■ NAD and NADP are the freely diffusible coenzymes of many dehydrogenases. Both NAD^+ and $NADP^+$ accept two electrons and one proton. In addition to its role in oxidation-reduction reactions, NAD^+ is the source of AMP in the bacterial DNA ligase reaction and of ADP-ribose in the cholera toxin reaction, and is hydrolyzed in the deacetylation of proteins by some sirtuins.

■ FAD and FMN, the flavin nucleotides, serve as tightly bound prosthetic groups of flavoproteins. They can accept either one or two electrons and one or two protons. Flavoproteins also serve as light receptors in cryptochromes and photolyases.

Key Terms

Terms in bold are defined in the glossary.

autotroph 491
heterotroph 491
metabolism 492
metabolic pathways 492
metabolite 492
intermediary metabolism 492
catabolism 492
anabolism 493
standard transformed constants 497
homolytic cleavage 502
radical 502
heterolytic cleavage 502
nucleophile 502
electrophile 502
carbanion 503
carbocation 503
aldol condensation 503
Claisen condensation 503
kinases 506
phosphorylation potential (ΔG_p) 508
thioester 510
adenylylation 513
inorganic pyrophosphatase 513
nucleoside diphosphate kinase 516
adenylate kinase 516
creatine kinase 516
phosphagens 516
polyphosphate kinase-1, kinase-2 517
electromotive force (emf) 518
conjugate redox pair 518
dehydrogenation 519
dehydrogenases 519
reducing equivalent 520
standard reduction potential (E'°) 520
pyridine nucleotide 522
oxidoreductase 523
flavoprotein 525
flavin nucleotides 525
cryptochrome 526
photolyase 526

Problems

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

2. Calculation of $\Delta G'^\circ$ from an Equilibrium Constant Calculate the standard free-energy change for each of the following metabolically important enzyme-catalyzed reactions, using the equilibrium constants given for the reactions at 25 °C and pH 7.0.

(a) Glutamate + oxaloacetate $\overset{\text{aspartate aminotransferase}}{\rightleftharpoons}$ aspartate + α-ketoglutarate $\quad K'_{eq} = 6.8$

(b) Dihydroxyacetone phosphate $\overset{\text{triose phosphate isomerase}}{\rightleftharpoons}$ glyceraldehyde 3-phosphate $\quad K'_{eq} = 0.0475$

(c) Fructose 6-phosphate + ATP $\overset{\text{phosphofructokinase}}{\rightleftharpoons}$ fructose 1,6-bisphosphate + ADP $\quad K'_{eq} = 254$

3. Calculation of the Equilibrium Constant from $\Delta G'^\circ$ Calculate the equilibrium constant K'_{eq} for each of the following reactions at pH 7.0 and 25 °C, using the $\Delta G'^\circ$ values in Table 13-4.

(a) Glucose 6-phosphate + $H_2O \overset{\text{glucose 6-phosphatase}}{\rightleftharpoons}$ glucose + P_i

(b) Lactose + $H_2O \overset{\beta\text{-galactosidase}}{\rightleftharpoons}$ glucose + galactose

(c) Malate $\overset{\text{fumarase}}{\rightleftharpoons}$ fumarate + H_2O

4. Experimental Determination of K'_{eq} and $\Delta G'^\circ$ If a 0.1 M solution of glucose 1-phosphate at 25 °C is incubated with a catalytic amount of phosphoglucomutase, the glucose 1-phosphate is transformed to glucose 6-phosphate. At equilibrium, the concentrations of the reaction components are

Glucose 1-phosphate $\rightleftharpoons$ glucose 6-phosphate
4.5×10^{-3} M $\qquad$ 9.6×10^{-2} M

Calculate K'_{eq} and $\Delta G'^\circ$ for this reaction.

5. Experimental Determination of $\Delta G'^\circ$ for ATP Hydrolysis A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^\circ$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favorable equilibrium constants:

Glucose 6-phosphate + $H_2O \longrightarrow$ glucose + $P_i \quad K'_{eq} = 270$
ATP + glucose $\longrightarrow$ ADP + glucose 6-phosphate $\quad K'_{eq} = 890$

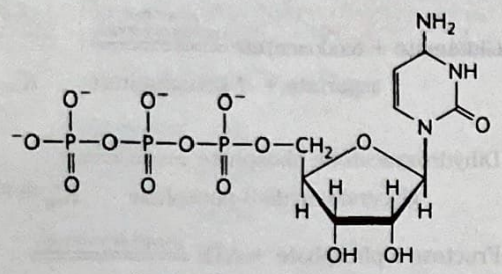
Using this information for equilibrium constants determined at 25 °C, calculate the standard free energy of hydrolysis of ATP.

6. Difference between $\Delta G'^\circ$ and ΔG Consider the following interconversion, which occurs in glycolysis (Chapter 14):

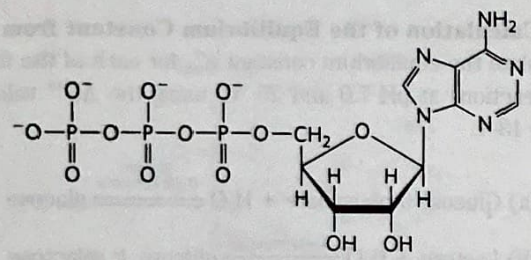
Fructose 6-phosphate $\rightleftharpoons$ glucose 6-phosphate $\quad K'_{eq} = 1.97$

(a) What is $\Delta G'^\circ$ for the reaction (K'_{eq} measured at 25 °C)?
(b) If the concentration of fructose 6-phosphate is adjusted to 1.5 M and that of glucose 6-phosphate is adjusted to 0.50 M, what is ΔG?
(c) Why are $\Delta G'^\circ$ and ΔG different?

7. Free Energy of Hydrolysis of CTP Compare the structure of the nucleoside triphosphate CTP with the structure of ATP.



Cytidine triphosphate (CTP)



Adenosine triphosphate (ATP)

Now predict the K'_{eq} and $\Delta G'^{\circ}$ for the following reaction:

$$ATP + CDP \longrightarrow ADP + CTP$$

8. Dependence of ΔG on pH The free energy released by the hydrolysis of ATP under standard conditions is −30.5 kJ/mol. If ATP is hydrolyzed under standard conditions except at pH 5.0, is more or less free energy released? Explain.

9. The $\Delta G'^{\circ}$ for Coupled Reactions Glucose 1-phosphate is converted into fructose 6-phosphate in two successive reactions:

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

Using the $\Delta G'^{\circ}$ values in Table 13-4, calculate the equilibrium constant, K'_{eq}, for the sum of the two reactions:

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

10. Effect of [ATP]/[ADP] Ratio on Free Energy of Hydrolysis of ATP Using Equation 13-4, plot ΔG against ln Q (mass-action ratio) at 25 °C for the concentrations of ATP, ADP, and P_i in the table below. $\Delta G'^{\circ}$ for the reaction is −30.5 kJ/mol. Use the resulting plot to explain why metabolism is regulated to keep the ratio [ATP]/[ADP] high.

	Concentration (mM)				
ATP	5	3	1	0.2	5
ADP	0.2	2.2	4.2	5.0	25
P_i	10	12.1	14.1	14.9	10

11. Strategy for Overcoming an Unfavorable Reaction: ATP-Dependent Chemical Coupling The phosphorylation of glucose to glucose 6-phosphate is the initial step in the catabolism of glucose. The direct phosphorylation of glucose by P_i is described by the equation

$$\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^{\circ} = 13.8 \text{ kJ/mol}$$

(a) Calculate the equilibrium constant for the above reaction at 37 °C. In the rat hepatocyte, the physiological concentrations of glucose and P_i are maintained at approximately 4.8 mM. What is the equilibrium concentration of glucose 6-phosphate obtained by the direct phosphorylation of glucose by P_i? Does this reaction represent a reasonable metabolic step for the catabolism of glucose? Explain.

(b) In principle, at least, one way to increase the concentration of glucose 6-phosphate is to drive the equilibrium reaction to the right by increasing the intracellular concentrations of glucose and P_i. Assuming a fixed concentration of P_i at 4.8 mM, how high would the intracellular concentration of glucose have to be to give an equilibrium concentration of glucose 6-phosphate of 250 μM (the normal physiological concentration)? Would this route be physiologically reasonable, given that the maximum solubility of glucose is less than 1 M?

(c) The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is used to phosphorylate glucose:

(1) $\text{Glucose} + P_i \longrightarrow \text{glucose 6-phosphate} + H_2O \qquad \Delta G'^{\circ} = 13.8 \text{ kJ/mol}$

(2) $ATP + H_2O \longrightarrow ADP + P_i \qquad \Delta G'^{\circ} = -30.5 \text{ kJ/mol}$

Sum: $\text{Glucose} + ATP \longrightarrow \text{glucose 6-phosphate} + ADP$

Calculate K'_{eq} at 37 °C for the overall reaction. For the ATP-dependent phosphorylation of glucose, what concentration of glucose is needed to achieve a 250 μM intracellular concentration of glucose 6-phosphate when the concentrations of ATP and ADP are 3.38 mM and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose in the cell? Explain.

(d) Although coupling ATP hydrolysis to glucose phosphorylation makes thermodynamic sense, we have not yet specified how this coupling is to take place. Given that coupling requires a common intermediate, one conceivable route is to use ATP hydrolysis to raise the intracellular concentration of P_i and thus drive the unfavorable phosphorylation of glucose by P_i. Is this a reasonable route? (Think about the solubility product, K_{sp}, of metabolic intermediates.)

(e) The ATP-coupled phosphorylation of glucose is catalyzed in hepatocytes by the enzyme glucokinase. This enzyme binds ATP and glucose to form a glucose-ATP-enzyme complex, and the phosphoryl group is transferred directly from ATP to glucose. Explain the advantages of this route.

12. Calculations of $\Delta G'^{\circ}$ for ATP-Coupled Reactions From data in Table 13-6, calculate the $\Delta G'^{\circ}$ value for the following reactions:

(a) Phosphocreatine + ADP ⟶ creatine + ATP

(b) ATP + fructose ⟶ ADP + fructose 6-phosphate

13. Coupling ATP Cleavage to an Unfavorable Reaction To explore the consequences of coupling ATP hydrolysis under physiological conditions to a thermodynamically unfavorable biochemical reaction, consider the hypothetical transformation X → Y, for which $\Delta G'^{\circ} = 20.0$ kJ/mol.

(a) What is the ratio [Y]/[X] at equilibrium?

(b) Suppose X and Y participate in a sequence of reactions during which ATP is hydrolyzed to ADP and P_i. The overall reaction is

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

Calculate [Y]/[X] for this reaction at equilibrium. Assume that the temperature is 25 °C and the equilibrium concentrations of ATP, ADP, and P_i are 1 M.

(c) We know that [ATP], [ADP], and [P_i] are *not* 1 M under physiological conditions. Calculate [Y]/[X] for the ATP-coupled reaction when the values of [ATP], [ADP], and [P_i] are those found in rat myocytes (Table 13-5).

14. Calculations of ΔG at Physiological Concentrations Calculate the actual, physiological ΔG for the reaction

Phosphocreatine + ADP ⟶ creatine + ATP

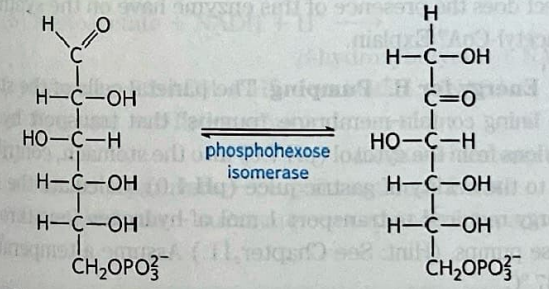
at 37 °C, as it occurs in the cytosol of neurons, with phosphocreatine at 4.7 mM, creatine at 1.0 mM, ADP at 0.73 mM, and ATP at 2.6 mM.

15. Free Energy Required for ATP Synthesis under Physiological Conditions In the cytosol of rat hepatocytes, the temperature is 37 °C and the mass-action ratio, Q, is

$$\frac{[ATP]}{[ADP][P_i]} = 5.33 \times 10^2\ \text{M}^{-1}$$

Calculate the free energy required to synthesize ATP in a rat hepatocyte.

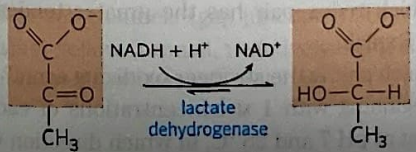
16. Chemical Logic In the glycolytic pathway, a six-carbon sugar (fructose 1,6-bisphosphate) is cleaved to form two three-carbon sugars, which undergo further metabolism (see Fig. 14-6). In this pathway, an isomerization of glucose 6-phosphate to fructose 6-phosphate (shown below) occurs two steps before the cleavage reaction (the intervening step is phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate (p. 539)).



Glucose 6-phosphate — Fructose 6-phosphate

What does the isomerization step accomplish from a chemical perspective? (Hint: Consider what might happen if the C—C bond cleavage were to proceed without the preceding isomerization.)

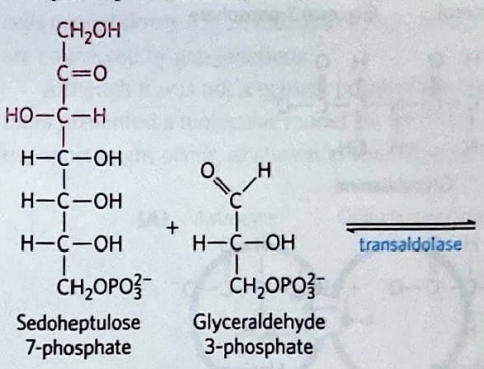
17. Enzymatic Reaction Mechanisms I Lactate dehydrogenase is one of the many enzymes that require NADH as coenzyme. It catalyzes the conversion of pyruvate to lactate:



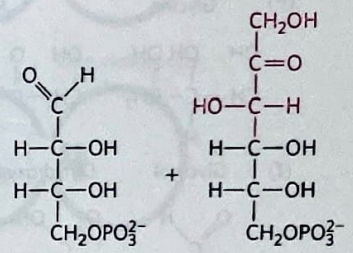
Pyruvate — L-Lactate

Draw the mechanism of this reaction (show electron-pushing arrows). (Hint: This is a common reaction throughout metabolism; the mechanism is similar to that catalyzed by other dehydrogenases that use NADH, such as alcohol dehydrogenase.)

18. Enzymatic Reaction Mechanisms II Biochemical reactions often look more complex than they really are. In the pentose phosphate pathway (Chapter 14), sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate react to form erythrose 4-phosphate and fructose 6-phosphate in a reaction catalyzed by transaldolase.



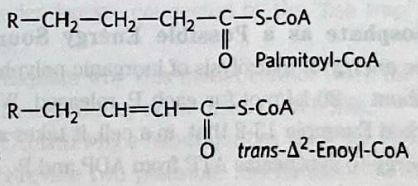
Sedoheptulose 7-phosphate — Glyceraldehyde 3-phosphate



Erythrose 4-phosphate — Fructose 6-phosphate

Draw a mechanism for this reaction (show electron-pushing arrows). (Hint: Take another look at aldol condensations, then consider the name of this enzyme.)

