ANSWERS TO PROBLEMS

Chapter 1

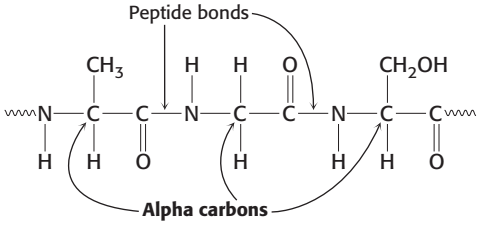
1. The hydrogen-bond donors are the NH and NH_2 groups. The hydrogen-bond acceptors are the carbonyl oxygen atoms and those ring nitrogen atoms that are not bonded to hydrogen or to deoxyribose.
2. Interchange the positions of the single and double bonds in the six-membered ring.
3. (a) Electrostatic interactions; (b) van der Waals interactions.
4. Processes *a* and *b*
5. $\Delta S_{system} = -661 \text{ J mol}^{-1} \text{ K}^{-1} (-158 \text{ kcal mol}^{-1} \text{ K}^{-1})$

$\Delta S_{surroundings} = +842 \text{ J mol}^{-1} \text{ K}^{-1} (+201 \text{ cal mol}^{-1} \text{ K}^{-1})$

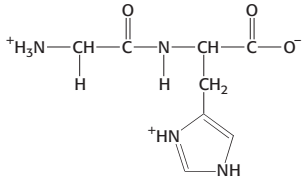
6. (a) 1.0; (b) 13.0; (c) 1.3; (d) 12.7
7. 2.88
8. 1.96
9. 11.83
10. 447; 0.00050
11. 0.00066 M
12. 6.0
13. 5.53
14. 6.48
15. 7.8
16. 100
17. (a) 1.6; (b) 0.51; (c) 0.16.
18. 0.1 M sodium acetate solution: 6.34; 6.03; 5.70; 4.75. 0.01 M sodium acetate solution: 5.90; 4.75; 3.38; 1.40.
19. 90 mM acetic acid; 160 mM sodium acetate, 0.18 moles acetic acid; 0.32 moles sodium acetate; 10.81 g acetic acid; 26.25 g sodium acetate.
20. 0.50 moles of acetic acid; 0.32 moles of NaOH; 30.03 g of acetic acid; 12.80 g of NaOH.
21. 250 mM; yes; no, it will also contain 90 mM NaCl.
22. 8.63 g Na_2HPO_4; 4.71 g NaH_2PO_4
23. 7.0; this buffer will not be very useful, because the pH value is far from the pK_a value.
24. 1.45 kJ mol^{-1} (0.35 kcal mol^{-1}); 57.9 kJ mol^{-1} (13.8 kcal mol^{-1})
25. There will be approximately 15 million differences.

Chapter 2

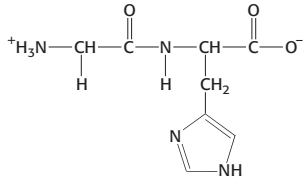
1. (A) Proline, Pro, P; (B) tyrosine, Tyr, Y; (C) leucine, Leu, L; (D) lysine, Lys, K.
2. (a) C, B, A; (b) D; (c) D, B; (d) B, D; (e) B.
3. (a) 6; (b) 2; (c) 3; (d) 1; (e) 4; (f) 5.
4. (a) Ala; (b) Tyr; (c) Ser; (d) His.
5. Ser, Glu, Tyr, Thr
6. (a) Alanine-glycine-serine; (b) Alanine; (c and d):



7.

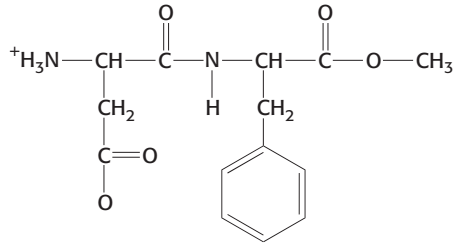


At pH 5.5, the net charge is +1



At pH 7.5, the net charge is 0

8. There are 20 choices for each of the 50 amino acids: 50^{20}, or 5×10^{21}.
9.



Aspartame at pH 7

10. The (nitrogen–α carbon–carbonyl carbon) repeating unit.
11. Side chain is the functional group attached to the α-carbon atom of an amino acid.
12. Amino acid composition refers simply to the amino acids that make up the protein. The order is not specified. Amino acid sequence is the same as the primary structure—the sequence of amino acids from the amino terminal to the carboxyl terminal of the protein. Different proteins may have the same amino acid composition, but amino acid sequence identifies a unique protein.
13. (a) Each strand is 35 kd and hence has about 318 residues (the mean residue mass is 110 daltons). Because the rise per residue in an α helix is 1.5 Å, the length is 477 Å. More precisely, for an α-helical coiled coil, the rise per residue is 1.46 Å; so the length is 464 Å. (b) Eighteen residues in each strand (40 minus 4 divided by 2) are in a β-sheet conformation. Because the rise per residue is 3.5 Å, the length is 63 Å.
14. The methyl group attached to the β-carbon atom of isoleucine sterically interferes with α-helix formation. In leucine, this methyl group is attached to the γ-carbon atom, which is farther from the main chain and hence does not interfere.
15. The first mutation destroys activity because valine occupies more space than alanine does, and so the protein must take a different shape, assuming that this residue lies in the closely packed interior. The second mutation restores activity because of a compensatory reduction of volume; glycine is smaller than isoleucine.
16. The native conformation of insulin is not the thermodynamically most stable form, because it contains two separate chains linked by disulfide bonds. Insulin is formed from proinsulin, a single-chain precursor, that is cleaved to form insulin, a 51-residue molecule, after the disulfide bonds have formed.
17. A segment of the main chain of the protease could hydrogen bond to the main chain of the substrate to form an extended parallel or antiparallel pair of β strands.
18. Glycine has the smallest side chain of any amino acid. Its size is often critical in allowing polypeptide chains to make tight turns or to approach one another closely.
19. Glutamate, aspartate, and the terminal carboxylate can form salt bridges with the guanidinium group of arginine. In addition, this group can be a hydrogen-bond donor to the side chains of glutamine, asparagine, serine, threonine, aspartate, tyrosine, and glutamate and to the main-chain carbonyl group. Histidine can form hydrogen bonds with arginine at pH7.
20. Disulfide bonds in hair are broken by adding a thiol-containing reagent and applying gentle heat. The hair is curled, and an oxidizing agent is added to re-form disulfide bonds to stabilize the desired shape.
21. Some proteins that span biological membranes are "the exceptions that prove the rule" because they have the reverse distribution of hydrophobic and hydrophilic amino acids. For example, consider

porins, proteins found in the outer membranes of many bacteria. Membranes are built largely of hydrophobic chains. Thus, porins are covered on the outside largely with hydrophobic residues that interact with the neighboring hydrophobic chains. In contrast, the center of the protein contains many charged and polar amino acids that surround a water-filled channel running through the middle of the protein. Thus, because porins function in hydrophobic environments, they are "inside out" relative to proteins that function in aqueous solution.

22. The amino acids would be hydrophobic in nature. An α helix is especially suited to crossing a membrane because all of the amide hydrogen atoms and carbonyl oxygen atoms of the peptide backbone take part in intrachain hydrogen bonds, thus stabilizing these polar atoms in a hydrophobic environment.

23. This example demonstrates that the pK_a values are affected by the environment. A given amino acid can have a variety of pK_a values, depending on the chemical environment inside the protein.

24. A possible explanation is that the severity of the symptoms corresponds to the degree of structural disruption. Hence, substitution of alanine for glycine might result in mild symptoms, but substitution of the much larger tryptophan might prevent little or no collagen triple-helix formation.

25. The energy barrier that must be crossed to go from the polymerized state to the hydrolyzed state is large even though the reaction is thermodynamically favorable.

26. Using the Henderson–Hasselbalch equation, we find the ratio of alanine-COOH to alanine-COO^- at pH 7 to be 10^{-4}. The ratio of alanine-NH_2 to alanine-NH_3^+, determined in the same fashion, is 10^{-1}. Thus, the ratio of neutral alanine to the zwitterionic species is $10^{-4} \times 10^{-1} = 10^{-5}$.

27. The assignment of absolute configuration requires the assignment of priorities to the four groups connected to a tetrahedral carbon atom. For all amino acids except cysteine, the priorities are: (1) amino group; (2) carbonyl group; (3) side chain; (4) hydrogen. For cysteine, because of the sulfur atom in its side chain, the side chain has a greater priority than does the carbonyl group, leading to the assignment of an *R* rather than *S* configuration.

28. ELVISISLIVINGINLASVEGAS

29. No, Pro–X would have the characteristics of any other peptide bond. The steric hindrance in X–Pro arises because the R group of Pro is bonded to the amino group. Hence, in X–Pro, the proline R group is near the R group of X, which would not be the case in Pro–X.

30. A, c; B, e; C, d; D, a; E, b.

31. The reason is that the wrong disulfides formed pairs in urea. There are 105 different ways of pairing eight cysteine molecules to form four disulfides; only one of these combinations is enzymatically active. The 104 wrong pairings have been picturesquely termed "scrambled" ribonuclease.

Chapter 3

1. (a) Phenyl isothiocyanate; (b) urea; β-mercaptoethanol to reduce disulfides; (c) chymotrypsin; (d) CNBr; (e) trypsin.

2. Each amino acid residue, except the carboxyl-terminal residue, gives rise to a hydrazide on reacting with hydrazine. The carboxyl-terminal residue can be identified because it yields a free amino acid.

3. The *S*-aminoethylcysteine side chain resembles that of lysine. The only difference is a sulfur atom in place of a methylene group.

4. A 1 mg ml^{-1} solution of myoglobin (17.8 kd; Table 3.2) corresponds to 5.62×10^{-5} M. The absorbance of a 1-cm path length is 0.84, which corresponds to an I_0/I ratio of 6.96. Hence 14.4% of the incident light is transmitted.

5. The sample was diluted 1000-fold. The concentration after dialysis is thus 0.001 M, or 1 mM. You could reduce the salt concentration by dialyzing your sample, now 1 mM, in more buffer free of $(NH_4)_2SO_4$.

6. If the salt concentration becomes too high, the salt ions interact with the water molecules. Eventually, there will not be enough water molecules to interact with the protein, and the protein will precipitate. If there is lack of salt in a protein solution, the proteins may interact with one another—the positive charges on one protein with the negative charges on another or several others. Such an aggregate becomes too large to be solublized by water alone. If salt is added, the salt neutralizes the charges on the proteins, preventing protein–protein interactions.

7. Tropomyosin is rod shaped, whereas hemoglobin is approximately spherical.

8. The frictional coefficient, *f*, and the mass, *m*, determine *s*. Specifically, *f* is proportional to *r* (see equation 2 on p. 71). Hence, *f* is proportional to $m^{1/3}$, and so *s* is proportional to $m^{2/3}$ (see the equation on p. 76). An 80-kd spherical protein undergoes sedimentation 1.59 times as rapidly as a 40-kd spherical protein.

9. The long hydrophobic tail on the SDS molecule (see p. 72) disrupts the hydrophobic interactions in the interior of the protein. The protein unfolds, with the hydrophobic R groups now interacting with SDS rather than with one another.

10. 50 kd.

11. The protein may be modified. For instance, serine, threonine, and tyrosine may have phosphoryl groups attached.

12. A fluorescence-labeled derivative of a bacterial degradation product (e.g., a formylmethionyl peptide) would bind to cells containing the receptor of interest.

13. (a) Trypsin cleaves after arginine (R) and lysine (K), generating AVGWR, VK, and S. Because they differ in size, these products could be separated by molecular exclusion chromatography. (b) Chymotrypsin, which cleaves after large aliphatic or aromatic R groups, generates two peptides of equal size (AVGW) and (RVKS). Separation based on size would not be effective. The peptide RVKS has two positive charges (R and K), whereas the other peptide is neutral. Therefore, the two products could be separated by ion-exchange chromatography.

14. Antibody molecules bound to a solid support can be used for affinity purification of proteins for which a ligand molecule is not known or unavailable.

15. If the product of the enzyme-catalyzed reaction is highly antigenic, it may be possible to obtain antibodies to this particular molecule. These antibodies can be used to detect the presence of product by ELISA, providing an assay format suitable for the purification of this enzyme.

16. An inhibitor of the enzyme being purified might have been present and subsequently removed by a purification step. This removal would lead to an apparent increase in the total amount of enzyme present.

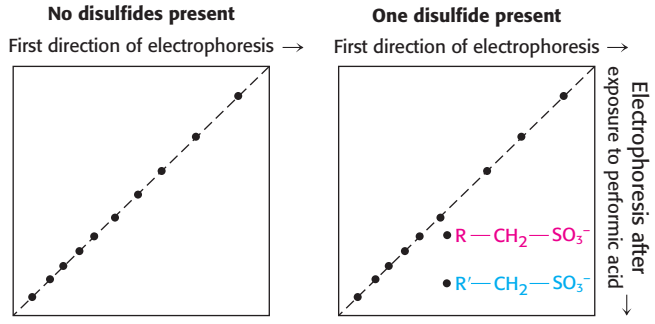
17. Many proteins have similar masses but different sequences and different patterns when digested with trypsin. The set of masses of tryptic peptides forms a detailed "fingerprint" of a protein that is very unlikely to appear at random in other proteins regardless of size. (A conceivable analogy is: "Just as similarly sized fingers will give different individual fingerprints, so also similarly sized proteins will give different digestion patterns with trypsin.")

18. Isoleucine and leucine are isomers and, hence, have identical masses. Peptide sequencing by mass spectrometry as described in this chapter is incapable of distinguishing these residues. Further analytical techniques are required to differentiate these residues.

19. See the table at the top of the facing page.

Purification procedure	Total protein (mg)	Total activity (units)	Specific activity (units mg^{-1})	Purification level	Yield (%)
Crude extract	20,000	4,000,000	200	1	100
$(NH_4)_2SO_4$ precipitation	5,000	3,000,000	600	3	75
DEAE–cellulose chromatography	1,500	1,000,000	667	3.3	25
Gel-filtration chromatography	500	750,000	1,500	7.5	19
Affinity chromatography	45	675,000	15,000	75	17

20. Protein crystal formation requires the ordered arrangement of identically positioned molecules. Proteins with flexible linkers can introduce disorder into this arrangement and prevent the formation of suitable crystals. A ligand or binding partner may induce an ordered conformation to this linker and could be included in the solution to facilitate crystal growth. Alternatively, the individual domains separated by the linker may be expressed by recombinant methods and their crystal structures solved separately.
21. Treatment with urea will disrupt noncovalent bonds. Thus the original 60-kd protein must be made of two 30-kd subunits. When these subunits are treated with urea and β-mercaptoethanol, a single 15-kd species results, suggesting that disulfide bonds link the 30-kd subunits.
22. (a) Electrostatic repulsion between positively charged ε-amino groups hinders α-helix formation at pH 7. At pH 10, the side chains become deprotonated, allowing α-helix formation.
(b) Poly-L-glutamate is a random coil at pH 7 and becomes α helical below pH 4.5 because the γ-carboxylate groups become protonated.
23. The difference between the predicted and the observed masses for this fragment equals 28.0, exactly the mass shift that would be expected in a formylated peptide. This peptide is likely formylated at its amino terminus, and corresponds to the most N-terminal fragment of the protein.
24. Light was used to direct the synthesis of these peptides. Each amino acid added to the solid support contained a photolabile protecting group instead of a *t*-Boc protecting group at its α-amino group. Illumination of selected regions of the solid support led to the release of the protecting group, which exposed the amino groups in these sites to make them reactive. The pattern of masks used in these illuminations and the sequence of reactants define the ultimate products and their locations.
25. Mass spectrometry is highly sensitive and capable of detecting the mass difference between a protein and its deuterated counterpart. Fragmentation techniques can be used to identify the amino acids that retained the isotope label. Alternatively, NMR spectroscopy can be used to detect the isotopically labeled atoms because the deuteron and the proton have very different nuclear-spin properties.
26. First amino acid: A
Last amino acid: R (not cleaved by carboxypeptidase).
Sequence of N-terminal tryptic peptide: AVR (tryptic peptide ends in K)
Sequence of N-terminal chymotryptic peptide: AVRY (chymotryptic peptide ends in Y)
Sequence: AVRYSR
27. First amino acid: S
Last amino acid: L
Cyanogen bromide cleavage: M is 10th position,
C-terminal residues are: (2S,L,W)
Amino-terminal residues: (G,K,S,Y), tryptic peptide, ends in K
Amino-terminal sequence: SYGK
Chymotryptic peptide order: (S,Y), (G,K,L), (F,I,S), (M,T), (S,W), (S,L)
Sequence: SYGKLSIFTMSWSL
28. If the protein did not contain any disulfide bonds, then the electrophoretic mobility of the trypsin fragments would be the same before and after performic acid treatment: all the fragments would lie along the diagonal of the paper. If one disulfide bond were present, the disulfide-linked trypsin fragments would run as a single peak in the first direction, then would run as two separate peaks after performic acid treatment. The result would be two peaks appearing off the diagonal:

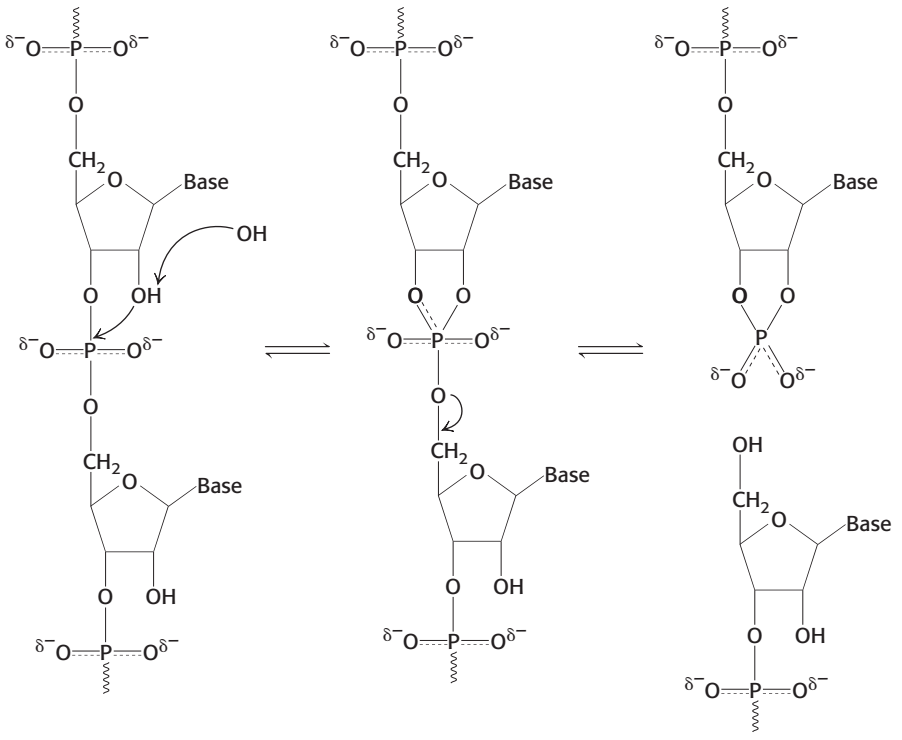


These fragments could then be isolated from the chromatography paper and analyzed by mass spectrometry to determine their amino acid composition and thus identify the cysteines participating in the disulfide bond.

Chapter 4

1. A nucleoside is a base attached to a ribose sugar. A nucleotide is a nucleoside with one or more phosphoryl groups attached to the ribose.
2. Hydrogen-bond pairing between the base A and the base T as well as hydrogen-bond pairing between the base G and the base C in DNA.
3. T is always equal to A, and so these two nucleotides constitute 40% of the bases. G is always equal to C, and so the remaining 60% must be 30% G and 30% C.
4. Nothing, because the base-pair rules do not apply to single-stranded nucleic acids.
5. (a) TTGATC; (b) GTTCGA; (c) ACGCGT; (d) ATGGTA.
6. (a) [T] + [C] = 0.46. (b) [T] = 0.30, [C] = 0.24, and [A] + [G] = 0.46.
7. Stable hydrogen bonding occurs only between GC and AT pairs. Moreover, two purines are too large to fit inside the double helix, and two pyrimidines are too small to form base pairs with each other.
8. The thermal energy causes the chains to wiggle about, which disrupts the hydrogen bonds between base pairs and the stacking forces between bases and thereby causes the strands to separate.
9. The probability that any sequence will appear is 4^n, where 4 is the number of nucleotides and n is the length of the sequence. The probability of any 15-base sequence appearing is $1/4^{15}$, or 1/1,073,741,824. Thus, a 15-nucleotide sequence would be likely to appear approximately three times (3 billion × probability of appearance). The probability of a 16-base sequence appearing is $1/4^{16}$, which is equal to 1/4,294,967,296. Such a sequence will be unlikely to appear more than once.

10. One end of a nucleic acid polymer ends with a free 5′-hydroxyl group (or a phosphoryl group esterified to the hydroxyl group), and the other end has a free 3′-hydroxyl group. Thus, the ends are different. Two chains of DNA can form a double helix only if the chains are running in different directions—that is, have opposite polarity.
11. Although the individual bonds are weak, the population of thousands to millions of such bonds provides much stability. There is strength in numbers.
12. There would be too much charge repulsion from the negative charges on the phosphoryl groups. These charges must be countered by the addition of cations.
13. The three forms are the A-DNA, the B-DNA and the Z-DNA, with B-DNA being the most common. There are many differences (see Table 4.2). Some key differences are: A-DNA and B-DNA are right-handed, whereas Z-DNA is left-handed. A-DNA forms in less-hydrated conditions than does B-DNA. The A form is shorter and wider than the B form.
14. 5.88×10^3 base pairs
15. In conservative replication, after 1.0 generation, half of the molecules would be ^{15}N-^{15}N, the other half ^{14}N-^{14}N. After 2.0 generations, one-quarter of the molecules would be ^{15}N-^{15}N, the other three-quarters ^{14}N-^{14}N. Hybrid ^{14}N-^{15}N molecules would not be observed in conservative replication.
16. (a) Tritiated thymine or tritiated thymidine.
(b) dATP, dGTP, dCTP, and TTP labeled with ^{32}P in the innermost (α) phosphorus atom.
17. Molecules in parts *a* and *b* would not lead to DNA synthesis, because they lack a 3′-OH group (a primer). The molecule in part *d* has a free 3′-OH group at one end of each strand but no template strand beyond. Only the molecule in part *c* would lead to DNA synthesis.
18. A retrovirus is a virus that has RNA as its genetic material. However, for the information to be expressed, it must first be converted into DNA, a reaction catalyzed by the enzyme reverse transcriptase. Thus, at least initially, information flow is opposite that of a normal cell: RNA ⟶ DNA rather than DNA ⟶ RNA.
19. A thymidylate oligonucleotide should be used as the primer. The poly(A) template specifies the incorporation of T; hence, radioactive thymidine triphosphate (labeled in the α phosphoryl group) should be used in the assay.
20. The ribonuclease serves to degrade the RNA strand, a necessary step in forming duplex DNA from the RNA–DNA hybrid.
21. Treat one aliquot of the sample with ribonuclease and another with deoxyribonuclease. Test these nuclease-treated samples for infectivity.
22. Deamination changes the original G · C base pair into a G · U pair. After one round of replication, one daughter duplex will contain a G · C pair and the other duplex will contain an A · U pair. After two rounds of replication, there will be two G · C pairs, one A · U pair, and one A · T pair.
23. (a) $4^8 = 65,536$. In computer terminology, there are 64K 8-mers of DNA.
(b) A bit specifies two bases (say, A and C) and a second bit specifies the other two (G and T). Hence, two bits are needed to specify a single nucleotide (base pair) in DNA. For example, 00, 01, 10, and 11 could encode A, C, G, and T. An 8-mer stores 16 bits ($2^{16} = 65,536$), the *E. coli* genome (4.6×10^6 bp) stores 9.2×10^6 bits, and the human genome (3.0×10^9 bases) stores 6.0×10^9 bits of genetic information.
(c) A standard CD can hold about 700 megabytes, which is equal to 5.6×10^9 bits. A large number of 8-mer sequences could be stored on such a CD. The DNA sequence of *E. coli,* could be written on a single CD with room to spare for a lot of music. One CD would not be quite enough to record the entire human genome.
24. (a) Deoxyribonucleoside triphosphates versus ribonucleoside triphosphates.
(b) 5′ ⟶ 3′ for both.
(c) Semiconserved for DNA polymerase I; conserved for RNA polymerase.
(d) DNA polymerase I needs a primer, whereas RNA polymerase does not.
25. Messenger RNA encodes the information that, on translation, yields a protein. Ribosomal RNA is the catalytic component of ribosomes, the molecular complexes that synthesize proteins. Transfer RNA is an adaptor molecule, capable of binding a specific amino acid and recognizing a corresponding codon. Transfer RNAs with attached amino acids are substrates for the ribosome.
26. (a) 5′-UAACGGUACGAU-3′
(b) Leu-Pro-Ser-Asp-Trp-Met
(c) Poly(Leu-Leu-Thr-Tyr)
27. The 2′-OH group in RNA acts as an intramolecular nucleophile. In the alkaline hydrolysis of RNA, it forms a 2′-3′ cyclic intermediate.
28.

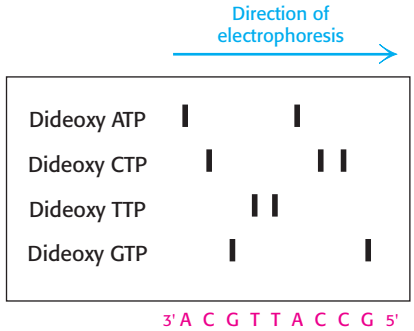


29. Gene expression is the process of expressing the information of a gene in its functional molecular form. For many genes, the functional information is a protein molecule. Thus, gene expression includes transcription and translation.
30. A nucleotide sequence whose bases represent the most-common, but not necessarily the only, members of the sequence. A consensus sequence can be thought of as the average of many similar sequences.
31. Cordycepin terminates RNA synthesis. An RNA chain containing cordycepin lacks a 3′-OH group.
32. Only single-stranded RNA can serve as a template for protein synthesis.
33. Degeneracy of the code refers to the fact that most amino acids are encoded by more than one codon.

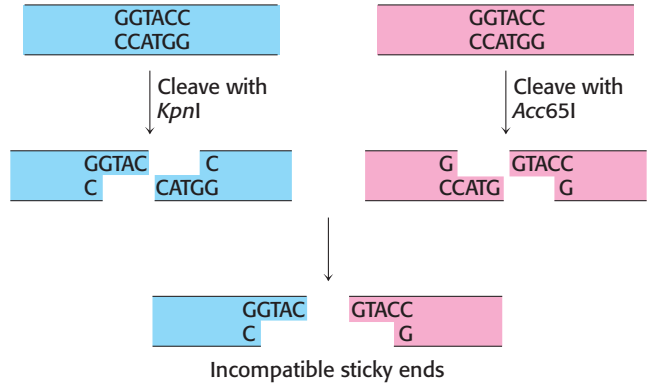
34. If only 20 of the 64 possible codons encoded amino acids, then a mutation that changed a codon would likely result in a nonsense codon, leading to termination of protein synthesis. With degeneracy, a nucleotide change might yield a synonym or a codon for an amino acid with similar chemical properties.
35. (a) 2, 4, 8; (b) 1, 6, 10; (c) 3, 5, 7, 9.
36. (a) 3; (b) 6; (c) 2; (d) 5; (e) 7; (f) 1; (g) 4.
37. Incubation with RNA polymerase and only UTP, ATP, and CTP led to the synthesis of only poly(UAC). Only poly(GUA) was formed when GTP was used in place of CTP.
38. A peptide terminating with Lys (UGA is a stop codon), another containing -Asn-Glu-, and a third containing -Met-Arg-.
39. Highly abundant amino acid residues have the most codons (e.g., Leu and Ser each have six), whereas the least-abundant amino acids have the fewest (Met and Trp each have only one). Degeneracy (1) allows variation in base composition and (2) decreases the likelihood that a substitution for a base will change the encoded amino acid. If the degeneracy were equally distributed, each of the 20 amino acids would have three codons. Both benefits (1 and 2) are maximized by the assignment of more codons to prevalent amino acids than to less frequently used ones.
40. Phe-Cys-His-Val-Ala-Ala
41. Exon shuffling is a molecular process that can lead to the generation of new proteins by the rearrangement of exons within genes. Because many exons encode functional protein domains, exon shuffling is a rapid and efficient means of generating new genes.
42. It shows that the genetic code and the biochemical means of interpreting the code are common to even very distantly related life forms. It also testifies to the unity of life; that all life arose from a common ancestor.
43. (a) A codon for lysine cannot be changed to one for aspartate by the mutation of a single nucleotide. (b) Arg, Asn, Gln, Glu, Ile, Met, or Thr.
44. The genetic code is degenerate. Of the 20 amino acids, 18 are specified by more than one codon. Hence, many nucleotide changes (especially in the third base of a codon) do not alter the nature of the encoded amino acid. Mutations leading to an altered amino acid are usually more deleterious than those that do not and hence are subject to more stringent selection.
45. GC base pairs have three hydrogen bonds compared with two for AT base pairs. Thus, the higher content of GC means more hydrogen bonds and greater helix stability.
46. C_0t value essentially corresponds to the complexity of the DNA sequence—in other words, how long it will take for a sequence of DNA to find its complementary strand to form a double helix. The more complex the DNA, the slower it reassociates to make the double-stranded form.

Chapter 5

1. (a) 5′-GGCATAC-3′
(b) The Sanger dideoxy method of sequencing would give the gel pattern shown here.



2. Ovalbumin cDNA should be used. *E. coli* lacks the machinery to splice the primary transcript arising from genomic DNA.
3. Consistent with its planar, aromatic structure, ethidium bromide is a DNA intercalator: it aligns itself between the paired bases in a DNA duplex.
4. The presence of the *Alu*I sequence would, on average, be $(1/4)^4$, or 1/256, because the likelihood of any base being at any position is one-fourth and there are four positions. By the same reasoning, the presence of the *Not*I sequence would be $(1/4)^8$, or 1/65,536. Thus, the average product of digestion by *Alu*I would be 250 base pairs (0.25 kb) in length, whereas that by *Not*I would be 66,000 base pairs (66 kb) in length.
5. No, because most human genes are much longer than 4 kb. A fragment would contain only a small part of a complete gene.
6. Southern blotting of an *Mst*II digest would distinguish between the normal and the mutant genes. The loss of a restriction site would lead to the replacement of two fragments on the Southern blot by a single longer fragment. Such a finding would not prove that GTG replaced GAG; other sequence changes at the restriction site could yield the same result.
7. Although the two enzymes cleave the same recognition site, they each break different bonds within the 6-bp sequence. Cleavage by *Kpn*I yields an overhang on the 3′ strand, whereas cleavage by *Acc*65I produces an overhang on the 5′ strand. These sticky ends do not overlap.



8. A simple strategy for generating many mutants is to synthesize a degenerate set of cassettes by using a mixture of activated nucleosides in particular rounds of oligonucleotide synthesis. Suppose that the 30-bp coding region begins with GTT, which encodes valine. If a mixture of all four nucleotides is used in the first and second rounds of synthesis, the resulting oligonucleotides will begin with the sequence XYT (where X and Y denote A, C, G, or T). These 16 different versions of the cassette will encode proteins containing either Phe, Leu, Ile, Val, Ser, Pro, Thr, Ala, Tyr, His, Asn, Asp, Cys, Arg, or Gly at the first position. Likewise, degenerate cassettes can be made in which two or more codons are simultaneously varied.
9. Because PCR can amplify as little as one molecule of DNA, statements claiming the isolation of ancient DNA need to be greeted with some skepticism. The DNA would need to be sequenced. Is it similar to human, bacterial, or fungal DNA? If so, contamination is the likely source of the amplified DNA. Is it similar to that of birds or crocodiles? This sequence similarity would strengthen the case that it is dinosaur DNA because these species are evolutionarily close to dinosaurs.
10. PCR amplification is greatly hindered by the presence of G–C-rich regions within the template. Owing to their high melting temperatures, these templates do not denature easily, preventing the initiation of an amplification cycle. In addition, rigid secondary

structures prevent the progress of DNA polymerase along the template strand during elongation.
11. At high temperatures of hybridization, only very close matches between primer and target would be stable because all (or most) of the bases would need to find partners to stabilize the primer–target helix. As the temperature is lowered, more mismatches would be tolerated; so the amplification is likely to yield genes with less sequence similarity. In regard to the yeast gene, synthesize primers corresponding to the ends of the gene, and then use these primers and human DNA as the target. If nothing is amplified at 54°C, the human gene differs from the yeast gene, but a counterpart may still be present. Repeat the experiment at a lower temperature of hybridization.
12. Digest genomic DNA with a restriction enzyme, and select the fragment that contains the known sequence. Circularize this fragment. Then carry out PCR with the use of a pair of primers that serve as templates for the synthesis of DNA away from the known sequence.
13. The encoded protein contains four repeats of a specific sequence.
14. Use chemical synthesis or the polymerase chain reaction to prepare hybridization probes that are complementary to both ends of the known (previously isolated) DNA fragment. Challenge clones representing the library of DNA fragments with both of the hybridization probes. Select clones that hybridize to one of the probes but not the other; such clones are likely to represent DNA fragments that contain one end of the known fragment along with the adjacent region of the particular chromosome.
15. The codon(s) for each amino acid can be used to determine the number of possible nucleotide sequences that encode each peptide sequence (see Table 4.5):

Ala–Met–Ser–Leu–Pro–Trp:
$4 \times 1 \times 6 \times 6 \times 4 \times 1 = 576$ total sequences

Gly–Trp–Asp–Met–His–Lys:
$4 \times 1 \times 2 \times 1 \times 2 \times 2 = 32$ total sequences

Cys–Val–Trp–Asn–Lys–Ile:
$2 \times 4 \times 1 \times 2 \times 2 \times 3 = 96$ total sequences

Arg–Ser–Met–Leu–Gln–Asn:
$6 \times 6 \times 1 \times 6 \times 2 \times 2 = 864$ total sequences

The set of DNA sequences encoding the peptide Gly-Trp-Asp-Met-His-Lys would be most ideal for probe design because it encompasses only 32 total oligonucleotides.
16. Within a single species, individual dogs show enormous variation in body size and substantial diversity in other physical characteristics. Therefore, genomic analysis of individual dogs would provide valuable clues concerning the genes responsible for the diversity within the species.
17. On the basis of the comparative genome map shown in Figure 5.27, the region of greatest overlap with human chromosome 20 can be found on mouse chromosome 2.
18. T_m is the melting temperature of a double-stranded nucleic acid. If the melting temperatures of the primers are too different, the extent of hybridization with the target DNA will differ during the annealing phase, which would result in differential replications of the strands.
19. Careful comparison of the sequences reveals that there is a 7-bp region of complementarity at the 3′ ends of these two primers:

```
5′-GGATCGATGCTCGCGA-3′
                |||||||
          3′-GAGCGCTGGGCTAGGA-5′
```

In a PCR experiment, these primers would likely anneal to one another, preventing their interaction with the template DNA. During DNA synthesis by the polymerase, each primer would act as a template for the other primer, leading to the amplification of a 25-bp sequence corresponding to the overlapped primers.
20. A mutation in person B has altered one of the alleles for gene *X*, leaving the other intact. The fact that the mutated allele is smaller suggests that a deletion has occurred in one copy of the gene. The one functioning copy is transcribed and translated and apparently produces enough protein to render the person asymptomatic.

