oxidation than there are aCoA molecules formed**. So, for example, if you send lauric acid through β -oxidation, you'll obtain six aCoA molecules in five turns of β -oxidation; likewise, if you send palmitic acid through β -oxidation, you'll obtain eight molecules of aCoA in seven turns of β -oxidation; likewise, if you send triacontic acid through β -oxidation, you'll obtain 15 molecules of aCoA through 14 turns of β -oxidation.

All of the aCoA that's generated in β -oxidation under cold-, exercise- or irisin-induced conditions are fed into the TCA for energy production. Gram for gram, lipids provide more than twice as much energy as either proteins or carbohydrates. This is why aerobic exercise is so important when exercising.

Special Topic: Ethyl Alcohol Metabolism

The catabolism of lipids from a strictly saturated fatty acid perspective is complete. We're preparing to enter the wacky world of fatty acid anabolism. To that end, we'll start the monologue with a brief visit with ethanol metabolism, graphic at right. Ethanol is one

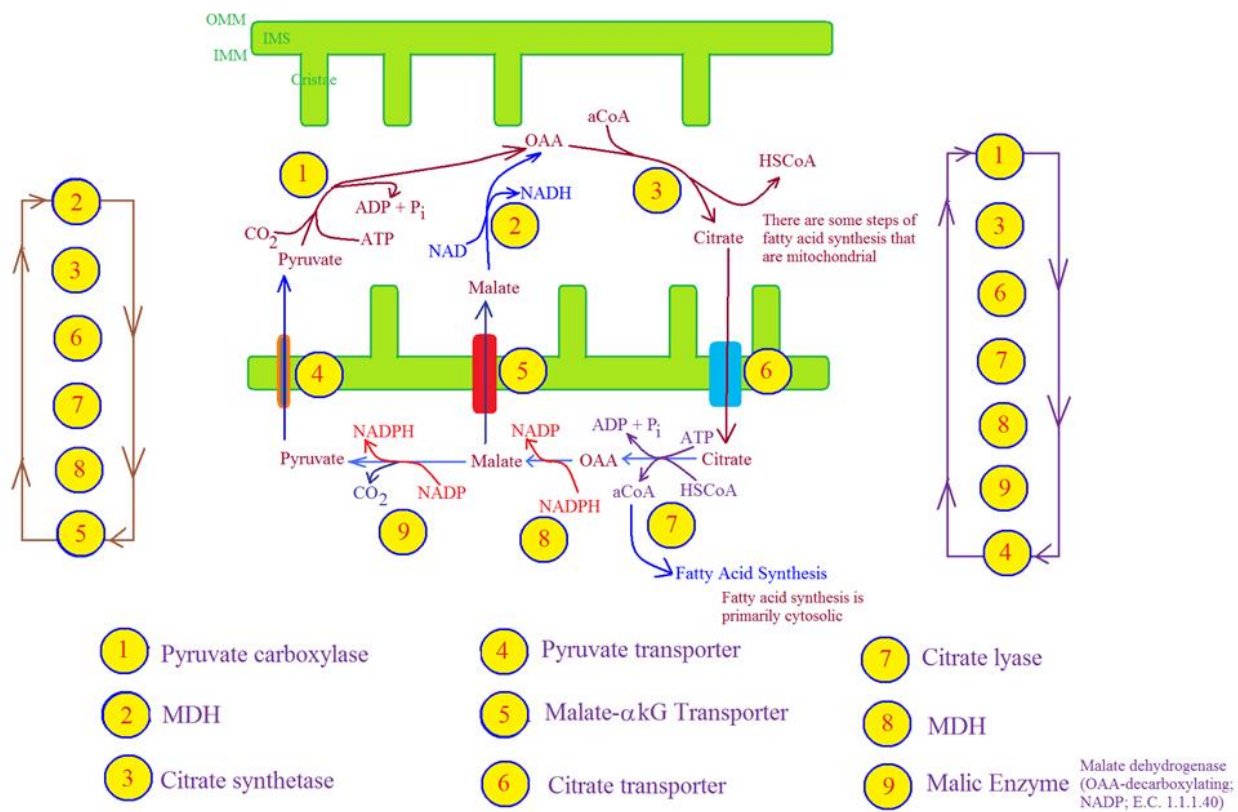


of the easiest molecules to absorb across the stomach wall and rapidly enters one’s bloodstream, where it heads to the liver.

Alcohol dehydrogenase, at the expense of NAD, oxidizes ethanol to ethanal (acetaldehyde) and also forms NADH as a co-product. Aldehyde dehydrogenase, again at the expense of NAD, oxidizes the ethanal to ethanoic acid (acetic acid – referred to as acetate as it’s ionized under cellular conditions) and NADH.

On a good day, the acetate will be oxidized to carbon dioxide and water. There aren’t very many of those where alcohol metabolism is concerned, which means that the acetate is thiolated to form aCoA. All of the NADH from this process makes the mitochondria an incredibly REDUCED environment; with lots of NADH, this also inhibits ALL NAD-requiring enzymes. Therefore, between the aCoA and NADH formed in this process, EtOH is typically used in fat **synthesis** rather than in fat oxidation. End of Special Topic.

The initiation of saturated fatty acid synthesis is illustrated below:



By now, you should be more than familiar with most (if not all) of the acronyms in the graphic above. Note that there are two (2) sequences of numbers on each side of the graphic. In either case, the sequence generates aCoA at step 7 to be used in fatty acid synthesis. Note also that the enzyme that catalyzes step 9 is identified using its EC nomenclature as a reminder that EC nomenclature is of great significance on molecular biology and biochemistry.

If we begin with the right sequence of steps, note that pyruvate is transported through the mitochondrial membranes by its own transporter at step 4. Once the pyruvate is in the matrix, it’s acted on by pyruvate carboxylase that requires ATP hydrolysis to ADP and P_i . It also requires carbon dioxide and biotin to generate OAA at step 1.

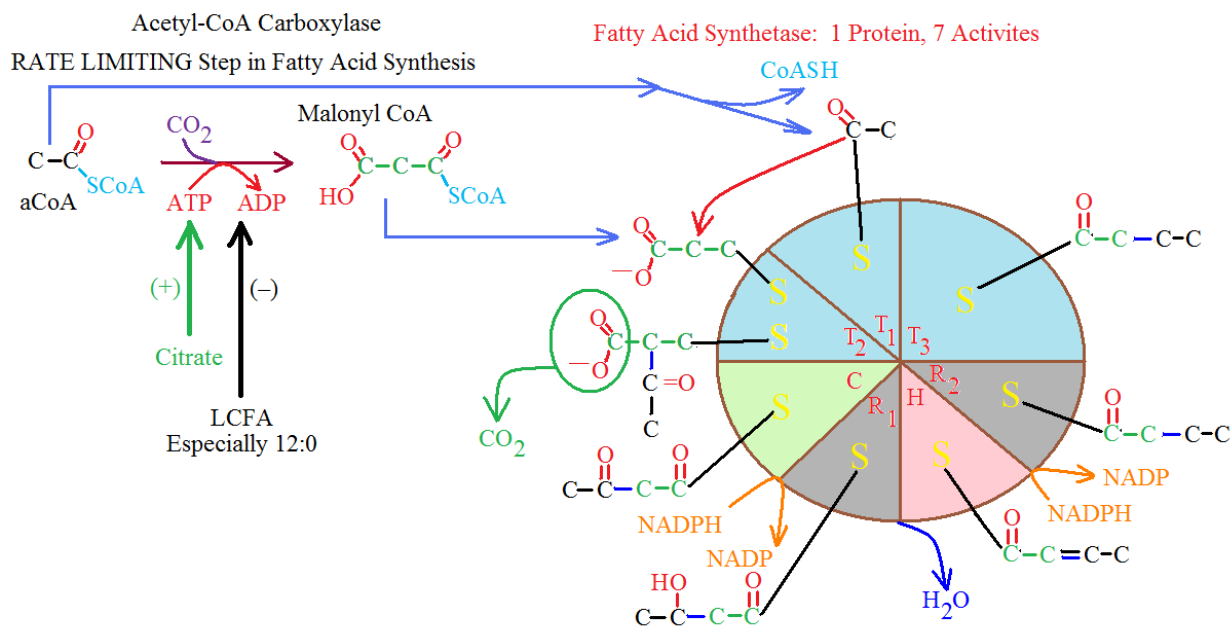
The OAA is thiolated by citrate synthetase to form citrate, as well as HSCoA as a co-product at step 3. The citrate transporter at step 6 transports the citrate into the cytosol from the matrix where citrate lyase with ATP and HSCoA produce OAA at step 7. aCoA and ADP and P_i are formed as co-products, as well.

