S. A. WAKSMAN

THE ACTINOMYCETES

Volume 1

THE ACTINOMYCETES

Nature, Occurrence and Activities

by

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THE ACTINOMYCETES

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Vol. II: Descriptions of Genera and Species of Actinomycetes

Vol. III: Antibiotics of Actinomycetes

PREFACE

In any attempt to classify and divide living systems, nay, even living *versus* nonliving systems, certain borderline bodies are encountered which may be considered as transition forms from one group to another. This was recognized by the early students of the microscopic forms of life, who considered the bacteria and similar organisms as "protista" or primitive bodies, related, on the one hand, to the plants and, on the other hand, to animals. Recently accumulated information points also to viruses as transitory between nonliving and living bodies.

The actinomycetes form such a borderline system, but on a much more specialized scale. Considered by some as bacteria ("higher bacteria"), or Eubacteriales, and by others as fungi ("lower fungi"), or Hyphomycetes, actinomycetes are often placed in a group by themselves, with some of the properties of both. There are found, among the actinomycetes, certain forms that are more closely related to the bacteria and others that are nearer to the fungi.

My personal attention was first directed to the actinomycetes about 45 years ago. In 1914, as a senior in college, specializing in soil microbiology, or, as it was designated at that time, "soil bacteriology," I was assigned by my professor, Jacob G. Lipman, the task of making a comparative monthly study of the bacterial population of certain soil types located on the experimental grounds of the college. The results obtained in this study were used for a thesis which I presented the following June for my B.Sc. degree.*

Throughout the year 1914–1915, I sampled, at monthly intervals, several different soil types. Samples taken under sterile conditions were obtained from various depths (from the surface to 30 inches). These I brought to the laboratory and plated out, using suitable dilutions and proper culture media. After varying periods of incubation, I counted the colonies of bacteria developing on the plates. I was soon struck by the fact that a fairly large number of the colonies that I could observe did not look exactly like the majority of the others, more typical of bacteria. These particular colonies were compact and leathery in nature, pyramidal in structure, penetrating deep into the agar medium, frequently covered with a surface fuzz that was distinct from the substrate growth. On examination of such colonies even with a low-power microscope, the fuzzy growth proved to be made up of an aerial, branching mycelium that resembled that of fungus colonies.

When I brought the plates to my professor, he shook his head, smiled, and said, "Yes, I have been aware of the occurrence of these types of bacteria. Frequently they are designated as a special group, under the name actinomyces. You had better go and see our botanist, Professor M. T. Cook. He may be able to tell you more about them." Professor Cook was indeed familiar with the group, but merely as causative agents of potato scab. He considered them, not as bacteria but as fungi. He referred me to various papers in which further information could be obtained on this group of organisms. I decided in my very early studies, that the organisms could be differentiated from both bacteria and fungi. To my great satisfaction, I learned later that similar suggestions had already been made previously by others.

Thus, at the very threshold of my scien-

* "Bacteria, Actinomyces and Fungi in the Soil." Selman A. Waksman, Thesis, Rutgers College, New Brunswick, N.J., 1915 (Abstract published in J. Bacteriol. 1: 101, 1916).

tific career, I came in touch with a group of microorganisms that were to occupy a major part of my future scientific life. The final year of my undergraduate studies of these organisms was followed by three years of graduate work,† and by many more years as scientific assistant and finally as microbiologist at the New Jersey Agricultural Experiment Station.

The following treatise is, in part, a summary of these investigations carried out for nearly half a century, mostly in the laboratories of Rutgers University, first at the College of Agriculture and Experiment Station, and more recently at the Institute of Microbiology. In a larger sense, however, I wish to give credit to the many other investigators who, by their careful and exhaustive studies, have so far advanced our knowledge of the actinomycetes during this first half of the Twentieth Century.

In the preparation of this volume, I have drawn freely from the various theses submitted by candidates for their Ph.D. degrees, working under my direct or indirect supervision. I wish to acknowledge the assistance of my colleagues and collaborators, notably Dr. Ruth E. Gordon, Dr. Hubert A. Lechevalier, Mr. Robert A. Day, and Mrs. Herminie B. Kitchen. I also wish to thank Dr. C. W. Emmons, of the National Institutes of Health, for reading Chapter 17, and Dr. L. A. Schaal, of the U. S. Department of Agriculture, for reading Chapter 18.

