Biochemistry 463, Summer II **Your Name:** Key

University of Maryland, College Park **Your SID #:**

Biochemistry and Physiology **Prof. Jason Kahn**

Final Exam (150 points total) **August 16, 2013**

You have 90 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is extra space on the last page if you need it.

You will not need a calculator for this exam, and no other study aids or materials are permitted.

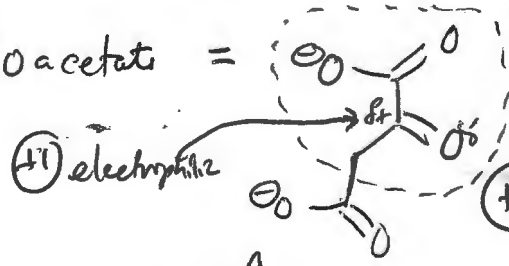
Partial credit will be given, *i.e.*, if you don't know, guess.

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

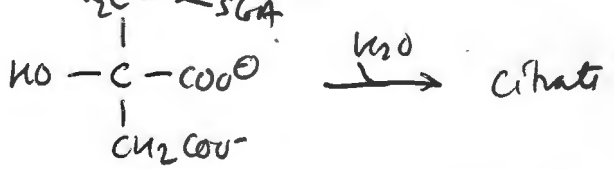
1. (15 pts) TCA Cycle Energetics and Regulation

   Citrate synthase condenses oxaloacetate (OAA) and acetyl-CoA. Draw the structure of OAA and indicate the electrophilic carbon. What is the biochemical function of the final hydrolysis step of the mechanism, which appears to be just a waste of a thioester? Explain why the concentration of citrate actually decreases when the flux through the TCA cycle increases upon removal of NADH.

OAA = oxaloacetate =  = pyruvate

(+1) electrophile (+5)

+1 for any structure
+2 for anything w/ 4 carbons and a $-COO^{\ominus}$
+3 for anything w/ a carbonyl + a carboxylate

Citroyl-CoA = 

(+5) thermodynamic driving force for the reaction is large and ⊖, so it + the [OAA] can thereby drives the preceding unfavorable malate dehydrogenase reaction.

[Citrate] ↓ because (+4) Isocitrate dehydrogenase is very active downstream, which depletes citrate, adds additional driving force for the cycle. (+2 for aconitase - that reaction actually held near equilibrium)

