BIOCHEMISTRY FINAL EXAMINATION **641/10** **FALL 1996**

NAME KEY

Notes: There are 13 pages on this exam - please check.
Please detach the metabolic charts carefully.

The point value of questions varies widely - take note.

Please make your answers brief and to the point.

Please write LEGIBLY. Draw clear diagrams where appropriate.

The course grade is "curved".

Grades will not be posted - they can be accessed as in the Registration booklet.
I will not give grades over the phone or via EMail.

Good luck and happy holidays.

8d .16 g/L
not .016 g l-1

Question 1 (20 pts) Calculate the total yield or equivalent from one molecule of the following. Unless otherwise indicated assume that the molecule undergoes complete oxidation.

a.	Pyruvate aerobically	15
b.	Fructose aerobically	~~26~~ 36
c.	Per acetate completely oxidized via the TCA cycle	10
d.	From fructose-6P to ethanol in yeast	3
e.	From citrate to succinate	7
f.	From lactate completely oxidized in aerobic tissue	17
g.	Per citrate in the presence of arsenite	3
h.	From fumarate to citrate in the presence of malonate	3
i.	Per glyceraldehyde-3-P aerobically	19
j.	Per maltose converted to ethanol	2x2 = (4)

Question 2 (6 pts). Ethanol provides a significant source of energy to a range of organisms (including beer drinkers). Clearly show (step by step) how ethanol is utilized for fuel in man. Finally calculate the net yield of ATP per molecule of ethanol during ~~metabolism~~ by liver.

assume cytoplasm → acetate

Yield of ATP per molecule of ethanol .... (14) (2)

+2ATP (1) $CH_3-CH_2-OH + NAD^+ \longrightarrow CH_3-CHO + NADH + H^+$

+2ATP (2) $CH_3-CHO + NAD^+ + H_2O \longrightarrow CH_3-COOH + NADH + H^+$

4

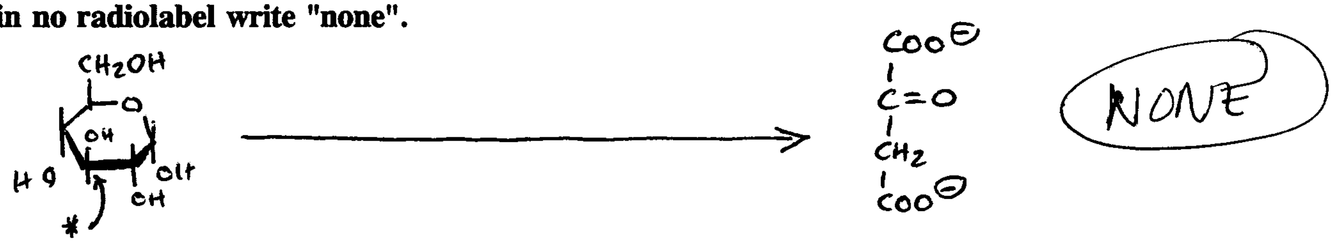
-2ATP (3) $CH_3-\overset{O}{\overset{\|}{C}}-OH + ATP + CoASH \longrightarrow CH_3-\overset{O}{\overset{\|}{C}}-SCoA + AMP + PP_i$ ($PP_i \rightarrow 2P_i$)

(4) $CH_3-\overset{O}{\overset{\|}{C}}-SCoA \longrightarrow TCA \equiv 12\ ATP$ told in class

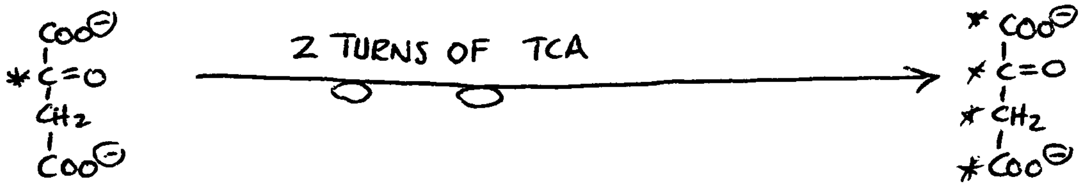
Don't accept pyruvate decarboxylase examples -- Not in humans

Question 3 (10 pts) **Indicate the position of the radiolabel in the following products. If the products contain no radiolabel write "none".**

(2) A.

