MCDB 108B - Biochemistry
Winter 2012

Final Examination

March 21, 2012

Answer Key.

Make sure your exam contains pages 1 through 12. Answer all questions.

Question	
1	_____
2	_____
3	_____
4	_____
5	_____
6	_____
7	_____
8	_____
9	_____
10	_____
11	_____
12	_____
total	_____/

1. Everyone loves a story..... That's because a story always tells the larger picture of a certain event, or a particular detail, and it always brings significance to something we do or believe. A great story always puts things in meaningful perspective, and without knowing the larger story, things often seem incomplete, random and meaningless.

a. Every major topic we discussed in the first weeks of this class, from chemical equilibrium to glycolysis, is part of a larger story of biological life. Tell how the following concepts/topics fit into the story of biological life: Equilibrium, work, steady-state, rate-determining steps, and enzymes. Tell a compelling story incorporating these terms **(~50 words)** (**10**).

All life, as we know it, requires the ability to perform work - the work of making ATP. To perform work, a biological system must be displaced away from equilibrium, because at equilibrium no work can be done. It is the approach to equilibrium from some initial condition is associated with the ability to do work. In order to displace a metabolic pathway away from equilibrium, the steady-state must be imposed, along with incorporation of a rate-determining step. In a metabolic pathway, steady-state flow is controlled by enzymes. Therefore understanding the control of enzymes is critical to understanding how a metabolic pathway is capable of performing work.

b. Explain, in thermodynamic terms, why wood, starch, fat, protein, gasoline and other similar materials can potentially produce large amounts of free energy when exposed to oxygen. We know that when the above materials are simply exposed to ambient oxygen, nothing happens unless they are ignited. By comparison, when we ingest CHOs or fats in our bodies, energy is released spontaneously. What is the precise chemical process that liberates this energy? Why does sugar yield less energy per weight than does fat? Finally, explain why CHOs or fats, when ingested, release energy spontaneously in the body. **(~50 words)** (**10**).

All these substances are highly reduced and can be oxidized by O2. The transfer of e's from these substances to O2 is associated with the release of a large amount of free energy. Sugar contains more oxygen than fat, and therefore fewer e's are available for oxidation by O2.

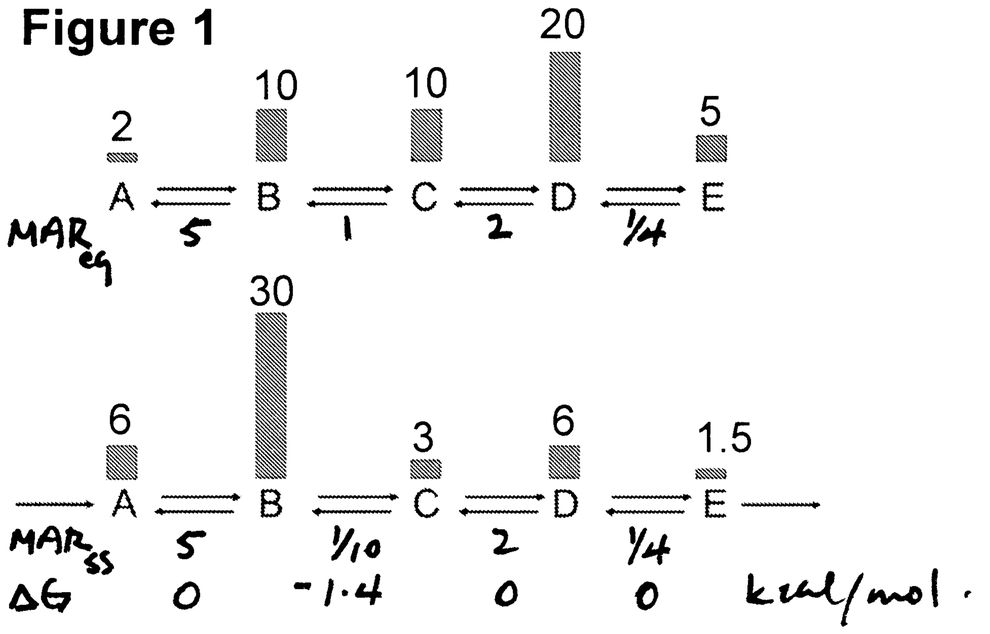
Oxidation of fuels is associated with a high activation energy, and therefore does not proceed without igniting the fuel. In the body, enzymes lower the activation energy, and thus oxidation proceeds spontaneously.

c. Construction workers at UCSB are building a new building – essentially, creating order out of randomness. Given that the universe tends toward maximum decay how is this possible? **Show step-by-step how energy from the sun is used to create a highly ordered structure built by humans**.... (You can use a flow diagram with arrows....) (**5**)

Energy from the sun + CO_2 ⟶ photosynthesis by plants ⟶ starch
Humans consume starch + O_2 ⟶ oxidation ⟶ $CO_2 + H_2O$ + Energy
Energy is used to build a building ie. to convert chaos into order.

