Insect Microbiology

AN ACCOUNT OF THE MICROBES ASSOCIATED WITH INSECTS AND TICKS WITH SPECIAL REFERENCE TO THE BIOLOGIC RELATIONSHIPS INVOLVED

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BY EDWARD A. STEINHAUS

TO MY FATHER AND MOTHER

PREFACE

SINCE today's world is one of extremely rapid progress on all scientific fronts, there is a tendency to allow our "lines of communication" to become overextended, permitting our practical accomplishments to lose the sturdy support of our reserves of basic knowledge and information. Especially is this true with the biologic sciences in which the "practical" or applied accomplishments so frequently appear to overshadow the results of academic or so-called pure research. Until World War II necessitated the sending of our troops into all regions of the world, including the tropics, few Americans realized the really great part played by insects in the transmission of disease agents affecting man. America's part in producing ever-increasing quantities of food during this period has focused our attention as never before on the role of insects in the spread of diseases of animals and plants. Yet in the handling of most of the new problems concerned in this activity we were fortified with knowledge and progress gained during the prewar years in the peaceful pursuit of the answers to similar but less pressing problems. With the renewed realization of the importance of insects and the microorganisms associated with them, it is not only safe but wise to continue the study of the basic biologic relationships concerned in order to provide continuously a firm foundation upon which future accomplishments may be built securely. As concerns the field of insect microbiology, we seem to have got ahead of ourselves in many respects. There has been very little effort to group our forces, so to speak, and for the most part the available knowledge and information is not properly known, organized, or appreciated. Research workers in particular have long been handicapped by the widely scattered, unorganized, and often inaccessible literature of the field. If we hope to meet the opportunity and the challenge which the future of this field is offering, this situation must be corrected. To help accomplish this is the writer's modest purpose behind the presentation of this publication.

In general, this book is an attempt to treat the various associations and relationships existing between all types of microbes and insects (including ticks

and mites) from a biologic standpoint, and, in a sense, to serve as a compendium of the data concerned. Many of these data have not been sufficiently studied to present adequate bases for discussions relating to the biologic relationships involved. While this is not a book on insect pathology or on medical entomology, an attempt has been made to include the biologic relationships existing between pathogenic agents and their arthropod hosts and vectors as well as all those between nonpathogenic agents and insects and ticks in general.

Although all types of microbes (bacteria, protozoa, yeasts, fungi, rickettsiae, viruses, and spirochetes) are concerned in the subject matter of the book, yet in no instance have we attempted to give the reader a treatment of any particular field of microbiology as such. In other words, this book does not include the fundamentals of bacteriology itself, or protozoology itself, etc. An effort has been made, however, to treat each of the groups of microbes in a fashion acceptable to the present authorities in each particular field, especially with reference to its association with insects. Brief treatments of the taxonomy of the various microbial groups are included both for the sake of order and to enable the reader to read in the group concerned even though he may not have an intimate knowledge of that particular branch of microbiology.

The writing of this book has been made difficult by the inaccessibleness at this time of many of the foreign references and by the fact that the nature of the subject matter is such that it calls for treatment in a variety of ways. For example, the bacteria could not very well be treated in the same manner as the protozoa, nor the intracellular bacterium- and yeastlike organisms in a manner similar to that used for the true rickettsiae. For this reason we have felt justified in considering each group of microorganisms in the way most suitable to that group, varying the method of treatment in different chapters of the book. In places, lists of microbes are given without much associated individual discussion. This has been done for two reasons: first, considering the dearth of information a discussion of each microbe is impossible; second, thinking such a listing desirable, the author tried to give at least the names of the microbes concerned. For example, hundreds of entomophilic protozoa have been discovered and named, but practically nothing is known of the nature of the biologic relationships between them and their hosts. A similar situation prevails with most of the entomogenous fungi. In the belief that the reader would like to have the names of these microorganisms, the writer has presented them at the risk of appearing to present parts of the book as merely annotated lists. A similar situation prevails concerning much of the general subject matter presented on these pages. In many cases an appreciable number of facts have been gathered, but very few generalizations or conclusions have been or can be made. The writer is fully aware that this is an unfortunate

deficiency. It is hoped that with an increase in our knowledge of this field appropriate deductions, conclusions, and generalizations can be made.

