



**Benchmark Papers
in Microbiology / 13**

A BENCHMARK® Books Series

**MICROBIAL
RESPIRATION**

Edited by

WALTER P. HEMPFLING



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University of Rochester



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SERIES EDITOR'S FOREWORD

Knowledge of the respiration of procaryotes is not as extensive as the knowledge of mitochondrial respiration. As Dr. Hempfling points out, this is partly because the procaryotic cell had potentially wide respiratory capability compared to the eucaryotic mitochondria, and that the mitochondrion, since it could be isolated in a functional condition, was an experimentally simpler system. It is, therefore, important to assemble what we do know about the respiration of procaryotes lest it be assumed, as it so often is, that the mitochondrial system is the respiratory system of all living cells. True, there are many similarities between the procaryotes and mitochondria, but there are distinctive and important differences which should not be neglected. If, indeed, the mitochondria are the result of the establishment of an endosymbiotic relationship between a procaryote (specifically suggested to be *Micrococcus denitrificans*) and a eucaryote at a time when eucaryotes had not yet achieved a respiratory capability, it would seem surprising that the establishment of this relationship should have occurred only once. It would seem worthwhile to examine the possibility that not all mitochondria follow the same pattern, and perhaps one may trace their origin by the presence of procaryotic respiratory processes.

The general lack of really good oxidative phosphorylation among the procaryotes makes rather a shambles of energy calculations based upon the assumption of three energy rich phosphates per mole of $2H$. Presumably, the endosymbiotic relationship permitted an increase in the phosphorylating ability.

Dr. Hempfling is an excellent guide to the field of microbial respiration and his selection of papers gives one a sampling of the major contributions to the field. It is a pleasure to have these scattered contributions available in one handy volume. And I feel certain that this volume will prove to be useful as well as a starting point for a new look at microbial respiration.

WAYNE W. UMBREIT

PREFACE

What are the mechanics of the electron transport-dependent esterification of inorganic phosphate? This is the greatest question facing fundamental biochemistry today. The unique properties of the procaryotes have been insufficiently employed in answering this question. These convictions prompted me to accept the invitation of Professor Wayne W. Umbreit to compile a Benchmark volume on *Microbial Respiration*, selecting the contributions that, in my view, formed the corpus of accomplishment in this vital area of bioenergetics. With this volume, I hoped to facilitate the future use of non-mitochondrial respiratory systems by the judicious selection of outstanding experimentation and exemplary exposition.

In assembling the articles for this volume I have naturally favored my own tastes in content and style of investigation and in manner of presentation, limited only by the reasonable constraints of space. Those inclinations were acquired largely during the years, too few in number, of my association with the general microbiologist Wolf V. Vishniac.

I acknowledge with pleasure the organizational talent of Rita Nielsen Rasmussen, without whose participation this volume would not have been completed. Sue Hall Hoke Hempfling, my wife, typed the final manuscript and provided liberal allotments of her immense reserves of patience. George Hoch and Craig Rice offered much-needed criticism and encouragement.

WALTER P. HEMPFLING

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INTRODUCTION

Life demands energy, and exists on this planet because of the ability of living organisms to couple the energy-requiring reactions of biosynthesis and maintenance to energy-liberating chemical transformations. The linkage between endergonic and exergonic reactions is achieved principally by the use of phosphate anhydride bonds. Reaction pathways that yield biologically conservable energy usually involve electron transfer. We define fermentation as an energy-yielding sequence of reactions in which the final electron acceptor arises from the initial substrate, and respiration as a series of reactions in which the final electron acceptor is exogenously supplied and is not itself an intermediate product of metabolism. The processes of respiration in procaryotes and the phenomena associated with trapping and converting energy liberated thereby to biologically useful forms concern us here.

Hypotheses have been offered which rationalize the mitochondrial localization of the eucaryotic respiratory apparatus as a result of establishment of an endosymbiotic relationship between a respiring procaryote and a nascent eucaryote.¹ Similarities between the respiratory systems of procaryotes and eucaryotes are extensive, and have prompted such notions, but important overt differences between the two kinds of respiration exist. While eucaryotes are restricted to oxygen-dependent respiration, many bacteria may employ oxygen or nitrate ion; for still other procaryotes, sulfate ion or carbon dioxide may serve as terminal oxidant. The nonphotosynthetic eucaryotes are limited to the use of organic reductants to supply respiration, but the chemoautotrophic bacteria make use of inorganic electron donors. The capability of adaptive response of procaryotes to changing edaphic conditions exceeds that of eucaryotes respiring with mitochondrial systems, in part due to the many choices of respiratory reductant and oxidant. *Escherichia coli*, for example, reacts to changes of dissolved oxygen concentration by adjusting the concentrations of multiple terminal oxidases (Paper 29), reacts to the absence of oxygen by

carrying out fermentation of suitable substrates or by respiring with nitrate² and responds to changes of the identity of the growth substrate by modifying the net amount of conserved energy available for biomass formation (Paper 31).

