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## PHOTOSYNTHESIS

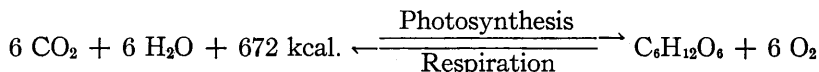
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During the activities of growth, maintenance, and repair, the living organism converts food materials into protoplasmic and structural constituents, or into energy. This assimilatory process applies to plants and animals alike, and it is accompanied by the process of respiration, that is, atmospheric oxygen is taken into the cells, the various organic substances are partially or completely oxidized, and carbon dioxide is produced and eliminated.

The green plant is capable of performing the reduction of carbon dioxide (the most depreciated form of carbon in nature) under liberation of free oxygen, and it can do so only in light. The carbon, which became available in the reaction, is used to synthesize the numerous constituents of the plant and the process is called "assimilation of carbon" or "photosynthesis." The reduction of carbon dioxide, as such, is not exclusive with green plants; besides normal photosynthesis, one has observed that certain purple bacteria and unicellular algae can reduce carbon dioxide in light with the simultaneous consumption of inorganic or organic hydrogen donors (Gaffron, 1933, 1944; Van Niel, 1931). In the dark, the reduction of carbon dioxide is often accompanying enzymatic processes, such as the metabolic reactions in animal tissues, or bacterial fermentation by methane and by propionic acid bacteria, oxidations in plants and unicellular algae, and bacterial oxidations, as in the sulfur bacteria (Krebs, 1943; Werkman and Wood, 1942). The three different types of biological reduction of carbon dioxide may be demonstrated with unicellular algae of the family *Scenedesmus*. Gaffron (1944) was able to have these organisms perform at will the chemo-reduction of carbon dioxide in the dark by burning hydrogen with oxygen to water—a photoreduction in which 2 molecules of hydrogen and one molecule of carbon dioxide disappear—and the normal photosynthesis, in which, for each reduced molecule of carbon dioxide, the equivalent amount of oxygen is liberated.

For the purpose of further discussion here, photosynthesis by green plants will be considered mainly. In light it is superimposed upon the respiration process; the two processes may be represented schematically as follows, when a hexose sugar is used for the purpose of illustration:



Photosynthesis is, of course, not limited to carbohydrate production. Its several steps involve enzymatic reactions; and one may define it as an enzymatically controlled oxidation-reduction, brought about by radiations from the visible region of the solar spectrum, and causing the reduction of carbon dioxide under simultaneous oxidation of water, or of another hydrogen donor.

#### THE ASSIMILATORY SYSTEM

The green parts of plants differ widely in structure throughout the plant kingdom. In most plants the cells contain the green pigments in the chloroplasts,

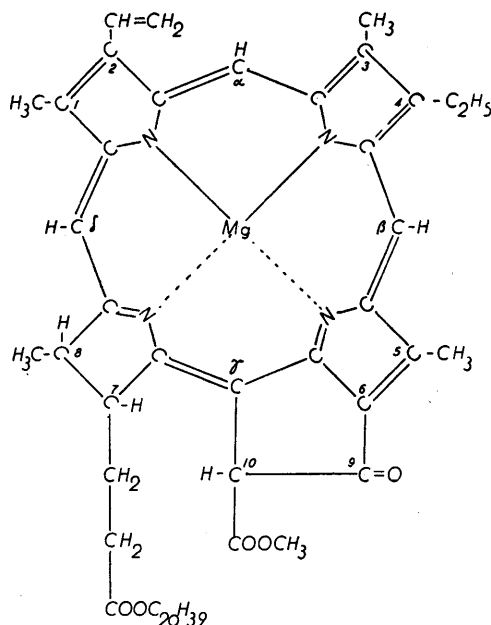


FIGURE 1. Chlorophyll *a*.

which are usually spherical or double convex protein-containing bodies. The cells of most plants contain a large number of chloroplasts, but in some of the algae only one single large chloroplast is found in each cell; in others the pigments are not localized, and are fairly evenly distributed throughout the cells.

The coloring matter in the chloroplasts of green plants is a mixture of pigments. By suitable extraction and separation methods one obtains usually two green pigments (chlorophyll *a* and chlorophyll *b*), a number of yellow and orange substances belonging to the carotinoids, and from some plants red and blue pigments, phycoerythrin and phycocyanin, respectively. In certain bacteria a special bacteriochlorophyll has been found (Fischer and Hasenkamp, 1935). All living cells also contain specific pigments, the cytochromes, which are important in biological oxidations.