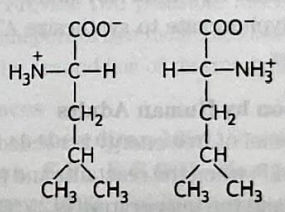
19. Recognizing Reaction Types For the following pairs of biomolecules, identify the type of reaction (oxidation-reduction, hydrolysis, isomerization, group transfer, or internal rearrangement) required to convert the first molecule to the second. In each case, indicate the general type of enzyme and cofactor(s) or reactants that would be required, and any other products that would result.



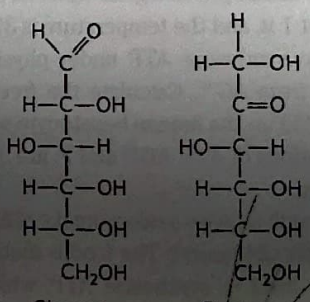
Palmitoyl-CoA

(a)

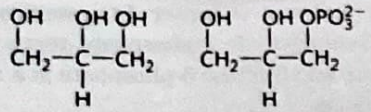
trans-Δ^2-Enoyl-CoA



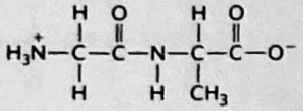
(b) L-Leucine — D-Leucine



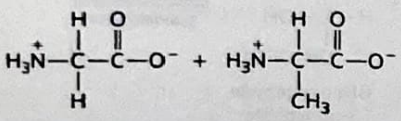
(c) Glucose — Fructose



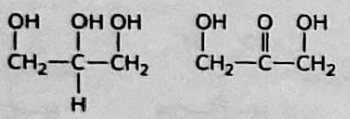
(d) Glycerol Glycerol 3-phosphate



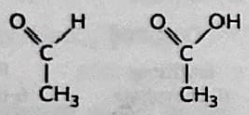
Glycylalanine



(e) Glycine Alanine

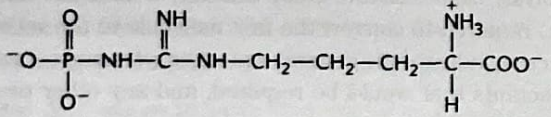


(f) Glycerol Dihydroxyacetone



(g) Acetaldehyde Acetic acid

20. Effect of Structure on Group Transfer Potential Some invertebrates contain phosphoarginine. Is the standard free energy of hydrolysis of this molecule more similar to that of glucose 6-phosphate or of ATP? Explain your answer.



Phosphoarginine

21. Polyphosphate as a Possible Energy Source The standard free energy of hydrolysis of inorganic polyphosphate (polyP) is about −20 kJ/mol for each P_i released. We calculated in Worked Example 13-2 that, in a cell, it takes about 50 kJ/mol of energy to synthesize ATP from ADP and P_i. Is it feasible for a cell to use polyphosphate to synthesize ATP from ADP? Explain your answer.

22. Daily ATP Utilization by Human Adults

(a) A total of 30.5 kJ/mol of free energy is needed to synthesize ATP from ADP and P_i when the reactants and products are at 1 M concentrations and the temperature is 25 °C (standard state). Because the actual physiological concentrations of ATP, ADP, and P_i are not 1 M, and the temperature is 37 °C, the free energy required to synthesize ATP under physiological conditions is different from $\Delta G'^\circ$. Calculate the free energy required to synthesize ATP in the human hepatocyte when the physiological concentrations of ATP, ADP, and P_i are 3.5, 1.50, and 5.0 mM, respectively.

(b) A 68 kg (150 lb) adult requires a caloric intake of 2,000 kcal (8,360 kJ) of food per day (24 hours). The food is metabolized and the free energy is used to synthesize ATP, which then provides energy for the body's daily chemical and mechanical work. Assuming that the efficiency of converting food energy into ATP is 50%, calculate the weight of ATP used by a human adult in 24 hours. What percentage of the body weight does this represent?

(c) Although adults synthesize large amounts of ATP daily, their body weight, structure, and composition do not change significantly during this period. Explain this apparent contradiction.

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

24. Cleavage of ATP to AMP and PP_i during Metabolism Synthesis of the activated form of acetate (acetyl-CoA) is carried out in an ATP-dependent process:

$$\text{Acetate} + \text{CoA} + \text{ATP} \longrightarrow \text{acetyl-CoA} + \text{AMP} + PP_i$$

(a) The $\Delta G'^\circ$ for hydrolysis of acetyl-CoA to acetate and CoA is −32.2 kJ/mol and that for hydrolysis of ATP to AMP and PP_i is −30.5 kJ/mol. Calculate $\Delta G'^\circ$ for the ATP-dependent synthesis of acetyl-CoA.

(b) Almost all cells contain the enzyme inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i to P_i. What effect does the presence of this enzyme have on the synthesis of acetyl-CoA? Explain.

25. Energy for H^+ Pumping The parietal cells of the stomach lining contain membrane "pumps" that transport hydrogen ions from the cytosol (pH 7.0) into the stomach, contributing to the acidity of gastric juice (pH 1.0). Calculate the free energy required to transport 1 mol of hydrogen ions through these pumps. (Hint: See Chapter 11.) Assume a temperature of 37 °C.

26. Standard Reduction Potentials The standard reduction potential, E'°, of any redox pair is defined for the half-cell reaction:

$$\text{Oxidizing agent} + n \text{ electrons} \longrightarrow \text{reducing agent}$$

The E'° values for the NAD^+/NADH and pyruvate/lactate conjugate redox pairs are −0.32 V and −0.19 V, respectively.

(a) Which redox pair has the greater tendency to lose electrons? Explain.

(b) Which pair is the stronger oxidizing agent? Explain.

(c) Beginning with 1 M concentrations of each reactant and product at pH 7 and 25 °C, in which direction will the following reaction proceed?

$$\text{Pyruvate} + \text{NADH} + H^+ \rightleftharpoons \text{lactate} + NAD^+$$

(d) What is the standard free-energy change ($\Delta G'^\circ$) for the conversion of pyruvate to lactate?

(e) What is the equilibrium constant (K'_{eq}) for this reaction?

27. Energy Span of the Respiratory Chain Electron transfer in the mitochondrial respiratory chain may be represented by the net reaction equation

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons H_2O + NAD^+$$

(a) Calculate $\Delta E'^\circ$ for the net reaction of mitochondrial electron transfer. Use E'° values in Table 13-7.

(b) Calculate $\Delta G'^\circ$ for this reaction.

(c) How many ATP molecules can *theoretically* be generated by this reaction if the free energy of ATP synthesis under cellular conditions is 52 kJ/mol?

28. Dependence of Electromotive Force on Concentrations Calculate the electromotive force (in volts) registered by an electrode immersed in a solution containing the following mixtures of NAD^+ and NADH at pH 7.0 and 25 °C, with reference to a half-cell of E'° 0.00 V.

(a) 1.0 mM NAD^+ and 10 mM NADH

(b) 1.0 mM NAD^+ and 1.0 mM NADH

(c) 10 mM NAD^+ and 1.0 mM NADH

29. Electron Affinity of Compounds List the following in order of increasing tendency to accept electrons: (a) α-ketoglutarate + CO_2 (yielding isocitrate); (b) oxaloacetate; (c) O_2; (d) $NADP^+$.

30. Direction of Oxidation-Reduction Reactions Which of the following reactions would you expect to proceed in the direction shown, under standard conditions, in the presence of the appropriate enzymes?

(a) Malate + NAD^+ ⟶ oxaloacetate + NADH + H^+

(b) Acetoacetate + NADH + H^+ ⟶ β-hydroxybutyrate + NAD^+

(c) Pyruvate + NADH + H^+ ⟶ lactate + NAD^+

(d) Pyruvate + β-hydroxybutyrate ⟶ lactate + acetoacetate

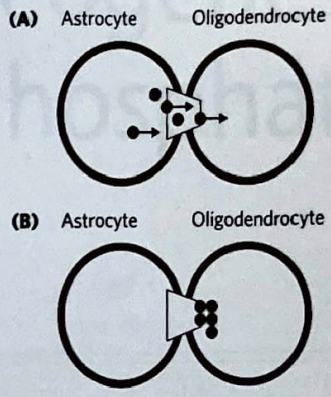
(e) Malate + pyruvate ⟶ oxaloacetate + lactate

(f) Acetaldehyde + succinate ⟶ ethanol + fumarate

Data Analysis Problem

31. Thermodynamics Can Be Tricky Thermodynamics is a challenging area of study and one with many opportunities for confusion. An interesting example is found in an article by Robinson, Hampson, Munro, and Vaney, published in *Science* in 1993. Robinson and colleagues studied the movement of small molecules between neighboring cells of the nervous system through cell-to-cell channels (gap junctions). They found that the dyes Lucifer yellow (a small, negatively charged molecule) and biocytin (a small zwitterionic molecule) moved in only one direction between two particular types of glia (nonneuronal cells of the nervous system). Dye injected into astrocytes would rapidly pass into adjacent astrocytes, oligodendrocytes, or Müller cells, but dye injected into oligodendrocytes or Müller cells passed slowly if at all into astrocytes. All of these cell types are connected by gap junctions.

Although it was not a central point of their article, the authors presented a molecular model for how this unidirectional transport might occur, as shown in their Figure 3:



The figure legend reads: "Model of the unidirectional diffusion of dye between coupled oligodendrocytes and astrocytes, based on differences in connection pore diameter. Like a fish in a fish trap, dye molecules (black circles) can pass from an astrocyte to an oligodendrocyte (A) but not back in the other direction (B)."

Although this article clearly passed review at a well-respected journal, several letters to the editor (1994) followed, showing that Robinson and coauthors' model violated the second law of thermodynamics.

(a) Explain how the model violates the second law. Hint: Consider what would happen to the entropy of the system if one started with equal concentrations of dye in the astrocyte and oligodendrocyte connected by the "fish trap" type of gap junctions.

(b) Explain why this model cannot work for small molecules, although it may allow one to catch fish.

(c) Explain why a fish trap *does* work for fish.

(d) Provide two plausible mechanisms for the unidirectional transport of dye molecules between the cells that do not violate the second law of thermodynamics.

References

Letters to the editor. 1994. *Science* 265:1017–1019.

Robinson, S.R., E.C.G.M. Hampson, M.N. Munro, and D.I. Vaney. 1993. Unidirectional coupling of gap junctions between neuroglia. *Science* 262:1072–1074.

Further Reading is available at www.macmillanlearning.com/LehningerBiochemistry7e.

more sensitive to a thiamine deficiency: even a moderate thiamine deficiency (tolerable in individuals with an unmutated transketolase) can drop the level of TPP below that needed to saturate the enzyme. The result is a slowing down of the whole pentose phosphate pathway. In people with Wernicke-Korsakoff syndrome this results in a worsening of symptoms, which can include severe memory loss, mental confusion, and partial paralysis. ■

Glucose 6-Phosphate Is Partitioned between Glycolysis and the Pentose Phosphate Pathway

Whether glucose 6-phosphate enters glycolysis or the pentose phosphate pathway depends on the current needs of the cell and on the concentration of $NADP^+$ in the cytosol. Without this electron acceptor, the first reaction of the pentose phosphate pathway (catalyzed by G6PD) cannot proceed. When a cell is rapidly converting NADPH to $NADP^+$ in biosynthetic reductions, the level of $NADP^+$ rises, allosterically stimulating G6PD and thereby increasing the flux of glucose 6-phosphate through the pentose phosphate pathway **(Fig. 14-28)**. When the demand for NADPH slows, the level of $NADP^+$ drops, the pentose phosphate pathway slows, and glucose 6-phosphate is instead used to fuel glycolysis.

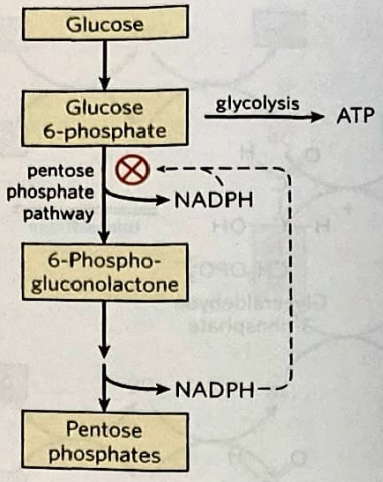


FIGURE 14-28 Role of NADPH in regulating the partitioning of glucose 6-phosphate between glycolysis and the pentose phosphate pathway. When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction (see Fig. 14-21), [NADPH] rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.

SUMMARY 14.5 Pentose Phosphate Pathway of Glucose Oxidation

■ The *oxidative* pentose phosphate pathway (phosphogluconate pathway, or hexose monophosphate pathway) brings about oxidation and decarboxylation at C-1 of glucose 6-phosphate, reducing $NADP^+$ to NADPH and producing pentose phosphates.

■ NADPH provides reducing power for biosynthetic reactions, and ribose 5-phosphate is a precursor for nucleotide and nucleic acid synthesis. Rapidly growing tissues and tissues carrying out active biosynthesis of fatty acids, cholesterol, or steroid hormones send more glucose 6-phosphate through the pentose phosphate pathway than do tissues with less demand for pentose phosphates and reducing power.

■ The first phase of the pentose phosphate pathway consists of two oxidations that convert glucose 6-phosphate to ribulose 5-phosphate and reduce $NADP^+$ to NADPH. The second phase comprises nonoxidative steps that convert pentose phosphates to glucose 6-phosphate, which begins the cycle again.

■ In the second phase, transketolase (with TPP as cofactor) and transaldolase catalyze the interconversion of three-, four-, five-, six-, and seven-carbon sugars, with the reversible conversion of six pentose phosphates to five hexose phosphates. In the carbon-assimilating reactions of photosynthesis, the same enzymes catalyze the reverse process, the *reductive* pentose phosphate pathway: conversion of five hexose phosphates to six pentose phosphates.

■ A genetic defect in transketolase that lowers its affinity for TPP exacerbates the Wernicke-Korsakoff syndrome.

■ Entry of glucose 6-phosphate either into glycolysis or into the pentose phosphate pathway is largely determined by the relative concentrations of $NADP^+$ and NADPH.

Key Terms

Terms in bold are defined in the glossary.

glycolysis 534
fermentation 534
lactic acid fermentation 537
hypoxia 537
ethanol (alcohol) fermentation 537
isozymes 539
acyl phosphate 542
substrate-level phosphorylation 543
respiration-linked phosphorylation 543
phosphoenolpyruvate (PEP) 544
aerobic glycolysis 545
mutases 550
isomerases 550
lactose intolerance 551
galactosemia 552
thiamine pyrophosphate (TPP) 555
gluconeogenesis 558
biotin 560
pentose phosphate pathway 565
phosphogluconate pathway 565
hexose monophosphate pathway 565

Problems

1. Equation for the Preparatory Phase of Glycolysis Write balanced biochemical equations for all the reactions in the catabolism of glucose to two molecules of glyceraldehyde 3-phosphate (the preparatory phase of glycolysis), including the standard free-energy change for each reaction. Then write

the overall or net equation for the preparatory phase of glycolysis, with the net standard free-energy change.

