CHEM-643 Biochemistry Name _____
Final Examination
3:30 – 6:30 PM, Monday, 15 December 2008
Dr. H. White – Instructor

There are 12 pages to this examination including this page and the final data sheet. **Write your name** on each new page. **Read every question** so that you understand what is being asked. If you feel any question is unclear or ambiguous, **clearly explain your answer or interpretation**. Please call my attention to any suspected errors you encounter.

The first part of this examination is with closed notes. You will get the second part when you hand in the first part. You can use your notes thereafter. You may then refer to your assignments and your lecture notes, but not textbooks. You may also refer to the metabolic pathway sheets printed from the course website.

This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is intended to be challenging even to the best students in the class. Some of the questions will deal with material you have not seen before and is not in your text; however, the questions can be answered by applying basic principles discussed in the course.

Do not expose your answers to the scrutiny of your neighbors. Please fold under each page before you go on to the next. You may use the backs of pages, if you need more space.

The maximum possible score is 123. Graded Exams can be picked up starting Thursday and will be held until Spring Semester.

Exam Statistics:

Class Range _____	Part 1	34 points
	Part 2	89 points
Class Mean _____	Total	123 points

Your Score _____

Your Rank in Class _____ out of 39

Course Grade _____

Part I - Short Answer Questions (1 point each)

_____ 1. What does the abbreviation PKU stand for?

_____ 2. In a C_3 plant, CO_2 gets incorporated into this compound first.

_____ 3. $\delta^{13}C$ value for carbon in the Peedee belemnite.

_____ 4. Amino acid with an indole ring system.

_____ 5. This amino acid lacks a chiral carbon.

_____ 6. What is the natural source of the carbon used in making nylon?

_____ 7. Isotope produced when ^{14}C decays.

_____ 8. Antifolate drug used in cancer chemotherapy.

_____ 9. Fatty acid precursor for prostaglandins.

_____ 10. Product of transamination of oxaloacetate.

_____ 11. Birds excrete waste nitrogen in this compound.

_____ 12. A typical human can survive approximately __ days without food.

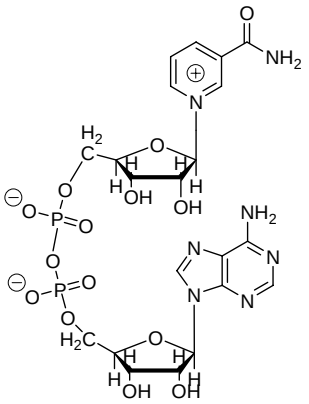
_____ 13. Major carbon energy source for brain metabolism.

_____ 14. Instead of creatine in their muscles, insects use this compound.

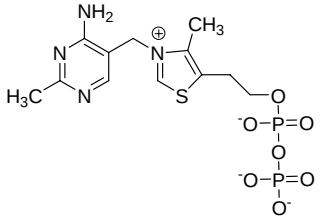
Please draw the structures of the compounds indicated. (1 point each)

15. Alanine	16. Methionine	17. Tyrosine
18. Valine	19. Glutamine	20. Threonine
21. Glucose	22. Ribose	23. Palmitic Acid
24. Oxaloacetate	25. Phosphoenol pyruvate	26. UMP

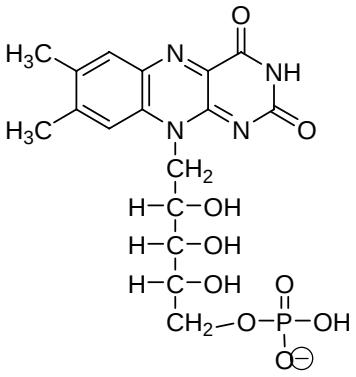
27-34 Identify the structures shown. (1 point each)



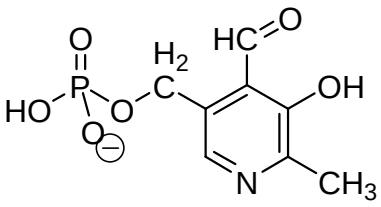
27._____



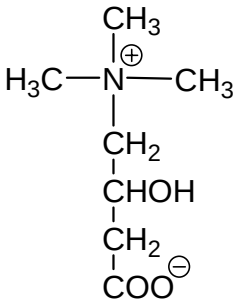
28. _____



1. _____



30. _____



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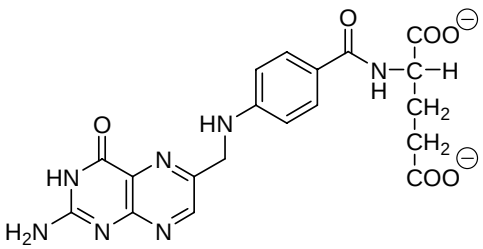

