

BIOCHEMISTRY AND MOLECULAR BIOLOGY

Problem Unit Seven 1999/2000

Nitrogen Metabolism

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Module 1: Amino Acid Metabolism, I. Nitrogen Metabolism

Module 2: Amino Acid Metabolism, II. Carbon Skeleton Metabolism

Module 3: Purine and Pyrimidine Metabolism

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Estimated Work Time: 40 hours.

Learning Resources:

A. This study guide is provided in two forms: printed and electronic (produced by Dr. E.C. Niederhoffer, Biochemistry and Molecular Biology). ***It is best viewed in electronic form as a pdf file which can be read on your computer using Adobe Acrobat Reader.*** See [Appendix I](#) for an introduction on how to view a pdf file. The pdf file can be downloaded from the biochemistry server (<http://www.siu.edu/departments/biochem>) and Acrobat Reader can be downloaded free from Adobe's web page (<http://www.adobe.com/acrobat>). They should also be installed on the student computers. There are a number of advantages to using the electronic version including color, a hypertext index, and hypertext links within the text. Hypertext links in the text body are in blue underlined characters ([such as this](#)). Clicking on these will lead to a jump to the linked material for further details. The destination material is indicated by red underlined characters ([such as this](#)). (Clicking on the black double arrows in the menu bar will allow you to "hyper-jump" back and forth.)

This and other study guides are provided to help you focus on the topics that are important in the biochemistry curriculum. These are designed to guide your studying and provide information that may not be readily available in other resources. They are not designed to replace textbooks, and are not intended to be complete. They are guides for starting your reading and reviewing the material at a later date. Some of the terms in Nomenclature and Vocabulary, and Keywords are linked to their reference in the Study Guide.

B. Textbooks:

1. Champ & Harvey, Lippincotts Illustrated Reviews: Biochemistry, 2nd ed. ('94), Lippincott. Efficient presentation of basic principles.
2. Murray et al., Harper's Biochemistry, 24th ed. ('96), Appleton & Lange. An excellent review text for examinations.
3. Devlin, Textbook of Biochemistry with Clinical Correlations, 4th ed. ('97), Wiley-Liss. Core text for Biochemistry & Molecular Biology.

4. Marks, Marks, and Smith, Basic Medical Biochemistry: A Clinical Approach, ('96), Williams & Wilkins. Good basic presentation with clinical relevance.

Secondary Resources:

1. Montgomery, et. al., Biochemistry: A Case-Oriented Approach, 5th ed., ('90), Mosby. The cases mentioned in Clinical Aspects.
2. Meisenberg, G., and Simmons, W. H., Principles of Medical Biochemistry, ('98), Mosby. Good basic presentation with clinical relevance.
3. Cohn, R. M., and Roth, K. S., Biochemistry and Disease: Bridging Basic Science and Clinical Practice, ('96), Williams & Wilkins. Clinically oriented text.

Most texts of biochemistry have sections on nitrogen metabolism. The content of the subject is much the same from text to text; the differences are basically in style and rigor. The Study Guide, Pretest, and Post Test in the Problem Unit will set the level of rigor expected of you. Read the sections on nitrogen metabolism in several texts. What differences there will be between these texts and the Study Guide will be helpful to you in gaining perspective on the subject. Additional material can be found on the web at the National Institutes of Health (<http://www.nih.gov>), the National Library of Medicine (<http://www.nlm.nih.gov>), and the free MEDLINE PubMed Search system at the National Library of Medicine (<http://www3.ncbi.nlm.nih.gov/PubMed/>).

You may find worthwhile reading in some of the more popular journals and review series (see also the searchable [SIU-SOM database](#)). These resources typically contain specific articles involving blood and hemoglobin. Suggestions for journals include *American Family Physician*, *Journal of Biological Chemistry*, *Nature*, *Science*, and *Scientific American* (and SA's *Science and Medicine*). Excellent reviews may be found in the *Annual Review of Biochemistry*, *Cell and Developmental Biology*, *Genetics*, *Medicine*, and *Microbiology*.

C. Practice Exams.

A combination of Practice Exams and Problem Sets are included.

D. Lecture/Discussions

All of the major points with emphasis on the more difficult concepts will be presented in lectures.

**Evaluation Criteria
and Testing Informa-
tion:**

These modules will be examined as part of Problem Unit 7. Answers to questions, discussions, solving of problems, and definitions will be judged against the learning resources. A written secure examination covering the objectives in Problem Unit 7 will be scheduled. The pass level is 70%. The examination will be confidential and will not be returned to the students. Students can make arrangements for reviewing their examinations by contacting faculty in charge of the Problem Unit.

Module 1: Amino Acid Metabolism, I. Nitrogen Metabolism

Objectives:

1. After reading a given passage from a medical journal or textbook on nitrogen metabolism (which may be a clinical investigation or a biochemical description), answer questions about the passage (which may involve the drawing of inferences or conclusions) or use the information given to solve a problem.

In order to accomplish objective 1, you will need to be able to do the following which are also objectives:

2. With the aid of a diagram, give an overall view of the metabolism of protein and amino acids.
3.
 - a. Using equations, describe the reaction catalyzed by a transaminase (aminotransferase).
 - b. Show the structure of the Schiff-base that forms during the reaction.
 - c. Identify the structure and function of pyridoxamine, pyridoxal, and pyridoxal phosphate.
4.
 - a. When given an α -keto acid (e.g., α -ketoglutarate or pyruvate), write the equation describing transamination involving it.
 - b. When given an amino acid, write the equation describing transamination involving it.
5.
 - a. Describe what the following abbreviations stand for: AST (GOT), SGOT, ALT (GPT), SGPT.
 - b. Give examples of, and identify examples of, reactions catalyzed by AST and ALT.

6. Using equations, describe how ammonia is formed by
 - a. the oxidative deamination of glutamate and other amino acids
 - b. the direct deamination of histidine
 - c. deamination by dehydration of serine and threonine
 - d. the hydrolytic deamination of glutamine and asparagine
7. a. For various coenzymes that are water-soluble vitamins or contain B vitamins as part of their structure, give:
 1. the name of the vitamin.
 2. the name and, where appropriate, the abbreviation of the coenzyme.
 3. the type of reaction in which the coenzyme functions.
 4. an example of the type of reaction in which the coenzyme is involved.b. Give the name of the coenzymes involved in various transamination, oxidative deamination and deamination by dehydration reactions.
8. List the major sources of ammonia production in the body.
9. Hyperammonemia can cause a coma. Describe possible mechanisms of ammonia toxicity. Describe causes of and management of hyperammonemia.
10. Describe how ammonia is converted into non-toxic forms (a) for transport from one cell to another and (b) for removal from the body.
11. a. Using structures and names, draw a diagram of the urea cycle including the formation of carbamoyl phosphate.
 - b. Describe the role of *N*-acetylglutamate in carbamoyl phosphate formation.
 - c. When given the name of an enzyme involved in urea formation and the structure of its substrates, draw the structure(s) of its products.
 - d. When given a partial diagram of the urea cycle, identify the missing components.
12. Describe how the urea cycle is regulated.
13. a. Using a diagram, show the relationship of the urea cycle to the TCA cycle.

- b. Indicate where the enzymes of the urea cycle are located in the cell.
 - c. Identify the enzymes that are defective in Type I and II hyperammonemia, citrullinuria, and argininosuccinic acidemia.
14. Define essential and nonessential amino acids. Explain what makes them essential or dispensable for humans. List the essential and nonessential amino acids.
 15. Describe how the determination of BUN, NPN and blood creatinine is used in clinical diagnosis.
 16. Describe, define and properly use the terms in the [NOMENCLATURE AND VOCABULARY](#) list as well as the [KEY WORDS](#) list.

Clinical Aspects:

- A. Montgomery, *et al*:
 1. Hereditary Hyperammonemia, p. 372
 2. Defective Urea Cycle, p. 375
- B. Devlin:
 1. Carbamoyl Phosphate Synthetase and *N*-Acetylglutamate Synthetase Deficiencies. *Clin. Corr.* 11.1, p. 456.
 2. Deficiencies of Urea Cycle Enzymes, *Clin. Corr.* 11.2, p. 457.

Nomenclature and Vocabulary:

ALT	Amino acid pool
Aminotransferase	Ammonia
AST	Biological value
Blood creatinine	BUN
Deamination	Essential amino acid
GDH	GOT
GPT	Half-life
Incomplete protein	Kwashiorkor
Marasmus	Metabolic pool
Negative nitrogen balance	Nitrogen balance
Nitrogen pool	Non-essential amino acid
NPN	Oxidative deamination
Ping-pong reaction	PLP
PMP	Positive nitrogen balance
Protein energy malnutrition	Schiff-base

SGOT
Transaminase
Turnover rate
Urea

SGPT
Transamination
Urea cycle

Key Words:

Amino acids
Aminotransferases
Biochemistry
Coenzymes
Diagnostic Tests, Routine
Metabolism
Proteins
Vitamin B Complex

Amino acids, essential
Ammonia
Blood urea nitrogen
Creatinine
Enzymes
Nitrogen
Urea
Vitamins

STUDY GUIDE-1

There are a great number of pathways of amino acid metabolism. Most of the amino acids have a pathway for synthesis, and all have one or more pathways of catabolism. This Study Guide will focus on the generalities of amino acid metabolism.

We ingest proteins, which are digested to their constituent amino acids. At the same time, tissue proteins are hydrolyzed to form amino acids which mix with those derived from food as an **amino acid pool** in body tissues (Figure 1).

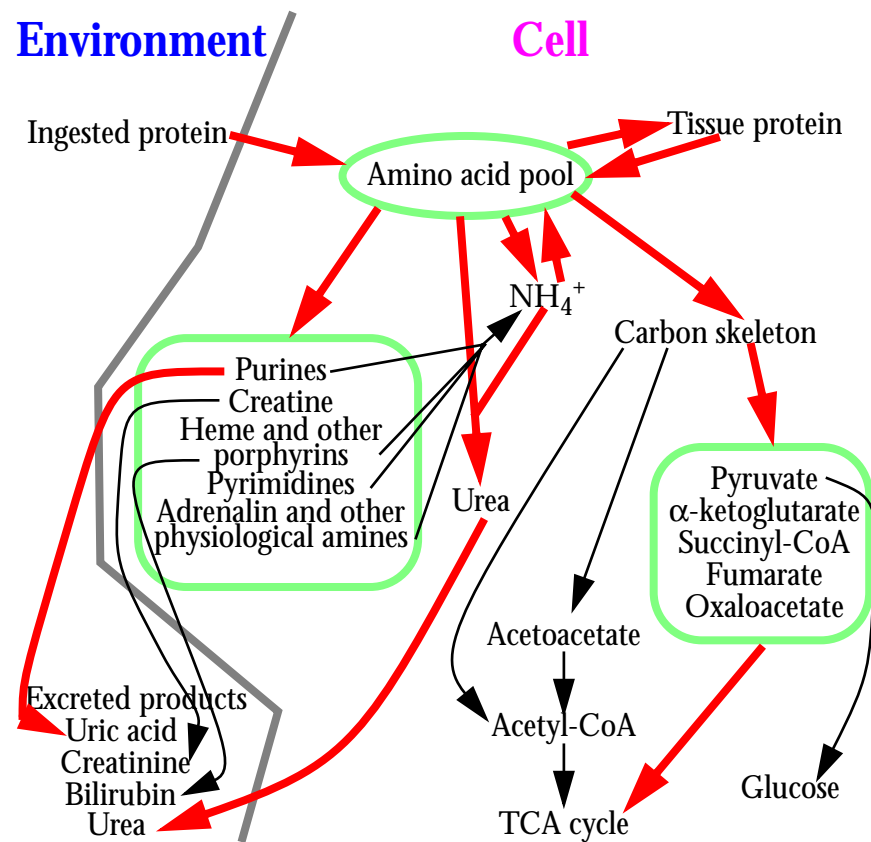


Figure 1. - Summary of nitrogen flow through the body.

A **metabolic pool** is a group of compounds that can provide a chemical entity for synthesis or can be metabolized to end products. A metabolic pool can be likened to a car pool, a group of cars that can be called upon for transportation when needed. In this case, the **nitrogen pool** consists of those compounds that can donate nitrogen for various syntheses or can be catabolized to excretory products. The components of the nitrogen pool vary from tissue to tissue, but

are primarily amino acids.

Part of the amino acid pool is used to rebuild tissue protein. In adults the total amount of protein remains relatively constant from day to day, thus, the quantity of amino acids used to make tissue protein is no greater than the quantity obtained by the breakdown of tissue proteins. Therefore, the usual adult will have a surplus of amino acids equivalent to the amount ingested. The excess amino acids are used as fuel. The metabolism of these amino acids requires the disposal of the nitrogen as urea and the degradation of the carbon backbone to intermediates of glycolysis and the TCA cycle.

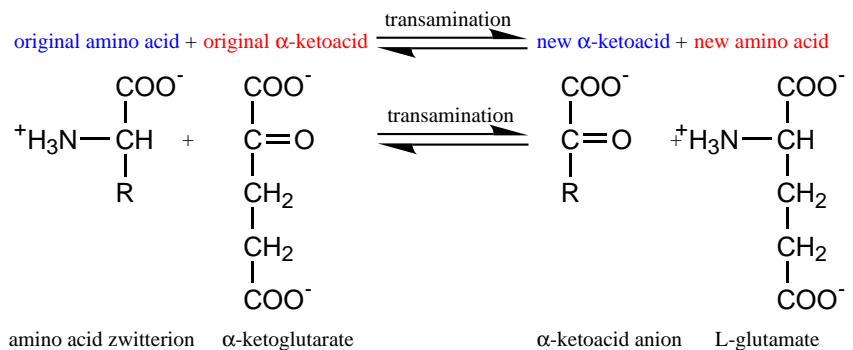
The transport of amino acids against a concentration gradient requires the expenditure of energy. A Na^+ -coupled symport mechanism for the absorption of neutral amino acids is present in the brush border of the intestinal mucosa and the kidney tubules. The passage of Na^+ down its concentration gradient effects simultaneous transport of amino acids against their concentration gradient in the same way glucose is transported in these cells.

Amino acids can be transported as small peptides. Since there are multiple mechanisms for transporting amino acids, the hereditary disorders affecting amino acids transport are relatively benign.

I. What is transamination? What is its role in amino acid metabolism?

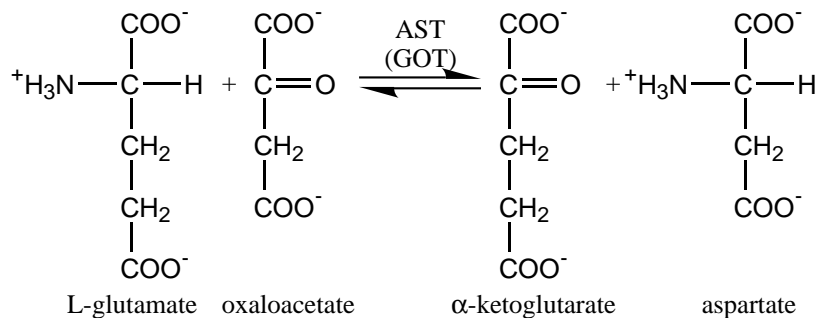
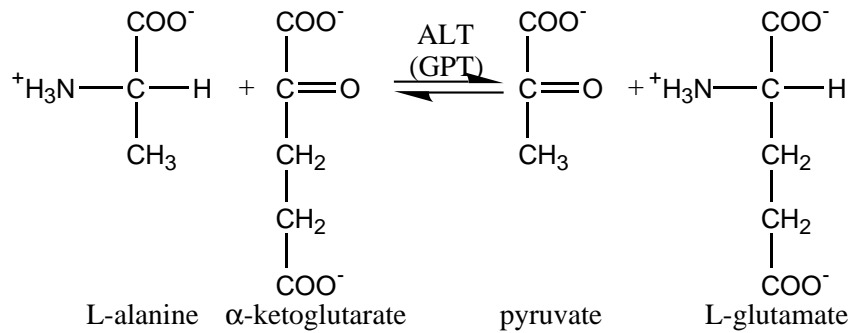
In some cases, an amino acid is made from another amino acid. For example, glycine is made from serine; proline is made from glutamic acid; cysteine is made from serine and homocysteine (from methionine), and tyrosine is made from phenylalanine.