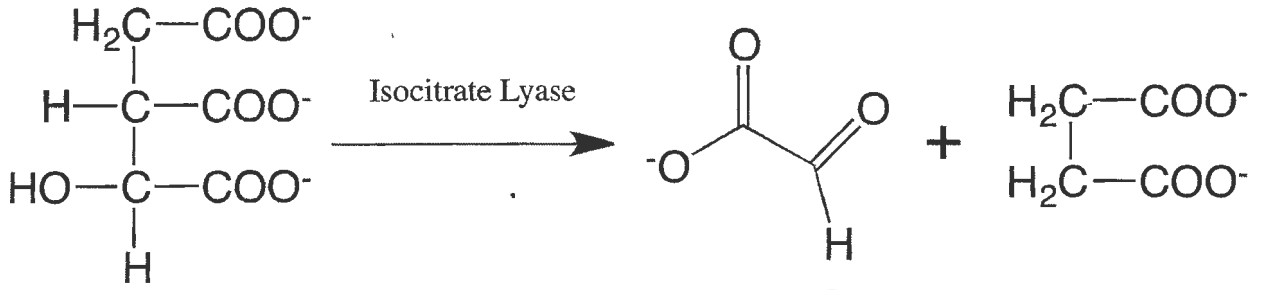
Score for the page_____

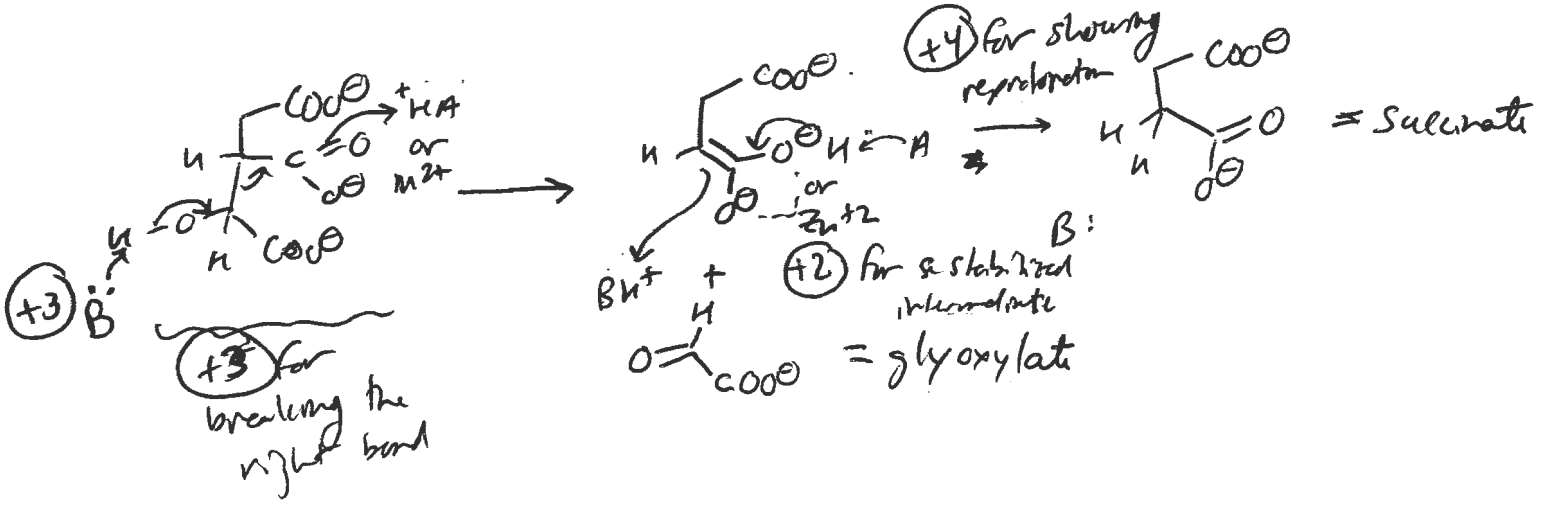
2. (15 pts) Enzyme Mechanisms

Mammals cannot incorporate net carbon from acetyl-CoA into glucose. Why not, biochemically?

(+3) The Two carbons are lost as CO_2 for each Acetyl-CoA that enters the cycle.

Other organisms have a glyoxylate shuttle, in which the enzyme isocitrate lyase converts isocitrate into succinate plus glyoxylate and then glyoxylate is condensed with acetyl-CoA to give malate. This allows net conversion of acetyl-CoA into OAA and hence glucose. This seems like a useful trick to me – it would let us avoid using ketone bodies. The isocitrate lyase mechanism resembles the Class II aldolase mechanism of glycolysis (the one with an enolate intermediate rather than a Schiff's base). Propose a retro-aldol (actually formally a retro-Claisen) mechanism for isocitrate lyase. Include AH+ and B: groups as needed.





3. (8 pts) Glycogen

Name a glycogen storage or breakdown disease and briefly describe the biochemical defect..

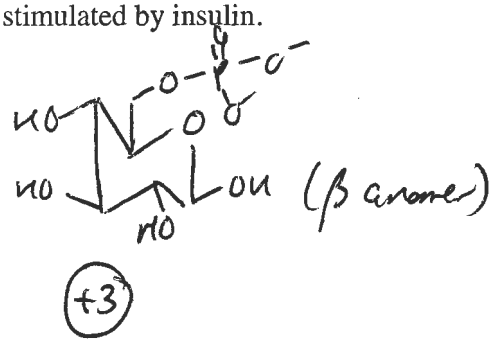
(+3) McArdle's disease - (or any other in the book)

(+5) Glycogen phosphorylase deficiency in muscle -> cramps due to exertion for mentioning biochemical cause

Score for the page_____

4. (15 pts) Metabolic Nodes

Glucose-6-phosphate is an important control point metabolite. Draw its structure in the chair form. List three of the four possible fates for G6P in liver cells, and indicate which pathways will be stimulated by insulin.



