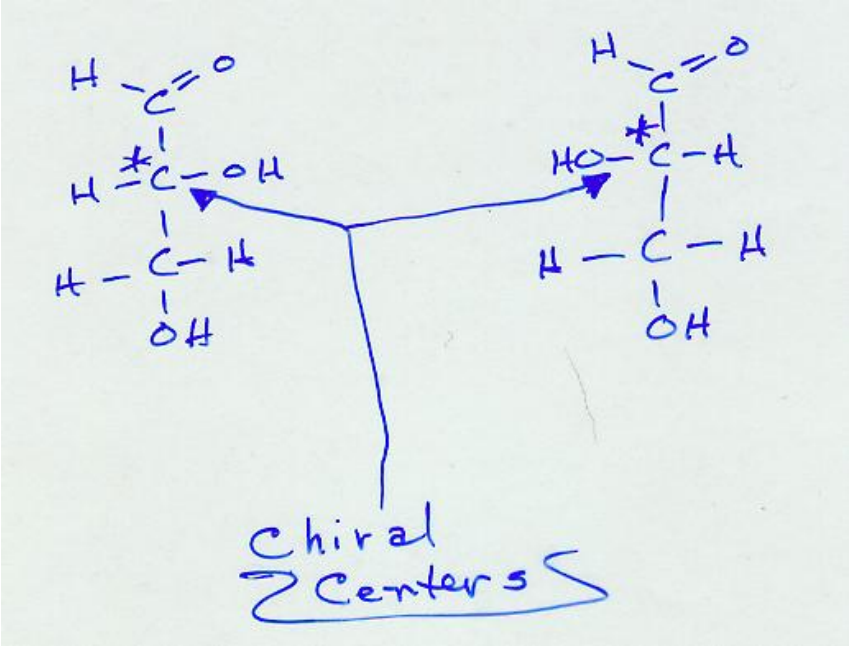
Biological Chemistry: Introduction to Carbohydrates and Carbohydrate Metabolism

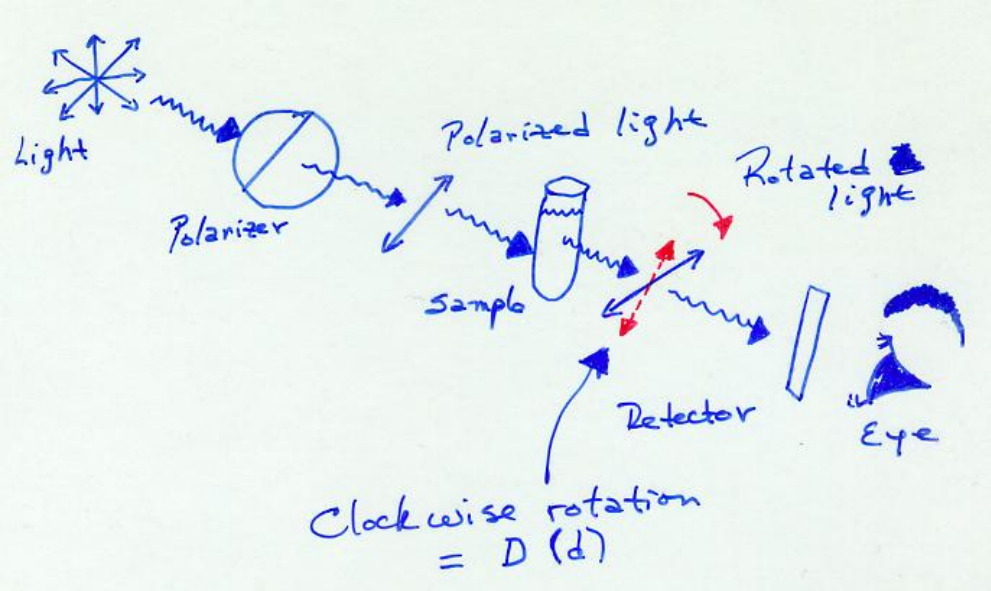
"Sugars"

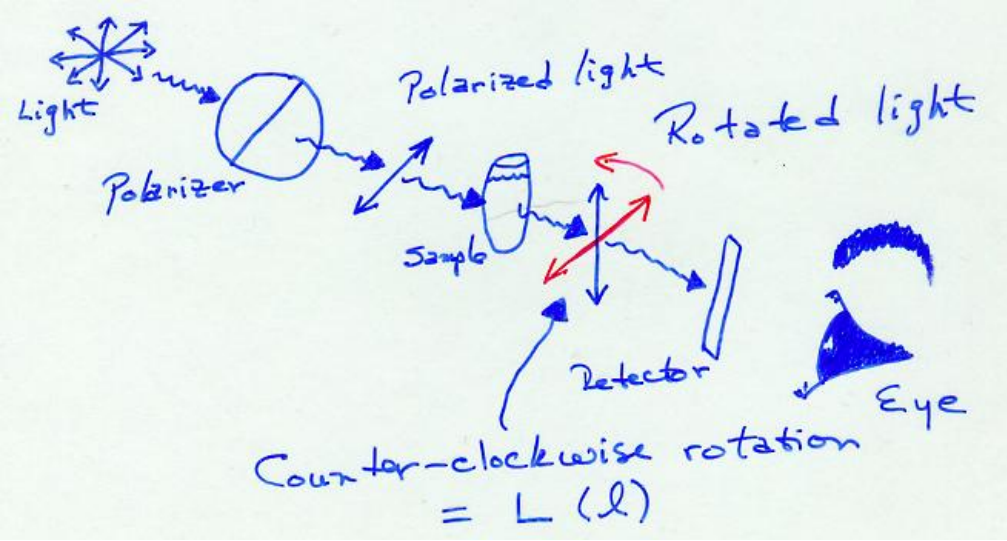
Carbohydrates: Optical Activity

- A carbon with 4 different R groups is said to be CHIRAL
- These compounds, i.e., with chiral carbons, rotate the plane of polarized light.

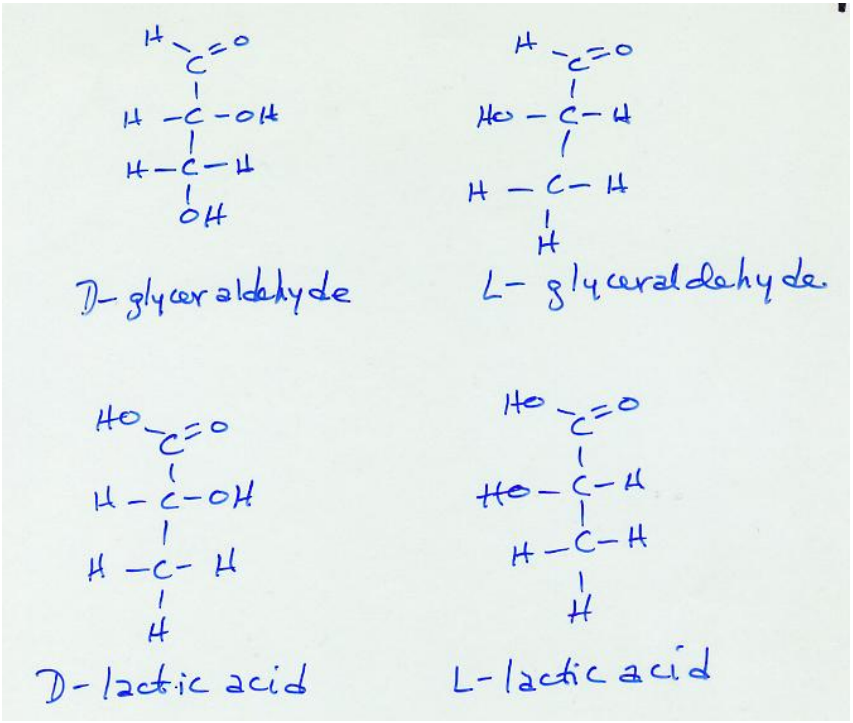


Polarized Light Rotation





Chiral Compounds: Examples



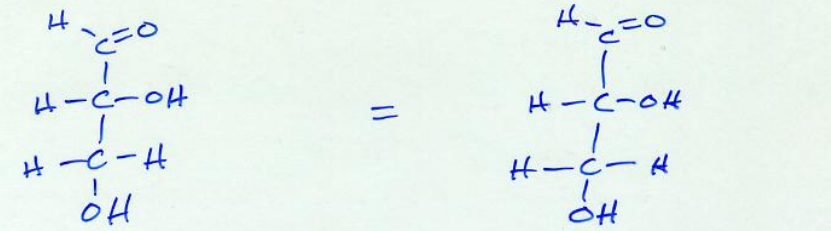
D & L are for configuration not rotation —

d & l are for rotation

For OUR purposes, we will consider:

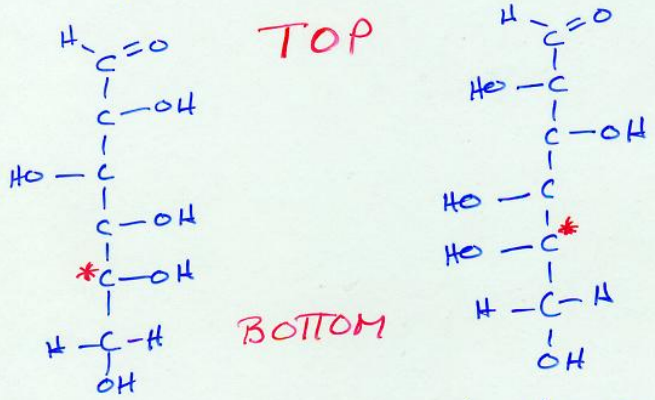
D = d & L = l

Hence



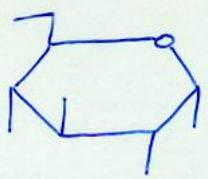
D-glyceraldehyde = d-glyceraldehyde

Why are D/d + L/l important?

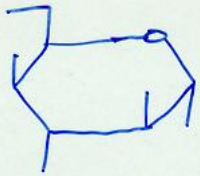


D-glucose L-glucose

"chain" forms



α-D-glucose



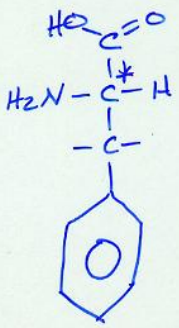
α-L-glucose

will α-L-glucose fit into the active site of hexokinase — substrate is α-D-glucose:

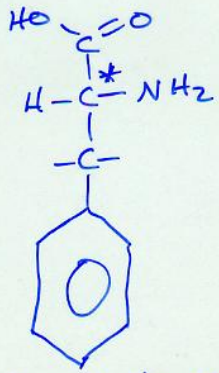
NO

Humans metabolize D sugars

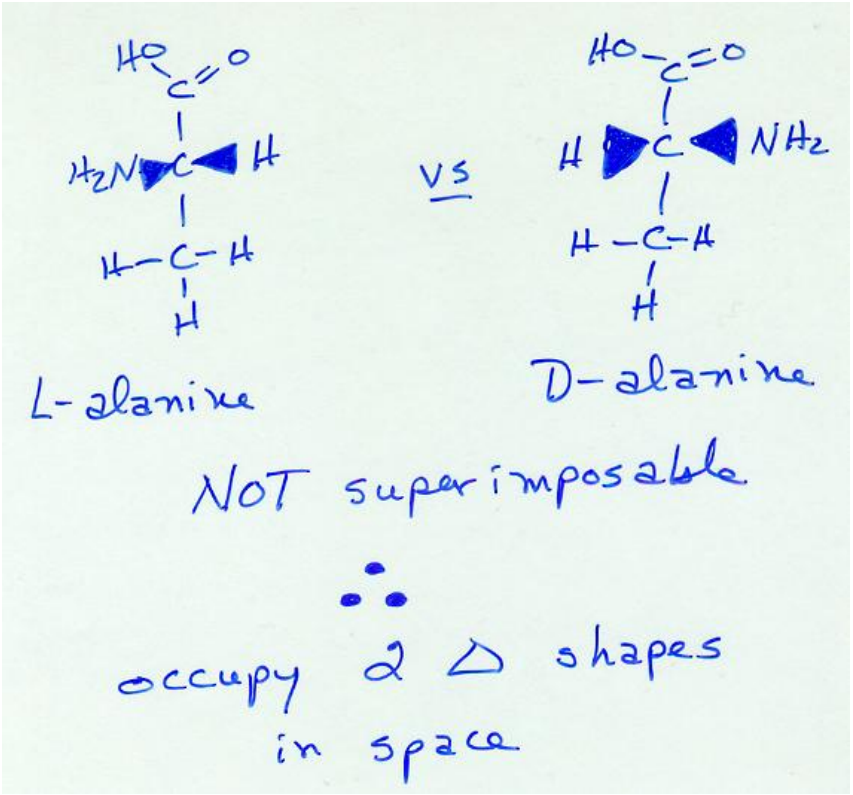
Humans metabolize L amino acids



L-phenylalanine



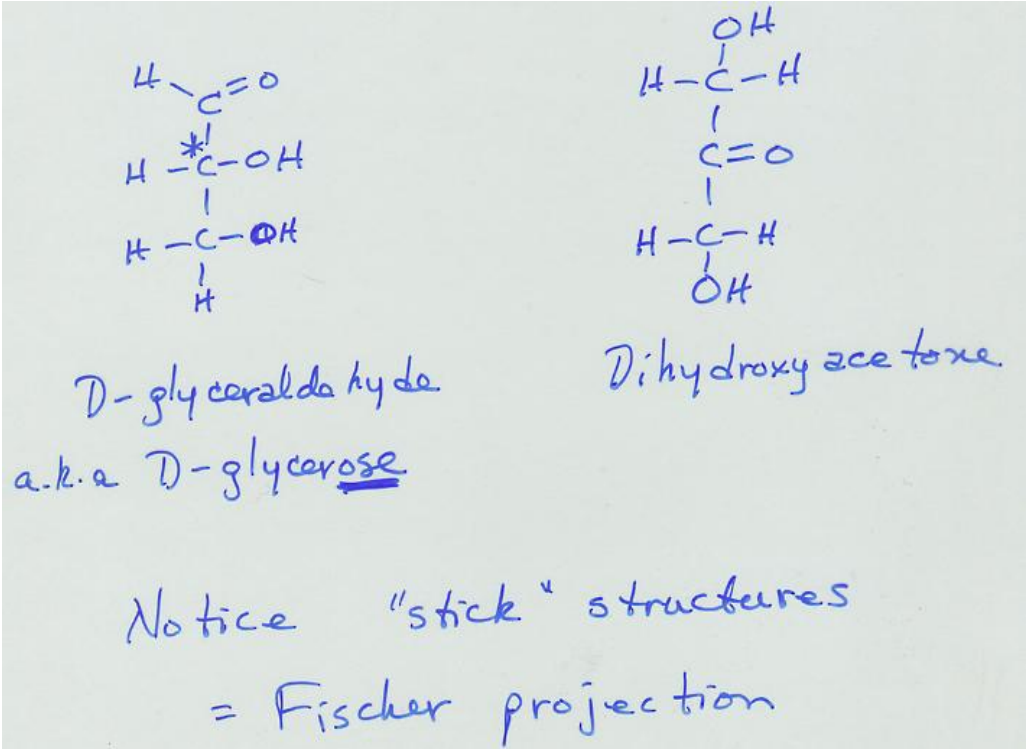
D-phenylalanine



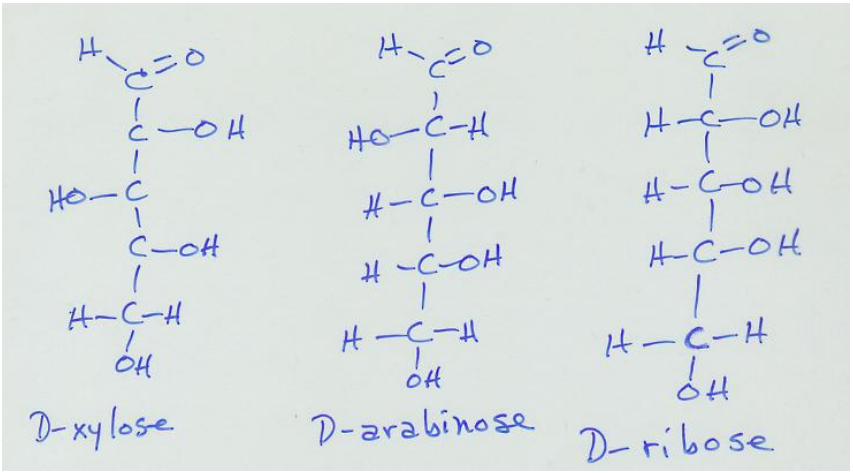
- It's like putting your right hand in a left hand glove
- D-ala does not fit the enzyme – L-ala DOES
- All AA's this way in mammals

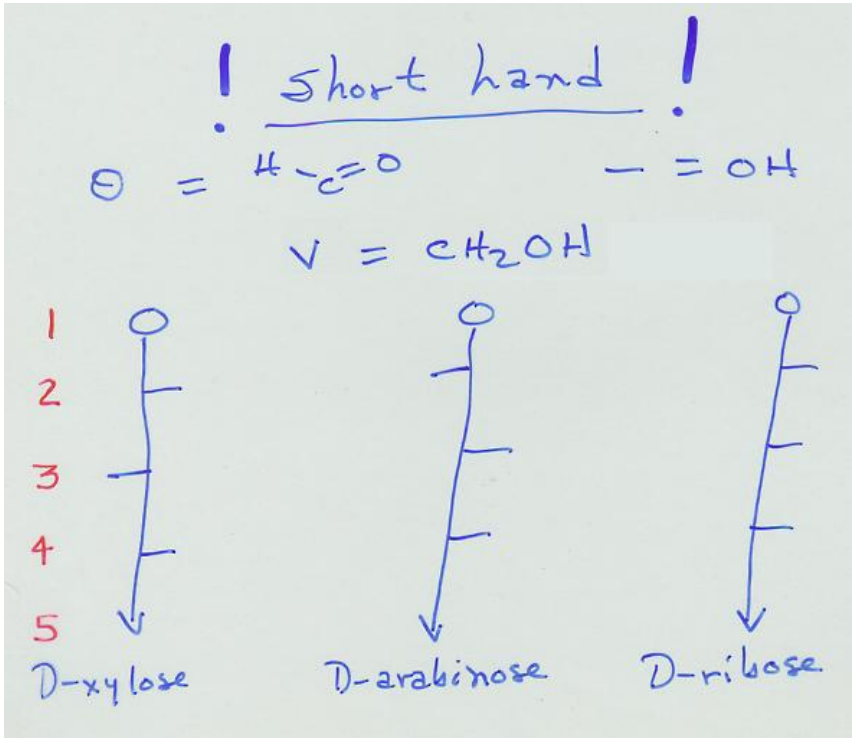
Carbohydrates – "Organic"ally

- Saccharide = Greek for sugar
- "ose" = suffix for a sugar



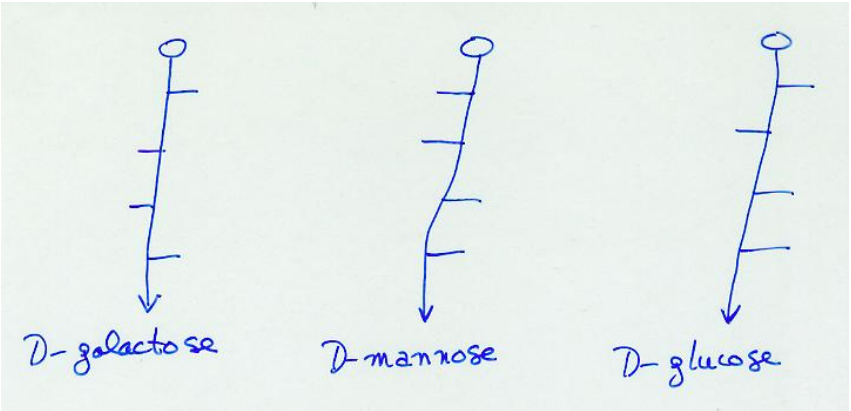
Pentoses


D-xylose
D-arabinose
D-ribose

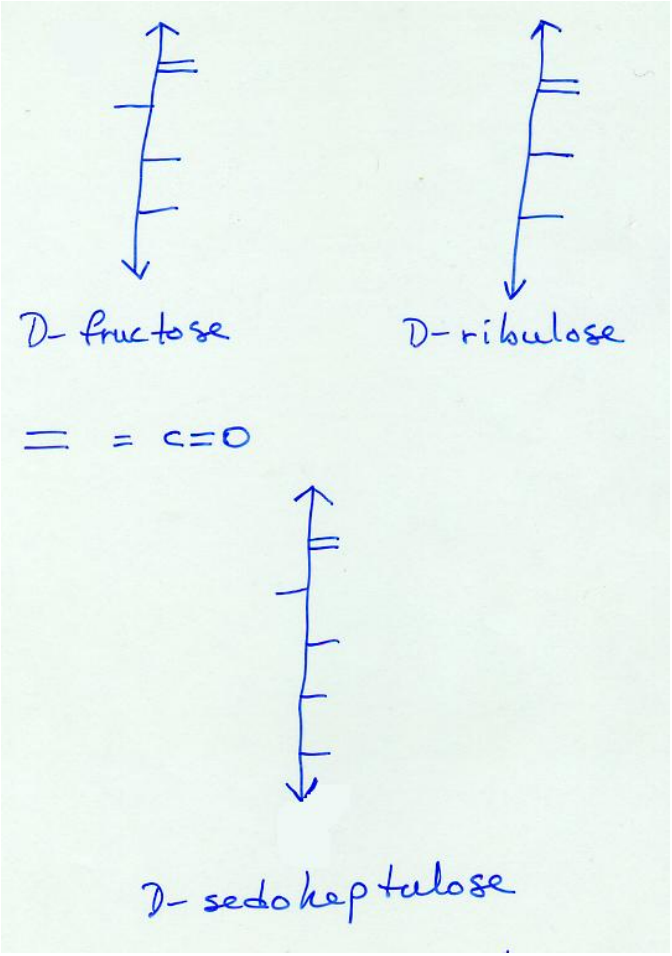

! Short hand !
= OH
= CH2OH
1
2
3
4
5
D-xylose
D-arabinose
D-ribose

Hexoses

- The pentoses (previous slide) and the hexoses (this slide) are ALDOSES ("aldehyde sugars")
- They are also mono-saccharides ("one sugars")

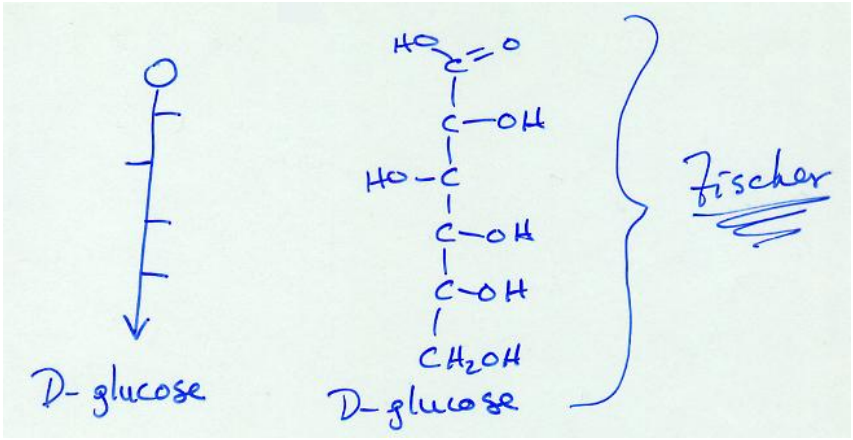


Ketoses -- monosaccharides

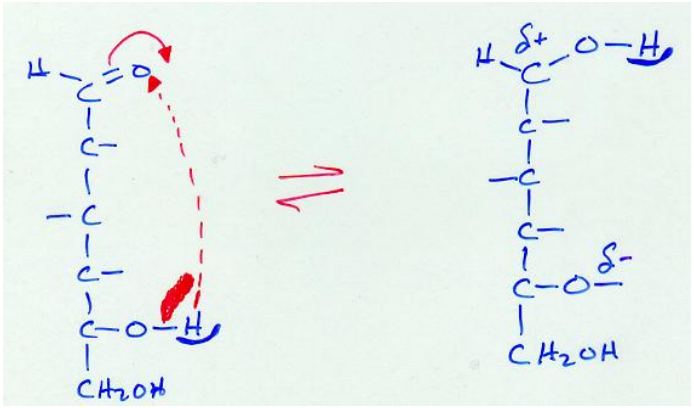


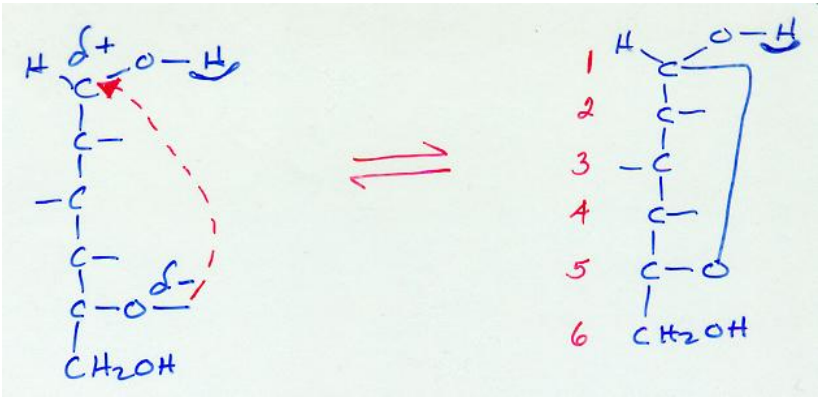
Fisher to Haworth Transition

- While pentoses and hexoses exist as ring structures almost exclusively (there ARE exceptions) in biological systems, O Chemists start from stick structures
- Biochemists close the sticks to form rings

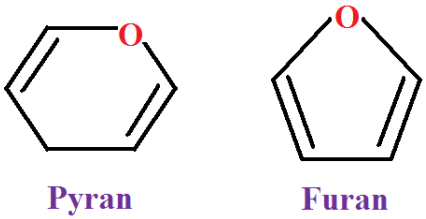


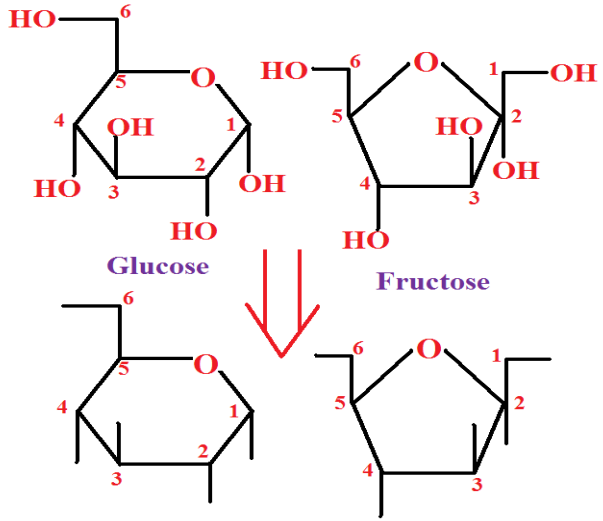
Glucose Haworth formation



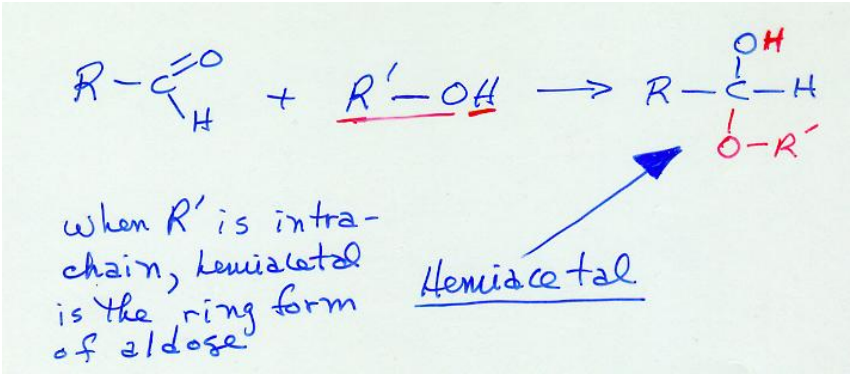


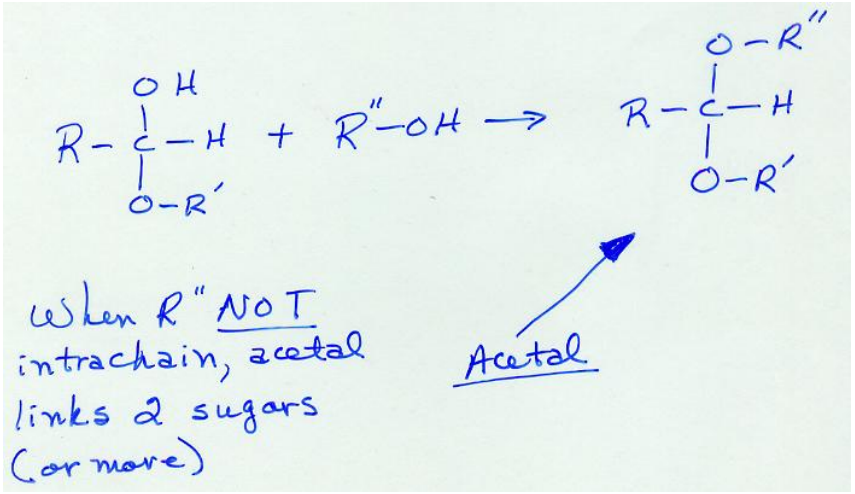
REMEMBER: organic molecules are NOT linear when the C is in sp^3 hybridization.



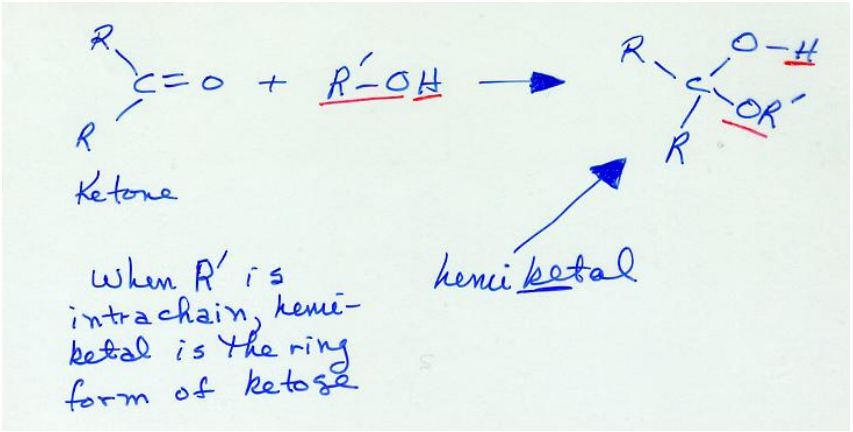


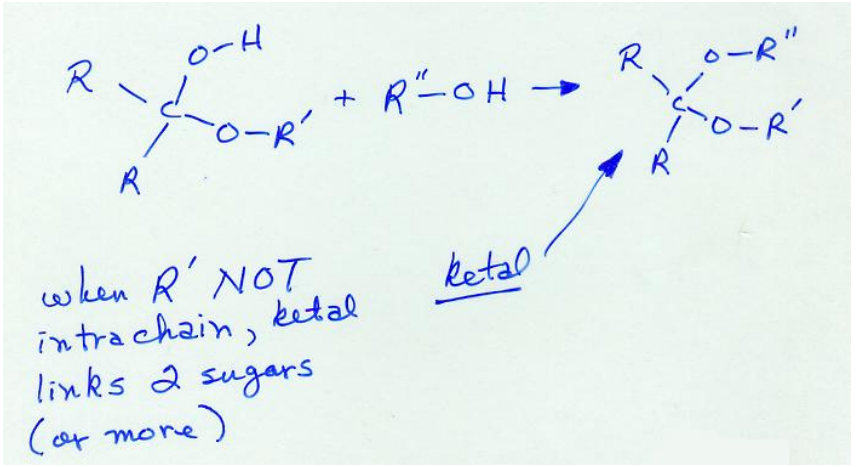
In General: Hemiacetal and Acetal





In General: Hemiketals and Ketals

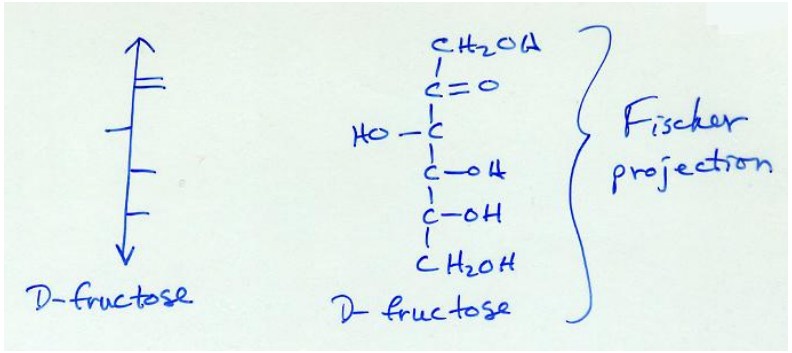


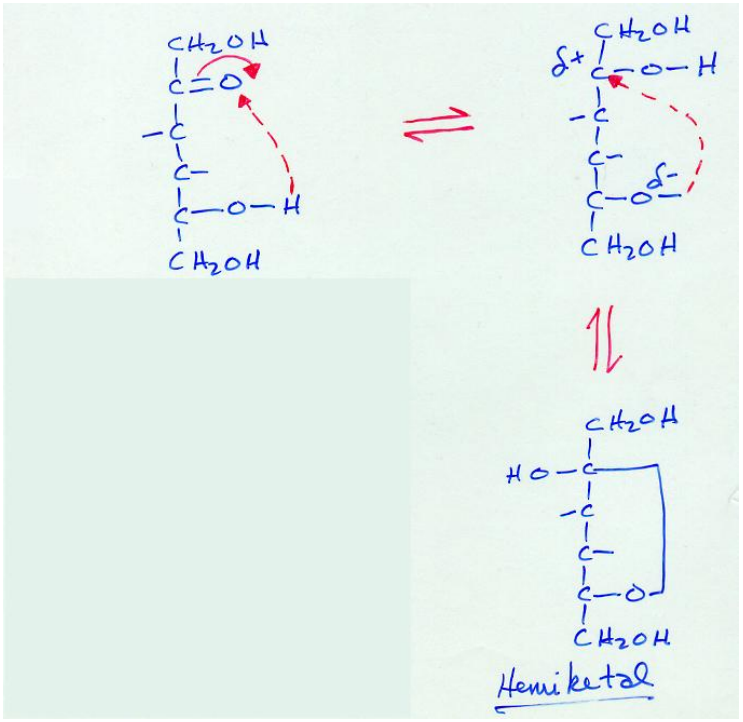


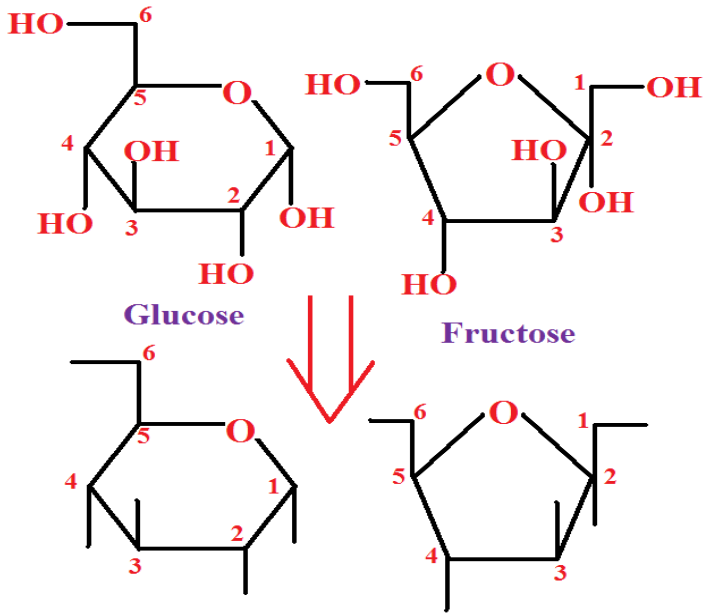
NOTE:

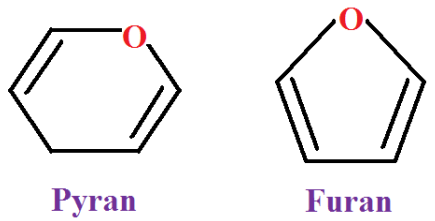
It's VERY difficult to tell acetals and ketals apart, therefore, we usually only talk about ACETALS.

