MCB 102 Midterm II

October 30, 2003

NAME ____________________________

Student ID _________________________

Signature ___________________________

TA _________________________________

Relax. There is ample time to finish everything.

Only exams answered by using non-erasable ink qualify for possible re-grading.

I. __________ (15)

II. __________ (10)

III. __________ (10)

IV. __________ (14)

V. ___________ (8)

VI. ___________ (9)

VII. __________ (12)

VIII. ___________ (8)

IX. __________ (9)

X. __________ (5)

Total _________ (100)

I. Fill in the blanks with the most appropriate words or phrases (15 pts).(1 point for each item)

1. The "Q cycle" hypothesis for the operation of Complex III is supported by the observation that sudden oxidation of cytochrome c results in the oxidation of __cytochrome c1___ and in the reduction of _cytochromes bH and bL (or b562 and b566, or simply bs).

2. Among the enzymes involved in the generation of glucose 6-phosphate from glycogen, there is one enzyme that becomes phosphorylated and dephosphorylated during each catalytic cycle. This enzyme is ___phosphoglucomutase_____________.

3. In the glycolytic pathway, substrate-level phosphorylation of a nucleoside diphosphate occurs via two enzymes, _phosphoglycerate kinase____ and __pyruvate kinase______________. In the citric acid cycle, there is only one enzyme, ___succinyl-CoA synthetase___________ that catalyzes substrate-level phosphorylation.

4. One complete turn of the γ subunit in the F1-ATPase results in the synthesis of __3__ ATP molecules. Proton influx is thought to rotate the ring-like arrangement of the _c_ subunits of F0 portion of the ATPase.

5. _Ubiquinone (Coenzyme Q, or CoQ)_ is a mobile hydrogen carrier that can normally diffuse within the lipid interior of the mitochondrial inner membrane.

6. Pyridoxal phosphate is a prosthetic group of the enzymes _(glycogen) phosphorylase___ and __transaminase___________.

7. In glycogen synthesis, glycogen synthase catalyzes the addition of new glucose units at the ___non-reducing___ ends of glycogen, from the donor molecule __UDPG (or -glucose).

8. Organisms that generate ATP by the oxidation of inorganic compounds are called __lithotrophs___________.

9. In gluconeogenesis, the step catalyzed by phosphofructokinase in glycolysis is bypassed by using the enzyme _FBPase (or fructose bisphosphatase)_.

II. Give the structural formulae for (all of the) following compounds. Be sure to draw **every atom** (including hydrogen) of the molecule (or the moiety) requested (10 pts).

1. dihydroxyacetone phosphate

2. citrulline

3. isocitrate

4. oxaloacetate

5. malonyl-CoA (the free form of CoA should be written as “CoASH”)

III. In each of the following sets of statements, there is one statement that is incorrect. Indicate this **incorrect** statement in each set by placing a check mark or an **X** on one of the letters **a** through **e**. No credit will be given if more than one statement is checked in each set. (10 points) (2 points each)

1. For anaerobic glycolysis to continue in a cell-free muscle extract, the following conditions must be fulfilled.
 (a) There should be an abundant supply of glucose.
 (b) NADH must be reoxidized to NAD^+.
 (c) ATP synthesized in the process must be hydrolyzed to ADP.
 X(d) NADPH must be reoxidized to $NADP^+$.
 (e) If lactic acid is the final product, there must be enough buffer to maintain the pH close to neutrality.

2. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, the following statements are correct.
 (a) The enzyme catalyzes the hydride transfer from the substrate to NAD^+.
 X(b) The enzyme reaction involves a nucleophilic attack on the phosphorus atom of inorganic phosphate.
 (c) The enzyme makes possible the endergonic formation of acid phosphate moiety by coupling it with an exergonic oxidation of aldehyde moiety.
 (d) The enzyme reaction involves the formation of a thiohemiacetal.
 (e) When the enzyme is a part of a series of enzymes catalyzing lactic acid fermentation, arsenate will not inhibit the production of 3-phosphoglyceric acid.

