CT part 75 pts.

CHEM 641 THORPE part FINAL EXAM FALL 2005

YOUR NAME: ____

SECTION (circle one) **Morning** **or** **Afternoon**

NOTES:

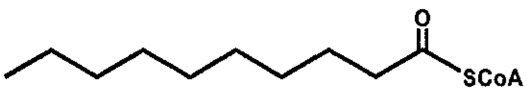
1. where appropriate please show work - if in doubt show it anyway.
2. pace yourself - you may want to do the easier questions first.
3. please note the point value of questions - adjust your answers and effort accordingly.
4. some questions may have more data than you need.
5. please be brief - unfocused, rambling answers won't receive as much credit as a few short appropriate phrases.
6. Please write CLEARLY - if I cannot read it - it is wrong.
7. Three metabolic charts are included at the back of this exam.
   Detach (carefully) if you wish.
8. Good luck

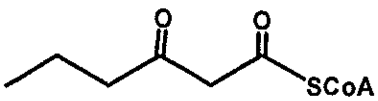
Question 1. (10 pts) What is the yield of ATP per molecule of the following. Insert a number from 0-100 in the space provided.

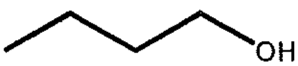
a. Pyruvate converted to CO_2 and water ____________

b. Dietary maltose converted to CO_2 and water ____________

c. Per isocitrate in the presence of malonate ____________

d.  converted to CO_2 and water ____________

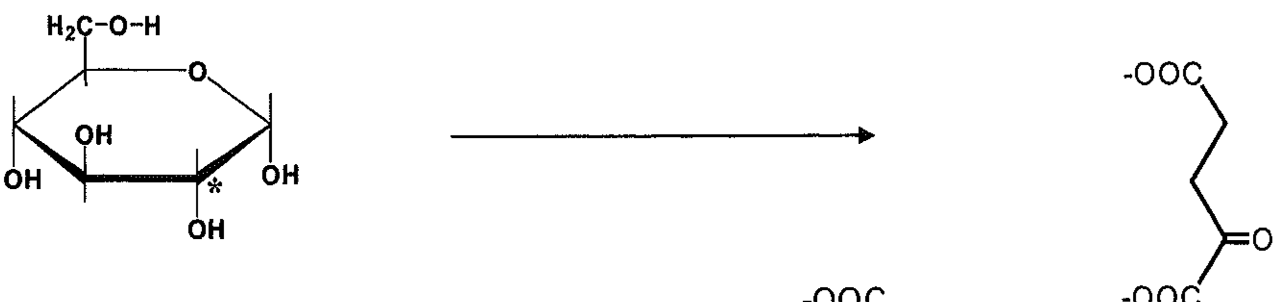
e.  converted to CO_2 and water ____________

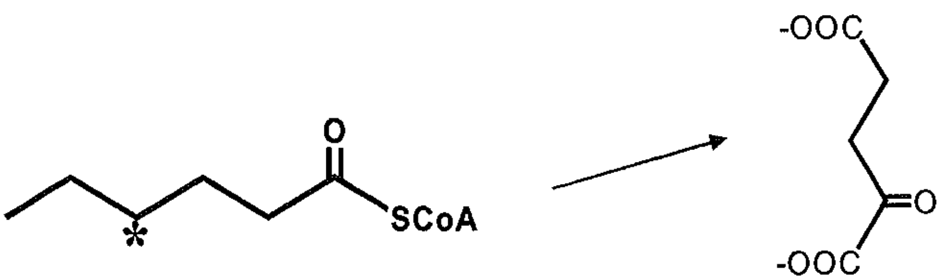
f.  converted to CO_2 and water ____________

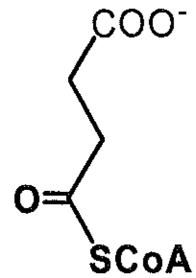
g. Dihydroxyacetone-P to lactate in the presence of arsenate ____________

h. Glucose completely oxidized to CO_2 and water ____________

Question 2 (6 pts) Tracing radiolabels. Place asterisks indicating the position of the radiolabel in the molecules shown to the right - if the product contains no radiolabel write "NONE".