Fructose Haworth Formation



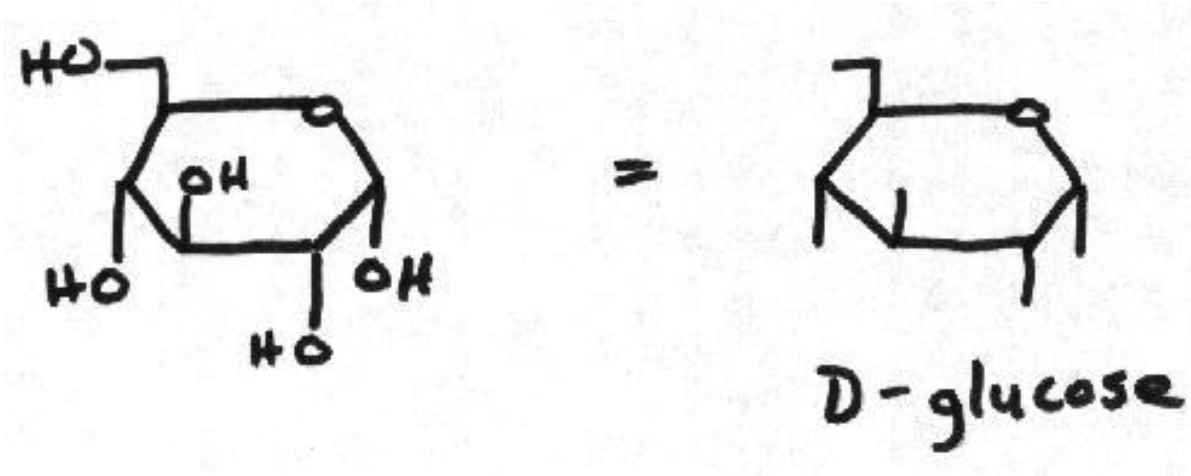


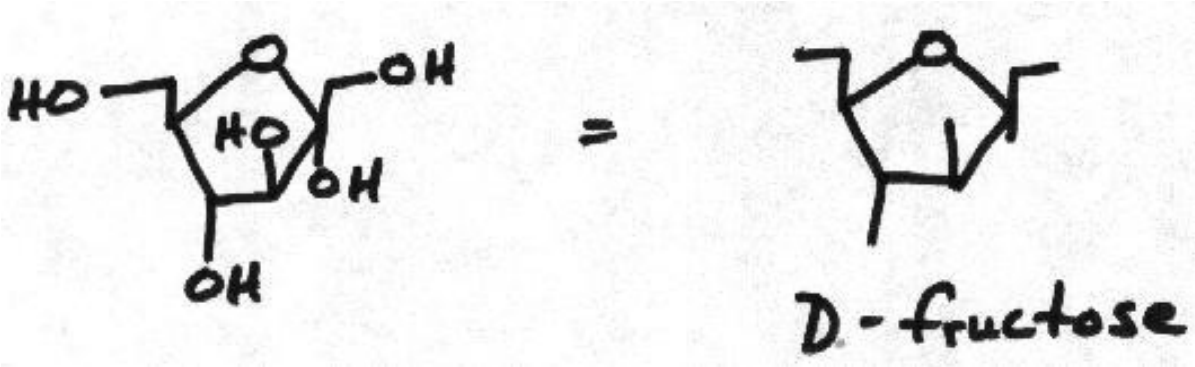




- Carbohydrates are generally seen as sources of quick energy.
- They consist of carbon, hydrogen and oxygen.
- In the old days, they were named carboHYDRATES as the ratio of hydrogen to oxygen was thought to be 2:1.
- We now know differently, although the name has stuck throughout time.
- There are three categories of carbohydrates in which we have interest: monosaccharides, disaccharides and polysaccharides.

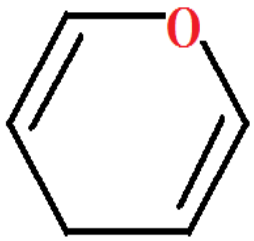
- Two of the simplest carbohydrates are glucose and fructose, the sugar in our blood and fruit sugar.



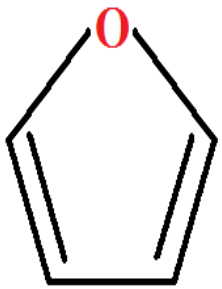


- As you can see, both have unique geometric shapes in their base structures and each has a unique orientation of the -OH groups upon the base structure.
- Remember that at each corner, there is a carbon atom and that carbon takes 4 bonds.
- The hydrogen atoms that are by themselves are not shown but are understood to be present.
- Biochemists developed a short-hand for quickly sketching carbohydrates called the Hayworth projections.
- The graphics on the right of each of the above sugars show the Hayworth projections, where the -OH's are replaced with lines going in the correspondingly correct directions.

- Each of the above carbohydrates has a base structure that contains one oxygen atom in its ring.
- Glucose and fructose are both hexoses (6-carbon sugars); glucose is a pyranose and fructose is a furanose.
- The sugars are so called because their base ring structures are based upon furan and pyran.



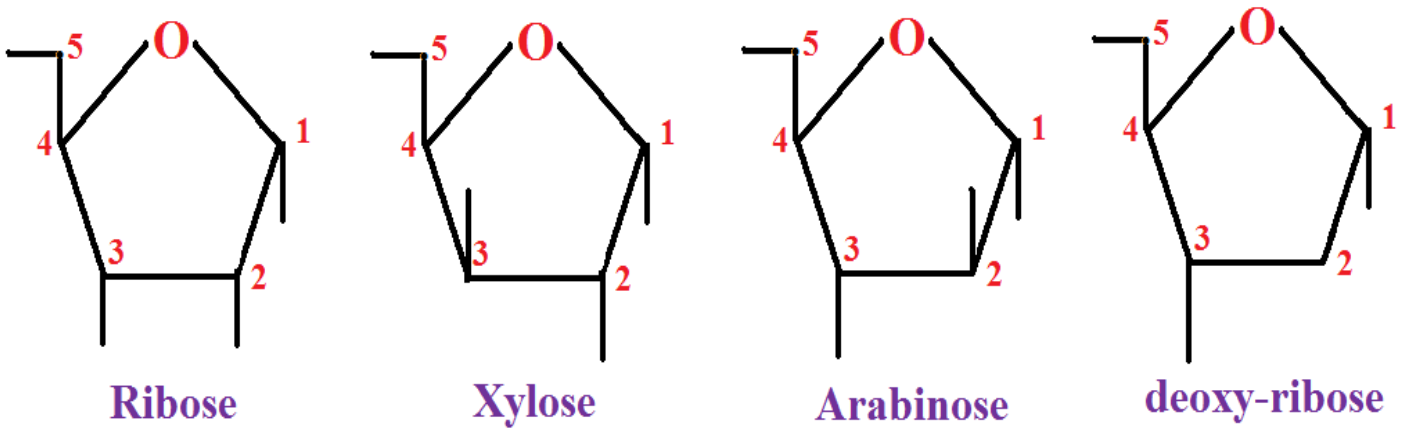
Pyran



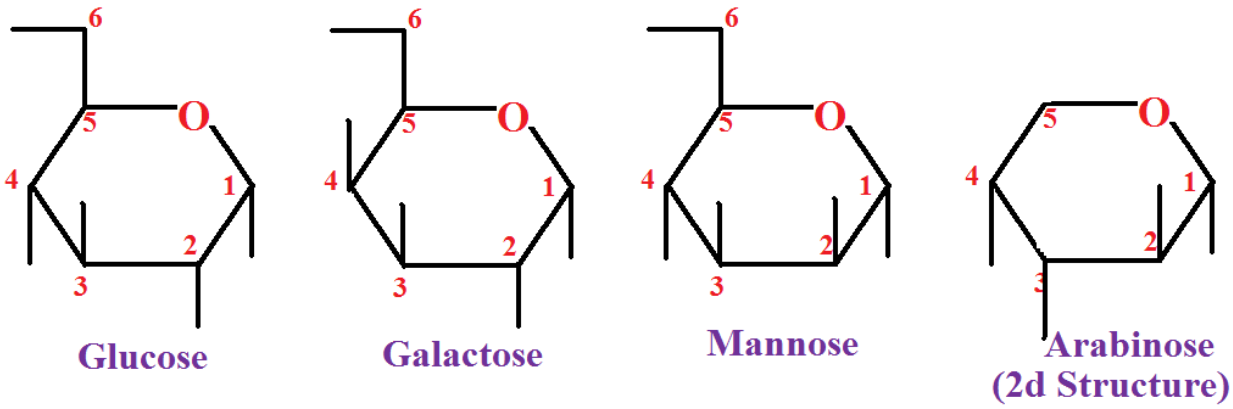
Furan

Monosaccharides -- Furanoses

- These two compounds (furan and pyran) provide the framework for numerous monosaccharides (single sugars).
- Ribose is found in ribonucleic acid (RNA); xylose is wood sugar and is non-fermentable; arabinose is gum sugar -- it may sometimes be found in urine.
- A form of ribose is found in deoxyribonucleic acid (DNA): deoxy-ribose. Ribose must lose the -OH on the 2' carbon to become deoxy-ribose.
- Note that ribose, xylose and arabinose are pentoses (5 carbon sugars).
- In addition, the proper names of the above furanoses are: D-ribofuranose, D-xylofuranose and D-arabinofuranose.



Pyranoses



- Glucose, we've briefly discussed.
- Galactose is of significance in that it forms half of the disaccharide (double sugar) lactose (milk sugar).
- Mannose is a plant sugar.
- Note that these sugars differ in their geometric structure simply by the orientation of the -OH groups.
- Indeed, the orientation of the -OH groups -- only differing by the position of 1 -OH group -- changes the sweetness of the sugar.
- To remember these sugars, I've got three mnemonics for you.
- They are based off the first 4 carbons (1,2,3,4) and #5 carbon has no -OH group on it and #6 carbon is always up and to the left for our purposes.
- To remember glucose, the mnemonic is DDUD: down, down, up, down.
- This describes the orientation of the -OH groups on the first four carbons.
- Galactose is DDUU, while mannose is DUUD.
- The proper names for the above pyranoses are: D-glucopyranose, D-galactopyranose and D-mannopyranose.

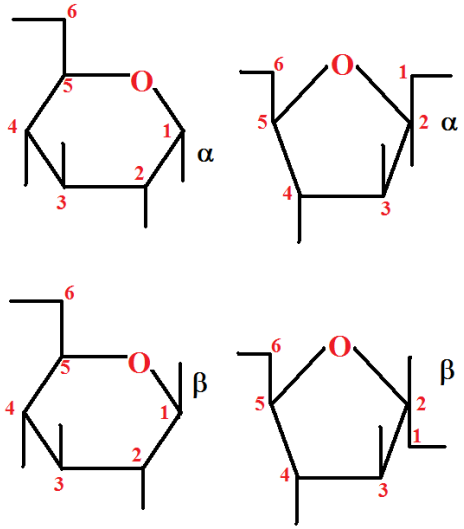
Terminology

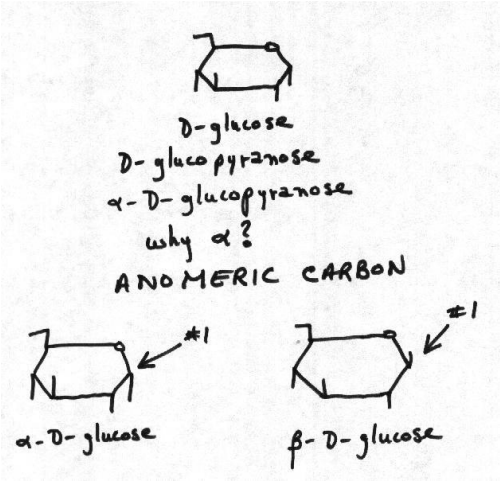
- Epimer: a class of isomers that are non-superposable, non-mirror images of one another.

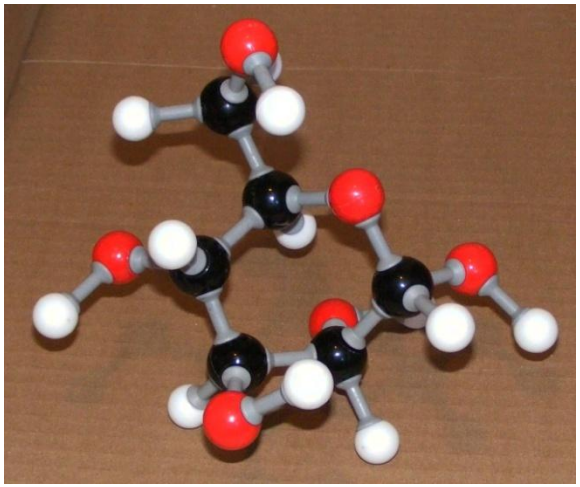
- Anomer: is a special type of epimer. It is an isomer of a saccharide in the cyclic form (Hayworth projection) that differs only in its configuration at the hemiacetal carbon in aldoses or hemiketal carbon in ketoses, which is also called the **anomeric carbon**. If the the hydroxyl group on the anomeric carbon is in the down position of glucose, then the sugar is an alpha (α) anomer. If, however, that hydroxyl group is in the up position, then the sugar is a beta (β) anomer. For example, α-D-glucopyranose and β-D-glucopyranose, the two cyclic forms of glucose, are anomers.

Anomeric Carbon

- We can go another step in naming monosaccharides based upon the position of the -OH on carbon #1.
- This carbon is called the anomeric carbon.
- An anomer is a special type of epimer (a class of isomers that are non-superposable, non-mirror images of one another). It is an isomer of a saccharide in the cyclic form (Hayworth projection) that differs only in its configuration at the hemiacetal carbon in aldoses or hemiketal carbon in ketoses, which is also called the **anomeric carbon**.
- When the -OH group is down on carbon #1, that is said to be in the α-configuration.
- When the -OH group is up on carbon #1, it is said to be in the β-configuration.
- Humans metabolize monosaccharides in the α-configuration.
- The graphic illustrates glucose in both configurations.





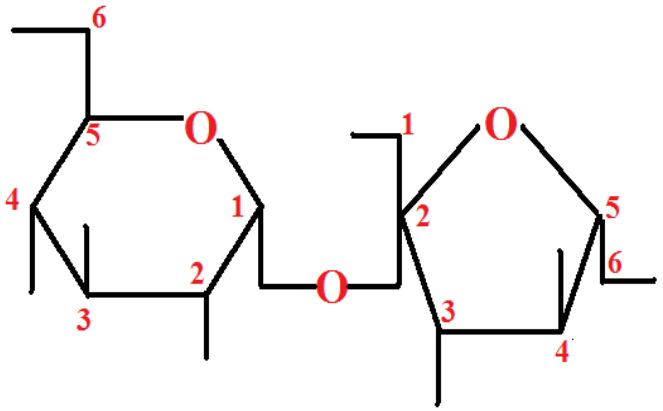


The Disaccharides

We are interested in only three of them:

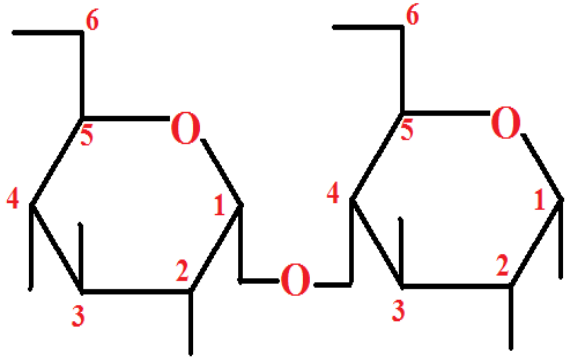
Sucrose

- Sucrose is table sugar.
- Sucrose consists of one molecule of glucose and one molecule of fructose bonded together.



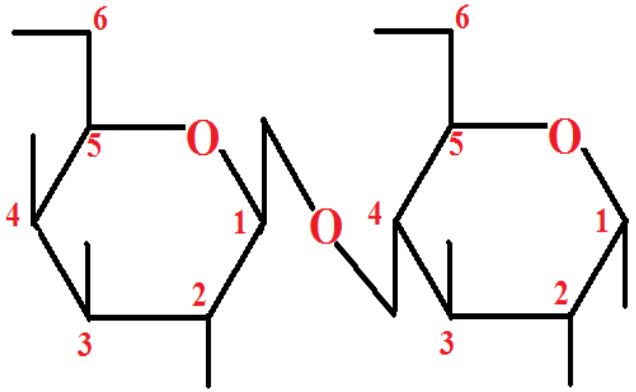
Maltose

- Maltose is also known as malt sugar;
- Maltose consists of two glucose molecules bonded together;



Lactose

- Lactose is milk sugar;
- Lactose consists of one molecule of galactose and one molecule of glucose – (pretty clever considering that young animals living on mother's milk use the glucose for quick energy and send the galactose to their livers where it will stored for future energy needs as glycogen) – are bonded together.



Glycoside Bonds

- The bonds that hold these sugars together are called glycoside bonds.
- An oxygen atom between the first and 4th carbons of each respective glucose molecule (see above) connects the two glucose molecules linked together in maltose.
- Since the linkage is from an -OH group on the left glucose molecule that is in the α-configuration, this is called an α1 to 4 link, or $\alpha 1 \rightarrow 4$ link.
- Since the linkage between the galactose molecule and the glucose molecule starts in the β-configuration and is also between the 1st and 4th carbons via an oxygen atom, this is called a β 1 $\rightarrow$ 4 link, or β 1 $\rightarrow$ 4 link.
- The linkage between the glucose and fructose molecules in sucrose occurs through the 1st and 2nd carbons of glucose and fructose, respectively.
- This is an α 1 to 2 link, or α 1 $\rightarrow$2 link.
- Refer to the above graphics for clarification of the shapes of these bonds.
- Remember, also, that there are NO carbons in the actual glycoside bond: ONLY an oxygen atom links the monosaccharides together.

The Polysaccharides

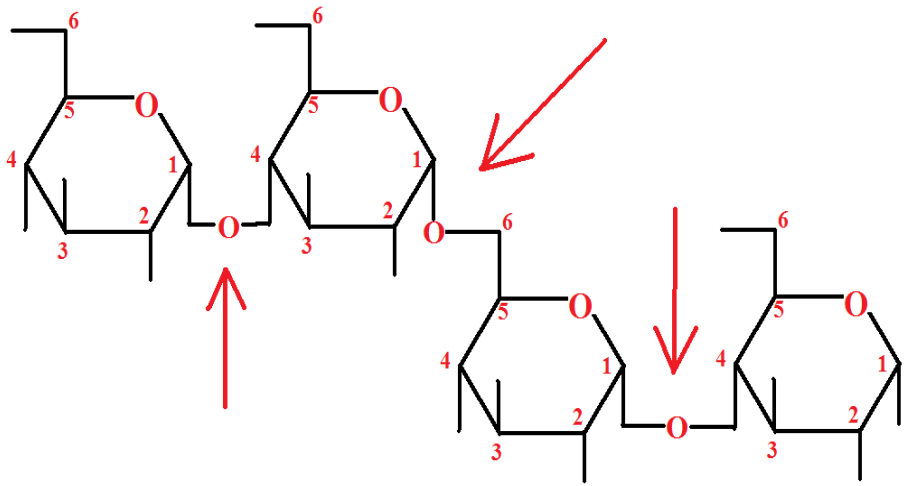
We are interested in three polysaccharides: starch, glycogen and cellulose.

Starch consists of two forms of complex carbohydrates: amylose and amylopectin

Amylose	Amylopectin
Is the less abundant form and forms a helix	The more abundant form in starch
Iodine "crawls" into the helix and forms <u>inclusion compounds</u> which turns a dark blue	Forms BOTH α 1$\rightarrow$ 4 and α 1$\rightarrow$ 6 links (shown in glycogen below)
Starch is found in PLANTS	Similar to glycogen due to the branching caused by the α 1$\rightarrow$ 6 links
Amylose is hydrolyzed by amylase in our mouths	Has lesser helix amount, hence less iodine binding; the color obtained is a red-violet color
	Found in PLANTS

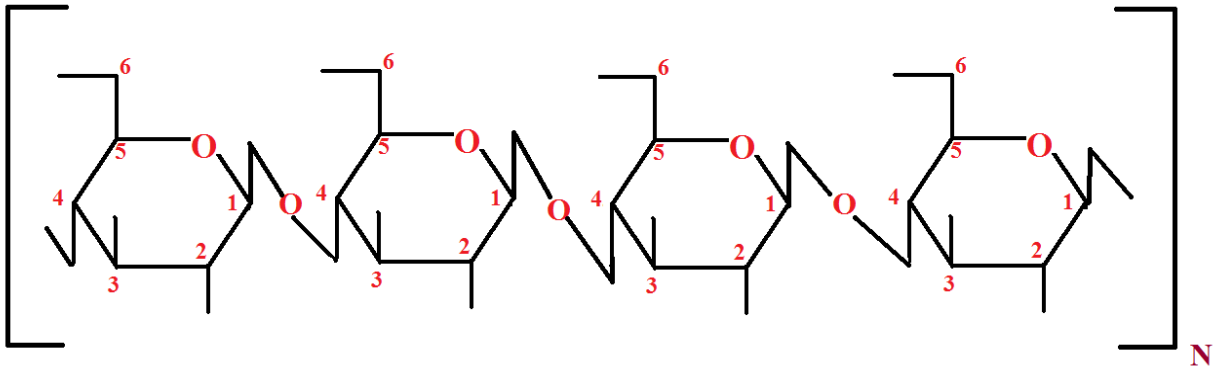
Glycogen

- Glycogen is found in ANIMALS, specifically in the skeletal muscle and liver of animals.
- It is also found in fetal hearts and fetal lungs.
- Fetal hearts run off glycogen while adult hearts run off lipids.
- The glycogen in fetal lungs is necessary to form surfactant to make oxygen passage into the body from the lungs easier when the fetus is in room air rather than the womb.
- Glycogen branches due to the same sort of linkages found in amylopectin (α 1$\rightarrow$6 links),



- Having both α 1 to 4 and α 1 to 6 links lets the glycogen molecule become very dense and be very efficient for storage.
- Approximately 1/3 of the weight of the human liver is glycogen.
- The branches that are formed are then "de-formed" by an enzyme called the "debranching enzyme" when glycogen is needed for energy.
- Although glycogen has some helix, it is more like amylopectin: it forms less inclusion compounds with iodine.
- The color obtained is amber red and may be stabilized with the addition of the dihydrate of calcium chloride.
- Each glycogen molecule contains approximately 100,000 molecules of glucose per molecule of glycogen.

Cellulose



- Cellulose, our last carbohydrate, is a bit different.
- In order to hold the glucose molecules together, they are linked by $\beta 1 \rightarrow 4$ linkages.
- Humans can not metabolize these β links, while ruminants can.
- Ruminants digest cellulose because they have bacteria in their stomachs that contain the enzyme cellulase that hydrolyzes these links.
- Cellulose is the most abundant carbohydrate on earth; humans utilize it as dietary fiber.

Reducing Sugars

- ANY CHO which can reduce an alkaline solution of Cu(II)
- ALL monosaccharides
- Lactose and Maltose
- NOT sucrose!!!!!!

IN GENERAL: if a free –OH is on the **ANOMERIC CARBON**, the CHO is a reducing sugar.

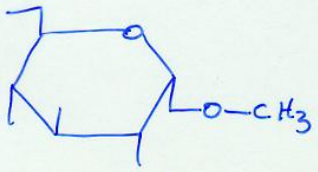
Benedict's/Fehling's Tests

Alkaline $Cu^{2+} \longrightarrow Cu_2O$ ppt

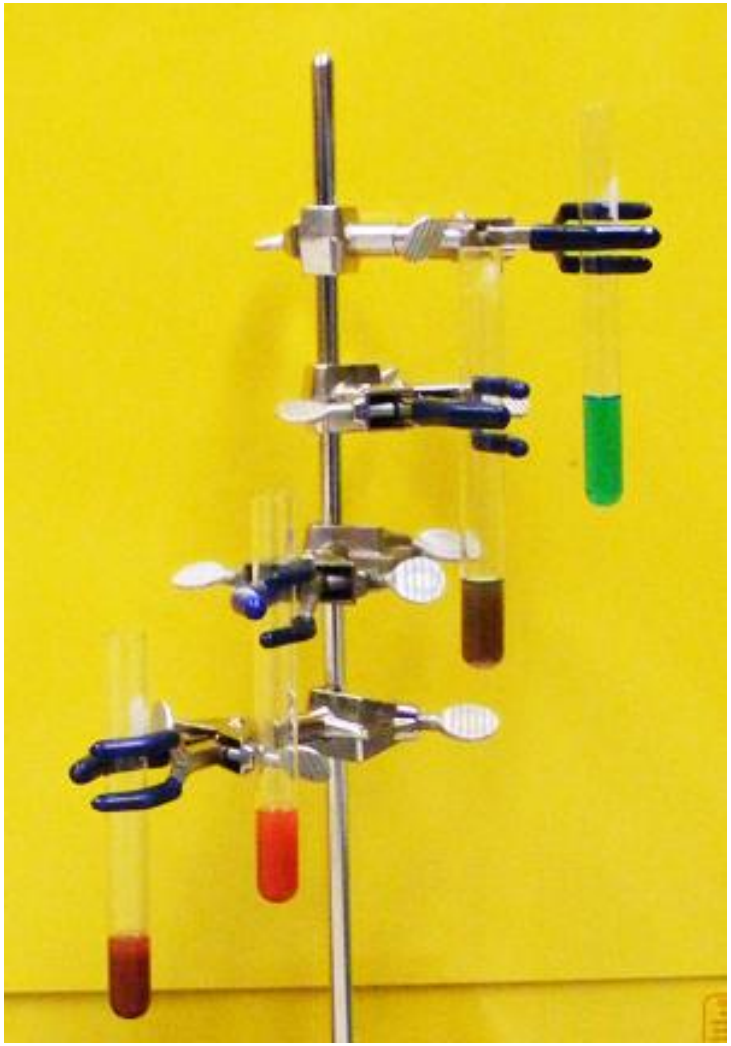
2° reducing sugars

Hemiacetal + $2Cu^{2+}$ $\xrightarrow{OH^-}$ Cu_2O red ppt

Hemiketal + $2Cu^{2+}$ $\xrightarrow{OH^-}$ Cu_2O red ppt

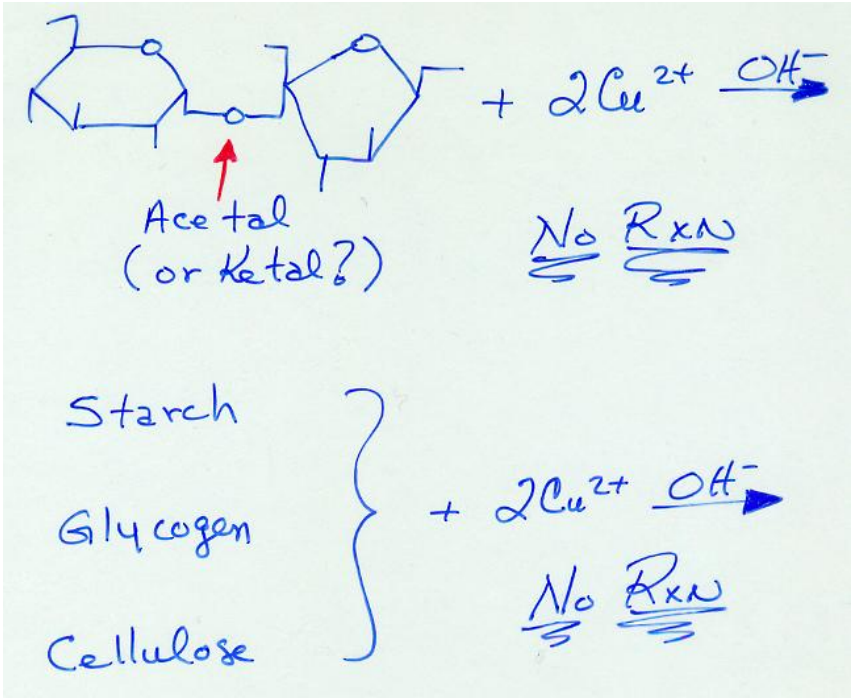


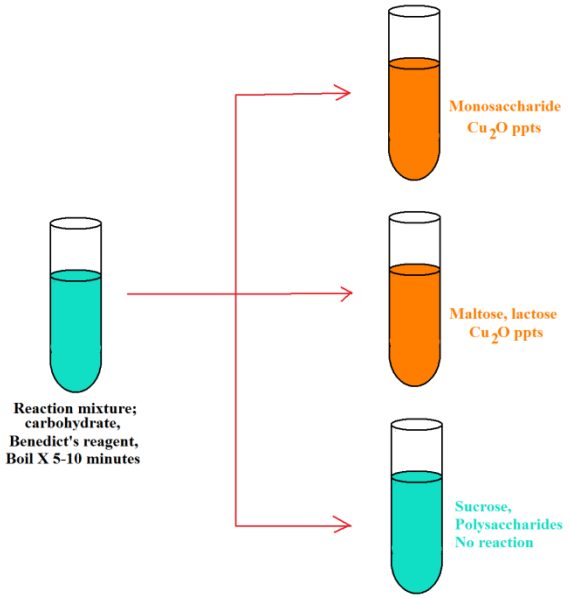
Glycoside + $2Cu^{2+}$ $\xrightarrow{OH^-}$ No Rxn



Cont'd

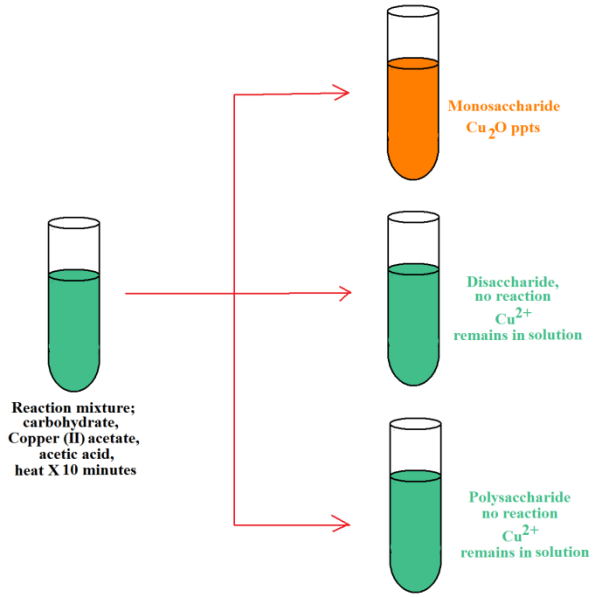
- Benedict's test is basis for Clinitest: urine test for urinary sugar excretion in diabetes
- Green: NO glucose in urine
- Brick Red: > 2 g glucose/100 mL urine