However, the last step in the synthesis and the first step in the breakdown of most amino acids is **transamination**, the transfer of an amino group from an amino acid to an α -keto acid to form a new amino acid, and vice versa.



Transamination is, for the most part, a cytoplasmic reaction, although there is aspartate **transaminase (AST)** [commonly referred to as glutamate-oxaloacetate transaminase (**GOT**)] in mitochondria. L-Glutamic acid is the most active amino acid in transamination.

We might ask, "How can L-alanine be used in the synthesis of aspartic acid? The answer is "Through transamination involving the enzymes alanine transaminase (**ALT**) and aspartate transaminase (AST)[respectively, glutamic-pyruvic transaminase (**GPT**) and glutamic-oxaloacetic transaminase (GOT) (glutamate-aspartate **aminotransferase**)]".



The greatest amounts of AST (GOT) and ALT (GPT) are present in the liver, followed by lesser amounts in heart and skeletal muscle. Small amounts are present in the kidneys, pancreas, erythrocytes, and the lungs. When cells in these tissues are injured, enzyme is released and increased serum levels result (increased **SGOT** and **SGPT**). Various diseases result in an increased production of these enzymes and an increased SGOT and SGPT. (S stands for serum.)

Elevated transaminase levels are most often associated with heart and liver disease. SGOT activity rises within the first 18 hours following an acute myocardial infarction. The degree of elevation is related to the amount of myocardial muscle that has become necrotic. An elevation of SGOT is particularly helpful to the physician when the

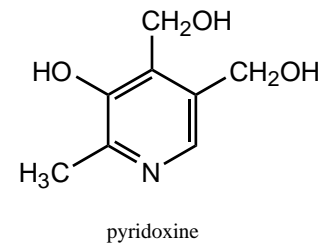
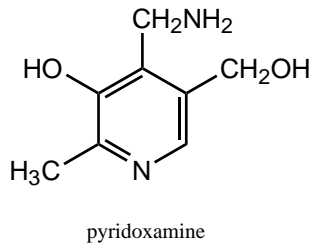
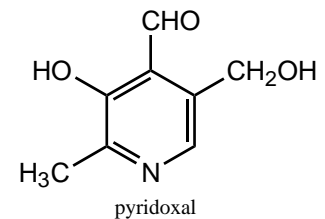
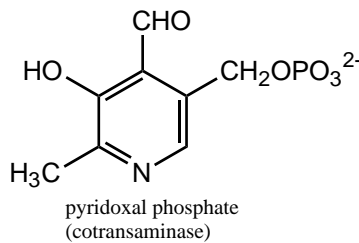
electrocardiogram does not indicate the presence of a myocardial infarct.

Since the liver contains the highest levels of both transaminases, any damage to the parenchyma cells of the liver will result in elevated levels of both SGOT and SGPT. The degree of elevation usually reflects the severity of hepatic damage.

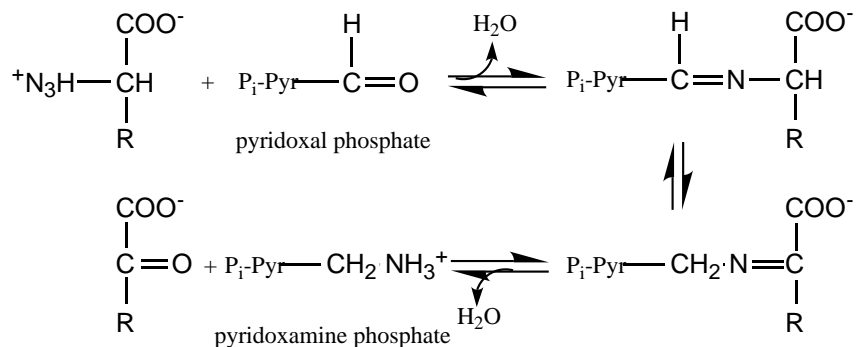
There are other transaminases. In fact, most amino acids can be converted to the corresponding α -keto acids by transamination and many can be made from the corresponding α -keto acid by transamination.

II. What is the function of pyridoxine?

Vitamin B₆ (pyridoxine) is converted into **pyridoxal phosphate**, the coenzyme for transamination, decarboxylation, **deamination**, racemization, and aldol-like condensations of amino acids.



In the presence of the enzyme, the coenzyme forms a **Schiff base** with the amino acid.



There are several water-soluble B vitamins that are coenzymes or form parts of coenzymes or prosthetic groups (TABLE 1). Pyridoxal phosphate is a coenzyme of reactions involving all amino acids. Folic acid and cobalamine are coenzymes for reactions, some of which involve amino acids (viz., glycine, serine, and methionine).

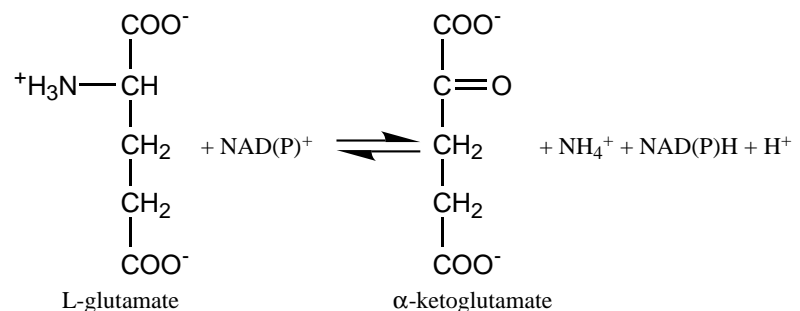
Table 1: Vitamins - Coenzyme Relationship for Various B Vitamins

Vitamin	Coenzyme or Prosthetic Group	Process
Thiamin	TPP (cocarboxylase)	decarboxylation
Nicotinic acid, nicotinamide (niacin)	NAD ⁺ , NADP ⁺ (transfer of 2e ⁻ + H ⁺)	oxidation-reduction
Riboflavin (B ₂)	FMN, FAD, (transfer of 2e ⁻ + 2H ⁺)	oxidation-reduction
Pantothenic acid (B ₃)	coenzyme A	acylation
Lipoic acid	itself	oxidation-reduction (transfer of 2e ⁻ + 2H ⁺)
Biotin	itself	carboxylation
Pyridoxal, pyridoxine, pyridoxamine (B ₆)	pyridoxal phosphate, pyridoxamine phosphate (cotransaminase)	transamination, decarboxylation and other reactions of amino acids
Folic acid	itself	one-carbon metabolism
Cobalamine (B ₁₂)	itself	C-C, C-O, C-N bond cleavages; methyl group activation

III. How is nitrogen (ammonia) ultimately removed from amino acids for the synthesis of urea?

L-Glutamic acid, being specifically permeable to the inner mitochondrial membrane, passes to the cytoplasm. There, other nonessential (dispensable) amino acids can be formed by transamination. Because transamination is reversible, the nitrogen of other amino acids can collect in L-glutamic acid. L-Glutamic acid can then return to the matrix of the mitochondria. There it can undergo transamination in the presence of oxaloacetate and mitochondrial GOT to form L-aspartic acid. Alternatively, it can be deaminated by mitochondrial **glutamic dehydrogenase (GDH)**. However, before we discuss **deamination** further, let us repeat that transaminases are found both in the cytoplasm and in the mitochondria of eukaryotic cells, that the enzymes in each region have characteristic properties, and that they are responsible for synthesis of nonessential amino acids, provided a source of nitrogen is available.

Most nitrogen gets removed from amino acids via L-glutamic acid. Specifically, ammonia is removed by **oxidative deamination** of L-glutamic acid, a reaction catalyzed by glutamic dehydrogenase.



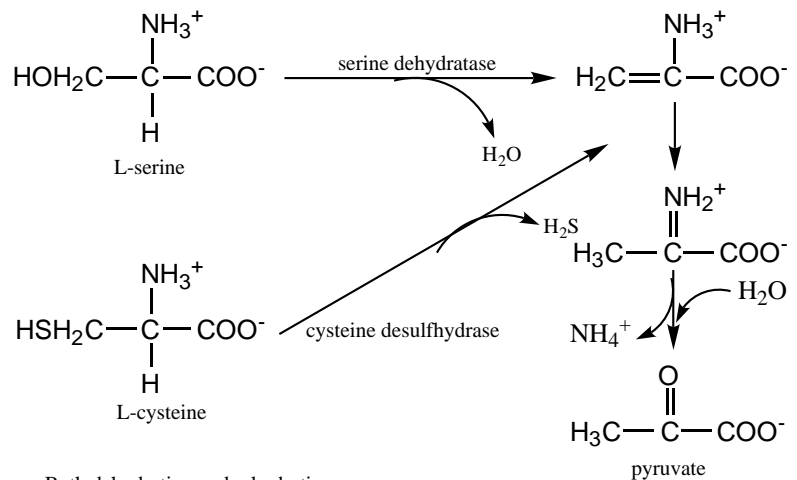
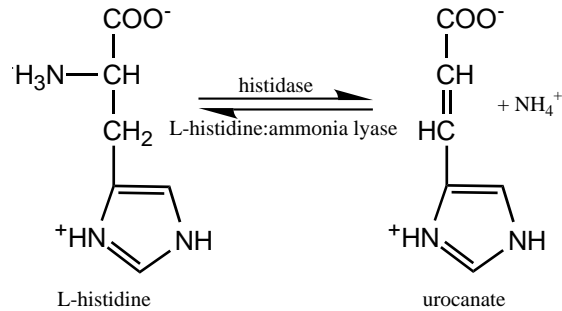
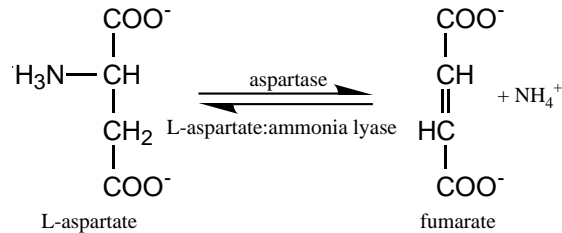
This reaction is reversible so nitrogen can also enter amino acid synthesis by **reductive amination** of α -ketoglutarate. Thus, L-glutamic acid is the first amino acid to be labeled after $^{15}\text{NH}_4^+$ is administered. However, this reaction is of much less importance in the total nitrogen pool than is transamination. It also specifically requires NADPH for reversal.

IV. How else are amino acids deaminated?

Other reactions of amino acids will be discussed in the same manner as has the synthesis of nonessential amino acids, i.e., in general terms, for there is at least one pathway of synthesis and one of catabolism for each amino acid, resulting in a large number of individual pathways. In the previous section, it was pointed out that the coupling of glutamic dehydrogenation and transamination can either make amino acids or deaminate them. It might be pointed out here that L-glutamic acid is also the direct precursor of proline and ornithine and, hence, the indirect precursor of hydroxyproline, citrulline and

arginine. As might be anticipated, glutamic dehydrogenase is an allosteric enzyme. And, although it is so obvious that it hardly needs to be pointed out, the interconversions of pyruvate and L-alanine, α -keto-glutarate and L-glutamate and oxaloacetate and L-aspartate make obvious the interrelationships of amino acid metabolism and carbohydrate and lipid metabolism. Other amino acids have this same relationship.

There are several kinds of nonoxidative deamination. Examples are given below.



Both dehydration and rehydration are catalyzed by serine dehydratase

V. What happens to the nitrogen that is removed from amino acids?

Nitrogen ingested (largely in the form of protein) needs to be excreted in order to maintain a **nitrogen balance**. This is accomplished in several ways, but primarily via the **urea cycle**.

Ammonia or the ammonium ion is toxic even in relatively low concentrations. Humans, therefore, convert it into **urea**. The pathway for its formation is given in Figure 2. (All intermediates are shown ionized.)

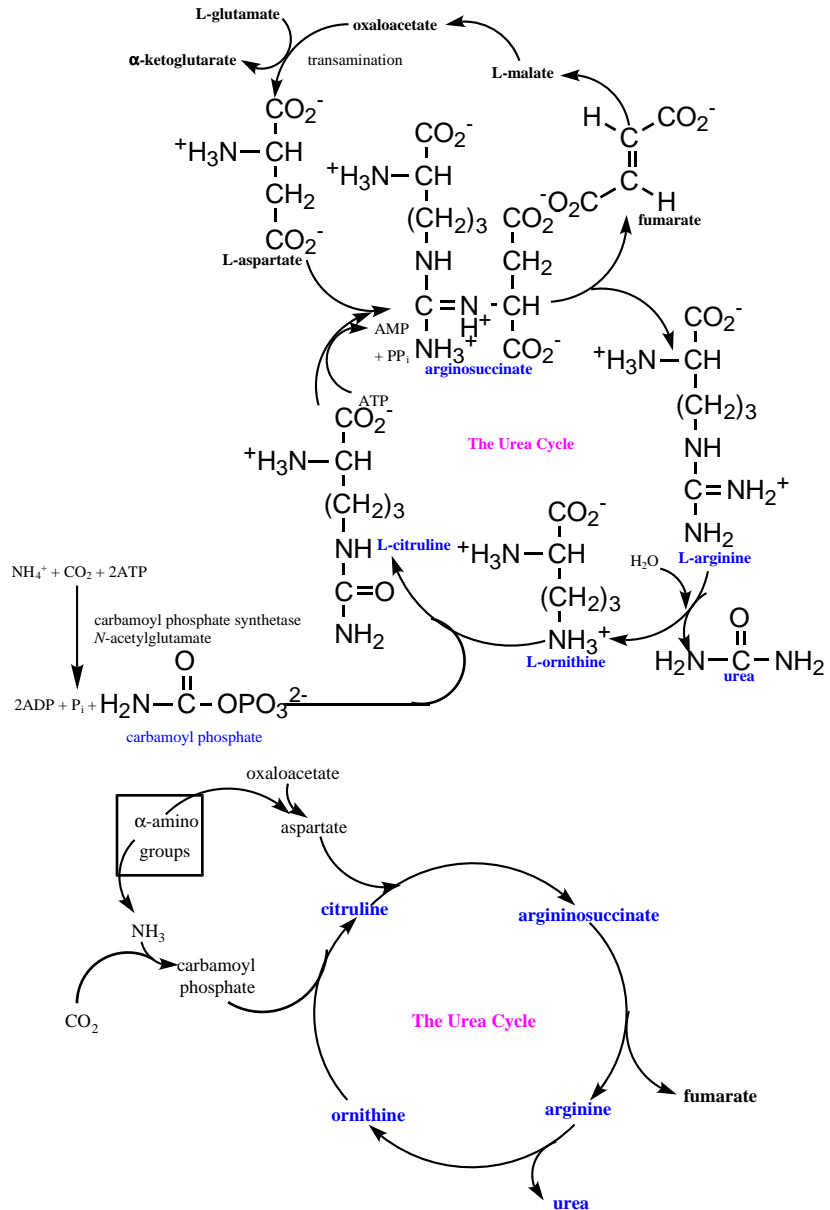
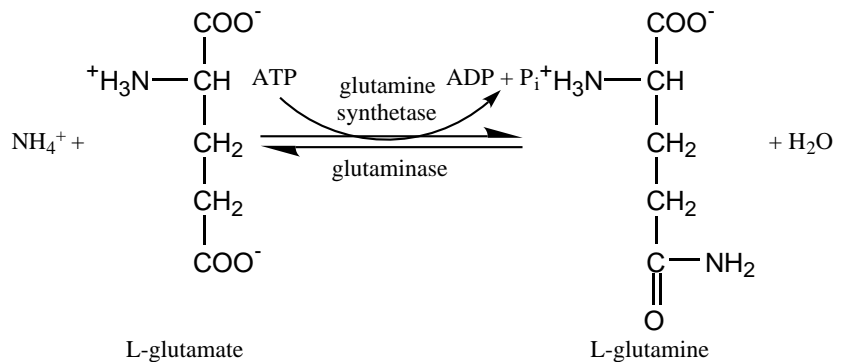


Figure 2. The urea cycle and its relationship to the TCA cycle.