The OAA is reduced to malate at step 8 by a cytosolic MDH that requires NADPH. The malate is decarboxylated to pyruvate by malic enzyme at step 9; NADPH is also formed at this step. Pyruvate propagates the cycle as the cell signals require.

The left sequence of reactions leave out steps 1, 4 and 9 in the right sequence and replaces them with steps 2 and 5 to speed up aCoA biosynthesis when the cell signals a more rapid process of biosynthesis is required.

The cell, now, is ready to begin synthesizing saturated fatty acids in the cytosol. The rate limiting step in fatty acid synthesis is the carboxylation of aCoA by aCoA-carboxylase to form malonyl-CoA at the expense of ATP hydrolysis to ADP and P_i . High levels of citrate turn on aCoA-carboxylase while high levels of long- and medium-chain fatty acids appear to inhibit aCoA-carboxylase.

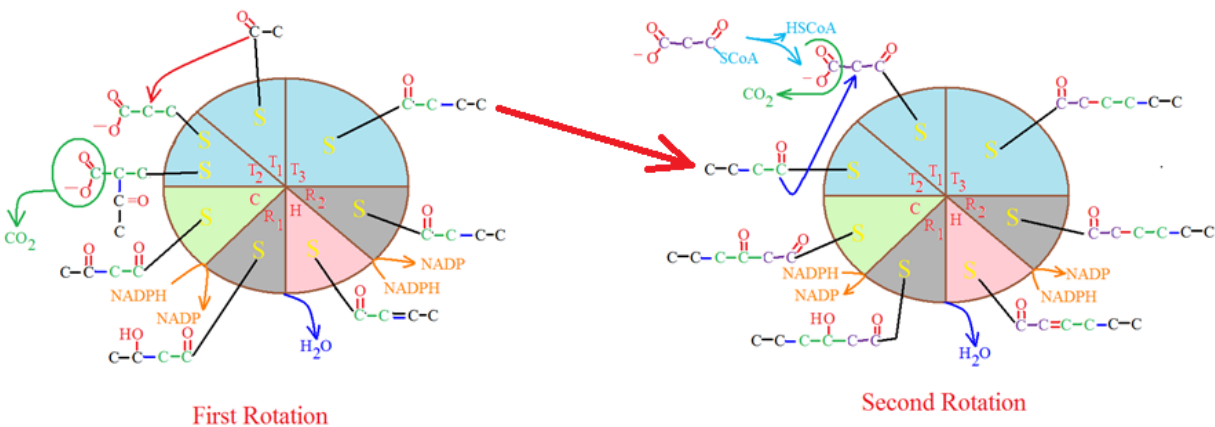
Fatty acid synthesis is partially illustrated in the image below:



The upper left portion of the image illustrates the rate limiting step (acetylCoA-carboxylase) in fatty acid synthesis. Note, though, that both aCoA and malonyl CoA (mCoA) are required to “push” fatty acid synthesis forward via fatty acid synthetase. Fatty acid synthetase is one protein with seven distinct activities: there are three transferase activities, one condensation activity, two reductase activities and one hydratase activity ... all on the same protein!!! The synthesis of fatty acids is referred to as “rotary synthesis” in that the synthesis appears to “go around” the enzyme.

The biosynthesis of saturated fatty acids begins with aCoA releasing its CoASH to provide the energy for the acetate to bind to the first transferase activity (**T₁** in the graphic, above). Concurrently mCoA releases its HSCoA to provide the energy for malonate to bind to the second transferase activity (**T₂** in the image, above). As rapidly as malonate binds, the acetate is transferred from **T₁** onto the malonate in **T₂**. **T₂** also has a decarboxylase activity that removes carbon dioxide from the acetyl-malonate intermediate (circled in green, above), to generate an acetoacetyl derivative on the condensation activity (**C** in the graphic, above). The acetoacetyl derivative is spun around to the first reductase activity (**R₁** in the graphic, above) where the β-ketone is reduced to an alcohol by NADPH. Once the alcohol derivative is formed, it’s rotated to the hydratase activity (at **H**, above), where water is removed between the α and the β carbons to produce an enoyl derivative that is reduced by NADPH at the second reductase activity (**R₂** in the image, above) to give a saturated four-carbon intermediate that’s transferred to the third transferase activity (**T₃**, above). This ends the first cycle in saturated fatty acid synthesis.

The second and succeeding cycles are slightly different:



For orientation purposes, the first rotation is illustrated on the left, above.

Note that the saturated four carbon acyl group is transferred from the third transferase in rotation one to the second transferase in rotation two, and that mCoA is transferred to the first transferase.

The carbonyl of the four carbon acyl group attacks at the middle carbon of the malonate and the distal carboxyl group on the malonate is removed as carbon dioxide. There is now a six carbon intermediate on the condensation activity that is reduced at the first reductase activity by NADPH to obtain the β-hydroxyacyl derivative from the β-ketoacyl derivative. The β-hydroxyacyl derivative is dehydrated by the hydratase activity to produce the enoyl derivative that is reduced by NADPH at the second reductase

activity to form the six carbon, saturated, acyl derivative that is moved to the third transferase activity in preparation for the third turn of the biosynthetic pathway.

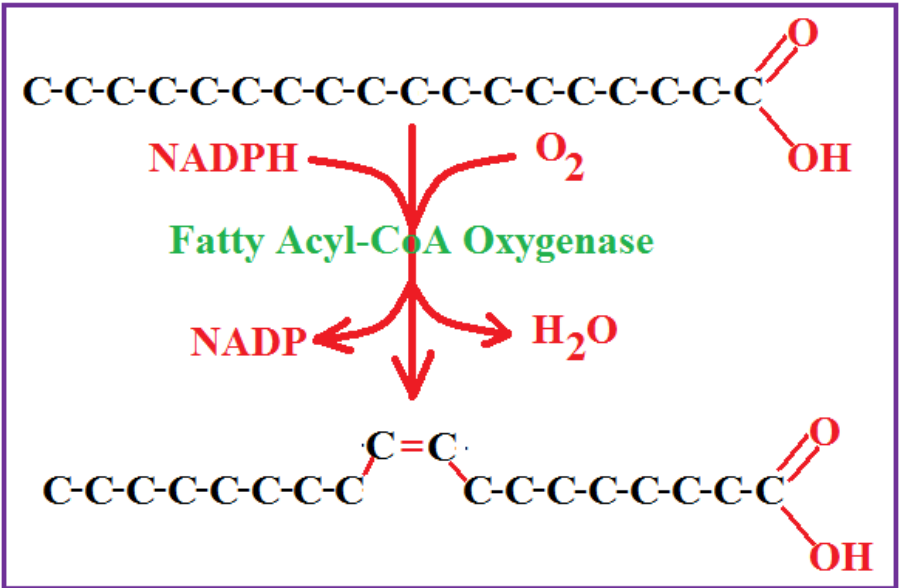
The protein continues rotations until palmitic acid is synthesized (16:0), at which point synthesis stops and palmitic acid is cleaved from the protein.

