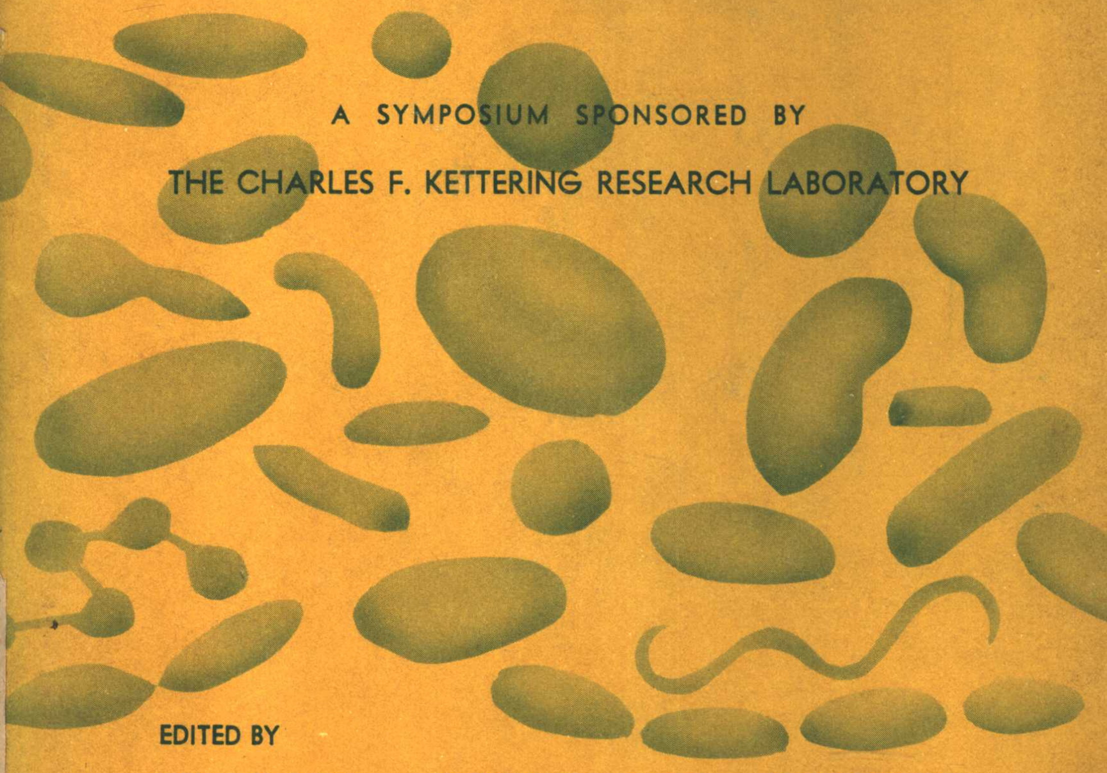
BACTERIAL PHOTOSYNTHESIS



A SYMPOSIUM SPONSORED BY

THE CHARLES F. KETTERING RESEARCH LABORATORY

EDITED BY

HOWARD GEST • ANTHONY SAN PIETRO • LEO P. VERNON

BACTERIAL PHOTOSYNTHESIS

Edited by

HOWARD GEST
Washington University
St. Louis, Missouri

ANTHONY SAN PIETRO
Charles F. Kettering Research Laboratory
Yellow Springs, Ohio

LEO P. VERNON
Charles F. Kettering Research Laboratory
Yellow Springs, Ohio

A SYMPOSIUM SPONSORED BY

The Charles F. Kettering Research Laboratory

INTRODUCTION

This book contains the papers presented at a small, invitational Symposium on Bacterial Photosynthesis held in Yellow Springs, Ohio on March 18-20, 1963. The Charles F. Kettering Research Laboratory was very pleased to serve as host for this conference. Research progress on bacterial systems is moving rapidly and a review of the present state of knowledge seemed appropriate. The organizing committee therefore invited some fifty-five overseas and American investigators to meet and exchange information at a small, informal meeting held under the Foundation's auspices. The staff of the Charles F. Kettering Research Laboratory was stimulated immensely by the conference; we hope these papers will serve as a point of departure for additional photosynthetic investigations.

E. W. Kettering

Left to right: H. Gest, H. Gaffron, C. B. van Niel, R. Hill, L. P. Vernon, A. San Pietro.