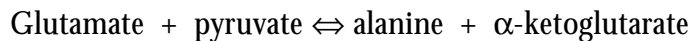
The reactions of the urea cycle are primarily found in the liver, the

principal site of urea formation. Some urea synthesis can also occur in the brain and the kidney. Ammonia is stored and transported in the form of L-glutamine which is formed from L-glutamate and as alanine which is formed by transamination from pyruvate.

L-Glutamine can diffuse through the plasma membrane of cells into the blood. The liver and kidney are rich in glutaminase which catalyzes the hydrolysis of L-glutamine into L-glutamic acid and ammonium ion which, in the liver, is converted into urea and, in the kidney, is excreted directly.



The flow of nitrogen from peripheral tissues to the liver via alanine could occur by the following transamination reactions.



Alanine can then be transported to the liver, where by reversal of these reactions as well as others, NH_4^+ and aspartate, the nitrogen donors to the urea cycle, will be produced.

Carbamoyl phosphate (also called carbamyl phosphate) is important in several biosynthetic reactions. In this case, the carbamoyl phosphate synthetase is a mitochondrial enzyme. There is also a cytoplasmic enzyme. The two differ in their properties and in the mechanism used for carbamoyl phosphate synthesis. There are also differences in the means of carbamoyl phosphate synthesis from organism to organism.

In urea formation, carbamoyl phosphate is formed from ammonia and carbon dioxide using the energy of 2 ATP molecules. N-Acetylglutamate is an allosteric activator of carbamoyl phosphate synthetase. It then reacts with ornithine to form citrulline and thereby donates the carbon atom and one nitrogen atom of urea. The second

nitrogen atom comes from glutamic acid via aspartic acid by a process that converts citrulline to arginine.

The enzyme arginase then acts on the guanidino group [-NH-(C=NH)-NH₂] to release urea and to replace ornithine, thus completing the cycle.

The urea cycle involves, as intermediates, amino acids (L-citrulline and L-ornithine) that are not constituents of proteins. There are other amino acids that are not found in proteins.

The enzymes of the urea cycle are carbamoyl phosphate synthetase and ornithine transcarbamoylase which are in the mitochondria and arginino-succinate synthetase, argininosuccinase, and arginase which are in the cytosol. Carbamoyl phosphate synthetase and ornithine transcarbamoylase are defective in Type I and II hyperammonemia, respectively. Arginino-succinate synthetase is defective in citrullinuria, and argininosuccinase is defective in argininosuccinic acidemia.

VI. Protein Nutrition

To synthesize proteins, both **essential** (or indispensable) and **nonessential** (or dispensable) **amino acids** are required. **Essential (indispensable) amino acids** are the amino acids that are not synthesized at all or are not synthesized rapidly enough for normal growth and maintenance and, therefore, must be supplied in the diet. **Nonessential (dispensable) amino acids** are the amino acids that may be synthesized in the body rapidly enough to meet the needs for protein synthesis. An amino acid is essential when the animal (in our case, the human) lacks the ability to make its corresponding α -keto acid. If the carbon skeleton is available, the amino acid can be formed by transamination. That is, the amino acid can be formed by the transfer of an amino group from another amino acid to its carbon skeleton (α -keto acid) to form the new amino acid.

The number of essential amino acids varies both from organism to organism and with the age of the organism. Those that are essential for humans are given in the following table.

Table 2: Essential and Nonessential Amino Acids (for Humans)

Essential*	Nonessential
Tryptophan	Alanine
Threonine	Aspartic acid
Histidine**	Asparagine
Arginine**	Cysteine
Lysine***	Glutamic acid
Leucine	Glutamine
Isoleucine	Glycine
Methionine***	Proline
Valine	Serine
Phenylalanine	Tyrosine
*Can be remembered by remembering the initials T.T. Hallim V. P. or Millpath T. V. or Pvt. Tim Hall.	
**Not necessary for nitrogen balance in the adult because some can be made, but necessary for growth.	
***Older people need twice as much as younger persons.	

An exact **nitrogen balance** occurs when the daily intake of nitrogen equals that lost in feces, urine, and sweat. However, the term **nitrogen balance** does not necessarily refer to an exact balance. Nutritionists speak of **positive nitrogen balance**, in which the amount of nitrogen ingested exceeds that excreted, and **negative nitrogen balance**, in which the intake is less than the loss.

A positive nitrogen balance is found in growing children (including a growing fetus) and in persons recovering from a disease in which there has been a loss of tissue mass. A negative nitrogen balance is found under the following conditions:

1. inadequate intake of protein
2. dietary deficiency of one or more essential amino acids
3. incomplete digestion of proteins or absorption of amino acids (high fecal nitrogen)
4. excessive catabolism of proteins in tissues (starvation)

5. loss of protein (for example, hemorrhage)

A dietary deficiency of one or more essential amino acids results in a negative nitrogen balance because cells cannot use other amino acids for protein synthesis if one is absent. For a cell to try to **make** protein molecules when an amino acid is missing would be like a typesetter trying to set the type for this sentence if the letter **m** were not available. The first 15 letters could be set, but the sentence could not be completed until a letter m was obtained. So protein molecules cannot be made if one of the amino acids is unavailable. Fragments could be started, but synthesis would stop until the missing amino acid was provided.

The **biological value** of protein is a measure of the value of the protein in supplying amino acids for tissue-protein synthesis. To make protein, a cell needs a balance of essential and nonessential amino acids. Essential amino acids must be part of the dietary protein since they cannot be synthesized at a rate necessary for normal growth or repair. Also, there must be an adequate supply of nonessential amino acids needed for protein synthesis. The biologic value of a protein takes into account the ratio of essential amino acids in the protein, and its ease of digestion. In order for dietary proteins to have biologic value, they must provide all essential amino acids. Animal proteins contain amino acids in about the same ratio as is found in human proteins. Therefore, animal proteins are, in general, well balanced and have a high biologic value. An exception is gelatin, which lacks the essential amino acid tryptophan. Gelatin is 100% deficient in tryptophan.

Plant proteins, in general, have a low biologic value because most plant proteins have a low level of one or more essential amino acids. That is, most plant proteins are deficient in one or more essential amino acids. Such proteins are called **incomplete proteins**. Zein, the predominant protein in corn, is 100% deficient in lysine and tryptophan. Corn contains other proteins in addition to zein. Whole grains (corn, wheat, rice, and sorghum) are 50-60% deficient in lysine. Soybean protein has the highest biologic value of the common plant proteins.

Two-thirds of the world's population subsists almost entirely on vegetable diets. Plant proteins in these diets should be mixed. A proper mixture of vegetables and grains can provide all the necessary protein. Unfortunately, the diet is not varied in most cases, and therefore, the proteins are not mixed. Many people in the world who live on vegetable diets live almost entirely on rice, corn, wheat, sorghum, or other cereal grains. In some areas of the world, 40% of all children die before reaching the age of four. One factor behind this statistic is the

lack of sufficient protein of good quality in their diets.

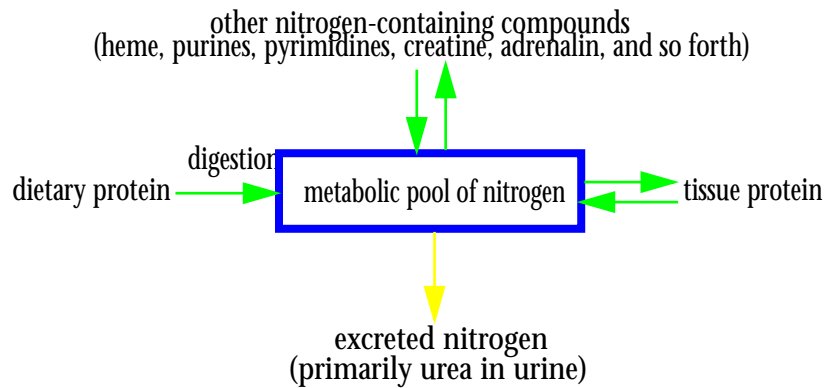
Even in this country, there are population segments that subsist on suboptimal protein intake for economic reasons or because of poor dietary practices. These groups may include old people; poor people; people on reducing diets of poor quality; alcoholics; and growing children who, because of food preferences, consume a low-protein diet consisting of largely cereal foods. Wheat is the primary cereal grain in the diet of most Americans.

In general, animal proteins are more rapidly and completely hydrolyzed than are plant proteins; and, in general, cooked (denatured) proteins are more rapidly and completely hydrolyzed than are uncooked proteins. This is one reason we eat cooked meat rather than raw meat. In addition, raw soybeans and raw egg white contain rather powerful inhibitors of the proteolytic enzyme trypsin. Raw egg white also contains a basic protein, avidin, that combines with and inactivates the B vitamin, biotin.

Why is dietary protein required daily? Does the source of protein make any difference?

As we have discussed several times before, proteins, like all other tissue components, are in what is called a dynamic state. Proteins are constantly being broken down and replaced by newly synthesized protein, a process that uses some of the energy (ATP) generated in mitochondria. Turnover of proteins occurs in plant tissues, but it is less dynamic and less dramatic than in animals. The **turnover rate** (the rate at which molecules are broken down or used up and replaced by new molecules) of tissue protein is measured as its half-life. **Half-life** is the time required for one-half of the protein molecules to be replaced by new molecules. Typical half-lives are as follows:

Tissue Protein	Half-life
Plasma	6 days
Liver	10 days
Muscle	180 days
Collagen	3 years



VII. Blood Urea Nitrogen (BUN), Nonprotein Nitrogen (NPN) and Blood Creatinine

The following is taken from The Biochemistry of Clinical Medicine, by W. S. Hoffman, Year Book Medical Publishers, Inc., 4th ed., 1970 (abridged).

In most parts of the U.S., the blood urea nitrogen concentration is reported in terms of urea nitrogen (**BUN** = blood urea nitrogen) expressed as mg/mL. The only value to this expression is that it allows comparison of the urea nitrogen concentration with the total nonprotein nitrogen (**NPN**) concentration, which also includes the creatinine concentration. Urea N concentrations can be converted into urea concentrations by multiplying by 2.14. Thus, 10 mg of urea N per 100 mL of blood equals 21.4 mg of urea per 100 mL. Yet another unit of expression is millimoles/liter; 1.0 millimole of urea = 60 mg of urea.

Urea is quantitatively the most important nonprotein nitrogenous constituent of blood. It is the chief end-product of protein metabolism and normally is excreted entirely by the kidneys. Hence, its blood concentration is directly related to the protein content of the diet and the renal excretory capacity. Because the rate of excretion of urea is only slightly influenced by the protein content, the BUN concentration in normal persons is primarily related to the protein intake. (In persons who are not taking food, the factor of protein intake is replaced by protein breakdown; see below.) As would be expected from the wide range of dietary protein intake in the normal population, the BUN concentration has a wide range, from 5 to 23 mg per 100 mL, or 11 to 50 mg of urea per 100 mL. The average concentration is about 11 mg of urea N per 100 mL. Most of the population has concentrations between 8 and 16 mg per 100 mL.

The table below gives general, expected values relating ingested protein to BUN.

Table 3: Relationship of Ingested Protein to BUN

Protein ingested			
Person with	Amount of protein (g)	N content of protein (g)	BUN conc (mg/100 ml)
Average diet	70	11.2	8-16
High-protein diet	140	22.4	~23
Low-protein diet	35	5.6	~5

Since the regular hospital diet contains not more than 1 g of protein per kg of body weight and since the appetite of hospital patients is usually below normal, the finding of a BUN concentration of more than 16 mg per 100 mL should be viewed with suspicion. It means either renal dysfunction or excessive bodily breakdown of protein. It may, of course, mean dehydration, which involves both of those factors. With severe renal insufficiency, the BUN concentration rises to 100 mg or more per 100 mL. In uremia, it may rise to 250 mg.

In the normal individual, the BUN concentration is less than half the NPN concentration. Because many of the nonprotein nitrogenous substances of whole blood are not excretory substances and because their concentration does not rise with renal insufficiency, it follows that, when the latter phenomenon develops, the urea N concentration becomes a higher fraction of the total NPN concentration. Thus, we might see the following progression of ratios in a patient passing from normal levels to those of uremia (numerators are urea N and denominators NPN):

$$12/32 \quad 50/80 \quad 100/140 \quad 200/250$$

Here the urea N percentage of the total has risen from 37.5 to 80.

Creatinine is a spontaneous decomposition product of creatine and phosphocreatine. It is a waste product and must be excreted by the kidneys. Because its daily production is directly proportional (about 2%) to the creatine content of the body, which remains constant if the muscle mass is unchanged, it is obvious that, with constant renal function, the **blood creatinine** concentration will vary little from day to day. For any individual, the concentration of blood creatinine is more constant than that of any of the common excretory substances.

The creatinine concentration of the whole blood of normal individu-

als ranges from 0.7 to 1.5 mg per 100 mL, with an average of about 1.2 mg. In plasma or serum concentrations are lower; the range is from 0.6 to 1.1 mg per 100 mL, with an average of about 0.8 mg.

The urea (or NPN) concentrations and the creatinine concentration frequently do not show a parallel rise in various types of renal insufficiency. To analyze the relationship between these two concentrations, several metabolic facts must be understood. (1) The blood urea concentration is determined by the protein intake if the patient is in protein equilibrium or by the degree of protein breakdown if the patient is in negative nitrogen balance. In acute renal insufficiency from any cause, the adrenocortical stimulation which occurs as part of the alarm reaction causes a rapid breakdown of body protein. Such an alarm reaction, however, has no influence on creatinine formation. (2) Simple dehydration, by producing concentrated urine, increases tubular reabsorption of urea without similarly influencing creatinine excretion. Thus, the blood urea concentration may rise without any significant rise in creatinine concentration. On the other hand, diminished glomerular filtration from any cause will decrease the excretion of both urea and creatinine. (3) In the terminal stage of chronic Bright's disease, the urea clearance approaches the creatinine clearance. In such cases, the blood creatinine concentration rises relatively faster than it did previously. (4) The blood creatinine concentration is determined by the muscle content of the body. If true renal failure develops rapidly in persons of strong muscular build, the creatinine concentration may rise to unusually high levels. In general, men have higher creatinine concentrations than women for the same degree of kidney damage. In protracted renal disease in which emaciation has fully developed, muscular wasting may reduce the rate of creatinine formation and thereby mitigate the rise in blood creatinine.

