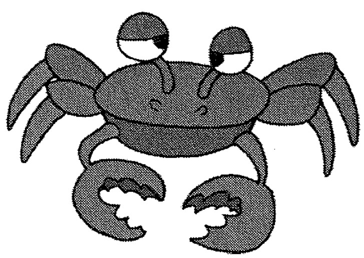
Problem 5D:
Do some research about the Crabtree effect online – can you give two possible explanations for why yeast makes ethanol under aerobic conditions?

The fermentation process does not require oxygen. If oxygen is present, some species of yeast (*Kluyveromyces lactis, Kluyveromyces lipolytica*) oxidize pyruvate completely to carbon dioxide and water. This process is called respiration. Thus, these yeasts produce ethanol only in an (5)________________environment.

Crabtree effect:
The yeast, *Saccharomyces cerevisiae*, prefers fermentation to respiration and produces ethanol aerobically in the (6)___________________ of high external glucose concentrations rather than producing biomass via (7)____________________, the usual process occurring aerobically in most yeast. Increasing concentrations of glucose accelerates glycolysis (the breakdown of glucose) which results in the production of appreciable amounts of ATP through (8)_________________________ phosphorylation. This reduces the need of oxidative phosphorylation done by the TCA cycle via the electron transport chain and therefore (9)__________________ oxygen consumption. The phenomenon is believed to have evolved as a (10)__________________ mechanism (due to the antiseptic nature of ethanol) around the time when the first fruits on Earth fell from the trees.

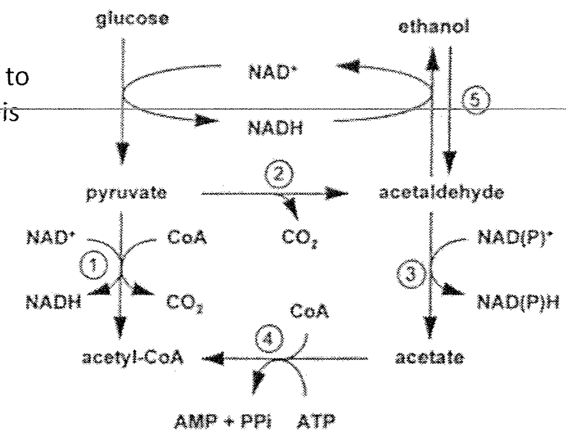


Word Bank for 5D (circle):
(5) aerobic | anaerobic
(6) absence | presence
(7) glycolysis | TCA cycle | oxidative phosphorylation | fermentation
(8) substrate-level | oxidative
(9) increases | decreases
(10) cooperation | competition

Kirk's contact email: knh093020@utdallas.edu

Problem Set #3: Due Friday 11/11 at 5:00PM in FO 3.602 or turn in class or at workshop
Exam #3 Review: TBA
Exam #3: Monday 11/14 at 10:00AM in normal classroom

Problem 5C:
Energetically, it would seem more favorable to convert ethanol to acetyl-CoA via acetaldehyde and pyruvate. Why do you think this pathway does not operate?



<u>Ethanol to Acetyl-CoA Pathway via Acetate</u>
5. Ethanol + NAD^+ → Acetaldehyde + NADH + H^+
3. Acetaldehyde + NAD^+ → Acetate + NADH + H^+
4. Acetate + CoA + ATP → Acetyl-CoA + AMP + PP_i

Compared to…

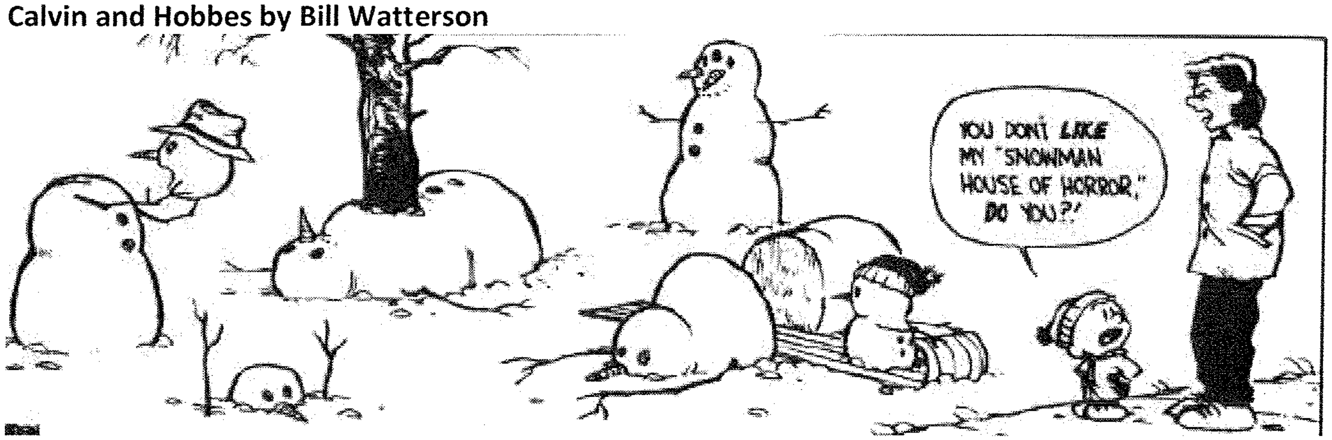
<u>Ethanol to Acetyl-CoA Pathway via Pyruvate</u>
5. Ethanol + NAD^+ → Acetaldehyde + NADH + H^+
2. Acetaldehyde → Pyruvate??
1. Pyruvate + NAD^+ + CoA → Acetyl-CoA + NADH + CO_2