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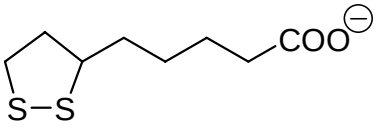
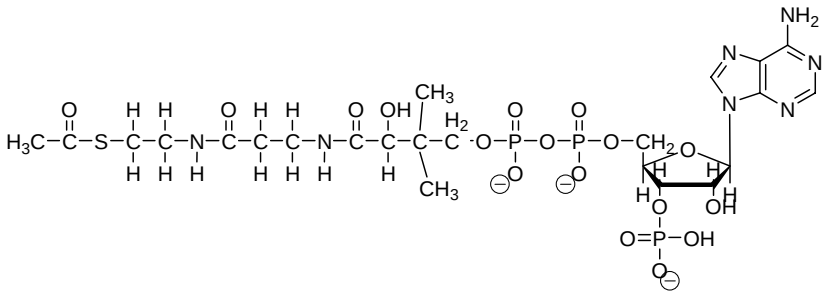


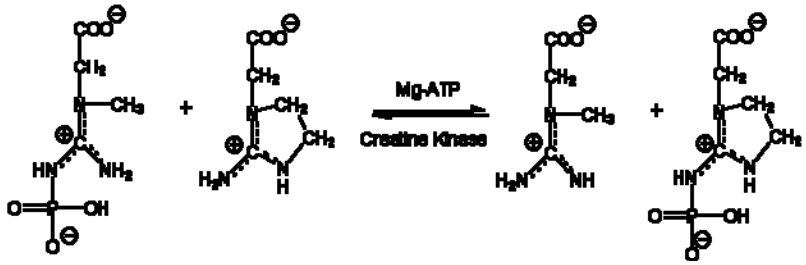
33. _____



34. _____

Part II Problems-Open Book:

1. (18 Points) Cyclocreatine (1-carboxy-2-iminoinidazolidine) is a substrate for creatine kinase in vertebrate muscle [Annesley & Walker, *Biochem. Biophys. Res. Comm. 74*, 185-190 (1977)]. Cyclocreatine-fed chicks accumulated up to 60 mM phosphocyclocreatine in the cytoplasmic water of breast muscle [Griffiths and Walker J. Biol. Chem. 251, 2049 (1976)]. The BBRC article was concerned with determining the equilibrium constant for the following reaction in the presence of small amounts of MgATP.



PCr cCr Cr PcCr

Data for three experiments are given below.

Expt. No.	Conditions	Substrate			
		PCr mM	cCr mM	Cr mM	PcCr mM
1	Initial	40	40	0	0
	Final*	6	7	31	32
2	Initial	0	0	40	40
	Final*	7	6	30	36
3	Initial	35	35	35	35
	Final*	11	13	59	60

* 6-8 hours at 37°C in the presence of trace amounts of MgATP and creatine kinase.

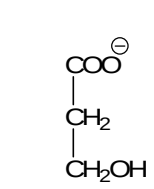
a. (6 points) Estimate the K_{eq} for the reaction using all of the data. [Wiseman & Kushmerick *J. Biol. Chem. 270*, 12428-12438 (1995)].

b. (3 points) Draw out the reaction catalyzed by creatine kinase?

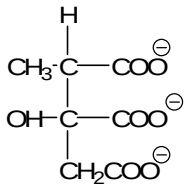
c. (3 points) Why are trace amounts of MgATP necessary for the reaction in part "a" to come to equilibrium?

d. (6 points) If the ratio of [PCr]/[Cr] in resting muscle is 2, what would you expect the ratio of [PcCr]/[cCr] to be in the muscles of birds fed cyclocreatine.

2. (15 points) The medical literature reports a sick infant who excretes excessive amounts of 3-hydroxypropionate and 2-methyl citrate in his urine. The structures are given below. These abnormal metabolites arise from a homozygous recessive mutation in a gene encoding propionylCoA carboxylase.



