The Biology of the Mycobacteria

Volume 1
Physiology, Identification and Classification

Edited by
COLIN RATLEDGE and JOHN STANFORD

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1982

ACADEMIC PRESS

A Subsidiary of Harcourt Brace Jovanovich, Publishers

London New York
Paris San Diego San Francisco São Paulo
Sydney Tokyo Toronto

ACADEMIC PRESS INC. (LONDON) LTD
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue,
New York, New York 10003

British Library Cataloguing in Publication Data

The Biology of the mycobacteria
Vol. 1
1. Mycobacteria
I. Ratledge, C. II. Stanford, J. L.
589.9'2 QR82.M8

ISBN 0–12–582301–0

LCCCN 81–68013

Filmset by Northumberland Press Ltd,
Gateshead, Tyne and Wear
Printed in Great Britain by
Fletcher and Son Ltd, Norwich

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Preface

For many years mycobacteria were a neglected group of organisms. They were extremely difficult to grow in the laboratory and posed many problems in their handling and subsequent study. Certainly they were nobody's favourite organism for laboratory research into bacterial physiology and biochemistry. It was probably only because their numbers included the pathogens, *Mycobacterium tuberculosis* and *Mycobacterium leprae*, that any attention at all was paid to this genus. This, of course, is reason enough and is the reason why mycobacteria have continued to receive attention on an ever-increasing scale over the past twenty years or so.

Tuberculosis and leprosy remain serious problems on a global scale. The situation is not likely to improve substantially in the foreseeable future and there is an urgent need to improve our understanding of these diseases and the organisms which cause them. We still need to know how to combat these organisms in the host and therefore need to understand their physiology and behaviour, first, in the laboratory and then by extension into the *in vivo* situation.

Whilst there has been a steady but intermittent number of books written about tuberculosis, and the occasional one on leprosy, there have been few books seeking to bring together most of the salient information about the mycobacteria themselves. It was with this concept in mind that we began to consider how best this deficiency could be remedied, and we quickly appreciated that such a task could only be undertaken by a team of authors. It was clearly beyond the skills of either, or both of us together, to cover the comprehensive amount of information which has been gained over the recent years in numerous laboratories throughout the world.

We were fortunate in being able to find considerable enthusiasm for this project amongst our colleagues who then responded in their own inimitable ways by covering the selected areas of their chapters so admirably. Any errors of substance may therefore be laid exclusively at our feet as our contributors, being the experts they are in their respective fields of study, have done all that we have asked of them. We would like to take this opportunity to thank them for bearing our occasional cajolings and for producing such excellent and informative reviews. We hope that their expert knowledge will be of benefit to all those who work with and seek to understand the mycobacteria, whether this be on the benches of academia, the

laboratories of hospitals or clinics, or out in the field where the frightening interface between man and disease occurs. We also hope that the book will be of value to the research student, teacher, bacteriologist, biochemist, immunologist, clinician and pathologist whose studies and work brings them into occasional contact, but not hazardously so, with the mycobacteria.

Do not seek in this book information on clinical pictures, clinical diagnosis or treatment of disease. We have tried to paint the world from a mycobacterial point of view in which man and other animals are seen as that special acre set aside for the least timorous of bacilli. The immunological and environmental aspects of mycobacteria will be dealt with in Volume 2.

Whilst it is customary to thank wives and other loved ones for bearing the editors' pre-occupation with their book so nobly and so well for too many years, we doubt in this case if they noticed much difference in our usual pattern of erratic behaviour. However should they ever pick up this book from our bookshelves let them see that, although not acid-fast, they are far from being forgotten.

Easter, 1981

Colin Ratledge
John Stanford

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Immunological and Environmental Aspects

