

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. A glycolysis chart is included at the back of this exam (on page 11).
Detach (carefully) if you wish. Please make sure you have all 11 pages.
8. Good luck

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

Fructose

Sucrose in the diet

Maltose in the diet

Dihydroxyacetone-P in the presence of arsenate

For a typical glucose molecule released from glycogen intracellularly

Question 2. (6 pts.) Short problems. Show work, but most credit goes to the correct numerical answer.

- a. an enzyme has a V_{max} of $0.55 \mu\text{mol}/\text{min}$ and a rate of $0.12 \mu\text{mol}/\text{min}$ as $260 \mu\text{M}$ substrate. What is the K_m for the substrate

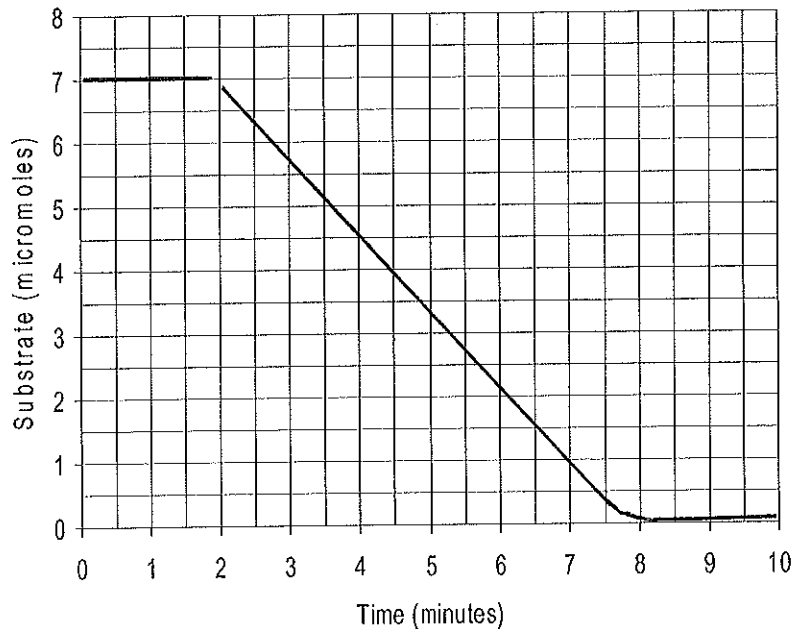
$K_m = \text{_____M}$

- b. a reaction: $A \leftrightarrow B + C$ has a standard free energy of $+4 \text{ kcal}$. If A is maintained at 10 mM , what concentration of B and C (assume $[B] = [C]$) would be in equilibrium with A? Assume 27°C , $R = 2 \text{ cal}/\text{mole}/^\circ$.

$[B]=[C]=\text{_____M}$

Question 3 (6 pts.) The graph to the right shows an enzyme assay converting a single substrate into a single product ($S \rightarrow P$). It was started at time 2 minutes by the addition of 7 micrograms of enzyme to a solution of 1 mL of substrate containing the amount of substrate shown in the graph. The pH was 7.5 and the temperature 25 °C.

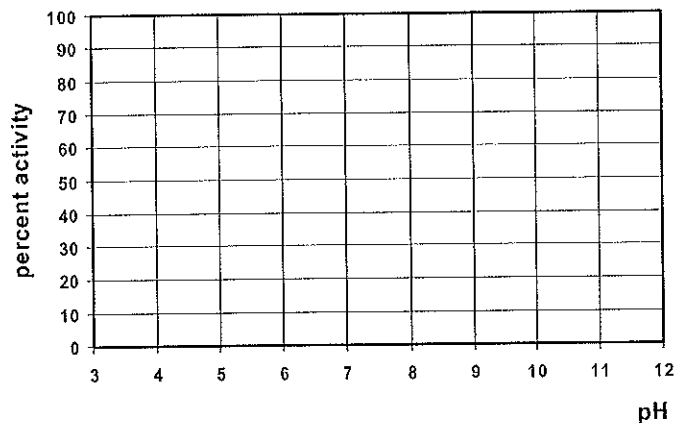
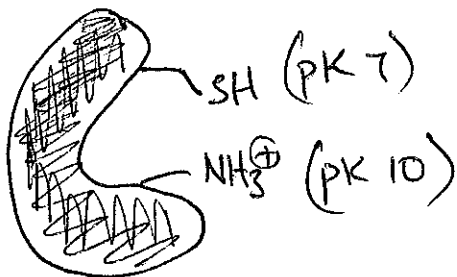
Answer the following questions - there is more information than you need.