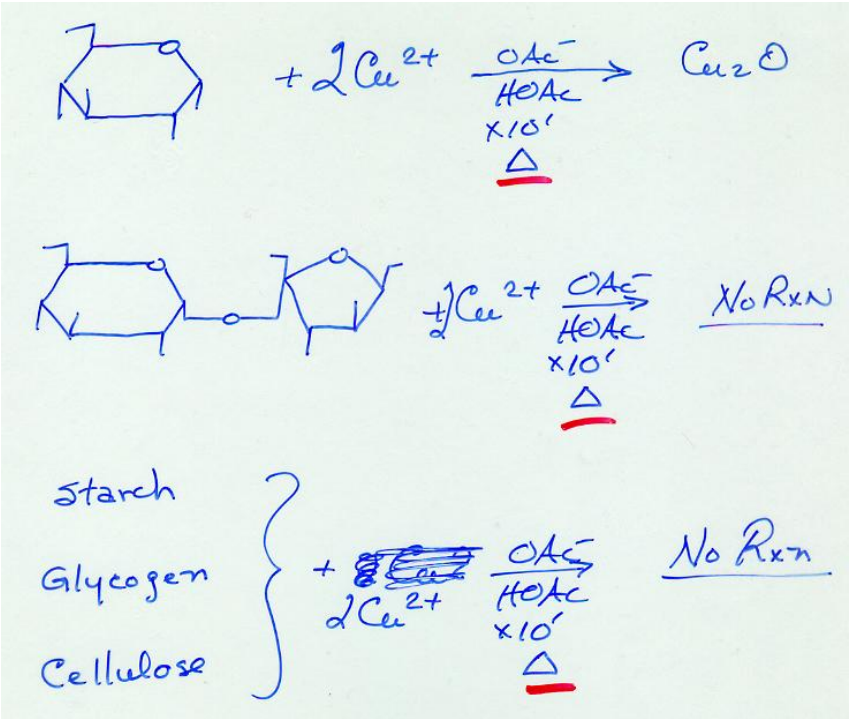




Barfoed Test

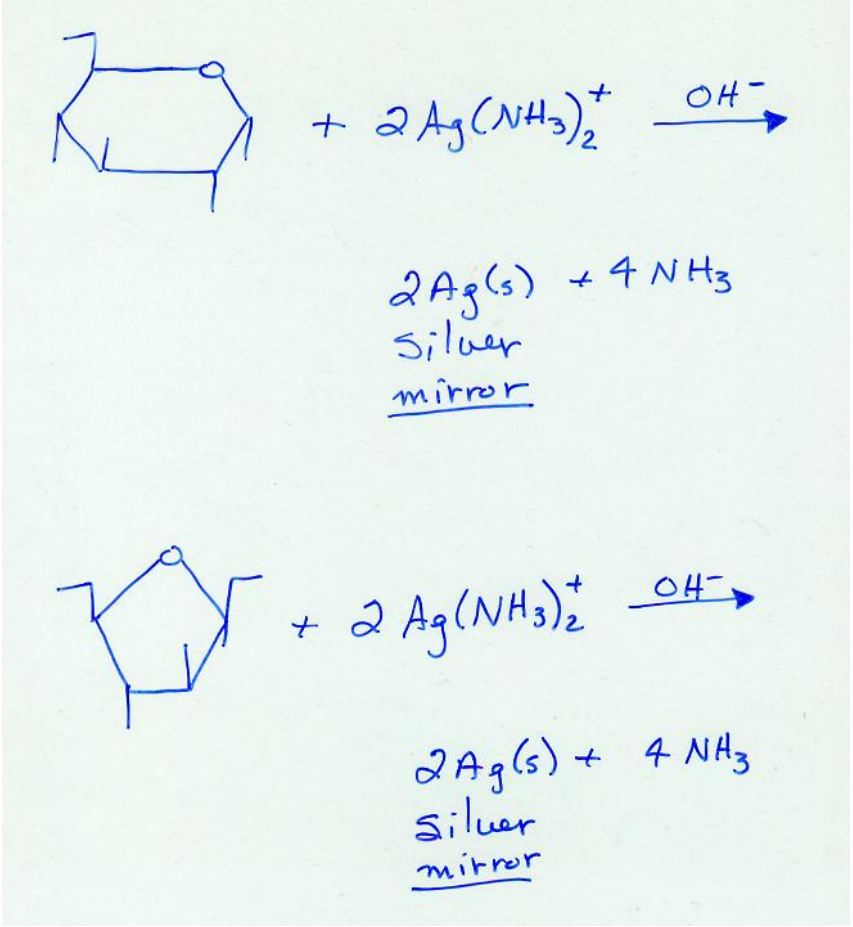
- In acid solution, Cu(II) is a WEAKER oxidizing agent
- Used to differentiate BETWEEN mono- and di-saccharides
- If the solutions are heated for > 10 minutes, false positive results will be obtained.

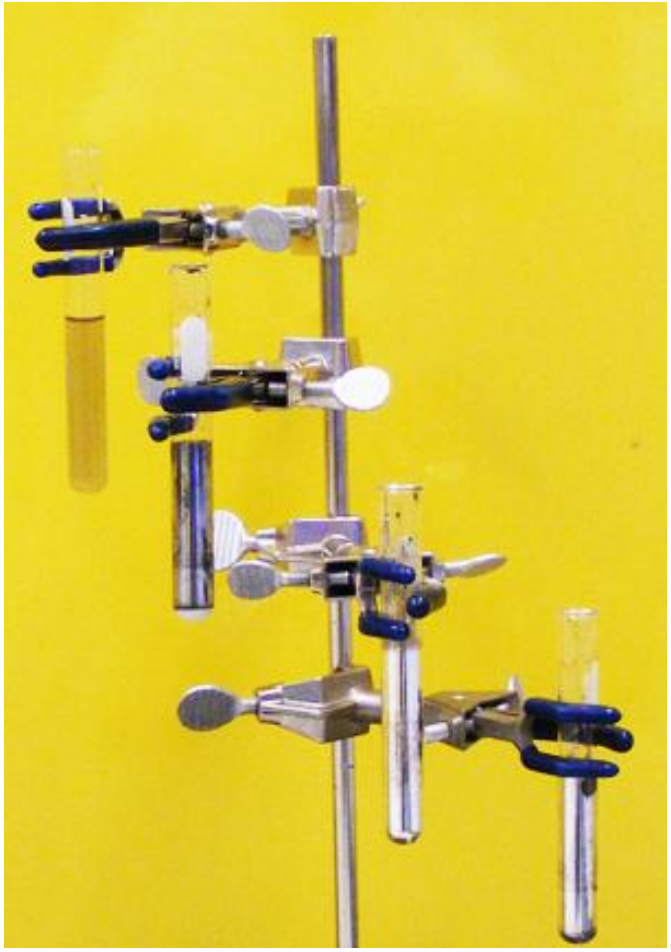




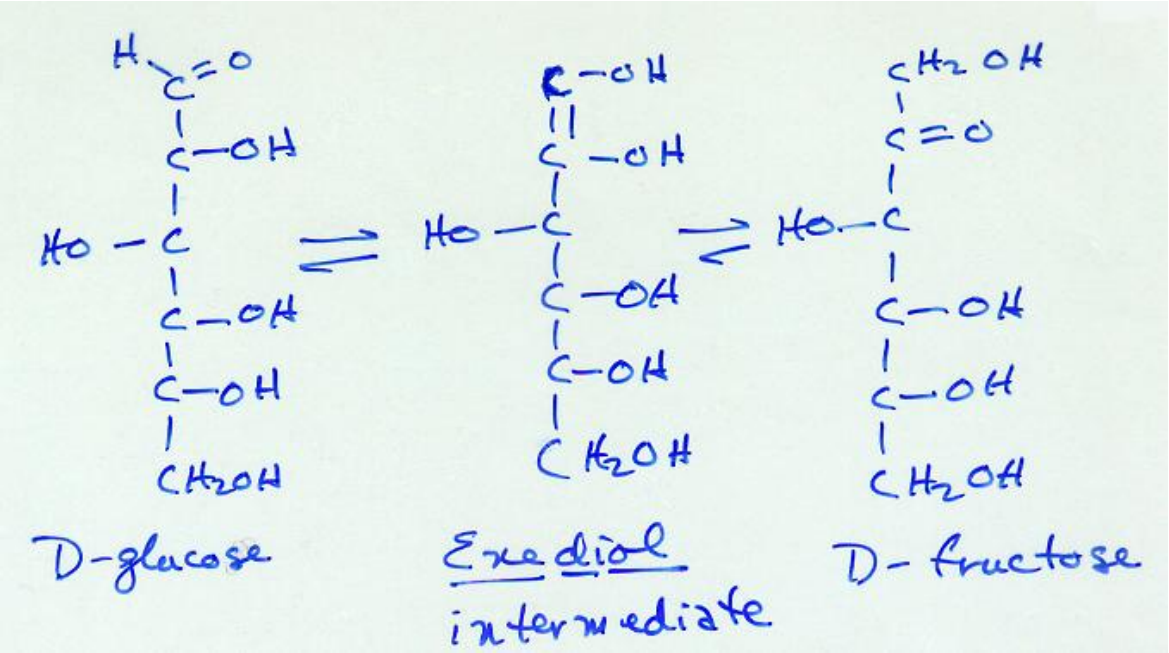
Tollen's Test

- Alkaline solution of Ag(I) with ammonia
- Why ALKALINE so important?





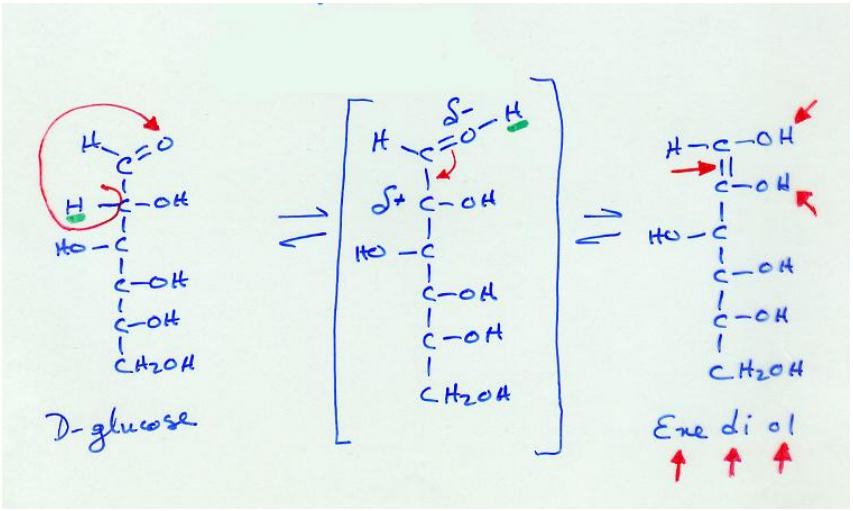
Tollen's, Cont'd

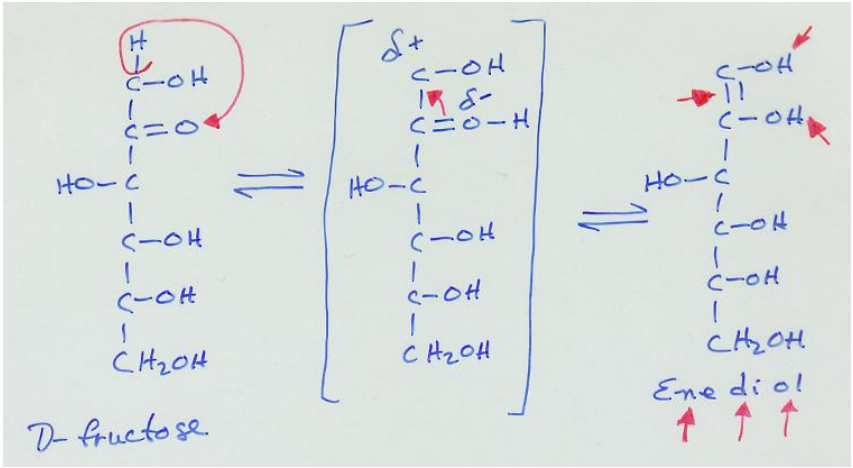


- Alkaline Conditions favor ENEDIOL formation of BOTH glucose and fructose

HOW??????

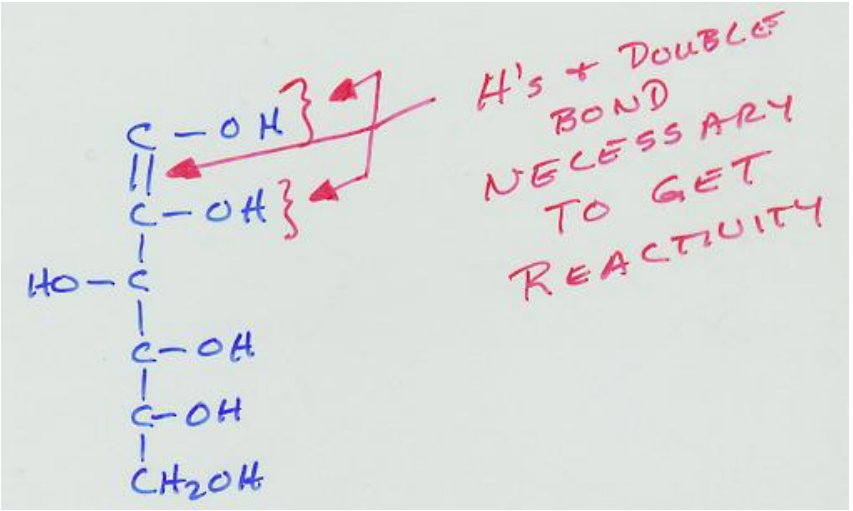
Enediol Formation





Base Catalyzes Enediol Formation

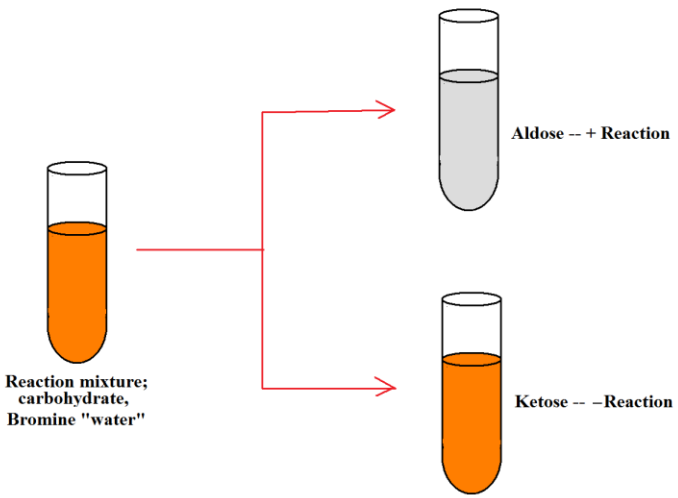
- Classically, aldehydes, but NOT ketones rxt with Tollen's Reagent
- Due to TAUTOMERIZATION, fructose CAN react with Tollen's Reagent.

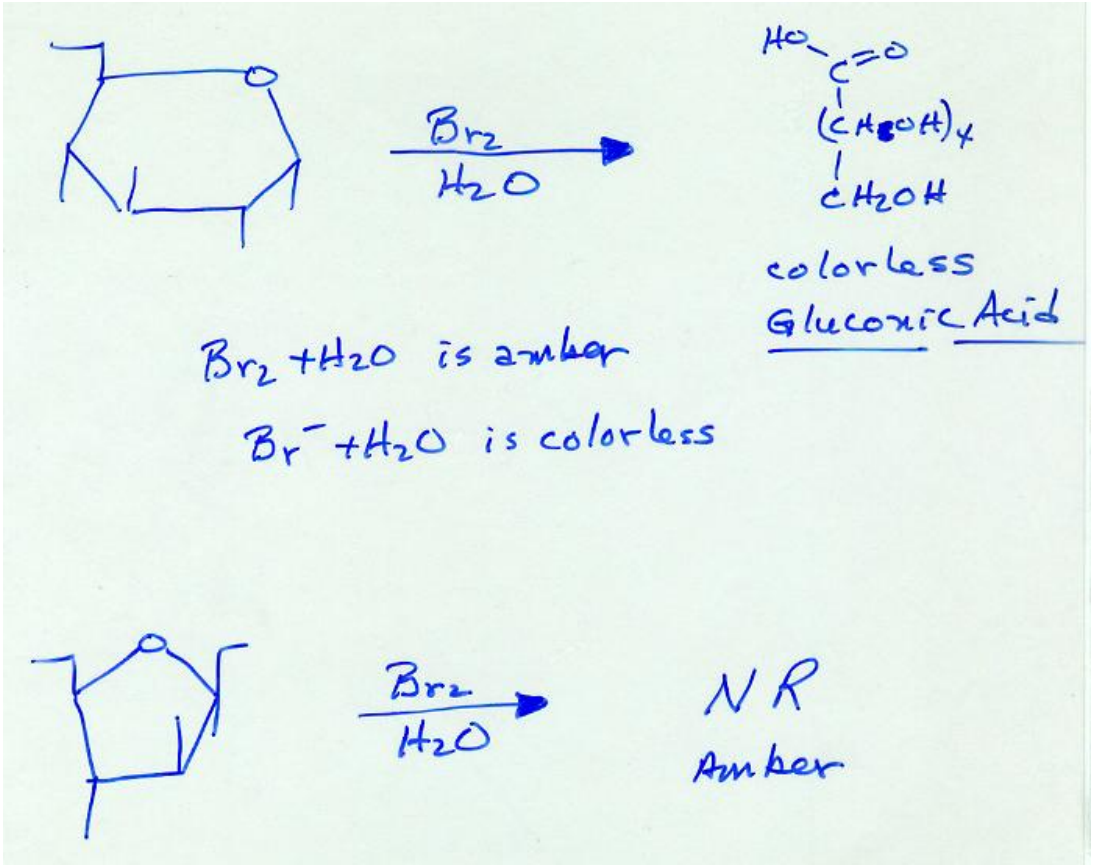


Tautomerization

- ≡ fast reorientation of isomers with movement of an “H” and a double bond.
  - Fructose (keto) and Enediol (enol) are tautomers
- Hence, ketoses react with Benedict’s, Fehling’s and Tollen’s reagents as well as do aldoses, anyways.
- Reiterate: Ketose $\rightarrow$ Enediol $\leftarrow$ Aldose

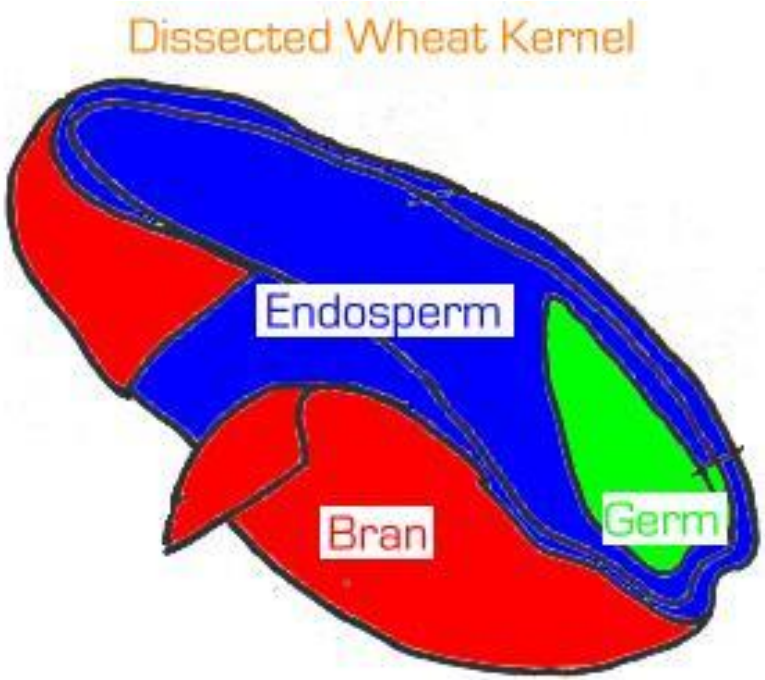
Bromine Water





Bromine water oxidizes aldoses but NOT ketoses.

Fiber in the Diet



- The main part of the **wheat** kernel is the starchy **endosperm**, which comprises more than 80 percent of the kernel weight and is the part of the kernel that eventually will be milled to wheat flour.
- **Wheat bran** is the papery brown coating of a whole grain of wheat. Removed during milling, wheat bran is sold packaged and in bulk. A quarter cup provides 6 g fiber.
- **Wheat germ** is the embryo of the wheat kernel. It is removed during the milling of white flour but is left intact in whole-wheat varieties. Wheat germ is sold raw or lightly toasted.

•http://arken.nlh.no/~ipfmol/hardness.html
•http://www.cooking.com/advice/adgloss.asp?GlossType=ingr&Item=Wheat+bran
•http://www.cooking.com/advice/adgloss.asp?glosstype=ingr&keywords=wheat+germ&x=18&y=9

Dietary Fiber

- Fiber
  - 1) increases satiety rate,
  - 2) reduces nutrient bioavailability,
  - 3) reduces energy density,
  - 4) alters hormonal response and
  - 5) alters thermogenesis in obesity.
- The mechanisms seem to be that fiber
  - 1) prolongs chewing and swallowing movements;
  - 2) increases fecal fat content;
  - 3) inhibits absorption of carbohydrate in high fiber foods;
  - 4) increases transit time and
  - 5) alters the action of insulin, gut glucagon and other intestinal hormones.

Fiber

- The common idea is that fiber gives bulk/roughage to aid in defecation.
- Burkitt noticed in 1983 in Africa that people eating high fiber diets had reduced incidences of GI disease.
- Epidemiological evidence shows the converse in developed countries (remember the paradox with pregnancy and lactation, too).
- Current research classifies fiber into 3 groups based upon their structure (chemistry) and properties.

Cellulose

- Cellulose comes from and is a constituent of the main cell wall.
- It is poly-β-glucopyranosyl-β-glucopyranoside.
- It is insoluble fiber and holds water.
- It acts as a laxative and reduces colonic intraluminal pressure.
- It also binds minerals.

Cellulose Fiber Sources

- Selected sources of cellulose fibers include grains (bran, whole wheat, whole rye), fruits (apples, pears) and vegetables (beans, peas, cabbage family, root vegetables and fresh tomatoes).

The Non-Cellulose Polysaccharides Summarized, Below

Dietary Fiber Class	Plant Part of Origin	Function
Hemicellulose	Secretions; cell wall material	Mostly insoluble; holds water, increases stool bulk; reduces colonic pressure; binds bile acids
Pectins	Intracellular cement material	Soluble, binds cholesterol and bile acids
Gums	Special cell secretions	Soluble; binds cholesterol and bile acids; slows gastric emptying; provide fermentable material for colonic bacteria with production of volatile fatty acids and gas
Mucilages	Cell secretions	Soluble; slows gastric emptying time; fermentable substrate for colonic bacteria; binds bile acids
Algal substances	Algae, seaweed	Soluble; slows gastric emptying time; fermentable substrate; binds bile acids

Non-Cellulose Polysaccharides' Sources

- Hemicelluloses may be obtained from bran, cereals and whole grains.
- Pectins may be obtained from fruits (apples, citrus, fruits, berries -- especially strawberries) and vegetables (green beans, carrots).
- Gums may be found in grains (oatmeal) and vegetables (dried beans and other legumes).
- Gums, mucilages and algal substances are used as food product thickeners and stabilizers.

Lignins

- Non-carbohydrate fiber includes lignin, which comes from the woody part of plants. It is insoluble, it is an anti-oxidant and it binds bile acids and metals.

Lignin Sources

- Lignins may be found in grains (whole wheat, whole rye), fruits (strawberries, peaches, pears, plums) and vegetables (mature vegetables).

There are 4 Physiological Effects of Dietary Fiber

- 1) water absorption,
  - The absorption of water leads to a bulkier stool and gives a laxative effect. This influences the transit time of the food mass through the bowel.
- 2) binding effect,
  - The rate of absorption of nutrients is effected by the amount of time in the bowel. Non-cellulose fibers bind bile salts and cholesterol, preventing their absorption. Some of these binding effects are undesirable: excessive amounts of dietary fiber bind iron, zinc and/or calcium. These binding effects also effect lipid levels.
- 3) relation to colonic bacteria
  - Some non-cellulose dietary fibers (gums) provide fermentation substances for colon bacteria. This produces short chain fatty acids and gas.
- 4) satiety
  - Satiety is enhanced by fiber, since there is extra bulk. These foods also take more time to eat. This property regulates the amount of food eaten, it increases transit time in the bowel and it contributes to the management of obesity and diabetes.

Fiber and Cancer

- For a number of years it was thought that fiber in the diet reduced the incidence of colon cancer by altering the kinds and amounts of bile acids and their metabolites in the bowel.
- It was thought that these chemicals altered the structure of the bowel, its cell turnover rate and the function of the resulting cell.
- An extensive study in late 1998 to mid-1999, though, has shown that this is not the case.
- It seems that fiber does NOT protect against bowel cancer.
- Interestingly enough, it appears that calcium may play a significant role in reducing the incidence of colon cancer.

Fiber and GI Disorders

- In other GI disorders (diverticular disease, constipation, hiatal hernia and hemorrhoids), fiber
  - 1) reduces pressure from within the intestinal lumen and
  - 2) increases the diameter of the intestinal lumen, thus allowing the intestinal tract to contract more, propelling its contents more rapidly and inhibiting segmentations.
- Fiber seems to do these by
  - 1) increasing transit time,
  - 2) increasing water absorption, resulting in a larger, softer stool and
  - 3) increasing pressure and weakness along the walls of the GI tract.

Diabetes and Fiber

- In the case of diabetes mellitus, it seems that there are at least 5 effects of fiber in the diet:
  - 1) it reduces fasting blood sugar levels;
  - 2) it reduces glycosuria;
  - 3) it reduces insulin requirements;
  - 4) it increases insulin sensitivity and
  - 5) it inhibits postprandial hyperglycemia.
- The modes of action are to, overall, slow carbohydrate absorption across the bowel.
- Fiber appears to do this by one of 5 mechanisms:
  - 1) it delays gastric emptying time;
  - 2) it forms gels with pectin or guar gum in the small bowel, thus impeding carbohydrate absorption;
  - 3) by "protecting" carbohydrates from enzymatic activity with a fibrous coat;
  - 4) by allowing "protected" carbohydrates to escape into the large bowel where they are digested by bacteria and
  - 5) they alter gut hormone levels (glucagon) to enhance glucose metabolism in the liver.

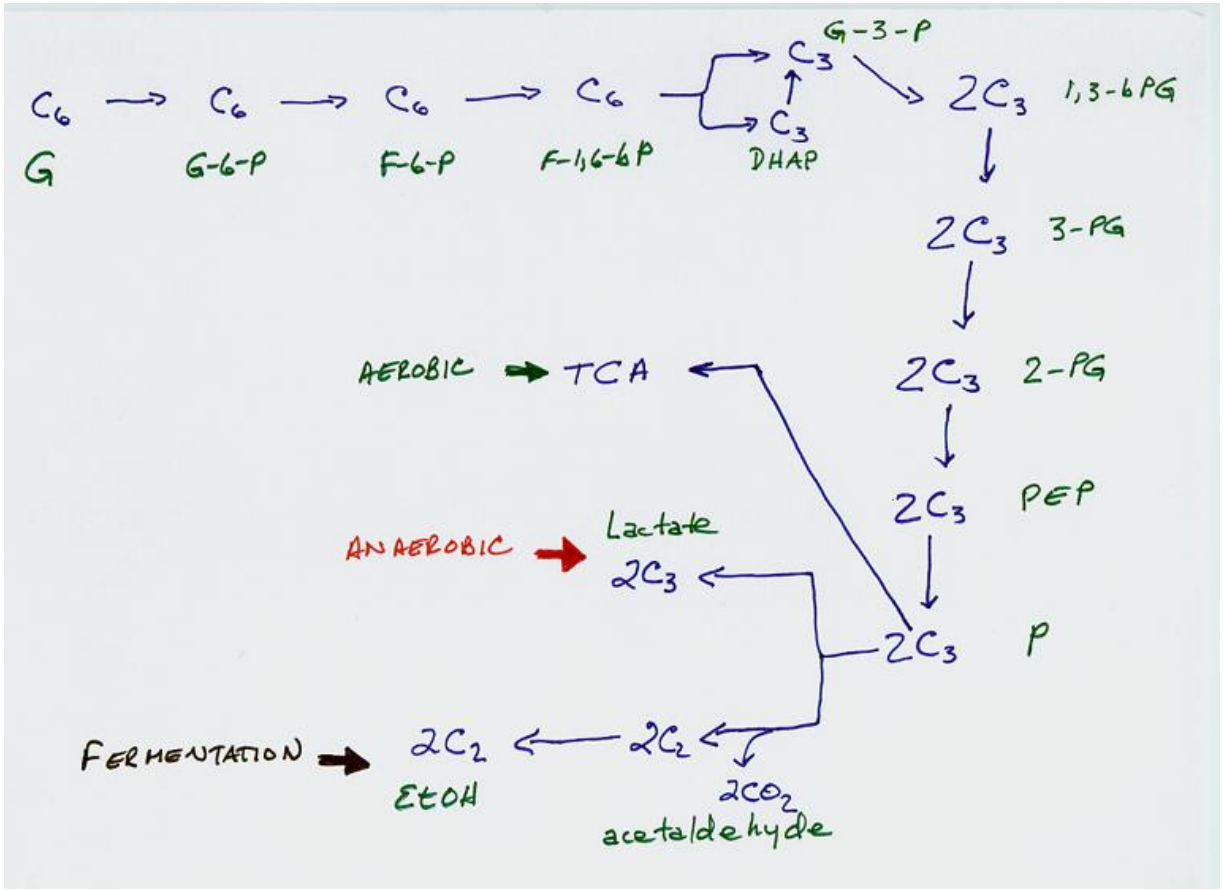
Fiber and Coronary Artery Disease

- In coronary artery disease (aka coronary heart disease), fiber
  - 1) inhibits recirculation of bile acids and
  - 2) reduces triglyceride and cholesterol levels (epidemiological evidence).
- The putative mechanisms that this takes include
  - 1) fiber alters bacterial metabolism of bile acids;
  - 2) fiber alters bacterial flora, resulting in a change in metabolic activity;
  - 3) fiber forms gels that bind bile acids;
  - 4) fiber alters the function of pancreatic and intestinal enzymes;
  - 5) fiber reduces insulin levels;
  - 6) fiber binds cholesterol, preventing absorption and
  - 7) fiber slows fat absorption by forming gel matrices in the intestine.

