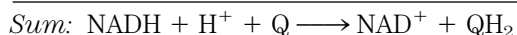
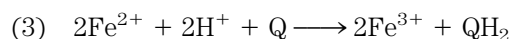


# Oxidative Phosphorylation and Photophosphorylation

## chapter

# 19

- 1. Oxidation-Reduction Reactions** The NADH dehydrogenase complex of the mitochondrial respiratory chain promotes the following series of oxidation-reduction reactions, in which  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  represent the iron in iron-sulfur centers, Q is ubiquinone,  $\text{QH}_2$  is ubiquinol, and E is the enzyme:



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

**Answer** Oxidation-reduction reactions require an electron donor and an electron acceptor. Recall that electron donors are reducing agents; electron acceptors are oxidizing agents.

(1) NADH is the electron donor **(a)** and the reducing agent **(d)**; E-FMN is the electron acceptor **(b)** and the oxidizing agent **(e)**;  $\text{NAD}^+/\text{NADH}$  and E-FMN/E-FMNH<sub>2</sub> are conjugate redox pairs **(c)**.

(2) E-FMNH<sub>2</sub> is the electron donor **(a)** and reducing agent **(d)**;  $\text{Fe}^{3+}$  is the electron acceptor **(b)** and oxidizing agent **(e)**; E-FMN/E-FMNH<sub>2</sub> and  $\text{Fe}^{3+}/\text{Fe}^{2+}$  are redox pairs **(c)**.

(3)  $\text{Fe}^{2+}$  is the electron donor **(a)** and reducing agent **(d)**; Q is the electron acceptor **(b)** and oxidizing agent **(e)**; and  $\text{Fe}^{3+}/\text{Fe}^{2+}$  and Q/QH<sub>2</sub> are redox pairs **(c)**.

- 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?

**Answer** The long isoprenoid side chain makes ubiquinone very soluble in lipids and allows it to diffuse in the semifluid membrane. This is important because ubiquinone transfers electrons from Complexes I and II to Complex III, all of which are embedded in the inner mitochondrial membrane.

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- 3. Use of FAD Rather Than NAD<sup>+</sup> in Succinate Oxidation** All the dehydrogenases of glycolysis and the citric acid cycle use NAD<sup>+</sup> ( $E'^{\circ}$  for NAD<sup>+</sup>/NADH is  $-0.32$  V) as electron acceptor except succinate dehydrogenase, which uses covalently bound FAD ( $E'^{\circ}$  for FAD/FADH<sub>2</sub> in this enzyme is  $0.050$  V). Suggest why FAD is a more appropriate electron acceptor than NAD<sup>+</sup> in the dehydrogenation of succinate, based on the  $E'^{\circ}$  values of fumarate/succinate ( $E'^{\circ} = 0.031$ ), NAD<sup>+</sup>/NADH, and the succinate dehydrogenase FAD/FADH<sub>2</sub>.

**Answer** From the difference in standard reduction potential ( $\Delta E'^{\circ}$ ) for each pair of half-reactions, we can calculate the  $\Delta G'^{\circ}$  values for the oxidation of succinate using NAD<sup>+</sup> and oxidation using E-FAD.

For NAD<sup>+</sup>:

$$\begin{aligned}\Delta G'^{\circ} &= -n\mathcal{F}\Delta E'^{\circ} \\ &= -2(96.5 \text{ kJ/V} \cdot \text{mol})(-0.32 \text{ V} - 0.031 \text{ V}) \\ &= 68 \text{ kJ/mol}\end{aligned}$$

For E-FAD:

$$\begin{aligned}\Delta G'^{\circ} &= -2(96.5 \text{ kJ/V} \cdot \text{mol})(0.050 \text{ V} - 0.031 \text{ V}) \\ &= -3.7 \text{ kJ/mol}\end{aligned}$$

The oxidation of succinate by E-FAD is favored by the negative standard free-energy change, which is consistent with a  $K'_{\text{eq}}$  of  $>1$ . Oxidation by NAD<sup>+</sup> would require a large, positive, standard free-energy change and have a  $K'_{\text{eq}}$  favoring the synthesis of succinate.

- 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<sub>2</sub> are abundant, the steady-state degree of reduction of the carriers decreases as electrons pass from the substrate to O<sub>2</sub>. 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*<sub>1</sub>, *c*, and *a* + *a*<sub>3</sub>.

- (a) Abundant NADH and O<sub>2</sub>, but cyanide added
- (b) Abundant NADH, but O<sub>2</sub> exhausted
- (c) Abundant O<sub>2</sub>, but NADH exhausted
- (d) Abundant NADH and O<sub>2</sub>

**Answer** As shown in Figure 19–6, the oxidation-reduction state of the carriers in the electron-transfer system varies with the conditions.

- (a) Cyanide inhibits cytochrome oxidase (*a* + *a*<sub>3</sub>); all carriers become reduced.
- (b) In the absence of O<sub>2</sub>, no terminal electron acceptor is present; all carriers become reduced.
- (c) In the absence of NADH, no carrier can be reduced; all carriers become oxidized.
- (d) These are the usual conditions for an aerobic, actively metabolizing cell; the early carriers (e.g., Q) are somewhat reduced, while the late ones (e.g., cytochrome *c*) are oxidized.

- 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.