The individual pigments are chemically identical, irrespective of the plant from which they were isolated, but their relative amounts vary greatly. In many higher plants, the ratio of the blue-green chlorophyll *a* to the yellow-green chlorophyll *b* is about 3 : 1. In some plants the ratio is greatly different and in certain green algae, as well as in the classes *Rhodophyceae*, *Phaeophyceae*, and *Cyanophyceae*, and in the family *Diatoms*, only chlorophyll *a* is found, but no chlorophyll *b* (Seibold, Egle, and Hülbrich, 1941). In the groups of purple and brown bacteria several derivatives of chlorophyll occur (Van Niel and Arnold, 1938). The green pigments (chlorophyll *a* and *b*) are the most important ones for the process of photosynthesis. They are complicated pyrrole pigments, and contain magnesium in complex linkage in the molecule. Figures 1 and 2 indicate the present

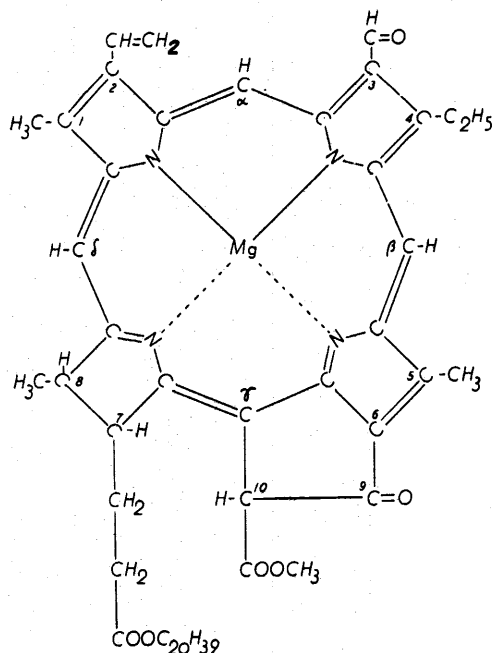


FIGURE 2. Chlorophyll *b*.

concept of the chemical structure of these two pigments, and the numbering system according to Hans Fischer. The two chlorophylls differ only with respect to the substituent in position 3 of the molecule; in chlorophyll *a*, a methyl group

( $\text{CH}_3$ ) is found, in chlorophyll *b*, the aldehyde group  $\left( \begin{array}{c} \text{H} \\ \diagup \text{C} \\ \diagdown \text{O} \end{array} \right)$ . This differ-

ence has caused much speculation, whether the two pigments could be partners in an oxidation-reduction system, but the absence of chlorophyll *b* in so many plants, and the complete lack of direct experimental evidence have eliminated this view. The structure of bacteriochlorophyll is closely related to that of the other two green pigments, as figure 3 indicates. The formulae of these pigments have not been confirmed by synthesis as yet, however their chemical properties are treated in detail in H. Fischer's book on pyrrole chemistry (Fischer and Orth, 1943). A review on chlorophyll may be found in Medical Physics (Rothmund,

1944); its technical production and uses are the subject of a recent summary (Rothemund, 1949).

#### MEASUREMENT OF PHOTOSYNTHESIS

A great number of methods for the determination of the yield, or of the rate of photosynthesis under natural and controlled conditions have been devised. They may be classified according to the kind of determination performed: (a) absorption of carbon dioxide, (b) evolution of oxygen, (c) production of carbohydrate, (d) determination of the fluorescence intensity of the pigments, and (e) use of stable (e.g.,  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{18}\text{O}$ ) or of radioactive isotopes (e.g.,  $^3\text{H}$ ,  $^{11}\text{C}$ ,  $^{14}\text{C}$ ). To all these methods one difficulty is common, namely, the influence of plant respiration on the result. For detailed descriptions of the different methods of

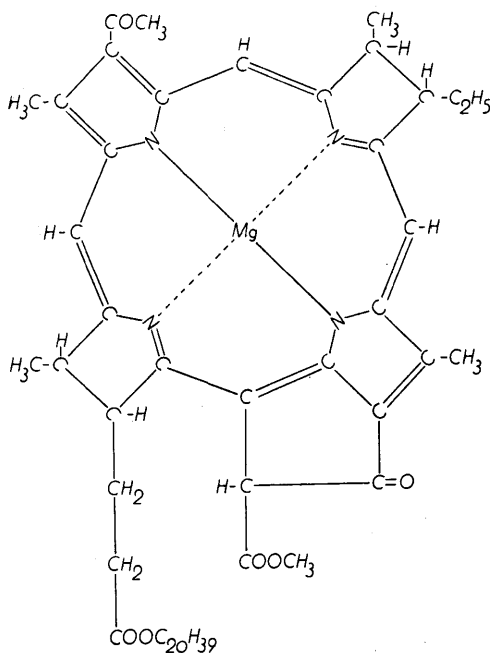


FIGURE 3. Bacteriochlorophyll a.

measurement and for their critical evaluation, the reader is referred to several monographs on photosynthesis (Spoehr, 1926; Rabinowitch, 1945, 1951; Franck and Loomis, 1949).