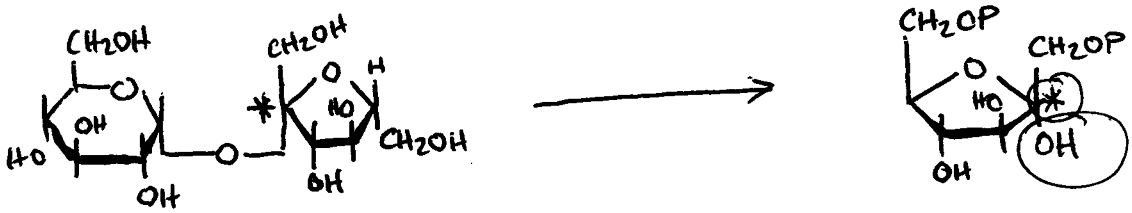


(2) B.



(2) C.

D.



E.

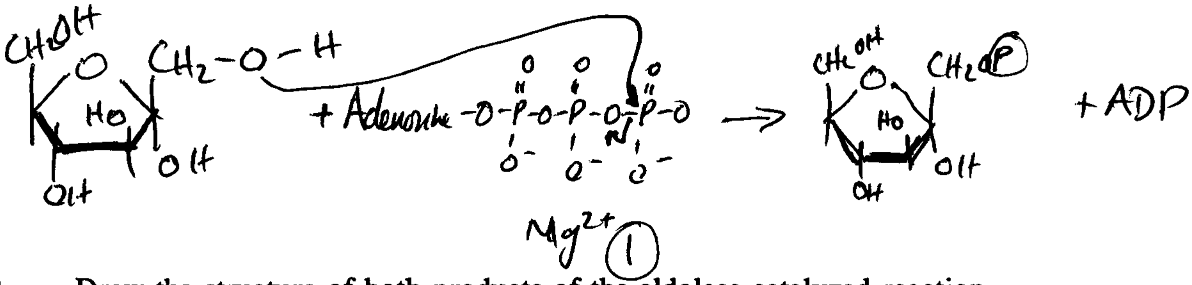
Question 4 (3 pts) Yeast growing on glucose with a plentiful supply of oxygen produce substantial quantities of ethanol. Explain briefly - put the "punch line" first.

Glycolysis has a much higher flux than TCA. Hence can make more ATP/sec with glycolysis than with GLYCOLYSIS + TCA. So yeast uses glucose partially at first & then returns to re-utilize ethanol more efficiently.

Question 5 (14 pts) Answer the following questions concisely. One peculiar way fructose enters glycolysis is via a direct phosphorylation to give fructose-1P followed by attack by an aldolase very similar to that shown in your chart.

A. Draw a reaction showing exactly how production of Fructose-1P occurs. Include any anticipated cofactors etc. Show the products.

(5)



B. Draw the structure of both products of the aldolase catalyzed reaction.

(2)

C. One of them is released from a covalent intermediate on the enzyme surface. Which one is it?

(2)

D. How was the information in "C" obtained?

(1) Borohydride trapping of dihydroxyacetone(P) treated enzyme.

E. How would both products of the aldolase reaction in "B" enter glycolysis?

II as normal

(2) I needs to be phosphorylated. How about glyceraldehyde kinase

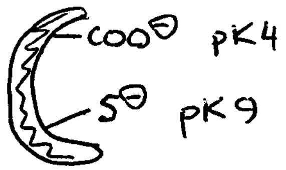
I + ATP ⇌ I-(P) + ADP

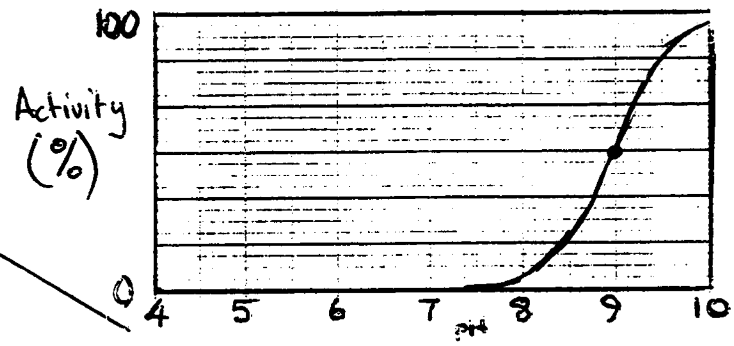
F. Outline another way fructose enters glycolyis.