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† "Proteolytic Activities of the Soil Fungi and Actinomycetes," Selman A. Waksman, Ph.D. Thesis, University of California, December 1917 (J. Bacteriol. 3: 475–492, 509–530, 1918).

INTRODUCTORY

No other group of microbes, and for that matter no other group of living systems, whether of plant, animal, or microbial origin, has been in recent years the focus of so much attention by the investigator, especially the microbiologist, the chemist, and the medical scientist, and by the pharmaceutical manufacturer, as the actinomycetes. Only 20 years ago scarcely a dozen laboratories in the whole world were devoting much attention to this group of organisms, and they were concerned largely with either disease-producing or soil-inhabiting forms. Today, literally thousands of investigators in numerous laboratories throughout the world are isolating cultures of actinomycetes from soils and other substrates and studying their physiological and biochemical activities. This increased attention is due primarily to the discovery that the actinomycetes comprise many forms that have the capacity to produce a large number of chemical substances capable of inhibiting the growth of microorganisms, especially disease-producing forms. These substances have come to be known as antibiotics. The discovery that certain actinomycetes can produce growth-promoting substances or vitamins and certain potent enzyme systems has added greatly to this interest. Many of the antibiotics produced by the actinomycetes have found extensive practical application in the control of infectious diseases of man, animals, and plants; also in animal nutrition; and in the preservation of biological products, including virus preparations, and of human foodstuffs.

Our first knowledge of the actinomycetes dates back to 1875, when Ferdinand Cohn named an organism he found in the tear duct of the human eye *Streptothrix Foersteri*. This was soon followed (1877 to 1878) by a description by Harz, of another organism, *Actinomyces bovis*, found in "lumpy jaw" of cattle. Since then, many actinomycetes have been isolated, and a number of genera and hundreds of species have been described. These include organisms causing animal and plant diseases and numerous saprophytes occurring in soils, in dust, in water basins, and in other natural substrates.

Because of the above two generic names and for other reasons, the systematic position of actinomycetes became highly confused. Animal and plant pathologists, botanists, zoologists, mycologists, bacteriologists, and biochemists were eager to introduce new names in describing as new species freshly isolated cultures of actinomycetes. New genera and new species were thus created, without due regard to previously established names or even previous descriptions. This tended to complicate greatly our knowledge of the taxonomy and classification of the actinomycetes.

A number of subsequent milestones in the history of actinomycetes should be noted. Among them were the isolation by Israel of a pure culture of an anaerobic organism, for which the generic name *Actinomyces* was reserved; the introduction of synthetic media by Krainsky and by Waksman and Curtis; the recognition of the sporulating mechanisms of actinomycetes by Ørskov; the classification systems of Waksman and Henrici and of Krassilnikov; the isolation of antibiotics from cultures of actinomycetes; and finally the study of the cell walls of actinomycetes. These and numerous other milestones have marked the development of our knowledge of the actinomycetes from the original concept that they were a small group of negligible organisms causing certain obscure diseases to the comprehensive recog-

nition that they represent a large and highly important microbial group of universal distribution, possessing numerous biochemical activities, and of great practical potentialities.

From an ecological point of view, the interest in the actinomycetes has centered largely upon the study of their occurrence in soils, in composts, in water basins, in the atmosphere, and in the infected tissues of living systems. Their role as causative agents of human, animal, and plant diseases at first attracted wide attention, but more recently this interest became of limited significance. Under some conditions, however, the actinomycetes may play a highly important role in the causation of certain plant diseases, such as potato scab.

