Graduate Biochemistry **Name:**
Exam 3

(Desai questions)

1. The first step of the urea cycle is the formation of carbamoyl phosphate. Answer following questions relevant to the above reaction: (0.5 points each)

a) Name the cellular compartment where the above reaction occurs?
Mitochondria

b) What are the two building blocks of carbamoyl phosphate?
NH^3 and HCO3-

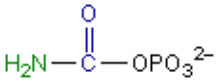
c) Which enzyme catalyzes formation of carbamoyl phosphate?
Carbamoyl phosphate synthatase I

d) Name the allosteric activator of the enzyme that catalyzes formation of carbamoyl phosphate?

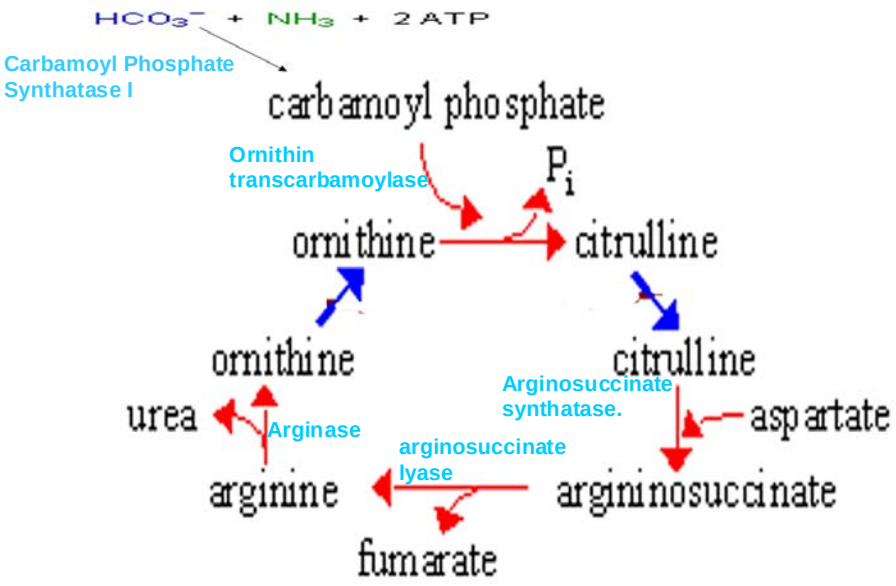
***N*-acetylglutamate**

e) This reaction is reversible-----True or False?
False

f) Draw a structure of carbamoyl phosphate.



2. Diagram the five steps of the urea cycle, giving the names of substrates, products, and enzyme required for each step. (5 points)



3. Briefly explain the role of the ubiquitin/26S proteasome in degrading cellular proteins to amino acids? (2 points)

Proteins selected for degradation by the ubiquitin-proteasome mechanism are first covalently attached to ubiquitin, a small, globular protein. Ubiquitination of the target substrate occurs through linkage of the α-carboxyl glycine of ubiquitin to a lysine ε-amino group on the protein substrate by a three-step, enzyme-catalyzed process. The consecutive addition of ubiquitin moieties generates a polyubiquitin chain. Proteins tagged with ubiquitin are then recognized by a large, barrel-shaped, macromolecular, proteolytic complex called a proteasome, which functions like a garbage disposal. The proteasome cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool.

4. Briefly answer following questions related to ketogenesis: (3 points)

a) What is ketosis?

Accumulation of excess Ketone Bodies

b) Name three ketone bodies.

Acetone, Acetoacetic acid and beta-hydroxybutyric acid.

c) Which amino acids are "exclusively" ketogenic?

Leucine and Lysine

5. **There are a series of human diseases involving deficiencies in enzymes that participate in the synthesis of amino acids. What are the consequences of a deficiency in phenylalanine hydroxylase? (2 points)**

It will lead to Phenylketonuria (PKU), caused by a deficiency of *phenylalanine hydroxylase*. Biochemically, it is characterized by accumulation of phenylalanine (and a deficiency of tyrosine).

