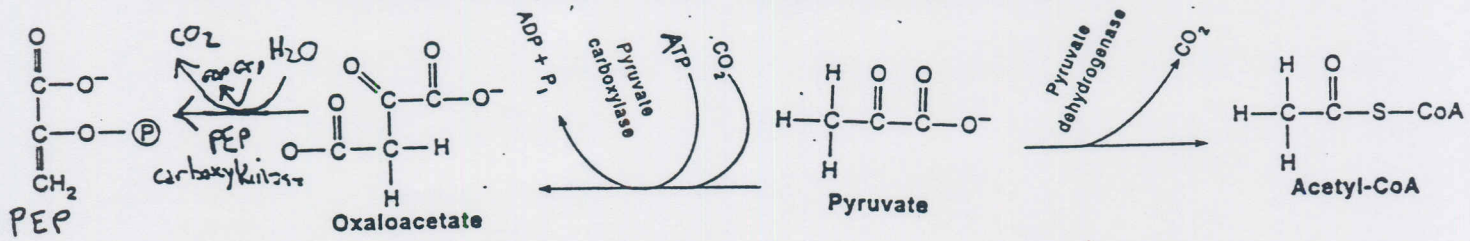
7

C) When oxygen is no longer limiting, the lactate that results from lactic acid fermentation is cleared from the body. Actually, most of it is converted back into glucose at the liver. (2)

i) In general terms (I don't need the series of reactions) how is this accomplished? (4 points)

ii) Why do you need oxygen to do this and how energetically expensive is it? (4 points)

2) We have seen that pyruvate is a key metabolic intermediate that has multiple fates depending on the needs of an organism. Two possible fates of pyruvate are shown below. How are the key enzymes in these two pathways regulated and in response to what? (8 points)



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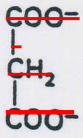
D) You find that malonate (shown below) inhibits this reaction. succinate dehydrogenase (4)

i) What type of inhibitor (competitive or noncompetitive) of succinate dehydrogenase do you think malonate is? Why? (3 points)



Malonate

ii) How could you prove your hypothesis about the type of inhibition caused by malonate? (3 points)

4) Leder's heriditary optic neuropathy is a disease that results from an inability to transfer electrons from NADH to complex I of the electron transport chain.

A) If this disease blocks the input of electrons into the first step of the electron transport chain why is it not lethal? (3 points)

B) What effect would this defect have on ATP production via oxidative phosphorylation? (3 points)

5

C) How would this defect impact on the citric acid cycle? Be specific. (3 points)

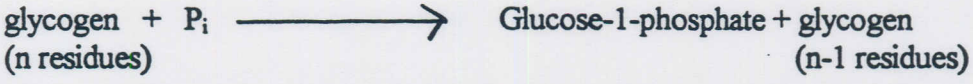
5) Oxidative phosphorylation is responsible for much of the ATP production in aerobic organisms, but ATP may also be synthesized by substrate-level phosphorylation. We have discussed two different substrate level phosphorylations.

A) Show all reactants and products and name the necessary cofactors and enzyme catalyzing **one** of these substrate-level phosphorylations. (5 points)

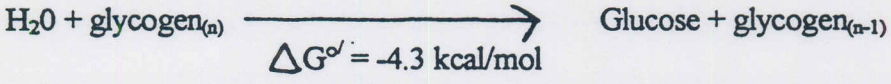
7

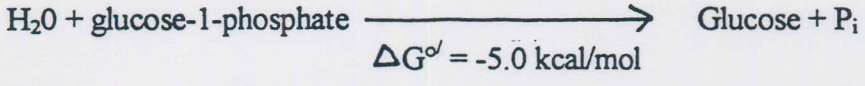
(7)

7) In our digestive tract we use the enzyme glycogen phosphorylase to break down glycogen through phosphorolysis (attack by P_i). The products of the phosphorolysis reaction are glucose-1-phosphate and glycogen (containing one less glucose residue).



a) Calculate the standard free-energy change for this reaction from the following data: (Show all calculations). (3 points)





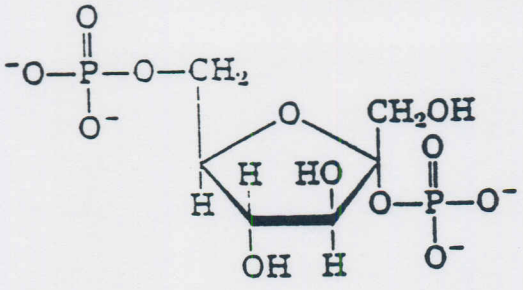
b) Calculate the ratio of products to reactants when the phosphorolysis reaction is at equilibrium. (3 points)