From a biochemical point of view, interest in the actinomycetes has centered largely upon their role in the transformation of organic matter in the soil and their ability to form antibiotics, vitamins, and enzymes. The interest in the antibiotics produced by actinomycetes has been phenomenal. It all began with the isolation of actinomycin in 1940. This was followed by the isolation of streptothricin in 1942 and of streptomycin in 1943, and later of chloramphenicol, the tetracyclines, the erythromycins, the neomycins, novobiocin, oleandomycin, nystatin, and numerous others. To date, more than 500 different antibiotics have been isolated from cultures of actinomycetes. Many of them have been obtained in the form of pure compounds, the chemical nature of which has been determined. Others are still of unknown composition. Nearly 25 of these antibiotics have already found extensive practical application as chemotherapeutic agents. Of the total 2,400,000 pounds of antibiotics produced in the United States in 1955, valued at more than a half a billion dollars, at least two-thirds have been obtained from cultures of actinomycetes.

The interest in the antibiotics evoked tremendous interest in these organisms, their distribution in nature, their growth and nutrition under controlled conditions, and finally their biochemical activities. Among the earlier treatises devoted to the subject of actinomycetes, note should be taken of the work of Lieske (1919), Duché (1935), Kriss (1937), Krassilnikov (1938), and Cope (1938). I have personally contributed to many phases of the study of actinomycetes. Following my work on "The cultural properties of actinomycetes," published in 1919, I edited the section on actinomycetes in the various editions of *Bergey's Manual*, beginning with the first in 1923 and including the seventh in 1958. My more recent books include a book on *The Actinomycetes* published in 1950 and various volumes and papers on the antibiotics of actinomycetes.

The rapid accumulation of basic knowledge concerning the actinomycetes justifies a comprehensive treatise at this time. In this work, I have made no attempt to review or even to list the extensive literature on this subject. Only certain pertinent references have been selected. In view of the fact that more than 6000 references on the subject of a single antibiotic, streptomycin, had been collected (as of 1952!) one can readily imagine the extensive literature covering the other antibiotics that have found practical application in the treatment of numerous human and animal diseases, in animal feeding, and in the preservation of various biological preparations and food materials. And all of these antibiotic references, of course, would be in addition to the thousands of papers that have been published relating to the organisms themselves.

This treatise is limited to a review of our knowledge of the true actinomycetes. It does not concern itself with the various bacterial forms frequently included among the *Actinomycetales*, namely, the mycobacteria, corynebacteria, and mycococci.

In view of the frequent references to mem-

bers of the various genera of the actinomycetes by vernacular designations, the following comments may be made here:

The terms "actinomycete" and "actinomycetes" will be used in this treatise as inclusive terms for any or all of the organisms now included in the Actinomycetales, exclusive of the mycobacteria and corynebacteria. The term "*Actinomyces*" will be used only when referring to the single genus of that name; "actinomyces" will be used as the vernacular expression only for members of this genus, both in singular and in plural senses. The term "streptomyces" will be used as a vernacular expression of the genus *Streptomyces*, both in singular and plural senses. The terms "nocardia" and "nocardias" will be used in the vernacular for members of the genus *Nocardia*, and "micromonospora" and "micromonosporas" for "*Micromonospora*."

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OCCURRENCE, NATURE, AND ACTIVITIES

CHAPTER 1

Historical Background

What Are Actinomycetes?

Actinomycetes are a group of branching unicellular organisms, which reproduce either by fission or by means of special spores or conidia. They are closely related to the true bacteria; frequently, they are considered as higher, filamentous bacteria. They usually form a mycelium which may be of a single kind, designated as substrate (vegetative), or of two kinds, substrate (vegetative) and aerial (in part sporogenous).

In the early descriptions, actinomycetes were often defined as "unicellular microorganisms, 1 μ in diameter, filamentous; branching monopodial, seldom dichotomous, producing colonies of radiating structure." Two forms of reproduction have commonly been recognized: (a) fragmentation, or oidia-formation, and (b) segmentation. Both kinds of spores grow in ordinary media to form a filamentous mycelium.

Frequently the actinomycetes have been looked upon as a separate group of organisms occupying a position between the filamentous fungi and the true bacteria. It has even been said that actinomycetes are the original prototypes from which both fungi and bacteria have been derived. Some forms of actinomycetes, such as members of the genus *Nocardia*, are known to have their counterparts among the bacteria; other forms, like some species of *Streptomyces*, *Micromonospora*, and some of the other genera, have their counterparts among the fungi. The similarity in diameter between bacteria and the mycelium and spores of actinomycetes and certain common chemical and biochemical properties, recently discovered, suggest that the actinomycetes should be classified with the bacteria. They are usually placed in a separate order, the Actinomycetales, which is said to be distinct from the Eubacteriales, or the true bacteria, although this relationship has recently been questioned.