Some authors claim that the continuous analysis by mass spectrography of gases containing oxygen isotopes may be used to establish the effect of light on the respiratory rate of plants. No increase of respiration in light as compared with the respiration in the dark has been reported so far, but photoinhibition of respiration up to 100 percent seems to occur in certain organisms [Personal communication, reported by Gaffron and Fager (1951)]. It must be kept in mind, however, that all experiments with isotopes in biological systems may suffer from secondary reactions, namely, the exchange of isotopes, and the possible discrimination for or against one or more isotopes. Thus, it has been reported that in the measuring of photosynthesis in *Chlorella* and barley, at about 1,000 foot candles the ratio of utilization of isotopes was:

$$^{18}\text{C}/^{12}\text{C} = 0.96, \quad ^{14}\text{C}/^{12}\text{C} = 0.85, \quad \text{and} \quad ^{14}\text{C}/^{13}\text{C} = 0.89$$

(Gaffron and Fager, 1951), showing discrimination against the heavier isotope in each case. (The discrimination against  $^{14}\text{C}$  was considered especially noteworthy.)

In order to determine the rate of photosynthesis, reliable measurements of the total incident light and of the radiation actually transformed into chemical energy in the plant must be obtained. The total energy received by the tissue is not entirely utilized for photosynthesis, but also in such other processes as transpiration, transmission, reflection, thermal emission, and fluorescence. Experimental difficulties in measuring the share of such losses of radiation exactly are responsible for much contradiction in the literature concerning the utilization of energy in photosynthesis.

#### FACTORS INFLUENCING PHOTOSYNTHESIS

The photosynthetic apparatus of the green plant is influenced in its operation by a number of factors, which may be conveniently classified as internal and external. The proper interaction of these factors leads for each organism to an optimum value for photosynthesis; variations in the relative share of each factor in the process may cause photosynthesis to cease entirely, or, on the other hand, reach a maximum of performance. The important internal factors are:

- (1) anatomical structure of the tissue
- (2) chlorophyll content of the cells
- (3) enzyme system in the protoplasm and in the chloroplasts
- (4) influence of reaction products of photosynthesis and their accumulation on the progress of the reaction
- (5) freedom from pathological conditions.

External factors may be listed as follows:

- (1) concentration of carbon dioxide
- (2) concentration of oxygen
- (3) intensity and frequency of incident light
- (4) temperature
- (5) water supply
- (6) supply of nutrient salts and trace elements
- (7) substances not encountered normally by the plant (irrespirable gases, anaesthetics, toxic materials, etc.)
- (8) effect of hydrostatic and of osmotic pressure in aquatic plants
- (9) influence of mechanical injuries
- (10) electrical conditions (atmospheric or applied intentionally).

No detailed discussion of each of these factors can be given here, but a few observations should be mentioned. Experiments with cell triturates show that the photosynthetic apparatus operates even though the entire structure of the cell has been destroyed so that microscopic examination reveals absence of intact cells.

Variations in the rate of photosynthesis may also occur under physiological conditions without the activity of a limiting factor simply by processes like "resting," or by phenomena like "aging" of the plant.

Many investigators perform photosynthesis experiments on cells or parts of plants, which are working under much higher concentrations of carbon dioxide than that normally encountered by the plant under study. For low carbon dioxide concentrations, there is approximate proportionality between rate of photosynthesis and concentration. For higher concentrations, the observers disagree—some find indirect proportionality; others report direct proportionality up to a certain point, and they assume that from then on some other factor becomes limiting. Such a factor may be the temperature. It has been shown that the rate of photosynthesis under excess carbon dioxide is strongly influenced by temperature. Continuation of the experiment with very high concentrations causes a decrease of the photosynthetic rate, most likely due to the unphysiological conditions used.