3. When epinephrine reaches muscle cells,
 (a) adenyl cyclase becomes stimulated.
 X(b) protein kinase A becomes phosphorylated.
 (c) phosphorylase kinase becomes phosphorylated.
 (d) glycogen synthase becomes phosphorylated.
 (e) phosphorylase b is converted into phosphorylase a.

4. In the resting skeletal muscle, the following compound is present at a higher concentration in comparison with the same muscle in the midst of a strenuous exercise.
(a) ATP
(b) Acetyl-CoA
(c) Citrate
X(d) AMP
(e) Creatine phosphate

5. DCCD inhibits oxidative phosphorylation because
X(a) it dissipates proton gradient.
(b) it inhibits the c subunit of ATPase by modifying the crucial acidic residue.
(c) it eventually decreases the rate of electron transport.
(d) it ends up increasing the proton-motive force, which eventually shuts off electron transport.
(e) it inhibits the production of ATP.

IV. Circle the correct choice(s). There may be more than one correct choice per question, and if so, all of them must be circled for full credit. Points will be subtracted if incorrect choices are circled. (14 points)(1 point for each correct answer; subtract 0.5 point for each wrong answer circled)

1. Each of the following compound *alone* (in the presence of the usual coenzymes, ATP, Pi, etc.) can produce the net generation of glucose 6-phosphate (and eventually glycogen) in mammalian liver.
○ (a) glycerol
○ (b) succinate
(c) formate ($HCOO^-$)
(d) acetate
○ (e) citrate

2. The following substance regularly acts always as an electron carrier (i. e. never as a hydrogen carrier), in the electron transport pathway in animal mitochondria:
(a) oxaloacetate
○ (b) iron-sulfur protein
○ (c) cytochrome b_{550}
(d) coenzyme Q
(e) FMN

3. You can conclude that ATP is being synthesized continuously from ADP and Pi by the F_1F_0 ATPase in the absence of energy input because:
(a) the isotopically labeled hydrogen of water exchanges with that of Pi when ADP and Pi are added to F_1 ATPase.
(b) the isotopically labeled oxygen of water exchanges with that of ATP when ATP is added to F_1 ATPase.
○ (c) the isotopically labeled oxygen of water exchanges with that of Pi when ADP and Pi are added to F_1 ATPase.
(d) the isotopically labeled oxygen of water exchanges with that of Pi when ADP is added to Pi in the absence of any enzyme.
(e) the isotopically labeled hydrogen of water exchanges with that of ADP when ADP and Pi are added to F_1 ATPase.

4. You added a newly synthesized organic compound to a preparation of mitochondria oxidizing NADH in the presence of ADP and Pi. When you find that this compound decreases the P/O ratio drastically without inhibiting the consumption of O_2, you can conclude that:
(a) this compound is an inhibitor of F_1 ATPase.
(b) this compound is an inhibitor of F_0 ATPase.
(c) this compound is an inhibitor of complex II.
(d) this compound is an inhibitor of complex I.
○ (e) this compound facilitates the passive equilibration of protons across the membrane.

5. NADH generated in the cytosol, for example through glycolysis, can generate ATP in mitochondria because:
(a) NADH can diffuse through the porin channels of mitochondrial outer membrane, and donates reducing equivalents to complex I from the intermembrane space.
(b) NADH enters into mitochondrial matrix through the NADH/NAD^+ exchanger protein located in the mitochondrial inner membrane.
(c) NADH enters into mitochondrial matrix through the NADH/ATP exchanger protein located in the mitochondrial inner membrane.
○ (d) NADH reduces some low-molecular-weight compounds, which enter the matrix and regenerate NADH there.
(e) NADH oxidizes glycerol phosphate and other compounds, which in turn oxidizes components of the mitochondrial electron transport chain.

