

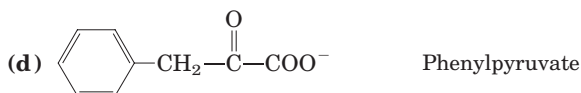
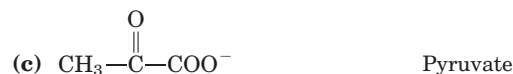
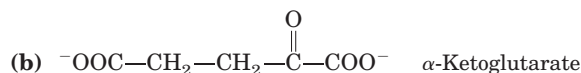
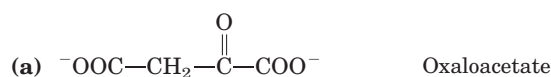
# Amino Acid Oxidation and the Production of Urea

## chapter

# 18

- 1. Products of Amino Acid Transamination** Name and draw the structure of the  $\alpha$ -keto acid resulting when each of the following amino acids undergoes transamination with  $\alpha$ -ketoglutarate: (a) aspartate, (b) glutamate, (c) alanine, (d) phenylalanine.

### Answer



- 2. Measurement of Alanine Aminotransferase Activity** The activity (reaction rate) of alanine aminotransferase is usually measured by including an excess of pure lactate dehydrogenase and NADH in the reaction system. The rate of alanine disappearance is equal to the rate of NADH disappearance measured spectrophotometrically. Explain how this assay works.

**Answer** The measurement of the activity of alanine aminotransferase by measurement of the reaction of its product with lactate dehydrogenase is an example of a “coupled” assay. The product of the transamination (pyruvate) is rapidly consumed in the subsequent “indicator reaction,” catalyzed by an excess of lactate dehydrogenase. The dehydrogenase uses the cofactor NADH, the disappearance of which is conveniently measured by observing the rate of decrease in NADH absorption at 340 nm. Thus, the rate of disappearance of NADH is a measure of the rate of the aminotransferase reaction, *if NADH and lactate dehydrogenase are added in excess.*

- 3. Alanine and Glutamine in the Blood** Normal human blood plasma contains all the amino acids required for the synthesis of body proteins, but not in equal concentrations. Alanine and glutamine are present in much higher concentrations than any other amino acids. Suggest why.

**Answer** Muscle tissue can convert amino acids to their keto acids plus ammonia, then oxidize the keto acids to produce ATP for muscle contraction. However, urea cannot be formed in

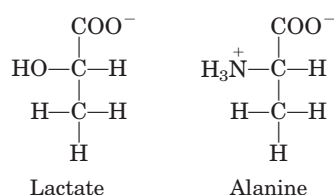
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muscle. Alanine and glutamine transport amino groups in the bloodstream to the liver (see Fig. 18–2) from muscle and other nonhepatic tissues. In muscle, amino groups from all amino acids are transferred to pyruvate or glutamate to form alanine or glutamine, and these latter amino acids are transported to the liver.

- 4. Distribution of Amino Nitrogen** If your diet is rich in alanine but deficient in aspartate, will you show signs of aspartate deficiency? Explain.

**Answer** No; aspartate is readily formed by the transfer of the amino group of alanine to oxaloacetate. Cellular levels of aminotransferases are sufficient to provide all of the amino acids in this fashion, if the  $\alpha$ -keto acids are available.

- 5. Lactate versus Alanine as Metabolic Fuel: The Cost of Nitrogen Removal** The three carbons in lactate and alanine have identical oxidation states, and animals can use either carbon source as a metabolic fuel. Compare the net ATP yield (moles of ATP per mole of substrate) for the complete oxidation (to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ ) of lactate versus alanine when the cost of nitrogen excretion as urea is included.



**Answer** Lactate and alanine are converted to pyruvate by their respective dehydrogenases, lactate dehydrogenase and alanine dehydrogenase, producing pyruvate and  $\text{NADH} + \text{H}^+$  and, in the case of alanine,  $\text{NH}_4^+$ . Complete oxidation of 1 mol of pyruvate to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  produces 12.5 mol of ATP via the citric acid cycle and oxidative phosphorylation (see Table 16–1). In addition, the  $\text{NADH}$  from each dehydrogenase reaction produces 2.5 mol of ATP per mole of  $\text{NADH}$  reoxidized. Thus oxidation produces 15 mol of ATP per mole of lactate. Urea formation uses the equivalent of 4 mol of ATP per mole of urea formed (Fig. 18–10), or 2 mol of ATP per mol of  $\text{NH}_4^+$ . Subtracting this value from the energy yield of alanine results in 13 mol of ATP per mole of alanine oxidized.