The relationship between photosynthesis and light intensity depends greatly upon the plant under investigation (shade plants and sun plants differ in their response to changes in light intensities). In general, the behavior of plants to variations of light intensity is similar to that under variations of carbon dioxide concentration—proportionality up to a certain point, leveling off smoothly or sharply at the “saturation level,” where probably another factor becomes limiting, and finally a decrease. The latter is interpreted as either mechanical response of the plant to high light intensities (or carbon dioxide concentrations), or as a consequence of the poisoning of the photosynthetic system. Some plants do not form starch continuously when exposed to light, but either stop after a certain length of time or even redissolve the starch already formed. Ursprung (1917) called this phenomenon “solarization”; it is not accompanied by permanent injury of the plant’s photosynthetic abilities.

While respiration is believed to be, for most plants, independent of light intensity, if experiments are performed of sufficiently short duration, the rate of photosynthesis increases up to a certain point. There exists then a light intensity at which the net gas exchange becomes zero, when the two processes—respiration and photosynthesis—furnish and use, respectively, identical volumes of carbon dioxide or oxygen. This light intensity is called the “light compensation point.” For some unknown reason, there exists a great variation in the value of the compensation point for different species of plants. It is lower in shade plants than in sun plants, and it does not seem to be a function of the respiratory activity of the plant.

For intermittent irradiation and high light intensities, the rate of photosynthesis is larger than for continuous illumination, referring to equal total time elapsed during the light periods. Under low light intensities, intermittent illumination does not exhibit such an influence on photosynthesis (Warburg, 1919). After a period of darkness, the cell, again exposed to light, does not operate immediately at the same intensity level at which it had ended its work during the preceding period of illumination. This phenomenon is called “photochemical induction.” Usually light and dark periods follow each other in rapid succession. Different plants vary in their responses to the same frequency of light and dark changes. By suitable choice of plant material and frequency of light and dark periods, the influence of photochemical induction may be brought to a minimum. The fluorescence of the chlorophylls under intermittent light also rises to its full value only gradually after a dark period.

Various chemical substances are used in order to influence selectively certain defined steps in the photosynthetic process. By using the proper inhibitor for a particular reaction, or a process like respiration, or an enzyme system, other unaffected sequences in photosynthesis may be studied independently. Many inhibitors are very specific; e.g., narcotics and cyanide. Through skillful selection of substances and experimental conditions many of the important results in our present knowledge of photosynthesis were established. Since many conclusions drawn from biological material are holding only for one family, or one species, selected algae or bacteria, grown in pure culture, are used. These organisms often adapt themselves to variations in living conditions, and may sometimes even be “trained” to accept conditions which were formerly unphysiological for them.

#### PRODUCTS OF PHOTOSYNTHESIS

The outstanding gaseous product of photosynthesis is oxygen. The ratio:

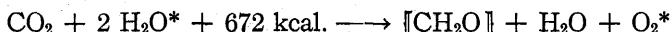
$$Q_P = \frac{\text{Moles O}_2 \text{ liberated}}{\text{Moles CO}_2 \text{ absorbed}}$$

is called the photosynthetic quotient, or assimilatory quotient. Its numerical

value is, with relatively few exceptions, unity or surprisingly close to it. The respiratory quotient is the inverse ratio of the above: or

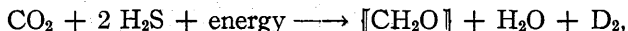
$$Q_R = \frac{CO_2}{O_2}$$

According to the schematic equation for photosynthesis, given on page 281, carbon dioxide is reduced, and a simple sugar is obtained under evolution of oxygen. For a long time it had been assumed that the reduction led to formaldehyde, which then condensed to the sugar. Polymerization of the latter causes the formation of starch in the leaf. Leaf starch was demonstrated as early as 1862 by J. Sachs. The formaldehyde hypothesis was suggested in 1870 by A. von Baeyer, who based it on a previous observation by A. Butlerow (1861). It was tested in experiments with isotopic carbon with the result that formaldehyde is now considered very unlikely as an intermediate in photosynthesis (Ruben, Hassid, and Kamen, 1939; Ruben, Kamen, and Hassid, 1940; Ruben, Kamen, and Perry, 1940). And the use of oxygen isotope  $^{18}O$  proved that all the oxygen evolved comes from water molecules, and not from the carbon dioxide (Ruben, Randall, Kamen, and Hyde, 1941; Dole and Jenks, 1944). Photosynthesis is actually making use of hydrogen from the water to reduce carbon dioxide, and the hydrogen is obtained by photolytic fission of the water molecule—it is not a hydration of carbon, as the term carbohydrate might indicate. The equation may be simplified by referring only to one molecule of carbon dioxide, and indicating the oxygen isotope by an asterisk, as follows:

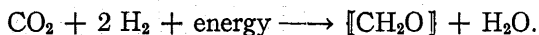


$[CH_2O]$  no longer represents a formaldehyde molecule but symbolizes here that simple structural unit, which through condensation and polymerization reactions, will ultimately build up the starch molecule in the plant.

