DEBRECENI EGYETEM

PRACTICAL EXERCISES FOR THE COURSE OF BIOCHEMISTRY

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A Debreceni Egyetem fejlesztése a felsőfokú oktatás minőségének és hozzáférhetőségének együttes javítása érdekében
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Exercise 1.

1. Acidity of gastric HCl

Question: In a hospital laboratory, a 10.0 mL sample of gastric juice, obtained several hours after a meal, was titrated with 0.1 M NaOH to neutrality; 7.2 mL of NaOH was required. The patient's stomach contained no ingested food or drink, thus assume that no buffers were present. What was the pH of the gastric juice?

Answer: pH=1.1.

2. Vitamin C: Is the synthetic vitamin as good as the natural one?

Question: A claim put forth by some purveyors of health foods is that vitamins obtained from natural sources are more healthful than those obtained by chemical synthesis. For example, pure L-ascorbic acid (vitamin C) extracted from rose hips is better than pure L-ascorbic acid manufactured in a chemical plant. Are the vitamins from the two sources different? Can the body distinguish a vitamin's source?

Answer: The vitamin molecules from the two sources are identical; the body can not distinguish the source, only associated impurities might vary with the source.

3. Separating biomolecules

Question: In studying a particular biomolecule (a protein, nucleic acid, carbohydrate, or lipid) in the laboratory, the biochemist first needs to separate it from other biomolecules in the sample—that is, to purify it. However, by looking at the monomeric subunits of a biomolecule, you should have some ideas about the characteristics of the molecule that would allow you to separate it from other molecules. For example, how would you separate (a) amino acids from fatty acids and (b) nucleotides from glucose?

Answer: **a.** Only the amino acids have amino groups; separation could be based on the charge or binding affinity of these groups. Fatty acids are less soluble in water than amino acids, and the two types of molecules also differ in size and shape – either of this property difference could be the basis for separation. **b.** Glucose is smaller molecule than a nucleotide; separation could be based on size. The nitrogenous base and/or the phosphate group also endow nucleotide with characteristics (solubility, charge) that could be used for separation from glucose.

4. Properties of a buffer

Question: The amino acid glycine is often used as the main ingredient of a buffer in biochemical experiments. The amino group of glycine, which has a pK_a of 9.6, can exist either in the protonated form (─NH_3^+) or as the free base (─NH_2), because of the reversible equilibrium. (a) In what pH range can glycine be used as an effective buffer due to its amino group? (b) In a 0.1 M solution of glycine at pH 9.0, what fraction of glycine has its amino group in the ─NH_3^+ form? (c) How much 5 M KOH must be added to 1.0 L of 0.1 M glycine at pH 9.0 to bring its pH to exactly 10.0? (d) When 99% of the glycine is in its ─NH_3^+ form, what is the numerical relation between the pH of the solution and the pK_a of the amino group?

Answer: **a.** pH 8.6 to 10.6. **b.** 4/5. **c.** 10 ml. **d.** pH=pK_s –2.

5. The effect of pH on solubility

Question: The strongly polar, hydrogen-bonding properties of water make it an excellent solvent for ionic (charged) species. By contrast, nonionized, nonpolar organic molecules, such as benzene, are relatively insoluble in water. In principle, the aqueous solubility of any organic acid or base can be increased by converting the molecules to charged species. For example, the solubility of benzoic acid in water is low. The addition of sodium bicarbonate to a mixture of water and benzoic acid raises the pH and deprotonates the benzoic acid to form benzoate ion, which is quite soluble in water. Are the following compounds more soluble in an aqueous solution of 0.1 M NaOH or 0.1 M HCl? Pyridine ion (pK_a=5); β-naphthol (pK_a=10); N-acetyltyrosine methyl ester (pK_a=10).

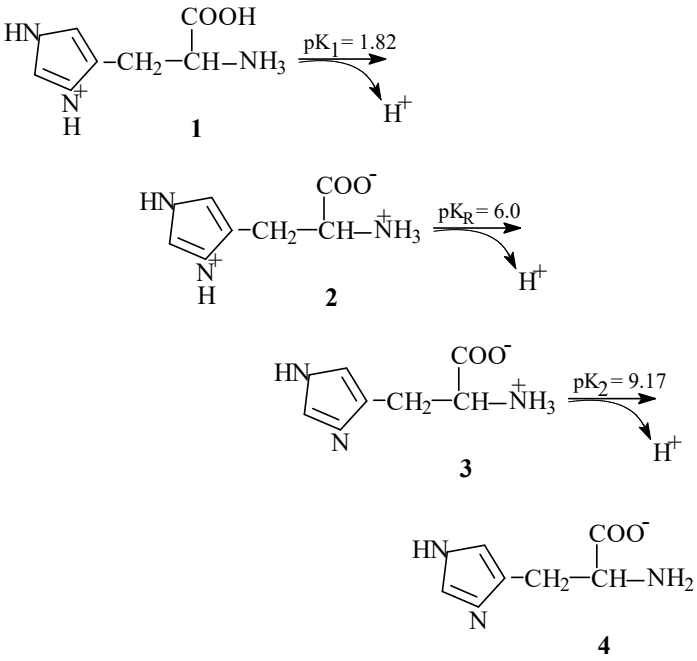
Answers: **a.** 0.1 M HCl. **b.** 0.1 M NaOH. **c.** 0.1 M NaOH.

6. Ionization state of amino acids

Question: Each ionizable group of an amino acid can exist in one of two states, charged or neutral. The electric charge on the functional group is determined by the relationship between its pK_a and the pH of the solution. This relationship is described by the Henderson-Hasselbalch equation. (a) Histidine has three ionizable functional groups.Write the equilibrium equations for its three ionizations and assign the proper pK_a for each ionization. Draw the structure of histidine in each ionization state. What is the net charge on the histidine molecule in each ionization state? (b) Draw the structures of the predominant ionization state of histidine at pH 1, 4, 8, and 12. Note that the ionization state can be approximated by treating each ionizable group independently. (c) What is

the net charge of histidine at pH 1, 4, 8, and 12? For each pH, will histidine migrate toward the anode (+) or cathode (-) when placed in an electric field?