Word Bank for 5C (circle):
(1) carboxylation | carbonation | decarboxylation
(2) reversible | irreversible
(3) probable | not probable
(4) acetate | pyruvate

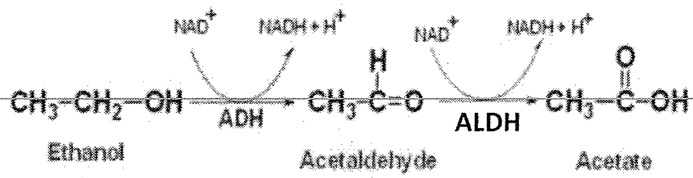
Reaction 2 is a (1)____________________ chemical reaction because pyruvate decarboxylase removes a carbon from pyruvate (3-carbon) to form acetaldehyde (2-carbon) and CO_2. The Gibbs free energy of reaction 2 in the forward direction is most likely largely negative. This makes the forward reaction from pyruvate to acetaldehyde (2)____________________ and makes the backward reaction from acetaldehyde to pyruvate (3)____________________ to occur under physiological conditions.

This means that the ethanol to acetyl-CoA pathway via (4)__________________ pathway does not operate.

Calvin and Hobbes by Bill Watterson



Happy Halloween!



<u>Acetaldehyde to Acetate Pathway</u>

2 Acetaldehyde + 2NAD$^+$ → 2 Acetate + <u>2NADH</u> + 2H$^+$

Net result: 2 Acetate, _____ NADH, _____ ATP

<u>Acetate to Acetyl-CoA Pathway</u>

2 Acetate + 2 CoA + <u>2ATP</u> → 2 Acetyl-CoA + 2AMP + 2 Pyrophosphate [cytosolic]

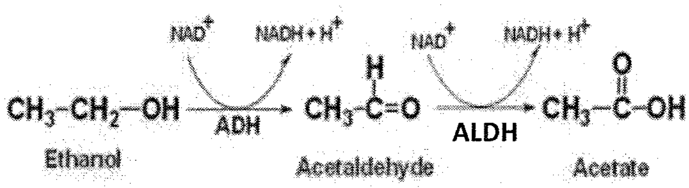
Net result: 2 acetyl-CoA, _____ NADH, _____ ATP

Overall net yield: _____ NADH yield per molecule of glucose, _____ ATP yield per molecule of glucose

Draw the pathway!

Problem 5B:

The inter-conversion of ethanol and acetaldehyde in *S. cerevisiae* is catalyzed by two isoenzymes of ethanol dehydrogenase. What predictions can you make about the kinetic properties of these enzymes?



Isozymes are enzymes that differ in amino acid sequence with <u>different subunits</u> but catalyze the same chemical reaction. Isozymes have different kinetic parameters and different regulatory properties.In other words, isozymes differ in terms of relative affinities to substrates and sensitivity to inhibition by their product. Examples are lactate dehydrogenase (LDH), ethanol dehydrogenase (EDH), and glucokinase. Different tissues express different isozyme forms. In this inter-conversion of ethanol and acetaldehyde in yeast, one isozyme form catalyzes the conversion of ethanol to acetaldehyde and the other isozyme form catalyzes in the reverse direction. What predictions can you make about the kinetic properties of these two isozyme forms?

____

____

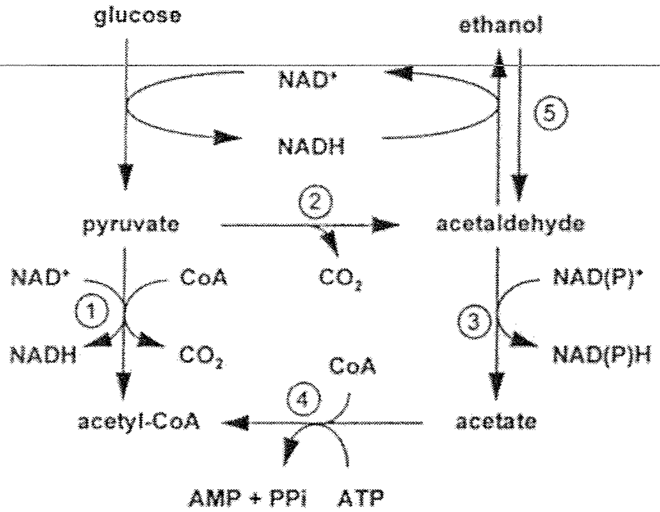
____

Another joke...

A mother complained to her doctor about her daughter's strange eating habits. "All day long she lies in bed and eats yeast and car wax. What will happen to her?"

"Eventually," said the Doctor, "she will rise and shine!"

Problem 5



In most organisms, oxygen slows down glycolysis, and pyruvate is directed towards the TCA cycle rather than fermentative pathways (this is called the **Pasteur Effect**). The brewers' yeast *Saccharomyces cerevisiae* displays the opposite effect, called the **Crabtree Effect**, in which high concentrations of glucose accelerate glycolysis and the production of ethanol, even in the presence of oxygen. In other words, this organism generates ATP by fermentation even when oxygen is available. When the glucose concentration falls, the ethanol is taken back up, and it is converted to acetyl-CoA via acetaldehyde and acetate (the acetyl-CoA can then enter the TCA cycle).

Problem 5A:

For each molecule of glucose that is converted to two molecules of acetyl-CoA via ethanol and acetate, what is the net yield of ATP and NADH?