a. 

b. 

c. $^{14}CO_2$ + pyruvate + acetyl-CoA ⟶ 

Question 3 (8 pts.) Suppose you have two enzymes (catalyzing steps A and B) involved in making acetyl-CoA by an unusual route:

STEP A: Acetate + ATP + CoASH ↔ Acetyl-CoA + ADP + Pi $\Delta G^{o'} = +3.6$ kcal

STEP B: ADP + H_2O ↔ AMP + Pi $\Delta G^{o'} = -7.3$ kcal

Calculate the standard free energy for the reaction as written below

Acetate + ATP + CoASH + H_2O ↔ acetyl-CoA +AMP+ 2 Pi $\Delta G^{o'}$ = ____________ kcal

In class we discussed two enzymes (here, called enzyme 1 and enzyme 2) that, together, catalyze exactly this overall reaction. Name these enzymes below:

Enzyme 1____________________ **Enzyme 2**____________________

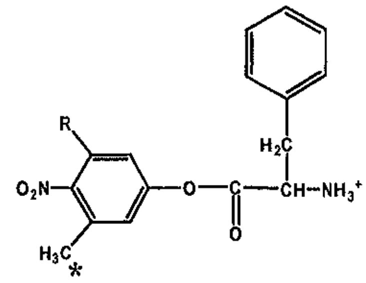
Write out the two reactions catalyzed by these two enzymes:

Enzyme 1:

Enzyme 2:

Explain, below, why the combination of steps A and B would not be used in Nature?

Question 4 (7 pts) Chymotrypsin is mixed with the radiolabeled ester substrate shown to the right (C^{14} at the asterisk) to give concentrations of 15 µM and 10 mM respectively.



The first product to be released shows a molar extinction coefficient of 8,000 $M^{-1}cm^{-1}$ at 410 nm under the conditions of the experiment (pH 8.5).

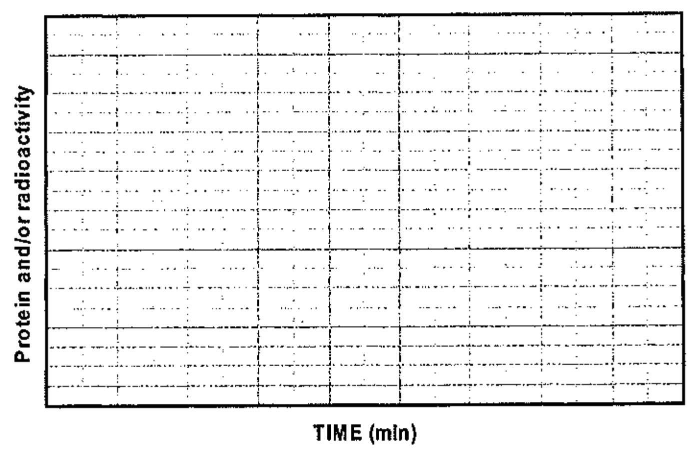
a. The burst phase is completed in less than 0.3 min. Calculate the <u>concentration</u> of first product released in the burst __________

b. calculate the corresponding **<u>absorbance</u>** increase in a 1 cm pathlength __________

c. Suppose the turnover number of the enzyme in the steady state was 5/min, what is the **<u>increase in absorbance</u>** at 410 nm that would be observed between 1 and 2 min after mixing?

