

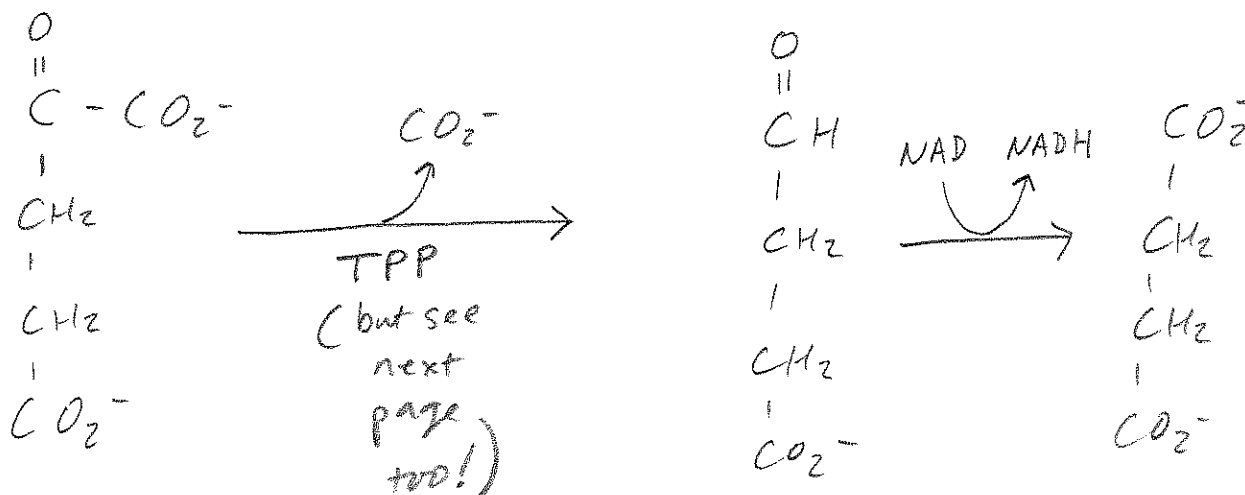
NAME: KEY

Unless indicated otherwise, each question is worth 2 points – Good luck!

1. THE REAL WORLD

A. The components that make-up the Citric Acid Cycle in a cyanobacteria were recently reported in a paper that appeared in the journal *Nature*. Interestingly, the organism lacks α -ketoglutarate dehydrogenase and succinyl-CoA synthetase. However, it still makes succinate. The authors of this paper then found that two enzymes existed in this organism in place of α -ketoglutarate dehydrogenase and succinyl-CoA synthetase. The first enzyme performs a non-oxidative decarboxylation and the second is a dehydrogenase.

Based on this information, propose a metabolic pathway by which the reactant for α -ketoglutarate dehydrogenase can be converted into succinate. Draw the structures of the reactants/products of these two enzymes, and if appropriate, denote the name of any cofactors (double credit).



B. Based on this information, approximately how many ATP equivalents are "lost" compared to mammals for each turn of this cycle? (In brief, please provide your reasoning)

In the absence of α -ketoglutarate dehydrogenase:

$-1 \text{ NADH} \rightarrow -3 \text{ ATP}$

In the absence of succinyl CoA synthetase $\rightarrow -1 \text{ ATP}$

AND, this reaction creates an NADH (+3 ATP)

So, only one ATP is "lost".

C. What is the full name of the cofactor that most likely performs the non-oxidative decarboxylation, and what is the name of the enzyme that we have already discussed that performs the same type of reaction?

- Thiamine pyrophosphate

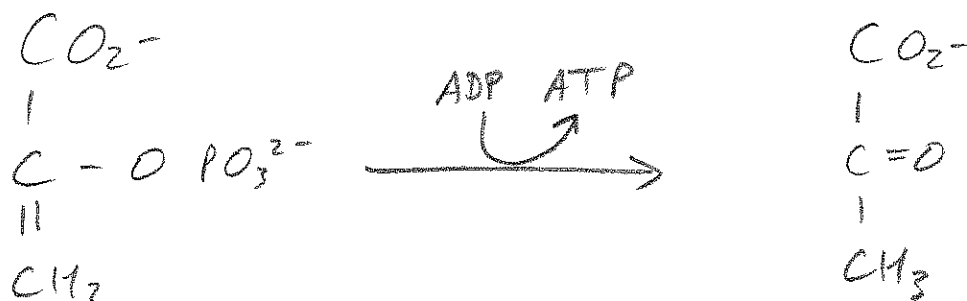
- pyruvate decarboxylase (in yeast)

(NOT pyruvate dehydrogenase)

2. It is becoming increasingly clear that cancer cells "prefer" to make ATP in a non-oxidative manner, thus generating lactic acid. One way this happens is by reduced activity of the enzyme that catalyzes the final favorable step in glycolysis.

