CHEM 527
Final exam, Fall 2003

NAME Key

NOTES:

1. Please stay calm.
2. Where appropriate, show work to receive full credit.
3. This exam contains 10 pages + metabolic charts (*detach gently, please*).
4. Pace yourself - you may want to do the easiest questions first.
5. Note the point value of questions varies widely - adjust your answers accordingly.
6. Please give concise answers - if there isn't much space allotted - a short answer is appropriate.
7. Questions may have more data than needed to tackle the problem.
8. PLEASE write clearly. If I cannot read it .... it is wrong.
9. As mentioned in class and EMails, you are allowed to refer to a single piece of 8.5 x 11" paper during this exam. It can feature any material distributed over both sides.

Question 1 (12 pts) Yield of ATP. In the space provided give the yield of ATP (or equivalent e.g. GTP) that would be formed in the following processes:

a. per molecule of glucose completely oxidized to CO_2 and water 30

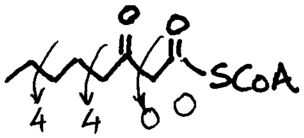
b. per pyruvate in the presence of arsenite 0

c. per molecule of fructose 6-P converted to lactate 3

d. per molecule of lactate completely oxidized to CO_2 and water 14

e. per citrate in the presence of malonate 6

f. per

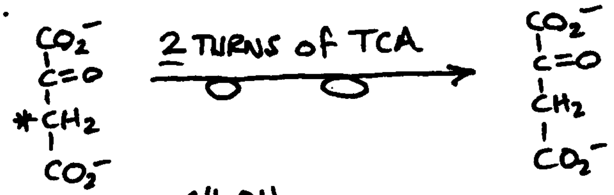


completely oxidized to CO_2 and water 48

4x10 + 8

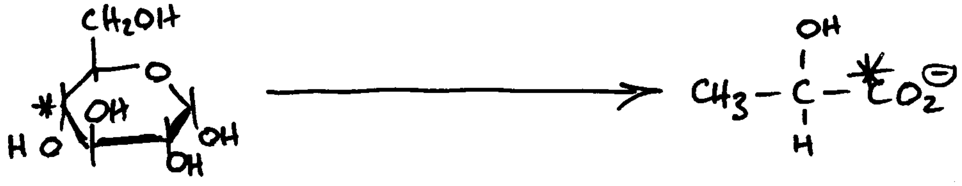
Question 2 (8 pts) Tracing radiolabels. Place asterisks indicating the position of the radiolabel in the molecules shown to the right - if the product contains no radiolabel write "NONE".

a.

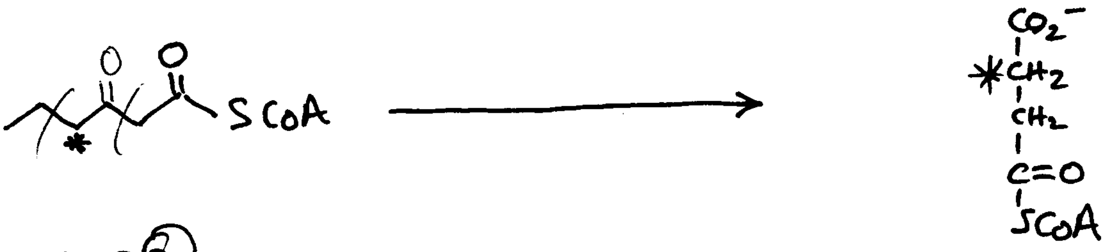


NONE

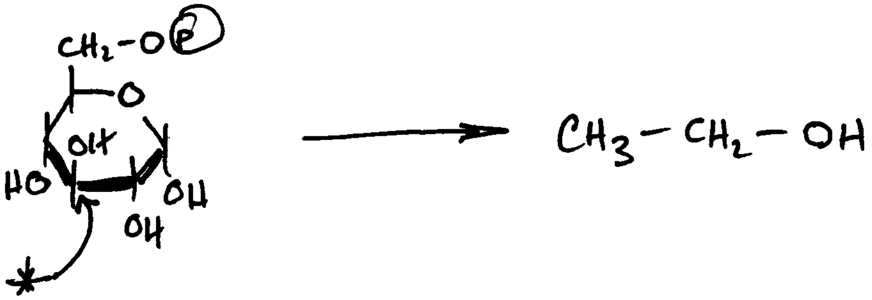
b.



c.



d.

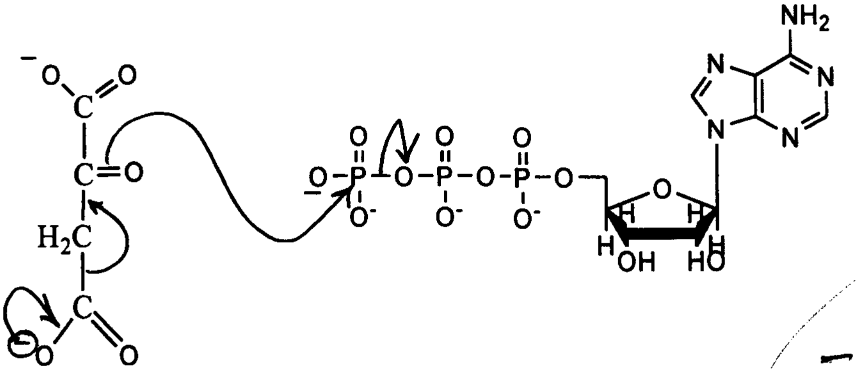


NONE

Question 3 (7 pts) Fill in the <u>initial series of curved arrows that start the reactions</u> of the following enzymes. The curved arrows should make chemical sense. Don't draw any more structures.

