

ZINSSER

BACTERIOLOGY

SMITH
and
CONANT

ELEVENTH
EDITION

Z I N S S E R

BACTERIOLOGY

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To the memory of
PHILIP HANSON HISS, JR.
HANS ZINSSER
and to
STANHOPE BAYNE-JONES

whose labors over more than one third of a
century have made this textbook a favorite
with students of medicine and public health.

Preface

In the preface to the first edition of this *Textbook of Bacteriology*, written in 1910, the authors emphasized the importance of a basic approach which would include not only the biologic characteristics of the organisms but also the reactions of the living tissues to the bacteria and their products. This approach made the volume of equal value to the bacteriologist and to the student of public health and clinical medicine. For over a third of a century with the development of microbiology, this text grew through eight editions under the wise guidance of Hiss, Zinsser, and Bayne-Jones.

The present authors studied this text when they were students and for many years have used it in their teaching of other students. It is their desire to keep inviolate the basic biologic approach to bacteriology and, at the same time, to emphasize the public health significance and the practical clinical importance of certain of the biologic characteristics of the organisms.

Almost twice as many significant publications have appeared in the field of microbiology during the past five years as appeared during the previous five year period. This made necessary extensive revisions or complete rewriting of almost every chapter in the eleventh edition. The most rapid development has been in the area of bacterial physiology, immunology, and the viruses. These sections have been completely rewritten and enlarged to accommodate the new material. A new chapter has been added on the blood groups and immunohematology. The new edition includes information about the susceptibility of each organism to the newer antibiotics. Where the information was available, we have presented the favorable and unfavorable effects

of ACTH and cortisone on each specific infection.

The chapters dealing with infections have an introductory paragraph which emphasizes the public health aspect of the disease. The important biologic and cultural characteristics of the organism have been emphasized by printing them in boldface type. Highly technical sections and descriptions of diseases of minor importance have been printed in small type. There are 121 new illustrations in this edition, making a total of 422.

We wish to thank our many colleagues and students for their critical advice and generous help in the preparation of this revision and especially our friends who have supplied us with new illustrative material.

We are indebted to Elon H. Clark, Robert L. Blake, Paul I. Fairchild, Henry F. Pickett, Thurmon M. Ellis, and Carl M. Bishop for the preparation of new illustrations. We wish to acknowledge the valuable assistance of our secretaries, Mrs. Joanne Barnhill, Miss Jane Parker, Mrs. Shirley Crumpton, Miss Jacquelyn Walters, and Miss Martha Hadley who have cheerfully typed and retyped the manuscript many times.

Special thanks are due to Miss Judith Farrar, medical librarian, for her valuable assistance with the literature lists. We wish also to express our appreciation to Mr. G. A. McDermott and his staff of Appleton-Century-Crofts, Inc. for their many helpful suggestions in the preparation of the manuscript.

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Preface to the First Edition

The volume here presented is primarily a treatise on the fundamental laws and technic of bacteriology, as illustrated by their application to the study of pathogenic bacteria.

So ubiquitous are the bacteria and so manifold their activities that bacteriology, although one of the youngest of sciences, has already been divided into special fields—medical, sanitary, agricultural, and industrial—having little in common, except problems of general bacterial physiology and certain fundamental technical procedures.

From no other point of approach, however, is such a breadth of conception attainable, as through the study of bacteria in their relation to disease processes in man and animals. Through such a study one must become familiar not only with the growth characteristics and products of the bacteria apart from the animal body, thus gaining a knowledge of methods and procedures common to the study of pathogenic and nonpathogenic organisms, but also with those complicated reactions taking place between the bacteria and their products on the one hand

and the cells and fluids of the animal body on the other—reactions which often manifest themselves as symptoms and lesions of disease or by visible changes in the test tube.

Through a study and comprehension of the processes underlying these reactions, our knowledge of cell physiology has been broadened, and facts of inestimable value have been discovered, which have thrown light upon some of the most obscure problems of infection and immunity and have led to hitherto unsuspected methods of treatment and diagnosis. Thus, through medical bacteriology—that highly specialized offshoot of general biology and pathology—have been given back to the parent sciences and to medicine in general methods and knowledge of the widest application.

It has been our endeavor, therefore, to present this phase of our subject in as broad and critical a manner as possible in the sections dealing with infection and immunity and with methods of biological diagnosis and treatment of disease, so that the student and practitioner of medicine, by becoming familiar with underlying laws and principles, may not only be in a position to realize the meaning and scope of some of these newer discoveries and methods, but may be in better position to decide for themselves their proper application and limitation.