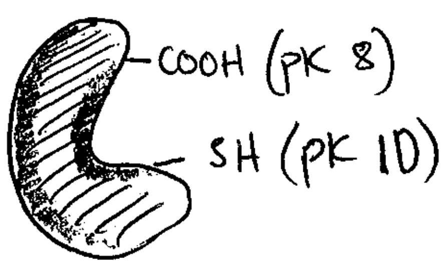
Absorbance increase__________

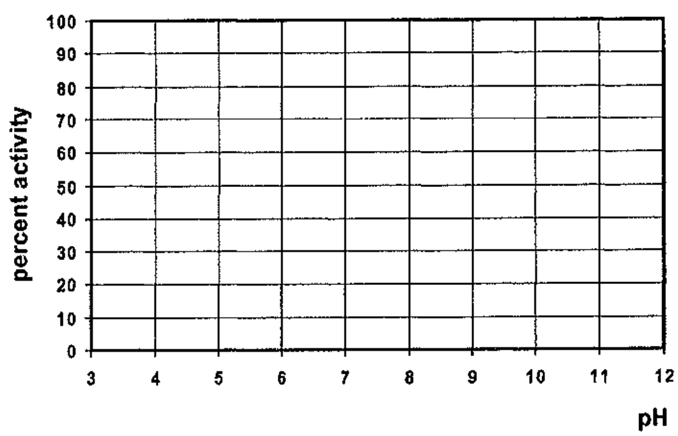
d. After 1 min the mixture is cooled rapidly and gel-filtered (size exclusion chromatography) at 4°C. Using the graph draw a representative trace of the chromatogram clearly LABEL where protein and radioactivity would emerge.



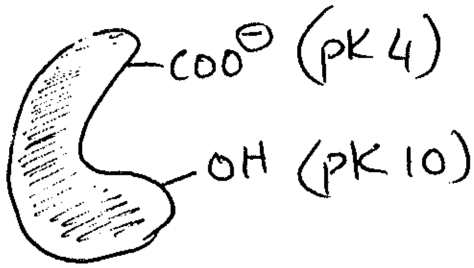
Question 5 (6 pts.) Draw the pH activity curves for the following situations. Accuracy matters.

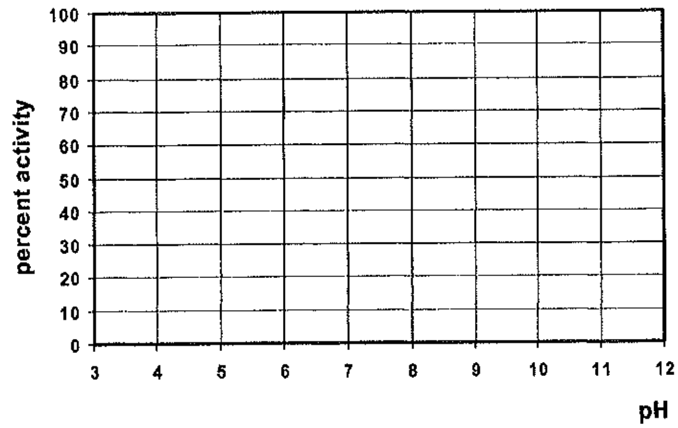
a. <u>Only</u> this protonic form is active.