3-Hydroxypropionate



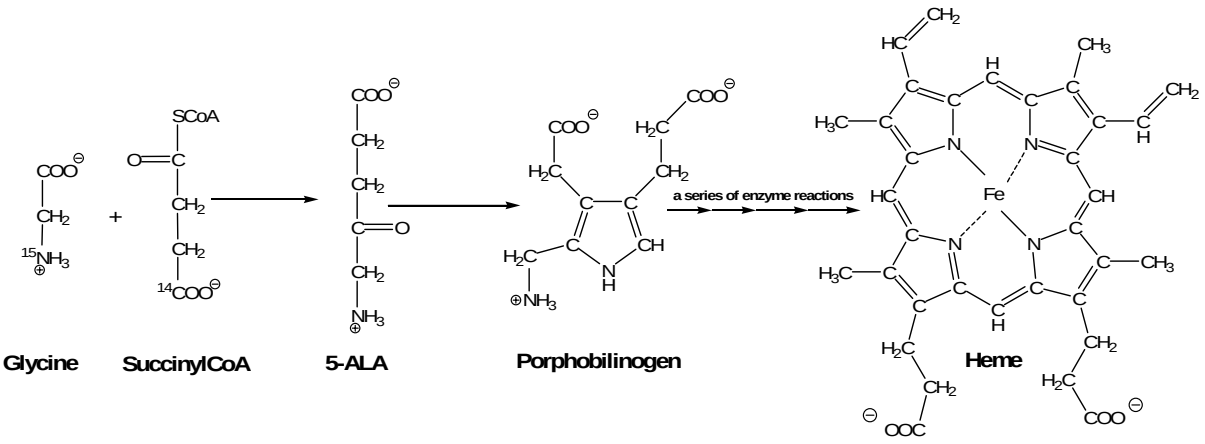
2-Methylcitrate

a. (4 points) What is the reaction catalyzed by propionylCoA carboxylase? Show substrates and any cofactors/coenzymes involved in the reaction.

b. (7 points) Propose a pathway for how the accumulation of the normal substrate, propionylCoA can be metabolized by well known enzymes to produce the abnormal compound 3-hydroxypropionate.

c. (4 points) Propose how the accumulation of the normal substrate, propionylCoA can be metabolized to produce the abnormal compound 2-methylcitrate.

3. (15 points) At this very moment, maturing red blood cells in your body's bone marrow are making about 10^{14} molecules of heme per sec! They are using succinylCoA from the TCA cycle and condensing it with glycine to form 5-aminolevulinic acid (ALA) shown below. It in turn condenses with another molecule of ALA to form porphobilinogen (PBG). PBG is converted to heme in a series of reactions. All of the atoms of heme are derived from glycine and succinylCoA, but not all of the atoms from glycine and succinylCoA end up in heme.



   a. (5 points) On the structures of ALA, porphobilinogen and heme above, circle all the carbon atoms derived from the ^{14}C-labeled carboxyl group of succinylCoA.

   b. (5 points) If this were a double-label experiment with both ^{15}N-glycine and [^{14}C-carboxy] succinylCoA, what would be the ^{15}N/^{14}C ratio of the resulting heme? [You need to show your work or justify your answer for credit.]

   c. (5 points) Current strategies for studying biosynthetic pathways generally use ^{13}C as a label, instead of ^{14}C, and determine the location of the label in the product using NMR. When ^{13}C atoms are bonded to each other, their spins couple and they "talk" to each other in ways that can be detected by NMR. Assuming that each of the carbon atoms in heme is distinguishable by NMR, which carbon in glycine or succinylCoA would you want to study to refine the heme biosynthetic pathway? Justify your choice.

4. (18 points) The following is a verbal description of the fermentation of glucose to butyric acid, carbon dioxide, and hydrogen gas by the strict anaerobic bacteria

Clostridium butylicum. Glucose is converted to pyruvate via normal reactions of the glycolytic pathway. The oxidative decarboxylation of pyruvate to form acetyl coenzyme A differs from the pyruvate dehydrogenase reaction in that ferredoxin instead of NAD accepts the electrons. Reduced ferredoxin in turn reacts with solvent protons to produce H_2 gas. Two moles of acetyl CoA condense to form acetoacetyl CoA (β-ketobutyryl CoA) which in turn undergoes β-reduction to butyryl CoA using NADH for both reduction steps. Finally, the energy liberated in the thioester cleavage to butyric acid is coupled to ATP formation.

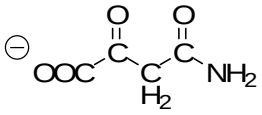
a. (10 points) Provide the structures of the chemical intermediates between pyruvate and butyrate showing which coenzymes are involved when they are needed.

b. Considering the entire pathway from glucose to butyrate,

   i. (4 points) How many net ATPs are formed? Compare this with yeast fermentation of glucose to ethanol.