Answer: **a.** The net charge on the histidine molecule in each ionization state is 2+, 1+, 0, 1–.



b. Structure 1: net charge: +2, migrates toward cathode. Structure 2: net charge: +1, migrates toward cathode, Structure 3: net charge: 0, does not migrate. Structure 4: net charge: –1, migrates toward anode.

Exercise 2

7. Separation of amino acids by ion exchange chromatography

Question: Mixtures of amino acids are analyzed by first separating the mixture into its components through ion exchange chromatography. Amino acids placed on a cation exchange resin containing sulfonate groups flow down the column at different rates because of two factors that influence their movement: (1) ionic attraction between the $-SO_3^-$ residues on the column and positively charged functional groups on the amino acids, and (2) hydrophobic interactions between amino acid side chains and the strongly hydrophobic backbone of the polystyrene resin. For each pair of amino acids listed, determine which will be eluted first from an ion-exchange column using a pH 7.0 buffer. (a) Asp and Lys, (b) Arg and Met, (c) Glu and Val, (d) Gly and Leu, (e) Ser and Ala.

Answer: **a**. Asp. **b**. Met. **c**. Glu. **d**. Ser.

8. The size of proteins

Question: What is the approximate molecular weight of a protein with 682 amino acid residues in a single polypeptide chain?

Answer: 75.000.

9. The number of tryptophan residues in bovine serum albumin

Question: A quantitative amino acid analysis reveals that bovine serum albumin (BSA) contains 0.58% tryptophan (Mr 204) by weight. (a) Calculate the minimum molecular weight of BSA (i.e., assuming there is only one tryptophan residue per protein molecule). (b) Gel filtration of BSA gives a molecular weight estimate of 70,000. How many tryptophan residues are present in a molecule of serum albumin?

Answer: a. 32.100. **b**. 2.

10. Isoelectric point of pepsin

Question: Pepsin is the name given to several digestive enzymes secreted (as larger precursor proteins) by glands that line the stomach. These glands also secrete hydrochloric acid, which dissolves the particulate matter in food, allowing pepsin to enzymatically cleave individual protein molecules. The resulting mixture of food, HCl, and digestive enzymes is known as chyme and has

a pH near 1.5. What pI would you predict for the pepsin proteins? What functional groups must be present to confer this pI on pepsin? Which amino acids in the proteins would contribute such groups?
Answer: pI=1.0, carboxylate groups, Asp and Glu.

11. The isoelectric point of histones

Question: Histones are proteins found in eukaryotic cell nuclei, tightly bound to DNA, which has many phosphate groups. The pI of histones is very high, about 10.8. What amino acid residues must be present in relatively large numbers in histones? In what way do these residues contribute to the strong binding of histones to DNA?
Answer: Lys, His, Arg, negatively charged phosphate groups in DNA interact with positively charged side groups of histones.

12. Solubility of polypeptides

Question: One method for separating polypeptides makes use of their differential solubilities. The solubility of large polypeptides in water depends upon the relative polarity of their R groups, particularly on the number of ionized groups: the more ionized groups there are, the more soluble the polypeptide. Which of each pair of the polypeptides that follow is more soluble at the indicated pH?
(a) (Gly)20 or (Glu)20 at pH 7.0
(b) (Lys–Ala)3 or (Phe–Met)3 at pH 7.0
(c) (Ala–Ser–Gly)5 or (Asn–Ser–His)5 at pH 6.0
(d) (Ala–Asp–Gly)5 or (Asn–Ser–His)5 at pH 3.0
Answer: **a.** (Glu)20. **b.** (Lys–Ala)3. **c.** (Asn–Ser–His)5. **d.** (Asn–Ser–His)5.

Exercise 3.

13. Sequence determination of the brain peptide leucine enkephalin

Question: A group of peptides that influence nerve transmission in certain parts of the brain has been isolated from normal brain tissue. These peptides are known as opioids, because they bind to specific receptors that also bind opiate drugs, such as morphine and naloxone. Opioids thus mimic some of the properties of opiates. Some researchers consider these peptides to be the brain's own pain killers. Using the information below, determine the amino acid sequence of the opioid leucine enkephalin. Explain how your structure is consistent with each piece of information. (a) Complete hydrolysis by 6 M HCl at 110 °C followed by amino acid analysis indicated the presence of Gly, Leu, Phe, and Tyr, in a 2:1:1:1 molar ratio. (b) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene followed by complete hydrolysis and chromatography indicated the presence of the 2,4-dinitrophenyl derivative of tyrosine. No free tyrosine could be found. (c) Complete digestion of the peptide with pepsin followed by chromatography yielded a dipeptide containing Phe and Leu, plus a tripeptide containing Tyr and Gly in a 1:2 ratio.

Answer: Tyr–Gly–Gly–PheLeu.

14. Disulfide bonds determine the properties of many proteins

Question: A number of natural proteins are very rich in disulfide bonds, and their mechanical properties (tensile strength, viscosity, hardness, etc.) are correlated with the degree of disulfide bonding. For example, glutenin, a wheat protein rich in disulfide bonds, is responsible for the cohesive and elastic character of dough made from wheat flour. Similarly, the hard, tough nature of tortoise shell is due to the extensive disulfide bonding in its α-keratin. (a) What is the molecular basis for the correlation between disulfide-bond content and mechanical properties of the protein? (b) Most globular proteins are denatured and lose their activity when briefly heated to 65 °C. However, globular proteins that contain multiple disulfide bonds often must be heated longer at higher temperatures to denature them. One such protein is bovine pancreatic trypsin inhibitor (BPTI), which has 58 amino acid residues in a single chain and contains three disulfide bonds. On cooling a solution of denatured BPTI, the activity of the protein is restored. What is the molecular basis for this property?

Answer: **a.** Disulphide bonds are covalent bonds, which are much stronger than the noncovalent interactions that stabilize most proteins. The crosslink protein chains, increasing their stiffness. mechanical strength, and hardness. **b.** Cystine residues (disulphide bonds) prevent the complete unfolding of the protein.

15. Amino acid sequence and protein structure

Question: Our growing understanding of how proteins fold allows researchers to make predictions about protein structure based on primary amino acid sequence data. (a) In the amino acid sequence above, where would you predict that bends or β-turns would occur? (b) Where might intrachain disulfide cross-linkages be formed? (c) Assuming that this sequence is part of a larger globular protein, indicate the probable location (the external surface or interior of the protein) of the following amino acid residues: Asp, Ile, Thr, Ala, Gln, Lys. Explain your reasoning.