The actinomycetes are generally recognized to represent a large and heterogeneous group of microorganisms, comprising several genera and numerous species. They vary greatly in their morphology, physiology, biochemical activities, and role in natural processes. They play an important part in the cycle of life in nature by bringing about the decomposition of complex plant and animal residues and the liberation of a continuous stream of available elements, notably carbon and nitrogen, essential for fresh plant growth. Some of the biochemical activities of the actinomycetes are now being utilized for the large-scale production of chemical substances essential for public health and human economy.

Early Concepts

The early history of the actinomycetes revolves around their role as causative agents of disease, especially a disease in cattle known as "actinomycosis" or "lumpy jaw."

Ferdinand Cohn's first description of an actinomycete was based upon his study of an

FIGURE 1. The first illustration of an actinomycete ever published, *Streptothrix Foersteri* (Reproduced from: Cohn, F. Untersuchungen über Bacterien II. Beitr. Biol. Pflanzen 1: 141–207, 1875).

organism found in concretions of the lachrymal ducts and which he named *Streptothrix Foersteri*. The concretions were transmitted to Cohn by Foerster for microscopic examination. Cohn says: "On April 15, 1874, he transmitted to me a mass which was whitish, like tallow, easily broken down and still consisting of fine, very thin colorless branch-

ing threads running parallel to one another or in various directions, curving and in places also wavy." This type of growth reminded Cohn of the curvatures of spirilla and spirochaetes, although it was more irregular. The threads were found to break up into fragments, some of which reached a length of 50 μ. The branching filaments were surrounded with masses of micrococci, filling the spaces between the threads. These filaments were distinctly different from the straight, thick, and unbranched (false-branching) *Leptothrix buccalis* commonly found in the mouth. The photographs of the organism published by Cohn leave no doubt that this was a true actinomycete. Cohn considered this organism to be a bacterial form with branching mycelium, though all attempts to cultivate the organism failed.

Two years later, Harz examined a pathologic specimen, obtained from "lumpy jaw" of cattle and submitted to him by Bollinger. He gave to the organism observed in this specimen the generic name *Actinomyces* and the specific name *bovis*. No pure culture was obtained. The masses of filaments were found to be arranged radially, which suggested the name "actino-myces" or "ray-fungus."

Neither of these two generic descriptions was universally accepted, largely because the first (*Streptothrix*) had been preempted in 1839 by Corda for a true fungus and the second (*Actinomyces*) had been meeting with much criticism, because the description of the organism was based on its etiology rather than on its morphology and cultural characteristics.

The first isolations of pure cultures of actinomycetes from human and animal infections involved some difficult problems in ecology and taxonomy. They were the primary causes of much confusion in the history of actinomycetes. O. Israel claims to have isolated in 1884, from a human infection, an aerobic filamentous organism, the hyphae undergoing ready fragmentation. Bostroem claims to have isolated in 1885, also from human cases, an aerobic, filamentous, spore-forming culture. Nocard isolated an aerobic culture in 1888 from an animal infection. This was followed (1889) by the isolation from a human infection of an aerobic culture by Afanassiev. In 1890, Eppinger isolated a nonsporulating aerobic organism, and Wolff and J. Israel isolated, the same year, a nonsporulating microaerophilic form.

These cultures came from different sources and, because of their filamentous nature, were considered to represent the isolates of Cohn and Harz. None of the above isolations were, however, the cause of as much confusion as the report made by Bostroem of his isolation in 1890 of a pure aerobic culture of an actinomycete from a case of actinomycosis. This culture, now known to be a *Streptomyces*, rapidly found a place among the various collections and was believed at first to be the true cause of actinomycosis. The general consensus now is that this culture did not represent the causative agent of the disease but was merely an air contaminant. Unfortunately, this error remained to plague the subsequent literature of the actinomycetes and became a cause of much confusion. First, the claim that *Actinomyces bovis* was an aerobe rather than an anaerobe was wrong; second, the wide distribution of the contaminant led many to assume that actinomycosis was caused by an aerobic organism similar to the group now designated as *Streptomyces*.