2. **Fig. 1** shows a metabolic pathway in equilibrium (top) or in steady-state (bottom).

Figure 1



a. What is the ΔG value for each reaction step during steady-state? **Show your work.** (**5**)

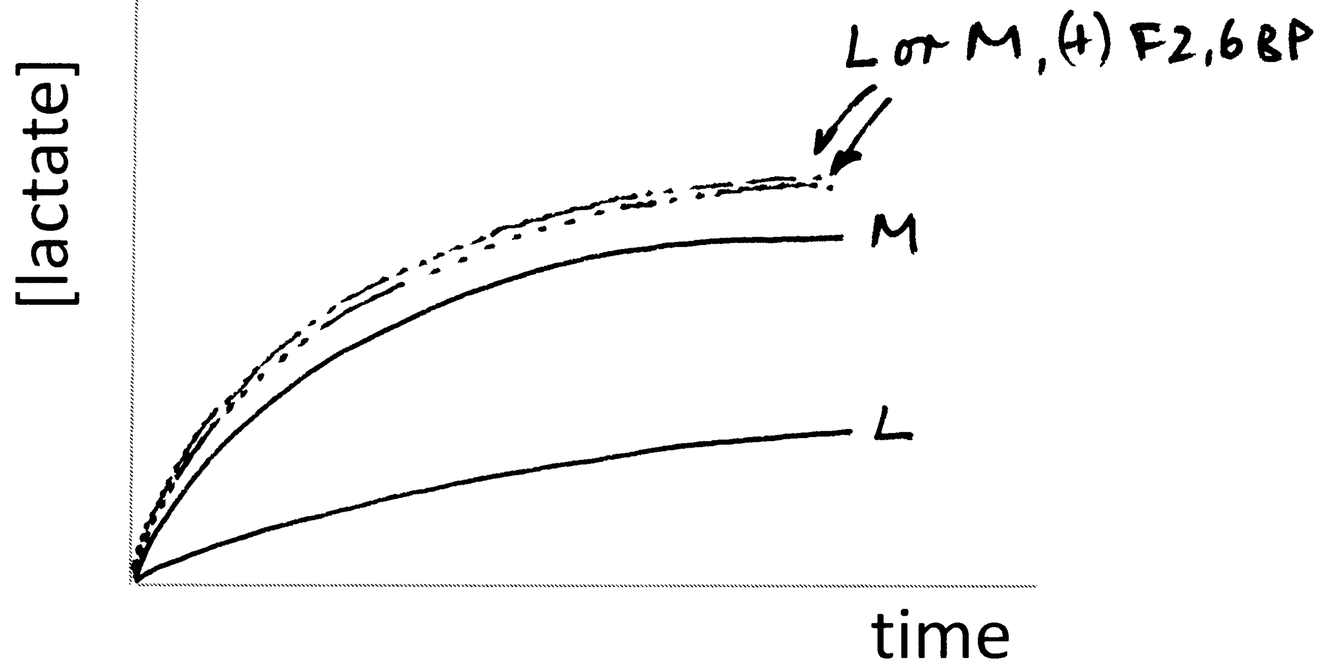
ΔG is the difference in MAR_{eq} and MAR_{ss}. The only step in which there is a difference is B→C. The difference is 10-fold. If the steady state were allowed to relax to equil^{m}, C would build up. Therefore ΔG is −1.4 kcal/mol.

b. If enzyme(s) in this pathway were subject to regulation by metabolites, **which step(s) would you expect to be subject to regulatory control? Explain why.** (**1 sentence**) (**3**)

B to C. b/c ΔG is large and negative compared to all other steps.

3. A rat is subjected to starvation conditions for 3 days. It is then killed, and extracts of the liver and skeletal muscle are prepared. All the enzymes and cofactors necessary for the conversion of glucose to lactate are present in both extracts and each extract can theoretically support glycolysis upon the addition of glucose. Glycolytic flux is measured by the rate of lactate production.