- 6. Ammonia Toxicity Resulting from an Arginine-Deficient Diet** In a study conducted some years ago, cats were fasted overnight then given a single meal complete in all amino acids except arginine. Within 2 hours, blood ammonia levels increased from a normal level of  $18 \mu\text{g/L}$  to  $140 \mu\text{g/L}$ , and the cats showed the clinical symptoms of ammonia toxicity. A control group fed a complete amino acid diet or an amino acid diet in which arginine was replaced by ornithine showed no unusual clinical symptoms.
- What was the role of fasting in the experiment?
  - What caused the ammonia levels to rise in the experimental group? Why did the absence of arginine lead to ammonia toxicity? Is arginine an essential amino acid in cats? Why or why not?
  - Why can ornithine be substituted for arginine?

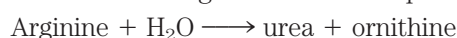
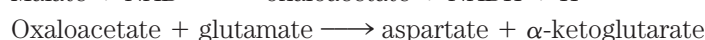
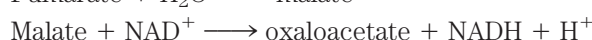
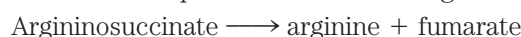
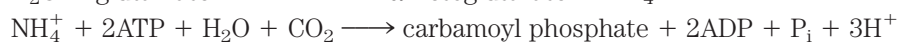
**Answer**

- Fasting resulted in lowering of blood glucose levels. Subsequent feeding of an arginine-free diet led to a rapid catabolism of all the ingested amino acids, especially the glucogenic ones. This catabolism was exacerbated by the lack of an essential amino acid, which prevented protein synthesis.
- Oxidative deamination of amino acids caused the elevation of ammonia levels. In addition, the lack of arginine (an intermediate in the urea cycle) slowed the conversion of ammonia to urea. Arginine (or ornithine) synthesis in the cat is not sufficient to meet the needs imposed by the stress of this experiment, suggesting that arginine is an essential amino acid.

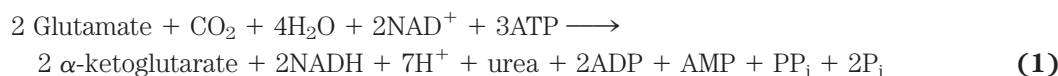
- (c) Ornithine (or citrulline) can be substituted for arginine because it also is an intermediate in the urea cycle.

**7. Oxidation of Glutamate** Write a series of balanced equations, and an overall equation for the net reaction, describing the oxidation of 2 mol of glutamate to 2 mol of  $\alpha$ -ketoglutarate and 1 mol of urea.

**Answer**



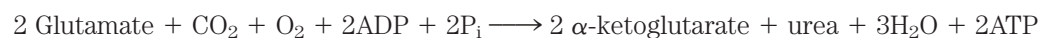
The sum of these reactions is



Three additional reactions need to be considered:



Summing the last four equations:



**8. Transamination and the Urea Cycle** Aspartate aminotransferase has the highest activity of all the mammalian liver aminotransferases. Why?

**Answer** The second amino group introduced into urea is transferred from aspartate. This amino acid is generated in large quantities by transamination between oxaloacetate and glutamate (and many other amino acids), catalyzed by aspartate aminotransferase. Approximately one half of all the amino groups excreted as urea must pass through the aspartate aminotransferase reaction, and liver contains higher levels of this aminotransferase than of any other.

**9. The Case against the Liquid Protein Diet** A weight-reducing diet heavily promoted some years ago required the daily intake of “liquid protein” (soup of hydrolyzed gelatin), water, and an assortment of vitamins. All other food and drink were to be avoided. People on this diet typically lost 10 to 14 lb in the first week.

- (a) Opponents argued that the weight loss was almost entirely due to water loss and would be regained very soon after a normal diet was resumed. What is the biochemical basis for this argument?
- (b) A number of people on this diet died. What are some of the dangers inherent in the diet and how can they lead to death?

**Answer**

- (a) A person on a diet consisting only of protein must use amino acids as the principal source of metabolic fuel. Because the catabolism of amino acids requires the removal of

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nitrogen as urea, the process consumes large quantities of water to dilute and excrete the urea in the urine. Furthermore, electrolytes in the “liquid protein” must be diluted with water and excreted. If this abnormally large daily water loss through the kidney is not balanced by a sufficient water intake, a net loss of body water results.