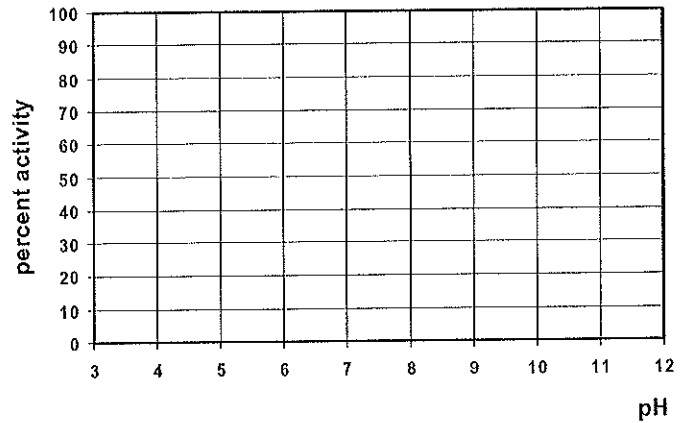
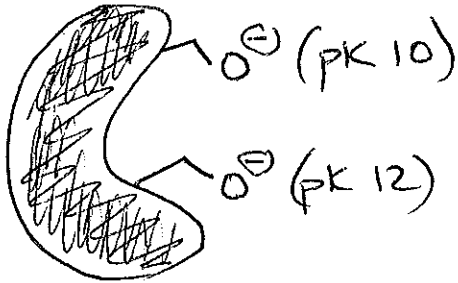
- Calculate the rate of the enzyme assay _____ micromole substrate/min
- What is the rate in the absence of enzyme? _____ micromoles/min
- what is the *concentration* of substrate in the assay before the addition of enzyme _____ [M]

Question 4 (9 pts.) Draw the pH activity curves for the following situations.

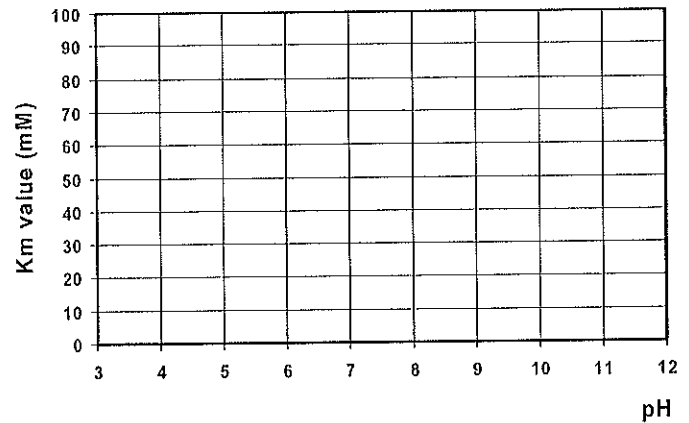
- Only this protonic form is active.



b. This enzyme is only active as shown



c. An enzyme has a lysine side chain (pK of 9 which solely determines the K_m for the substrate. The protonated side chain shows a K_m of 70 mM and the deprotonated form shows a K_m of 10 mM. Complete the graph.



Question 5 (5 pts) Draw an equation to illustrate the reaction stoichiometry of adenylate kinase:

