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Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: MODERN ASPECTS OF LIGHT IN PHOTOSYNTHESIS

Pages in Study: 37 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope of Study: The purpose of this study is three fold: (1) to provide a general knowledge of the function of light in the photosynthetic process so it may be easily communicated to the high school student; (2) to present a simple guide which may serve as resource material for other teachers; and (3) an endeavor to compile sufficient reference for the interested teacher to progress to further study.

Findings and Conclusions: A complete understanding of the energy changes which occur in photosynthesis must be left to the expert. However, there is a wealth of proven material that could be used to enrich the secondary school biological science curriculum.

Present evidence indicates that the role of light in the initial photochemical step is solely to increase the energy of the electrons in chlorophyll. Succeeding reactions transport these excited electrons along varying paths until the excititation energy is captured and converted into chemical energy.

This concept of energy transformation in plants should be introduced at an early level in an individual's schooling in order to develop his understanding of the relationship of all life to the sun.

ADVISER'S APPROVAL ______________________

MODERN ASPECTS OF LIGHT IN PHOTOSYNTHESIS

By

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1957

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1961

MODERN ASPECTS OF LIGHT IN PHOTOSYNTHESIS

Report Approved:

Report Adviser

Dean of the Graduate School

ACKNOWLEDGEMENTS

The writer is indebted to Dr. James Zant for critical reading of this study and to his wife, Martha, for her invaluable assistance in the preparation of the paper. He also is appreciative of the committee on the selection of Institute participants for providing the writer the opportunity to study at this University.

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CHAPTER I

THE PROBLEM

The Problem Stated

Almost everyone has heard of photosynthesis, the foodmaking process occurring in plants, and of the direct effect it has on his life. The average secondary school science curriculum includes the photosynthetic cycle beginning with the raw materials and concluding with the products of the process. But, unfortunately, the entire photosynthetic process is vastly oversimplified in most high school textbooks. The typical high school science teacher is acquainted with only the following chemical equation used to represent the process:

$$CO_2 + H_2O \xrightarrow[\text{(chlorophyll)}]{\text{(light)}} \text{carbohydrate} + O_2$$

What many teachers and students of biology do not realize is that there are numerous intricate reactions required to convert one molecule each of carbon dioxide and water to one molecule of carbohydrate and oxygen. Another puzzling aspect to the problem is how the radiant energy from the sun is converted to chemical energy and stored within the finished product.

Because the writer was confronted with such questions, he has decided to prepare a study suitable for a high school biology course about the function of light in photosynthesis.

Purpose of This Study

The purpose of this study is three fold: (1) to provide the writer with a satisfactory knowledge of the up-to-date effects of light in the photosynthetic process so that they may be more easily presented to high school students; (2) to provide a simple guide which can serve as resource material for other teachers in the teaching of the modern concepts of photosynthesis; and (3) to compile information about the role of light in photosynthesis which will provide sufficient reference for a teacher or student engaged in further study in this field.

Limitations of This Study

Several factors limit this study. First, because the literature is extensive, the study will be a survey of the field rather than an attempt to investigate it. Next, in order to better evaluate the role of light, it has been necessary to select a few factors out of many related ones which affect photosynthesis. Finally, with the development of the electron microscope the concept of chloroplast organization has become more exact. This structure must be considered to obtain the clearest picture of the effect of light.

Plan of Procedure

The information for this study has come from the published literature in this field. Numerous textbooks on photosynthesis were reviewed, as well as other books on plant physiology, magazine articles, and specific research studies in this area.

Part one of the study is about the historical development of photosynthesis research. An attempt has been made to trace, from the ancients to the moderns, the influence of each succeeding generation of investigators.

The remainder of the study is devoted to the effect that radiant energy has upon the photosynthetic process. Consideration has been given to the action of light upon the chloroplast pigments and to the energy transfer which occurs during the foodmaking process.

CHAPTER II

HISTORICAL INTRODUCTION

From the time of Aristotle until the eighteenth century, it was generally supposed that plants derived their nourishment from the soil. Early in the seventeenth century a Dutchman named Van Helmont became convinced that a plant was not nourished from the soil, but by water. In 1727, Aristotle's theory was again questioned by Stephen Hales. This investigation suggested that plants took their nourishment from the air and also suggested that possibly light played a part in the process.[1]

Further progress in the study of photosynthesis came only after chemists had learned to distinguish the different gases in air. It was Joseph Priestley, the discoverer of oxygen, who made one of the first advances in the field of photosynthesis. In 1772 he found that green plants had the ability to reverse the effect of respiration.[2]

By 1779 the Dutch physician Ingen-Housz demonstrated that light, as well as the green parts of a plant, played a role in the purification of air. He further observed that in the dark reaction of photosynthesis in plants the photosynthetic process caused the air to become foul. According

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[1] J. Terrien, G. Truffant, and J. Charles, Light, Vegetation and Chlorophyll (New York, 1957), p. 151.

[2] Robert Hill and C.P. Whittingham, Photosynthesis (New York, 1954), p. 1.

to Ingen-Housz, sunshine split apart the carbon dioxide (CO_2) that the plant absorbed from the air. The plant gave off oxygen, and retained the carbon for nourishment.[3]

Senebier, a Swiss naturalist, in 1782 demonstrated that an accelerating effect occurred in the production of CO_2 by plants in the dark and upon the production of oxygen in the light.

In 1804, the great Swiss physiologist Nicholas de Saussure, introduced quantitative experiments to show that the gain in weight of a plant during a period of photosynthesis plus the weight of the oxygen evolved exceeded the weight of the CO_2 consumed. This difference he attributed to an uptake of water. The overall reaction could be written as follows:

$$CO_2 + H_2O \xrightarrow[\text{(chlorophyll)}]{\text{(light)}} \text{organic matter} + O_2$$

He also found that the ratio of the CO_2 consumed and the oxygen produced was equal to one.[4]

Later other investigators proposed that the carbon from the broken CO_2 combined with water to form a product with the empirical formula (CH_2O). This photosynthetic hypothesis became a fixed principle of biology. Thus, by the early nineteenth century the green plant had become known as an autotropic organism. As a result, all living organisms were divided into the two following groups: (1) green plants, which at that time were considered to be the only organisms capable of assimilating carbon dioxide; and (2) all other forms of life which had to depend on the products produced by the photosynthetic group.

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[3] E. Rabinowitch, "Photosynthesis," Scientific American, August, 1948, p.25.

[4] Terrien, pp. 152-153.