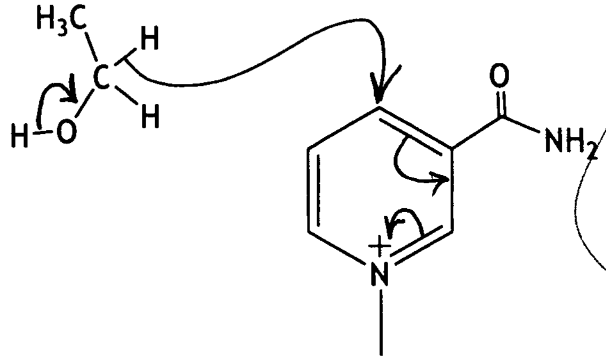
a. PEP carboxykinase

4 pts



b. Alcohol dehydrogenase

3 pts

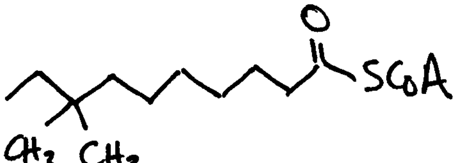


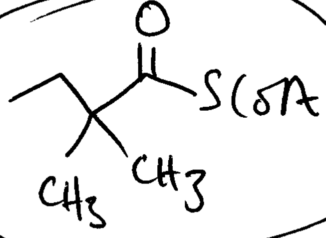
-1 for each sign error to 0

Question 4. (6 pts). Fatty acid oxidation handles unusual fatty acids as well as normal (e.g. straight-chain) ones. For each of these compounds shown to the left show what compound you would expect to accumulate after conventional β-oxidation has finished.

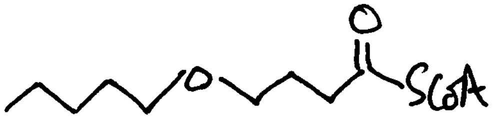
2ea

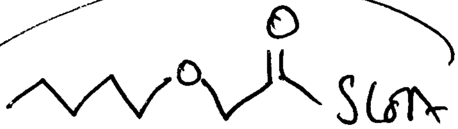
a.



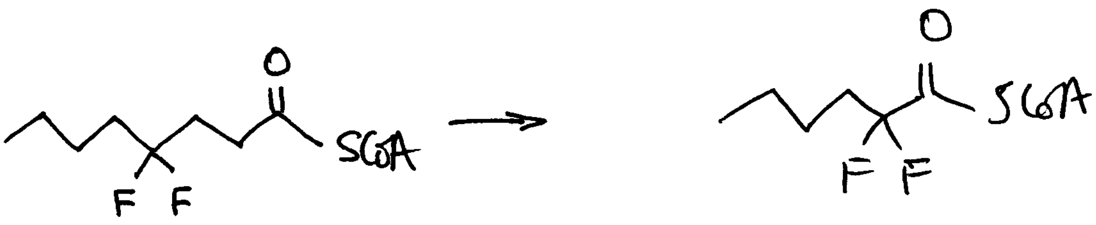


b.





c.



Question 5 (18 pts) Place in the space provided a <u>single number</u> from 0 - 18. Do not put enzyme or substrate product names.

a. Transamination of aspartic acid gives what TCA cycle intermediate 8

b. The biosynthesis of glucose from 2 molecules of pyruvate costs how many molecules of ATP 6

c. The complete oxidation of ethanol generates how many molecules of CO_2 2

d. What intermediate of the TCA cycle would accumulate at low phosphate concentration 4

2ea

e. Ethanol contains _ more electrons than acetaldehyde 2

f. The number of NADH molecules formed converting glucose to 2 lactates 2

g. placing a molecule of glucose on the growing end of a glycogen molecule costs how many ATP equivalents? 2

i. The number of electrons required to reduce one oxygen molecule to water 4

j. How many ATP (or equivalents) would you expect to need to make a molecule of acetyl-CoA from acetate and CoA 2

c

3

Question 6 (5 pts) About 2% of sudden infant death is caused by a mutation in E1 of fatty acid oxidation. Mutation leads to a decrease in enzyme activity observed in the cell. Patients with this genetic disease would be expected to suffer <u>all but one</u> of the following symptoms:

✓ a. an inability to utilize fats for fuel
✓ b. increased reliance on dietary carbohydrate and protein for energy
~~c.~~ Increased formation of ketone bodies
✓ d. Low blood glucose
✓ e. Low levels of glycogen granules in the liver.