Elongation beyond the 16-C length of the palmitate product of Fatty Acid Synthase is mainly catalyzed by enzymes associated with the endoplasmic reticulum (ER). ER enzymes lengthen fatty acids produced by Fatty Acyl Synthase as well as dietary polyunsaturated fatty acids. Fatty acids esterified to coenzyme A serve as substrates. Malonyl-CoA is the donor of 2-carbon units in a reaction sequence similar to that of Fatty Acid Synthase except that individual steps are catalyzed by separate proteins. A family of enzymes designated Fatty Acid Elongases or ELOVL (elongation of very long chain fatty acid) catalyze the initial condensation step.

Source accessed 22 July 2017, 1209 hours, PDT: <https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/fasyntesis.htm>

Special Topic: 18:1, n-9 Biosynthesis

Elongation of 18:1, n-9 in vertebrates does not seem to occur commonly: mammals (excepting herbivores and non-seafood eating animals) lack the enzymes to unsaturate FA's beyond the #9 position (image, below); plants don't seem to elongate 18:1, n-3 to 20:5, n-3, although it seems that herbivores, et al, do.

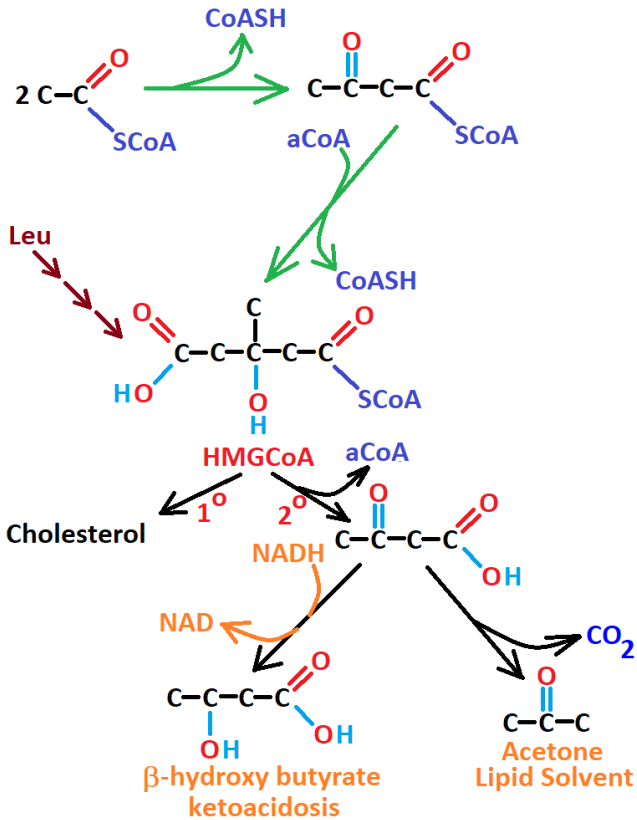


There's LOTS of confusing data on MUFA to PUFA (n-3) conversion in the literature. Unsaturation of 18:1, n-9 in plants DOES appear to occur in the synthesis of ALA (18:3, n-3). End of Special Topic.

In addition to fatty acid catabolism and anabolism, there are additional topics that are covered in lipid metabolism that are of importance to human health. The next topic will cover the uses of acetyl CoA, including cholesterol synthesis (and inhibition).

At right is an elementary graphic of some of the pathways in which aCoA is involved. Condensing three molecules of aCoA drives the synthesis of a molecule called HMGCoA (don't worry about the name – it's coming up shortly), which is, at once, a collecting point and a branching point, from a pathway perspective. Leu catabolism also forms HMGCoA. HMGCoA is a major metabolite in cholesterol synthesis (the primary pathway HMGCoA uses). In some patients, e.g., out of control diabetics, HMGCoA is de-thiolated, releasing aCoA, and forms ketone bodies.

The ketone bodies include acetoacetic acid (right side of graphic right after HMGCoA splits to the right), β-hydroxy butyrate and acetone. All of these ketone bodies lead to ketosis or ketoacidosis when



insulin inhibition of hormone sensitive lipase is withdrawn and TGS' are released and catabolized to ketone bodies.

In the case of cholesterol synthesis, the image at right begins the process of *de novo* cholesterol synthesis. Note that the first two steps to HMGCoA are identical to what we examined regarding aCoA.

It's the step where HMGCoA is reduced to mevalonate that clinicians have their greatest, consistently reproducible, control over cholesterol synthesis and preventing heart attacks as a result of hyperlipidemia/hypercholesterolemia.

This regulatory step is catalyzed by the enzyme β -hydroxy- β -methyl glutaryl CoA (HMGCoA) Reductase. It requires two molecules of NADPH for reductive power to form mevalonate – CoASH is released from HMGCoA to provide the energy in addition to the NADPH to drive the reaction forward.

HMGCoA Reductase inhibitors include the "statin" family of medications, image at right.

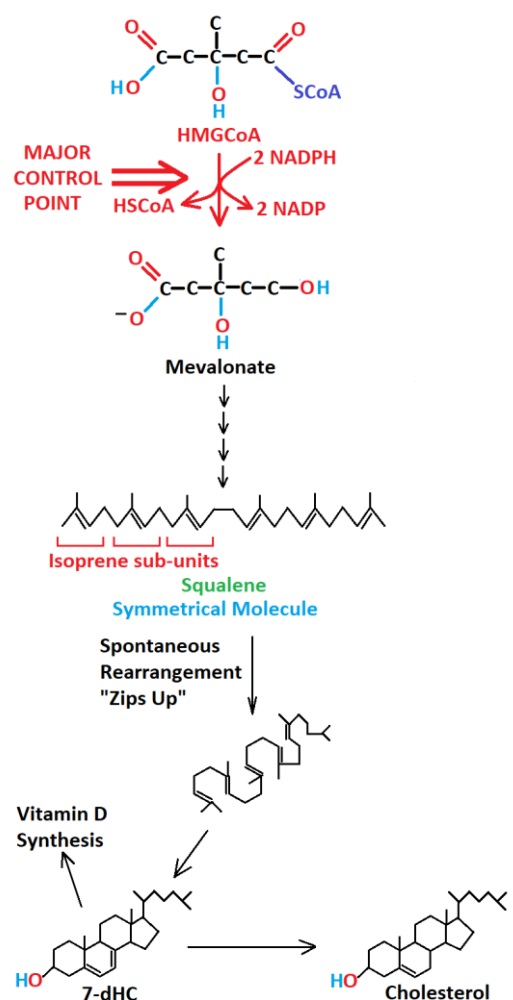
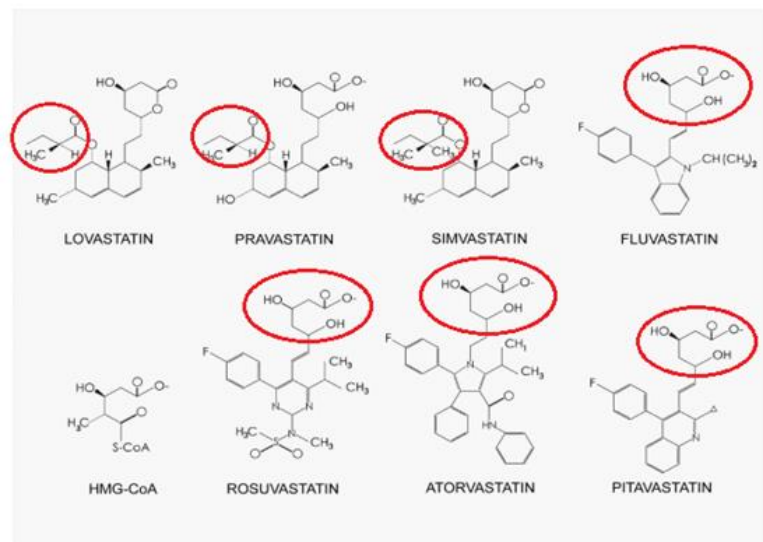
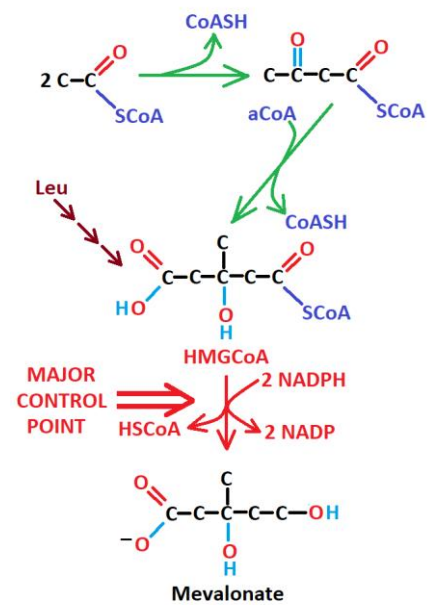
Note the areas circled in red: these are the regions that bind to HMGCoA reductase to competitively inhibit the enzyme and to lower one's blood cholesterol and, hence, reduce the risk of heart disease/attack. Do you notice the red regions' similarities to the structure of HMGCoA?