- (b) When considering the nutritional benefits of protein, keep in mind the total amount of amino acids needed for protein synthesis and the distribution of amino acids in the dietary protein. Gelatin contains a nutritionally unbalanced distribution of amino acids. As large amounts of gelatin are ingested and the excess amino acids are catabolized, the capacity of the urea cycle may be exceeded, leading to ammonia toxicity. This is further complicated by the dehydration that may result from excretion of large quantities of urea. A combination of these two factors could produce coma and death.

**10. Ketogenic Amino Acids** Which amino acids are exclusively ketogenic?

**Answer** Lysine and leucine are exclusively ketogenic. These amino acids are degraded entirely to acetyl-CoA and acetoacetyl-CoA, and no parts of their carbon skeletons can be used for glucose synthesis. Leucine is especially common in proteins. Its degradation makes a substantial contribution to ketosis under starvation conditions.

- 11. A Genetic Defect in Amino Acid Metabolism: A Case History** A two-year-old child was taken to the hospital. His mother said that he vomited frequently, especially after feedings. The child’s weight and physical development were below normal. His hair, although dark, contained patches of white. A urine sample treated with ferric chloride ( $\text{FeCl}_3$ ) gave a green color characteristic of the presence of phenylpyruvate. Quantitative analysis of urine samples gave the results shown in the table.

Substance	Concentration (mM)	
	Patient’s urine	Normal urine
Phenylalanine	7.0	0.01
Phenylpyruvate	4.8	0
Phenyllactate	10.3	0

- (a) Suggest which enzyme might be deficient in this child. Propose a treatment.  
 (b) Why does phenylalanine appear in the urine in large amounts?  
 (c) What is the source of phenylpyruvate and phenyllactate? Why does this pathway (normally not functional) come into play when the concentration of phenylalanine rises?  
 (d) Why does the boy’s hair contain patches of white?

**Answer**

- (a) Because phenylalanine (and its related phenylketones) accumulate in this patient, it is likely that the first enzyme in phenylalanine catabolism, phenylalanine hydroxylase (also called phenylalanine-4-monooxygenase), is defective or missing (see Fig. 18–23). The most appropriate treatment for patients with this disease, known as phenylketonuria (PKU), is to establish a low-phenylalanine diet that provides just enough of the amino acid to meet the needs for protein synthesis.
- (b) Phenylalanine appears in the urine because high levels of this amino acid accumulate in the bloodstream and the body attempts to dispose of it.

- (c) Phenylalanine is converted to phenylpyruvate by transamination, a reaction that has an equilibrium constant of about 1.0. Phenyllactate is formed from phenylpyruvate by reduction (see Fig. 18–25). This pathway is of importance only when phenylalanine hydroxylase is defective.
- (d) The normal catabolic pathway of phenylalanine is through tyrosine, a precursor of melanin, the dark pigment normally present in hair. Decreased tyrosine levels in patients with phenylketonuria result in varying degrees of pigment loss.

**12. Role of Cobalamin in Amino Acid Catabolism** Pernicious anemia is caused by impaired absorption of vitamin B<sub>12</sub>. What is the effect of this impairment on the catabolism of amino acids? Are all amino acids equally affected? (Hint: see Box 17–2.)

**Answer** The catabolism of the carbon skeletons of valine, isoleucine, and methionine is impaired because of the absence of a functional methylmalonyl-CoA mutase. This enzyme requires coenzyme B<sub>12</sub> as a cofactor, and a deficiency of this vitamin leads to elevated methylmalonic acid levels (methylmalonic acidemia). The symptoms and effects of this deficiency are severe (see Table 18–2 and Box 18–2).

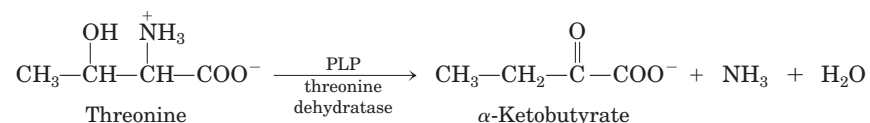
**13. Vegetarian Diets** Vegetarian diets can provide high levels of antioxidants and a lipid profile that can help prevent coronary disease. However, there can be some associated problems. Blood samples were taken from a large group of volunteer subjects who were vegans (strict vegetarians: no animal products), lactovegetarians (vegetarians who eat dairy products), or omnivores (individuals with a normal, varied diet including meat). In each case, the volunteers had followed the diet for several years. The blood levels of both homocysteine and methylmalonate were elevated in the vegan group, somewhat lower in the lactovegetarian group, and much lower in the omnivore group. Explain.