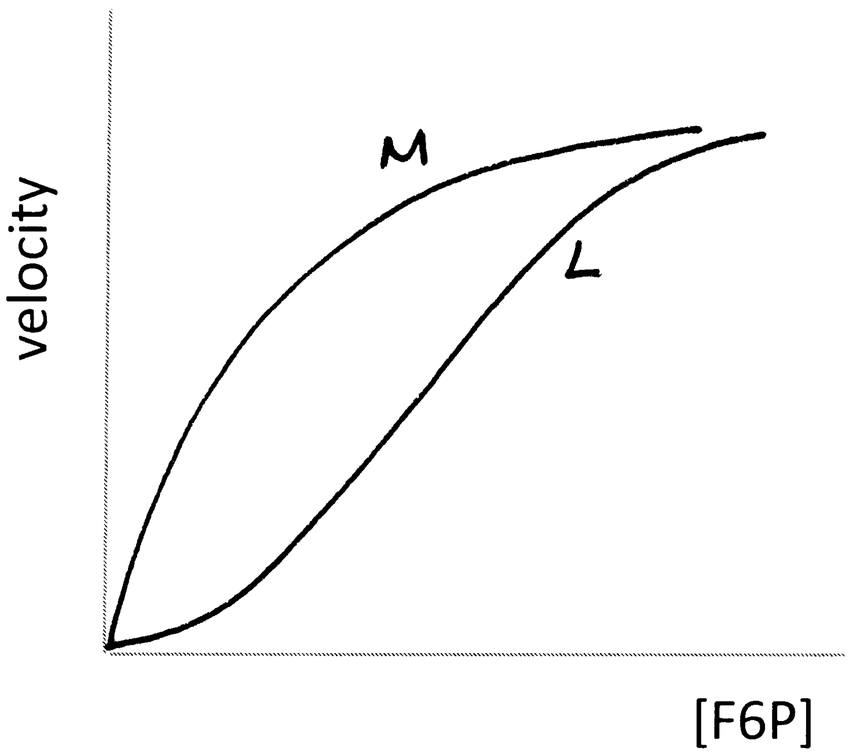
Figure 2



a. In Figure 2, draw the expected graphs of lactate production vs time for both the liver and skeletal muscle extract after the addition of glucose. Explain the physiological reason if there are differences. (**2 sentences**) (**4**)

In starvation, glycolysis in liver is low in activity to shunt glucose to the blood. Glycolysis in muscle is high in activity to provide power for locomotion to find food.

b. Draw the graphs of enzyme velocity vs **F6P** concentration for **PFK** from both extracts. Label your graphs. Briefly explain. (**2-3 sentences**). (**4**)



For the liver extract, glycolysis is inhibited principally at the level of PFK. Under these conditions PFK exists mainly in the inactive T-state, which exhibits sigmoidal behavior.

For the muscle extract, PFK is activated, exists mainly in the R-state, which exhibits M. M. behavior.

c. <u>In a separate experiment,</u> **F2,6BP**, is added to each extract at the same time as glucose. **In Figure 2**, superimpose the expected graphs for liver and muscle in the presence of **F2,6BP**. <u>Explain the biochemical basis.</u> **(2-3 sentences) (5)**

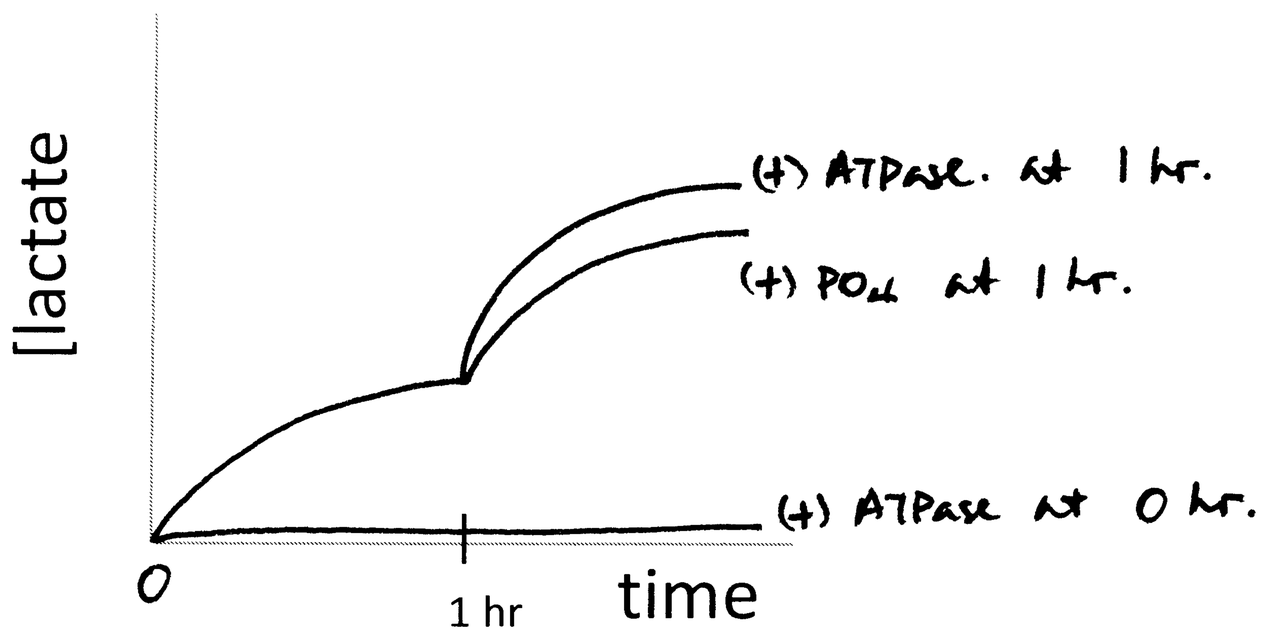
F2,6BP activates PFK. In liver, glycolysis is inhibited b/c the levels of F2,6BP are held low during starvation. Adding F2,6BP to liver extracts therefore results in activation. Adding F2,6BP to muscle extracts however has ~~little~~ less effect, b/c muscle already exhibits high levels of ~~PFK~~ F2,6BP hence high glycolytic activity.

