Chapter 17

The Enzymatic Paths from Water to Molecular Oxygen and from Carbon Dioxide to Carbohydrates

According to Figs. 5.4 and 14.4, the main products of the two primary photochemical reactions are a reduced intermediate, XH, and an oxidized intermediate, Z.

The oxidant, Z, must be strong enough to oxidize H_2O to molecular oxygen. The reductant XH (or XH_2) must be strong enough to reduce (either alone or with the help of ATP formed as a side product of the photochemical reaction) carbon dioxide to carbohydrate.

PATH TO MOLECULAR OXYGEN

We know next to nothing about the enzymatic mechanism of oxygen liberation. (Not too much more is known about the reverse process in respiration—the uptake of molecular oxygen!) That it *does* involve enzymes can be inferred, for example, from the similarity of the saturation

ratios (and the effects on them of temperature and certain poisons) of photosynthesis and of the Hill reaction (Chapters 6 and 7). These rates appear to be limited by an enzymatic reaction in the oxygen-evolving stage, common to both processses, rather than a reaction in the carbon dioxide-reducing stage, which is nonoperative in the Hill reaction.

The enzymatic system involved in the liberation of oxygen seems to require *manganese*—probably, as a heavy metal component of some enzyme. From a variety of experiments, it is now clear that manganous and chloride ions are associated with the operation of light reaction II. For example (see Chapter 16), manganese is present in higher concentration in the "heavy" fraction of chloroplast particles, which perform preferentially light reaction II, than in the "light" fraction, which sensitizes preferentially light reaction I. Manganese deficiency was found not to affect the chloroplast-sensitized photoreduction of pyridine nucleotide ($NADP^+$) by reduced dichlorophenol indophenol—a reaction apparently involving only PSI; while it did stop the complete Hill reaction (which requires the operation of both PSI and PSII). The high oxidation-reduction potential of the couple Mn^{3+}/Mn^{2+} (E_0' about 1.5 volts!) makes it a plausible speculation to suggest that Mn^{2+} is photooxidized to Mn^{3+} in light reaction II, and Mn^{3+} then oxidizes H_2O by a dark reaction ($2Mn^{3+} + H_2O \rightarrow \frac{1}{2}O_2 + 2Mn^{2+} + 2H^+$). Of course, Mn^{3+}, complexed with proteins in an enzyme, is likely to have a redox potential considerably less positive than that of the free Mn^{3+}/Mn^{2+} couple—but it may still be sufficient to oxidize water to oxygen (requiring $E_0' > +0.75$ volt).

Recent kinetic studies in P. Joliot's laboratory in Paris and in J. Rosenberg's laboratory in Pittsburgh have provided some information regarding details of the oxygen evolution process. They showed that the formation of *one* oxygen molecule requires the accumulation of *four* precursor molecules, formed by four light reactions.

THE JUNCTION OF THE PHOTOCHEMICAL PROCESS WITH THE ENZYMATIC SEQUENCE OF THE CO_2 REDUCTION

In schemes 5.4 and 14.4, we designated the compound located in the junction of the vertical arrow with the upper horizontal arrow as "X."

This compound is supposed to be reduced, in photochemical reaction I, to XH (or XH_2); the latter must bring about the reduction of CO_2 by an enzymatic reaction sequence.

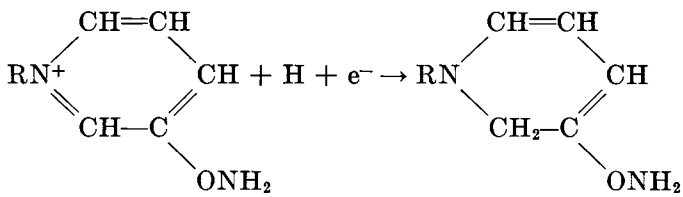
We do not know the chemical identity of X. Since the presence of $NADP^+$ in green cells is well established, and chloroplasts are known to be able to use this compound as a "Hill oxidant," it had been suggested that X is identical with $NADP^+$ (E_0' is -0.35 volt for the couple $NADP^+$/NADPH). However, more recently, it has been made likely that (at least) two NADPH-precursors—an iron-containing protein, "ferredoxin," and an enzyme, "ferredoxin-NADP-reductase" (mediating the reduction of $NADP^+$ by reduced ferredoxin), precede $NADP^+$ in the photosynthetic reaction sequence. Is ferredoxin ($E_0' = -0.4$ volt) then the primary photochemical oxidant, X? Bessel Kok (at the RIAS laboratory in Baltimore), found, that illuminated chloroplasts can reduce dyes with E_0'-values down to -0.6 volt; X may be a compound with a similarly low potential. Ferredoxin, Fd, would then be an intermediate between X and NADPH:

$$\begin{array}{lccccc} & \dfrac{X}{XH} & \rightarrow & \dfrac{Fd}{\text{reduced Fd}} & \rightarrow \begin{array}{c}\text{Fd-NADP}\\ \text{reductase}\end{array} \rightarrow & \dfrac{NADP^+}{NADPH} \\ E_0' = & -0.6 \text{ volt} & & -0.4 \text{ volt} & & -0.35 \text{ volt} \end{array} \qquad (17.1)$$

The reduction of $NADP^+$ involves, at pH 7, the transfer of one H-atom and one electron ($\bar{e}$):

$$NADP^+ + H + e^- \rightarrow NADPH$$

or



(17.2)

where R is an organic residue containing 3 phosphate groups, 2 ribose groups (ribose being a five-carbon sugar) and a purine base called adenine (see Chapter 18).

Perhaps, in the "downhill" redox reaction (17.1), enough energy becomes available to produce, without extra expenditure of light energy,

a third "bonus" ATP (in addition to the two provided by four electrons sliding down the intermediate enzyme reaction sequence, from Cyt *b* to Cyt *f*, that is, from $E_0' = 0.0$ to $E_0' +0.4$ volts). This would be a welcome possibility from the point of view of Calvin's scheme (to be described later in this chapter), since the latter calls for three ATP molecules to be invested in the reduction of each CO_2 molecule (see Chapter 18).