Carbohydrate Metabolism

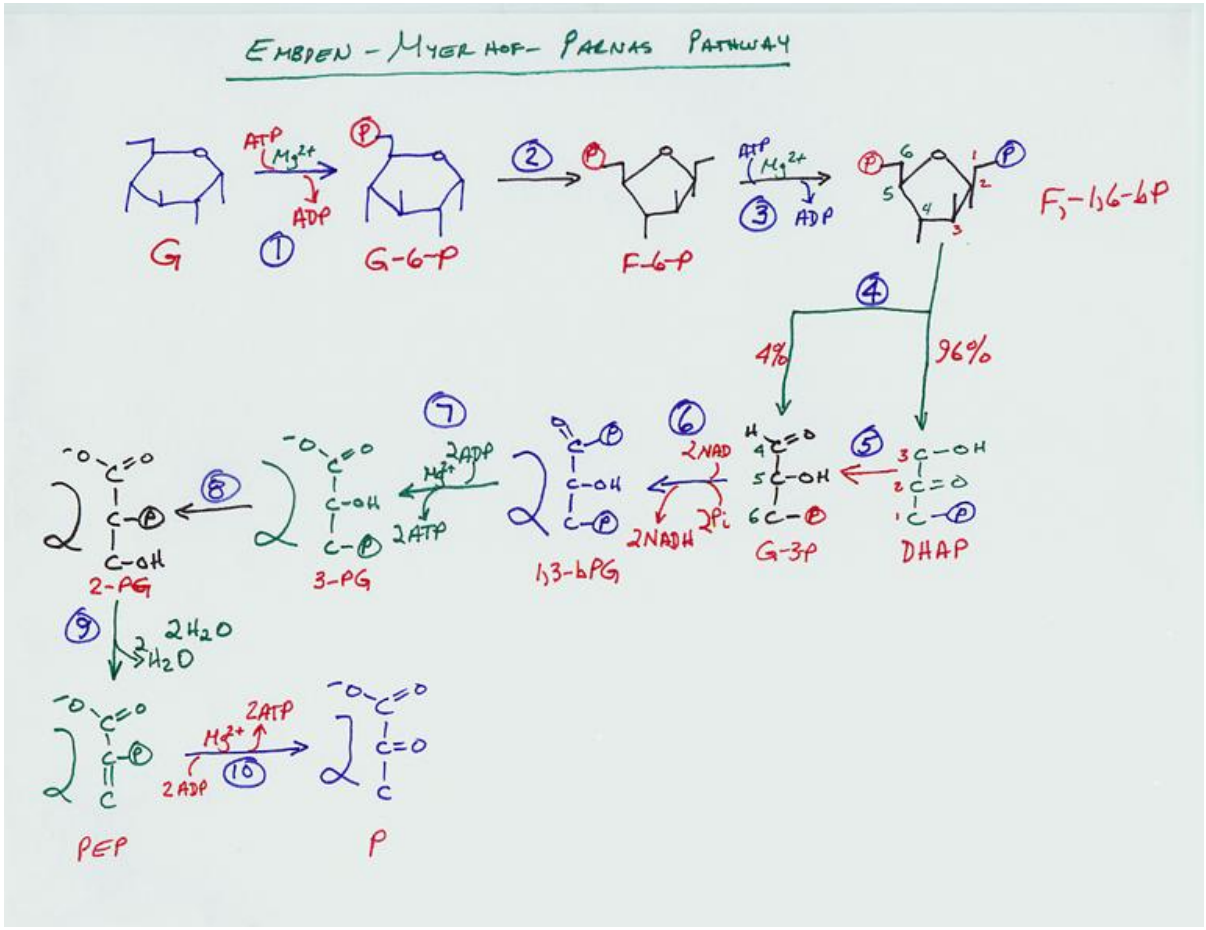
The Nickel Tour

Embden-Meyerhof-Parnas Overview



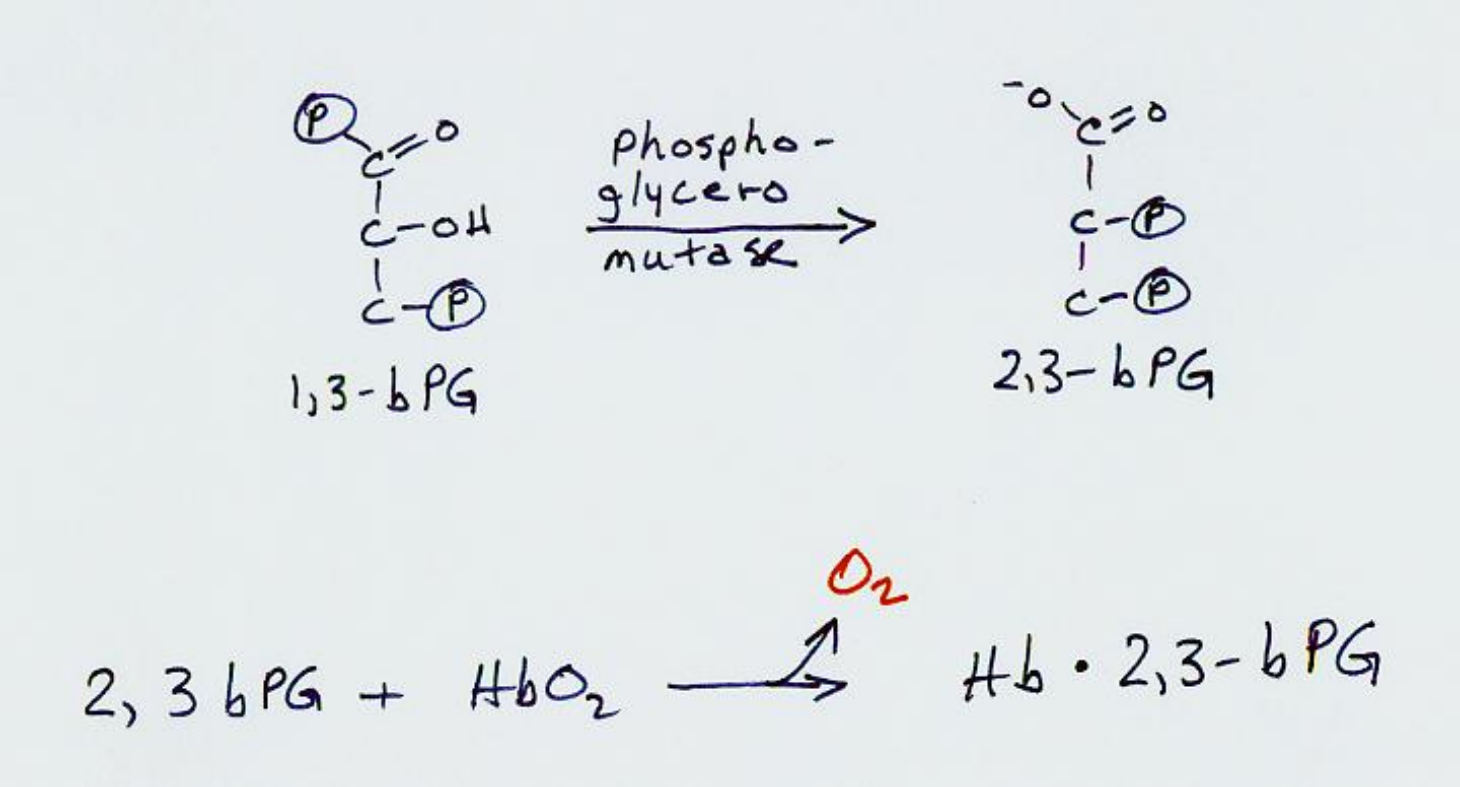
- Carbohydrate metabolism seems to get most of the time and attention throughout all of metabolism
- This pathway begins with a six-carbon sugar, glucose, and ends with 2 three-carbon intermediates (pyruvate) as glucose is oxidized.

Embden-Meyerhof-Parnas



- for glucose to be adequately catabolized, it has to be "trapped" in cells

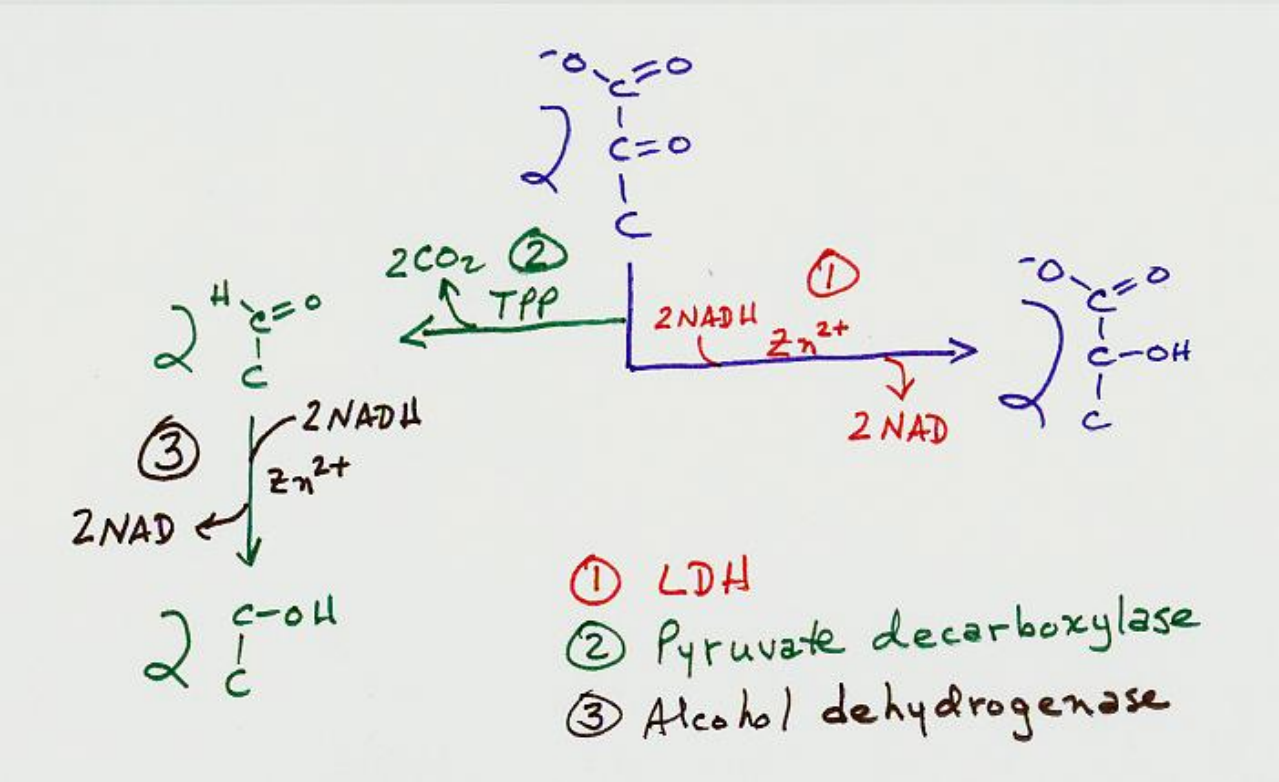
1. Hexokinase
2. Phosphogluco-isomerase
3. PFK
4. Aldolase
5. Triose phosphate isomerase
6. G-3-PDH
7. Phosphoglycerate kinase
8. Phosphoglycero-mutase
9. Enolase
10. Pyruvate kinase



2,3-bPG

- 2,3-bPG reduces the affinity of Hb for O_2 – is a primary compensatory factor in going to higher altitudes -- Is a side-rxn of EMP
- E.g., if live in San Francisco, to adapt (short term) to life at Lake Tahoe, body increases [2,3-bPG] so more oxygen is released to the cells in the body

Anaerobic/Fermentative Metabolism



- The stoichiometry – ***aerobic AND anaerobic*** -- (review your Chem 121) 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.

EMP – Stimulators and Inhibitors

Stimulators ("Activators")

- PFK: ADP and AMP
- Low energy turns on EMP

Inhibitors

- PFK: Citrate and ATP
- G-3-PDH: AsO_4^{3-}
- Enolase: fluoride ion
- Pyruvate kinase: ATP
- High energy turns off EMP – arsenate inhibits because it looks like phosphate

ATP Summary – Used and Gained -- AEROBIC

ATP Used

- Hexokinase: -1
- PFK: -1
- Total USED = 2 ATP

ATP Gained

- G-3-PDH: +6 ($\Leftrightarrow$)
- Phosphoglycerate kinase: +2
- Pyruvate kinase: +2
- Total GAINED: 10 ATP

Overall: ***8 ATP produced***

ATP Summary – Used and Gained – ANAEROBIC (FERMENTATIVE, too)

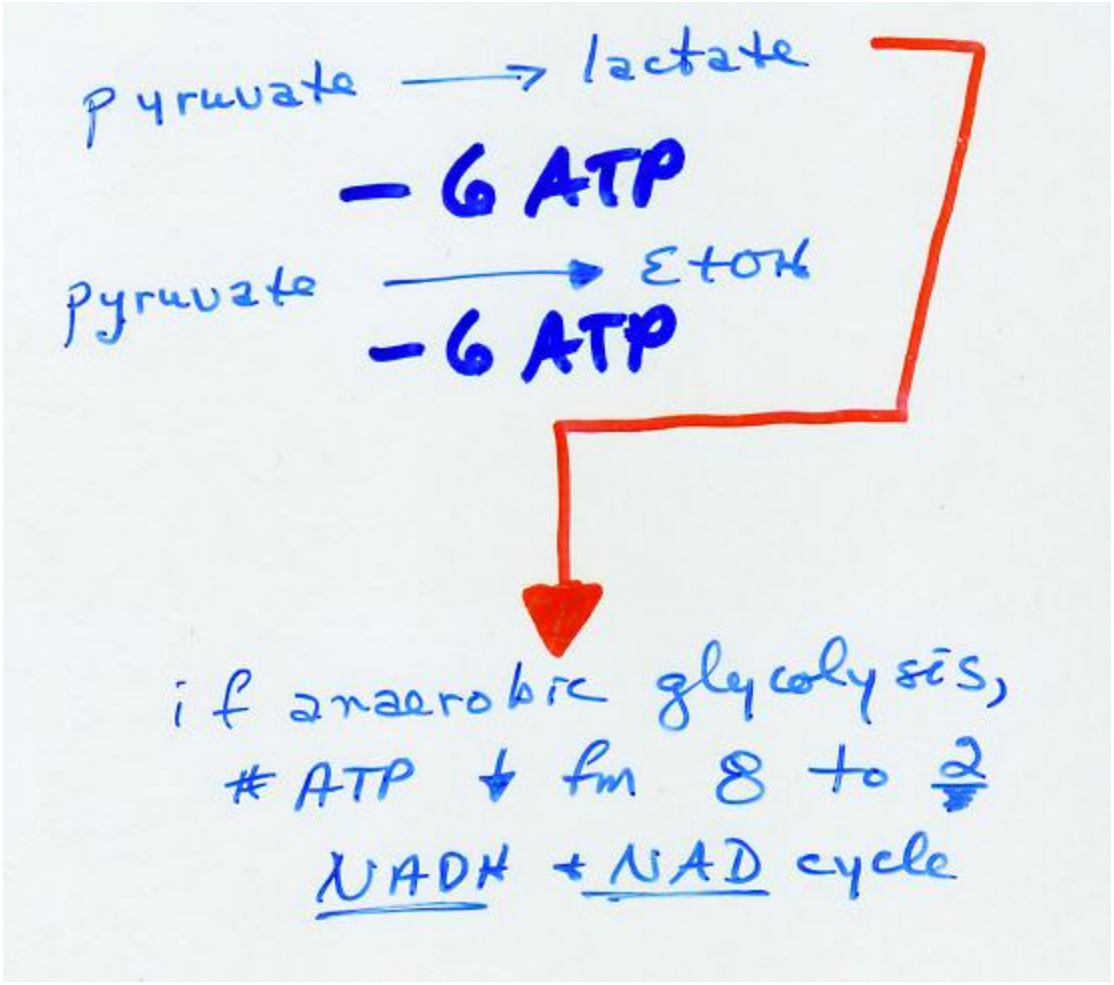
ATP Used

- Hexokinase: -1
- PFK: -1
- LDH (alcohol DH) : -6 (⇔)
- Total ATP USED: -8 ATP

ATP Gained

- G-3-PDH: +6 (⇔)
- Phosphoglycerate kinase: +2
- Pyruvate kinase: +2
- Total GAINED: 10 ATP

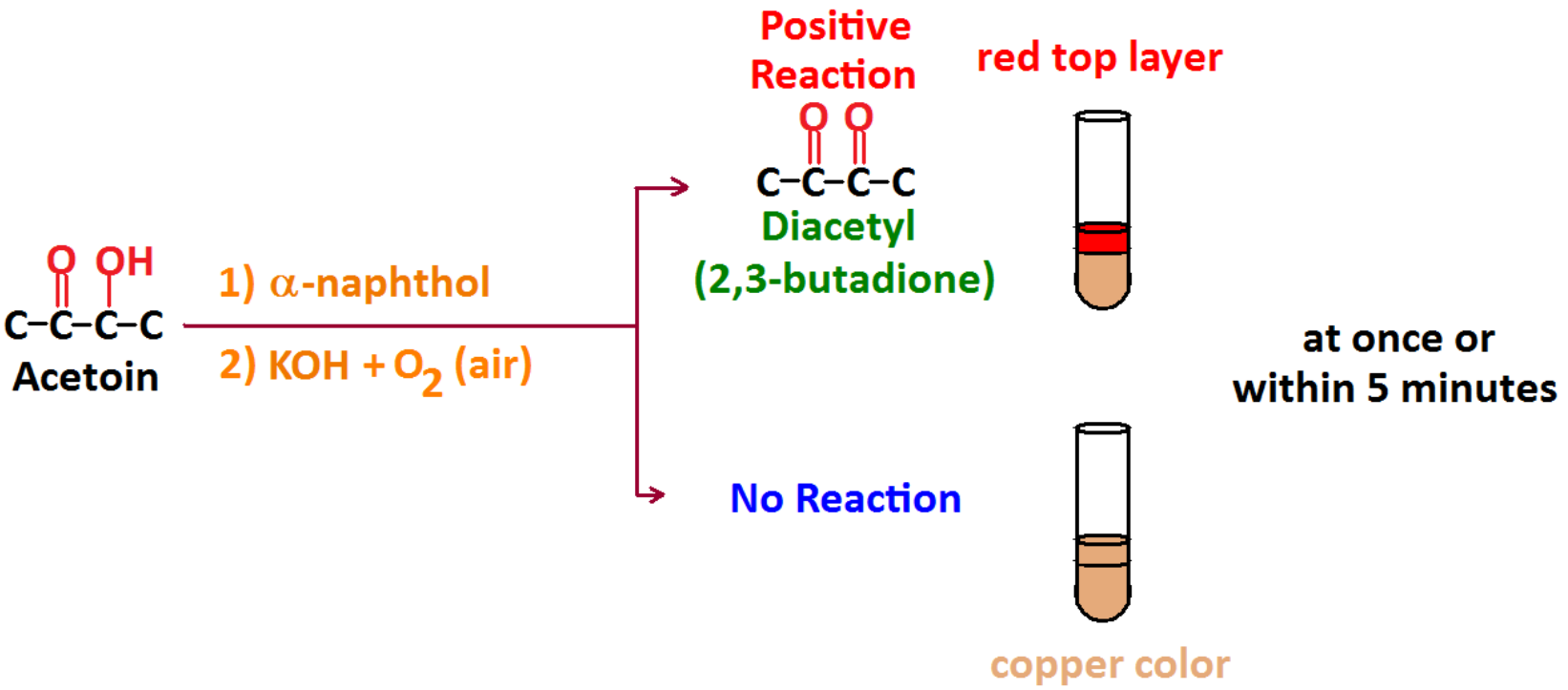
In anaerobic glycolysis (fermentation),
 # ATP go down from 8 to 2 because the NADH and NAD cycle


Pyruvate → lactate
−6 ATP
Pyruvate → EtOH
−6 ATP
if anaerobic glycolysis,
ATP ↓ fm 8 to 2
NADH + NAD cycle

Voges-Proskauer

Metabolism and Application

Principles Behind the VP Test



α-naphthol added first as it enhances the red color sensitivity – using second may give false negative; KOH and oxygen in the air oxidize the secondary alcohol to the ketone;
α-naphthol is also known as Barritt's Reagent A
KOH is also known as Barritt's Reagent B

Application

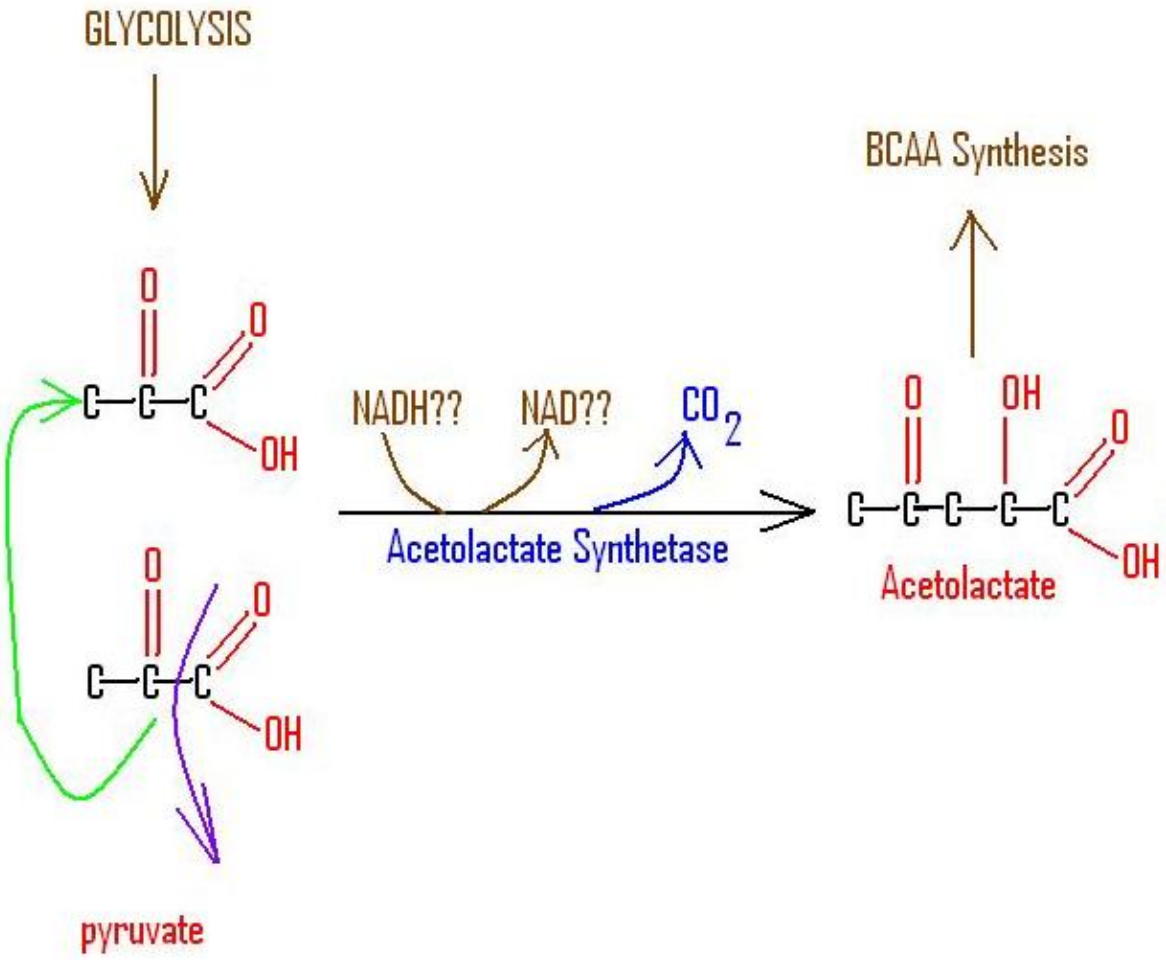
- Voges-Proskauer test is used in the identification of bacteria by identifying the presence of acetoin (3-keto-2-butanol) in the bacterial media:
- Voges-Proskauer Positive Bacteria Include:
  - E. aerogenes S. marcescens
  - K. pneumoniae
- Voges-Proskauer Negative Bacteria Include:
  - E. coli Shigella Citrobacter
  - C. freundii Yersinia
- Voges-Proskauer Inconclusive Bacteria Include:
  - Proteus

Butylene glycol (2,3-butanediol) pathway

Initiation of the butylene glycol pathway, as it's also known, derives from EMP: the formation of pyruvate.

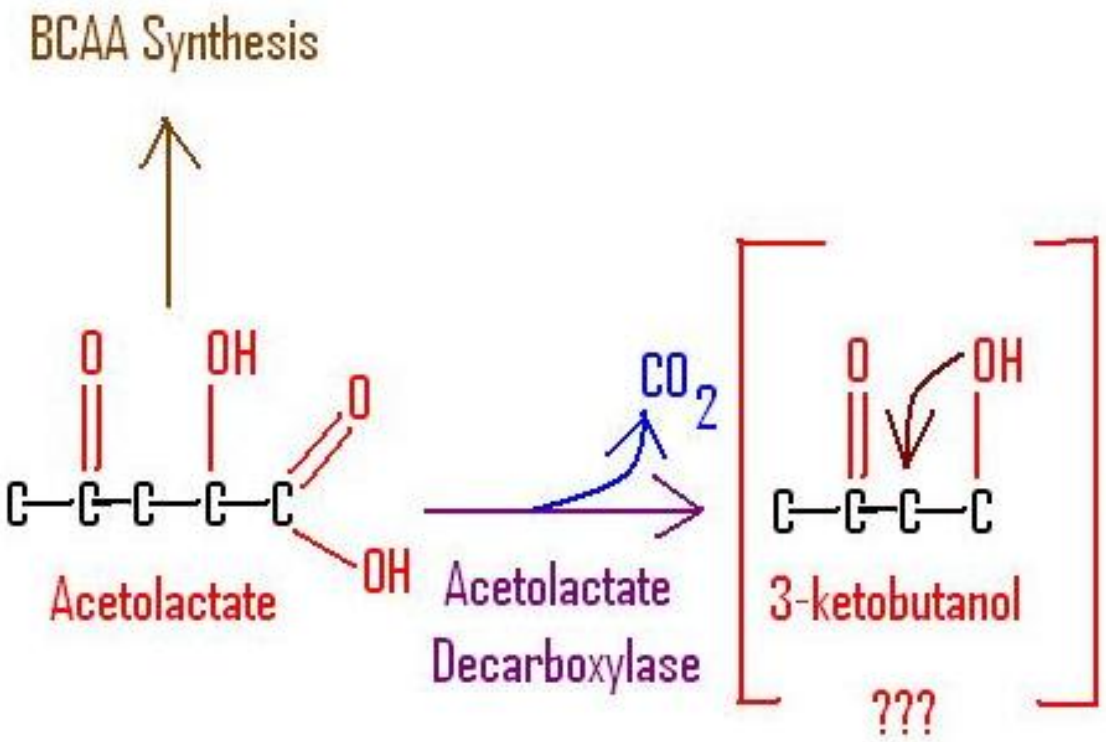
Two pyruvates are condensed with a concomitant loss of one CO_2 and the reduction of one of the ketones to an alcohol to form acetolactate.

The enzyme that catalyzes this reaction is acetolactate synthetase.



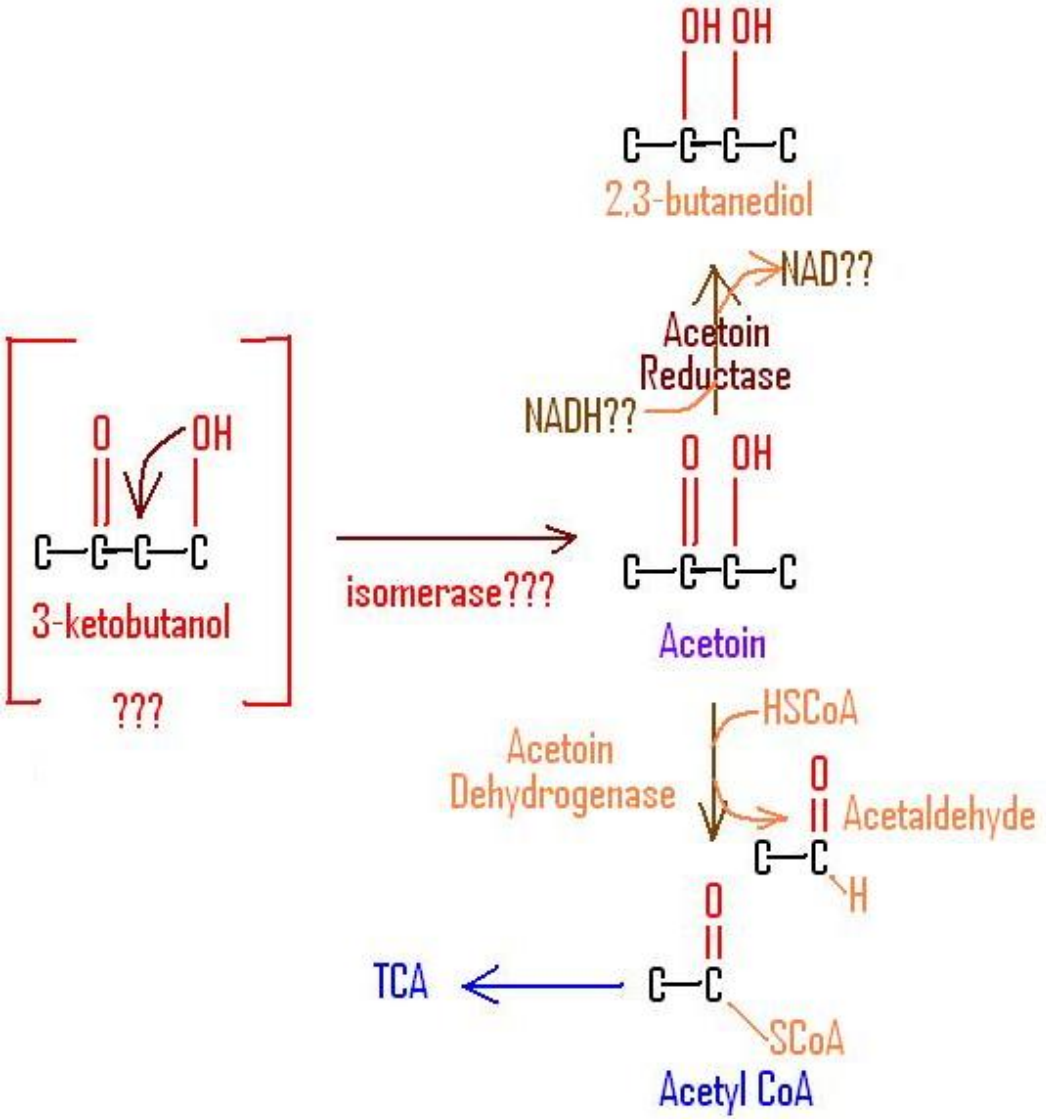
Acetolactate is commonly known as an intermediate in the synthesis of BCAA's.

If BCAA's are not needed by the prokaryote, acetolactate decarboxylase removes the carboxyl group to form [presumably] 3-ketobutanol.



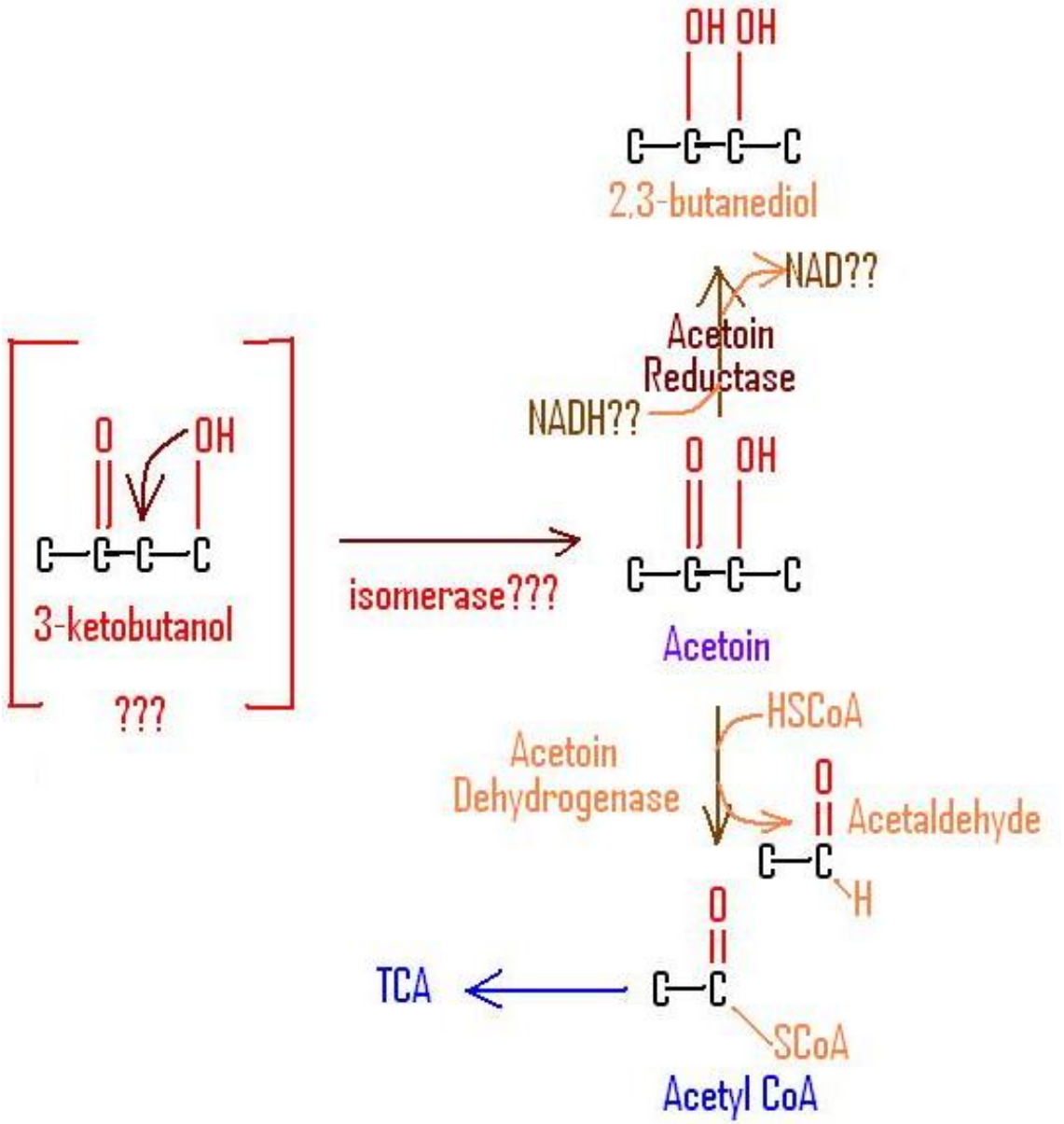
- Again, presumably,an isomerase rearranges the 1-ol to a 2-ol to form acetoin.

- It may very well be that acetoin is a branch point to regulate the bacterium or bacteria



Acetoin (top pathway) is reduced to 2,3-butanediol by, presumably, NADH.

The reaction is catalyzed by acetoin reductase.