Continuing on with cholesterol synthesis (image below right), note that many, many, many reactions later, mevalonate polymerizes to form squalene. Squalene consists of isoprene units (not unlike those found in latex rubber), which spontaneously undergo rearrangement of the symmetrical molecule into the familiar steroid shaped structure. Multiple reactions later, 7-dehydrocholesterol (7-dHC) is formed, which is a branch point. 7-dHC is used in the synthesis of vitamin D (more in A&P) as well as in the synthesis of cholesterol.

Remember that not all cholesterol is bad cholesterol: we need some cholesterol to synthesize our sex hormones, estrogens, progesterone and testosterone.

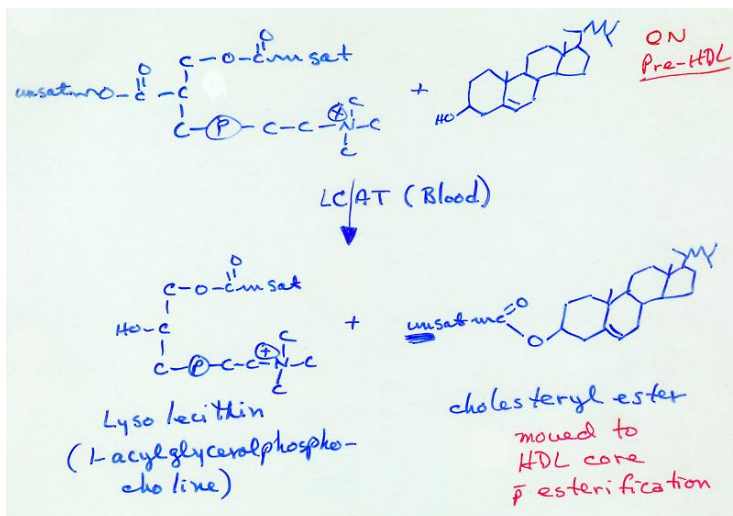
In addition, we also need cholesterol for HDL (so-called "good cholesterol") synthesis. Do keep in mind that there are two fractions of HDL-Cholesterol: HDL₂ and HDL₃. In general, the former is the cardioprotective form of HDL (and the one we look at for "good cholesterol" and heart health) and the latter, whilst unclear, appears to be not as cardioprotective.

For a long time, it was believed that the higher one's total-HDL was, the more protected from heart disease one was – unfortunately, that's not necessarily the case as there have been reports in the literature of people with HDL's of 100+ mg/dL and those patients having a heart attack (myocardial infarction – MI) ... because their HDL₃ was elevated instead of their HDL₂. In addition, whilst there is correlation between high HDL and reduced risk of MI, a brief search of the literature suggests that there are no medications that actually improve heart health in spite of



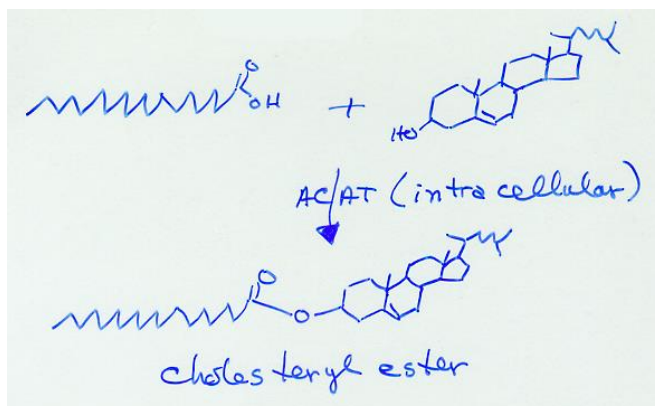
raising HDL levels. This remains a conundrum of sorts for researchers to continue exploring and teasing data from.

While the general synthesis of HDL is best covered in another course, there is one enzyme found on the surface of pre-HDL that fits into this discussion: Lecithin-Cholesterol Acyl Transferase: "LCAT". This enzyme is used in lipid **TRANSPORT**, see image at right, and is bound to HDL's and LDL's in the blood.



A lecithin reacts with cholesterol on the pre-HDL to form a cholesteryl ester that is moved into the HDL core upon esterification and makes HDL spherical. A lysolecithin is a co-product core of a developing lipoprotein. Note that the unsaturated fatty acid on carbon #2 of the glycerol backbone is the fatty acid esterified with the cholesterol. As a note in passing, LDL-Cholesterol appears to preferentially take up saturated fatty acids, ignoring for the most part, as it were, the unsaturated fatty acids for uptake.

Another cholesterol-involved enzyme of significance is Acyl CoA: Cholesterol Acyl Transferase: "ACAT", or as it's aka: Sterol-O-Acyl Transferase (SOAT). This enzyme prepares cholesterol for intracellular **STORAGE**.



Note that SOAT/ACAT biosynthesizes the cholesteryl ester with saturated fatty acids.

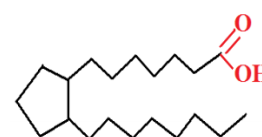
Foam cells are formed from macrophages transported to fatty deposits on the blood vessel walls. The macrophage phagocytizes, *in situ*, the deposit so as to destroy it. The macrophage fills with lipids, which, microscopically, gives the cell[s] a "foamy" appearance.

Why is Arachidonic acid important? It is important because it is the precursor fatty acid for prostaglandin and leukotriene biosynthesis. These compounds are known as eicosanoids, i.e., compounds based off 20 carbons. Therefore, the next “higher class” of lipids to go over are the prostaglandins. Prostaglandins mediate pain, fever, smooth muscle contraction, mucus production in the stomach, blood clotting, PMS and inflammation, to name a few.

Prostaglandins are based off prostanoic acid (image at right); representative PG's and leukotrienes (LT's) are shown in the image on the following page, as well. There are nomenclature rules that follow prostaglandins, too. PG is short for ProstaGlandin. The letter tells us about the ring constituents and the subscripted number tells us how many double bonds there are on the side chains. PG's may be inhibited at the level of synthesis with aspirin; anti-leukotriene agents are now available for treating airway diseases. The image at top right on the following page provides a succinct description, as well as the structures, of each of these eicosanoids.

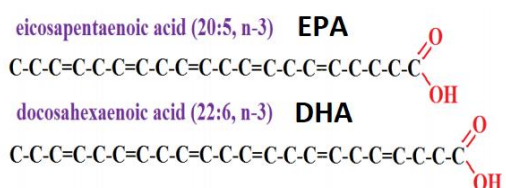


Prostanoic acid



Prostanoic acid

Note that arachidonic acid gives rise to the PG² series of prostaglandins – most of these are pro-inflammatory and can lead to serious consequences, e.g. myocardial infarction. Recently, two significant PUFA's (EPA and DHA) in fish oils have found more and more use for the treatment of various hyperlipidemias because they are n-3 fatty acids which produce the PG³ family which are primarily anti-inflammatory and, hence, heart healthy (EPA and DHA are illustrated above). There is some preliminary research (2010) that suggests that mammalian cells can elongate ALA (18:1, n-3) to 20:5, n-3 (EPA) – the conversion is not very efficient: 11



g of ALA (10 Tbsp of flaxseed meal a day!) are needed to biosynthesize 1 g EPA. It also seems that 20:5, n-3 is elongated to 22:6, n-3.