(pyruvate dehydrogenase)

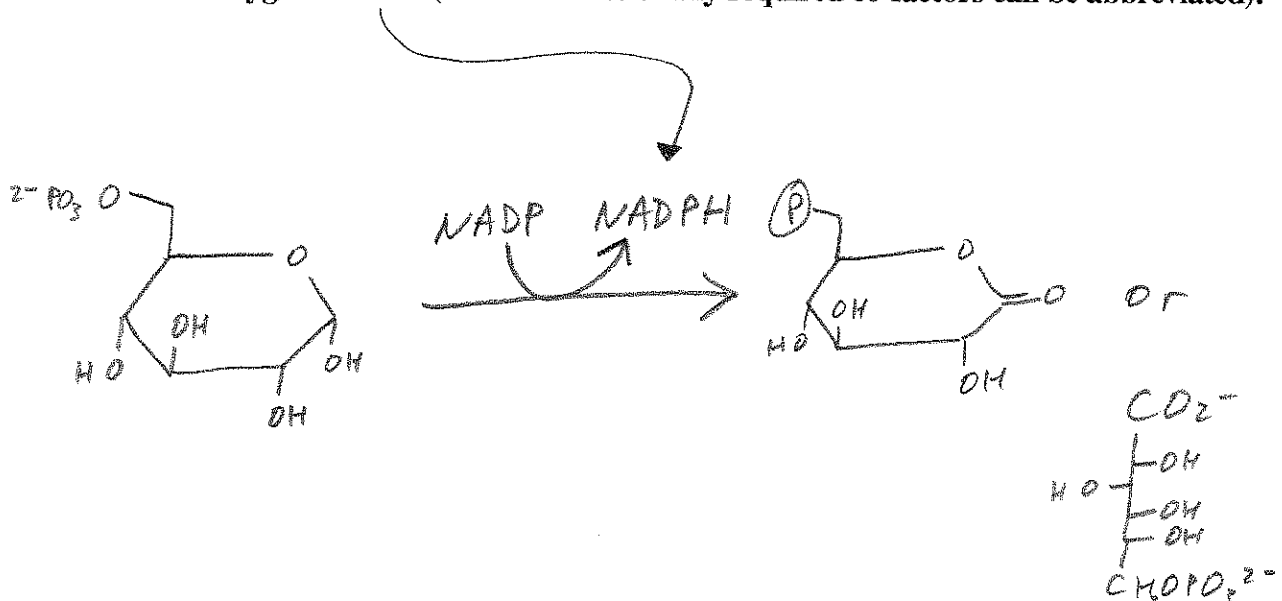
A. Draw the structures of the reactant and the product in the reaction catalyzed by this enzyme:



B. Based on the mechanism by which this enzyme is regulated in the liver, propose a mechanism that cancer cells use to reduce the enzyme's activity:

In the liver, the enzyme is activated when it is dephosphorylated, so perhaps cancer cells activate a kinase (or inhibitor + ~~the~~ phosphatase).

C. As a result of this altered metabolic "decision" in cancer cells, it is thought that more glucose-6-phosphate is available to the cells. This, in turn, reduces both oxidative damage and the generation of reactive oxygen radicals that might otherwise kill the cancer cells. Draw the structures of the reactants and products for the reaction that uses glucose-6-phosphate and generates a product that can then be used, indirectly, to relieve the cell of oxygen radicals (the structures of any required co-factors can be abbreviated):



3. Cholera leads to a horrible loss of body fluids and electrolytes through uncontrolled diarrhea. This is the result of the phosphorylation of an ion channel in the cells that line the small intestine, which when activated releases chloride into the lumen of the small intestine. When chloride leaves these cells, water also leaves the cells, and diarrhea results. Interestingly, the way that cholera triggers this event is by constitutively activating an α subunit in a G protein.