To KEES

In recognition of his pioneering research on bacterial photosynthesis, this volume is dedicated to Dr. C. B. van Niel, Herzstein Professor of Biology, Stanford University.

PREFACE

The past ten years have witnessed a rapidly increasing tempo of research on bacterial photosynthesis. This is perhaps attributable, in part, to the fact that modern developments in microbiology and biochemistry have demonstrated the potential advantages of using bacteria as the source of experimental systems for investigation of numerous basic biological phenomena. The relatively large, and possibly unique, range of metabolic capacities shown by the photosynthetic bacteria has added to their appeal for such studies. There is little doubt, however, that the main stimulus for closer scrutiny of these organisms stems from the desire to understand the "comparative biochemistry" of photosynthesis in greater depth. Although considerable evidence has accumulated showing close similarities between green plant and bacterial photosynthesis, investigators have long been intrigued with the reasons for, and possible implications of, the differences observed between the two processes. Indeed, when a fundamental research advance is made with either type of photosynthetic system, pertinent reexamination of the other soon follows. This pattern of cross-checking, which has become more prominent in recent years, has unquestionably facilitated progress in elucidation of the mysteries of photosynthesis.

In the past, however, symposia on this important topic have been concerned primarily with green plant systems and only secondarily with photosynthetic bacteria. This realization and the conviction that an up-to-date review of the problem would stimulate further progress led to organization of the present symposium, devoted exclusively to the bacterial process. Inevitably, we were faced with the usual dilemmas posed by the attempt to arrange a meeting at which all investigators actively working in the field would be present and able to exchange ideas and viewpoints freely under informal circumstances. It is our hope that any shortcomings in this respect will be ameliorated by our efforts to make the proceedings of the symposium available to the scientific community at the earliest possible time.

A number of animated controversies developed during the course of the meeting and this we interpret as one of the signs of its success. Groups of participants directly interested in the debated questions met informally, as time permitted, with the aim of resolving basic issues. Our deepest gratitude goes to Dr. Martin Kamen who undertook the formidable task of presenting their conclusions to the symposium audience during his summarizing remarks.

It is a pleasure to acknowledge the valuable services of the following conveners: Dr. W. Arnold, Dr. C. S. French, Dr. R. Y. Stanier, and Dr. C. B. van Niel. Special notes of gratitude are due to Dr. R. K. Clayton and Miss Jane Finney for their editorial assistance and to Mr. Justin C. Crawford for his capable efforts in making the necessary arrangements. The editors are also indebted to those who contributed to the appendices, which contain frequently required experimental data and bibliographies of areas which could not be adequately covered due to lack of time.

We are particularly grateful to Antioch College for providing an auditorium and other attractive facilities for the symposium, and to the Charles F. Kettering Foundation for financially supporting the symposium.

The Editors

SYMPOSIUM PARTICIPANTS

William Arnold
Daniel I. Arnon
Howard Bales
Herrick Baltscheffsky
Robert Bartsch
Helmut Beinert
C. C. Black
Lawrence Bogorad
Subir Bose
Thomas Brown
Bruce Burnham
Warren Butler
J. E. Carnahan
Britton Chance
R. K. Clayton
Germaine Cohen-Bazire
S. F. Conti
J. C. Crawford
C. Stacy French
Albert W. Frenkel
Keelin T. Fry
R. C. Fuller
Hans Gaffron
David Geller
Howard Gest
Martin Gibbs
J. C. Goedheer
Norman Good
Govindjee
John J. Heise
Robin Hill
G. Hind
S. Hood
Takekazu Horio
S. Ichimura
Andre T. Jagendorf
Synnøve Liaaen Jensen
Martin Kamen
Bacon Ke
D. L. Keister
Bessel Kok
H. V. Knorr
June Lascelles
R. A. Lazzarini
Gene Lindstrom
Berger Mayne
Bernard S. Meyer
R. M. Miller
S. Morita
Jack W. Newton
Mitsuo Nishimura
John Olson
John J. Ormerod
Norbert Pfennig
Jose Ramirez
Ronald Ruby
Harry Rudney
A. San Pietro
Kenneth Sauer
G. R. Seely
W. R. Sistrom
Robert Smillie
Lucille Smith
Roger Y. Stanier
Carroll A. Swanson
Christiaan Sybesma
Kunio Tagawa
R. W. Treharne
C. B. van Niel
E. H. Vause
L. P. Vernon
Wolf Vishniac
W. Zaugg

