Chapter 5

Energetics of Photosynthesis: A Closer Look

MOLECULAR ORIGIN OF PHOTOSYNTHETIC ENERGY

Why does the system "organic matter plus molecular oxygen" produced by photosynthesis contain much more energy than the raw material of photosynthesis, the system "carbon dioxide plus water"?

The two systems consist of the same atoms, C, H, and O. Each of these atoms is an assembly of positive nuclei and negative electrons swarming around them. When two different or identical atoms approach each other, electrical interactions result—attractions between positive and negative particles and repulsions between particles of the same sign. Overall attraction means that the electrons of the two atoms can arrange themselves more comfortably around the two nuclei held together than around the two separate nuclei. Overall repulsion means that the two electronic systems are most stable when the two nuclei are far apart.

The energy liberated or consumed in a chemical reaction is determined by the change of stability of the systems participating in it. In photosynthesis, we begin with a very stable arrangement of the atoms, C, H, and O, in the molecules CO_2 and H_2O, and end up with a much less stable arrangement of the same nuclei and electrons in the system $(CH_2O) + O_2$. The main reason for this decrease in stability is the fact

that oxygen atoms are little attracted to each other. The molecule O_2 is a relatively loose one; its two oxygen atoms much prefer to be bound to carbon or hydrogen atoms.

The energy *liberated* when two atoms (or atom groups) A and B become attached to each other is called *bond energy*. It is *negative*, because, when the bond is formed, the energy of the system $A + B$ decreases.

In the first approximation, one can consider the total energy of a molecule as a sum of the energies of a number of atom-to-atom bonds, neglecting the much weaker "cross interactions" between atoms *not* forming bonds. For example, the energy of the molecule H_2O can be treated as the sum of the energies of two O—H bonds. The energy of each of them is then —110 Kcal/mole—one half of the total binding energy of one atom of O and two atoms of H (—220 Kcal/mole) (Fig. 5.1). As mentioned above, the "cross interaction" between the two H atoms is neglected in this approximation. The total binding energy of CO_2, —380 Kcal/mole, can be similarly considered as the sum of the energies of four C—O bonds, since each oxygen is bound to carbon by a double
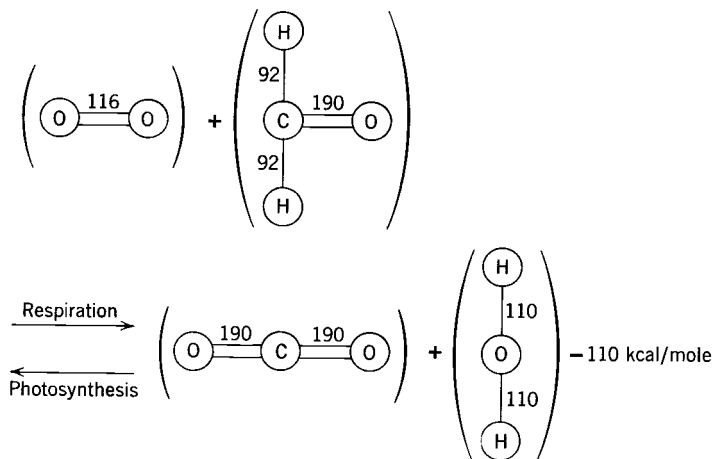

FIG. 5.1 Bond transformation in respiration and photosynthesis. The numbers are bond energies in Kcal/mole. They add up to 490 on the left and to 600 on the right side. The difference (about 110 Kcal/mole) is the energy stored in photosynthesis and liberated in respiration (see Eq. 2.1).

bond, C═O. The interaction energy of the two oxygen atom is neglected, and each single C—O bond is assigned the energy of —95 Kcal/mole.

The energy of each of the two O—O bonds in the oxygen molecule O═O is only —58 Kcal/mole (the total binding energy being —116 Kcal/mole). Therefore, every time an oxygen molecule is consumed by oxidation of an organic compound (in respiration or combustion), a considerable amount of energy is liberated by the more stable attachment of oxygen atoms to their new partners, carbon and hydrogen. This is the main reason for the large energy storage in photosynthesis, where O—O bonds (bond energy, —58 Kcal/mole) are *formed*, and O—H bonds (bond energy, —110 Kcal/mole) are *destroyed*. Other bond changes also occur in photosynthesis, such as replacement of O—H bonds by C—H bonds; but these contribute considerably less to the net energy change than the replacement of O—O bonds by C—O and O—H bonds (Fig. 5.1).

