This exam consists of 6 questions. A maximum of 100 points can be earned. Partial credit will be given. There are a total of 14 pages, including the cover page and one blank sheet at the end for notes. However, do not use the blank sheet for your final answers. If you need more space, use the back of pages 2-13. Write your name on top of each page! Petitions for re-grading will be considered only if you have used permanent ink, unless an addition error has occurred.

*IT IS YOUR RESPONSIBILITY TO WRITE LEGIBLE! No extra effort will be made to decipher your handwriting.

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T = 25 °C (298 K)
T = 37 °C (310 K)

R = 8.315 J mol⁻¹ K⁻¹
F = 96.5 kJ mol⁻¹ V⁻¹
n = equivalents of electrons
Z = charge of proton

\[ \Delta G^\circ = -RT\ln K_{eq} \]
\[ \Delta G = \Delta G^\circ + RT\ln[\text{Prod.}]/[\text{React.}] \]
\[ \Delta G = \Delta G^\circ + 2.303RT\Delta pH \]
\[ \Delta G^\circ = -nF\Delta E^\circ \]
\[ \Delta E^\circ = E^\circ_{\text{Oxidant}} - E^\circ_{\text{Reductant}} \]

I, ____________________________, authorize the University to distribute publicly this graded exam (e.g., handed out in class or left in a bin for pick up).

I am aware of the fact that violations of the Academic Code of Conduct¹ may be reported to UC Davis Student Judicial Affairs.

¹Examples of academic misconduct include: receiving or providing unauthorized assistance on examinations, using unauthorized materials during an examination, altering an exam and submitting it for re-grading, or using false excuses to obtain extensions of time (http://sja.ucdavis.edu/cac.htm).
1. (16 pts) Questions related to the Tricarboxylic Acid (TCA) or Citric Acid cycle.

a) Draw the structure of $\alpha$-ketoglutarate into the box. (2 pts)

b) The enzyme that uses $\alpha$-ketoglutarate as a substrate in the TCA cycle, $\alpha$-ketoglutarate dehydrogenase, has a requirement for two soluble and three tightly or covalently bound co-factors. Name these five co-factors. (5 pts)

Coenzyme A

NAD$^+$

FAD

Thiamine pyrophosphate (TPP)

Lipoic acid

c) The $\alpha$-ketoglutarate dehydrogenase-catalyzed reaction releases carbon dioxide. Which carbon of $\alpha$-ketoglutarate is converted to CO$_2$? Circle the carbon in the box above. (1 pt)

d) The $\alpha$-ketoglutarate dehydrogenase-catalyzed reaction is highly regulated by the availability of ATP and NADH. Briefly answer the following related questions:

Explain why $\alpha$-ketoglutarate dehydrogenase is a good target for regulation. (2 pts)

The reaction catalyzed by $\alpha$-ketoglutarate dehydrogenase is essentially irreversible (large negative $\Delta G$ and loss of CO$_2$).

Predict the effect (activation or inhibition) of ATP and NAD$^+$ (this is not a typo) on this enzyme. Circle. (2 pts)
e) In addition to being an intermediate of the TCA cycle, \( \alpha \)-ketoglutarate is also a building block for other compounds in metabolism, such as amino acids. If \( \alpha \)-ketoglutarate is removed from the TCA cycle for amino acid synthesis, oxaloacetate (OAA) cannot be regenerated from \( \alpha \)-ketoglutarate. Briefly answer the following related questions.

What is the consequence for the TCA cycle if OAA is not regenerated? (2 pts)

If OAA cannot be regenerated, which is the acceptor for incoming acetyl-CoA, the TCA cycle will slow down or even stop to operate.