$$\text{Fructose} + \text{ATP} \xrightarrow[\text{Mg}^{2+}]{\text{hexokinase}} \text{Fructose 6P} + \text{ADP}$$

Question 6 (10 pts) Draw accurate graphs for the following as a function of pH

a. An enzyme whose only active form is:



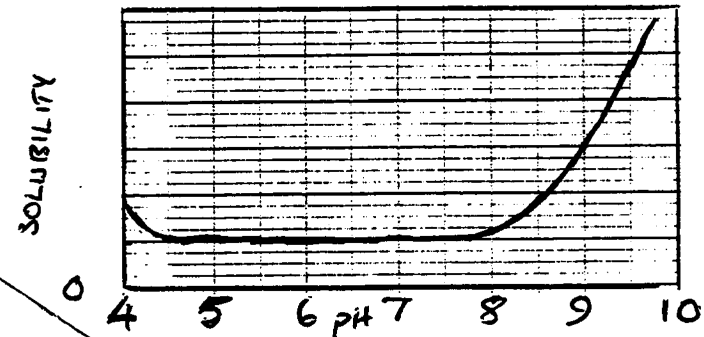


b. The solubility of this peptide in buffers of the indicated pH values

pK 9 pK 3

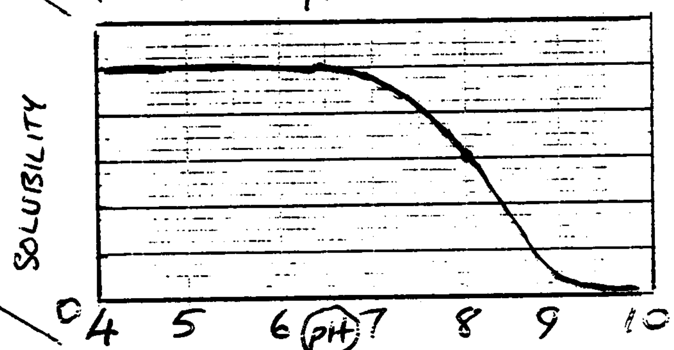
$NH_3^{\oplus}$ – LEU – ILE – MET – $COO^{\ominus}$

pI = 6

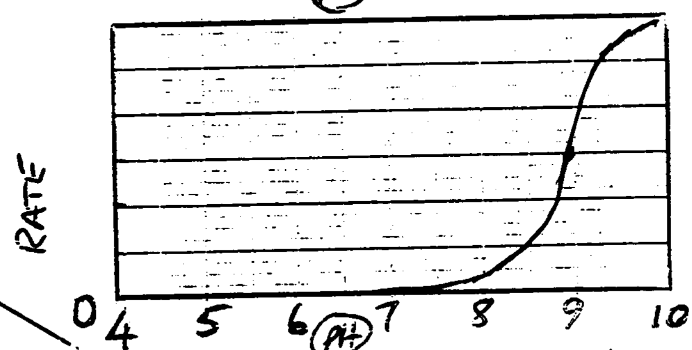


c. The solubility of this carboxylic acid

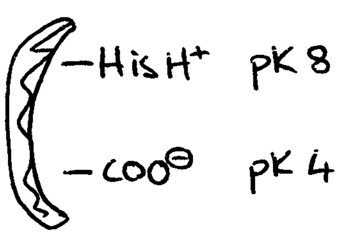
in oil. Its pK in oil is 8.