***Phenyllactate, phenylacetate, and phenylpyruvat (which are not normally produced in significant amounts in the presence of functional *phenylalanine hydroxylase)* are elavated.**

***Patients with phenylketonuria often show a deficiency of pigmentation (fair hair, light skin color, and blue eyes). The hydroxylation of tyrosine by *tyrosinase*, which is the first step in the formation of the pigment melanin, is competitively inhibited by the high levels of phenylalanine present in PKU.**

***** Tyrosine deficiency also leads to defective formation of tissue proteins and catecholamines.**

6. What are the seven intermediary metabolites that are produced during the catabolism of amino acids? Identify any two intermediates that are the catabolic products of the glucogenic amino acids and identify the amino acids associated with the intermediate. (3 points)

1) Oxaloacetate (Aspargine and Aspartate)
2) α-ketoglutarate (Glutamine, Proline, Arginine, Histidine)
3) Pyruvate (Alanine, Serine, Glycine, Cystine Threonine)

4) Fumarate (Phenylalanine and Tyrosine)
5) Succinyl coenzyme A (CoA) Valine, Isoleucine and Threonine
6) Acetyl CoA
7) Acetoacetate

7. A four year-old boy was noted by the parents to have darkening of the urine to an almost black color when it was left standing. Which of the following is most likely elevated in this patient? (1 point)

A) Methylmalonate
B) Homogentisate
C) Phenylpyruvate
D) α-Ketoisovalerate
E) Homocysteine

Briefly describe the reason for the above disorder? (1 point)

Alkaptonuria is a rare metabolic disease involving a deficiency in *homogentisic acid oxidase*, resulting in the accumulation of homogentisic acid.

This reaction occurs in the degradative pathway of tyrosine.

The illness has three characteristic symptoms: homogentisic aciduria (the patient's urine contains elevated levels of homogentisic acid, which is oxidized to a dark pigment on standing.

8. Many biomolecules are derived from amino acid precursors. Name the correct precursor for the following biomolecules. (2 points)

Tryptophan Serotonin

Histidine Histamine

Tyrosine Epinephrine

Glycine Heme

9. Explain why the synthesis of δ-aminolevulinic acid (ALA) is increased in patients treated with some antifungal and anticonvulsant drugs? (3 points)

Administration of some antifungal and anticonvulsant agents results in a significant increase in hepatic *ALA synthase* activity. These drugs are metabolized by the *microsomal cytochrome P450 monooxygenase* system—a hemeprotein oxidase system found in the liver. In response to these drugs, the synthesis of cytochrome P450 proteins increases, leading to an enhanced consumption of heme—a component of cytochrome P450 proteins. This, in turn, causes a decrease in the concentration of heme in liver cells. The lower intracellular heme concentration leads to an increase in the synthesis of *ALA synthase* (derepression), and prompts a corresponding increase in ALA synthesis.

10. What is porphyria? What are the two major classes of porphyrias and why are they named such? (5 points)

Porphyrias are defects in heme synthesis, resulting in the accumulation and increased excretion of porphyrins or porphyrin precursors

Each porphyria results in the accumulation of a unique pattern of intermediates caused by the deficiency of an enzyme in the heme synthetic pathway

The porphyrias are classified as erythropoietic or hepatic, depending on whether the enzyme deficiency occurs in the erythropoietic cells of the bone marrow or in the liver.

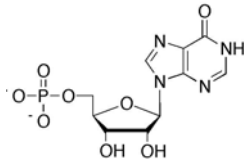
Hepatic porphyrias can be further classified as acute or chronic.

Individuals with an enzyme defect leading to the accumulation of tetrapyrrole intermediates show photosensitivity—These symptoms are thought to be a result of the porphyrin-mediated formation of superoxide radicals from oxygen. These reactive oxygen species can oxidatively damage membranes, and cause the release of destructive enzymes from lysosomes. Destruction of cellular components leads to the photosensitivity.