Although these manifold capabilities would seem to make the procaryotes ideal experimental subjects for the study of respiration and associated mechanisms of energy conservation, investigations of isolated mitochondria have furnished most of the information extant about such processes, and work with microbial respiration has been largely imitative. A comparison of the contributions of studies of procaryotic systems to bioenergetics and to the elucidation of the mechanism of heredity provides a sharp contrast. The simplicity of growth requirements, short generation times, and convenience of manipulation of bacteria made their patterns of inheritance indispensable as models of the heredity of evolutionarily higher creatures. A surrogate mitochondrion was unnecessary, however, since the organelle could be isolated in a functional state and in good yield from multicellular organisms at about the same time that suitable techniques were developed to exploit its availability. Above all, the ability of nucleotide-permeable mitochondrial preparations to carry out oxidative phosphorylation was markedly superior to that of extracts of bacteria. It has only recently become feasible to study oxidative phosphorylation in intact bacteria, the proper analogs of intact mitochondria.

To some extent the inability to measure phosphorylation efficiency during coupled procaryotic respiration has been circumvented by studies of respiration-driven membrane transport processes. It thereby has become practicable to examine the mechanisms by which respiration is linked to energy-requiring phenomena other than oxidative phosphorylation. Models have been provided in which the maintenance of transmembrane solute disequilibria and oxidative phosphorylation share a common intermediate derived from respiration. A portion of this work has already been summarized in the Benchmark Series,³ and the coupling to respiration of membrane transport processes will not be treated further here.

We will consider the present state of understanding of microbial respiration as the outcome of results obtained during three periods of investigation, each of which may be identified by their guiding hypotheses and by the techniques devised to test them. The first is the era of establishment of the role of intermediate electron-transferring compounds in respiratory processes, specifically the demonstration of the participation and characterization

of the pyridine nucleotides,⁴ flavoproteins,⁵ and "oxygen-transferring iron" by Otto Warburg (Papers 1 and 2) and the rediscovery of "cytochrome" by D. Keilin.⁶ Keilin has also prepared an account of this period entitled *The History of Cell Respiration and Cytochrome*,⁷ which includes a thoughtful and sympathetic appreciation of the contributions of C. A. MacMunn.⁸ Paper 3 is a contribution by A. Bertho and H. Glück, associates of Heinrich Wieland, one of Warburg's scientific adversaries.

During the third and fourth decades of this century the overt events of microbial respiration were described. Compounds serving as reductants and oxidants were identified, as described in the inimitable text, *Bacterial Metabolism*,⁹ by Marjory Stephenson, who, along with Keilin, J. H. Quastel, and their associates at Cambridge, obtained much of the information presented. Even though M. W. Beijerinck had pointed out as early as 1903 the importance of understanding biological energy conservation as a means of rationalizing microbial growth,¹⁰ it was not until the demonstration of oxidative phosphorylation (that is, esterification of inorganic phosphate obligatorily linked to respiration by V. A. Belitser and E. T. Tsybakova¹¹ in 1939 that experimental approaches to the mechanisms of respiratory energy conservation became possible. This and related contributions have been collected by H. Kalckar as *Biological Phosphorylations. Development of Concepts*.¹²

A major issue examined in the succeeding years was that of the mechanism of oxidative phosphorylation; and we identify the second era as that dominated by a view of oxidative phosphorylation modelled after substrate-level phosphorylation, or the "chemical intermediate" hypothesis as articulated by E. C. Slater (Paper 16).

As embodied in Lemberg's and Legge's *Haematin Compounds and Bile Pigments*,¹³ development of techniques in porphyrin chemistry had progressed far enough to facilitate the chemical characterization of the cytochromes of bacteria. Excellent examples of such work are found in Papers 4 and 10. Yet such procedures did not allow the investigator to probe the dynamic interactions *in situ* of the components of the respiratory sequence. Keilin, Warburg, and others had approached this task, but it was Britton Chance who developed an array of powerful techniques allowing the rapid sensing of the state of reduction of electron transport components in highly optically scattering suspensions of mitochondria and bacteria (Papers 7 and 9). These and related tools, as well as the procedures of microbial genetics as successfully applied by Butlin, Cox, Gibson, and their associates (Paper 27), are employed in the third and current period of investigation in which

the "chemiosmotic" model of P. Mitchell dominates as the regnant hypothesis to explain the coupling of respiration to energy-requiring functions (Papers 20, 21, and 22).