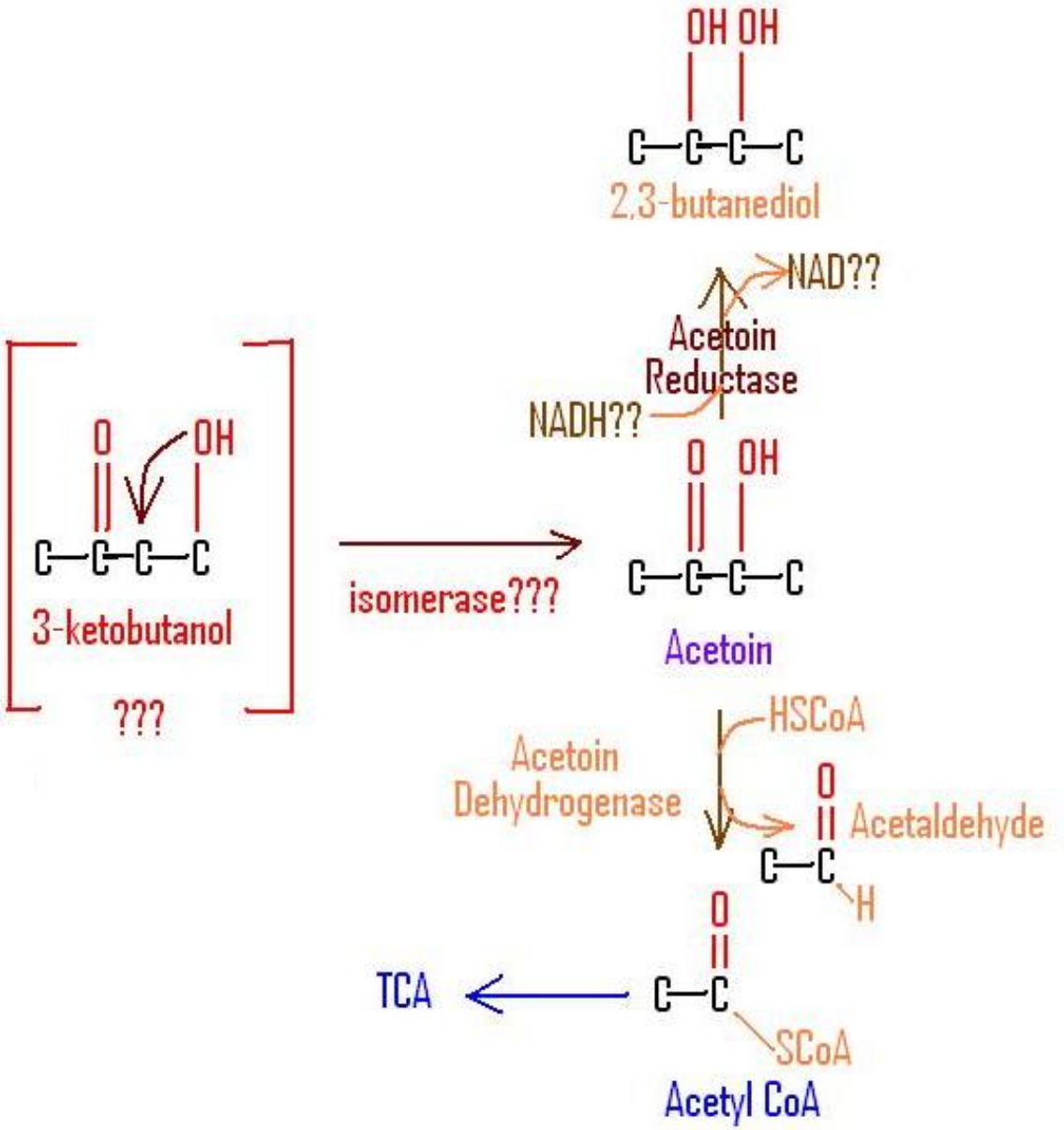


Acetoin (bottom pathway) is oxidized and thiolated to form acetyl-CoA.

The enzyme that catalyses this reaction is acetoin dehydrogenase.

An additional product formed is acetaldehyde. Its purpose is not known.

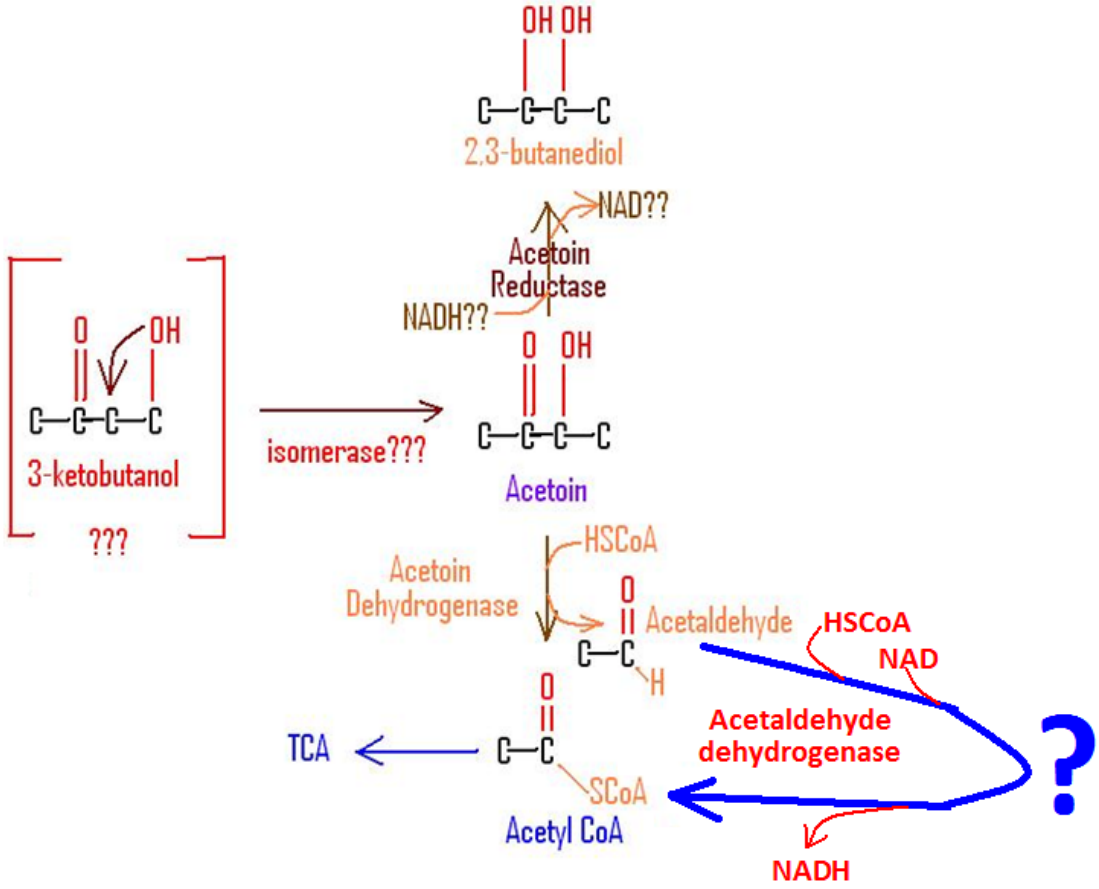
The acetyl CoA feeds into the TCA.



It seems likely that the 2,3-butanediol (butylene glycol) is probably synthesized during times when the bacteria has sources of energy and needs to regulate its population growth/decline. Top pathway.

It seems likely that the acetoin reduction to release acetaldehyde and acetyl-CoA are for when the bacterium lacks energy sources in its "diet" and uses a portion of the acetoin catabolically. Bottom pathway.

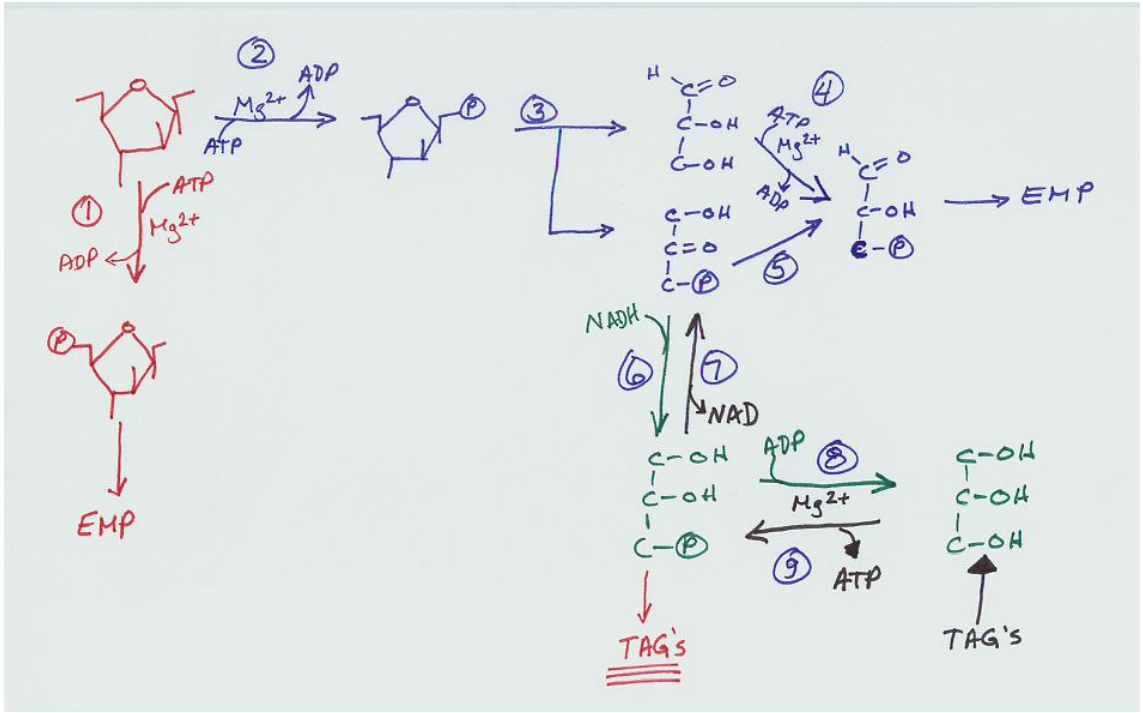
It also seems likely that the acetaldehyde that is generated is oxidized and thiolated to form more aCoA



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 follows.

Fructose Metabolism



1. Hexokinase (adipose tissue)
2. Fructokinase (liver)
3. F-1-P aldolase
4. Triose kinase
5. Triose phosphate isomerase (TPI)
6. DHAP DH
7. Glycerol phosphate DH
8. Phosphatase
9. Glycerol kinase

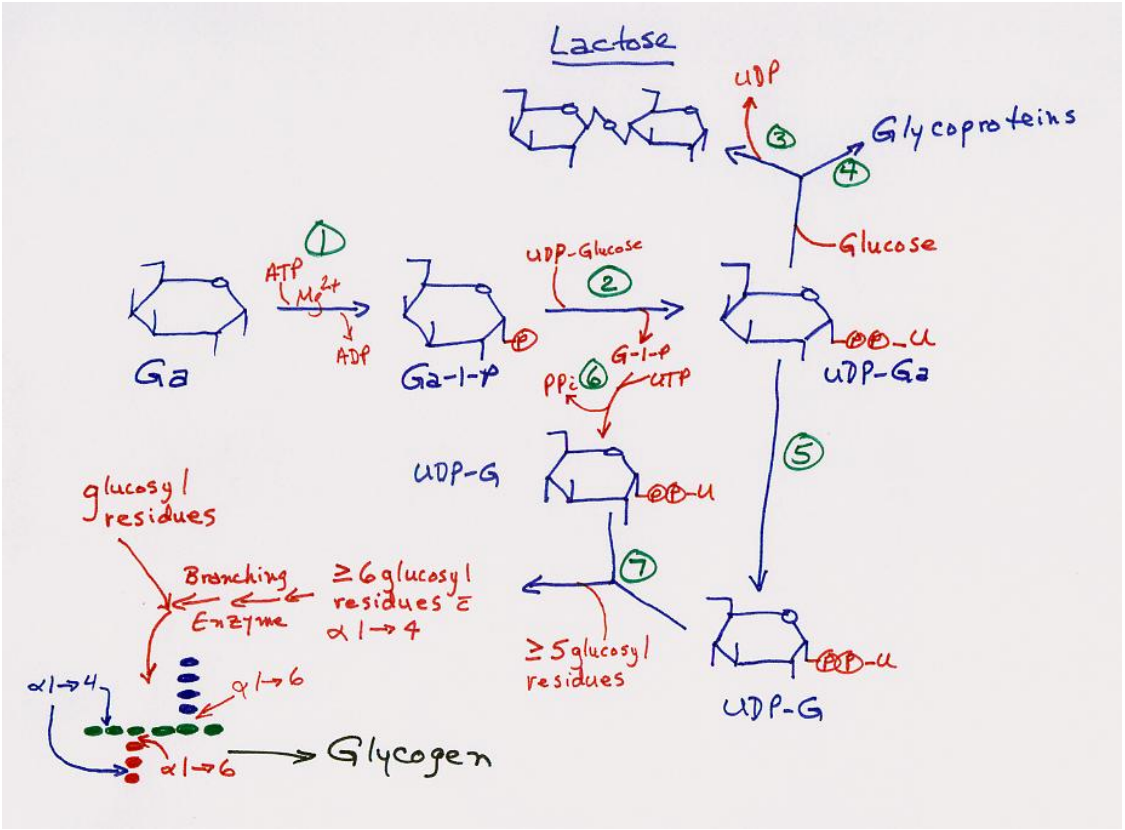
Hereditary Fructose Intolerance

- This disorder is inherited as an autosomal recessive disorder.
- There is a fructose-1-phosphate (F-1-P) aldolase deficiency.
- This disease causes hypoglycemia with increased accumulation of F-1-P in tissues.
- The patient fails to thrive and has nausea and vomiting (N/V), jaundice, an enlarged liver, which may develop into liver failure, proteins and amino acids in the urine and tyr in the urine, as well.
- Diagnostic testing is to "pre-load" the patient with fructose and observe for hypoglycemia and hypophosphatemia.
- Therapy is to discontinue cane sugar from the diet. Patients must double check over the counter (OTC) medications for sucrose additions as tablet binders.
- If the diet is discontinued, the patient has an increased risk of growth failure; on the diet, the patient will grow relatively normally.

Galactose Metabolism

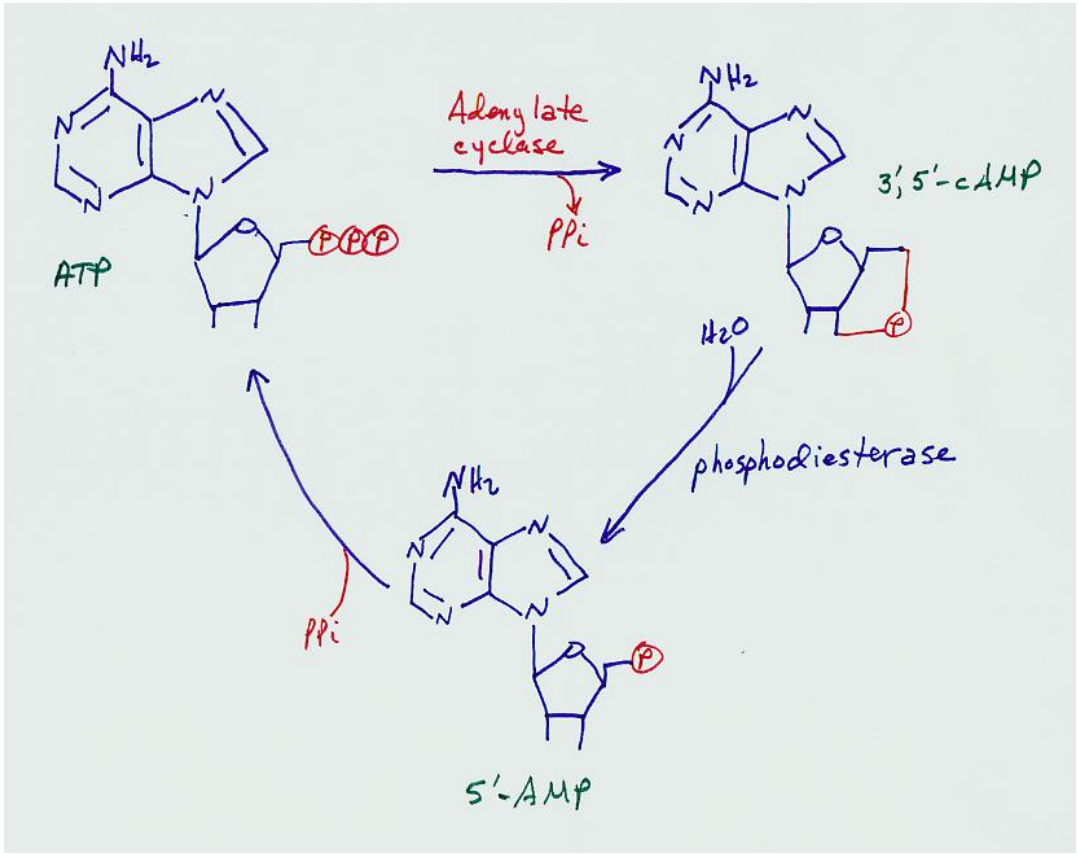
- Galactose is an important constituent of lactose,
- Galactose may be used in the synthesis of glycoproteins
- Galactose may be used in the synthesis of glycogen

Galactose Metabolism



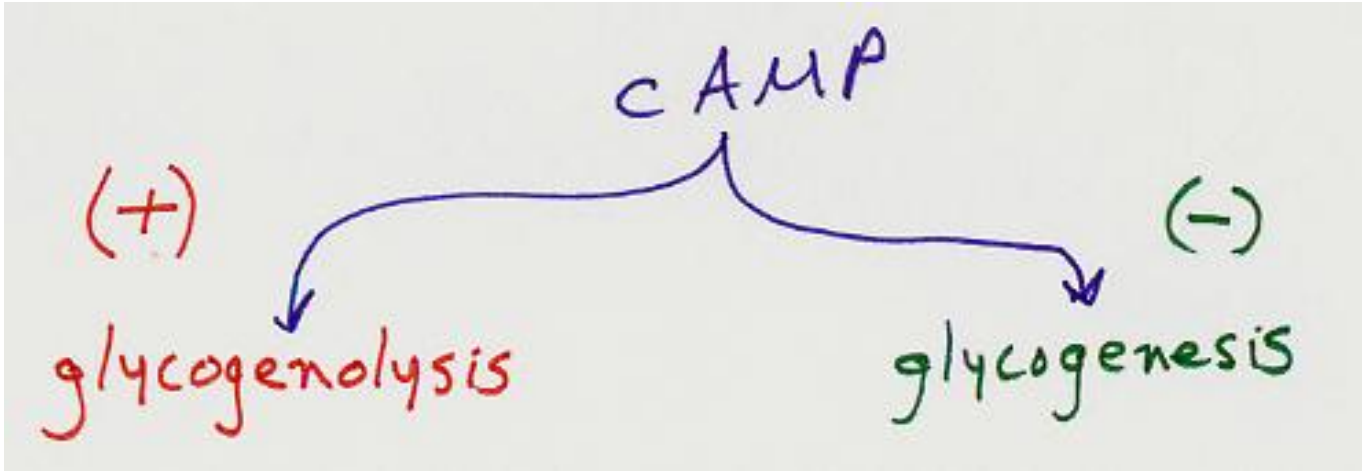
1. Galactokinase
2. Galactose-1-phosphate uridyl transferase
3. Lactose synthetase (BOTH catalytic unit and specificity protein)
4. Lactose synthetase (catalytic unit only)
5. UDP-galactose-4-epimerase
6. UDP-glucose pyrophosphorylase
7. Glycogen synthetase

Glycogen Metabolism Regulated by cAMP



- Phosphodiesterase (PDE) inhibited by:
  - Theophylline
  - Caffeine
  - Theobromine
- These compounds are called xanthines.
- When PDE is inactivated, cAMP levels build up, making it easier for patients to breathe.

In General

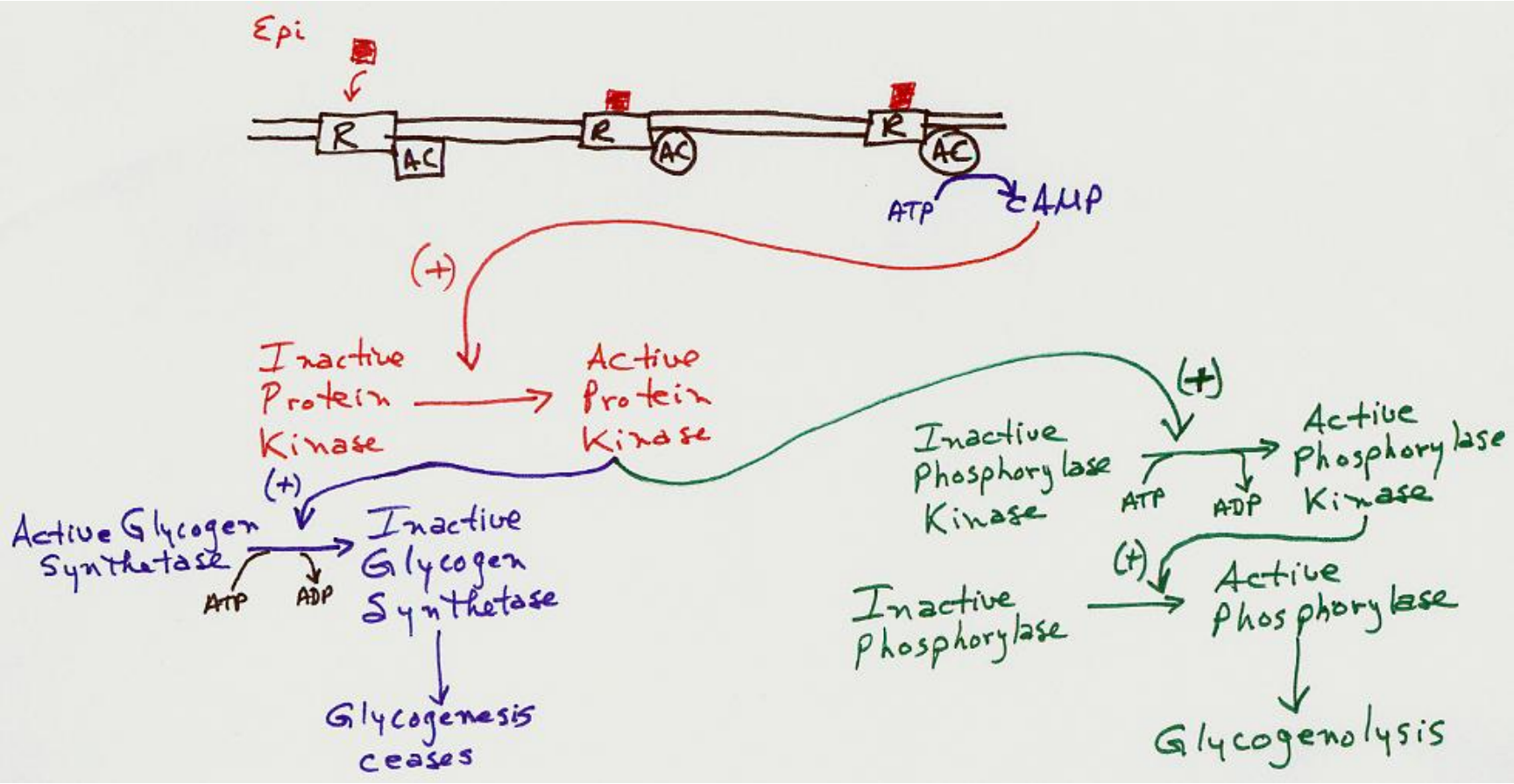


In General

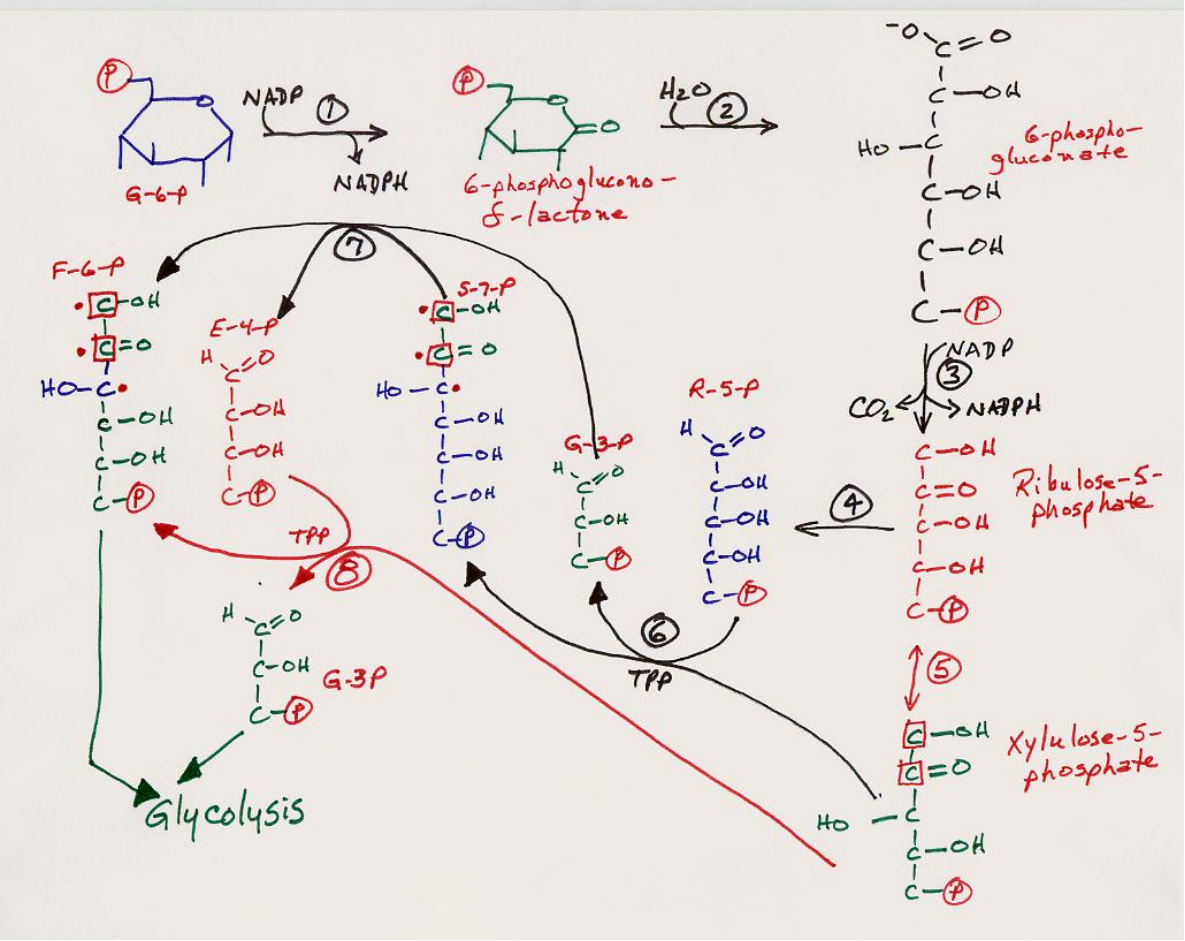
- Glycogenolysis (destruction of glycogen)
- Glycogenesis (production of glycogen)

- 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 Detail


Epi
R
AC
R
AC
R
AC
ATP
cAMP
(+)
Inactive Protein Kinase
Active Protein Kinase
(+)
Active Glycogen Synthetase
ATP
ADP
Inactive Glycogen Synthetase
Glycogenesis ceases
(+)
Inactive Phosphorylase Kinase
ATP
ADP
Active Phosphorylase Kinase
(+)
Inactive Phosphorylase
Active Phosphorylase
Glycogenolysis

Hexose Monophosphate Shunt (Pentose Phosphate Pathway)



1. G-6-PDH
2. Lactonase
3. 6-P-gluconate DH
4. Phosphopentose isomerase
5. Phosphopentose epimerase
6. Transketolase -- (2 C transfer)
7. Transaldolase -- (3 C transfer)
8. Transketolase -- (2 C transfer)

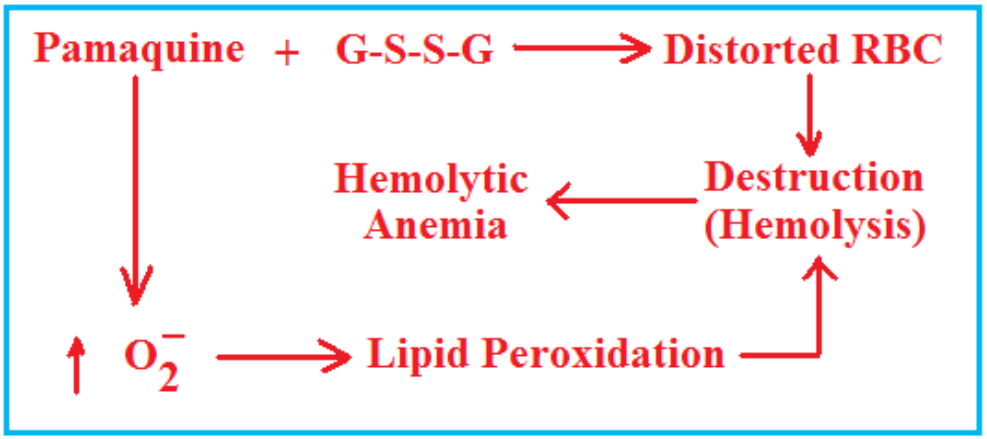
Hexose Monophosphate Shunt (Pentose Phosphate Pathway): Significance

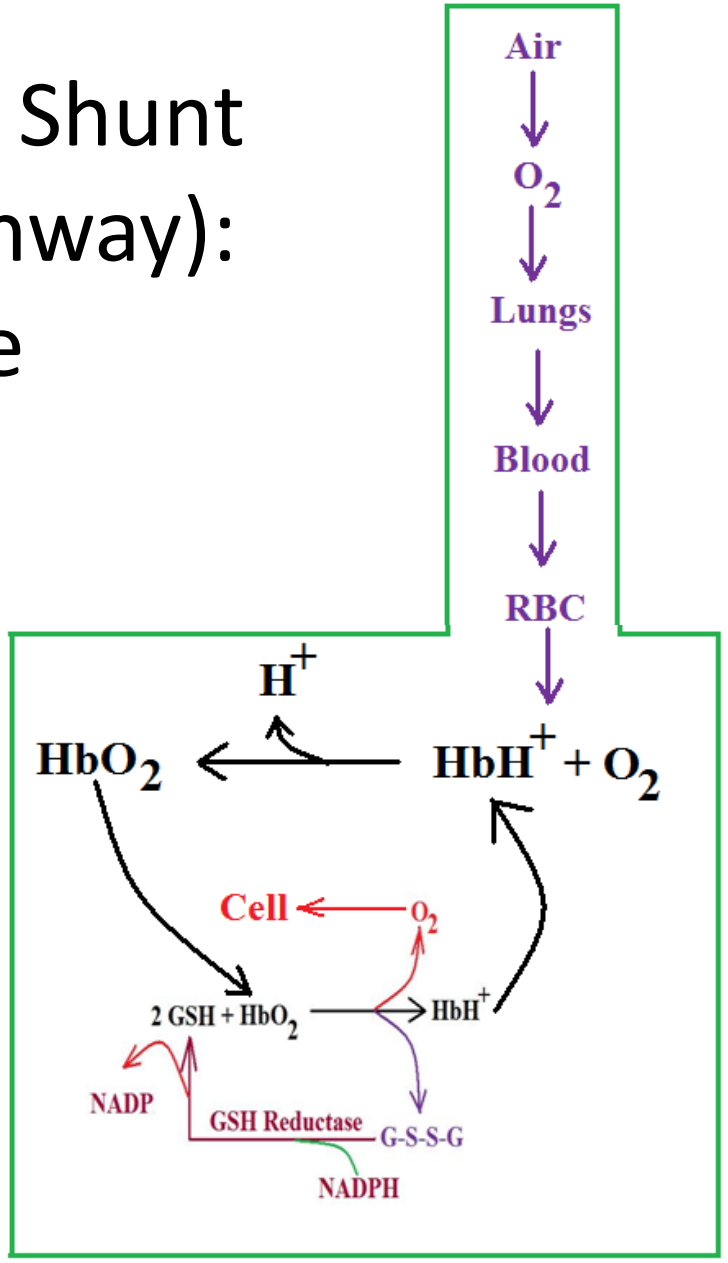
- 2 NADPH – reductive power
- Ribose – nucleoside/nucleotide synthesis

Hexose Monophosphate Shunt (Pentose Phosphate Pathway): Clinical Significance

- People with G-6-PDH deficiency don't make enough NADPH to reduce G-S-S-G and causes health problems

Hexose Monophosphate Shunt (Pentose Phosphate Pathway): Clinical Significance

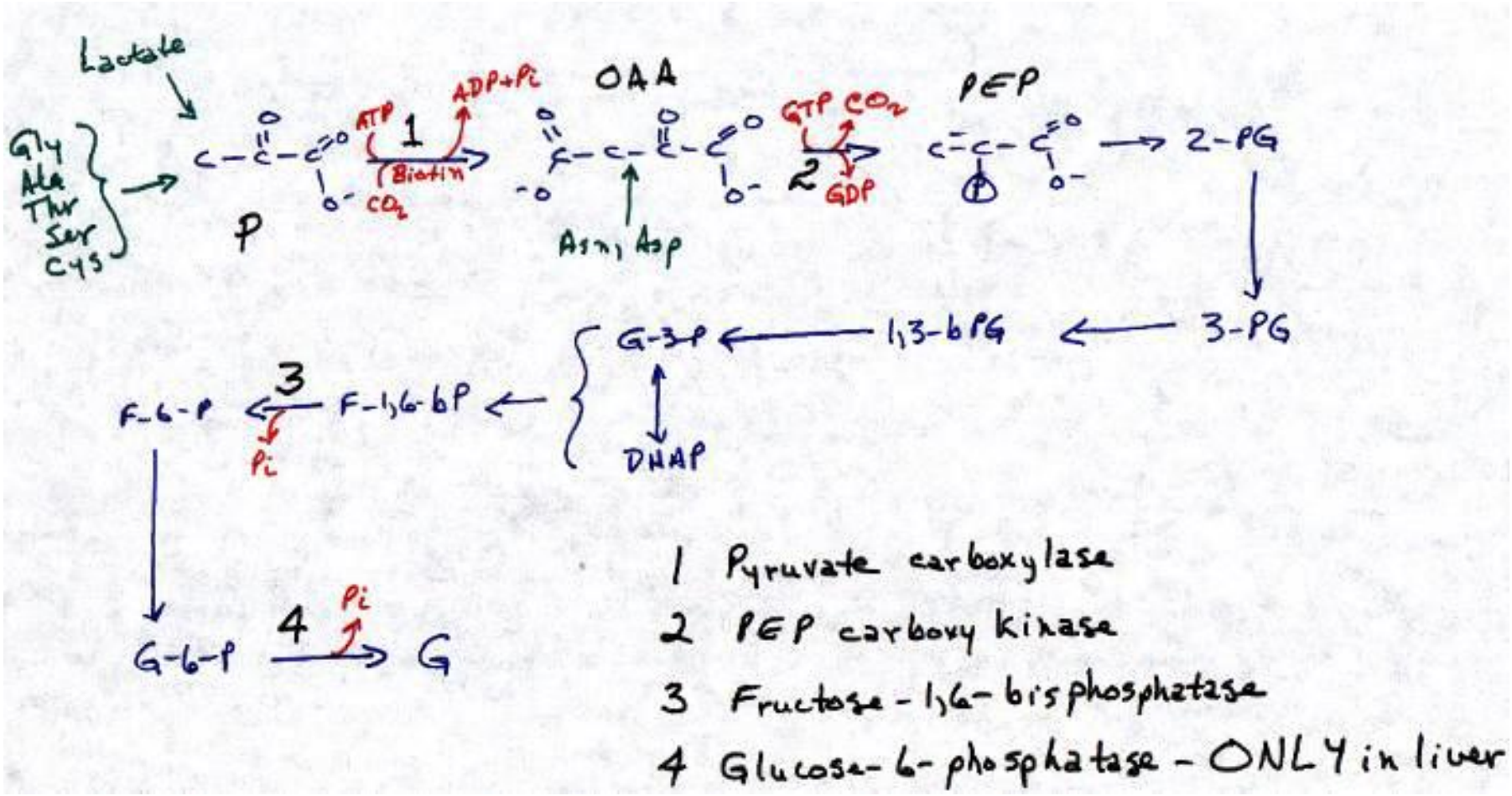




Gluconeogenesis

- 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.