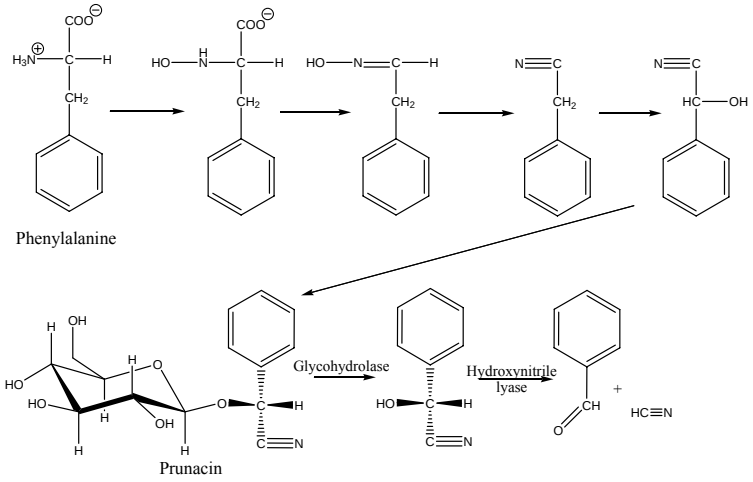
   ii. (4 points) To be a fermentation, there must be a net balance of NADH production and consumption. Show that that is the case here.

5. (5 points) α-Ketosuccinamide (shown below) has been detected as a metabolite in rat liver [Anal. Biochem. 167, 312-320 (1987)]. Show with chemical structures and reactions how this compound could be formed simply from common intermediates using reasonable side reactions of familiar enzymes.



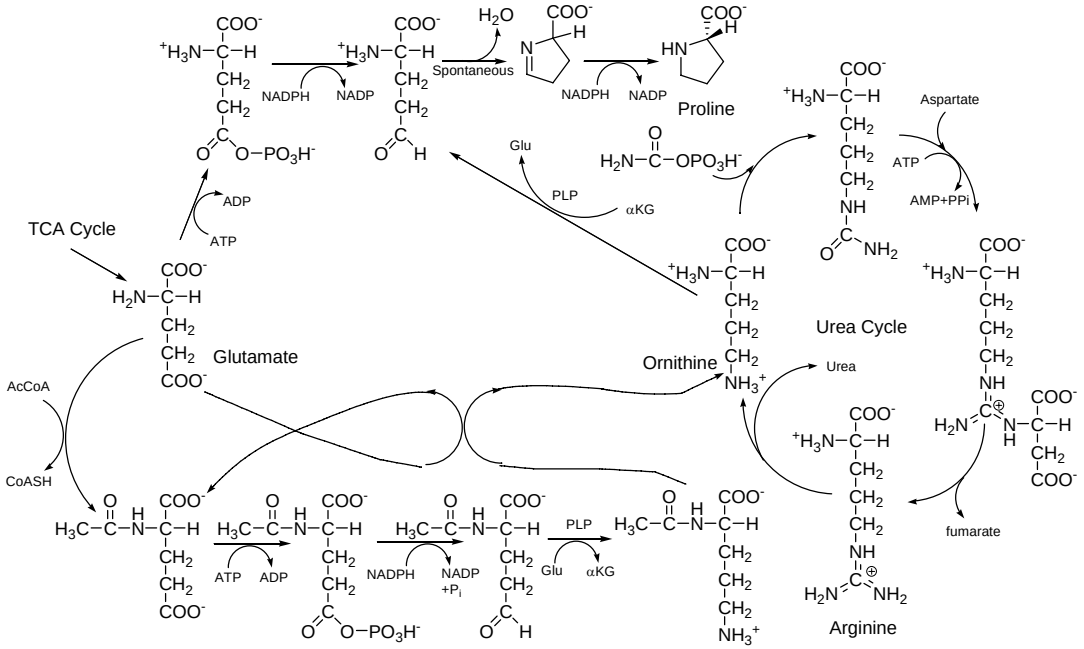
6. (6 points) A short news item in Science [**292**, 1831 (2001)] begins as follows: *After three weeks of feverish detective work, researchers late last month identified the likely cause of more than 500 stillbirths or deaths of newborn thoroughbred foals this spring in central Kentucky. The culprit: cyanide from wild black cherry leaves passed on to mares via caterpillars.*

Cherry leaves synthesize the cyanogenic glycoside prunacin from phenylalanine (as shown below) and store it. When the leaves are damaged by a caterpillar or a hungry horse, there is a glycohydrolase that releases mandelonitrile, a cyanohydrin, which in turn reacts with hydroxynitrile lyase to generate toxic cyanide. Different cyanogenic glycosides are produced by other plants. The structures vary depending on the number and identity of sugar units and the starting amino acid.



