STUDENT COMPANION

to accompany

Biochemistry, Fifth Edition

Richard I. Gumport
College of Medicine at Urbana-Champaign, University of Illinois

Frank H. Deis
Rutgers University

Nancy Counts Gerber
San Francisco State University

Expanded Solutions to Text Problems
contributed by

Roger E. Koeppe, II
University of Arkansas at Fayetteville

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13. Protein scientists have devised a competition called CASP, or Critical Assessment of Techniques for Protein Structure Prediction, which is held every other year. Laboratories that are working on determination of three-dimensional structure by x-ray crystallography (or nmr) announce that they expect to release the structure in a few months. They give a description of the sequence of the protein and its use in the cell, and withhold the actual structural coordinates until a certain date. In the meantime, laboratories with predictive algorithms publicly post the structure they think the protein will have. The success or failure of the prediction takes place in a public arena, and the better predictors have bragging rights. CASP-4 in 2000 showed that there are several effective programs available, notably ROSETTA, used by David Baker of the University of Washington. Results of the competition are published in the journal *Protein* and online (in technical language) at the website http://predictioncenter.llnl.gov/.

14. The primary advantage of precursor chain synthesis is that the production of related proteins can be coordinated. This could be important in viral infection, and it may also be important for coordinated synthesis of hormones with related activities. It is worth noting that there are other reasons for the synthesis of polyprotein precursors. For example, the genome of the poliovirus consists of a single RNA molecule that acts as a messenger on entering the cytoplasm of the host. In eukaryotic cells a messenger RNA molecule can be translated into only one polypeptide chain. Therefore the poliovirus can reproduce only by synthesizing its proteins by sequential cleavages.

15. The molarity of water equals the number of moles of water per liter. A liter of water weighs 1000 grams, and its molecular weight is 18, so the molarity of water is

$$M = \frac{1000}{18} = 55.6$$

At 25°C, K_w is 1.0×10^{-14}; at neutrality, the concentration of both hydrogen and hydroxyl ions is each equal to 10^{-7} M. Thus, the actual concentration of H_2O is (55.6 − 0.0000001) M; the difference is so small that it can be disregarded.

16. Because pH values are based on a logarithmic scale, every unit change in pH means a tenfold change in hydrogen ion concentration. When pH = 2.0, $[H^+] = 10^{-2}$ M; when pH = 3.0, $[H^+] = 10^{-3}$ M.

17. Assume that HCl in solution is completely ionized to H^+ and Cl^-. Then find the concentration of H^+, which equals the concentration of Cl^-.

$$\text{pH} = \log\left[H^+\right] = 2.1$$

$$\left[H^+\right] = 10^{2.1}$$

$$= 10^{0.9} \quad 10^{3}$$

$$= 7.94 \quad 10^{3}\,M$$

$$\text{Thus, } \left[H^+\right] = \left[Cl^-\right] = \left[HCl\right] = 7.94 \quad 10^{3}\,M$$

18. Use the Henderson-Hasselbalch equation to calculate the concentration of histidine, whose imidazole ring is ionized at neutral pH. The value of p*K* for the ring is 6.0 for a histidine residue in a protein (see Table 3.1).

30. Mitochondria can use a genetic code that differs from the standard code because mitochondrial DNA encodes a distinct set of tRNAs that are matched to the genetic code used in their mRNAs.
31. The mutations in a given gene of *E. coli* could be mapped by recombination analysis. The proteins encoded by the wild-type and the mutant genes could then be sequenced, and the location and nature of the amino acid substitution for each mutation identified. The result would be that the order of the mutations on the genetic map is the same as the order of the corresponding changes in the amino acid sequence of the polypeptide produced by the gene; these experiments established that genes and their polypeptide products are collinear in prokaryotes.
32. (a) The number of nucleotides in the gene was significantly greater than three times the number of amino acids in the protein. There were two stretches of extra nucleotides between the exon sequences that encode the amino acids in the β-chain.
    (b) The mRNA hydridized to the DNA under conditions where DNA-RNA hybrids are more stable than DNA-DNA hybrids, but there were sections of duplex DNA between the hybrid regions. This indicated that there are intron sequences in the DNA that have no corresponding sequences in the mRNA. (See Figure 5.33 in the text.)
    (c) The intervening sequences (introns) in the nascent or primary transcript, which are complementary to the template strand of the DNA of the gene but do not encode amino acids in the protein, must be removed by splicing to generate the mRNA that functions in translation.
33. The shuffling of exons that encode discrete functional domains, such as catalytic sites, binding sites, or structural elements, preserves the functional units but allows them to interact in new ways, thereby generating new kinds of proteins.
34. b, c, and d. (a) is incorrect because exon shuffling takes place at the DNA level through breakage and rejoining of DNA not RNA.

PROBLEMS

1. The genome of the mammalian virus SV40 is a circular DNA double helix containing 5243 base pairs. When a solution containing intact DNA molecules is heated, one observes an increase in the absorbance of ultraviolet light at 260 nm. When the solution is then cooled slowly, a decrease in absorbance is observed. If one or more breaks are made in the sugar-phosphate backbones of the SV40 double-strand circles, heating causes a similar hyperchromic effect. However, when the solution of nicked molecules is cooled, the reduction in absorbance is much slower than that observed in the solution containing intact molecules. Why do the two types of molecules behave differently when they are cooled after heating?
2. A number of factors influence the behavior of a linear, double-strand DNA molecule in a 0.25M sodium chloride solution. Considering this, explain each of the following observations.
    (a) The T_m increases in proportion to length of the molecule.
    (b) As the concentration of sodium chloride decreases, the T_m decreases.
    (c) Renaturation of single strands to form double strands occurs more rapidly when the DNA concentration is increased.
    (d) The T_m value is reduced when urea is added to the solution.