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Introduction

COLIN RATLEDGE and JOHN L. STANFORD

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From the very dawn of civilization, man appears to have suffered from mycobacterial infections. Organisms remarkably like present day mycobacteria have been seen in stained preparations from mummified bodies of great antiquity and evidence of mycobacterial invasions of the bone and joints have been seen in skeletons of prehistoric man. Similar examinations have shown the existence of tuberculosis in pre-Colombian America, although leprosy seems to have been an Old World disease which only reached America and Australasia in the last few centuries. Leprosy is, in fact, still spreading into the centre of South America and continental Australia. Even before man, mycobacteria were probably infecting animals just as they do today, and a study of mummified ibises and cats from Egyptian tombs might provide valuable evidence of the antiquity of their disease. Acid-fast bacteria, still retaining their acid-fastness as an indicative and permanent character of the mycobacterial cell, have been recovered from the long-extinct mammoth and woolly rhinoceros. Mycobacteria have probably existed since the very earliest times of biological life on this planet. Their high frequency and perhaps important ecological role in sphagnum vegetation, which is of exceedingly great antiquity, also suggests their great age. Although it might be argued that mycobacteria evolved as pathogens first, with the saprophytic species evolving subsequently, it seems more logical to assume that the free-living forms existed first and then, with the coming of the higher animals, these evolved into ecological niches not previously colonized by other bacteria and thus became the pathogens of the disease we know today as tuberculosis and leprosy.

Leprosy may be of much more recent origin than other mycobacterioses and definite evidence for its existence only goes back about 3000 years. It is almost certain that the leprosy bacillus then arose as a spontaneous mutation of some existing, and probably saprophytic, mycobacterium. The recent

discovery of "non-cultivatable" mycobacteria in sphagnum vegetation in Norway is a fascinating source of speculation. The question is more than just curiosity, for the discovery of the non-pathogenic progenitor of the leprosy bacillus could have considerable bearing on how a vaccine might be developed against leprosy. Considerable efforts are therefore being made to assess the similarities of *Mycobacterium leprae* with all the known mycobacteria and this highlights the importance of "knowing one's enemies".

For quite different reasons, but with the same underlying philosophy of knowing your enemy, the study of the tubercle bacillus, *Mycobacterium tuberculosis*, is of the utmost importance. Tuberculosis—the scourge of nations, of underprivileged peoples—has been one of the most persistent, dangerous and frightening diseases of man. Although it has none of the qualities of the super-rampaging diseases, the pandemics of the Black Death, of plague or of cholera, as the "White Death" it is with us continuously. Lest it be thought that tuberculosis or indeed leprosy are diseases of the past, the current global mortality rate due to tuberculosis is about 3000000 people per annum, and even in Great Britain today approximately 1000 people die each year with evidence of the disease. It is also reckoned that there are in excess of 20 million sufferers of the disease who may be placing at risk a further 50 to 100 million people every year. For leprosy, the current number of cases is estimated at between 15 to 20 million and, as a disease of high morbidity rather than high mortality, may be considered to be directly contributing to the deaths of as many as 1 million people per annum and to the misery of many more.

With diseases of this magnitude, and there are no other bacterial diseases which account for so many deaths or for so much misery and suffering, there must be international concern. Mycobacteria are no respecters of race or colour; the prevalence of tuberculosis has, of course, dramatically declined in developed countries in the comparatively recent past but the number of known infective cases of tuberculosis even here leaves no room for complacency. In the United Kingdom the number of notifiable cases of tuberculosis has never yet fallen below 10000 per annum and this represents the minimum number of cases as 20% of tubercular deaths are not diagnosed as such until the post-mortem examination. The numbers of cases of tuberculosis per million in other European countries are similar to those in the United Kingdom and the picture in North America is not substantially different.

Mycobacterioses can be, and are, caused by many species of mycobacteria though it has to be said that many of these infections are rare and tend to occur in the compromised host—either the patient has suffered previous tissue damage due to some disease, for example pneumonoconiosis or even tuberculosis itself, or has suffered from some breakdown in his immune system thus rendering him susceptible to invasion by organisms to which

previously he was resistant. Mycobacteria therefore cover a wide range of virulence and infectivity. With the exception of the tubercle and leprosy bacilli themselves, all other species of mycobacteria live free in the environment but a proportion of them can attack man and animals if introduced into their tissues. Thus the diseases they cause must be considered opportunistic and the control of the occurrence of such diseases poses almost insuperable problems.