(β anomer)

+3

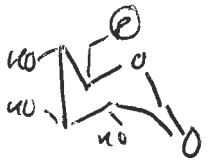
glucose ⇝ export

⊕Insulin ⇅ ⊕Insulin

pentose phosphate pathway (↑ indirectly by demand for NADPH) ← G6P ⇌ G1P ⇌ glycogen

↓ glycolysis – insulin inhibits –

F6P



optimal

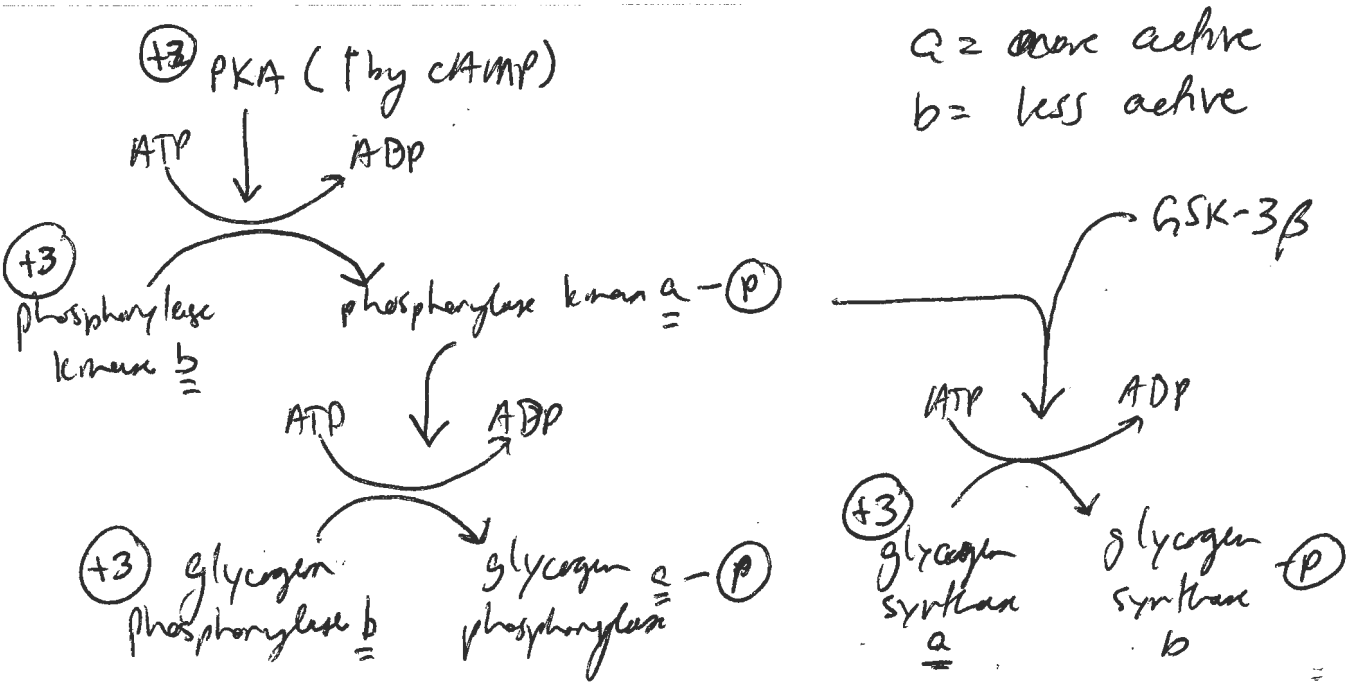
+3 for each of 3 correct fates = 9

+3 for correct insulin effects

5. (15 pts) Regulation

Sketch the bicyclic phosphorylation cascade that shuts off glycogen synthesis and activates glycogenolysis. Don't worry about the phosphatase side of things. Why is glycogen synthase also independently inhibited by other kinases like GSK-3β?.

a = more active
b = less active



– There are times when glycogenolysis should be inhibited but the cell does not need to make more glycogen – independent control

+3 – Short-term vs. long term regulation. Delay to avoid "jittering".

Score for the page_____

6. (8 pts) Hormone regulation

How does insulin stimulate glucose uptake by muscle cells? Why does it make physiological sense that muscle cells do not respond to glucagon?

- Insulin stimulates fusion of vesicles bearing the GLUT4 glucose transporter w/ the plasma membrane — muscle cells are impermeable to Glucose w/out insulin. (+4)

- Muscles cannot export glucose or do gluconeogenesis, so there is no reason that they should respond to glucagon → they are selfish. (+4)

7. (6 pts) Metabolite Pools

Why do cells maintain separate pools of NADH/NAD+ and NADPH/NADP+ even though the cofactor pairs have identical redox properties? Name an enzyme (or draw the reaction) that reduces NADP+ to give NADPH.