Answer: **a.** Bends are most likely at residue 7 and 19; Pro residues in the cis configuration accommodate turns well. **b.** The Cys residues at position 13 and 24 can form disulfide bonds. c. External surface: polar and charged residues (Asp, Gln, Lys) ; interior: nonpolar and aliphatic residues (Ala, Ile); Thr, though polar, has hydropathy index near zero and thus can be found on the external surface or interior of the protein.

16. Bacteriorhodopsin in purple membrane proteins

Question: Under the proper environmental conditions, the salt-loving bacterium *Halobacterium halobium* synthesizes a membrane protein (*M*r 26,000) known as bacteriorhodopsin, which is purple because it contains retinal. Molecules of this protein aggregate into “purple patches” in the cell membrane. Bacteriorhodopsin acts as a light-activated proton pump that provides energy for cell functions. X-ray analysis of this protein reveals that it consists of seven parallel α-helical segments, each of which traverses the bacterial cell membrane (thickness 45 Å). Calculate the minimum number of amino acid residues necessary for one segment of α-helix to traverse the membrane completely. Estimate the fraction of the bacteriorhodopsin protein that is involved in membrane-spanning helices. (Use an average amino acid residue weight of 110.)

Answer: 30 amino acid residues, 89%.

17. Keeping the sweet taste of corn

Question: The sweet taste of freshly picked corn (maize) is due to the high level of sugar in the kernels. Store-bought corn (several days after picking) is not as sweet, because about 50% of the free sugar is converted to starch within one day of picking. To preserve the sweetness of fresh corn, the husked ears can be immersed in boiling water for a few minutes ("blanched") then cooled in cold water. Corn processed in this way and stored in a freezer maintains its sweetness. What is the biochemical basis for this procedure?

Answer: The activity of the enzyme that converts sugar to starch is destroyed by heath denaturation.

18. Rate enhancement by urease

Question: The enzyme urease enhances the rate of urea hydrolysis at pH 8.0 and 20 ^{o}C by a factor of 1014. If a given quantity of urease can completely hydrolyze a given quantity of urea in 5.0 min at 20 ^{o}C and pH 8.0, how long would it take for this amount of urea to be hydrolyzed under the same conditions in the absence of urease? Assume that both reactions take place in sterile systems so that bacteria cannot attack the urea.

Answer: 9.5 x 10^{8} year.

19. Protection of an enzyme against denaturation by heat

Question: When enzyme solutions are heated, there is a progressive loss of catalytic activity over time due to denaturation of the enzyme. A solution of the enzyme hexokinase incubated at 45 ^{o}C lost 50% of its activity in 12 min, but when incubated at 45 ^{o}C in the presence of a very large concentration of one of its substrates, it lost only 3% of its activity in 12 min. Suggest why thermal denaturation of hexokinase was retarded in the presence of one of its substrates.

Answer. The enzyme-substrate complex is more stable than the enzyme alone.

Exercise 4

20. Determination of an empirical formula

Question: An unknown substance containing only C, H, and O was isolated from goose liver. A 0.423 g sample produced 0.620 g of CO_2 and 0.254 g of H_2O after complete combustion in excess oxygen. Is the empirical formula of this substance consistent with its being a carbohydrate? Explain.

Answer: Yes, the empirical formula of CH_2O, typical of carbohydrate.

21. Sugar alcohols

Question: In the monosaccharide derivatives known as sugar alcohols, the carbonyl oxygen is reduced to a hydroxyl group. For example, D-glyceraldehyde can be reduced to glycerol. However, this sugar alcohol is no longer designated D or L. Why?

Answer: With reduction of the carbonyl oxygen to a hydroxyl group, the chemistry at C1 and C3 is the same; the glycerol molecule is not chiral.

22. A taste of honey

Question: The fructose in honey is mainly in the β-D-pyranose form. This is one of the sweetest carbohydrates known, about twice as sweet as glucose. The β-D-furanose form of fructose is much less sweet. The sweetness of honey gradually decreases at a high temperature. Also, high-fructose corn syrup (a commercial product in which much of the glucose in corn syrup is converted to fructose) is used for sweetening cold but not hot drinks. What chemical property of fructose could account for both these observations?

Answer: Fructose cyclizes to either pyranose or the furanose structure. Increasing the temperature shifts the equilibrium in the direction of the furanose, the less sweet form.

23. Physical properties of cellulose and glycogen

Question: The almost pure cellulose obtained from the seed threads of *Gossypium* (cotton) is tough, fibrous, and completely insoluble in water. In contrast, glycogen obtained from muscle or liver disperses readily in hot water to make a turbid solution. Although they have markedly different physical properties, both substances are composed of (1→4)-linked D-glucose polymers of

comparable molecular weight. What structural features of these two polysaccharides underlie their different physical properties? Explain the biological advantages of their respective properties.

Answer: native cellulose consists of glucose units linked by (β1→4) glycosidic bonds, which force the polymer chain into an extended conformation. Parallel series of these extended chains from intermolecular hydrogen bonds, aggregating into long, though, insoluble fiber. Glycogen consists of glucose units linked by (α1→4) glycosidic bonds, which cause bends in the chain and prevent formation of the long fibers. In addition, glycogen is highly branched, and because many of its hydroxyl groups are exposed to water, is highly hydrated and disperses in water. Cellulose is a structural material in plants, consistent with its side-by-side aggregation into insoluble fibers. Glycogen is storage fuel in animals. Highly hydrated glycogen granules with their many nonreducing ends are rapidly hydrolyzed by glycogen phosphorylase to release glucose-1-phosphate.

24. Information content of oligosaccharides

Question: The carbohydrate portion of some glycoproteins may serve as a cellular recognition site. In order to perform this function, the oligosaccharide moiety of glycoproteins must have the potential to exist in a large variety of forms. Which can produce a greater variety of structures: oligopeptides composed of five different amino acid residues or oligosaccharides composed of five different monosaccharide residues? Explain.

Answer: Oligosaccharides, their subunits can bi combined in more ways than the amino acid subunits of olygopeptides. Each hydroxyl group can participate in glycosidic bonds, and the configuration of each glycosidic bond can be either α or β. The polymer can be linear or branched.