We have not hesitated, whenever necessary for a proper understanding of processes of bacterial nutrition or physiology, or for breadth of view in considering problems of the relation of bacteria to our food supply and environment, to make free use of illustrations from the more special fields of agricultural and sanitary bacteriology, and some special methods of the bacteriology of sanitation are given in the last division of the book, dealing with the bacteria in relation to our food and environment.

In conclusion it may be said that the scope and arrangement of subjects treated of in this book are the direct outcome of many years of experience in the instruction of students in medical and in advanced university courses in bacteriology, and that it is our hope that this volume may not only meet the needs of such students but may prove of value to the practitioner of medicine for whom it has also been written.

It is a pleasure to acknowledge the courtesy of those who furnished us with illustrations for use in the text, and our indebtedness to Dr. Gardner Hopkins and Professor Francis Carter Wood for a number of the photomicrographs taken especially for this work.

P. H. HISS, JR.

H. ZINSSER

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The General Biology of Bacteria and the General Methods of Bacteriology

1

OUTLINE OF THE HISTORY AND SCOPE OF BACTERIOLOGY

The history of many concepts now embodied in the doctrines of bacteriology is an account of attempts to solve the problems of the origin of life, the putrefaction of dead organic materials, and the nature of communicable changes in the bodies of living men and animals. The visible aspects of these phenomena were as apparent and as interesting to ancient observers as they are to modern biologists. In the past, notions of ultimate causes were derived from the available factual knowledge colored by the theological and philosophic tenets of the time. The early history of what has become the science of microbiology is to be found, therefore, in the writings of the priests, philosophers and scientists who studied and pondered these basic biologic problems.

Fortunately for the student of medical bacteriology, Bulloch (7) published in 1938 a small concisely written book entitled "The History of Bacteriology," containing the results of his extensive studies. We acknowledge a great debt to this volume which has served as a guide to information and as a stimulus to further study of the history of microbiology. In this historical outline we shall review only the theories and investigations which appear to have exerted the greatest influence upon the development of medical bacteriology.

Among ancient peoples epidemic and even endemic diseases were regarded as supernatural in origin and sent by the gods as punishment for the sins of man. The treatment and, more important, the prevention of these diseases was sought by sacrifices and lustrations to appease the anger of the gods.

Since man is willful, wanton and sinful by nature there was never any difficulty in finding a particular set of sins to justify a specific epidemic.

The Egyptians apparently had a vague notion that disease could be transmitted by touch; certainly the Hebrews at the time of Moses believed that leprosy was contagious. They thought it could be contracted by personal contact from the clothes of the patient and even by living in the home of a former leper (Leviticus, Chapters 13 and 14). Although recognizing that leprosy could be transmitted by direct contact, the Hebrews also believed it could be created *de novo* as in the case of Miriam who angered the Lord by speaking against Moses (Numbers 12:9). The establishment of quarantine by Marseilles and Venice in the fourteenth century was perhaps influenced as much by the religious feelings that a period of purification was needed, as by the pragmatic idea that such a period of isolation would prevent the spread of disease.

At the time of the siege of Troy the Greeks believed in the divine origin of epidemic diseases, but as they lost faith in their gods they also lost faith in the divine origin of disease. By 430 B.C. Thucydides had concluded that certain plagues were contagious.

Hippocrates rejected the theory of divine origin but did not consider the possibility of contagion. He recognized two factors that were involved in the development of disease—an intrinsic factor of "constitution" of the patient, and an extrinsic factor, or "miasm," air modified in some unknown way so that the air itself became deleterious. The belief

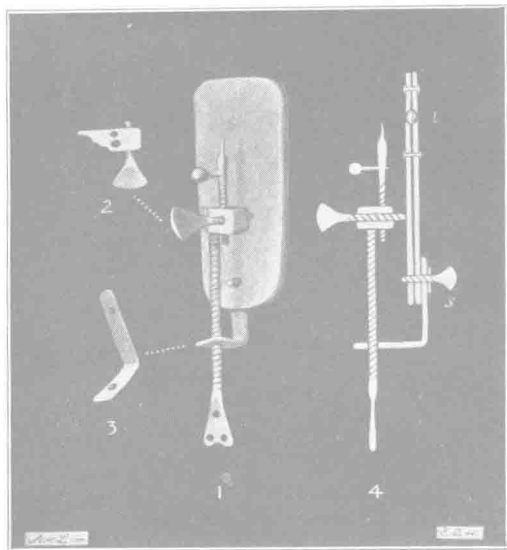


Fig. 1. Leeuwenhoek's "microscope." (From Dobell, 1932. Courtesy Harcourt, Brace & Co.)

in the disease-producing effect of bad air thrived in the Middle Ages and has persisted to modern times as shown by the medical words "influenza" and "malaria."

