Primary Processes in Photosynthesis

by

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Sorghum chloroplasts. Top: *Sorghum saccharatum* mesophyll chloroplasts. × 64,000. Lower left: Section from top. × 120,000. Lower right: Section from top. × 800,000. Areas which correspond are outlined and indicated by arrows.

Courtesy, Dr. J. D. McLean, see J. L. Farrant and J. D. McLean, *Proc. European Reg. Conf. Electron Microscopy, Delft, 1960,* 2, 1039 (1961).

Author's Preface

The shift in emphasis from the study of secondary to the study of primary processes in photosynthesis has generated a need for a text which can serve not only as an introduction for novices but also as an aid to those, expert in the older knowledge, who must accommodate themselves to rapid new developments in fields with the language of which they are not conversant. Such a text must not only present a clear account of present trends and underlying basic concepts, but must also relate these to older knowledge which still retains usefulness and validity. Moreover, it is essential that the resultant book be a small one.

I have approached this task in what I believe to be a wholly novel way, based on the emphasis of comparative aspects and relationships between the various sciences involved. By analysis of the photosynthetic process into successive "eras," the interrelation of the various disciplines is clearly outlined so that the reader, whether a novice or a sophisticate, can obtain a useful perspective of the process as a whole. This, together with a statement of a simple systematics, comprises the first chapter and sets the stage for the discussion of the photosynthetic apparatus in the second chapter, and the presentation of the picture of the primary processes given in the remaining two chapters.

This book ends where others begin. It deals only with events which precede the appearance of the more familiar secondary processes. The thesis is that much of the excitement is over by the time identifiable chemical products of photosynthesis appear. The bridge to the conventional text is supplied in a companion volume on biochemical aspects, written by Dr. A. San Pietro, the Editor of this series. These two volumes together should provide readers at all levels of sophistication with an adequate guide to the rapidly proliferating literature of modern photosynthetic research.

There is one hazard which plagues all writers on photosynthesis and which is best stated in terms of the famous saying, often quoted by C. F. Kettering and attributed to some early American sage: "It's not the things you know that kill you; it's the things you know that ain't so." My hope is that few "ain't so" statements have crept in.

With a few exceptions, no papers later than the end of 1960 were considered in the preparation of this text. This cutoff at 1960 coincides with the onset of a particularly active and fruitful period of photosynthesis research, which is still continuing and shows evidence of an acceleration in the rate of solid achievement. Although obsolescence of some material presented is inevitable, this should not handicap the reader because the emphasis is placed first on principles, rather than whatever factual aspects may be prominent at this particular stage in the development of the field.

As an aid in achieving continuity with the field as it develops, an extensive bibliography has been provided so that the reader will know the laboratories and investigators now active and the likely sources for modification of material in this text.

It is a pleasure to acknowledge the editorial assistance of Mrs. M. C. Bartsch and the suggestions of Beka Doherty Kamen in the preparations of the first draft, as well as the work of Miss S. Hosmer who typed portions of the final draft. I am most particularly indebted to Dr. M. Gonterman and Dr. D. Mauzerall for their careful reading of the text and for suggestions and corrections that they made. Various colleagues extended gracious permission to reproduce figures and illustrations. Acknowledgements are included at appropriate places in the text.

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University of California, San Diego
October, 1963

Editor's Preface

The vistas of biochemical research have been so greatly extended within recent years that it is virtually impossible to remain abreast of all present-day advances. The result has been that most scientists are forced to limit their attention only to that portion of the scientific literature which deals directly with their area of specialization. Of even more serious consequence is the fact that much of the graduate teaching in biochemistry in this country has become extremely specialized in its coverage.

Although the present series was designed primarily for the graduate student in the biological sciences, this does not preclude its usefulness to the research scientist. It is expected that each monograph will provide an introduction to one major area in biochemistry, with particular emphasis on the problems currently under investigation and the experimental procedures employed. The goal of the series is to provide the graduate student with insight and understanding of the scientific approach to problems of a biochemical nature. The extent to which this is achieved will determine the success of the series.

I wish to express my gratitude to all authors of individual monographs and especially to Dr. Martin Kamen for the conscientious preparation of this monograph, which is the first in the series. I am indebted to the staff of Academic Press for their unbounded patience and kindness during the preparation of this work.

ANTHONY SAN PIETRO

Yellow Springs, Ohio
August, 1963

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CHAPTER I

The Natural History of Photosynthesis

I. Definitions

Few phenomena in natural science equal photosynthesis in sweep and grandeur. It suffices to cite as evidence the annual yield of organic matter from photosynthesis which is estimated to be between 10^{10} and 10^{11} tons—an amount equivalent to the weight of a square slab of concrete some hundreds of miles on the side and several stories high, or the weight of metropolitan New York City.