25. Nucleotide structure

Question: Which positions in a purine ring of a purine nucleotide in DNA have the potential to form hydrogen bonds but are not involved in Watson-Crick base pairing?

Answer: N-3 and N-7.

26. Base sequence of complementary DNA strands

Question: One strand of a double-helical DNA has the sequence (5')GCGCAATATTTCTCAAAATATTGCGC(3'). Write the base sequence of the complementary

strand. What special type of sequence is contained in this DNA segment? Does the double-stranded DNA have the potential to form any alternative structures?

Answer: (5') GCGCAATATTTCTCAAAATATTGCGC(3'), it cointains a palindrome. The individual strands can form hairpin structure, the two strands can for a cruciform.

Exercise 5

27. Operational definition of lipids

Question: How is the definition of "lipid" different from the types of definitions used for other biomolecules that we have considered, such as amino acids, nucleic acids, and proteins?

Answer: The term "lipid" does not specify a particular chemical structure. Compounds are categorized as lipids based on their greter solubility in organic solvents than in water.

28. Melting points of lipids

Question: The melting points of a series of 18-carbon fatty acids are: stearic acid, 69.6 ºC; oleic acid, 13.4 ºC; linoleic acid, -5 ºC; and linolenic acid, -11 ºC. (a) What structural aspect of these 18-carbon fatty acids can be correlated with the melting point? Provide a molecular explanation for the trend in melting points. (b) Draw all the possible triacylglycerols that can be constructed from glycerol, palmitic acid, and oleic acid. Rank them in order of increasing melting point. (c) Branched-chain fatty acids are found in some bacterial membrane lipids. Would their presence increase or decrease the fluidity of the membranes (that is, give them a lower or higher melting point)? Why?

Answer: **a.** The number of cis double bouds. Each cis double bound cause a band in the hydrocarbon chain, lowering the melting temperature. **b.** Six different triacylglycerols can be constructed. Melting points: OOO < OOP = OPO < PPO = POP < PPP, where O = oleic acid, P = palmitic acid. The grater the content of saturated fatty acids, the higher is the melting point. c. Branched chain fatty acids increase the fluidity of membranes because they decrease the extent of membrane lipid packing.

29. Preparation of Béarnaise sauce

Question: During the preparation of béarnaise sauce, egg yolks are incorporated into melted butter to stabilize the sauce and avoid separation. The stabilizing agent in the egg yolks is lecithin (phosphatidylcholine). Suggest why this works.

Answer: Lecithin, an amphipathic compound, is an emulsifying agent, facilitating the solubilization of butter.

30. Alkali lability of triacylglycerols

Question: A common procedure for cleaning the grease trap in a sink is to add a product that contains sodium hydroxide. Explain why this works.

Answer: The triacylglycerols of animal fats (grease) are hydrolyze by sodium hydroxide (saponified) to form soaps, which are much more soluble in water than are triacylglycerols.

31. Storage of fat-soluble vitamins

Question: In contrast to water-soluble vitamins, which must be a part of our daily diet, fat-soluble vitamins can be stored in the body in amounts sufficient for many months. Suggest an explanation for this difference, based on solubilities.

Answer: Water-soluble vitamins are most rapidly excreted in the urine and are not stored effectively. Fat-soluble vitamins have very low solubility in water and stored in body lipids.

32. Ninhydrin to detect lipids on TLC plates

Question: Ninhydrin reacts specifically with primary amines to form a purplish-blue product. A thin-layer chromatogram of rat liver phospholipids is sprayed with ninhydrin, and the color is allowed to develop. Which phospholipids can be detected in this way?

Answer: Phosphatidylethanolamine and phosphatidylserine.

Exercise 6

33. Properties of lipids and lipid bilayers

Question: Lipid bilayers formed between two aqueous phases have this important property: they form two-dimensional sheets, the edges of which close upon each other and undergo self-sealing to form liposomes. (a) What properties of lipids are responsible for this property of bilayers? Explain. (b) What are the consequences of this property for the structure of biological membranes?

Answer: **a.** Lipids that from bilayers are amphipathic molecules: they contain hydrophilic and hydrophobic units. To minimize the hydrophobic area that is exposed to the water surface, these lipids form two dimensional sheets with the hydrophilic units buried in the interior of the sheet. Furthermore, to avoid exposing the hydrophobic edges of the sheet is perforated, the hole will seal because the membrane is semifluid. **b.** These sheets form the closed membrane surface that envelop cells and compartments within cells (organelles).

34. Lipid melting temperatures

Question: Membrane lipids in tissue samples obtained from different parts of the leg of a reindeer have different fatty acid compositions. Membrane lipids from tissue near the hooves contain a larger proportion of unsaturated fatty acids than those from tissue in the upper leg. What is the significance of this observation?

Answer: The temperature of the body tissues at the extremities, such as near the hooves, is generally lover, than that of the tissues closer to the center of the body. If lipid to remain fluid at this lower temperature, as required by the fluid mosaic model, it must contain a higher proportion of unsaturated fatty acids; unsaturated fatty acids lower the melting point of lipid mixtures.

35. Entropy changes during egg development

Question: Consider a system consisting of an egg in an incubator. The white and yolk of the egg contain proteins, carbohydrates, and lipids. If fertilized, the egg is transformed from a single cell to a complex organism. Discuss this irreversible process in terms of the entropy changes in the system, surroundings, and universe. Be sure that you first clearly define the system and surroundings.

Answer: Consider developing chick as a system, the nutrients, egg shell, and outside word are the surroundings. Transformation of single cell into a chick drastically reduces the entropy, of the

system. Initially, the part of the egg outside the embryo (the surroundings) contain complex fuel molecules (a low entropy condition). During incubation, some of these complex molecules are converted into large numbers of CO_2 and H_2O molecules (high entropy). This increase in the entropy of the surroundings is larger than the decrease of entropy of the chick (the system).

36. Rates of turnover of γ and β phosphates of ATP

Question: If a small amount of ATP labeled with radioactive phosphorus in the terminal position, [γ-32P]ATP, is added to a yeast extract, about half of the 32P activity is found in Pi within a few minutes, but the concentration of ATP remains unchanged. Explain. If the same experiment is carried out using ATP labeled with 32P in the central position, [β-32P]ATP, the 32P does not appear in Pi within such a short time. Why?