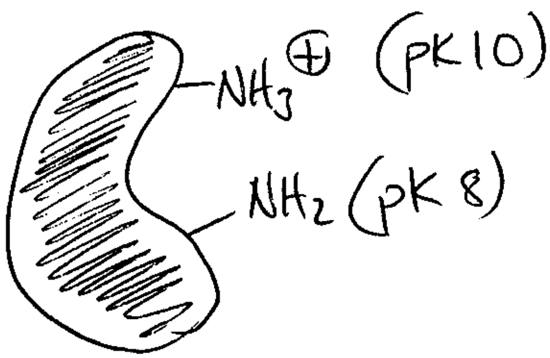


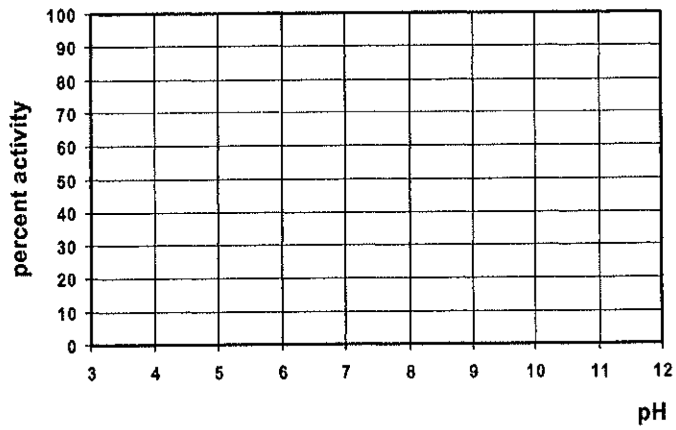
b. This enzyme is only active as shown



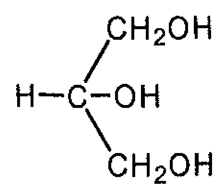


c. This enzyme is only active as shown





Question 6 (8 pts) Draw two equations to show clearly how glycerol (right) could enter the glycolytic pathway. The equations should be: compound A + B → C + D ..... Draw structures of A, B and etc, or provide acceptable names/abbreviations. Don't draw curved arrows or mechanisms. (Full credit given to any reasonable sets of equations - it is not necessary to reproduce the actual equations that we use to metabolize glycerol)



Reaction 1 (below) will be catalyzed by what class of enzyme? ______________________

reaction 1

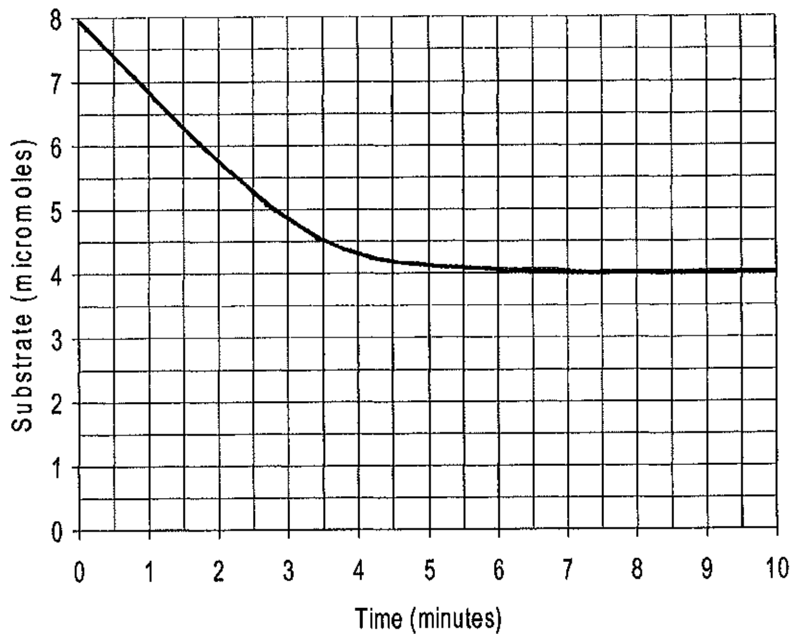
Reaction 2 (below) will be catalyzed by what class of enzyme? ____________________

reaction 2

How many molecules of ATP would you get in the oxidation of one molecule of glycerol to CO_2 and water? ____________

Question 7 (7 pts.) The graph to the right shows an enzyme assay converting a single substrate (L-alanine) into a single product (D-alanine). It was started at time 0 minutes by the addition of 7 nM enzyme to a solution of 1 mL of L-alanine. The pH was 7.5 and the temperature 25 °C.

Answer the following questions - there is more information than you need.



a. what is the concentration of enzyme in the assay?