Problem Set

1. Arginosuccinic aciduria was the first reported hereditary impairment of the urea cycle; in subsequent years, four additional recessively inherited disorders of this cycle have been recognized. Each causes hyperammonemia, which tends to be more severe the more proximal the defect is in the urea cycle, which in turn may determine the severity of signs and symptoms (convulsions, vomiting, failure to thrive, hepatomegaly) and degree of mental retardation. The concentration of blood urea and its renal excretion are both normal in each disease. The explanation for this paradox is found in the apparent phenotypic effect of the mutations on urea cycle enzymes. In citrullinemia, for example, arginosuccinic acid synthetase shows partial activity with a new K_m about 25 times above normal. Substrate accumulation in this circumstance sustains formation of product, and thus urea.
 - a. Why might signs and symptoms tend to be more severe the more proximal the defect is in the urea cycle? [answer](#)
 - b. Why would an increase in the K_m of arginosuccinic acid synthetase cause citrullinemia? [answer](#)
 - c. Does the mutation causing citrullinemia result in the loss of arginosuccinic acid synthetase? [answer](#)
2. "Catabolism or degradation of amino acids in the liver involves two major reactions: One of these is oxidative deamination. . . This reaction is catalyzed by L-amino acid oxidases, with but two exceptions: glycine oxidation is catalyzed by glycine oxidase and glutamic acid oxidation by glutamic dehydrogenase. Transamination is a far more important mechanism." (From D. H. Alpers and K. J. Isselbacher, "Derangements of Hepatic Metabolism," in *Principles of Internal Medicine*, 6th edition, 1970).
 - a. What is oxidative deamination? Give an example. [answer](#)
 - b. We now know that glycine oxidase is D-amino acid oxidase. Where is D-amino acid oxidase found? Why is it present? Why should it work with glycine? [answer](#)
 - c. What is the reaction catalyzed by glutamic dehydrogenase? [answer](#)
 - d. Explain the statement "transamination is a far more important mechanism". [answer](#)
3. "Proteins that occur in the blood serum have half-lives of about 10 days and the erythrocyte has a life span of about 125 days.

Thus, measurements of serum nitrogen and protein are useful indicators of a large number of disease states. Some common clinical tests measure serum proteins (including albumin and globulin), blood urea nitrogen (BUN), nonprotein nitrogen (NPN), serum creatinine, serum uric acid, and"

- a. Some minutes ago there was an aspartic acid molecule in the brain. Now the nitrogen from that molecule is in a urea molecule in the blood. How did it get there? Give the reactions that are involved and name the enzymes. [answer](#)
- b. What are likely candidates to make up the pool of NPN in blood serum? [answer](#)

4. The coenzyme for transamination is:

- a. NAD^+
- b. coenzyme A
- c. tetrahydrofolate
- d. thiamine pyrophosphate
- e. pyridoxal phosphate
- f. vitamin B_{12} derivative

[answer](#)

5. Most of the ammonia formed in the kidney is by the action of:

- a. urease
- b. uricase
- c. arginase
- d. glutaminase
- e. carbamoyl phosphate synthetase
- f. glutamine synthetase

[answer](#)

6. The two nitrogen atoms in urea arise from:

- a. ammonia and glutamine
- b. ammonia and aspartate
- c. glutamine and aspartate
- d. glutamine and glutamate
- e. glutamate and alanine
- f. ammonia and ornithine

[answer](#)

7. A patient undergoing convalescence is in positive nitrogen bal-

ance if

- a. The amount of nitrogen ingested equals the amount of nitrogen excreted in the urine, feces and sweat.
- b. The amount of nitrogen excreted in the urine, feces and sweat is less than the nitrogen ingested.
- c. The amount of nitrogen excreted in urine, feces, sweat is greater than the amount of nitrogen ingested.
- d. The patient is fed a protein-free diet.
- e. None of the above is true.

[answer](#)

8. In an individual on a 'fad' diet, where corn and gelatin were the only significant sources of protein, there would be:
- a. increased protein synthesis.
 - b. positive nitrogen balance.
 - c. sufficient amino acid pool for adequate protein synthesis.
 - d. deficiency of lysine and tryptophan.

[answer](#)

9. Amino transferases:
- a. catalyze freely reversible reaction
 - b. are present in both cytosol and mitochondria
 - c. do not cause a net synthesis or breakdown of total amino acids
 - d. require ATP as energy source

[answer](#)

10. Ammonia is a reaction product of:
- a. glutamate dehydrogenase
 - b. glutamate-oxaloacetate transaminase
 - c. histidase (histidinase)
 - d. glutamate-pyruvate transaminase

[answer](#)

11. Ammonium ions are directly used or produced by:
- a. carbamoyl phosphate synthetase
 - b. glutamate-oxaloacetate transaminase (GOT)
 - c. glutamine synthetase
 - d. serine dehydratase

- e. amino acid oxidase
- f. alanine aminotransferase (ALT)
- g. glutamate dehydrogenase
- h. *N*-acetylglutamate synthetase

[answer](#)

12. The essential amino acids:
- a. must be supplied in the diet because the organism has lost the ability or capacity to aminate the corresponding α -keto acids
 - b. must be supplied in the diet because the organism has an impaired ability to synthesize the carbon chains of the corresponding α -keto acids
 - c. are identical in all mammals
 - d. may be defined as "those amino acids which cannot be synthesized by the organism at a rate adequate to meet metabolic requirements"

[answer](#)

13. The two enzymatic activities that catalyze the bulk of the nitrogen flow from amino acids to ammonia are:
- a. transaminases and glutaminase
 - b. transaminases and glutamate dehydrogenase
 - c. transaminases and amino acid oxidases
 - d. glutaminase and glutamate dehydrogenase
 - e. glutaminase and arginase

[answer](#)

14. In the urea cycle:
- a. the supply of carbamoyl phosphate to the urea cycle is regulated by *N*-acetylglutamate
 - b. the immediate precursor of urea is ornithine
 - c. the formation of carbamoyl phosphate requires the expenditure of two molecules of ATP per molecule of ammonia incorporated
 - d. formate is formed

[answer](#)

15. Most of the ammonia is derivatized and transported in the blood as:
- a. glutamine

- b. glutamate
- c. alanine
- d. arginine

[answer](#)

16. The urea cycle:

- a. requires conversion of four moles of ATP to ADP to produce one mole of urea
- b. requires both mitochondrial and cytosolic enzymes
- c. produces both urea and fumarate
- d. occurs primarily in the liver

[answer](#)

17. Transaminations (aminotransferases)

- a. involve a net formation of keto acids
- b. have equilibrium constants near 1
- c. reduce the level of free ammonia in the cells
- d. result in the intermediate formation of a Schiff base between the aldehyde group of pyridoxal phosphate and the amino group of an amino acid

[answer](#)

18. In the urea cycle

- a. energy in the form of GTP is required
- b. the carbons of aspartate are partially incorporated into arginine
- c. arginine is the immediate precursor of citrulline
- d. only catalytic amounts of ornithine are required
- e. oxaloacetate is a direct product of the argininosuccinase reaction

[answer](#)

19. Removal of α -amino groups from amino acids in animals is carried out by:

- a. hydrolysis and reductive amination
- b. transamination only
- c. oxidative deamination and transamination
- d. anaerobic deamination only
- e. none of the above

answer

20. Hyperammonemia may be caused by a deficiency of:
- Aspartate transcarbamoylase
 - Glutamate dehydrogenase
 - Ornithine transcarbamoylase
 - Creatine kinase
 - Glutaminase
 - Urease

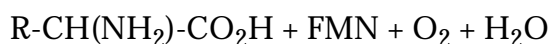
answer

21. Which of the following constitute essential amino acids; that is, must be ingested on a daily basis by humans?
- Leucine
 - Cysteine
 - Phenylalanine
 - Serine
 - Tryptophan
 - Tyrosine
 - Proline
 - Glutamic acid

answer

Answers

1.
 - a. Each causes hyperammonemia (elevated levels of ammonia in the blood). Ammonia is toxic. Where there is a block, the intermediates pile up. Apparently some intermediates can be excreted to get rid of some nitrogen (argininosuccinic aciduria). The closer the defect is to the beginning of the cycle, the less nitrogen would accumulate in intermediates and the more would accumulate in ammonia.
 - b. Citrulline is the immediate precursor of argininosuccinic acid, so a block in the enzyme catalyzing the conversion would cause a build up of the precursor citrulline, which would then be dumped into the blood stream. (Remember that a higher K_m means that it takes a higher substrate concentration to reach V_m and that the enzyme has a lower affinity for the substrate.)
 - c. No. The enzyme is simply altered in some way so that it has a much higher K_m and, hence, is less effective. (This probably owes to a change in a single amino acid causing a change in conformation and thereby altering binding or catalysis.)
2.
 - a. There is a general L-amino acid oxidase of liver and kidney which has a relatively low activity and acts as follows:



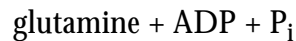
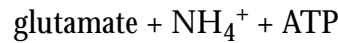
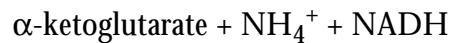
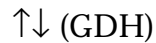
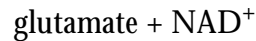
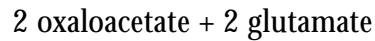
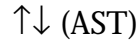
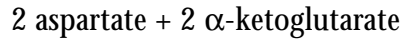
- b. A general D-amino acid oxidase is found in liver and kidney. It oxidizes D-amino acids should any show up. (Some like D-alanine are normal constituents of some bacterial cells walls.) D-Amino acids would undoubtedly be quite harmful. Glycine has no D and L optical isomers.
- c. $\text{L-glutamate} + \text{NAD}^+ + \text{H}_2\text{O} \leftrightarrow \alpha\text{-ketoglutarate} + \text{NADH} + \text{NH}_4^+$

This is the principle way that ammonia is removed since amino groups collect in glutamate by transamination.

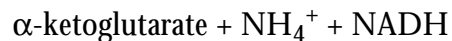
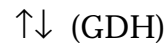
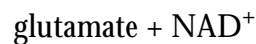
- d. Amino groups are removed from amino acids by transamination with α -ketoglutarate. In this way, amino nitrogen collects in glutamate. It is then removed with the aid of

glutamic dehydrogenase. (Of course, transamination is also an important synthetic reaction.)

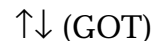
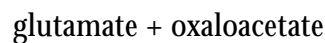
3. a. (This is one possible series of reactions.) In the brain:



The specific nitrogen from aspartate may be at any of the two places in glutamine. The glutamine is transported in the blood to the liver, where:



or



Both NH_4^+ and aspartate are nitrogen sources for urea which is formed in the liver and enters the blood.

b. Most are listed in the quoted paragraph, viz., urea, creatinine, and uric acid. Some smaller amounts of amino acids would add to the serum NPN.

- | | |
|--------------------------|-----------------------|
| 4. e | 13. d |
| 5. d | 14. a, c |
| 6. b | 15. a, c |
| 7. b | 16. a, b, c, d |
| 8. d | 17. b, d |
| 9. a, b, c | 18. d |
| 10. a, c | 19. c |
| 11. a, c, d, e, g | 20. c |
| 12. b, d | 21. a, c, e |

Module 2: Amino Acid Metabolism, II. Carbon Skeleton Metabolism

Objectives:

1. After reading a given passage from a medical journal or textbook on amino acid metabolism, which may be either a clinical investigation or a biochemical description, answer questions about the passage, which may involve the drawing of inferences or conclusions, or use the information given to solve a problem.

In order to accomplish objective 1, you will need to be able to do the following, which are also objectives:

2. a. Describe the characteristics of each of the following groups of amino acids (with respect to catabolic fate, i.e., glycolytic or TCA cycle intermediate).
 - i. alanine, glycine, cysteine, serine
 - ii. aspartate, asparagine
 - iii. glutamine, glutamate, proline, arginine, histidine
 - iv. threonine, methionine
 - v. phenylalanine, tyrosine
 - vi. valine, isoleucine, leucine
 - vii. lysine, tryptophan
- b. When given an amino acid, indicate to which group it belongs, i.e., what is the principal catabolic fate.
- c. Describe the catabolic pathway for each amino acid in groups i-iv.
3. a. Describe the relationships between amino acid catabolism and (i) pernicious anemia, (ii) propionic acidemia, (iii) methylmalonic acidemia, (iv) maple-syrup urine disease, (v) alcaptonuria, (vi) phenylketonuria (PKU), (vii) homocystinuria, (viii) cystathioninuria, (ix) albinism, and (x) tyrosinemia.
- b. Describe the enzymic defects in ii-x.
4. Outline the synthesis of various human non-essential amino acids.

5. Describe the roles of the following in amino acid metabolism
 - a. S-adenosylmethionine (SAM or AdoMet)
 - b. folic acid
 - c. tetrahydrofolate
 - d. tetrahydrobiopterin
 - e. Vitamine B₁₂
6. List various one-carbon carriers and describe their role in one-carbon metabolism.
7. Describe the pathways for the regeneration of methionine.
8. Explain the relationship between folic acid and vitamin B₁₂ metabolism.
9. Describe in general terms the mechanism of action of antifolates and sulfa drugs.
10. Describe and discuss
 - a. the formation of histamine
 - b. the conversion of tryptophan into serotonin
 - c. the conversion of tyrosine into L-DOPA, dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline).
 - d. the formation of γ -aminobutyric acid (GABA)
 - e. the formation of glutathione
 - f. the formation of melanin
11. Describe or define the terms in the [NOMENCLATURE AND VOCABULARY](#) as well as the [KEY WORDS](#) list of this module and be able to properly use them.

Clinical aspects:

- A. Montgomery *et al.*:
 1. Vitamin B12 Deficiency, p. 39.
 2. Amino Acid Metabolism in Starvation, p. 365.
 3. Hartnup Disease, p. 369.
 4. Methylmalonic Acidemia, p.373.
 5. Glutathionuria, p. 375.
 6. Tropical Sprue, p. 583.
 7. Defect in B12 coenzymes synthesis, p. 586.
 8. Folate deficiency in alcoholism, p. 594.

B. Devlin:

1. Nonketogenic Hyperglycinemia, *Clin. Corr.* 11.3, p. 461.
2. Folic acid deficiency, *Clin. Corr.* 11.4, p. 463.
3. Phenylketonuria, *Clin. Corr.* 11.5, p. 465.
4. Disorders, of tyrosine metabolism, *Clin. Corr.* 11.6, p. 467.
5. Parkinson's disease, *Clin. Corr.* 11.7, p. 467.
6. Hyperhomocysteinemia and Atherogenesis, *Clin. Corr.* 11.8, p. 471.
7. Other diseases of sulfur amino acids, *Clin. Corr.* 11.9, p. 471.
8. Diseases of metabolism of branched-chain amino acids, *Clin. Corr.* 11.10, p. 479.
9. Diseases of propionate and methyl-malonate metabolism, *Clin. Corr.* 11.12, p. 480.
10. Diseases involving lysine and ornithine, *Clin. Corr.* 11.12, p. 481.
11. Histidinemia, *Clin. Corr.* 11.13, p. 482.
12. Diseases of folate metabolism, *Clin. Corr.* 11.14, p. 483.

Nomenclature and Vocabulary:

[Aminoacidemia](#)

[Amphibolic reaction](#)

[Biogenic \(physiological\) amines](#)

[Glycogenic amino acid](#)

[Ketone body](#)

[Aminoaciduria](#)

[Anaplerotic reaction](#)

[Folate antagonist](#)

[Ketogenic amino acid](#)

[One-carbon metabolism](#)

Key Words:

Amino acids

Biochemistry

Dopamine

Epinephrine

Folic acid

Glutathione

Homocystinuria

Maple syrup urine disease

Metabolism

Neuroregulators

Norepinephrine

Phenylketonuria

Tetrahydrofolates

Vitamins

Anemia, pernicious

DOPA

Drugs

FIGLU

GABA

Histamine

Levodopa

Melanins

Methionine

Nitrogen

Pharmacology

Serotonin

Vitamin B₁₂

STUDY GUIDE-2

I. What happens to the carbon skeletons of amino acids after deamination? What are glycogenic and ketogenic amino acids?

Amino acids can be classified as **glycogenic** (or glucogenic) or **ketogenic** amino acids. The **glycogenic amino acids** are those that give rise to pyruvate or an intermediate of the TCA cycle and, hence, can be converted to glucose, although they usually are oxidized to carbon dioxide and water via the TCA cycle.