d. In all cases, the rate of lactate accumulation starts to slow soon after glucose is added. Give the 2 most likely reasons for this. (Assume that glucose is not running out.) **(4)**

1. Increased energy charge

2. PO4 becomes limiting

e) Graph the expected effects of adding PO4 at 1 hr. Compare this to adding an ATPase at the same time. Explain your graphs and the differences between the effects of PO4 vs ATPase. On the same graph, show the expected effects of adding ATPase at time 0. Explain the biochemical reason for your predictions **(2-3 sentences total). (5)**



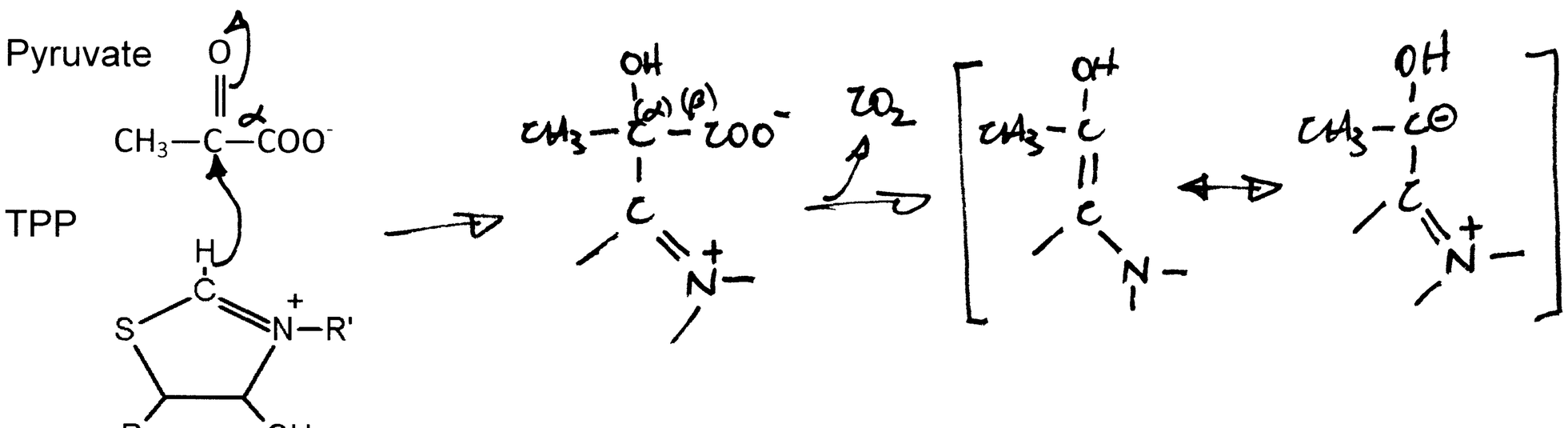
Addition of PO_4 at 1 hr ↑'s glycolysis b/c PO_4 is becoming limiting. Adding an ATPase replenishes both PO_4 AND ↑'s energy charge, which stimulates to a higher degree.

Addition of ATPase at time 0 destroys all ATP so that glucose cannot be P'd and glycolysis cannot be initiated.

f) Inhibition of an enzyme in any metabolic pathway is expected to result in accumulation of the substrate and depletion of the product. When an inhibitor of LDH was added to these extracts, the rate of lactate production slowed as expected – but pyruvate did not build up! **Explain why. (2 sentences) (3).**

Inhibition of LDH prevents the regeneration of NAD+ from NADH. Without NAD+, glycolysis becomes blocked at the level of GAPDH, which is well before pyruvate generation. Therefore pyruvate does not build up.

4. Thiamine pyrophosphate derived from vitamin B1 is essential for decarboxylation of pyruvate but not oxaloacetate. Show the chemical mechanism by which thiamine-PP facilitates α-decarboxylation of pyruvate by PDH. Explain your answer (2-3 sentences). You do not need to describe the oxidative arm of the mechanism. (5)



In general, α-decarboxylation is difficult, but β-decarboxylation is easy. The quaternary N of thiamine is analogous to a carbonyl O in a β-mechanism. Thus thiamine mimics a β-mechanism allowing pyruvate decarboxylation.