A theoretical alternative is direct use of XH_2 for reduction of a carbonyl group in phosphoglyceric acid (PGA) (avoiding altogether Fd and $NADP^+$ as intermediates!); this would eliminate the need for the two ATP molecules required for this key step of photosynthesis. (Since $NADP^+$ has an E_0' of only —.035 volt, while E_0' of the carboxyl/carbonyl couple is about —0.5 volt, the reduction of carboxyl to carbonyl by NADPH cannot take place without the assistance of an ATP molecule!) This alternative deserves to be kept in mind.

In certain blue-green algae, ferredoxin is replaced by a flavin-containing protein "flavodoxin." (Flavins are the well-known water-soluble yellow redox intermediates involved in the respirations of plants and animals.)

THE PATH OF CARBON IN PHOTOSYNTHESIS

The conversion of CO_2 to carbohydrate with the help of a reducing agent (such as NADPH) provided by the photochemical reaction, is the most thoroughly analyzed stage in photosynthesis. This success had been due to the availability of a convenient tracer—a radioactive carbon isotope. It is well known that such tracers can be used to follow the pathway of various elements in chemical reactions, by observing the appearance of their characteristic radiations in different reaction intermediates and products. The application of this method to photosynthesis has been one of its most spectacular successes, rewarded in 1961 by the Nobel Prize given to Melvin Calvin of Berkeley, California. Together with A. A. Benson, J. Bassham and other co-workers, he has achieved most important progress in this field.

The ordinary, nonradioactive, carbon isotope is ^{12}C (the superscript referring to the atomic mass). The first radioactive carbon isotope used

as tracer was ^{11}C; however, its application had only limited success, because of its very rapid decay (^{11}C has a half-time of 20.5 sec). The discovery of the long-lived ^{14}C (half-life, 5720 years) by Martin Kamen and Sam Ruben at Berkeley (California) just before World War II, made it possible to follow, at leisure as it were, the passage of the carbon tracer from a "tagged" substrate (such as carbon dioxide or bicarbonate) into one organic compound after another. The ^{11}C isotope was given up as an analytical tool, and ^{14}C became *the* all-important carbon tracer. We will use for it the simpler symbol C*, with the asterisk indicating its radioactive nature.

The first applications of C* to photosynthesis led to confusing results, until two facts were established; one, that photosynthesis in live cells is rapidly followed by secondary transformations, bringing C* into a variety of metabolic products; and two, that CO_2 is not such a biochemically inert compound as it had been previously thought to be. Because of the latter fact, the tracer, supplied as C^*O_2 or $HC^*O_3^-$, can enter certain metabolic products by dark enzymatic reactions. In particular, the carboxyl groups of certain organic acids, such as oxalacetic acid and pyruvic acid (which are important intermediates in respiration), undergo relatively rapid isotopic exchange with external carbon dioxide:

$$RCOOH + CO^*_2 \rightleftharpoons RC^*OOH + CO_2$$

(where the radical R is $HOOC—CH_2—C(=O)$ in oxalacetic acid, and $CH_3—C(=O)$ in pyruvic acid). Exchange reactions of this type cause a "nonreductive" incorporation of radioactive carbon into carboxylic acid, and thence into other metabolic compounds, such as amino acids.

Rapid progress first became possible when Calvin and co-workers recognized that in order to separate the fast photochemical incorporation of C* into organic material from slower incorporation by dark reactions, one must work very rapidly. They proceeded to expose already actively photosynthesizing plants (to avoid induction delays) to $HC^*O_3^-$ or C^*O_2 for seconds or minutes rather than for hours (as it was done before). Such fast experiments were made possible by two new, highly sensitive analytical techniques, paper chromatography and autoradiography.

In these experiments, a suspension of algae (or another plant material) is exposed to light in "ordinary," nonradioactive carbon dioxide or car-

bonate until a steady state of photosynthesis is established. Radioactive carbon is then supplied by sudden injection of C*-containing bicarbonate into the suspension medium (or of C^*O_2 into the circulating gas). Illumination is continued for a few seconds (or minutes); then, plant cells are rapidly killed—usually by dropping them into boiling alcohol. The cell material is extracted with various solvents, and the extract fractionated by so-called two-dimensional paper chromatography.

In this procedure, excess solvent is removed by evaporation, and the concentrated extract applied near one of the corners of a sheet of filter paper. Then, a suitable solvent is allowed to mount through the paper, driven upward by capillarity; different components of the solution are deposited at different heights, depending upon their properties, thus forming a one-dimensional sequence along one edge of the sheet. The paper is then dried, and the edge along which the compounds had been deposited is dipped into another solvent. The compounds are now carried up to different heights at right angle to the direction of their original diffusion, thus forming a two-dimensional "chromatogram."

This procedure can lead to a very effective fractionation. With one basic and one acidic solvent, neutral components (such as sugars or esters) may be deposited near the original corner of the sheet, while basic and acidic components deviate in opposite directions from the diagonal. How far the different components diffuse depends on their adsorbability on paper and their solubility in the solvents used. By using mixtures of known chemical compounds, one can prepare an empirical "chromatographic map," showing where these compounds will be found after a certain period of diffusion (at a certain temperature, on a certain kind of filter paper).

Because of the radioactivity of the components that form the subject of this research, their position on the paper chromatogram can be identified by "autoradiography," even if their amounts are so small as to be invisible to the eye. For this, the filter paper is pressed against a sheet of X-ray film, and the latter is developed after proper exposure. The radiation (β^- particles), emanating from the radioactive carbon present in certain spots on filter paper, blackens the opposite spots on the X-ray film (see Fig. 17.1).

Various improvements of this wonderfully sensitive and powerful analytical method have been introduced. The amount of C* in the various spots can be estimated from the intensity of radiation emitted by them.

