CHEMICAL ACTIVITIES OF FUNGI

BY

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Dedicated with much appreciation

to

My microbiological mentors

PROFESSOR S. A. WAKSMAN
PROFESSOR R. L. STARKEY
PROFESSOR C. B. VAN NIEL

and to numerous colleagues, especially at the New Jersey Agricultural Experimental Station, Hopkins Marine Station, Stanford University, University of California, Merck and Co., Inc., and the University of Texas, whose scientific fraternization with me through my career has better prepared me to undertake this work.

PREFACE

This book is the result of my bewilderment when as a beginning graduate student in 1936, I was assigned by Dr. Waksman to a problem in mold metabolism. The bewilderment was a direct consequence of the unavailability of a suitable treatise comprehensive enough to orientate a non-specialist in this field. What impressed me particularly was the tremendous amount of work that had been done, and the fact that it was widely scattered in innumerable literature sources, predominately foreign at that time. Furthermore, much of the work on the major phases of the subject was so contradictory that the beginner could scarcely avoid a sensation of confusion.

What was needed was an authoritative, critical book integrating and evaluating the field, and I was presumptuous enough to tell Dr. Waksman that he ought to write such a volume. Whereupon he suggested that I work five years in the field, then write it myself. It has taken me thirteen years to become so bold as to do it.

Considering the enormous developments during this time, the plight of the beginning student has, I should imagine, become a great deal worse. It is primarily for such persons that this book is designed, though obviously many other groups of individuals may find it valuable. Consequently the didactic approach is conspicuous throughout, perhaps to the point of being repetitious at times. In fact, development of the subject matter was made with an eye toward the use of this book as a text for college courses at the upperclass or graduate level. The giant developments in this field, both academic and technological, are convincing evidence that mold metabolism as a distinct entity "has arrived." It would appear that the inclusion of a college course on this subject is essential for a well-rounded curriculum in mycology or general microbiology, and this book is designed to meet the needs of such a course.

This book is not an exhaustive compilation, but, instead, is a treatise in which the main outlines of the field are developed around the theme of comparative biochemistry. A definite attempt to synthesize the field has been made. The long-known facts have been reinterpreted and presented in terms of modern concepts of biology and biochemistry. Oftentimes this has necessitated a discussion of the merits and flaws in theories contradicting each other; and there is no dearth of such instances!

The reader will seek in vain a list, for example, of all the pigments or antibiotics produced by fungi, for my purpose has been to stress the subject matter from the standpoint of dynamics, that is, *transformations* effected by fungi, and not merely to list all facts regardless of their significance.

Similarly, details of certain processes such as production of riboflavin by *Eremothecium asbyii* have been omitted on the grounds that they are largely empirical and illustrate no new principles—they merely reflect other principles discussed throughout the book. Admittedly, this is an arbitrary basis, but it was done advisedly, to keep the size of the book within bounds.

Also arbitrary has been the interpretation of "Fungi" as used in the title. In general this book is limited to the filamentous forms, the so-called "lower fungi" or "molds" according to the definition in Chapter 1. This ordinarily will mean fungi which are easily handled as experimental material in the laboratory.

Inasmuch as fungi are very much a part of the industrial scene nowadays, the evolution of a process to industrial scale is treated in connection with the particular transformation; yet this book is not one on applied microbiology. Nevertheless, factors involved in the industrial approach of necessity depend on and capitalize on principles of physiology of the organism, and this has provided me with an excellent opportunity to draw upon my own industrial experience. A great many of the industrial features presented here have never appeared in student texts.

Another feature deliberately incorporated is the historical development and evolution of each major line of work with a special aim to associate names and personalities with definite contributions. This policy I adopted when I commenced writing the book in November, 1945, and it was a source of great satisfaction to see the noted scientist-educator J. B. Conant espouse this very cause some two years later in his book "On Understanding Science."

I do not doubt that many important sins of omission and commission are embraced by this book. I am cognizant of many; and I would sincerely welcome any and all corrections, criticisms and different points of view from readers, so that if possible, the book can be amended in the future.

My acknowledgments and gratitude are due to innumerable people for expediting the preparation of this book in various ways. In the professional field, for advice and criticism of certain parts of the MS, I wish to thank Drs. R. P. Wagner, B. S. Gould, F. F. Nord, and also O. Wyss for exchanges of opinions on certain subjects. Thanks are due also to Dr. Otto Behrens for permission to utilize some information on artificial penicillins prior to its publication.