What are the reactions collectively called that regenerate OAA? (2 pt)

*Name of reactions:* ______ Anaplerotic reactions ________________

2. (25 pts) Questions related to the electron transport chain (ETC).

a) Given below are redox reactions catalyzed by the mitochondrial ETC. Depending on the given substrates, one complex or more complexes of the ETC (complexes I, II, III, IV) are required for each redox reaction to be completed as written. For each equation, circle the necessary complexes. (5 pts)

*Example:*

\[ A + B \rightarrow C + D \]

\[ \text{NADH} + H^+ + \text{CoQ} \rightarrow \text{NAD}^+ + \text{CoQH}_2 \]

\[ \text{FADH}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O} \]

\[ \text{NADH} + H^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O} \]

\[ \text{CoQH}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{CoQ} + \text{H}_2\text{O} \]

\[ 2\text{Cytc} \text{(red)} + \frac{1}{2} \text{O}_2 \rightarrow 2\text{Cytc} \text{(ox)} + \text{H}_2\text{O} \]
b) A bacterium uses nitrate (NO$_3^-$) as the terminal electron acceptor and acetoacetate as an initial electron donor to generate ATP by a process similar to the mitochondrial ETC/ATP synthase. Calculate the standard free energy change ($\Delta G^o'$) of the acetoacetate oxidation. Assuming an efficiency of 83% for ATP synthesis, how many moles of ATP could be produced under standard conditions per mole acetoacetate oxidized? (8 pts)

Use the following information:

- NO$_3^-$ + 2H$^+$ + 2e$^-$ $\rightarrow$ NO$_2^-$ + H$_2$O $\quad E^{o'} = +0.42$ V
- Acetoacetate + 2H$^+$ + 2e$^-$ $\rightarrow$ $\beta$-Hydroxybutyrate $\quad E^{o'} = –0.35$ V
- $\Delta G^{o'}$ of ATP formation: = +30.5 kJ mol$^{-1}$

$$\Delta E^{o'} = E^{o'}_{\text{Oxidant}} - E^{o'}_{\text{Reductant}}$$

$$\Delta E^{o'} = E^{o'}_{\text{Nitrate}} - E^{o'}_{\text{Acetoacetate}}$$

$$\Delta E^{o'} = +0.42$ V – (–0.35 $\text{V}$) = +0.77 $\text{V}$

$$\Delta G^{o'} (\text{bacterial ETC}) = +0.77 \text{ V}$$

$$\Delta G^{o'} = – nF \Delta E^{o'}$$

$$\Delta G^{o'} = – 2 \times 96.5 \text{ kJ mol}^{-1} \text{ V}^{-1} \times 0.77 \text{ V}$$

$$\Delta G^{o'} = – 148.6 \text{ kJ mol}^{-1}$$

$$\Delta G^{o'} (\text{bacterial ETC}) = – 148.6 \text{ kJ mol}^{-1}$$

$$148.6 \text{ kJ mol}^{-1} \times 0.83 / 30.5 \text{ kJ mol}^{-1} = 4.04$$

Moles of ATP : 4 moles of ATP
c) A pH difference ($\Delta pH$) of 0.76 units was determined across the inner mitochondrial membrane (matrix is more alkaline) and a membrane potential ($\Delta \Psi$) of 161 mV was measured (matrix side is more negative). The pH difference and the membrane potential contribute to the proton-motive force (PMF) across the mitochondrial membrane. Calculate the PMF ($\Delta G$) for these mitochondria at 37°C ($\Delta G^\circ$=0). For full credit you must show your work. (5 pts)

$$\Delta G = \Delta G^\circ + 2.303RT\Delta pH + ZF\Delta \Psi$$

$$\Delta G = (2.303 \times 8.315 \text{ J mol}^{-1} \text{ K}^{-1} \times 310 \text{ K} \times 0.76) + (1 \times 96.5 \text{ kJ mol}^{-1} \text{ V}^{-1} \times 0.161 \text{ V})$$