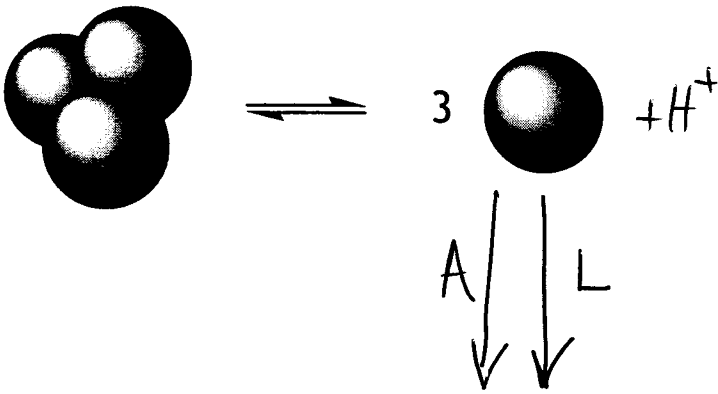
The mutant enzyme from the patients with this metabolic disorder (in E1) was purified and found to have exactly the same turnover number and Km for substrates as normal enzyme. Explain (in <u>one sentence</u>) how the activity in the cell could be found to be very low.

2 (It is a mutation which drastically decreases enzyme stability (so much less enzyme in cell).

KEY

Question 7 (12 pts) Linked equilibria.

Three identical subunits are in equilibrium with a trimer as shown to the right. A is an allosteric modulator which affects the binding of L. His_{50} is the only amino acid whose pK changes on trimer formation.



MONOMER	TRIMER
Binds **L** tightly	Binds **L** weakly
Binds **A** tightly	Binds **A** weakly
pK HIS_{50} = 6	pK HIS_{50} = 8

a. Changing the pH from 7-9 will __ the percentage of monomer - circle: [✓ increase] decrease not affect

b. Increasing the concentration of **A** will __ the affinity for **L** [✓ increase] decrease not affect

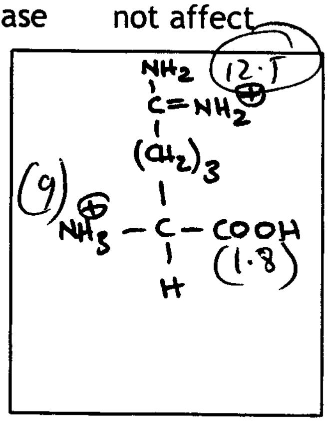
c. Diluting the solution of the protein will __ the affinity for **L**: [✓ increase] decrease not affect

d. In a very lightly buffered solution of protein at pH 7, adding **A** will ___ the pH: increase [✓ decrease] not affect

e. Changing the pH from 7 to 5 will ___ the affinity for **A**: increase [✓ decrease] not affect

f. On gel filtration, adding L to the buffer would ___ the time taken for the protein to elute [✓ increase] decrease not affect

Question 8 (6 pts) The structure of one form of arginine is shown at the right.



2ea

(pK 1.8, 9, 12.5_{R-})

You have 0.6 moles of arginine in the form shown at the right. How much KOH in moles do you need to take the original 0.6 moles ~~to a pH~~ of:

9 0.9

12.5 1.5

5.4 0.6

KEY

Question 9 (18 pts). Short problems. Most of the credit goes for the correct numerical answer

a. Aspirin (pK 3.5), a weak carboxylic acid, is dissolved in water to give a solution with a pH of 3.5. What is the proton concentration?

3.16×10^{-4} M

pH = 3.5 so $H^+ = 10^{-3.5}$

b. You add 0.12 moles of KOH to 0.8 L of 0.3 M formic acid (pK 3.7). What is the pH of the mixture?

pH = 3.7

0.12 moles KOH

0.24 moles formic acid

so after 0.12 mol formate / 0.12 mol formic

$pH = 3.7 + \log \frac{.12}{.12}$

c. the concentration of oxygen dissolved in 1L of buffer in equilibrium with air is 0.24 mM. You then add 8 g of myoglobin and stir gently in air until equilibrium is reached. What is the total concentration of oxygen (free and bound) now carried in the solution. (MW oxygen 32, myoglobin, 16,700, water 18).

$\frac{8g}{16,700g/mol} \equiv 0.479$ mM

[total oxygen] = .719 mM ②

[free oxygen concn.] = 0.24 mM ①

d. Mootase (10 µg) catalyzes the breakdown of 3 µmol of product formation per minute at room temperature. The molecular weight of the enzyme is 45,000 g/mol, the substrate 225 g/mol and the product 225 g/mol. What is the turnover number of Mootase?

Turnover number 13,500 /min

$\frac{3 \times 10^{-6} \text{ mol/min}}{10 \times 10^{-6} / 45,000 \text{ g/mole}} =$

g. the concentration of protein disulfides (in total) in chicken egg white is 20 mM. If 20 mL of egg white contains 2 g or protein (with an average MW of 50,000 g/mole) calculate the average number of disulfides per protein molecule.

$\frac{2g}{20\ mL} \times \frac{1000\ mL}{\cancel{[illegible]}}$ $100 g/L \equiv 2 mM$ prot

20 mM disulfides

Number of disulfides/molecule 10

✓

h. a negligible volume of aldolase was added to 0.02 M fructose-1,6-diP and, at equilibrium, the concentration of fructose-1,6-diP <u>declined by</u> 1 mM. Calculate the equilibrium constant for the aldolase reaction:

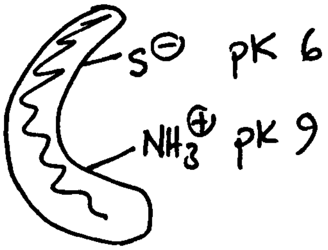
$$K_{eq} = \frac{(.001)(.001)}{(.019)}$$