PREFACE

Thanks are due of course to various authors and publishers for permission to use certain cuts and illustrations, and especially to Dr. G. W. Beadle because I used so many of his. In the physical task of preparation appreciation is due Merck and Co., Inc. for the unsurpassed services of their research library, and for the able typing services of the Misses Olga Myna and Jenny Yankow. At the University of Texas I am grateful to the Misses Marjorie Borel and Patricia Mackey for transcribing my longhand, and particularly to Miss Doris McGuire for much typing and for the odious task of making corrections and for "patching up" the MS. My thanks also to J. B. Davis for his service in reading and checking the proof.

No gratitude could be too much for my devoted wife who not only aided in the typing but spent many a long and lonely night and weekend vigil at home while I worked on the book at the laboratory.

Jackson W. Foster

Austin, Texas

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CHAPTER 1

INTRODUCTION, HISTORY, PERSPECTIVE

Historically, the initial developments in the organized study of fungi (mycology), very much like the classical approach to other systems of biology throughout the history of science, were primarily descriptive, centering at first mainly on what fungi are, including the evolutionary sequence within the group itself, and classification and identification (taxonomy), these being based on morphological characters, gross and microscopic. With the recognition that the reproductive structures and processes are the distinctive features about legions of otherwise indistinguishable organisms within the group, emphasis on these aspects led to rapid accumulation of knowledge about sporulation and reproduction mechanisms and complicated life cycles in the more highly evolved fungus forms. Particular attention was paid, naturally, to fungus forms of practical importance, and up until the last two or three decades this had come to mean traditionally those fungi responsible for diseases of cultivated crops and other plants, and of man and animals. It is not surprising, therefore, that until a recent era the study of fungi was almost exclusively the domain of the plant pathologist, who usually was a botanist, horticulturist, naturalist, mycologist, histologist, microscopist, agronomist, or combinations thereof, and whose generalized interest could best be described as plant biology. The study of molds was, then, a very logical extension of the classical botany, and, indeed, the early history of the study of fungi is closely associated with the names of the active botanists and plant pathologists of the day. Their influence is still widely felt in the study of fungi, for, judging from the texts available, the teaching of mycology is largely based on the classical themes of morphology, reproduction, and host-parasite relationships, and a good deal of art work, with only incidental attention to such aspects as modern concepts of ecology, artificial cultivation, nutrition, physiology, and biochemistry. Indeed, the impression is all too wide spread that mycology teaching today consists of the study of herbarium specimens and is handled jointly with plant pathology, if not actually considered synonymous with it. Nevertheless, the tremendous significance of plant pathology in mycology certainly cannot be underestimated, as E. C. Large has so scholarly revealed in his magnificent treatise *Advance of the*

Fungi (1940). The use of herbarium specimens is, in addition to revealing the characteristics of the infection, a consequence of the inability so far to cultivate many of the forms and sexual bodies apart from the host tissues. But this in turn is the result of lack of information on the cultural, physiological, and biochemical aspects of the fungus itself, as compared to what is known of the process of parasitism of the plant.

Mycology, then, as organized subject matter was for a long time thought of as being principally the province of plant pathology, directly or indirectly. However, this certainly was not due to any dearth of numbers and varieties of purely saprophytic fungi, whose very abundance and diversity, physiologically as well as morphologically, and whose ease of cultivation in pure culture, provide a tempting lure for an understanding of the intimate character of the organisms themselves.

While this has been true to a greater or lesser degree in the past, the emergence of the study of the behavior of the organism itself and of the factors contributing to it has become unmistakable and has been largely attributable to the application of physiological and biochemical approaches to the organism in the living state. Microbiology has spawned a new but substantial family member—Mold Metabolism.

A definition of terms is not out of place here, because the word mold is not clearly defined in standard treatises on fungi (in Henrici's *Molds, Yeasts and Actinomycetes* this definition is lacking). "Mold" has no strict taxonomic or even scientific meaning and its interpretation doubtless varies with different authorities. It is, perhaps, a colloquialism in microbiology. Nevertheless it is possible to shape the general connotation of the term, leaving disputable issues a matter of individual viewpoint.

The term "mold" connotes certain types of fungi. It is very frequently used by the English ("mould") who synonymize it with "lower fungi." Similarly the German "Schimmelpilze" connotes one type of fungi apart from all others, i.e., mold fungi, literally. It has come to have very narrow applications in some connections, mainly non-technical. Thus there is "black-mold," "white-mold," "bread-mold," "water-mold." Sometimes by "mold" is meant mildew and in other cases the term is used to denote any member of the Mucorales.