$$\Delta G = 4.5 \text{ kJ mol}^{-1} + 15.5 \text{ kJ mol}^{-1}$$

$$\Delta G = 20.0 \text{ kJ mol}^{-1} \quad (20.0481 \text{ kJ mol}^{-1})$$

PMF (Mitochondria): $\Delta G = 20.0 \text{ kJ mol}^{-1}$

d) Assume that the PMF of the mitochondria (calculated above) is not coupled to the synthesis of ATP but is coupled to the uptake of pyruvate from the cytosol into the mitochondria (one proton and one molecule of pyruvate are co-transported into the mitochondria). Calculate the gradient of pyruvate (i.e., the ratio of mitochondrial-to-cytosolic pyruvate that could be maintained by the mitochondrial PMF). Use the equation $\Delta G = \Delta G^\circ + RT\ln[\text{Prod.}] / [\text{React.}]$.

Keep in mind that the transport of pyruvate across the mitochondrial membrane can be considered a reaction (Pyruvate [cytosol] $\rightleftharpoons$ Pyruvate [mitochondria]) with a $K_{eq} = 1$ under standard conditions. This reaction or process, however, is displaced from equilibrium when coupled to the PMF.

Draw a sketch below to comprehend the question. (2 pts)

Something like this.
Calculate the ratio of pyruvate in the mitochondrion/cytosol that can be achieved. (5 pts)

*For full credit you must show your work. More space than needed is provided!!*

\[
\Delta G = \Delta G^\circ + RT \ln [\text{Prod.}]/[\text{React.}]
\]

\[
\Delta G = \Delta G^\circ + RT \ln \left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right] \quad K_{\text{eq}} = 1 \Rightarrow \Delta G^\circ = 0
\]

\[
\Delta G = RT \ln \left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right]
\]

\[
\left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right] = e^{\left(\frac{\Delta G}{RT}\right)}
\]

\[
\left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right] = e^{\left(\frac{20,000 \text{ J mol}^{-1}}{8.315 \text{ J mol}^{-1} \text{ K}^{-1}} \times 310 \text{ K}\right)}
\]

\[
\left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right] = e^{(7.759)} = e^{(7.778)} \quad \text{if 20,048 was used}
\]

\[
\left[\frac{\text{Pyruvate \_mito}}{\text{Pyruvate \_cyl}}\right] = 2343 = 2387
\]

*Ratio of mitochondrial-to-cytosolic pyruvate: 2343 : 1 (2387 : 1)*
3. (20 pts) Questions related to gluconeogenesis and glycolysis.

a) Two of the following metabolites cannot be converted by humans into glucose on a per net basis. **Circle** the two compounds. (2 pts)

\[ \text{Glycerol} \quad \text{Ethanol} \quad \text{Propionyl-CoA} \quad \text{Pyruvate} \quad \text{Acetyl-CoA} \]

b) Briefly explain why those compounds you circled above cannot be converted by humans into glucose on a per net basis. (2 pts)

Since ethanol is converted to acetyl-CoA and the reaction catalyzed by the PDH complex is irreversible, the two carbons of ethanol and of the acetyl-residue in acetyl-CoA will be lost as CO\(_2\) in the TCA cycle on a per net basis.

c) If you ingest \([^{14}\text{C}]\)-labeled compounds that cannot be converted by humans into glucose on a per net basis, you will find that radioactively labeled carbon atoms will eventually be incorporated into glucose and glycogen. How do you explain this paradox? (2 pts)

Once \([^{14}\text{C}]\)-labeled acetyl-CoA is incorporated into the TCA cycle, the two labeled carbons of the acetyl-residue “equilibrate” with the carbons of TCA cycle intermediates, including OAA, which is also an intermediate of gluconeogenesis.

d) Depending on your current state of activity, the lactate concentration in the blood can vary anywhere from 1 mM to 20 mM. What cell types, tissues or organs are the major sources of lactate in the blood? **Circle.** (2 pts)

\[ \text{Heart Muscle} \quad \text{Skeletal Muscle} \quad \text{Brain} \quad \text{Liver} \quad \text{Macrophages} \quad \text{Erythrocytes} \]
e) What biochemical pathway generates lactate? (2 pts, if you are specific)?