The spots can be treated by analytical reagents; they can be cut out, dissolved, and rechromatographed, or investigated by chemical methods.

The First Stable Product of CO_2 Fixation: Phosphoglyceric Acid[1]

The above-described combination of two ingenious techniques proved a unique boon to the study of the mechanism of carbon dioxide reduction

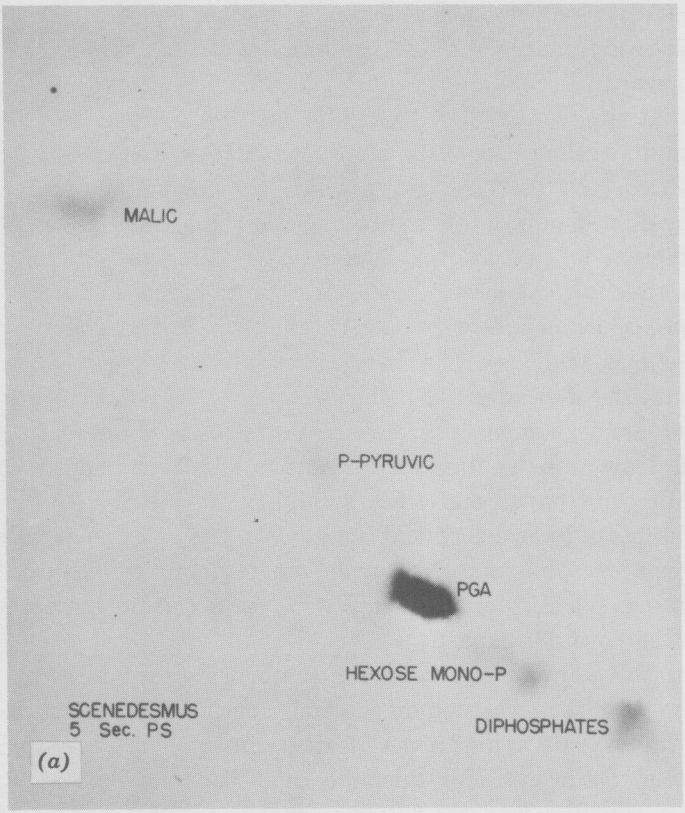


FIG. 17.1 Radiograms of products photosynthesized by Scenedesmus in CO_2 (see p. 227). (*a*) in 5 sec; (*b*) in 15 sec; (*c*) in 60 sec. The "neutral" products, such as sugar phosphates (designated by the letter *P*) remain nearest to the corner, while basic compounds are carried to the left and acidic ones to the right of the diagonal. (M. Calvin and co-workers, 1951.)

[1] The rest of this chapter requires some familiarity with organic chemistry and biochemistry.

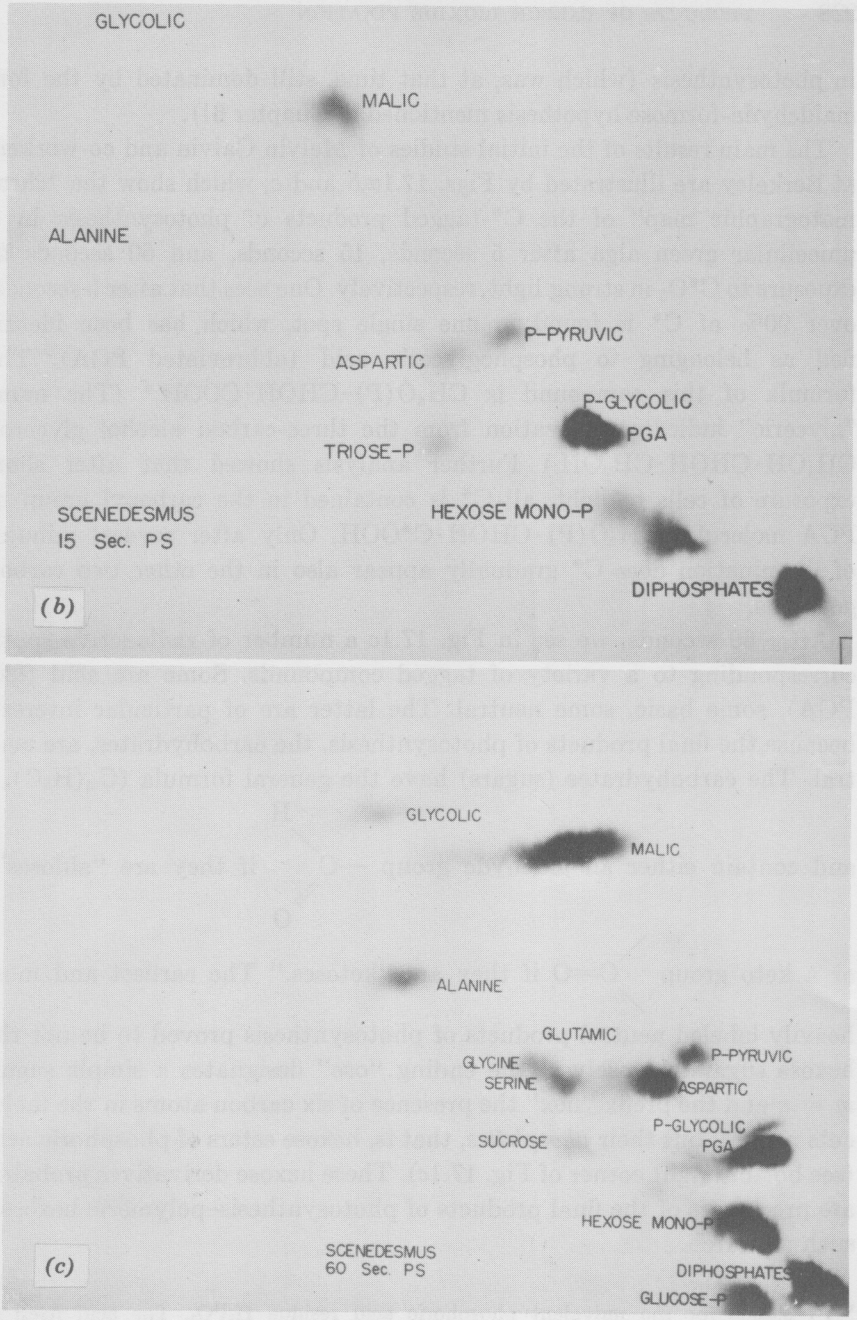
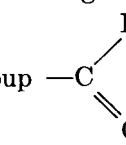
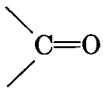