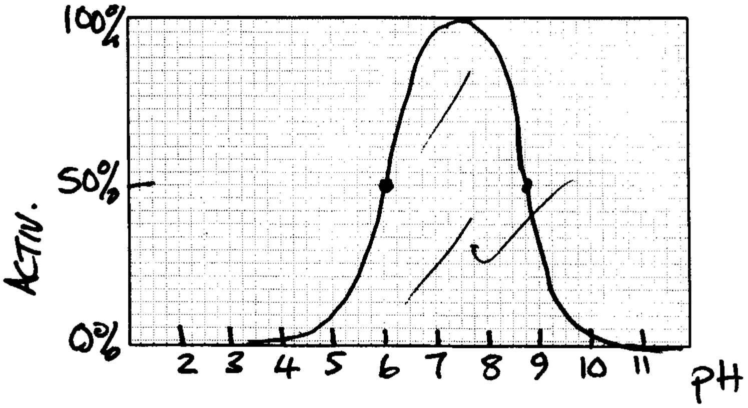
$K_{eq} = 5.26 \times 10^{-5}$ M

✓

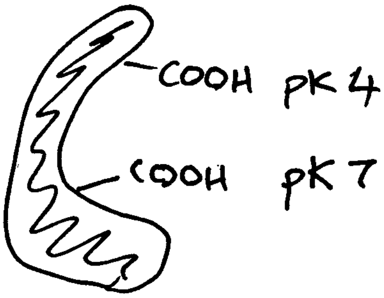
Question 10 (6 pts) Graphs. Draw clear accurate graphs to describe the behavior of the following systems. Clarity and accuracy rewarded.

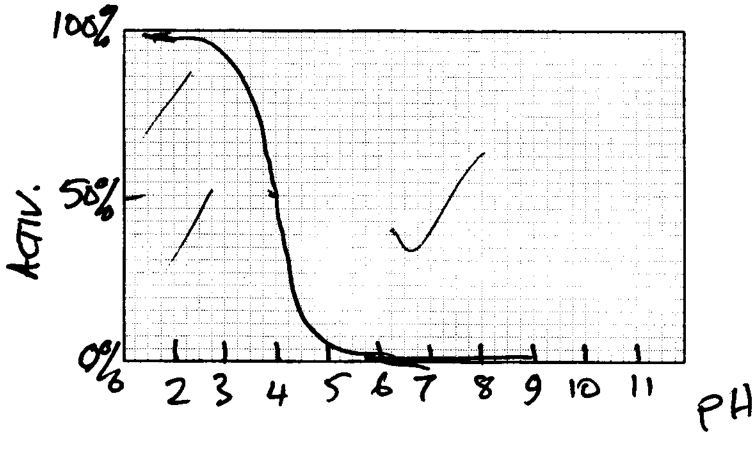
a. only this form of the enzyme show below is active. Show its pH dependence at the right.





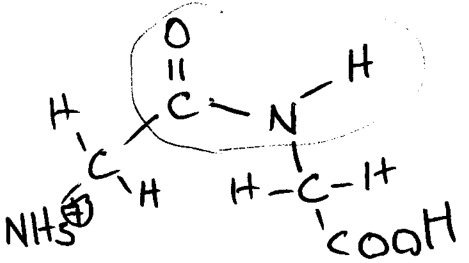
b. only this form of the enzyme show below is active. Show its pH dependence at the right.





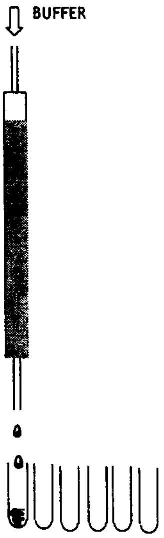
-0

Question 11 (5 pts) Draw a ~~clear~~ representation of the dipeptide GLY-GLY in the form that predominates at pH 1 and in a CIS peptide linkage.



Cis = 2pts

Question 12 (7 pts) (Thought question) You have a solution of buffer containing 100 μM NAD$^+$. This solution is used to thoroughly equilibrate a gel filtration column so that the concentration of NAD$^+$ emerging from the column becomes constant. Then, at time zero, you add a small sample of a 50,000 MW protein (P) dissolved in the same buffer to the top of the column. P binds NAD$^+$ tightly as follows:

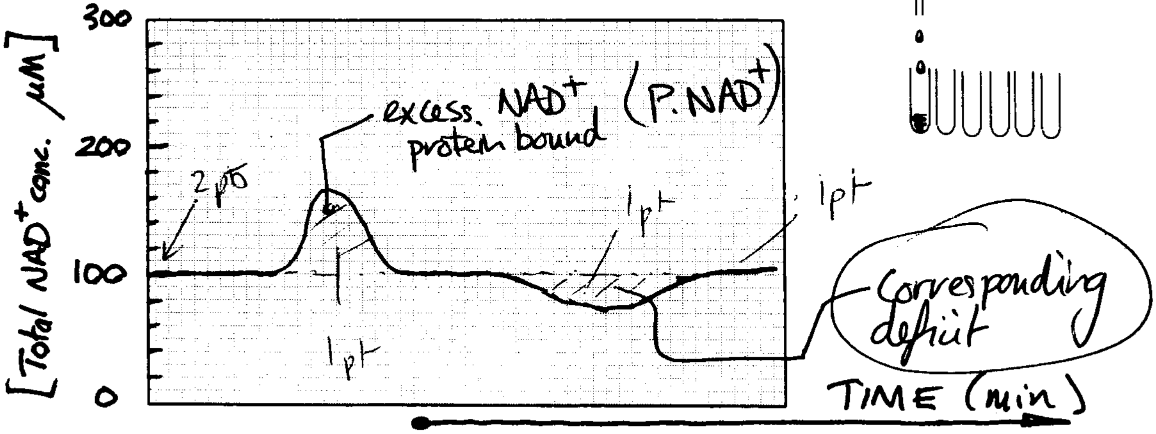


$$P + NAD^+ \leftrightarrow P.NAD^+$$

Sketch an accurate graph showing the NAD$^+$ concentration profile **before**, **during**, and **after** the protein elutes from the column.