For many years, investigators continued to believe either that the causative agent of actinomycosis was an aerobe or that there were two forms, one an aerobe and the other an anaerobe. There is no doubt now that Bostroem never succeeded in growing the true etiologic agent of actinomycosis but that some of his attempted isolates became contaminated with saprophytic actinomycetes from the dust in the air, and thus resulted in the mistaken isolation. Topley and Wilson

(1929) proposed that this isolate be named *Actinomyces graminis*. Vuillemin (1931) considered it to be identical with *Actinomyces sulphureus* Gasperini (1894).

In the absence of pure cultures of the causative agent of the disease for comparative studies, some of the early workers on actinomycetes had only a limited concept of the growth and life cycle of these organisms. This is illustrated, for example, in the description by MacFayden (1889) of the history of an actinomycete colony:

"It has its starting point in one or more cocci transported by the plasma currents or by the agency of a carrier cell (leucocyte). The cocci multiply by elongation and subsequent fission. By elongation some of the cocci give rise directly to short bacillary forms, and through these to long filaments. The further extension of the colony is effected by the growth and multiplication of both threads and cocci. The majority of the threads tend to develop clubs at their outer ends (involution forms)." For more phantasy and inaccuracy, one would have to search widely in microbiological literature.

Not much progress in the general understanding of these organisms seems to have been made during the next 20 years, as illustrated by reference to them in the Second Edition of H. W. Conn's (1909) *Agricultural Bacteriology*. In speaking of the actinomycetes, he says:

"Under this head are included a few forms of fungi which resemble other bacteria in some respects, but differ in others. They are composed of threads which are commonly larger than the threads of bacteria, and which may show frequent branching, a characteristic not usual in bacteria. They also have a peculiar method of forming reproducing bodies. The group is not one of very great importance. One type of *Streptothrix* is extremely abundant in soil and appears as round, white opaque colonies with an extensive brown halo upon the plates."

An important cause of confusion was the fact that the actinomycetes were grown on nitrogen rich organic media, now known to be totally unsuitable for them to form a characteristic growth, essential for comparative studies and for proper identification. As a result, a highly complex terminology was developed for the designation of actinomycetes; numerous descriptions of "new" species soon began to appear. This is illustrated by the summary made, as early as 1892 to 1894, by Gasperini (Table 1). There is no wonder, therefore, that the nature and classification of the actinomycetes soon appeared hopeless.

The adoption of the name "actinomycetes" was suggested by Gasperini and Lachner-Sandoval. Sanfelice, impressed by the analogy of the biological properties of the actinomycetes and those of the tuberculosis organism, suggested that the relationship of the actinomycetes to the bacteria was closer than to the fungi. Gasperini emphasized that the species or varieties belonging to the actinomycetes, included under one genus *Actinomyces*, show great variations in form and in behavior, especially in their ability to produce aerial spores and soluble pigments. Some of these properties were recognized to be inconstant and were found to depend on the conditions of culture and the composition of the medium; minor variations of the latter could bring about marked changes in growth and pigmentation.

Historical Periods

Before we consider in detail the historical background of our knowledge of the actinomycetes, we must recognize certain distinct periods in which the various concepts concerning the nature of these organisms and their importance in the cycle of life became crystallized. There is, of course, considerable overlapping of the various periods, since no one period came to an end before another