__________**M**

b. what is the concentration of L-alanine at time zero? __________**M**

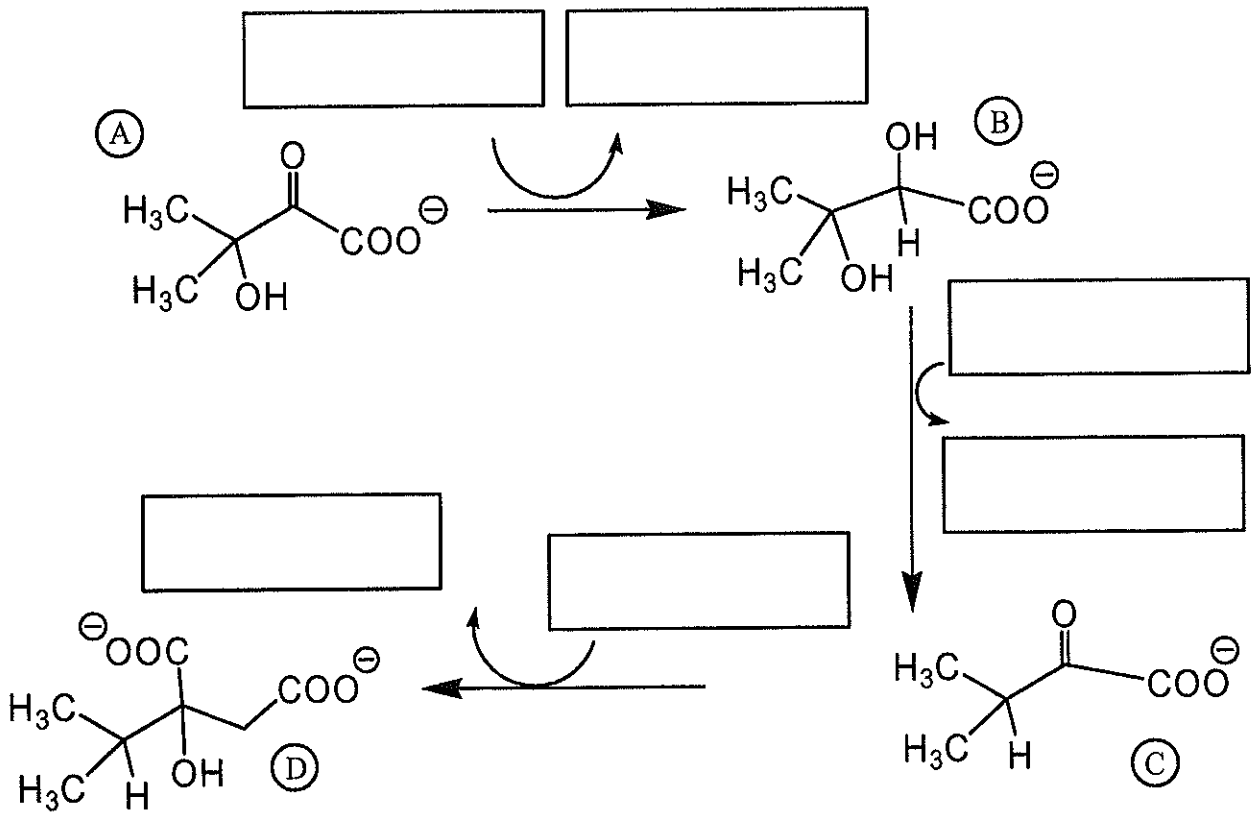
c. what is the initial rate of the assay? ______________**µmoles/min**

d. what is the corresponding turnover number? __________________**/min**

e. what is the value of K_{eq} = [D-ala]/[L-ala]? ____________________

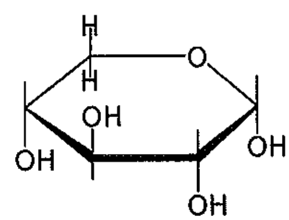
Question 8 (8 pts) The following is part of a metabolic pathway (here, converting A to D) that we did not discuss. Reason by analogy using your metabolic charts to **clearly indicate in the boxes every substrate and product missing for each reaction. Don't put enzyme names - a hypothetical example for one box is shown at right). If nothing is needed in the box put "NONE".**

NADH, H_2O, CO_2



Finally put an asterisk (indicating C-14) on the carboxylate carbon of compound A and trace it through to compound D. If there is no radiolabel on D write "**none**" on the line by the structure

Question 9 (3 pts) Alpha-D-xylose (shown) is dissolved in buffer and mixed with ATP/Mg^{2+} and hexokinase. The concentration of selected compounds is shown below at time zero and after 3 min



Time	xylose	ATP	ADP	AMP	Pi
0 min	1mM	10 mM	0 mM	0 mM	0 mM
3 min	1 mM	5 mM	5 mM	0 mM	5 mM

Below, show a chemical equation to describe this overall reaction in the presence of xylose

Questions 10 (6 pts). Fill in the blanks with a number from 0-20 - no words allowed

a. the number of <u>electrons</u> removed during the oxidation of lactate to CO_2 _____

b. the number of electrons removed during the complete oxidation of glucose _____

c. the number of electrons required to reduce one molecule of oxygen to water _____

Question 11 (8 pts) Fill in the blanks with not more than three legible words.