Linamarin is a cyanogenic glycoside produced from valine by cassava seedlings via a pathway basically the same as for prunacin. [*Arch. Biochem. Biophys*. **292**, 141- (1992)] In the space below, draw the structure of the cyanohydrin derived from linamarin and the products of its breakdown catalyzed by the lyase.

7. (12 point essay) The Lehninger textbook notes that in mammals the biosynthetic routes to proline and ornithine/arginine from glutamate may not be separate. Rather, the synthesis of proline would branch off of the pathway to arginine from the intermediate ornithine as shown below with the long diagonal line.



The data on the following page were collected well before the pathways above were worked out. However, those data should be consistent with one or the other of the hypothesized pathways to proline. Examine those data and use them selectively in constructing support for one of the two pathways and against the other.

Table 1. Specific radioactivity of amino acids biosynthesized from ^{14}C sucrose in three days by a mouse (Steele, 1952).

Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC	Amino Acid	nCi/mgC
Glutamate	19.0±1.9	Threonine	0.09±0.02	Valine	0.02±0.01	Lysine	0.0±0.02
Aspartate	15.8±0.9	Serine	8.4±0.1	Phenylalanine	0.02±0.07	Histidine	0.07±0.08
Alanine	26.5±3.3	Glycine	5.1±0.2	Tyrosine	0.0±0.07	Cystine	3.3±0.3
Proline	3.1±0.1	Isoleucine	0.06±0.05	Arginine	3.0±0.2	Methionine	1.03±0.06

Table 2. Growth of mouse L cells in media lacking the indicated amino acid (Eagle, 1955).

Amino Acid	Cell Growth	Amino Acid	Cell Growth	Amino Acid	Cell Growth	Amino Acid	Cell Growth
Glutamate	3.6 - 4.5	Threonine	0.2	Valine	0.06 - 0.2	Lysine	0.2 - 0.5
Aspartate	3.6 - 6.1	Serine	2.5 - 2.8	Phenylalanine	0.3 - 0.4	Histidine	0.3 - 0.4
Alanine	2.2 - 2.6	Glycine	3.6 - 3.7	Tyrosine	0.06 - 0.2	Cystine	0.1 - 0.3
Proline	2.4 - 6.8	Isoleucine	0.1 - 0.4	Arginine	0.4 - 0.9	Methionine	0.3 - 0.4
		Leucine	0.4 - 0.6	Tryptophan	0.3 - 0.4		

Table 3. Distribution of ^{15}N among the amino acids of liver proteins 8 hours after intravenous injection of various amino acid sources of ^{15}N. Values are normalized to the ^{15}N content of the source amino acid (100) incorporated into protein (Aqvist, 1951).

	Amino acids incorporated into rat liver proteins														
^{15}N-Amino Acid	Glu	Asp	Ala	Pro	Thr	Ser	Gly	Leu	Ile	Val	Phe	Tyr	Arg	Lys	His
Glutamate	100	50	74	12	3	46	19	31	nd	20	14	20	34	4	2
Aspartate	186	100	125	29	2	40	38	49	111	nd	26	38	60	15	25
Alanine	77	44	100	16	<1	23	21	38	40	29	9	10	33	4	3
Proline	23	14	18	100	1	5	3	4	5	5	1	2	11	2	<1
Threonine[1]	6	5	5	2	100	20	14	1	2	4	2	5	5	1	<1
Serine	9	9	12	2	14	100	50	3	2	2	2	6	9	1	1
Glycine	19	12	16	1	0	88	100	nd	nd	nd	3	nd	16	<1	2
Leucine	30	15	25	nd	<1	7	7	100	25	12	3	7	11	0	<1
Isoleucine	28	14	23	11	<1	9	8	34	100	15	8	12	10	4	3
Valine	34	19	29	7	0	12	10	46	41	100	5	6	14	1	2
Phenylalanine	24	12	18	2	<1	3	2	3	5	3	100	74	10	7	2
Tyrosine[2]	23	13	16	3	<1	4	4	4	5	4	44	100	9	1	1
Arginine[3]	34	23	20	18	2	6	1	nd	nd	nd	11	10	100	13	5
Lysine[3]	23	19	12	5	3	8	3	nd	nd	nd	6	nd	9	100	4
Histidine[3]	28	25	30	6	2	8	10	nd	nd	nd	9	nd	24	6	100

1. Slightly contaminated with ^{15}N serine. Data from one rat only.
2. Administered by a stomach tube. Animals killed after 12 hours.

3. ^{15}N excess significantly less than for other administered amino acids.