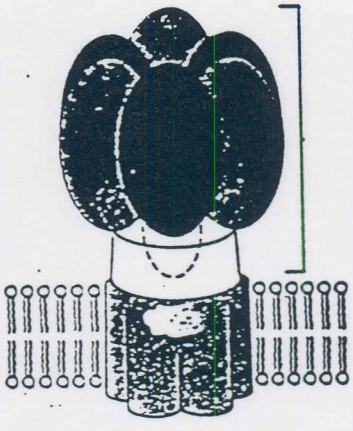
c) Explain the metabolic advantage to the cell of using glycogen phosphorylase to breakdown glycogen as opposed to simply hydrolyzing it to release glucose. (4 points)

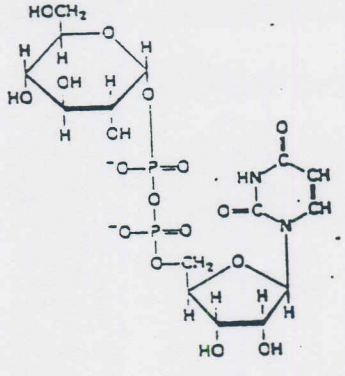
7

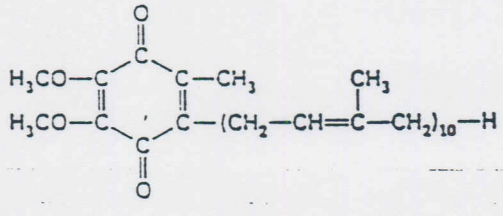
8) Name the following structures and in **ONE OR TWO SENTENCES** describe their function: (15 points)

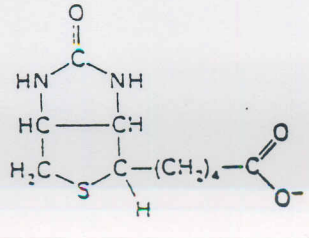
8









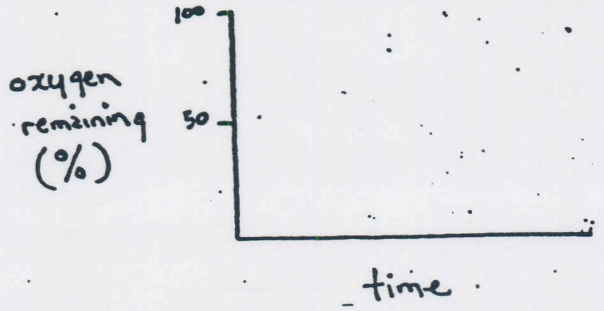


7

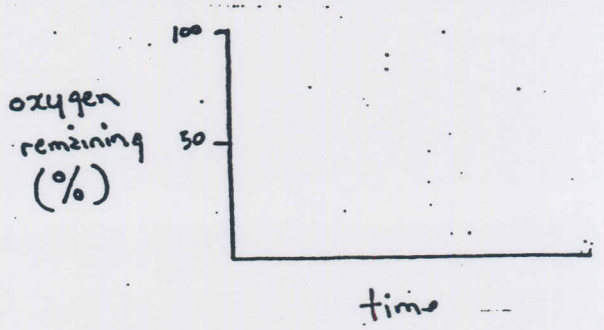
9) You are given an air tight container that contains intact and functional mitochondria, oxygen and an oxidizable substrate (NADH). On the following graphs of oxygen consumption versus time plot the curves for the consumption of oxygen that you would expect under the following experimental conditions. Provide a **ONE TO TWO SENTENCE** explanation for each observation. (12 points)

9

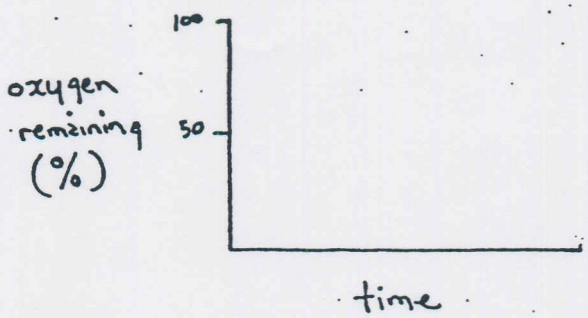
A) after addition of ADP and inorganic phosphate



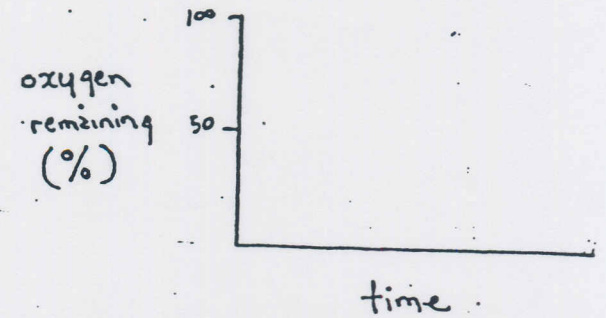
B) same as A but also with oligomycin (blocks ATP synthesis)