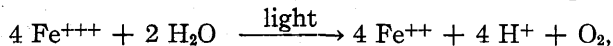
This view of the process is supported by photosynthesis experiments with purple bacteria (Van Niel, 1941; Franck and Gaffron, 1941), in which hydrogen sulfide furnishes the hydrogen for the reduction of carbon dioxide under elimination of sulfur, according to the equation:



and by experiments with specially conditioned algae, which are able to use gaseous hydrogen directly. In the latter case, no evolution of oxygen takes place, as shown in the scheme:



In the search for analogies *in vitro* for the formation of gaseous oxygen in light, with the help of chlorophyll or chlorophyllous materials, several working combinations have been reported. When water and carbon dioxide are exposed to light in the presence of chlorophyll, geraniol, glycerol, and methylene blue molecular oxygen and formaldehyde are formed; the production of percarbonic acid as reaction intermediate has been suggested (Baur, Gloor, and Künzler, 1938; Baur and Niggli, 1943). The so-called "Hill reaction" is a chlorophyll-sensitized reduction of ferric to ferrous iron under oxygen evolution according to the equation:



and can be brought about with chloroplast suspensions or leaf meal (Hill, 1937, 1939). Oxygen evolution also takes place by the action of light on quinone (Warburg and Lüttgens, 1944).

Starch, the other readily demonstrable product of photosynthesis, has been studied for a long time; but only during the past decade the fact was recognized that natural starch is a heterogeneous material. The two main components of ordinary starches are amylose, made up of long unbranched chains of glucose

residues, and amylopectin, consisting of highly branched short chains. (Schoch refers to the two components as fraction A, and fraction B, respectively.)

There is general agreement that starch formation in the plant is not part of the photosynthetic process proper, but rather the result of a secondary reaction. This view is supported by the fact that the leaves of many plants are starch-free, and that most plants are able to convert sugars into starch in the absence of light. The numerous biological transformations of starch are reviewed in a recent article (Peat, 1951).

The fixation of atmospheric nitrogen by plants is performed non-photosynthetically; this holds also for amino acids, fatty acids, and most other organic constituents of the plant.

#### MECHANISM OF PHOTOSYNTHESIS

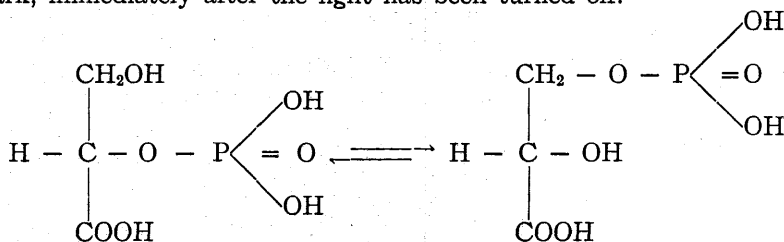
During the past two decades the method of chromatographic adsorption analysis, originally suggested by M. Tswett in 1906, has made great progress in technique. In form of paper chromatography it has, in recent years, become very useful in the analysis of complicated mixtures of organic substances, such as the ones found in extracts of cells after short periods of illumination. Numerous workers are using this technique in the hope of obtaining compounds which will help to elucidate the mechanism of photosynthesis; the results thus far have given a considerable amount of insight derived from the chemical structure of the substances isolated.

Ever since Liebig's time photosynthesis has been considered a process in which at least one photochemical reaction is coupled with one or more chemical reactions. The chemical steps precede or follow the photochemical reaction, and are also often called "dark reactions," "thermal reactions," or "Blackman reactions" (Blackman, 1905). They are entirely independent of irradiation, and their duration is obviously an important limiting factor for the photochemical part of photosynthesis. For the green alga *Chlorella pyrenoidosa*, Emerson and Arnold (1932) found the time required for the chemical step to be 0.02 sec. at 25° C, when light flashes of 10<sup>-5</sup> sec. duration were alternating with the dark periods. Some investigators stress the necessity of studying photosynthesis in the plant's natural environment, while others favor experiments under controlled artificial conditions, which are often very different from those the plants are accustomed to. Such "unphysiological" conditions are, for instance: the change of hydrogen ion concentration in experiments with algae or aquatic plants; the use of carbon dioxide concentrations far exceeding the 0.03 percent of this gas in the atmosphere; the substitution of steady illumination by flashing light; pre-illumination of the cells before measurements of photosynthesis; or the increase of the intensity of applied radiation to very high values. Under physiological conditions, the measurements must be extended often over long periods of time in order to be significant in comparison with apparatus "errors"; but then measurements may become faulty and useless due to fluctuating reaction rates, or secondary dark reactions. At present, the tendency in photosynthesis research is to apply short exposure times (seconds or minutes), and high light intensities. Thus, the results are necessarily based on compromises; and the results refer to special conditions only, often only to one species of plants, or to a particular culture of organisms.