Answer: The ATP system is a dynamic steady sate, ATP remains constant, because the rate of ATP consumption equals its rate of synthesis. ATP consumption involves release of the terminal (γ) phosphoryl group; synthesis of ATP from ADP involves replacement of this phosphoryl group. Hence, the terminal phosphate undergoes only relatively slow turnover. In contrast, the central (β) phosphate undergoes only relatively slow turnover.

37. Equation for the preparatory phase of glycolysis

Question: Write balanced biochemical equations for all the reactions in the catabolism of glucose to two molecules of glyceraldehyde 3-phosphate (the preparatory phase of glycolysis), including the standard free-energy change for each reaction. Then write the overall or net equation for the preparatory phase of glycolysis, with the net standard free-energy change.

Answer: Net equation: Glucose + 2 ATP → 2 glyceraldehyde 3-phosphate + 2 ADP + 2 H^+, $\Delta G^{o'}$ = 2.1 kJ/mol.

38. The payoff phase of glycolysis in skeletal muscle

Question: In working skeletal muscle under anaerobic conditions, glyceraldehyde 3-phosphate is converted to pyruvate (the payoff phase of glycolysis), and the pyruvate is reduced to lactate. Write balanced biochemical equations for all the reactions in this process, with the standard free-energy change for each reaction. Then write the overall or net equation for the payoff phase of glycolysis (with lactate as the end product), including the net standard free-energy change.

Answer: Net equation: 2 Glyceraldehyde 3-phosphate + 4 ADP + $2P_i$ → 2 lactate +2 NAD^+, $\Delta G^{o'}$ = –113.6 kJ/mol.

39. Fermentation to produce soy sauce

Question: Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

Answer: Soybean and wheat contain starch, a polymer of glucose, which is broken down to glucose by the microorganisms. The glucose is then broken down to pyruvate, via glycolysis. Because the process carried out in the absence of oxygen (it is a fermentation), pyruvate is reduced to lactic acid and ethanol by the microorganisms. If oxygen were present, pyruvate would be oxidized to acetyl-CoA and than to CO_2 and H_2O. Some of the acetyl-CoA, however, would also be hydrolyzed to acetate acid (vinegar) in the presence of oxygen.

Exercise 7

40. Efficiency of ATP production in muscle

Question: The transformation of glucose to lactate in myocytes releases only about 7% of the free energy released when glucose is completely oxidized to CO_2 and H_2O. Does this mean that anaerobic glycolysis in muscle is a wasteful use of glucose? Explain.

Answer: The transformation of glucose to lactate occurs when muscle cells are low in oxygen and provides a means of generating ATP under oxygen deficient conditions. Because lactate can be transform back to pyruvate, glucose is not wasted, pyruvate can be oxidized by aerobic reactions when oxygen becomes plentiful. This metabolic flexibility gives capacity to adapt to its environment.

41. Free-energy change for triose phosphate oxidation

Question: The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant (K_{eq} = 0.08; $\Delta G^{o\prime}$ = 6.3 kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

Answer: It rapidly removes the 1,3-biphosphoglycerate in a favorable subsequent step, catalyze by phosphoglycerate kinase.

42. Role of the vitamin niacin

Question: Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

Answer: Dietary niacin is used to synthesize NAD^+. Oxidation is carried out with NAD^+ as an electron carrier (reducing agent). Because of this cycling, one molecule can oxidize many thousand of molecules of glucose, and thus the dietary requirement for the precursor vitamin (niacin) is relatively small.

43. Muscle wasting in starvation

Question: One consequence of starvation is a reduction in muscle mass. What happens to the muscle proteins?

Answer: The muscle protein decompose to amino acids, the carbon skeleton of the amino acids step into the citrate cycle, and serve energy for the body.

44. Energy cost of a cycle of glycolysis and gluconeogenesis

Question: What is the cost (in ATP equivalents) of transforming glucose to pyruvate via glycolysis and back again to glucose via gluconeogenesis?

Answer:

The net equation of glycolysis:

Glucose + 2 Pi + 2 ADP + 2 NAD → 2pyruvate + 2 NADH + H^+ + 2 ATP + 2 H_2O

The net equation of the gluconeogenezis:

2 pyruvate + 4 ATP + 2 GTP + 2 NADH + H^+ + 6 H_2O →

glucose-6-phosphate + 4 ADP + 2 GDP + 2 NAD+ + 5P_i

Difference: 4 ATP between the two cycle: glycolysis and gluconeogenesis.

45. Glycogen breakdown in migrating birds

Question: Unlike the rabbit with its short dash, migratory birds require energy for extended periods of time. For example, ducks generally fly several thousand miles during their annual migration. The flight muscles of migratory birds have a high oxidative capacity and obtain the necessary ATP through the oxidation of acetyl-CoA (obtained from fats) via the citric acid cycle. Compare the regulation of muscle glycolysis during short term intense activity, as in the fleeing rabbit, and during extended activity, as in the migrating duck. Why must the regulation in these two settings be different?

Answer: The circulatory system of small vertebrates can carry oxygen to their muscle fast enough to avoid having to use muscle glycogen anaerobically. Migrating birds often fly great distances at high speed without rest and without incurring an oxygen debt. Many running animals of moderate size also maintain an essentially aerobic matabolism in their skeletal muscle. The circulatory system of larger animals can not completely sustain aerobic metabolism in skeletal muscle over long period of intense muscular activity.

Exercise 8

46. Balance sheet for the citric acid cycle

Question: The citric acid cycle has eight enzymes: citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, fumarase, and malate dehydrogenase. (a) Write a balanced equation for the reaction catalyzed by each enzyme. (b) Name the cofactor(s) required by each enzyme reaction. (c) For each enzyme determine which of the following describes the type of reaction(s) catalyzed: condensation (carbon–carbon bond formation); dehydration (loss of water); hydration (addition of water); decarboxylation (loss of CO_2); oxidation-reduction; substrate-level phosphorylation; isomerization. (d) Write a balanced net equation for the catabolism of acetyl-CoA to CO_2.