Although the Middle Ages were characterized by a series of destructive epidemics the belief that disease is caused by a living contagious agent grew slowly. However Boccaccio and his friends certainly believed that plague was a contagious disease when they fled from Florence in 1348 to escape its ravages and, incidentally, to entertain each other with the ribald stories later published as *The Decameron*. A knowledge of the natural history of syphilis influenced Fracastorius who clearly stated the theory that the agent of communicable diseases was living, a "contagium vivum." In his book on contagious diseases, published in 1546, Fracastorius (36) described the transmission of disease by direct contact, by intermediary inanimate fomites, and through the air "ad distans." He called the agents of disease "seminaria morbi," living germs, and expressed the opinion that the seeds of these agents, passing from one infected animal, produced the same disease in another which received them. These essentially true statements were unconvincing because they were not based upon a demonstration of the

physical reality of the hypothetical invisible organisms. Even after the discovery of bacteria, confirmation of the theories of Fracastorius was long delayed.

Although Fracastorius, as we now know, had indeed proposed the true germ theory of disease, no further progress could be made until microscopes were invented to visualize these germs and until the question of spontaneous generation of bacteria was settled.

According to Singer (74, 75, 76) and Disney (19) the compound microscope was invented independently by Zacharias Jansen in Holland and Galileo in Italy about 1609 (37).

Bacteria were discovered by Antony van Leeuwenhoek (1632-1723) in 1676. This statement has been placed beyond refutation through available historical records by the great biography of van Leeuwenhoek published by Dobell (21) in 1932. In addition to translations from the "Nether-Dutch" of all of the important parts of the letters of Leeuwenhoek in which bacteria and protozoa are described, this book contains convincing evidence that Leeuwenhoek was in fact the "father of protozoology and bacteriology" (20).

The "microscope" with which he saw bacteria was a simple biconvex lens, of short focal length, clamped between two metal plates. The preparation under examination in a drop of fluid or thin glass tube was fixed upon a metal point and moved into focus by means of screws. One type of the instrument is shown in Figure 1, copied from a drawing made by Dobell. It is probable that Leeuwenhoek's best glasses gave a magnification of 300 diameters. He never disclosed the secrets of all his methods and it is not known with certainty how he obtained the necessary illumination of his preparations. Dobell suggests that Leeuwenhoek probably hit upon some simple means of dark ground illumination. The first written descriptions of bacteria are in his letter to the Royal Society of London, dated October 9, 1676 (Dobell's No. 18). Here he gives recognizable descriptions of bacteria in various waters and in "pepper water" in which he was searching with his magnifying glass for the cause of the hotness of pepper. Clearer

descriptions of several forms of bacteria are given in his letter to the Royal Society, dated September 17, 1683 (Dobell's No. 39). In this he described the animalcules he found in the feces of man and animals and in the tartar from his teeth. A drawing of bacteria accompanied this letter. The original has been lost, but the record has been preserved in the plate illustrating the Latin translation of this letter published in 1695 (84). We reproduce a photograph of this famous drawing here, as Figure 2. It shows beyond question that Leeuwenhoek saw the chief forms of bacteria—cocci, rods, filaments and spirochetes (14). Leeuwenhoek believed that the air was the source of his microscopic creatures and that they existed in this medium in the form of seed or germs. It is doubtful if he ever heard of Fracastorius' book which was published 137 years before, and certainly neither he nor any of his immediate successors associated these "seed" with the "seed" which caused disease.

In 1678 Hooke confirmed Leeuwenhoek's discovery and during the early part of the eighteenth century the "little animals" were seen and described by a number of microscopists. The difficulties of assigning them a place and giving them a name are exemplified in Linnaeus' creation in 1758 of the genus *Vermes* which he called "Chaos." A few years later, Wrisberg named these organisms *Infusoria*. No doubt this helped Linnaeus to give them a name for in the eleventh edition of his *Systema naturae* (48), in 1767, according to Bulloch, he formed a class called *Chaos infusorium*. That he was still confused as to the nature of bacteria and protozoa is indicated by his listing them with "the ethereal nimbus floating in the month of flowering." In some respects the classification of bacteria is chaotic even now.