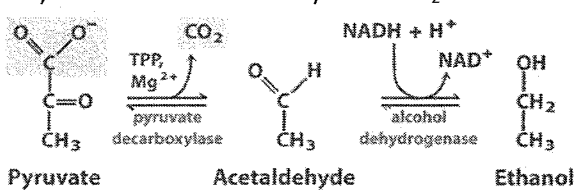
Glycolysis Pathway

Glucose + 2NAD$^+$ + 2ADP + 2P$_i$ → 2 Pyruvate + 2NADH + 2ATP + 2H$^+$ + 2H$_2$O

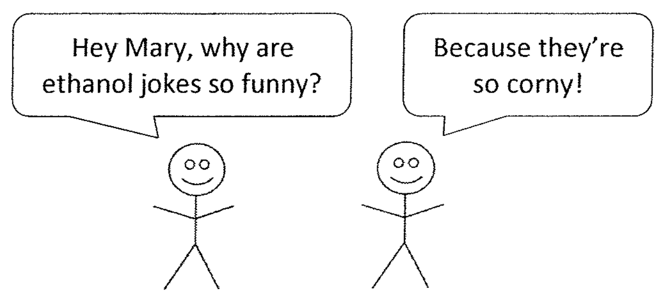
Net result: 2 Pyruvate, _____ NADH, _____ ATP

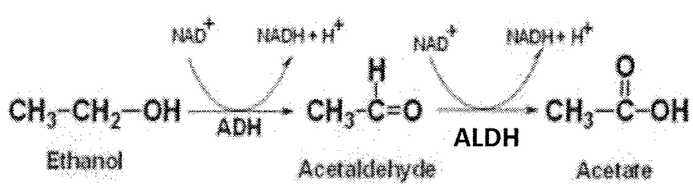
Pyruvate to Acetaldehyde Pathway

2 Pyruvate → 2 Acetaldehyde + 2CO$_2$



Net result: 2 Acetaldehyde, _____ NADH, _____ ATP





Acetaldehyde to Ethanol

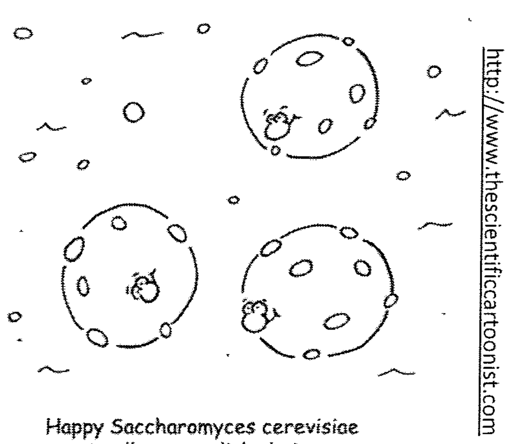
2 Acetaldehyde + 2NADH + 2H$^+$ → 2 Ethanol + 2NAD$^+$

Net result: 2 Acetaldehyde, _____ NADH, _____ ATP

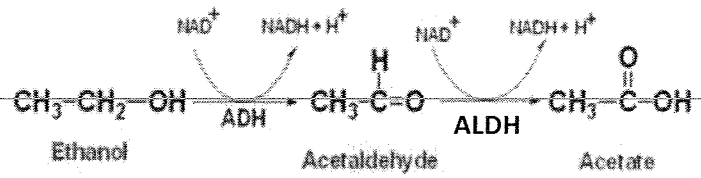
Ethanol to Acetylaldehyde

2 Ethanol + 2NAD$^+$ → 2 Acetaldehyde + 2NADH + 2H$^+$

Net result: 2 Acetaldehyde, _____ NADH, _____ ATP



Happy Saccharomyces cerevisiae yeast cells accomplish their purpose in life making beer for human misfits.



Problem 4B:
Alcohol intoxication is often accompanied by the accumulation of lactate in the bloodstream. Can you suggest a biochemical explanation for why this would occur?

How many molecules of NADH is produced from the conversion of ethanol to acetate?

_______ NADH produced per molecule of ethanol (NAD^+ is reduced to NADH)

The NADH must be (1)___________________.

This can be done by coupling it with a(n) (2)___________________ process: lactate fermentation.

Lactate dehydrogenase (LDH) reduces pyruvate to lactate with NADH as a coenzyme.

$$\text{Pyruvate} \xrightarrow[\text{LDH}]{\text{NADH} \quad \text{NAD}^+} \text{Lactate}$$

Word Bank for 4B (circle):
(1) reoxidized | re-reduced
(2) oxidation | reduction

How many molecules of NADH is oxidized from the conversion of pyruvate to lactate?

_______ NADH oxidized per molecule of pyruvate

Problem 4C:
Design an experiment to test the mechanism you suggested in 4B.

Here's your chance to be creative by coming up with your own super awesome experiment!

Problem 4D:
What are the possible metabolic fates of the acetate produced by these reactions?

Word Bank for 4D (circle):
(3) glycolysis | TCA cycle | oxidative phosphorylation | fermentation

Acetate to Acetyl-CoA Pathway
acetate + CoA + ATP → acetyl-CoA + AMP + pyrophosphate [cytosolic]

Acetate is converted to acetyl-CoA with the enzyme Coenzyme A (CoA). ATP is coupled in the reaction and broken down into AMP and PP_i. Fatty acids enter (3)_______________________ at the level of acetyl-CoA. Acetyl-CoA can then be metabolized to citrate. This cycle involves the citrate synthase catalyzing the condensation reaction b/w acetyl-CoA and a recycled molecule of oxaloacetate to form citrate and CoA-SH.