Before anything more is said about photosynthesis, it should be defined. But making a definition of so complex a process is not easy. Photosynthesis begins in the recesses of radiation physics and ends in the far reaches of ecology. Investigators from every branch of science can probe it, and must, if it is to be understood completely.

In the present state of knowledge, any definition tends to be an oversimplification which reveals more about the observer than the process. I may begin with this statement. *Photosynthesis is a series of processes in which electromagnetic energy is converted to chemical free energy which can be used for biosynthesis.*

This rather noncommittal definition, which hardly warrants italics, bears no obvious relation to those usually encountered in textbooks on general biology or chemistry. Most commonly, photosynthesis is thought of as a process in which green plants, with the aid of chlorophyll and light, convert carbon dioxide and water to carbohydrate and molecular oxygen. Variations on this theme occur in which the carbohydrate may be formaldehyde, glucose, or starch. This concept prevails among all sections of the literate public. In a few specialized monographs the occurrence of photosynthesis in bacteria and other living systems is recognized, but only rarely are such phenomena considered as other than atypical or of secondary importance. In more sophisticated treatises, the attempt is made to achieve some resolution of the various processes involved. Often the primary process is described as a "photolysis of water" in which assimilation of carbon dioxide is coupled with secondary non-photochemical processes.

II. A Small Aside on Traditional Attitudes

The Idols of the Tribe, the Idols of the Cave, the Idols of the Market Place, the Idols of the Theater—these, the reader will recall, were the demons Francis Bacon set out to exorcise over three centuries ago. These idols were his symbols for intellectual frailties, such as premature generalization, specious rationalization, and semantic confusion.

Among investigators of photosynthesis, as among humans in general, there are found many addicted to the worship of these idols. This idolatry is characterized by two massive syndromes which may be termed "mammalian chauvinism" and "temporal solipsism."

When mammalian chauvinism holds sway, the victims ascribe primary importance only to those aspects of photosynthesis which produce results directly beneficial to mammals, and, in particular, to that major aerobe, man. Hence, their analysis is dominated by awareness of the phenomenon of oxygen production which, in turn, leads to their neglect of processes in which photosynthesis is not characterized by oxygen evolution.

A leader in the reaction against mammalian chauvinism has been the distinguished microbiologist, C. B. Van Niel, whose vigorous espousal of the comparative biochemical viewpoint (1) has done much to liberate research from its unbalanced emphasis of green plant photosynthesis.

When temporal solipsism is dominant, its victims accord attention only to events which occur in intervals of time they can sense or measure directly. This condition is probably more widespread than mammalian chauvinism. To overcome this condition, it is necessary to "think exponentially," that is, one must learn to regard the time interval between 10^{-15} and 10^{-14} seconds as of an importance comparable to that between 10^{1} and 10^{2} seconds. Both involve equal changes in magnitude. As I will attempt to make clear in the next section, the habit of thinking in magnitudes of time will lead to a perception of the photosynthetic process which minimizes effects of such conditions as mammalian chauvinism and temporal solipsism, in which, to quote Bacon, "everyone has a cave or den of his own which defracts and discolours the light of nature."

III. The Time Sequence in Photosynthesis

I propose to start with an analysis of photosynthesis as a sequence of events which occur in successive "eras." Figure 1 shows a scheme for such an analysis. It is convenient to adopt, as an expression of time magnitude, the common logarithm of time in seconds. By analogy with the well-known symbol "p," which stands for "logarithm of reciprocal of" in symbolic expressions such as pH, p*K*, etc., "pt_s" will stand as an abbreviation for "logarithm of the reciprocal of time, expressed in seconds." [1]

Thus, the era between 10^{-15} and 10^{-9} seconds is expressed as pt_s +15 to pt_s +9. This happens to be the time interval which includes the first, or strictly physical, phase of photosynthesis. This era begins with the absorption of the radiant energy as a quantum of visible or infrared light. The limit of pt_s +15, as well as all the others shown between the successive eras, as pictured in Fig. 1, will be rationalized in succeeding chapters. It suffices to state here

[1] I am indebted to Professor D. Gutsche, Washington University, St. Louis, Missouri, for this suggestion.

that the time required for completion of the primary absorption act corresponds to the extreme limit of pt_s +15.