Man is but one of many animals which is invaded by the mycobacteria. Few vertebrates seem immune to them and individual species of mycobacteria such as *Mycobacterium bovis*, and *Mycobacterium avium*, may not only infect many different species of animal but more disconcertingly may be transmitted between animal species and on occasion into man himself. This, of course, is of considerable concern and also of some topical controversy as farm animals, particularly cows, may become infected with *M. bovis* carried by infected badgers and perhaps other wild animals. Such animals may leave viable and virulent bacteria behind them as they traverse through fields or even through cow byres themselves. The advocated cure is the elimination of infected badgers, a very difficult thing to achieve. In a similar manner, deer may be infected by *M. avium* carried by wild-fowl, though the elimination of infected wild-fowl seems even less practical than the eradication of diseased badgers. The danger is then that from the infected cow, man becomes infected through consumption of the milk. There is much less danger from the deer as *M. avium* is less virulent for man than *M. bovis* although deer carcasses may not be exposed to such rigorous meat inspection as are those of cattle.

Thus we see a spectrum of diseases caused by a spectrum of organisms, all bearing the epithet "mycobacteria". This is a book, however, written not about the diseases, how they may be recognized or treated, but about the organisms themselves—a few of which are our enemies. We believe, like many of our colleagues, that it is only by a thorough understanding of the mycobacteria—their make-up, their natural history, their physiology, biochemistry and immunological reactivities—that we can see where their weaknesses and strengths may lie. Upon such data will be based improved diagnosis, prophylaxis and treatment leading ultimately to effective control even if the opportunistic nature of the mycobacteria makes eradication an impossibility.

In this first volume, we cover the physiology of the organisms and the techniques and principles used for their identification and classification. The mycobacteria have long been thought of as a distinct and, in many ways, an unique group of bacteria. Their mode of growth, when in their rough form, by aggregating into clumps of cells gives some species their characteristic dry breadcrumb-like appearance on solid media and their pellicular form of growth on liquid media. This and other similarities with the actinomycetes group of bacteria has led many people to consider mycobacteria as being "evolutionary advanced" bacteria. The placing of the genus *Mycobacterium*

in the order of the Actinomycetales, together with the original view that the actinomycetes were mid-way in development between bacteria and fungi, reinforced the view that mycobacteria were fungal-like bacteria thus implying possession of characteristics not shared by other prokaryotic cells. Indeed even a current (1981) edition of one of the major student textbooks on microbiology still considers actinomycetes, which would include the mycobacteria, as 'fungal-like bacteria'. This, we now realize, is a complete misconception.

Much of our available evidence, which is presented in the various chapters of this book, comes repeatedly to the same basic conclusion that mycobacteria are no different from any other group of bacteria in their essential physiological and biochemical characteristics although as infective packages they have some subtleties almost without compare. Of course there are differences between mycobacteria and other bacteria; there has to be otherwise they would not be recognizable as a distinct and separate genus. But the essential difference that we appreciate today comes down to almost one single main point: the possession by the mycobacteria of a thick, lipid-rich envelope. It might be said that to understand this envelope is to understand the mycobacteria, or as one of the contributors to this volume has said, but not in his chapter, "mycobacteria might best be regarded as *Escherichia coli* wrapped up in a fur coat". (However, the first chapter of our second volume may challenge this contention.) This is obviously an over-simplification, but the envelope of the mycobacteria gives the cells their outward characteristics: their hydrophobicity, their resistance to chemical injury by acids, alkalis and many of the disinfectants used to kill other bacteria, their resistance to attack by the killing mechanisms of macrophages in which mycobacteria are then able to multiply to cause disease, their ability to survive long periods of starvation or aridity without dehydration, even their allergic and immunogenic properties mainly originate from materials within the cell envelope.

The envelope, through the materials it contains, then provides the means to understand the mycobacteria and also the means to identify and subsequently classify the numerous species that go to make up the genus. The basic matrix of the bacterial cell wall is similar to, but not identical with, the peptidoglycan backbone of other Gram-positive bacteria; it differs from it by the addition of a complex glycolipid. The lipids of this glycolipid are the mycolic acids which, being large molecules having molecular weights of about 1500, are then able to accommodate many other lipophilic molecules within the ensuing matrix. The agglomeration of molecules becomes the envelope which effectively encapsulates the rest of the cell.