Person C has only the smaller version of the gene. This gene is neither transcribed (negative northern blot) nor translated (negative western blot).

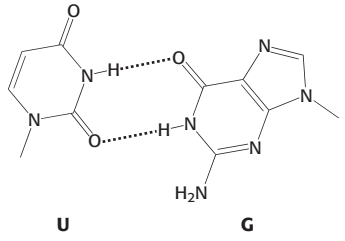
Person D has a normal-size copy of the gene but no corresponding RNA or protein. There may be a mutation in the promoter region of the gene that prevents transcription.

Person E has a normal-size copy of the gene that is transcribed, but no protein is made, which suggests that a mutation prevents translation. There are a number of possible explanations, including a mutation that introduced a premature stop codon in the mRNA.

Person F has a normal amount of protein but still displays the metabolic problem. This finding suggests that the mutation affects the activity of the protein—for instance, a mutation that compromises the active site of enzyme Y.
21. Chongqing: residue 2, L ⟶ R, CTG ⟶ CGG
Karachi: residue 5, A ⟶ P, GCC ⟶ CCC
Swan River: residue 6, D ⟶ G, GAC ⟶ GGC
22. This particular person is heterozygous for this particular mutation: one allele is wild type, whereas the other carries a point mutation at this position. Both alleles are PCR amplified in this experiment, yielding the "dual peak" appearance on the sequencing chromatogram.

Chapter 6

1. There are 26 identities and two gaps for a score of 210. The two sequences are approximately 26% identical. This level of homology is likely to be statistically significant.
2. They are likely related by divergent evolution, because three-dimensional structure is more conserved than is sequence identity.
3. (a) Identity score = −25; Blosum score = 14; (b) identity score = 15; Blosum score = 3.
4. U



U G

5. There are 4^{40}, or 1.2×10^{24}, different molecules. Each molecule has a mass of 2.2×10^{-20}, because 1 mol of polymer has a mass of 330 g mol^{-1} × 40, and there are 6.02×10^{23} molecules per mole. Therefore, 26.4 kg of RNA would be required.
6. Because three-dimensional structure is much more closely associated with function than is sequence, tertiary structure is more evolutionarily conserved than is primary structure. In other words, protein function is the most important characteristic, and protein function is determined by structure. Thus, the structure must be conserved but not necessarily a specific amino acid sequence.

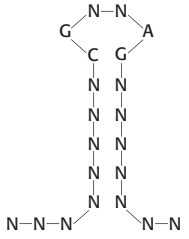
7. Alignment score of sequences (1) and (2) is $6 \times 10 = 60$. Many answers are possible, depending on the randomly reordered sequence. A possible result is

Shuffled sequence: (2) `TKADKAGEYL`

Alignment: (1) `ASNFLDKAGK`
(2) `TKADKAGEYL`

Alignment score is $4 \times 10 = 40$.

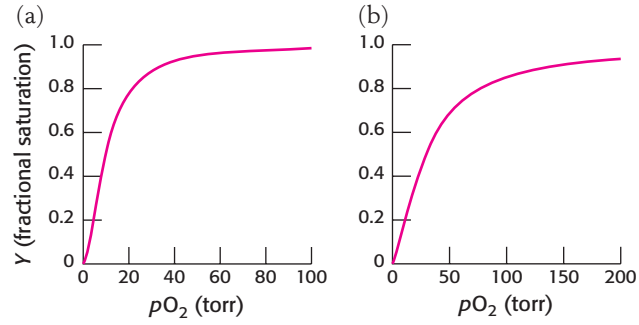
8. (a) Almost certainly diverged from a common ancestor. (b) Almost certainly diverged from a common ancestor. (c) May have diverged from a common ancestor, but the sequence alignment may not provide supporting evidence. (d) May have diverged from a common ancestor, but the sequence alignment is unlikely to provide supporting evidence.
9. Replacement of cysteine, glycine, and proline never yields a positive score. Each of these residues exhibits features unlike those of its other 19 counterparts: cysteine is the only amino acid capable of forming disulfide bonds, glycine is the only amino acid without a side-chain and is highly flexible, and proline is the only amino acid that is highly constrained through the bonding of its side chain to its amine nitrogen.
10. Protein A is clearly homologous to protein B, given 65% sequence identity, and so A and B are expected to have quite similar three-dimensional structures. Likewise, proteins B and C are clearly homologous, given 55% sequence identity, and so B and C are expected to have quite similar three-dimensional structures. Thus, proteins A and C are likely to have similar three-dimensional structures, even though they are only 15% identical in sequence.
11. The likely secondary structure is



12. To detect pairs of residues with correlated mutations, there must be variability in these sequences. If the alignment is over-represented by closely related organisms, there may not be enough changes in their sequences to allow the identification of potential base-pairing patterns.
13. After RNA molecules have been selected and reverse transcribed, PCR is performed to introduce additional mutations into these strands. The use of this error-prone, thermostable polymerase in the amplification step would enhance the efficiency of this random mutagenesis.
14. The initial pool of RNA molecules used in a molecular-evolution experiment is typically much smaller than the total number of possible sequences. Hence, the best possible RNA sequences will likely not be represented in the initial set of oligonucleotides. Mutagenesis of the initial selected RNA molecules allows for the iterative improvement of these sequences for the desired property.
15. 107 or 108 identities (depending on which annotated human sequence is chosen).

Chapter 7

1. The whale swims long distances between breaths. A high concentration of myoglobin in the whale muscle maintains a ready supply of oxygen for the muscle between breathing episodes.
2. (a) 2.96×10^{-11} g
(b) 2.74×10^{8} molecules
(c) No. There would be 3.17×10^{8} hemoglobin molecules in a red cell if they were packed in a cubic crystalline array. Hence, the actual packing density is about 84% of the maximum possible.
3. 2.65 g (or 4.75×10^{-2} mol) of Fe
4. (a) In human beings, 1.44×10^{-2} g (4.49×10^{-4} mol) of O_2 per kilogram of muscle. In sperm whale, 0.144 g (4.49×10^{-3} mol) of O_2 per kilogram.
(b) 128
5. The pK_a is (a) lowered; (b) raised; and (c) raised.
6. Deoxy Hb A contains a complementary site, and so it can add on to a fiber of deoxy Hb S. The fiber cannot then grow further, because the terminal deoxy Hb A molecule lacks a sticky patch.
7. 62.7% oxygen-carrying capacity
8. A higher concentration of BPG would shift the oxygen-binding curve to the right, causing an increase in P_{50}. The larger value of P_{50} would promote dissociation of oxygen in the tissues and would thereby increase the percentage of oxygen delivered to the tissues.
9. Oxygen binding appears to cause the copper ions and their associated histidine ligands to move closer to one another, thereby also moving the helices to which the histidines are attached (in similar fashion to the conformational change in hemoglobin).
10. The modified hemoglobin should not show cooperativity. Although the imidazole in solution will bind to the heme iron (in place of histidine) and will facilitate oxygen binding, the imidazole lacks the crucial connection to the particular α helix that must move so as to transmit the change in conformation.
11. Inositol pentaphosphate (part *c*) is highly anionic, much like 2,3-bisphosphoglycerate.
12.



13. Release of acid will lower the pH. A lower pH promotes oxygen dissociation in the tissues. However, the enhanced release of oxygen in the tissues will increase the concentration of deoxy-Hb, thereby increasing the likelihood that the cells will sickle.
14. (a) $Y = 0.5$ when $pO_2 = 10$ torr. The plot of Y versus pO_2 appears to indicate little or no cooperativity.
(b) The Hill plot shows slight cooperativity with $n \cdot 1.3$ in the central region.
(c) Deoxy dimers of lamprey hemoglobin could have lower affinity for oxygen than do the monomers. If the binding of the first oxygen atom to a dimer causes dissociation of the dimer to give two monomers, then the process would be cooperative. In this mechanism, oxygen binding to each monomer would be easier than binding the first oxygen atom to a deoxy dimer.
15. (a) 2; (b) 4; (c) 2; (d) 1.
16. The electrostatic interactions between BPG and hemoglobin would be weakened by competition with water molecules. The T state would not be stabilized.

Chapter 8

1. Rate enhancement and substrate specificity
2. A cofactor
3. Coenzymes and metals
4. Vitamins are converted into coenzymes.
5. Enzymes facilitate the formation of the transition state.
6. The intricate three-dimensional structure of proteins allows the construction of active sites that will recognize only specific substrates.
7. The energy required to reach the transition state (the activation energy) is returned when the transition state proceeds to product.
8. Protein hydrolysis has a large activation energy. Protein synthesis must require energy to proceed.
9. The enzymes help protect the fluid that surrounds eyes from bacterial infection.
10. Transition states are very unstable. Consequently, molecules that resemble transition states are themselves likely to be unstable and, hence, difficult to synthesize.
11. (a) 0; (b) 28.53; (c) −22.84; (d) −11.42; (e) 5.69.
12. (a) $\Delta G^{\circ\prime} = -RT \ln K'_{eq}$

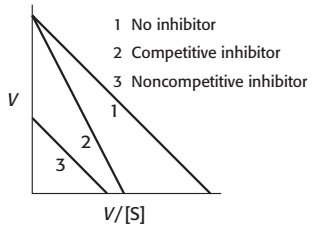
$$+1.8 = -(1.98 \times 10^{-3}\ \text{kcal}^{-1}\ \text{K}^{-1}\ \text{mol}^{-1})\ (298\ \text{K})\ (\ln[\text{G1P}]/[\text{G6P}])$$

$$-3.05 = \ln [\text{G1P}]/[\text{G6P}]$$

$$+3.05 = \ln [\text{G6P}]/[\text{G1P}]$$

$$K'^{-1}_{eq} = 21 \quad \text{or} \quad K'_{eq} = 4.8 \times 10^{-2}$$

Because [G6P]/[G1P] = 21, there is 1 molecule of G1P for every 21 molecules of G6P. Because we started with 0.1 M, the [G1P] is 1/22(0.1 M) = 0.0045 M and [G6P] must be 21/22(0.1 M) or 0.096 M. Consequently, the reaction does not proceed as written to a significant extent.
(b) Supply G6P at a high rate and remove G1P at a high rate by other reactions. In other words, make sure that the [G6P]/[G1P] is kept large.
13. $K_{eq} = 19$, $\Delta G^{\circ\prime} = -7.41$ kJ mol^{-1} (−1.77 kcal mol^{-1})
14. The three-dimensional structure of an enzyme is stabilized by interactions with the substrate, reaction intermediates, and products. This stabilization minimizes thermal denaturation.
15. At substrate concentrations near the K_M, the enzyme displays significant catalysis yet is sensitive to changes in substrate concentration.
16. A + S = 10 K_M, $V_0 = 0.91\ V_{max}$. I + S = 20 K_M, V_0 = 0.91 V_{max}.
So any Michaelis–Menten curves showing that the enzyme actually attains V_{max} are pernicious lies.
17. (a) 31.1 μmol; (b) 0.05 μmol; (c) 622 s^{-1}, a midrange value for enzymes (see Table 8.5).
18. (a) Yes, $K_M = 5.2 \times 10^{-6}$ M; (b) $V_{max} = 6.8 \times 10^{-10}$ mol minute^{-1}; (c) 337 s^{-1}.
19. Penicillinase, like glycopeptide transpeptidase, forms an acyl-enzyme intermediate with its substrate but transfers the intermediate to water rather than to the terminal glycine residue of the pentaglycine bridge.
20. (a) V_{max} is 9.5 μmol minute^{-1}. K_M is 1.1×10^{-5} M, the same as without inhibitor.
(b) Noncompetitive
(c) 2.5×10^{-5} M
(d) $f_{ES} = 0.73$, in the presence or absence of this noncompetitive inhibitor.
21. (a) $V = V_{max} - (V/[S])\ K_M$.
(b) Slope = $-K_M$, y-intercept = V_{max}, x-intercept = V_{max}/K_M.
(c) An Eadie–Hofstee plot



22. The rates of utilization of substrates A and B are given by

$$V_A = \left(\frac{k_{cat}}{K_M}\right)_A [\text{E}][\text{A}]$$

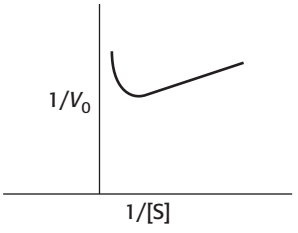
and

$$V_A = \left(\frac{k_{cat}}{K_M}\right)_B [\text{E}][\text{B}]$$

Hence, the ratio of these rates is

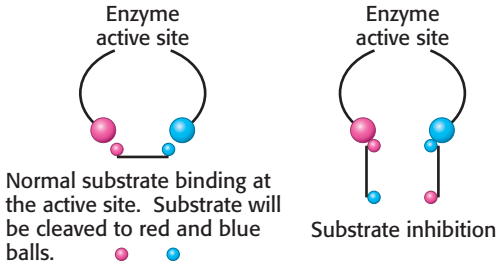
$$V_A/V_B = \left(\frac{k_{cat}}{K_M}\right)_B [\text{A}]/\left(\frac{k_{cat}}{K_M}\right)_A [\text{B}]$$

Thus, an enzyme discriminates between competing substrates on the basis of their values of k_{cat}/K_M rather than of K_M alone.
23. The mutation slows the reaction by a factor of 100 because the activation free energy is increased by 53.22 kJ mol^{-1} (12.72 kcal mol^{-1}). Strong binding of the substrate relative to the transition state slows catalysis.
24. 1.1 μmol minute^{-1}
25. (a) This piece of information is necessary for determining the correct dosage of succinylcholine to administer.
(b) The duration of the paralysis depends on the ability of the serum cholinesterase to clear the drug. If there were one-eighth the amount of enzyme activity, paralysis could last eight times as long, which is undesirable for two reasons. First, the respirator might break from extended use, which would not be good for the patient on the respirator; second, the doctors might miss their golf game.
(c) K_M is the concentration needed by the enzyme to reach $^1/_2 V_{max}$. Consequently, for a given concentration of substrate, the reaction catalyzed by the enzyme with the lower K_M will have the higher rate. The mutant patient with the higher K_M will clear the drug at a much lower rate.
26. If the total amount of enzyme (E_T) is increased, V_{max} will increase, because $V_{max} = k_2[E_T]$. But $K_M = (k_{-1} + k_2)/k_1$; that is, it is independent of substrate concentration. The middle graph describes this situation.
27. (a)

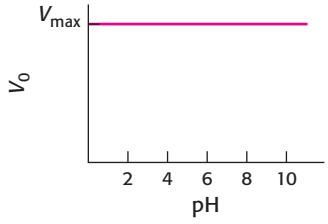


(b) This behavior is substrate inhibition: at high concentrations, the substrate forms unproductive complexes at the active site. The adjoining drawing shows what might happen. Substrate normally binds in a defined orientation, shown in the drawing

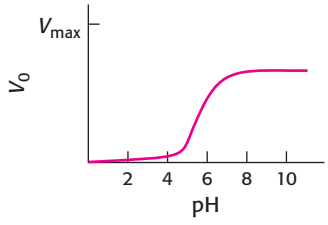
as red to red and blue to blue. At high concentrations, the substrate may bind at the active site such that the proper orientation is met for each end of the molecule, but two different substrate molecules are binding.



28. The first step will be the rate-limiting step. Enzymes E_B and E_C are operating at ½ V_{max}, whereas the K_M for enzyme E_A is greater than the substrate concentration. E_A would be operating at approximately $10^{-2}V_{max}$.
29. The fluorescence spectroscopy reveals the existence of an enzyme–serine complex and of an enzyme–serine–indole complex.
30. (a) When [S^+] is much greater than the value of K_M, pH will have a negligible effect on the enzyme because S^+ will interact with E^- as soon as the enzyme becomes available.



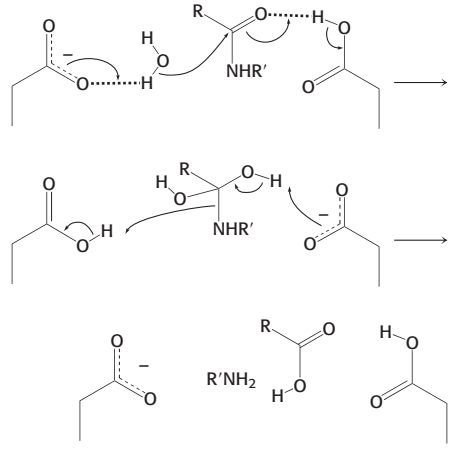
(b) When [S^+] is much less than the value of K_M, the plot of V_0 versus pH becomes essentially a titration curve for the ionizable groups, with enzyme activity being the titration marker. At low pH, the high concentration of H^+ will keep the enzyme in the EH form and inactive. As the pH rises, more and more of the enzyme will be in the E^- form and active. At high pH (low H^+), all of the enzyme is E^-.



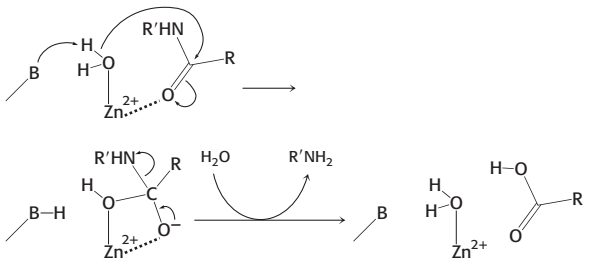
(c) The midpoint on this curve will be the pK_a of the ionizable group, which is stated to be pH 6.
31. (a) Incubating the enzyme at 37°C leads to a denaturation of enzyme structure and a loss of activity. For this reason, most enzymes must be kept cool if they are not actively catalyzing their reactions.
(b) The coenzyme apparently helps to stabilize enzyme structure, because enzyme from PLP-deficient cells denatures faster. Cofactors often help stabilize enzyme structure.

Chapter 9

1. For the amide substrate, the formation of the acyl-enzyme intermediate is slower than the hydrolysis of the acyl-enzyme intermediate, and so no burst is observed. A burst is observed for ester substrates; the formation of the acyl-enzyme intermediate is faster, leading to the observed burst.
2. The histidine residue in the substrate can substitute to some extent for the missing histidine residue of the catalytic triad of the mutant enzyme.
3. No. The catalytic triad works as a unit. After this unit has been made ineffective by the mutation of histidine to alanine, the further mutation of serine to alanine should have only a small effect.
4. The substitution corresponds to one of the key differences between trypsin and chymotrypsin, and so trypsinlike specificity (cleavage after lysine and arginine) might be predicted. In fact, additional changes are required to effect this specificity change.
5. Imidazole is apparently small enough to reach the active site of carbonic anhydrase. Buffers with large molecular components cannot do so, and the effects of the mutation are more evident.
6. No. The odds of such a sequence being present are approximately 1 in $4^{10} = 1{,}048{,}576$. Because a typical viral genome has only 50,000 bp, the target sequence would be unlikely to be present.
7. No, because the enzyme would destroy the host DNA before protective methylation could take place.
8. No. The bacteria receiving the enzyme would have their own DNA destroyed because they would likely lack the appropriate protective methylase.
9. EDTA will bind to Zn^{2+} and remove the ion, which is required for enzyme activity, from the enzyme.
10. (a) The aldehyde reacts with the active-site serine. (b) A hemiacetal is formed.
11. Trypsin
12. The reaction is expected to be slower by a factor of 10 because the rate depends on the pK_a of the zinc-bound water. $k_{cat} = 60{,}000\ s^{-1}$.
13. EDTA binds the magnesium necessary for the reaction.
14. ATP hydrolysis is reversible within the active site. ATP hydrolysis takes place within the active site with the incorporation of ^{18}O, ATP is re-formed, and the ATP is released back into solution.
15. If the aspartate is mutated, the protease is inactive and the virus will not be viable.
16. Water substitutes for the hydroxyl group of serine 236 in mediating proton transfer from the attacking water and the γ-phosphoryl group.
17. (a) Cysteine protease: The same as Figure 9.8, except that cysteine replaces serine in the active site and no aspartate is present.
(b) Aspartyl protease:



(c) Metalloprotease:

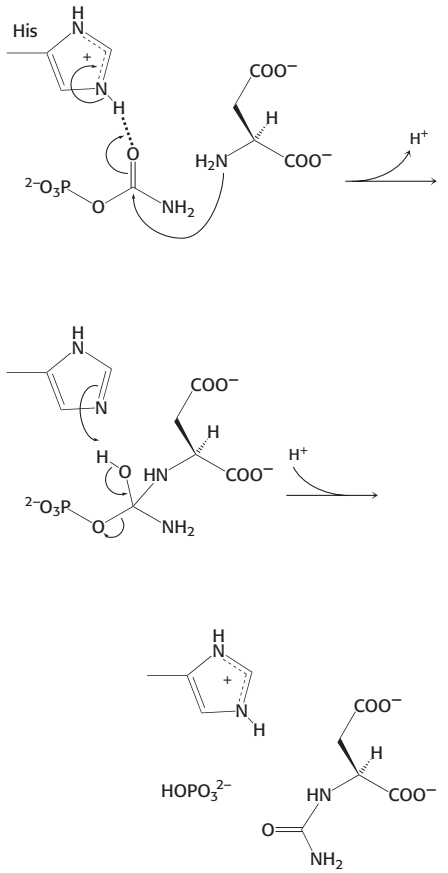


Chapter 10

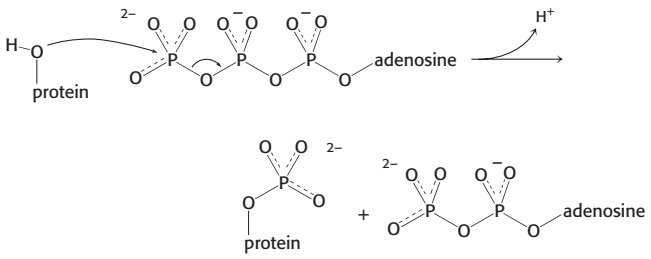
1. The enzyme catalyzes the first step in the synthesis of pyrimidines. It facilitates the condensation of carbamoyl phosphate and aspartate to form *N*-carbamoylaspartate and inorganic phosphate.
2. The protonated form of histidine probably stabilizes the negatively charged carbonyl oxygen atom of the scissile bond in the transition state. Deprotonation would lead to a loss of activity. Hence, the rate is expected to be half maximal at a pH of about 6.5 (the p*K* of an unperturbed histidine side chain in a protein) and to decrease as the pH is raised.
3. The inhibition of an allosteric enzyme by the end product of the pathway controlled by the enzyme. It prevents the production of too much end product and the consumption of substrates when product is not required.
4. High concentrations of ATP might signal two overlapping situations. The high levels of ATP might suggest that some *nucleotides* are available for nucleic acid synthesis, and consequently, CTP should be synthesized. The high levels of ATP indicate that *energy* is available for nucleic acid synthesis, and so CTP should be produced.
5. All of the enzyme would be in the R form all of the time. There would be no cooperativity. The kinetics would look like that of a Michaelis–Menten enzyme.
6. The enzyme would show simple Michaelis–Menten kinetics because it is essentially always in the R state.
7. CTP is formed by the addition of an amino group to UTP. Evidence indicates the UTP is also capable of inhibiting ATCase.
8. Homotropic effectors are the substrates of allosteric enzymes. Heterotropic effectors are the regulators of allosteric enzymes. Homotropic effectors account for the sigmoidal nature of the velocity versus substrate concentration curve, whereas heterotropic effectors alter the midpoint of K_M of the curve. Ultimately, both types of effectors work by altering the T/R ratio.
9. The reconstitution shows that the complex quaternary structure and the resulting catalytic and regulatory properties are ultimately encoded in the primary structure of individual components.
10. If substrates had been used, the enzyme would catalyze the reaction. Intermediates would not accumulate on the enzyme. Consequently, any enzyme that crystallized would have been free of substrates or products.
11. (a) 100. The change in the [R]/[T] ratio on binding one substrate molecule must be the same as the ratio of the substrate affinities of the two forms.
(b) 10. The binding of four substrate molecules changes the [R]/[T] by a factor of $100^4 = 10^8$. The ratio in the absence of substrate is 10^{-7}. Hence, the ratio in the fully liganded molecule is $10^8 \times 10^{-7} = 10$.
12. The fraction of molecules in the R form is 10^{-5}, 0.004, 0.615, 0.998, and 1 when 0, 1, 2, 3, and 4 ligands, respectively, are bound.
13. The sequential model can account for negative cooperativity, whereas the concerted model cannot.
14. The binding of PALA switches ATCase from the T to the R state because PALA acts as a substrate analog. An enzyme molecule containing bound PALA has fewer free catalytic sites than does an unoccupied enzyme molecule. However, the PALA-containing enzyme will be in the R state and, hence, have a higher affinity for the substrates. The dependence of the degree of activation on the concentration of PALA is a complex function of the allosteric constant L_0 and of the binding affinities of the R and T states for the analog and substrates.
15. The net outcome of the two reactions is the hydrolysis of ATP to ADP and P_i, which has a ΔG of -50 kJ mol^{-1} (-12 kcal mol^{-1}) under cellular conditions.
16. Isozymes are homologous enzymes that catalyze the same reaction but have different kinetic or regulatory properties.
17. Although the same reaction may be required in a variety of different tissues, the biochemical properties of tissues will differ according to their biological function. Isozymes allow the fine-tuning of catalytic and regulatory properties to meet the specific needs of the tissue.
18. (a) 7; (b) 8; (c) 11; (d) 6; (e) 1; (f) 12; (g) 3; (h) 4; (i) 5; (j) 2; (k) 10; (l) 9.
19. When phosphorylation takes place at the expense of ATP, sufficient energy is expended to dramatically alter the structure and hence activity of a protein. Moreover, because ATP is the cellular energy currency, protein modification is linked to the energy status of the cell.
20. Covalent modification is reversible, whereas proteolytic cleavage is irreversible.
21. Activation is independent of zymogen concentration because the reaction is intramolecular.
22. Although quite rare, cases of enteropeptidase deficiency have been reported. The affected person has diarrhea and fails to thrive because digestion is inadequate. In particular, protein digestion is impaired.
23. Add blood from the second patient to a sample from the first. If the mixture clots, the second patient has a defect different from that of the first. This type of assay is called a complementation test.
24. Activated factor X remains bound to blood-platelet membranes, which accelerates its activation of prothrombin.
25. Antithrombin III is a very slowly hydrolyzed substrate of thrombin. Hence, its interaction with thrombin requires a fully formed active site on the enzyme.
26. Residues *a* and *d* are located in the interior of an α-helical coiled coil, near the axis of the superhelix. Hydrophobic interactions between these side chains contribute to the stability of the coiled coil.
27. Leucine would be a good choice. It is resistant to oxidation and has nearly the same volume and degree of hydrophobicity as methionine has.
28. Inappropriate clot formation could block arteries in the brain, causing a stroke, or the heart, causing a heart attack.
29. Tissue-type plasminogen activator, or TPA, is a serine protease that leads to the dissolution of blood clots. TPA activates plasminogen that is bound to a fibrin clot, converting it into active plasmin, which then hydrolyzes the fibrin of the clot.
30. A mature clot is stabilized by amide linkages between the side chains of lysine and glutamine that are absent in a soft clot. The linkages are formed by transglutaminase.
31. The simple sequential model predicts that the fraction of catalytic chains in the R state, f_R, is equal to the fraction containing bound substrate, Y. The concerted model, in contrast, predicts that f_R increases more rapidly than Y as the substrate concentration is increased. The change in f_R leads to the change in Y on addition of substrate, as predicted by the concerted model.
32. The binding of succinate to the functional catalytic sites of the native c_3 moiety changed the visible absorption spectrum of nitrotyrosine residues in the *other* c_3 moiety of the hybrid enzyme. Thus, the binding of substrate analog to the active sites of one trimer altered the structure of the other trimer.

33. According to the concerted model, an allosteric activator shifts the conformational equilibrium of all subunits toward the R state, whereas an allosteric inhibitor shifts it toward the T state. Thus, ATP (an allosteric activator) shifted the equilibrium to the R form, resulting in an absorption change similar to that obtained when substrate is bound. CTP had a different effect. Hence, this allosteric inhibitor shifted the equilibrium to the T form. Thus, the concerted model accounts for the ATP-induced and CTP-induced (heterotropic), as well as for the substrate-induced (homotropic), allosteric interactions of ATCase.
34. In the R state, ATCase expands and becomes less dense. This decrease in density results in a decrease in the sedimentation value (see the formula on p. 76).
35. The interaction between trypsin and the inhibitor is so stable that the transition state is rarely formed. Recall that maximal binding energy is released when an enzyme binds to the transition state. If the substrate-enzyme interaction is too stable, the transition state rarely forms.

36.

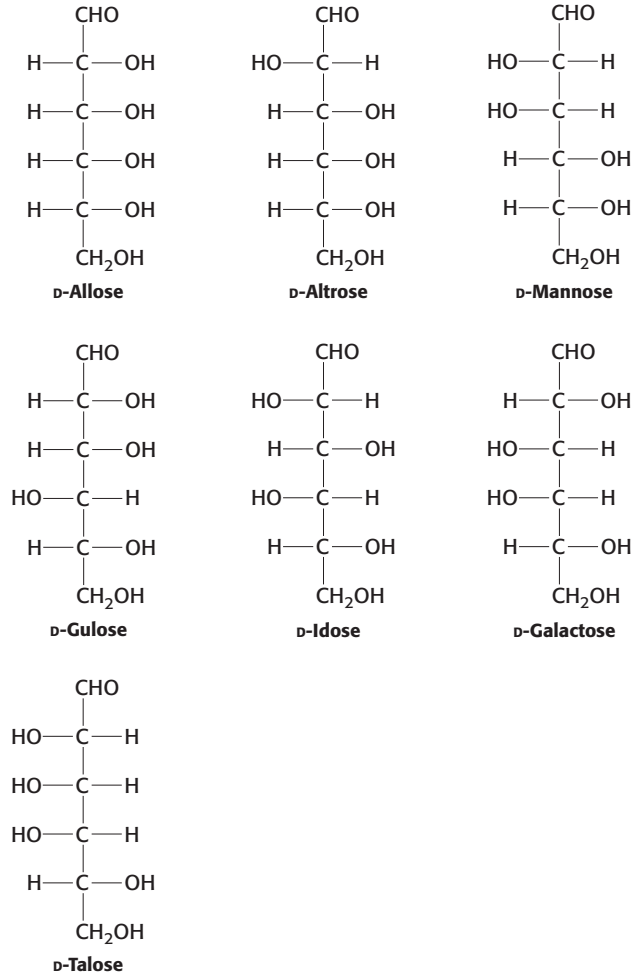


37.

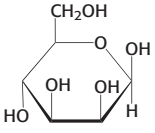


Chapter 11

1. Carbohydrates were originally regarded as *hydrates* of *carbon* because the empirical formula of many of them is $(CH_2O)_n$.
2. Three amino acids can be linked by peptide bonds in only six different ways. However, three different monosaccharides can be linked in a plethora of ways. The monosaccharides can be linked in a linear or branched manner, with α or β linkages, with bonds between C-1 and C-3, between C-1 and C-4, between C-1 and C-6, and so forth. In fact, the three monosaccharides can form 12,288 different trisaccharides.
3. (a) aldose-ketose; (b) epimers; (c) aldose-ketose (d) anomers; (e) aldose-ketose; (f) epimers.
4. Erythrose: tetrose aldose; Ribose: pentose aldose; Glyceraldehyde: triose aldose; Dihydroxyacetone: triose ketose; Erythrulose: tetrose ketose; Ribulose: pentose ketose; Fructose: hexose ketose.
5.



6. The proportion of the α anomer is 0.36, and that of the β anomer is 0.64.
7. Glucose is reactive because of the presence of an aldehyde group in its open-chain form. The aldehyde group slowly condenses with amino groups to form aldimine products of a type called Schiff-base adducts.
8. A pyranoside reacts with two molecules of periodate; formate is one of the products. A furanoside reacts with only one molecule of periodate; formate is not formed.
9. From methanol
10 (a) β-D-Mannose; (b) β-D-galactose; (c) β-D-fructose; (d) β-D-glucosamine.

11. The trisaccharide itself should be a competitive inhibitor of cell adhesion if the trisaccharide unit of the glycoprotein is critical for the interaction.
12. Reducing ends would form 1,2,3,6-tetramethylglucose. The branch points would yield 2,3-dimethylglucose. The remainder of the molecule would yield 2,3,6-trimethylglucose.
13. (a) not a reducing sugar; no open-chain forms are possible. (b) D-Galactose, D-glucose, D-fructose. (c) D-Galactose and sucrose (glucose + fructose).
14. 