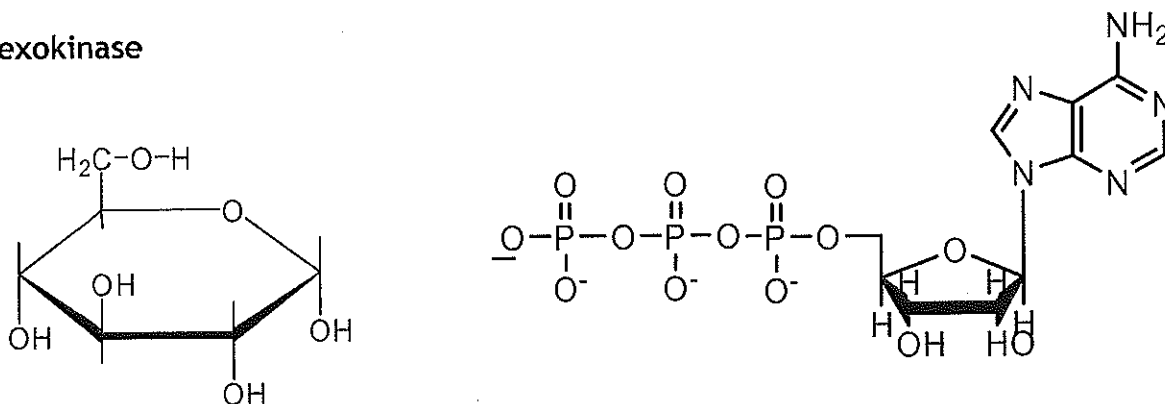


You add phosphofructokinase to a tube containing these (initial) concentrations of substrates/products: $[\text{Compound \#3}] = 5 \text{ mM}$ $[\text{Mg ATP}] = 5 \text{ mM}$

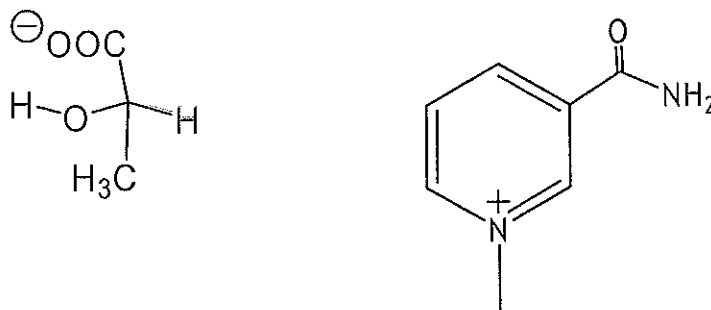
Why is the production of compound #4 accelerated as the reaction progresses by adding small amounts of adenylate kinase?

Question 6 (6 pts) Fill in the initial series of curved arrows that start the reactions of the following enzymes. The curved arrows should make chemical sense. Don't draw any more detailed structures. (If you need to deprotonate or protonate something draw general base/acids as appropriate). Add any additional critical catalytic groups as appropriate.