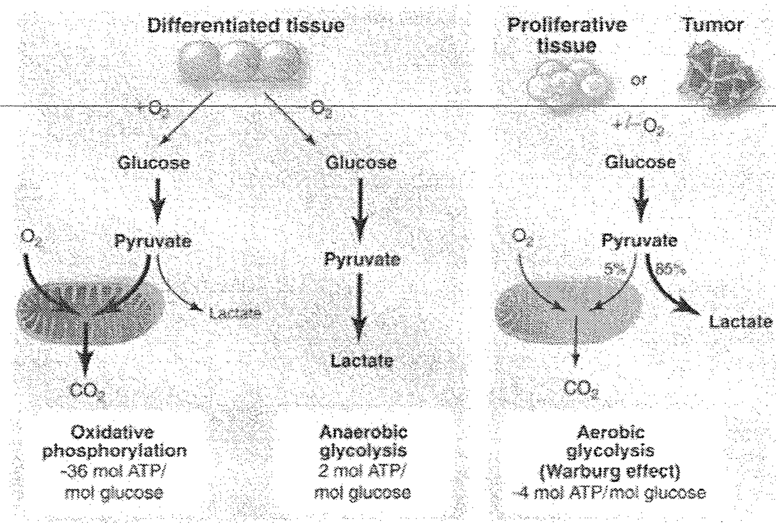
acetyl-CoA + oxaloacetate + H_2O → citrate + CoA-SH

Problem 4E:
Ethylene glycol ($CH_2OH.CH_2.CH_2.CH_2OH$) is a component of anti-freeze and a quite frequent cause of poisoning. An effective treatment for ethylene glycol poisoning is the administration of an intoxicating dose of ethanol. Explain how this treatment works.

Ethanol is a competitive (4)________________ of alcohol dehydrogenase (ADH). In cases where ethylene glycol is absorbed from anti-freeze, ethanol will compete and bind to ADH. ADH has a hundred-fold (5)______________ affinity for ethanol than for ethylene glycol. This prevents further accumulation of toxic metabolites such as glycoaldehyde (oxidized to glycolic acid) from ethylene glycol. Thus, ethylene glycol can be excreted from the body while ADH acts on ethanol.

Word Bank for 4E (circle):
(4) activator | inhibitor
(5) higher | lower

Show work and solution on your own paper!



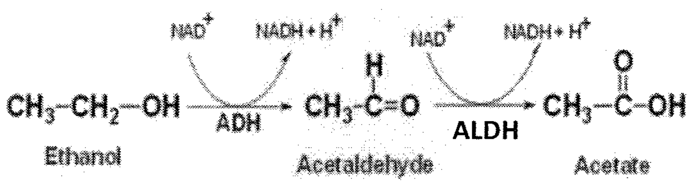
Problem 3D:
Compounds that inhibit glycolysis are attracting interest as potential treatments for cancer. Why is this?

The Warburg Effect: Cancer cells (and proliferating normal cells) take up large amounts of glucose, and metabolize it via glycolysis straight to lactate, largely bypassing the mitochondria, even in the presence of plenty of oxygen.

Main ATP Production:
Normal cells: TCA cycle and oxidative phosphorylation
Cancer cells: glycolysis and lactate fermentation

<u>Problem 4</u>
Alcohol is detoxified in the liver by conversion to acetaldehyde (by alcohol dehydrogenase) and then acetaldehyde is oxidized to acetate by aldehyde dehydrogenase. Both enzymes require NAD^+.

Problem 4A:
Write the balanced equations for the conversion of ethanol to acetate.



1. Ethanol is oxidized to acetaldehyde via alcohol dehydrogenase (ADH).
   $CH_3CH_2OH + NAD^+ \rightarrow CH_3CHO + NADH + H^+$

2. Acetaldehyde is a highly unstable compound that quickly breaks down into free radicals (acetate) with the help of aldehyde dehydrogenase (ALDH).
   $CH_3CHO + NAD^+ \rightarrow CH_3COOH + NADH + H^+$

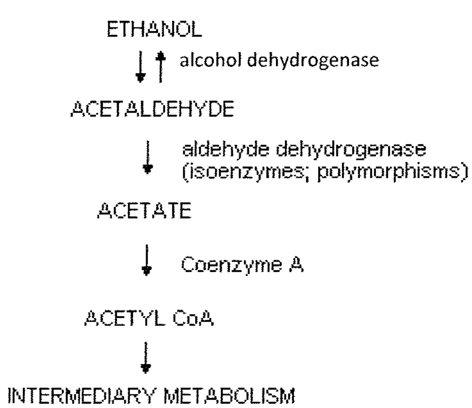
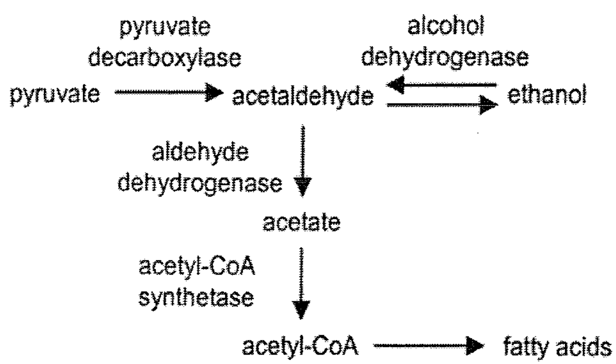