But there are many questions to which we do not yet have answers with the mycobacteria. The principal unanswered question is why do they grow so slowly. There does not seem to be a simple answer to this. It probably is not due to the slow uptake of nutrients which might be imagined to be a consequence of a thick lipoidal envelope; on the contrary, water-soluble

molecules like sugars and amino acids seem to cross the cell envelope with alacrity. Various proposals are given by several authors in this book but without any firm conclusion being produced. Nor do we understand why some mycobacteria have proved, and are still proving, so intractable to growing in the laboratory. The failure to cultivate the leprosy bacillus, and until recently *M. lepraemurium* the rat-leprosy bacillus, is completely inexplicable. The organisms on the one hand seem to be ordinary intact mycobacteria and yet they are incompetent to grow outside the host tissue for reasons we still struggle to comprehend. Clearly there is more to the complexity of mycobacteria than their outward appearance might suggest.

We hope that the following chapters will provide the basis for finding some of the answers to these questions.

* * * * *

The "Approved Lists of Bacterial Names" edited by V. B. D. Skerman, V. McGowan and P. H. A. Sneath, were published in the *International Journal of Systematic Bacteriology* volume 30 in January 1980. These lists are to act as a new starting point for bacterial nomenclature by presenting the names of all species accepted at that date and by replacing the previously accepted date for the establishment of priority of names which was 1 May 1753.

In the case of the genus *Mycobacterium* Lehmann and Neumann 1896, the type species *M. tuberculosis* is retained together with 40 other species for one of which an additional subspecies is named. These 41 valid species names replace the 128 validly published legitimate names listed in the Index Bergeyana (1956) and any other names validly published between the compilation of the Index Bergeyana and 1 January 1978. Remarkably, only 16 of the 128 species names in the Index Bergeyana have survived and appear in the "Approved list".

Unhappily the "Approved list" has already come in for considerable criticism for two reasons. Firstly, it lists as species organisms that are at best subspecies (for example, *M. africanum* and *M. bovis*, which should be subspecies of *M. tuberculosis*) and secondly, it omits a number of validly published species with type strains available in the international collections of type cultures (for example *M. diernhoferi* and five or six species described in Japan). Several authors in the present work use trinomials where they consider the relationship between two named organisms to be subspecific or use names erroneously omitted from the list. Presumably time will once again come to the aid of taxonomy and nomenclature, and a sensible list of species will emerge.

The List of Approved species of Mycobacteria (1980)

M. africanum
M. asiaticum
M. aurum
*M. avium**
M. bovis
*M. chelonei**
M. chitae
M. duvalii
M. farcinogenes
*M. flavescens**
*M. fortuitum**
M. gadium
M. gastri
M. gilvum
*M. gordonae**
M. haemophilum
M. intracellulare
*M. kansasii**
M. komossense
*M. leprae**
M. lepraemurium
M. malmoense
*M. marinum**
M. microti
*M. nonchromogenicum**
*M. neoaurum**
M. parafortuitum
M. paratuberculosis
*M. phlei**
*M. scrofulaceum**
M. senegalense
M. simiae
*M. smegmatis**
M. szulgai
M. terrae
M. thermoresistibile
M. triviale
*M. tuberculosis**
*M. ulcerans**
*M. vaccae**
*M. xenopi**

* Names marked thus are very well established and are unlikely to change (all but *M. neoarurum* are survivors from the Index Bergeyana list).

Some of the names which should have appeared in the approved list, but were omitted, include:

M. agri
M. aichiense
M. chubense
M. diernhoferi
M. obuense
M. rhodesiae
M. shimoidei

and there may well be others.

Suprafamilial classification of mycobacteria also remains in a state of flux. Although traditionally the *Mycobacteriaceae* form a family of the order *Actinomycetales*, the coryneform genera of the order *Eubacteriales* are in most respects more closely related to mycobacteria than are actinomyces. Definition of the genus *Mycobacterium* in a way that clearly distinguishes it from genera such as *Rhodococcus* and *Nocardia* is not easy and best advice we can offer to those seeking one must be—read on.

Part I

Physiology of Mycobacteria

2

The Anatomy of Mycobacteria

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I. Macroscopic Anatomy

A. PIGMENTATION

Two features of mycobacteria are readily visible to the naked eye: their colour and the morphology of their colonies grown on solid media. Most species are whitish or cream-coloured, the slight colour being presumably due to cytochromes or other coloured constituents of the biochemical machinery of the cell. McCready and Ratledge (1978) showed that *Mycobacterium smegmatis* contains porphyrins, and that the amount increases as the cells age. In such cultures and those of other 'non-pigmented' mycobacteria, por-