Anaerobic glycolysis

f) Why does excessive ethanol consumption interfere with gluconeogenesis from lactate? (2 pts)

Ethanol is metabolized into acetate by two oxidation steps. Each step requires NAD\(^+\) as a co-factor, which is also required for the conversion of lactate into pyruvate. Thus, ethanol competes with lactate for the reductant (NAD\(^+\)).

g) Gluconeogenesis from pyruvate and glycolysis to pyruvate share seven reactions. However, the three irreversible steps of glycolysis need to be “by-passed” in gluconeogenesis. How is this achieved? Provide balanced reactions (use common abbreviations) for the by-passes in gluconeogenesis. (8 pts)

First by-pass: \[\text{Pyr} + \text{ATP} + \text{HCO}_3^- \rightarrow \text{OAA} + \text{ADP} + \text{Pi}\]
\[\text{OAA} + \text{GTP} \rightarrow \text{PEP} + \text{GDP} + \text{CO}_2\]

Second by-pass: \[\text{Fructose-1,6-BP (F1,6BP)} + \text{H}_2\text{O} \rightarrow \text{Fructose-6P (F6P)} + \text{Pi}\]

Third by-pass: \[\text{Glucose-6-P (G6P)} + \text{H}_2\text{O} \rightarrow \text{Glucose (Glc)} + \text{Pi}\]
4. (11 pts) Questions related to the pentose phosphate pathway and the Calvin cycle.

a) Xylitol, an artificial sweetener sold under the brand name Xylosweet and a supplement in chewing gum, has been proven to reduce cavities. Xylitol (see structure below) is a sugar alcohol, which is converted into xylulose-5-P (Xu5P) by two reactions. Use the following information: ADP is a product of the first reaction, and the second step is a redox reaction. Draw the structure of Xu5P into the box on the right and fill in the blank lines. (5 pts)

![Structure of Xylitol and Xu5P](image)

Name of the intermediate: **Xylitol phosphate (Xylitol-5-phosphate)**

Common name of enzyme 1: **Xylitol kinase**

Co-substrate of reaction 2: **NAD⁺**

Common name of enzyme 2: **Xylitol phosphate dehydrogenase**

b) Assume that the xylitol in your chewing gum is completely oxidized to gain energy (ATP), which of the three phases of the pentose phosphate pathway would further metabolize the Xu-5-P. **Circle.** (2 pts)
c) What is the purpose of the Calvin cycle reactions that are similar to the pentose phosphate pathway? (2 pts)

To regenerate the acceptor molecule for CO2 fixation, ribulose-1,5-bisphosphate (Ru1,5BP)

d) The ATP- and NADPH-consuming reactions of the Calvin cycle are similar to reactions of what pathway discussed in class? (2 pts)

Gluconeogenesis

5. (10 pts) Short questions related to fatty acid degradation.

a) Draw the structure of oleic acid, which can be abbreviated as 18:1 (cisΔ9). (2 pts)

b) What is the general name of enzymes that produce free fatty acids from triglycerides? (1 pt)

Name: Lipases

c) These enzymes (see above in b) are members of which enzyme class? Circle. (1 pt)

Oxidoreductase Transferase Hydrolase
Lyase Isomerase Ligase

d) In what intracellular compartments are free fatty acids activated and degraded? (2 pts)

Activation: Cytosol Degradation: Mitochondria
e) Under conditions when the TCA cycle ceases to operate (severe starvation or diabetes), ATP can still be generated by the oxidation of NADH and FADH$_2$ produced during β-oxidation of fatty acids. Under these conditions, how many moles of NADH are formed during the degradation of one mole of octanoid acid (8:0)? (2 pts)

\[
\text{Moles of NADH per mole of 8:0 acid:} \quad 3 \text{ moles (1 mole per round, 3 rounds)}
\]

f) Under the conditions described in e) when the TCA cycle is not functional, what will be the metabolic fate of the acetyl-CoA generated by β-oxidation of octanoic acid? One word could be enough. (2 pts)

