



# *The* METABOLISM of The Tubercle Bacillus

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THE METABOLISM OF  
THE TUBERCLE BACILLUS

IN MEMORIAM

Gerald B. Webb

and

Alfred Boquet

## Foreword

**I**N BRINGING OUT a monograph on the metabolism of the tubercle bacillus at this time, characterized as it is by the practical application of new facts on the bacillus in diagnosis and chemotherapy, Drea and Andrejew have performed a highly useful service for bacteriological investigators. A great deal of information has accumulated in the last 30 years, much of which is still to be coordinated. After an exceptionally active period in the 1920's and a relative lull in the 1930's, the 1940's brought a sharp intensification in research on the tubercle bacillus, which led to the development of rapid procedures for growing the organism and ingenious methods of studying the phenomenon of bacteriostasis by antibiotics and other chemotherapeutic agents.

Drea and Andrejew have supplied an excellent review of the important literature leading to this late development. But this is only part of the benefit the reader will derive from their book. Both are pioneers in important phases of the general problem under consideration, and it was their personal investigations, and the need they discovered for a better understanding of the problem, that led them to undertake the monograph. Drea was an early student of subsurface growth of tubercle bacilli, and Andrejew of the mechanisms involved in respiration of the bacillus.

The first and third chapters of the book, respectively on nutrition and respiration, contain much information on the fundamental physiology of tubercle bacilli, and supply a comprehensive review of the older literature. The authors carry the story of oxidation and respiration from the earliest observations up to the modern work of Bernheim and Lehman on the effects on respiration of benzoic and salicylic acids and the substituted salicylic acids like the para-aminosalicylic form, or PAS. In the field of nutrition they show how recent workers, like Bance of the Pasteur Institute in Tunis, with modern technics and methods, have added substantially to the knowledge accumulated in the 70 years since Koch's discovery of the bacillus.

The chapter on depth growth of the tubercle bacillus is of almost paramount interest in the monograph. The basis for much of the modern work in this field was furnished ten years ago by Drea and Boissevain in studies at the Colorado Foundation for Research in Tuberculosis. The monograph provides a comprehensive review of the literature, extending back to Koch and leading up to the well known work of Dubos, Davis and Middlebrook on rapid cultivation of tubercle bacilli and primary isolation on tween-albumin media. With the passage of years and improvement in technic Drea himself has cultivated bacilli with striking rapidity from exceedingly minute inocula in the chemically pure Long's medium. Apparently small inocula of tubercle bacilli grow well in synthetic media, such as the latter, without addition of other substances.

Andrejew, through his special skills in the study of respiration, has helped to elucidate the problems of oxygen utilization in sub-surface growth. The authors quite properly point out in concluding this chapter that depth cultures of tubercle bacilli in synthetic media are advantageous for the study of metabolism of tubercle bacilli, because submerged bacilli in an environment of low oxygen and abundant water simulate bacilli in animal tissues much more closely than do bacilli growing in the more usual laboratory surface cultures.

The fourth chapter, on enzymatic systems in tubercle bacilli, while furnishing much information of specific as well as general interest on the subject of enzyme systems, indicates clearly the paucity of applicable information in the case of the tubercle bacillus. A recent remark of Dubos in this connection applies to the tubercle bacillus with special force: "The effect of the *in vivo* environment on the cellular structure and metabolic equipment of bacteria and therefore on their pathogenic behavior is one of the virgin fields of medical bacteriology." Fortunately the field here under consideration is not wholly obscure. The studies of Andrejew on the relation of oxygen tension to autolysis form one particularly bright spot.

The text closes with a chapter on the products of metabolism of the tubercle bacillus, i.e., its structural elements, including its proteins, lipids, carbohydrates and nucleic acid, and the specific

proteins of tuberculin. In this chapter the reader will find a detailed review of the elaborate chemical studies of the bacillus and its products by Rudolph J. Anderson and Florence B. Seibert, whose investigations, sponsored over a period of years by the National Tuberculosis Association, have made the tubercle bacillus more extensively investigated from the chemical point of view than any other microörganism.

The monograph is heavily documented with tables and graphs, and the bibliography is well selected for its purpose.

In conclusion it is of interest to point out that the combination of an American and a French author is a fitting one in this field. Of the vast number of scientific articles on the subject a large majority are either French or American in origin. When the personal contributions of the two authors are added to this fact, and the deep interest of both in further research, joint authorship seems particularly appropriate.

ESMOND R. LONG



## Preface

**G**RANTING the importance of the tubercle bacillus as a pathogenic microorganism and the very great complexity of the problems which it poses *in vitro* as well as *in vivo*, it appeared to us that it would be desirable to examine and record in a new book the results and ideas acquired up to the present time regarding the metabolism and the growth of these microorganisms. We have sought to examine these problems by studying, whenever possible, the publications concerned with them and to carefully prepare a useful book for the greatest possible number of investigators in tuberculosis (physicians, bacteriologists, immunologists, histologists, etc.) and not solely for some specialized biochemists.

In addition to the considerations necessary to understanding the physiology of the tubercle bacillus, this book contains some brief statements concerning the biological consequences of its activity. In order to really know a metabolic product of the tubercle bacillus, we think, that it does not suffice only to isolate it, purify it and establish its physico-chemical properties, but that it is necessary to know also the influence it exerts upon the tissues or the cells of the animal body. This is essential not only for a better understanding of the already acquired facts, but also for the purpose of eventually suggesting new, broad, basic investigations that are required by tuberculosis when considered as a disease. Viewed from this angle, the researches on the metabolism of the tubercle bacillus present then some important ramifications which, in certain cases, apparently belong outside the framework of what is conveniently called bacterial metabolism.

