

PHOTOPHYSIOLOGY

**CURRENT TOPICS IN PHOTOBIOLOGY
AND PHOTOCHEMISTRY**

ARTHUR C. GIESE

VOLUME VII

PHOTOPHYSIOLOGY

CURRENT TOPICS IN PHOTOBIOLOGY AND PHOTOCHEMISTRY

Edited by

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PREFACE

While the present series of *Photophysiology* reviews primarily current findings on fundamental problems in photochemistry and photobiology, such findings also provide the background for a better understanding of the practical problems resulting from man's interference with the photic environment of the earth to which life is adapted.

To an increasing degree it is shown that photobiology and photochemistry impinge on basic problems in science and practical ones as well. Thus, for example, "dark repair" which was first discovered in studies on recovery of cells from injury by ultraviolet (UV) radiation now is understood to repair injury caused by factors other than radiation and to correct mistakes made in normal replication of DNA in cells. Radiation studies also promise to reveal the nature of mutation in cells. As for the practical importance of photobiological studies, consider the supersonic transport (SST) controversy. It is thought that the exhaust from the SST will catalyze the destruction of ozone in the stratosphere where the SST flies. Any reduction in ozone of the stratosphere would decrease the capacity of the ozone layer to absorb the sun's incoming far-UV radiation which is lethal to all living things. Without further study it is difficult to predict how extensive damage would be to life on land, in fresh water, and in the sea, or on the ecological relationships in these environments. Increase in skin cancer in man is another likely result of increased far-UV radiation in sunlight reaching the earth.

For these reasons and others, continuation of the series is deemed worthwhile.

I wish to thank the authors for their cooperation and their contributions to the present volume, and to the outgoing editorial board goes my appreciation for their suggestions and help on the present volume and previous volumes in the series.

ARTHUR C. GIESE

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Chapter 1

ELECTRON RESONANCE STUDIES OF PHOTOSYNTHETIC SYSTEMS

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1. Introduction

Photosynthesis is a biological process which results in the abstraction of an electron from a reduced compound and the transfer of this electron to a pyridine nucleotide which can then furnish the reducing power to convert carbon dioxide to organic substances. The passage of the electron also generates adenosine triphosphate (ATP) and is driven by visible or near-infrared light. It is assumed that the reader is familiar with modern concepts of the mechanism of the process, which is basic to the existence of all living things on earth, and probably anywhere else

in the universe [see Clayton (1971)³ and Smith (1971) for discussions of general photosynthesis].

Electron paramagnetic resonance (called EPR, or ESR for electron spin resonance) spectroscopy is based on the fact that substances containing unpaired electrons are paramagnetic. This paramagnetism may be due to the presence of transition elements which have unfilled shells, or it may be due to the transitory presence of oxidized or reduced substances. In the case of photosynthetic materials, this oxidation and reduction (i.e., the transfer of an electron from one substance to another) is set in motion by light. It is this conversion of electromagnetic energy to chemical energy which can be used for biosynthesis which is at the heart of photosynthesis. The transitory paramagnetic state induced by light and methods of detecting it by EPR spectroscopy are the subjects of this chapter.

The difference in paramagnetism of photosynthetic materials in the light and in the dark was discovered in 1956 (Commoner *et al.*, 1956). In the next five years, the two prominent light-induced resonances in plants and the single one in bacteria were described, and numerous speculations on their origin and significance were advanced. It was also discovered that light would induce a paramagnetic state in concentrated solutions of chlorophyll and in crystalline chlorophyll. This seeming evidence that paramagnetism was associated with chlorophyll per se, and was not necessarily a function of the photosynthetic process, made it less interesting to those in search of an explanation for the mode of action of light on plants. This supposition, however, was not true; we now know that light-induced resonances in photosynthetic organisms or in subcellular preparations of them are indeed probes into both early and later events essential to the overall process of photosynthesis.

There are several intrinsic advantages to the EPR method for the study of photosynthesis. The detecting radiation of the spectrometer is in the microwave region of the spectrum. Most commercial spectrometers employ a frequency of about 9.5 GHz, which is a wavelength of about 3 cm. This is less energetic than light in the near ultraviolet by a factor of 10^5 ($3000 \text{ \AA} = 3 \times 10^{-5} \text{ cm}$) and is probably far too weak to induce any transitions in living matter. If high microwave powers are used, heating can result, but except for such thermal effects there is no known and specific effect on tissue. This means that the system being observed is not disturbed by the means of observation. An unavoidable drawback of light spectroscopy is that the measuring beam inevitably affects the observed system, and certain assumptions must be made about the magnitude of this effect. (This statement is not meant in any way to detract from the enormous and essential contributions of light

spectroscopy in all its aspects to the study of photosynthetic systems.)

A second advantage of the EPR method lies in the fact that only molecules undergoing oxidation-reduction reactions in response to light are detected. The great bulk of chlorophyll, about 99%, serves merely to intercept photons. These molecules are passive and do not participate in chemical reactions under normal photosynthetic conditions, and hence are "invisible" by EPR spectroscopy. Again, the situation may be contrasted to that of spectroscopy in the visible region, where the absorption of bulk pigments represents an enormous background against which absorption changes by a small percentage of molecules must be detected.

Water itself is a very strong absorber of microwave energy. For this reason, early EPR experiments on biological systems used lyophilized material. However, the sensitivity of the modern spectrometer is such that this attenuation can be tolerated and whole cells, chloroplasts, and subcellular preparations can be observed under physiologically favorable conditions.

This chapter will endeavor to give the reader an appreciation of the types of information to be gained by EPR spectroscopy in the study of photosynthesis, a statement on the basic phenomenon and some explanation of the reasons for instrumental design, an outline of experimental methods, an overview of current knowledge, and a glimpse of possible future developments. It will make no attempt to be exhaustive, but rather will be selective; it is planned as a sequel to Chapter 3 of the first volume in this series.

2. Electron Paramagnetic Resonance (EPR) Techniques

This section is concerned with a sketch of the characteristics of the paramagnetic resonance phenomenon and how these characteristics influence the design and performance of instrumentation. Blois and Weaver (Volume I of this series) described briefly the broad classification of matter as regards magnetic properties. There were materials exhibiting diamagnetism and paramagnetism and those exhibiting ferro- and anti-ferromagnetism. We will deal in this section **only** with the detection and analysis of very dilute paramagnetism in a diamagnetic matrix. The detection and characterization of a paramagnetic state in an intrinsically nonmagnetic medium is of prime interest here.

For a system to exhibit paramagnetism there must be present unpaired electrons whose recombination rates are slow enough to be observable; for biological systems of interest here, the observable rates are from a few microseconds (limited by the instrument) to essentially infinite in some cases in the frozen state. Reversible effects are the rule