The first important classification of the *Infusoria* was made by O. F. Müller (56) in 1773 and 1786. The terms *Vibrio* and *Monas* were introduced by Müller. Some 50 years later, Ehrenberg (27), without really understanding the nature of bacteria, began to publish classifications of them. In 1829 he established the genus *Bacterium*, using a term formed from the Greek word *Βακτήριον*, the diminutive of *Βάκτρον*, signifying a staff. The whole subject of bac-

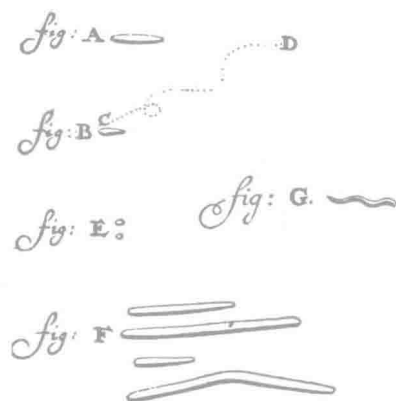


Fig. 2. Leeuwenhoek's picture of bacteria from the human mouth. Dobell's identifications are as follows: A, a motile *Bacillus*. B, *Selenomonas sputigena*. C, micrococci. F, *Leptothrix buccalis*. G, probably *Spirochaeta buccalis*. (From van Leeuwenhoek.)

teriology has taken its common name from the prominence of the rod forms. In his huge book on the infusion animals, published in 1838, Ehrenberg established more firmly the generic terms *Bacterium* and *Vibrio* and introduced others which are used today, notably *Spirillum* and *Spirochaeta*. Ehrenberg regarded the bacteria as complete animals, classing them with the polygastric protozoa. The transference of the bacteria to the plant kingdom was first proposed by Ferdinand Cohn in 1854. Cohn's (15) publications after 1872 marked a great advance in the systematic study of bacteria. His personal influence as a teacher and director of investigators equalled that of his written works. Under Cohn's guidance, the botanic point of view became dominant in bacteriology. In 1857 Nägeli had noted the relationship of bacteria to fungi and had introduced the term *Schizomycetes*, a term which has remained for the scientific designation of these organisms.

Primitive classifications of bacteria were established and accepted before they were associated with disease. One early concept of the origin of disease, however, involved a belief in a communicable agent such as that responsible for the leavening of bread, the making of wine and the brewing of beer. Robert Boyle suggested in 1663 that some diseases such as "Feavers and others" might

be a kind of fermentation. But fermentations and putrefactions often occurred without any known cause and were, therefore, attributed to spontaneous generation.

The problem of spontaneous generation is one facet of the larger problem of the origin of life. That phase of the problem which deals with small animals, worms and insects could be studied without the aid of a microscope but, backed by Aristotle's authority, this conception of spontaneous generation, or abiogenesis, dominated thought throughout the Middle Ages. Samson's riddle relating to the production of bees and honey from the carcass of a lion (Judges 14:8, 14) was matched in 1652 by van Helmont's directions for generating mice from pieces of dirty cloth and fermenting grain. What appeared to be particularly astonishing to van Helmont was that the mice generating from rags and grain were sexually mature and capable of copulating with mice descended from natural parents (83). Similar fanciful notions of the generation of insects were put to experimental test by Francesco Redi shortly after the middle of the seventeenth century. Redi proved conclusively that the maggots which appeared in decomposing meat were developed from the eggs of flies and showed by experiments that when meat was screened by gauze so that flies could not deposit their eggs in it no visible worms or insects were generated, although the meat putrefied. Redi's conclusion that life comes from life, supported by experiments which anyone could repeat, should have decided the issue.

The work of Redi and his followers (66) finally disposed of the theory of spontaneous generation of living things of macroscopic size, yet its advocates did not surrender but continued to believe in spontaneous generation at the microscopic level. The old controversy about spontaneous generation, now transferred to the microscopic level, had to be settled before acceptable conclusions could be reached regarding other problems. Louis Joblot, who opposed the idea of spontaneous generation, published his experiments in 1718, showing that hay infusions remained sterile as long as air was excluded but became filled with bacteria when air was admitted. The orderly development of the

subject was rudely disrupted by the studies of the Welshman, Needham (58), which were given wide publication by the Frenchman, Buffon. He found bacteria in his infusion of animal and vegetable materials even after boiling and excluding air from the vessels. Needham and Buffon were assailed by the great Italian naturalist, Abbé Spallanzani (77) who pointed out the technical errors in their work (1765-1776). He also discovered bacteria which grew in the absence of air, and forms which resisted boiling which we now know as spores. His work would have settled the question of spontaneous generation had Needham not shifted the grounds for the controversy by claiming that the excessive heating produced chemical changes in the solutions which made spontaneous generation impossible. Schulze (72), Schwann (73), Schröder and von Dusch (71) supported Spallanzani with numerous new experiments including the introduction of cotton stoppers to filter bacteria from the air.