ANSWERS TO PROBLEMS

1. Cleavage of a circular molecule at one specific site, followed by denaturation, will yield single-strand DNA molecules with a specific end-to-end base sequence; that is, the molecules have base sequences that are perfectly complementary. Such molecules will anneal to form double-strand linears, rather than circles. Random single cleavages of the original intact molecules also yield double-strand linears with a variety of end-to-end (or permuted) sequences. Denaturation and renaturation allow the random association of these linears, which results in the formation of double-strand linears with overlapping, complementary ends. Such molecules then form circles as their overlapping ends anneal.
2. During the early years of such experiments, few ways were available to determine what happened to the DNA during transformation attempts, so specific remedies could not be sought. Consequently, the fate of the test DNA could not be determined. Among the reasons that these transformation attempts were not successful were the failure of the cells to take up the DNA, the rapid degradation of the DNA inside the cell (restriction enzymes are a good example of a cause of this particular problem), the lack of accurate transcription or translation, and the inability of the host cells to replicate and maintain the foreign DNA as they divided.
3. Among the ways that a gene could be inactivated are the insertion of a stop codon in the sequence, which would prevent the complete translation of the protein; a mutation in the promoter region of the gene, which would prevent proper transcription; and other mutations that could prevent proper splicing or processing. To distinguish a functional gene from a pseudogene, you would have to determine the sequence of the protein and then compare it with the coding sequence for each of the gene sequences. These types of analyses remind us that protein sequencing remains a very necessary tool in molecular biology.
4. Whenever one attempts, using gel electrophoresis, to locate all the fragments produced by a particular enzyme, a chance exists that very small fragments generated by the cleavages may not be detected. Determining the sequences of a second set of fragments whose sequences extend across the junctions of the original set of fragments serves as a check on the overall assignment of sequence.
5. At high pH, protons dissociate from some of the bases, making them unable to participate in base pairing. One example is guanine, for which the pK_a of the proton on N-1 is 9.2. Removal of the hydrogen at this location disrupts the ability of guanine to pair with cytosine.

   If you mix the two types of double-strand molecules, you would expect to see linear molecules that are double-strand all along their length as well as some molecules that are only partially double-strand. These partially double-strand molecules will contain a single-strand loop that locates the position of the insertion; they are formed between one strand of the molecule containing the normal gene and one strand of the molecule containing the insertion.
6. Even if you were able to isolate intact, unbroken bacterial chromosomes, formation of intact heteroduplex molecules between the deletion and wild-type DNAs is difficult because the very long single strands become entangled as they pair with each other, making them impossible to analyze by electron microscopy. In addition, the time required for complete reassociation of the strands is very long. Generating shorter, randomly cleaved DNA frag-

16. The enzyme DNA ligase catalyzes the formation of a phosphodiester bond at a break (nick) in the phosphodiester backbone of a duplex DNA molecule. The enzyme from bacteriophage T4 uses the free energy of hydrolysis ATP as the energy source for the formation of the phosphodiester bond. A covalently modified form of the enzyme in which AMP is bound to a lysine side chain is an intermediate in the reaction. The intermediate is formed by the reaction of E + ATP to form E-AMP + PP_i. In the next step, the AMP is transferred from the enzyme to a phosphate on the DNA to form a pyrophosphate-linked DNA-AMP. In the last step of the reaction, the phosphodiester bond is formed by the free enzyme to seal the nick in the DNA and AMP is released.
    (a) Write chemical equations that show the individual steps that occur over the course of the overall reaction.
    (b) Does this enzyme catalyze a double-displacement reaction?
    (c) Do you think that if DNA were omitted from the reaction mixture, the enzyme would catalyze a partial reaction? If so, what reaction might it catalyze?

17. If you were studying an enzyme that catalyzed the reaction of ATP and fructose 1-phosphate to form fructose 1,6-bisphosphate and ADP and discovered that a plot of the initial velocity of formation of fructose 1,6-bisphosphate *versus* ATP concentration was not hyperbolic, but rather sigmoid, what would you suspect?