**Answer** The vegan diet lacks vitamin B<sub>12</sub>, leading to the increase in homocysteine and methylmalonate (reflecting the deficiencies in methionine synthase and methylmalonic acid mutase, respectively) in individuals on the diet for several years. Dairy products provide some vitamin B<sub>12</sub> in the lactovegetarian diet.

**14. Pernicious Anemia** Vitamin B<sub>12</sub> deficiency can arise from a few rare genetic diseases that lead to low B<sub>12</sub> levels despite a normal diet that includes B<sub>12</sub>-rich meat and dairy sources. These conditions cannot be treated with dietary B<sub>12</sub> supplements. Explain.

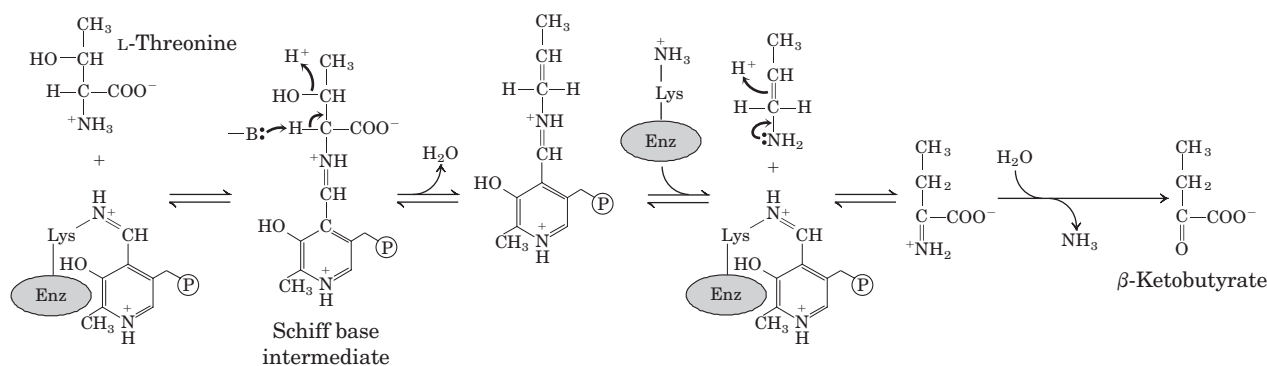
**Answer** The genetic forms of pernicious anemia generally arise as a result of defects in the pathway that mediates absorption of dietary vitamin B<sub>12</sub> (see Box 17–2, p. 658). Because dietary supplements are not absorbed in the intestine, these conditions are treated by injecting supplementary B<sub>12</sub> directly into the bloodstream.

**15. Pyridoxal Phosphate Reaction Mechanisms** Threonine can be broken down by the enzyme threonine dehydratase, which catalyzes the conversion of threonine to  $\alpha$ -ketobutyrate and ammonia. The enzyme uses PLP as a cofactor. Suggest a mechanism for this reaction, based on the mechanisms in Figure 18–6. Note that this reaction includes an elimination at the  $\beta$  carbon of threonine.

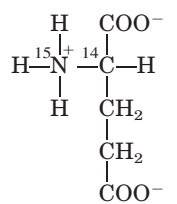


**Answer** The mechanism is identical to that for serine dehydratase (see Fig. 18–20a, p. 693) except that the extra methyl group of threonine is retained, yielding  $\alpha$ -ketobutyrate instead of pyruvate.

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- 16. Pathway of Carbon and Nitrogen in Glutamate Metabolism** When  $[2\text{-}^{14}\text{C}, \text{}^{15}\text{N}]$  glutamate undergoes oxidative degradation in the liver of a rat, in which atoms of the following metabolites will each isotope be found: **(a)** urea, **(b)** succinate, **(c)** arginine, **(d)** citrulline, **(e)** ornithine, **(f)** aspartate?



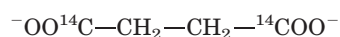
Labeled glutamate

**Answer**

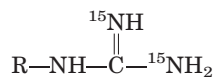
- (a)** The amino groups of urea contain  $^{15}\text{N}$ , a result of glutamate dehydrogenase producing  $^{15}\text{NH}_4^+$  or of a transaminase producing  $^{15}\text{N}$ -labeled aspartate.



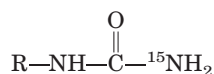
- (b)** After loss of the amino group, the  $[2\text{-}^{14}\text{C}]$   $\alpha$ -ketoglutarate is metabolized in the citric acid cycle. Succinate thus formed is labeled in the carboxyl groups.



- (c)** The arginine formed in the urea cycle contains  $^{15}\text{N}$  in both guanidino nitrogens.