A. Propose a candidate for the kinase that activates the intestinal chloride channel:

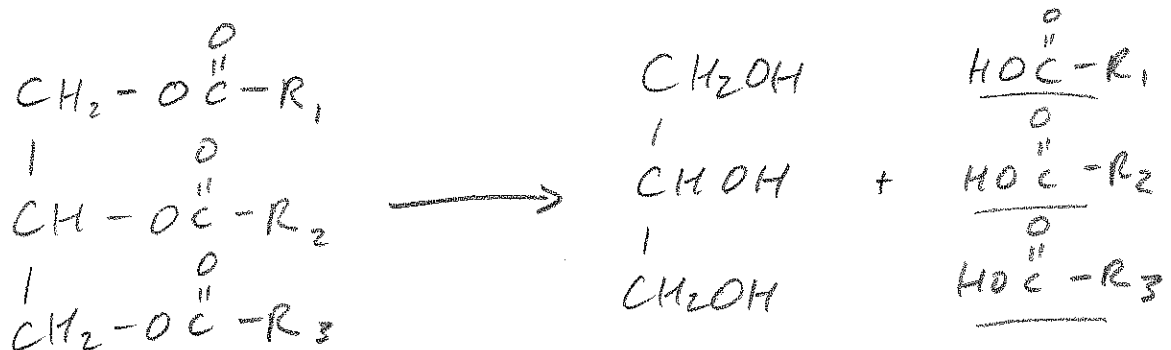
Protein Kinase A (or, the cAMP-dependent protein kinase)
 (Phosphorylase kinase is also OK) (BARK is also OK)

B. Recently, a protein known as "GoLoCo" was found to bind to and stabilize G α -GDP. Would an increase in the amount of this protein in intestinal cells make the effects of cholera better or worse? Why?

The protein would "hold" the G α subunit in the inactive conformation, ideally lessening the effects of cholera.

4. The activity of lipoprotein lipase is highly regulated, and in fact some diseases result from an inability to properly synthesize and secrete this enzyme to the extracellular surface of the plasma membrane.

A. Draw the structures of the reactants and the products of the reaction that is catalyzed by lipoprotein lipase (a generic substrate and the reactants can be depicted):



B. Suppose you wanted to do gene therapy and use a virus to introduce the enzyme into the endothelial cells of people who expressed only ~50% of the normal amount of lipoprotein lipase. Which metabolite might the transcriptional promoter respond to that you would want to use to drive the transcription of the message encoding the lipase?

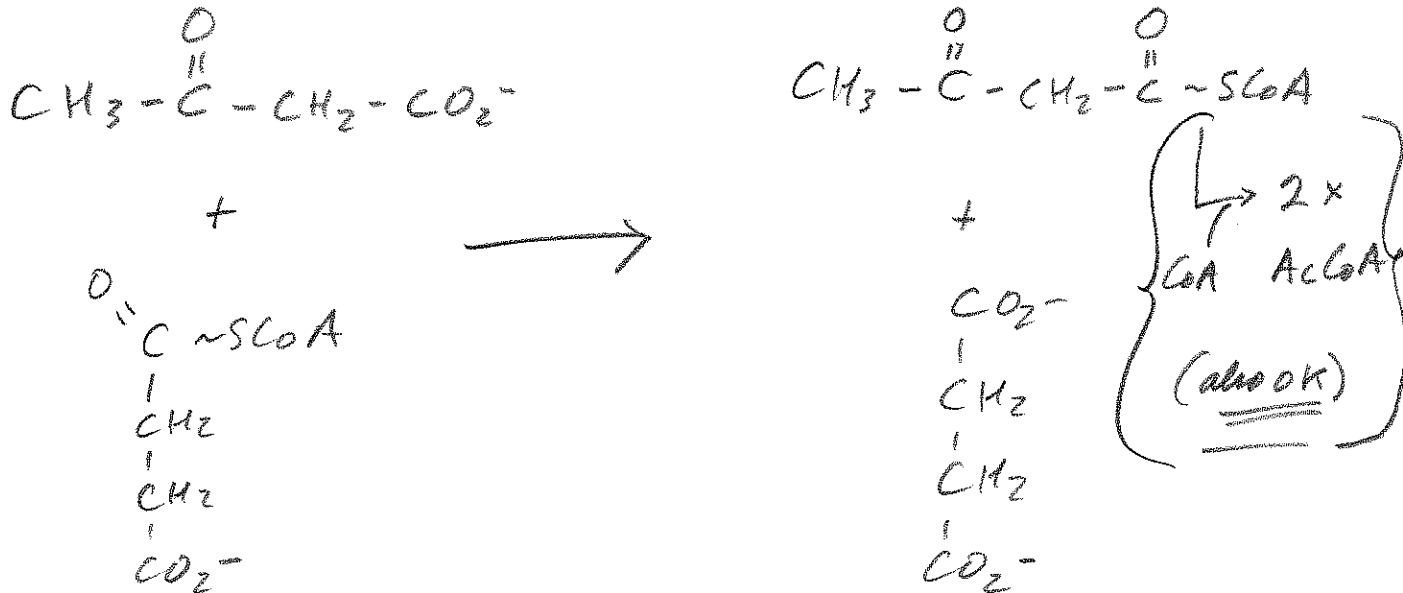
Fatty acids; this way, the enzyme would be synthesized when there was an increase in serum Fatty acids (e.g., after a meal).