All organic matter on earth is surrounded by a swarm of free oxygen molecules, like maidens in a castle wooed by a host of suitors. Only the castle walls, the activation barriers that have to be overcome for oxidation to get underway, assure the precarious existence of living matter in contact with air. The respiration enzymes stealthily open little doors in the activation walls, and lead the organic molecules, one by one, into the embrace of oxygen.

One can formulate an approximate relation between the oxidation or combustion energy of an organic molecule and the number of oxygen molecules used up in this process. For this purpose, we define an *average reduction level, R,* of carbon in a molecule with the general composition $C_cH_hO_o$ (such as a carbohydrate, $C_nH_{2n}O_n$):

$$R = \frac{c + 0.25h - 0.5o}{c} \tag{5.1}$$

R is the number of oxygen molecules needed to burn a compound to CO_2 and H_2O, divided by the number of C-atoms present in the molecule. (Each C-atom requires one molecule of oxygen to be converted to CO_2; each H-atom requires one quarter of a molecule of oxygen to be converted to H_2O, and each atom O, already present in the molecule, diminishes by one-half molecule the number of outside O_2 molecules needed for combustion.) The approximate rule is that the *energy liberated in*

combustion of a molecule $C_cH_hO_o$ (its "heat of combustion") is about 110 Kcal/mole per carbon atom per unit R.

For a carbohydrate, (CH_2O), R is equal to 1.0; while for the fully reduced one-carbon molecule, CH_4 (methane), $R = 2$. Photosynthesis thus lifts the raw material, CO_2 ($R = O$), roughly halfway up the range of reduction levels, and thus stores about one half of the maximum possible combustion energy per atom of carbon. (Actually, it is somewhat *more* than one half, because the plot of the heats of combustion as function of the reduction level is slightly concave. The energy storage slows down as the reduction progresses; the combustion energy of methane is 211 Kcal/mole, rather than $2 \times 110 = 220$ Kcal/mole.)

After photosynthesis has lifted carbon dioxide about halfway up the reduction scale, two kinds of follow-up processes can occur. These are *oxidations*, with the liberation of the stored energy, as in respiration; and organic *syntheses*, involving transformations of carbohydrates into other organic compounds, such as proteins, fats, etc. To the extent to which some of these compounds, particularly fats and oils, have a higher reduction level than the carbohydrates, their synthesis requires additional energy. (For example, R is about 1.4 for a simple fat, such as triglycerate of oleic acid, $C_{57}H_{104}O_6$.) Nevertheless, these syntheses do occur in the dark, without the supply of light energy. How is this possible? Nature does this by coupling an *oxidation*, which liberates energy, with an energy-accumulating *reduction*, making the net free energy change negative. Reactions of this type are called *dismutations*. The metabolic processes known as fermentations belong to this class. In alcoholic fermentation of glucose, $R = 1.0$, some of it is *reduced* to ethyl alcohol, C_2H_5OH ($R = 1.5$), while another part is *oxidized* to carbon dioxide ($R = O$). The net effect is the release of only 21 Kcal/mole, as compared with 670 Kcal/mole released when the same molecule is oxidized by O_2 to CO_2 and H_2O in respiration.

Processes in which the reduction level remains unchanged, such as conversion of one sugar into another, polymerization of a sugar to starch or cellulose, hydration (that is, addition of water), and dehydration (loss of water) by an organic molecule, proceed on an almost constant level of energy.