β-D-Mannose
The hemiketal linkage of the α anomer is broken to form the open form. Rotation about the C-1 and C-2 bonds allows the formation of the β anomer, and a mixture of isomers results.
15. Heating converts the very sweet pyranose form into the more-stable but less-sweet furanose form. Consequently, the sweetness of the preparation is difficult to accurately control, which also accounts for why honey loses sweetness with time. See Figure 11.5 for structures.
16. (a) Each glycogen molecule has one reducing end, whereas the number of nonreducing ends is determined by the number of branches, or α-1,6 linkages. (b) Because the number of nonreducing ends greatly exceeds the number of reducing ends in a collection of glycogen molecules, all of the degradation and synthesis of glycogen takes place at the nonreducing ends, thus maximizing the rate of degradation and synthesis.
17. No, sucrose is not a reducing sugar. The anomeric carbon atom acts as the reducing agent in both glucose and fructose but, in sucrose, the anomeric carbon atoms of fructose and glucose are joined by a covalent bond and are thus not available to react.
18. Glycogen is polymer of glucose linked by α-1,4-glycosidic bonds with branches formed approximately every 10 glucose units by α-1,6-glycosidic bonds. Starch consists of two polymers of glucose. Amylose is a straight-chain polymer formed by α-1,4-glycosidic bonds. Amylopectin is similar to glycogen but amylopectin has fewer branches, one branch per 30 or so glucose units.
19. Cellulose is a linear polymer of glucose joined by β-1,4 linkages. Glycogen is a branched polymer with the main chain being formed by α-1,4-glycosidic bonds. The β-1,4 linkages allow the formation of a linear polymer ideal for structural roles. The α-1,4 linkages of glycogen form a helical structure, which allows the storage of many glucose moieties in a small space.
20. Simple glycoproteins are often secreted proteins and thus play a variety of roles. For example, the hormone EPO is a glycoprotein. Usually, the protein component constitutes the bulk of the glycoprotein by mass. In contrast, proteoglycans and mucoproteins are predominantly carbohydrates. Proteoglycans have glycosaminoglycans attached, and play structural roles as in cartilage and the extracellular matrix. Mucoproteins often serve as lubricants and have multiple carbohydrates attached through an *N*-acetylgalactosamine moiety.
21. The attachment of the carbohydrate allows the EPO to stay in circulation longer and thus to function for longer periods of time than would a carbohydrate-free EPO.
22. The glycosaminoglycan, because it is heavily charged, binds many water molecules. When cartilage is stressed, such as when your heel hits the ground, the water is released, thus cushioning the impact. When you lift your heel, the water rebinds.
23. The lectin that binds the mannose 6-phosphate might be defective and not recognize a correctly addressed protein.
24. Different molecular forms of a glycoprotein that differ in the amount of carbohydrate attached or the location of attachment or both.
25. The total collection of carbohydrates synthesized by a cell at particular times and under particular environmental conditions.
26. The genome comprises all of the genes present in an organism. The proteome includes all of the possible protein products and modified proteins that a cell expresses under any particular set of circumstances. The glycome consists of all of the carbohydrates synthesized by the cell under any particular set of circumstances. Because the genome is static, but any given protein can be variously expressed and modified, the proteome is more complex than the genome. The glycome, which includes not only glycoforms of proteins, but also many possible carbohydrate structures, must be even more complex.
27. It suggests that carbohydrates are on the cell surfaces of all organisms for the purpose of recognition by other organisms or the environment.
28. A glycoprotein is a protein that is decorated with carbohydrates. A lectin is a protein that specifically recognizes carbohydrates. A lectin can also be a glycoprotein.
29. Each site either is or is not glycosylated, and so there are $2^6 = 64$ possible proteins.
30. As discussed in Chapter 9, many enzymes display stereochemical specificity. Clearly, the enzymes of sucrose synthesis are able to distinguish between the isomers of the substrates and link only the correct pair.
31. If the carbohydrate specificity of the lectin is known, an affinity column with the appropriate carbohydrate attached could be prepared. The protein preparation containing the lectin of interest could be passed over the column. The use of this method was indeed how the glucose-binding lectin concanavalin A was purified.
32. (a) Aggrecan is heavily decorated with glycosaminoglycans. If glycosaminoglycans are released into the media, aggrecan must be undergoing degradation.
(b) Another enzyme might be present that cleaves glycosaminoglycans from aggrecan without degrading aggrecan. Other experiments not shown established that glycosaminoglycan release is an accurate measure of aggrecan destruction.
(c) The control provides a baseline of "background" degradation inherent in the assay.
(d) Aggrecan degradation is greatly enhanced.
(e) Aggrecan degradation is reduced to the background system.
(f) It is an in vitro system in which not all the factors contributing to cartilage stabilization in vivo are present.

Chapter 12

1. 2.86×10^6 molecules, because each leaflet of the bilayer contains 1.43×10^6 molecules.
2. Essentially an "inside-out" membrane. The hydrophilic groups would come together on the interior of the structure, away from the solvent, whereas the hydrocarbon chains would interact with the solvent.
3. 2×10^{-7} cm, 6×10^{-6} cm, and 2×10^{-4} cm.
4. The radius of this molecule is 3.1×10^{-7} cm, and its diffusion coefficient is 7.4×10^{-9} cm^2 s^{-1}. The average distances traversed are 1.7×10^{-7} cm in 1 μs, 5.4×10^{-6} cm in 1 ms, and 1.7×10^{-4} cm in 1 s.
5. The membrane underwent a phase transition from a highly fluid to a nearly frozen state when the temperature was lowered. A carrier can shuttle ions across a membrane only when the bilayer is highly fluid. A channel, in contrast, allows ions to traverse its pore even when the bilayer is quite rigid.
6. The presence of a cis double bond introduces a kink in the fatty acid chain that prevents tight packing and reduces the number of atoms in van der Waals contact. The kink lowers the melting point compared with that of a saturated fatty acid. Trans fatty acids do not have the kink, and so their melting temperatures are higher, more similar to those of saturated fatty acids.

Because trans fatty acids have no structural effect, they are rarely observed.
7. Palmitic acid is shorter than stearic acid. Thus, when the chains pack together, there is less opportunity for van der Waals interaction and the melting point is thus lower than that of the longer stearic acid.
8. Hibernators selectively feed on plants that have a high proportion of polyunsaturated fatty acids with lower melting temperature.
9. The initial decrease in fluorescence with the first addition of sodium dithionite results from the quenching of NBD-PS molecules in the outer leaflet of the bilayer. Sodium dithionite does not traverse the membrane under these experimental conditions; hence, it does not quench the labeled phospholipids in the inner leaflet. A second addition of sodium dithionite has no effect, as the NBD-PS molecules in the outer leaflet remain quenched. However, after a 6.5 hour incubation, about half the NBD-PS has flipped over to the outer leaflet of the bilayer, resulting in the 50% decrease in fluorescence when sodium dithionite is added.
10. The addition of the carbohydrate introduces a significant energy barrier to the flip-flop because a hydrophilic carbohydrate moiety would need to be moved through a hydrophobic environment. This energetic barrier enhances membrane asymmetry.
11. The C_{16} alkyl chain is attached by an ether linkage. The C-2 carbon atom of glycerol has only an acetyl group attached by an ester linkage instead of a fatty acid, as is the case with most phospholipids.
12. In a hydrophobic environment, the formation of intrachain hydrogen bonds stabilizes the amide hydrogen atoms and carbonyl oxygen atoms of the polypeptide chain, and so an α helix forms. In an aqueous environment, these groups are stabilized by interaction with water, and so there is no energetic reason to form an α helix. Thus, the α helix would be more likely to form in a hydrophobic environment.
13. The protein may contain an α helix that passes through the hydrophobic core of the protein. This helix is likely to feature a stretch of hydrophobic amino acids similar to those observed in transmembrane helices.
14. The shift to the lower temperature would decrease fluidity by enhancing the packing of the hydrophobic chains by van der Waals interactions. To prevent this packing, new phospholipids having shorter chains and a greater number of cis double bonds would be synthesized. The shorter chains would reduce the number of van der Waals interactions, and the cis double bonds, which cause the kink in structure, would prevent the packing of the fatty acid tails of the phospholipids.
15. Each of the 21 v-SNARE proteins could interact with each of 7 t-SNARE partners. Multiplication gives the total number of different interacting pairs: $7 \times 21 = 147$ different v-SNARE–t-SNARE pairs.
16. (a) The graph shows that, as temperature increases, the phospholipid bilayer becomes more fluid. T_m is the temperature of the transition from the predominantly less fluid state to the predominantly more fluid state. Cholesterol broadens the transition from the less-fluid to the more-fluid state. In essence, cholesterol makes membrane fluidity less sensitive to temperature changes.
(b) This effect is important because the presence of cholesterol tends to stabilize membrane fluidity by preventing sharp transitions. Because protein function depends on the proper fluidity of the membrane, cholesterol maintains the proper environment for membrane-protein function.
17. The protein plotted in part *c* is a transmembrane protein from *C. elegans*. It spans the membrane with four α helices that are prominently displayed as hydrophobic peaks in the hydropathy plot. Interestingly, the protein plotted in part *a* also is a membrane protein, a porin. This protein is made primarily of β strands, which lack the prominent hydrophobic window of membrane helices. This example shows that, although hydropathy plots are useful, they are not infallible.
18. To purify any protein, the protein must first be solubilized. For a membrane protein, solubilization usually requires a detergent—hydrophobic molecules that bind to the protein and thus replace the lipid environment of the membrane. If the detergent is removed, the protein aggregates and precipitates from solution. Often, the steps in purification, such as ion-exchange chromatography, are difficult to perform in the presence of sufficient detergent to solubilize the protein. Crystals of appropriate protein–detergent complexes must be generated.

Chapter 13

1. In simple diffusion, the substance in question can diffuse down its concentration gradient through the membrane. In facilitated diffusion, the substance is not lipophilic and cannot directly diffuse through the membrane. A channel or carrier is required to facilitate movement down the gradient.
2. The two forms are (1) ATP hydrolysis and (2) the movement of one molecule down its concentration gradient coupled with the movement of another molecule up its concentration gradient.
3. The three types of carriers are symporters, antiporters, and uniporters. Symporters and antiporters can mediate secondary active transport.
4. The free-energy cost is 32 kJ mol^{-1} (7.6 kcal mol^{-1}). The chemical work performed is 20.4 kJ mol^{-1} (4.9 kcal mol^{-1}), and the electrical work performed is 11.5 kJ mol^{-1} (2.8 kcal mol^{-1}).
5. For chloride, $z = -1$; for calcium $z = +2$. At the concentrations given, the equilibrium potential for chloride is −97 mV and the equlibrium potential for calcium is +122 mV.
6. The concentration of glucose inside the cell is 66 times as great as that outside the cell $[(c_2/c_1) = 66]$ when the free-energy input is 10.8 kJ mol^{-1} (2.6 kcal mol^{-1}).
7. By analogy with the Ca^{2+} ATPase, with three Na^+ ions binding from inside the cell to the E_1 conformation and with two K^+ ions binding from outside the cell to the E_2 conformation, a plausible mechanism is as follows:

(i) A catalytic cycle could begin with the enzyme in its unphosphorylated state (E_1) with three sodium ions bound.
(ii) The E_1 conformation binds ATP. A conformational change traps sodium ions inside the enzyme.
(iii) The phosphoryl group is transferred from ATP to an aspartyl residue.
(iv) On ADP release, the enzyme changes its overall conformation, including the membrane domain. This new conformation (E_2) releases the sodium ions to the side of the membrane opposite that at which they entered and binds two potassium ions from the side where sodium ions are released.
(v) The phosphorylaspartate residue is hydrolyzed to release inorganic phosphate. With the release of phosphate, the interactions stabilizing E_2 are lost, and the enzyme everts to the E_1 conformation. Potassium ions are released to the cytoplasmic side of the membrane. The binding of three sodium ions from the cytoplasmic side of the membrane completes the cycle.

8. Establish a lactose gradient across vesicle membranes that contain properly oriented lactose permease. Initially, the pH should be the same on both sides of the membrane and the lactose concentration should be higher on the "exit" side of lactose

permease. As the lactose flows "in reverse" through the permease, down its concentration gradient, it can be tested whether or not a pH gradient becomes established as the lactose gradient is dissipated.

9. Ligand-gated channels open in response to the binding of a molecule by the channel, whereas voltage-gated channels open in response to changes in the membrane potential.

10. An ion channel must transport ions in either direction at the same rate. The net flow of ions is determined only by the composition of the solutions on either side of the membrane.

11. Uniporters act as enzymes do; their transport cycles include large conformational changes, and only a few molecules interact with the protein per transport cycle. In contrast, channels, after having opened, provide a pore in the membrane through which many ions may pass. As such, channels mediate transport at a much higher rate than do uniporters.

12. FCCP effectively creates a pore in the bacterial membrane through which protons can pass rapidly. Protons that are pumped out of the bacteria will pass through this pore preferentially (the "path of least resistance"), rather than participate in H^+/lactose symport.

13. Cardiac muscle must contract in a highly coordinated manner in order to pump blood effectively. Gap junctions mediate the orderly cell-to-cell propagation of the action potential through the heart during each beat.

14. The positively charged guanidinium group resembles Na^+ and binds to negatively charged carboxylate groups in the mouth of the channel.

15. SERCA, a P-type ATPase, uses a mechanism by which a covalent phosphorylated intermediate (at an aspartate residue) is formed. At steady state, a subset of the SERCA molecules are trapped in the E_2-P state and, as a result, radiolabeled. The MDR protein is an ABC transporter and does not operate through a phosphorylated intermediate. Hence, a radiolabeled band would not be observed for MDR.

16. The blockage of ion channels inhibits action potentials, leading to loss of nervous function. Like tetrodotoxin, these toxin molecules are useful for isolating and specifically inhibiting particular ion channels.

17. After repolarization, the ball domains of the ion channels engage the channel pore, rendering them inactive for a short period of time. During this time, the channels cannot be reopened until the ball domains disengage and the channel returns to the "closed" state.

18. Because sodium ions are charged and because sodium channels carry only sodium ions (but not anions), the accumulation of excess positive charge on one side of the membrane dominates the chemical gradients.

19. A mutation that impairs the ability of the sodium channel to inactivate would prolong the duration of the depolarizing sodium current, thus lengthening the cardiac action potential.

20. No. Channels will likely open or close in response to an external stimulus, but the unit conductance of the open channel will be influenced very little.

21. The ratio of closed to open forms of the channel is 10^5, 5000, 250, 12.5, and 0.625 when zero, one, two, three, and four ligands, respectively, are bound. Hence, the fraction of open channels is 1.0×10^{-5}, 2.0×10^{-4}, 4.0×10^{-3}, 7.4×10^{-2}, and 0.62.

22. These organic phosphates inhibit acetylcholinesterase by reacting with the active-site serine residue to form a stable phosphorylated derivative. They cause respiratory paralysis by blocking synaptic transmission at cholinergic synapses.

23. (a) The binding of the first acetylcholine molecule increases the open-to-closed ratio by a factor of 240, and the binding of the second increases it by a factor of 11,700. (b) The free-energy contributions are 14 kJ mol^{-1} (3.3 kcal mol^{-1}) and 23 kJ mol^{-1} (5.6 kcal mol^{-1}), respectively. (c) No; the MWC model predicts that the binding of each ligand will have the same effect on the open-to-closed ratio.

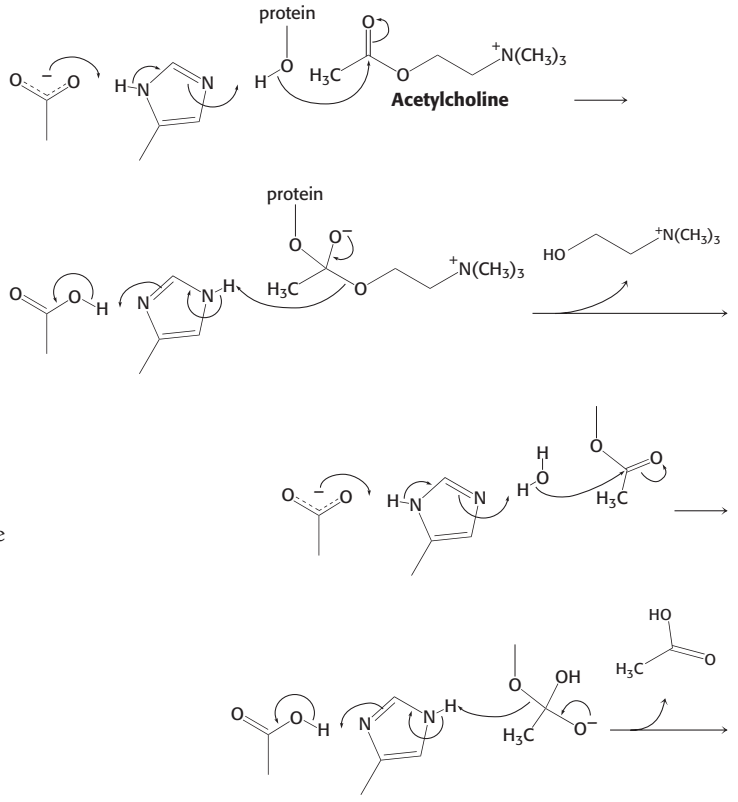
24. Batrachotoxin blocks the transition from the open to the closed state.

25. (a) Chloride ions flow into the cell. (b) Chloride flux is inhibitory because it hyperpolarizes the membrane. (c) The channel consists of five subunits.

26. After the addition of ATP and calcium, SERCA will pump Ca^{2+} ions into the vesicle. However, the accumulation of Ca^{2+} ions inside the vesicle will rapidly lead to the formation of an electrical gradient that cannot be overcome by ATP hydrolysis. The addition of calcimycin will allow the pumped Ca^{2+} ions to flow back out of the vesicle, dissipating the charge buildup, and enabling the pump to operate continuously.

27. The catalytic prowess of acetylcholinesterase ensures that the duration of the nerve stimulus will be short.

28. See reaction below.



29. (a) Only ASIC1a is inhibited by the toxin. (b) Yes; when the toxin was removed, the activity of the acid-sensing channel began to be restored. (c) 0.9 nM.

30. This mutation is one of a class of mutations that result in slow-channel syndrome (SCS). The results suggest a defect in channel closing; so the channel remains open for prolonged periods. Alternatively, the channel may have a higher affinity for acetylcholine than does the control channel.

31. The mutation reduces the affinity of acetylcholine for the receptor. The recordings would show the channel opening only infrequently.

32. Glucose displays a transport curve that suggests the participation of a carrier because the initial rate is high but then levels off

at higher concentrations, consistent with saturation of the carrier, which is reminiscent of Michaelis–Menten enzymes (Section 8.4). Indole shows no such saturation phenomenon, which implies that the molecule is lipophilic and simply diffuses across the membrane. Ouabain is a specific inhibitor the Na^+-K^+ pump. If ouabain were to inhibit glucose transport, then a Na^+-glucose cotransporter would be assisting in transport.

Chapter 14

1. The negatively charged glutamate residues mimic the negatively charged phosphoserine or phosphothreonine residues and stabilize the active conformation of the enzyme.
2. No. Phosphoserine and phosphothreonine are considerably shorter than phosphotyrosine.
3. The GTPase activity terminates the signal. Without such activity, after a pathway has been activated, it remains activated and is unresponsive to changes in the initial signal. If the GTPase activity were more efficient, the lifetime of the GTP-bound G_α subunit would be too short to achieve downstream signaling.
4. Two identical receptor molecules must recognize different aspects of the same signal molecule.
5. Growth-factor receptors can be activated by dimerization. If an antibody causes a receptor to dimerize, the signal-transduction pathway in a cell will be activated.
6. The mutated α subunit will always be in the GTP form and, hence, in the active form, which would stimulate its signaling pathway.
7. A G protein is a component of the signal-transduction pathway. GTPγS is not hydrolyzed by the G_α subunit, leading to prolonged activation.
8. Calcium ions diffuse slowly because they bind to many protein surfaces within a cell, impeding their free motion. Cyclic AMP does not bind as frequently, and so it diffuses more rapidly.
9. Fura-2 is a highly negatively charged molecule, with five carboxylate groups. Its charge prevents it from effectively crossing the hydrophobic region of the plasma membrane.
10. $G_{\alpha s}$ stimulates adenylate cyclase, leading to the generation of cAMP. This signal then leads to glucose mobilization (see Chapter 21). If cAMP phosphodiesterase were inhibited, then cAMP levels would remain high even after the termination of the epinephrine signal, and glucose mobilization would continue.
11. If the two kinase domains are forced to be within close proximity of each other, the activation loop of one kinase, in its inactivating conformation, can be displaced by the activation loop of the neighboring kinase, which acts as a substrate for phosphorylation.
12. The full network of pathways initiated by insulin includes a large number of proteins and is substantially more elaborate than indicated in Figure 14.25. Furthermore, many additional proteins take part in the termination of insulin signaling. A defect in any of the proteins in the insulin signaling pathways or in the subsequent termination of the insulin response could potentially cause problems. Therefore, it is not surprising that many different gene defects can cause type 2 diabetes.
13. The binding of growth hormone causes its monomeric receptor to dimerize. The dimeric receptor can then activate a separate tyrosine kinase to which the receptor binds. The signaling pathway can then continue in similar fashion to the pathways that are activated by the insulin receptor or other mammalian EGF receptors.
14. The truncated receptor will dimerize with the full-length monomers on EGF-binding, but cross-phosphorylation cannot take place, because the truncated receptor possesses neither the substrate for the neighboring kinase domain nor its own kinase domain to phosphorylate the C-terminal tail of the other monomer. Hence, these mutant receptors will block normal EGF signaling.
15. Insulin would elicit the response that is normally caused by EGF. Insulin binding will likely stimulate dimerization and phosphorylation of the chimeric receptor and thereby signal the downstream events that are normally triggered by EGF binding. Exposure of these cells to EGF would have no effect.
16. 10^5
17. The formation of diacylglycerol implies the participation of phospholipase C. A simple pathway would entail receptor activation by cross-phosphorylation, followed by the binding of phospholipase Cγ (through its SH2 domains). The participation of phospholipase C indicates that IP_3 would be formed and, hence, calcium concentrations would increase.
18. Other potential drug targets within the EGF signaling cascade include, but are not limited to, the kinase active sites of the EGF receptor, Raf, MEK, or ERK.
19. In the reaction catalyzed by adenylate cyclase, the 3′-OH group nucleophilically attacks the α-phosphorus atom attached to the 5′-OH group, leading to displacement of pyrophosphate. The reaction catalyzed by DNA polymerase is similar except that the 3′-OH group is on a different nucleotide.
20. ATP-competitive inhibitors are likely to act on multiple kinases because every kinase domain contains an ATP-binding site. Hence, these drugs may not be selective for the desired kinase target.
21. (a) $X \approx 10^{-7}$ M; $Y \approx 5 \times 10^{-6}$ M; $Z \approx 10^{-3}$ M. (b) Because much less X is required to fill half of the sites, X displays the highest affinity. (c) The binding affinity almost perfectly matches the ability to stimulate adenylate cyclase, suggesting that the hormone–receptor complex leads to the stimulation of adenylate cyclase. (d) Try performing the experiment in the presence of antibodies to $G_{\alpha s}$.
22. (a) The total binding does not distinguish binding to a specific receptor from binding to different receptors or from nonspecific binding to the membrane.
(b) The rationale is that the receptor will have a high affinity for the ligand. Thus, in the presence of excess nonradioactive ligand, the receptor will bind to nonradioactive ligand. Therefore, any binding of the radioactive ligand must be nonspecific.
(c) The plateau suggests that the number of receptor-binding sites in the cell membrane is limited.
23. Number of receptors per cell =

$$\frac{10^4 \text{ cpm}}{\text{mg of membrane protein}} \times \frac{\text{mg of membrane protein}}{10^{10} \text{ cells}} \times \frac{\text{mmol}}{10^{12} \text{ cpm}} \times \frac{6.023 \times 10^{20} \text{ molecules}}{\text{mmol}} = 600$$

Chapter 15

1. The highly integrated biochemical reactions that take place inside the cell.
2. Anabolism is the set of biochemical reactions that use energy to build new molecules and ultimately new cells. Catabolism is the set of biochemical reactions that extract energy from fuel sources or breakdown biomolecules.
3. Cellular movements and the performance of mechanical work; active transport; biosynthetic reactions.
4. 1. f; 2. h; 3. i; 4. a; 5. g; 6. b; 7. c; 8. e; 9. j; 10. d.
5. Charge repulsion, resonance stabilization, and stabilization by hydration.
6. Trick question. The answer is not known. Adenine appears to form more readily under prebiotic conditions; so ATP may have predominated initially.

7. Having only one nucleotide represent the available energy allows the cell to better monitor its energy status.
8. Increasing the concentration of ATP or decreasing the concentration cellular ADP or P_i (by rapid removal by other reactions, for instance) would make the reaction more exergonic. Likewise, altering the Mg^{2+} concentration could raise or lower the ΔG of the reaction.
9. The free-energy changes of the individual steps in a pathway are summed to determine the overall free-energy change of the entire pathway. Consequently, a reaction with a positive free-energy value can be powered to take place if coupled to a sufficiently exergonic reaction.
10. Reactions in parts *a* and *c,* to the left; reactions in parts *b* and *d,* to the right.
11. None whatsoever
12. (a) $\Delta G^{\circ\prime} = 31.4$ kJ mol^{-1} (7.5 kcal mol^{-1}) and $K'_{eq} = 3.06 \times 10^{-6}$; (b) 3.28×10^4.
13. $\Delta G^{\circ\prime} = 7.1$ kJ mol^{-1} (1.7 kcal mol^{-1}). The equilibrium ratio is 17.8.
14. (a) Acetate + CoA + H^+ goes to acetyl CoA + H_2O, $\Delta G^{\circ\prime} = -31.4$ kJ mol^{-1} (-7.5 kcal mol^{-1}). ATP hydrolysis to AMP and PP_i, $\Delta G^{\circ\prime} = -45.6$ kJ mol^{-1} (-10.9 kcal mol^{-1}). Overall reaction, $\Delta G^{\circ\prime} = -14.2$ kJ mol^{-1} (-3.4 kcal mol^{-1}).
(b) With pyrophosphate hydrolysis, $\Delta G^{\circ\prime} = -33.4$ kJ mol^{-1} (-7.98 kcal mol^{-1}). Pyrophosphate hydrolysis makes the overall reaction even more exergonic.
15. (a) For an acid AH,

$$AH \rightleftharpoons A^{-1} + H^+ \qquad K = \frac{[A^-]\,[H^+]}{[AH]}$$

The pK is defined as $pK = -\log_{10} K$. $\Delta G^{\circ\prime}$ is the standard free-energy change at pH 7. Thus, $\Delta G^{\circ\prime} = -RT \ln K = -2.303\,RT \log_{10} K = +2.303\,RT\,pK$.
(b) $\Delta G^{\circ\prime} = 27.32$ kJ mol^{-1} (6.53 kcal mol^{-1}).
16. Arginine phosphate in invertebrate muscle, like creatine phosphate in vertebrate muscle, serves as a reservoir of high-potential phosphoryl groups. Arginine phosphate maintains a high level of ATP in muscular exertion.
17. An ADP unit
18. (a) The rationale behind creatine supplementation is that it would be converted into creatine phosphate and thus serve as a rapid means of replenishing ATP after muscle contraction. (b) If creatine supplementation is beneficial, it would affect activities that depend on short bursts of activity; any sustained activity would require ATP generation by fuel metabolism, which, as Figure 15.7 shows, requires more time.
19. Under standard conditions, $\Delta G^{\circ\prime} = -RT \ln$ [products]/[reactants]. Substituting 23.8 kJ mol^{-1} (5.7 kcal mol^{-1}) for $\Delta G^{\circ\prime}$ and solving for [products]/[reactants] yields 7×10^{-5}. In other words, the forward reaction does not take place to a significant extent. Under intracellular conditions, ΔG is -1.3 kJ mol^{-1} (-0.3 kcal mol^{-1}). Using the equation $\Delta G = \Delta G^{\circ\prime} + RT \ln$ [products]/[reactants] and solving for [products]/[reactants] gives a ratio of 3.7×10^{-5}. Thus, a reaction that is endergonic under standard conditions can be converted into an exergonic reaction by maintaining the [products]/[reactants] ratio below the equilibrium value. This conversion is usually attained by using the products in another coupled reaction as soon as they are formed.
20. Under standard conditions,

$$K'_{eq} = \frac{[B]_{eq}}{[A]_{eq}} \times \frac{[ADP]_{eq}\,[P_i]_{eq}}{[ATP]_{eq}} = 10^{3.3/1.36} = 2.67 \times 10^2$$

At equilibrium, the ratio of [B] to [A] is given by

$$\frac{[B]_{eq}}{[A]_{eq}} = K'_{eq} \frac{[ATP]_{eq}}{[ADP]_{eq}\,[P_i]_{eq}}$$

The ATP-generating system of cells maintains the [ATP]/[ADP] [P_i] ratio at a high level, typically about 500 M^{-1}. For this ratio,

$$\frac{[B]_{eq}}{[A]_{eq}} = 2.67 \times 10^2 \times 500 = 1.34 \times 10^5$$

This equilibrium ratio is strikingly different from the value of 1.15×10^{-3} for the reaction A $\longrightarrow$ B in the absence of ATP hydrolysis. In other words, coupling the hydrolysis of ATP with the conversion of A into B has changed the equilibrium ratio of B to A by a factor of about 10^8.
21. Liver: -45.2 kJ mol^{-1} (-10.8 kcal mol^{-1}); muscle: -48.1 kJ mol^{-1} (-11.5 kcal mol^{-1}); brain: -48.5 kJ mol^{-1} (-11.6 kcal mol^{-1}). The ΔG is most negative in brain cells.
22. (a) Ethanol; (b) lactate; (c) succinate; (d) isocitrate; (e) malate; (f) 2-phosphoglycerate.
23. Recall that $\Delta G = \Delta G^{\circ\prime} + RT \ln$ [products]/[reactants]. Altering the ratio of products to reactants will cause ΔG to vary. In glycolysis, the concentrations of the components of the pathway result in a value of ΔG greater than that of $\Delta G^{\circ\prime}$.
24. Unless the ingested food is converted into molecules capable of being absorbed by the intestine, no energy can ever be extracted by the body.
25. NADH and $FADH_2$ are electron carriers for catabolism; NADPH is the carrier for anabolism.
26. The electrons of the C–O bond cannot form resonance structures with the C–S bond that are as stable as those that they can form with the C–O bond. Thus, the thioester is not stabilized by resonance to the same degree as an oxygen ester is stabilized.
27. Oxidation–reduction reactions; ligation reactions; isomerization reactions; group-transfer reactions; hydrolytic reactions; the addition of functional groups to double bonds to form single bonds or the removal of functional groups to form double bonds.
28. Controlling the amount of enzymes; controlling enzyme activity; controlling the availability of substrates.
29. Although the reaction is thermodynamically favorable, the reactants are kinetically stable because of the large activation energy. Enzymes lower the activation energy so that reactions take place on time scales required by the cell.
30. The activated form of sulfate in most organisms is 3′-phosphoadenosine-5′-phosphosulfate.
31. (a) As the Mg^{2+} concentration falls, the ΔG of hydrolysis rises. Note that pMg is a logarithmic plot, and so each number on the *x*-axis represents a 10-fold change in [Mg^{2+}].
(b) Mg^{2+} would bind to the phosphates of ATP and help to mitigate charge repulsion. As the [Mg^{2+}] falls, charge stabilization of ATP would be less, leading to greater charge repulsion and an increase in ΔG on hydrolysis.

Chapter 16

1. Two molecules of ATP are produced per molecule of glyceraldehyde 3-phosphate and, because two molecules of GAP are produced per molecule of glucose, the total ATP yield is four. However, two molecules of ATP are required to convert glucose into fructose 1,6-bisphosphate. Thus, the net yield is only two molecules of ATP.