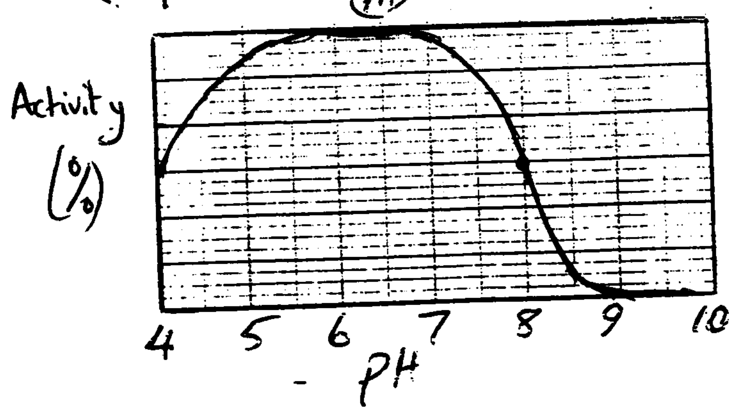


d. The rate of reaction of cysteine (pK_R=9) with iodoacetate

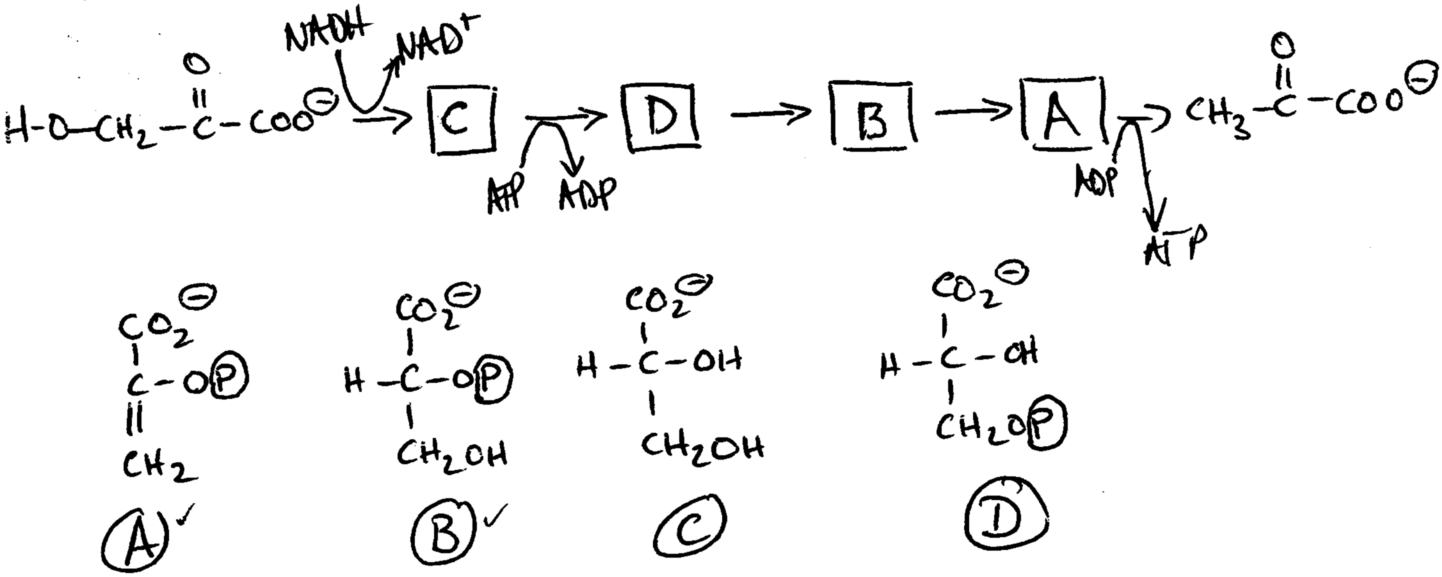


e. The activity of an enzyme whose only active form is





Question 7 (7 pts). Hydroxypyruvate is converted to pyruvate via the four intermediates listed below. Put A-D in the correct order in the boxes provided. Put in any other cofactors/coenzymes/prosthetic groups that would be involved



Question 8 (27 pts) Short Calculations. Most of the credit goes towards the correct numerical answer.

a. A negligible volume of aldolase is added to a solution of 0.02 M fructose 1,6-diP. After equilibration the concentration of fructose 1,6-diP was 0.019 M. Calculate the equilibrium constant for the aldolase catalyzed reaction.

