

There are 10 pages to this examination including this page. **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.

This is an **open-notes examination**. You may 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 116. Graded Exams can be picked up Friday after 3 pm.

Exam Statistics:

Class Range **34.5 – 99.5**

Class Mean **78.3**

Your Score _____

Your Rank in Class _____ out of 35

Course Grade _____

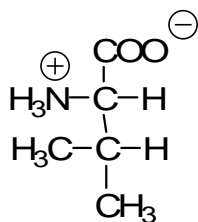
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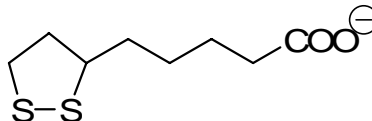
Part I - Short Answer Questions (1 point each)

- _____ 1. Another name for AMP.
- _____ 2. Organ that is the major site for gluconeogenesis.
- _____ 3. Primary source of muscle ATP for a 100 yard dash.
- _____ 4. Primary organ consuming glucose in humans.
- _____ 5. Reaction associated with β -ketoacids.
- _____ 6. Usual target of end-product inhibition.
- _____ 7. TCA intermediate that contains Sulfur and Phosphorus.
- _____ 8. How much ^{14}C will a fossil dinosaur bone contain?
- _____ 9. PLP is to vitamin B₆ as NAD is to?
- _____ 10. Atmospheric gas that plants consume at night.
- _____ 11. Class of macromolecules conserved during prolonged starvation.
- _____ 12. Coenzyme associated with C-C bond cleavage next to carbonyls.
- _____ 13. Name for six-membered ring that is part of purine.
- _____ 14. Name of five-member ring that is part of purine.

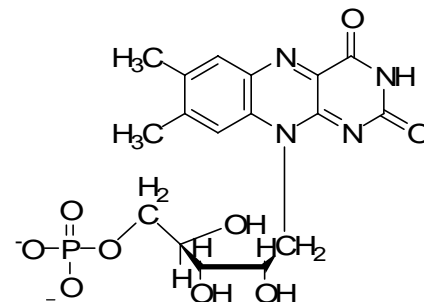
15-20 Identify the structures shown.



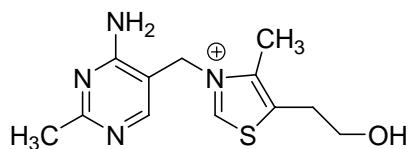
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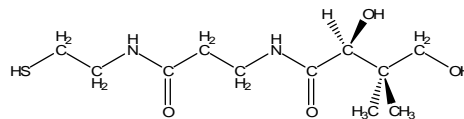
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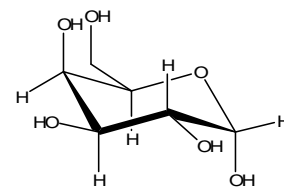
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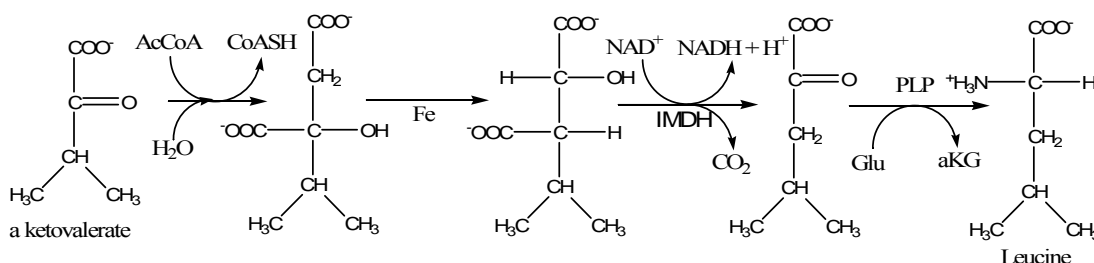
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Part II Problems:

1. (10 Points) The October 20 issue of *Science* this fall included an article in which the pyridine nucleotide specificity of an *E. coli* β -isopropylmalate dehydrogenase (IMDH) was changed from NAD to NADP through genetic engineering. [Miller et al. **314**, 458 (2006)]. Kinetic studies showed the following catalytic properties of the wild type and mutated enzyme.