ANSWERS TO PROBLEMS

1. The values for $\Delta G^{\circ\prime}$ are found by substituting the values for K'_{eq} into equation 6 on page 195 of the text.
    (a) $G^{\circ} = 2.303\, RT \log_{10} K_e$

    $= 2.303 \quad (1.98 \quad 10^{-3}) \quad 298 \log_{10} (1.5 \quad 10^{4})$

    $= 5.7$ kcal / mol

    (b) − 0.24 kcal/mol
    (c) + 1.1 kcal/mol
    (d) + 5.2 kcal/mol

2. Equation 8 in Section 8.2.2 is used to find the answers.
    (a) $K_e = 10 \quad G^{\circ}/1.36$

    $= 10^{(-10/1.36)}$

    $= 2.3 \quad 10^{7}$

    (b) 5.4
    (c) 0.18
    (d) 4.4×10^{-8}

3. (a) The applicable relationship is equation 1 in Section 8.2.2 of the text:

$$G = G^{\circ} + RT \ln \frac{[C][D]}{[A][B]}$$

$$= G^{\circ} + RT \ln \frac{[\text{glucose 6-phosphate}][ADP]}{[\text{glucose}][ATP]}$$

11. The structure of deoxyhemoglobin is stabilized by each of the following interactions *except* for
    (a) BPG binding.
    (b) salt bridges between acidic and basic side chains.
    (c) coordination of the hemes with the distal histidine.
    (d) hydrophobic interactions.
    (e) salt bridges involving N-terminal carbamates.
12. In the transition of hemoglobin from the oxy to the deoxy form, an aspartate residue is brought to the vicinity of His 146. This increases the affinity of this histidine for protons. Explain why.

Isozymes Provide a Means of Regulation Specific to Distinct Tissues and Developmental Stages

13. Which of the following would not be useful in distinguishing one isozyme from another?
    (a) electrophoretic mobility
    (b) gene sequence
    (c) kinetic rate constant
    (d) allosteric regulators

Covalent Modification Is a Means of Regulating Enzyme Activity

14. Protein kinases
    (a) transfer a phosphoryl group from one protein to another.
    (b) use AMP as a substrate.
    (c) use Thr, Ser, or Tyr as the acceptor groups for phosphoryl transfer.
    (d) transfer the α phosphorus atom of ATP.
    (e) are located on the external surface of cells.
15. Explain how a phosphoryl group can change the conformation of a protein.
16. Protein kinase A
    (a) is activated by ATP.
    (b) consists of two catalytic (c) and two regulatory (r) subunits in the absence of activator.
    (c) upon binding the activator dissociates into one c_2 and two r subunits.
    (d) contains a pseudosubstrate sequence in the c subunits.

Many Enzymes Are Activated by Specific Proteolytic Cleavage

17. The pancreas is the source of the proteolytic enzyme trypsin. Which of the following are reasons trypsin does not digest the tissue in which it is produced?
    (a) It is synthesized in the form of an inactive precursor that requires activation.
    (b) It is stored in zymogen granules that are enclosed by a membrane.
    (c) It is active only at the pH of the intestine, not at the pH of the pancreatic cells.
    (d) It requires a specific noncatalytic modifier protein in order to become active.

Fatty Acids Are Key Constituents of Lipids

3. Which of the following fatty acids is polyunsaturated?
   (a) arachididic
   (b) arachidonic
   (c) oleic
   (d) palmitic
   (e) stearic

There Are Three Common Types of Membrane Lipids

4. Which of the following are membrane lipids?
   (a) cholesterol
   (b) glycerol
   (c) phosphoglycerides
   (d) choline
   (e) cerebrosides

5. The phosphoinositol portion of the phosphatidyl inositol molecule is called which of the following?
   (a) the amphipathic moiety
   (b) the hydrophobic moiety
   (c) the hydrophilic moiety
   (d) the micelle
   (e) the polar head group

6. Acid hydrolysis will break all ester, amide, and acetal chemical linkages. Which of the following statements is incorrect about the acid hydrolysis of various lipids?
   (a) A cerebroside will release two fatty acids and one monosaccharide per mole of cerebroside.
   (b) Phosphatidylcholine will release two fatty acids and one glycerol molecule per mole of phosphatidylcholine.
   (d) Sphingomyelin and phosphatidylcholine will release equivalent molar amounts of choline and phosphoric acid.
   (e) Cerebrosides and sphingomyelin will each release one mole of sphingosine.

7. After examining the structural formulas of the four lipids in Figure 12.1, answer the following questions.
   (a) Which are phosphoglycerides?
   (b) Which is a glycolipid?
   (c) Which contain sphingosine?
   (d) Which contain choline?
   (e) Which contain glycerol?
   (f) Name the lipids.

5. The reaction

   phosphoenolpyruvate + ADP + H^+ $\longrightarrow$ pyruvate + ATP

   has a $\Delta G^{o\prime} = -7.5$ kcal/mol. Calculate $\Delta G^{o\prime}$ for the hydrolysis of phosphoenolpyruvate.

6. Inside cells, the ΔG value for the hydrolysis of ATP to ADP + P_i is approximately −12 kcal/mol. Calculate the approximate ratio of [ATP] to [ADP][P_i] found in cells at 37°C.

   (a) 5000/1
   (b) 4000/1
   (c) 2000/1
   (d) 1000/1
   (e) 200/1

7. Which of the following are ways by which two reactions can be coupled energetically to each other?

   (a) As common intracellular components of a compartment, two reactions become automatically coupled.
   (b) An ionic gradient across a membrane that is formed by one reaction can drive another reaction that uses the gradient to render it exergonic.
   (c) A shared, common intermediate can couple two reactions.
   (d) A protein that is activated by binding another molecule or by being covalently modified can provide energy to drive another reaction.

8. Which of the following statements about the structure of ATP are correct?

   (a) It contains three phosphoanhydride bonds.
   (b) It contains two phosphate ester bonds.
   (c) The sugar moiety is linked to the triphosphate by a phosphate ester bond.
   (d) The nitrogenous base is called *adenosine*.
   (e) The active form is usually in a complex with Mg^{2+} or Mn^{2+}.