Answer: **a.**

1.*Citrate synthase*: Acetyl-CoA + oxaloacetate +H_2O → citrate +CoA + H^+

2.*Aconitase*: Citrate → isocitrate

3.*Isocitrate dehydrogenese*: Isocitrate + NAD → α-ketoglutarate + CO_2 + NADH

4.α-*Ketoglutarate dehydrogenase*: α-Ketoglutarate + NAD^+ + CoA → Succinyl-CoA + CO_2 + NADH

5.*Succinyl-CoA synthetase:* Succinyl-CoA +P_i + GDP → succinate + GTP + CoA

6.*Succinate dehydrogenase:* Succinate + FAD → fumarate + H_2O → malate

7.*Fumarase*: Fumarate + H_2O → malate

8.*Malate dehydrogenase:* Malate + NAD^+ → oxaloacetate + NADH + H^+

b. c.

Step 1. CoA, condensation. 2. none, isomerization. 3. NAD^+, oxidative dacarboxylation, 4. NAD^+, CoA, and thiamin pyrophosphate, oxidative decarboxylation. 5. CoA, phosphorilation, 6. FAD, oxidation. 7. none, hydration. 8. NAD^+, oxidation.

d.

Acetyl-CoA + 3NAD^+ +FAD + GDP + P_i + 2H_2O → 2CO_2 + 3NADH + $FADH_2$ + GTP + 2H^+ + CoA

47. Stimulation of oxygen consumption by oxaloacetate and malate

Question: In the early 1930s, Albert Szent-Györgyi reported the interesting observation that the addition of small amounts of oxaloacetate or malate to suspensions of minced pigeon-breast muscle stimulated the oxygen consumption of the preparation. Surprisingly, the amount of oxygen consumed was about seven times more than the amount necessary for complete oxidation (to CO_2 and H_2O) of the added oxaloacetate or malate. Why did the addition of oxaloacetate or malate stimulate oxygen consumption? Why was the amount of oxygen consumed so much greater than the amount necessary to completely oxidize the added oxaloacetate or malate?

Answer: **a.** Oxygen consumption is a measure of the activity of the first two stages of cellular respiration: glycolysis and the citric acid cycle. The addition of oxaloacetate or malate stimulate the citric acid cycle, and thus stimulate respiration. **b.** The added oxaloacetate or malate serves a catalytic role, because it is regenerated in the latter part of citric acid cycle.

48. Respiration studies in isolated mitochondria

Question: Cellular respiration can be studied in isolated mitochondria by measuring oxygen consumption under different conditions. If 0.01 M sodium malonate is added to actively respiring mitochondria that are using pyruvate as fuel source, respiration soon stops and a metabolic intermediate accumulates. (a) What is the structure of this intermediate? (b) Explain why it accumulates.(c) Explain why oxygen consumption stops. (d) Aside from removal of the malonate, how can this inhibition of respiration be overcome? Explain.

Answer: **a.** $^{-}OOC–CH_2–CH_2–COO^{-}$ (succinate) **b.** Malonate is a competitive inhibitor of succinate dehydrogenase. **c.** A block in the citric acid cycle stops NADH formation, which stops electron transfer, which stops respiration. **d.** A large excess of succinate (substrate) overcomes the competitive inhibition.

49. Role of the vitamin thiamine

Question: People with beriberi, a disease caused by thiamine deficiency, have elevated levels of blood pyruvate and α-ketoglutarate, especially after consuming a meal rich in glucose. How are these effects related to a deficiency of thiamine?

Answer: Thiamine is required for the synthesis of thiamine pyrophosphate (TPP), a prostetic group in the pyruvate dehydrogenase and α-ketoglutarate dehydrogenese enzyme complexes. A thiamine

deficiency reduces the activity of these enzyme complexes, and cause the observed accumulation of precursors.

50. Synthesis of oxaloacetate by the citric acid cycle

Question: Oxaloacetate is formed in the last step of the citric acid cycle by the NAD^+-dependent oxidation of L-malate. Can a net synthesis of oxaloacetate from acetyl-CoA occur using only the enzymes and cofactors of the citric acid cycle, without depleting the intermediates of the cycle? Explain. How is oxaloacetate that is lost from the cycle (to biosynthetic reactions) replenished?

Answer: No. For every two carbons that enter as acetate, two leave the cycle as CO_2. Thus there is no net synthesis of oxaloacetate. Net synthesis of oxaloacetate occurs by the carboxylation of the pyruvate, an anaplerotic reaction.

51. Relationship between respiration and the citric acid cycle

Question: Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when O_2 is present. Why?

Answer: Oxygen is need to recycle NAD^+ from the NADH produced by the oxidative reactions of the citric acid cycle. Reoxidation of the NADH occurs during mitochondrial oxidative phosphorilation.

Exercise 9.

52. Energy in triacylglycerols

Question: On a per-carbon basis, where does the largest amount of biologically available energy in triacylglycerols reside: in the fatty acid portions or the glycerol portion? Indicate how knowledge of the chemical structure of triacylglycerols provides the answer.

Answer: The fatty acid portion; the carbon in fatty acids are more reduced than those in glycerol.

53. Fuel reserves in adipose tissue

Question: Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients. (a) If 15% of the body mass of a 70.0 kg adult consists of triacylglycerols, what is the total available fuel reserve, in both kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal = 4.18 kJ. (b) If the basal energy requirement is approximately 8,400 kJ/day (2,000 kcal/day), how long could this person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy? (c) What would be the weight loss in pounds per day under such starvation conditions (1 lb = 0.454 kg)?

Answer: a. 4.0 x 105 kJ,. b. 48 days. 0.5 lb/day.

54. Common reaction steps in the fatty acid oxidation cycle and Citric Acid Cycle

Question: Cells often use the same enzyme reaction pattern for analogous metabolic conversions. For example, the steps in the oxidation of pyruvate to acetyl-CoA and of α-ketoglutarate to succinyl-CoA, although catalyzed by different enzymes, are very similar. The first stage of fatty acid oxidation follows a reaction sequence closely resembling a sequence in the citric acid cycle. Use equations to show the analogous reaction sequences in the two pathways.

Answer: The first step in fatty acid oxidation is analogous to the conversion of succinate to fumarate; the second step, to the conversion of fumarate to malate; the third step, to the conversion of malate to oxaloacetate.

55. Compartmentation in β-oxidation

Question: Free palmitate is activated to its coenzyme A derivative (palmitoyl-CoA) in the cytosol before it can be oxidized in the mitochondrion. If palmitate and [^{14}C]coenzyme A are added to a

liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain.