Gluconeogenesis

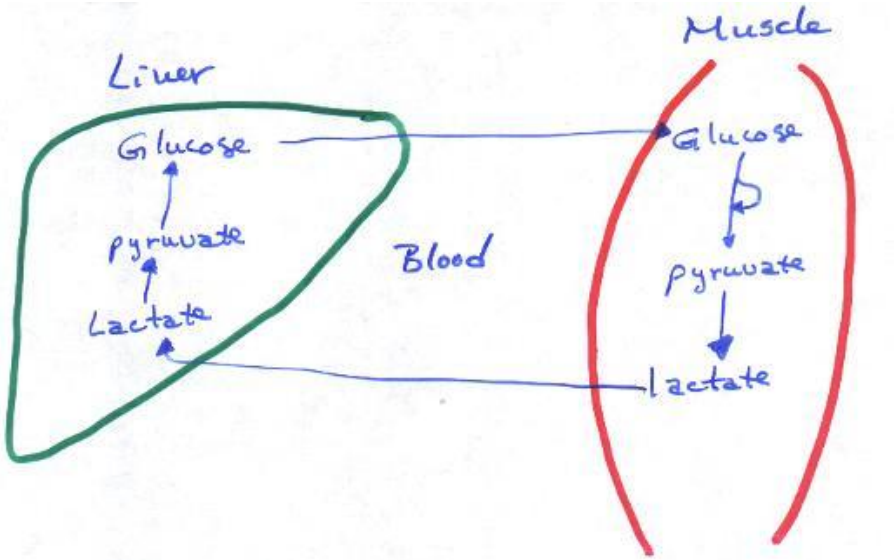


Gluconeogenesis

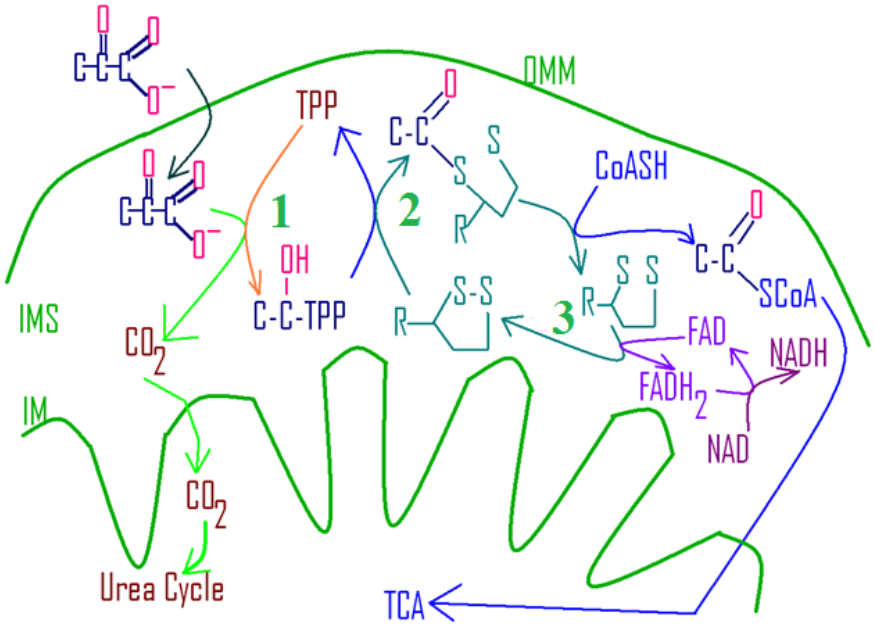
- Remember, though, that the purpose of phosphorylation of glucose in the first place is to trap it in the cell.
- Since that is a trapping mechanism, there has to be a way to remove the phosphate so that the newly formed glucose can get "dumped" into the blood.
- ONLY the liver cells contain G-6-phosphatase (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.



Pyruvate Dehydrogenase Complex -- AEROBIC



1: PDH, itself

2: Dihydrolipoyl transacetylase

3: Dihydrolipoyl dehdrogenase

NOTE: DOUBLE the Stoichiometry!

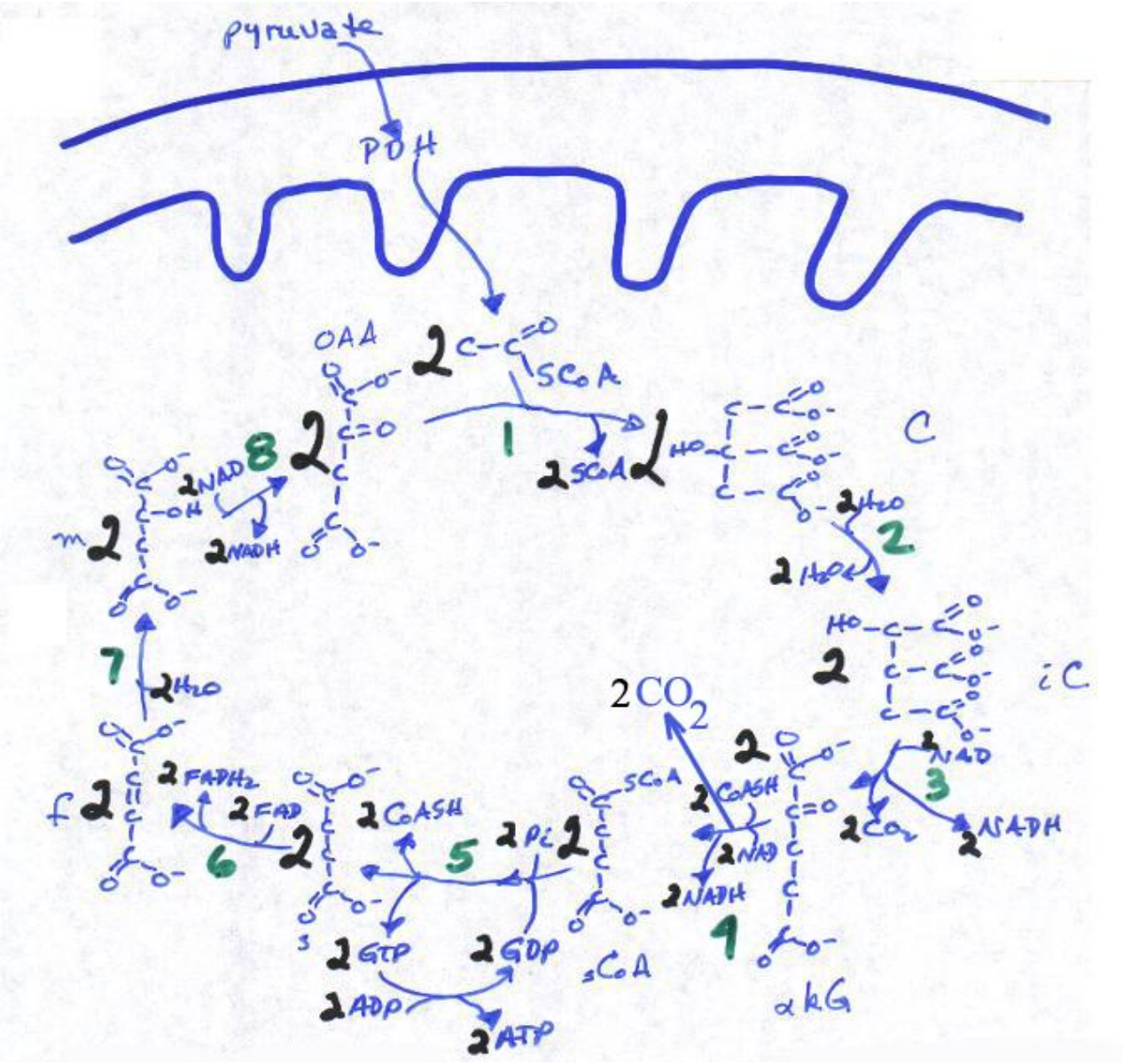
6 NADH formed!

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

Krebs' Cycle -- TCA

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

Krebs' Cycle -- TCA



1. Citrate synthetase
2. Aconitase
3. Isocitrate DH
4. αKGDH
5. Succinyl CoA synthetase
6. Succinate DH
7. Fumarase
8. Malate DH

TCA: ATP Summary – All MADE

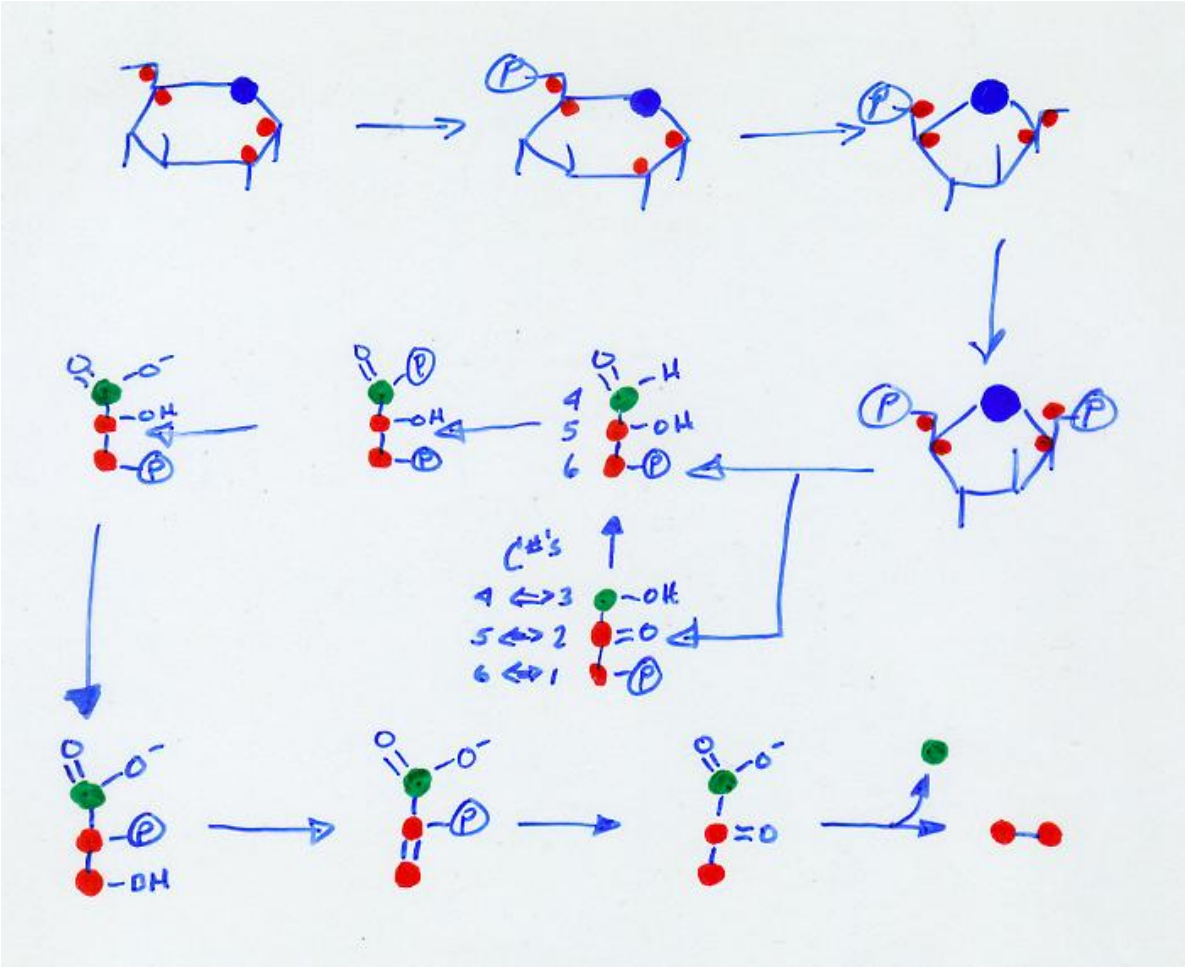
- PDH: +6 (⇔)
- iCDH: +6 (⇔)
- αKGDH: +6 (⇔)
- sCoA synthetase: +2
- SDH: +4 (⇔)
- MDH: +6 (⇔)
- TOTAL: +30
- Glycolysis: +8
- WAY TOTAL: = 38 ATP under aerobic conditions

TCA Regulators

- Energy is the **PRIME** regulator!!
- Enzyme Regulators
- Positive Effectors (stimulators)
  - ADP on iCDH
- Negative Effectors (inhibitors)
  - ATP, aCoA, NADH on PDH
  - ATP on CS
  - sCoA and NADH on α-kGDH

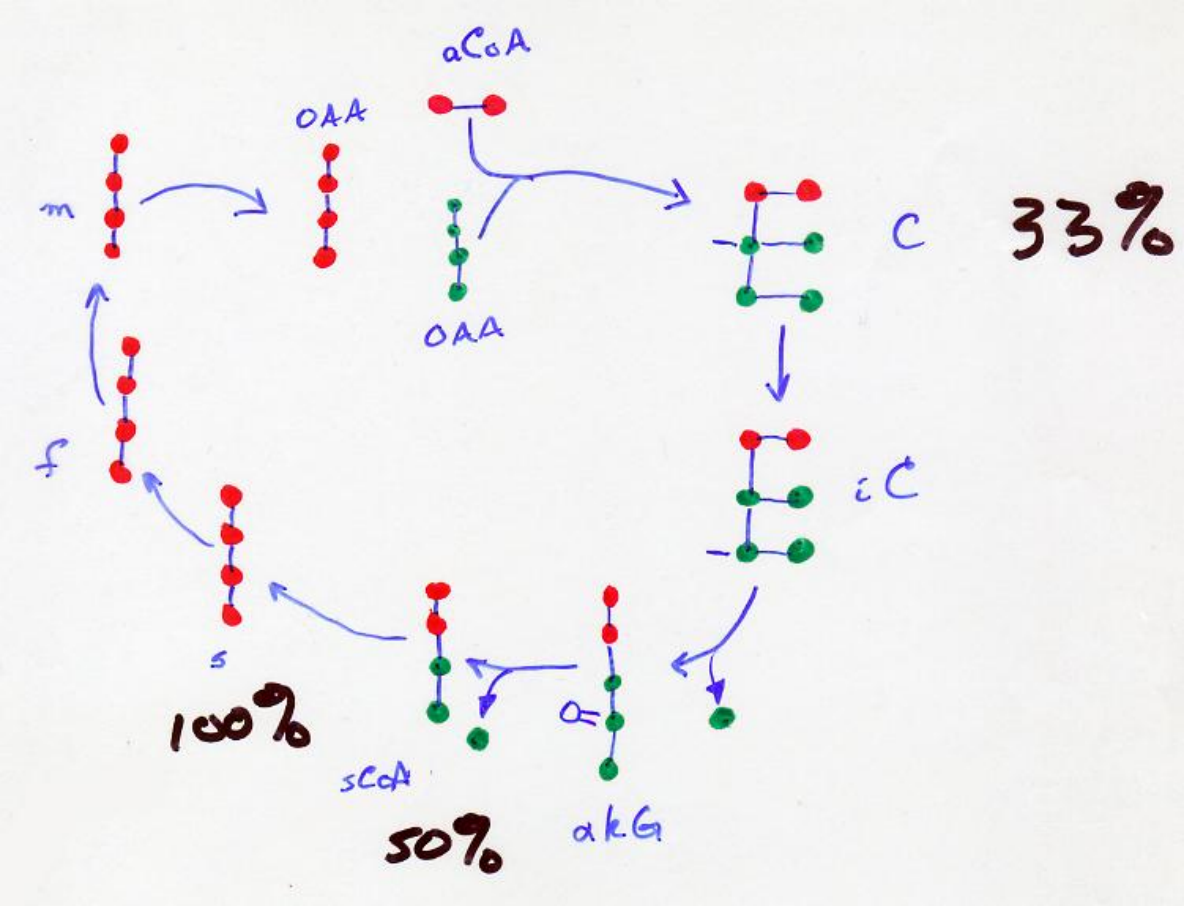
Carbon Tracing in Glycolysis

- Radioactive carbon
- Oxygen
- “Regular” carbon

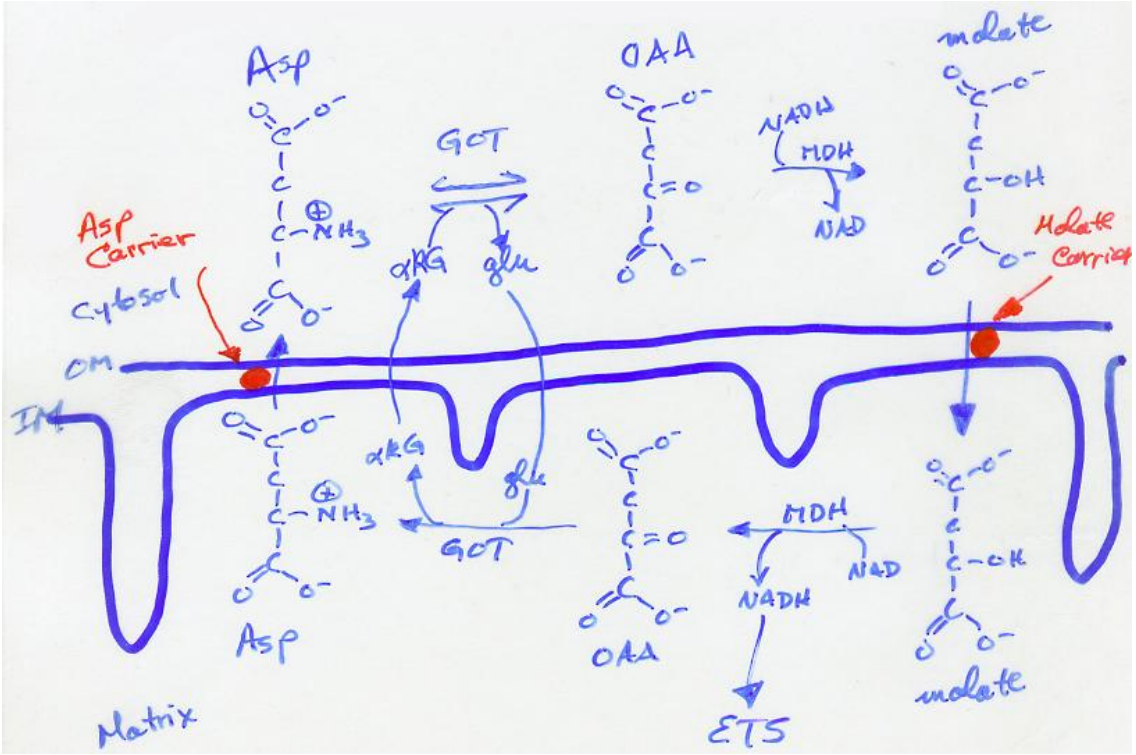


Carbon Tracing in the TCA

- "Regular Carbon
- Radioactive Carbon
- Why 100% labeling at succinate? SYMMETRY!

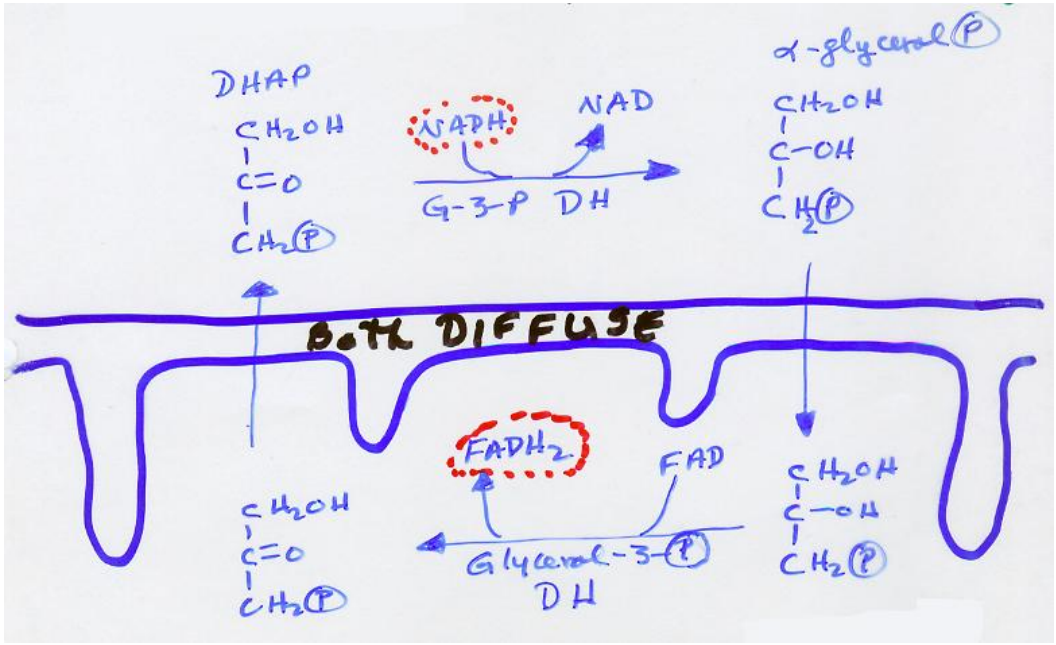


ATP Synthesis by Tissue: Clarification

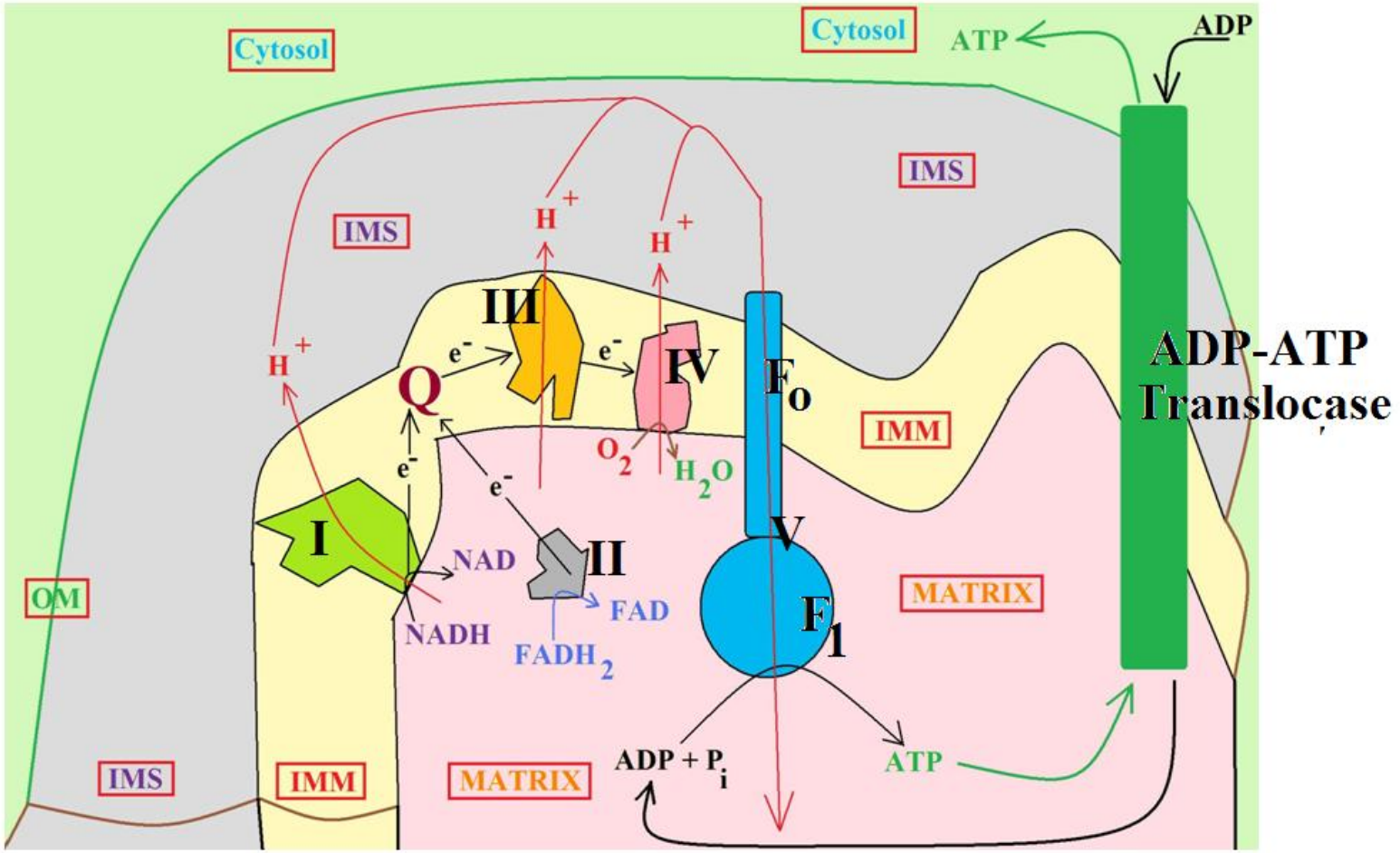


- NAD/NADH will NOT cross mito membranes – how get in there?
- **Aspartate-Malate Shuttle!**
- In HEART and LIVER = 38 ATP from aerobic glycolysis and TCA
- Note: OAA won't cross mito membrane, either

ATP Synthesis by Tissue: Clarification

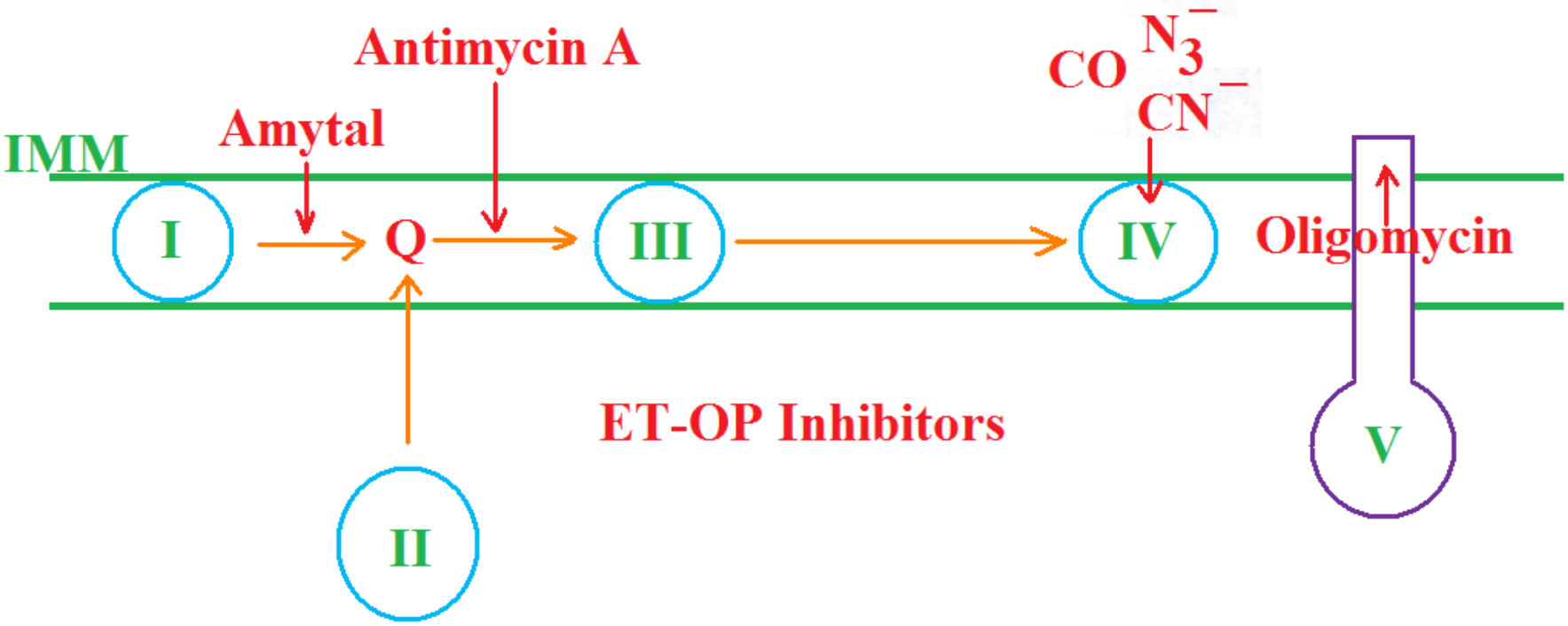


- Any other way? YES!!!!
- **Glycerol phosphate shuttle** in SKELETAL MUSCLE
- Has an effect on ATP production because NADH = 3 ATP and $FADH_2$ = 2 ATP
- Hence, 36 ATP produced in aerobic glycolysis and TCA in MUSCLE