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**Answer**

- (a) The inhibition of NADH dehydrogenase by rotenone decreases the rate of electron flow through the respiratory chain, which in turn decreases the rate of ATP production. If this reduced rate is unable to meet its ATP requirements, the organism dies.
- (b) Antimycin A strongly inhibits the oxidation of reduced Q in the respiratory chain, severely limiting the rate of electron transfer and ATP production.
- (c) Electrons flow into the system at Complex I from the  $\text{NAD}^+$ -linked reactions and at Complex II from succinate and fatty acyl-CoA through FAD (see Figs. 19–8 and 19–16). Antimycin A inhibits electron flow (through Q) from *all* these sources, whereas rotenone inhibits flow only through Complex I. Thus, antimycin A is a more potent poison.

**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, 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 lead to death?

**Answer** Uncouplers of oxidative phosphorylation stimulate the rate of electron flow but not ATP synthesis.

- (a) At relatively low levels of an uncoupling agent, P/O ratios drop somewhat, but the cell can compensate for this by increasing the rate of electron flow; ATP levels can be kept relatively normal. At high levels of uncoupler, P/O ratios approach zero and the cell cannot maintain ATP levels.
- (b) As amounts of an uncoupler increase, the P/O ratio decreases and the body struggles to make sufficient ATP by oxidizing more fuel. The heat produced by this increased rate of oxidation raises the body temperature. The P/O ratio is affected as noted in (a).
- (c) Increased activity of the respiratory chain in the presence of an uncoupler requires the degradation of additional energy stores (glycogen and fat). By oxidizing more fuel in an attempt to produce the same amount of ATP, the organism loses weight. If the P/O ratio nears zero, the lack of ATP will be lethal.

**7. Effects of Valinomycin on Oxidative Phosphorylation** When the antibiotic valinomycin is added to actively respiring mitochondria, several things happen: the yield of ATP decreases, the rate of  $\text{O}_2$  consumption increases, heat is released, and the pH gradient across the inner mitochondrial membrane increases. Does valinomycin act as an uncoupler or an inhibitor of oxidative phosphorylation? Explain the experimental observations in terms of the antibiotic's ability to transfer  $\text{K}^+$  ions across the inner mitochondrial membrane.

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**Answer** The observed effects are consistent with the action of an uncoupler—that is, an agent that causes the free energy released in electron transfer to appear as heat rather than in ATP. In respiring mitochondria,  $H^+$  ions are translocated out of the matrix during electron transfer, creating a proton gradient and an electrical potential across the membrane. A significant portion of the free energy used to synthesize ATP originates from this electric potential. Valinomycin combines with  $K^+$  ions to form a complex that passes through the inner mitochondrial membrane. So, as a proton is translocated out by electron transfer, a  $K^+$  ion moves in, and the potential across the membrane is lost. This reduces the yield of ATP per mole of protons flowing through ATP synthase ( $F_0F_1$ ). In other words, electron transfer and phosphorylation become uncoupled. In response to the decreased efficiency of ATP synthesis, the rate of electron transfer increases markedly. This results in an increase in the  $H^+$  gradient, in oxygen consumption, and in the amount of heat released.

- 8. Mode of Action of Dicyclohexylcarbodiimide (DCCD)** When DCCD is added to a suspension of tightly coupled, 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 is now added to the preparation,  $O_2$  consumption returns to normal but ATP production remains inhibited.
- What process in electron transfer or oxidative phosphorylation is affected by DCCD?
  - Why does DCCD affect the  $O_2$  consumption of mitochondria? Explain the effect of 2,4-dinitrophenol on the inhibited mitochondrial preparation.
  - Which of the following inhibitors does DCCD most resemble in its action: antimycin A, rotenone, or oligomycin?

**Answer**

- DCCD inhibits ATP synthesis. In tightly coupled mitochondria, this inhibition leads to inhibition of electron transfer also.
- A decrease in electron transfer causes a decrease in  $O_2$  consumption. 2,4-Dinitrophenol uncouples electron transfer from ATP synthesis, allowing respiration to increase. No ATP is synthesized and the P/O ratio decreases.
- DCCD and oligomycin inhibit ATP synthesis (see Table 19-4).

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

**Answer** Malate dehydrogenase catalyzes the conversion of malate to oxaloacetate in the citric acid cycle, which takes place in the mitochondrion, and also plays a key role in the transport of reducing equivalents across the inner mitochondrial membrane via the malate-aspartate shuttle (Fig. 19-29). This shuttle requires the presence of malate dehydrogenase in the cytosol and the mitochondrial matrix.

- 10. The Malate- $\alpha$ -Ketoglutarate Transport System** The transport system that conveys malate and  $\alpha$ -ketoglutarate across the inner mitochondrial membrane (see Fig. 19-29) 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.

**Answer** NADH produced in the cytosol cannot cross the inner mitochondrial membrane, but must be oxidized if glycolysis is to continue. Reducing equivalents from NADH enter the mitochondrion by way of the malate-aspartate shuttle. NADH reduces oxaloacetate to form malate and  $NAD^+$ , and the malate is transported into the mitochondrion. Cytosolic oxidation of glucose can continue, and the malate is converted back to oxaloacetate and NADH in the mitochondrion (see Fig. 19-29).

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- (a) If *n*-butylmalonate, an inhibitor of the malate- $\alpha$ -ketoglutarate transporter, is added to cells, NADH accumulates in the cytosol. This forces glycolysis to operate anaerobically, with reoxidation of NADH in the lactate dehydrogenase reaction.
- (b) Because reducing equivalents from the oxidation reactions of glycolysis do not enter the mitochondrion, oxygen consumption slows and eventually ceases.
- (c) The end product of anaerobic glycolysis, lactate, accumulates.
- (d) ATP is not formed aerobically because the cells have converted to anaerobic glycolysis. Overall, ATP synthesis decreases drastically, to 2 ATP per glucose molecule.