9. Which of the following factors contributes to the high phosphate group–transfer potential of ATP?

   (a) the greater resonance stabilization of ADP and P_i than of ATP
   (b) the increase in the electrostatic repulsion of oxygens upon hydrolysis of ATP
   (c) the interaction of the terminal phosphoryl group with the ribose group in ADP
   (d) the formation of a salt bridge between the base amino group and the negative charges of the phosphate oxygens in ATP

10. Which of the following are high-energy compounds?

    (a) glycerol 3-phosphate
    (b) adenosine diphosphate
    (c) glucose 1-phosphate
    (d) 1,3-bisphosphoglycerate
    (e) fructose 6-phosphate

11. ATP falls in the middle of the list of compounds having high phosphate group–transfer potentials. Explain why this is advantageous for energy coupling during metabolism.

12. Which of the following statements about the phosphoryl transfer potential of skeletal muscle are correct?

    (a) The ATP of muscle can sustain contraction for less than a second.
    (b) Creatine phosphate serves as a phosphoryl reservoir that replenishes the ATP pool.
    (c) Creatine phosphate can support contraction for up to four minutes.
    (d) The phosphoguanidino group of creatine phosphate has a large negative standard free energy of hydrolysis.
    (e) Creatine phosphate is formed by a reaction between creatine and ATP.

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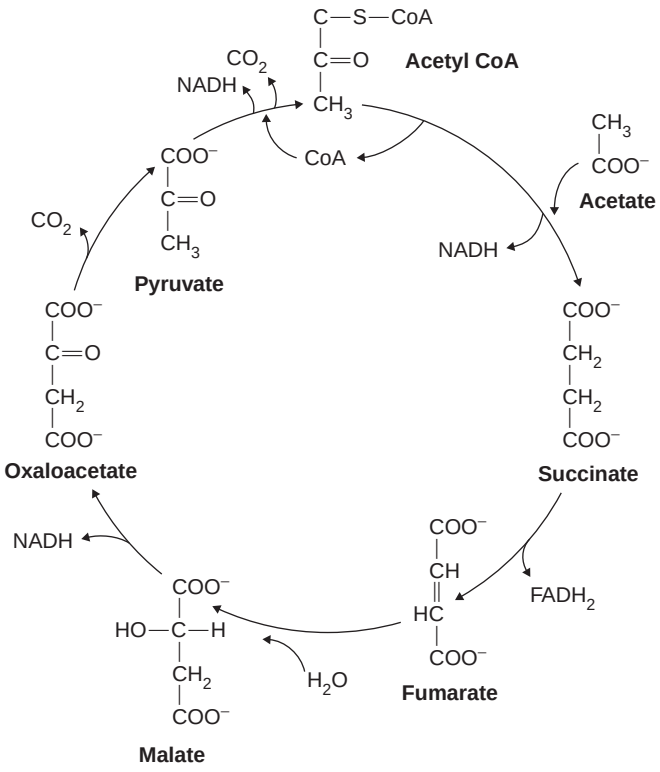
9. As shown in Figure 17.2, there are at least four steps that generate reduced electron carriers. For each acetate group consumed, 3 NADH and 1 $FADH_2$ are generated, and their subsequent reoxidation in the electron transport chain provides energy for the generation of nine molecules of ATP. The same number of reduced electron carriers is generated through the action of pyruvate dehydrogenase and the enzymes of the citric acid cycle, so that the energy liberated by both schemes is the same. Each of the reactions shown in Thunberg's scheme is known to occur, except for the condensation of two acetyl groups to form succinate.

FIGURE 17.2 Thunberg's cycle.



10. Pyruvate dehydrogenase phosphate phosphatase removes a phosphoryl group from pyruvate dehydrogenase, activating the enzyme complex and accelerating the rate of synthesis of acetyl CoA. Cells deficient in phosphatase activity cannot activate pyruvate dehydrogenase, so that the rate of entry of acetyl groups into the citric acid cycle will decrease, as will aerobic production of ATP. Under such conditions, stimulation of glycolytic activity and a subsequent increase in lactate production would be expected as the cell responds to a continued requirement for ATP synthesis. See the clinical note on page 481 of the text (Section 17.2.1).
11. As discussed in the previous problem, the phosphatase activates pyruvate dehydrogenase, stimulating the rate of both glycolysis and the citric acid cycle. Calcium-mediated activation of pyruvate dehydrogenase therefore promotes increased production of ATP, which is then available for muscle contraction.
12. Examination of the structures of the α-keto acid analogs of glutamate and aspartate shows that they are in fact both citric acid cycle intermediates, α-ketoglutarate and oxaloacetate.