5. The purpose of the TCA cycle is to remove electrons and discard the electron-depleted carbon atoms. The first metabolite committed to this process is citrate, which is the product of step 2 **(Fig. 3)**.

a. For every acetyl group that enters the TCA cycle, 2 atoms of "depleted" carbon leave.

Indicate which atoms in citrate correspond to acetyl CoA.

Explain the <u>chemical strategy</u> achieved in step 3 (both steps) to ultimately allow release of CO_2 **(1-2 sentences)**. **(4)**

The strategy is to move the OH group from the middle carbon to one of the methylene carbons. This OH group can then be oxidized to a carbonyl grp, which will facilitate β-decarboxylation.

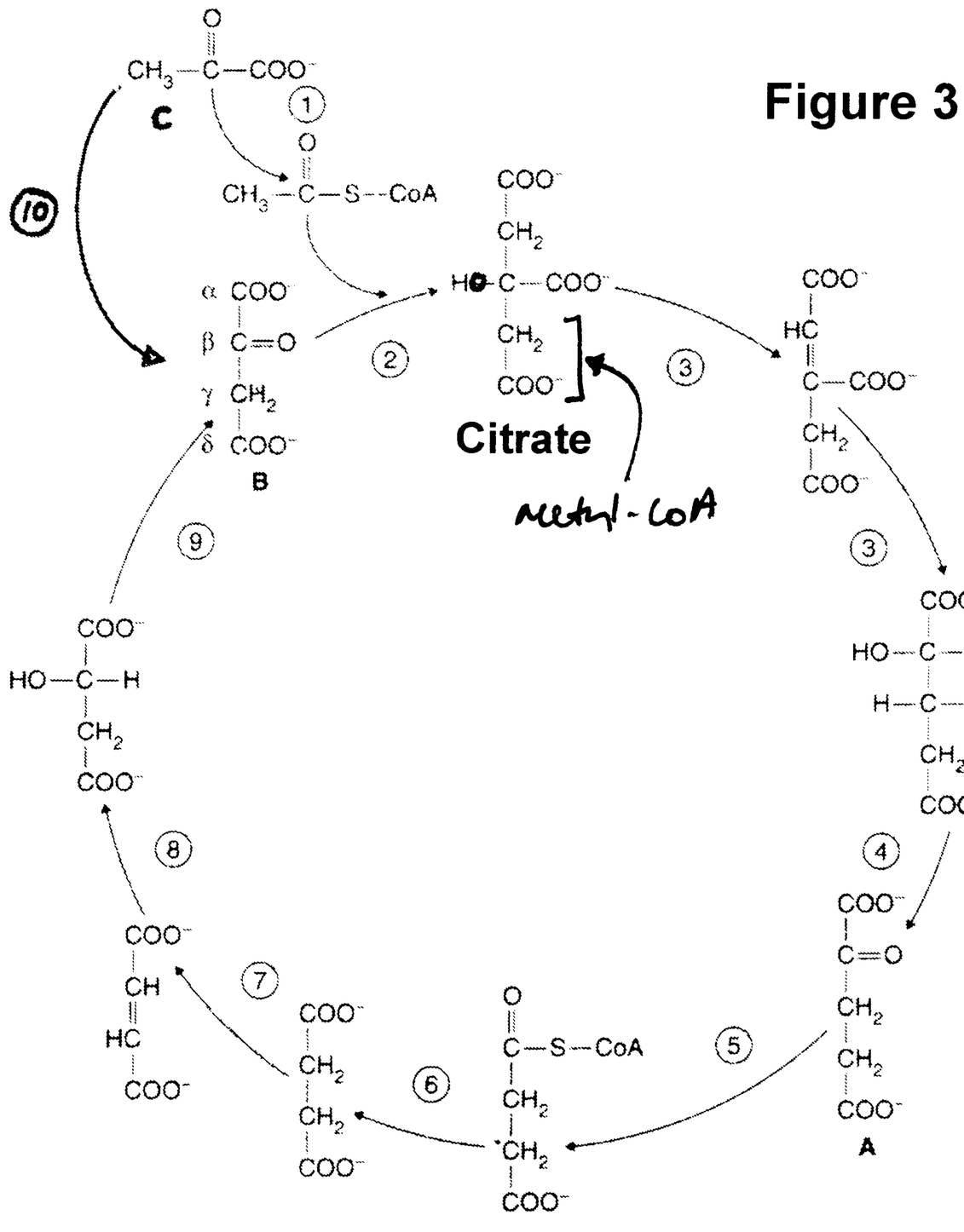
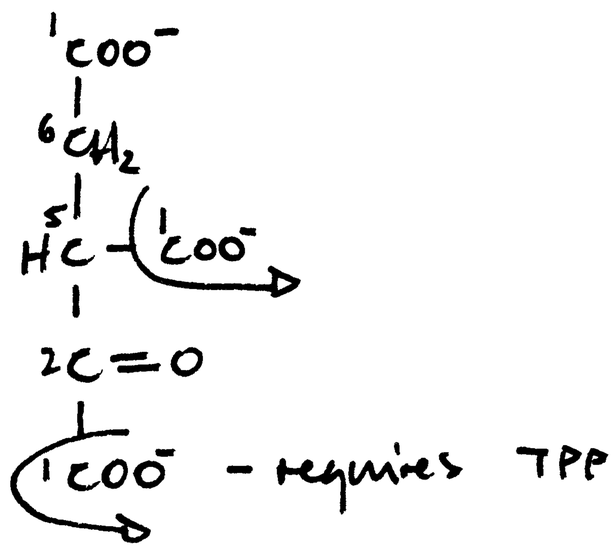