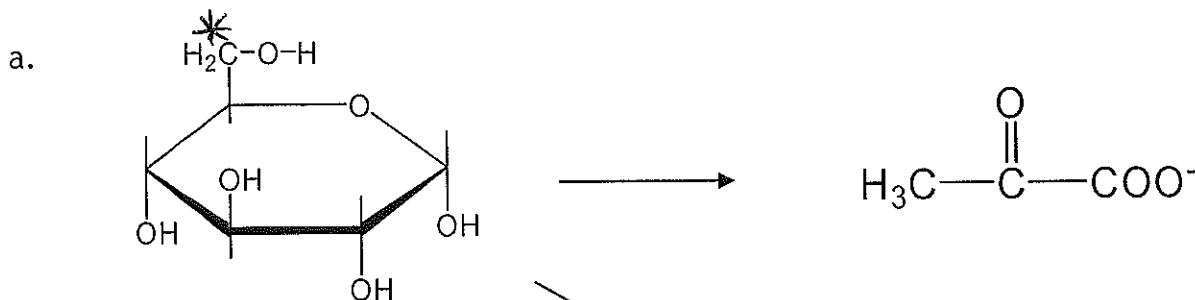
a. hexokinase



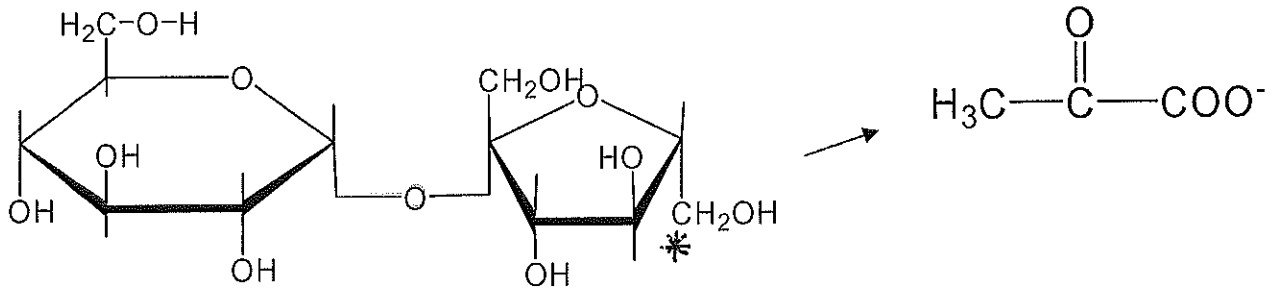
b. lactate dehydrogenase



Question 7 (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".

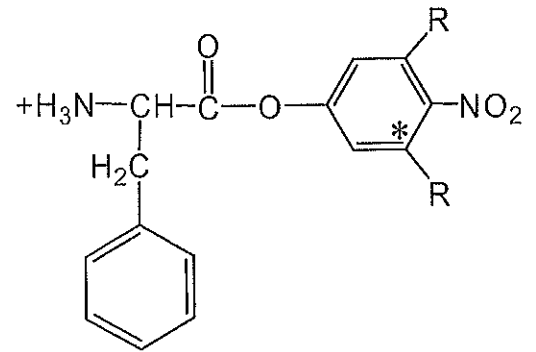


c.



Question 8 (12 pts) Chymotrypsin is mixed with the radiolabeled ester substrate shown to the right (C^{14} at the asterisk) to give concentrations of $30 \mu\text{M}$ and 10mM respectively.

The first product to be released shows a molar extinction coefficient of $10,000 \text{M}^{-1}\text{cm}^{-1}$ at 410nm under the conditions of the experiments ($\text{pH } 8.5$).



Answer the following questions:

a. In the box at the right draw the second product to be released from the enzyme:

b. The burst phase is completed in less than 1 min. Calculate the absorbance increase at 410nm in a 1cm pathlength expected for the burst phase:

Absorbance increase _____

c. Suppose the turnover number of the enzyme in the steady state was $20/\text{min}$ what is the increase in absorbance at 410nm that would be observed between 1 and 2 min after mixing?

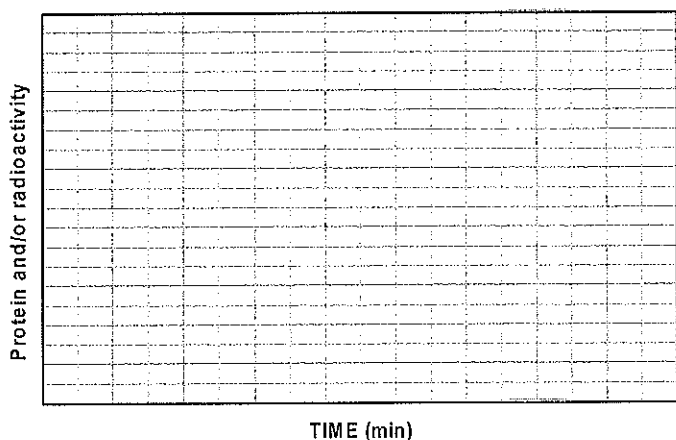
Absorbance increase _____

Question 8 (continued)

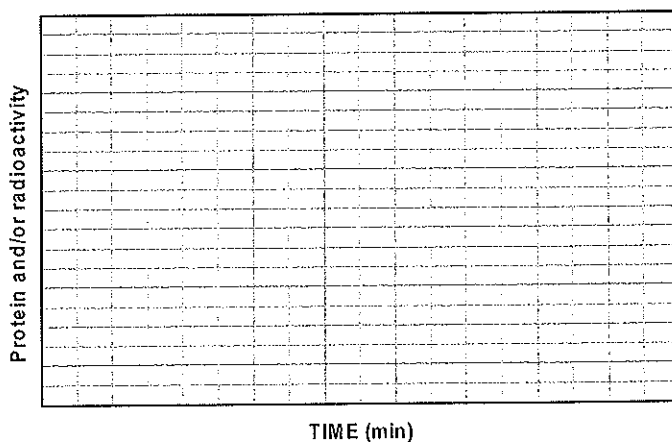
d. After 2 min the mixture is cooled rapidly and gel-filtered (size exclusion chromatography) at 4°C. Using the graph below **left** draw a representative trace of the chromatogram clearly indicating where protein and radioactivity would emerge.

e. The protein-containing fractions are collected and combined. They are allowed to warm to 25 C and then the chromatography is repeated. Again the fractions were followed for protein and radioactivity. Show the expected result at the **right** (above).