LIST OF ABBREVIATIONS

A(OD)	absorbance (optical density)
ADP	adenosine diphosphate
ATP	adenosine triphosphate
BChl	bacteriochlorophyll
Chl	chlorophyll
pCMB	p-chloromercuribenzoate
Cyt	cytochrome
DCMU	3-(3-4-dichlorophenyl)-1,1-dimethylurea
DPIP, $DPIPH_2$	2,6-dichlorophenolindophenol and its reduced form
EDTA	ethylenediaminetetraacetic acid
ev	electron-volt
ESR (EPR)	electron spin resonance
FAD, $FADH_2$	flavin adenine dinucleotide and its reduced form
FMN, $FMNH_2$	flavin mononucleotide and its reduced form
IDP	inosine diphosphate
ITP	inosine triphosphate
mv	millivolt
NAD, NADH (DPN, DPNH)	nicotinamide adenine dinucleotide and its reduced form
NADP, NADPH (TPN, TPNH)	nicotinamide adenine dinucleotide phosphate and its reduced form
PCMB	*p*-chloromercuribenzoate
Pi	orthophosphate
PMS (MPM)	phenazine methosulfate (methyl phenazonium methosulfate)
PPNR	photosynthetic pyridine nucleotide reductase
PQ	plastoquinone
RHP	*Rhodospirillum rubrum* heme protein (cytochromoid C)
Tris	tris(hydroxymethyl)aminomethane
UQ (CoQ)	ubiquinone

CONTENTS

OPENING ADDRESS

Opening Address

VAN NIEL'S THEORY: THIRTY YEARS AFTER

HANS GAFFRON
The Florida State University, Institute of Molecular Biophysics (Fels Fund)
Tallahassee, Florida

Ladies and Gentlemen:

Considering that we are, as I thought we would be, a gathering of experts, there is really not much excuse for an opening address. The purpose of such an address is probably to remind the participants of the few major problems which the meeting is about, in order that we do not lose sight of them when we begin to discuss the countless details and ramifications into which a topic like Bacterial Photosynthesis must of necessity be subdivided. On the other hand we have had lately symposia and meetings contributing to the problem of photosynthesis at the rate of one or two per year. It is unlikely, therefore, that any one of us could have lost sight of the major problem.

Even with modern teamwork, progress in terms of essential new discoveries is not so fast that a proposed meeting here and there could not be skipped. But the fact that we are all here shows that the idea of the organizing committee to hold this particular symposium must nevertheless have struck today's guests as an attractive proposition.

Two reasons can be pointed out immediately. Though we have been acquainted with the Kettering Foundation as a place of research in our field since the days of Inman, Albers and Knorr, Rothemund and later of Clendenning and Eyster, the Laboratory has lately undergone a rebuilding and an expansion which has moved it into the front line of modern research on the photochemistry in living cells.

One attraction must have been the desire to visit the Kettering Laboratory, and the second was the idea to single out the phototrophic bacteria for special consideration. This plan has automatically brought together not only the young keen minds for whom history begins after 1945, but also those of us who, in a much more leisurely way than is the fashion today, began once upon a time to investigate those reactions which still provide so much material for lively discussions.

A look at the elegantly done Symposium program has sharpened our anticipation of the coming intellectual pleasures. Mr. Kettering and Dr. Vernon deserve thanks indeed for having called us together.

And then there was the prospect that we might have among us our good colleague, the eminent and wise scholar Cornelis Benardus van Niel of Pacific Grove, whom his friends and pupils call Kees. Actually

I did not believe he would show up—too many of our meetings during past years had to be held without him. But to my surprise, and to everybody's pleasure, he arrived yesterday evening.

Our program promises us the description of quite a number of new observations, experimental techniques and contributions for or against certain hypotheses aimed at explaining the particular kind of metabolism that sets the purple bacteria apart from the green plant.

Hardly any one of us who were around twenty years ago would have believed that van Niel's idea of a photolysis of water as the core of the photosynthesis problem could still elicit a vivid discussion today. For the green plants it had been proven as correct by Hill's reaction. And as a reasonable interpretation also for the anaerobic photometabolism of purple bacteria there was the indirect evidence of the adaptable hydrogenase-containing algae.