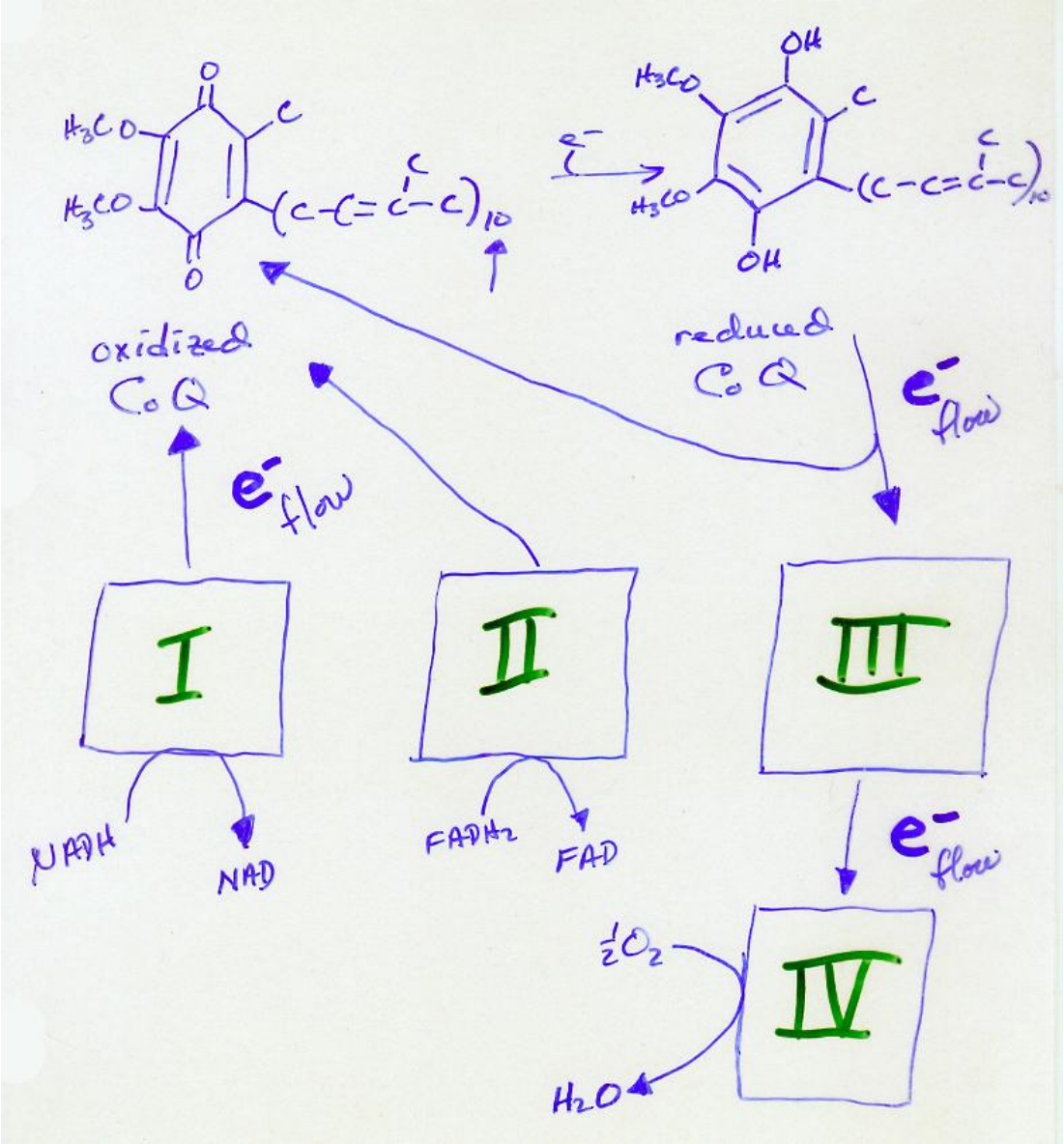
- In order to fully understand why
  - one mole of NADH is worth 3 ATP molecules and
  - why one mole of $FADH_2$ is worth 2 ATP molecules,
- we need to discuss how each is
  - oxidized by electron transport (ET)
  - to form ATP through oxidative phosphorylation (OP; the combination of the two is called ET-OP)
  - NADH to NAD and $FADH_2$ to FAD = **Oxidative**; ADP phosphorylated to ATP = **Phosphorylation**

ET-OP Inhibitors

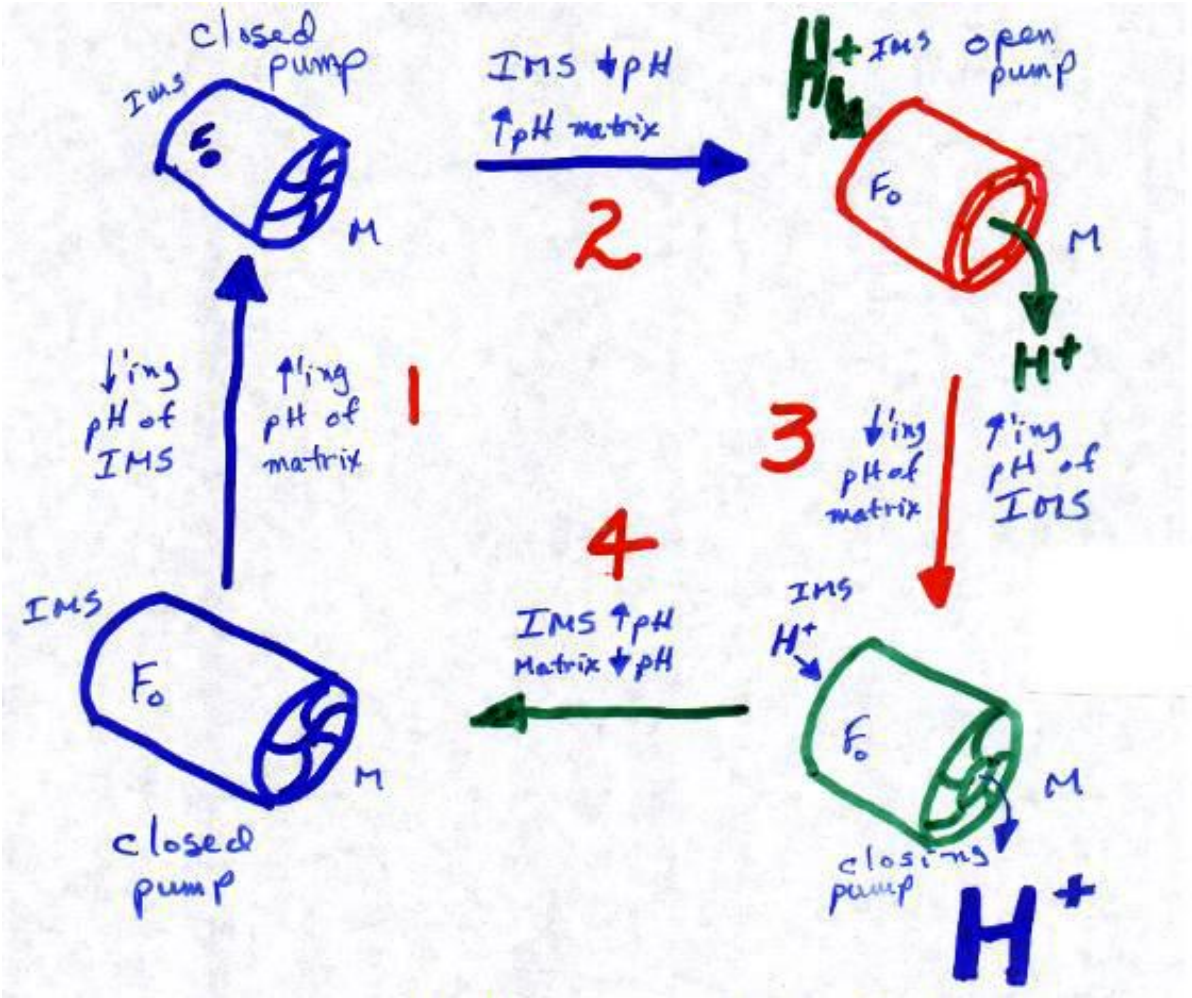


- Oligomycin inhibits the proton pump

Co-Q – Ubiquinone 10

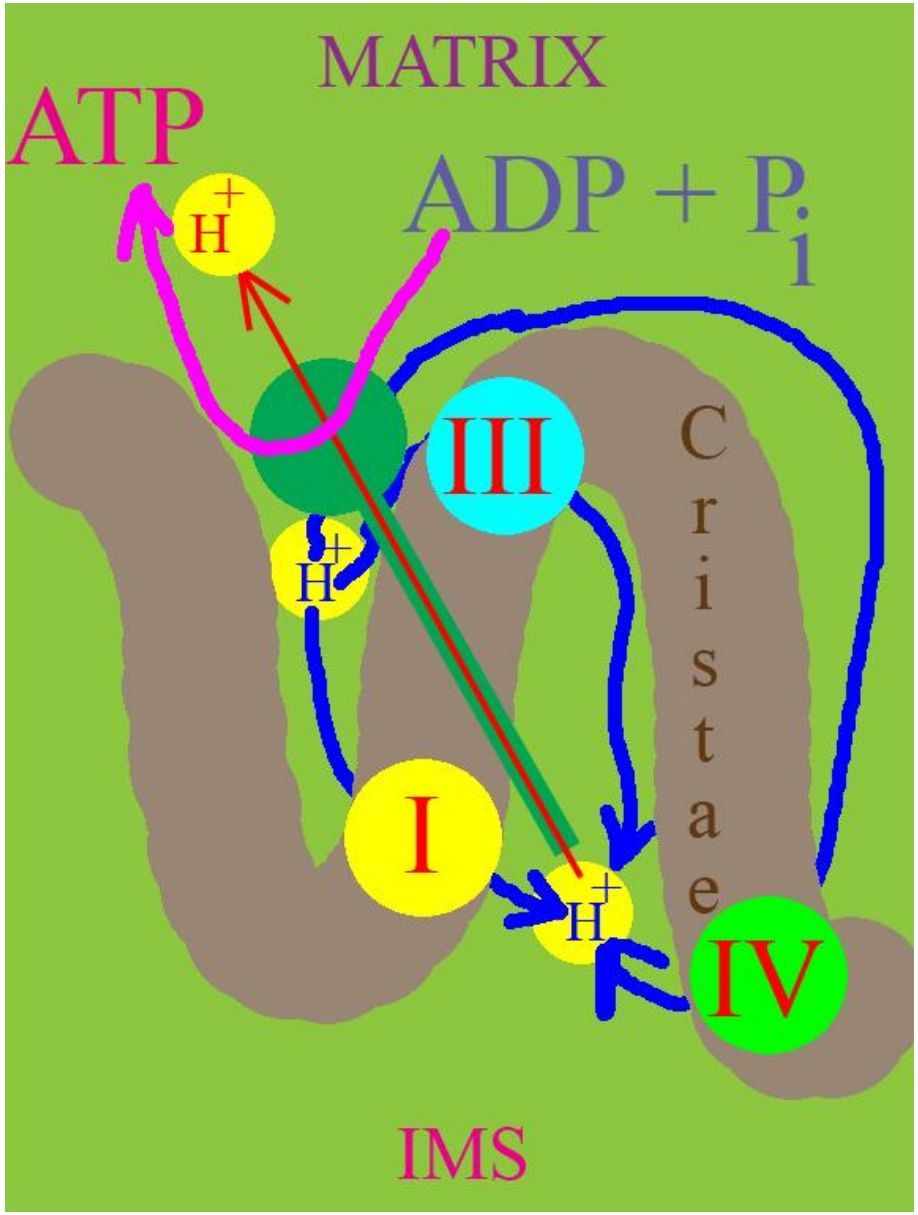


Proton Pump Functioning



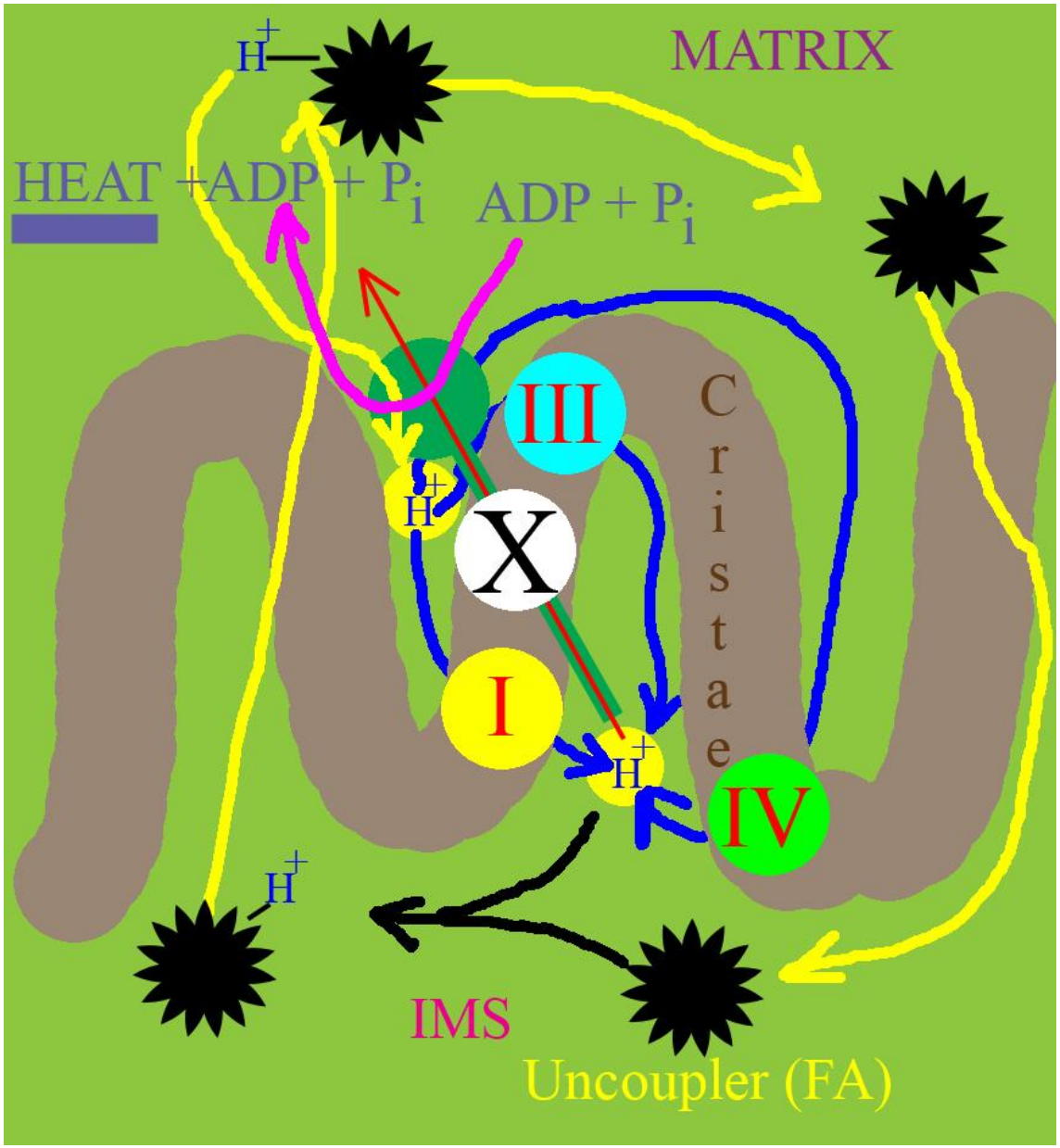
1. H^+ moving out of matrix at I, III, IV
2. pH gradient established – F_o opens and H^+ run through to drive ATP'ase
3. $[H^+]$ in IMS decreasing to point of maximal flow into matrix
4. H^+ being "recycled" and re-starting cycle – F_o fully closed

Application of Proton Pump in ETOP -- WAT



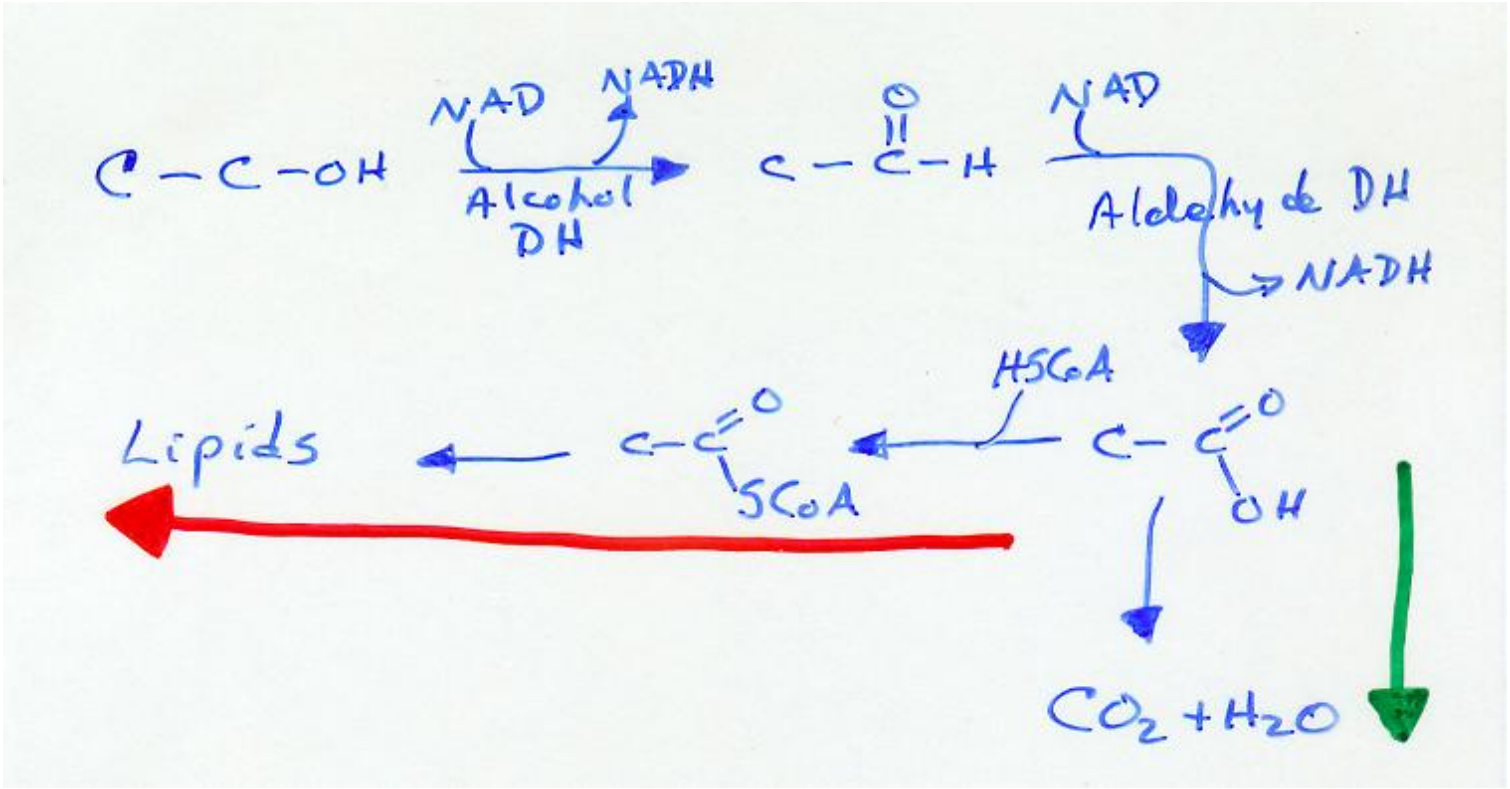
- White Adipose Tissue
- Not as many mito

Application of Proton Pump in ETOP -- BAT



- Brown Adipose Tissue
- Lots of Mito
- Thermogenesis
- T_4 and T_3 regulate
- Fatty Acids act as uncouplers
- NE regulates FA release in BAT

EtOH Metabolism



- **This sequence makes mito an incredibly REDUCED environment!**
- **With >>> ↑ NADH, this inhibits ALL NAD-requiring enzymes**
- **THEREFORE: EtOH typically used in fat synthesis rather than in oxidation**

Energy Sources from Intermediary Metabolism

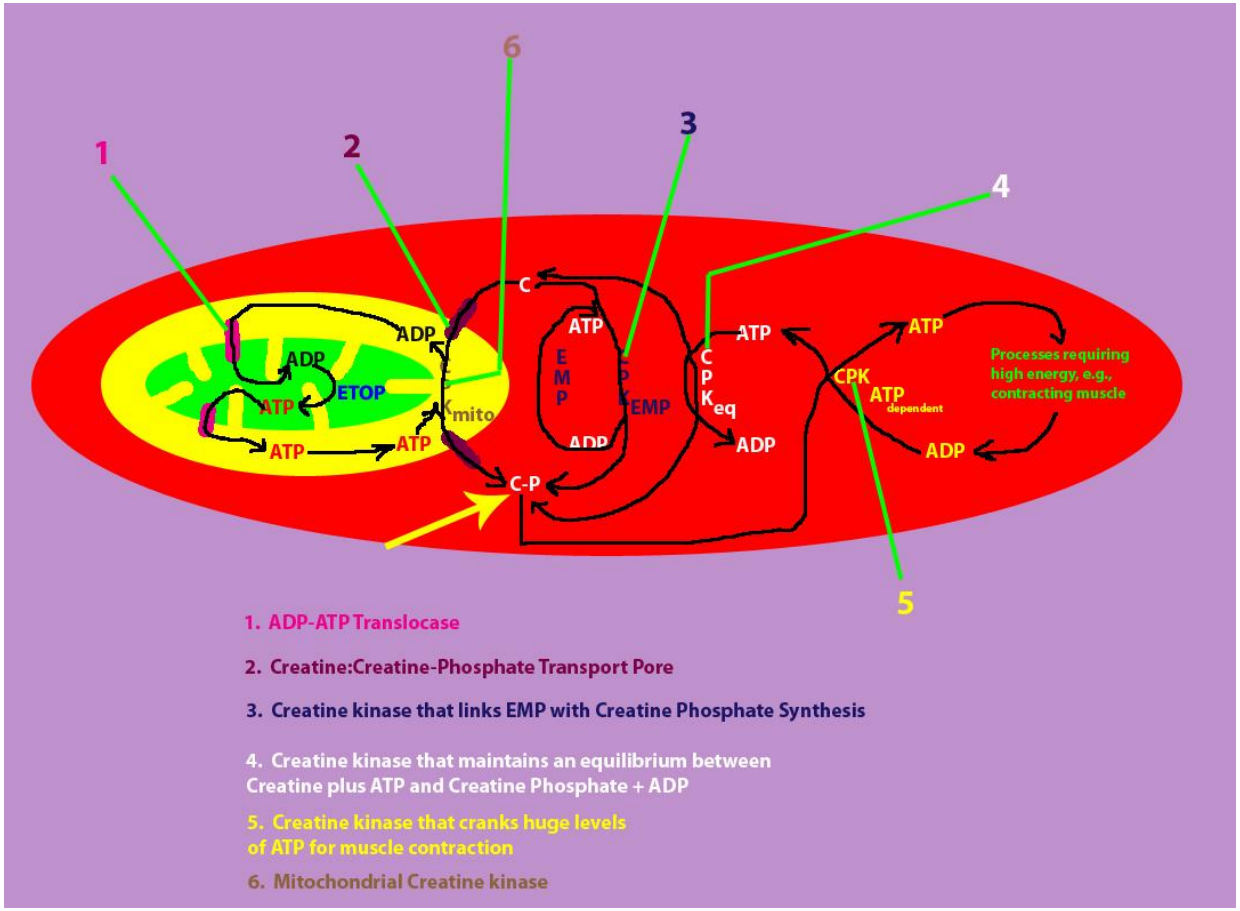
- The first system is the phosphagen system. In this system, the source of the energy is ATP. Remember that during muscular contraction, ATP is hydrolyzed to ADP, P_i and energy. When this happens, there is only enough energy for 5-6 seconds.

- So how do our cells get additional energy?

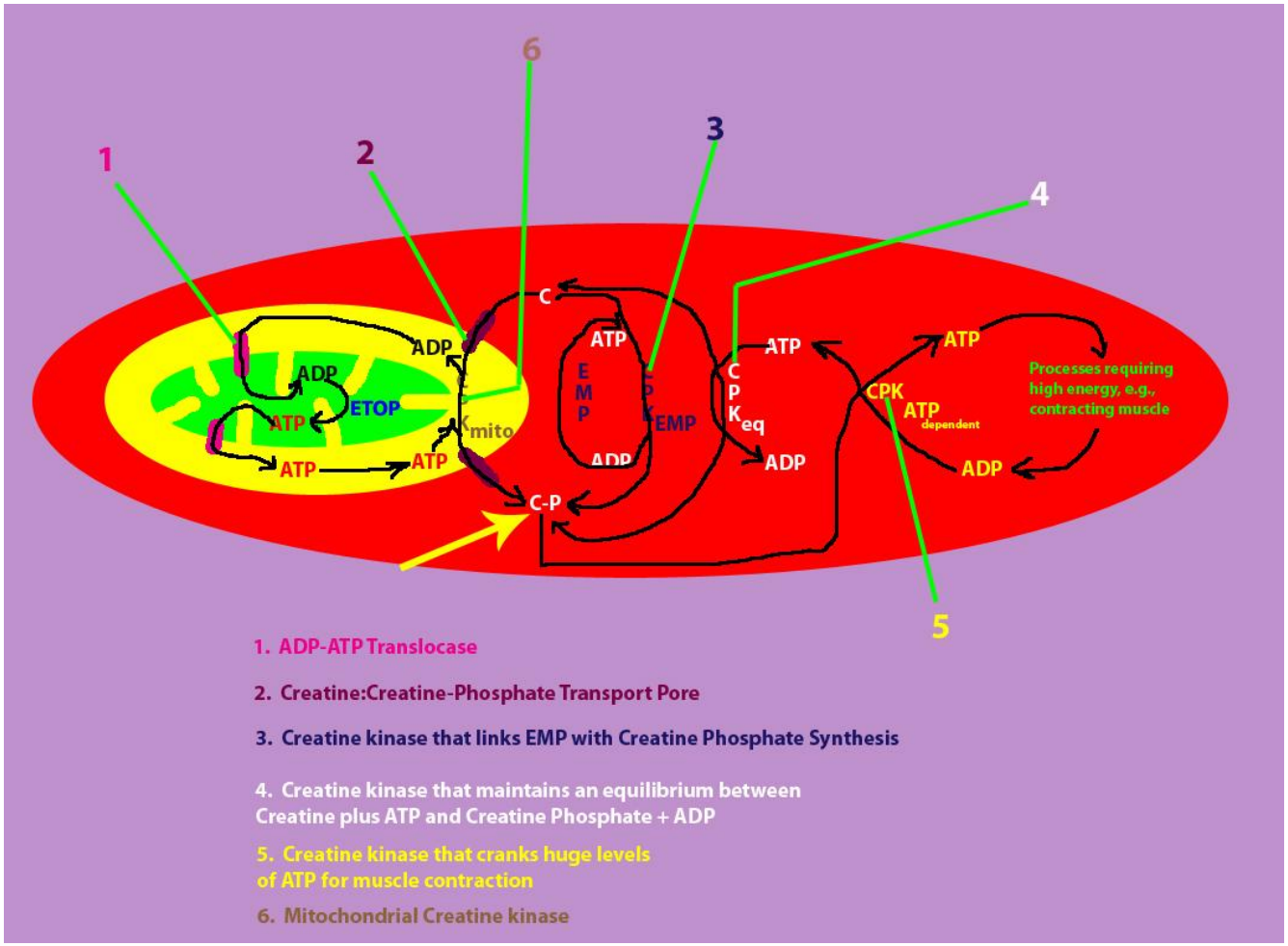
- Our cells get it via a compound called phosphocreatine (PCr). The concentration of PCr is about 2-3 times greater than the concentration of ATP. When PCr is available, it is hydrolyzed to Cr and P_i and energy. The P_i is used to re-phosphorylate ADP to make more ATP.

- This gives us about 15 seconds of maximal contractions and is used for short bursts.

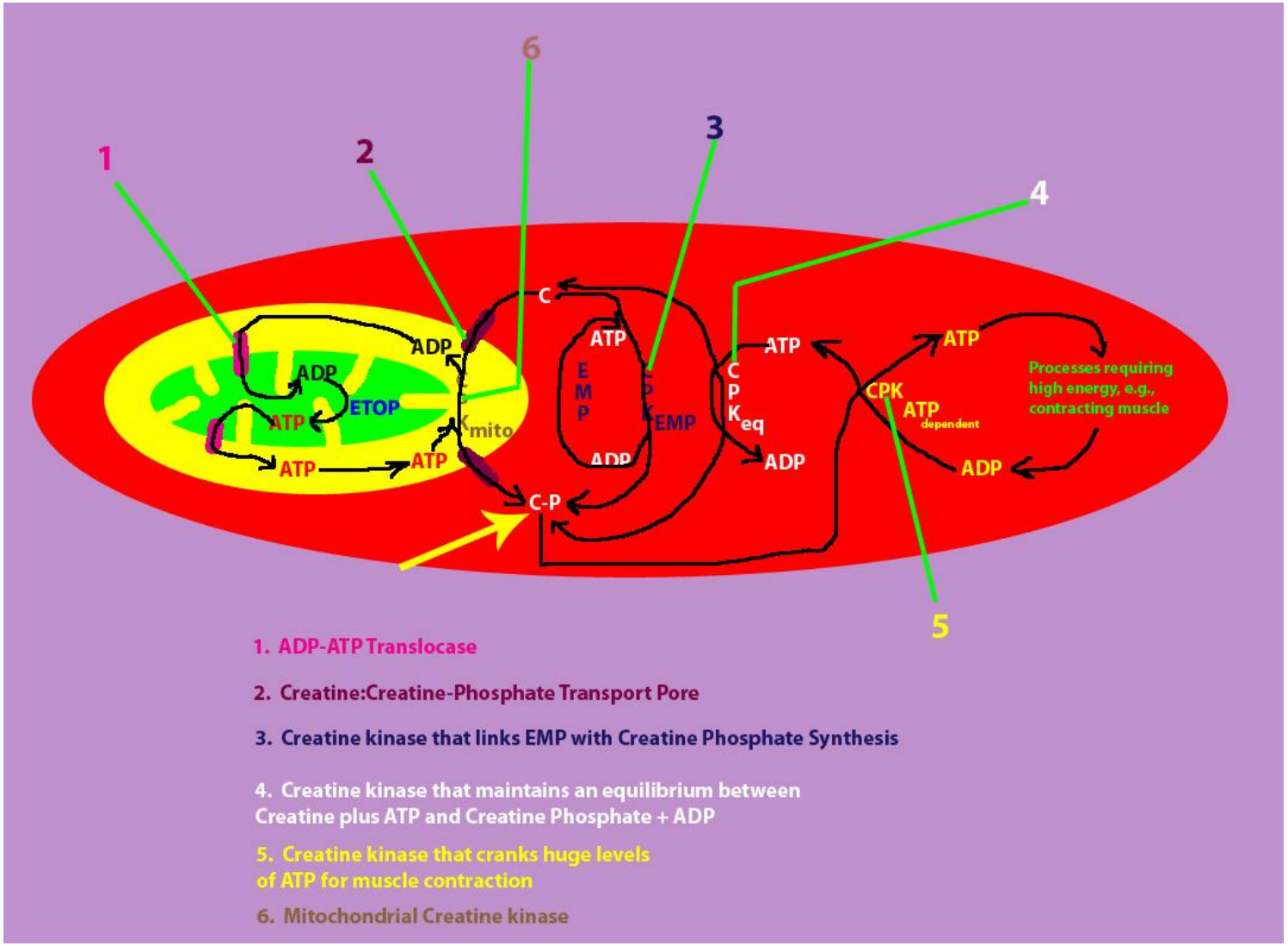
PCr Shuttle – Quick View



- 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. 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.



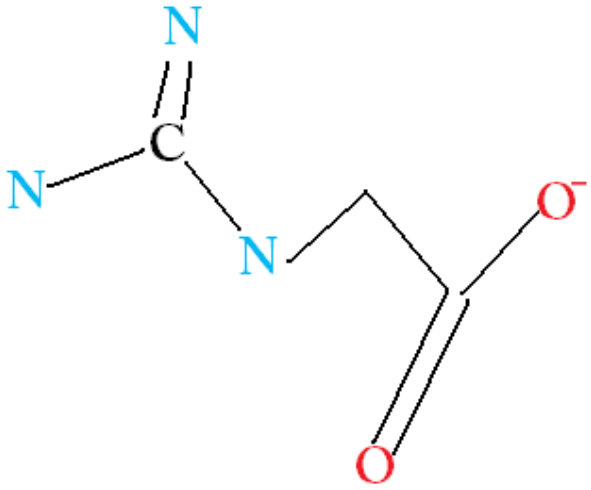
- The cytosolic PCr store is derived from an EMP-linked (glycolysis-linked) creatine kinase (CK) (3) and another CK that maintains the equilibrium between C and PCr (or ATP and ADP, if you prefer)(4). The utilization of the PCr from the cytosolic store occurs via an ATP dependent CK that cranks out hugely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

Of significance is that there are three isozymes
(enzymes with the same function that are found in different tissues
and are slightly different, structurally) of CK:

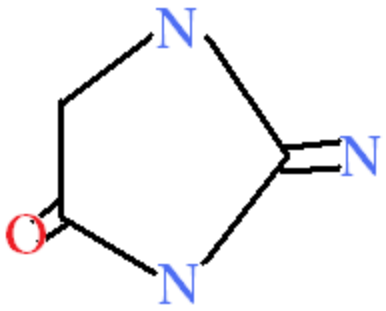
CK-BB	CK-MB	CK-MM
= CK-1	= CK-2	= CK-3
Brain, uterus, prostate, lung	Heart (and skeletal muscle)	Skeletal muscle (and cardiac, too)

Creatine, Urea, TCA and Malate-Aspartate Shuttle Interconnections

1. Creatine: 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.

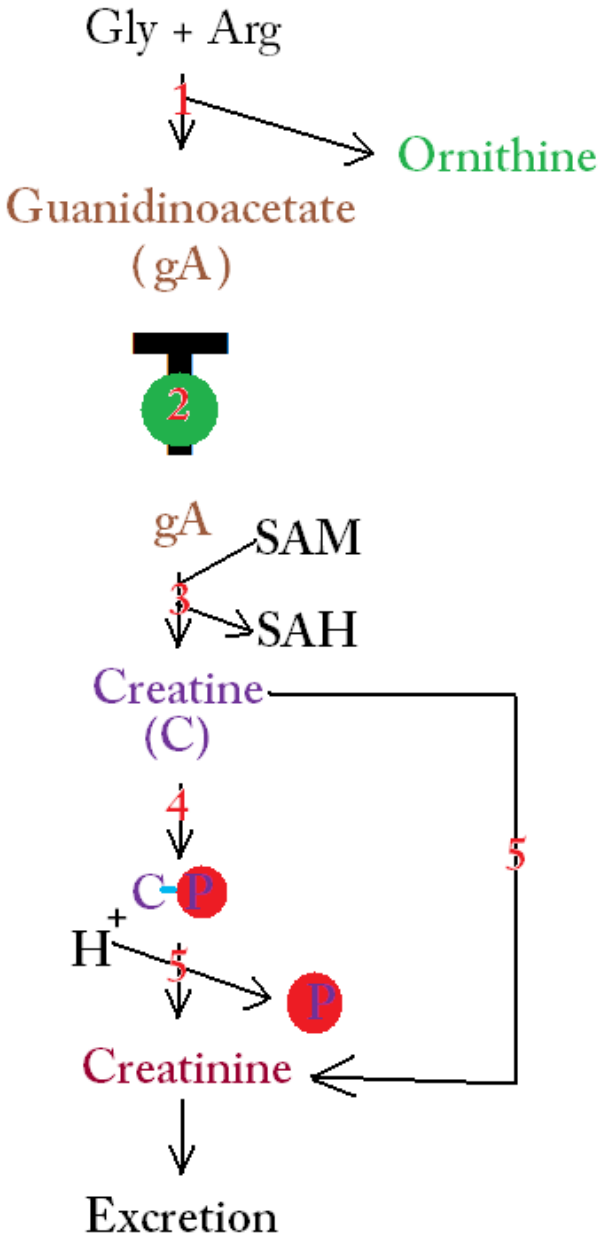


2. Creatinine: is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). Creatinine is chiefly filtered by the kidney, though a small amount is actively secreted. There is little-to-no tubular reabsorption of creatinine. 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.

