Name: Mary Beatrice Webb   Date of Degree: May 27, 1962

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Scope of Study: This report deals with three of the most important developments in the early history of Bacteriology: the improvement of microscopes, the disproof of the theory concerning spontaneous generation, and the verification that microorganisms may cause various diseases. These three events, and the men that contributed to them, are discussed separately and chronologically.

ADVISER'S APPROVAL ________________

HIGHLIGHTS IN THE EARLY HISTORY
OF BACTERIOLOGY

By

Mary B. Webb

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Drury College

Springfield, Missouri

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INTRODUCTION

In this so-called "age of science" history is seemingly emphasized in nearly every field but science. Although the central point of modern civilization has been the development of science, its history has been sadly neglected. When teaching science, often the tendency is to emphasize only the practical aspects of the subject. Naturally this is important; but, it is becoming more and more apparent that there is also a need to humanize science and to reveal the underlying influence of science on our civilization. Before this can be accomplished and the meaning of science interpreted, a study of its past is necessary. This is especially true when considering bacteriology, for it is often claimed that this subject has rendered more service to mankind than any other of the biological sciences.

Bacteriology is comparatively new as a science. Almost all of the knowledge is this field has been obtained since 1860, and the major developments have occurred since 1900. This is due to the fact that there were three big hurdles that had to be jumped before bacteriology could develop. The development of the microscope, the disproof of spontaneous generation in living things, and the development of the germ theory regarding disease were the accomplishments that laid the foundation for the science of bacteriology. These three key discoveries, or "highlights" in the early history of bacteriology, will be discussed in the following paper.

CHAPTER II

THE DEVELOPMENT OF THE MICROSCOPE

A knowledge of the principle of magnification is very old and it is difficult to say exactly when it was first discovered. According to most historical evidence, Euclid (300 B.C.) was the first to investigate the laws of refraction. The first written evidence of the discovery of magnification is recorded by Seneca in his Questiones Naturales, written about 63 B.C. Here he states that small letters are comparatively larger and distinct when seen through a glass globe filled with water.[1]

How far back in history the use of simple lenses goes is equally doubtful. It has sometimes been claimed that ancient gems could not have been cut into jewelry without an aid to the sight. All evidence tending to show rather indirectly that simple lenses must have been used at various early periods in history is of little importance. The real development of the knowledge of magnification came from the work of the mathematicians and the physicists beginning with Euclid. Ptolemy of Alexandria, about 153 A.D., studied the laws of the refraction of light

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[1]F. J. Munoz, The Microscope and its Use (New York, 1943), p. 1.

even more thoroughly. The first more or less scientific description of the magnifying power of shaped glass objects is found in the book of the Arabian mathematician Al Hăsan who died in 1038.[2] A hundred and fifty years later a Franciscon monk, Roger Bacon (1214-1294), cleared up many of the laws of reflection and refraction of light and suggested their use for bettering the vision. He is credited with being the founder of the science of optics and is sometimes called the "Father of Microscopy."[3]

George Hoefnagel published in 1592 a set of fifty plates of insects engraved in copper. These pictures were drawn by his son Jacob, and some indicate the use of magnification. These plates of Hoefnagel are, as far as known, the earliest printed figures of magnified objects. The naturalist Mouffet also probably worked with simple lenses. His Theator of Insects was prepared in manuscript as early as 1590, but was not published until 1634. Some of his illustrations show magnification.[4]

Simple microscopes or simple lenses attached to crude stands, are believed to have been first described by Descartes in 1637 in his Diotrique. This instrument had a magnifier and a concave mirror with the concavity towards

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[2]Gustave Fassin, "Something about the Early History of the Microscope," Scientific Monthly, 38 (1934), p. 452.

[3]S. E. Wedberg, Microbes and You (New York, 1954), p. 7.

[4]Locy, Growth of Biology (1925), p. 199.

the objects to be examined. He calls it "perspiculia pulicaria ex uno vitro" which means flie-glass with one lens.[5] In 1671 Athanasius Kircher, a Jesuit priest, made a crude simple microscope by attaching the object studied to a stage with a rest. This instrument gave an enlargement of about thirty-two diameters. One source stated that Kircher was apparently the first individual to note the use of simple lenses for the study of living materials. Another source claimed that he gave no detailed descriptions or figures of anything that magnification had revealed to him.[6] It is interesting to note that, although it is likely his instrument could not have revealed bacteria, he supposedly believed infections were produced by living organisms.[7] Later, as will be discussed, the germ theory of disease was verified.