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

**Answer** The steady-state concentration of  $P_i$  in the cell is much higher than that of ADP. As the ADP concentration rises as a result of ATP consumption, there is little change in  $[P_i]$ , so  $P_i$  cannot serve as a regulator.

**12. 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?

**Answer** In (a), respiratory control by ADP, the increase in respiratory rate is limited by the rate of diffusion of ADP, and the response would be expected to occur in fractions of a millisecond. The adjustment to (b), hypoxia mediated by HIF-1, requires a change in concentration of several proteins, the result of increased synthesis or degradation. The time scale for protein synthesis or degradation is typically many seconds to hours—much longer than the time required for changes in substrate concentration.

**13. 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 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.

**Answer** The addition of oxygen to an anaerobic suspension allows cells to convert from fermentation to oxidative phosphorylation as a mechanism for reoxidizing NADH and making ATP. Because ATP synthesis is much more efficient under aerobic conditions, the amount of glucose needed will decrease (the Pasteur effect). This decreased utilization of glucose in the presence of oxygen can be demonstrated in any tissue that is capable of aerobic and anaerobic glycolysis.

- (a) Oxygen allows the tissue to convert from lactic acid fermentation to respiratory electron transfer and oxidative phosphorylation as the mechanism for NADH oxidation.
- (b) Cells produce much more ATP per glucose molecule oxidized aerobically, so less glucose is needed.
- (c) As  $[ATP]$  rises, phosphofructokinase-1 is inhibited, thus slowing the rate of glucose entry into the glycolytic pathway.

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- 14. 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 13). 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?

**Answer** The absence of cytochrome oxidase prevents these mutants from oxidizing the products of fermentation (ethanol, acetate, lactate, or glycerol) via the normal respiratory route. These mutants do not have a working citric acid cycle because they cannot reoxidize NADH through the  $O_2$ -dependent electron-transfer chain. Thus, catabolism of glucose stops at the ethanol stage, even in the presence of oxygen. The ability to carry out these fermentations in the presence of oxygen is a major practical advantage because completely anaerobic conditions are difficult to maintain. The Pasteur effect—the decrease in glucose consumption that occurs when oxygen is introduced—is not observed in the absence of an active citric acid cycle and electron-transfer chain.

- 15. 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?

**Answer** When electron-carrying complexes are bound together in a supercomplex, electron flow between complexes occurs in a solid state; this electron movement is kinetically favored compared with the situation in which electron flow depends on each complex diffusing to and colliding with the next complex in the chain.

- 16. 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.
- Calculate  $[H^+]$  in the external medium and in the matrix under these conditions.
  - What is the outside-to-inside ratio of  $[H^+]$ ? Comment on the energy inherent in this concentration difference. (Hint: see Eqn 11-4, p. 396)
  - Calculate the number of protons in a respiring liver mitochondrion, assuming its inner matrix compartment is a sphere of diameter 1.5  $\mu\text{m}$ .
  - From these data, is the pH gradient alone sufficient to generate ATP?
  - If not, suggest how the necessary energy for synthesis of ATP arises.

**Answer**

- Using the equation  $\text{pH} = -\log [H^+]$ , we can calculate external  $[H^+] = 10^{-7.4} = 4.0 \times 10^{-8}$  M; and internal  $[H^+] = 10^{-7.7} = 2.0 \times 10^{-8}$  M.
- From (a), the ratio is 2:1. We can calculate the free energy inherent in this *concentration* difference across the membrane. Assuming a temperature of 25 °C:

$$\begin{aligned}\Delta G &= RT \ln (C_2/C_1) \\ &= (2.48 \text{ kJ/mol}) \ln 2 \\ &= -1.7 \text{ kJ/mol}\end{aligned}$$

- Given that the volume of the mitochondrion =  $\frac{4}{3}\pi(0.75 \times 10^{-3} \text{ mm})^3$  and  $[H^+] = 2.0 \times 10^{-8}$  M, the number of protons is

$$\frac{(1.33)(3.14)(0.75 \times 10^{-3} \text{ mm})^3(2.0 \times 10^{-8} \text{ mol/L})(6.02 \times 10^{23} \text{ protons/mol})}{(10^6 \text{ mm}^3/\text{L})} = 21 \text{ protons}$$

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- (d) No; the energy available from the  $H^+$  concentration gradient,  $2.3\Delta pH RT = 2.3(0.3)$  (2.48 kJ/mol) = 1.7 kJ/mol, is insufficient to synthesize 1 mol of ATP.
- (e) The total energy inherent in the pH gradient is the sum of the energy due to the concentration gradient and the energy due to the charge separation. The overall transmembrane electrical potential is the main factor in producing a sufficiently large  $\Delta G_t$  (see Eqns 19–8 and 19–9).

**17. 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  $10.0 \mu\text{mol}/\text{min}$ , 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  $5.0 \mu\text{mol}/\text{g}$  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.)

**Answer** ATP turns over very rapidly in all types of tissues and cells.

- (a) Glucose oxidation requires 6 mol of  $O_2$  per mol of glucose. Therefore, glucose is consumed at the rate of  $(10.0 \mu\text{mol}/\text{min} \cdot \text{g})/6 = 1.7 \mu\text{mol}/\text{min} \cdot \text{g}$  of tissue. If each glucose produces 32 ATP (see Table 19–5), the muscle produces ATP at the rate of  $(1.7 \mu\text{mol glucose}/\text{min} \cdot \text{g})(32 \text{ ATP}/\text{glucose}) = 54 \mu\text{mol}/\text{min} \cdot \text{g}$ , or  $0.91 \mu\text{mol}/\text{s} \cdot \text{g}$ .
- (b) It takes  $(5.0 \mu\text{mol}/\text{g})/(0.91 \mu\text{mol}/\text{s} \cdot \text{g}) = 5.5 \text{ s}$  to produce  $5.0 \mu\text{mol}$  of ATP per gram, so the entire pool of ATP must be regenerated (turned over) every 5.5 s. In order to do this, the cell must regulate ATP synthesis precisely.