6. NADPH is essential for the survival of all aerobic organisms
(a) because NADPH is a much stronger reductant than NADH.
(b) because the oxidation of NADPH produces more ATP molecules than that of NADH.
○ (c)Because NADPH is not easily reoxidized by using O_2 as the ultimate oxidant.
(d) Because $NADP^+$ is reduced by a wider variety of enzymes than NAD^+ is.
(e) Because isocitrate dehydrogenases of all aerobic organisms can use only $NADP^+$.

7. During the operation of the urea cycle,
(a) ammonia is added directly to citrulline.
(b) release of urea takes place in the mitochondrial matrix.
○ (c) a four-carbon compound is released in the cytosol.
○ (d) the amino group of aspartate is converted into one of the amide groups in urea.
(e) alanine always supplies one of the amide groups in urea.

8. In the oxidation of fatty acids,
(a) fatty acids must first be converted into acyl-ACP.
(b) oxidation of any fatty acids generates only acetyl-CoA as the sole product.
(c) unsaturated fatty acids cannot be degraded completely.
○ (d) both $FADH_2$ and NADH will be generated.
(e) acyl-CoA enters into mitochondrial matrix through a specific acyl-CoA carrier.

9. Elevated serum levels of glucagon results in
(a) the phosphorylation of PFK-1 in liver.
(b) accelerated glycolysis in skeletal muscles.
(c) the dephosphorylation of PFK-2/FBPase-2 in liver.
○ (d) the phosphorylation of acetyl-CoA carboxylase in liver.
(e) the phosphorylation of PFK-2/FBPase-2 in skeletal muscle.

10. High serum levels of glucose results in
○ (a) its increased conversion to glucose 6-phosphate in liver.
(b) increased production of ketone bodies in all cases.
(c) increased fatty acid oxidation in liver.
(d) increased glycolysis in muscle through the decreased secretion of glucagon.
(e) increased glycolysis in liver through the increased secretion of glucagon.

V. You heard of a group of bacteria which survive by using, as a sole source of energy, the oxidation of ethanol into acetaldehyde in the absence of air, with pyruvate as the electron acceptor. Reduction potentials of potentially relevant half-reactions are given below. (8 pts)

Half-reaction	Reduction potential (E_0')
$\frac{1}{2}O_2 + 2H^+ 2e^-$ ---------> H_2O	0.82V
$Fumarate^{2-} + 2H^+ + 2e^-$ ---------> $Succinate^{2-}$	0.03V
$Pyruvate^- + 2H^+ + 2e^-$ ---------> $Lactate^-$	-0.16V
$Acetaldehyde + 2H^+ + 2e^-$ ---------> Ethanol	-0.23V
$2H^+ + 2e^-$ ---------> H_2	-0.41V

Calculate the standard free energy change at pH 7 for this presumed energy-yielding reaction. Use 100 kJ/V/mol as the value of Faraday constant. There is no need to show calculations. No partial credit will be given even if calculations are shown. (4 pts)

- 14 kJ/mol

Explain whether this is enough to generate ATP. How can this organism survive by using these substrates and this reaction at pH 7 for ATP generation? (4 pts)

This is insufficient for ATP generation, as it requires at least 30.5 kJ/mol under standard conditions. However, the actual ΔG' could have a much larger negative value, if [ethanol] is far higher than [acetaldehyde] and if [pyruvate] is much higher than [lactate].

VI. 2,4-Dinitrophenol (pKa about 4.1) is a classical uncoupler of oxidative phosphorylation (9 pts). (3 pts for each item).

(a) Explain in one sentence why unsubstituted phenol (pKa about 10) does not work as an uncoupler.

Practically all of phenol would be protonated in the matrix, and will not release proton in this location.

(b) Explain in one sentence why 2,4,6-trinitrophenol (pKa about 1.0) does not work either as an uncoupler.

Practically none of this compound will exist in the protonated form in the intermembrane space, and thus no proton will be brought into the matrix by this compound.

(c) 2,4-Dinitrophenol was once used as a weight-reducing agent. Explain in one sentence why this compound could be effective for such a purpose.

Uncoupling will result in the excessive burning of fat and carbohydrate without the production of much ATP, and thus this will result in weight reduction.