Answer: Fatty acyl group condensed with CoA in the cytosol are first transferred to carnitine, releasing CoA, then transported into the mitochondrion, where they are again condensed with CoA. The cytosolic and mitochondrial pools of CoA are thus kept separate, and no radioactive CoA from the cytosolic pools enter the mitochondrion.

56. Fatty acids as a source of water

Question: Contrary to legend, camels do not store water in their humps, which actually consist of large fat deposits. How can these fat deposits serve as a source of water? Calculate the amount of water (in liters) that a camel can produce from 1.0 kg of fat. Assume for simplicity that the fat consists entirely of tripalmitoylglycerol.

Answer: Oxidation of fats release water; 1.4 liter of water per kg of tripalmitoylglycerol (ignores the small contribution of glycerol to the mass).

57. Petroleum as a microbial food source

Question: Some microorganisms of the genera *Nocardia* and *Pseudomonas* can grow in an environment where hydrocarbons are the only food source. These bacteria oxidize straight-chain aliphatic hydrocarbons, such as octane, to their corresponding carboxylic acids. How could these bacteria be used to clean up oil spills? What would be some of the limiting factors to the efficiency of this process?

Answer: The bacteria can be used to completely oxidized hydrocarbons to CO_2 and H_2O. However, contact between hydrocarbons and bacterial enzymes may be difficult to achieve. Bacterial nutrient such as nitrogen and phosphorus may be limiting and inhibit growth.

Exercise 10.

58. Fatty acid oxidation in uncontrolled diabetes

Question: When the acetyl-CoA produced during β-oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies—acetone, acetoacetate, and D-β-hydroxybutyrate. This occurs in severe, uncontrolled diabetes: because the tissues cannot use glucose, they oxidize large amounts of fatty acids instead. Although acetyl-CoA is not toxic, the mitochondrion must divert the acetyl-CoA to ketone bodies. What problem would arise if acetyl-CoA were not converted to ketone bodies? How does the diversion to ketone bodies solve the problem?

Answer: Because the mitochondrial pool of CoA is small, must be recycled from acetyl-CoA via the formation of ketone bodies. This allows the operation of the β-oxidation pathway, necessary for energy production.

59. Oxidation of arachidic acid

Question: How many turns of the fatty acid oxidation cycle are required for complete oxidation of arachidic acid to acetyl-CoA?

Answer: 9 turns; arachidic acid, a 20 carbon saturated fatty acid, yields ten molecules of acety-CoA, the last two both formed in the ninth turn.

60. Fate of labeled propionate

Question: If [3-^{14}C]propionate (14C in the methyl group) is added to a liver homogenate, ^{14}C-labeled oxaloacetate is rapidly produced. Draw a flow chart for the pathway by which propionate is transformed to oxaloacetate, and indicate the location of the ^{14}C in oxaloacetate.

Answer: [3-^{14}C]Succinyl-CoA is formed, which gives rise to oxaloacetate labeled at C-2 and C-3.

61. Biological importance of cobalt

Question: In cattle, deer, sheep, and other ruminant animals, large amounts of propionate are produced in the rumen through the bacterial fermentation of ingested plant matter. Propionate is the principal source of glucose for these animals, via the route propionate → oxaloacetate → glucose. In some areas of the world, notably Australia, ruminant animals sometimes show symptoms of

anemia with concomitant loss of appetite and retarded growth, resulting from an inability to transform propionate to oxaloacetate. This condition is due to a cobalt deficiency caused by very low cobalt levels in the soil and thus in plant matter. Explain.

Answer: Methylmalonyl-CoA mutase requires the cobalt-containing cofactor formed from vitamin B_{12}.

62. Fat loss during hibernation

Question: Bears expend about $25 \cdot 10^6$ J/day during periods of hibernation, which may last as long as seven months. The energy required to sustain life is obtained from fatty acid oxidation. How much weight loss (in kilograms) has occurred after seven months? How might ketosis be minimized during hibernation? (Assume the oxidation of fat yields 38 kJ/g.).

Answer: Mass lost per day is about 0,66 kg, or about 138 kg in 7 months. Ketosis could be avoided by degradation of nonessential body proteins to supply amino acid skeletons for gluconeogenesis.

Exercise 11.

63. Products of amino acid transamination

Question: Name and draw the structure of the α-keto acid resulting when each of the following amino acids undergoes transamination with α-ketoglutarate: (a) aspartate, (b) glutamate, (c) alanine, (d) phenylalanine.

Answer: **a.** oxaloacetate: $^{-}OOC–CH_2–C(O)–COO^{-}$

b. α-ketoglutarate: $^{-}OOC–CH_2–CH_2^{-}C(O)–COO^{-}$

c. pyruvate $CH_3–C(O)–COO^{-}$

d. Fenil–CH_2–C(O)–COO^{-}

64. Distribution of amino nitrogen

Question: If your diet is rich in alanine but deficient in aspartate, will you show signs of aspartate deficiency? Explain.

Answer: No; the nitrogen in Ala can be transferred to oxaloacetate via transamination, to form Asp.

65. Ammonia intoxication resulting from an arginine-deficient diet

Question: 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 µg/L to 140 µg/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. (a) What was the role of fasting in the experiment? (b) 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? (c) Why can ornithine be substituted for arginine?

Answer: **a.** Fasting resulted in low blood glucose, subsequent administration of the experimental diet led to rapid catabolism of glucogenic amino acids. **b.** Oxidative dezamination caused the rise in ammonia levels; the absence of arginine (an intermediate in urea cycle) prevented the conversion of ammonia to urea; Arg is not synthesized in sufficient quantities in the cat to meet the needs imposed by the stress of the experiment. This suggest, that Arg is an essential amino acid in the cat's diet.

66. Oxidation of glutamate

Question: 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 α-ketoglutarate and 1 mol of urea.