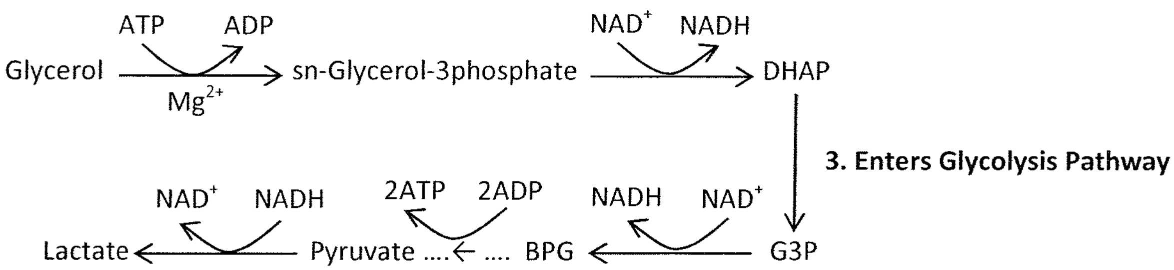
Fig. 1 Metabolism of acetaldehyde.



Kirk's Amazing Notes for Problem Set 3

Problem 3B:
Glycerol can enter glycolysis by the pathway described on pages 557-559. You might imagine that the lactic acid bacteria could grow on glycerol by converting it to lactate. What would the ATP yield of this pathway be? Draw out your proposed pathway from glycerol to lactate, and use it to explain why this pathway does not work as a means of sustaining growth.

1. Glycerol Kinase Reaction **2. Glycerol-P-dehydrogenase Reaction**



4. Lactate Fermentation

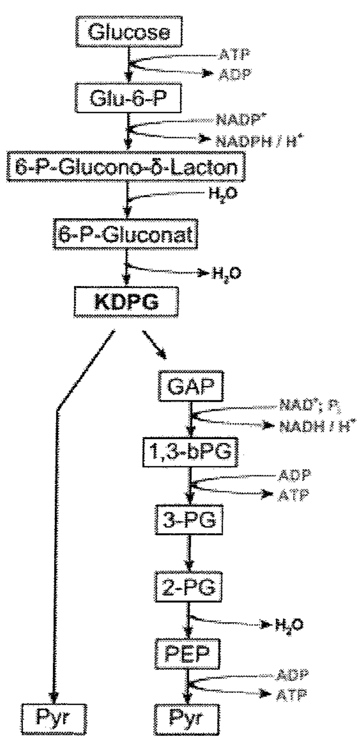
How much ATP is consumed in all four reactions? _______ ATP consumed
How much ATP is produced in all four reactions? _______ ATP produced
_______ ATP yield per molecule of glycerol

How much NADH is produced? _______ NADH produced
How much NADH is reoxidized? _______ NADH reoxidized
_______ NADH yield per molecule of glycerol

Why would this pathway not feasible as a means of sustaining growth? Is the reaction redox balanced (NADH)?

____

____



Problem 3C:
Entner-Doudoroff pathway (bacterium *Zymomonas mobilis*):
Converts glucose to ethanol:
Glucose + $NADP^+$ + NAD^+ + ADP + P_i → 2 Ethanol + NADPH + NADH + ATP + $2CO_2$
Net result: 2 Ethanol, 1 NADPH, 1 NADH, **1 ATP**

Compared to…

Glycolysis pathway (all life on earth):
Converts glucose to pyruvate:
Glucose + $2NAD^+$ + 2ADP + $2P_i$ → 2 Pyruvate + 2NADH + 2ATP + $2H^+$ + $2H_2O$
Net result: 2 Pyruvate, 2 NADH, **2 ATP**

What is the major energetic difference between these two pathways?

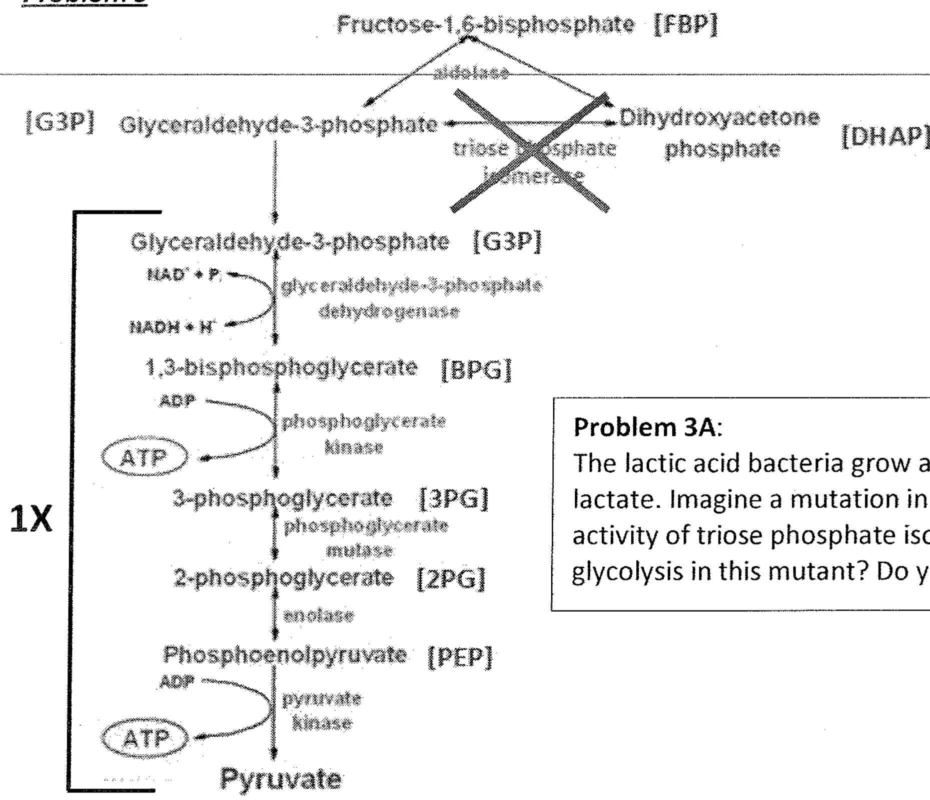
____

____

____

Show work and solution on your own paper!