Ketogenic amino acids are those which give rise to acetyl-CoA or to acetoacetate. Acetoacetate can be reduced to 3-hydroxybutyrate. These two compounds, termed ketone bodies, can be cleaved to acetyl-CoA and oxidized in the TCA cycle. They are actively oxidized by muscles and sometimes by the brain to produce energy.

Alanine, serine, cysteine, glycine, glutamic acid, glutamine, arginine, proline, histidine, methionine, valine, aspartic acid and asparagine are glycogenic amino acids whereas leucine and lysine are ketogenic. Phenylalanine, tyrosine, isoleucine and tryptophan are both ketogenic and glycogenic amino acids. Threonine is glycogenic when it is metabolized to succinyl-CoA and is both glycogenic and ketogenic when it is converted to glycine and acetyl-CoA.

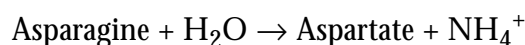
The degradation of individual amino acids is briefly summarized below and in Figure 1.

A. Precursors of pyruvate.

1. Alanine transaminated to pyruvate.
2. Serine directly deaminated by serine dehydratase, producing ammonia and pyruvate.
3. Glycine can be converted to serine by serine hydroxymethyl transferase. N^5, N^{10} -methylene-tetrahydrofolate is the 1-carbon donor.
4. Cysteine - one of the pathways for cysteine degradation is via cysteine desulhydrase which produces H_2S and pyruvate. Other pathways also produce pyruvate.

B. Precursors of oxaloacetate.

1. Aspartate transamination produces oxaloacetate.
2. Asparagine converted to aspartate by asparaginase.



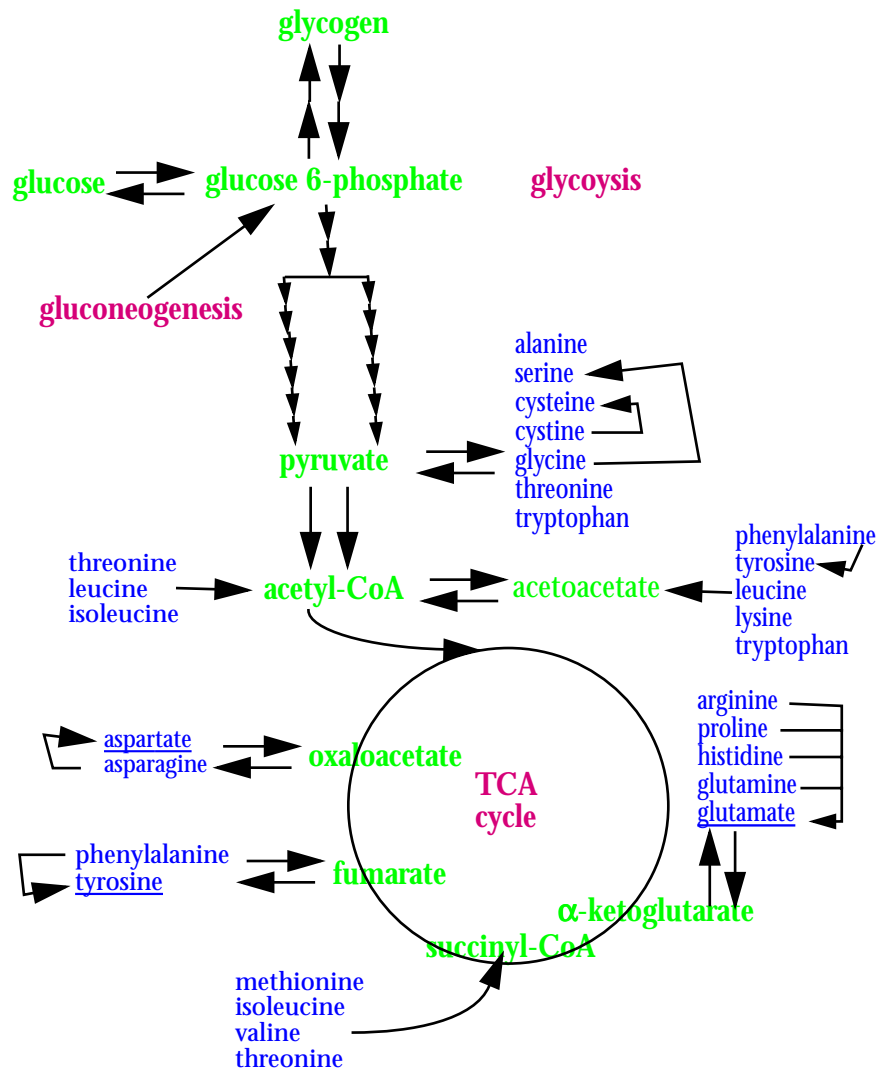


Figure 1.--Metabolic relationships between glycolysis, the TCA cycle, and amino acid metabolism.

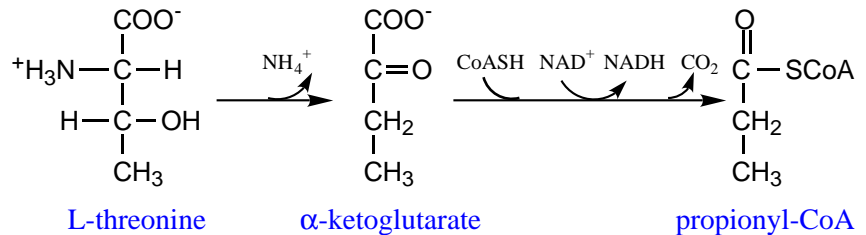
C. Precursors of α -ketoglutarate

1. Glutamate transamination and oxidative deamination yield α -ketoglutarate.
2. Glutamine converted to glutamate and NH_4^+ by glutaminase.
3. Arginine and 4.) proline both degraded via glutamate gamma-semialdehyde to glutamate.
5. Histidine also degraded to glutamate.

D. Precursors of succinyl-CoA.

1. Threonine-serine (threonine) dehydratase produces ammo-

nia and α -ketobutyrate. α -Ketobutyrate reacts with an enzyme comparable to pyruvate dehydrogenase giving propionyl-CoA. Propionyl-CoA is then carboxylated to methylmalonyl-CoA which is then racemized and mutased to give succinyl-CoA. Propionyl-CoA produced from odd-carbon fatty acids is converted to succinyl-CoA by the same reactions. Threonine can also be converted to glycine and acetyl-CoA by another pathway.



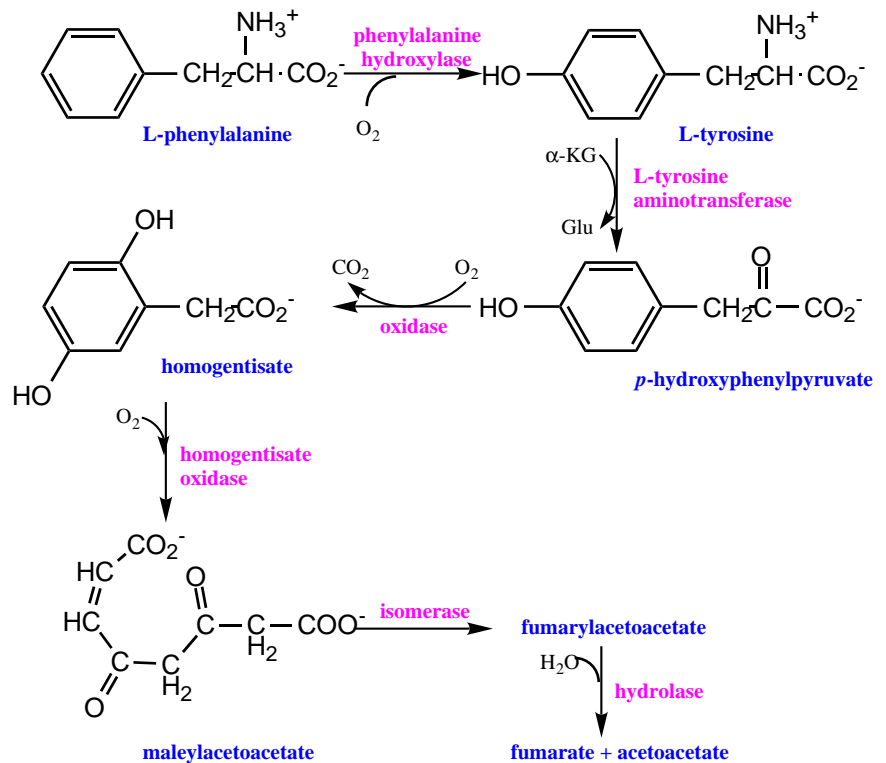
2. Valine transaminated and decarboxylated to give isobutyryl CoA which is converted to methylmalonyl-CoA.
3. Methionine converted to homocysteine, which as we will see later, condenses with serine to form cystathionine, which is cleaved to give cysteine and α -ketobutyrate. α -Ketobutyrate is then degraded as described for threonine.

E. Degradation of phenylalanine and tyrosine.

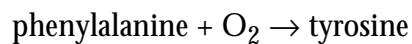
The pathway for phenylalanine and tyrosine degradation is given below. The reaction, catalyzed by phenylalanine hydroxylase requires the coenzyme tetrahydrobiopterin which is used as an electron carrier. There are two important genetic disorders associated with phenylalanine and tyrosine degradation, alcaptonuria and phenylketonuria.

One of the earliest genetic diseases to be studied was alcaptonuria. The most striking symptom is the dark color of the urine, which is caused by the presence of oxidized homogentisate, an intermediate in phenylalanine and tyrosine metabolism.

Alcaptonuria is caused by the absence of homogentisate oxidase, which leads to the accumulation of homogentisate. The disease is basically benign. Its significance is that it was one of the first demonstrations that a disease could be traced to a defect in a single enzyme.



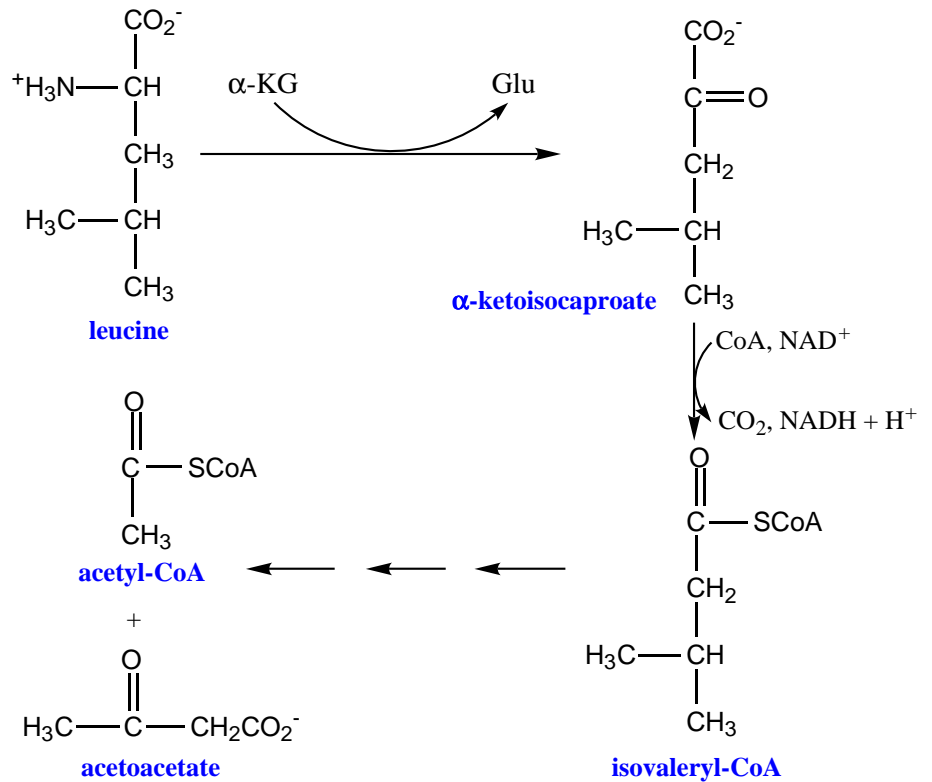
Another genetic disease traced to amino acid metabolism is phenylketonuria. PKU is a serious disease that occurs about once in every 10,000 births. The enzyme, phenylalanine hydroxylase, which catalyzes the reaction:



is absent or deficient. The accumulation of phenylalanine derivatives leads to mental retardation and a greatly shortened life-span. If diagnosed shortly after birth, extensive brain damage can be prevented by maintenance of a low phenylalanine diet. PKU will be discussed again later.

II. What is Maple Syrup Urine Disease?

Maple syrup urine disease is a rare disorder of the catabolism of the branched-chain amino acids, leucine, valine, and isoleucine. The catabolic pathway of each of these is exemplified by that of leucine.

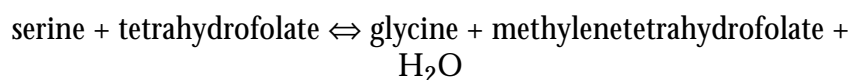


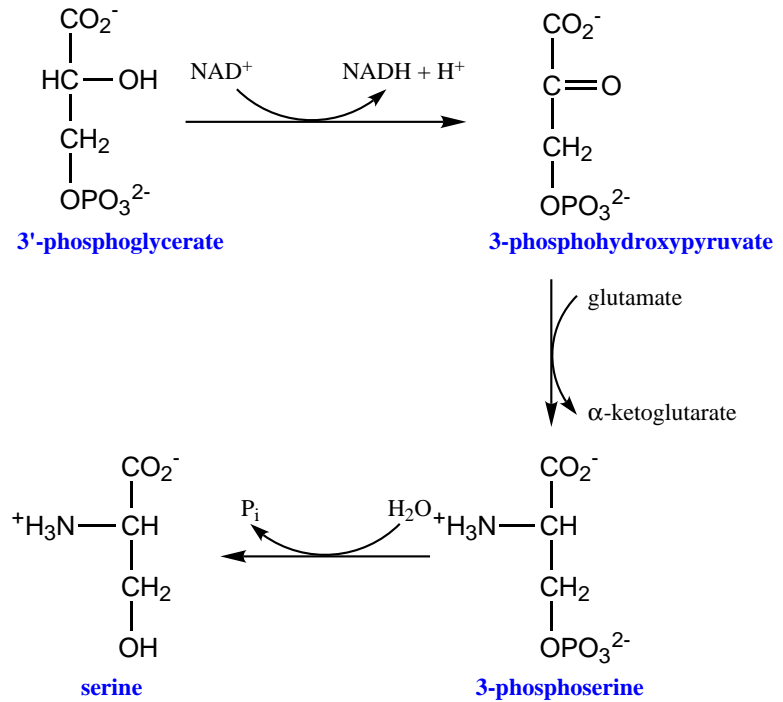
The enzyme catalyzing the second step, the oxidative decarboxylation of α -ketoisocaproate to yield isovaleryl-CoA, is deficient or absent in this disease (as are the analogous enzymes of the isoleucine and valine pathways). This defect occurs about once in every 100,000-250,000 births and leads to mental retardation.