Not all investigators shared similar views of this apparently logical picture of photosynthesis. In the 1880's two developments appeared which tended to weaken the earlier nineteenth century photosynthetic hypothesis. Sergei Winogradsky was the first to question the hypothesis, when he discovered chemosynthetic bacteria. These organisms contained no chlorophyll, but made organic material by assimilating carbon dioxide in the dark.

The second experimenter was Theodore Wilhelm Engleman who found that purple bacteria which metabolize sulfur compounds perform a type of photosynthesis without giving off oxygen. Until this time it was believed that oxygen was a by-product of the photosynthetic reaction.[5]

Since these new findings contradicted the then fundamental ideas about photosynthesis, the implications of Winogradsky's discovery were ignored by most of his contemporaries. An exception was the Russian microbiologist Lebedev. He insisted that carbon dioxide assimilation could no longer be considered limited to green, photosynthetic cells. He suggested that all cells possessed the ability. The big difference, Lebedev believed, was that the photosynthetic group used light as a source of energy for the process. This concept was much ahead of its time and, thus, found little support from others in the field.[6]

Even the observations made by Engleman had little effect on photosynthetic concepts of the time. Although Lebedev argued for the assimilation of carbon dioxide in the dark, he was convinced that photosynthesis took place by the splitting of the carbon dioxide molecule with the

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[5] D. I. Arnon, "The Role of Light in Photosynthesis," Scientific American, November, 1960, pp. 105-106.

[6] Ibid., p. 105.

eventual release of oxygen as a by-product.[7] It was not until forty years later that sufficient evidence indicated the existence of more than one kind of photosynthetic reaction in living organisms.

In the 1930's C. B. Van Niel convincingly demonstrated that bacteria could carry on photosynthesis without evolving oxygen. The splitting of carbon dioxide by light was no longer considered a valid hypothesis. Van Niel provided a new theory: he proposed that light splits water, not carbon dioxide.[8] According to this idea, which applied to both bacteria and green plants, light decomposed the water to produce the hydrogen needed to convert carbon dioxide to carbohydrate. In plants oxygen was released. Bacteria which lacked the enzymes to catalyze this reaction, combined the (OH) radical with hydrogen from an outside source to form water again. The reaction equations for the photosynthesis of green plants and of green bacteria show a close similarity:

$$CO_2 + H_2O \xrightarrow{\text{Light}} (CH_2O) + O_2$$

$$CO_2 + 2H_2S \xrightarrow{\text{Light}} (CH_2O) + H_2O + 2S$$

The general formula is the following:

$$CO_2 + 2H_2A \longrightarrow (CH_2O) + H_2 + 2A$$

Thus, the present hypothesis involved an oxidation-reduction reaction in which the carbon dioxide is reduced by an oxidizable hydrogen donor.[8]

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[7]Ibid., p. 106.

[8]Hill, pp. 75-77.

CHAPTER III

THE ROLE OF RADIANT ENERGY

The energy source of photosynthesis is light. Except in the case of chemosynthetic bacteria, plants deprived of light will not perform photosynthesis. Not only is light necessary for the formation of chlorophyll in most plants, it is also the source of energy which eventually becomes chemically bound in carbohydrate molecules. The ultimate source of the chemical energy bound in all foodstuffs is the sun.

One unsolved riddle which still confronts scientists is how radiant energy reaches the earth from the sun. One of the earliest investigators of the transmission of light was Pythagoras. During the sixth century B.C., this Greek philosopher proposed that light traveled in small particles. This came to be known as the particulate theory of light. Isaac Newton was also a proponent of the particle theory, but did admit that there was a possibility of wave motion. Newton proposed that light was composed of units called photons or corpuscles.[1]

In 1900, Max Planck re-expressed Newton's corpuscular theory of light by developing his quantum hypothesis of the nature of radiation. Where Newton defined light as consisting of photons, Planck's concept expressed the composition of light in terms of energy content.[2] According

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[1] Erston V. Miller, Within the Living Plant (New York, 1953), p. 114.

[2] D. Burk et al., "Efficient Transformation of Light into Chemical Energy in Photosynthesis," Scientific Monthly, 73 (1951), pp. 213-215.

to Planck's hypothesis, light is composed of discrete units called quanta. The energy value of the quanta depends on the wave length or the color of light. Red light of wave length 660 microns contains approximately 43,000 calories per mole quanta while blue light of wave length 430 microns contains 66,000 calories per mole quanta.

Albert Einstein in 1905 suggested that light was propagated as corpuscular radiation.[3] Each photon had an energy value (hv) measured in quanta. The h in the formula hv is Planck's constant and the v stands for the frequency of the emitted light. Thus, the energy value of quanta decreases along the color bands of the visible spectrum. For example, the quanta of radiant energy in the color bands of light range from violet with the most to red with the least.

The total of radiant energy is composed of many different colors, each having a particular energy level or wave length. The chart in Figure 1 illustrates the total and visible spectrums of radiant energy.

The light which we can see comprises the visible spectrum, and it makes up only a small part of the total spectrum. Beyond the violet portion are the invisible bands of radiant energy known as ultraviolet, X-rays, gamma rays, and cosmic rays. At the opposite end projecting beyond the red, are the invisible bands of infra-red and electromagnetic radiation.

It is in the region of the visible emission that light has the most important effects on a plant. The chief effect is the stimulation of photosynthesis. Plants, by this process transform radiant energy into chemical energy. Light also affects other features of plant growth such

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[3] Ibid., pp. 216-217.