The physical phase continues through the initial con-

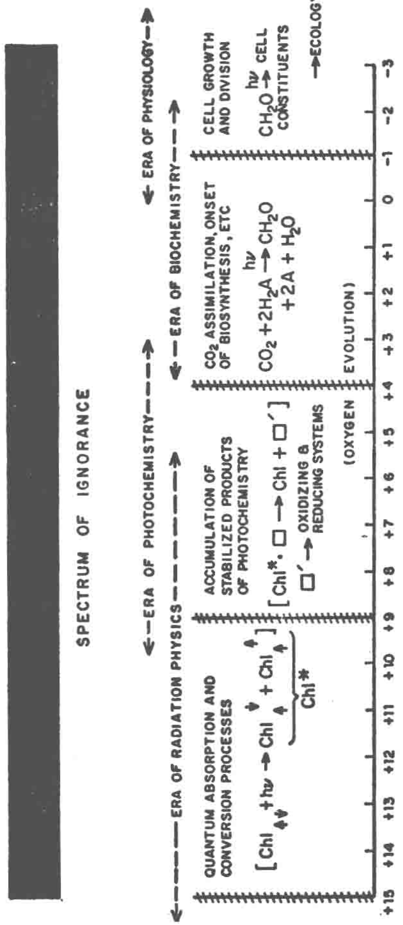

FIG. 1. The eras of photosynthesis (see text).

version of the quantum energy to its first level of stabilization as excitation energy for the primary photochemical process. This stabilization must occur before pt_s 9, as this is the limit set by the possibility that the initial quantum absorption may be reversed by loss of light energy through re-emission as fluorescence. There is possible a dispensation for a longer time period with a limit of pt_s 5, because the excitation energy may be stabilized further by formation of a so-called "metastable triplet" state. However, this possibility is set aside for the present, and pt_s 9 is chosen as the extreme limit on the long time side of the physical era. This gives six orders of magnitude in time for processes involving radiation and solid state physics.

Next, starting at pt_s 9, perhaps sooner, and extending to the time when enzyme-catalyzed chemical reactions inaugurate biochemistry, there is the era of photochemistry. Because enzyme reactions have turnover times with characteristic values no faster than pt_s 4 to 3, the photochemistry phase is set between the limits pt_s 9 and pt_s 4. The photochemistry which occurs in this era completes the conversion of quantum energy to free energy which is available for biochemistry and synthesis. This process lasts five orders of magnitude in time.

With the onset of the biochemical era, there are encountered for the first time processes which effect carbon dioxide assimilation, biosynthesis, and, in the particular case of green plant photosynthesis, evolution of molecular oxygen. Thus, only after passage through eleven orders of magnitude in time, eleven pt_s units, does one arrive at a point where most textbook expositions of photosynthesis begin!

The biochemical era lasts until it merges into the physiological era. The slowest biochemical reactions which correspond to cellular synthesis have turnover times which set the upper limit in time at pt_s -1. Somewhere in the era pt_s $+1$ to pt_s -1, one may discern the beginning of physiology—the interplay of biochemical metabolism and cellular organization. This physiological era may be said to end with the replication of the cell at pt_s -3 to pt_s -4. Beyond this, there is the domain, or era, of botany and ecology.

From the beginning to the end of this process, one notes a total time lapse extending from pt_s $+15$ to pt_s -4, some nineteen orders of magnitude in time expressed in seconds. It is sobering to note that the whole history of the cosmos, in years, is estimated to be only ten or eleven orders of magnitude in time. Thus, the reader can appreciate the vast sweep of photosynthesis, when expressed relative to the pt_s unit, even though such a unit is admittedly an artifice for telescoping events in time.

The problems of interest to contemporary research can be presented also in Fig. 1 by assigning a density of shading to each era shown. The density at each point is proportional to the ignorance about the nature of the process at that point. Greater density of shading implies greater ignorance. The reader will see that, relatively speaking, more is known at the extreme ends of the photosynthetic process and little in the middle. At pt_s 15, there is much factual and theoretical knowledge which belongs in the general area of radiation physics, and which can be applied to achieve some understanding of the act of quantum absorption. Almost immediately, say at pt_s 13, ignorance begins to increase, reflecting the small store of facts about the structure of the photoactive unit functional in the energy conversion process. Still, there is a great reservoir of solid state physics to lighten the gloom in this era.

The shades deepen on approach to pt_s 9, the era of photochemistry, for now there is inadequate theoretical knowledge and there are few facts. The emergence into light does not begin until somewhere in the biochemical era, pt_s 3, and becomes more pronounced, until one stands at pt_s −1 and beyond, in eras descriptions of which include all the vast amount of literature on photosynthesis compiled by botanists, plant physiologists, and farmers.

The passage through all these eras requires learning the languages of radiation physics, solid state physics, photochemistry of condensed systems, quantum chemistry, biochemistry, enzymology, plant physiology, and descriptive biology. It is hardly surprising that lack of communication often exists among different investigators in the many areas of photosynthetic research.