**18. Rate of ATP Breakdown in Flight Muscle** ATP production in the flight muscle of the fly *Lucilia sericata* results almost exclusively from oxidative phosphorylation. During flight,  $187 \text{ mL}$  of  $O_2/\text{hr} \cdot \text{g}$  of body weight is needed to maintain an ATP concentration of  $7.0 \mu\text{mol}/\text{g}$  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  $25^\circ\text{C}$  and  $101.3 \text{ kPa}$  (1 atm).

**Answer** Using the gas laws ( $PV = nRT$ ), we can calculate that  $187 \text{ mL}$  of  $O_2$  contains

$$n = PV/RT = (1 \text{ atm})(0.187 \text{ L})/(0.08205 \text{ L} \cdot \text{atm}/\text{mol} \cdot \text{K})(298 \text{ K}) = 7650 \mu\text{mol of } O_2$$

Thus, the rate of oxygen consumption by flight muscle is

$$(7650 \mu\text{mol}/\text{hr})/(1 \text{ g})(0.2)(3600 \text{ s}/\text{hr}) = 10.6 \mu\text{mol}/\text{s} \cdot \text{g}$$

Assuming a yield of 30 ATP per glucose (see Table 19–5; assume the use of the glycerol 3-phosphate shuttle), and given 6  $O_2$  consumed per glucose, the amount of ATP formed is

$$[(30 \text{ ATP}/\text{glucose})/(6 O_2/\text{glucose})](10.6 \mu\text{mol } O_2/\text{s} \cdot \text{g}) = 53 \mu\text{mol}/\text{s} \cdot \text{g}$$

Thus, a reservoir of  $7.0 \mu\text{mol}/\text{g}$  would last  $(7.0 \mu\text{mol}/\text{g})/(53 \mu\text{mol}/\text{s} \cdot \text{g}) = 0.13 \text{ s}$ .

**19. 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?

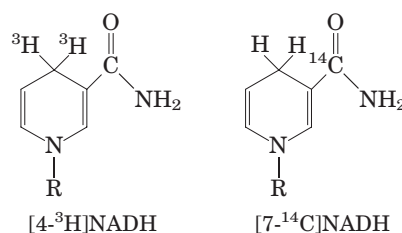
**Answer** Reactive oxygen species react with macromolecules, including DNA. If a mitochondrial defect leads to increased production of ROS, the nuclear genes that encode proto-oncogenes (pp. 473, 474) can be damaged, producing oncogenes and leading to unregulated cell division and cancer.

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- 20. Variable Severity of a Mitochondrial Disease** Individuals with a disease caused by a specific defect in the mitochondrial genome may have symptoms ranging from mild to severe. Explain why.

**Answer** The explanation is probably heteroplasmy. In some individuals, a mitochondrial mutation may affect only a small proportion of cells and tissues, because most mitochondria in these cells and tissues have normal genomes and the few mutant mitochondria do not significantly compromise the ability to produce ATP. In other individuals, the chance distribution of mitochondrial genomes during cell division has led to a high degree of heteroplasmy in which many cells have a majority of defective mitochondria, resulting in more severe symptoms.

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



**Answer** The malate-aspartate shuttle transfers electrons and protons from the cytoplasm into the mitochondrion. Neither  $\text{NAD}^+$  nor NADH passes through the inner membrane, thus the labeled NAD moiety of  $[7\text{-}^{14}\text{C}]\text{NADH}$  remains in the cytosol. The  $^3\text{H}$  on  $[4\text{-}^3\text{H}]\text{NADH}$  enters the mitochondrion via the malate-aspartate shuttle (see Fig. 19–29). In the cytosol,  $[4\text{-}^3\text{H}]\text{NADH}$  transfers its  $^3\text{H}$  to oxaloacetate to form  $[^3\text{H}]\text{malate}$ , which enters the mitochondrion via the malate- $\alpha$ -ketoglutarate transporter, then donates the  $^3\text{H}$  to  $\text{NAD}^+$  to form  $[4\text{-}^3\text{H}]\text{NADH}$  in the matrix.

- 22. 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.

**Answer** In these individuals, the usual route for pyruvate metabolism—conversion to acetyl-CoA and entry into the citric acid cycle—is slowed by the decreased capacity for carrying electrons from NADH to oxygen. Accumulation of pyruvate in the tissues shifts the equilibrium for pyruvate-alanine transaminase, resulting in elevated concentrations of alanine in tissues and blood.

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

**Answer** Pyruvate dehydrogenase is located in the mitochondrion, and glyceraldehyde 3-phosphate dehydrogenase in the cytosol. Because the mitochondrial and cytosolic pools of NAD are separated by the inner mitochondrial membrane, the enzymes do not compete for the same NAD pool. However, reducing equivalents are transferred from one nicotinamide coenzyme pool to the other via shuttle mechanisms (see Problem 21).

- 24. Diabetes as a Consequence of Mitochondrial Defects** Glucokinase is essential in the metabolism of glucose in pancreatic  $\beta$  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  $\beta$  cell.

