Title
CHAPTER 36. PHOTOSYNTHESIS: THE PATH OF CARBON

Permalink
https://escholarship.org/uc/item/55c0j3rs

Author
Bassham, J.A.

Publication Date
1964-02-01
CHAPTER 36

PHOTOSYNTHESIS: THE PATH OF CARBON

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

Berkeley, California
CHAPTER 36

PHOTOSYNTHESIS: THE PATH OF CARBON

J. A. Bassham

February 1964
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A.</td>
<td>Energetics and net reactions of photosynthesis</td>
<td>1</td>
</tr>
<tr>
<td>B.</td>
<td>Relations between energy conversion and carbon reduction</td>
<td>4</td>
</tr>
<tr>
<td>II.</td>
<td>Methods for mapping the path of carbon in photosynthesis</td>
<td>7</td>
</tr>
<tr>
<td>A.</td>
<td>Use of radioisotopes as tracers</td>
<td>7</td>
</tr>
<tr>
<td>B.</td>
<td>Analysis and identification of intermediate compounds</td>
<td>9</td>
</tr>
<tr>
<td>C.</td>
<td>Chemical degradation of labeled products</td>
<td>11</td>
</tr>
<tr>
<td>III.</td>
<td>Experimental results and their interpretation</td>
<td>12</td>
</tr>
<tr>
<td>A.</td>
<td>First labeled product</td>
<td>12</td>
</tr>
<tr>
<td>B.</td>
<td>Location of radiocarbon within molecules</td>
<td>13</td>
</tr>
<tr>
<td>C.</td>
<td>Light-dark transient changes</td>
<td>19</td>
</tr>
<tr>
<td>D.</td>
<td>High-low CO2 transient changes</td>
<td>20</td>
</tr>
<tr>
<td>IV.</td>
<td>The photosynthetic carbon reduction cycle</td>
<td>20A</td>
</tr>
<tr>
<td>A.</td>
<td>The complete cycle</td>
<td>20A</td>
</tr>
<tr>
<td>B.</td>
<td>Stoichiometry of the cycle</td>
<td>22</td>
</tr>
<tr>
<td>C.</td>
<td>Quantitative importance of cycle</td>
<td>23</td>
</tr>
<tr>
<td>D.</td>
<td>Probable energetic efficiency in the cycle</td>
<td>24</td>
</tr>
<tr>
<td>V.</td>
<td>Secondary carbon reduction pathways</td>
<td>25</td>
</tr>
<tr>
<td>A.</td>
<td>Carboxylic acids</td>
<td>25</td>
</tr>
<tr>
<td>B.</td>
<td>Fatty acids and fats</td>
<td>28</td>
</tr>
<tr>
<td>C.</td>
<td>Carbohydrates</td>
<td>30</td>
</tr>
<tr>
<td>D.</td>
<td>Amino acids and proteins</td>
<td>30</td>
</tr>
<tr>
<td>VI.</td>
<td>Areas of future discovery</td>
<td>33</td>
</tr>
</tbody>
</table>
Chapter 36

PHOTOSYNTHESIS: THE PATH OF CARBON

J. A. Bassham

Lawrence Radiation Laboratory, University of California,

Berkeley, California

I. Introduction

A. Energetics and net reactions of photosynthesis

Photosynthesis transforms light energy into an increased potential energy of chemical bonds. In living cells, all activities except photosynthesis expend this chemical potential energy. Thus, photosynthesis is the ultimate source of all energy used in plant or animal cells.

The products of photosynthesis are organic compounds and oxygen. These organic compounds and molecular oxygen together have a certain total free energy of formation. This is the energy which is reversibly released to the environment when these substances are formed from their elements. It is denoted by AF. In most cases it has a negative value, because stable compound formation from the elements is commonly accompanied by a loss of energy from the substance to the environment.

The organic compounds and oxygen are made by photosynthesis from the inorganic oxides, water, carbon dioxide, sulfate, and sometimes nitrate. These oxides, taken together, have a total AF of formation which has a more negative value than that of the products of photosynthesis. Consequently, a positive free energy change accompanies photosynthesis, because the products (organic compounds and oxygen) have a total AF of formation which is less negative than that of the reactant oxides. This increase
in free energy is supplied by the conversion of some of the absorbed light energy.

The simplest net reaction of photosynthesis [Eq. (1)] is the formation of a sugar such as glucose from water and carbon dioxide.

\[
6 \text{CO}_2 + 6 \text{H}_2\text{O} \xrightarrow{\text{Light}} \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \quad \Delta F^\circ = +675 \text{ Kcal (glucose)}
\]

The equation \(\Delta F^\circ = +675 \text{ Kcal}\) means that for each mole of glucose formed in this way, 675,000 calories of energy are stored as net decrease in the stability of the chemical bonds holding together the atoms of the product molecules. Thus, if the products of Eq. (1) were to react with each other to give back the reactants of Eq. (1), up to 675,000 calories of useful work could be performed in a "thermodynamically perfect" process. The released energy actually used for work by a respiring cell must always be less than 675,000 calories, since such processes are always less than 100% efficient.

Carbohydrate was long thought to be the unique organic product of photosynthesis. All other products formed in plants were assumed to be made from carbohydrates by reactions unrelated to photosynthesis.

Such syntheses of organic compounds coupled to the respiration of carbohydrates do occur in all non-photosynthetic cells and outside the chloroplasts of photosynthetic cells. But it now appears (Section V) that within the chloroplasts (Chapter 6) direct photosynthesis of compounds other than carbohydrates takes place. It appears that the chloroplast is a rather complete biosynthetic "factory". Chloroplasts can produce virtually all the substances required for the generation of new chloroplast material.
Additional amounts of photosynthetic products diffuse or are transported from the chloroplasts to other parts of the plant. The relative amount of photosynthetic products (photosynthate) used for new chloroplast material, as compared with the amounts exported from the chloroplasts, depends greatly on the plant species as well as on the age and state of development of the cell and of the plant. For example, a rapidly growing and dividing unicellular alga may use most of its photosynthate for the manufacture of more chloroplast material, but a mature sugar beet leaf may export most of its photosynthate to other parts of the plant.

Large variations in the nature of the photosynthate also occur. Smith (1944) demonstrated that under certain conditions sunflower leaves produce carbohydrates almost exclusively. Thus, their photosynthetic net reaction can be closely represented by Eq. (1').

\[
\text{light} \quad \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_12\text{O}_6 + \text{O}_2 \quad \Delta F^\circ = +112 \text{ Kcal}
\]

In contrast, the unicellular alga, Chlorella pyrenoidosa, under some conditions produces amino acids as 30% or more of its photosynthate (Smith, et al., 1961).

Amino acid synthetics incorporate sulfur and nitrogen. The oxides of these elements also are reduced by photosynthesis. Net photosynthetic reactions for the reduction of carbon dioxide to another organic compound, glyceric acid, is denoted by Eq. (4').

\[
\begin{align*}
(2') & \quad \text{CO}_2 + \text{H}_2\text{O} + 2 \text{H}^+ \rightarrow \text{H}_3\text{PO}_4 + 2 \text{O}_2 \quad \Delta F^\circ = +83 \text{ Kcal} \\
(3) & \quad 2\text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO}_3 + 2 \text{O}_2 \quad \Delta F^\circ = +190 \text{ Kcal} \\
(4) & \quad 3 \text{CO}_2 + 3 \text{H}_2\text{O} \rightarrow \text{C}_3\text{H}_6\text{O}_4 + 2-1/2 \text{O}_2 \quad \Delta F^\circ = +287 \text{ Kcal}
\end{align*}
\]

Many more photosynthetic reactions are required to convert the products of Eqs. (1'), (2), (3), and (4) to the multitude of substances formed.
photosynthetically in the chloroplast.