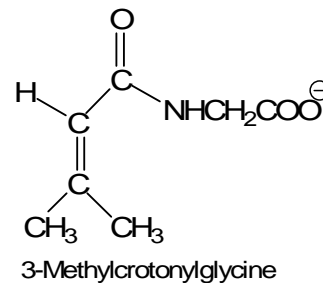
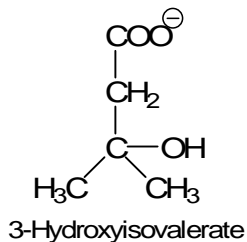
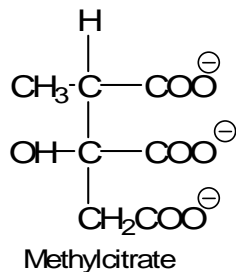
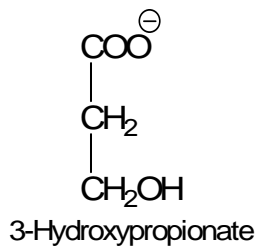
	K_{cat}/K_m for NAD ($M^{-1} s^{-1}$)	K_{cat}/K_m for NADP ($M^{-1} s^{-1}$)
Wild type IMDH	68×10^3	0.49×10^3
Mutant IMDH	0.2×10^3	34×10^3

IMDH catalyzes a reaction in the leucine biosynthetic pathway, as shown below.

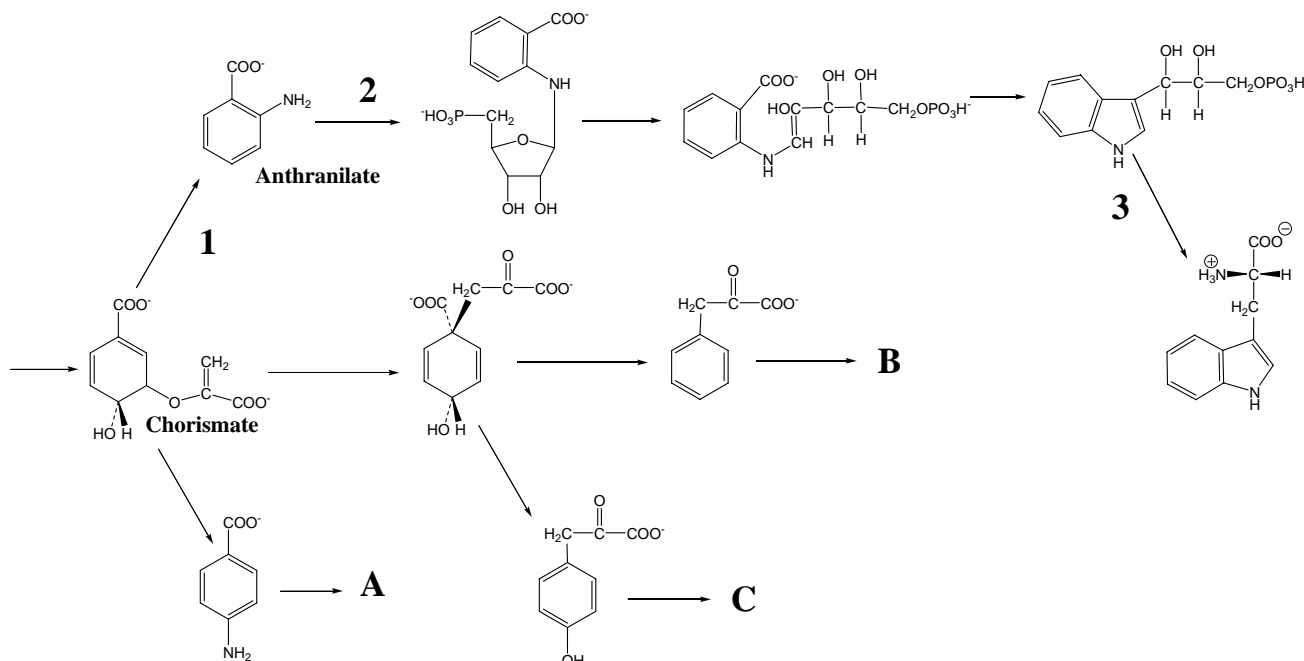


- (1 points) What reaction in the TCA cycle is most like IMDH?
- (1 point) Transamination of α -ketovalerate yields what amino acid?
- (3 points) Having engineered a mutant IMDH that was virtually as good with NADP as the wild type enzyme was with NAD, the authors then replaced the wild type enzyme with the mutant enzyme and studied the growth characteristics of *E. coli*.
 Will the mutant strain grow better, worse, or indistinguishably from the wild type?
- (5 points) Provide a well-reasoned explanation for your prediction above.

2. (10 Points) Multiple carboxylase deficiency, also known as biotinidase deficiency, knocks out biotin-dependent carboxylases. This leads to the accumulation of various carboxylase substrates that, due to their elevated concentration, begin reacting as substrate analogs in other metabolic reactions. As a result some unusual metabolites (shown below) appear in the blood and urine of patients with multiple carboxylase deficiency. Show clearly how **one** of the unusual molecules might form by modification of an accumulating carboxylase substrate via well known reactions.