Figure 3

b. Step 4 generates a key intermediate, which is not shown in Figure 3. Based on the substrate for step 4, **draw the structure of this intermediate**. Indicate the oxidation state of all carbons in this intermediate in terms of how many electrons each carbon "owns". Which carboxyl groups are removed in one turn of the TCA cycle? What co-factors are needed for which decarboxlylation reaction? **(4)**



d. After complete decarboxylation, reaction 6 shows the hydrolysis of succinyl-CoA to form succinate and free coenzyme A. Where does the water come from for hydrolysis, and what reaction is coupled to this step? **(1-2 sentences) (3)**

H_2O comes from the condensation of GDP + PO4, to form GTP, this reaction which is coupled to the hydrolysis of succinyl-CoA to succinate + CoA.

e. Explain the chemical strategy of reactions 7,8, and 9 describing all reactants, co-factors, and products involved at each step. **(1-2 sentence explanation for each reaction) (6)**

Step 7. Dehydrogenation of saturated carbon. Two hydrogen atoms are lost to FAD to form FADH2 and unsaturated carbon.

Step 8 Hydration of unsaturated carbon to generate a secondary alcohol which can eventually be oxidized to a carbonyl. Reaction requires H_2O.

Step 9 Oxidation of a secondary alcohol to a carbonyl. Two e's are lost to NAD+ to form NADH + H+.

6a. In Figure 3, when the carbonyl carbon of acetyl-CoA (product of step 1) is labeled with ^{14}C, multiple carbons become labeled in oxaloacetate (product of step 9) after one turn of the TCA cycle. Indicate with an asterisk which carbons in each intermediate of the TCA cycle becomes labeled after one complete turn. Explain how the radioactivity "migrates" to multiple carbons **(2-3 sentences). (6)**

At the level of succinate, carbon atoms 1 and 4 are indistinguishable by biochemical methods. Therefore radioisotope at one of these positions appears as 50% labeling at each position. When the isotope appears in malate, 50% of the malate molecules will in fact be labeled at position 1, and 50% will be labeled at position 4

b. Explain how such radiolabeling experiments proved that the carbons that go in to the TCA cycle from acetyl-CoA are not the same carbons that leave as CO_2 in the same cycle. **(2-3 sentences) (4)**

When radioisotope enters the TCA cycle from acetyl-CoA, the CO2 that evolves in the corresponding cycle is unlabeled. Therefore, these carbons are distinct from those of acetyl-CoA.

7. A Duracell battery powers a simple circuit consisting of a wire, a small electric toy motor and the battery itself. The electrodes of the alkaline "Copper Top" battery correspond to zinc (negative terminal, or reductant) and MnO_2 (positive terminal, or oxidant) which produce an E_o value of 1.5 volts. If this circuit serves as an analogy of oxidative phosphorylation in living cells:

a. What do the following electrical components correspond to in cells? **Explain. (4)**

the wire – the physical path of the electron

the motor – the proton pumps

the negative battery terminal – NADH or FADH2 (or ascorbate)

the positive battery terminal – O_2

b. If one were to physically block the electric motor, the motor would no longer turn, and current-flow would stop. This would be an analogy to what kind of inhibitor of oxidative phosphorylation? Give an example and explain the analogy. **(2-3 sentences) (4)**