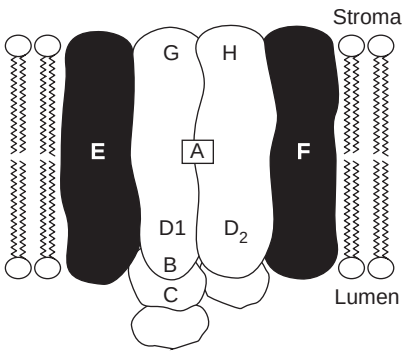
Light Absorption by Chlorophyll Induces Electron Transfer

5. Which of the following are constituents of chlorophylls?
   (a) substituted tetrapyrrole
   (b) plastoquinone
   (c) Mg^{2+}
   (d) Fe^{2+}
   (e) phytol
   (f) iron porphyrin
6. Why do chlorophylls absorb and transfer visible light efficiently?
7. Carefully read the description of the L, M, and H subunits of the bacterial reaction center and subunits D1 and D2 in photosystem II (pp. 532–535). How would you mark the locations of L, M, and H on the "box" structure of Figure 19.10? Where are D1 and D2 in Figure 19.13? Note that D1 contains the "loose" plastoquinone.

Two Photosystems Generate a Proton Gradient and NADPH in Oxygenic Photosynthesis

8. Which of the following statements about photosystem II are correct?
   (a) It is a multimolecular transmembrane assembly containing several polypeptides, several chlorophyll molecules, a special chlorophyll (P680), pheophytin, and plastoquinones.
   (b) It transfers electrons to photosystem I via the cytochrome *bf* complex.
   (c) It uses light energy to create a separation of charge whose potential energy can be used to oxidize H_2O and to produce a reductant, plastoquinol.
   (d) It uses an Fe^{2+}-Cu^{+} center as a charge accumulator to form O_2 without generating potentially harmful hydroxyl radicals, superoxide anions, or H_2O_2.
9. Which statement about the Mn center of photosystem II is INCORRECT?
   (a) The Mn center has four possible oxidation states.
   (b) Electrons are transferred from the Mn center to $P680^{+}$.
   (c) A tyrosine residue on the D1 protein is an intermediate in electron transfer.
   (d) The O_2 released by the Mn center comes from the oxidation of water.
   (e) Each photon absorbed by the reaction center leads to the removal of an electron from the Mn cluster.
10. Using the diagram of photosystem II (Figure 19.1), identify the appropriate components, sites, and functions listed below. The figure here is a greatly simplified version of Figure 19.12 on page 534 of the text, and a more complex version of Figure 19.13. Note that D1 contains the "loose" plastoquinone.

FIGURE 19.1



ANSWERS TO SELF-TEST

1. (a) A and G
   (b) G and E
   (c) All the bonds are α-1,4 glycosidic linkages except for the one between residues G and E.
   (d) No. The two branches are too short for phosphorylase cleavage. Phosphorylase stops cleaving four residues away from a branch point.
   (e) Yes. Residue G can be hydrolyzed by α-1,6-glucosidase (the debranching enzyme).
   (f) No. The branching enzyme transfers a block of about seven residues from a nonreducing end of a chain at least 11 residues long. Furthermore, the new α-1,6 glycosidic linkage must be at least four residues away from a preexisting branch point at a more internal site. The fragment of glycogen in Figure 21.1 does not fulfill these requirements.
2. b, c, d
3. Although the concentration of glycogen is higher in liver, the larger mass of muscle stores more glycogen in toto.
4. The phosphorolytic cleavage of glycogen produces glucose 1-phosphate, which can enter into the glycolytic pathway after conversion to glucose 6-phosphate. These reactions do not require ATP. On the other hand, the hydrolysis of glycogen would produce glucose, which would have to be converted to glucose 6-phosphate by hexokinase, requiring the expenditure of an ATP. Therefore, harvesting the free energy stored in glycogen by phosphorolytic cleavage rather than a hydrolytic one is more efficient because it decreases the ATP investment.
5. a, b
6. (a) 2, 4 (b) 1, 3 (c) 1, 2, 5. None of these enzymes requires ATP.
7. a. Answer (d) is incorrect because the phosphate group at one position on the small substrate molecule is transferred to, and remains for one cycle of reaction on, phosphoglucomutase and the phosphate at the other position on the small molecule product comes from a pre-existing phosphate on enzyme. For a given glucose 1-phosphate substrate, the phosphate on the product, glucose 6-phosphate, is not the same one that was present on the substrate; it came from the enzyme.
8. b
9. (a) Yes, phosphorylase would act on amylose by removing one glucose residue at a time from the nonreducing end.
   (b) No, the rate of degradation of amylose would be much slower than that of glycogen because amylose would have only a single nonreducing end available for reaction, whereas glycogen has many ends.
   (c) The increased the number of ends available to phosphorylase as a result of cleaving the chain into pieces with the endosaccharidase would allow a more rapid production of glucose 1-phosphate by phosphorylase.
10. c. A glucose molecule that is degraded in the glycolytic pathway to two pyruvate molecules yields two ATP; however, the formation of glucose-1-P from glycogen does not consume the ATP that would be required for the formation of glucose-6-P from glucose. Thus, the net yield of ATP for a glucose residue derived from glycogen is three ATP.
11. (a) A and D
    (b) B
    (c) B to A
    (d) protein phosphatase 1

Thirty g palmitoyl CoA is equivalent to 0.12 mole of palmitate, which generates $0.12 \times 123 = 14.8$ moles of water when oxidized. At 18 g mol^{-1}, 14.8 moles of water equal 266 g, or 266 ml, of water.