**Answer** In  $\beta$  cells in which both copies of glucokinase are defective, the rate of glycolytic ATP production does not increase when blood glucose rises, and thus these cells cannot produce high enough concentrations of ATP to affect the ATP-dependent  $K^+$  channel that indirectly regulates insulin secretion. With one functional copy of glucokinase,  $\beta$  cells can respond to very high glucose concentrations by producing suprathreshold concentrations of ATP, triggering insulin release.

- 25. 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.

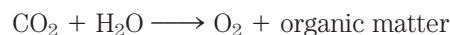
**Answer** Defects in Complex II result in increased production of ROS, damage to DNA, and mutations that lead to unregulated cell division (cancer). It is not clear why the cancer tends to occur in the midgut.

- 26. Photochemical Efficiency of Light at Different Wavelengths** The rate of photosynthesis, measured by  $O_2$  production, is higher when a green plant is illuminated with light of wavelength 680 nm than with light of 700 nm. However, illumination by a combination of light of 680 nm and 700 nm gives a higher rate of photosynthesis than light of either wavelength alone. Explain.

**Answer** Plants have two photosystems. Photosystem I absorbs light maximally at 700 nm and catalyzes cyclic photophosphorylation and  $NADP^+$  reduction (see Fig. 19-56). Photosystem II absorbs light maximally at 680 nm, splits  $H_2O$  to  $O_2$  and  $H^+$ , and donates electrons and  $H^+$  to PSI. Therefore, light of 680 nm is better in promoting  $O_2$  production, but maximum photosynthetic rates are observed only when plants are illuminated with light of both wavelengths.

- 27. Balance Sheet for Photosynthesis** In 1804 Theodore de Saussure observed that the total weights of oxygen and dry organic matter produced by plants is greater than the weight of carbon dioxide consumed during photosynthesis. Where does the extra weight come from?

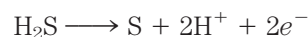
**Answer** Because the general reaction for plant photosynthesis is



the extra weight must come from the water consumed in the overall reaction.

- 28. Role of  $H_2S$  in Some Photosynthetic Bacteria** Illuminated purple sulfur bacteria carry out photosynthesis in the presence of  $H_2O$  and  $^{14}CO_2$ , but only if  $H_2S$  is added and  $O_2$  is absent. During the course of photosynthesis, measured by formation of [ $^{14}C$ ]carbohydrate,  $H_2S$  is converted to elemental sulfur, but no  $O_2$  is evolved. What is the role of the conversion of  $H_2S$  to sulfur? Why is no  $O_2$  evolved?

**Answer** Purple sulfur bacteria use  $H_2S$  as a source of electrons and protons:



The electrons are “activated” by a light energy-capturing photosystem. These cells produce their ATP by photophosphorylation and their NADPH from  $H_2S$  oxidation. Because  $H_2O$  is not split,  $O_2$  is not evolved (photosystem II is absent).



## Chapter 19 Oxidative Phosphorylation and Photophosphorylation S-233

- 33. Effect of Venturicidin on Oxygen Evolution** Venturicidin is a powerful inhibitor of the chloroplast ATP synthase, interacting with the  $CF_o$  part of the enzyme and blocking proton passage through the  $CF_oCF_1$  complex. How would venturicidin affect oxygen evolution in a suspension of well-illuminated chloroplasts? Would your answer change if the experiment were done in the presence of an uncoupling reagent such as 2,4-dinitrophenol (DNP)? Explain.

**Answer** Oxygen evolution requires continuing passage of electrons through PSII. Electrons will continue to flow through PSII and the cytochrome  $b_6f$  complex until the energetic cost of pumping a proton across the thylakoid membrane exceeds the energy available from absorption of a photon. This point is soon reached when proton flow through  $CF_oCF_1$  is blocked by venturicidin, and oxygen evolution ceases. Addition of an uncoupling agent provides a route for protons to move through the thylakoid membrane, dissipating the energy of the proton gradient. Electrons can now continue to move through PSII and the cytochrome  $b_6f$  complex, and oxygen is produced in the water-splitting reaction.

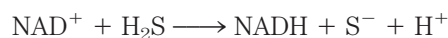
- 34. Bioenergetics of Photophosphorylation** The steady-state concentrations of ATP, ADP, and  $P_i$  in isolated spinach chloroplasts under full illumination at pH 7.0 are 120.0, 6.0, and 700.0  $\mu\text{M}$ , respectively.
- (a) What is the free-energy requirement for the synthesis of 1 mol of ATP under these conditions?
- (b) The energy for ATP synthesis is furnished by light-induced electron transfer in the chloroplasts. What is the minimum voltage drop necessary (during transfer of a pair of electrons) to synthesize ATP under these conditions? (You may need to refer to Eqn 13-7, p. 515.)

**Answer**

$$\begin{aligned} \text{(a)} \quad \Delta G &= \Delta G'^{\circ} + RT \ln \frac{[\text{ATP}]}{[\text{ADP}][P_i]} \\ &= 30.5 \text{ kJ/mol} + (2.48 \text{ kJ/mol}) \ln \frac{1.2 \times 10^{-4}}{(6.0 \times 10^{-6})(7.0 \times 10^{-4})} \\ &= 30.5 \text{ kJ/mol} + 25.4 \text{ kJ/mol} = 55.9 \text{ kJ/mol} \\ &= 56 \text{ kJ/mol (two significant figures)} \\ \text{(b)} \quad \Delta G &= -n\mathcal{F}\Delta E \\ \Delta E &= -\Delta G/n\mathcal{F} \\ &= \frac{-56 \text{ kJ/mol}}{-2(96.48 \text{ kJ/V} \cdot \text{mol})} \\ &= 0.29 \text{ V} \end{aligned}$$

- 35. Light Energy for a Redox Reaction** Suppose you have isolated a new photosynthetic microorganism that oxidizes  $\text{H}_2\text{S}$  and passes the electrons to  $\text{NAD}^+$ . What wavelength of light would provide enough energy for  $\text{H}_2\text{S}$  to reduce  $\text{NAD}^+$  under standard conditions? Assume 100% efficiency in the photochemical event, and use  $E'^{\circ}$  of  $-243 \text{ mV}$  for  $\text{H}_2\text{S}$  and  $-320 \text{ mV}$  for  $\text{NAD}^+$ . See Figure 19-46 for the energy equivalents of wavelengths of light.