For the establishment of the reaction mechanism, the isolation and identification of intermediates become of paramount importance. In experiments of very short duration, phosphoglyceric acid has been isolated (Calvin and Benson, 1948). It is thus far the only compound, the involvement of which, in photosynthesis, may be claimed with high probability. Tracer studies at 2° C yield mainly 2-phosphoglyceric acid which is converted into 3-phosphoglyceric acid so rapidly that at 20° C the equilibrium mixture of the two substances is isolated. These findings have been confirmed and extended (Fager and Rosenberg, 1950). It was

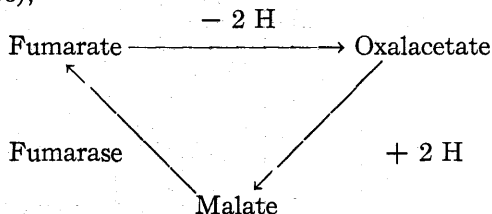


also shown that formation of phosphoglyceric acid is the major fixation reaction in the dark, immediately after the light has been turned off.



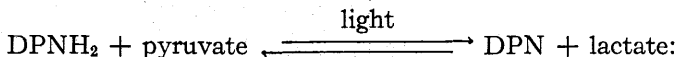
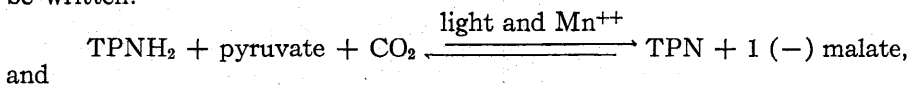
The next most likely step in the synthesis of a hexose—the formation of a triose, or of a triose-phosphate—has not been verified so far. If phosphoglyceric acid were reduced photochemically, as has been considered (Fager, Rosenberg, and Gaffron, 1950), another light reaction besides the established splitting of the water molecule would enter the mechanism of photosynthesis. A detailed discussion of this problem can be found in Gaffron and Fager (1951), and in two recent review articles (Calvin, 1949; Benson and Calvin, 1950). The mechanism of the formation of sucrose in the plant has not yet been established.

The positive identification of organic acids, or their salts, as intermediates, is rendered difficult by the fact that the respiratory mechanism involves the production of a number of such acids. The partners in the Szent-Györgyi cycle (Szent-Györgyi, 1936),

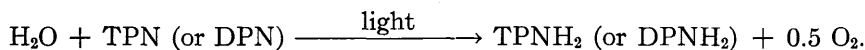


and participants in the Krebs or "citric acid" cycle (Krebs, 1940), figure 4, have been isolated from photosynthesizing cells. The diagrams of these cycles are given here to illustrate some of the reactions superimposed on photosynthesis in general, which are very important in assimilation experiments with certain microorganisms (Gest, Kamen, and Bregoff, 1950). The Szent-Györgyi cycle acts as a hydrogen carrier in the formation and oxidation of citric acid, and in the oxidation of triose to pyruvic acid. The oxidation of one triose molecule involves one Krebs cycle, and at least three repetitions of the Szent-Györgyi cycle.

Since all photosynthesis reactions involve enzymes and enzyme systems, considerable attention has been concentrated on the study of the enzymic mechanisms of carbon assimilation (Ochoa, 1946). Vishniac and Ochoa (1951) demonstrated that the mechanism of the citric acid cycle is used after a primary light reaction which involves pyridine nucleotides. The reactions studied may be written:



where TPN and DPN represent Triphosphopyridine nucleotide (Coenzyme II) and Diphosphopyridine nucleotide (Coenzyme I), respectively, and  $\text{TPNH}_2$  and  $\text{DPNH}_2$ , the corresponding reduced forms. The observations indicate that illuminated chloroplasts are able to catalyze the over-all reaction:



The availability of tracer elements, especially of isotopes of carbon, has greatly stimulated investigations on the distribution or location of the tracer in the molecules of suspected intermediates before and after illumination. In experiments with *Bryophyllum*, the malic acid, which had taken up the tracer in the dark, was found to form in light a high percentage of sugar tagged in positions 3 and 4 (Varner and Burrell, 1950); results of this kind may reveal the length of the carbon chain in reaction intermediates.