5. As we discussed, during starvation, ketone bodies serve as a means to provide energy _____ to cells in the central nervous system.

A. What is the name of the enzyme that performs the first step in ketone body synthesis?

thiolase

B. One ketone body is acetoacetate. Draw the structures of the reactants and the products by which acetoacetate is converted into a useful metabolite in the brain. Be sure to include the structures of both reactants and products:



C. If this reaction were running at a high rate in the brain, do you think the activity of the Citric Acid Cycle would increase or decrease? Why?

- OR:
- As the concentration of succinyl-CoA drops, then citrate synthase / α -KG dehydrogenase activity rises.
 - Acetyl CoA is delivered directly into the TCA cycle.

2. MULTIPLE CHOICE

i. Which of the following molecules leads to the inhibition of the activity of phosphofructokinase-1 in the liver?

- A. citrate
- B. AMP
- C. fructose-2,6-bisphosphate
- D. insulin
- E. More than one of the above is correct

ii. Which of the following vitamins/cofactors is not a component of the pyruvate dehydrogenase complex?

- A. thiamine pyrophosphate
- B. glutathione**
- C. lipoic acid
- D. niacin
- E. pantothenic acid

iii. An increase in the activity or the amount of each of the following would increase the mobilization of fatty acids in adipocytes, except:

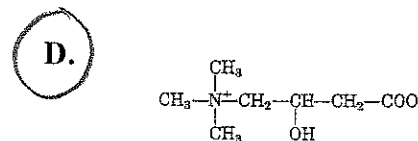
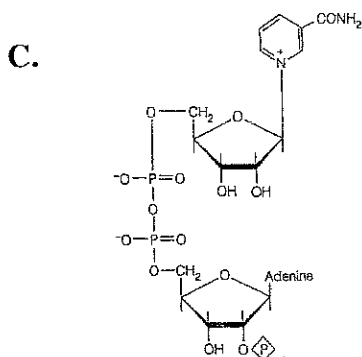
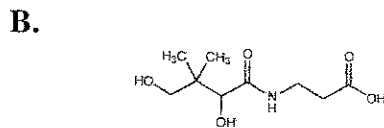
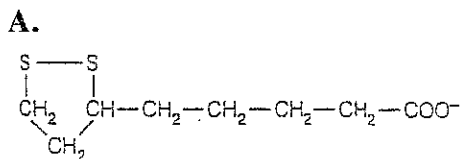
- A. monoacylglycerol lipase
- B. phosphorylated perilipin
- C. protein kinase A regulatory subunit ("R")**
- D. epinephrine
- E. adenylyl cyclase

iv. Which of the following sugars would not react with sodium borohydride (NaBH_4)?

- A. D-glucose
- B. D-fructose
- C. D-mannose
- D. inositol**
- E. L-lactate

(need a carbonyl at the 1 or 2 position)

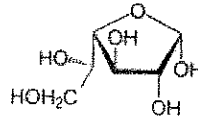
v. Which of the following molecules is conjugated onto fatty acids and is required for fatty acid transport into the mitochondria?



(carnitine)

vi. Which of the following terms best describes the classification of the depicted sugar?