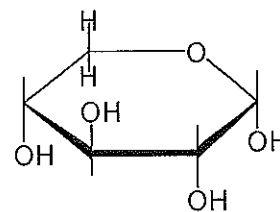
first chromatography



second chromatography



Question 9 (9 pts) Alpha-D-xylose (shown) is dissolved in buffer and mixed with ATP/Mg²⁺ and hexokinase. The concentration of selected compounds is shown below at time zero and after 5 min



Time	xylose	ATP	ADP	AMP	Pi
0 min	1mM	10 mM	0 mM	0 mM	0 mM
5 min	1 mM	6 mM	4 mM	0 mM	4 mM

a. show a chemical equation to describe this overall reaction in the presence of xylose:

b. Explain concisely what is happening in this example:

c. Finally, draw ["]D-xylose["] in a furanose ring form. Label the configuration of the anomeric carbon atom that you chose and circle the C atom in this furanose form that designates this as a D-sugar.

Question 10 (7 pts) Draw the catalytic triad of a thiol protease. Include side chain structures and interactions.

Suggest a simple covalent inhibitor (less than 12 atoms) of the enzyme that you depict.

Name _____ Chemical structure

Then in the space below draw the essential chemistry using appropriate curved arrows.

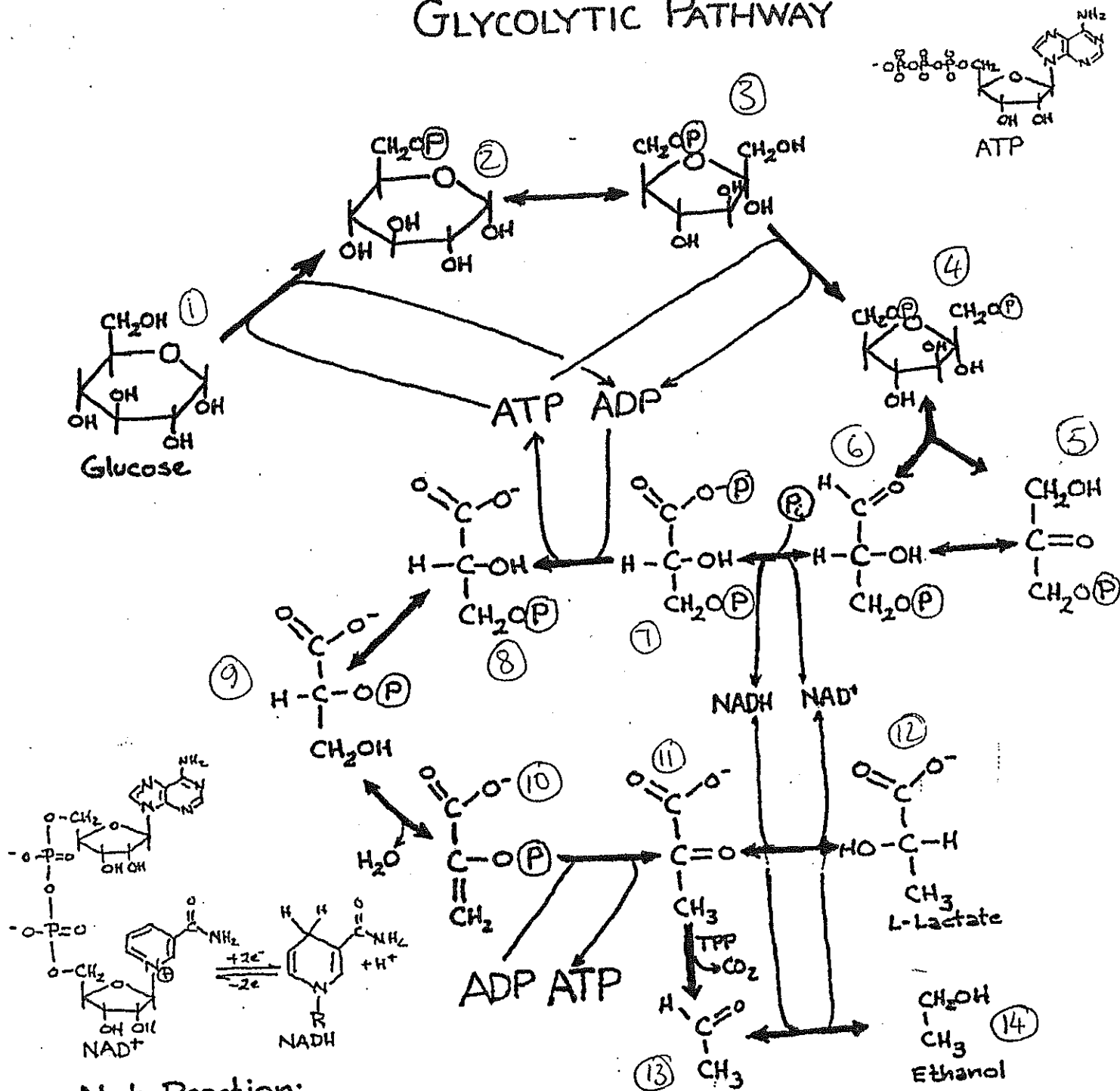
Question 11 (24 pts.) Fill in the blanks with not more than 3 legible words.