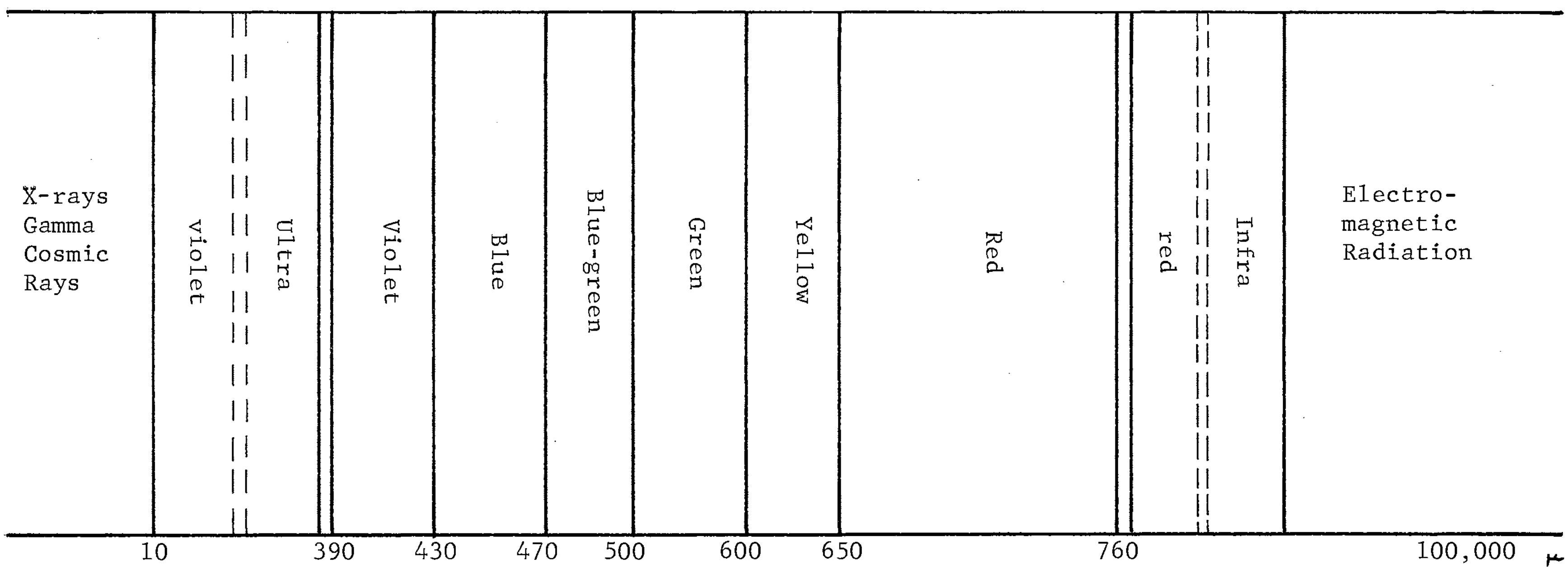

Figure 1. Radiant Energy Spectrum (Not to Scale)

as flowering, tuberization, and the opening and the closing of stomata.[4]

Infra-red light consists of all radiation of wave-lengths above 760 microns. The predominant action of infra-red radiation is to provide heat inside the leaf and thus stimulate the evaporation of water. This light is characterized by a smaller quantity of energy in each quanta than that of visible light which in turn makes its chemical activity much less. Experiments indicate that the chemical activity of infra-red radiation is not strong enough to disturb chemical structures. However, it does cause oscillation of atoms which results in heat.

The action of ultra-violet radiation is either harmful or inactive depending on its wave length. Harmful UV radiation has wave lengths shorter than 290 microns. These radiations are absent from the solar spectrum due to atmospheric absorption. However, if these waves are produced artifically, they do have a clearly injurious effect.

A second type of ultra-violet radiation nearer the visible spectrum or above 290 microns appears harmful. In response to the chemical activity of ultra-violet rays, plants cover themselves with apparently protective cells which are opaque to these radiations. It is thought that radiation action is too intense and dangerous for the complex substance of the cells.

Visible light of wave lengths between 400 microns and 760 microns plays a more complex role than all other radiations. The captured energy from visible light by chlorophyll is converted to chemical energy. This energy is used to oxidize water and reduce carbon dioxide.[5]

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[4]J. Terrien, G. Truffants, and J. Charles, p. 77.

[5]Ibid., pp. 60-79.

In 1923 the German cell-biologist Otto Warburg first attempted to measure the quantum yield or the efficiency of photosynthesis.[6] He found that with red light a maximum of 65 per cent of all the absorbed radiant energy was utilized in photosynthesis. This came to about four quanta of red light for the formation of one molecule of oxygen gas.

Not all biochemists were in agreement with Warburg's early findings. Other investigators began efficiency experiments. Many different experimental methods were used and different values were found for the efficiency of the photosynthetic process. The major impetus for further investigations was the seemingly too high efficiency of this natural process. Most investigators could not accept this high efficiency when the best man-made engines could operate only with an efficiency of 25-30 per cent.

By 1941 Robert Emerson and C. M. Lewis reported a possible error in Warburg's work. These investigators reported that photosynthesis required from ten to twelve quanta of red light per mole of oxygen evolved which thus makes the maximum possible efficiency 20-30 per cent. This was more in agreement with the best operating engines and biological processes known. Biochemists accepted this view until 1946 when Warburg published a confirmation of his earlier figures for four quanta of red light mole of oxygen given off.

Today there is still disagreement among investigators as to the quanta requirement in photosynthesis. Some insist on the ten to twelve quanta figure found by Emerson and Lewis while others feel that the smaller number of eight quanta is sufficient. Warburg still holds to

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[6] Cora G. Ryerson, "The Claim for High Efficiency in Photosynthetic Activity," Chemical and Engineering News, 27 (1949), pp. 3560-3561.

the three to four quanta figure. This problem has yet to be resolved by the authorities in the field.

Recent experiments by Warburg and Kruppahl using chlorella, a green algae, have yielded some interesting results.[7] It was discovered that the quantum requirement is affected by light intensity, the requirement decreasing with decreasing intensity. In his recent work Warburg arrived at a value of 2.85 quanta per mole of oxygen produced for the quantum requirement of the over-all reaction of photosynthesis. The new figure of 2.85 is very near the theoretical quantum requirement of 2.7 at 100 per cent efficiency. This figure produces an efficiency of 96 per cent in red light.

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[7]W. Bladergroen, Ph.D., Problems in Photosynthesis (Springfield, Illinois, 1960), pp. 187-188.

CHAPTER IV

CHLOROPLAST STRUCTURE AND CONSTITUENTS

The primary function of photosynthesis is to convert radiant energy into chemical energy and to store it in the glucose molecule. In order for the photochemical process to take place, light energy must be absorbed. The cellular inclusion in plants that absorbs radiant energy is the chloroplast which is composed of chlorophyll and other light absorbing pigments.

Chloroplast shapes and number vary according to the species of plant as well as the tissue in which it is located. In higher plants the chloroplast is ellipsoidal or saucer-shaped. It varies in size, the longest axis being from two to ten microns and the diameter four to six microns in thickness.[1] The number of chloroplasts per plant cell ranges from several hundred to only a few. In algae cells it is commonly found that each cell contains only one chloroplast, the shape of which could be the stellate chloroplast of zygonema, the cup-shape of chlorella, or the spiral ribbon of spirogyra.