- A. D-glucopyranose
- B. D-galactofuranose**
- C. L-aldopentose
- D. D-fructose
- E. Bob the Sugar



vii. Which of the following properties does not apply to ATP?

- A. The free energy change for hydrolysis to ADP is ~ 31.5 kJ/mol
- B. It has a low activation energy barrier for hydrolysis**
- C. The ionization of a phosphate in ADP contributes to the favorable free energy of hydrolysis
- D. In almost all cases Mg^{2+} is associated with ATP in cells
- E. None of the above, they all apply to this wacky molecule

3. THINK ABOUT IT....

A. Why does pyruvate carboxylase play a more important role than the malic enzyme as an anapleurotic reaction for the Citric Acid Cycle?

Pyruvate carboxylase produces oxaloacetate, which is limiting in the TCA cycle. In contrast, the malic enzyme produces malate, which would still need to be driven "up hill" to make oxaloacetate.

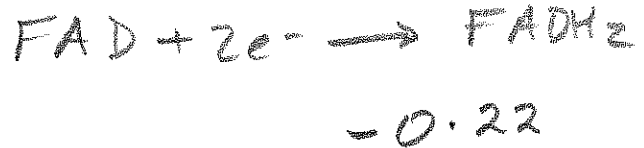
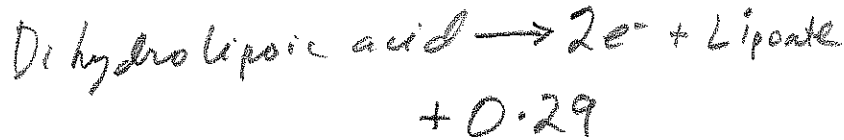
B. Which do you think would cause a more severe disease: People who lack glycogen phosphorylase or the debranching enzyme? Why?

Without glycogen phosphorylase, no glucose (via $G-1-P \rightleftharpoons 6-6-P$) would be produced when sugars are depleted.

In contrast, without debranching enzyme, at least some glucose could be produced via the action of phosphorylase

C. Based on the following Table, what is the ΔG^{10} for the transfer of 2 electrons from dihydrolipoamide acetyltransferase into the next enzyme within the pyruvate dehydrogenase complex? (You can assume that Faraday's constant, \mathcal{F} , is 100 kJ/V·mol) (double credit)

Half-reaction	E° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.816
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.421
Cytochrome <i>f</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>f</i> (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) + $e^- \rightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 (Fe^{3+}) + $e^- \rightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.295
Cytochrome <i>a</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>a</i> (Fe^{2+})	0.29
Cytochrome <i>c</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>c</i> (Fe^{2+})	0.254
Cytochrome c_1 (Fe^{3+}) + $e^- \rightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome <i>b</i> (Fe^{3+}) + $e^- \rightarrow$ cytochrome <i>b</i> (Fe^{2+})	0.077
Ubiquinone + $2H^+ + 2e^- \rightarrow$ ubiquinol + H_2	0.045
Fumarate $^{2-}$ + $2H^+ + 2e^- \rightarrow$ succinate $^{2-}$	0.031
$2H^+ + 2e^- \rightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA + $2H^+ + 2e^- \rightarrow$ butyryl-CoA	-0.015
Oxaloacetate $^{2-}$ + $2H^+ + 2e^- \rightarrow$ malate $^{2-}$	-0.166
Pyruvate $^-$ + $2H^+ + 2e^- \rightarrow$ lactate $^-$	-0.185
Acetaldehyde + $2H^+ + 2e^- \rightarrow$ ethanol	-0.197
FAD + $2H^+ + 2e^- \rightarrow FADH_2$	-0.219
Glutathione + $2H^+ + 2e^- \rightarrow$ 2 reduced glutathione	-0.23
$S + 2H^+ + 2e^- \rightarrow H_2S$	-0.243
Lipoic acid + $2H^+ + 2e^- \rightarrow$ dihydrolipoic acid	-0.29
$NAD^+ + H^+ + 2e^- \rightarrow NADH$	-0.320
$NADP^+ + H^+ + 2e^- \rightarrow NADPH$	-0.324
Acetoacetate + $2H^+ + 2e^- \rightarrow \beta$ -hydroxybutyrate	-0.346
α -Ketoglutarate + $CO_2 + 2H^+ + 2e^- \rightarrow$ isocitrate	-0.38
$2H^+ + 2e^- \rightarrow H_2$ (at pH 7)	-0.414
Ferredoxin (Fe^{3+}) + $e^- \rightarrow$ ferredoxin (Fe^{2+})	-0.432