The authorities for the specific names of the insects have been omitted in the text of the book but are given in the index. In the case of some of the microorganisms it occasionally has been expedient to include the names of their describers in the text.

Although the book's title specifies insects as the type of arthropods concerned, the microbiology of *ticks and mites* is also included. Hence, as it applies here, the term insect, when used in a general sense, includes the Acarina.

To the critical reviewer, who so often feels duty bound to find errors in a book, the author gives assurance that a detailed work of this kind is never entirely free from mistakes. The author has diligently tried to avoid them, however, and will welcome having them called to his attention. He has attempted to use the most recent names of insects and ticks but may not have done so in all cases. For the bacteria the systematics of the fifth edition of *Bergey's Manual of Determinative Bacteriology* have been followed, and similar standard works have been used for the other types of microorganisms. Cross references to synonyms have been kept to a minimum in the text. For the most part these are shown in the index. An effort has been made to list in the index the names of all the microbes associated with each insect.

The author has attempted to consult original papers and works as much as possible. Where the reference to the work would seem to be of special value to the reader, it has been cited. Although large numbers of references have been given, no attempt has been made to make the book completely bibliographic of the field of insect microbiology. The writer is greatly indebted to the many works read but not mentioned by name. The references cited in the text are listed together at the end of the book.

It is the earnest desire of the author that this volume shall be of use, not only as a reference book to workers in biologic research, but also as a textbook for the classroom. A specialized course in insect microbiology would serve as a basic study to those majoring in such fields as medical entomology, physiology of insects, insect pathology, plant pathology, bacteriology, or parasitology. Certainly there is abundant material available to support such a course.

The author is indebted to a great many people who have assisted him in the completion of this book. For thoughtful advice and inspiration I wish to express my appreciation to Professor Alvah Peterson of the Ohio State University and to my associates at the Rocky Mountain Laboratory and at the University of California. For kindly reading portions of the manuscript and for offering constructive criticisms on certain subjects, I am indebted to Prof. Lee Bonar of the University of California, Maj. Gordon E. Davis of the Army Medical

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Edward A. Steinhaus

Berkeley, California
September, 1945

INSECT MICROBIOLOGY

CONTENTS

❖ CHAPTER I ❖

INTRODUCTION

TO ANYONE who gives the matter some thought it is readily apparent that the field embracing the microbiology of insects is one that needs to be introduced to the microbiologist and entomologist alike. In a sense, it is somewhat superfluous to begin this book with a chapter titled "Introduction" when in reality the purpose of the entire book is to introduce the field to those it may concern. An entire book is necessary for such an introduction since, in addition to entomology, at least five sciences are concerned: bacteriology, protozoology, mycology, pathology, and immunology. These sciences encompass the following types of microbes which may be associated with insects and ticks: bacteria, protozoa, yeasts, molds (fungi), rickettsiae, viruses, and spirochetes.

The present day of specialization has a tendency to demand that one's attention be limited to a single subject. The unfortunate part of this demand is that it neglects important borderline fields which are badly in need of investigation and study. Insect microbiology is just such a field. It is a rare entomologist who has had extensive training in bacteriology, protozoology, or mycology. Similarly, the microbiologist has seldom seen fit to acquire an entomologic background. Hence, problems involving the co-operation of these two groups of sciences are sorely neglected. There is probably no field of biologic endeavor more in need of interscience co-operation than that of the microbiology of insects and ticks. The entomologist and the microbiologist greatly need to be introduced to each other.

