

March 11, 2002

Name _____

ID number _____

CH462/562 Exam 2

There are **4** short answer questions worth **6** points each.

There are **5** problems worth **18** points each:

If you are a CH462 student, you must answer **3** problems for full credit.

If you are a CH562 student, you must answer **4** problems for full credit.

(If you attempt all, I will drop the problem on which you score the lowest.)

The maximum amount of points is thus **78** (CH462) or **96** (CH562).

You are advised to look over the problems first before starting to work them.

Time should not be a factor, if you are well prepared.

Multiple choice		/24
Problems:	1	/18
	2	/18
	3	/18
	5	/18
	5	/18
Total		

No notes or books of any sort may be used during the exam.

I have neither given nor received aid on this exam.

(signature)

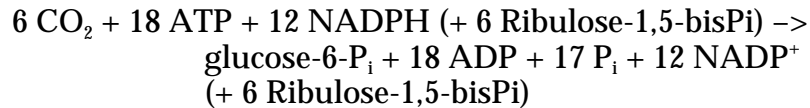
3) Which enzyme catalyzes the incorporation of nitrogen into organic molecules in all organisms? Show the reaction that it catalyzes.

4) Explain briefly how serine/glycine metabolism intersects with DNA synthesis.

Problems

1) Carbohydrate synthesis

Show the pathway producing carbohydrate by the reductive pentose-phosphate pathway (Calvin cycle). It can be summarized in this way:



(Note: not balanced for H⁺ or H₂O.)

Show each reaction and how many molecules go through it in order to make 1 glucose-6-P_i from 6 CO₂. Only structures are necessary; no names required. (If you give the names of intermediates instead of structures, you will receive only half credit.) Indicate any cofactors by the common abbreviations (e.g. ATP, etc.) Note: you may not be able to easily put it all on one page as a cycle. It may be easier to break it into individual reactions. (If necessary, use the back.)

2) **Lipids**

a) You have purified the enzyme fatty acid synthase from cow liver. Using this, you wish to create an *in vitro* system for synthesis of palmitate using this enzyme alone.

You add the following molecules:
ATP, NADPH, acetyl-CoA, malonyl-CoA, NAD⁺

Which are necessary and which are not?

b) Once you get your system working, you wish to characterize intermediates in the synthesis of palmitate. After starting the reaction by adding the necessary components, you periodically take a sample of the reaction, extract with hexane and run it on a GC/MS (gas chromatograph/mass spec). Like most such systems, it has an effective separation range of about 20 - 1000 Da. However, you find that the earliest time points have nothing but the components that you added. After a while, you see palmitic acid appearing. You **never** see any other intermediate molecule. (Actually, if you look hard you see a small amount of stearic acid and a tiny amount of myristic acid (C14) at around the same time as palmitic acid appears.)

Explain this result.

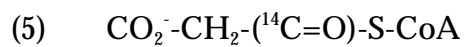
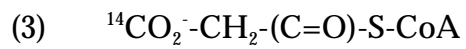
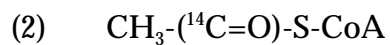
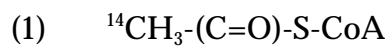
c) However, you find that if you extract with acid rather than hexane, you can see some intermediates. Explain why this should be the case.

You try the following experiment: you add a subset of components to the FA synthase, allow the reaction to proceed as far as it can go, and then dialyze the enzyme exhaustively to remove all molecules smaller than 5 kDa. You extract the enzyme with acid and then apply the extract to the GC/MS. (Note that this is a relatively gentle acid treatment - 1M HCl at room temperature. Not nearly enough to hydrolyze peptide bonds.)

(1) ATP, acetyl-CoA, malonyl-CoA

(2) NADPH, acetyl-CoA, malonyl-CoA

d) Show where you expect the label to appear in palmitic acid after running reaction with the following labeled molecules (the rest of the components are included but are unlabeled):



3) Amino acid synthesis

a) Show the synthesis of Tyr and Phe from chorismate.

b) You add ^{14}C -labeled pyruvate [$^{14}\text{CH}_3\text{-(C=O)-CO}_2^-$] to a culture of *E. coli* bacteria. They can take up pyruvate and feed it into pyruvate dehydrogenase and the TCA cycle. However, you add malonate to inhibit succinate dehydrogenase, which prevents the labeled carbon from being recycled back into oxaloacetate. (They will make oxaloacetate by carboxylation of pyruvate.) You extract the following molecules. Predict if and where they would be labeled. If unsure, show the pathway of synthesis and how the label would be incorporated.

(1) Valine

(2) Proline

(3) Asparagine

(4) Uracil

4) Nucleic acid synthesis

Choose either **AMP** or **GMP** and show the pathway of their *de novo* synthesis using some of the following molecules as starting points, cofactors, or atom donors (note: you will not use all of them).

PRPP (phosphoribosyl pyrophosphate)

Glu, Gln, Asp, Asn, Gly

ATP, GTP

10-formyl-tetrahydrofolate

5,10-methylene-tetrahydrofolate

NAD⁺, NADP⁺

NADH, NADPH

5) Mechanisms

Pick any **ONE** of the following enzymes and show its mechanism in as much detail as you can:

Nitrogenase

Ribonucleotide reductase

Transketolase

Rubisco (carboxylation reaction)

Thymidylate synthase