### Relations between energy conversion and carbon reduction

#### 1. Formulation of oxidative and reductive phases of photosynthesis

Experiments with isotopic oxygen showed that all of the $O_2$ evolved during photosynthesis comes instantaneously from water (Ruben, et al., 1941). When photosynthesizing plants were supplied with either water or $CO_2$, in which some of the oxygen atoms were $^{18}O$ instead of $^{16}O$, the $O_2$ evolved just after the addition of the heavy isotope agreed in isotopic composition with the water. Thus, photosynthesis in green plants should be represented by Eq. (5).

$CO_2 + 2 H_2O^{light} \rightarrow (CH_2O) + O_2 + H_2O$

Whereas green plants oxidize water and produce $O_2$, other types of photosynthetic organisms oxidize other substances. Green sulfur bacteria in the light oxidize $H_2S$ and produce elemental sulfur. In other organisms, the substance oxidized may be organic compounds such as secondary alcohols (purple bacteria, Foster, 1944) or even molecular hydrogen itself in the case of adapted green algae (Saffron, 1942). Van Niel formulated the general Eq. (6) for all types of photosynthesis. $H_2A$ represents the substance oxidized.

$CO_2 + 2 H_2A^{light} \rightarrow (CH_2O) + 2 A + H_2O$

In the green plant, where $H_2A$ is water, light energy is used to remove electrons and protons (equivalent to hydrogen atoms) from water, giving molecular $O_2$. A number of coenzymes are involved as electron carriers between water and $CO_2$ (and other oxides). Another result of the energy converting processes of photosynthesis is the storage of some of the light
energy through the formation of the biological acid anhydride, ATP, from ADP and inorganic phosphate. In Chapter 37, these processes which use light energy to oxidize water and to produce ATP will be discussed.

Of the various reduced cofactors formed by the conversion of light energy, the one with the most negative redox potential, and hence the one which is the strongest reducing agent, appears to be chloroplast ferredoxin (Togawa and Arnon, 1962). This substance contains protein and bound iron. It has a redox potential at pH 7 comparable to that of hydrogen gas, -0.42 V. Reduced chloroplast ferredoxin plus an enzyme in the chloroplasts brings about the reduction of NADP to NADPH₂, which has a redox potential of -0.32 V.

ATP and reduced cofactors are used during photosynthesis to accomplish the reduction of carbon dioxide, sulfate, and nitrate. The products of these reductions are many organic compounds, including carbohydrates, amino acids, and proteins, fatty acids, fats, and many other substances.

The most general formulation of the oxidative and reductive stages of photosynthesis for the formation of carbohydrates is given in Eqs. (7) and (8) respectively.

\[
\text{light} \quad (7) \quad 2 \text{H}_2\text{O} + 2 \text{X} + n(\text{ADP} + \text{P}1) \longrightarrow 2(\text{X} \cdot 2 \text{H}) + \text{O}_2 + n\text{ATP}
\]

\[
(8) \quad 2(\text{X} \cdot 2 \text{H}) + n\text{NTP} + \text{CO}_2 \longrightarrow (\text{CH}_2\text{O}) + 2 \text{X} + \text{H}_2\text{O} + n\text{ADP} + n\text{P}1
\]

We will now preview the reactions of the carbon reduction cycle which use the ATP and reduced cofactors. The details of the cycle and the evidence upon which it is formulated will be given in Sections III and IV. This cycle is the central feature of the path of photosynthetic carbon reduction.
2. Utilization of ATP

The chemical energy stored in the bonds of ATP is used at two points in the photosynthetic carbon reduction cycle. This energy provides additional driving force to make the cycle proceed in the direction of synthetase. In one of these reactions [Eq. (9)] ATP is used to esterify the hydroxyl group on the number 1 carbon atom of ribulose-5-phosphate (I). The resulting ribulose-1,5-diphosphate (II) is sufficiently reactive to undergo a carboxylation reaction with carbon dioxide. This activation of the sugar phosphate molecule toward carboxylation is of crucial importance to the operation of the photosynthetic carbon reduction cycle.

\[
\begin{align*}
\text{CH}_2\text{OH} & \quad \text{O} \quad \text{CH}_2\text{PO}_3\text{H}^- \\
\text{C}=\text{O} & \quad \text{R-O-P-CH} \quad \text{C}=\text{O} \\
\text{HCON} & \quad \text{C}^- \quad \text{HCON} \\
\text{H}_2\text{COPO}_3\text{H}^- & \quad \text{(ATP)} \quad \text{H}_2\text{COPO}_3\text{H}^- \\
\text{I} & \quad \text{II}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{PO}_3\text{H}^- & \quad \text{O} \quad \text{CH}_2\text{PO}_3\text{H}^- \\
\text{H}^+ & \quad \text{CH}_2\text{OH} \quad \text{R-O-P-CH} \quad \text{CH}_2\text{OH} + \text{ROH} \\
\text{C}^- & \quad \text{O} \quad \text{C-OPO}_3\text{H}^- \\
\text{C} & \quad \text{(ATP)} \quad \text{O} \\
\text{III} & \quad \text{IV}
\end{align*}
\]

R denotes all of the ATP molecule except its terminal phosphate group.

As will be seen later, the product of the carboxylation of ribulose-1,5-diphosphate is 3-phosphoglyceric acid (III). This compound is then
reduced to sugar phosphate, but first it must be transformed with ATP to phosphor-y-3 phosphoglyceric acid (IV). This reaction [Eq. (10)] may be viewed as the utilization of one anhydride compound (ATP) to make another acid anhydride. In this way the energy stored in the bonds of the acid anhydride is conserved. The resulting product is chemically more reactive, and hence it is more easily reduced than 3-phosphoglyceric acid.

3. Utilization of reduced cofactors

Reduced pyridine nucleotide, NADPH₂, is required for the reduction of phosphor-y-3-phosphoglyceric acid (IV) to glyceraldehyde-3-phosphate (V) with the enzyme triosephosphate dehydrogenase. [Eq. (11)].

This is the sole reductive step in the reduction of carbon dioxide by the carbon reduction cycle of photosynthesis, as that cycle is usually formulated.

\[
\begin{align*}
\text{CH₂OPO₃H}^+ & \quad \text{CH₂OPO₃H}^+ \\
\text{CHOH} & \quad \text{CHOH} \\
\text{C-OPO₃H}^- & \quad \text{C-OPO₃H}^- \\
\text{O} & \quad \text{O} \\
\end{align*}
\]

\[\quad \rightleftharpoons \]

\[
\begin{align*}
\text{CH₂OPO₃H}^+ & \quad \text{CH₂OPO₃H}^+ \\
\text{CHOH & NADPH₂} & \quad \text{CHOH + NADP + H₂O₃H}^- \\
\text{SH} & \quad \text{SH} \\
\text{0} & \quad \text{0} \\
\end{align*}
\]

IV \quad \rightarrow \quad V

The two steps utilizing ATP [Eqs. (9) and (10)] and Eq. (11) utilizing reduced pyridine nucleotide, comprise the mechanism by which the chemical energy from the energy converting reactions of photosynthesis is employed in the reduction of carbon dioxide to sugar phosphate. (Fig. 4).

II. Methods for mapping the path of carbon in photosynthesis

A. Use of radioisotopes as tracers

The basic carbon reduction cycle by which carbon dioxide is reduced to sugar phosphate involves at least twelve intermediate compounds. Some of these occur at very small concentrations in the plant. Many other
similar compounds are also present in the photosynthetic cell. In some cases they are closely linked by metabolites to the intermediates in the carbon reduction cycle. In order to understand the mechanism of photosynthetic carbon reduction, one must know the identity of the intermediates involved and the sequence in which these substances are made.