The need for the co-operative merging of abilities in entomology and microbiology and for adequate training of the individual in the fundamentals of both sciences has been well expressed by Leach (1940):

> The discovery of the nature of virus diseases of plants and the role of insects in the transmission of viruses has greatly stimulated the interest of both entomologist and pathologist in the general subject of insect transmission of plant diseases. The

appearance in Europe and America of the destructive and spectacular Dutch elm disease, which is so dependent upon insects for its spread and development, also served to focus the attention of both groups of workers on the problem. The necessity for cooperation between entomologist and plant pathologist in the solution of these borderline problems is now generally recognized by both groups, and the need has been expressed in the literature on numerous occasions. For various reasons, however, the present situation leaves much to be desired. It is one thing to talk of cooperation and another to cooperate. In fact, so much has been said and written in recent years about cooperation in research that the word, to some extent, has fallen into disrepute. Cooperation often works much better on paper than in practice. In order to avoid the difficulties of cooperation, we often plead for coordination of effort. This sounds much better but is often more difficult, for it requires a great deal of earnest cooperation before we can have successful coordination. But despite all these difficulties there is, and has been, a fair amount of real and successful cooperation in scientific research.

The failure or lack of cooperation may be caused by many different factors. The human or personal element is perhaps the most common of them all and the most difficult to overcome. Other causes may be administrative, political or, as indicated above, largely a matter of tradition. However, it is the author's belief that the greatest success in the solution of borderline problems cannot be achieved by the expedient of cooperation alone. Cooperative work is sometimes attempted on the principle of strict division of labor in which all "entomological" work is done by the entomologist and all "phytopathological" work by the phytopathologist. This type of cooperation nearly always is doomed to failure. In the study of the relation of insects to plant diseases, such strict division of labor is not practical. For the greatest success, the invisible, though very real, wall separating the two fields of research must be broken down. This may be rather difficult, but it can be done.

A first step in this direction would be a liberalization of the narrow professional viewpoint, which in effect often hangs out a sign reading, "This is the phytopathological field; entomologists encroach at their own risk" or "This is the entomological field; all phytopathologists keep off." Such a viewpoint may simplify some of the problems of organization, but it is not conducive to the solution of these neglected, but mutually important, problems. The necessity for well-defined fields of research with corresponding responsibility and authority is recognized. Such responsibility and authority are necessary, not only for efficient administration, but also for the existence of the guild spirit so important in scientific research. Nevertheless, when attempts are made to draw too sharp a line between related fields of activity, many problems of vital importance and significance usually are neglected.

A second step would be a modification of our educational procedure so that research workers would be given the viewpoint and training in techniques necessary for the solution of the problem in hand. The worker should have a thorough knowledge of the essentials of both entomological and microbiological techniques. Instead of placing the emphasis upon training entomologists or plant pathologists, some of the workers should be given the training and viewpoint necessary for the solution of this particular kind of problem, namely, the role of insects in the spread and development of plant diseases. It is not proposed that we train mental giants who can master both fields of knowledge, but rather workers who have a sufficient

grasp of the essentials of both sciences for the solution of this particular kind of problem. When these qualifications are combined in one man, many of the difficulties of cooperation will be avoided. No claim is made for the novelty of the idea, for already it has met with considerable success in the solution of other borderline problems.[1]

Historical. Medical historians tell us that one of the first to link the spread of disease with the activities of insects was Mercurialis in 1577. He believed that the cause of bubonic plague was carried from the ill or the dead to the well by flies. Naturally Mercurialis had no conception of microbes in his day, but his idea that the virus of infection could be conveyed by insects was essentially correct, although in the case of plague the mode of transmission is usually not by way of flies. During the following three hundred years a few others put forth similar views, but most of these were purely conjecture. In some cases, however, certain observers came extremely close to the truth. Thus in 1848 Josiah Nott expressed his belief that mosquitoes were responsible for the occurrence of both yellow fever and malaria. Similarly, Beauperthuy in 1854 published a theory on the transmission of yellow fever and other diseases by mosquitoes.

It was not until several decades after the discovery of microbes that men's suspicions were fully aroused as to the possible connection between these microscopic forms and insects. Noteworthy in this connection are the experiments by Raimbert, who in 1869 showed by the inoculation of guinea pigs that flies could be contaminated with anthrax bacilli and very probably could disseminate them.