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

3. GLUT Transporters Compare the localization of GLUT4 with that of GLUT2 and GLUT3, and explain why these localizations are important in the response of muscle, adipose tissue, brain, and liver to insulin.

4. Ethanol Production in Yeast When grown anaerobically on glucose, yeast *(S. cerevisiae)* converts pyruvate to acetaldehyde, then reduces acetaldehyde to ethanol using electrons from NADH. Write the equation for the second reaction, and calculate its equilibrium constant at 25 °C, given the standard reduction potentials in Table 13-7.

5. Energetics of the Aldolase Reaction Aldolase catalyzes the glycolytic reaction

Fructose 1,6-bisphosphate ⟶
glyceraldehyde 3-phosphate + dihydroxyacetone phosphate

The standard free-energy change for this reaction in the direction written is +23.8 kJ/mol. The concentrations of the three intermediates in the hepatocyte of a mammal are: fructose 1,6-bisphosphate, 1.4×10^{-5} M; glyceraldehyde 3-phosphate, 3×10^{-6} M; and dihydroxyacetone phosphate, 1.6×10^{-5} M. At body temperature (37 °C), what is the actual free-energy change for the reaction?

6. Pathway of Atoms in Fermentation A "pulse-chase" experiment using ^{14}C-labeled carbon sources is carried out on a yeast extract maintained under strictly anaerobic conditions to produce ethanol. The experiment consists of incubating a small amount of ^{14}C-labeled substrate (the pulse) with the yeast extract just long enough for each intermediate in the fermentation pathway to become labeled. The label is then "chased" through the pathway by the addition of excess unlabeled glucose. The chase effectively prevents any further entry of labeled glucose into the pathway.

(a) If [1-^{14}C]glucose (glucose labeled at C-1 with ^{14}C) is used as a substrate, what is the location of ^{14}C in the product ethanol? Explain.

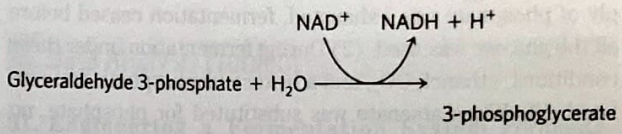
(b) Where would ^{14}C have to be located in the starting glucose to ensure that all the ^{14}C activity is liberated as $^{14}CO_2$ during fermentation to ethanol? Explain.

7. Heat from Fermentations Large-scale industrial fermenters generally require constant, vigorous cooling. Why?

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

9. Equivalence of Triose Phosphates ^{14}C-Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.

10. Glycolysis Shortcut Suppose you discovered a mutant yeast whose glycolytic pathway was shorter because of the presence of a new enzyme catalyzing the reaction



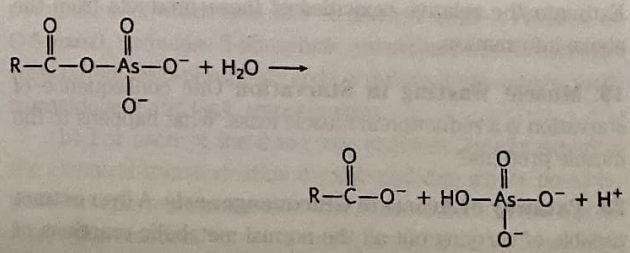
Would shortening the glycolytic pathway in this way benefit the cell? Explain.

11. Role of Lactate Dehydrogenase During strenuous activity, the demand for ATP in muscle tissue is vastly increased. In rabbit leg muscle or turkey flight muscle, the ATP is produced almost exclusively by lactic acid fermentation. ATP is formed in the payoff phase of glycolysis by two reactions, promoted by phosphoglycerate kinase and pyruvate kinase. Suppose skeletal muscle were devoid of lactate dehydrogenase. Could it carry out strenuous physical activity; that is, could it generate ATP at a high rate by glycolysis? Explain.

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

13. Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant (K'_{eq} = 0.08; $\Delta G'^{\circ}$ = 6.3 kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

14. Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:



On the other hand, acyl *phosphates*, such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

15. Requirement for Phosphate in Ethanol Fermentation In 1906 Harden and Young, in a series of classic studies on the fermentation of glucose to ethanol and CO_2 by extracts of brewer's yeast, made the following observations. (1) Inorganic phosphate was essential to fermentation; when the supply of phosphate was exhausted, fermentation ceased before all the glucose was used. (2) During fermentation under these conditions, ethanol, CO_2, and a hexose bisphosphate accumulated. (3) When arsenate was substituted for phosphate, no hexose bisphosphate accumulated, but the fermentation proceeded until all the glucose was converted to ethanol and CO_2.

(a) Why did fermentation cease when the supply of phosphate was exhausted?

(b) Why did ethanol and CO_2 accumulate? Was the conversion of pyruvate to ethanol and CO_2 essential? Why? Identify the hexose bisphosphate that accumulated. Why did it accumulate?

(c) Why did the substitution of arsenate for phosphate prevent the accumulation of the hexose bisphosphate yet allow fermentation to ethanol and CO_2 to go to completion? (See Problem 14.)

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

17. Synthesis of Glycerol Phosphate The glycerol 3-phosphate required for the synthesis of glycerophospholipids can be synthesized from a glycolytic intermediate. Propose a reaction sequence for this conversion.

18. Severity of Clinical Symptoms Due to Enzyme Deficiency The clinical symptoms of two forms of galactosemia—deficiency of galactokinase or of UDP-glucose:galactose 1-phosphate uridylyltransferase—show radically different severity. Although both types produce gastric discomfort after milk ingestion, deficiency of the transferase also leads to liver, kidney, spleen, and brain dysfunction and eventual death. What products accumulate in the blood and tissues with each type of enzyme deficiency? Estimate the relative toxicities of these products from the above information.

19. Muscle Wasting in Starvation One consequence of starvation is a reduction in muscle mass. What happens to the muscle proteins?

20. Pathway of Atoms in Gluconeogenesis A liver extract capable of carrying out all the normal metabolic reactions of the liver is briefly incubated in separate experiments with the following ^{14}C-labeled precursors.

(a) [^{14}C]Bicarbonate, $HO-{}^{14}C(=O)O^-$

(b) [1-^{14}C]Pyruvate, $CH_3-C(=O)-{}^{14}COO^-$

Trace the pathway of each precursor through gluconeogenesis. Indicate the location of ^{14}C in all intermediates and in the product, glucose.

21. Energy Cost of a Cycle of Glycolysis and Gluconeogenesis What is the cost (in ATP equivalents) of transforming glucose to pyruvate via glycolysis and back again to glucose via gluconeogenesis?

22. Relationship between Gluconeogenesis and Glycolysis Why is it important that gluconeogenesis is not the exact reversal of glycolysis?

23. Energetics of the Pyruvate Kinase Reaction Explain in bioenergetic terms how the conversion of pyruvate to phosphoenolpyruvate in gluconeogenesis overcomes the large, negative, standard free-energy change of the pyruvate kinase reaction in glycolysis.

24. Glucogenic Substrates A common procedure for determining the effectiveness of compounds as precursors of glucose in mammals is to starve the animal until the liver glycogen stores are depleted and then administer the compound in question. A substrate that leads to a *net* increase in liver glycogen is termed glucogenic, because it must first be converted to glucose 6-phosphate. Show by means of known enzymatic reactions which of the following substances are glucogenic.

(a) Succinate, $^-OOC-CH_2-CH_2-COO^-$

(b) Glycerol, $HO-CH_2-CH(OH)-CH_2-OH$

(c) Acetyl-CoA, $CH_3-C(=O)-S\text{-}CoA$

(d) Pyruvate, $CH_3-C(=O)-COO^-$

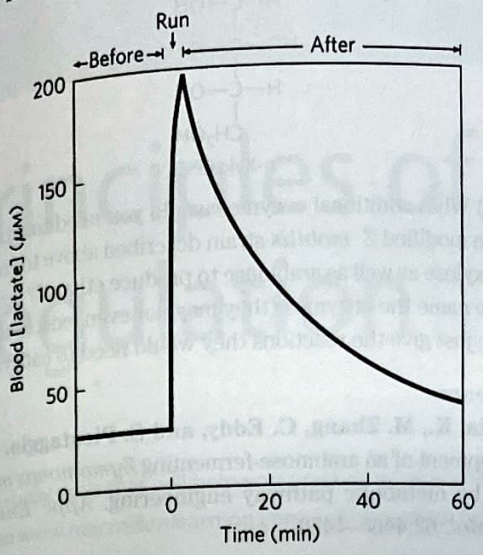
(e) Butyrate, $CH_3-CH_2-CH_2-COO^-$

25. Ethanol Affects Blood Glucose Levels The consumption of alcohol (ethanol), especially after periods of strenuous activity or after not eating for several hours, results in a deficiency of glucose in the blood, a condition known as hypoglycemia. The first step in the metabolism of ethanol by the liver is oxidation to acetaldehyde, catalyzed by liver alcohol dehydrogenase:

$$CH_3CH_2OH + NAD^+ \longrightarrow CH_3CHO + NADH + H^+$$

Explain how this reaction inhibits the transformation of lactate to pyruvate. Why does this lead to hypoglycemia?

26. Blood Lactate Levels during Vigorous Exercise The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.



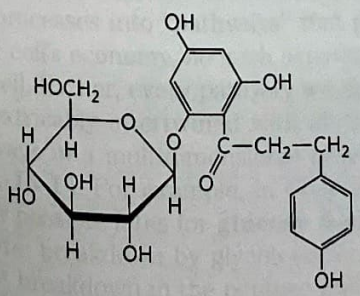
(a) What causes the rapid rise in lactate concentration?

(b) What causes the decline in lactate concentration after completion of the sprint? Why does the decline occur more slowly than the increase?

(c) Why is the concentration of lactate not zero during the resting state?

27. Relationship between Fructose 1,6-Bisphosphatase and Blood Lactate Levels A congenital defect in the liver enzyme fructose 1,6-bisphosphatase results in abnormally high levels of lactate in the blood plasma. Explain.

28. Effect of Phloridzin on Carbohydrate Metabolism Phloridzin, a toxic glycoside from the bark of the pear tree, blocks the normal reabsorption of glucose from the kidney tubule, thus causing blood glucose to be almost completely excreted in the urine. In an experiment, rats fed phloridzin and sodium succinate excreted about 0.5 mol of glucose (made by gluconeogenesis) for every 1 mol of sodium succinate ingested. How is the succinate transformed to glucose? Explain the stoichiometry.



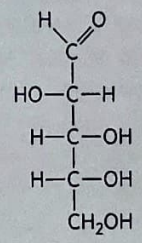
Phloridzin

29. Excess O_2 Uptake during Gluconeogenesis Lactate absorbed by the liver is converted to glucose, with the input of 6 mol of ATP for every mole of glucose produced. The extent of this process in a rat liver preparation can be monitored by administering [^{14}C]lactate and measuring the amount of [^{14}C] glucose produced. Because the stoichiometry of O_2 consumption and ATP production is known (about 5 ATP per O_2), we can predict the extra O_2 consumption above the normal rate when a given amount of lactate is administered. However, when the extra O_2 used in the synthesis of glucose from lactate is actually measured, it is always higher than predicted by known stoichiometric relationships. Suggest a possible explanation for this observation.

30. Role of the Pentose Phosphate Pathway If the oxidation of glucose 6-phosphate via the pentose phosphate pathway were being used primarily to generate NADPH for biosynthesis, the other product, ribose 5-phosphate, would accumulate. What problems might this cause?

Data Analysis Problem

31. Engineering a Fermentation System Fermentation of plant matter to produce ethanol for fuel is one potential method for reducing the use of fossil fuels and thus the CO_2 emissions that lead to global warming. Many microorganisms can break down cellulose then ferment the glucose to ethanol. However, many potential cellulose sources, including agricultural residues and switchgrass, also contain substantial amounts of arabinose, which is not as easily fermented.



D-Arabinose

Escherichia coli is capable of fermenting arabinose to ethanol, but it is not naturally tolerant of high ethanol levels, thus limiting its utility for commercial ethanol production. Another bacterium, *Zymomonas mobilis*, is naturally tolerant of high levels of ethanol but cannot ferment arabinose. Deanda, Zhang, Eddy, and Picataggio (1996) described their efforts to combine the most useful features of these two organisms by introducing the *E. coli* genes for the arabinose-metabolizing enzymes into *Z. mobilis*.

(a) Why is this a simpler strategy than the reverse: engineering *E. coli* to be more ethanol-tolerant?

Deanda and colleagues inserted five *E. coli* genes into the *Z. mobilis* genome: *araA*, coding for L-arabinose isomerase, which interconverts L-arabinose and L-ribulose; *araB*, L-ribulokinase, which uses ATP to phosphorylate L-ribulose at C-5; *araD*, L-ribulose 5-phosphate epimerase, which interconverts L-ribulose 5-phosphate and L-xylulose 5-phosphate; *talB*, transaldolase; and *tktA*, transketolase.

(b) For each of the three *ara* enzymes, briefly describe the chemical transformation it catalyzes and, where possible, name an enzyme discussed in this chapter that carries out an analogous reaction.

Key Terms

Terms in bold are defined in the glossary.

glucose 6-phosphate 575
homeostasis 577
cellular differentiation 577
transcription factor 577
response element 577
turnover 578
transcriptome 579
proteome 579
metabolome 579
metabolic regulation 580
metabolic control 580
mass-action ratio, *Q* 581
adenylate kinase 582
AMP-activated protein kinase (AMPK) 582
flux control coefficient, C 585
flux, J 585
elasticity coefficient, ε 585
response coefficient, R 587
gluconeogenesis 589
futile cycle 590
substrate cycle 590
hexokinase II 590
hexokinase I 591
hexokinase IV (glucokinase) 591
GLUT2 591
glucagon 593
fructose 2,6-bisphosphate 593
phosphofructokinase-2 (PFK-2) 593
fructose 2,6-bisphosphatase (FBPase-2) 593
carbohydrate response element binding protein (ChREBP) 597
sterol regulatory element binding protein (SREBP) 598
cyclic AMP response element binding protein (CREB) 598
forkhead box other (FOXO1) 598
glycogenolysis 601
glycogenesis 601
glucose 1-phosphate 601
debranching enzyme 601
oligo (α1→6) to (α1→4) glucantransferase 601
phosphoglucomutase 601
sugar nucleotides 603
UDP-glucose pyrophosphorylase 605
amylo (1→4) to (1→6) transglycosylase 606
glycogenin 607
glycogen phosphorylase *a* 608
glycogen phosphorylase *b* 608
enzyme cascade 609
phosphorylase *b* kinase 609
phosphoprotein phosphatase 1 (PP1) 609
glycogen synthase *a* 610
glycogen synthase *b* 610
glycogen synthase kinase 3 (GSK3) 610
casein kinase II (CKII) 611
priming 611
glycogen-targeting proteins 612

Problems

1. Measurement of Intracellular Metabolite Concentrations Measuring the concentrations of metabolic intermediates in a living cell presents great experimental difficulties—usually a cell must be destroyed before metabolite concentrations can be measured. Yet enzymes catalyze metabolic interconversions very rapidly, so a common problem associated with these types of measurements is that the findings reflect not the physiological concentrations of metabolites but the equilibrium concentrations. A reliable experimental technique requires all enzyme-catalyzed reactions to be instantaneously stopped in the intact tissue so that the metabolic intermediates do not undergo change. This objective is accomplished by rapidly compressing the tissue between large aluminum plates cooled with liquid nitrogen (−190 °C), a process called **freeze-clamping**. After freezing, which stops enzyme action instantly, the tissue is powdered and the enzymes are inactivated by precipitation with perchloric acid. The precipitate is removed by centrifugation, and the clear supernatant extract is analyzed for metabolites. To calculate intracellular concentrations, the intracellular volume is determined from the total water content of the tissue and a measurement of the extracellular volume.