The gross structure of a chloroplast may be observed to some extent with a light microscope. It usually consists of an outer semipermeable proteinacous membrane and enclosed cytoplasm which is composed of two phases: (1) a continuous lipid substratum, the stroma, and (2) a dark system of granules called grana. In higher plants the photosynthetic

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[1]"Chloroplast Structure and Its Relationship to Photosynthesis," Research in Photosynthesis (New York, 1957), pp. 459-461.

pigments are found only in the matrix of the grana.[2]

The number of grana in a single chloroplast varies from about ten to several hundred. Electron microscopy studies indicate that individual grana have an elaborate and orderly structure. Each granum appears as a disc-like body consisting of protein membranous laminations at right angles to the short axis. Complete agreement has not been reached about the ultrastructure of the chloroplast.

One interpretation of grana structures has resulted from algae studies conducted by E. Steinmann and F. S. Sjostrand. Figure 2 pictures grana organized in double membranous discs with each membrane about 65 Angstroms (Å) thick, and enclosing a narrow space of about 65 Å. The double membranous discs of adjacent grana are mutually connected through 30 Å thick membranes extending through the stroma. Proceeding through the granum as pictured in Figure 2, the following regions are met in succession: (1) interlamellar membrane--30 Å, (2) upper disc membrane--35 Å, (3) interdisc space or matrix--65 Å, (4) lower disc membrane--35 Å, (5) interlamellar membrane--30 Å, and (6) intradisc space--65 Å.

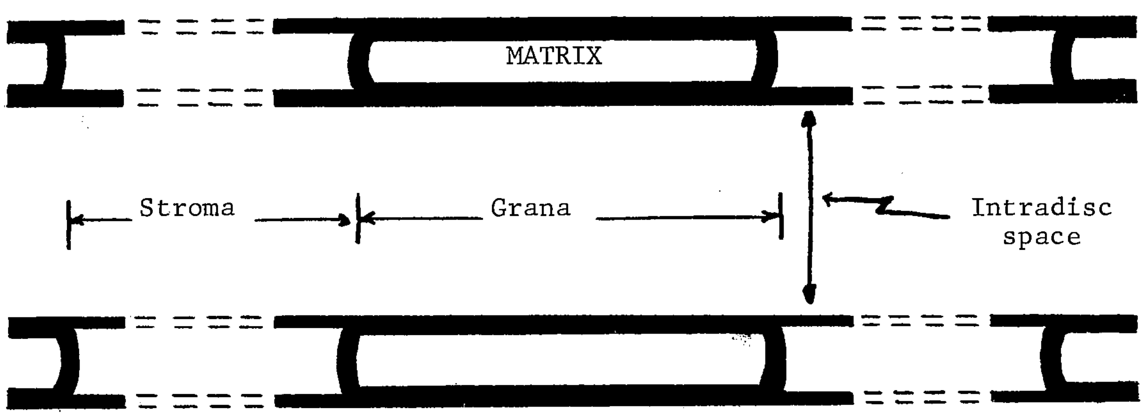

Figure 2. Grana Model

[2]Hans Gaffron, "Energy Storage: Photosynthesis," Plant Physiology, ed. F. C. Steward (New York, 1960), p. 29.

The photosynthetic pigments, principally chlorophyll, are distributed in monolayers on the disc membranes in the interdisc space. This type of arrangement is believed to provide increased surface area.[3]

In 1936, B. Hubert suggested that the polar chlorophyll molecules are attached by their hydrophilic porphil "heads" to the protein layers while their lipophilic phytol tails are associated with lipid molecules.[4] The fat soluble carotenoid pigments are then aligned between the lipid molecules. This hypothetical structure of chloroplast is shown in Figure 3. In this interpretation the chlorophyll-containing lamallae are embedded in lipoid layers which are situated between protein-water layers.

Not all photosynthetic organisms possess chloroplasts similar to those of higher plants. Photosynthetic bacteria show only local concentrations of pigments which resemble grana.[5] Further study has shown that the photosynthetic bacteria contain free-grana. Thus, it appears that the larger lamelated chloroplast of higher plants is not essential for the function of chloroplast pigments.

The pigments in the chloroplast are of fundamental importance since photolysis, the chemical decomposition of water due to light, depends on at least chlorophyll a. Chloroplast pigments belong to the two following chemical classes: tetraphyrrolic compounds to which the chlorophylls belong, and the carotenoids which include yellow xanthophylls and the orange carotenes. Leaf color is sometimes modified by the presence of red or purple anthocyanin which is dissolved in the cell sap and is absent

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[3]E. Steinmann and F. S. Sjostrand, "The Ultrastructure of Chloroplasts," Experimental Cell Research, VIII (1955), pp. 15-23.

[4]Eugene I. Rabinowitch, Photosynthesis and Related Processes (New York, 1945), pp. 367-368.

[5]A. E. Vatter and R. S. Wolf, "The Structure of Photosynthetic Bacteria," Journal of Bacteriology, 75 (1958), pp. 367-368.

from the cytoplasm thereby playing no direct part in photosynthesis. In red algae additional pigments may be present which mask the chlorophyll that is present. These are related to the chlorophylls and are also tetrapyrrolic compounds. Table I summarizes chloroplast pigments.

The seven types of chlorophyll which are known today are the chlorophylls *a*, *b*, *c*, *d*, and *e*; bacteriochlorophyll; and bacterioviridin. Blue-green chlorophyll *a* is the most abundant and can be found in all auto-