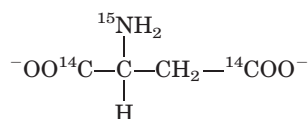


- (d)** Citrulline formed in the urea cycle contains  $^{15}\text{N}$  in the carboxamide group.



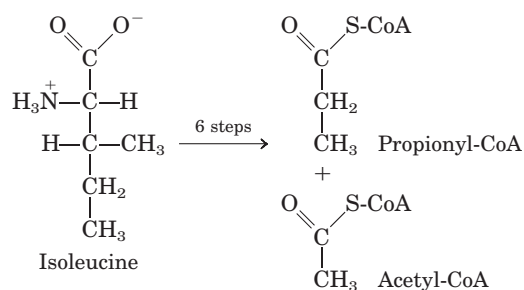
## Chapter 18 Amino Acid Oxidation and the Production of Urea S-217

- (e) No labeled N is found in ornithine.  
 (f) Aspartate contains  $^{15}\text{N}$  in its amino group as a result of transamination from glutamate. It also contains  $^{14}\text{C}$  in its carboxyl groups as a result of succinate conversion to oxaloacetate (as in (b)).

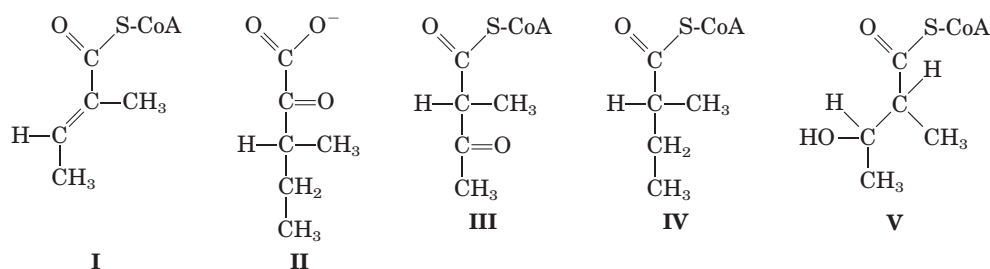


Note: in (c), (d), and (e), these urea cycle intermediates will contain low levels of  $^{14}\text{C}$  as a result of a very weak synthesis of ornithine from glutamate.

**17. Chemical Strategy of Isoleucine Catabolism** Isoleucine is degraded in six steps to propionyl-CoA and acetyl-CoA.



- (a) The chemical process of isoleucine degradation includes strategies analogous to those used in the citric acid cycle and the  $\beta$  oxidation of fatty acids. The intermediates of isoleucine degradation (I to V) shown below are not in the proper order. Use your knowledge and understanding of the citric acid cycle and  $\beta$ -oxidation pathway to arrange the intermediates in the proper metabolic sequence for isoleucine degradation.



- (b) For each step you propose, describe the chemical process, provide an analogous example from the citric acid cycle or  $\beta$ -oxidation pathway (where possible), and indicate any necessary cofactors.