K_{eq} = 5.26×10^{-5} M

.019 M = .001M + .001M

$$\frac{(.001)^2}{.019}$$

b. In a 1 mL assay mixture you place 20 μmol substrate and 0.25 mL of a 1mg/mL solution of enzyme MW 90,000. After 3 min 12 μmol of substrate remain. What is the average turnover number of the enzyme?

TN = 960 /min

$$\frac{8\ \mu mol / 3\ min}{\frac{.25 \times 10^{-3}\ g}{90{,}000\ g/mole}} = 960$$

2880 for 3 min

c. The pK of acetic acid is 4.7. What is the standard free energy change for the dissociation of the acid at 25°C? R= 2cal/mole/degree.

$HA \rightleftharpoons H^+ + A^-$ $K_{eq} = 10^{-4.7}$

$\Delta G^{\circ\prime} =$ + 6.45 kcal

$\Delta G^{0\prime} = -2.303 \times R \times T \times \log 10^{-4.7}$

$= -2.303 \times 2 \times 298 \times (-4.7)$

d. How much iron (in grams) is contained in the average person's hemoglobin. You will need some of the following pieces of information. MW hemoglobin, 64,000; atomic weight of iron, 56; number of red cells in a human, 2.5×10^{18}; volume of blood 7L; concentration of hemoglobin in blood, 0.~~0~~16g/mL.

$7L \times .016\ g/mL \times 1000\ mL/L \times \frac{4}{64{,}000\ g/mol} \times 56\ g/mole$ (3.92 g)

e. Our laboratory is working on a sulfhydryl oxidase that seems to act in the maturation of proteins in egg white forming disulfides as indicated:

2 (-CYS-SH) + O_2 → (-CYS-S-S-CYS-) + H_2O_2

The concentration of protein disulfides in egg white is 15 mM and we estimate the concentration of the enzyme in egg white as 50 nM. Assume the turnover number of the enzyme is 1200 molecules of hydrogen peroxide formed/min. How many minutes would it take to completely generate these disulfide bridges?

$\frac{15 \times 10^{-3}}{50 \times 10^{-9}} \times \frac{1}{1200/min} =$ (250 min.)

f. What is the amount of NaOH in moles required to bring 0.5 moles of the histidine dihydrochloride (pKs 1.8, 9.2, 6) to the following pH values.

pH 6.0 0.75

pH 7.6 1.0

pH 9.2 1.25

(9.2) HisH$^+$ (pK 6)

$NH_3^{\oplus}$ — COOH (1.8)

0.5 mole

g. An enzyme shows a Km of 23 mM with substrate S. What concentration of S would be needed to get to exactly 80% of the maximal possible rate with S?

$$0.8\,V_{max} = \frac{V_{max} \cdot S}{23\,mM + S}$$

18.4 + 0.8 S = S

0.2S = 18.4

S = 92 mM

h. A lab makes 100 mM phosphate buffer by mixing 100 mM stock solutions of the acid form KH_2PO_4 with the required volume of the basic form K_2HPO_4. You have 1L of the acid form (pK 7.2) and need a pH of 6.8. What volume of the basic form will you need to add?

$pH = pK + \log \frac{A^-}{HA}$

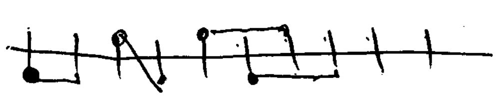
$6.8 = 7.2 + \log \frac{A^-}{HA}$

$\frac{A^-}{HA} = .398$

Vol 0.398 L

i, A protein contains 10 cysteine residues and enough oxidant is added to convert all but 20% of them to disulfide bridges. On average how many combinations of disulfides in the oxidized material will be found?