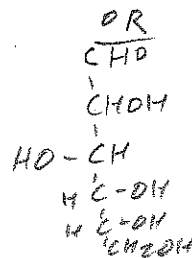
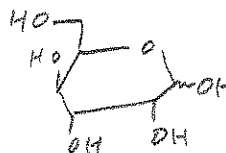
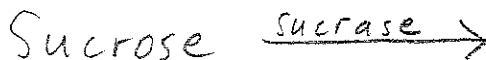


$0.29 - 0.22 = 0.07 V$

$\Delta G^{10} = -n \mathcal{F} \Delta E^{10} = -(2)(100)(0.07)$
 $= -14 \text{ kJ/mol}$

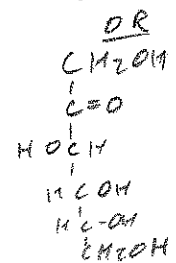
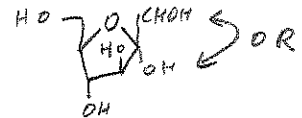
4. THOSE CRAZY REACTIONS (or, WHY I BOUGHT A BUNCH OF ^\$#@! INDEX CARDS)—as always, draw the structures AND give the names of the products of the following reactions:

A.



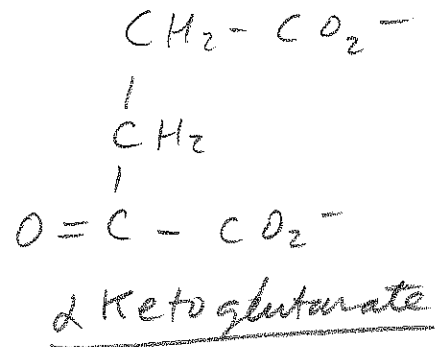
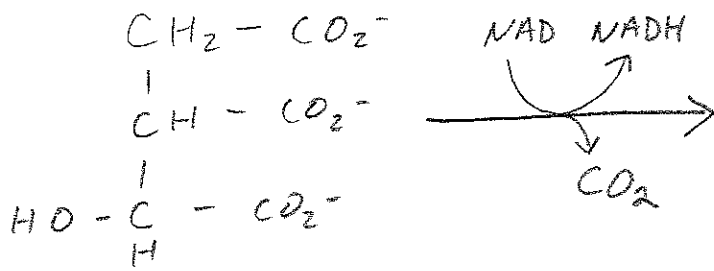
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glucose

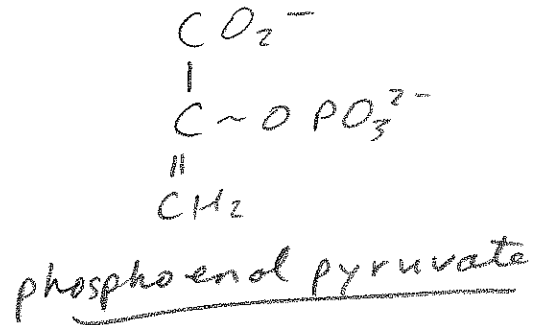
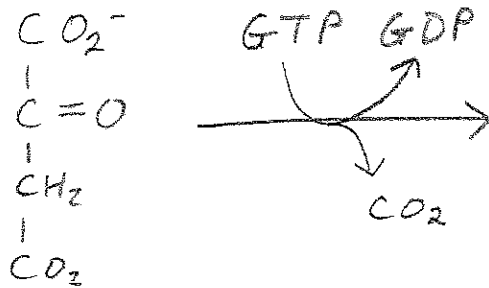


Fructose

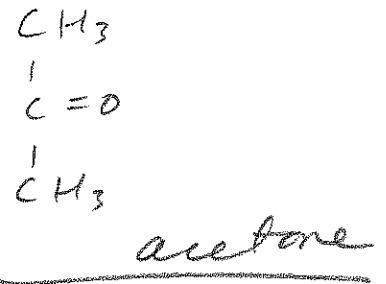
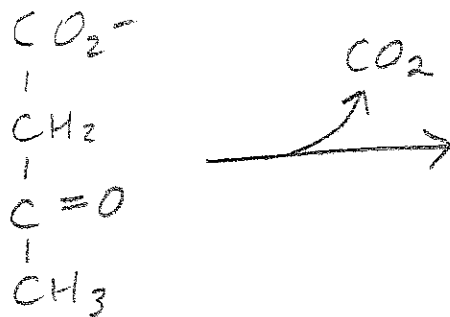
B.