The question was not definitely settled until the years immediately following 1860 when Pasteur conducted a series of experiments which were important not only in incontrovertibly refuting the doctrine of spontaneous generation of bacteria, but also in establishing the principles of scientific investigation which have influenced bacteriologic research since his time.

Pasteur attacked the problem from two points of view. In the first instance he demonstrated that when air was filtered through cotton wool innumerable microorganisms were deposited upon the filter. A single shred of such a contaminated filter dropped into a flask of previously sterilized nutritive fluid sufficed to initiate a rapid and luxuriant growth of microorganisms. In the second experiment he succeeded in showing that similar, sterilized "putrescible" liquids, when left in contact with air, would remain uncontaminated provided the entrance of dust particles was prevented. This he accomplished by devising flasks, the necks of which had been drawn out into fine tubes bent in the form of a U. The ends of these U-tubes, being left open, permitted the sedimentation of dust from the air as far as the lowest angle of the tube, but, in the absence of an

air current, no dust was carried up the second arm into the liquid. In such flasks he showed that no contamination occurred but that it could be immediately induced by slanting the entire apparatus until the liquid was allowed to run into the bent arm of the U-tube. Finally, by exposing a series of flasks containing sterile yeast infusion at different atmospheric levels, in places in which the air was subject to varying degrees of dust contamination, he showed an inverse relationship between the purity of the air and the contamination of his flasks with microorganisms (62, 63) (Fig. 3).

The doctrine of spontaneous generation of bacteria had thus received its final refutation, except in one particular. It was still puzzling why complete sterility was sometimes not obtained by the application of definite degrees of heat. In England, as in France, the chief opponent of the doctrine of spontaneous generation was a man of ideas, great experimental skill, keen insight and exact method. This was John Tyndall (81) who, starting from experiments to remove motes from air, made many important discoveries in bacteriology. He demonstrated independently the great heat-resistance of spores previously discovered by Cohn in 1871, and devised the method of fractional sterilization. By means of lectures and demonstrations, and by a notable book published in 1882, Tyndall, as Bulloch states, "gave the final blow to the doctrine of spontaneous generation" and opened the road for the advancement of the germ theory of disease.

Meanwhile Pasteur, while investigating spontaneous generation, had been carrying on experiments on the subject of fermentation along the lines suggested by Cagniard-Latour (9). As a result of these experiments he not only confirmed the opinions both of this author and of Schwann concerning the fermentation of beer and wine by yeast, but also showed that a number of other fermentations, such as those of lactic and butyric acid, as well as the putrefactive decomposition of organic matter were directly due to the action of microorganisms. The importance of his contribution to bacteriology can hardly be overemphasized; indeed, as René Dubos (24) has so aptly said in his "Louis Pasteur. Free Lance of Science"

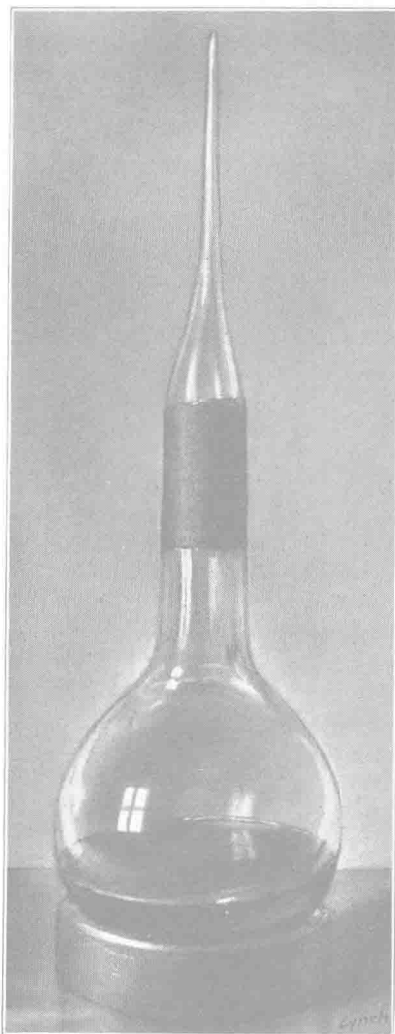


Fig. 3. One of Pasteur's original flasks. (From Bulloch.)