5pts

anything reasonable



How could you use this method to determine the K$_d$ of binding NAD$^+$ to P.

Since know free NAD+ conc = 100μM & know how much NAD+ bound to (a known amount of) enzyme. Could use $Keq = \frac{[NAD^+][P]}{[P.NAD^+]}$ can calculate ratio from ⊖

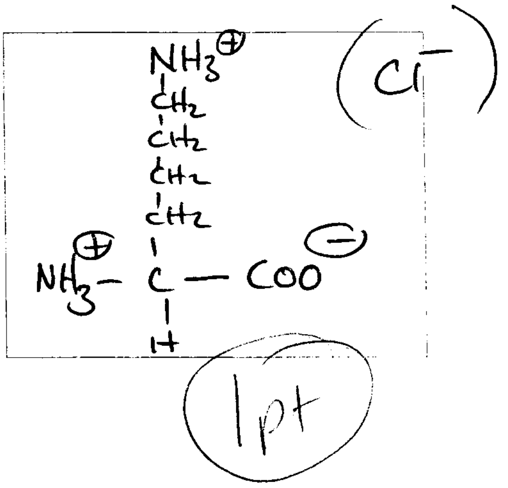
2pts

C

Question 13 (3 pts) What is the most appropriate answer?

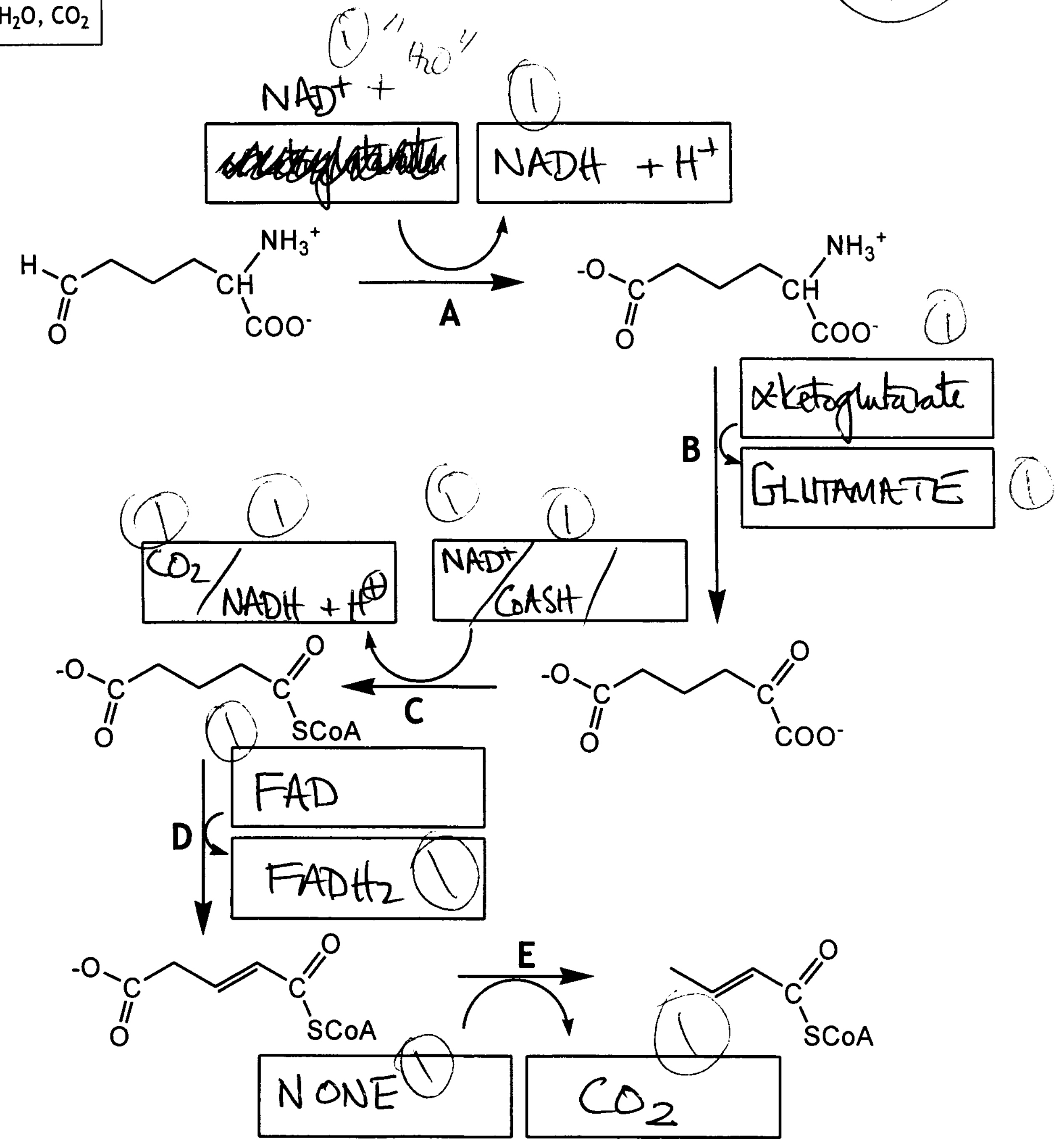
a. the higher the K$_m$ the higher the affinity
b. at [S] = 2 K$_m$, v = V$_{max}$
[✓ c.] at [S] = 2 K$_m$, doubling the enzyme concentration would exactly double the rate
d. K$_m$ is exactly one half of the maximal velocity
e. all of the above are false.