**Answer**

1 2 3 4 5 6

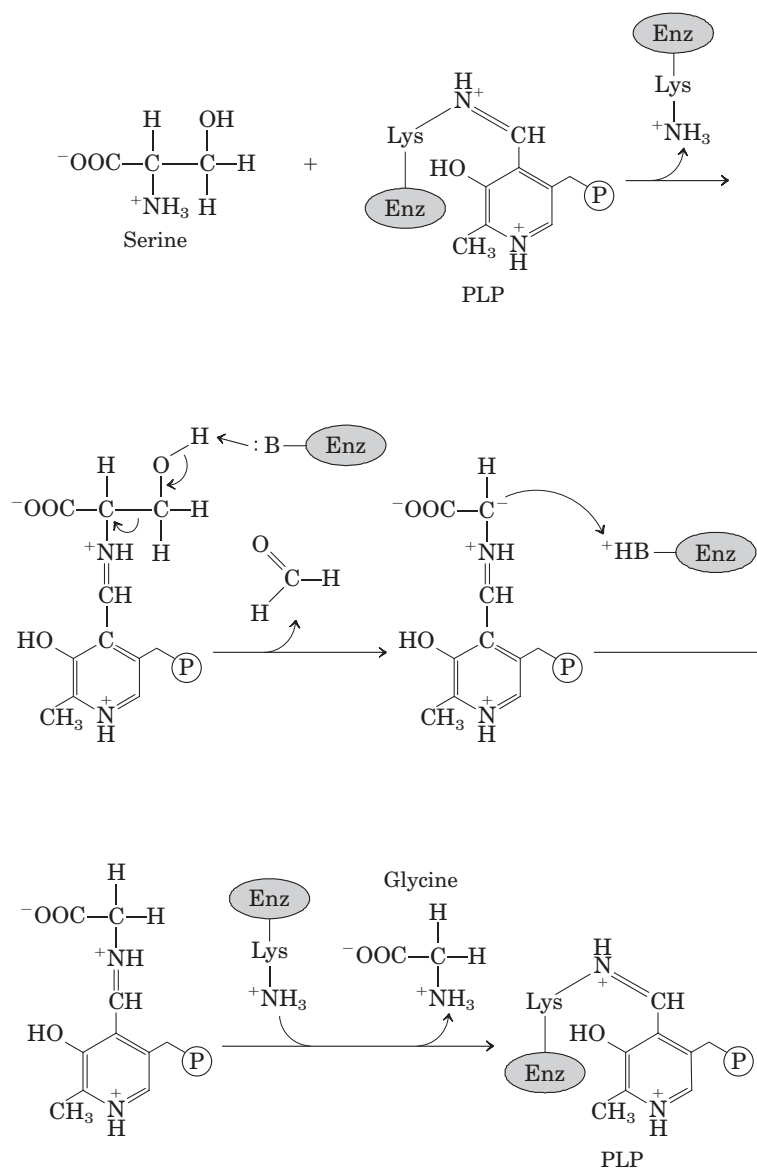
- (a) Isoleucine  $\rightarrow$  II  $\rightarrow$  IV  $\rightarrow$  I  $\rightarrow$  V  $\rightarrow$  III  $\rightarrow$  acetyl-CoA + propionyl-CoA

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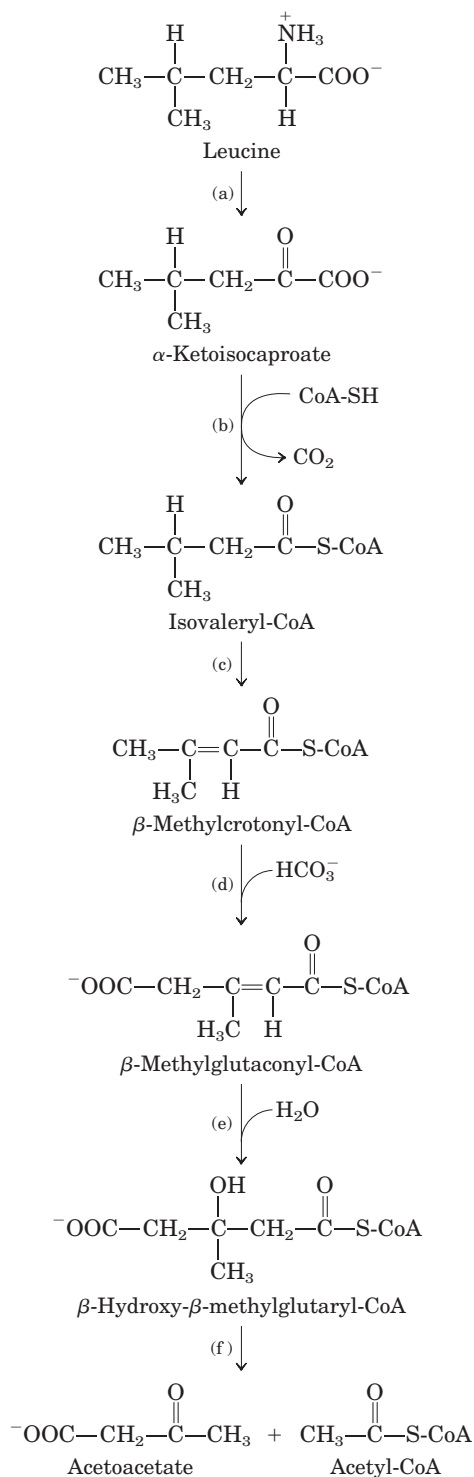
- (b) Step 1 is a transamination that has no analogous reaction; it requires PLP. Step 2 is an oxidative decarboxylation similar to the pyruvate dehydrogenase reaction; it requires  $\text{NAD}^+$ , TPP, lipoate, FAD. Step 3 is an oxidation similar to the succinate dehydrogenase reaction; it requires FAD. Step 4 is a hydration analogous to the fumarase reaction; no cofactor is required. Step 5 is an oxidation analogous to the malate dehydrogenase reaction of the citric acid cycle; it requires  $\text{NAD}^+$ . Step 6 is a thiolysis analogous to the final cleavage step of  $\beta$  oxidation catalyzed by thiolase; it requires CoA.