High [NADPH]/[NADP+] → drive reductive biosynthetic reactions. (+1)

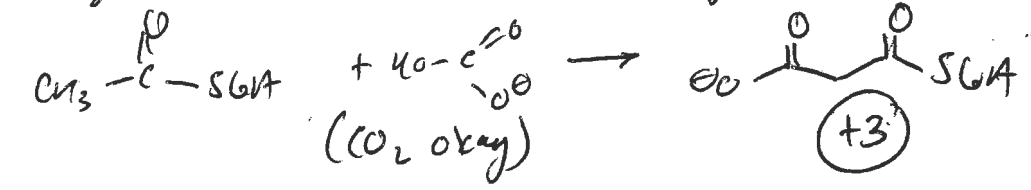
Low [NADH]/[NAD+] → drive catabolism (+1) / can do both at once (+2)

(+2) Glucose-6-phosphate dehydrogenase [+1 for PPP] or cytosolic malate dehydrogenase

8. (12 pts) Commitment to Fat

Draw the reaction (not the mechanism, just reactants and products), name the enzyme, and name the cofactor for the committed step for fatty acid biosynthesis. Where else have we seen a carboxylate installed just to be decarboxylated and provide a driving force for the following uphill reaction?

Acetyl-CoA (+2) + HCO_3^- (+2) → Malonyl CoA [+1 for just the name]



(CO_2 okay)

(+3)

(+2) Acetyl-CoA carboxylase (ACC) (+1)

(+1) Biotin cofactor

(+2) PEPCK reaction

Score for the page_____

9. (8 pts) Building Fat

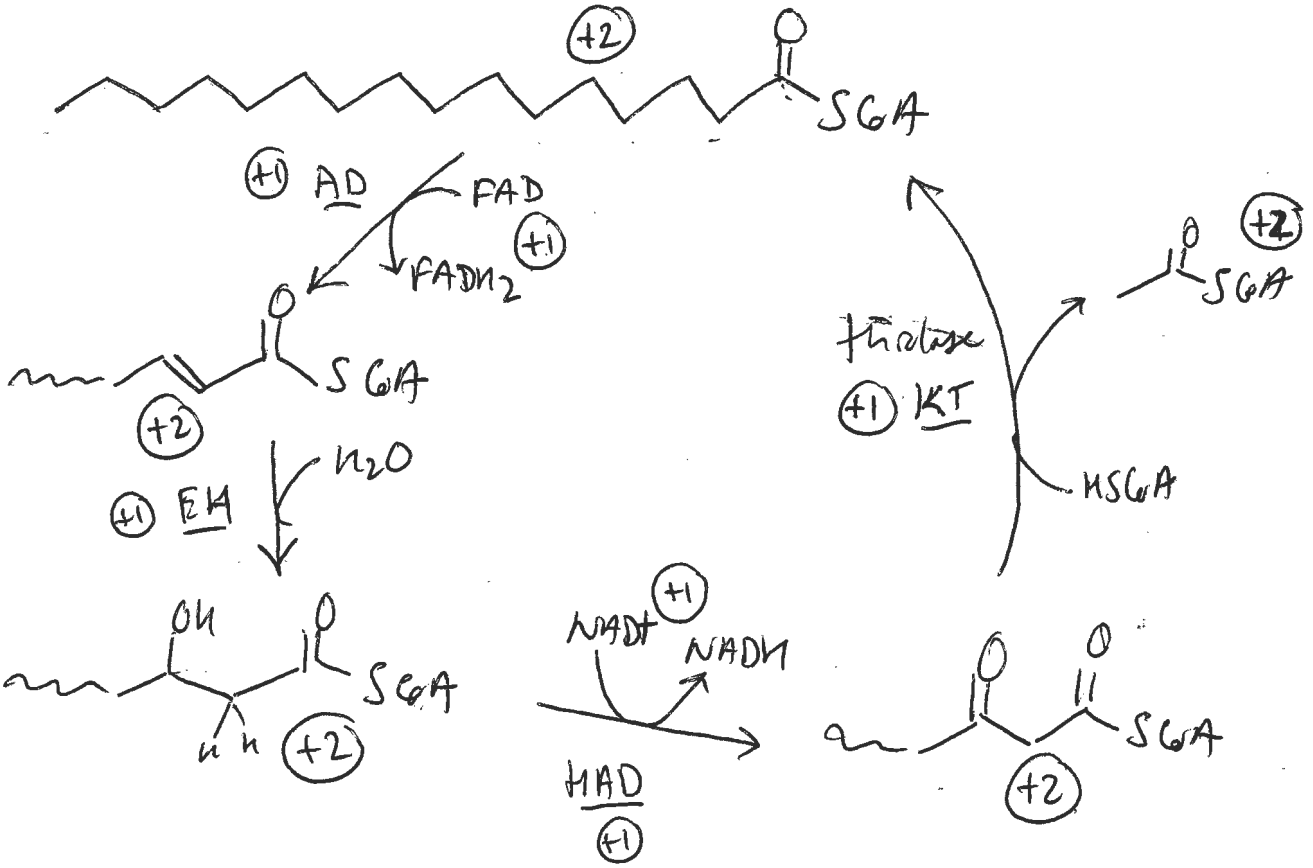
Mammalian and yeast fatty acid synthases are both megasynthases, protein complexes with many linked active sites. What is the biochemical rationale for this? What speculative reason did we give for the fact the two complexes have very different architectures?