The greatest development of simple microscopes, and we even say their perfection, came at the hand of Antony van Leeuwenhoek (1632-1723) of Delft, Holland. He was perhaps the first man to seriously study objects with the microscope in a very persistent and thorough manner. He was descended from a good Dutch family of brewers and at the age of sixteen went to Amsterdam and became bookkeeper

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[5]Gustave Fassin, "Something about the Early History of the Microscope," Scientific Monthly, 38 (1934), p. 452.

[6]L. L. Woodruff, "Microscopy Before the Nineteenth Century," American Naturalist, 73 (1939), p. 489.

[7]A. J. Salle, Fundamental Principles of Bacteriology (New York, 1948), p. 692.

and cashier in a clothing store. He returned to Delft after a few years, and remained there where he owned a dry goods store and was appointed janitor of the City Hall of Delft. Sandwiched in between these duties he began his persistent lens-grinding activities which must have drained off most, if not all, of his spare time. Fortunately Leeuwenhoek was not a well-read man and therefore was unbiased by the printed word which was so often based on fallacy instead of fact. Because of this, he was not distracted by the nonsense adhered to by the so-called learned professions and was able to build his knowledge by using the scientific method.[8]

He manufactured lenses by grinding them with diamond dust which greatly improved their usefulness. He used a very small single lens which was about one eighth of an inch in diameter and had a strong curve that produced a fairly high magnification. They had a screw-like device which brought the object close to the lens for proper observation.[9] There is much disagreement as to the number of microscopes he possessed and the magnification power they gave. Two sources agreed that he possessed 247 simple microscopes. Charles Singer believes that the high-

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[8] S. E. Wedberg, <u>Microbes and You</u> (New York, 1954), pp. 9-12.

[9] F. J. Munoz, <u>The Microscope and its Use</u> (New York, 1943), p. 6.

est magnification obtained in Leeuwenhoek's microscopes was 160 times, and it varied from 40 to 133 times.[10] He observed a great variety of things, far too numerous to mention here. Had it not been for Regnier de Graaf's suggestion that the Royal Society of London request a record of Leeuwenhoek's work, it might possibly have been undiscovered. Our concern here is not only with his contribution to microscopy but his accurate descriptions of what he termed animalcules but are now known to be bacteria. The first record where bacteria were described was written on October 9th, 1676. In another letter written in 1783 he sketched the three principal shapes of bacteria we accept today: the rods, the spheres, and the spirals.[11]

Although he was generous with his microscopes, his particular method of observation was left for himself alone. Two explanations have been given as to the reason for his incredible success with his simple lenses. Dobell, who made a very thorough study of Leeuwenhoek, believes that he used some method of dark-ground illumination.[12] Barnett Cohen in 1937 suggested he used the inherent optical properties of the spherical drop of fluid

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[10] C. Singer, "Steps Leading to the Invention of the First Optical Apparatus," Studies in the History and Method of Science, II (1921), p. 385.

[11] S. E. Wedberg, Microbes and You (New York, 1954), p. 13.

[12] L. L. Woodruff, "Microscopy Before the Nineteenth Century," American Naturalist, 73 (1939), p. 494.

containing the objects he was observing.[13] No one has ever seen as much as he did with simple microscopes, and soon after his work appeared compound microscopes began to be improved and offered greater advantages to the scientist and particularly the bacteriologist. Leeuwenhoek is sometimes called the "Father of Microbiology", a title some leave for Louis Pasteur.

The discovery of the compound microscope is actually associated with the discovery of the telescope, and there are three men who claim the honors. Singer states that these are Zacharias Jansen, Jan Lippershey, and James Metius. The date of the discovery of this microscope may be placed between 1591 and 1608. By this time both convex and concave lenses were well known and constantly used in the manufacture of spectacles.