Problem 3



Problem 3A:
The lactic acid bacteria grow anaerobically converting glucose to lactate. Imagine a mutation in this organism that eliminates the activity of triose phosphate isomerase. What is the ATP yield of glycolysis in this mutant? Do you expect the mutant to be viable?

Glycolysis Pathway (normal)		Glycolysis Pathway (mutant)
Phase 1: 1 Glucose → 2 G3P		Phase 1: 1 Glucose → _____ G3P
2 ATP consumed	elimination of triose phosphate isomerase →	2 ATP consumed
2 NAD^+ consumed		2 NAD^+ consumed
Phase 2: 2 G3P → 2 Pyruvate		Phase 2: _____ G3P → _____ Pyruvate
4 ATP produced		_____ ATP produced
2 NADH produced		_____ NADH produced
Net result: 2 Pyruvate, 2 NADH, 2 ATP		Net result: _____ Pyruvate, _____ NADH, _____ ATP

Normal glycolysis converts one FBP to one G3P and one DHAP. Only G3P is capable of converting to BPG in phase II so DHAP must be converted to G3P via triose phosphate isomerase. Thus, normal glycolysis produces two molecules of each substrate and product in Phase II. Elimination of triose phosphate isomerase leaves only one G3P to react in Phase II.

What happens to the ATP yield in this mutant if triose phosphate isomerase is eliminated?

____

____

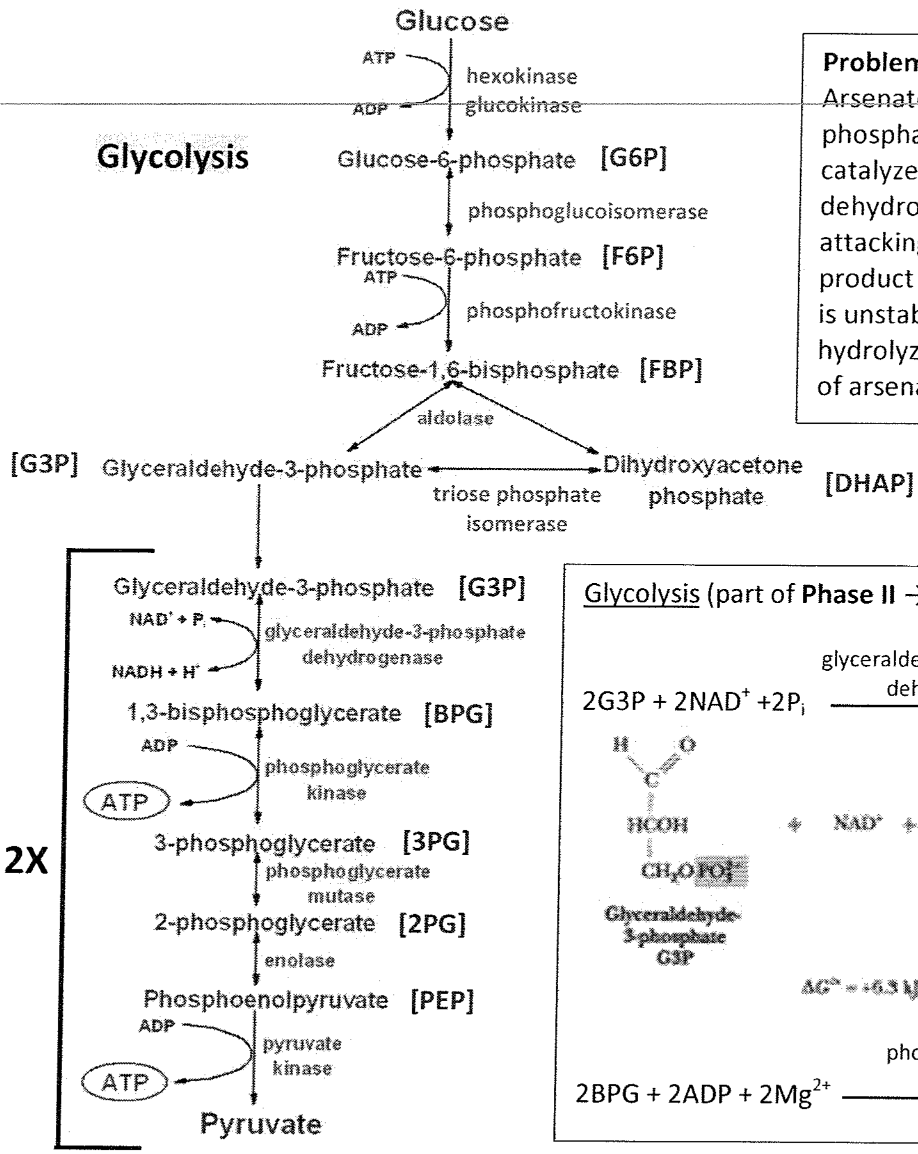
Do you expect this mutant to be viable?

____

____

Show work and solution on your own paper!