(+2) – Substrate channeling increases rate of reaction and sequesters possible (+2) for either reactive intermediates

(+4) – Yeast FAS makes only saturated lipids, whereas mammalian FAS is related to more versatile polyketide synthases.

10. (16 pts) Utility of Fat

Starting with palmitoyl (saturated C16)-CoA, draw the intermediates in the sequential oxidation, hydration, oxidation, and retro-Claisen reactions of one cycle of β-oxidation, with names of cofactors, abbreviations for enzymes, but no mechanisms.



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11. (18 pts) Connections

Succinate dehydrogenase is uniquely situated among TCA cycle enzymes. How so, and what is its other name? What cofactors does it use, and why doesn't it use NADH? SDH deficiency can predispose cells to cancer. What was our speculation on how this works (a bird's eye view, not specific targets)?

(+2)

- Succinate dehydrogenase is the only membrane-bound TCA cycle enzyme - it is a.k.a. Complex II of the ETC. (+2)

(+2) (+2)

- It uses FAD/$FADH_2$ (and CoQ/$CoQH_2$) because the ~~reduct~~ oxidation of an alkane to an alkene is not exergonic enough to drive NAD^+ reduction. (+3)

q ↑

q ↓ - The inadequacy of the TCA cycle and ETC means that SDH deficiency should cause the cell to rely more on glycolysis for energy. This mimics the situation for (+3) tumor cells that are often anoxic (the Warburg effect). (+3)

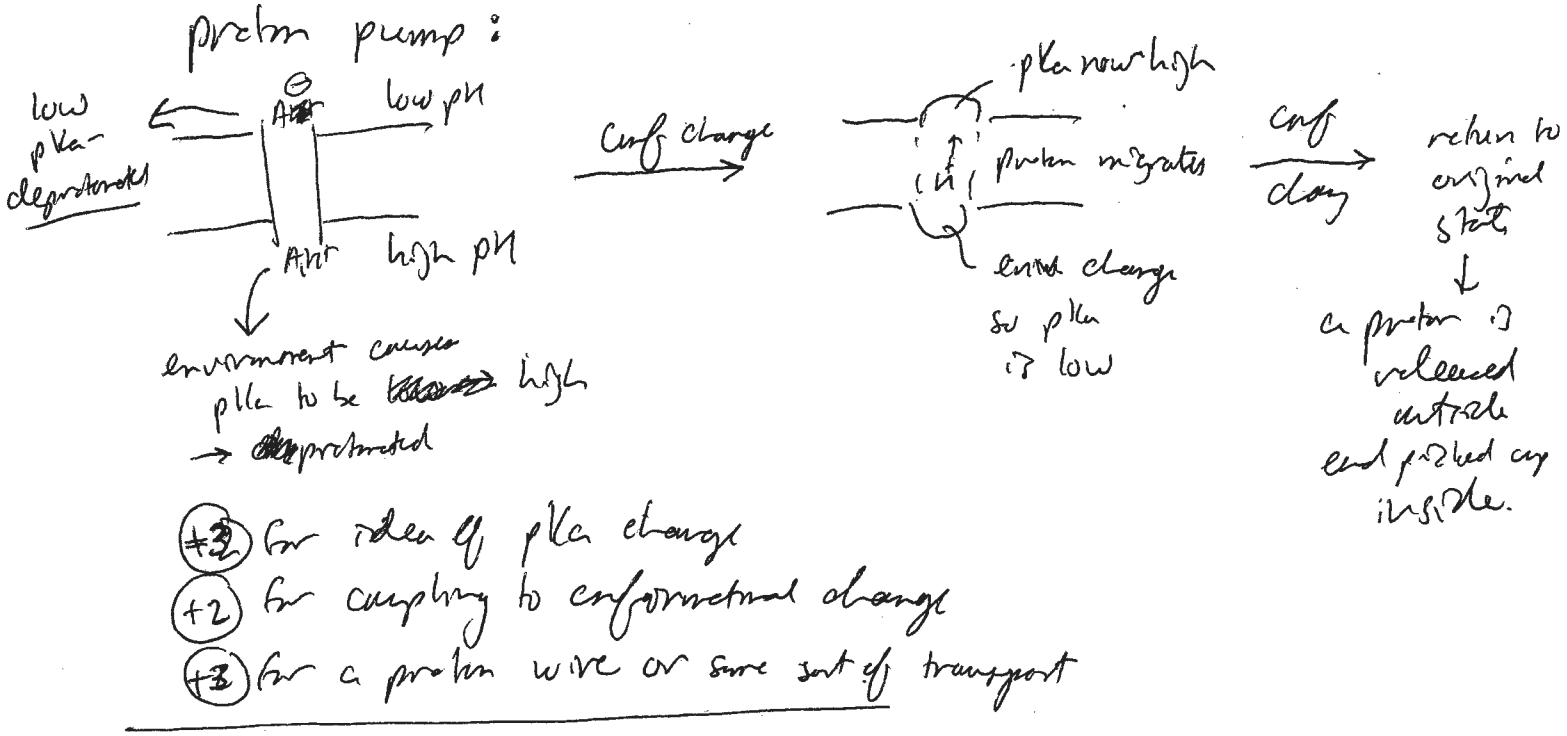
(+3) So the cellular metabolism is "~~pre~~ pre-adapted" to become cancerous if other mutations occur.