However, to workers in the field the designation has broader significance, meaning fungi (Eumycetes) whose characteristic nature is distinctively filamentous, but which do not form any organized specialized fleshy fruiting structures of macroscopic dimensions. It at once rules out the great numbers of so-called ascocarpous and mushroom-type organisms. Mushroom spawn, for example, exclusively mycelial, usually is not called mold because the organism typically forms a fleshy fruiting body. On the other hand, it does not exclude all fungi which bear sexual spores in specialized structures, only the macroscopic fleshy types. Thus many filamentous genera like Penicillium, Aspergillus, etc., bear spores in specialized structures under certain conditions, but mainly in microscopic fruiting structures, at least not in structures that could be called fleshy.

In mycology, the larger and more complex the fruiting body is, the "higher" the fungus is said to be. This stems from the fact that in an evolutionary sense those forms are more highly specialized, adapted, or evolved. Conversely, organisms with relatively simple and inconspicuous fruiting bodies, or with none known, are considered "lower" forms or lower fungi.

Historical Development of Mold Metabolism

It can well be said that bacteriology as a science in the sense that we know it today stems from the fact that bacteria are of extremely great practical importance in the economy of man and civilization. Indeed, the "Golden Age of Bacteriology," perhaps not yet in its stage of highest development, was a direct result of Pasteur's elegant demonstration in a convincing and unequivocal scientific manner, of the role of bacteria as causative instruments of disease in animals, and of the undesirable changes in spoilage of fermented beverages. The impetus this work bestowed upon the field and upon the subsequent autocatalytic development of medical and agricultural bacteriology is too well known to dwell upon here. Sanitary and industrial bacteriology next became the high points in the field. Out of these pursuits came understanding of the profound and intimate characters of the bacteria themselves. Particularly did this work take a tremendous spurt with the revelation of the stepwise chemical and enzymic nature of the mechanisms of respiration, fermentation, and biological oxidations through the pioneering work of Harden and Young, Neuberg, Meyerhof, Wieland, Warburg, and many others. Not only did yeast and muscle biochemistry find ready confirmation and corraboration with bacteria but, not long after, the very reverse became true—bacteria, because of their simplicity, ease of handling, etc. became fair game for the study of phenomena of fundamental biological significance and application to biological systems at large. Indeed, from this has been synthesized the beautiful pattern of the doctrine of comparative biochemistry (van Niel, 1943) first clearly enunciated in 1926 by Kluyver and Donker in their epochal article, *Die Einheit in der Biochemie.*

Detailed consideration is given here to the development of bacterial metabolism because it is a perfect orientation for the development of mold metabolism, standing as the latter does in striking parallelism to it, and affording, as it were, a precedent. As emphasized above, molds first and for a long time were the property of the descriptive botanist and mycologist, and earned their first importance in plant pathology. Nevertheless, in a less spectacular way, the role of fungi in diseases of plants was as important a stimulus for the study of mold metabolism as human and animal diseases were for bacteriology.

The development was not, however, exclusively from the standpoint of mycology and pathology. What may be called mold physiology was very much in evidence, as far back as a half century ago, but here again physiology was of the classic variety, based a good deal on physiology in a dynamic and physical sense—response to stimuli of various kinds—a carry-over from the plant physiology of the day. In fact, the mold physiologists were the plant physiologists of the day (Pfeffer, Wehmer, Bertrand, Brefeld, etc.). This does not mean that the problems of the activities and workings of molds were not being attacked at all before this field became well-defined. Indeed, numerous isolated historical examples could be given of molds being studied for what they do and what they produce, and of a practical utilization of the findings. In fact, practically all of the cornerstones of the study of mold metabolism as we know it today can be traced back to pioneering researches of the last century. The point is that more and more representative processes were revealed, but they failed to develop further because they were ahead of their times: the tools, mechanisms, and concepts of the newer knowledge of physiology, biochemistry, and nutrition were yet undiscovered and the approaches were not far from empirical. One is reminded of the comment of the great German biochemist Otto Warburg when, some twenty years ago, he decided to abandon his researches on tumor metabolism simply because the tools and concepts essential for the successful prosecution of the problem were not available and because it would not be worthwhile to resume the problem until they were. Twenty years later Warburg did return to the problem with modern techniques and theories (largely developed by himself!). In the same way some aspects of mold metabolism were already well defined considerably before the close of the last century and never realized the fullest academic exploitation in the sense we now know is possible simply because of limited experimental means. It is worth-while to mention a few of these, for they were the forerunners of the science of mold metabolism as we know it today, and occupy a unique historical significance in the development of this now definite branch of microbiology. (1) Van Tieghem, 1867: Conversion of tannin in gall nuts to gallic acid by fungi. (2) Raulin, 1867: Mineral nutrition of fungi. (3) Wehmer, 1891: Organic acid production from sugar by fungi.