IV. Another Look at Definitions

Now, imagine the sorts of definitions the various eras of photosynthesis might inspire. The temporal solipsist, making a living as a radiation physicist, will see only from pt$_s$ 15 to pt$_s$ 9, or perhaps to pt$_s$ 5—not such a small vista! He may propose to define photosynthesis as "a process in which quanta absorbed in a semi-ordered aggregate are converted and trapped with high efficiency in various metastable states of electronic excitation." If he is pressed to present a formulation of this process, he will write an expression such as that shown in the box for the physical era in Fig. 1, i.e.,

$$\mathrm{Chl}_{\uparrow\downarrow} \xrightarrow{h\nu} \mathrm{Chl}_{\uparrow}^{\downarrow} \quad \text{and/or} \quad \mathrm{Chl}_{\uparrow}^{\uparrow} \tag{1}$$

where "Chl" stands for the photoactive compound, that is, chlorophyll, bacteriochlorophyll, bacterioviridin, etc. The meaning of this equation, with its various symbols, will be elaborated in Chapter III.

The temporal solipsist, whose profession is that of photochemist, will see little but the era from pt$_s$ 9 to pt$_s$ 4. This, again, is no small reach in time. His definition might be that photosynthesis is a process "in which electronic excitation states of chlorophyll (bacteriochlorophyll, etc.) are selectively quenched in a series of chemical reactions leading to production of stabilized (ground-state) energy-rich systems characterized both by electron excess and by electron deficiency." A formulation based on the era of photochemistry could look like the symbolic reaction shown in Fig. 1, i.e.,

$$\mathrm{Chl}^{*}\cdot\Box \rightarrow \mathrm{Chl} + \Box' \begin{cases} \nearrow \text{oxidants} \\ \searrow \text{reductants} \end{cases} \tag{2}$$

An explanation of the meaning of these symbols must be deferred to Chapter IV.

The biochemist, whose temporal solipsism constrains his attention to pt_s +4 to pt_s −1, will see the reactions involved in carbon dioxide assimilation, oxygen evolution, and metabolism of organic substrates. He will be many eras removed from the quantum process. Most of what he sees will be the nonphotochemical phases of photosynthesis. His definition of photosynthesis will convey the notion of a process in which a photochemical act causes "dehydrogenation of hydrogen donors and reduction of carbon dioxide, mediated by simultaneous synthesis of 'energy-rich' catalysts, such as adenosine triphosphate, acylated coenzymes, etc." A simple formulation will be the reaction, originally proposed by Van Niel, shown in Fig. 1, i.e.,

$$CO_2 + 2H_2A \xrightarrow{h\nu} CH_2O + 2A + H_2O \qquad (3)$$

where "H_2A" is a general hydrogen donor, "A" is a radical (organic or inorganic) generated by oxidation of H_2A, and "CH_2O" is cellular material in which carbon is at the oxidation level of carbohydrate.

The plant physiologist, constrained to pt_s 1 and beyond, will think of photosynthesis as a process in which "cellular growth is supported at the expense of light energy absorbed by chlorophyll." He will be addicted to proposals such as the classic equation,

$$6CO_2 + 6H_2O \xrightarrow{h\nu} 6O_2 + C_6H_{12}O_6 \qquad (4)$$

or perhaps its less committal generalization,

$$nCO_2 + nH_2O \xrightarrow{h\nu} nO_2 + (CH_2O)_n \qquad (5)$$

The bacterial physiologist, seeing as products no oxygen and often little carbohydrate, will be no less a temporal solipsist and will revert to some form of Eq. (3).

The reader, by now, will feel that I have labored this matter of definition sufficiently. He will also feel that a general definition, satisfactory to all concerned, must remain no more specific than some general statement such as that proposed at the beginning of this chapter.

V. Systematics of Photosynthesis

The various photosyntheses are classified traditionally on the basis of nutritional requirements for growth. Usually, the *minimal requirement for photosynthetic growth* is chosen as a decisive criterion. This means that the point of reference is taken somewhere at the end of the natural history of the process, i.e., at $pt_s \sim -3$). Only a limited systematics can result from the use of such a reference point.

A more fundamental system of classification requires that a point of reference be picked as near to the onset of chemistry as is possible, to permit a generally valid statement in terms of the simplest possible stoichiometry. In the present state of knowledge, as one may infer from the spectrum of ignorance in Fig. 1, such a point will be somewhere at the start of the biochemical era ($pt_s \sim +3$). Future progress will see the point of reference pushed further toward the left in Fig. 1.

Before attempting to set up a system for classifying the various photosyntheses, it will be convenient to use the concept of a chemical process as a sum of partial reversible processes, and, in particular, of processes which can be expressed as partial reversible "electrode" reactions. These can be imagined to occur at the electrodes of a galvanic cell set up so that the summation of the electrode reactions, as written, yields the over-all process in question, as well as the free energy change involved. In applying such a procedure to photosynthesis, one must identify reactants and products in some stoichiometric relations and in defined standard states. Further, one must find a reaction in terms of which all others that are known can be included, at least insofar as total energy requirements are concerned.

Photosynthesis, as performed by green plants, has a special advantage with respect to energy requirement, because it alone exhibits two simultaneous energy-storing processes,