Moreover, the virulent nature of the pathogenic tubercle bacilli makes some manipulations dangerous and limits the scope of the investigations. This fact explains, perhaps, some important gaps which still exist in our knowledge of their metabolism, especially of their intermediary metabolism. The slow growth of tubercle bacilli and the great variety of their strains also make some

kinds of experiments inoperable and some results difficult to compare. At the same time, it should be noted that the studies inspired by the tubercle bacillus even when strictly theoretical, have been influenced, in a great number of cases, by considerations of a practical nature. Even when certain of these studies were more or less rigorously on a biochemical plane, we thought it was very useful to include and discuss them. The problem of tuberculosis is not solely a problem of biochemistry; the close collaboration of different specialists is an absolute necessity in this field of investigation. This is especially so when the problem is the study of the growth and respiration *in vitro* of the tubercle bacilli under conditions simulating their environment *in vivo*.

Each time that it was possible or seemed desirable, we have sought to compare the tubercle bacillus with other acid-fast or non-acid-fast microorganisms. This is why we have resorted to numerous parallels and differences which exist between the metabolic properties of virulent and avirulent tubercle bacilli and the corresponding properties of other microorganisms.

In order to better determine the properties of the metabolic products of the tubercle bacilli and give the reasons for their alterations due to manipulations, it appears indispensable to us to indicate, in certain essential cases, the exact ways of their isolation, giving preference to modern procedures.

With some exceptions, until quite recently, the phenomena associated with the growth of tubercle bacilli in the laboratory were studied with the use of surface cultures. It is now becoming more and more evident that depth or submerged cultures of tubercle bacilli are being used from choice in many kinds of investigations. The great difference in the two types of culture is largely due to the greatly different available amounts of molecular oxygen at the disposal of the microorganisms. With depth cultures, especially when well established, there is a paucity of oxygen as there is in the depth of infected animal tissues. If fresh supplies of oxygen are withheld from the bacilli in either case, the bacilli die. This is in contrast to the situation with surface cultures exposed to the air, where the cultures finally cease to increase, and begin to diminish because of the exhaustion of a necessary nutrient and the increasing concentration of meta-

bolic products. With both surface and depth cultures, however, as with tissue lesions not completely walled off from the rest of the body, some bacilli, though few in number, may continue to be viable for indeterminate periods of time. Also, the aqueous nature of the tubercle bacillus is made evident by its ability to grow submerged in liquid media as well as in animal tissues composed in part of relatively large amounts of water. It is largely for these reasons that a separate chapter has been used for the discussion of depth cultures of tubercle bacilli.

The late Dr. Charles T. Ryder, an Associate Research Director of the Colorado Foundation for Research in Tuberculosis, reviewed the second chapter and commented upon the then unfinished book. His advice was good.

With the same élan the late Dr. Alfred Boquet, formerly Chief of the Service for Research in Tuberculosis at the Pasteur Institute in Paris, inspired us.

We wish also to express our appreciation to Dr. John H. Hanks of the Leonard Wood Memorial Research Laboratory in the Harvard Medical School and to Dr. Guy P. Youmans of the Northwestern University Medical School for valuable criticism and advice. Dr. Florence B. Seibert's reviews of metabolic products of tubercle bacilli were very helpful.

Dr. Esmond R. Long not only studied the manuscript and wrote a tentative foreward before the manuscript in its final form was submitted to the publisher, but also restudied the galleys for the text before writing the slightly revised final introduction. We deeply appreciate Dr. Long's action, not only because of his eminent position as a research worker, director of research in tuberculosis, and author, but also because he took time out of a very busy life to do this for us.

A large number of the original papers from which the information was secured for this work were not at hand, and we are especially grateful to Miss Louise F. Kampf, Librarian of Coburn Library at Colorado College for securing them for us through the Bibliographical Center for Research in Denver, Colorado by means of an inter-library loan system. With such assistance, it is truly remarkable how accessible become relatively rare, older and original sources of information, as well as those of recent date

which, because of expense, cannot be included in small libraries.

Mlle. Ch. Benex, an assistant librarian at the Pasteur Institute in Paris, helped very much by checking many of the references in the Bibliography.

Miss Anna C. Holt, Librarian at the Harvard University Schools of Medicine and Public Health, and Miss Barbara Hurley, Librarian of the Library of the Medical Society of the City and County of Denver, were likewise most cooperative.

Mrs. H. E. Mathias, Mrs. Romona B. Teason, Dr. Robert E. Ehlert, and Mr. Abe Mogilner gave valuable assistance. One of our secretaries, Miss Dorcas Brady Reed, was very helpful during the final preparation of the manuscript.

The book owes its existence to a suggestion by the late Dr. Gerald B. Webb that we, the authors, proceed to write a monograph upon this subject. At that time Dr. Webb was President of the Colorado Foundation for Research in Tuberculosis and also Director of Research. We have tried to justify Dr. Webb's confidence in us, and at the same time to pay a tribute to the many earnest investigators in the field of research in tuberculosis. We regret that, because of certain limitations, we are unable to include in this book all of the contributions to our knowledge in this field.

Dr. James J. Waring, who has taken over the mantle of leadership from Dr. Webb, has been unfailing in his encouragement and support toward completion of the task begun while Dr. Webb was alive.

We are grateful to the authors, editors, and publishers who have granted us permission to use restricted materials. We think we have given credit in the text to all persons to whom it is due. Any errors or omissions are the results of oversights for which we express regret. All figures are from redrawings of the originals.

Charles C Thomas • Publisher, through their representatives, Mr. Payne E L Thomas and Mr. Warren H. Green, have done their usual good job of producing a fine, easily readable type of book. We are grateful for their generous cooperation.

W. F. DREA

A. ANDREJEW

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