Question (13 pts) Draw the complete structure of lysine in the form that predominates at pH 6.



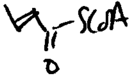
The following is part of the degradation pathway for LYSINE. Reason by analogy to **clearly indicate in the boxes every** substrate and product **missing for each reaction A-E. Don't put enzyme names - a hypothetical example for one box is shown below). If nothing is needed in the box put "NONE".**

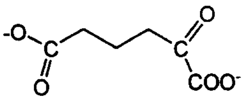
NADH, H_2O, CO_2



2.5
2x acetyl-CoA ≡ 20

$FADH_2$ 1.5





What is the yield of ATP from one molecule of oxidized in mitochondrion to carbon dioxide and water?

26.5 **mol ATP**

Question (14 pts) Fill in the blanks with not more than 3 legible words.

a. reactions that replenish TCA cycle intermediates are called anaplerotic

b. This technique rapidly stops metabolism allowing mass action ratios to be determined freeze clamping

c. Name an enzyme that is involved in thermogenesis in bumble bees PFK (or) Fructose 1,6 diP phosphatase

d. Adenine, guanine, cytosine and ___ (other base in RNA) URACIL

e. Adenine, guanine, cytosine and ___ (other base in DNA) THYMINE

f. Locally folded units within a polypeptide chain are called DOMAIN

g. One enzyme that cleaves α(1-6) glycosides is called DE-BRANCHING

h. the organ most sensitive to thiamine deficiency BRAIN

i. this fellow developed the Chemiosmotic hypothesis MITCHELL, Peter

j. this enzyme initiates the biological effects of methanol alcohol dehydrogenase

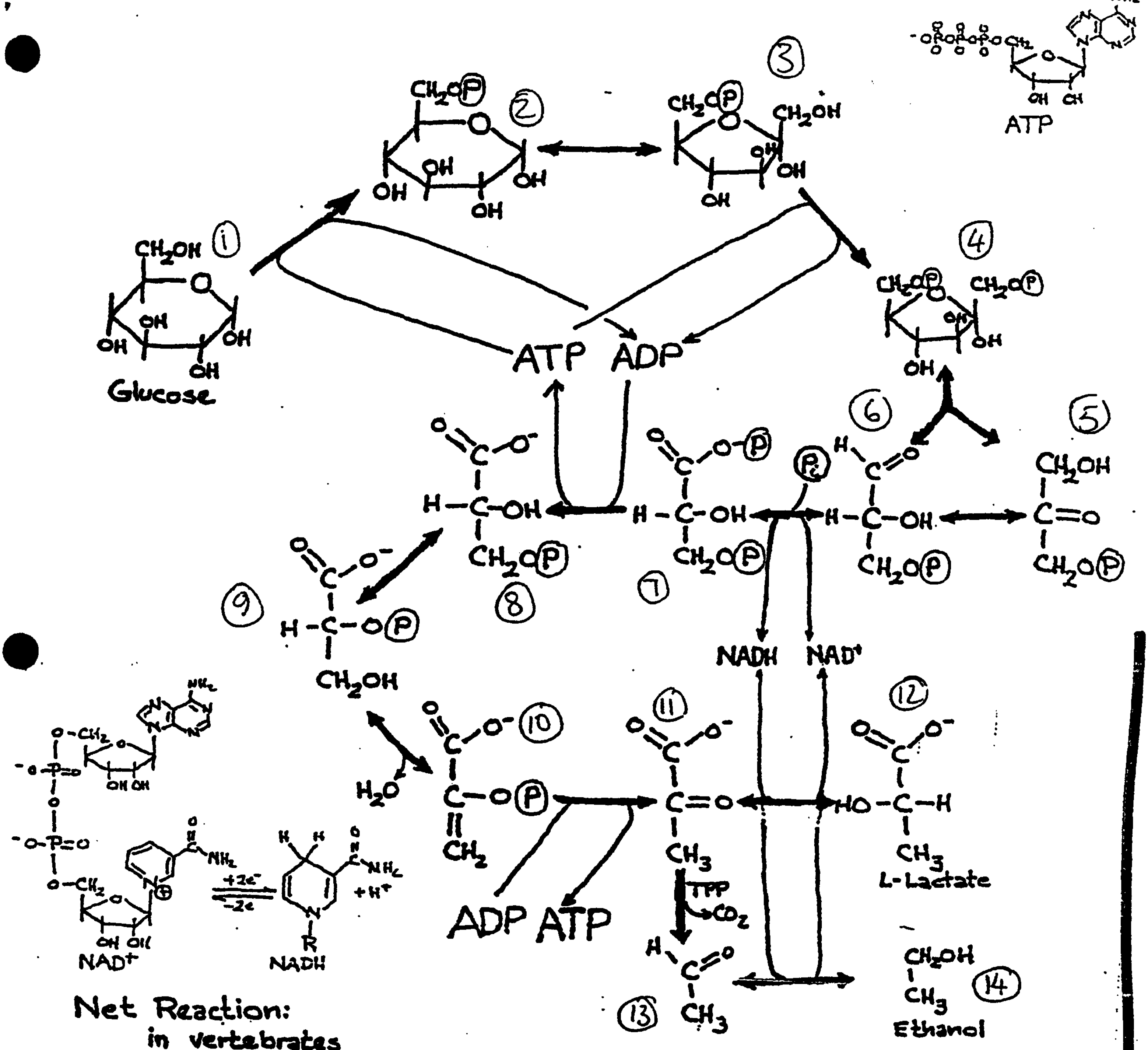
k. the reagent that cleaves peptides at MET CNBr