The intracellular concentrations of the substrates and products of the phosphofructokinase-1 reaction in isolated rat heart tissue are given in the table below.

Metabolite	Concentration (μM)[a]
Fructose 6-phosphate	87.0
Fructose 1,6-bisphosphate	22.0
ATP	11,400
ADP	1,320

Source: Data from J. R. Williamson, *J. Biol. Chem.* 240:2308, 1965.
[a]Calculated as μmol/mL of intracellular water.

(a) Calculate Q, [fructose 1,6-bisphosphate][ADP]/[fructose 6-phosphate][ATP], for the PFK-1 reaction under physiological conditions.

(b) Given a $\Delta G'^{\circ}$ for the PFK-1 reaction of −14.2 kJ/mol, calculate the equilibrium constant for this reaction.

(c) Compare the values of Q and K'_{eq}. Is the physiological reaction near or far from equilibrium? Explain. What does this experiment suggest about the role of PFK-1 as a regulatory enzyme?

2. Are All Metabolic Reactions at Equilibrium?

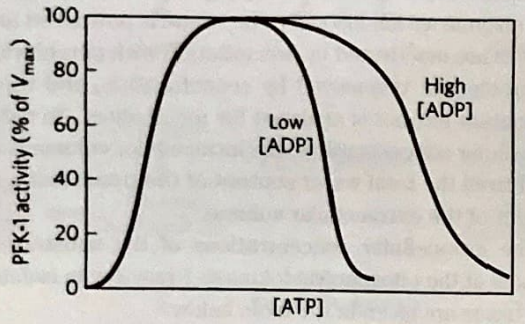
(a) Phosphoenolpyruvate (PEP) is one of the two phosphoryl group donors in the synthesis of ATP during glycolysis. In human erythrocytes, the steady-state concentration of ATP is 2.24 mM, that of ADP is 0.25 mM, and that of pyruvate is 0.051 mM. Calculate the concentration of PEP at 25 °C, assuming that the pyruvate kinase reaction (see Fig. 13-13) is at equilibrium in the cell.

(b) The physiological concentration of PEP in human erythrocytes is 0.023 mM. Compare this with the value obtained in (a). Explain the significance of this difference.

3. Effect of O_2 Supply on Glycolytic Rates The regulated steps of glycolysis in intact cells can be identified by studying the catabolism of glucose in whole tissues or organs. For example, the glucose consumption by heart muscle can be measured by artificially circulating blood through an isolated intact heart and measuring the concentration of glucose before and after the blood passes through the heart. If the circulating blood is deoxygenated, heart muscle consumes glucose at a steady rate. When oxygen is added to the blood, the rate of glucose consumption drops dramatically, then is maintained at the new, lower rate. Explain.

4. Regulation of PFK-1 The effect of ATP on the allosteric enzyme PFK-1 is shown below. For a given concentration of fructose 6-phosphate, the PFK-1 activity increases with increasing

concentrations of ATP, but a point is reached beyond which increasing the concentration of ATP inhibits the enzyme.



(a) Explain how ATP can be both a substrate and an inhibitor of PFK-1. How is the enzyme regulated by ATP?

(b) In what ways is glycolysis regulated by ATP levels?

(c) The inhibition of PFK-1 by ATP is diminished when the ADP concentration is high, as shown in the graph. How can this observation be explained?

5. Cellular Glucose Concentration The concentration of glucose in human blood plasma is maintained at about 5 mM. The concentration of free glucose inside a myocyte is much lower. Why is the concentration so low in the cell? What happens to glucose after entry into the cell? Glucose is administered intravenously as a food source in certain clinical situations. Given that the transformation of glucose to glucose 6-phosphate consumes ATP, why not administer intravenous glucose 6-phosphate instead?

6. Enzyme Activity and Physiological Function The V_{max} of the glycogen phosphorylase from skeletal muscle is much greater than the V_{max} of the same enzyme from liver tissue.

(a) What is the physiological function of glycogen phosphorylase in skeletal muscle? In liver tissue?

(b) Why does the V_{max} of the muscle enzyme need to be greater than that of the liver enzyme?

7. Glycogen Phosphorylase Equilibrium Glycogen phosphorylase catalyzes the removal of glucose from glycogen. The $\Delta G'^{\circ}$ for this reaction is 3.1 kJ/mol.

(a) Calculate the ratio of $[P_i]$ to [glucose 1-phosphate] when the reaction is at equilibrium. (Hint: The removal of glucose units from glycogen does not change the glycogen concentration.)

(b) The measured ratio $[P_i]$/[glucose 1-phosphate] in myocytes under physiological conditions is more than 100:1. What does this indicate about the direction of metabolite flow through the glycogen phosphorylase reaction in muscle?

(c) Why are the equilibrium and physiological ratios different? What is the possible significance of this difference?

8. Regulation of Glycogen Phosphorylase In muscle tissue, the rate of conversion of glycogen to glucose 6-phosphate is determined by the ratio of phosphorylase *a* (active) to phosphorylase *b* (less active). Determine what happens to the rate of glycogen breakdown if a muscle preparation containing glycogen phosphorylase is treated with (a) phosphorylase kinase and ATP; (b) PP1; (c) epinephrine.

9. Glycogen Breakdown in Rabbit Muscle The intracellular use of glucose and glycogen is tightly regulated at four points. To compare the regulation of glycolysis when oxygen is plentiful and when it is depleted, consider the utilization of glucose and glycogen by rabbit leg muscle in two physiological settings: a resting rabbit, with low ATP demands, and a rabbit that sights its mortal enemy, the coyote, and dashes into its burrow. For each setting, determine the relative levels (high, intermediate, or low) of AMP, ATP, citrate, and acetyl-CoA and describe how these levels affect the flow of metabolites through glycolysis by regulating specific enzymes. In periods of stress, rabbit leg muscle produces much of its ATP by anaerobic glycolysis (lactate fermentation) and very little by oxidation of acetyl-CoA derived from fat breakdown.

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

11. Enzyme Defects in Carbohydrate Metabolism Summaries of four clinical case studies follow. For each case determine which enzyme is defective and designate the appropriate treatment, from the lists provided at the end of the problem. Justify your choices. Answer the questions contained in each case study. (You may need to refer to information in Chapter 14.)

Case A The patient develops vomiting and diarrhea shortly after milk ingestion. A lactose tolerance test is administered. (The patient ingests a standard amount of lactose, and the glucose and galactose concentrations in blood plasma are measured at intervals. In individuals with normal carbohydrate metabolism, the levels increase to a maximum in about 1 hour, then decline.) The patient's blood glucose and galactose concentrations do not increase during the test. Why do blood glucose and galactose increase and then decrease during the test in healthy individuals? Why do they fail to rise in the patient?

Case B The patient develops vomiting and diarrhea after ingestion of milk. His blood is found to have a low concentration of glucose but a much higher than normal concentration of reducing sugars. The urine tests positive for galactose. Why is the concentration of reducing sugar in the blood high? Why does galactose appear in the urine?

Case C The patient complains of painful muscle cramps when performing strenuous physical exercise but has no other symptoms. A muscle biopsy indicates a muscle glycogen concentration much higher than normal. Why does glycogen accumulate?

Case D The patient is lethargic, her liver is enlarged, and a biopsy of the liver shows large amounts of excess glycogen. She also has a lower than normal blood glucose level. What is the reason for the low blood glucose in this patient?

Defective Enzyme

(a) Muscle PFK-1
(b) Phosphomannose isomerase
(c) Galactose 1-phosphate uridylyltransferase
(d) Liver glycogen phosphorylase
(e) Triose kinase
(f) Lactase in intestinal mucosa
(g) Maltase in intestinal mucosa
(h) Muscle debranching enzyme

Treatment

1. Jogging 5 km each day
2. Fat-free diet
3. Low-lactose diet
4. Avoiding strenuous exercise
5. Large doses of niacin (the precursor of NAD^+)
6. Frequent feedings (smaller portions) of a normal diet

12. Effects of Insufficient Insulin in a Person with Diabetes A man with insulin-dependent diabetes is brought to the emergency room in a near-comatose state. While vacationing in an isolated place, he lost his insulin medication and has not taken any insulin for two days.

(a) For each tissue listed below, is each pathway faster, slower, or uncharged in this patient, compared with the normal level when he is getting appropriate amounts of insulin?

(b) For each pathway, describe at least one control mechanism responsible for the change you predict.

Tissue and Pathways

1. Adipose: fatty acid synthesis
2. Muscle: glycolysis; fatty acid synthesis; glycogen synthesis
3. Liver: glycolysis; gluconeogenesis; glycogen synthesis; fatty acid synthesis; pentose phosphate pathway

13. Blood Metabolites in Insulin Insufficiency For the patient described in Problem 12, predict the levels of the following metabolites in his blood *before* treatment in the emergency room, relative to levels maintained during adequate insulin treatment: (a) glucose; (b) ketone bodies; (c) free fatty acids.

14. Metabolic Effects of Mutant Enzymes Predict and explain the effect on glycogen metabolism of each of the following defects caused by mutation: (a) loss of the cAMP-binding site on the regulatory subunit of protein kinase A (PKA); (b) loss of the protein phosphatase inhibitor (inhibitor 1 in Fig. 15-42); (c) overexpression of phosphorylase *b* kinase in liver; (d) defective glucagon receptors in liver.

15. Hormonal Control of Metabolic Fuel Between your evening meal and breakfast, your blood glucose drops and your liver becomes a net producer rather than consumer of glucose. Describe the hormonal basis for this switch, and explain how the hormonal change triggers glucose production by the liver.

16. Altered Metabolism in Genetically Manipulated Mice Researchers can manipulate the genes of a mouse so that a single gene in a single tissue either produces an inactive protein (a "knockout" mouse) or produces a protein that is always (constitutively) active. What effects on metabolism would you predict for mice with the following genetic changes: (a) knockout of glycogen debranching enzyme in the liver; (b) knockout of hexokinase IV in liver; (c) knockout of FBPase-2 in liver; (d) constitutively active FBPase-2 in liver; (e) constitutively active AMPK in muscle; (f) constitutively active ChREBP in liver?

Data Analysis Problem

17. Optimal Glycogen Structure Muscle cells need rapid access to large amounts of glucose during heavy exercise. This glucose is stored in liver and skeletal muscle in polymeric form as particles of glycogen. The typical glycogen particle contains about 55,000 glucose residues (see Fig. 15-35b). Meléndez-Hevia, Waddell, and Shelton (1993) explored some theoretical aspects of the structure of glycogen, as described in this problem.

(a) The cellular concentration of glycogen in liver is about 0.01 μM. What cellular concentration of free glucose would be required to store an equivalent amount of glucose? Why would this concentration of free glucose present a problem for the cell?

Glucose is released from glycogen by glycogen phosphorylase, an enzyme that can remove glucose molecules, one at a time, from one end of a glycogen chain. Glycogen chains are branched (see Figs 15-28 and 15-35b), and the degree of branching—the number of branches per chain—has a powerful influence on the rate at which glycogen phosphorylase can release glucose.

(b) Why would a degree of branching that was too low (i.e., below an optimum level) reduce the rate of glucose release? (Hint: Consider the extreme case of no branches in a chain of 55,000 glucose residues.)

(c) Why would a degree of branching that was too high also reduce the rate of glucose release? (Hint: Think of the physical constraints.)

Meléndez-Hevia and colleagues did a series of calculations and found that two branches per chain (see Fig. 15-35b) was optimal for the constraints described above. This is what is found in glycogen stored in muscle and liver.

To determine the optimum number of glucose residues per chain, Meléndez-Hevia and coauthors considered two key parameters that define the structure of a glycogen particle: t = the number of tiers of glucose chains in a particle (the molecule in Fig. 15-35b has five tiers); g_c = the number of glucose residues in each chain. They set out to find the values of t and g_c that would maximize three quantities: (1) the amount of glucose stored in the particle (G_T) per unit volume; (2) the number of unbranched glucose chains (C_A) per unit volume (i.e., number of chains in the outermost tier, readily accessible to glycogen phosphorylase); and (3) the amount of glucose available to phosphorylase in these unbranched chains (G_{PT}).

(d) Show that $C_A = 2^{t-1}$. This is the number of chains available to glycogen phosphorylase before the action of the debranching enzyme.

(e) Show that C_T, the total number of chains in the particle, is given by $C_T = 2^t - 1$. Thus $G_T = g_c(C_T) = g_c(2^t - 1)$, the total number of glucose residues in the particle.

(f) Glycogen phosphorylase cannot remove glucose from glycogen chains that are shorter than five glucose residues. Show that $G_{PT} = (g_c - 4)(2^{t-1})$. This is the amount of glucose readily available to glycogen phosphorylase.

Key Terms

Terms in bold are defined in the glossary.

respiration 619
cellular respiration 619
citric acid cycle 619
tricarboxylic acid (TCA) cycle 619
Krebs cycle 619
mitchondrial pyruvate carrier (MPC) 620
pyruvate dehydrogenase (PDH) complex 620
oxidative decarboxylation 620
thioester 621
lipoate 621
substrate channeling 623
iron-sulfur center 627
moonlighting enzymes 628
α-ketoglutarate dehydrogenase complex 630
synthases 631
synthetases 631
ligases 631
lyases 631
kinases 631
phosphorylases 631
phosphatases 631
nucleoside diphosphate kinase 632
prochiral molecule 634
amphibolic pathway 636
glyoxylate cycle 636
anaplerotic reaction 636
biotin 638
avidin 639
metabolon 642

Problems

1. Balance Sheet for the Citric Acid Cycle The citric acid cycle has eight enzymes: citrate synthase, aconitase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinyl-CoA synthetase, succinate dehydrogenase, fumarase, and malate dehydrogenase.

(a) Write a balanced equation for the reaction catalyzed by each enzyme.