**Answer** First, calculate the standard free-energy change ( $\Delta G'^{\circ}$ ) of the redox reaction



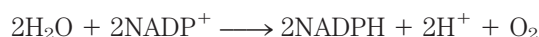
Because  $\Delta E'^{\circ} = -320 \text{ mV} - (-243 \text{ mV}) = -77 \text{ mV}$ ,

$$\begin{aligned} \Delta G'^{\circ} &= -n\mathcal{F}\Delta E'^{\circ} \\ &= (-2)(96.48 \text{ kJ/V} \cdot \text{mol})(-0.077 \text{ V}) = 15 \text{ kJ/mol} \end{aligned}$$

This is the minimum energy needed to drive the reduction of  $\text{NAD}^+$  by  $\text{H}_2\text{S}$ . Inspection of Figure 19-46 shows that the energy in a “mole” of photons (an einstein) in the visible part of the spectrum ranges from 170 to 300 kJ. Any visible light should have sufficient energy to drive the reduction of  $\text{NADH}$  by  $\text{H}_2\text{S}$ . In principle, and assuming 100% efficiency, even infrared light should have enough energy to drive this reaction.

## S-234 Chapter 19 Oxidative Phosphorylation and Photophosphorylation

- 36. Equilibrium Constant for Water-Splitting Reactions** The coenzyme  $\text{NADP}^+$  is the terminal electron acceptor in chloroplasts, according to the reaction



Use the information in Table 19–2 to calculate the equilibrium constant for this reaction at 25 °C. (The relationship between  $K'_{\text{eq}}$  and  $\Delta G'^{\circ}$  is discussed on p. 492.) How can the chloroplast overcome this unfavorable equilibrium?

**Answer** Using standard reduction potentials from Table 19–2,  $\Delta E'^{\circ}$  for the reaction is  $-0.324 \text{ V} - 0.816 \text{ V} = -1.140 \text{ V}$ .

$$\begin{aligned}\Delta G'^{\circ} &= -n\mathcal{F}\Delta E'^{\circ} \\ &= -4(96.48 \text{ kJ/V} \cdot \text{mol})(-1.140 \text{ V}) \\ &= 440 \text{ kJ/mol}\end{aligned}$$

(Note that  $n = 4$  because 4 electrons are required to produce 1 mol of  $\text{O}_2$ .)

$$\begin{aligned}\Delta G'^{\circ} &= -RT \ln K'_{\text{eq}} \\ \ln K'_{\text{eq}} &= -\Delta G'^{\circ}/RT \\ &= (-440 \text{ kJ/mol})/(2.48 \text{ kJ/mol}) \\ &= -177 \\ K'_{\text{eq}} &= e^{-177} = 1.35 \times 10^{-77}\end{aligned}$$

The equilibrium is clearly very unfavorable. In chloroplasts, the input of light energy overcomes this barrier.

- 37. Energetics of Phototransduction** During photosynthesis, eight photons must be absorbed (four by each photosystem) for every  $\text{O}_2$  molecule produced:

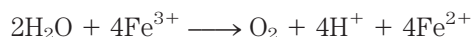


Assuming that these photons have a wavelength of 700 nm (red) and that the absorption and use of light energy are 100% efficient, calculate the free-energy change for the process.

**Answer** From Problem 36,  $\Delta G'^{\circ}$  for the production of 1 mol of  $\text{O}_2$  in this reaction is 440 kJ/mol. A light input of 8 photons (700 nm) is equivalent to  $(8)(170 \text{ kJ/einstein}) = 1360 \text{ kJ/einstein}$  (see Fig. 19–46). (An einstein is a “mole” of photons.) The overall standard free-energy change of the reaction is

$$\Delta G'^{\circ} = (440 - 1360) \text{ kJ/mol} = -920 \text{ kJ/mol}$$

- 38. Electron Transfer to a Hill Reagent** Isolated spinach chloroplasts evolve  $\text{O}_2$  when illuminated in the presence of potassium ferricyanide (a Hill reagent), according to the equation



where  $\text{Fe}^{3+}$  represents ferricyanide and  $\text{Fe}^{2+}$ , ferrocyanide. Is NADPH produced in this process? Explain.

**Answer** No NADPH is produced. Artificial electron acceptors can remove electrons from the photosynthetic system and stimulate  $\text{O}_2$  production. Ferricyanide competes with the cytochrome  $b_6f$  complex for electrons and removes them from the system. Consequently, P700 (of photosystem I) does not receive any electrons that can be activated for  $\text{NADP}^+$  reduction. However,  $\text{O}_2$  is evolved because all components of photosystem II are oxidized (see Fig. 19–56).