TABLE 1

Species of actinomycetes recognized in 1892 to 1894 by Gasperini

Name	Observer	Name	Observer
Act. bovis sulphureus	Rivolta	*Act. bovis* (?)	—
Act. Foersteri	Cohn	*Streptothrix Foersteri*	—
Act. canis	Vachetta	*Act. pleuriticus canis familiaris*	Rivolta
		Act. canis	Rabe
Act. bovis farcinicus	Nocard	*Bacillus farcinicus*	—
Act. cati	Rivolta	—	—
Act. bovis albus	Gasperini	*Streptothrix* 1, 2, 3	Almquist
		Streptothrix Albus	Rossi-Doria
Act. asteroides	Eppinger	*Cladothrix asteroides*	—
		Strept. asteroides	Gasperini
		Strept. Eppingerii	Rossi-Doria
Act. chromogenus	Gasperini	*Strept. chromogenus*	—
		Strept. niger	Rossi-Doria
		Oospora Metschnikowi (?)	Sauvageau & Radais
		Oospora Guignardi (?)	Sauvageau & Radais
Act. bovis luteo-roseus	Gasperini		—
Act cuniculi	Schmorl	*Streptothrix cuniculi*	—
Act. Hoffmanni	Gruber	*Micromyces Hoffmanni*	—
Act. albido-flavus	Rossi-Doria	*Streptothrix albido-flava*	—
Act. violaceus	Rossi-Doria	*Streptothrix violacea*	—
Act. carneus	Rossi-Doria	*Streptothrix carnea*	—
Act. citreus	Gasperini	—	—
Act. pluricolor (?)	Terni	—	—
Act. arborescens	Edington	—	—
Act. ferrugineus	Naunyn	—	—

one began. These periods can be briefly outlined as follows:

1. *Causation of disease.* This period began in 1875 and continued to the end of the 19th century. The predominant interest in the actinomycetes during these years was in their role as pathogens, first in human and animal diseases, especially actinomycosis in cattle, and later in plant diseases, particularly potato scab (R. Thaxter).

2. *Occurrence and importance in soil.* During the next two decades, beginning about 1900, with the work of Beijerinck, and ending about 1919, with the work of Krainsky, Conn, and Waksman and Curtis, the interest in the actinomycetes was predominantly concerned with their occurrence in soils and in other natural environments. The introduction of synthetic media served to broaden greatly our knowledge of the nature and occurrence of the actinomycetes.

3. *Biological period.* Between 1919 and 1940, intensive knowledge accumulated concerning the cultural properties of the actinomycetes, their physiology, and their biochemical activities, notably their antagonistic effects upon bacteria and fungi. This period may be said to have begun with the work of Waksman in 1919 and Lieske in 1921. It continued with the studies of Gratia and his group on the bacteriolytic effects of certain actinomycetes and of Krassilnikov and his associates on the antibacterial properties of actinomycetes. Problems of variability (Schaal, Tempel, Kriss), decomposition of plant and animal residues (Conn, Waksman