FIG. 17.1 (Continued)

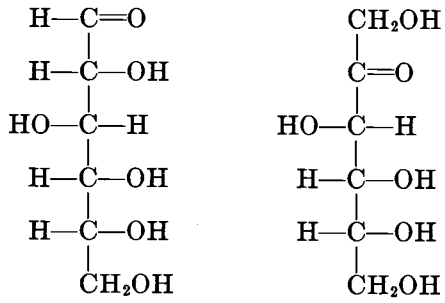
in photosynthesis (which was, at that time, still dominated by the formaldehyde-formose hypothesis mentioned in Chapter 6!).

The main results of the initial studies of Melvin Calvin and co-workers at Berkeley are illustrated by Figs. 17.1*a,b* and *c*, which show the "chromatographic map" of the C*-tagged products of photosynthesis in a unicellular green alga after 5 seconds, 15 seconds, and 60 seconds of exposure to C^*O_2 in strong light, respectively. One sees that after 5 seconds, over 90% of C* is found in one single spot, which has been identified as belonging to phosphoglyceric acid (abbreviated PGA). The formula of this compound is $CH_2O(P)\cdot CHOH\cdot COOH$.[2] (The name "glyceric" indicates derivation from the three-carbon alcohol glycerol, $CH_2OH\cdot CHOH\cdot CH_2OH$.) Further analysis showed that after short exposure of cells to light, all C* is contained in the carboxyl group of PGA molecule; $CH_2O(P)\cdot CHOH\cdot C^*OOH$. Only after several minutes of illumination does C* gradually appear also in the other two carbon positions.

After 60 seconds, we see in Fig. 17.1*c* a number of radioactive spots, corresponding to a variety of tagged compounds. Some are acid (like PGA), some basic, some neutral. The latter are of particular interest, because the final products of photosynthesis, the carbohydrates, are neutral. The carbohydrates (sugars) have the general formula $(C_m(H_2O)_n)$, and contain either an aldehyde group  if they are "aldoses"; or a keto group  if they are "ketoses." The earliest and most heavily labeled neutral products of photosynthesis proved to be not the hexose sugars themselves (the ending "ose" designates a simple sugar, $m = n$ and the prefix "hex" the presence of six carbon atoms in the molecule $n = 6$), but their *phosphates*, that is, hexose esters of phosphoric acid (see bottom right corner of Fig. 17.1*c*). These hexose derivatives probably are precursors of the final products of photosynthesis–polymeric hexoses, such as starch.

[2] (P) stands for the univalent phosphoric acid residue H_2PO_3. The acid itself is (P)OH; the notation Pi is often used instead to denote "inorganic" phosphate (as contrasted to "high energy phosphates," such as ATP, see Chapter 18).

Among hexoses, the most important are glucose (an aldose) and fructose (a ketose), shown in Eq. 17.3.[3]



Glucose (aldose) Fructose (ketose) (17.3)

Cane sugar (sucrose), $C_{12}H_{22}O_{11}$, is the product of condensation of one molecule of glucose with one molecule of fructose (with loss of a water molecule); starch is a high-polymeric form of glucose.

One sugar phosphate spot on the chromatogram of early products of photosynthesis proved to be peculiarly important. Identification of this product as a *pentose phosphate* (that is, a phosphate of a sugar containing five rather than six carbon atoms)—more specifically, as *ribulose diphosphate* (for short, RDP), by A. A. Benson and co-workers in 1951, was the second fundamental discovery resulting from the application of radiocarbon tracer to photosynthesis (after the identification of PGA). Ribulose diphosphate turned out to be the main or only carbon dioxide "acceptor" in photosynthesis.

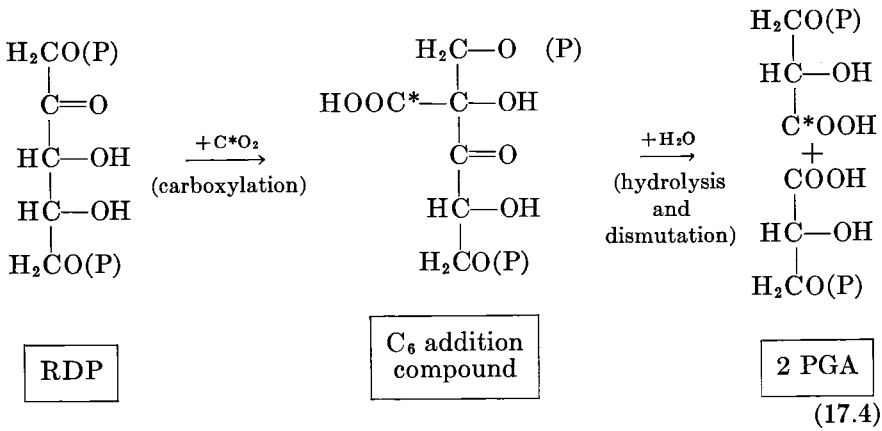
The Primary CO_2 Acceptor: Ribulose Diphosphate

It is a priori unlikely that carbon dioxide could undergo reduction as such (or as a carbonate ion); rather, it was long surmised that CO_2 is first bound in photosynthesis by a nonphotochemical reaction to some organic "acceptor."