C) same as A but also with antimycin A (blocks electron transfer in cytochrome reductase)



D) same as B but also with dinitrophenol (an uncoupler)



10) You have isolated a never before seen bacterium from a coffee pot at A.L. Van Houte. Analysis of the electron transport pathway of this organism reveals the presence of six electron-transport molecules with the redox potentials listed below.

oxidant	reductant	e^- transferred	E'_o
cytochrome f (+3)	cytochrome f (+2)	1	-0.29
cytochrome x (+3)	cytochrome x (+2)	1	+0.22
NAD^+	NADH	2	-0.32
ferroprotein a (oxidized)	ferroprotein a (reduced)	2	+0.77
ferroprotein b (oxidized)	ferroprotein b (reduced)	2	-0.58
flavoprotein	flavoprotein	2	+0.14

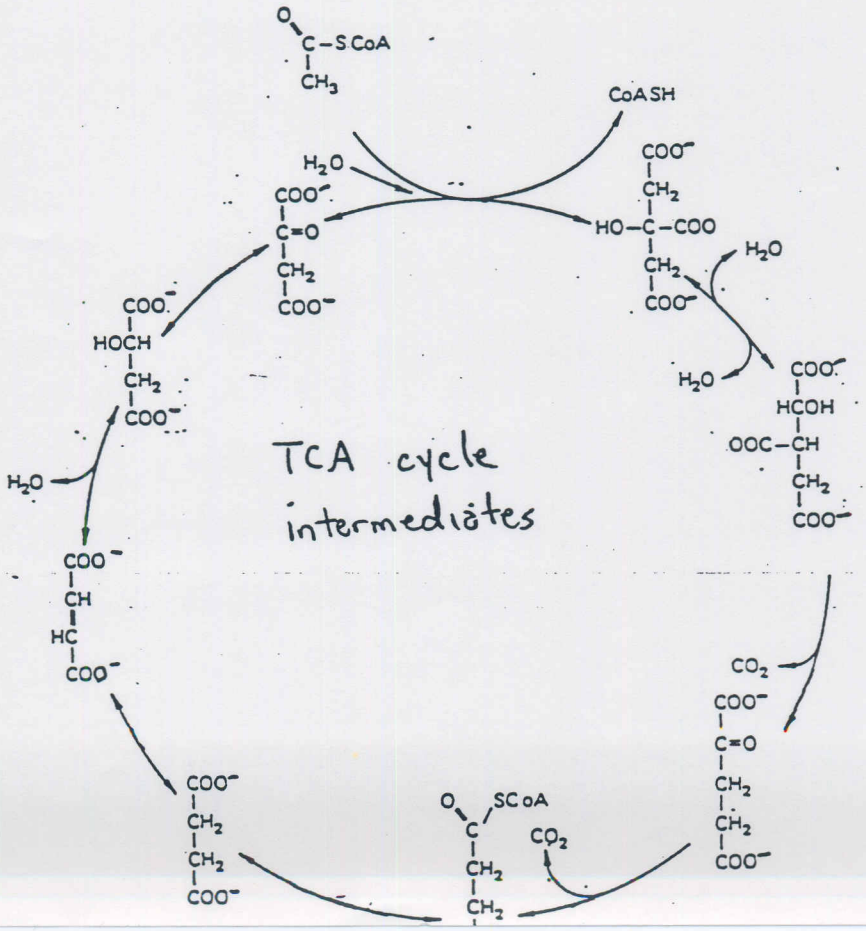
A) Predict the sequence of carriers in the electron-transport chain of this bacterium. (3 points)

B) How many molecules of ATP can be generated under standard conditions when a pair of electrons is transported along the pathway? (Hint: ATP hydrolysis yields -7.3 kcal/mol). (5 points)

7

11) You add pyruvate radiolabelled with ^{14}C at the 3 position to actively metabolizing rat liver cells. After a few seconds you find that carbon 3 of oxaloacetate is radioactive. You wait a few minutes and find that the radioactive label is now shared between carbons 1, 2, 3 and 4 of oxaloacetate. How do you explain these two observations? (8 points)

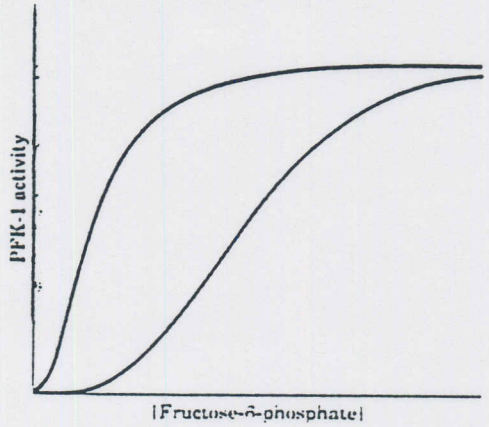
(11)