2. In both cases, the electron donor is glyceraldehyde 3-phosphate. In lactic acid fermentation, the electron acceptor is pyruvate, converting it into lactate. In alcoholic fermentation, acetaldehyde is the electron acceptor, forming ethanol.
3. (a) 3 ATP; (b) 2 ATP; (c) 2 ATP; (d) 2 ATP; (e) 4 ATP.
4. Glucokinase enables the liver to remove glucose from the blood when hexokinase is saturated, ensuring that glucose is captured for later use.
5. Glycolysis is a component of alcoholic fermentation, the pathway that produces alcohol for beer and wine. The belief was that understanding the biochemical basis of alcohol production might lead to a more-efficient means of producing beer.
6. The conversion of glyceraldehyde 3-phosphate into 1,3-bisphosphoglycerate would be impaired. Glycolysis would be less effective.
7. Glucose 6-phosphate must have other fates. Indeed, it can be converted into glycogen (Chapter 21) or be processed to yield reducing power for biosynthesis (Chapter 20).
8. The energy needs of a muscle cell vary widely, from rest to intense exercise. Consequently, the regulation of phosphofructokinase by energy charge is vital. In other tissues, such as the liver, ATP concentration is less likely to fluctuate and will not be a key regulator of phosphofructokinase.
9. The $\Delta G^{\circ\prime}$ for the reverse of glycolysis is $+96$ kJ mol^{-1} ($+23$ kcal mol^{-1}), far too endergonic to take place.
10. The conversion of glucose into glucose 6-phosphate by hexokinase; the conversion of fructose 6-phosphate into fructose 1,6-bisphosphate by phosphofructokinase; the formation of pyruvate from phosphoenolpyruvate by pyruvate kinase.
11. Lactic acid is a strong acid. If it remained in the cell, the pH of the cell would fall, which could lead to the denaturation of muscle protein and result in muscle damage.
12. GLUT2 transports glucose only when the blood concentration of glucose is high, which is precisely the condition in which the β cells of the pancreas secrete insulin.
13. Fructose + ATP ⟶ fructose 1-phosphate + ADP: Fructokinase

Fructose 1-phosphate ⟶ dihydroxyacetone phosphate + glyceraldehyde: Fructose 1-phosphate aldolase

Glyceraldehyde + ATP ⟶ glyceraldehyde 3-phosphate + ADP: Triose kinase

The primary controlling step of glycolysis catalyzed by phosphofructokinase is bypassed by the preceding reactions. Glycolysis will proceed in an unregulated fashion.
14. Without triose isomerase, only one of the two three-carbon molecules generated by aldolase could be used to generate ATP. Only two molecules of ATP would result from the metabolism of each glucose. But two molecules of ATP would still be required to form fructose 1,6-bisphosphate, the substrate for aldolase. The net yield of ATP would be zero, a yield incompatible with life.
15. Glucose is reactive because its open-chain form contains an aldehyde group.
16. (a) The label is in the methyl carbon atom of pyruvate.
(b) 5 mCi mM^{-1}. The specific activity is halved because the number of moles of product (pyruvate) is twice that of the labeled substrate (glucose).
17. (a) Glucose + 2 P_i + 2 ADP ⟶ 2 lactate + 2 ATP.
(b) $\Delta G = -114$ kJ mol^{-1} (-27.2 kcal mol^{-1}).
18. 3.06×10^{-5}
19. The equilibrium concentrations of fructose 1,6-bisphosphate, dihydroxyacetone phosphate, and glyceraldehyde 3-phosphate are 7.8×10^{-4} M, 2.2×10^{-4} M, and 2.2×10^{-4} M, respectively.
20. All three carbon atoms of 2,3-BPG are ^{14}C labeled. The phosphorus atom attached to the C-2 hydroxyl group is ^{32}P labeled.
21. Hexokinase has a low ATPase activity in the absence of a sugar because it is in a catalytically inactive conformation. The addition of xylose closes the cleft between the two lobes of the enzyme. However, xylose lacks a hydroxymethyl group, and so it cannot be phosphorylated. Instead, a water molecule at the site normally occupied by the C-6 hydroxymethyl group acts as the acceptor of the phosphoryl group from ATP.
22. (a) The fructose 1-phosphate pathway forms glyceraldehyde 3-phosphate.
(b) Phosphofructokinase, a key control enzyme, is bypassed. Furthermore, fructose 1-phosphate stimulates pyruvate kinase.
23. The reverse of glycolysis is highly endergonic under cellular conditions. The expenditure of six NTP molecules in gluconeogenesis renders gluconeogenesis exergonic.
24. Lactic acid is capable of being further oxidized and is thus useful energy. The conversion of this acid into glucose saves the carbon atoms for future combustion.
25. In glycolysis, the formation of pyruvate and ATP by pyruvate kinase is irreversible. This step is bypassed by two reactions in gluconeogenesis: (1) the formation of oxaloacetate from pyruvate and CO_2 by pyruvate carboxylase and (2) the formation of phosphoenolpyruvate from oxaloacetate and GTP by phosphoenolpyruvate carboxykinase. The formation of fructose 1,6-bisphosphate by phosphofructokinase is bypassed by fructose 1,6-bisphosphatase in gluconeogenesis, which catalyzes the conversion of fructose 1,6-bisphosphate into fructose 6-phosphate. Finally, the hexokinase-catalyzed formation of glucose 6-phosphate in glycolysis is bypassed by glucose 6-phosphatase, but only in the liver.
26. Reciprocal regulation at the key allosteric enzymes in the two pathways. For instance, PFK is stimulated by fructose 2,6-bisphosphate and AMP. The effect of these signals is opposite that of fructose 1,6-bisphosphatase. If both pathways were operating simultaneously, a futile cycle would result. ATP would be hydrolyzed, yielding only heat.
27. Muscle is likely to produce lactic acid during contraction. Lactic acid is a strong acid and cannot accumulate in muscle or blood. Liver removes the lactic acid from the blood and converts it into glucose. The glucose can be released into the blood or stored as glycogen for later use.
28. Glucose produced by the liver could not be released into the blood. Tissues that rely on glucose as an energy source would not function as well unless glucose was provided in the diet.
29. Glucose is an important energy source for both tissues and is essentially the only energy source for the brain. Consequently, these tissues should never release glucose. Glucose release is prevented by the absence of glucose 6-phosphatase.
30. 6 NTP (4 ATP and 2 GTP); 2 NADH.
31. (a) None; (b) none; (c) 4 (2 ATP and 2 GTP); (d) none.
32. If the amino groups are removed from alanine and aspartate, the ketoacids pyruvate and oxaloacetate are formed. Both of these molecules are components of the gluconeogenic pathway.
33. (a) Increased; (b) increased; (c) increased; (d) decreased.
34. Fructose 2,6-bisphosphate, present at high concentration when glucose is abundant, normally inhibits gluconeogenesis by blocking fructose 1,6-bisphosphatase. In this genetic disorder, the phosphatase is active irrespective of the glucose level. Hence, substrate cycling is increased. The level of fructose 1,6-bisphosphate is

consequently lower than normal. Less pyruvate is formed and thus less ATP is generated.
35. Reactions in parts *b* and *e* would be blocked.
36. There will be no labeled carbons. The CO_2 added to pyruvate (formed from the lactate) to form oxaloacetate is lost with the conversion of oxaloacetate into phosphoenolpyruvate.
37. The net reaction in the presence of arsenate is

$$\text{Glyceraldehyde 3-phosphate} + NAD^+ + H_2O \longrightarrow \text{3-phosphoglycerate} + NADH + 2\,H^+$$

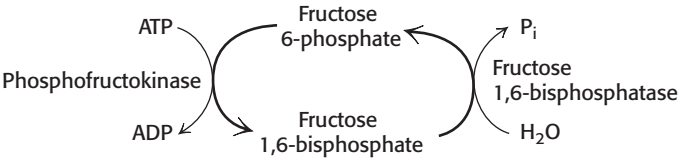
Glycolysis proceeds in the presence of arsenate, but the ATP normally formed in the conversion of 1,3-bisphosphoglycerate into 3-phosphoglycerate is lost. Thus, arsenate uncouples oxidation and phosphorylation by forming a highly labile acyl arsenate.
38. This example illustrates the difference between the *stoichiometric* and the *catalytic* use of a molecule. If cells used NAD^+ stoichiometrically, a new molecule of NAD^+ would be required each time a molecule of lactate was produced. As we will see, the synthesis of NAD^+ requires ATP. On the other hand, if the NAD^+ that is converted into NADH could be recycled and reused, a small amount of the molecule could regenerate a vast amount of lactate, which is the case in the cell. NAD^+ is regenerated by the oxidation of NADH and reused. NAD^+ is thus used catalytically.
39. Consider the equilibrium equation of adenylate kinase:

$$K_{eq} = [ATP]\,[AMP]/[ADP]^2 \quad (1)$$

or

$$AMP = K_{eq}\,[ADP_2]/[ATP] \quad (2)$$

Recall that [ATP] > [ADP] > [AMP] in the cell. As ATP is utilized, a small decrease in its concentration will result in a larger percentage increase in [ADP] because its concentration is greater than that of ADP. This larger percentage increase in [ADP] will result in an even greater percentage increase in [AMP] because the concentration of AMP is related to the square of [ADP]. In essence, equation 2 shows that monitoring the energy status with AMP magnifies small changes in [ATP], leading to tighter control.
40. The synthesis of glucose during intense exercise provides a good example of interorgan cooperation in higher organisms. When muscle is actively contracting, lactate is produced from glucose by glycolysis. The lactate is released into the blood and absorbed by the liver, where it is converted by gluconeogenesis into glucose. The newly synthesized glucose is then released and taken up by the muscle for energy generation.
41. The input of four additional high-phosphoryl-transfer-potential molecules in gluconeogenesis changes the equilibrium constant by a factor of 10^{32}, which makes the conversion of pyruvate into glucose thermodynamically feasible. Without this energetic input, gluconeogenesis would not take place.
42. The mechanism is analogous to that for triose phosphate isomerase (Figure 16.5). It proceeds through an enediol intermediate. The active site would be expected to have a general base (analogous to Glu 165 in TPI) and a general acid (analogous to His 95 in TPI).
43. Galactose is a component of glycoproteins. Possibly, the absence of galactose leads to the improper formation or function of glycoproteins required in the central nervous system. More generally, the fact that the symptoms arise in the absence of galactose suggests that galactose is required in some fashion.
44. Fructose 2,6-bisphosphate stabilizes the R state of the enzyme.
45. (a) Curiously, the enzyme uses ADP as the phosphoryl donor rather than ATP.
(b) Both AMP and ATP behave as competitive inhibitors of ADP, the phosphoryl donor. Apparently, the *P. furiosus* enzyme is not allosterically inhibited by ATP.
46. (a) If both enzymes operated simultaneously, the following reactions would take place:



The net result would be simply:

$$ATP + H_2O \longrightarrow ADP + P_i$$

The energy of ATP hydrolysis would be released as heat.
(b) Not really. For the cycle to generate heat, both enzymes must be functional at the same time in the same cell.
(c) The species *B. terrestris* and *B. rufocinctus* might show some futile cycling because both enzymes are active to a substantial degree.
(d) No. These results simply suggest that simultaneous activity of phosphofructokinase and fructose 1,6-bisphosphatase is unlikely to be employed to generate heat in the species shown.

Chapter 17

1. Pyruvate dehydrogenase catalyzes the decarboxylation of pyruvate and the formation of acetyllipoamide. Dihydrolipoyl transacetylase catalyzes the formation of acetyl CoA. Dihydrolipoyl dehydrogenase catalyzes the reduction of the oxidized lipoic acid. The kinase associated with the complex phosphorylates and inactivates the complex, whereas the phosphatase dephosphorylates and activates the complex.
2. Thiamine pyrophosphate plays a role in the decarboxylation of pyruvate. Lipoic acid (as lipoamide) transfers the acetyl group. Coenzyme A accepts the acetyl group from lipoic acid to form acetyl CoA. FAD accepts the electrons and hydrogen ions when reduced lipoic acid is oxidized. NAD^+ accepts electrons from $FADH_2$.
3. Catalytic coenzymes (TPP, lipoic acid, and FAD) are modified but regenerated in each reaction cycle. Thus, they can play a role in the processing of many molecules of pyruvate. Stoichiometric coenzymes (coenzyme A and NAD^+) are used in only one reaction because they are the components of products of the reaction.
4. The advantages are as follows:
   The reaction is facilitated by having the active sites in proximity.
   The reactants do not leave the enzyme until the final product is formed.
   Constraining the reactants minimizes loss due to diffusion and minimizes side reactions.
   All of the enzymes are present in the correct amounts.
   Regulation is more efficient because the regulatory enzymes—the kinase and phosphatase—are part of the complex.
5. (a) After one round of the citric acid cycle, the label emerges in C-2 and C-3 of oxaloacetate. (b) The label emerges in CO_2 in the formation of acetyl CoA from pyruvate. (c) After one round of the citric acid cycle, the label emerges in C-1 and C-4 of oxaloacetate. (d) and (e) Same fate as that in part *a*.

6. (a) Isocitrate lyase and malate synthase are required in addition to the enzymes of the citric acid cycle.
(b) 2 Acetyl CoA + 2 NAD^+ + FAD + 3 H_2O ⟶ oxaloacetate + 2 CoA + 2 NADH + $FADH_2$ + 3 H^+.
(c) No. Hence, mammals cannot carry out the net synthesis of oxaloacetate from acetyl CoA.
7. -41.0 kJ mol^{-1} (-9.8 kcal mol^{-1})
8. Enzymes or enzyme complexes are biological catalysts. Recall that a catalyst facilitates a chemical reaction without the catalyst itself being permanently altered. Oxaloacetate can be thought of as a catalyst because it binds to an acetyl group, leads to the oxidative decarboxylation of the two carbon atoms, and is regenerated at the completion of a cycle. In essence, oxaloacetate (and any cycle intermediate) acts as a catalyst.
9. Thiamine thiazolone pyrophosphate is a transition-state analog. The sulfur-containing ring of this analog is uncharged, and so it closely resembles the transition state of the normal coenzyme in thiamine-catalyzed reactions (e.g., the uncharged resonance form of hydroxyethyl-TPP).
10. A decrease in the amount of O_2 will necessitate an increase in anaerobic glycolysis for energy production, leading to the generation of a large amount of lactic acid. Under conditions of shock, the kinase inhibitor is administered to ensure that pyruvate dehydrogenase is operating maximally.
11. Acetyllipoamide and acetyl CoA
12. In muscle, the acetyl CoA generated by the complex is used for energy generation. Consequently, signals that indicate an energy-rich state (high ratios of ATP/ADP and $NADH/NAD^+$) inhibit the complex, whereas the reverse conditions stimulate the enzyme. Calcium as the signal for muscle contraction (and, hence, energy need) also stimulates the enzyme. In liver, acetyl CoA derived from pyruvate is used for biosynthetic purposes, such as fatty acid synthesis. Insulin, the hormone denoting the fed state, stimulates the complex.
13. (a) Enhanced kinase activity will result in a decrease in the activity of the PDH complex because phosphorylation by the kinase inhibits the complex.
(b) Phosphatase activates the complex by removing a phosphate. If the phosphatase activity is diminished, the activity of the PDH complex also will decrease.
14. She might have been ingesting, in some fashion, the arsenite from the peeling paint or the wallpaper. Also, she might have been breathing arsine gas from the wallpaper, which would be oxidized to arsenite in her body. In any of these circumstances, the arsenite inhibited enzymes that require lipoic acid—notably, the PDH complex.
15. The TCA cycle depends on a steady supply of NAD^+, which is typically generated from NADH by reaction of the NADH with oxygen. If there is no oxygen to accept the electrons, the citric acid cycle will cease to operate.
16. (a) The steady-state concentrations of the products are low compared with those of the substrates. (b) The ratio of malate to oxaloacetate must be greater than 1.57×10^4 for oxaloacetate to be formed.
17.

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \xrightarrow[\text{complex}]{\text{Pyruvate dehydrogenase}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH}$$

$$\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \xrightarrow[\text{carboxylase}]{\text{Pyruvate}} \text{oxaloacetate} + \text{ADP} + \text{P}_i + \text{H}^+$$

$$\text{Oxaloacetate} + \text{acetyl CoA} + \text{H}_2\text{O} \xrightarrow[\text{synthase}]{\text{Citrate}} \text{citrate} + \text{CoA} + \text{H}^+$$

$$\text{Citrate} \xrightarrow{\text{Aconitase}} \text{isocitrate}$$

$$\text{Isocitrate} + \text{NAD}^+ \xrightarrow[\text{dehydrogenase}]{\text{Isocitrate}} \alpha\text{-ketoglutarate} + \text{CO}_2 + \text{NADH}$$

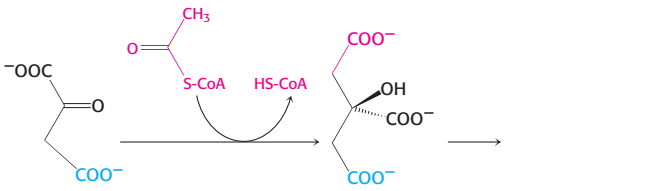
Net: 2 Pyruvate + 2 NAD^+ + ATP + H_2O ⟶
α-ketoglutarate + CO_2 + ADP + P_i + 2 NADH + 3 H^+

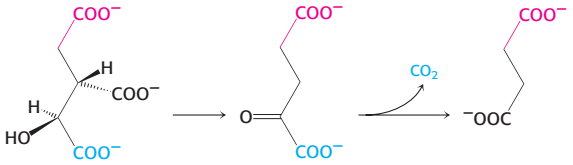
18. Succinate will increase in concentration, followed by α-ketoglutarate and the other intermediates "upstream" of the site of inhibition. Succinate has two methylene groups that are required for the dehydrogenation, whereas malonate has but one.
19. Pyruvate carboxylase should be active only when the acetyl CoA concentration is high. Acetyl CoA might accumulate if the energy needs of the cell are not being met, because of a deficiency of oxaloacetate. Under these conditions the pyruvate carboxylase catalyzes an anapleurotic reaction. Alternatively, acetyl CoA might accumulate because the energy needs of the cell have been met. In this circumstance, pyruvate will be converted back into glucose, and the first step in this conversion is the formation of oxaloacetate.
20. The energy released when succinate is reduced to fumarate is not sufficient to power the synthesis of NADH but is sufficient to reduce FAD.
21. Citrate is a tertiary alcohol that cannot be oxidized, because oxidation requires a hydrogen atom to be removed from the alcohol and a hydrogen atom to be removed from the carbon atom bonded to the alcohol. No such hydrogen exists in citrate. The isomerization converts the tertiary alcohol into isocitrate, which is a secondary alcohol that can be oxidized.
22. Because the enzyme nucleoside diphosphokinase transfers a phosphoryl group from GTP (or any nucleoside triphosphate) to ADP according to the reversible reaction:

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP}$$

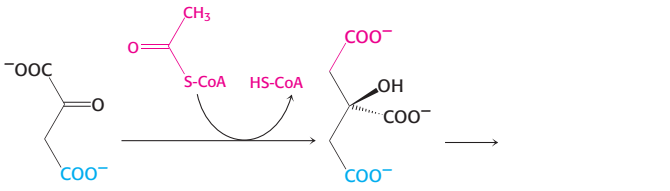
23. The reaction is powered by the hydrolysis of a thioester. Acetyl CoA provides the thioester that is converted into citryl CoA. When this thioester is hydrolyzed, citrate is formed in an irreversible reaction.
24. We cannot get the net conversion of fats into glucose, because the only means to get the carbon atoms from fats into oxaloacetate, the precursor of glucose, is through the citric acid cycle. However, although two carbon atoms enter the cycle as acetyl CoA, two carbon atoms are lost as CO_2 before oxaloacetate is formed. Thus, although some carbon atoms from fats may end up as carbon atoms in glucose, we cannot obtain a *net* synthesis of glucose from fats.
25. Acetyl CoA will inhibit the complex. Glucose metabolism to pyruvate will be slowed because acetyl CoA is being derived from an alternative source.
26. The enol intermediate of acetyl CoA attacks the carbonyl carbon atom of glyoxylate to form a C–C bond. This reaction is like the condensation of oxaloacetate with the enol intermediate of acetyl CoA in the reaction catalyzed by citrate synthase. Glyoxylate contains a hydrogen atom in place of the $-CH_2COO^-$ group of oxaloacetate; the reactions are otherwise nearly identical.
27. Citrate is a symmetric molecule. Consequently, the investigators assumed that the two $-CH_2COO^-$ groups in it would react identically. Thus, for every citrate molecule undergoing the reactions shown in path 1, they thought that another citrate

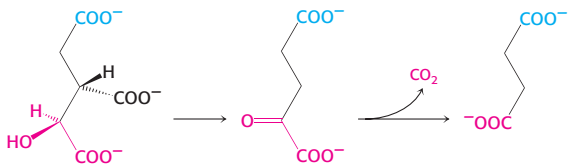
molecule would react as shown in path 2. If so, then only *half* the label should have emerged in the CO_2.





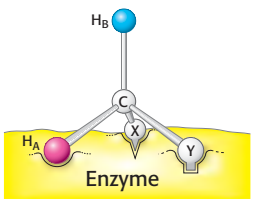
Path 1





Path 2 (does not occur)

28. Call one hydrogen atom A and the other B. Now suppose that an enzyme binds three groups of this substrate—X, Y, and H—at three complementary sites. The adjoining diagram shows X, Y, and H_A bound to three points on the enzyme. In contrast, X, Y, and H_B cannot be bound to this active site; two of these three groups can be bound, but not all three. Thus, H_A and H_B will have different fates.



Sterically nonequivalent groups such as H_A and H_B will almost always be distinguished in enzymatic reactions. The essence of the differentiation of these groups is that the enzyme holds the substrate in a specific orientation. Attachment at three points, as depicted in the diagram, is a readily visualized way of achieving a particular orientation of the substrate, but it is not the only means of doing so.

29. (a) The complete oxidation of citrate requires 4.5 μmol of O_2 for every micromole of citrate.

$$C_6H_8O_7 + 4.5\ O_2 \longrightarrow 6\ CO_2 + 4\ H_2O$$

Thus, 13.5 μmol of O_2 would be consumed by 3 μmol of citrate.
(b) Citrate led to the consumption of far more O_2 than can be accounted for simply by the oxidation of citrate itself. Citrate thus facilitated O_2 consumption.

30. (a) In the absence of arsenite, the amount of citrate remained constant. In its presence, the concentration of citrate fell, suggesting that it was being metabolized.
(b) The action of arsenite is not altered. Citrate still disappears.
(c) Arsenite is preventing the regeneration of citrate. Recall (pp. 517–518) that arsenite inhibits the pyruvate dehydrogenase complex.

31. (a) The initial infection is unaffected by the absence of isocitrate lyase, but the absence of this enzyme inhibits the latent phase of the infection.
(b) Yes
(c) A critic could say that, in the process of deleting the isocitrate lyase gene, some other gene was damaged, and it is the absence of this other gene that prevents latent infection. Reinserting the isocitrate lyase gene into the bacteria from which it had been removed renders the criticism less valid.
(d) Isocitrate lyase enables the bacteria to synthesize carbohydrates that are necessary for survival, including carbohydrate components of the cell membrane.

Chapter 18

1. In fermentations, organic compounds are both the donors and the acceptors of electrons. In respiration, the electron donor is usually an organic compound, whereas the electron acceptor is an inorganic molecule, such as oxygen.

2. Biochemists use E'_0, the value at pH 7, whereas chemists use E_0, the value in 1 M H^+. The prime denotes that pH 7 is the standard state.

3. The reduction potential of $FADH_2$ is less than that of NADH (see Table 18.1). Consequently, when those electrons are passed along to oxygen, less energy is released. The consequence of the difference is that electron flow from $FADH_2$ to O_2 pumps fewer protons than do the electrons from NADH.

4. The $\Delta G^{\circ\prime}$ for the reduction of oxygen by $FADH_2$ is -200 kJ mol^{-1} (-48 kcal mol^{-1}).

5. $\Delta G^{\circ\prime}$ is + 67 kJ mol^{-1} (+16.1 kcal mol^{-1}) for oxidation by NAD^+ and -3.8 kJ mol^{-1} (-0.92 kcal mol^{-1}) for oxidation by FAD. The oxidation of succinate by NAD^+ is not thermodynamically feasible.

6. Pyruvate accepts electrons and is thus the oxidant. NADH gives up electrons and is the reductant.

7. $\Delta G^{\circ\prime} = -nF\Delta E'_0$

8. The $\Delta E'_0$ value of iron can be altered by changing the environment of the ion.

9. c, e, b, a, d.

10. (a) 4; (b) 3; (c) 1; (d) 5; (e) 2.

11. The 10 isoprene units render coenzyme Q soluble in the hydrophobic environment of the inner mitochondrial membrane. The two oxygen atoms can reversibly bind two electrons and two protons as the molecule transitions between the quinone form and quinol form.

12. Rotenone: NADH, NADH-Q oxidoreductase will be reduced. The remainder will be oxidized. Antimycin A: NADH, NADH-Q oxidoreductase and coenzyme Q will be reduced. The remainder will be oxidized. Cyanide: All will be reduced.

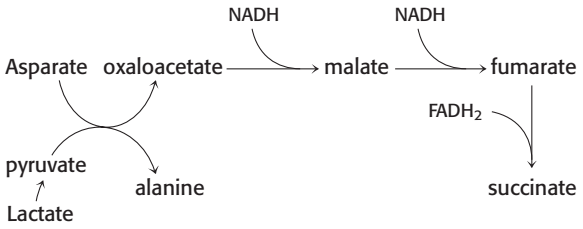
13. Complex I would be reduced, whereas Complexes II, III, and IV would be oxidized. The citric acid cycle would become reduced because it has no way to oxidize NADH.

14. The respirasome is another example of the use of supramolecular complexes in biochemistry. Having the three complexes that are proton pumps associated with one another will enhance the efficiency of electron flow from complex to complex, which in turn will cause more-efficient proton pumping.

15. Hydroxyl radical ($OH\cdot$), hydrogen peroxide (H_2O_2), superoxide ion ($O_2^{\cdot-}$), and peroxide (O_2^{2-}). These small molecules react

with a host of macromolecules—including proteins, nucleotides, and membranes—to disrupt cell structure and function.
16. The ATP is recycled by ATP-generating processes, most notably oxidative phosphorylation.
17. (a) 12.5; (b) 14; (c) 32; (d) 13.5; (e) 30; (f) 16.
18. (a) It blocks electron transport and proton pumping at Complex IV. (b) It blocks electron transport and ATP synthesis by inhibiting the exchange of ATP and ADP across the inner mitochondrial membrane. (c) It blocks electron transport and proton pumping at Complex I. (d) It blocks ATP synthesis without inhibiting electron transport by dissipating the proton gradient. (e) It blocks electron transport and proton pumping at Complex IV. (f) It blocks electron transport and proton pumping at Complex III.
19. If the proton gradient is not dissipated by the influx of protons into a mitochondrion with the generation of ATP, eventually the outside of the mitochondrion develops such a large positive charge that the electron-transport chain can no longer pump protons against the gradient.
20. The subunits are jostled by background thermal energy (Brownian motion). The proton gradient makes clockwise rotation more likely because that direction results in protons flowing down their concentration gradient.
21. In the presence of poorly functioning mitochondria, the only means of generating ATP is by anaerobic glycolysis, which will lead to an accumulation of lactic acid in blood.
22. If ADP cannot get into mitochondria, the electron-transport chain will cease to function because there will be no acceptor for the energy. NADH will build up in the matrix. Recall that NADH inhibits some citric acid cycle enzymes and that NAD^+ is required by several citric acid cycle enzymes. Glycolysis will stop functioning aerobically but will switch to anaerobic glycolysis so that the NADH can be reoxidized to NAD^+ by lactate dehydrogenase.
23. (a) No effect; mitochondria cannot metabolize glucose.
(b) No effect; no fuel is present to power the synthesis of ATP.
(c) The $[O_2]$ falls because citrate is a fuel and ATP can be formed from ADP and P_i.
(d) Oxygen consumption stops because oligomycin inhibits ATP synthesis, which is coupled to the activity of the electron-transport chain.
(e) No effect, for the reasons given in part *d*.
(f) $[O_2]$ falls rapidly because the system is uncoupled and does not require ATP synthesis to lower the proton-motive force.
(g) $[O_2]$ falls, though at a lower rate. Rotenone inhibits Complex I, but the presence of succinate will enable electrons to enter at Complex II.
(h) Oxygen consumption ceases because Complex IV is inhibited and the entire chain backs up.
24. (a) The P : O ratio is equal to the product of $(H^+/2\ e^-)$ and (P/H^+). Note that the P : O ratio is identical with the P : $2\ e^-$ ratio.
(b) 2.5 and 1.5, respectively.
25. Cyanide can be lethal because it binds to the ferric form of cytochrome oxidase and thereby inhibits oxidative phosphorylation. Nitrite converts ferrohemoglobin into ferrihemoglobin, which also binds cyanide. Thus, ferrihemoglobin competes with cytochrome oxidase for cyanide. This competition is therapeutically effective because the amount of ferrihemoglobin that can be formed without impairing oxygen transport is much greater than the amount of cytochrome oxidase.
26. Such a defect (called Luft syndrome) was found in a 38-year-old woman who was incapable of performing prolonged physical work. Her basal metabolic rate was more than twice normal, but her thyroid function was normal. A muscle biopsy showed that her mitochondria were highly variable and atypical in structure. Biochemical studies then revealed that oxidation and phosphorylation were not tightly coupled in these mitochondria. In this patient, much of the energy of fuel molecules was converted into heat rather than ATP.
27. Triose phosphate isomerase converts dihydroxyacetone phosphate (a potential dead end) into glyceraldehyde 3-phosphate (a mainstream glycolytic intermediate).
28. This inhibitor (like antimycin A) blocks the reduction of cytochrome c_1 by QH_2, the crossover point.
29. If oxidative phosphorylation were uncoupled, no ATP could be produced. In a futile attempt to generate ATP, much fuel would be consumed. The danger lies in the dose. Too much uncoupling would lead to tissue damage in highly aerobic organs such as the brain and heart, which would have severe consequences for the organism as a whole. The energy that is normally transformed into ATP would be released as heat. To maintain body temperature, sweating might increase, although the very process of sweating itself depends on ATP.
30. If ATP and ADP cannot exchange between the matrix and the mitochondria, ATP synthase will cease to function because its substrate ADP is absent. The proton gradient will eventually become so large that the energy released by the electron-transport chain will not be great enough to pump protons against the larger-than-normal gradient.
31. Add the inhibitor with and without an uncoupler, and monitor the rate of O_2 consumption. If the O_2 consumption increases again in the presence of inhibitor and uncoupler, the inhibitor must be inhibiting ATP synthase. If the uncoupler has no effect on the inhibition, the inhibitor is inhibiting the electron-transport chain.
32. Presumably, because the muscle has greater energy needs, especially during exercise, it will require more ATP. This requirement means that more sites of oxidative phosphorylation are called for, and these sites can be provided by an increase in the amount of cristae.
33. The arginine residue, with its positive charge, will facilitate proton release from aspartic acid by stabilizing the negatively charged aspartate.
34. 4; 4.7
35. The ATP synthase would pump protons at the expense of ATP hydrolysis, thus maintaining the proton-motive force. The synthase would function as an ATPase. There is some evidence that damaged mitochondria use this tactic to maintain, at least temporarily, the proton-motive force.
36. It suggests that malfunctioning mitochondria may play a role in the development of Parkinson disease. Specifically, it implicates Complex I.
37. The extra negative charge on ATP relative to that on ADP accounts for ATP's more-rapid translocation out of the mitochondrial matrix. If the charge differences between ATP and ADP were lessened by the binding of Mg^{2+}, ADP might more readily compete with ATP for transport to the cytoplasm.
38. When all of the available ADP has been converted into ATP, ATP synthase can no longer function. The proton gradient becomes large enough that the energy of the electron-transport chain is not enough to pump against the gradient, and electron transport and, hence, oxygen consumption falls.
39. The effect on the proton gradient is the same in each case.
40. ATP export from the matrix. Phosphate import into the matrix.
41. Recall from the discussion of enzyme-catalyzed reactions that the direction of a reaction is determined by the ΔG difference between substrate and products. An enzyme speeds up the rate of both the forward and the backward reactions. The hydrolysis of ATP is exergonic, and so ATP synthase will enhance the hydrolytic reaction.

42. The cytoplasmic kinases thereby obtaining preferential access to the exported ATP.
43. The organic acids in the blood are indications that the mice are deriving a large part of their energy needs through anaerobic glycolysis. Lactate is the end product of anaerobic glycolysis. Alanine is an aminated transport form of lactate. Alanine formation plays a role in succinate formation, which is caused by the reduced state of the mitochondria.



The electron-transport chain is slowed because the inner mitochondrial membrane is hyperpolarized. Without ADP to accept the energy of the proton-motive force, the membrane becomes polarized to such an extent that protons can no longer be pumped. The excess H_2O_2 is probably due to the fact that the superoxide radical is present in higher concentration because the oxygen can no longer be effectively reduced.

$$O_2 \cdot^- + O_2 \cdot^- + 2\,H^+ \longrightarrow O_2 + H_2O_2$$

Indeed, these mice display evidence of such oxidative damage.
44. (a) Vitamins C and E.
(b) Exercise induces superoxide dismutase, which converts ROS in hydrogen peroxide and oxygen.
(c) The answer to this question is not fully established. Two possibilities are (1) the suppression of ROS by vitamins prevents the expression of more superoxide dismutase and (2) some ROS may be signal molecules required to stimulate insulin-sensitivity pathways.
45. (a) Succinate is oxidized by Complex II, and the electrons are used to establish a proton-motive force that powers ATP synthesis.
(b) The ability to synthesize ATP is greatly reduced.
(c) Because the goal was to measure ATP hydrolysis. If succinate had been added in the presence of ATP, no reaction would have taken place, because of respiratory control.
(d) The mutation has little effect on the ability of the enzyme to catalyze the hydrolysis of ATP.
(e) They suggest two things: (1) the mutation did not affect the catalytic site on the enzyme, because ATP synthase is still capable of catalyzing the reverse reaction, and (2) the mutation did not affect the amount of enzyme present, given that the controls and patients had similar amounts of activity.
46. The absolute configuration of thiophosphate indicates that inversion at phosphorus has taken place in the reaction catalyzed by ATP synthase. This result is consistent with an in-line phosphoryl-transfer reaction taking place in a single step. The retention of configuration in the Ca^{2+}-ATPase reaction points to two phosphoryl-transfer reactions—inversion by the first and a return to the starting configuration by the second. The Ca^{2+}-ATPase reaction proceeds by a phosphorylated enzyme intermediate.