Answer: H_2O + Glutamate + NAD → α-ketoglutarate + NH^4_+ + NADH + H^+

NH_4^+ + 2ATP + H_2O + CO_2 → carbamoyl phosphate + 2ADP + P_i + $3H^+$

Carbamoyl phosphate + ornithine → citrullin + P_i + H^+

Citrullin + aspartate + ATP → arginosuccinate + AMP + PP_i + H^+

Arginosuccinate → arginine + fumarate

Fumarate + H_2O → malate

Malate + NAD^+ → Oxaloacetate + NADH + H^+

Ocaloacetate + glutamate → aspartate + α-ketoglutarate

Arginine + H_2O → urea + ornithine

Σ: 2Glutamate + CO_2 + $4H_2O$ + $2NAD^+$ + 3ATP →

2 α-ketoglutarate + 2NADH + $7H^+$ + urea + 2ADP + AMP + $2P_i$

Additional reactions that need to considered are:

AMP + ATP → 2ADP

O_2 + $8H^+$ + 2NADH + 6ADP + $6P_i$ → $2NAD^+$ + 6ATP + $8H_2O$

H_2O + PP_i → $2P_i$ + H^+

ΣΣ: 2Glutamate + CO_2 + O_2 + 2ADP + $2P_i$ → 2α-ketoglutarate + urea + $3H_2O$ + 2ATP

67. Alanine and glutamine in the blood

Question: 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: Ala and Gln play special roles in the transport of amino groups from muscle and from mother nonhepatic tissues, respectively, to the liver.

Exercise 12.

68. Compartmentalization of citric acid cycle components

Question: Isocitrate dehydrogenase is found only in the mitochondrion, but malate dehydrogenase is found in both the cytosol and mitochondrion. What is the role of cytosolic malate dehydrogenase?

Answer: Cytosolic malate dehydrogenase plays a key role in the transport of reducing equivalents across the inner mitochondrial membrane via the malate-aspartate suttle.

69. Cellular ADP concentration controls ATP formation

Question: Although both ADP and P_i are required for the synthesis of ATP, the rate of synthesis depends mainly on the concentration of ADP, not P_i. Why?

Answer: The steady-state concentration of P_i in the cell is much higher than that of ADP. P_i released by ATP hydrolysis changes total [P_i] very little.

70. Synthesis of fatty acids from glucose

Question: After a person has ingested large amounts of sucrose, the glucose and fructose that exceed caloric requirements are transformed to fatty acids for triacylglycerol synthesis. This fatty acid synthesis consumes acetyl-CoA, ATP, and NADPH. How are these substances produced from glucose?

Answer: Both glucose and fructose degraded to pyruvate in glycolysis. The pyruvate is converted to acetyl-CoA by the pyruvate dehydrogenase complex. Some of this acetyl-CoA enters the citric acid cycle, which produces reducing equivalents (NAD and NADPH). Mitochondrial electron transfer to O_2 yields ATP.

Exercise 13.

71. Regulation of cholesterol biosynthesis

Question: Cholesterol in humans can be obtained from the diet or synthesized de novo. An adult human on a low-cholesterol diet typically synthesizes 600 mg of cholesterol per day in the liver. If the amount of cholesterol in the diet is large, de novo synthesis of cholesterol is drastically reduced. How is this regulation brought about?

Answer. The rate-determining step in the biosynthesis of cholesterol is the synthesis of mevalonate catalyzed by hydroxymethylglutaryl-CoA reductase. This enzyme is allosterically regulated by mevalonate and cholesterol derivatives. High level of intracellular cholesterol also reduce transcription of the gene encoding HMG-CoA reductase.

72. ATP consumption by root nodules in legumes

Question: Bacteria residing in the root nodules of the pea plant consume more than 20% of the ATP produced by the plant. Suggest why these bacteria consume so much ATP.

Answer: In their symbiotic relationship with the plant, bacteria supply ammonium ion by reducing atmospheric nitrogen, which requires large quantities of ATP.

73. Transformation of aspartate to asparagine

Questions: There are two routes for transforming aspartate to asparagine at the expense of ATP. Many bacteria have an asparagine synthetase that uses ammonium ion as the nitrogen donor. Mammals have an asparagine synthetase that uses glutamine as the nitrogen donor. Given that the latter requires an extra ATP (for the synthesis of glutamine), why do mammals use this route?

Answer: Toxic ammonium ions are transformed to glutamine in the mammalian route, reducing toxic effect on the brain.

Exercise 14.

74. Equation for the synthesis of aspartate from glucose

Question: Write the net equation for the synthesis of aspartate (a nonessential amino acid) from glucose, carbon dioxide, and ammonia.

Answer: Glucose + 2 CO_2 + 2NH_4^+ → 2aspartate + 2H^+ + 2H_2O

75. Phenylalanine hydroxylase deficiency and diet

Question: Tyrosine is normally a nonessential amino acid, but individuals with a genetic defect in phenylalanine hydroxylase require tyrosine in their diet for normal growth. Explain.

Answer: If phenylalanine hydroxylase is defective, the biosynthesis route to Tyr is blocked, and Tyr must be obtained from the diet.

76. Nucleotides as poor sources of energy

Question: Under starvation conditions, organisms can use proteins and amino acids as sources of energy. Deamination of amino acids produces carbon skeletons that can enter the glycolytic pathway and the citric acid cycle to produce energy in the form of ATP. Nucleotides, on the other hand, are not similarly degraded for use as energy-yielding fuels. What observations about cellular physiology support this statement? What aspect of the structure of nucleotides makes them a relatively poor source of energy?

Answer: Organisms do not store nucleotides to be used as fuel and do not completely degrade them, but rather hydrolyze them to release the bases, which can be recovered in salvage pathways. The low C:N ratio of nucleotide makes them poor source of energy.

77. Treatment of gout

Question: Allopurinol, an inhibitor of xanthine oxidase, is used to treat chronic gout. Explain the biochemical basis for this treatment. Patients treated with allopurinol sometimes develop xanthine stones in the kidneys, although the incidence of kidney damage is much lower than in untreated gout. Explain this observation in the light of the following solubilities in urine: uric acid, 0.15 g/L; xanthine, 0.05 g/L; and hypoxanthine, 1.4 g/L.

Answer: Treatment with allopurinol has two biochemical consequences. 1. Conversion of hypoxanthine to uric acid is inhibited, causing accumulation of hypoxanthine, which is more soluble and more readily excreted. This alleviates the clinical problems associated with AMP degradation. 2. Conversion of guanine to uric acid is also inhibited, causing accumulation of xanthine, which is, unfortunately even less soluble than uric acid. This is the source of xanthine stones . Because the amount of GMP degradation is low relative to AMP degradation, the kidney damage caused by xanthine stone is less than that caused by untreated gout.