The greatest impetus given the study of microbes in relation to their arthropod hosts occurred during the years from 1890 to 1900. During this period several epoch-making discoveries were made. The first of these was Waite's (1891) discovery that bees and wasps were vectors of fire blight, a bacterial disease of pears and other orchard fruits. Then Smith and Kilbourne's (1893) discovery that the cattle tick, *Boöphilus annulatus,* is the invertebrate host of *Babesia bigemina,* the cause of Texas cattle fever, was of great fundamental importance. Of great significance also was their observation that the protozoan was transmitted to the next generation through the egg. In rapid succession were reported the discoveries that trypanosomes were carried by tsetse flies (Bruce, 1895), that mosquitoes carry the malaria parasite (Ross, 1897), that the plague bacillus may be transmitted to rats by infected fleas (Simond, 1898), and that the virus of yellow fever is transmitted by the mosquito *Aëdes aegypti* (Finlay, 1881; Reed, 1900).

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[1] Quoted from J. G. Leach, *Insect Transmission of Plant Diseases,* by permission of McGraw-Hill Book Co., New York.

Similar discoveries followed throughout the decades after 1900 until the transmission of all types of microorganisms (bacteria, fungi, protozoa, spirochetes, rickettsiae, and viruses) by arthropods was clearly established.

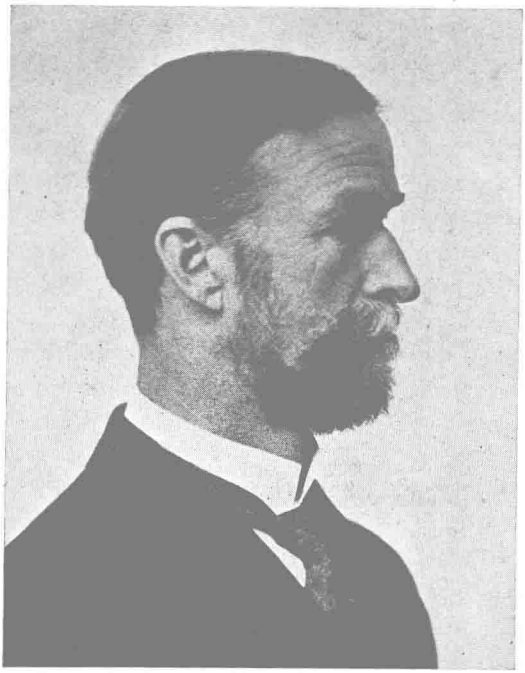

Fig. 1. Theobald Smith. One of the first investigators to show experimentally that an arthropod may transmit a disease agent. (Courtesy Dr. Carl Ten Broeck.)

Thus the discoveries concerning the arthropod-transmitted diseases of animals, man, and plants dramatically called attention to the association of microbes with insects and ticks. During the course of such investigations other entomophilic microbes, unrelated to disease, were also observed. In the hurry to work out the relationships of the microbes of medical importance, observations on the nonpathogenic microorganisms were largely neglected. Fortunately a few biologists, braving the wrath of those who scorn academic or "pure" science, laboriously sought the secrets of some of these supposedly less glamorous associations. As will be pointed out in later paragraphs, some of these little-known relationships may ultimately make greater contributions to our knowledge of life than have some of the more dramatic discoveries.

The historical data relative to each of the groups of organisms constituting the subject matter of this book will be considered in the chapters dealing with these groups. This history consists of a continuing parade of many interesting personalities, institutions, discoveries, and mistakes.

Biologic Relationships. The reader may wonder where lies the essence of our story—what is the plot? Essentially it has to do with the phenomena concerned wherever and whenever microbe and insect happen to meet. We shall throughout be concerned chiefly with the *biologic relationships* existing between microbes and insects. We shall want to know the effects each of these forms of life has upon the other. We shall inquire into the adaptations and

physiologic processes involved in these associations and their effect upon the respective ecologies and biologies.

To support such a plot, it will be necessary frequently to list or catalogue the microbes concerned. This, however, is done out of necessity, since without introducing the characters it would be difficult to carry out the theme, which, as we have just said, is concerned with the biologic relationships involved.