After the identification of PGA as the most important early-labeled intermediate of photosynthesis, the nature of the CO_2 "acceptor" suggested itself by the following consideration: Since at first, PGA carries

[3] The OH and H groups are placed to suggest the spatial arrangement of the substituents in the specific forms of the sugars found in nature ("dextro" forms). Their mirror image stereomers ("levo" forms) do not occur in nature.

the "label" entirely in its carboxyl group, it must have been formed by *carboxylation,* according to the equation: $RH + C^*O_2 \rightarrow RC^*OOH$ (a reaction in which CO_2 inserts itself between an organic radical R, and an H-atom). It seems that to produce PGA by such a reaction, the acceptor should be *glycol phosphate,* $CH_2O(P) \cdot CH_2OH$ (glycol is the two-carbon di-alcohol, $CH_2OH—CH_2OH$). This presumption could not be confirmed; but the riddle solved itself by identification of ribulose diphosphate (RDP) as an early-tagged product of photosynthesis. When RDP (a five-carbon compound) adds a CO_2 molecule, an acid containing six carbon atoms is formed ($C_5 + C_1 = C_6$). If a molecule of this acid breaks into two parts, each containing three carbon atoms, both can be PGA molecules (as indicated in Eq. 17.4)!



(17.4)

Reaction 17.4 involves, in addition to *carboxylation* (addition of CO_2) and splitting of the six-carbon chain in two by hydrolysis, also a *"dismutation"*—oxidation of one carbonyl group to carboxyl, and reduction of another one to alcohol. (Otherwise, a single CO_2 could not produce *two* carboxyl groups!) The enzyme that brings about this combined reaction is called a *carboxy-dismutase.*

If RDP is the primary acceptor of CO_2, and PGA is formed as the product of this carboxylation, a sudden depletion of C^*O_2 in a steadily photosynthesizing system should result in an immediate drop of the PGA concentration, and corresponding rise of that of RDP. This was confirmed in Calvin's laboratory (see Fig. 17.2*a*).

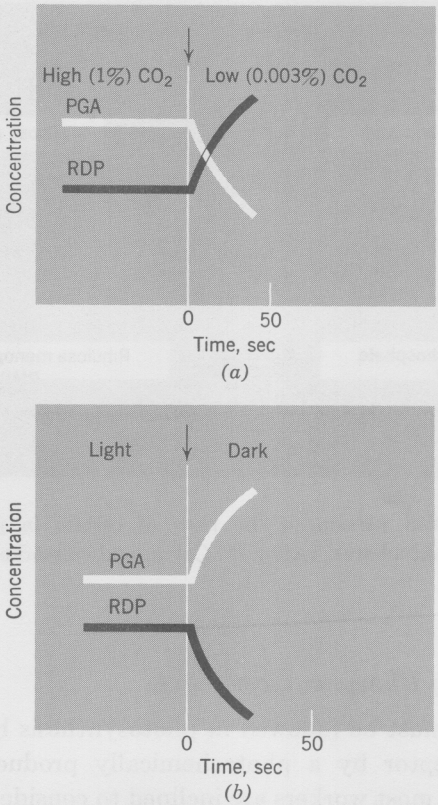


FIG. 17.2 Diagram showing the effect of lowering the CO_2 level (*a*) and turning off the light (*b*) on the concentration of phosphoglyceric acid (PGA) and ribulose diphosphate (RDP). (M. Calvin and co-workers, 1957.)

The reduction of PGA by a reductant supplied by the photochemical primary process leads in part to the formation of sugar, and in part to regeneration of the CO_2 acceptor used up in the reaction. More specifically, five sixths of reduced PGA must be reconverted to RDP and one sixth—corresponding to the one molecule CO_2 added in carboxylation (17.4)—can be converted to the final product, hexose. The resulting reaction sequence is known as the "Calvin cycle." It is represented in Figs. 17.3 and 17.4.

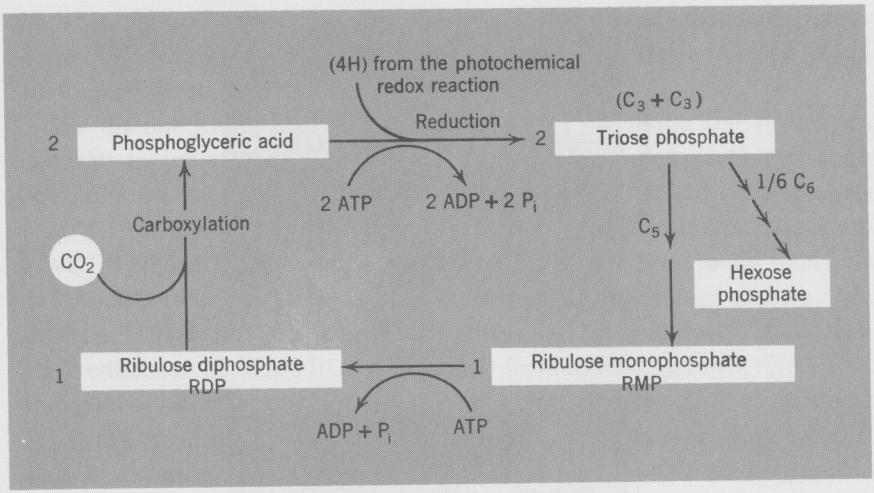


FIG. 17.3 A simplified version of the path of carbon in photosynthesis (Pi = (P)OH = inorganic phosphate). (After M. Calvin and co-workers.)

The Reduction of Phosphoglyceric Acid

Carboxylation must be followed in photosynthesis by *reduction* of the carboxylated acceptor by a photochemically produced reductant. As mentioned before, most workers are inclined to consider reduced pyridine nucleotide, NADPH, as the actual reductant. As pointed out before, reduced pyridine nucleotide does not have enough reducing power (its potential, $E_0' = -0.35$, is not sufficiently negative), to reduce a carboxyl group to a carbonyl group (the E_0' of the couple carboxyl-carbonyl is about -0.5 volt). It has been suggested that this reduction becomes possible if it is coupled with the energy-releasing hydrolysis of a molecule of a "high energy phosphate": ATP $\xrightarrow{+H_2O}$ ADP + P(OH) (see Chapter 18).