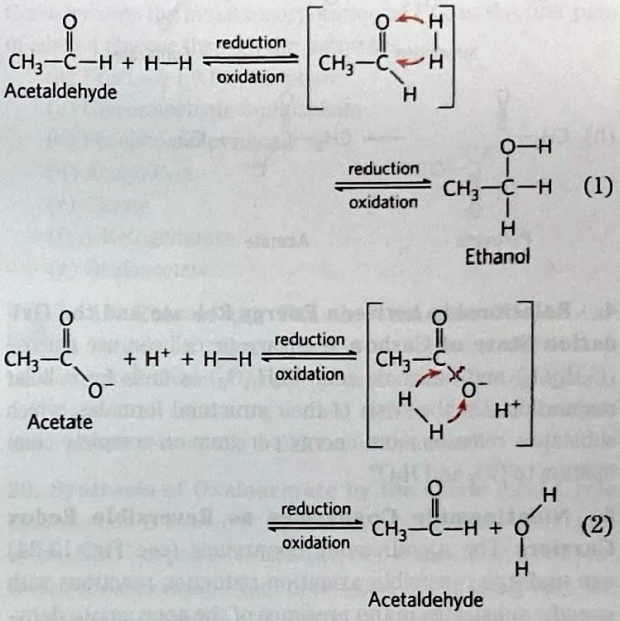
(b) Name the cofactor(s) required by each enzyme reaction.

(c) For each enzyme determine which of the following describes the type of reaction(s) catalyzed: condensation (carbon–carbon bond formation); dehydration (loss of water); hydration (addition of water); decarboxylation (loss of CO_2); oxidation-reduction; substrate-level phosphorylation; isomerization.

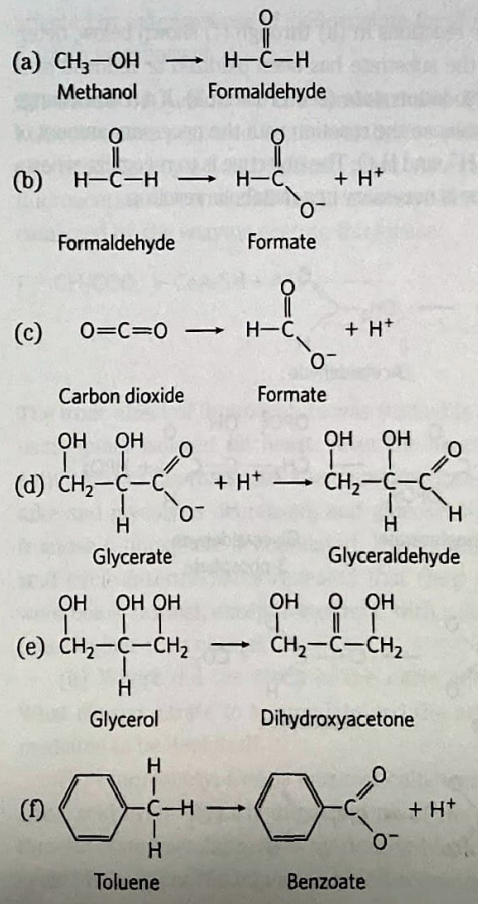
(d) Write a balanced net equation for the catabolism of acetyl-CoA to CO_2.

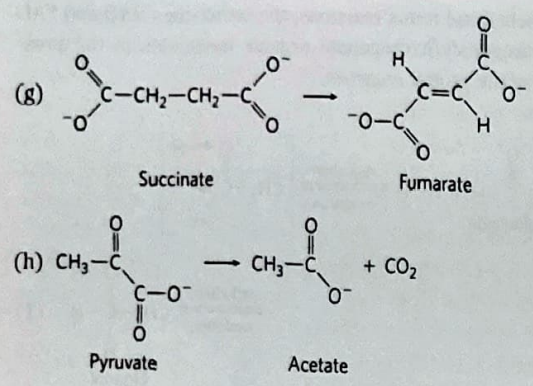
2. Net Equation for Glycolysis and the Citric Acid Cycle Write the net biochemical equation for the metabolism of a molecule of glucose by glycolysis and the citric acid cycle, including all cofactors.

3. Recognizing Oxidation and Reduction Reactions One biochemical strategy of many living organisms is the stepwise oxidation of organic compounds to CO_2 and H_2O and the conservation of a major part of the energy thus produced in the form of ATP. It is important to be able to recognize oxidation-reduction processes in metabolism. Reduction of an organic molecule results from the hydrogenation of a double bond (Eqn 1, below) or of a single bond with accompanying cleavage (Eqn 2). Conversely, oxidation results from dehydrogenation. In biochemical redox reactions, the coenzymes NAD and FAD dehydrogenate/hydrogenate organic molecules in the presence of the proper enzymes.



For each of the metabolic transformations in (a) through (h), determine whether oxidation or reduction has occurred. Balance each transformation by inserting H—H and, where necessary, H_2O.



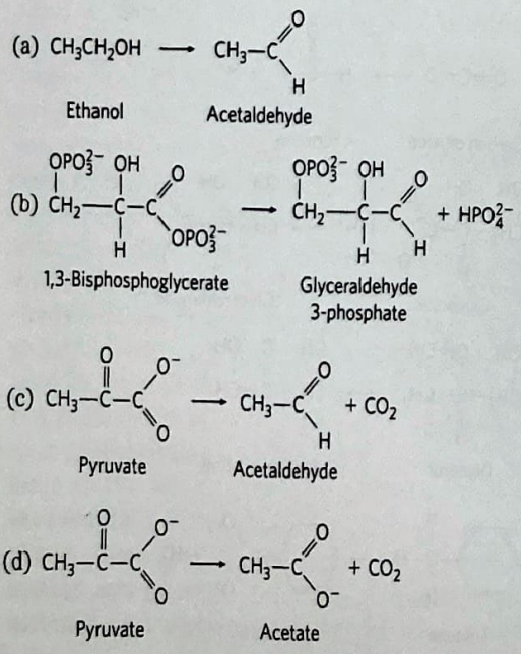


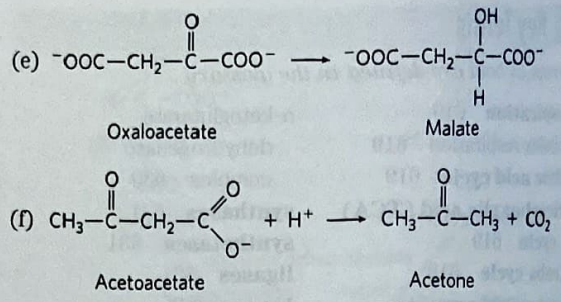
4. Relationship between Energy Release and the Oxidation State of Carbon A eukaryotic cell can use glucose ($C_6H_{12}O_6$) and hexanoic acid ($C_6H_{14}O_2$) as fuels for cellular respiration. On the basis of their structural formulas, which substance releases more energy per gram on complete combustion to CO_2 and H_2O?

5. Nicotinamide Coenzymes as Reversible Redox Carriers The nicotinamide coenzymes (see Fig. 13-24) can undergo reversible oxidation-reduction reactions with specific substrates in the presence of the appropriate dehydrogenase. In these reactions, NADH + H^+ serves as the hydrogen source, as described in Problem 3. Whenever the coenzyme is oxidized, a substrate must be simultaneously reduced:

$$\underset{\text{Oxidized}}{\text{Substrate}} + \underset{\text{Reduced}}{\text{NADH}} + H^+ \rightleftharpoons \underset{\text{Reduced}}{\text{product}} + \underset{\text{Oxidized}}{NAD^+}$$

For each of the reactions in (a) through (f) shown below, determine whether the substrate has been oxidized or reduced or is unchanged in oxidation state (see Problem 3). If a redox change has occurred, balance the reaction with the necessary amount of NAD^+, NADH, H^+, and H_2O. The objective is to recognize when a redox coenzyme is necessary in a metabolic reaction.





6. Pyruvate Dehydrogenase Cofactors and Mechanism Describe the role of each cofactor involved in the reaction catalyzed by the pyruvate dehydrogenase complex.

7. Thiamine Deficiency Individuals with a thiamine-deficient diet have relatively high levels of pyruvate in their blood. Explain this in biochemical terms.

8. Isocitrate Dehydrogenase Reaction What type of chemical reaction is involved in the conversion of isocitrate to α-ketoglutarate? Name and describe the role of any cofactors. What other reaction(s) of the citric acid cycle are of this same type?

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

10. Formation of Oxaloacetate in a Mitochondrion In the last reaction of the citric acid cycle, malate is dehydrogenated to regenerate the oxaloacetate necessary for the entry of acetyl-CoA into the cycle:

$$\text{L-Malate} + NAD^+ \longrightarrow \text{oxaloacetate} + \text{NADH} + H^+$$
$$\Delta G'^\circ = 30.0\ \text{kJ/mol}$$

(a) Calculate the equilibrium constant for this reaction at 25 °C.

(b) Because $\Delta G'^\circ$ assumes a standard pH of 7, the equilibrium constant calculated in (a) corresponds to

$$K'_{eq} = \frac{[\text{oxaloacetate}][\text{NADH}]}{[\text{L-malate}][NAD^+]}$$

The measured concentration of L-malate in rat liver mitochondria is about 0.20 mM when $[NAD^+]/[\text{NADH}]$ is 10. Calculate the concentration of oxaloacetate at pH 7 in these mitochondria.

(c) To appreciate the magnitude of the mitochondrial oxaloacetate concentration, calculate the number of oxaloacetate molecules in a single rat liver mitochondrion. Assume the mitochondrion is a sphere of diameter 2.0 μm.

11. Cofactors for the Citric Acid Cycle Suppose you have prepared a mitochondrial extract that contains all the soluble enzymes of the matrix but has lost (by dialysis) all the low molecular weight cofactors. What must you add to the extract so that the preparation will oxidize acetyl-CoA to CO_2?

12. Riboflavin Deficiency How would a riboflavin deficiency affect the functioning of the citric acid cycle? Explain your answer.

13. Oxaloacetate Pool What factors might decrease the pool of oxaloacetate available for the activity of the citric acid cycle? How can the pool of oxaloacetate be replenished?

14. Energy Yield from the Citric Acid Cycle The reaction catalyzed by succinyl-CoA synthetase produces the high-energy compound GTP. How is the free energy contained in GTP incorporated into the cellular ATP pool?

15. Respiration Studies in Isolated Mitochondria Cellular respiration can be studied in isolated mitochondria by measuring oxygen consumption under different conditions. If 0.01 M sodium malonate is added to actively respiring mitochondria that are using pyruvate as fuel, respiration soon stops and a metabolic intermediate accumulates.

(a) What is the structure of this intermediate?

(b) Explain why it accumulates.

(c) Explain why oxygen consumption stops.

(d) Aside from removal of the malonate, how can this inhibition of respiration be overcome? Explain.

16. Labeling Studies in Isolated Mitochondria The metabolic pathways of organic compounds have often been delineated by using a radioactively labeled substrate and following the fate of the label.

(a) How can you determine whether glucose added to a suspension of isolated mitochondria is metabolized to CO_2 and H_2O?

(b) Suppose you add a brief pulse of [3-^{14}C]pyruvate (labeled in the methyl position) to the mitochondria. After one turn of the citric acid cycle, what is the location of the ^{14}C in the oxaloacetate? Explain by tracing the ^{14}C label through the pathway. How many turns of the cycle are required to release all the [3-^{14}C]pyruvate as CO_2?

17. Pathway of CO_2 in Gluconeogenesis In the first bypass step of gluconeogenesis, the conversion of pyruvate to phosphoenolpyruvate (PEP), pyruvate is carboxylated by pyruvate carboxylase to oxaloacetate, which is subsequently decarboxylated to PEP by PEP carboxykinase (Chapter 14). Because the addition of CO_2 is directly followed by the loss of CO_2, you might expect that in tracer experiments, the ^{14}C of $^{14}CO_2$ would not be incorporated into PEP, glucose, or any intermediates in gluconeogenesis. However, investigators find that when a rat liver preparation synthesizes glucose in the presence of $^{14}CO_2$, ^{14}C slowly appears in PEP and eventually at C-3 and C-4 of glucose. How does the ^{14}C label get into the PEP and glucose? (Hint: During gluconeogenesis in the presence of $^{14}CO_2$, several of the four-carbon citric acid cycle intermediates also become labeled.)

18. [1-^{14}C]Glucose Catabolism An actively respiring bacterial culture is briefly incubated with [1-^{14}C]glucose, and the glycolytic and citric acid cycle intermediates are isolated. Where is the ^{14}C in each of the intermediates listed below? Consider only the initial incorporation of ^{14}C, in the first pass of labeled glucose through the pathways.

(a) Fructose 1,6-bisphosphate

(b) Glyceraldehyde 3-phosphate

(c) Phosphoenolpyruvate

(d) Acetyl-CoA

(e) Citrate

(f) α-Ketoglutarate

(g) Oxaloacetate

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

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

21. Oxaloacetate Depletion Mammalian liver can carry out gluconeogenesis using oxaloacetate as the starting material (Chapter 14). Would the operation of the citric acid cycle be affected by extensive use of oxaloacetate for gluconeogenesis? Explain your answer.

22. Mode of Action of the Rodenticide Fluoroacetate Fluoroacetate, prepared commercially for rodent control, is also produced by a South African plant. After entering a cell, fluoroacetate is converted to fluoroacetyl-CoA in a reaction catalyzed by the enzyme acetate thiokinase:

$$F{-}CH_2COO^- + CoA\text{-}SH + ATP \longrightarrow F{-}CH_2C(=O){-}S\text{-}CoA + AMP + PP_i$$

The toxic effect of fluoroacetate was studied in an experiment using intact isolated rat heart. After the heart was perfused with 0.22 mM fluoroacetate, the measured rate of glucose uptake and glycolysis decreased, and glucose 6-phosphate and fructose 6-phosphate accumulated. Examination of the citric acid cycle intermediates revealed that their concentrations were below normal, except for citrate, with a concentration 10 times higher than normal.

(a) Where did the block in the citric acid cycle occur? What caused citrate to accumulate and the other cycle intermediates to be depleted?

(b) Fluoroacetyl-CoA is enzymatically transformed in the citric acid cycle. What is the structure of the end product of fluoroacetate metabolism? Why does it block the citric acid cycle? How might the inhibition be overcome?

(c) In the heart perfusion experiments, why did glucose uptake and glycolysis decrease? Why did hexose monophosphates accumulate?

(d) Why is fluoroacetate poisoning fatal?

23. Synthesis of L-Malate in Wine Making The tartness of some wines is due to high concentrations of L-malate. Write a sequence of reactions showing how yeast cells synthesize L-malate from glucose under anaerobic conditions in the presence of dissolved CO_2 (HCO_3^-). Note that the overall reaction for this fermentation cannot involve the consumption of nicotinamide coenzymes or citric acid cycle intermediates.

24. Net Synthesis of α-Ketoglutarate α-Ketoglutarate plays a central role in the biosynthesis of several amino acids. Write a sequence of enzymatic reactions that could result in the net synthesis of α-ketoglutarate from pyruvate. Your proposed sequence must not involve the net consumption of other citric acid cycle intermediates. Write an equation for the overall reaction and identify the source of each reactant.

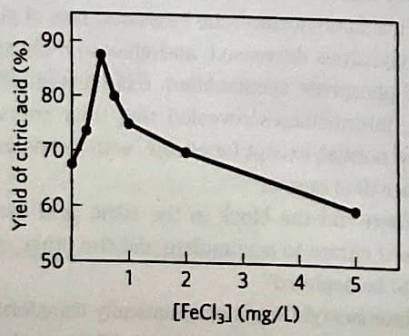
25. Amphibolic Pathways Explain, giving examples, what is meant by the statement that the citric acid cycle is amphibolic.