Chapter 19

1. Photosystem I generates ferredoxin, which reduces $NADP^+$ to NADPH, a biosynthetic reducing power. Photosystem II activates the manganese complex, an oxidant capable of oxidizing water, generating electrons for photosynthesis, and generating protons to form a proton gradient and to reduce $NADP^+$ and O_2.
2. The light reactions take place on thylakoid membranes. Increasing the membrane surface increases the number of ATP- and NADH-generating sites.
3. These complexes absorb more light than can a reaction center alone. The light-harvesting complexes funnel light to the reaction centers.
4. $NADP^+$ is the acceptor. H_2O is the donor. Light energy.
5. The charge gradient, a component of the proton-motive force in mitochondria, is neutralized by the influx of Mg^{2+} into the lumen of the thylakoid membranes.
6. Chlorophyll is readily inserted into the hydrophobic interior of the thylakoid membranes.
7. Protons released by the oxidation of water; protons pumped into the lumen by the cytochrome *bf* complex; protons removed from the stroma by the reduction of $NADP^+$ and plastoquinone.
8. 700-nm photons have an energy content of 172 kJ mol^{-1}. The absorption of light by photosystem I results in a $\Delta E_0'$ of -1.0 V. Recall that $\Delta G_0' = -nF\,\Delta E_0'$, where $F = 96.48$ kJ $mol^{-1}\,V^{-1}$. Under standard conditions, the energy change for the electrons is 96.5 kJ. Thus, the efficiency is $96.5/172 = 56\%$.
9. The electron flow from PS II to PS I is uphill, or exergonic. For this uphill flow, ATP would need to be consumed, defeating the purpose of photosynthesis.
10. $\Delta E_0' = 10.11$ V, and $\Delta G^{\circ\prime} = -21.3$ kJ mol^{-1} (-5.1 kcal mol^{-1}).
11. (a) All ecosystems require an energy source from outside the system, because the chemical-energy sources will ultimately be limited. The photosynthetic conversion of sunlight is one example of such a conversion.
(b) Not at all. Spock would point out that chemicals other than water can donate electrons and protons.
12. DCMU inhibits electron transfer in the link between photosystems II and I. O_2 can evolve in the presence of DCMU if an artificial electron acceptor such as ferricyanide can accept electrons from Q.
13. DCMU will have no effect, because it blocks photosystem II, and cyclic photophosphorylation uses photosystem I and the cytochrome *bf* complex.
14. (a) 120 kJ $einstein^{-1}$ (28.7 kcal $einstein^{-1}$)
(b) 1.24 V
(c) One 1000-nm photon has the free energy content of 2.4 molecules of ATP. A minimum of 0.42 photon is needed to drive the synthesis of a molecule of ATP.
15. At this distance, the expected rate is one electron per second.
16. The distance doubles, and so the rate should decrease by a factor of 64 to 640 ps.
17. The cristae.
18. In eukaryotes, both processes take place in specialized organelles. Both depend on high-energy electrons to generate ATP. In oxidative phosphorylation, the high-energy electrons originate in fuels and are extracted as reducing power in the form of NADH. In photosynthesis, the high-energy electrons are generated by light and are captured as reducing power in the form of NADPH. Both processes use redox reactions to generate a proton gradient, and the enzymes that convert the proton gradient into ATP are very similar in both processes. In both systems, electron transport takes place in membranes inside organelles.
19. We need to factor in the NADPH because it is an energy-rich molecule. Recall from Chapter 18, that NADH is worth 2.5 ATP if oxidized by the electron-transport chain. 12 NADPH = 30 ATP. Eighteen molecules of ATP are used directly, and so the equivalent of 48 molecules of ATP is required for the synthesis of glucose.
20. Both photosynthesis and cellular respiration are powered by high-energy electrons flowing toward a more-stable state. In cellular respiration, the high-energy electrons are derived from the

oxidation of carbon fuels as NADH and $FADH_2$. They release their energy as they reduce oxygen. In photosynthesis, high-energy electrons are generated by absorbing light energy, and they find stability in photosystem I and ferridoxin.
21. The electrons flow through photosystem II directly to ferricyanide. No other steps are required.
22. (a) Thioredoxin
(b) The control enzyme is unaffected, but the mitochondrial enzyme with part of the chloroplast γ subunit increases activity as the concentration of DTT increases.
(c) The increase was even larger when thioredoxin was present. Thioredoxin is the natural reductant for the chloroplast enzyme, and so it presumably operates more efficiently than would DTT, which probably functions to keep the thioredoxin reduced.
(d) They seem to have done so.
(e) The enzyme is susceptible to control by the redox state. In plant cells, reduced thioredoxin is generated by photosystem I. Thus, the enzyme is active when photosynthesis is taking place.
(f) Cysteine
(g) Group-specific modification or site-specific mutagenesis.

Chapter 20

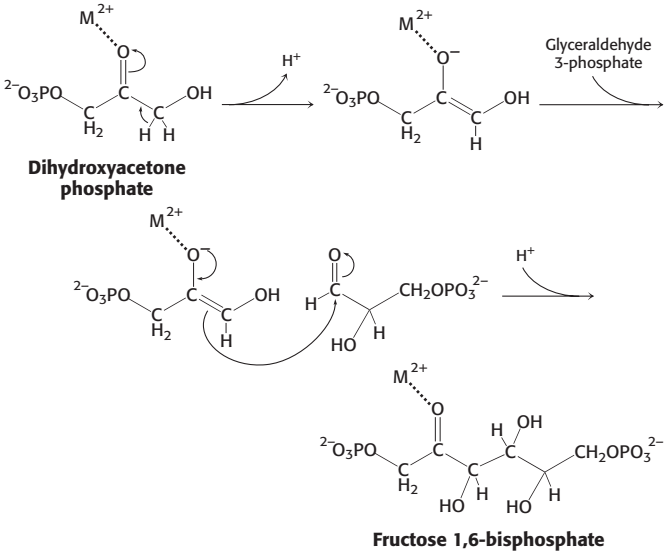
1. The Calvin cycle is the primary means of converting gaseous CO_2 into organic matter—that is, biomolecules. Essentially, every carbon atom in your body passed through rubisco and the Calvin cycle at some time in the past.
2.

Calvin cycle	Krebs cycle
Stroma	Matrix
Carbon chemistry for photosynthesis	Carbon chemistry for oxidative phosphorylation
Fixes CO_2	Releases CO_2
Requires high-energy electrons (NADPH)	Generates high-energy electrons (NADPH)
Regenerates starting compound (ribulose 1.5-bisphosphate	Regenerates starting compound (oxaloacetate)
Requires ATP	Generates ATP or GTP
Complex stoichiometry	Simple stoichiometry

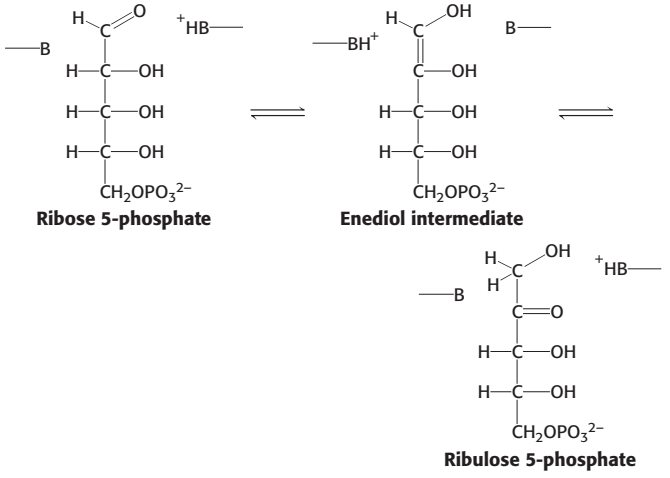
3. (a) 3-Phosphoglycerate. (b) The other members of the Calvin cycle.
4. Stage 1 is the fixation of CO_2 with ribulose 1,5-bisphosphate and the subsequent formation of 3-phosphoglycerate. Stage 2 is the conversion of some of the 3-phosphoglycerate into hexose. Stage 3 is the regeneration of ribulose 1,5-bisphosphate.
5. It catalyzes a crucial reaction, but it is highly inefficient. Consequently, it is required in large amounts to overcome its slow catalysis.
6. Because carbamate forms only in the presence of CO_2, this property prevents rubisco from catalyzing the oxygenase reaction exclusively when CO_2 is absent.
7. Because NADPH is generated in the chloroplasts by the light reactions.
8. The concentration of 3-phosphoglycerate would increase, whereas that of ribulose 1,5-bisphosphate would decrease.
9. The concentration of 3-phosphoglycerate would decrease, whereas that of ribulose 1,5-bisphosphate would increase.
10. Aspartate + glyoxylate $\longrightarrow$ oxaloacetate + glycine
11. The oxygenase activity of rubisco increases with temperature. Crabgrass is a C_4 plant, whereas most grasses lack this capability. Consequently, the crabgrass will thrive at the hottest part of the summer because the C_4 pathway provides an ample supply of CO_2.
12. The C_4 pathway allows the CO_2 concentration to increase at the site of carbon fixation. High concentrations of CO_2 inhibit the oxygenase reaction of rubisco. This inhibition is important for tropical plants because the oxygenase activity increases more rapidly with temperature than does the carboxylase activity.
13. ATP is required to form phosphoenolpyruvate (PEP) from pyruvate. The PEP combines with CO_2 to form oxaloacetate and, subsequently, malate. Two ATP molecules are required because a second ATP molecule is required to phosphorylate AMP to ADP.
14. Photorespiration is the consumption of oxygen by plants with the production of CO_2, but it does not generate energy. Photorespiration is due to the oxygenase activity of rubisco. It is wasteful because, instead of fixing CO_2 for conversion into hexoses, rubisco is generating CO_2.
15. As global warming progresses, C_4 plants will invade the higher latitudes, and C_3 plants will retreat to cooler regions.
16. The light reactions lead to an increase in the stromal concentrations of NADPH, reduced ferredoxin, and Mg^{2+}, as well as an increase in pH.
17. The enzymes catalyze the transformation of the five-carbon sugar formed by the oxidative phase of the pentose phosphate pathway into fructose 6-phosphate and glyceraldehyde 3-phosphate, intermediates in glycolysis (and gluconeogenesis).
18. The label emerges at C-5 of ribulose 5-phosphate.
19. Oxidative decarboxylation of isocitrate to α-ketoglutarate. A β-ketoacid intermediate is formed in both reactions.
20. (a) 5 Glucose 6-phosphate + ATP $\longrightarrow$
6 ribose 5-phosphate + ADP + H^+.
(b) Glucose 6-phosphate + 12 $NADP^+$ + 7 H_2O $\longrightarrow$
6 CO_2 + 12 NADPH + 12 H^+ + P_i.
21. The nonoxidative phase of the pentose phosphate pathway can be used to convert three molecules of ribose 5-phosphate into two molecules of fructose 6-phosphate and one molecule of glyceraldehyde 3-phosphate. These molecules are components of the glycolytic pathway.
22. The conversion of fructose 6-phosphate into fructose 1,6-bisphosphate by phosphofructokinase requires ATP.
23. When much NADPH is required. The oxidative phase of the pentose phosphate pathway is followed by the nonoxidative phase. The resulting fructose 6-phosphate and glyceraldehyde 3-phosphate are used to generate glucose 6-phosphate through gluconeogenesis, and the cycle is repeated until the equivalent of one glucose molecule is oxidized to CO_2.
24. Fava beans contain pamaquine, a purine glycoside that can lead to the generation of peroxides—reactive oxygen species that can damage membranes as well as other biomolecules. Glutathione is used to detoxify the ROS. The regeneration of glutathione depends on an adequate supply of NADPH, which is synthesized by the oxidative phase of the pentose phosphate pathway. People with low levels of the dehydrogenase are especially susceptible to pamaquine toxicity.
25. Because red blood cells do not have mitochondria and the only means to obtain NADPH is through the pentose phosphate pathway. There are biochemical means to convert mitochondrial NADH into cytoplasmic NADPH.
26. Reactive peroxides are a type of reactive oxygen species. The enzyme glutathione peroxidase uses reduced glutathione to neutralize peroxides by converting them into alcohols while generating oxidized glutathione. Reduced glutathione is regenerated by glutathione reductase with the use of NADPH, the product of the oxidative phase of the pentose phosphate pathway.

27. $\Delta E_0'$ for the reduction of glutathione by NADPH is + 0.09 V. Hence, $\Delta G°'$ is -17.4 kJ mol^{-1} ($-$ 4.2 kcal mol^{-1}), which corresponds to an equilibrium constant of 1126. The required [NADPH]/[NADP$^+$] ratio is 8.9×10^{-5}.

28.



29.



30. Incubate an aliquot of a tissue homogenate with glucose labeled with ^{14}C at C-1, and incubate another with glucose labeled with ^{14}C at C-6. Compare the radioactivity of the CO_2 produced by the two samples. The rationale of this experiment is that only C-1 is decarboxylated by the pentose phosphate pathway, whereas C-1 and C-6 are decarboxylated equally when glucose is metabolized by the glycolytic pathway, the pyruvate dehydrogenase complex, and the citric acid cycle. The reason for the equivalence of C-1 and C-6 in the latter set of reactions is that glyceraldehyde 3-phosphate and dihydroxyacetone phosphate are rapidly interconverted by triose phosphate isomerase.

31. The reduction of each mole of CO_2 to the level of a hexose requires two moles of NADPH. The reduction of NADP$^+$ is a two-electron process. Hence, the formation of two moles of NADPH requires the pumping of four moles of electrons by photosystem I. The electrons given up by photosystem I are replenished by photosystem II, which needs to absorb an equal number of photons. Hence, eight photons are needed to generate the required NADPH. The energy input of eight moles of photons is 1594 kJ (381 kcal). Thus, the overall efficiency of photosynthesis under standard conditions is at least 477/1594, or 30%.

32. It is neither a violation nor a miracle. The equation on page 580 requires not only 18 ATP, but also 12 NADPH. These electrons, if transferred to NAD$^+$ and used in the electron-transport chain, would yield 30 ATP. Thus, the synthesis of glucose requires the equivalent of 48 ATP.

33. (a) The curve on the right in graph A was generated by the C_4 plant. Recall that the oxygenase activity of rubisco increases with temperature more rapidly than does the carboxylase activity. Consequently, at higher temperatures, the C_3 plants would fix less carbon. Because C_4 plants can maintain a higher CO_2 concentration, the rise in temperature is less deleterious.

(b) The oxygenase activity will predominate. Additionally, when the temperature rise is very high, the evaporation of water might become a problem. The higher temperatures can begin to damage protein structures as well.

(c) The C_4 pathway is a very effective active-transport system for concentrating CO_2, even when environmental concentrations are very low.

(d) With the assumption that the plants have approximately the same capability to fix CO_2, the C_4 pathway is apparently the rate-limiting step in C_4 plants.

Chapter 21

1. Glycogen is an important fuel reserve for several reasons. The controlled breakdown of glycogen and release of glucose increase the amount of glucose that is available between meals. Hence, glycogen serves as a buffer to maintain blood-glucose levels. Glycogen's role in maintaining blood-glucose levels is especially important because glucose is virtually the only fuel used by the brain, except during prolonged starvation. Moreover, the glucose from glycogen is readily mobilized and is therefore a good source of energy for sudden, strenuous activity. Unlike fatty acids, the released glucose can provide energy in the absence of oxygen and can thus supply energy for anaerobic activity.

2. As an unbranched polymer, α-amylose has only one nonreducing end. Therefore, only one glycogen phosphorylase molecule could degrade each α-amylose molecule. Because glycogen is highly branched, there are many nonreducing ends per molecule. Consequently, many phosphorylase molecules can release many glucose molecules per glycogen molecule.

3. The patient has a deficiency of the branching enzyme.

4. In muscle, the *b* form of phosphorylase is activated by AMP. In the liver, the *a* form is inhibited by glucose. The difference corresponds to the difference in the metabolic role of glycogen in each tissue. Muscle uses glycogen as a fuel for contraction, whereas the liver uses glycogen to maintain blood-glucose levels.

5. Cells maintain the [P_i]/[glucose 1-phosphate] ratio at greater than 100, substantially favoring phosphorolysis. We see here an example of how the cell can alter the free-energy change to favor a reaction taking place by altering the ratio of substrate and product.

6. The high level of glucose 6-phosphate in von Gierke disease, resulting from the absence of glucose 6-phosphatase or the transporter, shifts the allosteric equilibrium of phosphorylated glycogen synthase toward the active form.

7. The phosphoryl donor is glucose 1,6-bisphosphate, which is formed from glucose 1-phosphate and ATP in a reaction catalyzed by phosphoglucokinase.

8. The different manifestations correspond to the different roles of the liver and muscle. Liver glycogen phosphorylase plays a crucial role in the maintenance of blood-glucose levels. Recall that glucose

is the primary fuel for the brain. Muscle glycogen phosphorylase provides glucose only for the muscle and, even then, only when the energy needs of the muscle are high, as during exercise. The fact that there are two different diseases suggests that there are two different isozymic forms of the glycogen phosphorylase—a liver-specific isozyme and a muscle-specific isozyme.

9. Water is excluded from the active site to prevent hydrolysis. The entry of water could lead to the formation of glucose rather than glucose 1-phosphate. A site-specific mutagenesis experiment is revealing in this regard. In phosphorylase, Tyr 573 is hydrogen bonded to the 2′-OH group of a glucose residue. The ratio of glucose 1-phosphate to glucose product is 9000 : 1 for the wild-type enzyme, and 500 : 1 for the Phe 573 mutant. Model building suggests that a water molecule occupies the site normally filled by the phenolic OH group of tyrosine and occasionally attacks the oxocarbonium ion intermediate to form glucose.

10. The amylase activity was necessary to remove all of the glycogen from the glycogenin. Recall that glycogenin synthesizes oligosaccharides of about eight glucose units, and then activity stops. Consequently, if the glucose residues are not removed by extensive amylase treatment, glycogenin will not function.

11. The substrate can be handed directly from the transferase site to the debranching site.

12. During exercise, [ATP] falls and [AMP] rises. Recall that AMP is an allosteric activator of glycogen phosphorylase *b*. Thus, even in the absence of covalent modification by phosphorylase kinase, glycogen is degraded.

13. Although glucose 1-phosphate is the actual product of the phosphorylase reaction, glucose 6-phosphate is a more versatile molecule with respect to metabolism. Among other fates, glucose-6-phosphate can be processed to yield energy or building blocks. In the liver, glucose 6-phosphate can be converted into glucose and released into the blood.

14. Epinephrine binds to its G-protein-coupled receptor. The resulting structural changes activate a G_α protein, which in turn activates adenyl cyclase. Adenyl cyclase synthesizes cAMP, which activates protein kinase A. Protein kinase A partly activates phosphoryl kinase, which phosphorylates and activates glycogen phosphorylase. The calcium released during muscle contraction further activates the phosphorylase kinase, leading to further stimulation of glycogen phosphorylase.

15. First, the signal-transduction pathway is shut down when the initiating hormone is no longer present. Second, the inherent GTPase activity of the G protein converts the bound GTP into inactive GDP. Third, phosphodiesterases convert cyclic AMP into AMP. Fourth, PP1 removes the phosphoryl group from glycogen phosphorylase, converting the enzyme into the usually inactive *b* form.

16. It prevents both from operating simultaneously, which would lead to a useless expenditure of energy. See the answer to Problem 24.

17. All these symptoms suggest central nervous system problems. If exercise is exhaustive enough or the athlete has not prepared well enough or both, liver glycogen also can be depleted. The brain depends on glucose derived from liver glycogen. The symptoms suggest that the brain is not getting enough fuel.

18. Liver phosphorylase *a* is inhibited by glucose, which facilitates the R ⟶ T transition. This transition releases PP1, which inactivates glycogen breakdown and stimulates glycogen synthesis. Muscle phosphorylase is insensitive to glucose.

19. The presence of high concentrations of glucose 6-phosphate indicates that glucose is abundant and that it is not being used by glycolysis. Therefore, this valuable resource is saved by incorporation into glycogen.

20. Free glucose must be phosphorylated at the expense of a molecule of ATP. Glucose 6-phosphate derived from glycogen is formed by phosphorolytic cleavage, thus sparing one molecule of ATP. Thus, the net yield of ATP when glycogen-derived glucose is processed to pyruvate is three molecules of ATP compared with two molecules of ATP from free glucose.

21. Breakdown: Phosphoglucomutase converts glucose 1-phosphate, liberated from glycogen breakdown, into glucose 6-phosphate, which can be released as free glucose (liver) or processed in glycolysis (muscle and liver). Synthesis: Converts glucose 6-phophosphate into glucose 1-phosphate, which reacts with UTP to form UDP-glucose, the substrate for glycogen synthase.

22. $\text{Glycogen}_n + P_i \longrightarrow \text{glycoge}_{n-1} + \text{glucose 6-phosphate}$

$\text{Glucose 6-phosphate} \longrightarrow \text{glucose 1-phosphate}$

$\text{UTP} + \text{glucose 1-phosphate} \longrightarrow \text{UDP-glucose} + 2\ P_i$

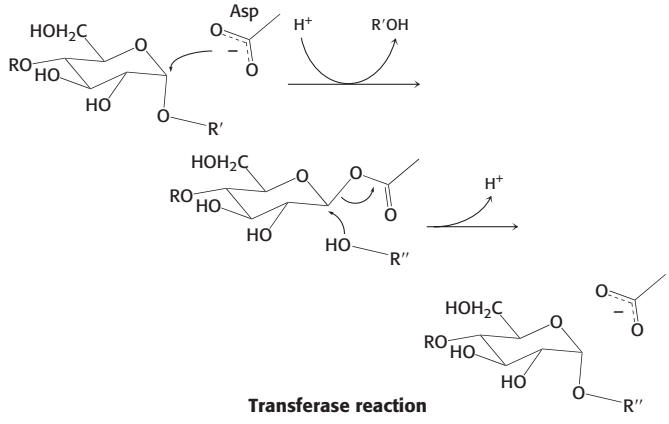
$\text{Glycogen}_{n-1} + \text{UDP-glucose} \longrightarrow \text{glycogen}_n + \text{UDP}$

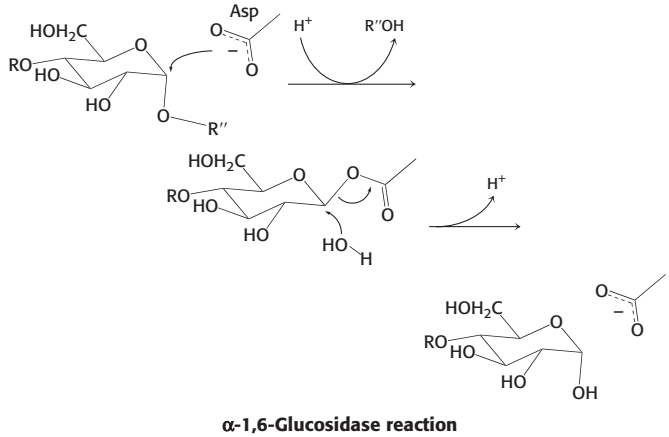
$\text{Sum: Glycogen}_n + \text{UTP} \longrightarrow \text{glycogen}_n + \text{UDP} + P_i$

23. In principle, having glycogen be the only primer for the further synthesis of glycogen should be a successful strategy. However, if the glycogen granules were not evenly divided between daughter cells, glycogen stores for future generations of cells might be compromised. Glycogenin synthesizes the primer for glycogen synthase.

24. Insulin binds to its receptor and activates the tyrosine kinase activity of the receptor, which in turn triggers a pathway that activates protein kinases. The kinases phosphorylate and inactivate glycogen synthase kinase. Protein phosphatase 1 then removes the phosphate from glycogen synthase and thereby activates the synthase.

25.





26. Galactose + ATP + UTP + H_2O + glycogen$_n$ ⟶ glycogen$_{n+1}$ + ADP + UDP + 2 P_i + H^+.
27. Phosphorylase, transferase, glucosidase, phosphoglucomutase, and glucose 6-phosphatase.
28. Glucose is an allosteric inhibitor of phosphorylase *a*. Hence, crystals grown in its presence are in the T state. The addition of glucose 1-phosphate, a substrate, shifts the R-to-T equilibrium toward the R state. The conformational differences between these states are sufficiently large that the crystal shatters unless it is stabilized by chemical cross-links.
29. Galactose is converted into UDP-galactose to eventually form glucose 6-phosphate.
30. This disease can also be produced by a mutation in the gene that encodes the glucose 6-phosphate transporter. Recall that glucose 6-phosphate must be transported into the lumen of the endoplasmic reticulum to be hydrolyzed by phosphatase. Mutations in the other three essential proteins of this system can likewise lead to von Gierke disease.
31. (a) Glycogen was too large to enter the gel and, because analysis was by western blot with the use of an antibody specific to glycogenin, we would not expect to see background proteins.
(b) α-Amylase degrades glycogen, releasing the protein glycogenin, which can be visualized by a western blot.
(c) Glycogen phosphorylase, glycogen synthase, and protein phosphatase 1. These proteins might be visible if the gel were stained for protein, but a western analysis reveals the presence of glycogenin only.
32. (a) The smear was due to molecules of glycogenin with increasingly large amounts of glycogen attached to them.
(b) In the absence of glucose in the medium, glycogen is metabolized, resulting in a loss of the high-molecular-weight material.
(c) Glycogen could have been resynthesized and added to the glycogenin when the cells were fed glucose again.
(d) No difference between lanes 3 and 4 suggests that, by 1 hour, the glycogen molecules had attained maximum size in this cell line. Prolonged incubation does not apparently increase the amount of glycogen.
(e) α-Amylase removes essentially all of the glycogen, and so only the glycogenin remains.

Chapter 22

1. Glycerol + 2 NAD^+ + P_i + ADP ⟶ pyruvate + ATP + H_2O + 2 NADH + H^+

Glycerol kinase and glycerol phosphate dehydrogenase
2. The ready reversibility is due to the high-energy nature of the thioester in the acyl CoA.
3. To return the AMP to a form that can be phosphorylated by oxidative phosphorylation or substrate-level phosphorylation, another molecule of ATP must be expended in the reaction:

$$\text{ATP} + \text{AMP} \rightleftharpoons 2\ \text{ADP}$$

4. b, c, a, g, h, d, e, f.
5. The citric acid cycle. The reactions that take succinate to oxaloacetate, or the reverse, are similar to those of fatty acid metabolism (Section 17.2).
6. The next-to-last degradation product, acetoacetyl CoA, yields two molecules of acetyl CoA with the thiolysis by only one molecule of CoA.
7. Palmitic acid yields 106 molecules of ATP. Palmitoleic acid has a double bond between carbons C-9 and C-10. When palmitoleic acid is processed in β oxidation, one of the oxidation steps (to introduce a double bond before the addition of water) will not take place, because a double bond already exists. Thus, $FADH_2$ will not be generated, and palmitoleic acid will yield 1.5 fewer molecules of ATP than palmitic acid, for a total of 104.5 molecules of ATP.
8.

Activation fee to form the acyl CoA	−2 ATP
Seven rounds of yield:	
7 acetyl CoA at 10 ATP/acetyl CoA	+ 70 ATP
7 NADH at 2.5 ATP/NADH	+ 17.5 ATP
7 $FADH_2$ at 1.5 ATP/$FADH_2$	+ 10.5 ATP
Propionyl CoA, which requires an ATP to be converted into succinyl CoA	− 1 ATP
Succinyl CoA ⟶ succinate	+ 1 ATP (GTP)
Succinate ⟶ fumarate + $FADH_2$ $FADH_2$ at 1.5 ATP/$FADH_2$	+ 1.5 ATP
Fumarate ⟶ malate	
Malate ⟶ oxaloacetate + NADH NADH at 2.5 ATP/NADH	+ 2.5 ATP
Total	120 ATP

9. You might hate yourself in the morning, but at least you won't have to worry about energy. To form stearoyl CoA requires the equivalent of 2 molecules of ATP.

Stearoyl CoA + 8 FAD + 8 NAD^+ + 8 CoA + 8 H_2O ⟶ 9 acetyl CoA + 8 $FADH_2$ + 8 NADH + 8 H^+

9 acetyl CoA at 10 ATP/acetyl CoA	+ 90 ATP
8 NADH at 2.5 ATP/NADH	+ 20 ATP
8 $FADH_2$ at 1.5 ATP/$FADH_2$	+ 12 ATP
Activation fee	−2.0
Total	122 ATP

10. Keep in mind that, in the citric acid cycle, 1 molecule of $FADH_2$ yields 1.5 ATP, 1 molecule of NADH yields 2.5 ATP, and 1 molecule of acetyl CoA yields 10 ATP. Two molecules of ATP are produced when glucose is degraded to 2 molecules of pyruvate. Two molecules of NADH also are produced, but the electrons are transferred to $FADH_2$ to enter the mitochondria. Each molecule of $FADH_2$ can generate 1.5 ATP. Each molecule of pyruvate will produce 1 molecule of NADH. Each molecule of acetyl CoA generates 3 molecules of NADH, 1 molecule of $FADH_2$, and 1 molecule of ATP. So, we have a total of 10 ATP per acetyl CoA, or 20 for the 2 molecules of acetyl CoA. The total for glucose is 30 ATP. Now, what about hexanoic acid? Caprioic acid is activated to caprioic CoA at the expense of 2 ATP, and so we are 2 ATP in the hole. The first cycle of β oxidation generates 1 $FADH_2$, 1 NADH, and 1 acetyl CoA. After the acetyl CoA has been run through the citric acid cycle, this step will have generated a total of 14 ATP. The second cycle of β oxidation generates 1 $FADH_2$ and 1 NADH but 2 acetyl CoA. After the acetyl CoA has been run through the citric acid cycle, this step will have generated a total of 24 ATP. The total is 36 ATP. Thus, the foul-smelling caprioic acid has a net yield of 36 ATP. So on a per carbon basis, this fat yields 20% more ATP than does glucose, a manifestation of the fact that fats are more reduced than carbohydrates.
11. Stearate + ATP + 13.5 H_2O + 8 FAD + 8 NAD^+ ⟶ 4.5 acetoacetate + 14.5 H^+ + 8 $FADH_2$ + 8 NADH + AMP + 2 P_i.
12. Palmitate is activated and then processed by β oxidation according to the following reactions.

$$\text{Palmitate} + \text{CoA} + \text{ATP} \longrightarrow \text{palmitoyl CoA} + \text{AMP} + 2\ P_i$$

$$\text{Palmitoyl CoA} + 7\ \text{FAD} + 7\ \text{NAD} + 7\ \text{CoASH} + H_2O \longrightarrow 8\ \text{acetyl CoA} + 7\ FADH_2 + 7\ NADH + 7\ H^+$$

The eight molecules of acetyl CoA combine to form four molecules of acetoacetate for release into the blood, and so they do not contribute to the energy yield in the liver. However, the $FADH_2$ and NADH generated in the preparation of acetyl CoA can be processed by oxidative phosphorylation to yield ATP.

$$1.5\ \text{ATP}/FADH_2 \times 7 = 10.5\ \text{ATP}$$

$$2.5\ \text{ATP}/\text{NADH} \times 7 = 17.5\ \text{ATP}$$

The equivalent of 2 ATP were used to form palmitoyl CoA. Thus, 26 ATP were generated for use by the liver.

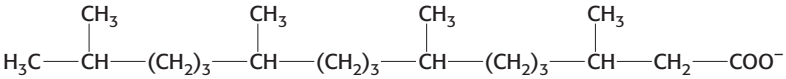
13. NADH produced with the oxidation to acetoacetate = 2.5 ATP. Acetoacetate is converted into acetoacetyl CoA.

Two molecules of acetyl CoA result from the hydrolysis of acetoacetyl CoA, each worth 10 ATP when processed by the citric acid cycle. Total ATP yield is 22.5.

14. Because a molecule of succinyl CoA is used to form acetoacetyl CoA. Succinyl CoA could be used to generate one molecule of ATP (GTP), and so someone could argue that the yield is 21.5.

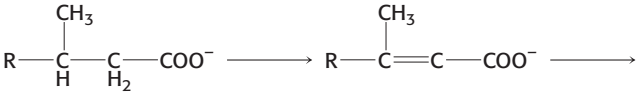
15. For fats to be combusted, not only must they be converted into acetyl CoA, but the acetyl CoA must be processed by the citric acid cycle. In order for acetyl CoA to enter the citric acid cycle, there must be a supply of oxaloacetate. Oxaloacetate can be formed by the metabolism of glucose to pyruvate and the subsequent carboxylation of pyruvate to form oxaloacetate.

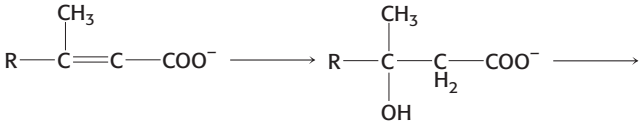
16. (a)

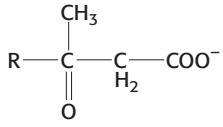


Phytanic acid

The problem with phytanic acid is that, as it undergoes β oxidation, we encounter the dreaded pentavalent carbon atom. Because the pentavalent carbon atom doesn't exist, β oxidation cannot take place and phytanic acid accumulates.

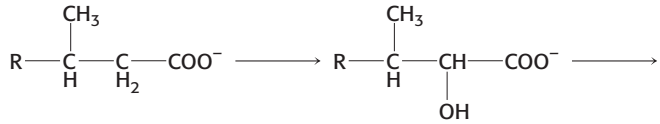


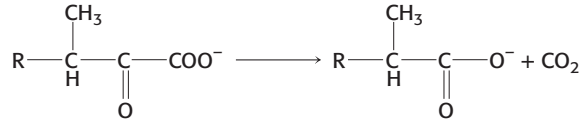




The dreaded pentavalent carbon atom

(b) Removing methyl groups, though theoretically possible, would be time consuming and, lacking in elegance. What would we do with the methyl groups? Our livers solve the problem by inventing α oxidation.





One round of α oxidation rather than β oxidation converts phytanic acid into a β-oxidation substrate.

17. The first oxidation removes two tritium atoms. The hydration adds nonradioactive H and OH. The second oxidation removes another tritium atom from the β-carbon atom. Thiolysis removes an acetyl CoA with only one tritium atom; so the tritium-to-carbon ratio is 1/2. This ratio will be the same for two of the acetates. The last one, however, does not undergo oxidation, and so all tritium remains. The ratio for this acetate is 3/2. The ratio for the entire molecule is then 5/6.

18. In the absence of insulin, lipid mobilization will take place to an extent that it overwhelms the ability of the liver to convert the lipids into ketone bodies.

19. (a) Oxidation in mitochondria; synthesis in the cytoplasm. (b) Coenzyme A in oxidation; acyl carrier protein for synthesis. (c) FAD and NAD^+ in oxidation; NADPH for synthesis. (d) the L isomer of 3-hydroxyacyl CoA in oxidation; the D isomer in synthesis. (e) From carboxyl to methyl in oxidation; from methyl to carboxyl in synthesis. (f) The enzymes of fatty acid synthesis, but not those of oxidation, are organized in a multienzyme complex.

20. $7\ \text{acetyl CoA} + 6\ \text{ATP} + 12\ \text{NADPH} + 12\ H^+ \longrightarrow \text{myristate} + 7\ \text{CoA} + 6\ \text{ADP} + 6\ \text{Pi} + 12\ \text{NADP}^+ + 5H_2O$.

21. We will need six acetyl CoA units. One acetyl CoA unit will be used directly to become the two carbon atoms farthest from the acid end. The other five units must be converted into malonyl CoA. The synthesis of each malonyl CoA molecule costs a molecule of ATP; so 5 molecules of ATP will be required. Each round of elongation requires 2 molecules of NADPH, 1 molecule to reduce the keto group to an alcohol and 1 molecule to reduce the double bond. As a result, 10 molecules of NADPH will be required. Therefore, 5 molecules of ATP and 10 molecules of NADPH are required to synthesize lauric acid.

22. e, b, d, a, c.

23. Such a mutation would inhibit fatty acid synthesis because the enzyme cleaves cytoplasmic citrate to yield acetyl CoA for fatty acid synthesis.