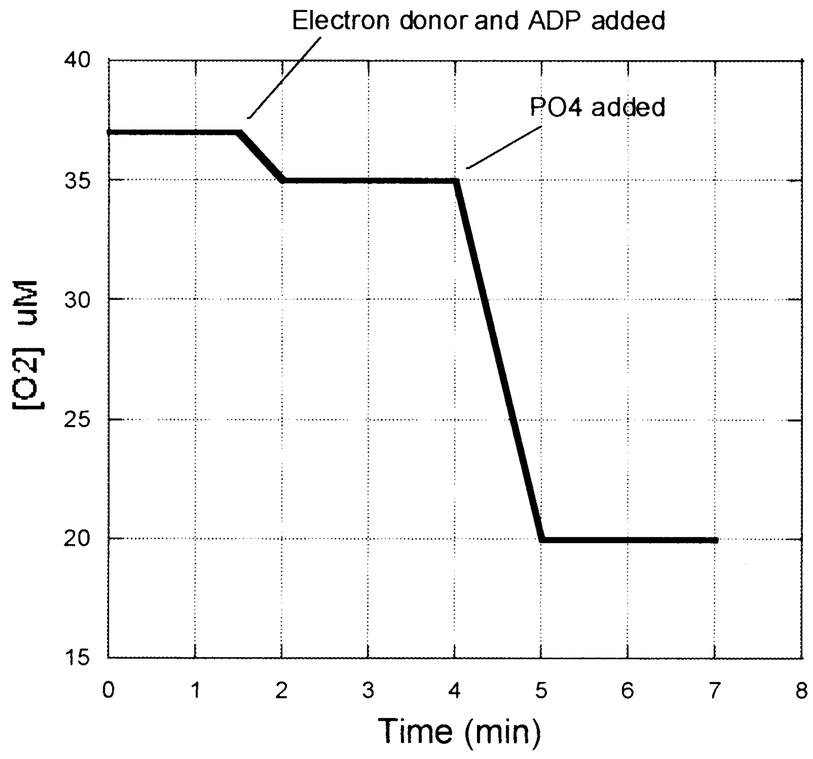
This would correspond to a proton pump inhibitor (rotenone, antimycin, etc), since the proton pumps are analogous to the motor, and their inhibition blocks both proton pumping as well as election flow.

c. If an alternate wire were connected from the negative terminal directly to the positive terminal of the battery, this situation would cause a "short circuit" and the motor would stop turning. What would be a reasonable *hypothetical* analogy in the mitochondria? *(In reality, this would not happen. Think carefully!)* **(1 sentence) (4)**

The closest analogy would be if O2 directly oxidized NADH or FADH2 without any electron flow through the proton pumps.

8. The O_2 concentration in a sample of isolated mitochondria is monitored over time. After 1.5 min, an electron donor (100 uM) and ADP (100 uM), are added to the sample. PO_4 is not present.

Figure 4



a) According to Figure 4, what is the small amount of O_2 consumption attributable to upon adding the electron donor? Why does O_2 consumption stop at minute 2? *(a few words is enough)* **(4)**

The small amount of O2 consumption between min 1.5 – 2, is the amount of oxidation required to generate the proton gradient. In the absence of PO4, oxidation stops because the proton gradient backs up, preventing further electon flow.

b) At minute 4, 30 uM PO_4 is added. Explain why O_2 consumption resumes. Why does O_2 consumption stop at minute 5? **Explain (1-2 sentences) (4)**

In presence of PO4, H+s can now flow through the ATP synthase machinery allowing the H+ gradient to dissipate and oxidation to resume. PO4 is limiting, therefore oxidation stops when PO4 runs out.

c) Calculate the P/O ratio for this electron donor. Where in the electron transport chain do these electrons enter? **Explain (1-2 sentences) (4).**

15 uM O2 (30 uM O) is consumed by 30 uM PO4 transferred to ADP. P/O is therefore 30 uM/30 uM = 1. These electrons must enter at complex IV. If they entered at complex I or III, the P/O ratio would be correspondingly higher.

d) In Figure 4, superimpose the corresponding graphs if the following compounds were added at time 0: oligomycin, antimycin A, CN^- **Explain your answers (1 sentence each) (6).**

CN- inhibits complex IV, therefore there would be no electron flow at all.

Oligomycin inhibits ATP synthase, therefore you would expect oxidation associated with generating the H+ gradient only.

Antimycin inhibits complex III, therefore electrons are expected to flow freely through complex IV.

9. You are given isolated intact mitochondria and 3 unknown compounds (A, B and C) that each inhibit oxidative phosphorylation when the electron donor is NADH. You find that A or B by themselves prevent O_2 consumption, but compound C does not. A and C together inhibit oxidation, but B and C together do not. What class of inhibitor are compounds A, B and C, respectively? **Explain your rationale (9).**

Compound A - "A" cannot be an uncoupler, b/c uncouplers never prevent oxidation. If an ATP synthase inhibitor, A + C (an uncoupler, see below) would not inhibit oxidation. "A" must be an inhibitor of electron transport.

Compound B – "B" cannot be an inhibitor of electron transport b/c oxidation is observed in the presence of B + C. 'Cannot be an uncoupler, b/c uncouplers never prevent oxidation "B" must be an ATP synthase inhibitor.