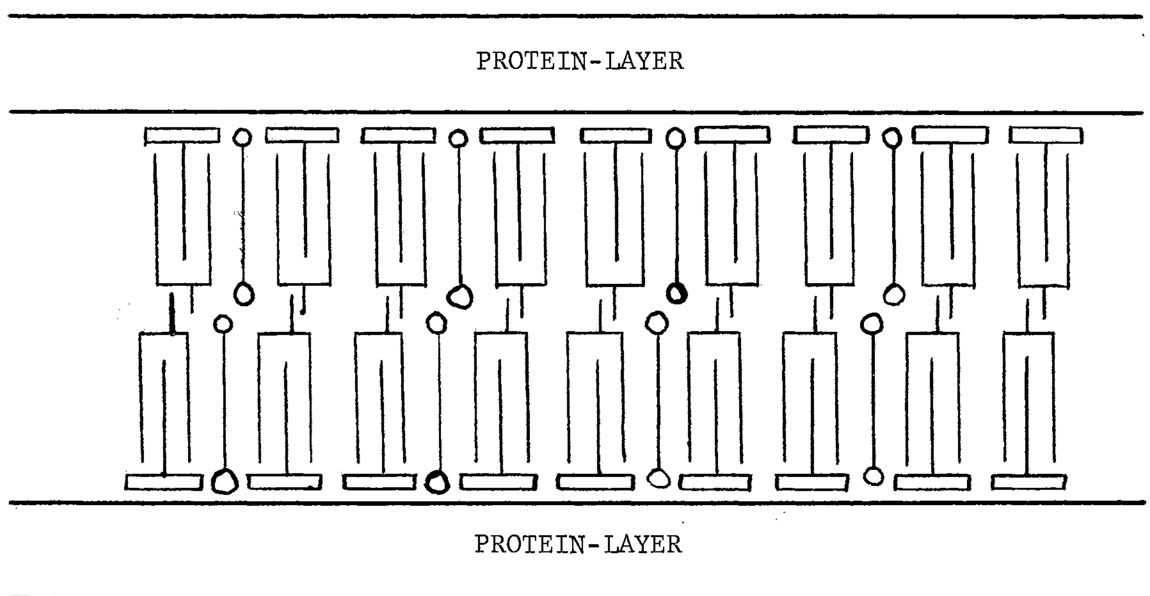

Figure 3. Model of Chloroplast Structure--Hubert. Protein Sheets Alternate with Bifoliar Layers Consisting of Chlorophyll (T), Phospholipid ( ) and Carotenoids ( ).

trophic organisms except pigmented bacteria. Chlorophyll *b*, yellow-green in color, is found in certain algae and higher plants. The remaining chlorophylls occur only with chlorophyll *a* in the algae. Bacteriochlorophyll is found in purple sulfur bacteria, and bacterioviridin, in green sulfur bacteria.

The chlorophylls absorb a maximum of light in the red and blue-violet regions of the spectrum. There is also absorption in other areas of the visible spectrum. Bacteriochlorophyll has maximum absorption in the infra-red and blue-violet regions while bacterioviridin absorbs in the red and violet areas.

Occurring in plants with the chlorophylls are the yellow carotenoids. Among the carotenoids only the oxygen containing members, the carotenols, appear to function as light absorbing agents. Carotene is always present in normal cells in the form of B-carotene. This pigment protects against photo-oxidation of chlorophyll, the destruction of chlorophyll. Light absorption by the carotenols is in the blue and blue-green area of the spectrum.

Accompanying the chlorophylls and carotenoids in some algae are red and blue phycobilins. These pigments are also associated with the photosynthetic mechanism. One member of the group of pigments, phycocyanin, has maximum absorption of red-orange. Phycoerythrin, the second member, absorbs in the green region of the spectrum.[6]

Table II gives a survey of most substances found in chloroplasts which are known or assumed to take part in photosynthetic reactions. It includes additionally the enzymes and coenzymes that activate numerous photochemical reactions.

There is substantial evidence that light absorbed by pigments other than chlorophyll can be utilized for photosynthesis. Since chlorophyll a is the sole pigment common to all photosynthetic organisms, it is believed that this is the only pigment which donates energy to photosynthetic reactions. Other pigments, such as the carotenoids and phycobilins, may absorb light first. This energy is not utilized directly in photosynthesis because these pigments transfer their excitation energy to chlorophyll a.[7]

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[6]Hill and Whittingham, pp. 16-38.

[7]Ibid., p. 67.

TABLE I

CHLOROPLAST PIGMENTS

	Location	Maximum Absorption
Chlorophylls		
Chlorophyll *a*	All green plants	
b	Not brown, red algae, diatoms blue-green	Red and blue-violet
c	Brown algae, diatoms	
d	Red algae	
Bacteriochlorophyll	Purple S bacteria	Infra-red and blue-violet
Bacterioviridin	Green S bacteria	Red and blue-violet
Phycobilins		
Phycocyanin	Blue-greens, also red algae	Orange red
Phycoerythrin	Red algae, blue-greens	Green
Carotenoids	Variable in different plants	Blue and blue-green
Xanthophyll (Carothenols)		
Carotenes		

TABLE II

SOME SUBSTANCES KNOWN OR ASSUMED TO TAKE PART IN PHOTOSYNTHETIC REACTIONS

A. Pigments present in large amounts (visible coloring matter)	
Chlorophyll *a* Bacteriochlorophyll *a*	Main light-energy converting agent
Chlorophyll *b* (*c*, *d*) Phycoerythrin Phycocyanin Fucoxanthol Xanthophyll	Absorbed energy transferred to chlorophyll *a*
Carotene	Protection against photo-oxidation
B. Coenzymes and similar factors	
Adenosine triphosphate (ATP) Flavin nucleotide Thioctic acid Pyridine nucleotides (TPN, DPN)	Factors needed for reduction
Adenosine diphosphate (ADP)	Factors for photophosphorytion
Vitamin K (or other quinone Cytochromes (f, c) Manganous ion (Mn^{++})	Factors for release of oxygen
Potassium Vanadium Iron Chloride anion	Function unknown
C. Catalytic proteins	
Reductases	Methemoglobin, cytochromes flavins DPN, TPN, carboxylic acids, NO_2^-, NO_3^-, N_2
Oxidases or photooxidases	Cytochrome c, ascorbic acid
Hydrogenase	Free hydrogen in purple bacteria and adapted algae
Photodehydrogenases	Fatty acid and alcohol utilization in heterotrophic purple bacteria
Sulfur-activating enzymes	Photoreduction in purple and green sulfur bacteria
Phosphorylating enzyme	Photophosphorylation in green plants and photosynthetic bacteria

Eugene I. Rabinowitch has shown that other plastid pigments may also absorb light which may be used photosynthetically.[8] It may be that chlorophyll is present, but masked.

That chlorophyll is the energy absorbing substance can be verified in several ways. The absorption spectrum of chlorophyll is the same as the action spectrum of photosynthesis. Chlorophyll absorption of green radiation is least and this light permits only a very slow rate of photosynthesis.[9]

Another property of chlorophyll which shows its absorbing potential is fluorescence. The chlorophyll molecule which absorbs light is excited by it. This added energy may be passed on to other energy carriers, dissipated as heat, or lost by emission as a photon which is observed as fluorescence.[10]

Haematin compounds called cytochromes absorb light in addition to the other pigments. In photosynthetic cells these pigments become oxidized when the cell is illuminated. They assist in the transfer of energy during photosynthesis.[11]

Therefore, it can be said that the function of light in photosynthesis is to supply energy, and the function of chlorophyll is to trap the energy. The role of the chloroplast pigment is to absorb radiant energy in the visible spectrum which then must be converted either into wave lengths to be used in photosynthesis or transferred directly to the compounds involved in the reaction. A second role of the chloroplast pigment is that it may

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[8]Rabinowitch, p. 31.

[9]Jacob Levitt, Plant Physiology (New York, 1954), pp. 126-127.

[10]Terrien, p. 171.