- 39. How Often Does a Chlorophyll Molecule Absorb a Photon?** The amount of chlorophyll *a* ( $M_r$  892) in a spinach leaf is about  $20 \mu\text{g}/\text{cm}^2$  of leaf. In noonday sunlight (average energy reaching the leaf is  $5.4 \text{ J}/\text{cm}^2 \cdot \text{min}$ ), the leaf absorbs about 50% of the radiation. How often does a single chlorophyll molecule absorb a photon? Given that the average lifetime of an excited chlorophyll molecule in vivo is 1 ns, what fraction of the chlorophyll molecules are excited at any one time?

**Answer** The leaf absorbs light in units of photons that vary in energy from 170 to 300 kJ/einstein, depending on wavelength (see Fig. 19-46). The leaf absorbs light energy at the rate of  $0.5(5.4 \text{ J}/\text{cm}^2 \cdot \text{min}) = 2.7 \text{ J}/\text{cm}^2 \cdot \text{min}$ . Assuming an average energy of 270 kJ/einstein, this rate of light absorption is

$$(2.7 \times 10^{-3} \text{ kJ}/\text{cm}^2 \cdot \text{min}) / (270 \text{ kJ}/\text{einstein}) = 1 \times 10^{-5} \text{ einstein}/\text{cm}^2 \cdot \text{min}$$

The concentration of chlorophyll in the leaf is

$$(20 \times 10^{-6} \text{ g}/\text{cm}^2) / (892 \text{ g}/\text{mol}) = 2 \times 10^{-8} \text{ mol}/\text{cm}^2$$

Thus, 1 mol of chlorophyll absorbs 1 einstein of photons every

$$(2 \times 10^{-8} \text{ mol}/\text{cm}^2) / (1 \times 10^{-5} \text{ einstein}/\text{cm}^2 \cdot \text{min}) = 2 \times 10^{-3} \text{ min} = 0.1 \text{ s}$$

Because excitation lasts about  $1 \text{ ns} = 1 \times 10^{-9} \text{ s}$ , the fraction of chlorophylls excited at any one time is  $(1 \times 10^{-9} \text{ s}) / (0.1 \text{ s}) = 1 \times 10^{-8}$ , or one in every  $10^8$  molecules.

- 40. Effect of Monochromatic Light on Electron Flow** The extent to which an electron carrier is oxidized or reduced during photosynthetic electron transfer can sometimes be observed directly with a spectrophotometer. When chloroplasts are illuminated with 700 nm light, cytochrome *f*, plastocyanin, and plastoquinone are oxidized. When chloroplasts are illuminated with 680 nm light, however, these electron carriers are reduced. Explain.

**Answer** Light at 700 nm activates electrons in P700 and  $\text{NADP}^+$  is reduced (see Fig. 19-56). This drains all the electrons from the electron-transfer system between photosystems II and I, because light at 680 nm is not available to replace electrons by activating PSII. When light at 680 nm activates PSII (but not PSI), all the carriers between the two systems become reduced because no electrons are excited in PSI.

- 41. Function of Cyclic Photophosphorylation** When the  $[\text{NADPH}]/[\text{NADP}^+]$  ratio in chloroplasts is high, photophosphorylation is predominantly cyclic (see Fig. 19-56). Is  $\text{O}_2$  evolved during cyclic photophosphorylation? Is NADPH produced? Explain. What is the main function of cyclic photophosphorylation?

**Answer** Neither  $\text{O}_2$  nor NADPH is produced. At high  $[\text{NADPH}]/[\text{NADP}^+]$  ratios, electron transfer from reduced ferredoxin to  $\text{NADP}^+$  is inhibited and the electrons are diverted into the cytochrome  $b_6f$  complex. These electrons return to P700 and ATP is synthesized by photophosphorylation. Because electrons are not lost from P700, none are needed from PSII. Thus,  $\text{H}_2\text{O}$  is not split and  $\text{O}_2$  is not produced. In addition, NADPH is not formed because the electrons return to P700. The function of cyclic photophosphorylation is to produce ATP.

### Data Analysis Problem

- 42. Photophosphorylation: Discovery, Rejection, and Rediscovery** In the 1930s and 1940s, researchers were beginning to make progress toward understanding the mechanism of photosynthesis. At the time, the role of “energy-rich phosphate bonds” (today, “ATP”) in glycolysis and cellular respiration was just becoming known. There were many theories about the mechanism of photosynthesis, especially about the role of light. This problem focuses on what was then called the “primary photochemical process”—that is, on what it is, exactly, that the energy from captured light produces in the photosynthetic cell. Interestingly, one important part of the modern model of photosynthesis was proposed early on, only to be rejected, ignored for several years, then finally revived and accepted.

## S-236 Chapter 19 Oxidative Phosphorylation and Photophosphorylation

In 1944, Emerson, Stauffer, and Umbreit proposed that “the function of light energy in photosynthesis is the formation of ‘energy-rich’ phosphate bonds” (p. 107). In their model (hereafter, the “Emerson model”), the free energy necessary to drive both CO<sub>2</sub> fixation *and* reduction came from these “energy-rich phosphate bonds” (i.e., ATP), produced as a result of light absorption by a chlorophyll-containing protein.

This model was explicitly rejected by Rabinowitch (1945). After summarizing Emerson and coauthors’ findings, Rabinowitch stated: “Until more positive evidence is provided, we are inclined to consider as more convincing a general argument against this hypothesis, which can be derived from energy considerations. Photosynthesis is eminently a problem of energy *accumulation*. What good can be served, then, by converting light quanta (even those of red light, which amount to about 43 kcal per Einstein) into ‘phosphate quanta’ of only 10 kcal per mole? This appears to be a start in the wrong direction—toward *dissipation* rather than toward accumulation of energy” (Vol. I, p. 228). This argument, along with other evidence, led to the abandonment of the Emerson model until the 1950s, when it was found to be correct—albeit in a modified form.