- a. name the glycosidic bond formed in amylose _____
- b. name the glycosidic bond at the branch points in amylopectin _____
- c. These compounds bind equally to both free enzyme and the enzyme substrate complex _____
- d. This process sets a physical upper limit on an enzymes catalytic efficiency _____
- e. The reagent used to diagnose *Helicobacter pylori* infections discussed in class _____
- f. The method for determining the in vivo concentrations of metabolites discussed in class _____
- g. A chemical inhibitor of glycolysis discussed in class _____
- h. And the enzyme it inhibits _____
- i. Inactive precursors of proteolytic enzymes _____
- j. Give an example of an affinity label for an enzyme _____
- k. And the enzyme it inhibits _____
- l. The epimerization step converting a galactose derivative to a glucose derivative utilizes what coenzyme _____
- m. this glycolytic enzyme generates a thioester intermediate _____

- n. One form of this enzyme uses a Schiff base intermediate _____
- o. if you could make yeast thiamine deficient, what compound would accumulate when yeast are fed fructose _____
- p. Name a metalloprotease _____
- q. Name an aspartyl protease _____
- r. Name a thiol protease _____
- s. reactions with positive free energies are called _____
- t. Name one component responsible for the brown color of aerobic avian muscle _____
- u. the absence of which enzyme in humans prevents us from converting dietary starch to ethanol _____
- v. the vitamin incorporated into NAD⁺ is called _____
- w. The human deficiency disease caused by the vitamin in "v" _____
- zz. the word that best describes this exam _____

"Life is a struggle with equilibrium that we all eventually lose"

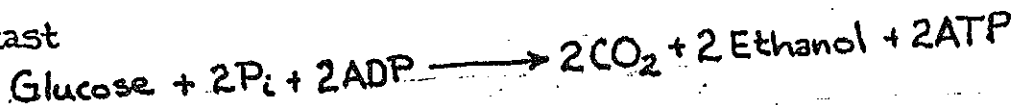
GLYCOLYTIC PATHWAY



Net Reaction:
in vertebrates



in yeast



- 1/2 hexokinase
- 2/3 phosphoglucosomerase
- 3/4 phosphofruktokinase
- 4/5+6 aldolase
- 5/6 triosephosphate isomerase
- 6/7 glyceraldehyde 3P dehydrogenase

- 7/8 phosphoglycerate kinase
- 8/9 phosphoglyceromutase
- 9/10 enolase
- 10/11 pyruvate kinase
- 11/12 lactate dehydrogenase
- 11/13 pyruvate decarboxylase
- 13/14 alcohol dehydrogenase

B