**18. Role of Pyridoxal Phosphate in Glycine Metabolism** The enzyme serine hydroxymethyltransferase requires pyridoxal phosphate as cofactor. Propose a mechanism for the reaction catalyzed by this enzyme, in the direction of serine degradation (glycine production). (Hint: see Figs 18–19 and 18–20b.)

**Answer** See the mechanism below. The formaldehyde produced in the second step reacts rapidly with tetrahydrofolate at the enzyme active site to produce  $N^5, N^{10}$ -methylene tetrahydrofolate (see Fig. 18–17).



- 19. Parallel Pathways for Amino Acid and Fatty Acid Degradation** The carbon skeleton of leucine is degraded by a series of reactions closely analogous to those of the citric acid cycle and  $\beta$  oxidation. For each reaction, **(a)** through **(f)**, indicate its type, provide an analogous example from the citric acid cycle or  $\beta$ -oxidation pathway (where possible), and note any necessary cofactors.



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**Answer**

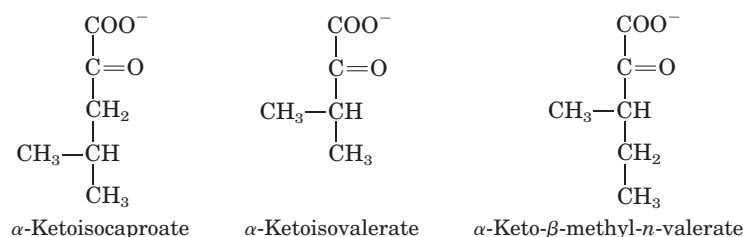
- (a) Transamination; no analogies in either pathway; requires PLP.
- (b) Oxidative decarboxylation; analogous to oxidative decarboxylation of pyruvate to acetyl-CoA prior to entry into the citric acid cycle, and of  $\alpha$ -ketoglutarate to succinyl-CoA in the citric acid cycle; requires  $\text{NAD}^+$ , FAD, lipoate, thiamine pyrophosphate.
- (c) Dehydrogenation (oxidation); analogous to dehydrogenation of succinate to fumarate in the citric acid cycle and of fatty acyl-CoA to enoyl-CoA in  $\beta$  oxidation; requires FAD.
- (d) Carboxylation; no analogous reaction in the citric acid cycle or  $\beta$  oxidation; requires ATP and biotin.
- (e) Hydration; analogous to hydration of fumarate to malate in the citric acid cycle and of enoyl-CoA to 3-hydroxyacyl-CoA in  $\beta$  oxidation; no cofactors.
- (f) Reverse aldol reaction; analogous to reverse of citrate synthase reaction in the citric acid cycle and identical to cleavage of  $\beta$ -hydroxy- $\beta$ -methylglutaryl-CoA in formation of ketone bodies; no cofactors.

**Data Analysis Problem**

**20. Maple Syrup Urine Disease** Figure 18–28 shows the pathway for the degradation of branched-chain amino acids and the site of the biochemical defect that causes maple syrup urine disease. The initial findings that eventually led to the discovery of the defect in this disease were presented in three papers published in the late 1950s and early 1960s. This problem traces the history of the findings from initial clinical observations to proposal of a biochemical mechanism.

Menkes, Hurst, and Craig (1954) presented the cases of four siblings, all of whom died following a similar course of symptoms. In all four cases, the mother's pregnancy and the birth had been normal. The first 3 to 5 days of each child's life were also normal. But soon thereafter each child began having convulsions, and the children died between the ages of 11 days and 3 months. Autopsy showed considerable swelling of the brain in all cases. The children's urine had a strong, unusual "maple syrup" odor, starting from about the third day of life.

Menkes (1959) reported data collected from six more children. All showed symptoms similar to those described above, and died within 15 days to 20 months of birth. In one case, Menkes was able to obtain urine samples during the last months of the infant's life. When he treated the urine with 2,4-dinitrophenylhydrazine, which forms colored precipitates with keto compounds, he found three  $\alpha$ -keto acids in unusually large amounts:



- (a) These  $\alpha$ -keto acids are produced by the deamination of amino acids. For each of the  $\alpha$ -keto acids above, draw and name the amino acid from which it was derived.

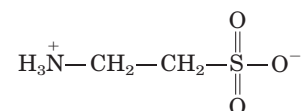
Dancis, Levitz, and Westall (1960) collected further data that led them to propose the biochemical defect shown in Figure 18–28. In one case, they examined a patient whose urine first showed the maple syrup odor when he was 4 months old. At the age of 10 months (March 1956), the child was admitted to the hospital because he had a fever, and he showed grossly retarded motor development.