III. How are the Non-essential Amino Acids Synthesized?

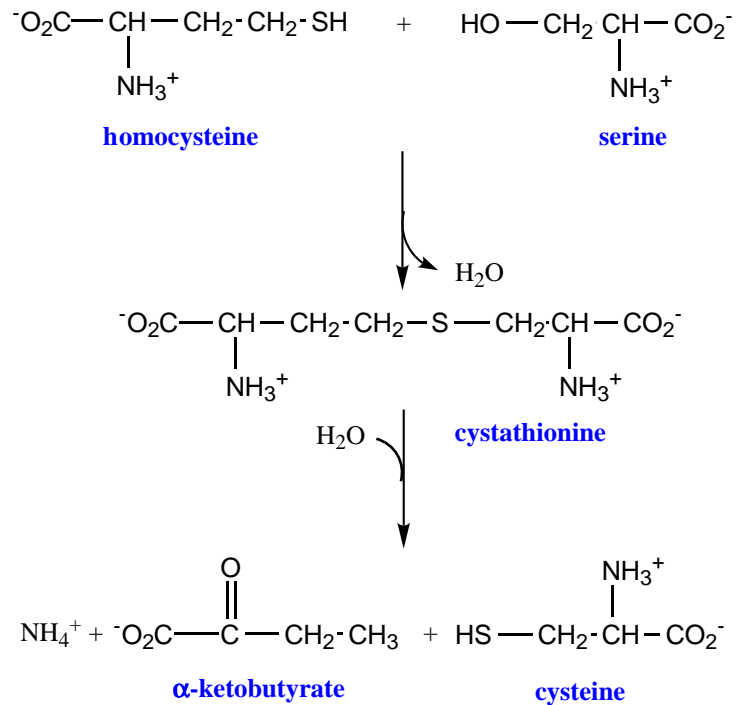
The biosynthetic pathway of most nonessential amino acids is quite simple. Glutamate is produced by reductive amination of α -ketoglutarate. Alanine and aspartate are synthesized by the transamination reactions of pyruvate and oxaloacetate, respectively, with glutamate. Glutamine and asparagine are produced by amination of glutamate and aspartate, respectively.

The synthesis of serine is complicated. It starts with 3'-phosphoglycerate, an immediate of the glycolytic pathway. Serine is the immediate precursor of both glycine and cysteine. Glycine is derived from serine in a reaction in which tetrahydrofolate accepts a methylene group from serine.



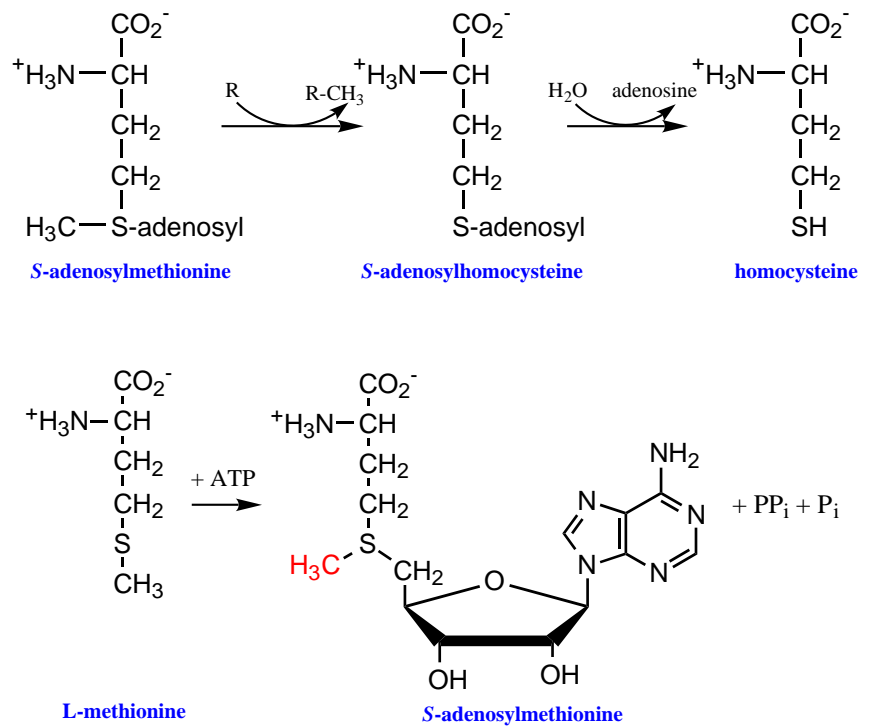


Cysteine is made by the reaction of homocysteine and serine.



The precursor of cysteine, homocysteine, is synthesized from *S*-adenosyl-methionine, which in turn is synthesized from the essential

amino acid methionine and ATP.

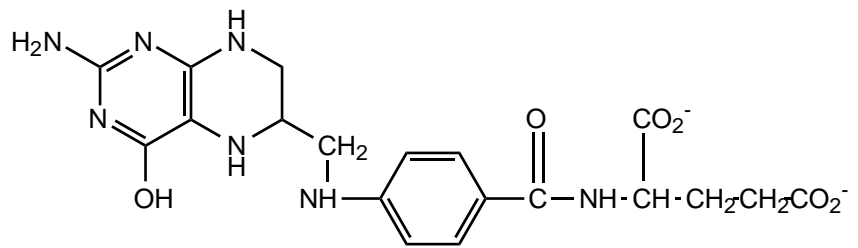


The methyl group attached to the sulfur atom (indicated in red in the above figure) can be donated to suitable acceptors (such as phosphatidyl ethanolamine).

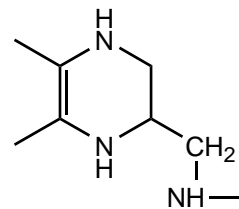
In summary, **glutamate** can be formed by reversal of the glutamate dehydrogenase reaction; **aspartate** and **alanine** are made by transamination of oxaloacetate and pyruvate; **arginine** is continuously made by the reactions of the urea cycle but, if arginine is removed for protein synthesis, ornithine from some other source must be supplied to replace it. **Proline** can be synthesized by an NADPH-dependent reduction of the cyclic form of glutamate semialdehyde. The source of the semialdehyde is not known with certainty. **Serine** and **glycine** are made from glucose and glutamate as described previously. **Glutamine** is made from glutamate and NH_4^+ at the expense of a high-energy phosphate bond. **Asparagine** is made from aspartate by the transfer of the glutamine amide nitrogen at the expense of a high-energy phosphate bond. **Tyrosine** can be made from phenylalanine, and **cysteine** can be made from methionine.

IV. What is the general function of tetrahydrofolate?

The structure of tetrahydrofolate is shown below:

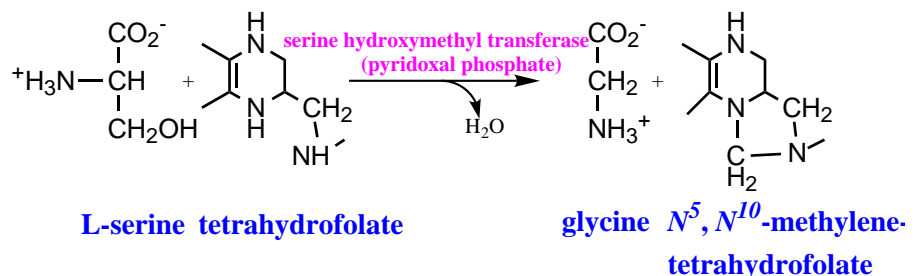


The reactive portion of the molecule is:



This reactive portion can accept one-carbon units viz., methyl (-CH₃), formyl (-CHO), methylene (-CH₂-), hydroxymethyl (-CH₂OH), methylidene (=CH-), and formimino (-CH₂=NH) groups; and conversely the one-carbon derivatives of tetrahydrofolate can transfer these units to various metabolic intermediates. One-carbon transfers of this type are involved in the synthesis of many compounds in living cells. Among the reactions involving one-carbon transfers are: 1) interconversions of amino acids, 2) the biosynthesis of purines and pyrimidines, 3) the biosynthesis of creatine, and 4) the biosynthesis of choline, and thereby acetylcholine and phosphatidylcholine (lecithin).

These one-carbon transfers are exemplified by the synthesis of glycine from serine.



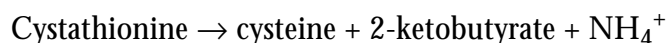
Because of the involvement of folic acid in numerous metabolic path-

ways, analogs of folic acid are of significant clinical interest. The microorganisms cannot absorb folic acid but require *p*-aminobenzoic acid (PABA) to synthesize folates. PABA makes up part of the folic acid structure. Therefore, sulfonamides, such as sulfanilamide, that mimic the structure of PABA, can be competitive inhibitors for the enzyme involved in this reaction, and, therefore, antibacterial agents. But they also have some toxicity to humans.

Compounds which competitively inhibit the dehydrogenases catalyzing the reduction of folic acid to dihydrofolic acid and subsequently to tetrahydrofolic acid (found in the liver) are also folic acid antagonists.

V. What is the cause of homocystinuria and cystathioninuria?

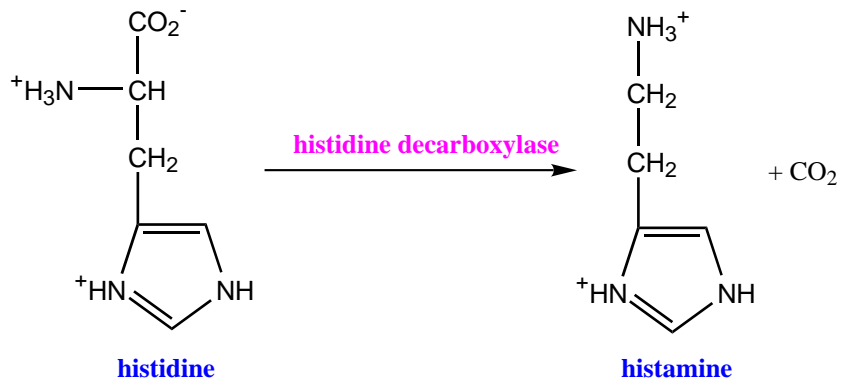
Genetic diseases also affect amino acid synthesis. Both homocystinuria and cystathioninuria result from blocks in the formation of cysteine and lead to neurological disturbances.



The first reaction is catalyzed by cystathionine synthase. When this enzyme is defective, homocysteine accumulates and its oxidized form, homocystine, is excreted. The second reaction is catalyzed by cystathioninase which when defective causes accumulation and excretion of cystathionine. Both of these enzymes require pyridoxal phosphate as a coenzyme. About half of the individuals with these disorders respond to high doses of vitamin B₆. Apparently, in these patients, the genetic defect alters the affinity of the coenzyme for the apoenzyme. High levels of coenzyme favor its binding to the defective enzyme, thus allowing some metabolism to proceed.

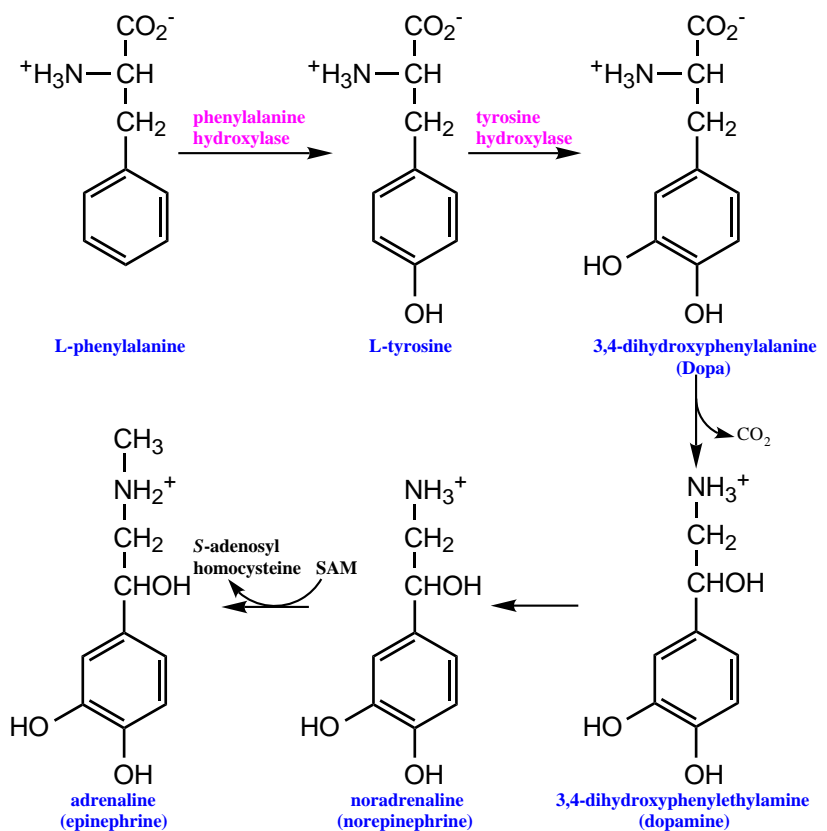
VI. What are some of the products of decarboxylation?

In addition to deamination, amino acids can undergo decarboxylation. These reactions require pyridoxal phosphate as a coenzyme. Many of the amines formed in these reactions have potent physiological effects. Examples follow.



Histamine stimulates gastric secretion and is a circulatory depressor.

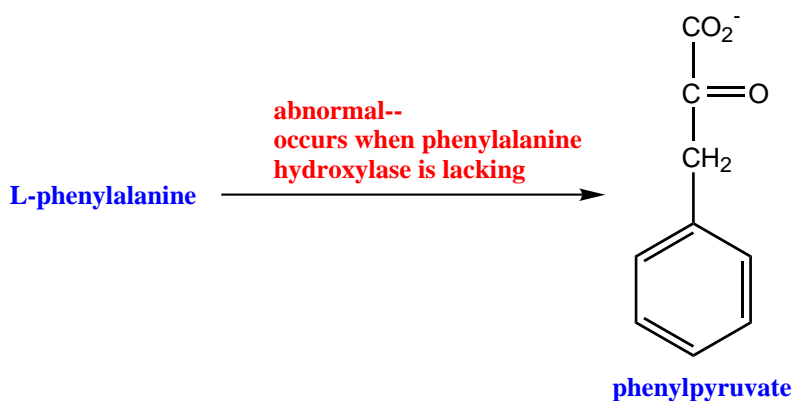
The following shows the synthesis of adrenalin (epinephrine) from phenylalanine and/or tyrosine by a pathway involving amino acid decarboxylation.



Adrenaline and noradrenaline are synthesized and stored in chromaffin cells of the adrenal medulla. Upon stimulation by the central nervous system, they are released into the blood.

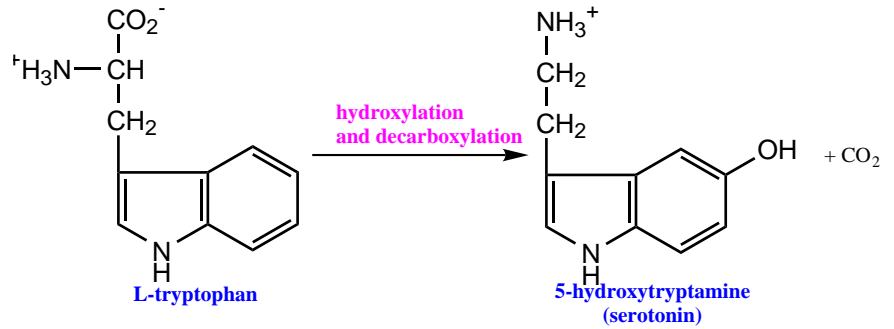
(In organic nomenclature, the prefix "nor" indicates either a change from the trivial name of a branched-chain compound into that of a straight-chain compound or a lower homolog. A homolog is a member of a series of compounds whose structures differ regularly by some radical, for example $-\text{CH}_2-$, from those of its adjacent neighbors in the series. Homo- is a prefix meaning similarity. In organic nomenclature, it indicates a difference of $-\text{CH}_2-$ but an otherwise identical structure.)