24. (a) False. Biotin is required for acetyl CoA carboxylase activity.
(b) True.
(c) False. ATP is required to synthesize malonyl CoA.
(d) True.
(e) True.
(f) False. Fatty acid synthase is a dimer.
(g) True.
(h) False. Acetyl CoA carboxylase is stimulated by citrate, which is cleaved to yield its substrate acetyl CoA.

25. Fatty acids with odd numbers of carbon atoms are synthesized starting with propionyl ACP (instead of acetyl ACP), which is formed from propionyl CoA by acetyl transacetylase.

26. All of the labeled carbon atoms will be retained. Because we need 8 acetyl CoA molecules and only 1 carbon atom is labeled in the acetyl group, we will have 8 labeled carbon atoms. The only acetyl CoA used

directly will retain 3 tritium atoms. The 7 acetyl CoA molecules used to make malonyl CoA will lose 1 tritium atom on addition of the CO_2 and another one at the dehydration step. Each of the 7 malonyl CoA molecules will retain 1 tritium atom. Therefore, the total retained tritium is 10 atoms. The ratio of tritium to carbon is 1.25.

27. With a diet rich in raw eggs, avidin will inhibit fatty acid synthesis by reducing the amount of biotin required by acetyl CoA carboxylase. Cooking the eggs will denature avidin, and so it will no longer bind biotin.

28. The only acetyl CoA used directly, not in the form of malonyl CoA, provides the two carbon atoms at the ω end of the fatty acid chain. Because palmitic acid is a C_{16} fatty acid, acetyl CoA will have provided carbons 15 and 16.

29. HCO_3^- is attached to acetyl CoA to form malonyl CoA. When malonyl CoA condenses with acetyl CoA to form the four-carbon keto acyl CoA, the HCO_3^- is lost as CO_2.

30. Phosphofructokinase controls the flux down the glycolytic pathway. Glycolysis functions to generate ATP or building blocks for biosynthesis, depending on the tissue. The presence of citrate in the cytoplasm indicates that those needs are met, and there is no need to metabolize glucose.

31. C-1 is more radioactive.

32. The mutant enzyme will be persistently active because it cannot be inhibited by phosphorylation. Fatty acid synthesis will be abnormally active. Such a mutation might lead to obesity.

33. (a) Palmitoleate; (b) linoleate; (c) linoleate; (d) oleate; (e) oleate; (f) linolenate.

34. Decarboxylation drives the condensation of malonyl ACP and acetyl ACP. In contrast, the condensation of two molecules of acetyl ACP is energetically unfavorable. In gluconeogenesis, decarboxylation drives the formation of phosphoenolpyruvate from oxaloacetate.

35. Fat mobilization in adipocytes is activated by phosphorylation. Hence, overproduction of the cAMP-activated kinase will lead to an accelerated breakdown of triacylglycerols and a depletion of fat stores.

36. Carnitine translocase deficiency and glucose 6-phosphate transporter deficiency.

37. In the fifth round of β oxidation, *cis*-Δ^2-enoyl CoA is formed. Dehydration by the classic hydratase yields D-3-hydroxyacyl CoA, the wrong isomer for the next enzyme in β oxidation. This dead end is circumvented by a second hydratase that removes water to give *trans*-Δ^2-enoyl CoA. The addition of water by the classic hydratase then yields L-3-hydroxyacyl CoA, the appropriate isomer. Thus, hydratases of opposite stereospecificities serve to *epimerize* (invert the configuration of) the 3-hydroxyl group of the acyl CoA intermediate.

38. The probability of synthesizing an error-free polypeptide chain decreases as the length of the chain increases. A single mistake can make the entire polypeptide ineffective. In contrast, a defective subunit can be spurned in the formation of a noncovalent multienzyme complex; the good subunits are not wasted.

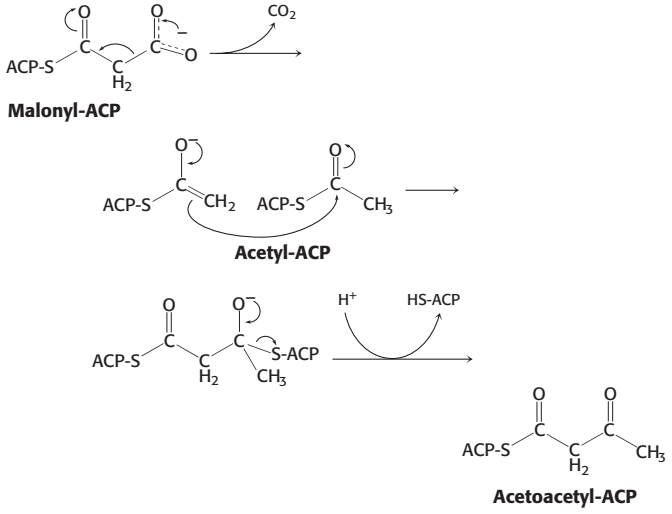
39. The absence of ketone bodies is due to the fact that the liver, the source of ketone bodies in the blood, cannot oxidize fatty acids to produce acetyl CoA. Moreover, because of the impaired fatty acid oxidation, the liver becomes more dependent on glucose as an energy source. This dependency results in a decrease in gluconeogenesis and a drop in blood-glucose levels, which is exacerbated by the lack of fatty acid oxidation in muscle and a subsequent increase in glucose uptake from the blood.

40. Peroxisomes enhance the degradation of fatty acids. Consequently, increasing the activity of peroxisomes could help to lower levels of blood triglycerides. In fact, clofibrate is rarely used because of serious side effects.

41. Citrate works by facilitating the formation of active filaments from inactive monomers. In essence, it increases the number of active sites available, or the concentration of enzyme. Consequently, its effect is visible as an increase in the value of V_{max}. Allosteric enzymes that alter their V_{max} values in response to regulators are sometimes called V-class enzymes. The more common type of allosteric enzyme, in which K_m is altered, comprises K-class enzymes. Palmitoyl CoA causes depolymerization and thus inactivation.

42. The thiolate anion of CoA attacks the 3-keto group to form a tetrahedral intermediate. This intermediate collapses to form acyl CoA and the enolate anion of acetyl CoA. Protonation of the enolate yields acetyl CoA.

43.



44. (a) Fats burn in the flame of carbohydrates. Without carbohydrates, there would be no anapleurotic reactions to replenish the components of the citric acid cycle. With a diet of fats only, the acetyl CoA from fatty acid degradation would build up.
(b) Acetone from ketone bodies
(c) Yes. Odd-chain fatty acids would lead to the production of propionyl CoA, which can be converted into succinyl CoA, a citric acid cycle component. It would serve to replenish the citric acid cycle and mitigate the halitosis.

45. A labeled fat can enter the citric acid cycle as acetyl CoA and yield labeled oxaloacetate, but only after two carbon atoms have been lost as CO_2. Consequently, even though oxaloacetate may be labeled, there can be no net synthesis of oxaloacetate and hence no net synthesis of glucose or glycogen.

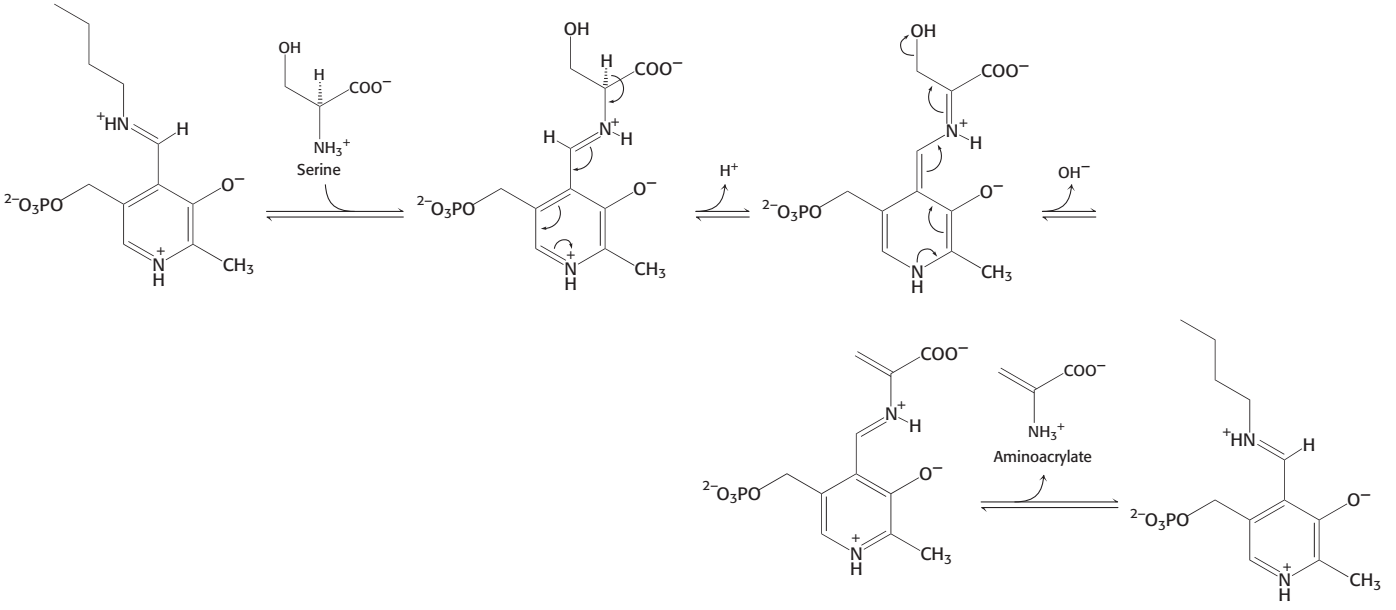
46. (a) The V_{max} is decreased and the K_m is increased. V_{max} (wild type) = 13 nmol minute^{-1} mg^{-1}; K_m (wild type) = 45 μM; V_{max} (mutant) = 8.3 nmol minute^{-1} mg^{-1}; K_m (mutant) = 74 μM.
(b) Both the V_{max} and the K_m are decreased. V_{max} (wild type) = 41 nmol minute^{-1} mg^{-1}; K_m (wild type) = 104 μM; V_{max} (mutant) = 23 nmol minute^{-1} mg^{-1}; K_m (mutant) = 69 μM.
(c) The wild type is significantly more sensitive to malonyl CoA.
(d) With respect to carnitine, the mutant displays approximately 65% of the activity of the wild type; with respect to palmitoyl CoA, approximately 50% activity. On the other hand, 10 μM of malonyl CoA inhibits approximately 80% of the wild type but has essentially no effect on the mutant enzyme.

(e) The glutamate appears to play a more prominent role in regulation by malonyl CoA than in catalysis.

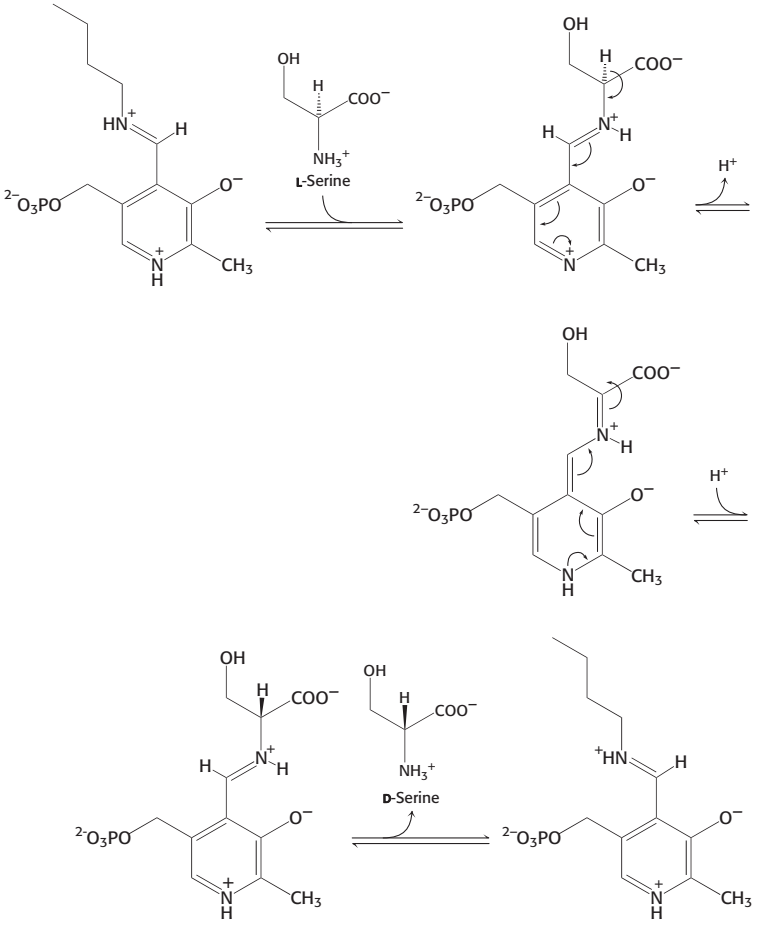
Chapter 23

1. When the proteins are denatured, all of the peptide bonds are accessible to proteolytic enzymes. If the three-dimensional structure of a protein is maintained, access to many peptide bonds is denied to the proteolytic enzymes.
2. First, the ubiquitin-activating enzyme (E1) links ubiquitin to a sulfhydryl group on E1 itself. Next, the ubiquitin is transferred to a cysteine residue on the ubiquitin-conjugating enzyme (E2) by E2. The ubiquitin–protein ligase (E3), using the ubiquitinated E2 as a substrate, transfers the ubiquitin to the target protein.
3. (a) 7; (b) 4; (c) 2; (d) 10; (e) 5; (f) 3; (g) 9; (h) 1; (i) 6; (j) 8.
4. (a) The ATPase activity of the 26S proteasome resides in the 19S subunit. The energy of ATP hydrolysis could be used to unfold the substrate, which is too large to enter the catalytic barrel. ATP may also be required for translocation of the substrate into the barrel.
(b) Substantiates the answer in part *a*. Because they are small, the peptides do not need to be unfolded. Moreover, small peptides could probably enter all at once and not require translocation.
5. (a) Pyruvate; (b) oxaloacetate; (c) α-ketoglutarate; (d) α-ketoisocaproate; (e) phenylpyruvate; (f) hydroxyphenylpyruvate.
6. (a) Aspartate + α-ketoglutarate + GTP + ATP + 2 H_2O + NADH + H^+ ⟶ ½ glucose + glutamate + CO_2 + ADP + GDP + NAD^+ + 2 P_i.
The required coenzymes are pyridoxal phosphate in the transamination reaction and NAD^+/NADH in the redox reactions.
(b) Aspartate + CO_2 + NH_4^+ + 3 ATP + NAD^+ + 4 H_2O ⟶ oxaloacetate + urea + 2 ADP + 4 P_i + AMP + NADH + H^+.
7. In the eukaryotic proteasome, the distinct β subunits have different substrate specificities, allowing proteins to be more thoroughly degraded.
8. The six subunits probably exist as a heterohexamer. Cross-linking experiments could test the model and help determine which subunits are adjacent to one another.
9. Thiamine pyrophosphate
10. Aminotransferases transfer the α-amino group to α-ketoglutarate to form glutamate. Glutamate is oxidatively deaminated to form an ammonium ion.
11. Aspartate (oxaloacetate), glutamate (α-ketoglutarate), alanine (pyruvate).
12. Serine and threonine
13. They are either fuels for the citric acid cycle, components of the citric acid cycle, or molecules that can be converted into a fuel for the citric acid cycle in one step.
14. It acts as an electron sink.
15. Carbamoyl phosphate and aspartate
16. (a) 4; (b) 5; (c) 1; (d) 6; (e) 7; (f) 3; (g) 2.
17. A, arginine; B, citrulline; C, ornithine; D, arginosuccinate. The order of appearance: C, B, D, E.
18. CO_2 + NH_4^+ + 3 ATP + NAD^+ + aspartate + 3 H_2O ⟶ urea + 2 ADP + 2 P_i + AMP + PP_i + NADH + H^+ + oxaloacetate.
Four high-transfer-potential phosphoryl groups are spent. Note, however, that an NADH is generated if fumarate is converted into oxaloacetate. NADH can generate 2.5 ATP in the electron-transport chain. Taking these ATP into account, only 1.5 high-transfer-potential phosphoryl groups are spent.
19. The synthesis of fumarate by the urea cycle is important because it links the urea cycle and the citric acid cycle. Fumarate is hydrated to malate, which, in turn, is oxidized to oxaloacetate. Oxaloacetate has several possible fates: (1) transamination to aspartate, (2) conversion into glucose by the gluconeogenic pathway, (3) condensation with acetyl CoA to form citrate, or (4) conversion into pyruvate. You can collect.
20. Ornithine transcarbamoylase (analogous to PALA; see Chapter 10).
21. Ammonia could lead to the amination of α-ketoglutarate, producing a high concentration of glutamate in an unregulated fashion. α-Ketoglutarate for glutamate synthesis could be removed from the citric acid cycle, thereby diminishing the cell's respiration capacity.
22. The mass spectrometric analysis strongly suggests that three enzymes—pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and the branched-chain α-ketoacid dehydrogenase—are deficient. Most likely, the common E_3 component of these enzymes is missing or defective. This proposal could be tested by purifying these three enzymes and assaying their ability to catalyze the regeneration of lipoamide.
23. Benzoate, phenylacetate, and arginine would be given to supply a protein-restricted diet. Nitrogen would emerge in hippurate, phenylacetylglutamine, and citrulline.
24. The liver is the primary tissue for capturing nitrogen as urea. If the liver is damaged (for instance, by hepatitis or the excessive consumption of alcohol), free ammonia is released into the blood.
25. This defect can be partly bypassed by providing a surplus of arginine in the diet and restricting the total protein intake. In the liver, arginine is split into urea and ornithine, which then reacts with carbamoyl phosphate to form citrulline. This urea-cycle intermediate condenses with aspartate to yield argininosuccinate, which is then excreted. Note that two nitrogen atoms—one from carbamoyl phosphate and the other from aspartate—are eliminated from the body per molecule of arginine provided in the diet. In essence, argininosuccinate substitutes for urea in carrying nitrogen out of the body. The formation of argininosuccinate removes the nitrogen, and the restriction on protein intake relieves the aciduria.
26. Aspartame, a dipeptide ester (L-aspartyl-L-phenylalanine methyl ester), is hydrolyzed to L-aspartate and L-phenylalanine. High levels of phenylalanine are harmful in phenylketonurics.
27. *N*-Acetylglutamate is synthesized from acetyl CoA and glutamate. Once again, acetyl CoA serves as an activated acetyl donor. This reaction is catalyzed by *N*-acetylglutamate synthase.
28. Not all proteins are created equal: some are more important than others. Some proteins would be degraded to provide the missing amino acid. The nitrogen from the other amino acids would be excreted as urea. Consequently, more nitrogen would be excreted than ingested.
29. The carbon skeletons of ketogenic amino acids can be converted into ketone bodies or fatty acids. Only leucine and lysine are purely ketogenic. Glucogenic amino acids are those whose carbon skeletons can be converted into glucose.
30. The branched-chain amino acids leucine, isoleucine, and valine. The required enzyme is the branched-chain α-ketoacid dehydrogenase complex.
31. Pyruvate (glycolysis and gluconeogenesis), acetyl CoA (citric acid cycle and fatty acid synthesis), acetoacetyl CoA (ketone-body formation), α-ketoglutarate (citric acid cycle), succinyl CoA (citric acid cycle), fumarate (citric acid cycle), and oxaloacetate (citric acid cycle and gluconeogenesis).

32.



33.



The equilibrium constant for the interconversion of L-serine and D-serine is exactly 1.

34. Exposure of such a domain suggests that a component of a multiprotein complex has failed to form properly or that one component has been synthesized in excess. This exposure leads to rapid degradation and the restoration of appropriate stoichiometries.

35. (a) Depletion of glycogen stores. When they are gone, proteins must be degraded to meet the glucose needs of the brain. The resulting amino acids are deaminated, and the nitrogen atoms are excreted as urea.
(b) The brain has adapted to the use of ketone bodies, which are derived from fatty acid catabolism. In other words, the brain is being powered by fatty acid breakdown.
(c) When the glycogen and lipid stores are gone, the only available energy source is protein.

36. Deamination to α-keto-β-methylvalerate; oxidative decarboxylation to α-methylbutyryl CoA; oxidation to tiglyl CoA; hydration, oxidation, and thiolysis yield acetyl CoA and propionyl CoA; propionyl CoA to succinyl CoA.

37. Glycogen phosphorylase. The coenzyme serves as an acid–base catalyst.

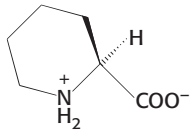
38. In the Cori cycle, the carbon atoms are transferred from muscle to liver as lactate. For lactate to be of any use, it must be reduced to pyruvate. This reduction requires high-energy electrons in the form of NADH. When the carbon atoms are transferred as alanine, transamination yields pyruvate directly.

39. (a) Virtually no digestion in the absence of nucleotides. (b) Protein digestion is greatly stimulated by the presence of ATP. (c) AMP-PNP, a nonhydrolyzable analog of ATP, is no more effective than ADP. (d) The proteasome requires neither ATP nor PAN to digest small substrates. (e) PAN and ATP hydrolysis may be required to unfold the peptide and translocate it into the proteasome. (f) Although *Thermoplasma* PAN is not as effective with the other proteasomes, it nonetheless results in threefold to fourfold stimulation of digestion. (g) In light of the fact that the archaea and eukarya diverged several billion years ago, the fact that

Thermoplasma PAN can stimulate rabbit muscle suggests homology not only between the proteasomes, but also between PAN and the 19S subunit (most likely the ATPases) of the mammalian 26S proteasome.

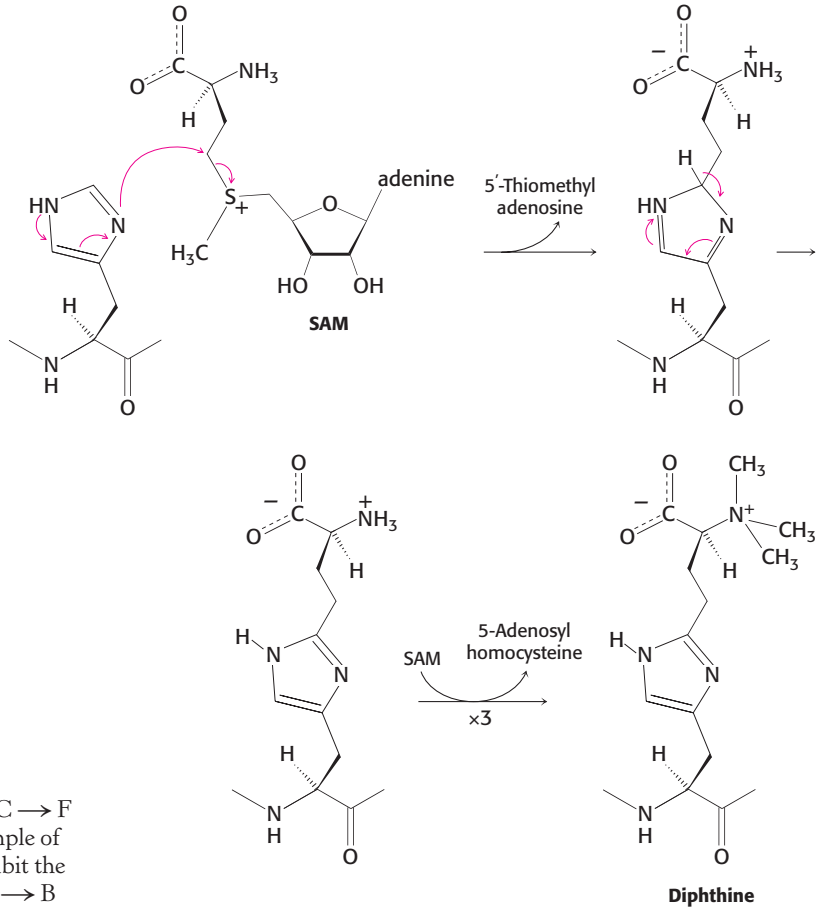
Chapter 24

1. Nitrogen fixation is the conversion of atmospheric N_2 into NH_3^+. Diazotrophic (nitrogen-fixing) microorganisms are able to fix nitrogen.
2. Oxaloacetate, pyruvate, ribose-5-phosphate, phosphoenolpyruvate, erythrose-4-phosphate, α-ketoglutarate, and 3-phosphoglycerate.
3. Human beings do not have the biochemical pathways to synthesize certain amino acids from simpler precursors. Consequently, these amino acids are "essential" and must be obtained from the diet.
4. Glucose + 2 ADP + 2 P_i + 2 NAD^+ + 2 glutamate ⟶ 2 alanine + 2 α-ketoglutrate + 2 ATP + 2 NADH + 2 H_2O + 2 H^+.
5. N_2 ⟶ NH_4^+ ⟶ glutamate ⟶ serine ⟶ glycine ⟶ δ-aminolevulinate ⟶ porphobilinogen ⟶ heme.
6. False. Nitrogen fixation is thermodynamically favorable. Nitrogenase is required because the process is kinetically disfavored.
7. Pyridoxal phosphate (PLP)
8. *S*-Adenosylmethionine, tetrahydrofolate, and methylcobalamin.
9. (a) N^5,N^{10}-Methylenetetrahydrofolate; (b) N^5-methyltetrahydrofolate.
10. γ-Glutamyl phosphate is a likely reaction intermediate.
11. The synthesis of asparagine from aspartate passes through an acyl-adenylate intermediate. One of the products of the reaction will be ^{18}O-labeled AMP.
12. The administration of glycine leads to the formation of isovalerylglycine. This water-soluble conjugate, in contrast with isovaleric acid, is excreted very rapidly by the kidneys.
13. The nitrogen atom shaded red is derived from glutamine. The carbon atom shaded blue is derived from serine.
14. They carry out nitrogen fixation. The absence of photosystem II provides an environment in which O_2 is not produced. Recall that the nitrogenase is very rapidly inactivated by O_2.
15. The cytoplasm is a reducing environment, whereas the extracellular milieu is an oxidizing environment.
16. (a) None; (b) D-glutamate and oxaloacetate.
17. Succinyl CoA is formed in the mitochondrial matrix.
18. Alanine from pyruvate; aspartate from oxaloacetate; glutamate from α-ketoglutarate.
19. Lysine cyclodeaminase converts L-lysine into the six-membered ring analog of proline, also referred to as L-homoproline or L-pipecolate:



Pipecolate

20. Y could inhibit the C ⟶ D step, Z could inhibit the C ⟶ F step, and C could inhibit A ⟶ B. This scheme is an example of sequential feedback inhibition. Alternatively, Y could inhibit the C ⟶ D step, Z could inhibit the C ⟶ F step, and the A ⟶ B step would be inhibited only in the presence of both Y and Z. This scheme is called concerted feedback inhibition.
21. The rate of the A ⟶ B step in the presence of high levels of Y and Z would be 24 s^{-1} ($0.6 \times 0.4 \times 100\ s^{-1}$).
22. Lysine 258 is absolutely essential for the activity of aspartate aminotransferase, as it is responsible both for the formation of the internal aldimine with the pyridoxal phosphate cofactor and for transferring the proton between the ketimine and quinonoid intermediates. Mutation of this residue to cysteine would be expected to dramatically impair catalysis, as cysteine cannot occupy the same space as lysine and also exhibits differing pK_a properties. Upon treatment with 2-bromoethylamine, however, the resulting thioether now has a shape and pK_a similar to the original lysine side chain. Hence, some catalytic activity is restored.
23. An external aldimine forms with SAM, which is deprotonated to form the quinonoid intermediate. The deprotonated carbon atom attacks the carbon atom adjacent to the sulfur atom to form the cyclopropane ring and release methylthioadenosine, the other product.
24. An external aldimine forms with L-serine, which is deprotonated to form the quinonoid intermediate. This intermediate is reprotonated on its opposite face to form an aldimine with D-serine. This compound is cleaved to release D-serine. The equilibrium constant for a racemization reaction is 1 because the reactant and product are exact mirror images of each other.
25. (a) In the first step, histidine attacks the methylene group from the methionine subgroup of SAM (rather than the usual methyl substituent), resulting in the transfer of an aminocarboxypropyl group. Three subsequent conventional SAM-mediated methylations of the primary amine yield diphthine.



(b) In this chapter, we have observed two examples of an ATP-dependent conversion of a carboxylate into an amide: glutamine synthetase, which uses an acyl-phosphate intermediate, and asparagine synthetase, which uses an acyl-adenylate intermediate. Either mechanism is possible in formation of diphthamide from diphthine.
26. Synthesis from oxaloacetate and α-ketoglutarate would deplete the citric acid cycle, which would decrease ATP production. Anapleurotic reactions would be required to replenish the citric acid cycle.
27. SAM is the donor for DNA methylation reactions that protect a host from digestion by its own restriction enzymes. A lack of SAM would render the bacterial DNA susceptible to digestion by the cell's own restriction enzymes.
28. Acetate ⟶ acetyl-CoA ⟶ citrate ⟶ isocitrate ⟶ α-ketoglutarate ⟶ succinyl-CoA.
29. (a) Asparagine is much more abundant in the dark. More glutamine is present in the light. These amino acids show the most dramatic effects. Glycine also is more abundant in the light.
(b) Glutamine is a more metabolically reactive amino acid, used in the synthesis of many other compounds. Consequently, when energy is available as light, glutamine will be preferentially synthesized. Asparagine, which carries more nitrogen per carbon atom and is thus a more-efficient means of storing nitrogen when energy is short, is synthesized in the dark. Glycine is more prevalent in the light because of photorespiration.
(c) White asparagus has an especially high concentration of asparagine, which accounts for its intense taste. All asparagus has a large amount of asparagine. In fact, as suggested by its name, asparagine was first isolated from asparagus.

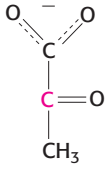
Chapter 25

1. In de novo synthesis, the nucleotides are synthesized from simpler precursor compounds, in essence from scratch. In salvage pathways, preformed bases are recovered and attached to riboses.
2. Carbon 2 and nitrogen 3 come from carbamoyl phosphate. Nitrogen 1 and carbons 4, 5, and 6 are derived from aspartate.
3. Nitrogen 1: aspartate; carbon 2: N^{10}-formyltetrahydrofolate; nitrogen 3: glutamine; carbons 4 and 5 and nitrogen 7: glycine; carbon 6: CO_2; carbon 8: N^{10}-formyltetrahydrofolate; nitrogen 9: glutamine.
4. Energy currency: ATP; signal transduction: ATP and GTP; RNA synthesis: ATP, GTP, CTP, and UTP; DNA synthesis: dATP, dCTP, dGTP, and TTP; components of coenzymes: ATP in CoA, FAD, and NAD(P)$^+$; carbohydrate synthesis: UDP-glucose. They are just some of the uses.
5. A nucleoside is a base attached to ribose. A nucleotide is a nucleoside with the ribose bearing one or more phosphates.
6. (a) 9; (b) 7; (c) 6; (d) 10; (e) 2; (f) 4; (g) 1; (h) 11; (i) 8; (j) 3; (k) 5.
7. Substrate channeling is the process whereby the product of one active site moves to become a substrate at another active site without ever leaving the enzyme. A channel connects the active sites. Substrate channeling greatly enhances enzyme efficiency and minimizes the diffusion of a substrate to an active site.
8. Glucose + 2 ATP + 2 $NADP^+$ + H_2O ⟶ PRPP + CO_2 + ADP + AMP + 2 NADPH + 3 H^+.
9. Glutamine + aspartate + CO_2 + 2 ATP + NAD^+ ⟶ orotate + 2 ADP + 2 P_i + glutamate + NADH + H^+.
10. (a, c, and d) PRPP; (b) carbamoyl phosphate.
11. PRPP and formylglycinamide ribonucleotide
12. dUMP + serine + NADPH + H^+ ⟶ dTMP + $NADP^+$ + glycine.
13. There is a deficiency of N^{10}-formyltetrahydrofolate. Sulfanilamide inhibits the synthesis of folate by acting as an analog of *p*-aminobenzoate, one of the precursors of folate.
14. (a) Cell A cannot grow in a HAT medium, because it cannot synthesize TMP either from thymidine or from dUMP. Cell B cannot grow in this medium, because it cannot synthesize purines by either the de novo pathway or the salvage pathway. Cell C can grow in a HAT medium because it contains active thymidine kinase from cell B (enabling it to phosphorylate thymidine to TMP) and hypoxanthine guanine phosphoribosyltransferase from cell A (enabling it to synthesize purines from hypoxanthine by the salvage pathway).
(b) Transform cell A with a plasmid containing foreign genes of interest and a functional thymidine kinase gene. The only cells that will grow in a HAT medium are those that have acquired a thymidylate kinase gene; nearly all of these transformed cells will also contain the other genes on the plasmid.
15. The reciprocal substrate relation refers to the fact that AMP synthesis requires GTP, whereas GMP synthesis requires ATP. These requirements tend to balance the synthesis of ATP and GTP.
16. Ring carbon 6 in cytosine will be labeled. In guanine, only carbon 5 will be labeled with ^{13}C.
17. The enzyme that uses ammonia synthesizes carbamoyl phosphate for a reaction with ornithine, the first step of the urea cycle. The enzyme that uses glutamine synthesizes carbamoyl phosphate for use in the first step of pyrimidine biosynthesis.
18. These patients have a high level of urate because of the breakdown of nucleic acids. Allopurinol prevents the formation of kidney stones and blocks other deleterious consequences of hyperuricemia by preventing the formation of urate.
19. The free energies of binding are −57.7 (wild type), −49.8 (Asn 27), and −38.1 (Ser 27) kJ mol^{-1} (−13.8, −11.9, and −9.1 kcal mol^{-1}, respectively). The loss in binding energy is 7.9 kJ mol^{-1} (1.9 kcal mol^{-1}) and 19.7 kJ mol^{-1} (4.7 kcal mol^{-1}).
20. Inosine or hypoxanthine could be administered.
21. N-1 in both cases, and the amine group linked to C-6 in ATP.
22. Nitrogen atoms 3 and 9 in the purine ring
23. Allopurinol, an analog of hypoxanthine, is a suicide inhibitor of xanthine oxidase.
24. An oxygen atom is added to allopurinol to form alloxanthine.
25.