26. Regulation of the Pyruvate Dehydrogenase Complex In animal tissues, the rate of conversion of pyruvate to acetyl-CoA is regulated by the ratio of active, phosphorylated to inactive, unphosphorylated PDH complex. Determine what happens to the rate of this reaction when a preparation of rabbit muscle mitochondria containing the PDH complex is treated with (a) pyruvate dehydrogenase kinase, ATP, and NADH; (b) pyruvate dehydrogenase phosphatase and Ca^{2+}; (c) malonate.

27. Commercial Synthesis of Citric Acid Citric acid is used as a flavoring agent in soft drinks, fruit juices, and many other foods. Worldwide, the market for citric acid is valued at hundreds of millions of dollars per year. Commercial production uses the mold *Aspergillus niger*, which metabolizes sucrose under carefully controlled conditions.

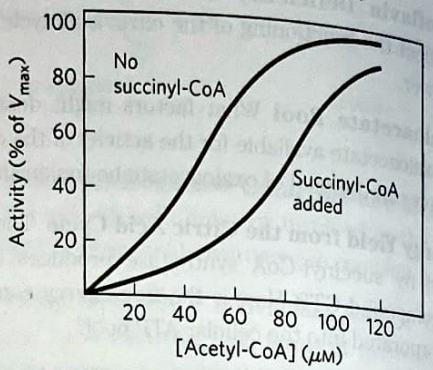
(a) The yield of citric acid is strongly dependent on the concentration of $FeCl_3$ in the culture medium, as indicated in the graph. Why does the yield decrease when the concentration of Fe^{3+} is above or below the optimal value of 0.5 mg/L?

(b) Write the sequence of reactions by which *A. niger* synthesizes citric acid from sucrose. Write an equation for the overall reaction.



(c) Does the commercial process require the culture medium to be aerated—that is, is this a fermentation or an aerobic process? Explain.

28. Regulation of Citrate Synthase In the presence of saturating amounts of oxaloacetate, the activity of citrate synthase from pig heart tissue shows a sigmoid dependence on the concentration of acetyl-CoA, as shown in the graph below. When succinyl-CoA is added, the curve shifts to the right and the sigmoid dependence is more pronounced.



On the basis of these observations, suggest how succinyl-CoA regulates the activity of citrate synthase. (Hint: See Fig. 6-35.) Why is succinyl-CoA an appropriate signal for regulation of the citric acid cycle? How does the regulation of citrate synthase control the rate of cellular respiration in pig heart tissue?

29. Regulation of Pyruvate Carboxylase The carboxylation of pyruvate by pyruvate carboxylase occurs at a very low rate unless acetyl-CoA, a positive allosteric modulator, is present. If you have just eaten a meal rich in fatty acids (triacylglycerols) but low in carbohydrates (glucose), how does this regulatory property shut down the oxidation of glucose to CO_2 and H_2O but increase the oxidation of acetyl-CoA derived from fatty acids?

30. Relationship between Respiration and the Citric Acid Cycle Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when O_2 is present. Why?

31. Effect of [NADH]/[NAD$^+$] on the Citric Acid Cycle How would you expect the operation of the citric acid cycle to respond to a rapid increase in the [NADH]/[NAD$^+$] ratio in the mitochondrial matrix? Why?

32. Thermodynamics of Citrate Synthase Reaction in Cells Citrate is formed by the condensation of acetyl-CoA with oxaloacetate, catalyzed by citrate synthase:

$$\text{Oxaloacetate} + \text{acetyl-CoA} + H_2O \rightleftharpoons \text{citrate} + \text{CoA} + H^+$$

In rat heart mitochondria at pH 7.0 and 25 °C, the concentrations of reactants and products are: oxaloacetate, 1 μM; acetyl-CoA, 1 μM; citrate, 220 μM; and CoA, 65 μM. The standard free-energy change for the citrate synthase reaction is −32.2 kJ/mol. What is the direction of metabolite flow through the citrate synthase reaction in rat heart cells? Explain.

33. Reactions of the Pyruvate Dehydrogenase Complex Two of the steps in the oxidative decarboxylation of pyruvate (steps ❹ and ❺ in Fig. 16-6) do not involve any of the three carbons of pyruvate yet are essential to the operation of the PDH complex. Explain.

34. Citric Acid Cycle Mutants There are many cases of human disease in which one or another enzyme activity is lacking due to genetic mutation. However, cases in which individuals lack one of the enzymes of the citric acid cycle are extremely rare. Why?

Data Analysis Problem

35. How the Citric Acid Cycle Was Discovered The detailed biochemistry of the citric acid cycle was determined by several researchers over a period of decades. In a 1937 article, Krebs and Johnson summarized their work and the work of others in the first published description of this pathway.

The methods used by these researchers were very different from those of modern biochemistry. Radioactive tracers were not commonly available until the 1940s, so Krebs and other researchers had to use nontracer techniques to work out the pathway. Using freshly prepared samples of pigeon breast muscle, they determined oxygen consumption by suspending minced muscle in buffer in a sealed flask and measuring the volume (in μL) of oxygen consumed under different conditions. They measured levels of substrates (intermediates) by treating samples with acid to remove contaminating proteins, then assaying the quantities of various small organic molecules. The two key observations that led Krebs and colleagues to propose a citric acid *cycle* as opposed to a *linear pathway* (like that of glycolysis) were made in the following experiments.

Experiment I. They incubated 460 mg of minced muscle in 3 mL of buffer at 40 °C for 150 minutes. Addition of *citrate* increased O_2 consumption by 893 μL compared with samples without added citrate. They calculated, based on the O_2 consumed during respiration of other carbon-containing compounds, that the expected O_2 consumption for complete respiration of this quantity of citrate was only 302 μL.

Experiment II. They measured O_2 consumption by 460 mg of minced muscle in 3 mL of buffer when incubated with *citrate* and/or with *1-phosphoglycerol* (glycerol 1-phosphate; this was known to be readily oxidized by cellular respiration) at 40 °C for 140 minutes. The results are shown in the table.

Sample	Substrate(s) added	μL O_2 absorbed
1	No extra	342
2	0.3 mL 0.2 M 1-phosphoglycerol	757
3	0.15 mL 0.02 M citrate	431
4	0.3 mL 0.2 M 1-phosphoglycerol and 0.15 mL 0.02 M citrate	1,385

(a) Why is O_2 consumption a good measure of cellular respiration?

(b) Why does sample 1 (unsupplemented muscle tissue) consume some oxygen?

(c) Based on the results for samples 2 and 3, can you conclude that 1-phosphoglycerol and citrate serve as substrates for cellular respiration in this system? Explain your reasoning.

(d) Krebs and colleagues used the results from these experiments to argue that citrate was "catalytic"—that it helped the muscle tissue samples metabolize 1-phosphoglycerol more completely. How would you use their data to make this argument?

(e) Krebs and colleagues further argued that citrate was not simply consumed by these reactions, but had to be *regenerated*. Therefore, the reactions had to be a *cycle* rather than a linear pathway. How would you make this argument?

Other researchers had found that *arsenate* (AsO_4^{3-}) inhibits α-ketoglutarate dehydrogenase and that *malonate* inhibits succinate dehydrogenase.

(f) Krebs and coworkers found that muscle tissue samples treated with arsenate and citrate would consume citrate only in the presence of oxygen; under these conditions, oxygen was consumed. Based on the pathway in Figure 16-7, what was the citrate converted to in this experiment, and why did the samples consume oxygen?

In their article, Krebs and Johnson further reported the following. (1) In the presence of arsenate, 5.48 mmol of citrate was converted to 5.07 mmol of α-ketoglutarate. (2) In the presence of malonate, citrate was quantitatively converted to large amounts of succinate and small amounts of α-ketoglutarate. (3) Addition of oxaloacetate in the absence of oxygen led to production of a large amount of citrate; the amount was increased if glucose was also added.

Other workers had found the following pathway in similar muscle tissue preparations:

$$\text{Succinate} \longrightarrow \text{fumarate} \longrightarrow \text{malate} \longrightarrow \text{oxaloacetate} \longrightarrow \text{pyruvate}$$

(g) Based only on the data presented in this problem, what is the order of the intermediates in the citric acid cycle? How does this compare with Figure 16-7? Explain your reasoning.

(h) Why was it important to show the *quantitative* conversion of citrate to α-ketoglutarate?

The Krebs and Johnson article also contains other data that filled in most of the missing components of the cycle. The only component left unresolved was the molecule that reacted with oxaloacetate to form citrate.

Reference

Krebs, H.A., and W.A. Johnson. 1937. The role of citric acid in intermediate metabolism in animal tissues. *Enzymologia* 4:148–156. Reprinted in *FEBS Lett.* 117(Suppl.):K2–K10, 1980.

Further Reading is available at www.macmillanlearning.com/LehningerBiochemistry7e.

synthesis by gluconeogenesis, for example, oxidation of cycle intermediates slows—and so does acetyl-CoA oxidation. Moreover, the liver contains only a limited amount of coenzyme A, and when most of it is tied up in acetyl-CoA, β oxidation slows for want of the free coenzyme. The production and export of ketone bodies frees coenzyme A, allowing continued fatty acid oxidation.

Ketone Bodies Are Overproduced in Diabetes and during Starvation

Starvation and untreated diabetes mellitus lead to overproduction of ketone bodies in the liver, with several adverse effects on health. During starvation, gluconeogenesis depletes citric acid cycle intermediates, diverting acetyl-CoA to ketone body production (**Fig. 17-20**). In untreated diabetes, when the insulin level is insufficient, extrahepatic tissues cannot take up glucose efficiently from the blood, either for fuel or for conversion to fat. Under these conditions, levels of malonyl-CoA (the starting material for fatty acid synthesis in the liver; see Fig. 21-1) fall, inhibition of carnitine acyltransferase 1 is relieved, and fatty acids enter mitochondria to be degraded to acetyl-CoA—which cannot pass through the citric acid cycle because cycle intermediates have been drawn off for use as substrates in gluconeogenesis. The resulting accumulation of acetyl-CoA accelerates the formation of ketone bodies and their release into the blood beyond the capacity of extrahepatic tissues to oxidize them. The increased blood levels of acetoacetate and D-β-hydroxybutyrate lower the blood pH, causing the condition known as **acidosis**. Extreme acidosis can lead to coma and in some cases death. Ketone bodies in the blood and urine of individuals with untreated diabetes can reach extraordinary levels—a blood concentration of 90 mg/100 mL (compared with a normal level of <3 mg/100 mL) and urinary excretion of 5,000 mg/24 hr (compared with a normal rate of ≤125 mg/24 hr). This condition is called **ketosis** or, when combined with acidosis, **ketoacidosis**.

Individuals on very low-calorie diets, using the fats stored in adipose tissue as their major energy source, also have increased levels of ketone bodies in their blood and urine. These levels must be monitored to avoid the dangers of ketoacidosis. ■

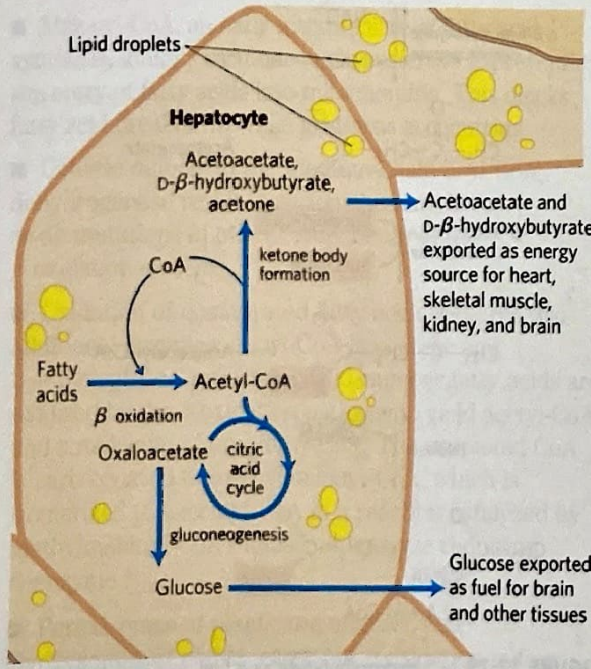


FIGURE 17-20 Ketone body formation and export from the liver. Conditions that promote gluconeogenesis (untreated diabetes, severely reduced food intake) slow the citric acid cycle (by drawing off oxaloacetate) and enhance the conversion of acetyl-CoA to acetoacetate. The released coenzyme A allows continued β oxidation of fatty acids.

SUMMARY 17.3 Ketone Bodies

■ The ketone bodies—acetone, acetoacetate, and D-β-hydroxybutyrate—are formed in the liver when fatty acids are the principal fuel supporting whole-body metabolism. Acetoacetate and D-β-hydroxybutyrate serve as fuel molecules in extrahepatic tissues, including the brain, through oxidation to acetyl-CoA and entry into the citric acid cycle.

■ Overproduction of ketone bodies in uncontrolled diabetes or severely reduced calorie intake can lead to acidosis or ketosis or both (ketoacidosis).

Key Terms

Terms in bold are defined in the glossary.

β oxidation 649
chylomicron 651
apolipoprotein 651
lipoprotein 651
perilipin 651
free fatty acids 651
serum albumin 651
carnitine shuttle 652
carnitine acyltransferase 1 653
acyl-carnitine/carnitine transporter 653
carnitine acyltransferase 2 653
trifunctional protein (TFP) 656
methylmalonyl-CoA mutase 661
coenzyme B_{12} 661
malonyl-CoA 661
pernicious anemia 663
intrinsic factor 663
PPAR (peroxisome proliferator-activated receptor) 664
medium-chain acyl-CoA dehydrogenase (MCAD) 664
multifunctional protein (MFP) 666
ω oxidation 666
mixed-function oxygenases 667
α oxidation 667
acidosis 670
ketosis 670
ketoacidosis 670

Problems

1. Energy in Triacylglycerols On a per-carbon basis, where does the largest amount of biologically available energy in triacylglycerols reside: in the fatty acid portions or the

glycerol portion? Indicate how knowledge of the chemical structure of triacylglycerols provides the answer.

2. Fuel Reserves in Adipose Tissue Triacylglycerols, with their hydrocarbon-like fatty acids, have the highest energy content of the major nutrients.

(a) If 15% of the body mass of a 70.0 kg adult consists of triacylglycerols, what is the total available fuel reserve, in both kilojoules and kilocalories, in the form of triacylglycerols? Recall that 1.00 kcal = 4.18 kJ.

(b) If the basal energy requirement is approximately 8,400 kJ/day (2,000 kcal/day), how long could this person survive if the oxidation of fatty acids stored as triacylglycerols were the only source of energy?

(c) What would be the weight loss in pounds per day under such starvation conditions (1 lb = 0.454 kg)?