Here, then, is one way to look at the multitude of synthetic and degrading processes in the organic world. Photosynthesis lifts the reduction level of carbon from 0 to 1, and respiration brings it back to zero.
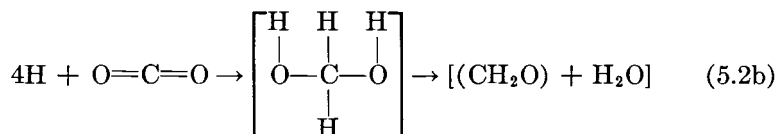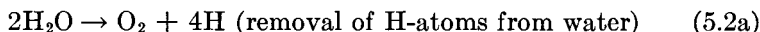
Other metabolic transformations keep the *average* reduction level constant; if one part of a substrate is oxidized, the other part is reduced.

OXIDATION-REDUCTION POTENTIALS

It is useful to introduce here another way of describing the energetics of oxidation and reduction processes. The original meaning of the term "oxidation" was *addition of oxygen* (as in rusting) and the original meaning of "reduction" was *removal of oxygen* (as in the preparation of a metal by smelting of an oxide ore). In organic chemistry, this picture was replaced by that of the *loss or acquisition of hydrogen atoms*. Thus, photosynthesis, once described as subtraction of oxygen from carbon dioxide and hydration of the remaining carbon:
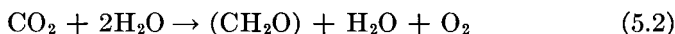
$$CO_2 \rightarrow C + O_2; \ C + H_2O \rightarrow (CH_2O)$$
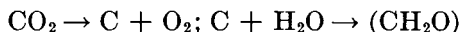
can be better represented as transfer of hydrogen atoms from water to carbon dioxide:

$$2H_2O \rightarrow O_2 + 4H \ \text{(removal of H-atoms from water)} \qquad (5.2a)$$

$$4H + O{=}C{=}O \rightarrow \begin{bmatrix} \overset{\displaystyle H}{\underset{\displaystyle |}{}} \ \overset{\displaystyle H}{\underset{\displaystyle |}{}} \ \overset{\displaystyle H}{\underset{\displaystyle |}{}} \\ O{-}C{-}O \\ \underset{\displaystyle H}{|} \end{bmatrix} \rightarrow [(CH_2O) + H_2O] \qquad (5.2b)$$

(addition of H-atoms to carbon dioxide, followed by dehydration)

which adds up to

$$CO_2 + 2H_2O \rightarrow (CH_2O) + H_2O + O_2 \qquad (5.2)$$

The dehydration of the immediate hydrogenated product, $HO{-}CH_2{-}OH$, indicated in parentheses in (5.2b) is of little significance energetically.

This new description permitted the inclusion, under the heading of oxidations and reductions, of processes in which oxygen takes no part at all. For example, the oxidation of a hydroquinone to a quinone

$$\text{Hydroquinone} \xrightarrow{-2H} \text{Quinone}$$
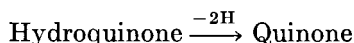
can be represented (in the case of benzohydroquinone) by:
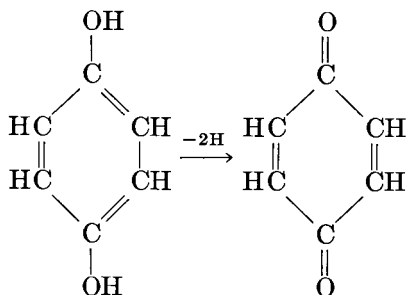
Generally, all processes of the type

$$AH + B \rightarrow A + BH \qquad (5.3a)$$

or

$$AH_2 + B \rightarrow BH_2 + A \qquad (5.3b)$$

are called reductions of B and oxidations of A. Obviously there can be no such oxidation without reduction. Hydrogen atoms are transferred from the reductant (or hydrogen donor) which is thus oxidized to the oxidant (or hydrogen acceptor) which is reduced. It is thus better to speak of "oxidation-reduction," rather than of oxidation *or* reduction. Every oxidation-reduction reaction requires two oxidation-reduction "couples," that is, two compounds that can exist in a hydrogenated (reduced) and a dehydrogenated (oxidized) state. In Eq. 5.3a the two participating couples are A/AH and B/BH. In photosynthesis, one oxidation-reduction couple is $CO_2/[(CH_2O) + H_2O]$, the other, O_2/H_2O. Photosynthesis is thus a reduction of CO_2 to CH_2O, and an oxidation of H_2O to O_2—however strange it may seem at first to call oxidation a process that leads to *liberation* of oxygen!