19. The oxidation of a C_{16} fatty acid (palmitate) leads to the formation of eight molecules of acetyl CoA. Acetyl CoA, which contains two carbon atoms, is oxidized to two CO_2 in the citric acid cycle, so that the net number of carbons entering and leaving the cycle is zero. Thus, no net carbons are available to enter the gluconeogenic pathway. On the other hand, oxidation of a C_{15} fatty acid generates seven acetyl CoA molecules, plus one molecule of propionyl CoA. This compound is converted by carboxylation, epimerization, and conversion to succinyl CoA, a *four*-carbon compound that is an intermediate in the citric acid cycle. Succinyl CoA contributes two extra carbons to the gluconeogenic pathway, leading to the net synthesis of glucose.

20. High levels of citrate signal that glucose utilization is no longer necessary and that adequate carbon atoms are available for synthesis of palmitoyl CoA. Citrate inhibits phosphofructokinase 1 activity, decelerating the rate of glycolysis. On the other hand, citrate stimulates the activity of acetyl CoA carboxylase, so that increased production of malonyl CoA leads to stimulation of fatty acid synthesis. The transport of citrate from the mitochondrial matrix to the cytosol is important because both phosphofructokinase 1 and acetyl CoA carboxylase are located in the cytosol.

21. The most likely deficiency is a lack of 2,4-dienoyl CoA reductase, an enzyme that is essential for the degradation of unsaturated fatty acids with double bonds at even-numbered carbons. Such fatty acids include linoleate (9-*cis*,12-*cis* 18:2). Four rounds of oxidation of linoleoyl CoA generate a 10-carbon acyl CoA that contains a *trans*-Δ^2 and a *cis*-Δ^4 double bond. This intermediate is a substrate for the reductase, which converts the 2,4-dienoyl CoA to *cis*-Δ^3-enoyl CoA. A deficiency of 2,4-dienoyl reductase leads to an accumulation of *trans*-Δ^2,*cis*-Δ^4-decadienoyl CoA molecules in the mitochondrion. The observation that carnitine derivatives of the 2,4-dienoyl CoA are found in blood and urine provides evidence that these molecules accumulate in the mitochondrion and are then attached to carnitine. Formation of carnitine decadienoate allows the acyl molecules to be transported across the inner mitochondrial membrane into the cytosol, and then into the circulation.

    Mitochondria from the patient function normally, taking up oxygen as they carry out oxidation of various substrates including palmitate, a saturated fatty acid. However, incubation of those mitochondria with linoleate results in reduced oxygen uptake, because the absence of the reductase molecule allows only a limited number of rounds of β oxidation to occur before the 2,4-dienoyl molecule is formed. Lack of muscle tone could mean that there are difficulties in oxidizing fuel molecules needed to provide energy for muscle contraction. If carnitine levels in cells are lower because many of them are esterified to decadienoate molecules, the result is a virtual deficiency of carnitine. The ability of the cell to transport other long-chain fatty acids across the inner mitochondrial membrane is limited under these conditions. Impairment of fatty acid oxidation means that fewer ATP molecules are available for muscular activity.

    One immediate strategy for dealing with this disorder is to limit linoleate in the diet. However, linoleate is a starting point for other unsaturated fatty acids including arachidonate, a precursor of eicosanoid hormones. Limiting linoleate in the diet of a person with the reductase deficiency would have to be carried out carefully, in order to avoid a deficiency of an essential fatty acid.

10. Plants synthesize all 20 common amino acids de novo. Glyphosate, a weed killer sold under the trade name Roundup, is an analog of phosphoenolpyruvate that specifically inhibits 3-enolpyruvylshikimate 5-phosphate synthase, a key enzyme of the pathway for chorismate biosynthesis. This compound is a very effective plant herbicide, but has virtually no effect on mammals. Why?
11. In *B. subtilis,* the pathway from chorismate to tryptophan is feedback-inhibited by tryptophan, which suppresses anthranilate synthase activity. Mutant *B. subtilis* that lacks tryptophan synthetase can grow on minimal medium only when supplemented with exogenous tryptophan. Under these conditions, none of the intermediates in the tryptophan biosynthetic pathway from anthranilate to indole 3-glycerol phosphate are produced. However, when the bacteria have depleted the medium of tryptophan, the levels of those intermediates increase, even though there is no net production of tryptophan. Why?
12. Both genetic and biochemical methods have been used to establish the biosynthetic pathways for essential amino acids in bacteria and other microorganisms. One classic approach is isotope competition, which begins with the use of radioactive glucose as the sole source of carbon for growing bacteria. Under these conditions, all the intermediates in a particular pathway will be uniformly labeled, but if a nonradioactive intermediate in the pathway is added to the growing cells, it will reduce or dilute the radioactivity of that intermediate and others following it in a pathway.

    Britten and his coworkers (R. B. Roberts, P. H. Abelson, D. B. Cowie, E. T. Bolton, and R. J. Britten, *Studies in Biosyntheses in* Escherichia coli. Carnegie Institution of Washington, Pub. No. 607, 1955), used isotope competition to examine the biosynthetic pathway for threonine, methionine, and other amino acids. They grew *E. coli* in a minimal medium containing labeled glucose and nonlabeled homoserine, which was known to relieve auxotrophy for several amino acids. Under these conditions, isoleucine, threonine, and methionine isolated from the cells had little or no radioactivity. In contrast, the radioactivity of aspartate and lysine was unchanged whether cells were grown with or without the addition of nonradioactive homoserine.