[11]Bladergroen, p. 8, p. 131.

act as a catalyst at some stage of the photosynthetic process.

CHAPTER V

PHASES OF PHOTOSYNTHESIS

The answer as to the how of photosynthesis has long been sought. Although much advancement has been made during the twentieth century, the means by which this process occurs is still not totally solved.

In 1905 the English plant physiologist Blackman demonstrated that photosynthesis was not a single photochemical reaction, but that it was composed of two major reactions. The first reaction was quite rapid and required light energy for its acceleration. The second reaction did not require light energy, and appeared to proceed equally well in either light or darkness. Blackman suggested that the light reaction produced unstable intermediate compounds which were converted by enzymes of the dark reaction into stable substances. The phase requiring light was called the luminous phase. The second, the dark phase.[1]

It was not until 1937 that Robert Hill illustrated the possible nature of the light reaction by using isolated chloroplasts suspended in water. Hill demonstrated that in the presence of light and the proper oxidizing agent (hydrogen acceptor), the chloroplasts caused a photodecomposition of water and reduction of compounds other than carbon dioxide. This is shown by the general equation:

$$2A + 2H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} 2H_2A + O_2$$

[1]Ferry, p. 135.

Reducible substances serving as hydrogen acceptors are denoted by A. This system produced no carbohydrate.[2]

The results of the Hill reaction indicated that the light reaction of photosynthesis was a decomposition of water into hydrogen and oxygen. Thus, the photochemical production of oxygen required the presence of a suitable hydrogen acceptor and not the presence of carbon dioxide which was reduced.

Research with isotopic oxygen (O^{18}) has confirmed that the function of light is the photodecomposition of water by the chloroplasts. It also demonstrated that the oxygen in carbohydrates comes from carbon dioxide, not water, and that the oxygen removed from the water is released as free oxygen. This established the fact that the first act in photosynthesis is photolysis, the decomposition of water.

After photolysis of the water the dark stage of photosynthesis occurs. This period of photosynthesis involves the chemical aspects of photosynthesis which include energy transformation as well as carbon dioxide fixation.

Studies have shown that carbon dioxide is reduced by the hydrogen derived from the photolysis of water. This reduced carbon dioxide is then transformed, independent of light, into carbohydrate molecules. Essentially this process consists of using hydrogen acceptors to drive backward the dark reactions of respiration in the direction of synthesis.[3]

Hill's discovery of the chloroplast reaction brought about the abandonment of the concept that chloroplasts were sites of the complete

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[2]Hill, p. 114.

[3]Terrien, pp. 113-115.

process of photosynthesis. However, the question of energy transfer to the carbohydrate still persisted. Investigation of this problem proceeded along two lines.

Investigators searching for the means of energy transfer found that cells lacking chlorophyll, e.g., liver cells, could synthesize carbohydrates from carbon dioxide. This occurred if the cells were furnished with the necessary energy in the form of adenosinetriphosphate (ATP) and reduced diphosphopyridine nucleotide ($DPNH_2$). Apparently the breakdown of carbohydrates could be reversed. If this happened in liver cells, the experimenters reasoned that the same reaction might occur in plants.

Speculation grew that there was no special photosynthetic way for assimilating carbon dioxide and that all cells, whether they contained chlorophyll or not, might accomplish it by reversing respiration.

This theory drew support from the work of Calvin and Benson who traced the path of carbon in photosynthesis from carbon dioxide to carbohydrate. They found that many intermediate products were identical with those formed when carbohydrate is burned in respiration. Each of the reactions in this photosynthetic carbon-cycle were later found in various cells that assimilated carbon dioxide in the dark. Energy was provided by ATP and $DPNH_2$ in all the dark reactions.[4]

Other research began to indicate a link between photolysis of water and carbon dioxide fixation reactions. Further investigation showed that under proper conditions, illuminated isolated chloroplasts could reduce the well-known physiological electron carriers, triphosphonucleotide (TPN) and DPN. This discovery permitted the linkage of the Hill reaction to numerous

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[4]Arnon, Scientific American, p. 108.

enzymatic reactions in photosynthesis which depended on reduced nucleotides.[5]

Early investigators also illustrated that the photochemically reduced DPN could be easily reoxidized by the mitochondria present in the cell which could then bring about the synthesis of ATP by oxidative phosphorylation of the type observed by Lehninger. This suggested a link between chloroplasts and mictochondria.

This concept limited the chloroplast functions to the photolysis of water and the transfer of electrons and hydrogen to suitable acceptors. Carbon dioxide fixation and the conversion of light energy were accomplished by mitochondria and soluble enzymes situated outside the chloroplasts. It was believed by some that the mitochondria obtained $DPNH_2$ from the chloroplasts and manufactured ATP with it. This theory appeared reasonable and was widely accepted.

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[5] D. I. Arnon, M. B. Allen, F. R. Wheatley, "Photosynthesis by Isolated Chloroplasts," Nature, August 28, 1954, p. 394.

CHAPTER VI

PHOTOSYNTHETIC PHOSPHORYLATION

One researcher, Daniel I. Arnon, did not believe that mitochondria manufactured ATP with the $DPNH_2$ produced by the chloroplasts. In 1954 his research results showed that chloroplasts alone carry out complete photosynthesis. His findings demonstrated that both intact chloroplasts and fragmentary chloroplasts are able to produce ATP without being able to fix CO_2 and without the multienzyme system of the mitochondria. Further study revealed that the assimilation of carbon dioxide by chloroplasts was definitely a reversal of carbohydrate breakdown reactions. These experiments proved that the role of light in photosynthesis was two fold: (1) the photolysis of water and (2) the formation of ATP.[1]

One problem remained--how light energy formed ATP and $TPNH_2$. Arnon tried to visualize a mechanism for the formation of ATP in the framework of Van Niel's water-splitting scheme. With little success along this course, he decided to look for an entirely new process.

Arnon found a process which he named photosynthetic phosphorlylation to distinguish it from oxidative phosphorylation accomplished by mitochondria. In photosynthetic phosphorylation chloroplasts are able to synthesize ATP from inorganic phosphate and adenosine mono or diphosphate (AMP or ADP). When conditions are proper, the chloroplasts use light energy to esterify inorganic phosphate according to the over-all reaction

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[1]D. I. Arnon, "The Chloroplast as a Complete Photosynthetic Unit," Science, 122 (1955), pp. 13-14.

shown in the following equation:

$$\text{Light} + P + ADP \longrightarrow ATP$$

In it P represents inorganic phosphate, ADP, adenosine disphosphate, and ATP, adenosine triphosphate. [2]

The enzymes responsible for phosphorylation are closely bound to the chlorophyll pigment system. Enzyme action is controlled by soluble co-factors such as vitamin K and flavin mononucleotide (FMN). Loss of these soluble factors renders these enzymes functionless.