All oxidation-reduction ("redox") couples can be arranged on a linear scale, placing on top the strongest oxidants and weakest reductants, and at the bottom the strongest reductants and weakest oxidants. The couple $O_2/2H_2O$, or—if we use Eq. 5.3b—$\frac{1}{2}O_2/H_2O$, will be near the top, since oxygen is a strong oxidant. The couple $CO_2/[(CH_2O + H_2O)]$ will be low on this scale, since sugar is a fairly strong reductant.

A given redox couple can be expected to oxidize any couple below it on the scale and to be oxidized by every couple above it. This, however, is only true in the first approximation, because the direction of a chemical reaction depends on the sign of the change in *free* energy, and not that

of the change in *enthalpy*. The change in free energy depends not only on the *nature* of the participating and resulting compounds, but also on their relative *concentration*. A weak oxidant, present in a very high concentration (relative to that of its reduced form), will oxidize the reduced form of a stronger oxidant, particularly if the latter is present in a very low concentration compared to *its* reduced form. To characterize unambiguously the relative oxidative and reductive powers of different redox couples, they are usually compared with each other under the so-called "normal conditions," that is, when the two forms, the reduced and the oxidized, are present in equal concentrations.

Having made the step from defining oxidation and reduction as acquisition or loss of *oxygen,* to defining it as loss or acquisition of *hydrogen* atoms, we now make a second step and generalize the concept of oxidation and reduction still further, by defining them as loss or acquisition of *electrons*. A hydrogen atom can be considered as consisting of an H^+-ion and an electron, $H = H^+ + e$. It is the addition or subtraction of the electron that matters in oxidation-reduction. The addition or subtraction of an H^+-ion does not contribute to it; it defines a different kind of reaction, the *acid-base* transformation, such as $H_2SO_4 \rightarrow H^+ + HSO_4^-$, or $HCl \rightarrow H^+ + Cl^-$. The general equation of a redox reaction is, according to this new definition:
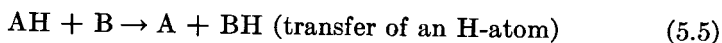
$$A^- + B \rightarrow B^- + A \text{ (electron transfer from A to B)} \qquad (5.4)$$
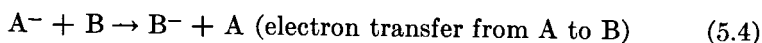
For ease of presentation, the reductant in Eq. 5.4 was assumed to be a negative ion, A^-; but it may as well be a neutral atom, or even a positive ion, able to lose a further electron, as in $Fe^{2+} \rightarrow Fe^{3+} + e$.
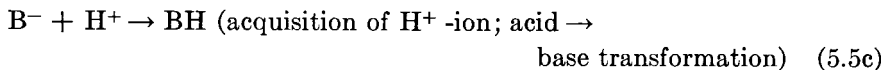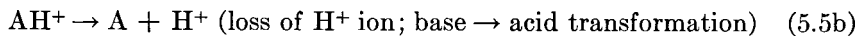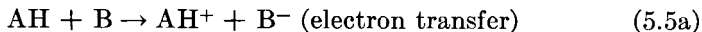
Equation 5.4 represents the actual mechanism of some oxidation-reductions. For example, when ferrous salts, containing the ions Fe^{2+}, are oxidized by ceric salts, containing the ions Ce^{4+}, the actual reaction is

$$2Fe^{2+} + Ce^{4+} \rightarrow 2Fe^{3+} + Ce^{2+}$$

$$\text{(transfer of two electrons from iron to cerium)}$$
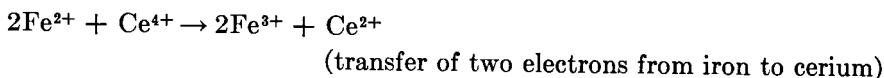
In other cases, the actual result may be transfer of hydrogen atoms, rather than of electrons. However, since in all aqueous systems, hydrogen ions, H^+, are present, we can interpret hydrogen atom transfers as electron transfers combined with acquisition (or loss) of H^+-ions. The general equation (5.5), for example, can be divided into steps (5.5a, b, and c).