These problems resisted scientific investigation until the advent of modern biochemical methods. One of the most valuable of these new methods proved to be the use of radiocarbon as a tracer element to follow the newly incorporated carbon dioxide during photosynthesis in a green plant. Photosynthesizing plants do not discriminate significantly between ordinary carbon dioxide, $^{12}$CO$_2$, and radioactive carbon dioxide, $^{14}$CO$_2$. The plants incorporate $^{14}$CO$_2$ into the intermediates in the carbon reduction pathway.

Huben, et al., first used radiocarbon in studies of the path of carbon in photosynthesis (1939-40). They found that CO$_2$ fixation in the dark is greater following preillumination. This is in accord with the concept that CO$_2$ fixation occurs by "dark reactions" which use relatively stable chemical species formed in the light. They discovered that the radioactive product formed from labeled CO$_2$ after preillumination was a carboxylic acid. These findings suggested that the path of carbon reduction in photosynthesis might very well include CO$_2$ fixation mechanisms similar to those found in non-photosynthetic plant tissues.

Calvin and Benson and their co-workers used the radiocycle method to study carbon reduction in photosynthesis (Calvin and Benson, 1948, 1949). They allowed the plants to photosynthesize intermediate compounds from
$^{14}$CO$_2$ for short periods of time (a few seconds) and under a variety of experimental conditions. The plants were then killed, stopping the biochemical reactions. The radioactive products in the plant material were analyzed and identified. A careful study was made to determine the amounts of radiocarbon incorporated into chemical substances as a function of the experimental conditions. From the results of this study, they were able to map the path of carbon in photosynthesis.

5. Analysis and identification of intermediate compounds

1. Paper chromatography

The separation and identification of the minute amounts of radioactive organic compounds formed by photosynthesis with $^{14}$CO$_2$ was a difficult analytical problem. This problem was solved through the use of two-dimensional paper chromatography (Benson, et al., 1950). In this method, after the plant has photosynthesized organic compounds from $^{14}$CO$_2$, it is killed, and the soluble compounds are extracted with such solvents as alcohol and water. Then one removes the excess solvent by evaporation and dries the concentrated extracts on a large sheet of filter paper near the corner. After that one allows suitable mixtures of organic solvents wet with water to traverse the paper by capillarity. These solvents dissolve and carry along the various compounds at different rates of travel. The rate for each compound depends on its physical properties.

When this development by a chromatographic solvent is complete, the paper is dried. At this point the substances have been separated into a row near one edge of the paper. That edge of the paper is then dipped in a second solvent which has different solvent properties from the first one. This time the compounds are carried in a direction at a right angle to the
First direction. As a result, the compounds which have been separated in a row following the first development, separate into a two-dimensional pattern over the paper after the second stage of chromatography.

2. Radioautography

The compounds of immediate interest contain atoms of $^{14}$C which emit $\beta$ particles. When a sheet of unexposed medical X-ray film is placed in the dark in contact with the dry paper chromatogram, the areas of the paper holding radioactive compounds are a source of $\beta$ particles which expose the X-ray film. After a suitable period of exposure, the X-ray film is developed. Dark spots on the film reveal corresponding areas of radioactive substances on the paper chromatogram. Such a radioautograph made from a unicellular plant which had photosynthesized with $^{14}$CO$_2$ for a few seconds is shown in Fig. 1.

3. Identification of radioactive products

Small amounts of many known chemical compounds have been chromatographed one at a time, using the same chromatographic procedure. Chemists found the locations of these substances on the paper chromatograms by spraying the papers with chemical reagents which react with the substance to give colored compounds. The substances are revealed as colored spots on the paper. From the locations of the compounds, a chromatographic map of known compounds was made. This map helped in the identification of the unknown radioactive compounds from the photosynthesis experiments.

Comparison of the location of an unknown radioactive compound on the paper with this map gives a preliminary indication of the possible identity of the substance. One may then elute the radioactive compound from the paper chromatogram with water. Next, one mixes the labeled
substance with an unlabeled sample of the suspected compound and rechromatographs the two together. Following this co-chromatography and radioautography, the paper is sprayed to give a colored spot. If the radioactive spot coincides precisely with the colored spot, the radioactive substance is tentatively identified. Further chemical tests, and chromatography with different solvent systems, verify the identification. In this way the of compounds labeled during photosynthesis with $^{14}CO_2$ were discovered (Benson, et al., 1950, 1951; Benson, 1951; Buchanan, et al., 1952).

C. Chemical degradation of labeled products

Information about the derivation of one intermediate substance from another along a metabolic path can be obtained by degrading the substances chemically. One determines the amount of labeling in each carbon atom position of the molecules. For example, Bassham, et al. (1950) hydrolyzed 3-phosphoglyceric acid and then allowed the glyceric acid to react with periodate to give three different substances, carbon dioxide, fumaric acid, and formaldehyde, which were derived respectively from the carboxyl group, the α carbon atom, and the β carbon atom of the original molecules. They determined the radioactive content of each of these derivatives. From this content of $^{14}C$, the distribution of tracer in the original molecule could be calculated. Comparison of this distribution of label within the molecule with the distribution of label within a sugar molecule such as a molecule of glucose, give information about the possible biochemical relations between the two substances (see Fig. 4).
III. Experimental results and their interpretation

A. First labeled product

1. 3-Phosphoglyceric acid

The labeled products of photosynthetic reduction of $^{14}$CO$_2$ for 60 seconds (Fig. 1) are several sugar phosphates and diphosphates, 3-phosphoglyceric acid, phosphoenolpyruvic acid, and small amounts of other carboxylic acids and amino acids (Calvin and Benson, 1949). Photosynthesis with $^{14}$CO$_2$ for 7 seconds produces sugar phosphates and diphosphates and 3-phosphoglyceric acid as the most prominently labeled products (Fig. 2). At only 2 seconds (Fig. 3) by far the most prominently labeled product is 3-phosphoglyceric acid (PGA).

Calvin, et al. (1951) degraded labeled PGA formed during 5 seconds' photosynthesis and showed that 95% of the radiocarbon was located in the carboxyl carbon atom. This finding supported their conclusion that the first reaction of carbon dioxide reduction in photosynthesis is a carboxylation reaction in which the carbon dioxide is incorporated into the carboxyl group of PGA. The nature of the substance which supplies the other two carbon atoms of PGA remained for some time unknown.

2. Sugar phosphates

Among the first sugar phosphates identified were fructose-6-phosphate and glucose-6-phosphate. Soon thereafter dihydroxyacetone phosphate and fructose-1,6-diphosphate, both of which occur in rather small concentrations, were identified. The finding of these compounds led Calvin and Benson (1948, 1949) to conclude that the path of carbon dioxide reduction in photosynthesis included a reversal of several steps of the glycolytic pathway which leads from hexose phosphate to PGA.
After a time several other sugar phosphates were identified. Most important among these were the 7-carbon compounds, sedoheptulose-7-phosphate and sedoheptulose-1,7-diphosphate (EDP), and the 5-carbon compound, ribulose-1,5-diphosphate (RuDP) and ribose-5-phosphate, xylulose-5-phosphate, and ribulose-5-phosphate (Benson, et al., 1952). The roles of these compounds in the path of carbon in photosynthesis became more clear after they had been degraded to locate the position of radiocarbon atoms within the individual molecules (Paschen, et al., 1954).

B. Location of radiocarbon within molecules

1. Carbon dioxide to hexose diphosphate

As already mentioned, most of the radiocarbon found in PGA was located in the carboxyl carbon following short periods of photosynthesis. The remaining radioactivity was found equally distributed at all times between the two other carbon atoms denoted as the α and β carbons. For example, after 5 seconds of photosynthesis with $^{14}$CO$_2$, the distribution of $^{14}$C among carboxyl, α, and β carbons respectively was 95%, 2.5%, 2.5%. After 30 seconds' photosynthesis the distribution was 50%, 25%, 25%.