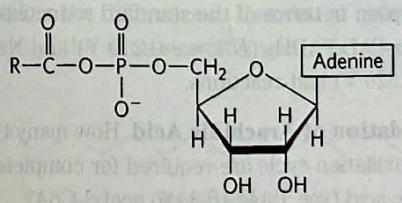
3. Common Reaction Steps in the Fatty Acid Oxidation Cycle and Citric Acid Cycle Cells often use the same enzyme reaction pattern for analogous metabolic conversions. For example, the steps in the oxidation of pyruvate to acetyl-CoA and of α-ketoglutarate to succinyl-CoA, although catalyzed by different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

4. β Oxidation: How Many Cycles? How many cycles of β oxidation are required for the complete oxidation of activated oleic acid, 18:1(Δ^9)?

5. Chemistry of the Acyl-CoA Synthetase Reaction Fatty acids are converted to their coenzyme A esters in a reversible reaction catalyzed by acyl-CoA synthetase:

$$R{-}COO^- + ATP + CoA \rightleftharpoons R{-}\overset{\displaystyle O}{\overset{\|}{C}}{-}CoA + AMP + PP_i$$

(a) The enzyme-bound intermediate in this reaction has been identified as the mixed anhydride of the fatty acid and adenosine monophosphate (AMP), acyl-AMP:



Write two equations corresponding to the two steps of the reaction catalyzed by acyl-CoA synthetase.

(b) The acyl-CoA synthetase reaction is readily reversible, with an equilibrium constant near 1. How can this reaction be made to favor formation of fatty acyl–CoA?

6. Intermediates in Oleic Acid Oxidation What is the structure of the partially oxidized fatty acyl group that is formed when oleic acid, 18:1(Δ^9), has undergone three cycles of β oxidation? What are the next two steps in the continued oxidation of this intermediate?

7. β Oxidation of an Odd-Number Fatty Acid What are the direct products of β oxidation of a fully saturated, straight-chain fatty acid of 11 carbons?

8. Oxidation of Tritiated Palmitate Palmitate uniformly labeled with tritium (^3H) to a specific activity of 2.48×10^8 counts per minute (cpm) per micromole of palmitate is added to a mitochondrial preparation that oxidizes it to acetyl-CoA. The acetyl-CoA is isolated and hydrolyzed to acetate. The specific activity of the isolated acetate is 1.00×10^7 cpm/μmol. Is this result consistent with the β-oxidation pathway? Explain. What is the final fate of the removed tritium?

9. Compartmentation in β Oxidation Free palmitate is activated to its coenzyme A derivative (palmitoyl-CoA) in the cytosol before it can be oxidized in the mitochondrion. If palmitate and [^{14}C]coenzyme A are added to a liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain.

10. Comparative Biochemistry: Energy-Generating Pathways in Birds One indication of the relative importance of various ATP-producing pathways is the V_{max} of certain enzymes of these pathways. The values of V_{max} of several enzymes from the pectoral muscles (chest muscles used for flying) of pigeon and pheasant are listed below.

	V_{max} (μmol substrate/min/g tissue)	
Enzyme	**Pigeon**	**Pheasant**
Hexokinase	3.0	2.3
Glycogen phosphorylase	18.0	120.0
Phosphofructokinase-1	24.0	143.0
Citrate synthase	100.0	15.0
Triacylglycerol lipase	0.07	0.01

(a) Discuss the relative importance of glycogen metabolism and fat metabolism in generating ATP in the pectoral muscles of these birds.

(b) Compare oxygen consumption in the two birds.

(c) Judging from the data in the table, which bird is the long-distance flyer? Justify your answer.

(d) Why were these particular enzymes selected for comparison? Would the activities of triose phosphate isomerase and malate dehydrogenase be equally good bases for comparison? Explain.

11. Mutant Carnitine Acyltransferase What changes in metabolic pattern would result from a mutation in the muscle carnitine acyltransferase 1 in which the mutant protein has lost its affinity for malonyl-CoA but not its catalytic activity?

12. Effect of Carnitine Deficiency An individual developed a condition characterized by progressive muscular weakness and aching muscle cramps. The symptoms were aggravated by fasting, exercise, and a high-fat diet. The homogenate of a skeletal muscle specimen from the patient oxidized added oleate more slowly than did control homogenates, consisting of muscle specimens from healthy individuals.

When carnitine was added to the patient's muscle homogenate, the rate of oleate oxidation equaled that in the control homogenates. The patient was diagnosed as having a carnitine deficiency.

(a) Why did added carnitine increase the rate of oleate oxidation in the patient's muscle homogenate?

(b) Why were the patient's symptoms aggravated by fasting, exercise, and a high-fat diet?

(c) Suggest two possible reasons for the deficiency of muscle carnitine in this individual.

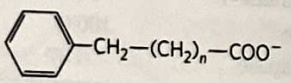
13. Fatty Acids as a Source of Water Contrary to legend, camels do not store water in their humps, which actually consist of large fat deposits. How can these fat deposits serve as a source of water? Calculate the amount of water (in liters) that a camel can produce from 1.0 kg of fat. Assume for simplicity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

14. Petroleum as a Microbial Food Source Some microorganisms of the genera *Nocardia* and *Pseudomonas* can grow in an environment where hydrocarbons are the only food source. These bacteria oxidize straight-chain aliphatic hydrocarbons, such as octane, to their corresponding carboxylic acids:

$$CH_3(CH_2)_6CH_3 + NAD^+ + O_2 \rightleftharpoons CH_3(CH_2)_6COOH + NADH + H^+$$

How could these bacteria be used to clean up oil spills? What would be some of the limiting factors in the efficiency of this process?

15. Metabolism of a Straight-Chain Phenylated Fatty Acid A crystalline metabolite was isolated from the urine of a rabbit that had been fed a straight-chain fatty acid containing a terminal phenyl group:



A 302 mg sample of the metabolite in aqueous solution was completely neutralized by 22.2 mL of 0.100 M NaOH.

(a) What is the probable molecular weight and structure of the metabolite?

(b) Did the straight-chain fatty acid contain an even or an odd number of methylene (—CH_2—) groups (i.e., is n even or odd)? Explain.

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

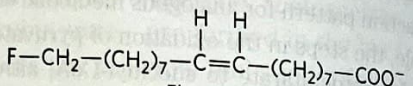
17. Consequences of a High-Fat Diet with No Carbohydrates Suppose you had to subsist on a diet of whale blubber and seal blubber, with little or no carbohydrate.

(a) What would be the effect of carbohydrate deprivation on the utilization of fats for energy?

(b) If your diet were totally devoid of carbohydrate, would it be better to consume odd- or even-number fatty acids? Explain.

18. Even- and Odd-Number Fatty Acids in the Diet In a laboratory experiment, two groups of rats are fed two different fatty acids as their sole source of carbon for a month. The first group gets heptanoic acid (7:0), and the second gets octanoic acid (8:0). After the experiment, a striking difference is seen between the two groups. Those in the first group are healthy and have gained weight, whereas those in the second group are weak and have lost weight as a result of losing muscle mass. What is the biochemical basis for this difference?

19. Metabolic Consequences of Ingesting ω-Fluorooleate The shrub *Dichapetalum toxicarium*, native to Sierra Leone, produces ω-fluorooleate, which is highly toxic to warm-blooded animals.



ω-Fluorooleate

This substance has been used as an arrow poison, and powdered fruit from the plant is sometimes used as a rat poison (hence the plant's common name, ratsbane). Why is this substance so toxic? (Hint: Review Chapter 16, Problem 22.)

20. Mutant Acetyl-CoA Carboxylase What would be the consequences for fat metabolism of a mutation in acetyl-CoA carboxylase that replaced the Ser residue normally phosphorylated by AMPK with an Ala residue? What might happen if the same Ser were replaced by Asp? (Hint: See Fig. 17-13.)

21. Effect of PDE Inhibitor on Adipocytes How would an adipocyte's response to epinephrine be affected by the addition of an inhibitor of cAMP phosphodiesterase (PDE)? (Hint: See Fig. 12-4.)

22. Role of FAD as Electron Acceptor Acyl-CoA dehydrogenase uses enzyme-bound FAD as a prosthetic group to dehydrogenate the α and β carbons of fatty acyl–CoA. What is the advantage of using FAD as an electron acceptor rather than NAD^+? Explain in terms of the standard reduction potentials for the Enz-FAD/$FADH_2$ ($E'^\circ = -0.219$ V) and NAD^+/NADH ($E'^\circ = -0.320$ V) half-reactions.

23. β Oxidation of Arachidic Acid How many turns of the fatty acid oxidation cycle are required for complete oxidation of arachidic acid (see Table 10-1) to acetyl-CoA?

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

25. Phytanic Acid Metabolism When phytanic acid uniformly labeled with ^{14}C is fed to a mouse, radioactivity can be detected in malate, a citric acid cycle intermediate, within minutes. Draw a metabolic pathway that could account for this. Which of the carbon atoms in malate would contain ^{14}C label?

26. Sources of H_2O Produced in β Oxidation The complete oxidation of palmitoyl-CoA to carbon dioxide and water is represented by the overall equation

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 16CO_2 + 108\text{ATP} + 23H_2O$$

Water is also produced in the reaction

$$\text{ADP} + P_i \longrightarrow \text{ATP} + H_2O$$

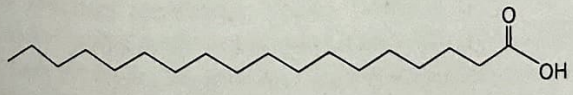
but is not included as a product in the overall equation. Why?

27. Biological Importance of Cobalt In cattle, deer, sheep, and other ruminant animals, large amounts of propionate are produced in the rumen through the bacterial fermentation of ingested plant matter. Propionate is the principal source of glucose for these animals, via the route propionate $\longrightarrow$ oxaloacetate $\longrightarrow$ glucose. In some areas of the world, notably Australia, ruminant animals sometimes show symptoms of anemia with concomitant loss of appetite and retarded growth, resulting from an inability to transform propionate to oxaloacetate. This condition is due to a cobalt deficiency caused by very low cobalt levels in the soil and thus in plant matter. Explain.

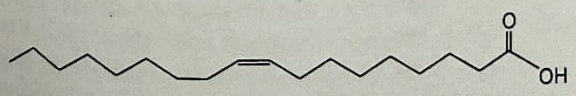
28. Fat Loss during Hibernation Bears expend about 25×10^6 J/day during periods of hibernation, which may last as long as seven months. The energy required to sustain life is obtained from fatty acid oxidation. How much weight loss (in kilograms) has occurred after seven months? How might ketosis be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

Data Analysis Problem

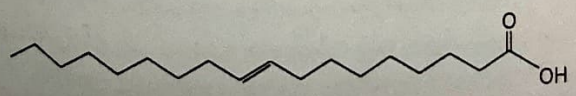
29. β Oxidation of Trans Fats Unsaturated fats with trans double bonds are commonly referred to as "trans fats." There has been much discussion about the effects of dietary trans fats on health. In their investigations of the effects of trans fatty acid metabolism on health, Yu and colleagues (2004) showed that a model trans fatty acid was processed differently from its cis isomer. They used three related 18-carbon fatty acids to explore the difference in β oxidation between cis and trans isomers of the same-size fatty acid.



Stearic acid
(octadecenoic acid)

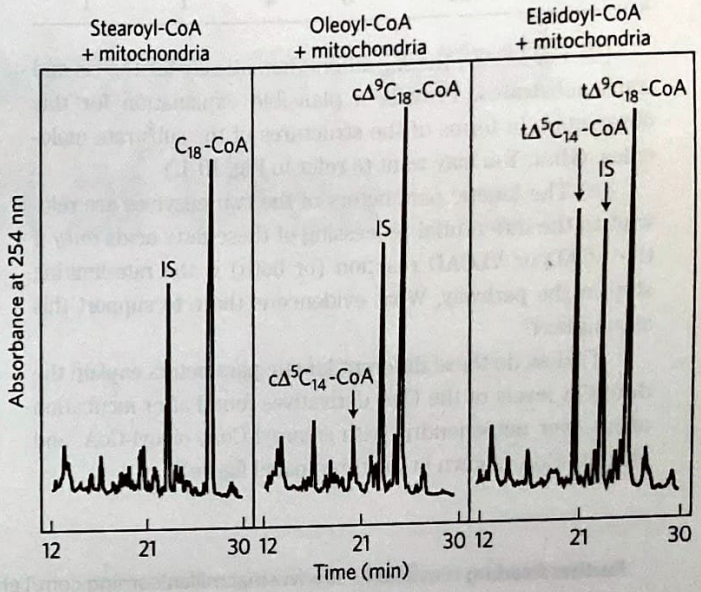


Oleic acid
(*cis*-Δ^9-octadecenoic acid)

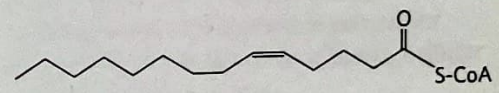


Elaidic acid
(*trans*-Δ^9-octadecenoic acid)

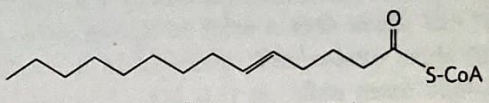
The researchers incubated the coenzyme A derivative of each acid with rat liver mitochondria for 5 minutes, then separated the remaining CoA derivatives in each mixture by HPLC (high-performance liquid chromatography). The results are shown below, with separate panels for the three experiments.



In the figure, IS indicates an internal standard (pentadecanoyl-CoA) added to the mixture, after the reaction, as a molecular marker. The researchers abbreviated the CoA derivatives as follows: stearoyl-CoA, C_{18}-CoA; *cis*-Δ^5-tetradecenoyl-CoA, $c\Delta^5C_{14}$-CoA; oleoyl-CoA, $c\Delta^9C_{18}$-CoA; *trans*-Δ^5-tetradecenoyl-CoA, $t\Delta^5C_{14}$-CoA; and elaidoyl-CoA, $t\Delta^9C_{18}$-CoA.



cis-Δ^5-Tetradecenoyl-CoA



trans-Δ^5-Tetradecenoyl-CoA

(a) Why did Yu and colleagues need to use CoA derivatives rather than the free fatty acids in these experiments?

(b) Why were no lower molecular weight CoA derivatives found in the reaction with stearoyl-CoA?

(c) How many rounds of β oxidation would be required to convert the oleoyl-CoA and the elaidoyl-CoA to *cis*-Δ^5-tetradecenoyl-CoA and *trans*-Δ^5-tetradecenoyl-CoA, respectively?

Yu and coworkers measured the kinetic parameters of two forms of the enzyme acyl-CoA dehydrogenase: long-chain acyl-CoA dehydrogenase (LCAD) and very-long-chain acyl-CoA dehydrogenase (VLCAD). They used the CoA derivatives of three fatty acids: tetradecanoyl-CoA (C_{14}-CoA), *cis*-Δ^5-tetradecenoyl-CoA ($c\Delta^5C_{14}$-CoA), and *trans*-Δ^5-tetradecenoyl-CoA ($t\Delta^5C_{14}$-CoA). The results are shown below. (See Chapter 6 for definitions of the kinetic parameters.)