a. give the name of a naturally occurring inhibitor of fatty acid oxidation _______________

b. the name of a direct inhibitor of ATP synthase _______________

c. the name of the compound that might be expected to accumulate during fatty acid oxidation in thiamine deficiency _______________

d. this <u>class</u> of compounds inhibits ATP formation but not electron transport _______________

e. an inhibitor of cytochrome oxidase _______________

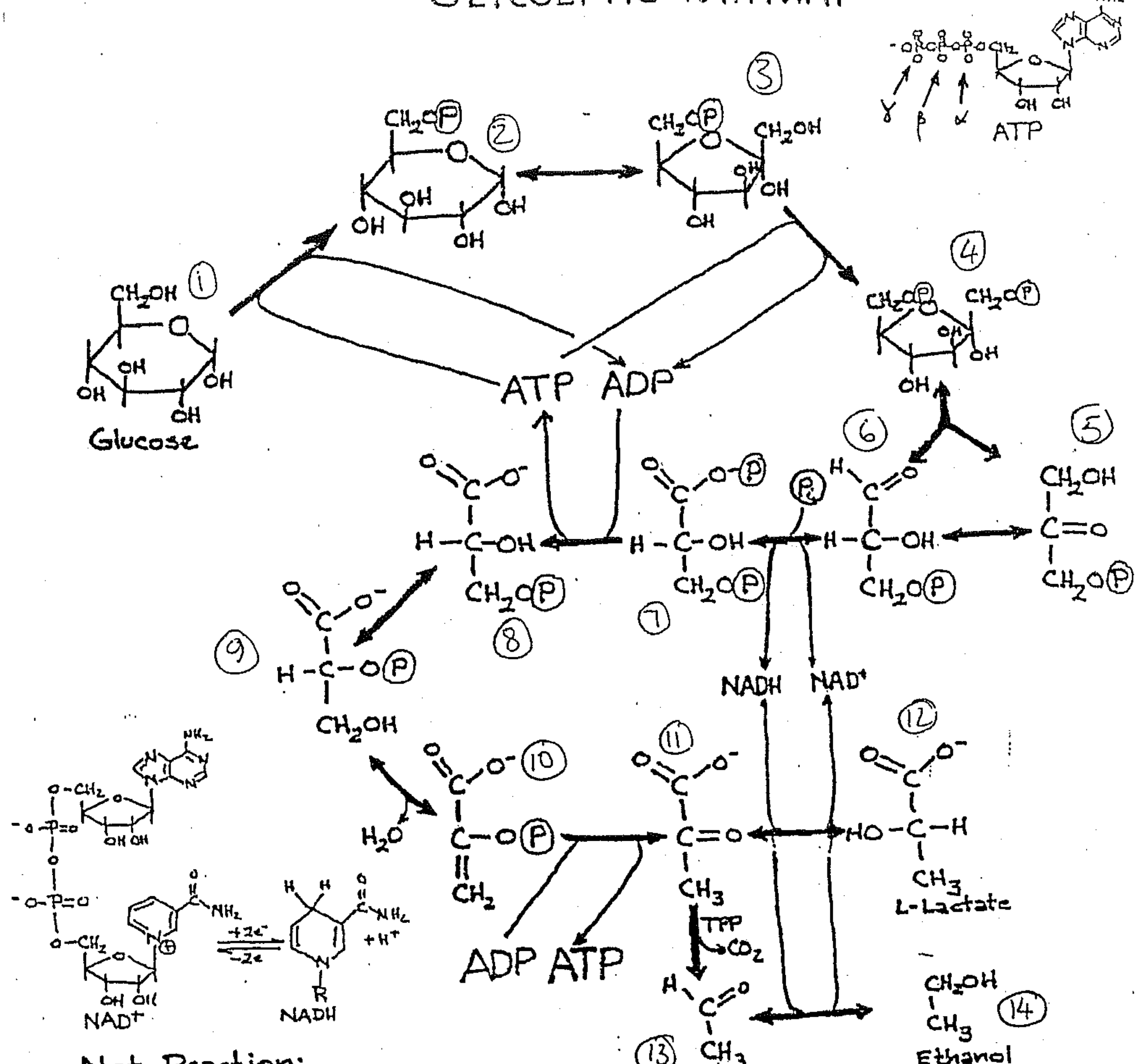
f. what is/are the <u>product(s)</u> of the cytochrome oxidase enzyme reaction _______________