(1950) the domestication of microbial life began with Pasteur.

Proof that putrefaction is caused by living agents stimulated Lister (38, 49), who was a bacteriologist as well as a surgeon, to apply carbolic acid dressings to operative wounds. Thus, through bacteriology were instituted the antiseptic principles later largely replaced by aseptic methods which have made modern surgery possible. The history of bacteriology in surgery from the time of Lister to the present era has been reviewed by Meleney (54).

Almost 150 years passed after Leeu-

wenhoek's discovery of bacteria and protozoa before any definite applications of this new knowledge were made in the study of the cause of disease. In a remarkable series of investigations during the first quarter of the nineteenth century Agostino Bassi proved almost conclusively that a fungus, later named in his honor *Botrytis bassiana*, was the cause of a disease of silkworms called "mal segno" in Italy and "muscardine" in France. Bassi's biographer, Calandruccio (10), called Bassi the founder of the theory of parasitism.

Bassi's work was confirmed and extended. In 1839 Schoenlein (70) found the causative fungus in the lesions of favus, and in 1846 Eichstedt (31) discovered a fungus in the skin-scrapings from patients with pityriasis versicolor and noted the contagiousness of the disease. Rayer and Davaine (65) had seen rod-shaped organisms in the blood of animals dead of anthrax in 1850. Rayer recalled experiments made by Barthélemy in 1825 showing that anthrax was inoculable in series in sheep. By 1863 Davaine (17) had proved experimentally that the disease could be transmitted by blood and the sediment of laked blood containing these rods, but could not be transmitted by blood from which the rods were absent. This work exerted a marked influence upon the development of a bacterial doctrine of infection in contrast to the prevailing belief that fungi were the chief agents of contagious diseases. Between 1868 and 1873 Obermeier (61) at the Charité in Berlin, under Virchow's eye, was carefully confirming his discovery of the relationship of a spirillum to relapsing fever. He demonstrated for the first time the presence of a pathogenic microorganism in the blood of man.

It must be remembered that during these early years of etiologic research investigators had pure cultures to work with only by accident and did not know when contaminants were present in the materials they used. There was much speculation and loose thinking and a considerable amount of equivocal work that hindered the development of bacteriology. A firm corrective of this tendency was the expression of a logical and critical point of view by Jacob Henle (40). In his theoretical discussion of miasmatic and con-

tagious diseases, Henle, the future teacher of Robert Koch, affirmed his belief in the animate nature and specific action of the agents of contagion. Referring particularly to the investigations of Jenner, Bassi, Audouin, Schoenlein and Ricord, he anticipated Koch's postulates by insisting that the proof that an organism causes a disease must be accompanied by demonstration of its constant presence in the lesions, the isolation of this microorganism and the reproduction of the disease by inoculation. With much the same point of view in 1865 Villemin (85) proved by experiments on rabbits that the tubercle was inoculable. Villemin interpreted his experiments as evidence that tuberculosis was the effect of a virus. He thought that the virus must be present in the morbid products and when introduced into a susceptible animal must be able to reproduce itself and at the same time reproduce the disease.

In 1876 Robert Koch, who had been a pupil of Jacob Henle, came to Breslau at the invitation of Cohn to demonstrate the results of his studies on anthrax. In Cohn's laboratory, Koch exhibited his culture methods, showed the life cycle of the anthrax bacillus from spore to spore, and proved beyond doubt the ability of cultures of this organism to produce the disease. Koch's classic paper on the etiology of anthrax, published in 1876, was the first of a great series of enlightening contributions by him and his pupils. It inaugurated a new era of research in bacteriology (44).

Koch realized the importance of method and technic and was not long in introducing many of the procedures which are now everyday, indispensable practices in laboratories of bacteriology. From Weigert, Ehrlich and Salomonsen he learned methods of staining bacteria with aniline dyes. In his work on anthrax his chief culture medium had been sterile aqueous humor from the eyes of animals. He had seen the advantages of the older opaque solid media made from potato, beets, starch, bread, egg-white and flesh, but realized that they were incapable of giving all the desired information about bacterial colonies. In order to separate one species of bacterium from another, in 1881 Koch devised a transparent solid medium by mixing gelatin with Löffler's peptone solution. Dur-