If the formation of hexose phosphates occurred via a reversal of the glycolytic pathway from PGA, both carbon atoms 3 and 4 of the hexose should be formed from the carboxyl of PGA. Carbon atoms 2 and 5 would come from the α carbons of PGA, while carbon atoms 1 and 6 would derive from the β carbons of PGA. When Calvin, et al. (1951) degraded hexose molecules in such a way as to obtain tetra pairs of carbon atoms, they found the distribution of radiocarbon in the hexose to be as predicted by this pathway (see Fig. 4). They concluded that during photosynthesis,
the PGA formed by the primary carboxylation reaction is reduced to glyceraldehyde phosphate (V) [Eqs. (10) and (11)], which isomerizes to dihydroxyacetone phosphate (VI). These two triose phosphates then condense end to end to make fructose-1,6-diphosphate (VII), and eventually fructose and glucose monophosphates.

\[ \begin{align*}
  \text{CH}_2\text{CO}_2\text{H}^- & \quad \text{CH}_2\text{CO}_2\text{H}^- \\
  \text{C} & \quad \text{C} \\
  \text{O} & \quad \text{CH}_2\text{O} \\
  \text{V} \quad \text{VI} \\
\end{align*} \]

\[ \begin{align*}
  \text{H}_2\text{COPO}_3\text{H}^- & \quad \text{C}_2\text{H}_4\text{O}_4 \\
  \text{C} & \quad \text{C} \\
  \text{HOCH} & \quad \text{HOCH} \\
  \text{HCOOH} & \quad \text{HCOOH} \\
  \text{VII} \quad \text{VIII} \\
\end{align*} \]

It is possible that these reactions are catalyzed by enzymes similar to those of the glycolytic pathway (see Chapter 10). Many such enzymes have been isolated from plant tissue, including photosynthetic tissue (Peterkovsky and Racker, 1961). Such tissues usually are capable of glycolysis, so that isolation of the enzymes involved does not in itself prove their role in photosynthesis. Some photosynthetic organisms appear to lack sufficient amounts of some of these enzymic activities (as assayed
in cell-free systems) to catalyze their assigned step in the path of carbon in photosynthesis (Richter, 1959; Pawson, et al., 1962). This might mean that these organisms perform carbon reduction by a different path. Since there is no direct evidence for such an alternate path, a more likely explanation for the apparent enzyme deficiencies is that the photosynthetic path is mediated by enzymes whose activity is in some way lost or diminished during the preparation of a cell-free system. Perhaps the photosynthetic carbon reduction cycle enzymes are more active in a particulate, or organized enzyme system in vivo, than they are following isolation.

Equations (10-14) bring about the conversion of PGA to fructose-6-phosphate (VIII). The corresponding glycolytic enzymes would be:
(10) phosphoglycerol kinase; (11) triose phosphate dehydrogenase; (12) triose phosphate isomerase; (13) aldolase. Equation (14) would require a phosphatase.

2. Hexose phosphates to pentose phosphates

The 7-carbon sugar phosphate, sedoheptulose-7-phosphate, was isolated from plants which had photosynthesized in $^{14}$CO$_2$ for a few seconds. Degradation of the sugar revealed that its label of $^{14}$C was located in the three middle carbon atoms, numbers 3, 4, and 5 (Bassham, et al., 1954). We have already seen that the 6-carbon sugars were labeled in their two center carbon atoms (numbers 3 and 4) and that the trioses were labeled in their unphosphorylated terminal carbon atom. Condensation of a triose phosphate with either carbon atoms 1-4 or 3-6 of the hexose would give sedoheptulose labeled as found experimentally.
The enzyme transketolase (Fackler, et al., 1953; Norecker, et al., 1953) mediates the removal of carbon atoms 1 and 2 of the ketose phosphate such as fructose-6-phosphate, forming a thiolene pyrophosphate-glycolaldehyde (IX) compound with the 2-carbon piece and producing at the same time an aldose phosphate with two fewer carbon atoms in its chain [Eq. (15)].

\[
\begin{align*}
\text{CH}_2\text{CH} & \\
\text{C}=\text{O} & \\
\text{H}_2\text{COPO}_3\text{H}^- & + \text{TPP} \xrightarrow{\text{BP}} \text{TPP} \xrightarrow{\text{H}_2\text{CO}_2\text{H}^+} \text{H}_2\text{COPO}_3\text{H}^-
\end{align*}
\]

\(\text{IX}\)

In this case, the resulting aldose phosphate is erythrose-4-phosphate (X), labeled in carbon atom positions 1 and 2. When this 4-carbon sugar phosphate is condensed by aldolase with dihydroxyacetone phosphate (VI), Eq. (16), the resulting sedoheptulose-1,7-diphosphate (XI) is labeled in positions 3, 4, and 5, as found experimentally. Removal of the phosphate one \[\text{E}^1, (17)\] on the number/carbon atom by a specific phosphatase then gives sedoheptulose-7-phosphate (XII).

\[
\begin{align*}
\text{CH}_2\text{CH} & \\
\text{C}=\text{O} & \\
\text{H}_2\text{COPO}_3\text{H}^- & + \text{H}_2\text{COPO}_3\text{H}^- \xrightarrow{\text{BP}} \text{H}_2\text{CO}_2\text{H}^+ \xrightarrow{\text{H}_2\text{CO}_2\text{H}^+} \text{H}_2\text{COPO}_3\text{H}^-
\end{align*}
\]

\(\text{XII}\)
Transketolase catalyzes a reaction [Eq. (18)] between thiamine pyrophosphate and sedoheptulose-7-phosphate to produce thiamine pyrophosphate-glycolaldehyde and a 5-carbon compound, ribose-5-phosphate (XIII), labeled in carbon atoms 1, 2, and 3.

\[
\begin{align*}
\text{H}_2\text{COH} & + \text{TPP} \rightarrow \text{CH}_2\text{CH} \\
\text{H}_2\text{COH} & + \text{TPP} \rightarrow \text{CH}_2\text{CH} \\
\end{align*}
\]

Phosphoribose isomerase (Axelrod and Jano, 1954) converts this compound to ribulose-5-phosphate (I) [Eq. (19)].

The two molecules of thiamine pyrophosphate-glycolaldehyde produced by Eqs. (15) and (18) could react with any of the aldose monophosphates mentioned so far. We shall see in the next section that the sugar phosphate which is used up in the carboxylation is a pentose phosphate. The product of the carboxylation, PGA, reduced to triose phosphate. Thus, there must be a net flow of carbon from triose to pentose under steady-state conditions of photosynthesis in order to complete the cycle.

This net flow is accomplished by a net reaction of thiamine pyrophosphate-glycolaldehyde molecules with glyceroldehyde phosphate molecules to produce xylulose-5-phosphate (XIV) [Eq. (20)]. These are converted by the action of
ribulose-phosphate-xylose-phosphate isomerase (Croner, et al., 1955) to ribulose-5-phosphate (I) [Eqs. (20)-(21)].

\[
\text{(20)} \quad \text{IX} \quad \text{CH}_2\text{OH} \quad + \quad \text{CH}_2\text{OH} \quad \rightarrow \quad \text{CH}_2\text{OH} \quad + \quad \text{TPP} \quad \text{CH}
\]

\[
\text{(21)} \quad \text{XIV} \quad \text{CH}_2\text{OH} \quad + \quad \text{CH}_2\text{OH} \quad \rightarrow \quad \text{CH}_2\text{OH} \quad + \quad \text{H}_2\text{CO}_3
\]