3. (23 Points +5) Chorismate, a product of the shikimate pathway, is a biosynthetic precursor of several important molecules including tryptophan as is shown below.



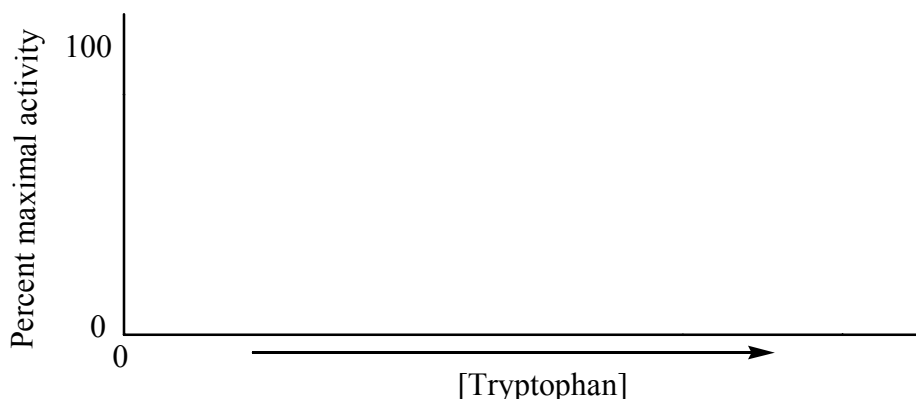
Fill in the answers to parts a-d & f on the figure above.

- (2 pt) What is the name of Cofactor A?
 - (2 pt) What are the names of familiar compounds B and C?
 - (2 pt) The reactions forming B and C use same cofactor and additional substrate. What are they?
 - (2 pt) What reacts with anthranilate in reaction 2 to form the product?
 - (5 pt bonus) Show mechanistically how B or C is formed from the precursor shown.
- f. (3 pt) The enzyme catalyzing reaction 3 was a topic in a problem set earlier in the semester. What familiar three carbon compounds are also substrates and products in this reaction and what cofactor is involved?

- g. (6 pt) The following quotation comes from the abstract of an article in *Journal of Experimental Botany* [57(12):3069-3078] published this year. It also applies to the preceding figure.

*Metabolic manipulation of plants to improve their nutritional quality is an important goal of plant biotechnology. Expression in rice (*Oryza sativa* L.) of a transgene (OASAIID) encoding a feedback-insensitive α subunit of rice anthranilate synthase [Reaction 1] results in the accumulation of tryptophan (Trp) in calli and leaves. It is shown here that the amount of free Trp in the seeds of such plants is increased by about two orders of magnitude compared with that in the seeds of wild-type plants. The total Trp content in the seeds of the transgenic plants was also increased.*

How is the mutant anthranilate synthase different than the wild type enzyme? Draw a graph that would be appropriate for the figure title indicated below. Be sure to identify which line is which enzyme.

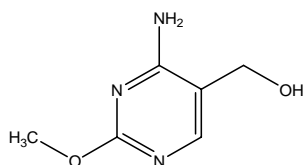


Comparison of the activity of wildtype and mutant antranilate synthase as a function of tryptophan concentration.

- h. (6 pt) Knowing that the transgenic rice plant now produces ~100 fold more free tryptophan in seeds, what questions would you ask about the resulting rice strain? Provide 3 substantive questions?
- 1.
 - 2.
 - 3.

4. (10 pt) There are a number of antifolate drugs including methotrexate, trimethoprim, and sulfanilamide. Select **one** of these. Describe how it works. What medical conditions is it used to treat?