\textbf{Ketogenesis, formation of ketone bodies}

6. (18 + 2 pts) Multiple-choice questions. Circle the best answer. There is only one best answer per question. Each question is worth 2 pts.

a. Gluconeogenic enzymes include all of the following EXCEPT:

\begin{itemize}
  \item[i] Lactate dehydrogenase
  \item[ii] Glyceraldehyde-3-P dehydrogenase
  \item[iii] \textbf{Glucose-6-P dehydrogenase}
  \item[iv] Triose phosphate isomerase
  \item[v] Phosphoglucoisomerase
\end{itemize}

b. Which one of the following enzymes does NOT catalyze a decarboxylation reaction?

\begin{itemize}
  \item[i] Pyruvate dehydrogenase
  \item[ii] α-Ketoglutarate dehydrogenase
  \item[iii] \textbf{Succinate dehydrogenase}
  \item[iv] Isocitrate dehydrogenase
  \item[v] 6-Phosphogluconate dehydrogenase
\end{itemize}
c. Which one of the following compounds is \textit{NOT} a product or intermediate of the pentose phosphate pathway?

\begin{itemize}
  \item[i] ATP
  \item[ii] NADPH
  \item[iii] CO₂
  \item[iv] Seduheptulose-7-P
  \item[v] Glyceraldehyde-3-P
\end{itemize}

d. \textit{Photosynthetic} organisms produce ATP by which of the following processes?

\begin{itemize}
  \item[i] Substrate level phosphorylation
  \item[ii] Oxidative phosphorylation
  \item[iii] Photophosphorylation
  \item[iv] All of the above
  \item[v] None of the above
\end{itemize}

e. Which of the following enzymes of the pentose phosphate pathway requires TPP as a coenzyme?

\begin{itemize}
  \item[i] Transaldolase
  \item[ii] \textbf{Transketolase}
  \item[iii] Phosphopentose isomerase
  \item[iv] Phosphopentose epimerase
  \item[v] Glucose-6-P dehydrogenase
\end{itemize}

f. Carbon flux through any part of the pentose phosphate pathway will increase \textit{EXCEPT}:

\begin{itemize}
  \item[i] in dividing cells
  \item[ii] in cells responding to oxidative stress
  \item[iii] in cells utilizing ribose as the sole carbon source
  \item[iv] \textbf{in muscle cells switching from aerobic to anaerobic glycolysis}
  \item[v] in cells producing reactive oxygen species for pathogen defense
\end{itemize}
g. **Photosystem II** of the ETC in *chloroplasts* is analogous to what complex of the mitochondrial ETC?

i. Complex I  
ii. Complex II  
iii. Complex III  
iv. Complex IV  
v. Complex V

h. The complete oxidation of 1 mole pyruvate via the PDH complex, TCA cycle and ETC yields how many moles of ATP? (NADH=3ATP; FADH$_2$=2ATP)

i. 12 ATP  
ii. 13 ATP  
iii. 14 ATP  
iv. **15 ATP**  
v. 16 ATP

i. Which one of the following enzymes does **NOT** catalyze substrate-level phosphorylation of ADP (or GDP) to ATP (or GTP)?

i. Pyruvate kinase  
ii. **Glycogen phosphorylase**  
iii. Succinyl-CoA synthetase  
iv. Phosphoglycerate kinase  
v. All of the above

j. **Bonus question (2 extra points)!** Who received the Nobel Prize in 1978 for the chemiosmotic theorie (coupling of an electron-motive force a to a proton-motive force in ATP synthesis)?  
... *at least here it pays off to have stuck your nose into a textbook* ...

i. Gerty and Carl Cori  
ii. Hans Krebs and Fritz Lipmann  
iii. Otto Warburg  
iv. Emil Fischer  
v. **Peter Mitchell**
BIS103-002 (Spring 2009)
Midterm #2 (May 19)  Name__________________________________________

Use blank sheet as scratch paper, if needed.