95

2. Phosphofructokinase-1 (PFK-1) catalyzes the committed step in glycolysis and its activity can be regulated by a number of allosteric effectors. **(12 pts)**

a) On the following graph identify the curves for PFK-1 activity versus [fructose-6-phosphate] at **low** and **high** [ATP].



b) ATP also serves as a substrate for this enzyme. How can it function both as a substrate and a modulator of enzyme activity? What feature(s) of the PFK-1 is(are) responsible for this?

c) What effect would an increase in [fructose-2,6-bisphosphate] have on these curves? Explain.

d) What effect would you expect fructose-2,6-bisphosphate to have on **fructose-1,6-bisphosphatase**? Why is this important in the liver? Explain.

95

3. Pure reduced cytochrome c is added to carefully prepared mitochondria along with ADP, P_i, and oxygen. **(12 pts)**

a) Would any ATP be produced in this system if you also added antimycin A (blocks electron transport between ubiquinone and cytochrome c)? Explain why or why not?

b) Would any ATP be produced in this system if you also added dinitrophenol (an uncoupler)? Explain why or why not?

c) Would any ATP be produced in this system if you also added dicyclohexylcarbodiimide (blocks proton flow through F_o)? Explain why or why not?

d) In each case (above) indicate the probable flow of electrons in the system.

95

4. **EITHER DO PART A OR PART B BUT NOT BOTH (6 pts)**

A) We have discussed both the thermodynamics and the kinetics of a number of biochemical reactions. Explain why thermodynamics and kinetics are each important in the reactions going on in your body. (Provide an example of each for full marks).

~~B) Louis Pasteur noted that if you added oxygen to what had been an anaerobic culture of fermenting grape juice there was a tremendous drop in the rate of glucose consumption.~~

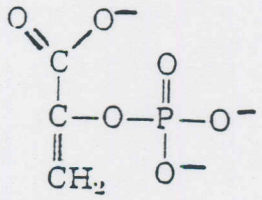
~~a) Why would the yeast cells consume less glucose in the presence of oxygen?~~

~~b) Approximately how much less glucose would they consume?~~

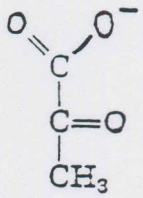
~~c) How would adding dinitrophenol with the oxygen affect glucose consumption? Why?~~

5. The standard reduction potential for ubiquinone is +0.04 V, and the standard reduction potential for flavin adenine dinucleotide (FAD) is -0.22 V. Show all calculations to indicate how the oxidation of $FADH_2$ by ubiquinone theoretically liberates enough energy to drive the synthesis of ATP from ADP and P_i under standard conditions. **(6 pts)**

8. How is it possible that both the conversion of phospoenolpyruvate to pyruvate and the reverse process, formation of phosphoenolpyruvate from pyruvate, are energetically favorable? Show all reactants and products and name required cofactors and necessary enzymes to illustrate your answer. **(9 pts)**



Phosphoenolpyruvate



Pyruvate

95

9. Phosphate in metabolism: (**12 pts**)

A) Phosphorylation is an example of a covalent modification that activates some enzymes and inhibits others. Describe in detail how glycogen synthesis and degradation are regulated in coordinated but opposite manners by phosphorylation and dephosphorylation.

EITHER DO PART B OR PART C BUT NOT BOTH

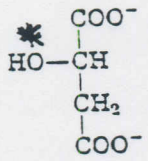
B) Harden and Young showed that inorganic phosphate was necessary for glycolysis. Show a specific reaction (including structures of reactants and products) that requires inorganic phosphate but does not involve direct ATP synthesis or hydrolysis.

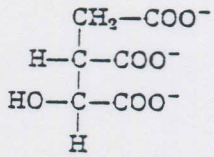
C) Oxidative phosphorylation is responsible for much of the ATP production in aerobic organisms, but ATP may also be synthesized by substrate-level phosphorylation. Show all reactants and products and name the necessary cofactors and enzyme catalyzing any reaction which provides an example of substrate-level phosphorylation.

95

10. **(16 pts)** a) If malate (labeled as indicated below) was fed into the TCA cycle where would the labelled oxygen be found in isocitrate? Show all reactants, products and name the necessary cofactors and enzymes required to illustrate your answer. (You only need to go from malate to isocitrate).

Malate





Isocitrate

b) The first step of this reaction, the conversion of malate to oxaloacetate has a standard free energy change of + 29.7 kJ/mol. How does this reaction proceed in the forward direction against this apparent large standard free energy change?

c) What effect would an increase in the concentration of available ATP have on this series of reactions? What about an increase in the level of citrate? In each case why?