VII. Citrate is an important ingredient in many canned drinks. One method of production of citrate involves the nearly stoichiometric conversion of glucose to citrate by a mold *Aspergillus*. This conversion proceeds poorly when the level of iron in the medium exceeds 0.5 mg/L. (12 pts).
(a) This conversion is achieved by the glycolytic pathway plus three additional enzymatic steps. Write balanced equations for these three additional enzymatic steps, by following the example for glycolysis below. (Disregard the consumption and generation of protons) Give the names of the enzymes catalyzing steps 2 through 4.. (1 pt each for the name; 2 pts each for the reaction).

1. Glycolysis: Glucose + 2ADP + 2 Pi + 2 NAD^+ → 2 Pyruvate + 2 ATP + 2 NADH

2.Pyruvate Dehydrogenase: Pyruvate + CoA + NAD^+ → Acetyl-CoA + NADH +CO_2_________

3.Pyruvate Carboxylase: Pyruvate + CO_2 + ATP → Oxaloacetate + ADP + Pi_______________

4.Citrate Synthase: Acetyl-CoA + Oxaloacetate → Citrate + CoA________________________

(b) Explain in one sentence why the excessive iron levels decrease the yield of citrate.

Iron is a component of aconitase, which will convert citrate into isocitrate, which in turn will be degraded via the citric acid cycle into CO_2. (2 pts)

(c) This conversion reaction does not proceed under anaerobic conditions. Explain (in one sentence) why. (1 pt)

Both glycolysis and pyruvate dehydrogenase produce NADH, which needs to be reoxidized.

VIII. Answer the following questions on lipid metabolism. (8 points)
(a) If you add unlabeled acetyl-CoA and an excess of malonyl-CoA that is radioactively labeled in all of its carbon atoms, how many labeled carbon atoms will you find in the myristate (C14:0) molecule synthesized? Where in the molecule will you find these labeled carbon atoms?

12 (1pt)
In all of the carbon atoms except the two that are farthest away from the carboxyl end. (1 pt)

(b) Fatty acids are converted into monounsaturated fatty acids in animal tissues either as a part of the degradation pathway or for the biosynthesis of unsaturated fatty acids as a component of membrane lipids. Compare and contrast these two enzymatic processes in a form of a table. Limit each entry to a few words; unorganized essays will receive no credit. (1 pt for each; for a total of 6 pts)

	Degradative pathway	Biosynthesis
Name of Enzyme:	____Acyl-CoA dehydrogenase__	____(Fatty acid) desaturase__
Electron Acceptor:	_________FAD______________	__________O_2_______________

Product:

___trans-β-enoyl-CoA________ ______cis-9-enoyl-(CoA)________

IX. Answer the following questions. (9 points)
(a) Name three enzymes that catalyze reactions similar in mechanism to the enolase reaction. (4.5 pts)

Aconitase

Fumarase

Enoyl-CoA hydratase

(β-hydroxylacyl-CoA dehydratase)

(b) Name three enzymes that catalyze reactions that are driven (at least in part) by the utilization of the exergonic hydrolysis of PPi. (4.5 pts)

UDPG pyrophosphorylase

Acyl-CoA synthetase

Adenyl cyclase

(Arginosuccinate synthetase)

X. Prof. Nikaido said in the class that F-2,6BP-mediated regulation of PFK was important only in the liver. However, a student with a scientific and skeptical mind examines the PFK from brain, and finds that the brain enzyme actually is activated by F-2,6BP with the half-maximal activation occurring at 0.1 mM F-2,6BP. You, being a kind and helpful student, want to defend the (perhaps oversimplified) conclusion Prof. Nikaido presented. What experiments would you do and what results do you expect? Please answer in three sentences or less (5 pts).

I would determine the level of F-2,6BP in brain. If it is in the micromolar range as expected, then this "regulation" is a laboratory artifact and has no physiological relevance. (3 pts)
If the level is much higher, then I would determine if it is affected by hormonal regulation. (2 pts)