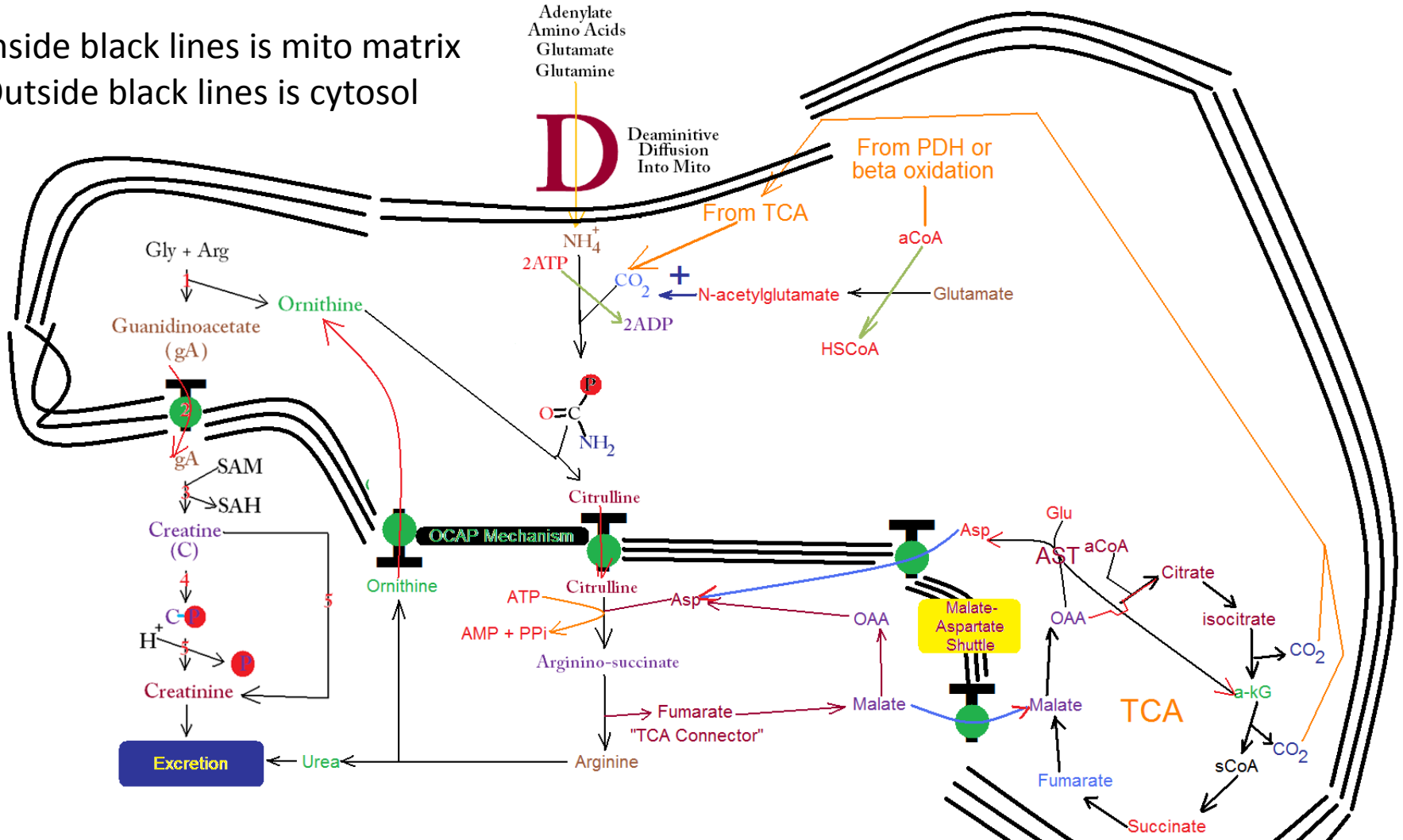


Creatine/Creatin**IN**e

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**



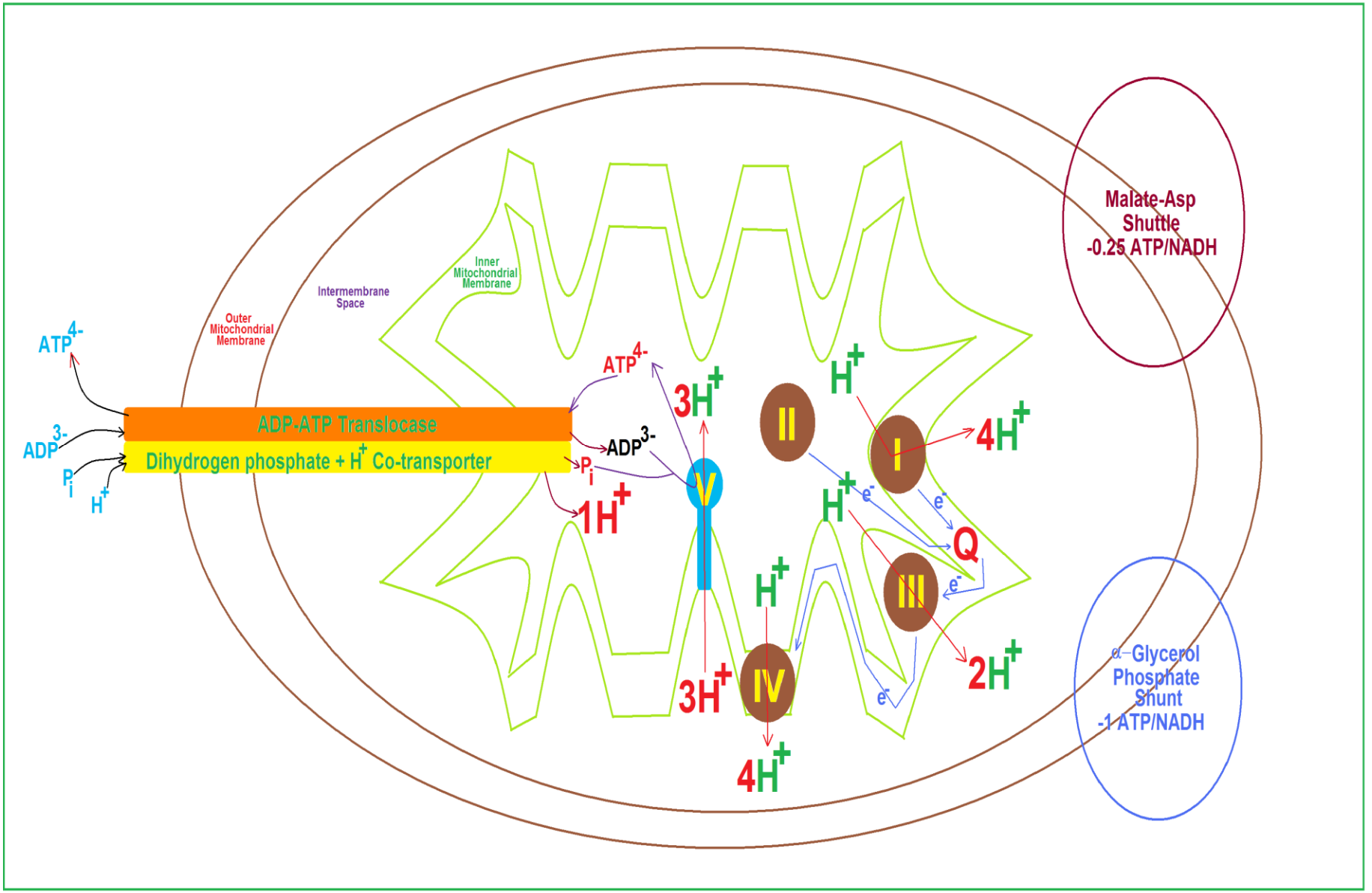
Inside black lines is mito matrix
Outside black lines is cytosol



N-acetylglutamate is an obligatory “on switch” for CPS-I.

Note relationships between Urea cycle, TCA, Creatine Synthesis and Asp-Malate Shuttle.

ATP Fine Tune



NADH: through ETS

- ADP-ATP Translocase coupled with Dihydrogen phospate/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
- 10 protons exported per NADH to make an ATP

$FADH_2$: through ETS

- ADP-ATP Translocase coupled with Dihydrogen phospate/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
- 6 protons exported per $FADH_2$ to make an ATP

Complex V and Co-Transporter

- 3 protons required to turn on F_o
- 1 proton co-transported with P_i
- 4 protons imported to synthesize 1 ATP molecule

Currently Accepted Stoichiometry

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

$$\frac{\#\,ATP}{1\,FADH_2} = \frac{\frac{6\ protons}{FADH_2}}{\frac{4\ protons}{ATP}} = 1.5\,\frac{ATP}{FADH_2}$$

Suggested Source: http://www.life.uiuc.edu/crofts/bioph354/ATP_2e_ratios.html

ATP Fine Tune – Part 2

Type I Muscle Fibers

- Red Fibers
- Slow Twitch
- For Endurance
- Small Diameter
- Aerobic
- Lots of Mitochondria
- Malate-Aspartate Shuttle
- Liver, Heart, Kidney
- Back Muscles, too

Type II Muscle Fibers

- 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

Special Topic

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:

$$CO_2(g) + H_2O(l) \rightarrow H^+ + HCO_3^-$$

- (Remember the trick with phenolphthalein and blowing into it during titrations in CHEM 121 Lab?)

Carbon Dioxide: CO_2

- 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:

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

- IMPORTANT in acid/base balance

- CO_2 origin from metabolic activity of the cells – TCA!!!
- Normal breathing and cellular metabolism cause a steady state of CO_2 anabolism and catabolism
  - Alter breathing, then alter CO_2 content
- Approximately 250 mL O_2 produces approximately 200 mL CO_2
- Divide latter by former and get an RQ of about 0.8
  - RQ = Respiratory Quotient, a measure of metabolism
  - About as efficient as a gasoline engine

Buffers

- Consist of a weak acid and its conjugate base.
  - In this case: H_2CO_3 and HCO_3^-
- The acid-base pair serves to maintain an essentially constant pH.
- The acid-base pair can NOT regulate excessive amounts of acid/alkaline substances secondary to a pathology of respiration or of metabolism.

Buffers, Cont'd

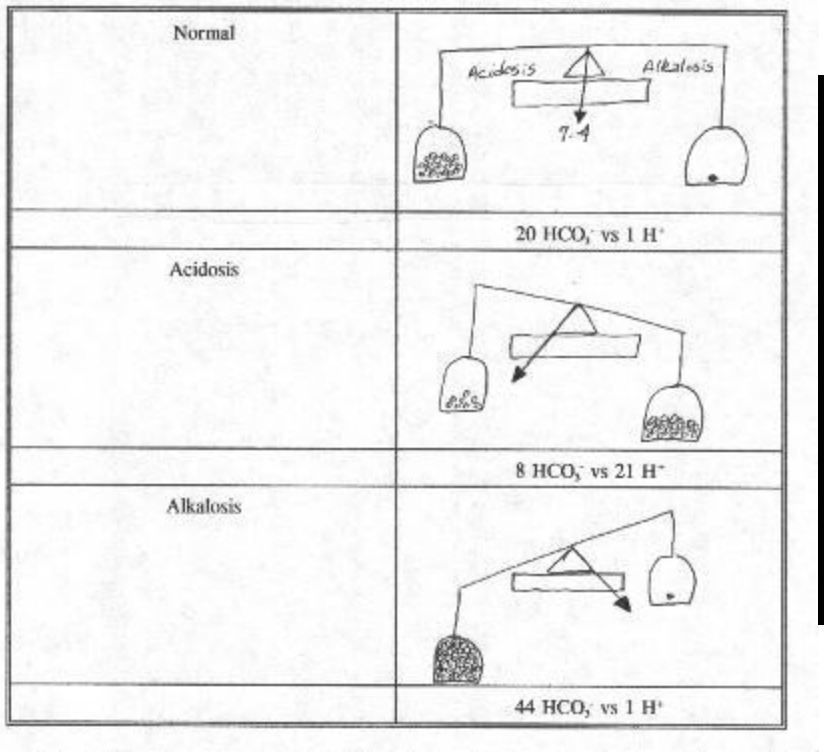
- Buffers work to:
  - Neutralize excess acid to elevate (raise; elevate) the pH
  - Neutralize excess base to reduce (lower) the pH
  - End result: keep pH within normal (7.35-7.45 in human arterial blood) limits under normal circumstances
- This is simply a physiological TITRATION!!
- It just doesn't use phenolphthalein.

- Under normal conditions, the bicarbonate to proton ratio is about 20 to 1 and the hydrogen ion concentration may be calculated by multiplying 24 times the ratio of pCO_2 to the bicarbonate ion concentration:

$$[H^+] = 24 * \frac{pCO_2}{[HCO_3^-]}$$

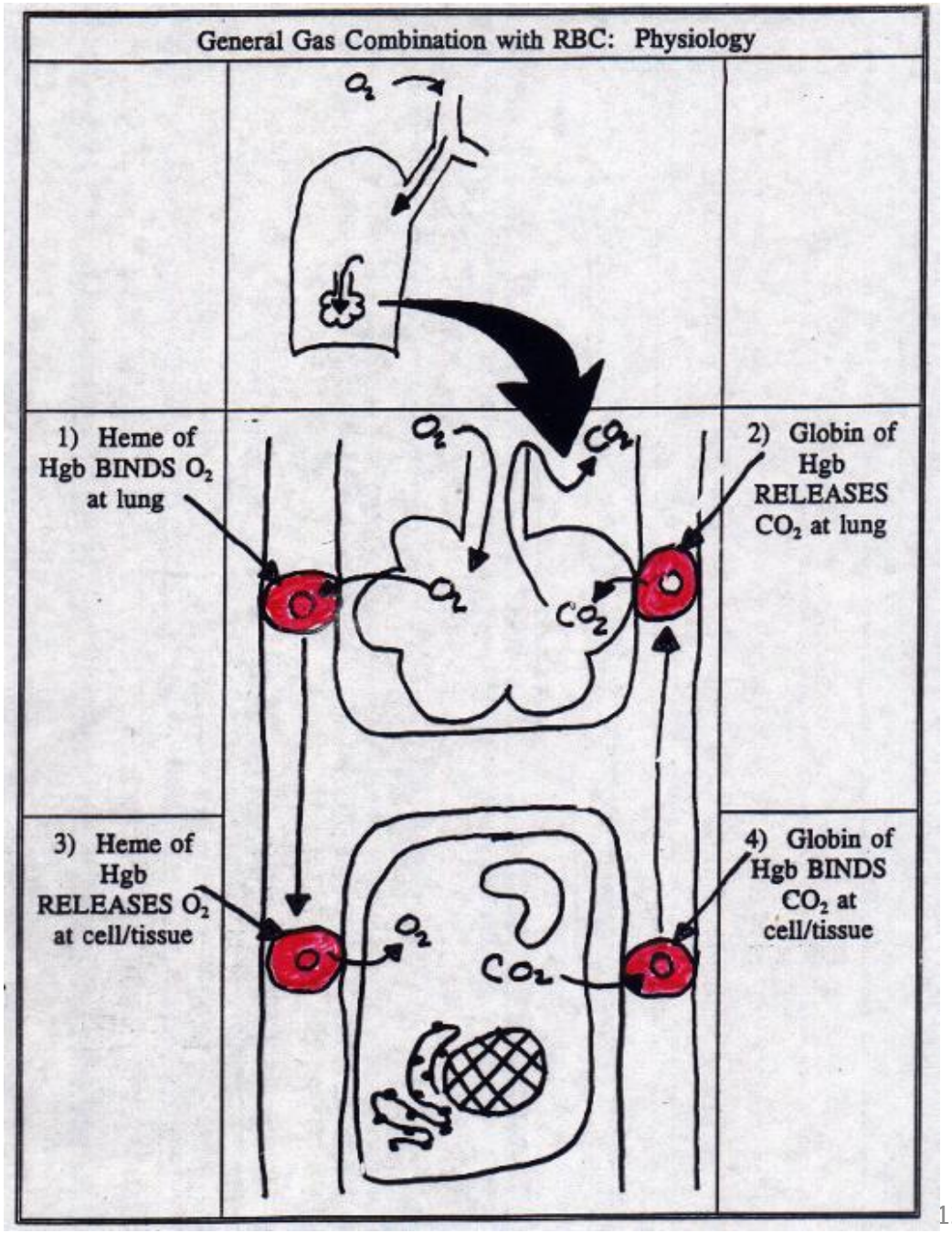
- That means, then, that the pH is proportional to the ratio of the pCO_2 (the respiratory contributor to pH balance) to bicarbonate (the metabolic contributor to pH balance) ion concentration.

Bicarbonate, Protons and Their Relationship



Condition:	Acidic	Normal	Alkaline
Bicarbonate to proton ratio:	8 to 21	20 to 1	44 to 1
Shifts to:	Left making the blood acidic	7.35-7.45 or normal balance	Right making the blood alkaline

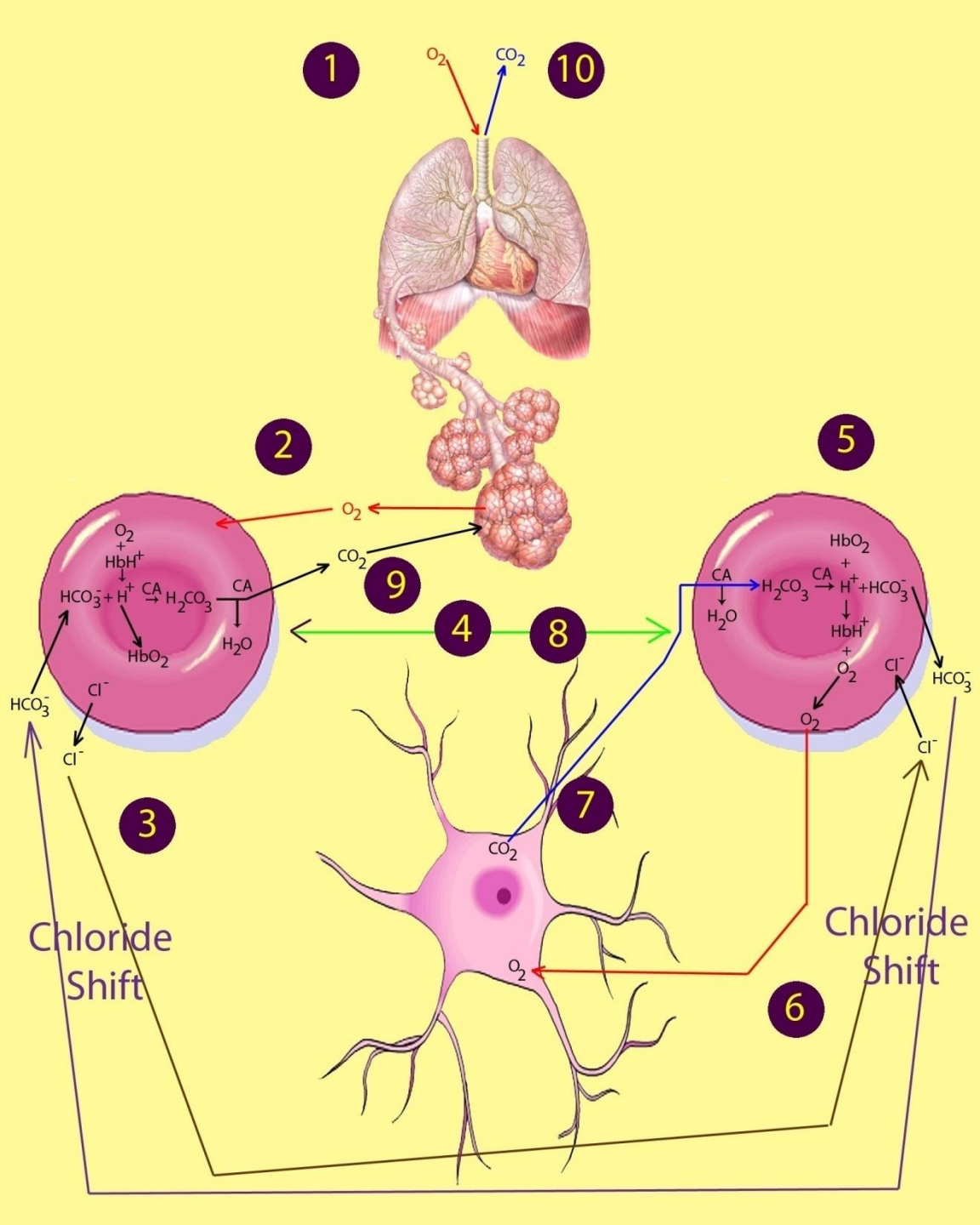
Red Cells and Acid-Base Balance



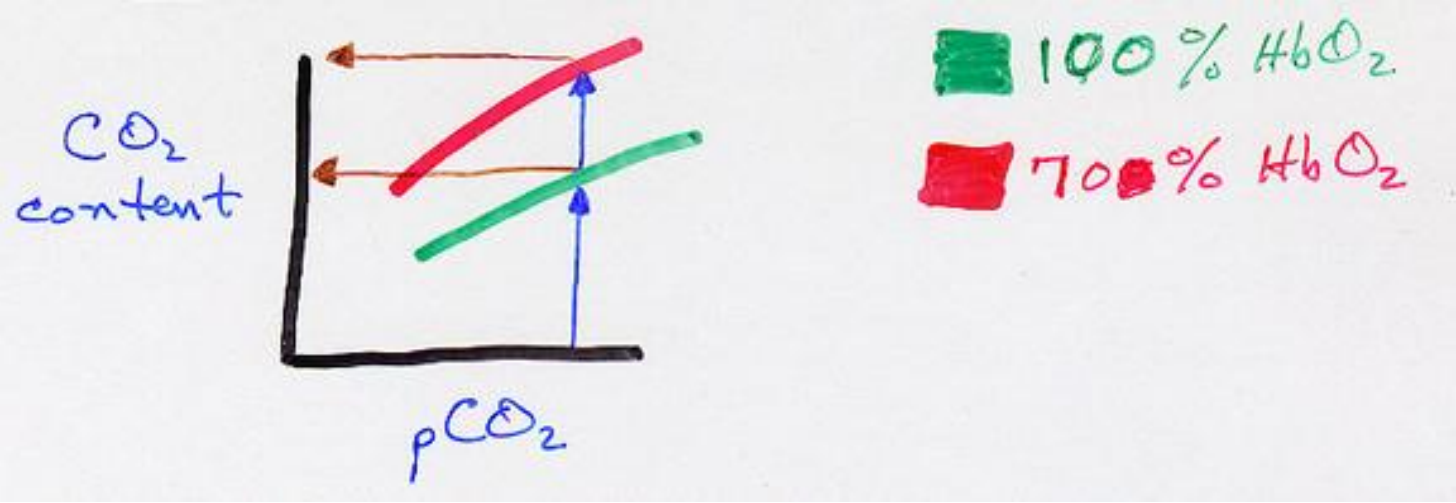
Cl^- and CO_2 are intertwined.

Note direction of movement of CO_2 and Cl^- at Lung (9) AND at RBC (5).

Transport protein (antiport) moves Cl^- and HCO_3^-.



Haldane Effect

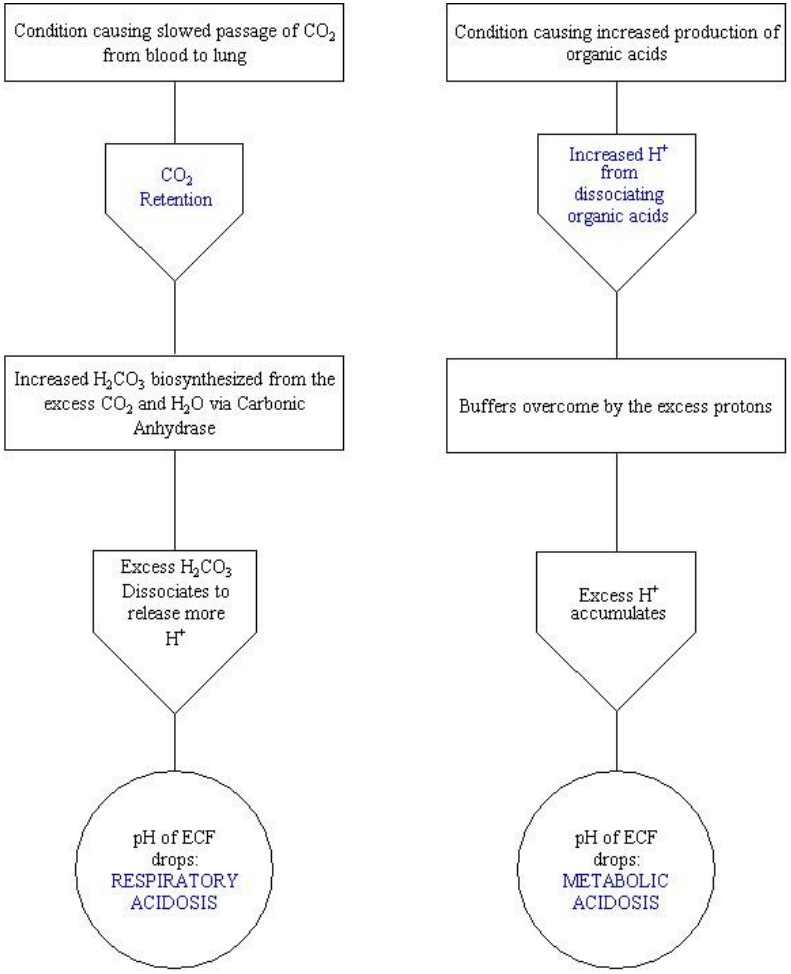


- With ↑$Hb(O_2)_4$, at some pCO_2 → ↓CO_2 content of blood
- With ↓ $Hb(O_2)_4$ at some pCO_2 → ↑CO_2 content of blood

pCO_2 Levels Above 70 mm Hg

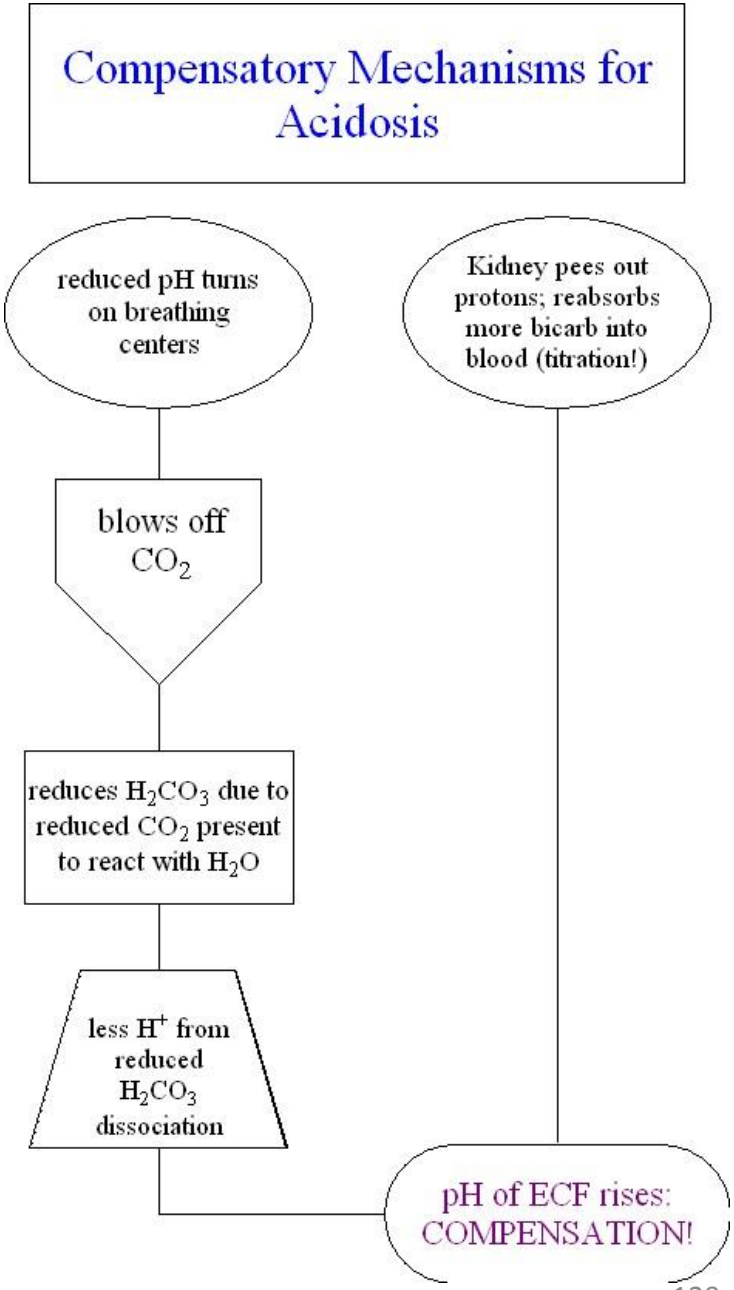
- May decrease respirations
- May cause stupor, coma (CO_2 narcosis)
- May cause hypoxemia

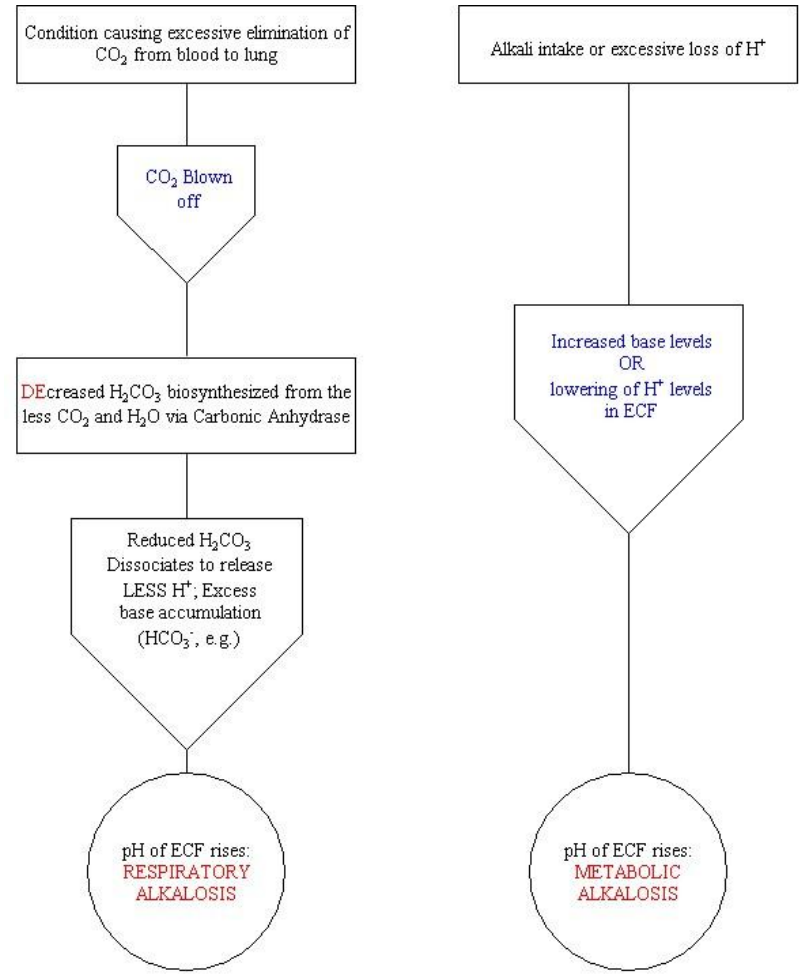
- SLOWLY decrease the pCO_2 so as to not cause posthypercapnic metabolic alkalosis



ACIDOSIS

pH go down, HCO_3^- go down, pCO_2 go up





ALKALOSIS

pH go up, HCO_3^- go up, pCO_2 go down

Compensatory Mechanisms for Alkalosis