The end result of Eqs. (12-21) is the conversion of five molecules of glyceraldehyde-3-phosphate to three molecules of ribulose-5-phosphate (see Fig. 4). Two of these molecules formed by Eqs. (20) and (21) are labeled in carbon atom position 3, while the third one, from Eqs. (18) and (19), is labeled in positions 1, 2, and 3. The resultant average labeling of ribulose phosphate is heavy in position 3 and lighter in positions 1 and 2. When the ribulose molecules, labeled after a few seconds' photosynthesis with $^{14}\text{CO}_2$, were degraded (Bassham, et al., 1954), this pattern of labeling was found (see Fig. 4). Thus, the mechanism of the conversion of five molecules of triose phosphate to three molecules of pentose phosphate was established.
C. Light-dark transient changes

Quite a different type of experiment was required to reveal the nature of the reaction which converts ribulose-5-phosphate to ribulose-1,5-diphosphate [Eq. (9)]. Calvin and Bassani devised a system which recirculated a stream of $^{14}CO_2$ through a suspension of photosynthesizing algae. The supply of $^{14}CO_2$ was such that it did not change appreciably during the course of the experiment (Calvin and Bassani, 1952). Small aliquot samples of the algae were taken and killed from time to time. Subsequent analysis by paper chromatography and radioautography showed that the $^{14}C$ content of intermediate compounds in the carbon reduction pathway no longer increased after about 5 minutes of photosynthesis. By this time, enough $^{14}C$ had passed through these intermediates on the way to end products to "saturate" each carbon atom position with the same degree of labeling (specific radioactivity) as the $^{14}CO_2$. Since both the $^{14}CO_2$ specific radioactivity (2) and the total radioactivity of an intermediate compound (R) could be experimentally determined, Calvin and Bassani could calculate the concentration of carbon in the compound as $C = R/2$.

As long as the algae photosynthesized under constant conditions, the concentration of intermediate compounds remained constant. Then the light was turned off and more samples were taken. Since certain steps in the path of carbon must require co-factors produced by light, one would expect such steps to be blocked by darkness. Calvin and Bassani found that the concentration of PGA rose quickly. This was expected, since light is needed to form the ATP and NADPH required for the reduction
of FBA to sugar phosphates. At the same time, the concentration of ribulose-1,5-diphosphate fell rapidly to zero, indicating that its formation requires ATP produced by light [Eq. (9)]. This reaction is mediated by the enzyme phosphoribulokinase (Grant, et al., 1956).

D. High-low CO₂ transient changes

These studies were continued by Berson and Calvin (1955), who left the light on but rapidly lowered the tension of CO₂ to 0.003%. Since FBA is a product of the carboxylation reaction, it was expected that its concentration would fall. This is exactly what happened. At the same time, the concentration of ribulose diphosphate rose rapidly and then fell. This behavior is to be expected if ribulose diphosphate is a substrate for the carboxylation reaction, since lowering the CO₂ pressure will stop the reaction which uses up RuBP. Thus, the carboxylation of ribulose diphosphate to give FBA as a first step in the carbon reduction pathway was discovered [Eq. (22)].

\[
\begin{align*}
\text{H}_2\text{COPO}_3^- & \rightarrow \\
\text{CO} & \rightarrow \\
\text{HCOH} + ^{14}\text{CO}_2 + \text{HCOH} & \rightarrow \rightarrow \\
\text{II} & \rightarrow \\
\text{H}_2\text{COPO}_3^- & \rightarrow \\
\text{CO}_2 & \rightarrow \\
\text{III} & \rightarrow \\
\end{align*}
\]

The carboxylation of ribulose diphosphate by cell-free extracts of Chlorella was demonstrated by Quayle, et al. (1954), who named the enzyme carboxydismutase. The enzyme has been purified from spinach leaves (Weissbach, et al., 1956; Rayenon, et al., 1957). It was
demonstrated that the purified enzyme catalyzes the addition of water and CO₂ to ribulose diphosphate, with an intramolecular oxidation-reduction reaction, or dismutation, leading to the formation of two molecules of PGA. In the carboxylation part of the reaction the carbon atom of CO₂ becomes bonded to the number 2 carbon atom of ribulose-diphosphate.

IV. The photosynthetic carbon reduction cycle

A. The complete cycle

The complete photosynthetic carbon reduction cycle is shown in detail in Fig. 4. A somewhat idealized distribution of label found experimentally
following, a short period of photosynthesis with $^{14}CO_2$ is indicated by the
actinum.

D ... details of the distribution of label, not previously dis-
cussed, should be mentioned. The concentration of dihydroxyacetone phos-
phate is considerably greater than that of glyceraldehyde phosphate. After
very short periods of photosynthesis with $^{14}CO_2$, the dihydroxyacetone
phosphate has a smaller fraction of $^{14}C/^{12}C$ than the glyceraldehyde from
which it is made. Thus, the dihydroxyacetone phosphate has a lower specific
activity in its terminal carbon atom than does phosphoglyceraldehyde. When
these two dissimilarly labeled triose phosphates condense the result is a
hexose phosphate labeled more heavily in the number 4 carbon atom than in
the number 3 carbon atom position. This distribution was observed experi-
mentally by Kandler and gibb (1956).

Labeling of sugars is also affected by the fact that the transketolase-
1 reaction [(1) and (19)] are highly reversible. A glyceraldehyde
thiamine pyrophosphate molecule has about as good a probability of
reacting with the aldose sugar phosphate from which it has just split
as it has of reacting with a different aldose sugar phosphate. There is
a common pool of glyceraldehyde thiamine pyrophosphate which interacts
directly with fructose-6-phosphate, sedoheptulose-7-phosphate, and
xylulose-5-phosphate. This permits a feedback of label from the number
1 and 2 carbon atoms of the pentose phosphates to the number 1 and 2 carbon
atoms of hexose phosphate. Under certain physiological conditions this
feedback is sufficiently great to result in greater labeling of carbon
atoms 1 and 2 of hexose than the corresponding carbon atoms 5 and 6.
This effect was also noted by Kandler and gibb (1956).
3. Stoichiometry of the cycle

Under conditions of steady photosynthesis, the concentrations of the intermediates of the carbon reduction cycle remain constant. Suppose that n molecules of CO₂ enter the cycle by the carboxylation reaction. Then n atoms of carbon incorporated into organic compounds are taken from the cycle by secondary reactions. These reactions utilize cycle intermediates as a starting point for the synthesis of various end products.

For example, the hexose phosphates may be converted to sucrose, oligosaccharides, and polysaccharides such as starch and cellulose. Another example is the conversion of 3-phosphoglyceric acid to phosphoglyceraldehyde acid and pyruvic acid and thence to alanine, an amino acid.

Consider one complete revolution of the carbon cycle, shown in Fig. 4. Such reaction occurs at least once. Three molecules of ribulose diphosphate (15 carbon atoms) react with 3 molecules of carbon dioxide, giving 6 molecules of P₃H or 18 carbon atoms in all. Of the 18 carbon atoms, 15 are required to regenerate the 3 molecules of ribulose phosphate while 3 are used in the formation of various end products. Besides 3 molecules of carbon dioxide, a complete cycle uses 9 molecules of ATP and 6 molecules of NADPH₂ (2 electrons per molecule).

These requirements are for the cycle as written in Fig. 4. It has been suggested that in vivo, the carboxylation of ribulose-1,5-diphosphate might be a reductive carboxylation (Wilson and Calvin, 1955). Broken isolated chloroplasts and cell free systems perform only the non-reductive carboxylation of ribulose diphosphate. It is a hypothesis, at present unproved, that in vivo enzyme systems capable of using electrons more
directly from the light reactions could catalyze reductive carboxylation [Eq. (23)]. Such a system might be disrupted when the chloroplasts are removed from the cells. In a cycle with a reductive carboxylation, the electron requirements might be different. For each complete cycle (3 molecules of CO₂ taken up) 3 of the ATP molecules would not be needed if 3 of the NADPH₂ molecules could be replaced by molecules of reduced ferredoxin. The total requirement would then be 6 ATP, 3 NADPH₂, and 6 reduced ferredoxin molecules, per 3 CO₂ molecules taken up.