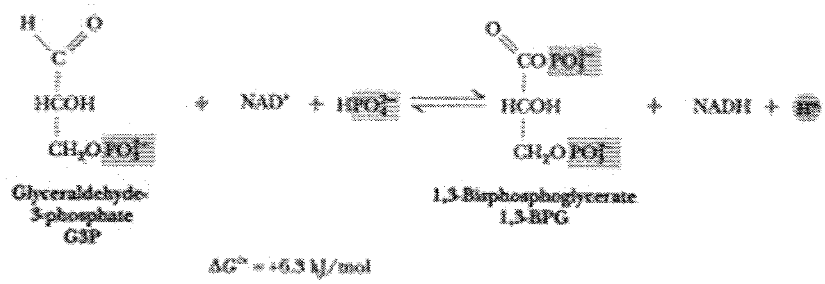
Kirk's Amazing Notes for Problem Set 3



Problem 2C:
Arsenate (AsO_4^{3-}) closely resembles P_i (inorganic phosphate) in structure and reactivity. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, arsenate can replace phosphate in attacking the energy-rich thioester intermediate. The product of this reaction, 1-arseno-3-phosphoglycerate, is unstable and it is rapidly and spontaneously hydrolyzed to 3-phosphoglycerate. What is the effect of arsenate on energy generation in a cell?

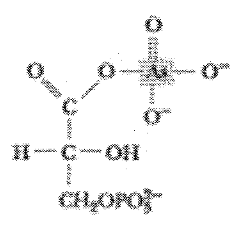
<u>Glycolysis</u> (part of **Phase II** → 2x reactants/products) – Normal

$$2G3P + 2NAD^+ + 2P_i \xrightarrow{\text{glyceraldehyde-3-phosphate dehydrogenase}} 2BPG + 2NADH + 2H^+$$



$$2BPG + 2ADP + 2Mg^{2+} \xrightarrow{\text{phosphoglycerate kinase}} 2\ 3PG + 2ATP$$

Effects of Arsenate on Aerobic Phosphorylation:
Arsenate is an anion analogous to phosphate and glyceraldehyde-3-phosphate dehydrogenase incorporates arsenate into G3P rather than phosphate. Arsenate attacks the energy-rich thioester intermediate and GAPDH catalyzes the reaction to form an arsenate ester called 1-arseno-3-phosphoglycerate.



1-Arseno-3-phosphoglycerate

Reaction looks like this: (2x reactants/products)
$G3P + NAD^+ + AsPO_4^{3-} \rightarrow$ 1-arseno-3-PG $\rightarrow 3PG + NADH + 2H^+$

Which step is bypassed?

____

____

What happens to the ATP yield?

____

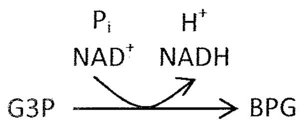
____

Show work and solution on your own paper!

Kirk's Amazing Notes for Problem Set 3

Problem 2

Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) catalyzes the following reaction from glycolysis:
$G3P + P_i + NAD^+ \rightarrow BPG + NADH + H^+$



In a spectrophotometer, NADH absorbs light at 340 nm but NAD^+ does not.

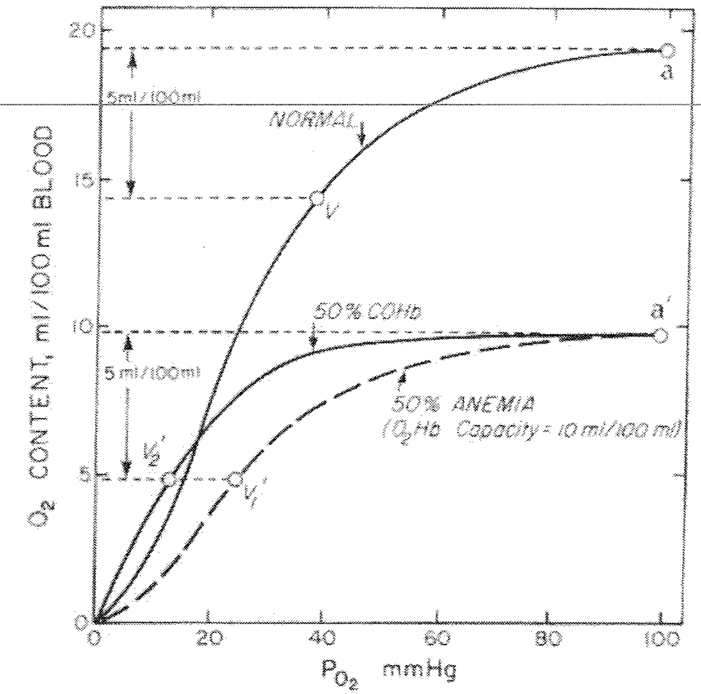
Beer-Lambert Law: A = ε.c.L
A = change in absorbance: 0.73 min^{-1}
ε = molar extinction coefficient for NADH: 6,300 L/mol cm^{-1}
c = change in concentration: __________ moles/L/min
L = length: 1 cm
Reaction volume of cuvette: 1 mL
Volume of G3PDH: 0.2 mL
Molality of G3PDH: 8.68 mg/mL

Problem 2A:
Finding the rate of production of NADH in the assay:
First, solve for the concentration:
c = A/εL
$\Delta c = \Delta A/\varepsilon L$
Δc = __________ min^{-1} / (__________ L/mol cm^{-1} x __________ cm)
Δc = __________ mol/L/min x 10^6 (convert to µmol/L/min)
Δc = __________ µmol/L/min

Second, solve for the rate of production:
Reaction volume of cuvette: 1 mL
__________ µmol/L/min x 1/1000 L/mL = __________ µmol/min (mL goes away)

Problem 2B:
Calculating the specific activity of G3PDH in the assay:
First, solve for the mass of G3PDH:
Volume of G3PDH: 0.2 mL
Molality of G3PDH: 8.68 mg/mL
__________ mL x __________ mg/ml = __________ mg

Second, solve for the specific activity:
Rate of production of NADH / mass of G3PDH:
__________ µmol/min / __________ mg = __________ µmol/min/mg



This diagram shows the oxygen binding curve of hemoglobin in the absence and presence of 50% COHb (curves I and IV on the previous Figure), but now oxygen binding is expressed as the oxygen content of whole blood.