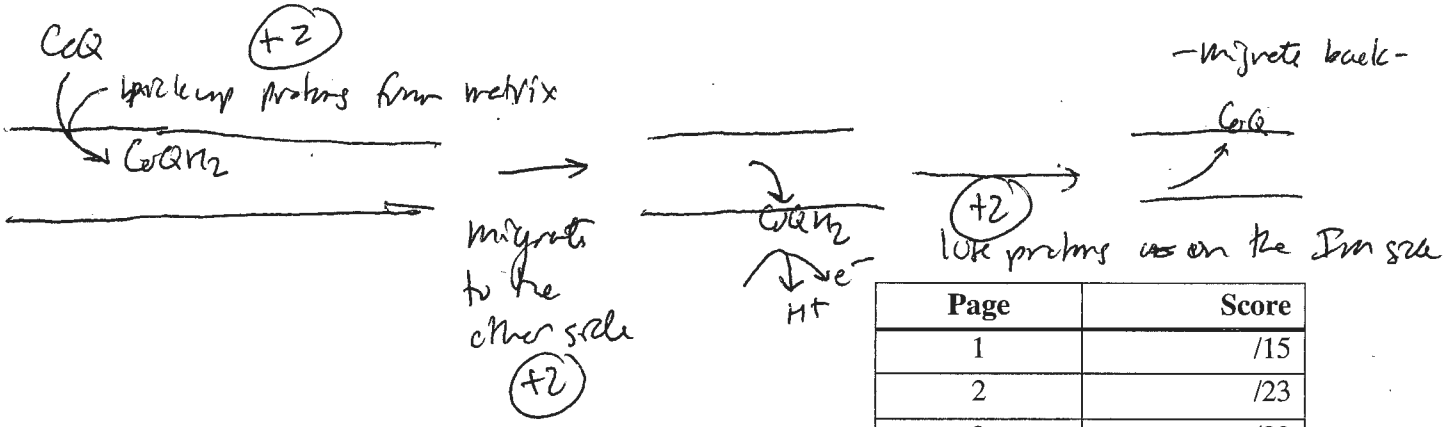
[Also - succinate kalvage ↑ destruction of HIF factor that helps adapt to anoxia]

Score for the page_____

12. (14 pts) Transport

We described two fundamentally different ways that protons are pumped ~~protons~~ across membranes in the ETC. Sketch them briefly (no need to describe the theory of relativity or the Q cycle in detail).





Page	Score
1	/15
2	/23
3	/30
4	/26
5	/24
6	/18
7	/14
Total	**/150**

Score for the page_____