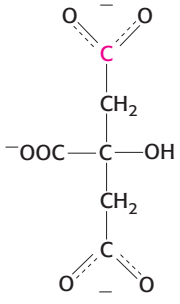
The synthesis of carbamoyl phosphate requires 2 ATP	2 ATP
The formation of PRPP from ribose 5-phosphate yields an AMP*	2 ATP
The conversion of UMP to UTP requires 2 ATP	2 ATP
The conversion of UTP to CTP requires 1 ATP	1 ATP
Total	7 ATP

*Remember that AMP is the equivalent of 2 ATP because an ATP must be expended to generate ADP, the substrate for ATP synthesis.
26. (a) Carboxyaminoimidazole ribonucleotide; (b) glycinamide ribonucleotide; (c) phosphoribosyl amine; (d) formylglycinamide ribonucleotide.
27. The first reaction proceeds by phosphorylation of glycine to form an acyl phosphate followed by nucleophilic attack by the amine of phosphoribosylamine to displace orthophosphate. The second reaction consists of adenylation of the carbonyl group of xanthylate followed by nucleophilic attack by ammonia to displace AMP.

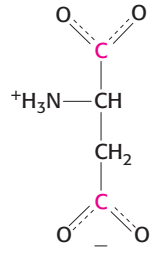
28. The $-NH_2$ group attacks the carbonyl carbon atom to form a tetrahedral intermediate. Removal of a proton leads to the elimination of water to form inosinate.
29. PRPP is the activated intermediate in the synthesis of phosphoribosylamine in the de novo pathway of purine formation; of purine nucleotides from free bases by the salvage pathway; of orotidylate in the formation of pyrimidines; of nicotinate ribonucleotide; of phosphoribosyl ATP in the pathway leading to histidine; and of phosphoribosylanthranilate in the pathway leading to tryptophan.
30. (a) cAMP; (b) ATP; (c) UDP-glucose; (d) acetyl CoA; (e) NAD^+, FAD; (f) dideoxynucleotides; (g) fluorouracil; (h) CTP inhibits ATCase.
31. In vitamin B_{12} deficiency, methyltetrahydrofolate cannot donate its methyl group to homocysteine to regenerate methionine. Because the synthesis of methyltetrahydrofolate is irreversible, the cell's tetrahydrofolate will ultimately be converted into this form. No formyl or methylene tetrahydrofolate will be left for nucleotide synthesis. Vitamin B_{12} is also required to metabolize propionyl CoA generated in the oxidation of odd-chain fatty acids and in the degradation of methionine.
32. Because folate is required for nucleotide synthesis, cells that are dividing rapidly would be most readily affected. They would include cells of the intestine, which are constantly replaced, and precursors to blood cells. A lack of intestinal cells and blood cells would account for the symptoms often observed.
33. The cytoplasmic level of ATP in the liver falls and that of AMP rises above normal in all three conditions. The excess AMP is degraded to urate.
34. Succinate ⟶ malate ⟶ oxaloacetate by the citric acid cycle. Oxaloacetate ⟶ aspartate by transamination, followed by pyrimidine synthesis. Carbons 4, 5, and 6 are labeled.
35. Glucose will most likely be converted into two molecules of pyruvate, one of which will be labeled in the 2 position:



Now consider two common fates of pyruvate—conversion into acetyl CoA and subsequent processing by the citric acid cycle or carboxylation by pyruvate carboxylase to form oxaloacetate. Formation of citrate by condensing the labeled pyruvate with oxaloacetate will yield labeled citrate:

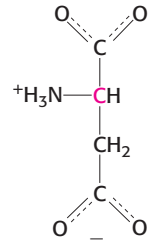


The labeled carbon will be retained through one round of the citric acid cycle but, on the formation of the symmetric succinate, the label will appear in two different positions. Thus, when succinate is metabolized to oxaloacetate, which may be aminated to form aspartate, two carbons will be labeled:



When this aspartate is used to form uracil, the labeled COO^- attached to the α-carbon is lost and the other COO^- becomes incorporated into uracil as carbon 4.

Suppose, instead, that labeled 2-[^{14}C]pyruvate is carboxylated to form oxaloacetate and processed to form aspartate. In this case, the α-carbon of aspartate bears the label.

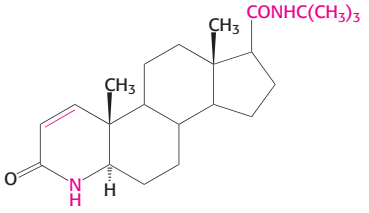


When this aspartate is used to synthesize uracil, carbon 6 bears the label.
36. (a) Some ATP can be salvaged from the ADP that is being generated. (b) There are equal numbers of high-phosphoryl-transfer-potential groups on each side of the equation. (c) Because the adenylate kinase reaction is at equilibrium, the removal of AMP would lead to the formation of more ATP. (d) Essentially, the cycle serves as an anaplerotic reaction for the generation of the citric acid cycle intermediate fumarate.
37. (i) The formation of 5-aminoididazole-4-carboxamide ribonucleotide from 5-aminoimidazole-4-(*N*-succinylcarboxamide) ribonucleotide in the synthesis of IMP. (ii) The formation of AMP from adenylosuccinate. (iii) The formation of arginine from argininosuccinate in the urea cycle.
38. Allopurinol is an inhibitor of xanthine oxidase, which is on the pathway for urate synthesis. In your pet duck, this pathway is the means by which excess nitrogen is excreted. If xanthine oxidase were inhibited in your duck, nitrogen could not be excreted, with severe consequences such as the formation of a dead duck.

Chapter 26

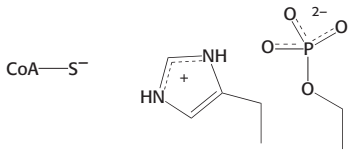
1. Glycerol 3-phosphate is the foundation for both triacylglycerol and phospholipid synthesis. Glycerol 3-phosphate is acylated twice to form phosphatidate. In triacylglycerol synthesis, the phosphoryl group is removed from glycerol 3-phosphate to form diacylglycerol, which is then acylated to form triacylglycerol. In phospholipid synthesis, phosphatidate commonly reacts with CTP to form CDP-diacylglycerol, which then reacts with an alcohol to form a phospholipid. Alternatively, diacylglycerol may react with a CDP-alcohol to form a phospholipid.
2. Glycerol 3-phosphate is formed primarily by the reduction of dihydroxyacetone phosphate, a gluconeogenic intermediate, and to a lesser extent by the phosphorylation of glycerol.

3. Glycerol + 4 ATP + 3 fatty acids + 4 H_2O ⟶ triacylglycerol + ADP + 3 AMP + 7 P_i + 4 H^+.
4. Glycerol + 3 ATP + 2 fatty acids + 2 H_2O + CTP + ethanolamine ⟶ phosphatidylethanolamine + CMP + ADP + 2 AMP + 6 P_i + 3 H^+.
5. Three. One molecule of ATP to form phosphorylethanolamine and two molecules of ATP to regenerate CTP from CMP.
6. All are synthesized from ceramide. In sphingomyelin, the terminal hydroxyl group of ceramide is modified with phosphorylcholine. In a cerebroside, the hydroxyl group has a glucose or galactose attached. In a ganglioside, oligosaccharide chains are attached to the hydroxyl group.
7. (i) Activate the diacylglycerol as CDP-DAG. (ii) Activate the alcohol as CDP-alcohol. (iii) Use the base-exchange reaction.
8. (a) CDP-diacylglycerol; (b) CDP-ethanolamine; (c) acyl CoA; (d) phosphatidylcholine; (e) UDP-glucose or UDP-galactose; (f) UDP-galactose; (g) geranyl pyrophosphate.
9. Such mutations are seen in mice. The amount of adipose tissue would decrease severely because diacylglycerol could not be formed. Normally, diacylglycerol is acylated to form triacylglycerols. If there were deficient phosphatidic acid phosphatase activity, no triacylglycerols would form.
10. (i) The synthesis of activated isoprene units (isopentyl pyrophosphate), (ii) the condensation of six of the activated isoprene units to form squalene, and (iii) cyclization of the squalene to form cholesterol.
11. The amount of reductase and its activity control the regulation of cholesterol biosynthesis. Transcriptional control is mediated by SREBP. Translation of the reductase mRNA also is controlled. The reductase itself may undergo regulated proteolysis. Finally, the activity of the reductase is inhibited by phosphorylation by AMP kinase when ATP levels are low.
12. (a and b) None, because the label is lost as CO_2.
13. The hallmark of this genetic disease is elevated cholesterol levels in the blood of even young children. The excess cholesterol is taken up by marcrophages, which eventually results in the formation of plaques and heart disease. There are many mutations that cause the disease, but all result in malfunctioning of the LDL receptor.
14. The categories of mutations are: (i) no receptor is synthesized; (ii) receptors are synthesized but do not reach the plasma membrane, because they lack signals for intracellular transport or do not fold properly; (iii) receptors reach the cell surface, but they fail to bind LDL normally because of a defect in the LDL-binding domain; (iv) receptors reach the cell surface and bind LDL, but they fail to cluster in coated pits because of a defect in their carboxyl-terminal regions.
15. "None of your business" and "I don't talk biochemistry until after breakfast" are appropriate but rude and uninformative answers. A better answer might be: "Although it is true that cholesterol is a precursor to steroid hormones, the rest of the statement is oversimplified. Cholesterol is a component of membranes, and membranes literally define cells, and cells make up tissues. But to say that cholesterol 'makes' cells and tissues is wrong."
16. Statins are competitive inhibitors of HMG-CoA reductase. They are used as drugs to inhibit cholesterol synthesis in patients with high levels of cholesterol.
17. No. Cholesterol is essential for membrane function and as a precursor for bile salts and steroid hormones. The complete lack of cholesterol would be lethal.
18. Deamination of cytidine to uridine changes CAA (Gln) into UAA (stop).
19. The LDL contains apolipoprotein B-100, which binds to an LDL receptor on the cell surface in a region known as a coated pit. On binding, the complex is internalized by endocytosis to form an internal vesicle. The vesicle is separated into two components. One, with the receptor, is transported back to the cell surface and fuses with the membrane, allowing continued use of the receptor. The other vesicle fuses with lysosomes inside the cell. The cholesteryl esters are hydrolyzed, and free cholesterol is made available for cellular use. The LDL protein is hydrolyzed to free amino acids.
20. Benign prostatic hypertrophy can be treated by inhibiting 5α-reductase. Finasteride, the 4-azasteroid analog of dihydrotestosterone, competitively inhibits the reductase but does not act on androgen receptors. Patients taking finasteride have a markedly lower plasma level of dihydrotestosterone and a nearly normal level of testosterone. The prostate gland becomes smaller, whereas testosterone-dependent processes such as fertility, libido, and muscle strength appear to be unaffected.



Finasteride

21. Patients who are most sensitive to debrisoquine have a deficiency of a liver P450 enzyme encoded by a member of the *CYP2* subfamily. This characteristic is inherited as an autosomal recessive trait. The capacity to degrade other drugs may be impaired in people who hydroxylate debrisoquine at a slow rate, because a single P450 enzyme usually handles a broad range of substrates.
22. Many hydrophobic odorants are deactivated by hydroxylation. Molecular oxygen is activated by a cytochrome P450 monooxygenase. NADPH serves as the reductant. One oxygen atom of O_2 goes into the odorant substrate, whereas the other is reduced to water.
23. Recall that dihydrotestosterone is crucial for the development of male characteristics in the embryo. If a pregnant woman were to be exposed to Propecia, the 5α-reductase of the male embryo would be inhibited, which could result in severe developmental abnormalities.
24. The oxygenation reactions catalyzed by the cytochrome P450 family permit greater flexibility in biosynthesis. Because plants are not mobile, they must rely on physical defenses, such as thorns, and chemical defenses, such as toxic alkaloids. The larger P450 array might permit greater biosynthetic versatility.
25. This knowledge would enable clinicians to characterize the likelihood of a patient's having an adverse drug reaction or being susceptible to chemical-induced illnesses. It would also permit a personalized and especially effective drug-treatment regime for diseases such as cancer.
26. The honey bees may be especially sensitive to environmental toxins, including pesticides, because these chemicals are not readily detoxified, owing to the minimal P450 system.
27. The core structure of a steroid is four fused rings: three cyclohexane rings and one cyclopentane ring. In vitamin D, the B ring is split by ultraviolet light.
28. The negatively charged phosphoserine residue interacts with the positively charged protonated histidine residue and decreases its ability to transfer a proton to the thiolate.



His

29. The methyl group is first hydroxylated. The hydroxymethylamine eliminated formaldehyde to form methylamine.
30. Note that a cytidine nucleotide plays the same role in the synthesis of these phosphoglycerides as a uridine nucleotide does in the formation of glycogen (Section 21.4). In all of these biosyntheses, an activated intermediate (UDP-glucose, CDP-diacylglycerol, or CDP-alcohol) is formed from a phosphorylated substrate (glucose 1-phosphate, phosphatidate, or a phosphorylalcohol) and a nucleoside triphosphate (UTP or CTP). The activated intermediate then reacts with a hydroxyl group (the terminus of glycogen, the side chain of serine, or a diacylglycerol).
31. The attachment of isoprenoid side chains confers hydrophobic character. Proteins having such a modification are targeted to membranes.
32. 3-Hydroxy-3-methylglutaryl CoA is also a precursor for ketone-body synthesis. If fuel is needed elsewhere in the body, as might be the case during a fast, 3-hydroxy-3-methylglutaryl CoA is converted into the ketone acetoacetate. If energy needs are met, the liver will synthesize cholesterol.
33. One way in which phosphatidylcholine can be synthesized is by the addition of three methyl groups to phosphatidylethanolamine. The methyl donor is a modified form of methionine, *S*-adenosylmethionine or SAM (Section 24.2).
34. Citrate is transported out of the mitochondria in times of plenty. ATP-citrate lyase yields acetyl CoA and oxaloacetate. The acetyl CoA can then be used to synthesize cholesterol.
35. (a) There is no effect. (b) Because actin is not controlled by cholesterol, the amount isolated should be the same in both experimental groups; a difference would suggest a problem in the RNA isolation. (c) The presence of cholesterol in the diet dramatically reduces the amount of HMG-CoA reductase protein. (d) A common means of regulating the amount of a protein present is to regulate transcription, which is clearly not the case here. (e) The translation of mRNA could be inhibited, and the protein could be rapidly degraded.

Chapter 27

1. Adipose tissue is now known to be an active endocrine organ, secreting signal molecules called adipokines.
2. Caloric homeostasis is the condition in which the energy expenditure of an organism is equal to the energy intake.
3. Leptin and insulin
4. CCK produces a feeling of satiety and stimulates the secretion of digestive enzymes by the pancreas and the secretion of bile salts by the gall bladder. GLP-1 also produces a feeling of satiety; in addition, it potentiates the glucose-induced secretion of insulin by the β cells of the pancreas.
5. Obviously, something is amiss. Although the answer is not known, the leptin-signaling pathway appears to be inhibited by suppressors of cytokine signaling, the regulatory proteins.
6. 1: a, b; 2: f; 3: c, d, f; 4: c, d; 5: c; 6: f; 7: e; 8: e; 9: e.
7. Phosphorylation of dietary glucose after it enters the liver; gluconeogenesis; glycogen breakdown.
8. Type 1 diabetes is due to autoimmune destruction of the insulin-producing cells of the pancreas. Type 1 diabetes is also called insulin-dependent diabetes because affected people require insulin to survive. Type 2 diabetes is characterized by insulin resistance. Insulin is produced, but the tissues that should respond to insulin, such as muscle, do not.
9. Leptin stimulates processes impaired in diabetes. For instance, leptin stimulates fatty acid oxidation, inhibits triacylglycerol synthesis, and increases the sensitivity of muscle and the liver to insulin.
10. (a) A watt is equal to 1 joule (J) per second (0.239 calorie per second). Hence, 70 W is equivalent to 0.07 kJ s^{-1} (0.017 kcal s^{-1}).
(b) A watt is a current of 1 ampere (A) across a potential of 1 volt (V). For simplicity, let us assume that all the electron flow is from NADH to O_2 (a potential drop of 1.14 V). Hence, the current is 61.4 A, which corresponds to 3.86×10^{20} electrons per second (1 A = 1 coulomb s^{-1} = 6.28×10^{18} charge s^{-1}).
(c) About 2.5 molecules of ATP are formed per molecule of NADH oxidized (two electrons). Hence, 1 molecule of ATP is formed per 0.8 electron transferred. A flow of 3.86×10^{20} electrons per second therefore leads to the generation of 4.83×10^{20} molecules of ATP per second, or 0.80 mmol s^{-1}.
(d) The molecular weight of ATP is 507. The total body content of ATP of 50 g is equal to 0.099 mol. Hence, ATP turns over about once in 125 seconds when the body is at rest.
11. (a) The stoichiometry of the complete oxidation of glucose is

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

and that of tripalmitoylglycerol is

$$C_{51}H_{98}O_2 + 72.5\ O_2 \longrightarrow 51\ CO_2 + 49\ H_2O$$

Hence, the RQ values are 1.0 and 0.703, respectively.
(b) An RQ value reveals the relative use of carbohydrates and fats as fuels. The RQ of a marathon runner typically decreases from 0.97 to 0.77 in the course of a race. The lowering of the RQ indicates the shift in fuel from carbohydrates to fat.
12. One gram of glucose (molecular weight 180.2) is equal to 5.55 mmol, and one gram of tripalmitoylglycerol (molecular weight 807.3) is equal to 1.24 mmol. The reaction stoichiometries (see Problem 11) indicate that 6 mol of H_2O is produced per mole of glucose oxidized, and 49 mol of H_2O is produced per mole of tripalmitoylglycerol oxidized. Hence, the H_2O yields per gram of fuel are 33.3 mmol (0.6 g) for glucose and 60.8 mmol (1.09 g) for tripalmitoylglycerol. Thus, complete oxidation of this fat gives 1.82 times as much water as does glucose. Another advantage of triacylglycerols is that they can be stored in essentially anhydrous form, whereas glucose is stored as glycogen, a highly hydrated polymer. A hump consisting mainly of glycogen would be an intolerable burden—far more than the straw that broke the camel's back.
13. The starved–fed cycle is the nightly hormonal cycle that humans experience during sleep and on eating. The cycle maintains adequate amounts of blood glucose. The starved part—sleep—is characterized by increased glucagon secretion and decreased insulin secretion. After a meal, glucagon concentration falls and insulin concentration rises.
14. Ethanol is oxidized to yield acetaldehyde by alcohol dehydrogenase, which is subsequently oxidized to acetate acetaldehyde. Ethanol is also metabolized to acetaldehyde by the MEOS, with the subsequent depletion of NADPH.
15. First, fatty liver develops owing to the increased amounts of NADH that inhibit fatty acid oxidation and stimulate fatty acid

synthesis. Second, alcoholic hepatitis begins owing to oxidative damage and damage due to excess acetaldehyde that results in cell death. Finally, fibrous tissues form, creating scars that impair blood flow and biochemical function. Ammonia cannot be converted into urea, and its toxicity leads to coma and death.

16. A typical macadamia nut has a mass of about 2 g. Because it consists mainly of fats (~37 kJ g^{-1}, ~9 kcal g^{-1}), a nut has a value of about 75 kJ (18 kcal). The ingestion of 10 nuts results in an intake of about 753 kJ (180 kcal). As stated in the answer to Problem 10, a power consumption of 1 W corresponds to 1 J s^{-1} (0.239 cal s^{-1}), and so 400-W running requires 0.4 kJ s^{-1} (0.0956 kcal s^{-1}). Hence, a person would have to run 1882 s, or about 31 minutes, to spend the calories provided by 10 nuts.

17. A high blood-glucose level triggers the secretion of insulin, which stimulates the synthesis of glycogen and triacylglycerols. A high insulin level would impede the mobilization of fuel reserves during the marathon.

18. A lack of adipose tissue leads to an accumulation of fats in the muscle, with the generation of insulin resistance. The experiment shows that adipokines secreted by the adipose tissue, here leptin, facilitate in some fashion the action of insulin in muscle.

19. Such a mutation would increase the phosphorylation of the insulin receptor and IRS in muscle and would improve insulin sensitivity. Indeed, PTP1B is an attractive therapeutic target for type 2 diabetes.

20. Lipid mobilization can be so rapid that it exceeds the ability of the liver to oxidize the lipids or convert them into ketone bodies. The excess is reesterified and released into the blood as VLDLs.

21. A role of the liver is to provide glucose for other tissues. In the liver, glycolysis is used not for energy production but for biosynthetic purposes. Consequently, in the presence of glucagon, liver glycolysis stops so that the glucose can be released into the blood.

22. The urea cycle and gluconeogenesis

23. (a) Insulin inhibits lipid utilization.
(b) Insulin stimulates protein synthesis, but there are no amino acids in the children's diet. Moreover, insulin inhibits protein breakdown. Consequently, muscle proteins cannot be broken down and used for the synthesis of essential proteins.
(c) Because proteins cannot be synthesized, blood osmolarity is too low. Consequently, fluid leaves the blood. An especially important protein for maintaining blood osmolarity is albumin.

24. During strenuous exercise, muscle converts glucose into pyruvate through glycolysis. Some of the pyruvate is processed by cellular respiration. However, some of it is converted into lactate and released into the blood. The liver takes up the lactate and converts it into glucose through gluconeogenesis. Muscle may process the carbon skeletons of branched-chain amino acids aerobically. The nitrogens of these amino acids are transferred to pyruvate to form alanine, which is released into the blood and taken up by the liver. After the transamination of the amino group to α-ketoglutarate, the resulting pyruvate is converted into glucose. Finally, muscle glycogen may be mobilized, and the released glucose can be used by muscle.

25. This conversion allows muscle to function anaerobically. NAD^+ is regenerated when pyruvate is reduced to lactate, and so energy can continue to be extracted from glucose during strenuous exercise. The liver converts the lactate into glucose.

26. Fatty acids and glucose, respectively.

27. This practice is called carbo-loading. Depleting the glycogen stores will initially cause the muscles to synthesis a large amount of glycogen when dietary carbohydrates are provided and will lead to the supercompensation of glycogen stores.

28. The oxygen consumption at the end of exercise is used to replenish ATP and creatine phosphate and to oxidize any lactate produced.

29. Oxygen is used in oxidative phosphorylation to resynthesize ATP and creatine phosphate. The liver converts lactate released by the muscle into glucose. Blood must be circulated to return the body temperature to normal, and so the heart cannot return to its resting rate immediately. Hemoglobin must be reoxygenated to replace the oxygen used in exercise. The muscles that power breathing must continue working at the same time as the exercised muscles are returning to resting states. In essence, all the biochemical systems activated in intense exercise need increased oxygen to return to the resting state.

30. Ethanol may replace water that is hydrogen bonded to proteins and membrane surfaces. This alteration of the hydration state of the protein would alter its conformation and hence function. Ethanol may also alter phospholipid packing in membranes. The two effects suggest that integral membrane proteins would be most sensitive to ethanol, as indeed seems to be the case.

31. Cells from the type I fiber would be rich in mitochondria, whereas those of the type II fiber would have few mitochondria.

32. (a) The ATP expended during this race amounts to about 8380 kg, or 18,400 pounds. (b) The cyclist would need about $1,260,000,000 to complete the race.

33. 55 pounds = 25 kg = 25,000 g = total weight gain

$$40 \text{ years} \times 365 \text{ days year}^{-1} = 14{,}600 \text{ days}$$

$$25{,}000 \text{ g}/14{,}600 \text{ days} = 1.7 \text{ g day}^{-1}$$

which is equivalent to an extra pat of butter per day. Her BMI is 26.5, and she would be considered overweight but not obese.

34. Exercise greatly enhances the ATP needs of muscle cells. To more efficiently meet these needs, more mitochondria are synthesized.

35. The inability of muscle mitochondria to process all of the fatty acids produced by overnutrition leads to excessive levels of diacylglycerol and ceramide in the muscle cytoplasm. These second-messenger molecules activate enzymes that impair insulin signaling.

36. Both are due to a lack of thiamine (vitamin B_1). Thiamine, which is sometimes called aneurin, is required most notably for the proper functioning of pyruvate dehydrogenase.

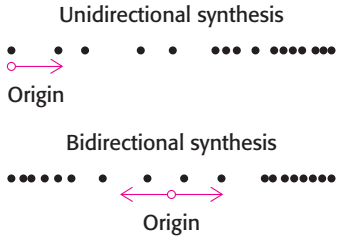
37. (a) Red blood cells always produce lactate, and fast-twitch muscle fibers (see Problem 31) also produce a large amount of lactate.
(b) At that point, the athlete is beginning to move into anaerobic exercise, in which most energy is produced by anaerobic glycolysis.
(c) The lactate threshold is essentially the point at which the athlete switches from aerobic exercise, which can be done for extended periods, to anaerobic exercise, essentially sprinting, which can be done for only short periods. The idea is to race at the extreme of his or her aerobic capacity until the finish line is in sight and then to switch to anaerobic.
(d) Training increases the amount of blood vessels and the number of muscle mitochondria. Together, they increase the ability to process glucose aerobically. Consequently, a greater effort can be expended before the switch to anaerobic energy production.

Chapter 28

1. DNA polymerase I uses deoxyribonucleoside triphosphates; pyrophosphate is the leaving group. DNA ligase uses DNA–adenylate (AMP joined to the 5′-phosphoryl group) as a reaction partner; AMP is the leaving group. Topoisomerase I uses a DNA–tyrosyl intermediate (5′-phosphoryl group linked to the phenolic OH group); the tyrosine residue of the enzyme is the leaving group.

2. Positive supercoiling resists the unwinding of DNA. The melting temperature of DNA increases in proceeding from negatively supercoiled to relaxed to positively supercoiled DNA. Positive supercoiling is probably an adaptation to high temperature.

3. The nucleotides used for DNA synthesis have the triphosphate attached to the 5′-hydroxyl group with free 3′-hydroxyl groups. Such nucleotides can be utilized only for 5′-to-3′ DNA synthesis.
4. DNA replication requires RNA primers. Without appropriate ribonucleotides, such primers cannot be synthesized.
5. This close contact prevents the incorporation of ribonucleotides rather than 2′-deoxyribonucleotides.
6. (a) 96.2 revolutions per second (1000 nucleotides per second divided by 10.4 nucleotides per turn for B-DNA gives 96.2 rps).
(b) 0.34 $\mu m\ s^{-1}$ (1000 nucleotides per second corresponds to 3400 $Å\ s^{-1}$ because the axial distance between nucleotides in B-DNA is 3.4 Å).
7. Eventually, the DNA would become so tightly wound that movement of the replication complex would be energetically impossible.
8. Linking number $Lk = Tw + Wr = 48 + 3 = 51$. If $Tw = 50$, the $Wr = 1$.
9. A hallmark of most cancer cells is prolific cell division, which requires DNA replication. If the telomerase were not activated, the chromosomes would shorten until they became nonfunctional, leading to cell death.
10. No.
11. Treat the DNA briefly with endonuclease to occasionally nick each strand. Add the polymerase with the radioactive dNTPs. At the broken bond, or nick, the polymerase will degrade the existing strand with its 5′ ⟶ 3′ exonuclease activity and replace it with a radioactive complementary copy by using its polymerase activity. This reaction scheme is referred to as nick translation because the nick is moved, or translated, along the DNA molecule without ever becoming sealed.
12. If replication were unidirectional, tracks with a low grain density at one end and a high grain density at the other end would be seen. On the other hand, if replication were bidirectional, the middle of a track would have a low density, as shown in the diagram below. For *E. coli*, the grain tracks are denser on both ends than in the middle, indicating that replication is bidirectional.



13. (a) Pro (CCC), Ser (UCC), Leu (CUC), and Phe (UUC). Alternatively, the last base of each of these codons could be U.
(b) Nitrous acid.
14. Potentially deleterious side reactions are prevented. The enzyme itself might be damaged by light if it could be activated by light in the absence of bound DNA harboring a pyrimidine dimer.
15. The free DNA ends that appear in the absence of telomeres are repaired by DNA fusion.
16. The free energy of ATP hydrolysis under standard conditions is $-30.5\ kJ\ mol^{-1}$ ($-7.3\ kcal\ mol^{-1}$). In principle, it could be used to break three base pairs.
17. The oxidation of guanine could lead to DNA repair: DNA strand cleavage could allow looping out of the triplet repeat regions and triplet expansion.
18. The release of DNA topoisomerase II after the enzyme has acted on its DNA substrate requires ATP hydrolysis. Negative supercoiling requires only the binding of ATP, not its hydrolysis.
19. (a) Size; the top is relaxed and the bottom is supercoiled DNA.
(b) Topoisomers. (c) The DNA is becoming progressively more unwound, or relaxed, and thus slower moving.
20. (a) It was used to determine the number of spontaneous revertants—that is, the background mutation rate.
(b) To firmly establish that the system was working. A known mutagen's failure to produce revertants would indicate that something was wrong with the experimental system.
(c) The chemical itself has little mutagenic ability but is apparently activated into a mutagen by the liver homogenate.
(d) Cytochrome P450 system.

Chapter 29

1. The sequence of the coding (+, sense) strand is

5′-ATGGGGAACAGCAAGAGTGGGGCCCTGTCCAAGGAG-3′

and the sequence of template (–, antisense) strand is

3′-TACCCCTTGTCGTTCTCACCCCGGGACAGGTTCCTC-5′

2. An error will affect only one molecule of mRNA of many synthesized from a gene. In addition, the errors do not become a permanent part of the genomic information.
3. At any given instant, only a fraction of the genome (total DNA) is being transcribed. Consequently, speed is not essential.
4. The active sites are related by convergent evolution.
5. Heparin, a glycosaminoglycan, is highly anionic. Its negative charges, like the phosphodiester bridges of DNA templates, allow it to bind to lysine and arginine residues of RNA polymerase.
6. This mutant σ will competitively inhibit the binding of holoenzyme and prevent the specific initiation of RNA chains at promoter sites.
7. The core enzyme without σ binds more tightly to the DNA template than does the holoenzyme. The retention of σ after chain initiation would make the mutant RNA polymerase less processive. Hence, RNA synthesis would be much slower than normal.
8. A 100-kd protein contains about 910 residues, which are encoded by 2730 nucleotides. At a maximal transcription rate of 50 nucleotides per second, the mRNA would be synthesized in 54.6 s.
9. The RNA polymerase slides along the DNA rapidly rather than simply diffusing through three-dimensional space.
10. The start site is in red type:

5′-GCCGTTGACACCGTTCGGCGATCGATCCGCTATAATGTGTGGATCCGCTT-3′

11. Initiation at strong promoters takes place every 2 s. In this interval, 100 nucleotides are transcribed. Hence, centers of transcription bubbles are 34 nm (340 Å) apart.
12. (a) The lowest band on the gel will be that of strand 3 alone (*i*), whereas the highest will be that of stands 1, 2, and 3 and core polymerase (*v*). Band *ii* will be at the same position as band *i* because the RNA is not complementary to the nontemplate strand, whereas band *iii* will be higher because a complex is formed between RNA and the template strand. Band *iv* will be higher than the others because strand 1 is complexed to 2, and strand 2 is complexed to 3. Band *v* is the highest because core polymerase associates with the three strands.
(b) None, because rifampicin acts before the formation of the open complex.

(c) RNA polymerase is processive. When the template is bound, heparin cannot enter the DNA-binding site.
(d) When GTP is absent, synthesis stops when the first cytosine residue downstream of the bubble is encountered in the template strand. In contrast, with all four nucleoside triphosphates present, synthesis will continue to the end of the template.
13. RNA polymerase must backtrack before cleavage, leading to dinucleotide products.
14. The base-pairing energy of the di- and trinucleotide DNA–RNA hybrids formed at the very beginning of transcription is not sufficient to prevent strand separation and loss of product.
15. (a) Because cordycepin lacks a 3′-OH group, it cannot participate in 3′ ⟶ 5′ bond formation. (b) Because the poly(A) tail is a long stretch of adenosine nucleotides, the likelihood that a molecule of cordycepin would become incorporated is higher than with most RNA. (c) Yes, it must be converted into cordycepin 5′-triphosphate.
16. There are $2^8 = 256$ possible products.
17. The relation between the −10 and −35 sequences could be affected by torsional strain. The fact that topoisomerase II introduces negative supercoils in DNA prevents this enzyme from overstimulating the expression of its own gene.
18. Ser-Ile-Phe-His-Pro-Stop
19. A mutation that disrupted the normal AAUAAA recognition sequence for the endonuclease could account for this finding. In fact, a change from U to C in this sequence caused this defect in a thalassemic patient. Cleavage was at the AAUAAA 900 nucleotides downstream of this mutant AACAAA site.
20. One possibility is that the 3′ end of the poly(U) donor strand cleaves the phosphodiester bond on the 5′ side of the insertion site. The newly formed 3′ terminus of the acceptor strand then cleaves the poly(U) strand on the 5′ side of the nucleotide that initiated the attack. In other words, a uridine residue could be added by two transesterification reactions. This postulated mechanism is similar to the one in RNA splicing.
21. Alternative splicing and RNA editing. Covalent modification of the proteins subsequent to synthesis further enhances the complexity.
22. Attach an oligo(dT) or oligo(U) sequence to an inert support to create an affinity column. When RNA is passed through the column, only poly(A)-containing RNA will be retained.
23. (a) Different amounts of RNA are present for the various genes.
(b) Although all of the tissues have the same genes, the genes are expressed to different extents in different tissues.
(c) These genes are called housekeeping genes—genes that most tissues express. They might include genes for glycolysis or citric acid cycle enzymes.
(d) The point of the experiment is to determine which genes are initiated in vivo. The initiation inhibitor is added to prevent initiation at start sites that may have been activated during the isolation of the nuclei.
24. DNA is the single strand that forms the trunk of the tree. Strands of increasing length are RNA molecules; the beginning of transcription is where growing chains are the smallest; the end of transcription is where chain growth stops. Direction is left to right. Many enzymes are actively transcribing each gene.