$$AH + B \rightarrow A + BH \text{ (transfer of an H-atom)} \qquad (5.5)$$

can be divided into

$$AH + B \rightarrow AH^+ + B^- \text{ (electron transfer)} \quad (5.5a)$$

$$AH^+ \rightarrow A + H^+ \text{ (loss of } H^+ \text{ ion; base} \rightarrow \text{acid transformation)} \quad (5.5b)$$

$$B^- + H^+ \rightarrow BH \text{ (acquisition of } H^+ \text{-ion; acid} \rightarrow$$
$$\text{base transformation)} \quad (5.5c)$$

In biological oxidation-reduction processes, some stages may be electron transfers and others, H-atom transfers. The former is the case in one important part of the reaction sequence of respiration, in which several so-called "cytochromes" act as intermediates. These are protein molecules carrying a porphyrin group with an iron atom in either the ferrous (Fe^{2+}) or the ferric (Fe^{3+}) state. The stage in respiration involving a sequence of several cytochromes is often referred to as the "electron transfer chain." A corresponding, even if apparently shorter, chain has been recently discovered in photosynthesis (see Chapters 13–16).

What makes representation of oxidations and reductions as electron losses and acquisitions particularly useful, is the possibility it offers to replace the somewhat abstract magnitudes, the *free energies* of oxidation-reduction reactions, by the (often directly measurable) *oxidation-reduction potentials* (redox potentials).

Since we will repeatedly use the term redox potential in the following discussion, some explanation is in order.

One way in which electrons can be added or subtracted from many atoms or molecules in aqueous solution is by interaction with the electrode in a galvanic cell. As an example, we can consider the so-called Daniel cell (Fig. 5.2). Placing a copper electrode in the solution of a copper salt, and a zinc electrode in the solution of a zinc salt, separating the two solutions by a porous wall to prevent mixing, and joining the two electrodes by an outside wire, we will find copper ions, Cu^{++}, being discharged and precipitated at the copper electrode, and zinc atoms going into solution from the zinc electrode as zinc ions, Zn^{++}.

In dissolving, zinc atoms leave their electrons in the electrode, from where they are conveyed, through the external wire, to the copper electrode. The result is *reduction* of copper ions, and *oxidation* of zinc atoms.

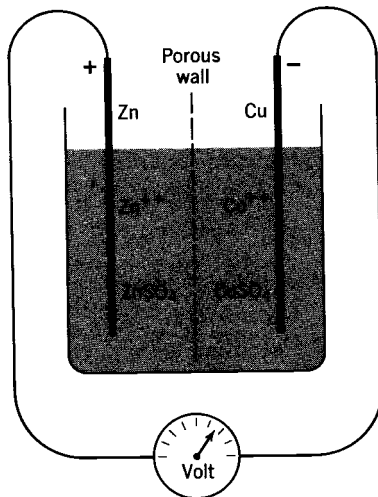$$Cu^{++} + Zn \rightarrow Cu + Zn^{++}$$
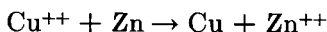
FIG. 5.2 A galvanic cell (Daniel cell). Electrons flow in the outside circuit from the zinc to the copper electrode.

The difference between the oxidative powers of the Zn^{++}/Zn and Cu^{++}/Cu couples is given directly by the galvanic potential of the cell, that is, by the counterpotential that has to be applied to *prevent* the electrons from moving from zinc to copper in the outer circuit. This potential can be measured by a voltmeter. One can plot the so-determined *oxidation-reduction potentials* (redox potentials) of different redox couples, instead of their free energies of oxidation-reduction, on a linear scale (Fig. 5.3) and use them to characterize the relative strength of oxidants and reductants. Of course, these potentials, like the corresponding free energies, will depend on the *ratios of the concentrations* of the oxidized and the reduced forms. We define as *normal* potentials, E_0, those obtained when these concentrations are equal.[1] Furthermore, if the redox reaction is associated with a loss or acquisition of H^+-ions, the potential will depend on the concentration of the latter (the pH of the solution[2]). The normal potential in *neutral* solution (pH7) is often denoted by E_0'.