Problems

1. Oxidation-Reduction Reactions Complex I, the NADH dehydrogenase complex of the mitochondrial respiratory chain, promotes the following series of oxidation-reduction reactions, in which Fe^{3+} and Fe^{2+} represent the iron in iron-sulfur centers, Q is ubiquinone, QH_2 is ubiquinol, and E is the enzyme:

(1) $NADH + H^+ + E\text{-}FMN \longrightarrow NAD^+ + E\text{-}FMNH_2$

(2) $E\text{-}FMNH_2 + 2Fe^{3+} \longrightarrow E\text{-}FMN + 2Fe^{2+} + 2H^+$

(3) $2Fe^{2+} + 2H^+ + Q \longrightarrow 2Fe^{3+} + QH_2$

Sum: $NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$

For each of the three reactions catalyzed by Complex I, identify (a) the electron donor, (b) the electron acceptor, (c) the conjugate redox pair, (d) the reducing agent, and (e) the oxidizing agent.

2. All Parts of Ubiquinone Have a Function In electron transfer, only the quinone portion of ubiquinone undergoes oxidation-reduction; the isoprenoid side chain remains unchanged. What is the function of this chain?

3. Use of FAD Rather Than NAD^+ in Succinate Oxidation All the dehydrogenases of glycolysis and the citric acid cycle use NAD^+ (E'° for NAD^+/NADH is −0.32 V) as electron acceptor except succinate dehydrogenase, which uses covalently bound FAD (E'° for FAD/$FADH_2$ in this enzyme is 0.050 V). Suggest why FAD is a more appropriate electron acceptor than NAD^+ in the dehydrogenation of succinate, based on the E'° values of fumarate/succinate (E'° = 0.031 V), NAD^+/NADH, and the succinate dehydrogenase FAD/$FADH_2$.

4. Degree of Reduction of Electron Carriers in the Respiratory Chain The degree of reduction of each carrier in the respiratory chain is determined by conditions in the mitochondrion. For example, when NADH and O_2 are abundant, the steady-state degree of reduction of the carriers decreases as electrons pass from the substrate to O_2. When electron transfer is blocked, the carriers before the block become more reduced and those beyond the block become more oxidized (see Fig. 19-6). For each of the conditions below, predict the state of oxidation of ubiquinone and cytochromes b, c_1, c, and $a + a_3$.

(a) Abundant NADH and O_2, but cyanide added

(b) Abundant NADH, but O_2 exhausted

(c) Abundant O_2, but NADH exhausted

(d) Abundant NADH and O_2

5. Effect of Rotenone and Antimycin A on Electron Transfer Rotenone, a toxic natural product from plants, strongly inhibits NADH dehydrogenase of insect and fish mitochondria. Antimycin A, a toxic antibiotic, strongly inhibits the oxidation of ubiquinol.

(a) Explain why rotenone ingestion is lethal to some insect and fish species.

(b) Explain why antimycin A is a poison.

(c) Given that rotenone and antimycin A are equally effective in blocking their respective sites in the electron-transfer chain, which would be a more potent poison? Explain.

6. Uncouplers of Oxidative Phosphorylation In normal mitochondria, the rate of electron transfer is tightly coupled to the demand for ATP. When the rate of use of ATP is relatively low, the rate of electron transfer is low; when demand for ATP increases, the electron-transfer rate increases. Under these conditions of tight coupling, the number of ATP molecules produced per atom of oxygen consumed when NADH is the electron donor—the P/O ratio—is about 2.5.

(a) Predict the effect of a relatively low and a relatively high concentration of uncoupling agent on the rate of electron transfer and the P/O ratio.

(b) Ingestion of uncouplers causes profuse sweating and an increase in body temperature. Explain this phenomenon in molecular terms. What happens to the P/O ratio in the presence of uncouplers?

(c) The uncoupler 2,4-dinitrophenol was once prescribed as a weight-reducing drug. How could this agent, in principle, serve as a weight-reducing aid? Uncoupling agents are no longer prescribed, because some deaths occurred following their use. Why might the ingestion of uncouplers cause death?

7. Effects of Valinomycin on Oxidative Phosphorylation When the antibiotic valinomycin (see Fig. 11-42) is added to actively respiring mitochondria, several things happen: the yield of ATP decreases, the rate of O_2 consumption increases, heat is released, and the pH gradient across the inner mitochondrial membrane increases. Does valinomycin act as an uncoupler or as an inhibitor of oxidative phosphorylation? Explain the experimental observations in terms of the antibiotic's ability to transfer K^+ ions across the inner mitochondrial membrane.

8. Cellular ADP Concentration Controls ATP Formation Although both ADP and P_i are required for the synthesis of ATP, the rate of synthesis depends mainly on the concentration of ADP, not P_i. Why?

9. Advantages of Supercomplexes for Electron Transfer There is growing evidence that mitochondrial Complexes I, II, III, and IV are part of a larger supercomplex. What might be the advantage of having all four complexes within a supercomplex?

10. How Many Protons in a Mitochondrion? Electron transfer translocates protons from the mitochondrial matrix to the external medium, establishing a pH gradient across the inner membrane (outside more acidic than inside). The tendency of protons to diffuse back into the matrix is the driving force for ATP synthesis by ATP synthase. During oxidative phosphorylation by a suspension of mitochondria in a medium of pH 7.4, the pH of the matrix has been measured as 7.7.

(a) Calculate $[H^+]$ in the external medium and in the matrix under these conditions.

(b) What is the outside-to-inside ratio of $[H^+]$? Comment on the energy inherent in this concentration difference. (Hint: See Eqn 11-4, p. 413.)

(c) Calculate the number of protons in a respiring liver mitochondrion, assuming its inner matrix compartment is a sphere of diameter 1.5 μm.

(d) From these data, is the pH gradient alone sufficient to generate ATP?

(e) If not, suggest how the necessary energy for synthesis of ATP arises.

11. Rate of ATP Turnover in Rat Heart Muscle Rat heart muscle operating aerobically fills more than 90% of its ATP needs by oxidative phosphorylation. Each gram of tissue consumes O_2 at the rate of 10.0 μmol/min, with glucose as the fuel source.

(a) Calculate the rate at which the heart muscle consumes glucose and produces ATP.

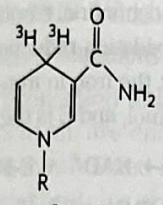
(b) For a steady-state concentration of ATP of 5.0 μmol/g of heart muscle tissue, calculate the time required (in seconds) to completely turn over the cellular pool of ATP. What does this result indicate about the need for tight regulation of ATP production? (Note: Concentrations are expressed as micromoles per gram of muscle tissue because the tissue is mostly water.)

12. Rate of ATP Breakdown in Insect Flight Muscle ATP production in the flight muscle of the fly *Lucilia sericata* results almost exclusively from oxidative phosphorylation. During flight, 187 mL of O_2/h·g of body weight is needed to maintain an ATP concentration of 7.0 μmol/g of flight muscle. Assuming that flight muscle makes up 20% of the weight of the fly, calculate the rate at which the flight-muscle ATP pool turns over. How long would the reservoir of ATP last in the absence of oxidative phosphorylation? Assume that reducing equivalents are transferred by the glycerol 3-phosphate shuttle and that O_2 is at 25°C and 101.3 kPa (1 atm).

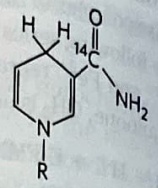
13. High Blood Alanine Level Associated with Defects in Oxidative Phosphorylation Most individuals with genetic defects in oxidative phosphorylation are found to have relatively high concentrations of alanine in their blood. Explain this in biochemical terms.

14. Compartmentalization of Citric Acid Cycle Components Isocitrate dehydrogenase is found only in mitochondria, but malate dehydrogenase is found in both the cytosol and mitochondria. What is the role of cytosolic malate dehydrogenase?

15. Transmembrane Movement of Reducing Equivalents Under aerobic conditions, extramitochondrial NADH must be oxidized by the mitochondrial respiratory chain. Consider a preparation of rat hepatocytes containing mitochondria and all the cytosolic enzymes. If [4-^{3}H]NADH is introduced, radioactivity soon appears in the mitochondrial matrix. However, if [7-^{14}C]NADH is introduced, no radioactivity appears in the matrix. What do these observations reveal about the oxidation of extramitochondrial NADH by the respiratory chain?



[4-^{3}H]NADH



[7-^{14}C]NADH

16. NAD Pools and Dehydrogenase Activities Although both pyruvate dehydrogenase and glyceraldehyde 3-phosphate dehydrogenase use NAD^+ as their electron acceptor, the two enzymes do not compete for the same cellular NAD pool. Why?

17. The Malate–α-Ketoglutarate Transport System The transport system that conveys malate and α-ketoglutarate across the inner mitochondrial membrane (see Fig. 19-31) is inhibited by *n*-butylmalonate. Suppose *n*-butylmalonate is added to an aerobic suspension of kidney cells using glucose exclusively as fuel. Predict the effect of this inhibitor on (a) glycolysis, (b) oxygen consumption, (c) lactate formation, and (d) ATP synthesis.

18. Time Scales of Regulatory Events in Mitochondria Compare the likely time scales for the adjustments in respiratory rate caused by (a) increased [ADP] and (b) reduced pO_2. What accounts for the difference?

19. The Pasteur Effect When O_2 is added to an anaerobic suspension of cells consuming glucose at a high rate, the rate of glucose consumption declines greatly as the O_2 is used up, and accumulation of lactate ceases. This effect, first observed by Louis Pasteur in the 1860s, is characteristic of most cells capable of both aerobic and anaerobic glucose catabolism.

(a) Why does the accumulation of lactate cease after O_2 is added?

(b) Why does the presence of O_2 decrease the rate of glucose consumption?

(c) How does the onset of O_2 consumption slow down the rate of glucose consumption? Explain in terms of specific enzymes.

20. Respiration-Deficient Yeast Mutants and Ethanol Production Respiration-deficient yeast mutants (p^-; "petites") can be produced from wild-type parents by treatment with mutagenic agents. The mutants lack cytochrome oxidase, a deficit that markedly affects their metabolic behavior. One striking effect is that fermentation is not suppressed by O_2—that is, the mutants lack the Pasteur effect (see Problem 19). Some companies are very interested in using these mutants to ferment wood chips to ethanol for energy use. Explain the advantages of using these mutants rather than wild-type yeast for large-scale ethanol production. Why does the absence of cytochrome oxidase eliminate the Pasteur effect?

21. Mitochondrial Disease and Cancer Mutations in the genes that encode certain mitochondrial proteins are associated with a high incidence of some types of cancer. How might defective mitochondria lead to cancer?

22. Variable Severity of a Mitochondrial Disease Different individuals with a disease caused by the same specific defect in the mitochondrial genome may have symptoms ranging from mild to severe. Explain why.

23. Diabetes as a Consequence of Mitochondrial Defects Glucokinase is essential in the metabolism of glucose in pancreatic β cells. Humans with two defective copies of the glucokinase gene exhibit a severe, neonatal diabetes, whereas those with only one defective copy of the gene have a much milder form of the disease (mature onset diabetes of the young, MODY2). Explain this difference in terms of the biology of the β cell.

24. Effects of Mutations in Mitochondrial Complex II Single nucleotide changes in the gene for succinate dehydrogenase (Complex II) are associated with midgut carcinoid tumors. Suggest a mechanism to explain this observation.

Data Analysis Problem

25. Identifying a Protein Central to the Activity of ATP Synthase Much of our knowledge about the steps in the respiratory chain and the mechanism of ATP synthase came about by dissecting the pathway, using various inhibitors and uncouplers (see Table 19-4) and bacterial mutants. In this problem, we see how Robert Fillingame used dicyclohexylcarbodiimide (DCCD) and *E. coli* mutants resistant to its effects to identify the components that came to be known as the c subunits of the F_o portion of ATP synthase.

DCCD reacts with carboxyl groups in the side chains of Asp and Glu residues. When DCCD is added to a suspension of intact, actively respiring mitochondria, the rate of electron transfer (measured by O_2 consumption) and the rate of ATP production dramatically decrease. If a solution of 2,4-dinitrophenol (DNP) is now added to the preparation, O_2 consumption returns to normal, but ATP production remains inhibited.

(a) Explain the effect of DNP on the inhibited mitochondrial preparation.

(b) Which process is directly affected by DCCD, electron transfer or ATP synthesis?

E. coli carries out oxidative phosphorylation with machinery remarkably similar to that in mammals, and *E. coli* is far more amenable to mutant selection. Addition of DCCD to a culture of wild-type *E. coli* (strain AN180) growing aerobically blocks further growth in a time- and dose-dependent fashion.

Fillingame selected a DCCD-resistant mutant of *E. coli* (RF-7) for which aerobic growth was only slightly diminished in the presence of DCCD. Next, he needed to demonstrate that the DCCD-resistant component in his *E. coli* strains was the ATP synthase. He isolated the membrane fraction from the wild-type and RF-7 strains and assayed them for ATPase activity in the presence and absence of DCCD. He found time- and dose-dependent inhibition of the ATPase activity in the membrane fraction of the wild-type, but not in the RF-7 membrane fraction.

(c) Why did Fillingame assay ATPase activity instead of ATP synthase activity?

(d) Is the DCCD-binding protein missing in the mutant RF-7, or just altered?

Fillingame wanted to know whether the DCCD-sensitive protein was an integral part of the membrane or could be solubilized into the fraction that contained the ATPase activity. He prepared "stripped membrane" and "soluble ATPase" fractions from both wild-type cells and RF-7 mutants, by treating intact membranes with dithiothreitol. He measured the ATPase activity in the native membranes, in the stripped membranes, and in systems reconstituted by mixing the stripped membranes with the soluble fraction from the wild-type or RF-7 mutant strain. The native membranes and reconstituted systems all had ATPase activity; the stripped membrane fractions had very little ATPase activity. Having established that all combinations of reconstituted systems had similar ATPase activity, Fillingame then added DCCD to see which combinations were inhibited.

(e) What results would you expect if the DCCD-binding protein were in the stripped membranes? What would you expect if it were in the soluble fraction?

The results were clear. For the stripped membranes from wild-type cells, the reconstituted ATPase was sensitive to DCCD, regardless of the source of the soluble fraction. For the stripped membranes from mutant cells, the reconstituted ATPase was insensitive to DCCD. So, DCCD sensitivity is due to a protein in the stripped membrane fraction, not to a protein in the fraction solubilized with dithiothreitol.

To identify the DCCD-sensitive protein, Fillingame exposed intact membranes of the wild-type (AN180) and RF-7 *E. coli* to ^{14}C-labeled DCCD, then used SDS-PAGE to separate the proteins. He cut the gel into thin slices from bottom to top and determined the ^{14}C content of each slice, measured as disintegrations per minute (dpm) per 2 mm gel slice, normalized to the amount of protein applied to the gel. The distance migrated is equal to the slice number times 2 mm. The results are plotted below. The arrows denote cytochrome *c*, used as a molecular mass marker; I and II, peaks of interest; and BPB, bromphenol blue, a tracking dye to indicate the front of the sample as it moves through the gel. Many proteins from each sample were labeled with [^{14}C] DCCD (measured in dpm, disintegrations per minute).