The enzymes catalyzing the conversion of tyrosine to noradrenaline are also contained in organelles near synapses. Noradrenaline is bound and stored in particles associated with these organelles. The active structure of the synapse apparently contains a small amount of the amine which is replenished from these stores after discharge during transmission. *S*-Adenosylmethionine is the methyl donor for the conversion of noradrenaline to adrenalin.

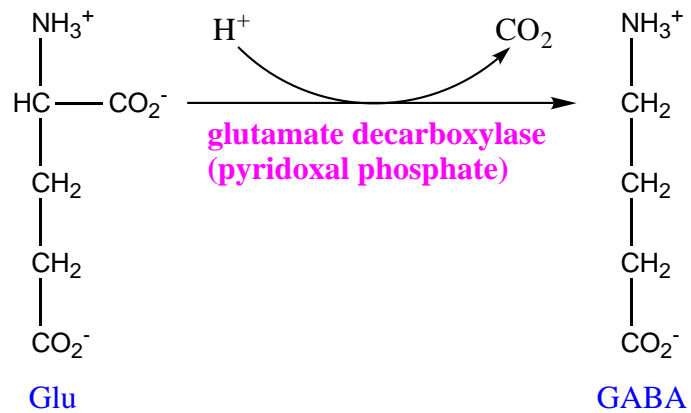


Adrenaline increases energy producing reactions. It and noradrenaline do this by binding with an **alpha receptor** and activate adenylate cyclase which causes an increased formation of cyclic AMP. This sets off a cascade of events which results in a rise in concentration of blood glucose and mobilization of fatty acids from adipose tissue. These hormones also effect an increase in the rate and depth of contraction of heart muscle and suppress contraction of many smooth muscles. 3,4-Dihydroxyphenylethylamine (dopamine) is an intermediate in this pathway. Dopamine increases blood pressure, mainly by enhancing cardiac contraction. It also effects renal vasodilation. In Parkinsonism, there is a marked decrease in the content of dopamine in the substantia nigra and the corpus striatum. L-Dopa is employed rather than dopamine, however, in the treatment of Parkinson's syndrome because dopamine does not readily penetrate the blood-brain barrier. L-Dopa (levodopa) presumably increases the concentration of dopamine in basal ganglia.

There are a number of other amines that are made by decarboxylation. One other is serotonin. It is an important neurotransmitter and causes contraction of smooth muscles in arterioles and bronchioles.



Another is γ -amino butyrate (GABA) which is concentrated in the brain and synaptic vesicles and is an important neurotransmitter.



Problem Set

1. Deficiency of vitamin B₁₂ leads to accumulation of:

- a. dTMP
- b. purines
- c. methionine
- d. 5,10 methylene tetrahydrofolate
- e. methylmalonate
- f. S-adenosyl methionine (SAM)

[answer](#)

2. Maple syrup urine disease is a defect in the metabolism of branched-chain amino acids due to deficiency of:

- a. branched-chain amino acid transaminase
- b. branched-chain α -keto acid dehydrogenase (decarboxylase)
- c. propionyl CoA carboxylase
- d. pyruvate dehydrogenase (decarboxylase)
- e. enzymes which oxidize acyl CoA esters derived from those amino acids
- f. none of the above

[answer](#)

3. Vitamin B₁₂ and folic acid are often interrelated in medicine. This is because:

- a. both are required for biosynthesis of branched-chain amino acids
- b. both are involved in transfer of one carbon fragment
- c. B₁₂ is needed for folate biosynthesis
- d. both are involved in synthesis of methionine from homocysteine

[answer](#)

4. An amino acid which is strictly ketogenic will be able to yield:

- a. energy
- b. ketone bodies
- c. fatty acids
- d. glucose

answer

5. In phenylketonuria (PKU):
- generally phenylalanine hydroxylase is deficient
 - tyrosine becomes an essential amino acid
 - blood level of phenylpyruvate rises
 - there is black urine

answer

6. A deficiency of tetrahydrobiopterin, or its abnormal metabolism, would affect the
- biosynthesis of cysteine
 - catabolism of methionine
 - biosynthesis of tyrosine
 - catabolism of branch-chain amino acids

answer

7. Homocystinuria
- may be due to a deficiency of cystathionine synthetase
 - may be due to an inability to methylate homocysteine to methionine
 - may be associated with methylmalonic aciduria due to an inability to form active B₁₂ coenzyme
 - may be treated, in some cases, with dietary supplements of cysteine or with vitamin B₆

answer

8. α -ketoglutarate can be formed by the metabolism of:
- | | |
|------------------|---------------|
| a. aspartic acid | e. histidine |
| b. glutamic acid | f. proline |
| c. arginine | g. leucine |
| d. alanine | h. isoleucine |

answer

9. Methionine regeneration involves:
- homocysteine
 - vitamin B₁₂
 - 5-methyltetrahydrofolate
 - 5,10-methylene tetrahydrofolate

answer

10. Alcaptonuria, an inborn error of metabolism, is due to the absence of the enzyme:
- tyrosinase
 - phenylalanine hydroxylase
 - DOPA decarboxylase
 - homogenistic acid oxidase
 - DOPA oxidase

answer

11. Succinyl-CoA can be formed by the metabolism of:
- | | |
|---------------|--------------|
| a. methionine | e. leucine |
| b. glutamine | f. glycine |
| c. valine | g. threonine |
| d. isoleucine | h. proline |

answer

12. Which of the following are both ketogenic and glucogenic amino acid:
- | | |
|------------------|---------------|
| a. isoleucine | e. tryptophan |
| b. leucine | f. tyrosine |
| c. glycine | g. valine |
| d. glutamic acid | h. methionine |

answer

13. The sulfa drugs, such as sulfanilamide, act
- to inhibit single-carbon transfers from tetrahydrofolic acid (FH4) compounds
 - to inhibit the production of tetrahydrofolic acid (FH4) from dietary folate
 - to overcome feedback control of purine nucleotide biosynthesis
 - as pseudo-feedback inhibitors of purine nucleotide biosynthesis
 - to inhibit tetrahydrofolic acid (FH4) *de novo* synthesis in certain bacteria

answer

14. Pyruvate can be formed by the metabolism of:

- a. glycine
- b. serine
- c. cysteine
- d. alanine
- e. aspartic acid
- f. methionine
- g. leucine
- h. histidine

[answer](#)

15. The metabolism of glycine is important in the formation and utilization of one carbon units; A vitamin functioning in glycine metabolism is:

- a. folic acid
- b. thiamine
- c. pantothenic acid
- d. vitamin E
- e. vitamin B₁₂
- f. ascorbic acid

[answer](#)

16. In the presence of dietary tyrosine in humans

- a. phenylalanine is a nutritionally nonessential amino acid
- b. phenylketonuria cannot occur
- c. less phenylalanine is required in the diet
- d. tyrosine can be reduced to phenylalanine
- e. none of the above

[answer](#)

Answers

- | | |
|----------------------|-----------------------|
| 1. e | 9. a, b, c |
| 2. b | 10. d |
| 3. b, d | 11. a, c, d, g |
| 4. a, b, c | 12. a, e, f |
| 5. a, b, c | 13. e |
| 6. c | 14. a, b, c, d |
| 7. a, b, c, d | 15. a |
| 8. b, c, e, f | 16. c |

Module 3: Purine and Pyrimidine Metabolism

Objectives:

1. After reading a given passage from a medical journal or textbook on purine or pyrimidine metabolism (which may be either a clinical investigation or a biochemical description), answer questions about the passage (which may involve the drawing of inferences or conclusions) or use the information given to solve a problem.

In order to accomplish objective 1, you will need to be able to do the following which are also objectives:

2. Draw the structure of purine, pyrimidine, adenine, guanine, hypoxanthine, xanthine, uric acid, orotic acid, cytosine, uracil, and thymine.
3. Draw the structure of the nucleosides, deoxynucleosides, nucleotides, and deoxynucleotides of the bases listed in objective 2.
4. *De novo* and salvage pathways for purine and pyrimidine nucleotide synthesis and NAD synthesis all utilize PRPP. Using an equation, describe the synthesis of PRPP.
5. Using an equation, describe the committed step in the *de novo* synthesis of purine nucleotides and show how this enzyme is regulated.
6. Inosine 5'-monophosphate (IMP) is the precursor purine nucleotide. Using equations describe how AMP and GMP are formed from IMP.
7. Describe the interconversion of purine nucleotides and show how these reactions are regulated.
8. Allopurinol is a drug used for the treatment of gouty arthritis. Using equations, explain
 - a. how uric acid is formed in purine metabolism
 - b. how allopurinol prevents uric acid formation.
9. Compare and contrast the location of, and the reaction for, the synthesis of carbamoyl phosphate in pyrimidine formation and

in the urea cycle.

10. Using equations, describe the various steps involved in the *de novo* synthesis of pyrimidine nucleotides. How are these reactions regulated?
11. Name the sources of various carbon and nitrogen atoms in the purine and pyrimidine rings.
12. What are the major differences between the synthesis of purine and pyrimidine nucleotides?
13. Describe the salvage pathways for purines and pyrimidines.
14. Describe the interconversion of pyrimidine nucleotides and show how these nucleotides are degraded.
15.
 - a. Explain how purine ring and thymidylate synthesis is inhibited by folate antagonists such as methotrexate.
 - b. Describe the clinical uses of methotrexate, sulfa drugs and azaserine.
16. Describe the molecular basis for the Lesch-Nyhan syndrome and orotic acidemia.
17. Describe how deoxyribonucleotides needed for DNA synthesis are formed.
18. Using equations, describe the different kinase reactions involved in the formation of various nucleotides.
19. Describe or define the terms in the [NOMENCLATURE AND VOCABULARY](#) list as well as the [KEY WORDS](#) list and be able to use them properly.

Clinical Aspects:

- A. Montgomery, *et al.*
 1. Gout, p. 578.
 2. Adenosine deaminase in immunodeficiency, p. 588.
 3. Orotic Aciduria, p. 590.
 4. Excessive Purine Synthesis in Gout, p. 591.
 5. Lesch-Nyhan Syndrome, p. 591.
 6. Chemotherapy of Breast Cancer, p. 593.
 7. Methotrexate Treatment of Adenocarcinoma, p. 593.
 8. Purine Nucleoside Phosphorylase Deficiency, p. 595.

9. Adenine Phosphoribosyltransferase Deficiency, p. 595.

B. Devlin:

1. Gout, *Clin. Corr.* 12.1, p. 498.
2. Lesch-Nyhan Syndrome, *Clin. Corr.* 12.2, p. 499.
3. Immunodeficiency diseases associated with defects in purine nucleoside metabolism, *Clin Corr.* 12.3, p. 503.
4. Hereditary Orotic Aciduria, *Clin. Corr.* 12.11, p. 505.

**Nomenclature and
Vocabulary:**

Gout

PRPP

Purine metabolism

Pyrimidine metabolism

Uric acid

Key Words:

Adenosine Monophosphate

Biochemistry

Drugs

Lesch-Nyhan Syndrome

Nitrogen

Nucleotides

Purines

Uric Acid

Allopurinol

DNA

Gout

Metabolism

Nucleosides

Pharmacology

Pyrimidines

Problem Set

1. A nucleoside may contain:
 - a. a purine
 - b. 2-deoxyribose
 - c. a pyrimidine
 - d. a phosphate
 - e. a ribose

[answer](#)

2. The normal end product of purine catabolism in humans is
 - a. urea
 - b. uric acid
 - c. creatinine
 - d. xanthine
 - e. hypoxanthine

[answer](#)

3. Deoxyribonucleotides needed for DNA synthesis are formed by reduction of:
 - a. ribose
 - b. phosphoribosyl pyrophosphate (PRPP)
 - c. ribonucleosides
 - d. ribonucleoside monophosphates
 - e. ribonucleoside diphosphates
 - f. ribonucleoside triphosphates

[answer](#)

4. The "committed step" in pyrimidine biosynthesis:
 - a. provides an excellent example of positive feedback by an allosteric modifier
 - b. results in the formation of dihydroorotic acid
 - c. is the formation of N-carbamyl aspartic acid
 - d. is catalyzed by orotic decarboxylase
 - e. requires ATP
 - f. is the formation of carbamoyl phosphate
 - g. is the formation of PRPP

answer

5. Which of the following is (are) required for the conversion of dUMP to dTMP by thymidylate synthetase?
- thioredoxin
 - SAM (*S*-adenosylmethionine)
 - THF (tetrahydrofolate)
 - ATP as source of energy
 - a flavin derivative (FMN)

answer

6. Methotrexate inhibits:
- CTP synthetase
 - dihydrofolate reductase
 - xanthine oxidase
 - thymidylate synthetase
 - ribonucleotide reductase
 - ribose phosphate pyrophosphokinase (PRPP synthetase)

answer

7. Lesch-Nyhan syndrome is caused by deficiency of:
- adenosine phosphoribosyl transferase
 - pyrimidine phosphoribosyl transferase
 - hypoxanthine-guanine phosphoribosyl transferase
 - ribonucleotide reductase
 - adenosine deaminase
 - purine nucleoside phosphorylase

answer

8. The formation of uric acid from purines is catalyzed by:
- adenylic acid deaminase
 - uricase
 - allantoinase
 - urease
 - xanthine oxidase

answer

9. Phosphoribosyl pyrophosphate (PRPP):
- is required in both purine and pyrimidine nucleotide synthe-

sis

- b. is produced by phosphorylation of ribose-1-pyrophosphate
- c. is required in salvage pathway for both purine and pyrimidine nucleotide formation
- d. formation is the committed step in *de novo* purine synthesis

[answer](#)

10. Direct precursors for the synthesis of both purine and pyrimidine nucleotides are:

- a. aspartate
- b. CO₂
- c. glutamine
- d. glycine
- e. fumarate
- f. formate
- g. ribose-1-phosphate
- h. PRPP

[answer](#)

11. Correct statement(s) regarding the regulation of the cellular concentration of 5'-phospho-ribosyl-1-pyrophosphate (PRPP) include which of the following?

- a. The formation of PRPP is catalyzed by PRPP synthetase, an allosteric enzyme with an absolute requirement for inorganic phosphate (P_i).
- b. The usual low levels of P_i in cells keep PRPP synthetase activity low.
- c. PRPP synthetase is allosterically inhibited by nucleoside di- and triphosphates.
- d. PRPP synthetase is allosterically inhibited by 2,3-bisphosphoglycerate, which is important in erythrocytes.

[answer](#)

12. Orotic aciduria:

- a. is a disorder of purine metabolism
- b. can be caused by reduced activity of dihydroorotate dehydrogenase
- c. would be expected to occur when there is a deficiency of PRPP synthetase
- d. is characterized by an increase in urinary orotidylic acid
- e. can be alleviated by the administration of uridine or cytidine to the patient

[answer](#)

13. The amino group of GMP during its formation from IMP is derived from:
- free ammonium ion
 - glutamate
 - glutamine
 - aspartate
 - asparagine

[answer](#)

14. de novo synthesis pathways of both purine and pyrimidine nucleotides:
- have all intermediates as derivative of ribose-5'-phosphate
 - are exclusively cytosolic
 - form a nitrogenous base which is then converted to a nucleotide
 - require ATP

[answer](#)

15. Allopurinol is generally used in treating gout. Allopurinol
- reduces serum uric acid level
 - is an activator of uricase
 - is an inhibitor of xanthine oxidase
 - is an inhibitor of guanase

[answer](#)

16. The committed step in purine nucleotide synthesis is the synthesis of
- 5-phosphoribosyl-1-amine
 - PRPP (5-phosphoribosyl-1-pyrophosphate)
 - inosinic acid (IMP)
 - glycinamide ribotide

[answer](#)

17. Methotrexate inhibits formation of substrates which are required in:
- PRPP synthesis
 - purine ring synthesis
 - pyrimidine ring synthesis
 - thymidylate synthesis

[answer](#)

18. The energy source for the conversion of XMP to GMP is:
- a. GTP
 - b. ATP
 - c. UTP
 - d. CTP
 - e. TTP

[answer](#)

Answers

1. a, b, c, e

2. b

3. e

4. c

5. c

6. b

7. c

8. e

9. a, c

10. a, b, c, h

11. a, b, c, d

12. c, e

13. c

14. d

15. a, c

16. a

17. b, d

18. b

Practice Exam

(NOTE: This exam was given years ago. The current exam format is mostly of multiple choice questions.