The last group of polyunsaturated fatty acid derivatives are called lipoxins (image beneath PG's and LT's, this page, lower right).

Folks taking aspirin (Acetylsalicylic acid; ASA) get the benefit of the ASA between the reduced production of TXA2 and the increased production of lipoxins. ASA changes COX-2 activity to produce anti-inflammatory lipoxins ("LX's"; derived from ω 3 fatty acids (EPA) as well as ω 6 fatty acids such as 20:4^{A5,8,11,14}).

Nucleic Acid Metabolism

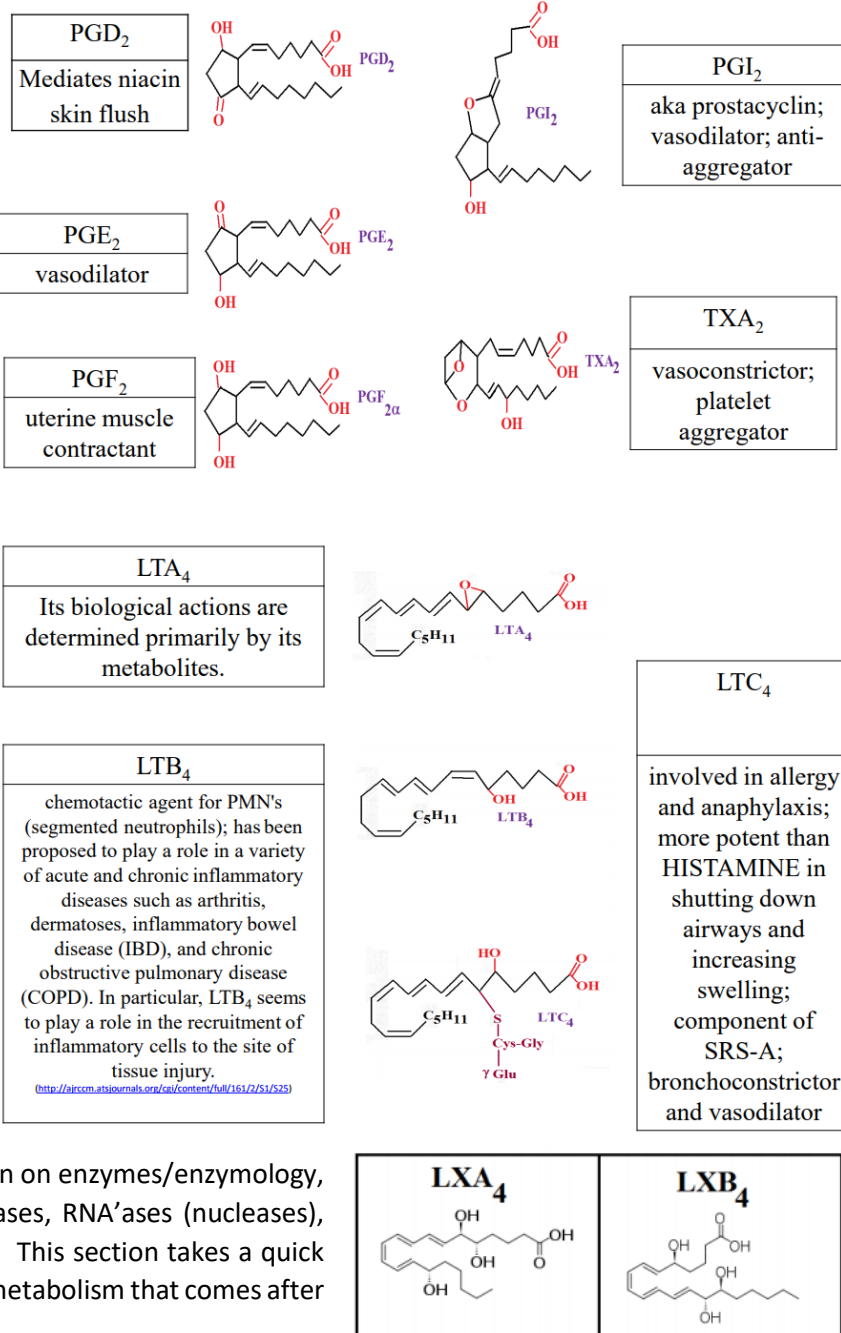
Lipoxins closed out lipid metabolism. The final section on metabolism is a very succinct section on nucleic acid metabolism. A portion of nucleic acid metabolism has already been covered in the section on enzymes/enzymology, i.e., the brief discussion on DNA'ases, RNA'ases (nucleases), nucleotidases and nucleosideases. This section takes a quick look at the purine and pyrimidine metabolism that comes after these digestive enzymes.

Something to keep in mind is that each of these four sections on metabolism are very brief: one can take 16 week (one semester) courses covering each kind of metabolism and spend a lifetime studying parts of (researching!) just one kind of metabolism.

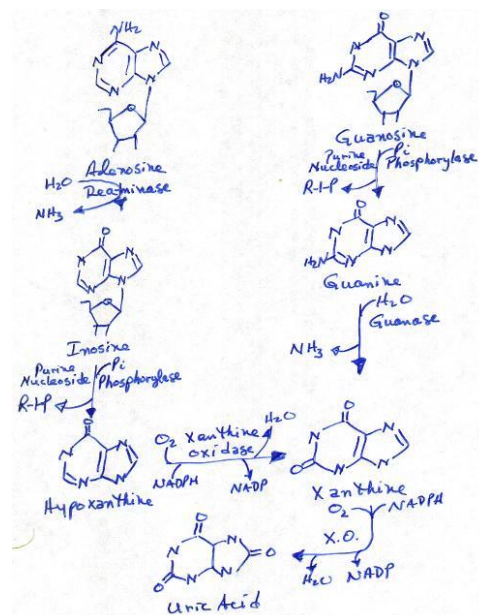
Purine metabolism is the first topic for this section (image below right). Adenosine is metabolized a little differently than guanosine simply because adenosine has an amine (on adenine) instead of a ketone (on guanine). Adenosine deaminase adds water across the amine bonded to carbon #6 to remove ammonia and to add a ketone in its place (at the expense of losing the double bond between nitrogen #1 and carbon #6) to produce inosine.

Purine nucleoside phosphorylase removes ribose-1-phosphate (R-1-P) from the inosine to produce hypoxanthine; the reaction requires inorganic phosphate. Hypoxanthine is oxidized to xanthine by xanthine oxidase, which requires molecular oxygen and NADPH; water and NADP are co-products of this step.

We're going to stop at xanthine for now and move to the metabolism of guanosine. Purine nucleoside phosphorylase strips off the R-1-P from guanosine; again, inorganic phosphate is required for this reaction. The product is guanine, which is hydrolyzed by guanase to remove the amine on carbon #2, replacing it with a ketone, producing xanthine. Xanthine, then is a collecting point intermediate for both adenosine and guanosine metabolism.

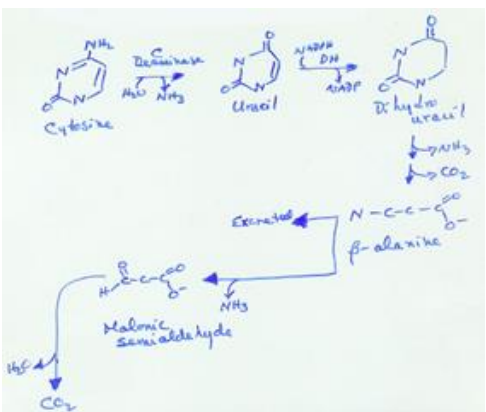


Lipoxins
anti-inflammatory



Xanthine is oxidized, again, by xanthine oxidase, which requires molecular oxygen and NADPH to produce uric acid and to co-produce water and NADP. Uric acid elevation in the blood can cause crystals to form within joints. This causes gout.