g. reactions with positive free energy changes are called _______________

h. the water soluble vitamin incorporated into NAD^+ is called _______________

zzz. The word that best describes today _______________

GLYCOLYTIC PATHWAY



Net Reaction:

in vertebrates

$$\text{Glucose} + 2P_i + 2\text{ADP} \longrightarrow 2\text{Lactate} + 2\text{ATP}$$

in yeast

$$\text{Glucose} + 2P_i + 2\text{ADP} \longrightarrow 2CO_2 + 2\text{Ethanol} + 2\text{ATP}$$

1/2	hexokinase	7/8	phosphoglycerate kinase
2/3	phosphoglucoisomerase	8/9	phosphoglyceromutase
3/4	phosphofructokinase	9/10	enolase
4/5+6	aldolase	10/11	pyruvate kinase
5/6	triosephosphate isomerase	11/12	lactate dehydrogenase
6/7	glyceraldehyde 3P dehydrogenase	11/13	pyruvate decarboxylase
		13/14	alcohol dehydrogenase

HB

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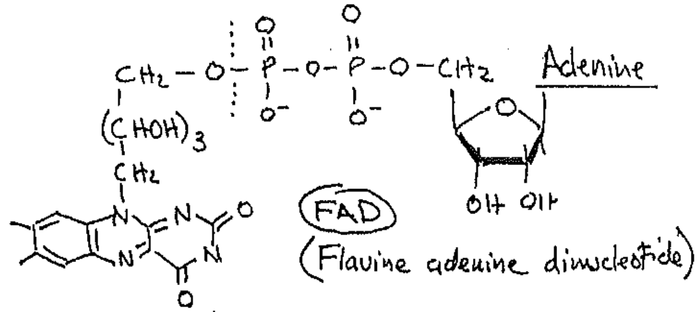
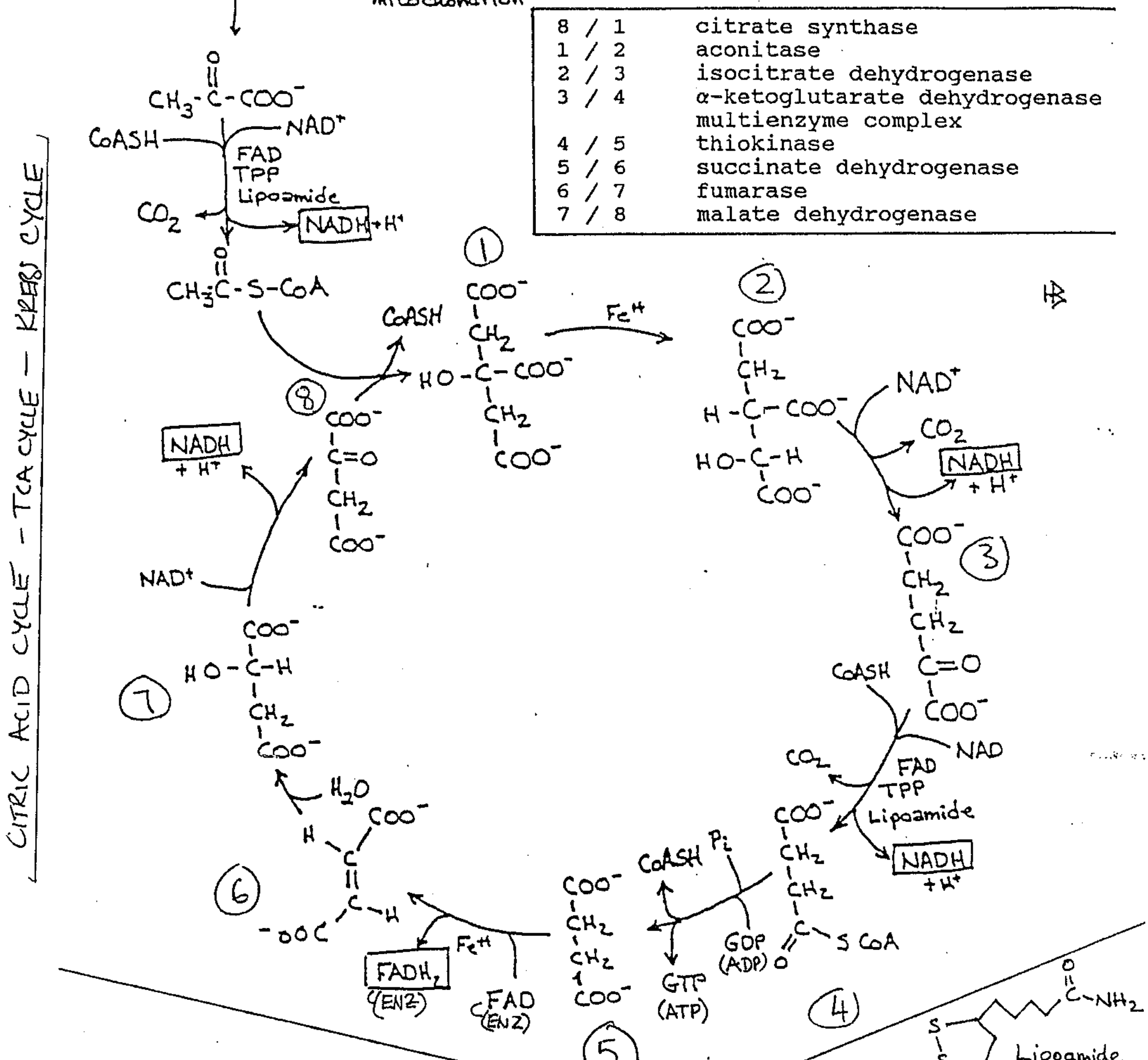
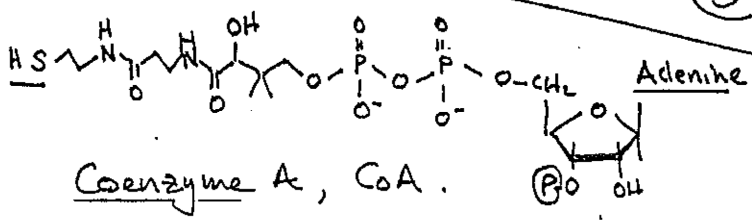