Part One, Multiple Choice

For each of the following multiple choice questions, choose the most appropriate answer.

1. Alcaptonuria is a:
 - A. defect in tyrosine metabolism characterized by urine that darkens on exposure to oxygen
 - B. defect in tryptophan metabolism characterized by urine that darkens on exposure to oxygen
 - C. defect in tyrosine metabolism that does not involve excretion of metabolites in the urine
 - D. deficiency of phenylalanine hydroxylase
 - E. deficiency of tyrosine aminotransferase

[answer](#)

2. Maple syrup urine disease is a consequence of a deficiency of:
 - A. branched-chain amino acid transaminase
 - B. pyridoxal phosphate
 - C. biotin
 - D. acyl-CoA dehydrogenase
 - E. branched-chain α -keto acid decarboxylase

[answer](#)

3. Of these statements on 1-carbon metabolism:
 1. the carrier at the level of carboxyl groups is SAM
 2. SAM is used directly in purine biosynthesis
 3. tetrahydrofolate carries 1-carbon groups at all oxidation levels
 4. biotin is the cofactor for serine hydroxymethyl transferase
 - A. only 2 is correct
 - B. only 3 is correct
 - C. 2 and 3 are correct
 - D. all are correct
 - E. none are correct

[answer](#)

4. Analyses of NORMAL tissue for transaminases give the following results in relative units:

	Heart	Liver
glutamate-oxaloacetate transaminase (GOT)	7800	7100
glutamate-pyruvate transaminase (GPT)	450	2850

Using the above table to assist you, what serum changes would you expect to observe in hepatic (liver) cirrhosis?

- A. Serum-GOT only is elevated.
 - B. Serum-GPT only is elevated.
 - C. Both enzymes are elevated.
 - D. Serum-GPT only is depressed.
 - E. Both enzymes are depressed.
5. In an uncontrolled diabetic which of the following amino acids would yield both glucose and ketone bodies?
- A. alanine
 - B. tyrosine
 - C. proline
 - D. glycine
 - E. leucine

[answer](#)

6. A toxic product of reactions catalyzed by amino acid oxidases and xanthine oxidase is:
- A. catalase
 - B. hydrogen peroxide
 - C. formate
 - D. peroxisome
 - E. HCHO

[answer](#)

7. A deficiency of Vitamin B₁₂ and folic acid causes megaloblastic anemia. The defect could be associated with:

- A. cystathionine \rightarrow cysteine + homoserine
- B. homoserine \rightarrow α -ketobutyrate
- C. serine + homocysteine \rightarrow cystathionine
- D. homocysteine \rightarrow methionine
- E. methionine + ATP \rightarrow SAM

[answer](#)

8. Which of the following is a membrane-bound enzyme?

- A. glutathione synthetase
- B. γ -glutamyl transpeptidase
- C. cysteinyl-glycinase
- D. 5-oxoprolinase
- E. γ -glutamyl cyclotransferase

[answer](#)

9. In mitochondria, the formation of carbamoyl phosphate requires splitting which of the following number of ATPs?

- A. 0
- B. 1
- C. 2
- D. 3
- E. 4

[answer](#)

10. The 6-amino group of AMP is donated by:

- A. Asp
- B. Gln
- C. Ser
- D. Lys
- E. Arg

[answer](#)

11. In *de novo* pyrimidine biosynthesis, which of the following compounds is an IMMEDIATE precursor in the reaction in which a cytosine nucleotide is produced?

- A. UTP
- B. UDP
- C. UMP
- D. orotidine 5'-phosphate

E. none of the above

[answer](#)

12. Which of the following intermediates directly links the urea cycle with the TCA cycle?

A. acetyl-CoA

B. arginine

C. fumarate

D. glutamate

E. none of these

[answer](#)

13. The first purine in the *de novo* biosynthesis of purine nucleotides is:

A. adenylic acid

B. xanthylic acid

C. inosinic acid

D. guanylic acid

E. none of the above

[answer](#)

14. If insufficient quantities of 5-phosphoribosyl 1-pyrophosphate (PRPP) are present, an accumulation of which intermediate in the pyrimidine biosynthetic pathway will occur?

A. carbamoyl phosphate

B. carbamoyl aspartate

C. dihydroorotate

D. orotidine-5'-monophosphate

E. orotic acid

[answer](#)

15. Which statement below best describes the substance cystathionine?

A. A compound resulting from the condensation of a molecule of serine and a molecule of cysteine.

B. A compound resulting from hydrolysis of *S*-adenosyl homocysteine.

C. A thioether resulting from the condensation of two molecules of cysteine.

D. A compound formed by condensation of a molecule of

homocysteine and a molecule of serine.

[answer](#)

16. Which of the following is involved in formation of deoxyribonucleotides from ribonucleotides?
- A. thioredoxin
 - B. dihydrofolate reductase
 - C. hypoxanthine phosphoribosyl transferase
 - D. 5-phosphoribosyl-1-pyrophosphate
 - E. glutamine

[answer](#)

17. Donald MacRonald, a hamburger manufacturer, has been informed by a government agency that his "Big Don" product is grossly deficient in folic acid. A teenaged student on an exclusive diet of "Big Don", French fries and Cola would be most likely to have an impaired ability for the synthesis of:
- A. choline
 - B. creatine
 - C. thymine of tRNA
 - D. thymine of DNA
 - E. epinephrine

[answer](#)

18. A major theory explaining the observation that a deficiency of vitamins B₁₂ and folic acid both lead to the symptoms of pernicious anemia states that:
- A. B₁₂ is required for the regeneration of free tetrahydrofolate from 5-methyltetrahydrofolate
 - B. B₁₂ and folic acid are both required for the methylmalonyl-CoA mutase reaction
 - C. folic acid is required for the conversion of dietary B₁₂ (cyanocobalamine) to its biologically active form
 - D. both tetrahydrofolate and B₁₂ are required for purine biosynthesis
 - E. B₁₂ and folic acid are both required for the protein synthesis

[answer](#)

19. Which amino acid is the precursor of serotonin?
- A. phenylalanine

- B. tryptophan
- C. tyrosine
- D. glutamic acid
- E. arginine

[answer](#)

20. In serine → glycine which of the following is most directly involved?

- A. N^5, N^{10} methylene tetrahydrofolate
- B. N^5 -methyl tetrahydrofolate
- C. formiminotetrahydrofolate
- D. serine dehydrase
- E. biotin

[answer](#)

21. The committed step in purine synthesis is the step in which:

- A. phosphoribosylamine is synthesized from PRPP and glutamic acid
- B. glutamine is incorporated intact into the molecule on the sugar phosphate unit
- C. N -formylglycinamide ribonucleotide is formed from glycina-mide ribonucleotide with a methyl group transferred from N^5, N^{10} -methylene THFA
- D. phosphoribosylamine is formed enzymatically from PRPP and glutamine
- E. bicarbonate is utilized to carboxylate aminoimidazole ribo-nucleotide

[answer](#)

22. The enzyme defect in homocystinuria is:

- A. cysteine synthase
- B. cystathionine synthase
- C. cystathionase
- D. S -adenosylmethionine sulfatase
- E. S -adenosylhomocysteine sulfatase

[answer](#)

Answer the following questions using the key outlined below:

- A. If 1, 2, and 3 are correct

- B. If 1 and 3 are correct
 - C. If 2 and 4 are correct
 - D. If only 4 is correct
 - E. If all four are correct
23. Conversion of propionyl-CoA to succinyl-CoA:
- 1. is important in the metabolism of some amino acids and certain fatty acids
 - 2. requires a vitamin B₁₂ coenzyme
 - 3. involves a carboxylation and an isomerization
 - 4. generates NADH

[answer](#)

24. The anticancer drug methotrexate:
- 1. inhibits the enzyme thymidylate synthetase
 - 2. inhibits *de novo* purine biosynthesis
 - 3. is a thymine analogue
 - 4. causes a depletion of the tetrahydrofolate pool in cells actively engaged in DNA synthesis

[answer](#)

25. The role(s) of methionine or its derivative include(s):
- 1. acting as a precursor of cysteine
 - 2. acting as a methyl donor in transmethylation
 - 3. contributing to gluconeogenesis
 - 4. transamination to pyruvate

[answer](#)

26. Ammonia or ammonium ions entering the liver via the portal circulation could be incorporated into nontoxic substances by the action of:
- 1. glutamate dehydrogenase
 - 2. carbamoyl phosphate synthetase I
 - 3. glutamine synthetase
 - 4. glutaminase

[answer](#)

27. In the conversion of ammonium ion to urea by the liver:
- 1. carbamoyl phosphate is synthesized in the mitochondria

2. aspartate is a required substrate
3. more than one ATP is required
4. the overall process proceeds in only one direction

[answer](#)

28. Pyrimidine biosynthesis *de novo* resembles purine biosynthesis *de novo* in that:

1. neither require folic acid coenzymes
2. both utilize the amide nitrogen atom of glutamine
3. ribose phosphate is introduced at the first step in each
4. both require aspartate

[answer](#)

29. Transamination:

1. is involved in the synthesis of some amino acids
2. is involved in the breakdown of some amino acids
3. requires pyridoxal 5-phosphate
4. yields ammonium ion

[answer](#)

30. Which of the following mammalian enzymes react(s) with substrate to produce free ammonia or ammonium ion?

1. glutamate dehydrogenase
2. serine dehydratase
3. glutaminase
4. glutamate-oxalacetate transaminase

[answer](#)

31. Which of the following amino acids is/are a direct source of carbon atoms for the *de novo* synthesis of either purines or pyrimidines?

1. glutamine
2. glycine
3. alanine
4. aspartic acid

[answer](#)

32. Carbamoyl phosphate is required for the synthesis of:

1. pyrimidines
2. citrulline

3. urea
4. ornithine

[answer](#)

33. The major carriers of nitrogen from extrahepatic tissues to the liver are most probably:

1. alanine
2. aspartate
3. glutamine
4. glutamate

[answer](#)

Answer the following question using the key outlined below:

- A. If both statement and reason are true and related as to cause and effect
- B. If both statement and reason are true but not related as to cause and effect
- C. If statement is true but reason is false
- D. If statement is false but reason is true
- E. If both statement and reason are false

34. Intermediates of the Krebs cycle provide the carbon skeleton for some non-essential amino acids

BECAUSE

transamination converts an α -keto acid intermediate of the cycle into an α -amino acid.

[answer](#)

Answer the following question using the key outlined below:

- A. if the item is associated with (A) only
 - B. if the item is associated with (B) only
 - C. if the item is associated with both (A) and (B)
 - D. if the item is associated with neither (A) nor (B)
35. Which of the following enzymes catalyzes an irreversible reaction?
- A. glutamate dehydrogenase
 - B. glutamate-pyruvate transaminase (glutamate-alanine aminotransferase)

[answer](#)

36. Epinephrine:
- A. derived from tryptophan
 - B. derived from tyrosine

[answer](#)

37. PRPP is involved in the:
- A. *de novo* synthesis of purine nucleotides
 - B. "salvage" of purine nucleotides

[answer](#)

Part 2, Short Answer

1. Hereditary hyperammonemia is generally caused by a defect in either of two enzymes. Which are these? What are the reactions catalyzed by these enzymes?
2. One of the common forms of phenylketonuria (PKU) is due to deficiency of an enzyme in phenylalanine (Phe) catabolism. These children generally have light skin color (due to less melanin), show increased Phe in blood and excrete a phenylketone (phenylpyruvate) in urine.
 - a. Name the deficient enzyme and write the reaction catalyzed by it.
 - b. How is phenylpyruvate formed? (Hint: How is pyruvate formed from alanine?)
 - c. Melanin is produced from a nonessential amino acid, tyrosine. Why is there a deficiency of melanin in PKU cases?
3. Lesch Nyhan Syndrome is generally caused by severe or complete lack of Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) activity. It is associated with hyperuricemia, excessive uric acid production (due to increased *de novo* synthesis of purine nucleotides), deposit of sodium urate crystals in joints (gout) and kidney, neurological symptoms, e.g., spasticity, mental retardation, self-mutilation, etc. Treatment with Allopurinol decreases the amount of uric acid formed relieving some of the problems caused by urate deposits. There is no known treatment of the neurological symptoms.
 - a. Write the reactions catalyzed by HGPRTase.
 - b. Using equations explain how uric acid is formed in purine metabolism.
 - c. Explain how the lack of HGPRTase activity causes increased synthesis of purine nucleotides.
 - d. What is the mechanism of action of allopurinol in reducing

serum uric acid.

- e. Which purines would be excreted in urine in excess after administration of allopurinol?

ANSWERS

Part One

- | | |
|--------------|--------------|
| 1. A | 20. A |
| 2. E | 21. D |
| 3. E | 22. B |
| 4. C | 23. A |
| 5. B | 24. C |
| 6. B | 25. A |
| 7. D | 26. A |
| 8. B | 27. E |
| 9. C | 28. C |
| 10. A | 29. A |
| 11. A | 30. A |
| 12. C | 31. C |
| 13. C | 32. A |
| 14. E | 33. B |
| 15. D | 34. A |
| 16. A | 35. D |
| 17. D | 36. B |
| 18. A | 37. C |
| 19. B | |

APPENDIX I: Using Acrobat Reader with pdf Files

Portable Document Format (PDF) files can be read by Acrobat Reader, a free program which can be downloaded from the Adobe Web site (<http://www.adobe.com/acrobat>). If Acrobat Reader is installed on your system, it will automatically open simply by double-clicking on the pdf file that you wish to read.

Acrobat Window

The document will be displayed in the center of your window and an index will appear at the left side of the screen. Each entry in the index is a hypertext link to the associated topic in the text.

Using hypertext links in a pdf document is exactly like that in a web page or html document. When you place the cursor over a hypertext link, it changes to a hand with the index finger pointing to the underlying text. Clicking the mouse causes the text window to jump to that location. The index does not change. Magnification may need to be adjusted using the menu option in the lower part of the screen to optimize the view and readability. The best magnification is usually around 125%.

Subheadings in the index can be viewed by clicking on the open diamonds to the left of appropriate entries to cause them to point downwards. Clicking again will close the subheadings lists.

Hypertext links

Hypertext links in the text (not in the index) are indicated by blue underlined text. The cursor should change to a hand with the index finger pointing to this text when it passes over it. Clicking will cause the text page to move to the associated or linked text which will be highlighted in red underlined text. Red underlined text is not a hyperlink, only a destination.

How to back up to a previous window:

If you wish to return to a previous text window after following a hypertext link, use the black double solid arrow key at the top of the Acrobat window (or use the key equivalent "command - "). Acrobat keeps a record of your last 20 or so windows so that multiple steps back can be made by repeating the command.

Links to web sites

A number of url links to web sites are located in the pdf file and appear in blue underlined type starting with http:// (e.g. <http://>

www.som.siu.edu). Clicking on these should open a web browser such as Netscape and take you to those web sites. You may need to resize the Acrobat Window to view the web browser window displayed underneath it.

COMMENTS

I hope that you find this pdf file useful. Comments on how to make it better would be greatly appreciated. Please notify me in person or by email (enieder@som.siu.edu) of any errors so that they can be removed. The online version on the Biochem server can be easily updated.