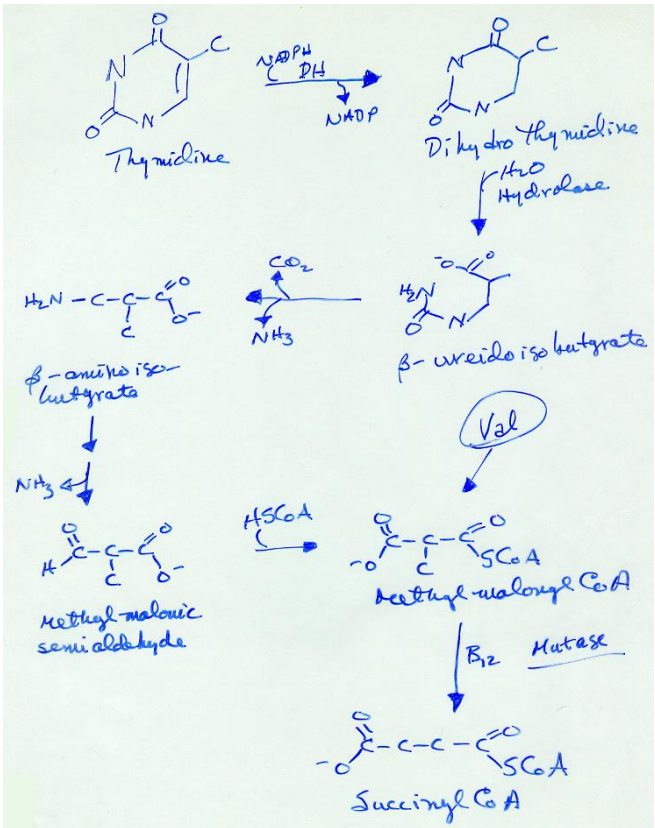
Up next are the pyrimidines, image at upper right. Cytosine is hydrolytically deaminated by cytosine deaminase to form uracil and to co-produce ammonia. Uracil dehydrogenase, using NADPH, reduces the double bond between carbons #5 and #6 to form di-hydrouracil and co-produce NADP.



Multiple reactions later, β-alanine is formed. In some cases, it's excreted un-changed, whilst in others, it's deaminated and oxidized to form malonic semi-aldehyde which is further catabolized to water and carbon dioxide via 3-hydroxy-pyruvate.

Additionally, Cytosine deaminase is highly elevated in some solid tumor cells. Inhibition of the enzyme with tetrahydrouridine improves therapy due to reduced drug degradation. Examples of solid tumors are sarcomas (connective tissue tumors), carcinomas (malignant cancers), and lymphomas (Lymphomas are blood cancers that develop in the lymphatic system. The two main types are Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL).).

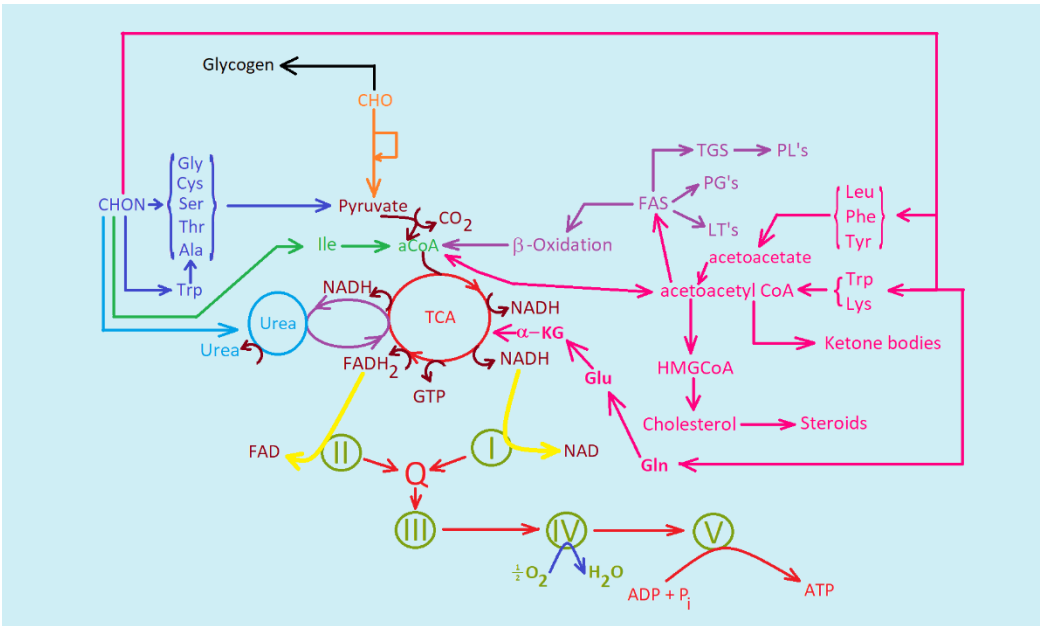
The last metabolic scheme of pyrimidines to be described is that of thymidine (Image at right). Thymidine dehydrogenase, with NADPH, reduces the double bond between carbons #5 and #6 to produce dihydro-thymidine and co-produce NADP.



Dihydro-thymidine hydrolase adds water across the N-C bond at N #3 and C #4 to open the ring up. β-ureidoisobutyrate is formed from this reaction.

Multiple reaction steps later, β-ureidoisobutyrate has been decarboxylated and deaminated to form β-aminoisobutyrate. Again, multiple steps later, β-aminoisobutyrate is deaminated and results in the biosynthesis of methyl malonic semialdehyde. CoEnzyme A is added to the methyl malonic semialdehyde to form methyl malonylCoA (which is also formed during valine catabolism). Methyl malonylCoA, acted on by methyl malonyl CoA mutase with vitamin B₁₂, opens the chain to let the α-methyl group flip into the chain, elongating it by one carbon to generate succinyl CoA. The sCoA, of course, ends up in the TCA.

This brings us back to the initial graphic that started us on this journey (image at right). Everything that was introduced as we began metabolism is covered in this "forest" picture. We've looked at each tree and a few branches.

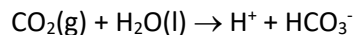


Lastly, we need to visit about another special topic that has a great deal of significance to humans from a cellular perspective, from an organ perspective and from a clinical perspective: carbon dioxide and an introduction to arterial blood gases in preparation for further courses in and out of the nursing program.

Carbonic Acid-Bicarbonate Ion: Physiological Interactions

Carbon dioxide: CO_2

CO_2 is fairly soluble in water (more soluble in cold water like in cold soda; less soluble in warm water like in "flat" soda). A saturated solution at 1 atm and 25°C is approximately 0.033M. At equilibrium only 0.17% of dissolved CO_2 is in the form of carbonic acid (H_2CO_3). An aqueous solution of CO_2 is typically acidic:



The carbon dioxide we're going to focus on is generated in PDH and TCA.