Compound C – "C" alone does not inhibit oxidation. Only an uncoupler exhibits this property.

10a. After 2-3 days of fasting, liver glycogen is depleted and thus no longer directly provides blood glucose for other tissues. Describe the key metabolic adaptations, the biochemical mechanisms responsible, and the physiological significance, in response to glucagon/epinephrine signaling in the following tissues: i) Liver, ii) Brain, iii) Muscle, iv) Adipose tissue. **Explain fully. Be concise! (15)**

ADIPOSE TISSUE
- Glucagon activates HSL; Triacylglycerols are broken down to FFAs which are transported to liver. The idea is to provide acetyl-CoA for fuel.

LIVER
- Glucagon inhibits glycogen synthase, activates phosphorylase.
- Glycogen synthesis is inhibited; Glycogenolysis is activated to produce glucose.
– Glycolysis is inhibited (PFK, PK) to prevent glucose breakdown.
- FA synthesis is inhibited (ACCase); FFAs undergo β-oxidation to produce acetyl CoA. NADH levels rise and TCA cycle stops.
- Acetyl-CoA forms ketone bodies to be exported to other tissues.
– Gluconeogenesis is activated to produce glucose from amino acids.

BRAIN
- Glucose is normally used to make oxaloacetate and acetyl-CoA.
- cannot use fats to make acetyl-CoA, cannot use protein to make oxaloacetate.
- Blood-born ketone bodies are now used to make acetyl-CoA sparing this need for glucose.
- Glucose is still required to make oxaloacetate.

MUSCLE
- proteins are broken down to amino acids for substrates for gluconeogenesis in liver.
- glucagon activates phosphorylase promoting glycogen breakdown to make G6P for glycolysis
- PFK is activated; glycolysis is turned on for locomotion.
- Blood-born ketone bodies are used to make acetyl-CoA of oxidation.

b. Similarly describe the key metabolic adaptations, the biochemical mechanisms responsible, and the physiological significance in response to the fed state (insulin signaling) in the following tissues: i) Liver, ii) Muscle, iii) Adipose tissue. **Explain fully. Be concise! (10)**

In response to insulin:

LIVER
glycogen synthase is activated: Glycogen is synthesized to store CHO
PFK, PK activated: Glycolysis is activated
Acetyl-CoA carboxylase is activated: FAs are synthesized to store fat

MUSCLE
glycogen synthase is activated: Glycogen is synthesized to store CHO
Acetyl-CoA carboxylase is activated: FAs are synthesized to store fat (they don't need to have this

ADIPOSE TISSUE
HSL inhibited: TAGs are synthesized to store fat

11. Answer the following questions regarding the inter-relationships between carbohydrate and fat metabolism:

a. Explain the biochemical basis for the common adage that "fats burn in the flame of carbohydrates". **(2-3 sentences) (6)**

Oxidation of acetyl-CoA requires oxaloacetate in order to run the TCA cycle. Oxaloacetate is normally made only from glucose. Oxaloacetate cannot be made from fat. (see 11b) Thus burning fat (acetyl-CoA) requires CHO (oxaloacetate).

b. When muscle glycogen stores run down, they cannot be replenished by fatty acids – "glucose cannot be made from fat". **Explain why. (2-3 sentences) (6)**

For every acetyl-CoA (2Cs) that enters the TCA cycle, 2 carbons leave as CO2. Therefore even though an enzymatic pathway from acetyl-CoA to glucose exists, a net increase in oxaloacetate (necessary for gluconeogenesis) is not possible from acetyl-CoA.

12. Fad diets all have in common a severe restriction of carbohydrate intake. Although it is not known conclusively how these diets work to reduce percent body fat, provide a reasonable explanation based on your knowledge of human metabolism. Describe normal TAG synthesis and breakdown, and how this may relate to the effects of fad dieting. A consequence of fad dieting is chronic blood acidity. Explain the metabolic basis for this. **(Short paragraph) (10)**

Normal TAG synthesis in adipose tissue requires glucose to make glycerol-3-P to combine with acyl-CoAs to make TAGs. TAGs are broken down to glycerol and FAs. The fatty acids are resynthesized to TAGs, which requires glucose b/c glycerol-3-P cannot be made from glycerol in adipose tissue. FA are always in a steady state of synthesis and breakdown. If glucose is low, then FA breakdown prevails.

Excess FAs are taken to the liver and oxidized to acetyl-CoA. Glucagon inhibits ACC, therefore no FA synthesis in liver. Acetyl-CoA is not needed in the TCA cycle b/c energy charge is high. Therefore ketone bodies are made, secreted into the blood, and excreted in the urine. Thus under CHO restriction, the blood is chronically acidified by ketones.