C. Quantitative importance of cycle

NADP and the sugar phosphates clearly account for most of the ¹⁴C found in individual compounds following a few seconds of photosynthesis with ¹⁴CO₂. Nonetheless, one might ask whether or not other important pathways of CO₂ reduction not involving these compounds have been overlooked. For example, there might be a pathway from CO₂ to sucrose which does not include the intermediate compounds of the carbon reduction cycle. If so, this path would have to include substances which are so small in concentration as not to be seen, or which are so unstable as not to be isolated by the methods of paper chromatography.

These possibilities were tested by Russhen and Kirk (1960), who refined the steady-state photosynthesis studies to permit direct comparison of the externally measured rates of ¹⁴C and ¹²CO₂ uptake with the rates of appearance of ¹⁴C in individual compounds. They demonstrated that in Chlorella the rate of labelling of sucrose (the most rapidly labeled carbohydrate) is only a few percent of the total rate of ¹⁴C uptake. In fact, its rate of labelling is no greater than that of som
other secondary products such as alanine. The sucrose labeling rate is insignificant during the first few seconds. These experimental results rule out the possibility of significant sucrose formation via a sequence of unknown intermediate compounds, all occurring at very small concentrations.

It was also found that labeling of RIA and the sugar phosphates accounts for at least 70% of the externally measured $^{14}C$ uptake between 10 and 40 seconds after the introduction of $^{14}CO_2$. The pool size of unstable intermediates preceding these stable compounds was not more than the equivalent of 5 seconds of photosynthesis. It is likely that even this small pool is nothing more than intracellular $CO_2$ and enzyme-bound $CO_2$. (An ability of the carboxylation enzyme to bind significant amounts of $CO_2$ has been reported by Aoyanagiou and Calvin, 1963.) It is clear that even if pools of unstable intermediate compounds do exist, they must be far too small to be involved in an unknown path to carbohydrates. This conclusion follows from the fact that such carbohydrates would become labeled much more rapidly than the experiments show if they were formed from $^{14}C$ via compounds of such small pool sizes.

3. Probable energetic efficiency in the cycle

The 3 moles of NADH and the 3 moles of ATP required for the reduction of 1 mole of $CO_2$ to carbohydrate represent an expenditure of stored chemical energy which can be calculated from the energy required to make these cofactors [Eqs. (24) and (25)].

\[ (24) \quad \text{NADP} + H_2C \rightarrow \text{NADPH}_2 + 1/2 \text{CO}_2 \Delta P' = +52.6 \text{ kcal} \]
(25) $\text{ADP}^{-3} + \text{HPO}_4^{2-} + H^+ = \text{ATP}^{-4} + H_2O \quad \Delta F^\circ = +11 \text{ kcal}$

The symbol $\Delta F^\circ$ indicates that the physiological $\Delta F^\circ = +6.8 \text{ kcal}$ has been corrected for an assumed inorganic phosphate concentration of $10^{-3} \text{ M}$.

This correction is given by $\Delta F^\circ = -RT \ln (10^{-3}) = +4.2 \text{ kcal}$, where $R$ is the gas constant and $T$ the absolute temperature (see Chapter 16).

$\Delta F^\circ = \Delta F^\circ + \Delta F^\circ = +6.8 + 4.2 = +11.0 \text{ kcal}$. The total stored chemical energy expended in reducing $\text{CO}_2$ to carbohydrate is thus $2 \times 52.6 + 3 \times 11$, or $140.2 \text{ kcal}$. The total energy stored by $\text{H}_2 \text{O}$ (1a) was $112 \text{ kcal}$. The efficiency of the cycle may thus be calculated as $112/140.2 = 81\%$

If the hypothetical reductive carboxylation [24, (23)] actually occurs and uses reduced ferredoxin as a reducing agent, the cycle efficiency may also be calculated. The redox potential for ferredoxin was $E^\circ = -0.430 \text{ v}$. From this the $\Delta F^\circ$ for $\text{H}_2 \text{O}$ (26) may be calculated according to $\Delta F^\circ = nF E^\circ$ (Chapter 16).

(26) $\text{H}_2 \text{O} + 2 \text{Na}^+ \rightarrow 2\text{Na}^{+2} + 1/2 \text{O}_2 + 2\text{H}^+$ \quad $\Delta F^\circ = -2 \times 23.07 \times (-0.43) = +57.5 \text{ kcal}$

The requirement for the reduction of 1 mole of $\text{CO}_2$ in this case would be 2 moles of reduced ferredoxin, 1 mole of $\text{NADPH}_2$, and 2 moles of $\text{ATP}$.

The chemical energy consumed would be $57.5 + 52.6 + 22 = 133.1 \text{ kcal}$.

The energy efficiency would be $84\%$. In either case, it is clear that the carbon reduction cycle of photosynthesis operates with a remarkably high energy efficiency, considering the many individual steps involved.

V. Secondary carbon reduction pathways

A. Carboxylic acids

From the earliest studies of photosynthesis with $^{14}\text{CO}_2$ by Calvin and Benson (1948, 1949), it was clear that many substances besides PGA.
and sugar phosphates were quickly labeled with $^{14}$C. Among the more important early products were carboxylic acids, such as malic acid, succinic acid, and glycolic acid. Also labeled at very short times were certain amino acids, such as alanine, aspartic acid, serine, and glycine (Stepka, et al., 1948). The rate of labeling of such compounds during the first few seconds of photosynthesis with $^{14}$CO$_2$ is often greater than the labeling rate of carbohydrates such as sucrose. Such findings suggest that these non-carbohydrate secondary compounds are formed directly from intermediates of the carbon reduction cycle rather than from unphosphorylated carbohydrates.

By analogy with known respiratory reactions, malic acid might be formed by reductive carboxylation of phosphoenolpyruvic acid (PEPA) (see Fig. 4) according to Eq. (27).

$$\begin{align*}
\text{PEPA} + ^{14}\text{CO}_2 + \text{NADPH}_2 & \rightarrow \text{CH}_3 + \text{HOCH}_2 + \text{HOPO}_3\text{H}^+ + \text{NADP} \\
\text{PEPA} & \rightarrow \text{CH}_3 + \text{HOCH}_2 + \text{HOPO}_3\text{H}^+ + \text{NADP}\end{align*}$$

During photosynthesis with $^{14}$CO$_2$, carboxylation of PEPA immediately labels the 3-carboxyl group of malic acid. Since the carboxyl group of PEPA, like that of PCA, is very quickly labeled, the C$_1$-C$_3$ carboxylation also results in malic acid with the α-carboxyl group labeled.

Further reversible reactions of the tricarboxylic acid cycle (see Chapter 10) convert labeled malic acid to labeled fumaric and succinic acids.
The rapid labeling of these dicarboxylic acids in plants photosynthesizing with $^{14}$CO$_2$ at first suggested that these compounds might be involved in the basic carbon reduction cycle (Calvin and Benson, 1949). However, experiments in which malonate was used to inhibit the formation of these labeled acids in photosynthesis in Chlorella showed that these compounds were not involved in the basic carbon cycle (Bassham, et al., 1953). Despite suppression of malic acid formation by the inhibitor, the cycle continued to operate at normal rates.

Citric acid is rapidly labeled by plants photosynthesizing in the presence of $^{14}$CO$_2$. Presumably it is formed by the condensation of acetyl coenzyme A with oxaloacetic acid, as in the tricarboxylic acid cycle (Chapter 10). The oxaloacetic acid might be formed by the direct carboxylation of PEP via the carbon reduction cycle [Eq. (28)].

\[
\begin{align*}
\text{CO}_2 + \text{COPO}_3\text{H}^- & \rightarrow \text{CH}_2 + \text{HOPO}_3\text{H}^- + \text{H}^+ \\
\text{CH}_2 + \text{PEP} & \rightarrow \text{CO}_3
\end{align*}
\]

The possible source of acetyl coenzyme A in photosynthesis is discussed in the next section.