NUMBER 945

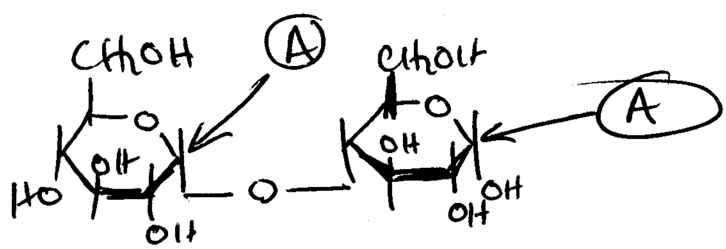


9×7×5×3

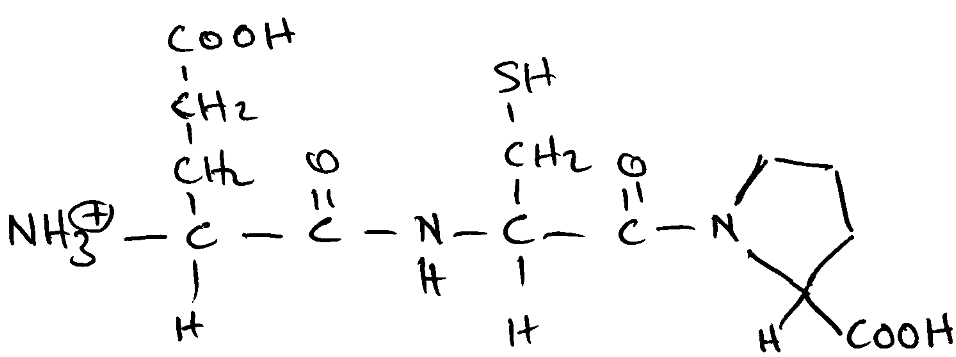
Question 9 (7 pts) Predict what will happen to the pK values of the following side chains. Circle UP, DOWN or NO CHANGE.

a. ARG driven into the interior of a globular protein	UP	**DOWN**	NC
b. GLU desolvated on binding substrate	**UP**	DOWN	NC
c. LYS pushed close to a carboxylate group	**UP**	DOWN	NC
d. HIS moving from a hydrophobic to a polar solvent	**UP**	DOWN	NC
e. ASP brought close to a coordinating Mg^{2+} ion	UP	**DOWN**	NC
f. ASP after it is incorporated into the C-terminus of a small peptide	**UP**	DOWN	NC
g. LYS after it is incorporated into the N-terminus of a small peptide	UP	**DOWN**	NC

Question 10 (4 pts) Draw a clear structure of MALTOSE. Show the anomeric carbon atom(s) by "A(s)"



Question 11 (4 pts) Draw the structure of the peptide GLU-CYS-PRO in the form that predominates at pH 1.



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Question 12 (22 pts) Fill in the blanks with not more than 3 legible words

a. Aconitase is colored because of this feature ___Fe/S___

b. A reducing agent used to trap Schiffs base ___Borohydride___

c. The name of prosthetic group with long mobile swinging arm ___Lipoic Acid (Biotin)___

d. A stimulant which inhibits phosphodiesterase ___CAFFEINE___

e. What is the key product of adenyl cyclase ___cAMP___

f. A protease with a low pH optimum ___PEPSIN___

g. An allosteric enzyme in which allosteric and catalytic sites are on different subunits ___Aspartate transcarbamylase___

h. Reactions with + $\Delta G^{o\prime}$ are called ___ENDERGONIC___

i. A negative allosteric modulator of phosphorylase b ___ATP___

j. Two enzymes involved in a "futile cycle" ___PFK___

k. and the second.... ___phosphatase___

l. What is the most extended single helix in protein secondary structures? ___3^{10} HELIX___

m. Which is the most conformationally accomodating amino acid in protein structures? ___GLY___

n. Asp is one substrate of aspartate transcarbamoylase, what is the second? ___carbamyl phosphate___

o. Name an allosteric inhibitor of the enzyme in "n" CTP

p. A chemical reagent can relieve this inhibition, it is (Mercurial) pOH mercuribenzoate

q. A protein toxin Botulinum (ricin)

r. An isomerase that utilizes NAD^+ UDP-galactose isomerase

s. Inactive precursors of proteolytic enzymes Zymogen

t. Increasing solubility with increasing ionic strength is called Salting in

u. A naturally occuring inhibitor of the TCA cycle Fluoroacetate

v. The compound that accumulates during the action of the compound "u" Citrate

THE END