Many, in fact most, organic oxidation-reduction couples are "electrode

[1] In the case of one form being a solid or liquid (rather than a solution), only the other one has to be present in unit concentration; in the case of one form being a gas, its normal condition is one atmosphere pressure.

[2] pH is defined as the negative logarithm of the H^+-ion concentration; pH = $- \log [H^+]$.

inactive," so that their oxidation-reduction potentials cannot be determined in a galvanic cell. In this case, to put them in proper position in Fig. 5.3, one must return to the determination of *free energy* change (ΔF), and calculate the potential difference (ΔE), by multiplication with an appropriate proportionality factor, F, according to the equation

$$\Delta F = nF \, \Delta E \tag{5.6}$$

where n = number of electrons moved, and F the so-called Faraday's constant (the total electric charge carried by a mole of electrons).

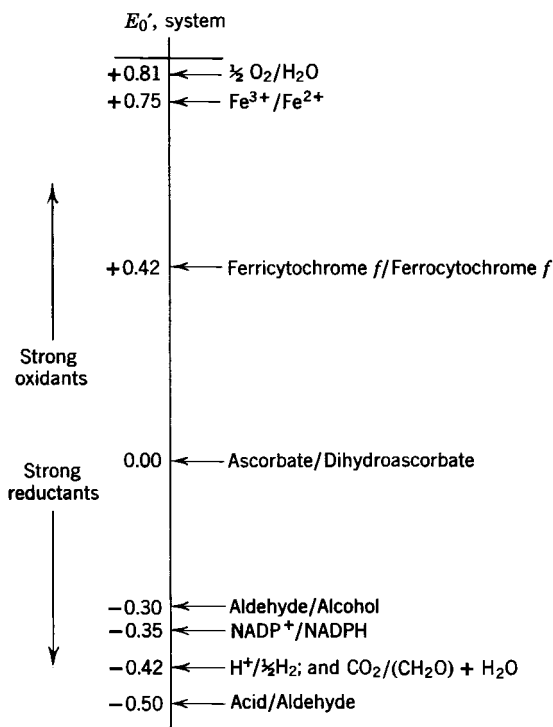E_0', system

+0.81	½ O_2/H_2O
+0.75	Fe^{3+}/Fe^{2+}
+0.42	Ferricytochrome f/Ferrocytochrome f

Strong oxidants

Strong reductants

0.00	Ascorbate/Dihydroascorbate
−0.30	Aldehyde/Alcohol
−0.35	$NADP^+/NADPH$
−0.42	H^+/½H_2; and CO_2/(CH_2O) + H_2O
−0.50	Acid/Aldehyde

FIG. 5.3 Scale of normal redox potentials (E_0') (in volts; in neutral solution, pH7). The potentials of the couple H^+/½H_2 at pH0 (i.e., in one-normal acid) is arbitrarily set at zero; this couple has a potential of −0.42 volt at pH7. $NADP^+$ and NADPH mean oxidized and reduced adenine dinucleotide phosphate, respectively. The calculated redox potential of the couple carbon dioxide/carbohydrate is approximately −0.40 volt. The potential of the couple ferricytochrome b/ferrocytochrome b is −0.40 volt, and that of the couple ferricytochrome c/ferrocytochrome c is +0.26 volt.

Speaking of the normal potential of a given redox couple is embarrassing; we said above that each oxidation-reduction must involve *two* couples! In fact, the only "real" thing is the difference between the two redox potentials. It is, however, convenient to define—quite arbitrarily—the oxidation-reduction potential of a certain standard couple as zero. One can then ascribe single potentials to all other couples—the potentials one would obtain by making these couples react with the standard couple in a galvanic cell. The standard is the couple $H^+/\frac{1}{2}H_2$, where H^+ is the oxidant and H_2 the reductant. This system can be realized, in practice, by bubbling hydrogen through acid solution at a platinum electrode covered with finely dispersed platinum. At such an electrode, hydrogen ions are discharged and converted into hydrogen gas smoothly (that is, without having to overcome an energy barrier, as on many other electrode-solution interfaces). The pressure of hydrogen must be one atmosphere, and the concentration of H^+ ions in solution, 1 mole/liter (a so-called one-normal solution). In neutral solution (pH7), the normal potential, E_0', of the hydrogen electrode is —0.42 volt (Fig. 5.3).