One of the most interesting, widely studied, and incompletely understood aspects of photosynthetic carbon reduction is the formation of glycolic acid. The formation of glycolic acid during photosynthesis is favored by low CO$_2$ pressures (0.1%) slightly greater than those to which plants are exposed under natural environments (Fritschard, et al., 1961). High concentrations of O$_2$ also increase glycolic acid formation (Bassham and Kirk, 1962). It appears that glycolic acid is formed from carbon...
stones 1 and 2 of the sugar phosphates of the carbon reduction cycle. It may be formed by oxidation of glyceraldehyde-phosphate-thiamine pyrophosphate (Calvin and Benson, 1962) (see Fig. 4).

The role of glycolic acid formation is in glycine and serine synthesis. Tullert has suggested (1953) that glycolic acid diffuses from the chloroplast to be oxidized to glyoxylic acid outside the chloroplast. The glyoxylic acid then reacts with other chloroplast where it would be reduced again by NADPH₂. In this way electrons from the light reaction could be transported to other parts of the cell where they could be employed in reductive reactions.

B. Fatty acids and lipids

If a rapidly growing photosynthetic cell such as an algae cell is exposed to ¹⁸CO₂ for 1 to 2 minutes and then killed, as much as 30% of the radioactive compounds formed behave as lipid-like substances when partitioned between aqueous and organic solvents. A considerable portion of the chloroplast structure consists of lipid materials, and rapid lipid synthesis is required for chloroplast growth and division.

The starting point for synthesis of fatty acids and other lipid substances such as carotenoids and terpenes is acetyl coenzyme A. Very little labeled acetic acid can be isolated from the photosynthesizing cell. It is presumed that the concentration of acetyl coenzyme A is very small and that the small pool turns over very rapidly.

Two paths have been suggested for the conversion of photosynthetic carbon reduction cycle intermediates to acetyl coenzyme A. One such path would be the hydrolysis of PEP to pyruvic acid. The pyruvic acid would then be oxidized by a pyruvic acid oxidase in the presence of
lipoic acid and coenzyme A, giving ultimately reduced lipoic acid, carbon dioxide, and acetyl coenzyme A (see Chapter 10). This sequence of reactions is of great importance in respiration. It seems less likely to occur in the chloroplast during photosynthesis because of the essentially "reducing" atmosphere of the chloroplast. The cofactors which carry electrons are being continually reduced by the process of photosynthesis, in contrast to respiratory systems in which they are transferring electrons to oxygen and thereby are being oxidized.

Another pathway to acetyl coenzyme A from the carbon cycle would be via a phosphorolysis splitting of the thiamine-pyrophosphate glyceraldehyde compound (IX) formed by the transketolase reaction. Such a split [Eq. (29)] could be mediated by an enzyme similar in part to phosphoketolase (Heath, et al., 1958).

\[
\begin{align*}
\text{CH}_2\text{OH} & \xrightarrow{\text{ATP}} \text{CH}_2\text{OH} + \text{HOPO}_3^2\text{H}\text{I} \rightarrow \text{CH}_3 \text{C(OH)}_2 + \\text{HPO}_3\text{H}^+ + \text{H}_2\text{O} \\
(29) & \quad \text{CH}_2\text{OH} + \text{HOPO}_3\text{H}^+ + \text{H}_2\text{O} 
\end{align*}
\]

The resulting acetyl phosphate could undergo a transacylation reaction with coenzyme A to give inorganic phosphate and acetyl coenzyme A. In the absence of definite biochemical evidence for either of these pathways in chloroplasts, the phosphoketolase reaction seems more probable and it does not involve an oxidative step.

Once acetyl coenzyme A has been formed, subsequent reactions leading to fatty acid synthesis are probably similar to those which occur in other biosynthetic systems. In the chloroplast these reactions may be photosynthetic reactions in that they employ ATP and reduced pyridine nucleotide cofactors formed by the photochemical reactions of photosynthesis.
The fatty acids thus photosynthesized are then esterified with glycerol or glycerol phosphate formed directly from ribose phosphate of the carbon reduction cycle [Ref. (30)]. Calvin et al. (1958) used the following reactions:

\[
\begin{align*}
\text{H}_2\text{CO}_3 & \quad + \text{NADPH}_2 \quad \rightarrow \quad \text{H}_2\text{CO}_2 \quad + \quad \text{NADP} \\
\text{H}_2\text{CO}_3 & \quad + \text{ADP} \quad \rightarrow \quad \text{H}_2\text{CO}_2 \quad + \quad \text{ATP}
\end{align*}
\]

1958) may be formed by reactions of these compounds with UDPG-sugar (Newall and Hall, 1964). UDPG-sugar is formed photodynamically in the chloroplast by the process of photosynthesis with UDPG-sugar epimerase.

C. Carbohydrates

Richman (1953) reported that sucrose is photosynthesized from fructose-6-phosphate of the carbon reduction cycle. One molecule of fructose-6-phosphate is converted to glucose-6-phosphate, which reacts with uridine triphosphate (UTP) to form uridine diphosphoglucose (UDPG). UDPG is always labeled with \(^{14}C\) during short periods of photosynthesis with \(^{14}CO_2\). The UTP was presumed to be formed from uridine diphosphate and photosynthetically produced ATP. UDPG then reacts with fructose-6-phosphate to produce sucrose phosphate and eventually sucrose. More recently, ADPG has been identified as an early labeled product of photosynthesis (Kandler, 1953). Both UDPG and ADPG are considered to be involved in the synthesis of other oligosaccharides and polysaccharides. As mentioned earlier, the synthesis of sucrose can account for nearly all of the uptake of carbon dioxide during photosynthesis in the mature leaves of certain green plants. On the other hand, a rapidly growing and dividing unicellular alga, such as Chlorella pyrenoidosa, may utilize 5% or less of the photosynthetic \(^{14}CO_2\) uptake for the synthesis of sucrose.

D. Amino acids and proteins

The earliest separations of the products of photosynthesis of \(^{14}CO_2\) by two-dimensional paper chromatography revealed certain amino acids
to be rapidly labeled products of $^{14}$CO$_2$ reduction (Stephan, et al., 1963).

Most important among these are alanine, aspartic acid, serine, glutamic acid, and glycine. Using quantitative steady-state tracer studies, Smith, et al. (1961) were able to show that Chlorella pyrenoidosa incorporate as much as 30% of the $^{14}$CO$_2$ taken up photosynthetically directly into these amino acids. The rate of incorporation of $^{14}$CO$_2$ into alanine by Chlorella pyrenoidosa may exceed the rate of labeling of sucrose.