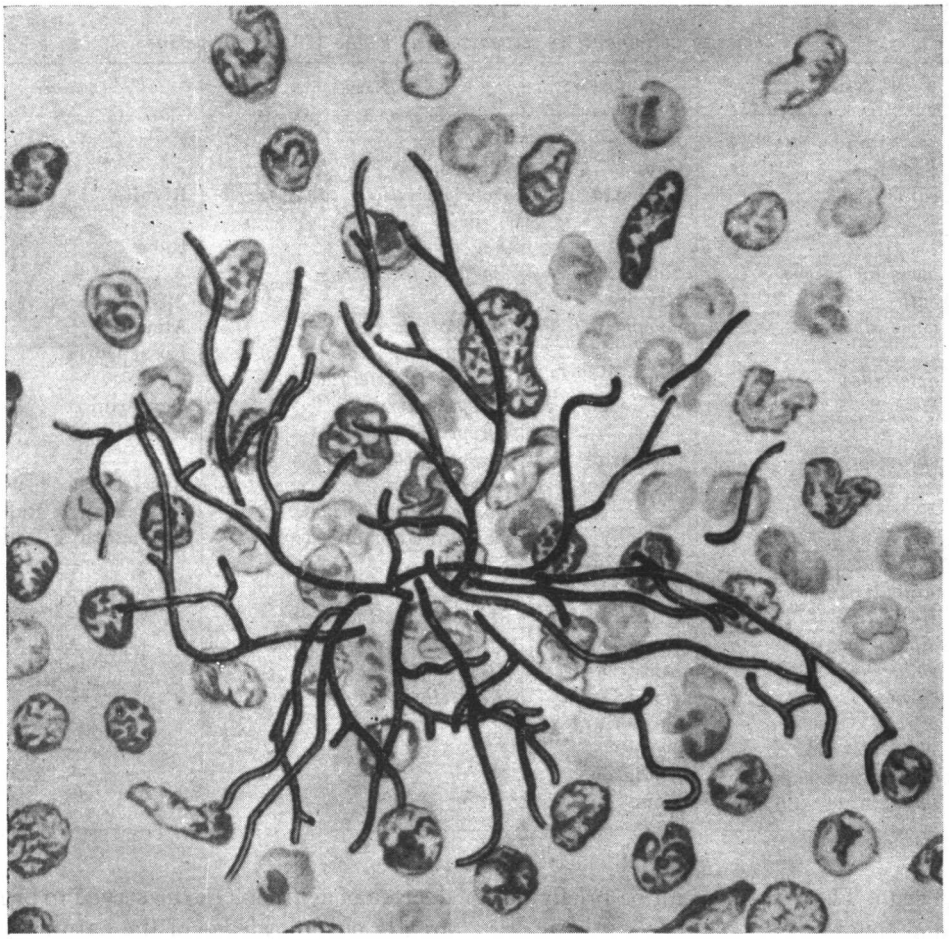

FIGURE 2. Growth of an actinomycete in animal tissue (Reproduced from: Butterfield, E. E. J. Infectious Diseases **2:** 430, 1905).

et al.), and the importance of actinomycetes in natural processes were given ever-growing consideration.

4. *The biochemical or, more precisely, the antibiotic period.* A new era in the study of actinomycetes began about 1940. Then, it was established that a large number of these organisms are capable of producing a great variety of chemical substances that have the capacity to inhibit the growth of various microorganisms, and that some of these substances can find chemotherapeutic applications in the treatment of numerous infectious diseases of man, animals, and plants. This resulted in extensive investigations devoted to the nutrition of actinomycetes, the biosynthesis by them of various chemical compounds, their chemical structure, life cycle, and numerous biochemical activities.

1. *Actinomycetes as Causative Agents of Disease (1875–1900)*

The initial work on the actinomycetes was done by two eminent botanists, F. Cohn in 1874 and C. O. Harz in 1877. Unfortunately, two circumstances soon shifted the interest in these organisms from the botanists to clinicians and veterinarians.

1. Cohn's work on actinomycetes, and for that matter on bacteria in general, was completely neglected by nearly all botanists following him. Even so outstanding a botanist as Roland Thaxter, who about 15 years later studied another group of actinomycetes, namely, the organisms causing potato scab, called them fungi (*Oospora scabies*), completely overlooking their close relationship to the bacteria.

2. The second circumstance had to do with the fact that the role of microbes as causative agents of infectious diseases had just come to be recognized as a result of the brilliant work of Louis Pasteur, Robert Koch, and numerous others. It was but natural that diseases caused by actinomycetes should also soon begin to attract attention. In 1876, Bollinger observed branching mycelium in the diseased jaw of a cow and recognized that a microbe was the causative agent of the disease. He handed this material to Harz, who examined the granules and observed the characteristic radiation, with the result described above. Simultaneously, J. Israel examined granules containing similar mycelium in two pathologic specimens of man; unfortunately, he was confused by the presence of secondary infections due to staphylococci. It was Ponfick, in 1879, who definitely established the role of actinomycetes as causative agents of human diseases. Israel's first clinical account appeared in 1885. Wolff working in collaboration with Israel soon established the anaerobic nature of the organism.

These pioneering studies were followed by the careful work of Gasperini and others who interpreted clearly the nature of the disease of actinomycosis and the role of actinomycetes in its causation.

The study of diseases caused by aerobic actinomycetes in animals and in man also began to receive attention, with the observations of Nocard and Trevisan. Unfortunately, the nature of the causative agents of these diseases and the complex nomenclature that soon evolved continued to cause confusion for years. As late as 1925, Dresel suggested that the term "actinomycosis" be reserved for those diseases that are caused by the anaerobe (*Actinomyces israeli*) and that another name be selected for the diseases caused by aerobes, in case the name "Streptothrix" should finally be disqualified.