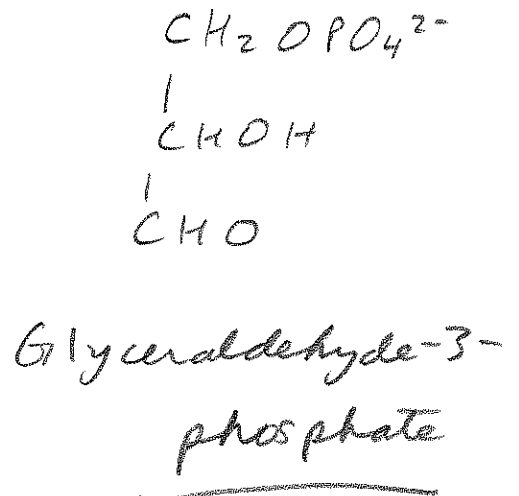
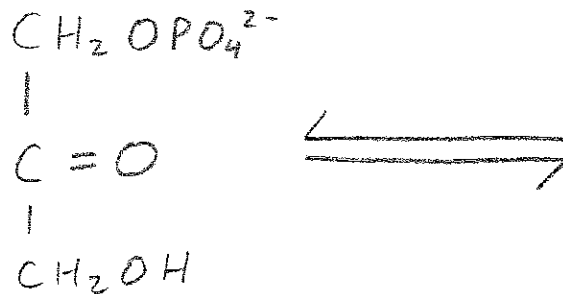
C.



D.

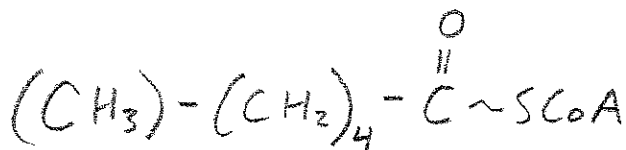


E.



5. SHORT ANSWER

A. How many ATPs could, in theory, be produced from the complete oxidation of the following fatty acid (assume that $\text{NADH} = 3 \text{ ATPs}$ and $\text{FADH}_2 = 2 \text{ ATPs}$)?



46 ATPs

6 carbons
(already "activated")
So, 2 β oxidation cycles
 $2 \times 5 = 10 \text{ ATP}$
3 acetyl CoAs
 $3 \times 12 = 36 \text{ ATP}$

B. If a liver cell expressed a mutant form of Protein Kinase A that was constitutively active, would phosphofructokinase-2 be active or inactive? And, what would the effect be on glycolysis in that cell?

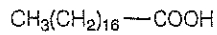
This would keep PFK-2 in the phosphorylated (OFF) state, so the levels of F-2,6-Bis Phosphate would drop and glycolysis would decrease.

What are the names of the two enzymes that we have discussed that have a phosphorylated histidine as a key component of their reaction mechanisms?

c. Phosphoglycerate mutase

d. Succinyl CoA synthetase

E. What is the name of the following fatty acid?

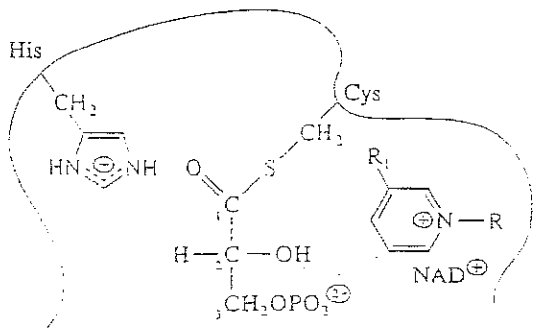


Stearic acid

F. This enzyme is activated by an insulin-dependent phosphatase and catalyzes the committed step in the synthesis of fatty acids:

acetyl CoA carboxylase

G. The following depicts an intermediate in the reaction catalyzed by which enzyme?



glyceraldehyde-3-phosphate dehydrogenase

Number incorrect:

$$75 - (2 \times \underline{\quad}) = \square$$