    In a similar experiment, nonradioactive aspartate was used in the growth medium; aspartate from protein hydrolysates was virtually nonradioactive, as were lysine, methionine, threonine, and isoleucine. When nonlabeled threonine was used along with radioactive glucose, only threonine and isoleucine from protein hydrolysates had reduced radioactivity. And when either nonradioactive isoleucine or methionine was used, they affected only their own levels of radioactivity in protein hydrolysates.

    On the basis of these observations, write an outline of the biosynthetic pathway for the amino acids isoleucine, threonine, homoserine, methionine, and lysine.

ANSWERS TO PROBLEMS

1. Glycine is an obligatory precursor of heme; in the reaction catalyzed by δ-aminolevulinate synthase, glycine condenses with succinyl CoA to form δ-aminolevulinate. The reduction in the concentration of glycine in the cell caused by the *glyA*$^-$ mutation will cause a decrease in the rate of heme synthesis.
2. (a) The studies led White and Beach to conclude that methionine, which is another sulfur-containing amino acid, is a biosynthetic precursor of cysteine. Later work elucidated the roles of methionine in the active methyl cycle and in the synthesis of cysteine and confirmed their conclusion.

18. (a) 2, 5, 7 (b) 1, 6, 7, 9 (c) 1, 4, 8 (d) 3, 4, 10

19. All the events except (c) occur in the LDL pathway. The proper sequence is d, b, a, e.

20. c, f

21. The main source of cholesterol for cells outside the liver and intestine is from circulating LDL. Cholesterol released during the degradation of LDL suppresses the formation of new LDL receptors, thereby decreasing the uptake of exogenous cholesterol by the cell.

22. A defect in apoprotein B-100 that prevents the binding of LDL to the cell-surface receptor would result in the stimulation of the synthesis of endogenous cholesterol and LDL receptors and a decrease in the synthesis of cholesterol esters via the ACAT reaction. Indeed, the cellular and physiological consequences of such a mutation may be similar to those seen in familial hypercholesterolemia.

23. a, d, e

24. b, e

25. Bile salts are effective detergents because they contain both polar and nonpolar regions. They have several hydroxyl groups, all on one side of the ring system, and a polar side chain that allow interactions with water. The ring system itself is nonpolar and can interact with lipids or other nonpolar substances. Bile salts are planar, amphipathic molecules, in contrast with such detergents as sodium dodecyl sulfate (text, p. 84), which are linear.

26. (a) cortisol
    (b) progesterone
    (c) six
    (d) true. A deficiency of 21-hydroxylase will impair hydroxylation at C-21 of progesterone, which will prevent the normal synthesis of cortisol and mineralocorticoids from progesterone.

27. b, d, e

28. In mammals, foreign aromatic molecules are hydroxylated by the cytochrome P450-dependent monooxygenases that are present in the endoplasmic reticulum of the liver cells. The hydroxylated derivatives are more water-soluble and have functional groups for the attachment of very polar substances, such as glucuronate, that allow them to be excreted in urine. The action of the cytochrome P450 system sometimes converts potential carcinogenic compounds into highly carcinogenic derivatives.

29. (a) 3, 5 (b) 1, 4 (c) 2

30. The water-soluble bile salt glycocholate is a major cholesterol breakdown product.

31. a

32. d

SELF-TEST

Transcription Is Catalyzed by RNA Polymerase

1. Give the subunit composition of the RNA polymerase of *E. coli* for both the holoenzyme and the core enzyme.
2. Match the subunit of the RNA polymerase of *E. coli* in the left column with its putative function during catalysis from the right column.

(a) α	(1) binds the DNA template
(b) β	(2) binds regulatory proteins and sequences
(c) β′	(3) binds NTPs and catalyzes bond formation
(d) σ70	(4) recognizes the promoter and initiates synthesis

3. Which of the following statements about *E. coli* promoters are correct?
   (a) They may exhibit different transcription efficiencies.
   (b) For most genes they include variants of consensus sequences.
   (c) They specify the start sites for transcription on the DNA template.
   (d) They have identical and defining sequences.
   (e) They are activated when C or G residues are substituted into their −10 regions by mutation.
   (f) Those that have sequences that correspond closely to the consensus sequences and are separated by 17 base pairs are very efficient.
4. The sequence of a duplex DNA segment in a DNA molecule is

   5′-ATCGCTTGTTCGGA-3′

   3′-TAGCGAACAAGCCT-5′

   When this segment serves as a template for *E. coli* RNA polymerase, it gives rise to a segment of RNA with the sequence 5′-UCCGAACAAGCGAU-3′