Word Bank for 1B (circle):
(7) left | right
(8) left | right
(9) more | less

Problem 1B:
What are the **two** major differences between the oxygen binding curves in the presence and absence of 50% COHb?

In the presence of 50% COHb, the oxygen binding curve shifts to the (7)__________________. Sigmoidal properties correspond to more cooperativity so a shift to the (8)__________________ means that there is (9)__________________ cooperativity.

What happens to the total O_2 content in the presence of 50% COHb? ________________________________

Problem 1C:
Can you suggest **two** reasons why CO restricts the delivery of O_2 to tissues?

Word Bank for 1C (circle):
(10) increases | decreases
(11) increases | decreases

Binding affinity between O_2 and Hb (10)_________________ in presence of CO.

How does this affect the delivery of O_2 to body tissues? ____

Overall binding capacity of O_2 to Hb (11)________________ in presence of CO because CO competitively binds to the same site on Hb as O_2.

Problem 1D:
Is CO poisoning (to a level of 50% COHb) more or less severe than 50% anemia, in terms of the ability of blood to deliver O_2 to tissues?

Anemia is a deficiency in hemoglobin or a <u>decreased</u> binding affinity of O_2 and Hb. This usually leads to hypoxia (lack of oxygen) in organs and tissues. Compare the binding affinity of O_2 in presence of 50% HbCO to the binding affinity of O_2 in 50% anemia. Which case is more severe?

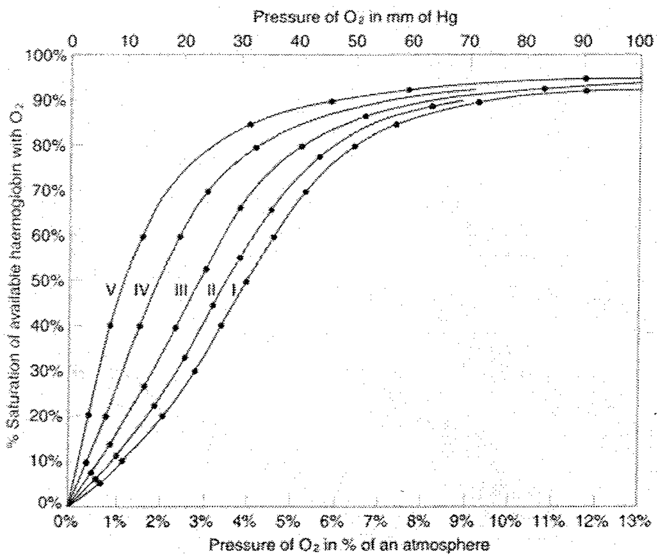
____

____

Kirk Huynh
Contact email:
knh093020@utdallas.edu

Kirk's Amazing Notes for Problem Set 3

Problem 1



Carbon monoxide (CO) is a potentially fatal poison. Amongst other things, CO exposure causes anoxemia, a reduced oxygen concentration in blood. CO binds avidly to hemoglobin (Hb), to form carboxyhemoglobin, COHb. For answering these questions, you can assume that if a sample of blood or Hb is described as containing (for example) 50% COHb, this means that 50% of the total available O_2 binding sites are occupied by CO. In this case, individual Hb molecules will be bound by, **on average**, two molecules of CO.

This diagram shows oxygen-binding curves for Hb, in the presence of increasing proportions of COHb (I = 0% COHb, II = 10% COHb, III = 25% COHb, IV = 50% COHb, V = 75% COHb). Here, oxygen binding on the y-axis is expressed as the % of available O_2 binding sites in Hb that are bound by O_2 (so each curve is normalized to 100%).

Problem 1A:
What is the effect of CO on the oxygen binding properties of hemoglobin? On the basis of the shapes of these curves, how could you best describe the behavior of CO with regards to its effect on O_2 binding?

Carbon monoxide inhibits hemoglobin's ability to carry oxygen. Hb is a tetramer with four oxygen binding sites. CO binds to the same location as O_2 and it has a (1)_______________ binding affinity than O_2 for Hb. When CO binds to one of the binding sites to create COHb, it creates a higher O_2 binding affinity to the remaining binding sites on Hb. This causes the Hb molecule to retain O_2 rather than delivering the necessary O_2 to the body tissues. Remember that there is increased binding affinity between O_2 and Hb in the presence of CO. This means that CO is an enzyme regulator, or more specifically, it is an allosteric (2)_________________. However, the overall binding capacity of O_2 to Hb (3)_________________ because CO competitively binds to the same site on Hb as O_2.

How quickly carboxyhemoglobin (COHb) forms is dependent on the concentration of inhaled CO and duration of exposure. At increasing CO levels, the affinity of O_2 for Hb changes by shifting the curve to the (4)_________________. Sigmoidal properties correspond to more cooperativity so a shift to the (5)_________________ means that there is (6)_________________ cooperativity.

Word Bank for 1A (circle):
(1) stronger | weaker
(2) activator | inhibitor
(3) increases | decreases
(4) right | left
(5) right | left
(6) more | less