For each piece of information from Emerson and coauthors’ article presented in (a) through (d) below, answer the following three questions:

1. How does this information support the Emerson model, in which light energy is used directly by chlorophyll *to make ATP*, and the ATP then provides the energy to drive CO<sub>2</sub> fixation and reduction?
2. How would Rabinowitch explain this information, based on his model (and most other models of the day), in which light energy is used directly by chlorophyll *to make reducing compounds*? Rabinowitch wrote: “Theoretically, there is no reason why *all* electronic energy contained in molecules excited by the absorption of light should not be available for oxidation-reduction” (Vol. I, p. 152). In this model, the reducing compounds are then used to fix and reduce CO<sub>2</sub>, and the energy for these reactions comes from the large amounts of free energy released by the reduction reactions.
3. How is this information explained by our modern understanding of photosynthesis?
  - (a) Chlorophyll contains a Mg<sup>2+</sup> ion, which is known to be an essential cofactor for many enzymes that catalyze phosphorylation and dephosphorylation reactions.
  - (b) A crude “chlorophyll protein” isolated from photosynthetic cells showed phosphorylating activity.
  - (c) The phosphorylating activity of the “chlorophyll protein” was inhibited by light.
  - (d) The levels of several different phosphorylated compounds in photosynthetic cells changed dramatically in response to light exposure. (Emerson and coworkers were not able to identify the specific compounds involved.)

As it turned out, the Emerson and Rabinowitch models were both partly correct and partly incorrect.

- (e) Explain how the two models relate to our current model of photosynthesis.

In his rejection of the Emerson model, Rabinowitch went on to say: “The difficulty of the phosphate storage theory appears most clearly when one considers the fact that, in weak light, eight or ten quanta of light are sufficient to reduce one molecule of carbon dioxide. If each quantum should produce one molecule of high-energy phosphate, the accumulated energy would be only 80–100 kcal per Einstein—while photosynthesis requires *at least* 112 kcal per mole, and probably more, because of losses in irreversible partial reactions” (Vol. 1, p. 228).

- (f) How does Rabinowitch’s value of 8 to 10 photons per molecule of CO<sub>2</sub> reduced compare with the value accepted today? You need to consult Chapter 20 for some of the information required here.
- (g) How would you rebut Rabinowitch’s argument, based on our current knowledge about photosynthesis?

**Answer**

- (a)** (1) The presence of  $Mg^{2+}$  supports the hypothesis that chlorophyll is directly involved in catalysis of the phosphorylation reaction:  $ADP + P_i \rightarrow ATP$ . (2) Many enzymes (or other proteins) that contain  $Mg^{2+}$  are not phosphorylating enzymes, so the presence of  $Mg^{2+}$  in chlorophyll does not prove its role in phosphorylation reactions. (3) The presence of  $Mg^{2+}$  is essential to chlorophyll's photochemical properties: light absorption and electron transfer.
- (b)** (1) Enzymes catalyze reversible reactions, so an isolated enzyme that can, under certain laboratory conditions, catalyze removal of a phosphoryl group could probably, under different conditions (such as in cells), catalyze addition of a phosphoryl group. So it is plausible that chlorophyll could be involved in the phosphorylation of ADP. (2) There are two possible explanations: the chlorophyll protein is a phosphatase only and does not catalyze ADP phosphorylation under cellular conditions, or the crude preparation contains a contaminating phosphatase activity that is unconnected to the photosynthetic reactions. (3) It is likely that the preparation was contaminated with a nonphotosynthetic phosphatase activity.
- (c)** (1) This light inhibition is what one would expect if the chlorophyll protein catalyzed the reaction  $ADP + P_i + \text{light} \rightarrow ATP$ . Without light, the reverse reaction, a dephosphorylation, would be favored. In the presence of light, energy is provided and the equilibrium would shift to the right, reducing the phosphatase activity. (2) This inhibition must be an artifact of the isolation or assay methods. (3) It is unlikely that the crude preparation methods in use at the time preserved intact chloroplast membranes, so the inhibition must be an artifact.
- (d)** (1) In the presence of light, ATP is synthesized and other phosphorylated intermediates are consumed. (2) In the presence of light, glucose is produced and is metabolized by cellular respiration to produce ATP, with changes in the levels of phosphorylated intermediates. (3) In the presence of light, ATP is produced and other phosphorylated intermediates are consumed.
- (e)** Light energy is used to produce ATP (as in the Emerson model) *and* is used to produce reducing power (as in the Rabinowitch model).
- (f)** The approximate stoichiometry for photophosphorylation (Chapter 19) is that 8 photons yield 2 NADPH and about 3 ATP. Two NADPH and 3 ATP are required to reduce 1  $CO_2$  (Chapter 20). Thus, at a minimum, 8 photons are required per  $CO_2$  molecule reduced. This is in good agreement with Rabinowitch's value.
- (g)** Because the energy of light is used to produce *both* ATP and NADPH, each photon absorbed contributes more than just 1 ATP for photosynthesis. The process of energy extraction from light is more efficient than Rabinowitch supposed, and plenty of energy is available for this process—even with red light.

**Reference**

**Emerson, R.L., Stauffer, J.F., & Umbreit, W.W.** (1944) Relationships between phosphorylation and photosynthesis in *Chlorella*. *Am. J. Botany* **31**, 107–120.

**Rabinowitch, E.I.** (1945) *Photosynthesis and Related Processes*, Interscience Publishers, New York.