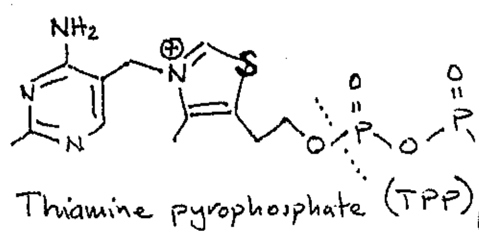




8	/	1	citrate synthase
1	/	2	aconitase
2	/	3	isocitrate dehydrogenase
3	/	4	α-ketoglutarate dehydrogenase multienzyme complex
4	/	5	thiokinase
5	/	6	succinate dehydrogenase
6	/	7	fumarase
7	/	8	malate dehydrogenase





FATTY ACID OXIDATION

a. Neutral fat (triglycerides) converted to free fatty acids via lipases.

b. Free fatty acids (R-COOH) enter cell and activated via:

$$R\text{-}COO^- + ATP + CoASH \longrightarrow R\text{-}CO\text{-}SCoA + AMP + P\text{-}P_i$$

[Note this reaction makes AMP and is equivalent to the consumption of 2 ATP molecules if they were converted to ADP]

c. Then the CoA thioester (R-CO-SCoA above) is degraded via the β-oxidation cycle as shown below. Note each turn releases acetyl-CoA which can enter the TCA cycle. 7 Turns of this pathway releases 8 molecules of acetyl-CoA.

MITOCHONDRIAL FATTY ACID OXIDATION (or β-oxidation)

E-1 acyl-CoA dehydrogenase E-2 hydratase

E-3 hydroxyacyl-CoA dehydrogenase E-4 thiolase