l. an amino acid without a chiral center GLYCINE

m. aconitase is colored because of this feature Fe/S centers

zzz. The word that best describes this exam One

GLYCOLYTIC PATHWAY



Net Reaction:

in vertebrates

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

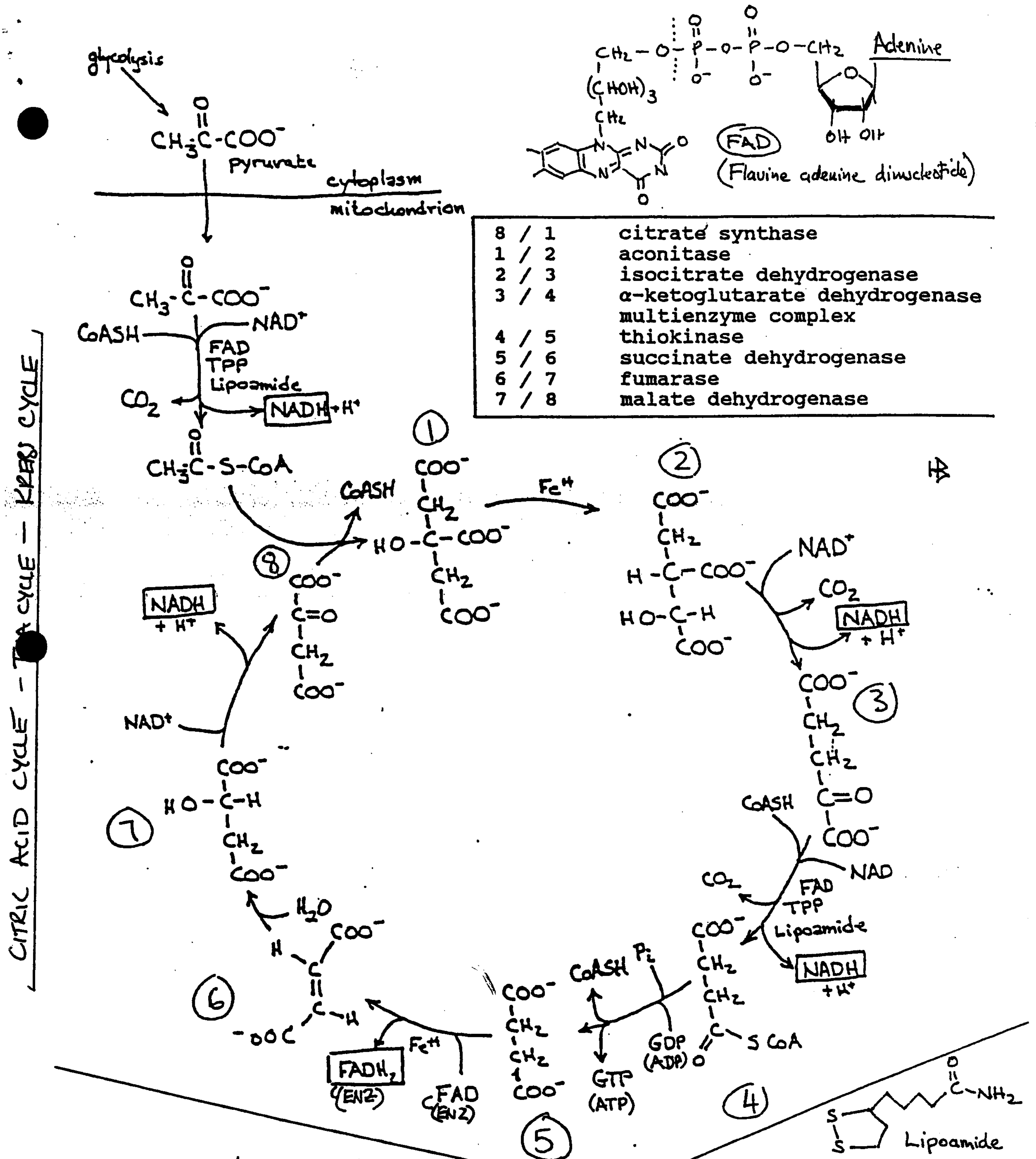
in yeast

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

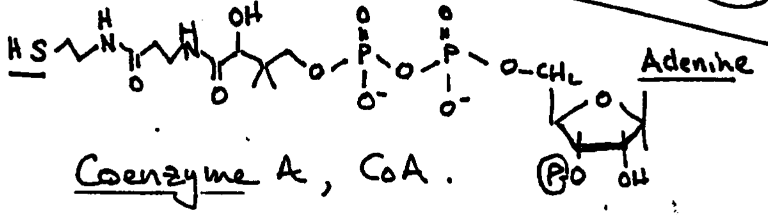
1/2	hexokinase
2/3	phosphoglucoisomerase
3/4	phosphofructokinase
4/5+6	aldolase
5/6	triosephosphate isomerase
6/7	glyceraldehyde 3P dehydrogenase
7/8	phosphoglycerate kinase
8/9	phosphoglyceromutase
9/10	enolase
10/11	pyruvate kinase
11/12	lactate dehydrogenase
11/13	pyruvate decarboxylase