Experiments of other investigators, namely Frenkel, exhibited the presence of a phosphorylating system which was confined to the chromatophores of photosynthetic bacteria. Evidence indicated that in these bacteria, the enzymes of photosynthetic phosphorylation are bound to the particles containing the photosynthetic pigments.[3]

There are two important differences between photosynthetic phosphorylation and oxidative phosphorylation. First, in photosynthetic phosphorylation ATP, which is synthesized by chloroplasts, represents a direct anaerobic synthesis of inorganic bonds at the expense of light energy. Secondly, Arnon found that TPN, not DPN, acts as a catalyst in photosynthetic phosphorylation which likewise demonstrated the difference between the two types of phosphorylation.

Photoreduction of TPN is expressed by the equation:

$$2TPN + 2H_2O \longrightarrow 2TPNH_2 + O_2 \quad \text{(a)}$$

In order for the reaction to occur, a TPN reducing factor located in the

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[2]D. I. Arnon, F. R. Wheatley, M. B. Allen, "Assimilatory Power in Photosynthesis," Science 127 (1958), p. 1029.

[3]A. W. Frenkel, "Photophosphorylation of Adenine Nucleotides by Cell-Free Preparation of Purple Bacteria," Journal of Biological Chemistry, 222 (1956), p. 833.

chloroplasts must be present. During investigations it was found that when one mole of O_2 is produced, two moles of TPN are reduced and two moles of inorganic phosphate are esterified, as shown in the following equation:

$$2ADP + 2P + 2TPN + 2H_2O \xrightarrow{Light} 2ATP + 2TPNH_2 + O_2 \qquad (b)$$

This reaction, which includes both light reactions, demonstrates that only a part of the light energy is used for ATP production while the remaining part is used for the reduction of TPN.

Thus, light energy provides both ATP and $TPNH_2$. Both substances form what Arnon calls "assimilating power".[4] He considers them the first true products of photosynthesis.

Recently Arnon developed a hypothesis which involved two phases of photophosphorylation: (1) cyclic photophosphorylation and (2) non-cyclic photophosphorylation. This hypothesis accounted for the electron donor and electron acceptor present in the process.

Cyclic Photophosphorylation

Photosynthetic phosphorylation in which ATP is the only product of light action is defined as cyclic photophosphorylation. This process was so named because the electron donor and acceptor is chlorophyll and because no outside donor is required. This type of photophosphorylation shown in reaction (a) is found in all photosynthetic bacteria since their chromatophores cannot carry out non-cyclic photophosphorylation.[5] This system is also found in green plants in a modified form.

In this process promoted by Arnon, the initial photochemical act involves the absorption of a photon of light. The quantum of energy excites a chlorophyll molecule with enough energy to remove an electron from it.

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[4]Bladergroen, p. 129.

[5]Ibid. p. 130.

The molecule having lost the electron is then in a position to act as an electron acceptor. The expelled electron may simply re-enter the chlorophyll molecule and cause it to fluoresce. This alone does not permit phosphorylation to occur.

In order for the absorbed light energy to be changed into chemical energy, the excited electron is captured by a molecule such as vitamin K. These electrons are then returned to the chlorophyll in a series of graded steps resembling those found in respiration. This path, the vitamin K pathway, takes the electron from vitamin K through a cytochrome component (CYT_I) and eventually back to the chlorophyll. (See Figure 4.) During this process enzymatic transformations occur which result in the formation of two molecules of ATP. The return of the electron restores the chlorophyll molecule to its unexcited state after which the system is ready to function again.[6]

Both photosynthetic bacteria and green plants exhibit cyclic photophosphorylation involving the vitamin K pathway for converting light into a single form of chemical energy ATP. All other energy conversion reactions or transport systems are considered to be modifications of this basic design.[7] This is significant from the aspect of biochemical evolution.

Non-cyclic Photophosphorylation

A second type of photophosphorylation found in green plants results in the production of molecular oxygen and reduced triphosphonucleotide ($TPNH_2$). The non-cyclic electron transport system is shown in Figure 4.

This process is similar to cyclic photophosphorylation in that it

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[6]D. I. Arnon, "Conversion of Light into Chemical Energy in Photosynthesis," Nature, 184 (1959), p.14.

[7]Ibid., p.15.