Calvin and co-workers suggested that phosphoglyceric acid (PGA), formed in reaction 17.1, is phosphorylated, in the next reaction step, to diphosphoglyceric acid (DPGA) (see Eq. 17.5). The latter is a "high energy" phosphate, because the second (P) radical is attached to the carboxyl group (see Chapter 18). This reaction is catalyzed by an enzyme of the type called "kinases." DPGA can be reduced by NADPH (see Eq. 17.6), if its high energy phosphate group is simultaneously split

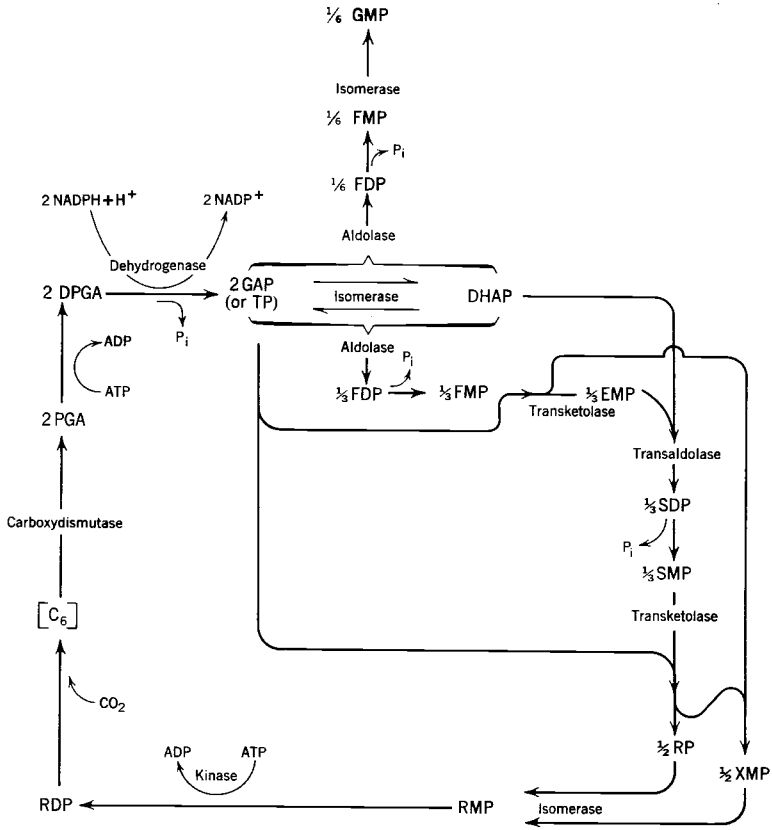


FIG. 17.4 The Calvin-Benson-Bassham cycle. (Pi = (P)OH = inorganic phosphate).

C_3 Compounds

PGA: 3-phosphoglyceric acid

DPGA: 1,3-diphosphoglyceric acid

GAP (or TP): glyceraldehyde-3-phosphate mono phosphate

DHAP: dihydroxyacetone phosphate

C_4 Compounds

EMP: erythrose-4-mono phosphate

C_5 Compounds

RP: ribose-5-mono phosphate

RMP: ribulose-5-mono phosphate

RDP: ribulose-1,5-diphosphate

XMP: xylulose-5-mono phosphate

C_6 Compounds

FMP: fructose-6-mono phosphate

FDP: fructose-1,6-diphosphate

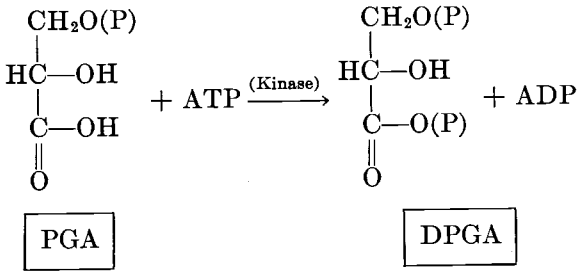
GMP: glucose-6-phosphate

C_7 Compounds

SMP: sedoheptulose-7-mono phosphate

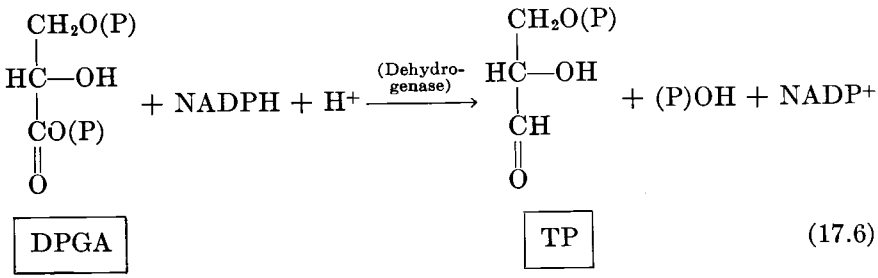
SDP: sedoheptulose-1,7-diphosphate

off as a "low energy" phosphate, (P)OH. The product of this reduction is a triose—a sugar with three carbon atoms (or rather, a triose phosphate, TP).



(17.5)

Reaction 17.6 requires an enzyme called triose phosphate dehydrogenase. (Note that the names of enzymes usually reveal the substrates they act on, and the kind of ractions they catalyze.)



(17.6)

Reaction 17.6 utilizes the reducing power of NADPH, while reaction 17.5 makes use of the high energy content of ATP—both acquired in the light reactions of photosynthesis.

If these reactions occur as suggested, turning off the light illuminating a steadily photosynthesizing cell suspension should lead to an immediate transitory increase in the concentration of PGA, and a decrease in that of RDP, because the reactions 17.4 will continue for a while, while reactions 17.5 and 17.6 must stop when no more ATP and NADPH are supplied by light. This expectation was confirmed (see Fig. 17.2*b*).