Purple bacteria furnished van Niel the key to the first generally convincing picture of the photosynthetic process in terms of modern metabolic ideas. And purple bacteria are now believed to provide clear evidence that a photolysis of water—water as an intermediate hydrogen donor—should not be accepted as part of the hypothetical picture for bacterial photosynthesis. That is, van Niel's generalization of 1935 is disallowed.

It is about this question mainly that I would like to speak to you. Usually after thirty years a theory ought to have been transformed into fact or replaced by a better one. With van Niel's theory it so happened that after ten years there were no doubts left that the oxygen of photosynthesis originates from water. This we have accepted as fact.

I propose to show that, like any good scientific theory which managed to live in these hectic times for thirty years, van Niel's extended version is still useful. A truly good theory never dies—it only becomes more refined. This may make it more difficult to explain and to teach—but it does not render the simpler version wrong.

It is often repeated that one new fact which does not fit destroys a hypothesis. This is not true. As long as this new observation does not give birth to a better theory—and better is by definition the more encompassing view—it should be noted but treated as if with a little more thought and patience it may soon find its place within the existing order.

We have accepted the proposition that light will split, oxidize, dehydrogenate, or photolyze water in green plants, because on the face of so much evidence we cannot explain from where else the oxygen could originate. On the other hand, purple bacteria do not evolve oxygen. Why should we assume that water is involved in the photochemical process, even as an intermediate and incomplete process, when there is as yet no incontrovertible evidence that the assumption is warranted? How sound a viewpoint—and what a dull one. As I pointed out recently somewhere else, the mechanism to release oxygen from water with eight quanta is too remarkable and complex a mechanism not to have a long evolutionary history. And there are too many parallels in the behavior of photosynthetic bacteria and plants not to be intrigued by what I am willing to call the more interesting and therefore more rewarding hypothetical proposition. And perhaps I am biased because it took me once so long to recognize its elegance.

Certainly thirty years ago I simply could not see why I should accept van Niel's proposition that organic substances serve purple bacteria exclusively as hydrogen donors (just like H_2S, sulfur or hydrogen) for the reduction of carbon dioxide to carbohydrate, and thence to bacterial substance.

My own results with purple bacteria did not show this at all. Quite independently (never having heard of van Niel) I had started about 1929 on investigations on purple bacteria after Warburg had mentioned to me that Stalfeld had told him of these strange organisms. As a chemist I had never looked at a microbe before and knew only Warburg's great discovery—the alga *Chlorella*. Soon I discovered that the reddish microbes behaved quite differently from green plants. They refused to do photosynthesis but evidently ate organic acids in the light without further ado, either with a stoichiometrically determined amount of carbon dioxide, or, if available, also with hydrogen. The product of the photometabolism was partly a substance ($C_4H_6O_2$) (which Hans Fisher later depolymerized into crotonic acid) and for the greater part just more bacteria. Later, when working with *Chromatium*, the purple sulfur bacteria, I found that they produced lots of H_2S in the dark, particularly when previously illuminated in presence of butyrate. So I concluded that the light reactions with sulfur were reversible and that this was the mechanism by which they were able to attack organic substances. Many of you will remember that van Niel challenged this vigorously. Years later Henley in my laboratory confirmed the fermentative sulfide formation from internally stored sulfur but not from sulfate. My observation of a particular accelerating effect of added sulfate was indeed, as van Niel had shown, a nonspecific salt effect.

In 1935 van Niel extended his special theory so that it included also the metabolism of the heterotrophic purple bacteria. In this paper he quotes Gaffron's statement that photosynthesis of the purple bacteria involves the cooperation of a larger number of molecules and that several intermediate reactions occur before the first stable reaction products appear. Van Niel then wrote, "This statement seems to contain an argument against a unified concept of photosynthesis in green plants and purple bacteria." Because I could not see eye to eye with a Dr. Roelofsen, working in Kluyver's laboratory, van Niel had spent a year in Holland devising a good number of experiments to prove convincingly that sulfur bacteria can use organic substances directly as hydrogen donors, just like the Athiorhodaceae. He then came to Berlin to see me, we set up one or two experiments, they were absolutely