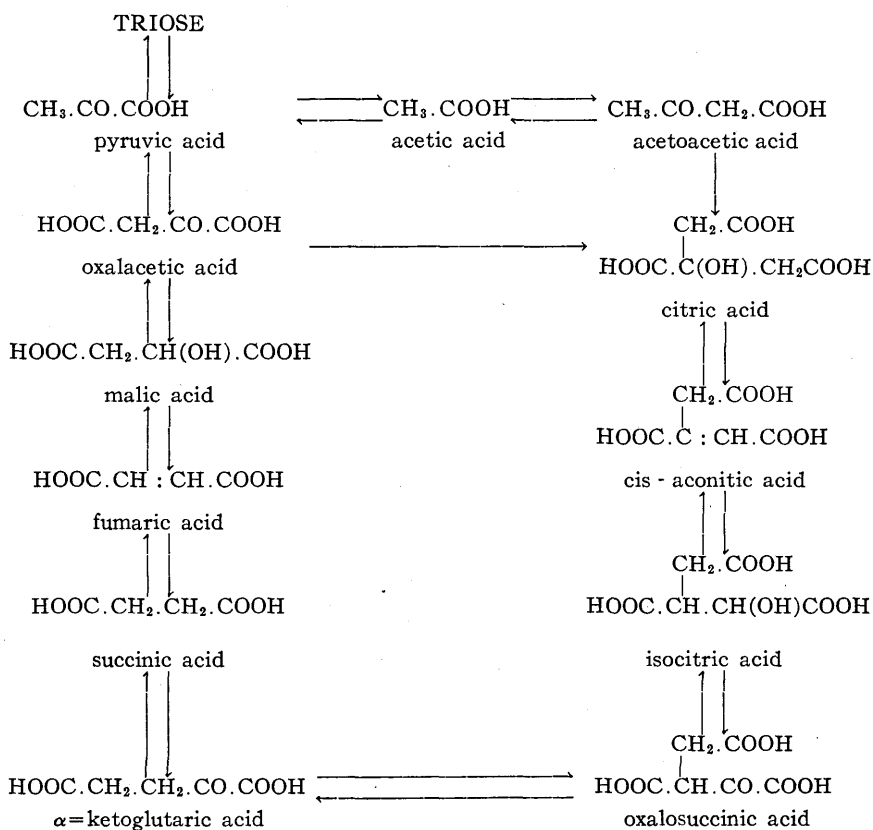


FIGURE 4. Citric Acid Cycle

#### MEASUREMENTS OF THE QUANTUM YIELD

Since the photosynthesis represents the conversion of light into chemical energy, the energy conversion factor of this transformation becomes of great importance. This factor, which is the measure for the efficiency of the process, is:

$$\epsilon = \frac{\text{chemical energy gained}}{\text{energy of light absorbed}} = \frac{H_c}{h\nu}$$

The chemical energy is determined by measuring the heat of combustion,  $H_c$ , of the reaction product—in this case carbohydrate— $h$  is Planck's constant— $6.624 \times 10^{-27}$  erg-seconds—and  $\nu$  is the frequency, when light of wavelength  $\lambda$  (in  $m\mu$ ) is used:

$$\nu = 3.00 \times 10^{17} / \lambda_{m\mu}$$

For one of the structural units  $[\text{CH}_2\text{O}]$ , the "molar" heat of combustion ( $H_m$ ) has been found experimentally to be approximately 112 kcal., or  $4.69 \times 10^{12}$  ergs.

Based on Planck's concept of energy quanta, Einstein postulated for photochemical processes the requirement that ideally the number of quanta (photons) of energy  $h\nu$ ,  $N_{h\nu}$  be equal to the number of molecules  $N$ , which had undergone a photochemical change by absorbing these quanta. In this case, the "quantum yield" or "quantum efficiency" would be unity.