CO_2 plays a major role in maintaining the pH of blood (and sea water). CO_2 is not normally transported as such, rather as HCO_3^- . This occurs via an enzymatic reaction catalyzed by carbonic anhydrase:

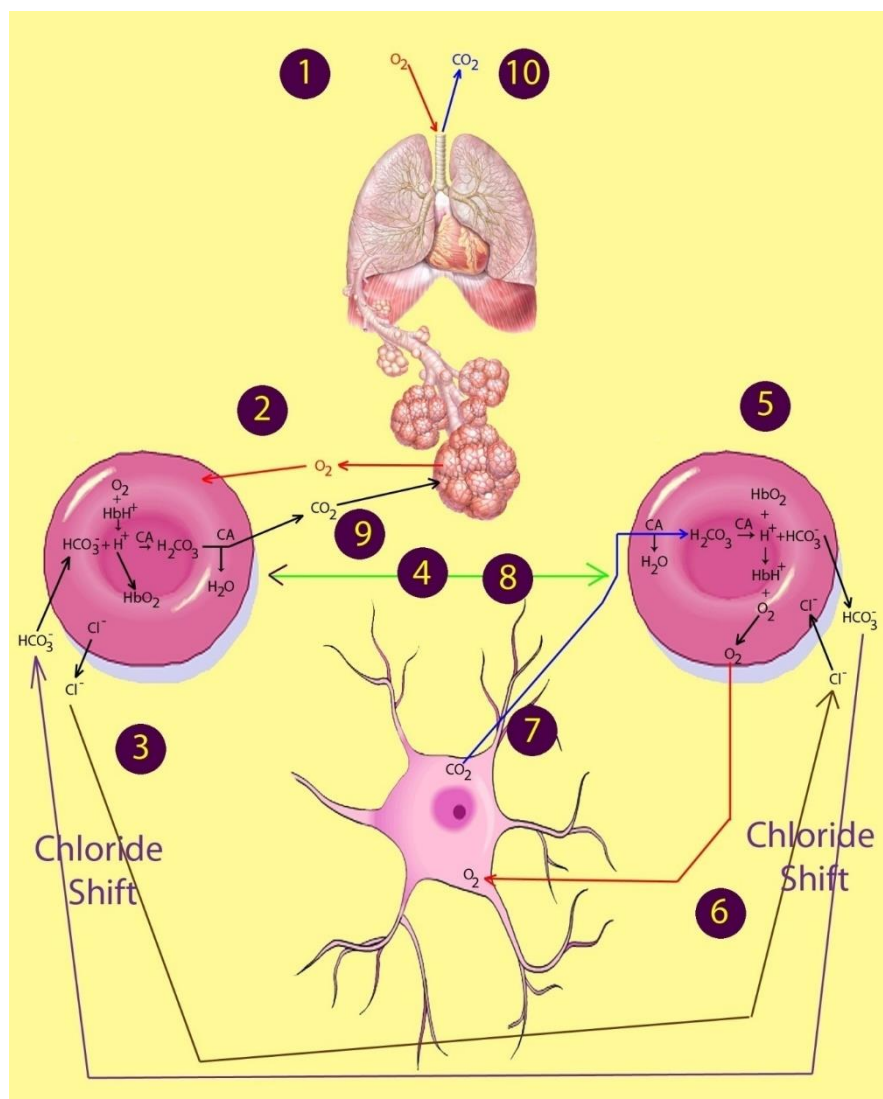


This reaction is incredibly IMPORTANT in acid/base balance.

Red Cells and Acid-Base Balance

The image at right illustrates the interconnection between human lungs, red blood cells (RBC) and the cells in the human body.

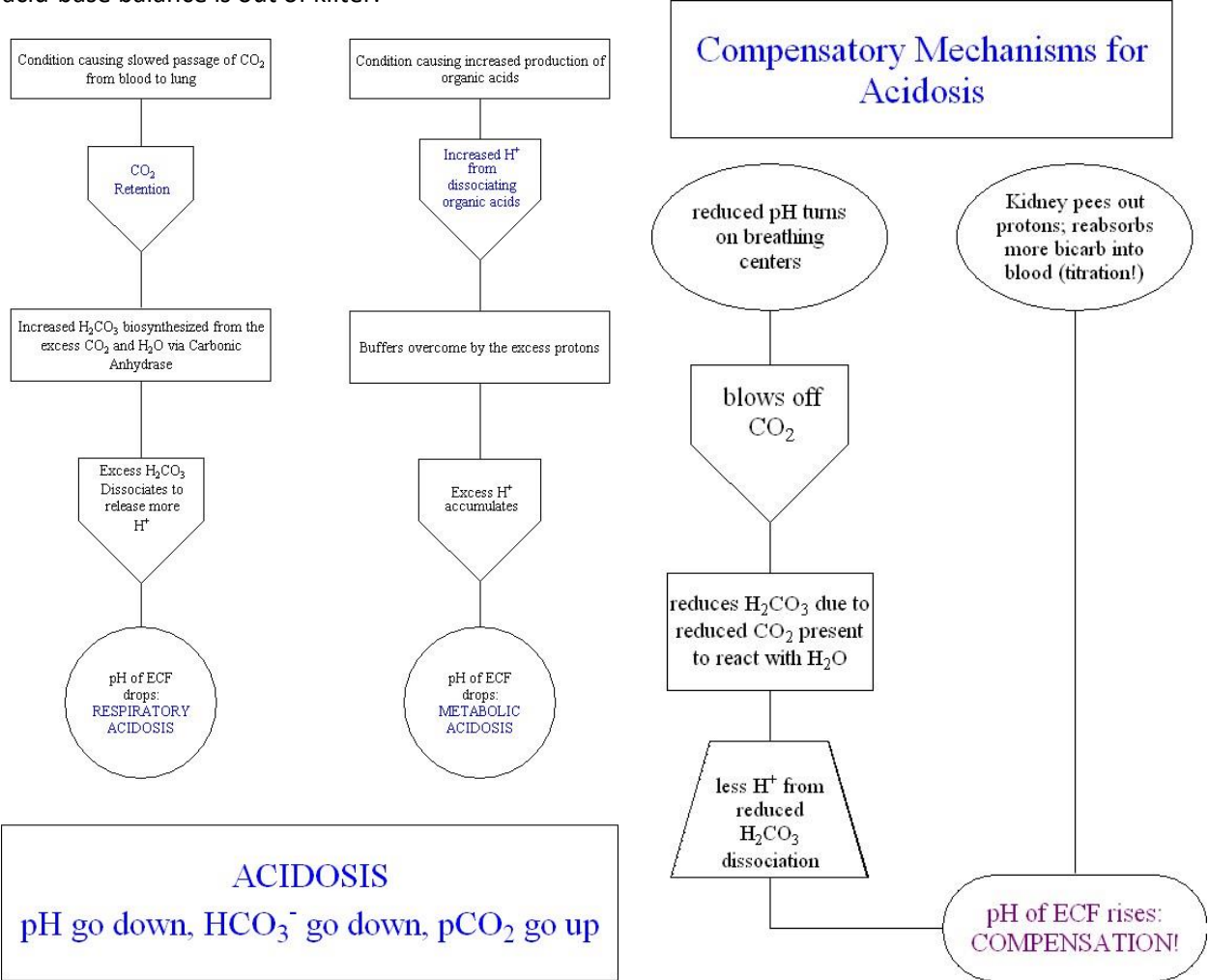
Humans inspire the "good air" at #1 in the image. Oxygen crosses the air sacs (alveoli) into the blood (#2), where it bonds with deoxyhemoglobin (HbH^+) to release the proton and form oxyhemoglobin (Hb-O_2) in the red blood cell (RBC) in the left of the image. The removed proton condenses with bicarbonate ion and, catalyzed by carbonic anhydrase, forms carbonic acid in the red cell. The carbonic acid is eventually dehydrated so that the carbon dioxide diffuses from the RBC into the alveolus to be expelled at #10.



In order for bicarbonate to be present in the RBC, it must be transported from the blood into the RBC (left RBC – far left of the graphic). The problem with this is that bicarbonate is a monovalent anion and an excess of anions in RBS (or a reduction in the blood) is physiologically frowned upon. Therefore, as bicarbonate is transported into the RBC, a chloride ion is pumped out of the RBC into the blood to balance the negative charge. This is called the "Chloride Shift".