13/14	alcohol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

HB

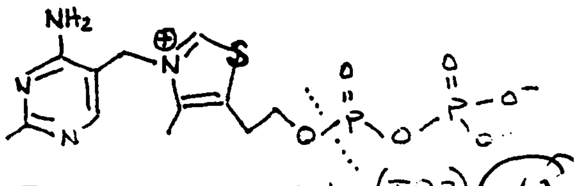
CITRIC ACID CYCLE – TCA CYCLE – KREBS CYCLE



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



Coenzyme A, CoA.



Thiamine pyrophosphate (TPP)

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:

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

[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 ß-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.

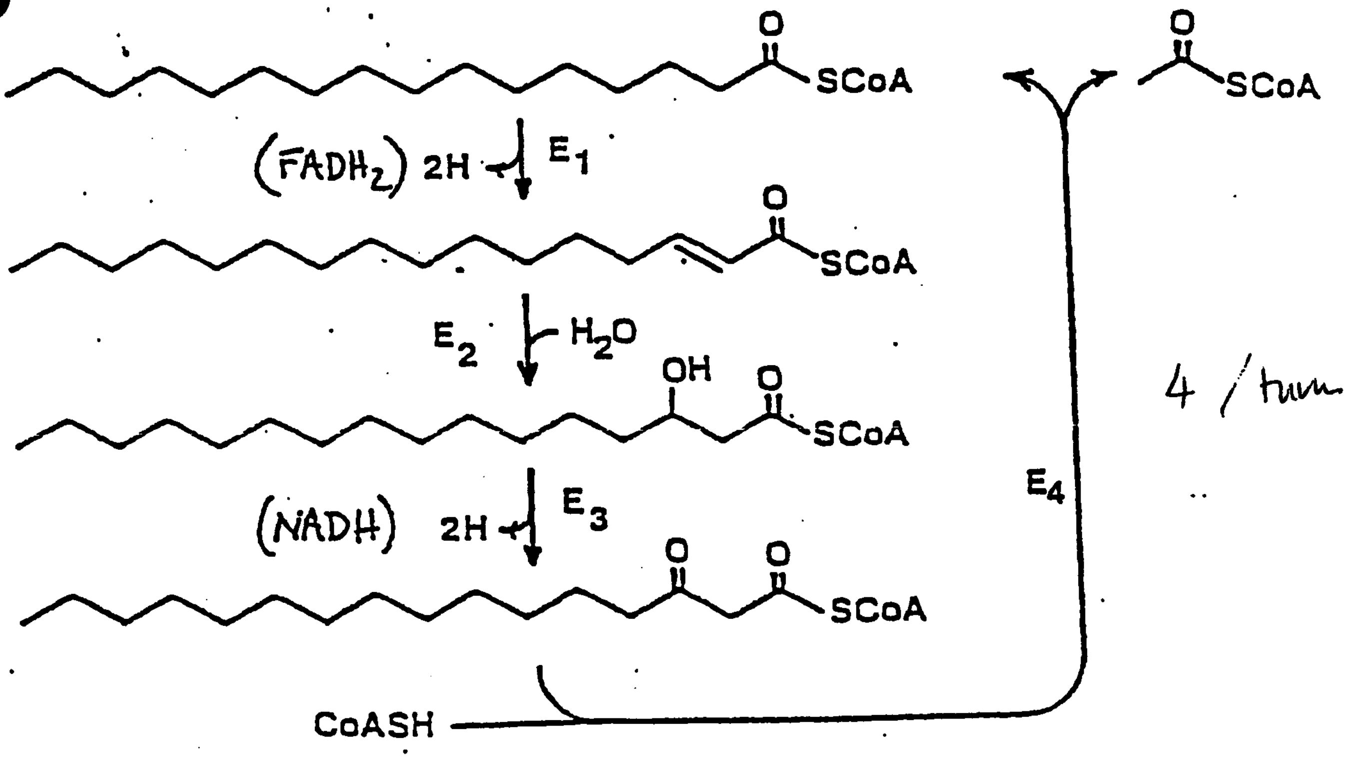
MITOCHONDRIAL FATTY ACID OXIDATION (or ß-oxidation)

E-1 acyl-CoA dehydrogenase

E-2 hydratase

E-3 hydroxyacyl-CoA dehydrogenase

E-4 thiolase



4 / turn