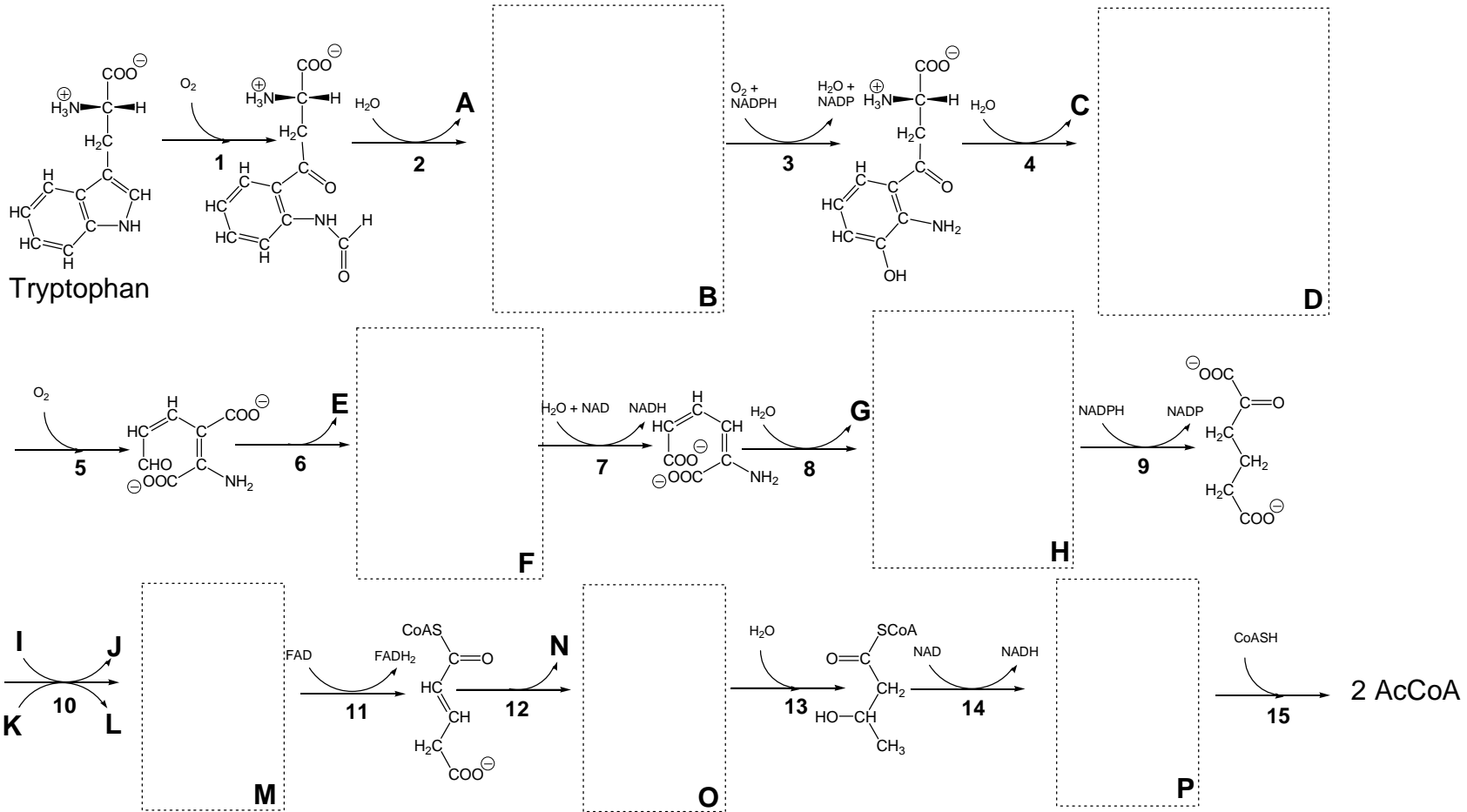
5. (5 points) When Dr. Tadhg Begley visited class, he told several stories in response to student questions. One was about an ill-fated 1861 British exploration to Australia.¹ Another was about the toxic mechanism of bacimethrin, a natural product shown below.²



In a paragraph or two, with discussion of the relevant chemistry where appropriate, summarize the important elements of one **or** the other of these stories.

¹ Earl, J. W., McCleary, B. V. (1994) Mystery of the poisoned expedition. *Nature* **368**: 683-684.

² Reddick, J. J.; Saha, S.; Lee, J.; Melnick, J. S.; Perkins, J.; Begley, T. P.. (2001) The mechanism of action of bacimethrin, a naturally occurring thiamin antimetabolite. *Bioorg. Med. Chem. Lett.*, **11**: 2245-2248.



6. (23 Points) The figure above shows the catabolic pathway for tryptophan in mammals. Every other intermediate is omitted. Please deduce and draw the structures of the missing intermediates in the appropriate boxes (2 points for each correct structure.) In the spaces below, give the common name or abbreviation for the coenzymes and other lettered compounds (1 point each)

- A. _____ G. _____ K. _____
- C. _____ I. _____ L. _____
- E. _____ J. _____ N. _____

7. (10 points) Essay. Select one or the other of the following questions to answer clearly. Circle the letter of the question you intend to answer.
- ATP is Nature's dehydrating agent.
 - Clearly explain the following quotation: During human starvation the "high rates of urea synthesis are simply a consequence of the need for glucose."¹

¹ Watford, M. (2003) The Urea Cycle: Teaching Intermediary Metabolism In a Physiological Setting, *Biochem and Mol. Biol. Educ.* **31**: 289-297