Once Hb-O_2 is formed (RBC on right side of graphic), the oxygen has to be removed from the hemoglobin to provide the oxygen to the cell. To do this, carbon dioxide generated inside the cell is transported into the RBC, where it's condensed with water to form carbonic acid. Carbonic anhydrase catalyzes this reaction, as well as the following reaction, where carbonic anhydrase partially deprotonates carbonic acid to bicarbonate and the proton. The proton reacts with the Hb-O_2 to remove the oxygen for diffusion into the cell, while forming deoxy-hemoglobin. Again, bicarbonate and chloride ions have to exchange in the "flip side" of the "Chloride Shift" and this cycle continues so that carbon dioxide is expelled and oxygen is taken up into the cells.

In terms of what the human body does when its acid-base balance is “off” (and, by the way, neither acid nor alkaline diets are of any substantial use from a dietary perspective: water, carbon dioxide, the bicarbonate ion and carbonic acid all work to maintain a very tight arterial pH range of 7.35-7.45), the following images provide you with a flowchart approach to acid-base balance and the compensation when acid-base balance is out of kilter:



Blood is slightly alkaline. Arterial blood runs a pH between 7.35 and 7.45. In venous blood, it runs less than 7.35 due to the high amount of carbon dioxide in it. Protons (hydrogen ions) come from aerobic metabolism of glucose, from hydrolysis of carbonic acid, from the oxidation of sulfur containing amino acids, from the anaerobic metabolism of glucose, from lactate, from ketone bodies and from phosphate containing proteins and nucleic acids.

Acid-Base Disturbances Come in two types: respiratory or metabolic. Each type may be further subdivided into acidosis and alkalosis, hence, we now have 4 possible pure acid-base disturbances to examine.

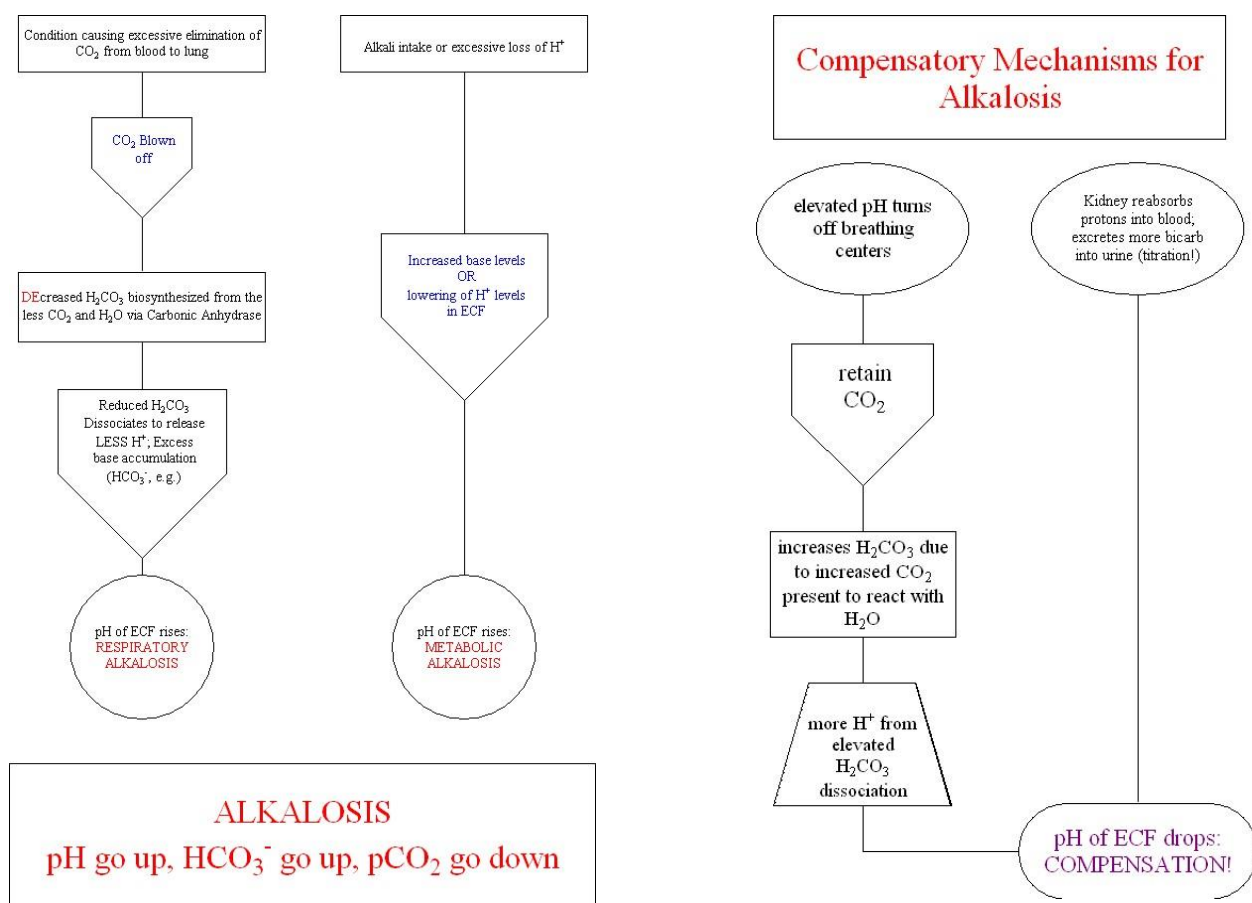
The top two graphics summarize acidosis development (left graphic) and compensatory mechanisms (right graphic). Starting with the left side of the left graphic, some condition develops where carbon dioxide is retained in the blood (stopping breathing is a good example of this). Once the carbon dioxide isn't being blown off, the levels increase, which increases the amount of carbonic acid produced. With increasing carbonic acid comes increasing protons dissociating from the carbonic acid which makes the blood acidic. Let's stop there for a minute. This causes respiratory acidosis.

On the right side of the left graphic, some condition develops where there are increasing organic acids accumulating, e.g., lactic acid. These acids produce elevated levels of protons which over-power the body's buffer system and cause a build-up of hydrogen ions, thereby lowering the pH of the blood. This causes metabolic acidosis.

Regardless of the acidosis, the body still compensates the same – respiratory compensation is faster; metabolic compensation is substantially slower.

Either way, reduced pH turns on the breathing centers in the brain which makes a person blow off the excess carbon dioxide. With increasing carbon dioxide being blown off, there is less carbon dioxide in the blood to make carbonic acid, which leads to fewer hydrogen ions and the pH begins to rise. That's the respiratory part. The metabolic part is that the kidneys pee out protons and retain bicarbonate ions in a physiological titration. This lets the pH of the blood rise, as well.

This ends the acidosis part. The alkalosis part begins at the top of the following page.



In the case of respiratory alkalosis, some condition that increases the expelling of carbon dioxide is the issue, e.g., hyperventilation. As the carbon dioxide is blown off, there is less carbonic acid capable of being produced. With less carbonic acid, there are fewer hydrogen ions and the pH of the blood goes up.

When a person has excessive alkali intake or disproportionate proton loss, this increases base levels and/or reduces proton levels and the pH of the blood rises.

As in acidosis, compensatory mechanisms for alkalosis are the same. In the case if alkalosis, the elevated pH turns off the breathing centers in the brain/stem. This is quite common in hyperventilating patients and they will pass out as a result. They'll start breathing, again, this is just their body saying, "you need more carbon dioxide!", so that their body can generate more carbonic acid for proton dissociation to lower the pH. Once that's happened, the patient will begin breathing, again. This is respiratory compensation. Metabolic compensation is for the kidneys to pee out excess bicarbonate ion and retain hydrogen ions – another physiological titration! Eventually, the pH in the blood drops back into the normal range under "simple circumstances". The more complex circumstances are discussed in second semester anatomy and physiology.

Finis

We have now reached the end of the metabolism journey for this course. There is more to come in anatomy and physiology: this section is/was only an introduction to get you ready for your advanced coursework.