As mentioned above, one sixth of triose phosphate formed by reduction of PGA must be converted, by a series of enzymatic reactions, into a hexose, which is the final product of photosynthesis; while five sixths

must be tranformed back into ribulose phosphate, to close the cycle (Fig. 17.3). Each time the cycle in Fig. 17.3 revolves once, one sixth of a hexose molecule is formed, and one molecule CO_2 and four hydrogen atoms are consumed

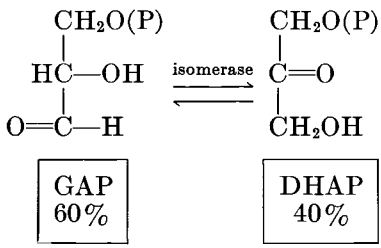
$$CO_2 + (4H) \rightarrow (CH_2O) + H_2O$$

After the cycle had rotated six times, one hexose molecule is formed, and six CO_2 molecules are used up.

The Transformation of Carbohydrates

It was stated above that the triose, $C_3H_6O_3$ (glyceraldehyde) is a carbohydrate: $(CH_2O)_3$. Its transformation into other carbohydrates, such as hexoses (glucose or fructose), or into a pentose (such as ribulose), which all have the same reduction level, $R = 1$ (see Chapter 5), can occur by enzymatic conversions with no additional energy requirement. However, experience shows that one ATP molecule is needed for the conversion of ribulose monophosphate into ribulose diphosphate.

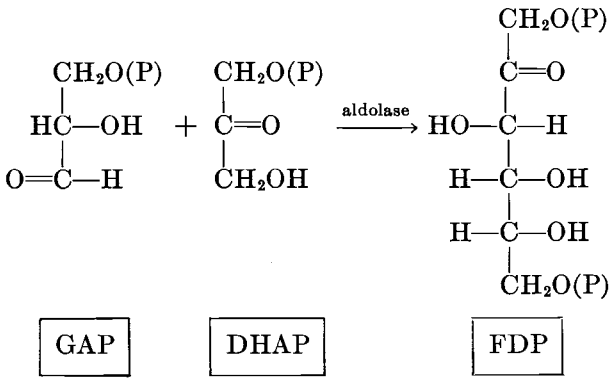
The specific mechanism by which triose phosphate (TP or GAP, glyceraldehyde phosphate), is converted in photosynthesis into hexose phosphates, has been established by tracer studies. Glyceraldehyde phosphate (GAP) first undergoes partial isomerization (with the help of an enzyme called isomerase) into dihydroxyacetone phosphate (DHAP) as in Eq. 17.7. The latter is another three-carbon sugar phosphate, which contains a ketone (C=O) group instead of the aldehyde (CHO) group present in glyceraldehyde. An equilibrium is established with 60% glyceraldehyde and about 40% DHAP.



(17.7)

One molecule of GAP and one molecule of DHAP combine (under the action of an enzyme called "aldolase") to form a molecule of fruc-

tose diphosphate (FDP).



(17.8)

The so-formed fructose diphosphate (FDP) loses a phosphate group (by action of the enzyme *phosphatase*), forming fructose monophosphate (FMP). With the help of an *isomerase*, the latter isomerizes partially to glucose monophosphate. Once glucose and fructose monophosphates are available, higher-molecular carbohydrates, sucrose or starch, can be formed. We do not need to enter here into the details of this synthesis. The process leading from ribulose diphosphate to glucose monophosphate is indicated in Fig. 17.4 (which is an elaboration of Fig. 17.3).

Regeneration of Ribulose Diphosphate

The conversion of one sixth of the triose formed in photosynthesis to hexose was described previously as a "straighforward" process (17.9*a*); but regeneration of a pentose, which must use up the remaining five sixths (17.9*b*), is much more involved. The net results are:

$$2C_3 \rightarrow C_6 \qquad (17.9a)$$

$$10C_3 \rightarrow 6C_5 \qquad (17.9b)$$

To carry out the conversion (17.9*b*) in steps, each involving one or two sugar molecules, required from nature real ingenuity! Biochemists working with C*-indicator, were able to identify these steps. The conclusions are illustrated in Scheme 17.10 (Fig. 17.5). A key role is played here by vitamin B_1 (thiamine), which can transfer C_2 groups from one sugar to another. It is used in two ways: it first takes a C_2 group from a C_6-sugar (formed according to 17.9*a*), forming a C_4 sugar (erythrose); and then a second one from a C_7 sugar (sedoheptulose),

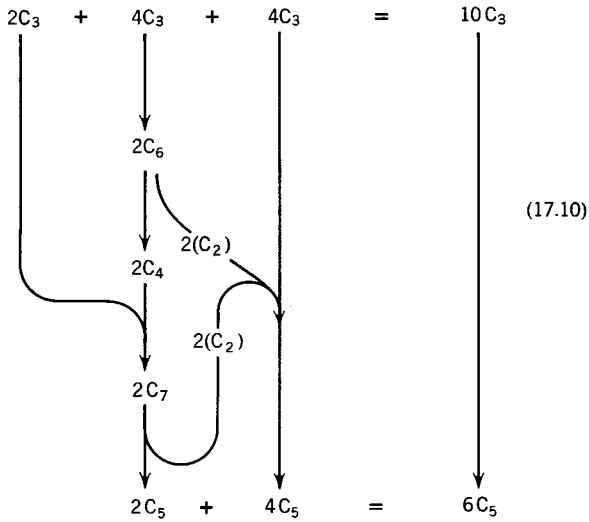


FIG. 17.5 The interconversions of carbon compounds; the subscript refers to the number of carbon atoms in particular molecules (see text).

formed by combining erythrose with a triose (C_3). This gives one pentose molecule; two more are formed by addition of the two thiamine-carried C_2-groups to triose molecules. Some additional details of scheme 17.10 are shown in Fig. 17.4. The transformation of C_3-chains into C_5-chains is shown there to include "transketolizations" and "transaldolizations," as well as a phosphorylation, producing as intermediates, in addition to erythrose (a C_4 sugar) and sedoheptulose (a C_7 sugar), also xylulose (a C_5 sugar, isomeric with ribulose). Scheme 17.10 (Fig. 17.5) describes, however, the essence of this complicated series of transformations—conversion of ten C_3-sugar molecules into six C_5-sugar molecules, by reactions involving only pairs of molecules, and a catalyst—thiamine—busily carrying C_2 groups between them.