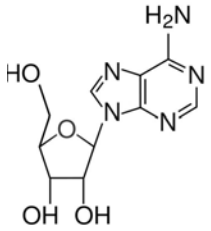
Name	Deficient enzyme	Accumulated Intermediates	Photosensitivity
Hepatic Porphyrias			
Acute intermittent porphria (Acute)	Hydroxymethylbiilane synthtase	Protoporphyrin and ALA in the urine	-
Variegate porphyria (Acute)	Protoporphyrinogen oxidase	Protoporphyrinogen IX and other intermedites prior to the block in the urine	+
Heriditary Coproporphyria (Acute)	Coproporphyrinogen oxidase	Coproporphyrinogen III other intermedites prior to the block in the urine	+
Erythropoietic porphyria			
Erythropoietic protoporphyria	Ferrochelatase	Protoporphyrins accumulate in the Bone marrow, erythrocytes and plasma	+
Congenital Erythropoietic porphyria	Uroporphyrinogen III synthatase	Uroporphyrinogen I and coporphyrinogen I urine	+
Hepatic and Erythropoietic porphyria			
Porphyria Cutanea Tarda (Chronic)	Uroporphyrinogen decarboxylase	Uroporphyrinogen I and coporphyrinogen Iin urine	+

Please read all questions carefully and answer as directed. I will only read the number of sentences I ask for and if your answer does not appear in the first sentence(s) you will not get credit for your answer.

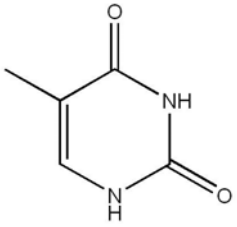
1. Using the appropriate nomenclature to distinguish between free bases, nucleosides, or nucleotides, name the following compounds on the line below the structure: (1 pt. each a-e)



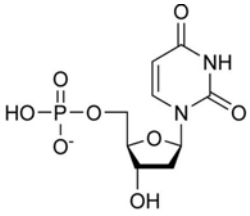
a. _Inosine monophosphate or Inosate____



b. _Adenosine_____



c. _Thymine_____



d. _Deoxyuridylate or Deoxyuridine monophosphate_

e. Name the functional group and its source on the number 2 atom of the guanine ring.
It is an amine group donated by glutamine.

2.

a. In **one sentence or diagram** describe what reaction adenosine deaminase catalyzes? (Be specific about the identity of the substrate and the products.) (2 pt)

Adenosine deaminase catalyzes the deamination of adenine producing an ammonia ion and inosine.

b. Inherited adenosine deaminase deficiency is one cause of Severe Combined Immunodeficiency (SCID) or "Boy in the Bubble Disease." In **no more than three sentences** what is the mechanism by which this enzymatic defect causes such immunodeficiency? (3pt)

Adenosine deaminase deficiency leads to the build up of dATP. dATP is an inhibitor of, ribonucleotide reductase, the enzyme responsible for converting nucleotides into the deoxynucleotides needed for DNA. A deficiency in deoxynucleotides is especially detrimental to rapidly dividing cells such as the cells that comprise the immune system, thus severely debilitating the immune response.

3. In **one sentence**, what is the pharmacological rationale for targeting thymidylate synthase for cancer therapies? In **one sentence**, when targeting rapidly dividing cancer cells and trying to minimize the effects to normal cells, why is thymidylate synthase a better target than dihydrofolate reductase, if they both inhibit thymidlate synthesis? (3pt)

Thymidylate is the only nucleotide unique to DNA, and thus is an effective target of actively dividing cells without affecting normal cells' capacities to synthesize the nucleotides needed for RNA synthesis. Thymidylate synthase is a better target, because folate derivatives are involved in purine synthesis as well as thymidylate synthesis thus inhibition of dihydrofolate reductase would hamper normal cells' abilities to synthesize nucleotides for RNA synthesis.