Chapter 30

1. The Oxford English Dictionary defines translation as the action or process of turning from one language into another. Protein synthesis converts nucleic acid sequence information into amino acid sequence information.
2. An error frequency of 1 incorrect amino acid every 10^4 incorporations allows for the rapid and accurate synthesis of proteins as large as 1000 amino acids. Higher error rates would result in too many defective proteins. Lower error rates would likely slow the rate of protein synthesis without a significant gain in accuracy.
3. (i) Each is a single chain. (ii) They contain unusual bases. (iii) Approximately half of the bases are base-paired to form double helices. (iv) The 5′ end is phosphorylated and is usually pG. (v) The amino acid is attached to the hydroxyl group of the A residue of the CCA sequence at the 3′ end of the tRNA. (vi) The anticodon is located in a loop near the center of the tRNA sequence. (vii) The molecules are L-shaped.
4. First is the formation of the aminoacyl adenylate, which then reacts with the tRNA to form the aminoacyl-tRNA. Both steps are catalyzed by aminoacyl-tRNA synthetase.
5. Unique features are required so that the aminoacyl-tRNA synthetases can distinguish among the tRNAs and attach the correct amino acid to the proper tRNA. Common features are required because all tRNAs must interact with the same protein-synthesizing machinery.
6. An activated amino acid is one linked to the appropriate tRNA.
7. (a) No; (b) no; (c) yes.
8. The ATP is cleaved to AMP and PP_i. Consequently, a second ATP is required to convert AMP into ADP, the substrate for oxidative phosphorylation.
9. Amino acids larger than the correct amino acid cannot fit into the active site of the tRNA. Smaller but incorrect amino acids that become attached to the tRNA fit into the editing site and are cleaved from the tRNA.
10. Recognition sites on both faces of the tRNAs may be required to uniquely identify the 20 different tRNAs.
11. The first two bases in a codon form Watson–Crick base pairs that are checked for fidelity by bases of the 16S rRNA. The third base is not inspected for accuracy, and so some variation is tolerated.
12. Four bands: light, heavy, a hybrid of light 30S and heavy 50S, and a hybrid of heavy 30S and light 50S
13. Two hundred molecules of ATP are converted into 200 AMP + 400 P_i to activate the 200 amino acids, which is equivalent to 400 molecules of ATP. One molecule of GTP is required for initiation, and 398 molecules of GTP are needed to form 199 peptide bonds.
14. (a, d, and e) Type 2; (b, c, and f) type 1.
15. The reading frame is a set of contiguous, nonoverlapping three-nucleotide codons that begins with a start codon and ends with a stop codon.
16. A mutation caused by the insertion of an extra base can be suppressed by a tRNA that contains a fourth base in its anticodon. For example, UUUC rather than UUU is read as the codon for phenylalanine by a tRNA that contains 3′-AAAG-5′ as its anticodon.
17. One approach is to synthesize a tRNA that is acylated with a reactive amino acid analog. For example, bromoacetyl-phenylalanyl-tRNA is an affinity-labeling reagent for the P site of *E. coli* ribosomes.
18. The sequence GAGGU is complementary to a sequence of five bases at the 3′ end of 16S rRNA and is located several bases upstream of an AUG start codon. Hence, this region is a start signal for protein synthesis. The replacement of G by A would be expected to weaken the interaction of this mRNA with the 16S rRNA and thereby diminish its effectiveness as an initiation signal. In fact, this mutation results in a 10-fold decrease in the rate of synthesis of the protein specified by this mRNA.
19. The peptide would be Phe-Cys-His-Val-Ala-Ala. The codons UGC and UGU encode cysteine but, because the

cysteine was modified to alanine, alanine is incorporated in place of cysteine.
20. Proteins are synthesized from the amino to the carboxyl end on ribosomes, whereas they are synthesized in the reverse direction in the solid-phase method. The activated intermediate in ribosomal synthesis is an aminoacyl-tRNA; in the solid-phase method, it is the adduct of the amino acid and dicyclohexylcarbodiimide.
21. The error rates of DNA, RNA, and protein synthesis are of the order of 10^{-10}, 10^{-5}, and 10^{-4}, respectively, per nucleotide (or amino acid) incorporated. The fidelity of all three processes depends on the precision of base-pairing to the DNA or mRNA template. Few errors are corrected in RNA synthesis. In contrast, the fidelity of DNA synthesis is markedly increased by the $3' \longrightarrow 5'$ proofreading nuclease activity and by postreplicative repair. In protein synthesis, the mischarging of some tRNAs is corrected by the hydrolytic action of aminoacyl-tRNA synthetase. Proofreading also takes place when aminoacyl-tRNA occupies the A site on the ribosome; the GTPase activity of EF-Tu sets the pace of this final stage of editing.
22. GTP is not hydrolyzed until aminoacyl-tRNA is delivered to the A site of the ribosome. An earlier hydrolysis of GTP would be wasteful because EF-Tu–GDP has little affinity for aminoacyl-tRNA.
23. The translation of an mRNA molecule can be blocked by antisense RNA, an RNA molecule with the complementary sequence. The antisense–sense RNA duplex cannot serve as a template for translation; single-stranded mRNA is required. Furthermore, the antisense–sense duplex is degraded by nucleases. Antisense RNA added to the external medium is spontaneously taken up by many cells. A precise quantity can be delivered by microinjection. Alternatively, a plasmid encoding the antisense RNA can be introduced into target cells.
24. (a) A_5. (b) $A_5 > A_4 > A_3 > A_2$. (c) Synthesis is from the amino terminus to the carboxyl terminus.
25. These enzymes convert nucleic acid information into protein information by interpreting the tRNA and linking it to the proper amino acid.
26. The rate would fall because the elongation step requires that the GTP be hydrolyzed before any further elongation can take place.
27. Protein factors modulate the initiation of protein synthesis. The role of IF1 and IF3 is to prevent premature binding of the 30S and 50S ribosomal subunits, whereas IF2 delivers Met-$tRNA_f$ to the ribosome. Protein factors are also required for elongation (EF-G and EF-Tu), for termination (release factors, RFs), and for ribosome dissociation (ribosome release factors, RRFs).
28. The signal sequence, signal-recognition particle (SRP), the SRP receptor, and the translocon.
29. The formation of peptide bonds, which in turn are powered by the hydrolysis of the aminoacyl-tRNAs.
30. The Shine–Dalgarno sequence of the mRNA base-pairs with a part of the 16S rRNA of the 30S subunit, which positions the subunit so that the initiator AUG is recognized.
31.

	Prokaryote	**Eukaryote**
Ribosome size	60S	80S
mRNA	polycistronic	Not polycistronic
Initiation	Shine–Dalgarno is required	First AUG is used
Protein factors	Required	Many more required
Relation to transcription	Translation can start before transcription is completed	Transcription and translation are spatially separated
First amino acid	fMet	Met

32. The SRP binds to the signal sequence and inhibits further translation. The SRP ushers the inhibited ribosome to the ER, where it interacts with the SRP receptor (SR). The SRP–SR complex binds the translocon and simultaneously hydrolyzes GTP. On GTP hydrolysis, SRP and SR dissociate from each other and from the ribosome. Protein synthesis resumes and the nascent protein is channeled through the translocon.
33. The alternative would be to have a single ribosome translating a single mRNA molecule. The use of polysomes allows more protein synthesis per mRNA molecule in a given period of time and thus the production of more protein.
34. (a) 1, 2, 3, 5, 6, 10; (b) 1, 2, 7, 8,; (c) 1, 4, 8, 9.
35. Transfer RNAs have roles in several recognition processes. A tRNA must be recognized by the appropriate aminoacyl-tRNA synthetase, and the tRNA must interact with the ribosome and, in particular, with the peptidyl transferase.
36. The nucleophile is the amino group of the aminoacyl-tRNA. This amino group attacks the carbonyl group of the ester of peptidyl-tRNA to form a tetrahedral intermediate, which eliminates the tRNA alcohol to form a new peptide bond.
37. The aminoacyl-tRNA can be initially synthesized. However, the side-chain amino group attacks the ester linkage to form a six-membered amide, releasing the tRNA.
38. EF-Ts catalyzes the exchange of GTP for GDP bound to EF-Tu. In G-protein cascades, an activated 7TM receptor catalyzes GTP–GDP exchange in a G protein.
39. The α subunits of G proteins are inhibited by a similar mechanism in cholera and whooping cough (Section 14.5).
40. Glu-$tRNA^{Gln}$ is formed by misacylation. The activated glutamate is subsequently amidated to form Gln-$tRNA^{Gln}$. Ways in which glutamine is formed from glutamate were discussed in Section 24.2. In regard to *H. pylori,* a specific enzyme, Glu-$tRNA^{Gln}$ amidotransferase, catalyzes the following reaction:

$$\text{Gln} + \text{Glu-tRNA}^{\text{Gln}} + \text{ATP} \longrightarrow \text{Gln-tRNA}^{\text{Gln}} + \text{glu} + \text{ADP} + P_i$$

Glu-$tRNA^{Glu}$ is not a substrate for the enzyme; so the transferase must also recognize aspects of the structure of $tRNA^{Gln}$.
41. The primary structure determines the three-dimensional structure of the protein. Thus, the final phase of information transfer from DNA to RNA to protein synthesis is the folding of the protein into its functional state.
42. (a) eIF-4H has two effects: (1) the extent of unwinding is increased and (2) the rate of unwinding is increased, as indicated by the increased rise in activity at early reaction times.
(b) To firmly establish that the effect of eIF-H4 was not due to any inherent helicase activity.
(c) Half-maximal activity was achieved at 0.11 μM of eIF-4H. Therefore, maximal stimulation would be achieved at a ratio of 1:1.
(d) eIF-4H enhances the rate of unwinding of all helices, but the effect is greater as the helices increase in stability.
(e) The results in graph C suggest that eIF-4H increases the processivity.
43. (a) The three peaks represent, from left to right, the 40S ribosomal subunit, the 60S ribosomal subunit, and the 80S ribosome.
(b) Not only are ribosomal subunits and the 80S ribosome present, but polysomes of various lengths also are apparent. The individual peaks in the polysome region represent polysomes of discrete length.
(c) The treatment significantly inhibited the number of polysomes while increasing the number of free ribosomal subunits. This outcome could be due to inhibited protein-synthesis initiation or inhibited transcription.

Chapter 31

1. (a) Cells will express β-galactosidase, *lac* permease, and thiogalactoside transacetylase even in the absence of lactose. (b) Cells will express β-galactosidase, *lac* permease, and thiogalactoside transacetylase even in the absence of lactose. (c) The levels of catabolic enzymes such as β-galactosidase and arabinose isomerase will remain low even at low levels of glucose.
2. The concentration is $1/(6 \times 10^{23})$ moles per 10^{-15} liter $= 1.7 \times 10^{-9}$ M. Because $K_d = 10^{-13}$ M, the single molecule should be bound to its specific binding site.
3. The number of possible 8-bp sites is $4^8 = 65{,}536$. In a genome of 4.6×10^6 base pairs, the average site should appear $(4.6 \times 10^6)/65{,}536 = 70$ times. Each 10-bp site should appear 4 times. Each 12-bp site should appear 0.27 times (many 12-bp sites will not appear at all).
4. The *lac* repressor does not bind DNA when the repressor is bound to a small molecule (the inducer), whereas the *pur* repressor binds DNA only when the repressor is bound to a small molecule (the corepressor). The *E. coli* genome contains only a single *lac* repressor-binding region, whereas it has many sites for the *pur* repressor
5. Anti-inducers bind to the conformation of repressors, such as the *lac* repressor, that are capable of binding DNA. They occupy a site that overlaps that for the inducer and, therefore, compete for binding to the repressor.
6. The inverted repeat may be a binding site for a dimeric DNA-binding protein or it may correspond to a stem-loop structure in the encoded RNA.
7. Bacteriophage λ would be more likely to enter the lytic phase because the cooperative binding of the λ repressor to O_R2 and O_R1, which supports the lysogenic pathway, would be disrupted.
8. λ repressor gene −10 region GATTTA −35 region TAGATA
   Cro gene −10 region TAATGG −35 region TTGACT
There are four differences in the −10 region and three differences in the −35 region.
9. Increased Cro concentration reduces the expression of the λ repressor gene. Increased λ repressor concentration reduces the expression of the Cro gene. At low λ repressor concentration, increased λ repressor concentration increases the expression of the λ repressor gene. At higher λ repressor concentrations, increased λ repressor concentration decreases the expression of the λ repressor gene.
10. Normally, bacterial mRNAs have a leader sequence in which a Shine–Delgarno sequence precedes the AUG start codon. The absence of a leader would be expected to lead to inefficient translation.
11. Add each compound to a culture of *V. fischeri* at low density and look for the development of luminescence.
12. ACC, 7; ACA, 1, ACU, 0; ACG, 0.
13. The reaction takes place with overall retention of configuration. Each step likely takes place with inversion of configuration, which suggests that the reaction consists of two (or some other even number of) steps. A possible mechanism is nucleophilic attack by the carboxylate group of Glu 537 on the C-1 carbon atom of the galactose moiety within glucose, releasing glucose and forming an intermediate with the galactose linked to the enzyme through an ester linkage. Water then attacks this carbon atom, displacing the glutamate carboxylate and releasing galactose.
14. The binding appears to be half complete at a concentration of λ repressor near 3.7 nM. Thus, K_d is approximately 3.7 nM and $\Delta G^\circ = -48$ kJ/mol (−11 kcal/mol) at 298 K.

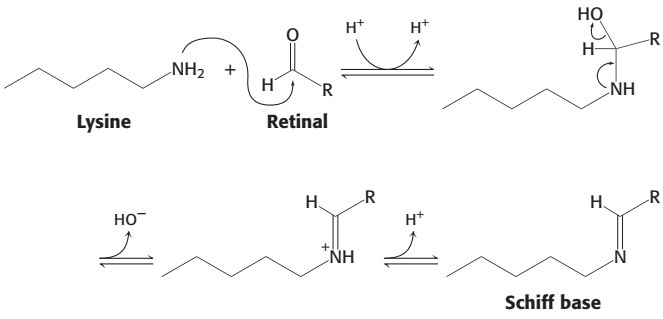
Chapter 32

1. The distribution of charged amino acids is H2A (13 K, 13 R, 2 D, 7 E, charge = +17), H2B (20 K, 8 R, 3 D, 7 E, charge = +18), H3 (13 K, 18 R, 4 D, 7 E, charge = +20), H4 (11 K, 14 R, 3 D, 4 E, charge = +18). The total charge of the histone octamer is estimated to be $2 \times (17 + 18 + 20 + 18) = +146$. The total charge on 150 base pairs of DNA is −300. Thus, the histone octamer neutralizes approximately one-half of the charge.
2. The presence of a particular DNA fragment could be detected by hybridization, by PCR, or by direct sequencing.
3. The total length of the DNA is estimated to be 145 bp × 3.4 Å/bp = 493 Å, which represents 1.75 turns or $1.75 \times 2\pi r = 11.0r$. Thus, the radius is estimated to be $r = 493$ Å/11.0 = 44.8 Å.
4. 5-Azacytidine cannot be methylated. Some genes, normally repressed by methylation, will be active.
5. Proteins containing these domains will be targeted to methylated DNA in repressed promoter regions. They would likely bind in the major groove because that is where the methyl group is located.
6. Gene expression is not expected to respond to the presence of estrogen. However, genes for which expression normally responds to estrogen will respond to the presence of progesterone.
7. The acetylation of lysine will reduce the charge from +1 to 0. The methylation of lysine will not reduce the charge.
8. On the basis of the pattern of cysteine and histidine residues, this region appears to contain three zinc-finger domains.
9. 10/4000 = 0.25%. 0.25% of 12 Mb = 30 kilobase pairs.
10. The addition of an IRE to the 5′ end of the mRNA is expected to block translation in the absence of iron. The addition of an IRE to the 3′ end of the mRNA is not expected to block translation, but it might affect mRNA stability.
11. The sequences of all of the mRNAs would be searched for sequences that are fully or nearly complementary to the sequence of the miRNA. These sequences would be candidates for regulation by this mRNA.
12. The amino group of the lysine residue, formed from the protonated form by a base, attacks the carbonyl group of acetyl CoA to generate a tetrahedral intermediate. This intermediate collapses to form the amide bond and release CoA.
13. In mouse DNA, most of the *Hpa*II sites are methylated and therefore not cut by the enzyme, resulting in large fragments. Some small fragments are produced from CpG islands that are unmethylated. For *Drosophila* and *E. coli* DNA, there is no methylation and all sites are cut.

Chapter 33

1. The transgenic nematode would avoid the compound. The identity of the ligand is determined by the receptor, whereas the behavioral response is dictated by the neuron in which the receptor is expressed.
2. Only a mixture of compounds C_5-COOH and HOOC-C_7-COOH is predicted to yield this pattern.
3. Bitter and sweet sensations are mediated by G proteins coupled to 7TM receptors, leading to millisecond time resolution. Salty and sour sensations are mediated directly by ion channels, which may lead to faster time resolution.
4. Sound travels 0.15 m in 428 μs. The human hearing system is capable of sensing time differences of close to a microsecond, and so the difference in arrival times at the two ears is substantial. A system based on G proteins is unlikely to be able to reliably distinguish between signals arriving at the two ears, because G proteins typically respond in milliseconds.
5. If a plant tastes bitter, animals will avoid eating it even if it is nontoxic, which may provide a selective advantage to the plant.
6. Using mice in which either the gene for T1R1 or the gene for T1R3 has been disrupted, test the taste responses of these mice to glutamate, aspartate, and a wide variety of other amino acids.

7. These women have four functional color receptors: blue, red, green, and a red–green hybrid. The additional color receptor allows some colors that appear identical to most people to be distinguished.
8. 380 (one for each receptor); there are $(380 \times 379)/2! = 72{,}010$ combinations of two receptors; $(380 \times 379 \times 378)/3! = 9{,}073{,}260$ combinations of three receptors.
9. The absorption of light converts 11-*cis*-retinal into all-*trans*-retinal.
10. These compounds are enantiomers and must bind to protein receptors to elicit a smell. Even these subtle structural differences can affect relative receptor binding affinities and, hence, the elicited odor.
11. Vision: cGMP-gated channel; taste: amiloride-sensitive sodium channel; hearing; tip-link channel.
12. For all senses, ATP hydrolysis is required to generate and maintain ion gradients and membrane potential. Olfaction: ATP is required for the synthesis of cAMP. Gustation: ATP is required for the synthesis of cyclic nucleotides, and GTP is required for the action of gustducin in the detection of bitter and sweet tastes. Vision: GTP is required for the synthesis of cGMP and for the action of transducin. Hearing and touch: ATP hydrolysis is required to generate and maintain ion gradients and membrane potential and may be required for other roles as well.
13.



Chapter 34

1. The innate immune system responds rapidly to common features present in many pathogens. The genes for the innate immune system's key molecules are expressed without substantial modification. In contrast, the adaptive immune system responds to specific features present only in a given pathogen. Its genes undergo significant rearrangement and mutation to enable specific recogition of a vast number of potential binding surfaces.
2. VJ and V(D)J recombination; variability in segment joining by the action of terminal deoxyribonucleotidyl transferase; somatic mutation.
3. *Affinity* refers to the strength of a single interaction; avidity refers to the cumulative strength of multiple independent binding interactions. *Avidity* may play a significant role in the interaction between IgM and antigen because this immunoglobulin class features 10 binding sites.
4. The intracellular signaling domain common to each of the TLRs is responsible for docking other proteins and reporting that a targeted pathogen-associated molecular pattern (PAMP), such as LPS, has been detected. If a mutation within this domain interfered with the intracellular docking and signal transduction, then TLR-4 would not respond to LPS.
5. Viruses that contain dsRNA genomes would be expected to stimulate a TLR-3-mediated immune response.
6. (a) $\Delta G^{\circ\prime} = -37$ kJ mol^{-1} (-8.9 kcal mol^{-1})
(b) $K_a = 3.3 \times 10^6$ M^{-1}
(c) $k_{on} = 4 \times 10^8$ M^{-1} s^{-1}. This value is close to the diffusion-controlled limit for the combination of a small molecule with a protein (see p. 245). Hence, the extent of structural change is likely to be small; extensive conformational transitions take time.
7. The fluorescence enhancement and the shift to blue indicate that water is largely excluded from the combining site when the hapten is bound. Hydrophobic interactions contribute significantly to the formation of most antigen–antibody complexes.
8. (a) An antibody combining site is formed by CDRs from both the H and the L chains. The V_H and V_L domains are essential. A small proportion of F_{ab} fragments can be further digested to produce F_v, a fragment that contains just these two domains. C_H1 and C_L contribute to the stability of F_{ab} but not to antigen binding.
(b) A synthetic F_v analog 248 residues long was prepared by expressing a synthetic gene consisting of a V_H gene joined to a V_L gene through a linker. See J. S. Huston et al., *Proc. Natl. Acad. Sci. U. S. A.* 85:5879–5883, 1988.
9. (a) Multivalent antigens lead to the dimerization or oligomerization of transmembrane immunoglobulins, an essential step in their activation. This mode of activation is reminiscent of that of receptor tyrosine kinases (Section 14.2).
(b) An antibody specific for a transmembrane immunoglobulin will activate a B cell by cross-linking these receptors. This experiment can be carried out by using, for example, a goat antibody to cross-link receptors on a mouse B cell.
10. B cells do not express T-cell receptors. The hybridization of T-cell cDNAs with B-cell mRNAs removes cDNAs that are expressed in both cells. Hence, the mixture of cDNAs subsequent to this hybridization are enriched in those encoding T-cell receptors. This procedure, called subtractive hybridization, is generally useful in isolating low-abundance cDNAs. Hybridization should be carried out by using mRNAs from a closely related cell that does not express the gene of interest. See S. M. Hedrick, M. M. Davis, D. I. Cohen, E. A. Nielsen, and M. M. Davis, *Nature* 308:149–153, 1984, for an interesting account of how this method was used to obtain genes for T-cell receptors.
11. TLR-4 is the receptor for LPS, a toxin found specifically in the walls of Gram-negative bacteria. Mutations that inhibit the function of TLR4 impair an affected person's defenses against this class of bacteria.
12. If the HLA alleles are not matched, then the recipient's T cell receptors will identify the MHC proteins of the transplanted tissue as nonself and transplant rejection is likely.
13. Purify an antibody with a specificity to one antigen. Unfold the antibody and allow it to re-fold either in the presence of the antigen or in the absence of the antigen. Test the re-folded antibodies for antigen-binding ability.
14. In some cases, V–D–J rearrangement will result in combining V, D, and J segments out of frame. mRNA molecules produced from such rearranged genes will produce truncated molecules if translated. This possibility is excluded by degrading the mRNA.
15. The mutant bacteria may still stimulate an immune response without causing disease. Hence, they may be valuable starting points for the design of a live attenuated vaccine for the original pathogenic strain.
16. The peptide is LLQATYSAV (L in second position, V in last).
17. Catalysis is likely to require a base for removing a proton from a water molecule. A histidine, glutamate, or aspartate residue is most likely. In addition, a potential hydrogen-bond donor may be present and will interact with the negatively charged oxygen atom that forms in the transition state.
18. A phosphotyrosine residue in the carboxyl terminus of Src and related protein tyrosine kinases binds to its own SH2 domain to generate the inhibited from of Src (Section 14.5). Removal of the phosphoryl group from this residue will activate the kinase.

19. (a) $K_d = 10^{-7}$ M; (b) $K_d = 10^{-9}$ M. The gene was probably generated by a point mutation in the gene for antibody A rather than by de novo rearrangement.

Chapter 35

1. (a) Skeletal muscle and eukaryotic cilia derive their free energy from ATP hydrolysis; the bacterial flagellar motor uses a proton-motive force.
(b) Skeletal muscle requires myosin and actin. Eukaryotic cilia require microtubules and dynein. The bacterial flagellar motor requires MotA, MotB, and FliG, as well as many ancillary components.
2. 6400 Å/80 Å = 80 body lengths per second. For a 10-foot automobile, this body-length speed corresponds to a speed of 80 × 10 feet = 800 feet per second, or 545 miles per hour.
3. 4 pN = 8.8×10^{-13} pounds. The weight of a single motor domain is 100,000 g mol^{-1}/(6.023×10^{23} molecules mol^{-1}) = 1.7×10^{-19} g = 3.7×10^{-22} pounds. Thus, a motor domain can lift ($8.8 \times 10^{-13}/3.7 \times 10^{-22}$) = 2.4×10^{9} times its weight.
4. Both actin filaments and microtubles are built from subunits and these subunits bind and hydrolyze nucleoside triphosphates. Actin filaments are built of a single type of subunit and these subunits bind ATP. Microtubules are built of two different types of subunits and these subunits bind GTP.
5. The light chains in myosin stiffen the lever arm. The light chains in kinesin bind cargo to be transported.
6. After death, the ratio of ADP to ATP increases rapidly. In the ADP form, myosin motor domains bind tightly to actin. Myosin–actin interactions are possible because the drop in ATP concentration also allows the calcium concentration to rise, clearing the blockage of actin by tropomyosin through the action of the troponin complex.
7. Above its critical concentration, ATP-actin will polymerize. The ATP will hydrolyze through time to form ADP-actin, which has a higher critical concentration. Thus, if the initial subunit concentration is between the critical concentrations of ATP-actin and ADP-actin, filaments will form initially and then disappear on ATP hydrolysis.
8. A one-base step is approximately 3.4 Å = 3.4×10^{-4} μm. If a stoichiometry of one molecule of ATP per step is assumed, this distance corresponds to a velocity of 0.017 μm s^{-1}. Kinesin moves at a velocity of 6400 Å per second, or 0.64 μm s^{-1}.
9. A proton-motive force across the plasma membrane is necessary to drive the flagellar motor. Under conditions of starvation, this proton-motive force is depleted. In acidic solution, the pH difference across the membrane is sufficient to power the motor.
10. The mean distance between tumbles would be longer when the bacterium is moving up a gradient of a chemoattractant.
11. (a) 1.13×10^{-9} dyne
(b) 6.8×10^{14} erg
(c) 6.6×10^{-11} erg per 80 molecules of ATP. A single kinesin motor provides more than enough free energy to power the transport of micrometer-size cargoes at micrometer-per-second velocities.
12. The spacing between identical subunits on microtubules is 8 nm. Thus, a kinesin molecule with a step size that is not a multiple of 8 nm would have to be able to bind at more than one type of site on the microtubule surface.
13. KIF1A must be tethered to an additional microtubule-binding element that retains an attachment to the microtubule when the motor domain releases.
14. Filaments built from subunits can be arbitrarily long, can be dynamically assembled and disassembled, and require only a small amount of genetic information to encode.
15. Protons still flow from outside to inside the cell. Each proton might pass into the outer half-channel of one MotA–MotB complex, bind to the MS ring, rotate clockwise, and pass into the inner half-channel of the neighboring MotA–MotB complex.
16. At a high concentration of calcium ion, Ca^{2+} binds to calmodulin. In turn, calmodulin binds to a protein kinase that phosphorylates myosin light chains and activates it. At low calcium ion concentration, the light chains are dephosphorylated by a Ca^{2+}-independent phosphatase.
17. (a) The value of k_{cat} is approximately 13 molecules per second, whereas the K_M value for ATP is approximately 12 μM.
(b) The step size is approximately (380 – 120)/7 = 37 nm.
(c) The step size is very large, which is consistent with the presence of six light-chain-binding sites and, hence, very long lever arms. The rate of ADP release is essentially identical with the overall k_{cat}; so ADP release is rate limiting, which suggests that both motor domains can bind to sites 37 nm apart simultaneously. ADP release from the hindmost domain allows ATP to bind, leading to actin release and lever-arm motion.

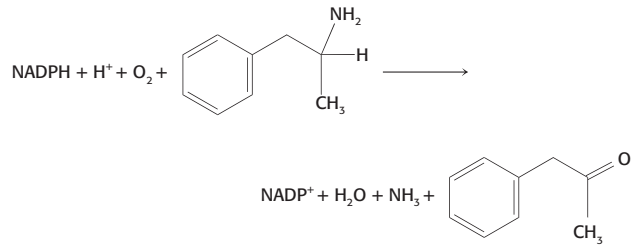
Chapter 36

1. (a) Before; (b) after; (c) after; (d) after; (e) before; (f) after.
2. (a) Yes; (b) yes; (c) no (MW > 600).
3. If computer programs could estimate log(*P*) values on the basis of chemical structure, then the required laboratory time for drug development could be shortened. The determination of the relative solubilities of pharmaceutical candidates by allowing each compound to equilibrate between water and an organic phase would no longer be necessary.
4. Perhaps *N*-acetylcysteine would conjugate to some of the *N*-acetyl-*p*-benzoquinone imine that is produced by the metabolism of acetaminophen, thereby preventing the depletion of the liver's supply of glutathione.
5. In phase 1 clinical trials, approximately 10 to 100 healthy volunteers are typically enrolled in a study designed to assess safety. In contrast, a larger number of subjects are enrolled in a typical phase 2 trial. Moreover, these persons may benefit from the drug administered. In a phase 2 trial, efficacy, dosage, and safety can be assessed.
6. The binding of other drugs to albumin could cause extra coumadin to be released. (Albumin is a general carrier for hydrophobic molecules.)
7. A drug that inhibits a P450 enzyme may dramatically affect the disposition of another drug that is metabolized by that same enzyme. If this inhibited metabolism is not accounted for when dosing, the second drug may reach very high, and sometimes toxic, levels in the blood.
8. Unlike competitive inhibition, noncompetitive inhibition cannot be overcome with additional substrate. Hence, a drug that acts by a noncompetitive mechanism will be unaffected by changing levels of the physiological substrate.
9. An inhibitor of MDR could prevent the efflux of a chemotherapeutic drug from tumor cells. Hence, this type of an inhibitor could be useful in averting resistance to cancer chemotherapy.
10. Agents that inhibit one or more enzymes of the glycolytic pathway could act to deprive trypanosomes of energy and thus be useful for treating sleeping sickness. A difficulty is that glycolysis in the host cells also would be inhibited.
11. Imatinib is an inhibitor of the Bcr-Abl kinase, a mutant kinase present only in tumor cells that have undergone a translocation between chromosomes 9 and 22 (see Figure 14.33). Before initiating treatment with imatinib, we could sequence the DNA of the tumor cells and determine (a) whether this translocation has taken place and (b) whether the sequence of *bcr-abl* carries any mutations

that would render the kinase resistant to imatinib. If the translocation has not taken place or if the gene carries resistance mutations, then imatinib would likely not be an effective treatment for the patient carrying this particular tumor.

12. Sildenafil increases cGMP levels by inhibiting the phosphodiesterase-mediated breakdown of cGMP to GMP. Intracellular cGMP levels can also be increased by activating its synthesis. This activation can be achieved with the use of NO donors (such as sodium nitroprusside and nitroglycerin) or compounds that activate guanylate cyclase activity. Drugs that act by the latter mechanism are currently in clinical trials.

13. A reasonable mechanism would be an oxidative deamination following an overall mechanism similar to that in Figure 36.9, with release of ammonia.



14. $K_I \approx 0.3$ nM. $IC_{50} \approx 2.0$ nM. Yes, compound A should be effective when taken orally because 400 nM is much greater than the estimated values of K_I and IC_{50}.

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Note: Page numbers followed by f, t, and b refer to figures, tables, and boxed material, respectively. **Boldface** page numbers indicate structural formulas and ribbon diagrams.

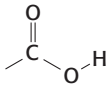
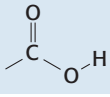
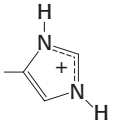
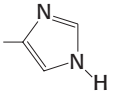
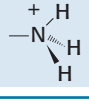
ACIDITY CONSTANTS

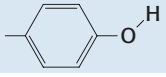
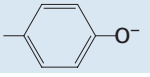
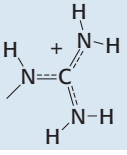
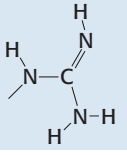
pK_a values of some acids

Acid	pK' (at 25°C)	Acid	pK' (at 25°C)
Acetic acid	4.76	Malic acid, pK_1	3.40
Acetoacetic acid	3.58	pK_2	5.11
Ammonium ion	9.25	Phenol	9.89
Ascorbic acid, pK_1	4.10	Phosphoric acid, pK_1	2.12
pK_2	11.79	pK_2	7.21
Benzoic acid	4.20	pK_3	12.67
n-Butyric acid	4.81	Pyridinium ion	5.25
Cacodylic acid	6.19	Pyrophosphoric acid, pK_1	0.85
Citric acid, pK_1	3.14	pK_2	1.49
pK_2	4.77	pK_3	5.77
pK_3	6.39	pK_4	8.22
Ethylammonium ion	10.81	Succinic acid, pK_1	4.21
Formic acid	3.75	pK_2	5.64
Glycine, pK_1	2.35	Trimethylammonium ion	9.79
pK_2	9.78	Tris (hydroxymethyl) aminomethane	8.08
Imidazolium ion	6.95	Water*	15.74
Lactic acid	3.86		
Maleic acid, pK_1	1.83		
pK_2	6.07		

*$[H^+]\,[OH^-] = 10^{-14}$; $[H_2O] = 55.5$ M.

Typical pK_a values of ionizable groups in proteins

Group	Acid	Base	Typical pK_a
Terminal α-carboxyl group			3.1
Aspartic acid			4.1
Glutamic acid			
Histidine			6.0
Terminal α-amino group			8.0

Group	Acid	Base	Typical pK_a
Cysteine			8.3
Tyrosine			10.4
Lysine			10.0
Arginine			12.5

Note: pK_a values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

STANDARD BOND LENGTHS

Bond	Structure	Length (Å)
C—H	R_2CH_2	1.07
	Aromatic	1.08
	RCH_3	1.10
C—C	Hydrocarbon	1.54
	Aromatic	1.40
C═C	Ethylene	1.33
C≡C	Acetylene	1.20
C—N	RNH_2	1.47
	O═C—N	1.34
C—O	Alcohol	1.43
	Ester	1.36
C═O	Aldehyde	1.22
	Amide	1.24
C—S	R_2S	1.82
N—H	Amide	0.99
O—H	Alcohol	0.97
O—O	O_2	1.21
P—O	Ester	1.56
S—H	Thiol	1.33
S—S	Disulfide	2.05