For molar quantities,  $N$  becomes Avogadro's number— $N_A = 6.02 \times 10^{23}$  (g-mole) $^{-1}$ —and for a quantum yield of one, there will be  $6.02 \times 10^{23}$  quanta necessary. This number of quanta corresponds to an energy of  $N_A \times h\nu = N_A \times$

$h \frac{c}{\lambda}$  ergs, that is:

$$\frac{6.02 \times 10^{23} \times 6.62 \cdot 10^{-27} \text{ erg-sec.} \times 3.00 \times 10^{10} \text{ cm/sec.}}{\lambda_{\text{cm}}}$$

or, for  $\lambda$  in  $m\mu$  ( $= 10^{-7}$  cm):

$$\frac{6.02 \times 10^{23} \times 1.986 \times 10^{-9}}{10^{-7}} \text{ ergs} =$$

$$\frac{6.02 \times 10^{23} \times 1.986 \times 10^{-9}}{\lambda_{m\mu}} \text{ ergs} =$$

$$\frac{1.196 \times 10^{15}}{\lambda_{m\mu}} \text{ ergs} = 1 \text{ Einstein.}$$

If  $P$  moles of carbon dioxide are reduced (or  $P$  moles of oxygen evolved) in a photochemical reaction, and if the energy ( $I_a$ ) of the quanta absorbed is given in Einsteins, then the quantum yield  $\gamma$  is:

$$\gamma = \frac{P}{I_a}.$$

This factor modifies the equation for the efficiency of the photosynthetic process, and one obtains:

$$\epsilon = \frac{H_m}{N_A h\nu} \times \gamma = \frac{4.69 \times 10^{12}}{1.196 \times 10^{15}} \times \lambda_{m\mu} \times \gamma$$

$$= 3.93 \times 10^{-3} \times \lambda_{m\mu} \times \gamma,$$

or approximately  $4 \times 10^{-3} \times \lambda_{m\mu} \times \gamma$ .

It has been mentioned that the quantum yield  $\gamma$  will be unity, if one molecule of a substance, upon receiving one quantum, breaks up into component parts irreversibly. If this is not the case, then the quantum yield may be smaller or larger than one. High quantum yields are found in reactions where a single molecule absorbs one quantum and causes a sequence of reactions to start, in which many molecules are involved. The quantum yield is low when a considerable number of absorbed quanta are given off as heat or as fluorescence.

About 30 years ago Warburg and Negelein (1923) studied the photosynthetic efficiency of monochromatic light on a species of *Chlorella*, and found, for different wavelengths, quantum yields between 0.20 and 0.23. The results indicated a reaction mechanism in which four (or possibly five) quanta were required for the reduction of one mole of carbon dioxide. This finding was widely accepted. It represents, indeed, the simplest way to account for the four hydrogen atoms necessary to reduce the carbon dioxide molecule. Four individual absorption acts could easily furnish the four quanta needed. Many reinvestigations, by a great number of authors and with a variety of organisms, followed, but resulted

in values spreading from about 0.25 to 0.04 (Rothemund, 1944; Emerson and Nishimura, 1949; Moore and Duggar, 1949; Rieke, 1949; Arnold, 1949). Results with  $\gamma$  approximately 0.08 to 0.1, corresponding to 10 to 12 quanta, seemed to be prevailing, although they were obtained with three different and independent techniques: manometry, polarography, and calorimetry. There was agreement as to the observed phenomena, but serious disagreements in the interpretations of the experimental results. Fluorescence presents a difficult problem in this connection, and Franck (1951) gave it a great deal of attention. Transient intensity changes in fluorescence during photosynthesis were first reported by Kautsky (1934). The relationship between chlorophyll fluorescence and photosynthesis is the subject of a recent review (Wassink, 1951). Its author concludes that there is, at present, no predictable general relationship between the two. In many cases, increased intensity of fluorescence and decrease of photosynthetic yield go together. The formulation of theories on the mechanism of photosynthesis, on the basis of reported quantum yields, is, under these circumstances, a useless undertaking.

During the past four or five years, Warburg and co-workers repeated Warburg's earlier work. They found at first, in a large number of determinations of the quantum yield, a confirmation of the value 0.25 (Burk, Hendricks, Korzenovsky, Schocken, and Warburg, 1949); but later, under changed conditions, Burk and Warburg obtained for *Chlorella* in monochromatic light of one minute duration, alternating with one minute dark periods, quantum yields of 1.0 to 1.25 (Burk and Warburg, 1950; 1951). Independently in each dark period a combustion process, almost proportional to the light intensity of the light periods, took place. This combustion could be increased to about 10 to 12 times the ordinary dark respiration. It is claimed that in a novel 2-phase cycle the two energy transformations are interacting so that red light, for example, furnishes only 40 kcal., and the combustion 80 kcal. for the reduction of one mole of carbon dioxide. This ratio of 1 : 3 is interpreted to mean that three or four revolutions of the photosynthetic energy cycle suffice to allow the fixation of one mole of carbon dioxide under evolution of one mole of oxygen.

Burk and Warburg consider the one-quantum mechanism and energy cycle the solution to the long-standing quantum problem in photosynthesis (Burk and Warburg, 1951).

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