The Calvin Cycle: Concluding Remarks

Figure 17.3 is a condensed version of the Calvin cycle—an elaboration of the upper arrow in Figs. 5.4 and 14.4; each arrow in it represents a sequence of several reactions. The three major reactions are:

1. *Carboxylation* of RDP (that is, addition of CO_2 to the 5-carbon keto sugar phosphate, RDP) and formation of PGA, a 3-carbon acid;
2. *Reduction* of PGA to a 3-carbon aldose—triose phosphate (TP)—and its polymerization to C_6-sugars;
3. *Regeneration* of RDP from TP.

As mentioned earlier, the reductant generated by the light reactions (usually presumed to be NADPH) is utilized for the reduction of PGA (reaction 2 above), while ATP (adenosine triphosphate), another product of the light reaction, is utilized at two sites: in the phosphorylation of the five-carbon keto sugar ribulose monophosphate, RMP, to diphosphate, RDP; and in the phosphorylation of phosphoglyceric acid, PGA, to diphosphoglyceric acid, DPGA, prior to its reduction by NADPH.

As suggested earlier, this second phosphorylation could be dispensed with if XH ($E_0' \simeq -0.6$ volt) could be used directly for the reduction of PGA, without some reducing power being first lost by electron transfer to $NADP^+$ ($E_0' = -0.35$ volt).

Despite the many remaining uncertainties (see below), the discoveries of Calvin, Benson, Bassham, and co-workers, particularly the identification of the two key compounds, PGA and RDP, stand as the most spectacular achievements in the study of metabolic reaction sequences by means of radioactive carbon tracer.

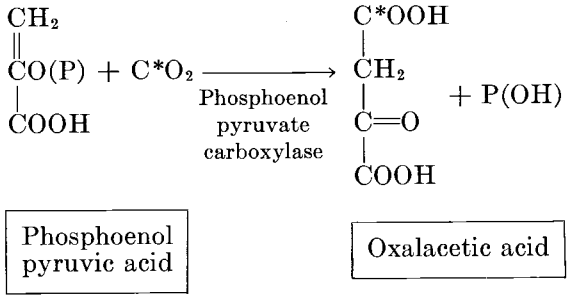
Alternate Pathways

Several unsolved, or only tentatively solved questions remain. Some early tagged products are alien to the cycle in Figs. 17.3 and 17.4. Among them are *carboxylic acids* (particularly malic acid, succinic acid, and glycolic acid) and *amino acids* (alanine, aspartic acid, serine, and glycine). Their appearance suggests that the cycle is complicated by various side reactions which become significant already in the first minutes of illumination. In many experiences, *malic acid* has been found heavily labeled after brief photosynthesis. Malic acid is known to be related to one of the central compounds of the Krebs cycle, pyruvic acid, $CH_3COCOOH$, by carboxylation and reduction:

$$\underset{}{CO_2} + \underset{\text{pyruvic acid}}{CH_3COCOOH} \xrightarrow{+2H} \underset{\text{malic acid}}{HOOCCH_2CH(OH)COOH} \qquad (17.11)$$

(Krebs cycle is the cycle involved in the oxidation of pyruvic acid to CO_2 and H_2O during respiration.) Are we to assume that reaction 17.11 provides a second "port of entry" of CO_2 into photosynthesis? Or is malic acid merely a rapidly formed by-product of the transformation of PGA?

M. D. Hatch and C. R. Slack (1966, 1968) suggested an alternate pathway of C^*O_2 fixation in corn leaves and several other plants. In this pathway, phosphoenolpyruvic acid is carboxylated as follows:



(17.12)

(Malic acid is formed as a side product from oxalacetic acid by NADH; malic acid dehydrogenase is the catalyst for this reaction.) Thus, RDP is not the direct acceptor of CO_2 in this pathway. However it is suggested that RDP and the 4-carbon acid formed above (see Eq. 17.12) react together to form PGA (that enters the Calvin cycle) and pyruvic acid; the latter is then phosphorylated to phosphoenol pyruvic acid, which continues the cycle (see Equations 17.13 and 17.14).

$$\text{4-Carbon acid} + \text{RDP} \rightarrow 2\text{PGA} + \text{Pyruvic acid} \qquad (17.13)$$

$$\text{Pyruvic acid} + \text{ATP} + \text{P(OH)} \xrightarrow[\text{synthetase}]{\text{Pyruvate}} \text{Phosphoenol pyruvic acid} + \text{AMP} + \text{P(P)OH} \qquad (17.14)$$

All the enzymes and the intermediates suggested by Equations 17.12–17.14 have been found in large enough quantities in corn and some, but not all, other plants studied. The author's surmised that in the latter, the cycle operates in Calvin's original form.

Formation of tagged glycolic acid ($CH_2OH \cdot C^*OOH$) in light has been noted in several laboratories. Under certain conditions (low CO_2 pressure, high concentration of O_2), it is much enhanced. The mechanism of this glycolate formation is, as yet, not properly understood.

It has been long suspected that direct photosynthesis of compounds

other than carbohydrates can take place in photosynthetic systems. It has been shown, for example, that under certain conditions, 30% or more of the photosynthate in the green alga *Chlorella* appears in the form of amino acids. Under other conditions, when algal cells are exposed to C^*O_2 for only 1–2 minutes, as much as 30% of the fixed carbon is found in lipidlike substances.

One likes the simplicity of Calvin's cycle—a single carboxylation and a single reduction!—but nature is not under obligation to be simple.