A study of the kinetics of the labeling of alanine show that its rate of labeling reaches a maximum as soon as the intermediates of the carbon reduction cycle are "saturated" with $^{14}$C. Since no secondary products of carbon photosynthesis such as sucrose are approaching saturation at this time (3-5 min), it appears that alanine is formed directly from intermediates of the cycle. This theory gains further support from the parallel behavior in the labeling of alanine and of PEP in many experiments. Conditions which result in the increase or decrease in the level of PEP invariably result in a corresponding trend in the rate of labeling of alanine. Presumably, alanine is formed from PEP by the transamination of pyruvic acid derived from phosphoenolpyruvic acid which in turn is derived from PEP [Eq. (31)].

\[
\begin{align*}
\text{HCO}_2 & \quad \text{glutamic acid} \\
\text{CO}_2 & \quad \text{HCO}_2 + \text{a-ketoglutaric acid}
\end{align*}
\]

(31) PEA → PEP

The photosynthetic formation of glycine appears to depend upon the prior formation of glycolic acid. Presumably, glycine acid is oxidized to glyoxylate acid, which is then transaminated to give glycine [Eq. (32)].
Serine formation appears to occur via the alternate pathways in
photosynthesis. One of them is indicated by a parallel behavior in
serine labeling, and PCA level. Serine may be formed from hydroxy-
glycine acid derived from the 3-PCA of the cycle [Eq. (33)].

\[
\begin{align*}
\text{H}_2\text{CO}_3 & \quad \mid \text{[O]} \quad \mid \text{glutamic acid} \\
\mid \text{CO}_2 & \quad \mid \text{CO}_2
\end{align*}
\]

\[
\text{H}_2\text{CO}_3 + \text{CO}_2 \rightarrow \text{H}_2\text{O} + \text{CO}_2
\]

This pathway may be more important at relatively high levels of CO₂
(1-2%). A second pathway to serine begins with glycine, and is probably
more important at low CO₂ pressure while favor the formation of
glycolic acid. In this pathway glycine is hydroxymethylated by hydroxy-
methyltetrahydrofolic acid (THFA-Ch₃OH) [Eq. (34)].

\[
\begin{align*}
\text{H}_2\text{CO}_3 & \quad \mid \text{THFA-Ch₃OH} \\
\mid \text{CO}_2 & \quad \mid \text{CO}_2
\end{align*}
\]

\[
\text{H}_2\text{CO}_3 + \text{THFA-Ch₃OH} \rightarrow \text{THFA} + \text{CO}_2
\]

Aspartic acid is formed by the transamination of oxaloacetic acid.
The formation of this dicarboxylic acid by carboxylation of PPGA was
discussed above.

Glutamic acid is of primary importance in photosynthesis of labeled
amino acids. It is probably formed as in non-photosynthetic tissues by
reductive elimination of a ketoglutaric acid [Eq. (35)].
In the chloroplast the reductive assimilation utilizes electrons formed by the light reactions of photosynthesis. The a-ketoglutaric acid is formed from citric and oxaloacetic acids via the Krebs cycle (Chapter 10). The reservoir sizes of these acids along the photosynthetic pathway to glutamic acid must be rather small, and the rate of labeling of these compounds towards less than that of glutamic acid. This led to the suggestion (Smith, et al., 1961) that glutamic acid may arise photosynthetically by a different pathway. However, Miller (1964) has used fluoroacetic acid to inhibit the conversion of citric acid to oxaloacetic acid. When Chlorella, photosynthesizing in the presence of $^{14}CO_2$ were so inhibited, glutamic acid labeling steps and/or labeling of citric and fluoroacetic acids become as great as that of glutamic acid in the uninhibited algae. Apparently there is a small but very rapidly turning over pool of citric acid in the chloroplast. This pool must be quickly saturated with $^{14}C$ during photosynthesis with $^{14}CO_2$. Thus, $^{14}C$ is passed through this pool and on to glutamic acid.

V. Areas of future discovery

In the past quarter century we have learned much about the mechanism of carbon reduction during photosynthesis. Nevertheless, it is evident
from the foregoing discussions that many unanswered questions remain.
Many details of the reactions leading from the carbon reduction cycle
to various secondary biosynthetic pathways are still unclear. The
mechanisms by which acetyl CoA and glycolic acid are formed from the
carbon reduction cycle are but two of the important problems requiring
further investigation.

As for the carbon reduction cycle, probably the most important
unanswered questions have to do with the mechanism of the carboxylation
reaction. Although all of the biochemical evidence from isolated enzyme
systems suggests that the carboxylation reaction is a non-reductive
carboxylation of ribulose diphosphate leading to the formation of two
molecules of ribulose, kinetic evidence with whole cells indicates the possi-
bility of a reductive carboxylation leading to the formation of one
molecule of ribulose and one molecule of triose phosphate. If this reductive
carboxylation does occur, it may be that electrons are somehow conveyed
(Sassham, 1964),
directly from the light reaction to the carbon reduction cycle. If there
is such a difference between the in vivo system and the in vitro system
this difference may reside in some intricate structural arrangement in
the living cell which is easily disrupted.

The finding of multifunctional enzyme systems for biosynthetic
pathways, such as fatty acid synthesis, suggests that such organized
systems may have importance elsewhere. Whether they exist in photo-
synthesis and how they operate if they do exist is a very important
question for the future. The answer will come from a combination of
tracer studies, investigation of the enzymes isolated by a variety of
sophisticated techniques, and the gathering of better and more detailed knowledge of the structure of the chloroplast through the application of electron microscopy and various techniques of chemical and physical analysis.
LITERATURE CITED

Beul's and Laidlaw


Original Research Articles:


J. Biol. Chem. 185, 761.


Benson, A. A., Raschke, J. A., Calvin, M., Goodwin, T. C., Haas, V. A.,

Benson, A. A., Raschke, J. A., Calvin, M., Hall, A. C., Hirsch, R. S.,
196, 703.

J. Am. Chem. Soc. 80, 4730.


Buchanan, J. G., Raschke, J. A., Benson, A. A., Bradley, D. P., Calvin, M.,
Daus, E. L., Goodman, H., Mayor, P. H., Lynch, V. H., Norris, L. T.,
and Wilson, A. T. (1952). In "Photosynthetic Pigments" (William D.
Hopkins Press, Baltimore, Md.


Calvin, M., Raschke, J. A., Benson, A. A., Lynch, V. H., Ouillet, G.,
10, V, p. 234.


*J. Biol. Chem.* 231, 1009.


21, 342.


14, 503.


J. Am. Chem. Soc. 76, 3610.

75, 1010.


62, 3443.

Chem. Soc. 63, 877.

Acta 48, 299.


Biochem. Biophys. 52, 535.


and 


Interscience Publishers, New York, N. Y.


Fig. 1. Radioautogram of products of 60 seconds' photosynthesis with $^{14}$CO$_2$.
Radioautograph of two-dimensional paper chromatogram of products formed by Chlorella pyrenoidosa during 60 seconds of photosynthesis with $^{14}$CO$_2$.
Abbreviations: $P_i$, PO$_3$H$_2$; UDPG, uridine diphosphoglucone; PGA, 3-phosphoglyceric acid; PEPA, phosphoenolpyruvic acid.
Sugar diphosphate, includes ribulose-1,5-diphosphate, sedoheptulose-1,7-diphosphate, and fructose-1,6-diphosphate.

Fig. 2. Radioautograph of two-dimensional paper chromatogram of products formed by Chlorella pyrenoidosa during 7 seconds of photosynthesis with $^{14}$CO$_2$.
Abbreviations: Same as for Fig. 1.

Fig. 3. Radioautograph of two-dimensional paper chromatogram of products formed by Chlorella pyrenoidosa during 2 seconds of photosynthesis with $^{14}$CO$_2$.
Abbreviations: Same as for Fig. 1.

Fig. 4. The carbon reduction cycle of photosynthesis. Solid arrows indicate reactions of the carbon reduction cycle as formulated by Calvin and co-workers. Dashed line represents hypothetical reductive carboxylation reaction discussed in text. Open arrows indicate start of some of the biosynthetic paths leading from intermediate compounds of the cycle. Asterisks indicate approximate relative degree of labeling after a few seconds of photosynthesis. They reflect the results of degradation studies by various workers as discussed in the text.
Fig. 4 (Continued)

Abbreviations: P_, PO_3H_2; RuDP, ribulose-1,5-diphosphate; FDP, fructose-1,6-diphosphate; SDP, sedoheptulose-1,7-diphosphate; TPP, thiamine pyrophosphate; G6P, glucose-6-phosphate; PEPA, phosphoenolpyruvic acid.

Numbers indicate equations as listed in text; Roman numerals are the same as used in text to identify structural formulae.