Foulerton wrote in 1899 that the disease known as "actinomycosis" in cattle and man had long been recognized clinically to be caused by more than one species of actinomyces, infections themselves being very similar. Gasperini described three such varieties or species. Wolff and Israel isolated from human actinomycosis an organism, "a streptothrix fungus," which differed from "Streptothrix actinomycotica" in that the growth under anaerobic conditions was very free, whilst in the presence of oxygen it was very scanty. Levy isolated from five actinomyces cases in man an organism which resembled that of Wolff and Israel in its free growth under anaerobic conditions. Kruse recognized two species as causing actinomycosis: (1) "*Streptothrix actinomyces*" of Rossi-Doria, said to be an "aerobic fungus;" and (2) "*Streptothrix israeli*," an "anaerobic fungus." A number of other investigators de-

FIGURE 3. Club formation by a culture of *A. bovis* grown in human blood serum (Wright, J. H. J. Med. Research 13: 349–404, 1905).

scribed, according to Foulerton, cases "which clinically present the features of actinomycosis, but which are caused by parasites which differ sufficiently from streptothrix actinomycotica to entitle them to be regarded as separate species." Bruns noted a culture which he believed to be similar to that described by Berestnew as occurring in a case of "pseudoactinomycosis." Bruns objected to the use of this term and considered the organism in question to belong to a new species.

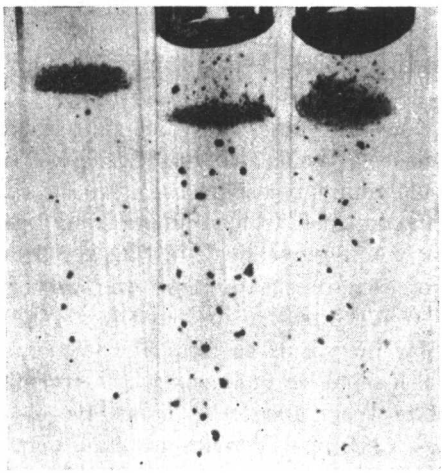

FIGURE 4. Appearance of cultures of *A. bovis* in agar tubes (Reproduced from: Wright, J. H. J. Med. Research 13: 349–404, 1905).

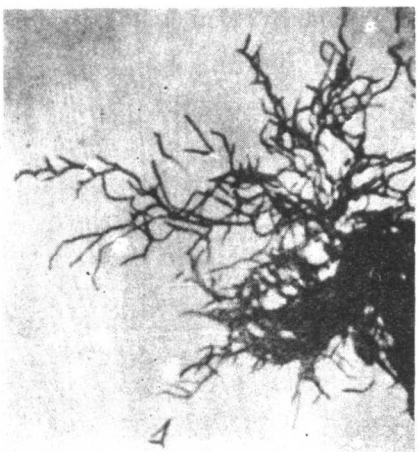

FIGURE 5. A cross-section of a colony of *A. bovis* in agar (Wright, J. H. J. Med. Research 13: 349-404, 1905).

Thus, the differentiation between aerobic and anaerobic forms as causative agents of specific diseases gradually became established, particularly through the work of Foulerton and Price-Jones (1902), Wright (1905), and others.

The first historical period is thus characterized by serious difficulties that were a direct result of the complications involved in the isolation and identification of the causative agents of disease conditions in animals and man, and by the problems of proper nomenclature, which will be discussed in detail in Chapter 4. Attention has already been drawn to the confusion introduced by Bostroem, in 1890, who isolated from infected lesions aerobic air contaminants, which he designated as the causative agent of the disease. Another cause of confusion was the introduction of the term "streptothricosis," based on Cohn's original designation, as a synonym—not always recognized as such—for "actinomycosis," or a disease caused by actinomycetes. Later suggestions that such names as "nocardiosis" and "maduramycosis" be used did not help to straighten out the ensuing confusion.

The study of the causation of plant diseases by actinomycetes also falls within this period. As has been noted, Thaxter elucidated, in 1891, the nature of the pathogenic organism concerned in potato scab. He called it *Oospora scabies*. The culture was isolated and carefully studied. This soon led to extensive investigations by numerous botanists and plant pathologists, which continued into the subsequent periods.

Outstanding work on the occurrence of actinomycetes, their morphology and systematic position, was also carried out during this period. It is sufficient to mention such names as Rossi-Doria, Lachner-Sandoval, and soon after Neukirch, and various other bacteriologists.