Although the chemiosmotic hypothesis could reasonably account for the coupling of respiration to active transport processes by means of electrical and proton transmembrane gradients, the intimate chemical details of the phosphorylation of adenosine diphosphate catalyzed by the membrane adenosine triphosphatase were not equally well provided. Papers 23 through 26 represent the most recent trend toward amplification of the role of the membrane ATPase in the mechanistic account of oxidative phosphorylation. This approach has produced a significant modification of the chemiosmotic mechanism in the view of some investigators, and the vitality and excitement attending the resultant controversy is depicted nicely in Paper 23, a communication in the series of exchanges between P. D. Boyer and P. Mitchell, in which the major features of Boyer's "conformational" hypothesis are outlined.

The final group of selections (Papers 29-31) illustrates the plasticity of respiratory mechanisms and their associated energy-conserving systems in bacteria. Greater usage should be made of the unique properties of procaryotic bioenergetic phenomena in working toward solutions of the problems of respiratory energy conservation.

REFERENCES

1. Broda, E. *The Evolution of the Bioenergetic Processes*. Pergamon Press, New York, pp. 123-125, 1975.
2. Taniguchi, S. Z. *Allg. Mikrobiol.*, **1**, 341-375, 1961.
3. Reeves, J. P., ed. *Microbial Permeability* (Benchmark Papers in Microbiology). Dowden, Hutchinson & Ross, Inc. Stroudsburg, Pa., 1973.
4. Warburg, O., W. Christian, and A. Griesse. *Biochem. Z.*, **282**, 157-164, 1935 (Paper 16 in reference 14).
5. Warburg, O., and W. Christian. *Biochem. Z.*, **254**, 444-454, 1932 (Paper 15 in reference 14).
6. Keilin, D. *Proc. Roy. Soc. London*, ser. B, **98**, 312-339, 1925 (pp. 178-191 in reference 12).
7. Keilin, D. *The History of Cell Respiration and Cytochrome* (J. Keilin, ed.), Cambridge Univ. Press, Cambridge, 1966.
8. MacMunn, C. A. *Phil. Trans. Roy. Soc. London*, **177**, 267-298, 1886.
9. Stephenson, M. *Bacterial Metabolism*, 3rd ed. Longmans, Green and Co., Ltd., London, 1949.
10. Beijerinck, M. W., *Handelingen van het G. Nederlandsch. Natuuren Geneeskundig. Congres*, 195, 1903. See also *Maly Jahresbericht* **33**, 1014, 1904.

11. Belitser, V. A., and E. T. Tsybakova. *Biokhimiya*, **4**, 516–534, 1939 (pp. 211–227 in reference 12).
12. Kalckar, H. M. *Biological Phosphorylations. Development of Concepts*. Prentice-Hall, Englewood Cliffs, N. J., 1969.
13. Lemberg, R., and J. W. Legge. *Haematin Compounds and Bile Pigments*. Interscience, New York, 1949. (See also Lemberg, R., and J. Barrett. *Cytochromes*. Academic Press, New York, 1973.)
14. Doelle, H. W., ed. *Microbial Metabolism* (Benchmark Papers in Microbiology). Dowden, Hutchinson & Ross, Inc. Stroudsburg, Pa. 1974.



Part I

HISTORICAL BACKGROUND

Editor's Comments

on Papers 1, 2, and 3

- 1 **WARBURG**
Iron, the Oxygen Transporting Constituent of the Respiration Enzyme
- 2 **WARBURG**
Cytochrome
- 3 **BERTHO and GLÜCK**
The Formation of Hydrogen Peroxide by Lactic Acid Bacteria

Otto Warburg dominated the field of respiration during most of the period prior to the discovery of oxidative phosphorylation. Even before David Keilin's epochal rediscovery of MacMunn's "histohaematin" and "myohaematin," Warburg had established the function of pyridine nucleotides and flavoprotein enzymes in oxidative metabolism, posited and obtained evidence for the participation of iron-containing compounds in respiration, and contended for a decade with Heinrich Wieland concerning the chemical nature of oxygen reduction in respiration.

Warburg's views of the development of the concepts of the functions of "oxygen-transporting" iron and of cytochrome at the end of World War II are presented in Papers 1 and 2. They are two chapters from his book *Schwermetalle als Wirkungsgruppe von Fermenten*. The Kaiser-Wilhelm-Institut für Zellphysiologie in Berlin-Dahlem, wherein much of Warburg's work was carried out, was destroyed by an Allied bombing attack in 1943.

The dispute with Wieland need never have arisen. As now accepted, either hydrogen peroxide (as predicted by Wieland) or water may arise as the terminal product of oxygen reduction depending upon the nature of the respiratory system employed. In the words of Warburg:

Oxygen transporting iron and oxidation by dehydrogenation are not incompatible. On the contrary, "oxidation by dehydrogenation" can almost be regarded as a consequence of oxygen transporting iron, for when molecular oxygen has oxidized the ferro-iron to the ferri-state, it has played its part in respiration. All further oxidations must proceed anaerobically and