4.

a. An oncologist says he is going to give your loved one a sulfonamide as a chemotherapy agent to treat a cancerous tumor, citing that is should inhibit the ability of the rapidly dividing cancer cells to synthesize the required nucleotides. In **one sentence**, should you switch doctors and why?(3 pt)

You should switch doctors because this doctor has forgotten that sulfonamides inhibit folate synthesis and humans obtain folate from the diet, therefore the drug would be ineffective on the cancerous cells.

b. Can you think of additional drug that would target the same pathway that sulfonamides target, but in a different manner? (1 pt)

Methotrexate is a folic acid analog and would inhibit the same pathway in a different manner, and would be effective in humans.

5. While strolling down a street, you observe a man that appears to be in his mid-40's eating foie gras and mussels and drinking what appears to be a pint of beer. You notice that the man is rubbing his swollen ankle and big toe on the same foot and appears to be in great discomfort. In **one** word, what do you deduce is the cause of this gentleman's discomfort? In **no more than two** succinct sentences, describe the cause of what you have identified as the gentleman's problem.(3 pt)

Gout

Gout is caused by increased serum levels of uric acid, likely due to the gentleman's gender, age and diet. When serum levels of uric acid, a product of pyrimidine catabolism, reach levels above 6.8 mg/dL uric acid begins to crystalize and deposit in joints and other areas, causing great pain and discomfort.

Graduate Biochemistry Name:
Exam 3
Haas questions

1. Sudden infant death syndrome (SIDS) is a devastating disease that affects neonates within their first 6 months after birth. Typically, the child expires in the early hours of the morning and is found dead in their crib with no underlying post-mortem autopsy indications. However, SIDS deaths are frequently correlated with coincident viral or bacterial infection. A significant fraction of SIDS deaths have been conclusively linked to a defect in Medium Chain Acyl CoA Dehydrogenase (MCAD), which is required for the β-oxidation of medium chain length fatty acids. Speculate on why a defect in MCAD could result in SIDS. (10 points)

This questions relates to the role of fatty acid b-oxidation during short term fast that occurs overnight. Because the total β-oxidation of fatty acids requires the concerted action of three acyl-CoA dehydrogenases that differ in chain length substrate specificity, a defect in MCAD leads to incomplete fatty acid β-oxidation, resulting in a deficiency in acetyl-CoA. The latter has two consequences as the sleeping infant's metabolism attempts to cope with diminishing blood glucose levels as the limited stores of liver glycogen are depleted: (1) Inadequate acetyl-CoA attenuates the allosteric activation of mitochondrial pyruvate carboxylase, the committed step of gluconeogenesis (no one noted this connection), leading to an inability to maintain adequate blood glucose from gluconeogenesis and (2) Inadequate acetyl-CoA as a precursor for ketone body formation as an alternate energy source. Viral infection and the resulting greater demand for metabolic energy accentuates this metbaolic downward spiral in children that might otherwise be asymptomatic, explicating the increased correlation of SIDS incident with viral infection.

BONUS QUESTION

Answer the following series of questions. A bonus of 10 points will be awarded only for correctly answering all parts of the question:

Glucose is labeled at carbon 1 with ^{14}C and incubated with mouse liver cells.

1. Where will the label appear in the immediate product(s) of Aldolase?

The label will appear in C1 of DHAP (the one with the phosphate monoester) corresponding to C1 of fructose 1,6-bisphosphate. The action of triose phosphate isomerase (TIM) with result in scrambling of the label to the C3 of glyceraldehyde 3-phosphate. [The trick here is to "follow the phosphates".]

2. Where will the label appear in pyruvate?

The label will appear in C3 of pyruvate.

3. Where will the label appear in ribose-1 phosphate?

If one considers only the oxidative phase of the pentose phosphate pathway, then label will not appear in ribose 1-phosphate, having been lost as $^{14}CO_2$ by the action of 6-phosphogluconate dehydrogenase. [However, if one considers the concerted action of nonoxidative phase of the pathway, label will scramble from the C3 position of glyceraldehyde 3-phosphate into C5 of ribose 5-phosphate by the action of transketolase.]