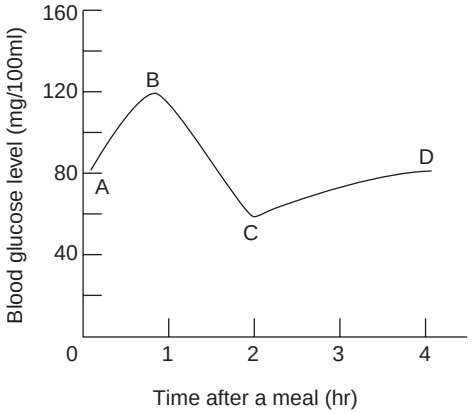
   Which of the following statements about the DNA segment are correct?
   (a) The top strand is the coding strand.
   (b) The bottom strand is the sense strand.
   (c) The top strand is the template strand.
   (d) The bottom strand is the antisense strand.
5. Which of the following statements about the σ subunit of RNA polymerase are correct?
   (a) It enables the enzyme to transcribe asymmetrically.
   (b) It confers on the core enzyme the ability to initiate transcription at promoters.
   (c) It decreases the affinity of RNA polymerase for regions of DNA that lack promoter sequences.
   (d) It facilitates the termination of transcription by recognizing hairpins in the transcript.
6. When growing *E. coli* are subjected to a rapid increase in temperature, a new and characteristic set of genes is expressed. Explain how this alteration in gene expression occurs.
7. Match the regions of a ρ-independent transcription termination signal in a DNA template in the left column with the structures or the functions performed by the encoded transcript segments in the right column.

(a) GC-rich palindromic region	(1) oligo(U) stretch in RNA
(b) AT-rich region	(2) hairpin in RNA
	(3) promotes the dissociation of RNA–DNA hybrid helix
	(4) causes the enzyme to pause

the inflection points is shown in Figure 30.1. After examining the figure, complete the following sentences:

(a) The increase in the glucose level from A to B is due to
(b) The decrease in the glucose level from B to C is due to
(c) The leveling off of the glucose level from C to D is due to
(d) The slight overshoot that is sometimes observed at C can be explained by

FIGURE 30.1 Blood-glucose levels after a meal rich in carbohydrate.



17. Match the fuel storage forms in the left column with the most appropriate characteristics from the right column.

(a) glycogen	(1) largest storage form of calories
(b) triacylglycerols	(2) most readily available fuel during muscular activity
(c) protein	(3) major source of precursors for glucose synthesis during starvation
	(4) depleted most rapidly during starvation
	(5) not normally used as a storage form of fuel

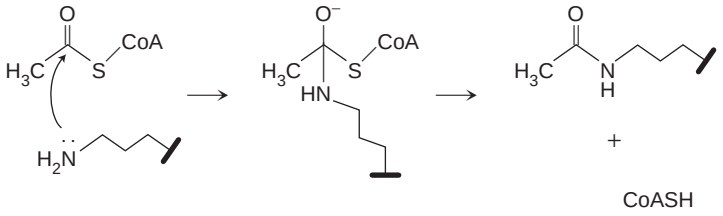
18. Relative to the well-fed state, fuel utilization after three days of starvation shifts in which of the following ways?

(a) More glucose is consumed by the brain.
(b) Adipose tissue triacylglycerols are degraded to provide fatty acids to most tissues.
(c) The brain begins to use ketone bodies as fuels.
(d) Proteins are degraded in order to provide three-carbon precursors of glucose.
(e) Glycogen is stored as a reserve fuel.

19. Metabolic adaptations to prolonged starvation include which of the following changes relative to the metabolic picture after three days of starvation?

(a) The rate of lipolysis (mobilization of triacylglycerols) in the adipose tissue increases.
(b) The glucose output by the liver decreases.
(c) The ketone body output by the liver decreases.
(d) The utilization of glucose by the brain decreases as the utilization of ketone bodies increases.
(e) The rate of degradation of muscle protein decreases.

9. The anti-inducer could be a competitive inhibitor of the inducer. As such, the anti-inducer would bind to the repressor at a similar or overlapping site to that of the inducer, but would not cause the conformational change necessary to release the repressor from the operator DNA. Higher concentrations of inducer would then be needed to displace the competitively bound anti-inducer from its site on the repressor.
10. Because symmetry is a recurring theme for protein–DNA interactions, the DNA sequence may have functional importance. One possibility is that the DNA sequence could be a binding site for a dimeric regulatory protein. Alternatively, inverted repeat sequences sometimes serve as hot spots for genetic rearrangements because they may form hairpin secondary structures that block DNA polymerases or are processed by structure-specific endonucleases.
11. The lysine amino group can make a nucleophilic attack on the carbonyl carbon of the thioester of acetyl-CoA to give a tetrahedral intermediate. The tetrahedral intermediate then could eliminate CoASH as a leaving group to yield acetyl-lysine.



12. The injected DNA fragments may bind competitively to CREB and thereby prevent CREB from binding to its true physiological target sites. For this reason, CREB would be unable to perform its role in stimulating the synthesis of new proteins. A proposed pathway for the stimulation of long-term memory by serotonin would be: (1) Serotonin binds to a receptor on the surface of a neuron cell and activates a G protein. (2) The G protein activates adenylate cyclase, which increases the intracellular concentration of cAMP. (3) cAMP activates protein kinase A. (4) Protein kinase A phosphorylates CREB. (5) Phosphorylated CREB binds the coactivator CBP. (6) The CREB/CBP complex activates the transcription of new proteins for long-term memory. (Step 6 would be inhibited by the DNA fragments that contain binding sites for CREB.)
13. A large percentage of the cytosine residues in mouse DNA are methylated, whereas very few C's in *Drosophila* or *E. coli* DNA are methylated. Therefore, the *Drosophila* and *E. coli* DNA are cut by HpaII into pieces of average size about 256 base pairs, while the mouse DNA is cut into pieces of average size about 50,000 base pairs.