Every couple that has a redox potential more negative than that of the hydrogen electrode is thermodynamically able to evolve gaseous hydrogen from a normal acid solution, and every couple with a more positive potential than that of the hydrogen electrode should be reduced, in one normal acid solution, by hydrogen under atmospheric pressure. Whether these reactions will actually go or not will depend on whether the couple can be made to react at a reversible, that is, "frictionless" electrode, as hydrogen ions do at a platinized platinum electrode, zinc ions at a zinc electrode, or copper ions at a copper electrode. With most organic couples, this is not the case. Nevertheless, it is convenient to calculate from thermodynamic data their redox potentials with respect to the hydrogen electrode, and place them in their proper position on the redox potential scale.

In photosynthesis, the situation is as follows: the normal potential of the system $\frac{1}{2}O_2/H_2O$ is +0.81 volt (oxygen gas is supposed to be under one atmosphere pressure, pH to be 7, and water to be liquid). The potential of the system $CO_2/[(CH_2O) + H_2O)]$, calculated from thermodynamic data, is about —0.40 volt. The difference between the two potentials, 1.21 volt, is a measure of the free energy of photosynthesis.

One may object that volts are a measure of electric *potential*, not energy. In order to convert potentials into energies, one has to multiply them by the charge moved up or down over the potential difference, as the energy of falling water is obtained by multiplication of the height of the fall by the amount of water carried over it. Thus, a 110 volt lamp, through which a current of 0.5 ampere, or 0.5 coulomb/second passes, consumes 55 watt (that means 55 joules/second, joules being a measure of energy, one joule = 10^7 erg). In dealing with electrons, the usual energy unit is *electron-volt*. One electron-volt is the energy consumed or released when a single electron is moved up or down a potential difference of one volt. Thus, the scale of redox *potentials* in *volts* can be treated as a scale of redox *free energies* in *electron-volts*.

It is important to note that oxidation-reduction processes involving the transfer of several electrons or hydrogen atoms, require or liberate proportionally more energy. For example, in photosynthesis, *four* hydrogen atoms have to be transferred from H_2O to CO_2 in order to reduce the latter to the reduction level of a carbohydrate:

$$CO_2 + 2H_2O \rightarrow (CH_4O_2) = [(CH_2O) + H_2O] + O_2$$
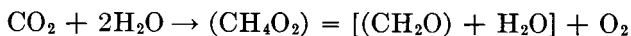
That the compound (CH_4O_2) is not stable, but loses one H_2O molecule and becomes (CH_2O), is unimportant from the point of view of energy, because water loss does not change the reduction level; in organic chemistry, whenever two OH groups find themselves attached to the same carbon atom, the tendency is for the structure $RC(OH)_2$ to transform itself into $R—C{=}O + H_2O$, with little change in energy.

To calculate the free energy of photosynthesis, one has to multiply the above-quoted potential difference of 1.21 volts by a factor of 4 (for 4 hydrogens) giving 4.84 electron-volts. Conversion table for various energy units gives, for the caloric equivalent of one electron-volt, 23.0 Kcal/mole. Multiplying 4.84 by this factor, we obtain 112 Kcal/mole as an approximate free energy of photosynthesis under normal conditions. (The true standard free energy of photosynthesis, as calculated from thermodynamic considerations, is a few Kcal higher.)

We can summarize this section by saying that plant photosynthesis is an oxidation-reduction reaction between an oxidant (CO_2) with a potential of about -0.4 volt, and a reductant (H_2O) with a potential of about $+0.8$ volt, in which *four* electrons (or four H-atoms) are transferred "uphill," against a potential gradient of about 1.2 volts. This

picture will be particularly helpful to use when discussing the likely steps in the overall processes.