In chapter IV we shall have occasion to define in detail our acceptance of the terms "symbiosis," "mutualism," "parasitism," "commensalism," and the other terms used to denote manners in which organisms associate with one another. Suffice it to say here that we feel obligated to use the word "symbiosis" as its originator, De Bary, originally used it: as a general term referring simply to the living together of dissimilar organisms and not excluding parasitism or commensalism. Thus, in a sense, we may also say that our theme has to do with the factors involved in the symbiotic associations prevailing between insects and microorganisms, i.e., between macrosymbiote and microsymbiote. Regardless of the association between the insect and microbe—whether the latter is parasitic, mutualistic, or commensal; pathogenic or nonpathogenic to vertebrates or to invertebrates; necessary or beneficial to the life of the insect, or only an adventitious associate—it shall make no difference in our treatment of it. All relationships existing between insects and microbes will receive our equal consideration.

To some extent the type of association or relationship involved determines the location of the microbes with respect to the insect. The association may be one in which the insect may voluntarily bring about or encourage the microbes to grow (e.g., those insects having fungus gardens). Usually, however, there is no such freedom of choice for the insect. The microbes may be found in the interior of the insect or externally on its chitinous covering. They may live endogenously and penetrate to the outside of the insect, or they may originate or grow exogenously and penetrate to within the insect's body. Internally, the microbes may abide extracellularly in the alimentary tract or in the hemocoele, or they may live intracellularly in the epithelial lining of the alimentary tract, Malpighian tubes, salivary glands, or in the cells of other tissues of the insect's body. They may live extracellularly in definite tubes, pouches, or ceca, or they may inhabit only certain specialized cells in certain specialized organs.

The microbes themselves may be highly specialized organisms capable of living only in association with specific insects or groups of insects, or they may be common, saprophytic, adventitious microbes existing only in a fortuitous association with insects. They may be harmless, nonpathogenic forms, or they may be disease parasites of animals or of plants, the insect acting as a necessary

or accidental carrying agent or vector. The microbes may parasitize the insect, causing it to become diseased, or they may actually benefit the insect by serving as food or as a source of enzymes and vitamins.

Thus it is apparent that almost every type of biologic relationship which is known to exist may be found among the many associations prevailing between microbes and insects. As concerns our knowledge of the extent of these associations—only the surface has been scratched!

Applications. The knowledge and information gained through the study of the microbes associated with insects and arachnids may be applied or put to use in numerous ways. These applications may fall into any one of three domains: agriculture, medicine, and general biology.

In *agriculture,* for instance, there exists one of the outstanding examples of the practical effects of co-operation between entomologist and microbiologist, namely the realization that insects (and ticks) transmit diseases of animals and plants. The insect-transmitted diseases, of the latter especially, are many and important, and the biologic relationships are as yet little understood. Another application of insect microbiology has to do with the biologic control of destructive insects by means of controlled bacterial and fungous diseases. The real possibilities of this application, in the past, have been considered mostly with a certain amount of dilettantism. Along with this goes the demand for still more knowledge of the microbial diseases which plague our useful insects such as the bee and the silkworm.

The applications of insect microbiology in *medicine* are likewise numerous and extremely important. An understanding of the biologic relationships between pathogenic microbes and their vectors forms the basis for the important field of medical entomology. Only through a thorough understanding of the general microbiology of insects and ticks can we acquire a clear picture of the manner in which these arthropods transmit disease-producing organisms. Our new interest in the arthropod-borne diseases of the tropics is making a heavy demand upon insect microbiology for fundamental information and data relating to the association of microbes and their tropical vectors.

In its applications to *general biology* the field of insect microbiology has an extremely promising future. It is now recognized that the academic and "pure" biology of today pays great dividends years and decades hence. It is not to be doubted, therefore, that present studies on the basic biologic principles underlying the many associations between microbes and insects will bring forth great scientific profit in the years to come. Who knows what biologic discoveries lie behind a knowledge of the mysterious ability of certain insects to harbor in their tissue cells large numbers of living microorganisms? Are certain of the intracellular symbiotes of today the pathogenic rickettsiae of