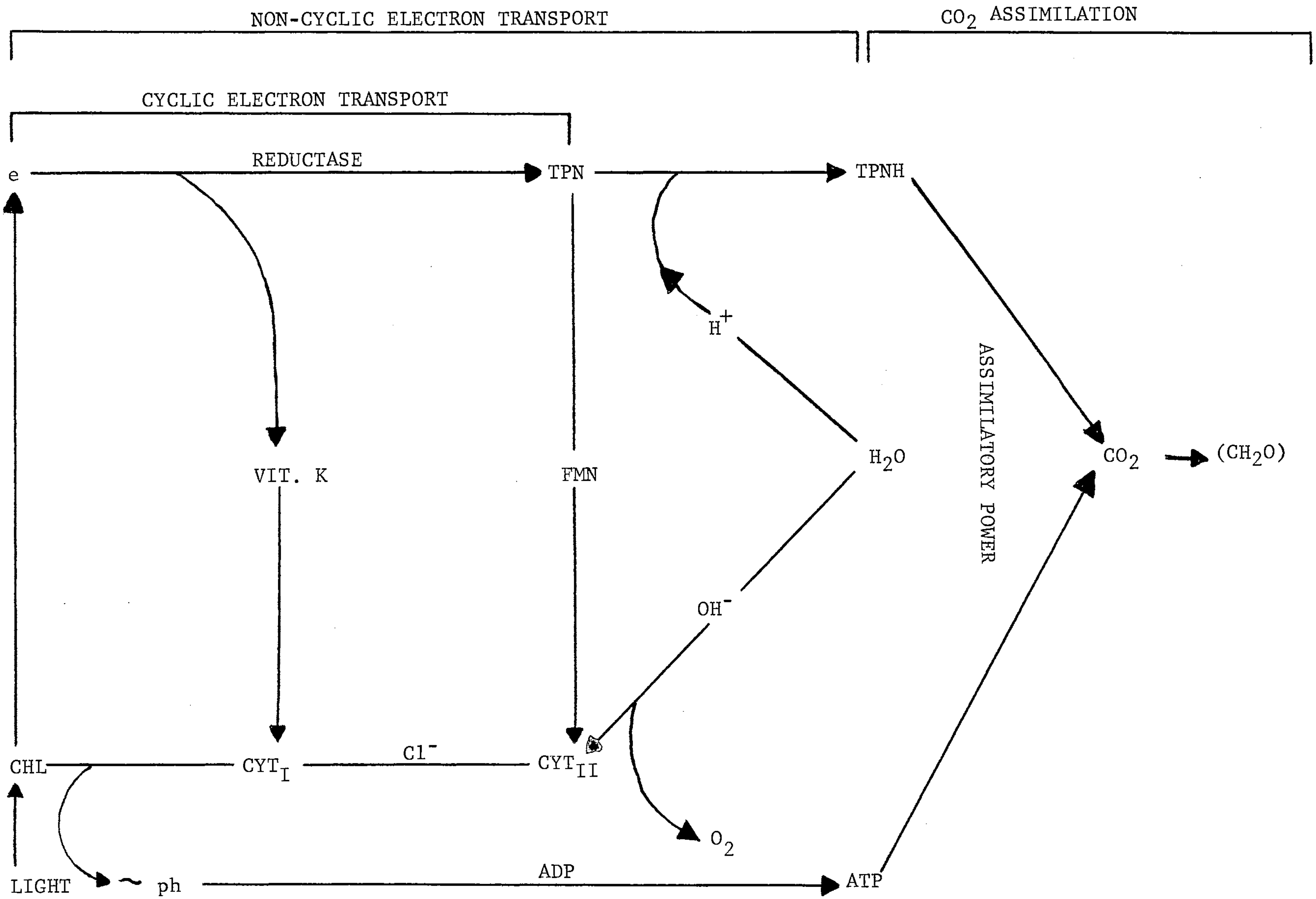

Figure 4. Photosynthetic Phosphorylation

shares the primary photochemical reaction and the phosphorylating site. It differs from the cyclic electron transport system in that the electron expelled from the chlorophyll molecule does not return to the chlorophyll system. This electron is removed by TPN together with H ions originating from the water in the presence of a light dependent reductase.

Replacement of the electron removed by non-cyclic transport is accomplished by the interaction of the hydroxyl ions (OH^-) with a cytochrome (CYT_{II}). This reaction produces molecular oxygen and donates electrons via the cytochrome chain to the chlorophyll which is then returned to its normal state.[8] The entire process of non-cyclic photophosphorylation is represented by reaction (b) which provides molecular O_2, characteristic of green plants, and $TPNH_2$ for the reduction of carbon dioxide.

Investigation by both Warburg and Arnon indicate the necessity of chloride (Cl) ions for the photosynthesis of green plants. Experiments point out that Cl ions are required for non-cyclic photophosphorylations and for the FMN pathway of cyclic phosphorylation. Chloride ions are not needed in the vitamin K pathway which is the only type of bacterial photosynthesis. In the absence of Cl ions chloroplast behave like chromatophores in being able to follow only the vitamin K passageway.

According to Van Niel's work, photosynthetic bacteria can also make use of external hydrogen dondors. These donors may be inorganic materials such as thiosulfate and organic acids such as succinate. The hydrogen in these substances does not have sufficient reducing capacity to convert pyridine nucleotide (PN) to PNH_2. More energy is needed.

A recently developed theory suggests that the electrons are transferred from the cytochromes to the chlorophyll replacing the ones removed

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[8]Ibid., pp. 14-15.

from the chlorophyll by the action of light. The oxidized cytochromes are then reduced by the action of thiosulfate and succinate and electrons are transferred by way of the cytochromes to the chlorophyll system. In the chloroplast they are raised by light energy to a reducing potential sufficient to reduce pyridine nucleotide.

In this example of non-cyclic photophosphorylation the activated electrons are eventually transferred to external receptors such as nitrogen gas which is converted to ammonia, PN which is converted to PNH_2 and protons which become hydrogen gas. It is evident in this non-cyclic process that light energy is used either to produce PNH_2 or to fix atmospheric nitrogen.[9] Table III shows a summary of the similarities and differences in photosynthesis of green plants and bacteria.

According to Arnon, the common denominator of photosynthesis in green plants and bacteria is the conversion of light energy absorbed by chlorophyll into assimilatory power in the form of phosphate bonds, not the photochemical formation of a carbon dioxide reductant. It also appears that the light reaction of photosynthesis serves only to produce ATP and $TPNH_2$ which are used in the dark reaction for carbon dioxide fixation.

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[9] Arnon, Scientific American, p. 115.

TABLE III

COMPARISON OF PHOTOSYNTHESIS IN GREEN PLANTS AND BACTERIA

	Green Plants	Photosynthetic Bacteria
Light	Cyclic Phosphorylation: $ADP + ph \longrightarrow ATP$ Non-Cyclic Phosphorylation: $2TPNH + 2H_2 + O + 2ADP + 2ph \longrightarrow$ $2TPNH + 2H_2 + O_2 + 2ATP$	Cyclic Phosphorylation: $ADP + ph \longrightarrow ATP$
Dark	Assimilation of CO_2: $CO_2 + 2TPNH + 2H^+ + nATP \longrightarrow$ $(CH_2O) + H_2O + 2TPN^+ + nADP + nph$	Reduction of PN: $2PN + 2H_2 \longrightarrow 2PNH_2 + 2H_2$ Assimilation of CO_2: $CO_2 + 2PNH_2^+ + 2H_2 + nATP \longrightarrow$ $(CH_2O) + H_2O + 2PN + nADP + nph$
Over-all	$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + O_2 + H_2O$	$CO_2 + 2H_2 \xrightarrow{\text{light}} (CH_2O) + H_2O$

CHAPTER VII

CONCLUSIONS

Recent research in the field of photosynthesis has indicated that the role of light in the initial photochemical step is simply to increase the energy of the electrons in chlorophyll. The succeeding reactions simply transport these excited electrons along varying paths until their energy is captured and changed to chemical energy.

Fundamentally, then, photosynthesis is chiefly concerned with the conversion of light into chemical energy. This is accomplished by cyclic photophosphorylation which results in the formation of adenosine triphosphate with no consumption of material substances. Carbon dioxide assimilation, oxygen evolution, nitrogen fixation, and hydrogen evolution are processes which the cell may or may not perform during photosynthesis.

The value of these studies is to bring about an understanding of the complex mechanism of forming chemical energy for it is plant-life which supplies all animal-life with its source of energy. If the present population boom continues, it may be necessary for scientists to find an inexpensive commercial process for food production. It is research of the type discussed in this paper which may someday bring about such a process.

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