We can thus represent photosynthesis by the scheme shown in Fig. 5.4 (compare with Fig. 5.3, where O_2/H_2O couple is near the top of the figure and CO_2/CH_2O couple near the bottom). The vertical arrow in this scheme shows the "uphill" hydrogen transfer from the potential level of $+0.8$ volt to a level of -0.4 volt. This uphill transfer of H-atoms is the essence of the energy-storing photochemical stage
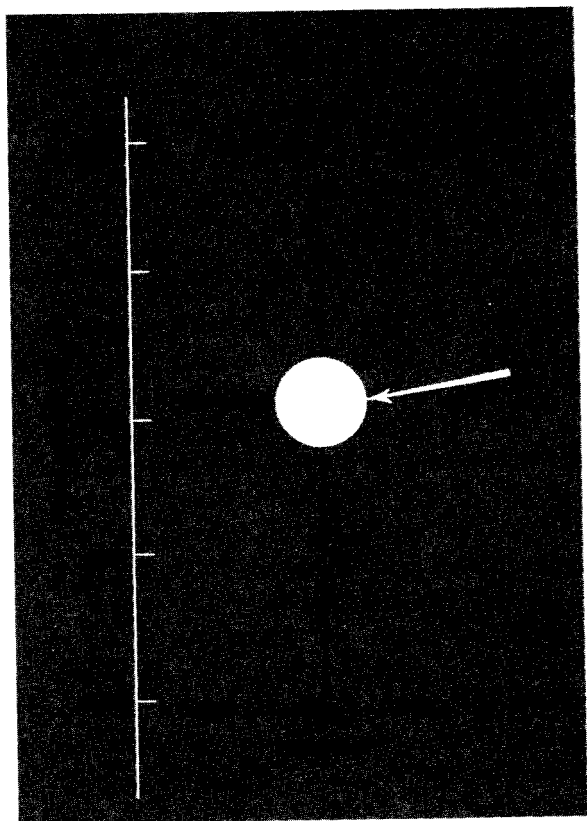

FIG. 5.4 Photosynthesis divided in three parts. (1) Enzymatic transformation of H_2O to O_2. (2) Hydrogen transfer from an intermediate (Z/ZH_2) in enzymatic sequence (1), to an intermediate (X/XH_2) in enzymatic sequence with the help of light-activated chlorophyll. (3) Enzymatic transformation of CO_2 to (CH_2O).

in photosynthesis.[3] The two series of horizontal arrows in Fig. 5.4 indicate nonphotochemical reactions occurring on an approximately horizontal (or downward-sloping) level on the energy scale. The upper arrow represents the sequence of enzymatic reactions by which the oxidant, CO_2 (or an organic molecule into which CO_2 had been incorporated by an enzyme-catalyzed reaction; see Chapter 17) is transformed into a carbohydrate. The lower arrow similarly represents the enzymatic reactions by which the reductant, water (or a compound into which water had been incorporated by a dark reaction) is converted into the final oxidation product, molecular oxygen. Figure 5.4 will serve as the basis for our subsequent discussions of the mechanisms of photosynthesis.

Bacterial photosynthesis, mentioned in Chapters 2 and 3, is an oxidation-reduction reaction in which compounds other than water serve as reductants. The amount of free energy stored in bacterial photosynthesis is much smaller (sometimes, there is even a loss of free energy) because the oxidation-reduction potentials (E_0') of bacterial reductants are much lower than that of the system $H_2O/\frac{1}{2}O_2$. For example, E_0' of the couple $H^+/\frac{1}{2}H_2$ is as low as -0.42 volt (at pH7); that of the couple H_2S/S is approximately -0.2 V, (as contrasted to $+0.8$ volt for the couple $H_2O/\frac{1}{2}O_2$).

[3] That negative levels appear in this scheme higher than the positive ones, is due to the convention, common to biologists the world over (as well as to European physical chemists), which makes strong oxidants *positive,* and strong reductants *negative;* American physicochemical convention used to assign, instead, positive potentials to strong reductants. The difference of potentials measures in one case the free energy of oxidation, in the other case, the free energy of reduction.