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At the age of 20 months (January 1957), he was readmitted and was found to have the degenerative neurological symptoms seen in previous cases of maple syrup urine disease; he died soon after. Results of his blood and urine analyses are shown in the table below, along with normal values for each component.

Amino acid(s)	Urine (mg/24 h)			Plasma (mg/ml)	
	Normal	Patient		Normal	Patient
		Mar. 1956	Jan. 1957		
Alanine	5–15	0.2	0.4	3.0–4.8	0.6
Asparagine and glutamine	5–15	0.4	0	3.0–5.0	2.0
Aspartic acid	1–2	0.2	1.5	0.1–0.2	0.04
Arginine	1.5–3	0.3	0.7	0.8–1.4	0.8
Cystine	2–4	0.5	0.3	1.0–1.5	0
Glutamic acid	1.5–3	0.7	1.6	1.0–1.5	0.9
Glycine	20–40	4.6	20.7	1.0–2.0	1.5
Histidine	8–15	0.3	4.7	1.0–1.7	0.7
Isoleucine	2–5	2.0	13.5	0.8–1.5	2.2
Leucine	3–8	2.7	39.4	1.7–2.4	14.5
Lysine	2–12	1.6	4.3	1.5–2.7	1.1
Methionine	2–5	1.4	1.4	0.3–0.6	2.7
Ornithine	1–2	0	1.3	0.6–0.8	0.5
Phenylalanine	2–4	0.4	2.6	1.0–1.7	0.8
Proline	2–4	0.5	0.3	1.5–3.0	0.9
Serine	5–15	1.2	0	1.3–2.2	0.9
Taurine	1–10	0.2	18.7	0.9–1.8	0.4
Threonine	5–10	0.6	0	1.2–1.6	0.3
Tryptophan	3–8	0.9	2.3	Not measured	0
Tyrosine	4–8	0.3	3.7	1.5–2.3	0.7
Valine	2–4	1.6	15.4	2.0–3.0	13.1

- (b) The table includes taurine, an amino acid not normally found in proteins. Taurine is often produced as a by-product of cell damage. Its structure is:



Based on its structure and the information in this chapter, what is the most likely amino acid precursor of taurine? Explain your reasoning.

- (c) Compared with the normal values given in the table, which amino acids showed significantly elevated levels in the patient's blood in January 1957? Which ones in the patient's urine?

Based on their results and their knowledge of the pathway shown in Figure 18–28, Dancis and coauthors concluded: “although it appears most likely to the authors that the primary block is in the metabolic degradative pathway of the branched-chain amino acids, this cannot be considered established beyond question.”

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- (d) How do the data presented here support this conclusion?
- (e) Which data presented here do *not* fit this model of maple syrup urine disease? How do you explain these seemingly contradictory data?
- (f) What data would you need to collect to be more secure in your conclusion?

**Answer**

- (a) Leucine; valine; isoleucine
- (b) Cysteine (derived from cystine). If cysteine were decarboxylated as shown in Figure 18-6, it would yield  $\text{H}_3\text{N}^+-\text{CH}_2-\text{CH}_2-\text{SH}$ , which could be oxidized to taurine.
- (c) The January 1957 blood shows significantly elevated levels of isoleucine, leucine, methionine, and valine; the January 1957 urine, significantly elevated isoleucine, leucine, taurine, and valine.
- (d) All patients had high levels of isoleucine, leucine, and valine in both blood and urine, suggesting a defect in the breakdown of these amino acids. Given that the urine also contained high levels of the keto forms of these three amino acids, the block in the pathway must occur after deamination but before dehydrogenation (as shown in Fig. 18-28).
- (e) The model does not explain the high levels of methionine in blood and taurine in urine. The high taurine levels may be due to the death of brain cells during the end stage of the disease. However, the reason for high levels of methionine in blood are unclear; the pathway of methionine degradation is not linked with the degradation of branched-chain amino acids. Increased methionine could be a secondary effect of buildup of the other amino acids. It is important to keep in mind that the January 1957 samples were from an individual who was dying, so comparing blood and urine results with those of a healthy individual may not be appropriate.
- (f) The following information is needed (and was eventually obtained by other workers): (1) The dehydrogenase activity is significantly reduced or missing in individuals with maple syrup urine disease. (2) The disease is inherited as a single-gene defect. (3) The defect occurs in a gene encoding all or part of the dehydrogenase. (4) The genetic defect leads to production of inactive enzyme.

**References**

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