Zacharias Jansen (1580-16 ?) discovered by accident that if he put two lenses in a tube they increased the size of the objects. He also found that by elongating the tube he could greatly enlarge objects.[14] One source stated the instrument was provided with two convex lenses, the lower one having a short focal length to look at near objects.[15] Another source stated this compound microscope

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[13] S. E. Wedberg, Microbes and You (New York, 1954), p. 15.

[14] F. J. Munoz, The Microscope and its Use (New York, 1943), p. 3.

[15] S. H. Gage, The Microscope, 17th ed. (New York, 1892) p. 23.

was provided with a concave ocular and a convex objective.[16] This instrument was called a flea-glass or fly-glass, because it was used to examine small objects like fleas or flies. The Jansens began to manufacture both types of instruments, flea-glasses or fly-glasses (microscopes) and spy-glasses (telescopes) in the early part of the seventeenth century. Practical uses of compound lenses were not put to use in biological science until the middle of the seventeenth century by Robert Hooke and others who will be discussed later.[17]

Jan Lippershey, a spectacle maker, is recorded to have petitioned the Assembly of the States at the Hague in 1608, with the invention of an instrument for seeing at a distance. Singer believes that his instrument was provided with a convex objective and a concave eyepiece.[18] In the same month James Metius also petitioned the Assembly of States for the right to sell an instrument to make distant objects appear larger and more distinct. He also accidentally put two lenses together in a tube and found that he could distinguish distant objects.

The scientific discoverer of the telescope was Galileo (1564-1642), but some doubt still exists as to his

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[16] S. E. Wedberg, Microbes and You (New York, 1954), p. 9.

[17] Ibid., p. 8.

[18] C. Singer, "Steps Leading to the Invention of the First Optical Apparatus", Studies in the History and Method of Science, II (1921), p. 208.

relationship to the discovery of the microscope. John Wodderman, a Scotch student who attended his lectures, stated in 1610 that he had often heard Galileo describe the use of an instrument for examining insects.[19] Once in Rome, he stated he had made instruments which magnified things as much as 50,000 times but we have no records of him making such instruments. The term "microscope" was introduced by Giovanni Faber in a letter of April 26, 1625 to the Acadamy of the Lincei and was applied to one of Galileo's instruments for looking at small things.[20]

As mentioned earlier, it was not until the middle of the seventeenth century that the compound microscope was used for biological purpose. At this period, it was not a very accurate instrument for scientific observations. Robert Hooke, a British scientist, developed a compound microscope with which he studied several tiny objects. In 1665 he published the book The Micrographia describing his observations. The fact that his microscopes were quite unsatisfactory is revealed in his statement:

> I have endeavored to discover with my microscope whether this green (in the water) were like Moss, or long striped Sea-weed, or any other peculiar form, yet so ill and imperfect are our microscopes, that I could not certainly discriminate any.[21]

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[19]Fahie, "The Scientific Works of Galileo", Studies in the History and Method of Science, II (1921), p. 206.

[20]S. E. Wedberg, Microbes and You (New York, 1954) p. 9.

[21]Robert E. Buchanan, Bacteriology (New York, 1951), pp. 5,6.

Yet Robert Hooke appears to be the first to have realized the full impact of what studying nature with the microscopes would bring. In The Micrographia he writes:

> By the help of microscopes, there is nothing so small as to escape our inquiry....By this the Earth it self shews quite a new thing to us, and in every particle of its matter, we now behold almost as great a variety of Creatures, as we were able before to reckon up in every part of the whole Universe it self. It seems not improbably, but by these helps the subtilty of the composition of bodies, the structure of their parts, the various texture of their matter, the instruments and manner of their inward motions, and all other possible appearances of things, may come to be fully discovered...[22]

There were very few improvements made in the microscopes during the eighteenth century. During this time however, the fundamental physical structure of the latter instruments was worked out and the foundation for the coming lens work was laid. The manufacturer followed the lines laid down by the Jansens which gave a magnification of 300 diameters in the compound microscope. This increase over that given by Leeuwenhoek's lens, about 160 diameters, marked the doom of the simple microscope, certainly for the study of bacteria.

In 1754, Joblot, a Frenchman, wrote an extensive treatise on many microscopic objects. Some of his peculiar statements can only be due to the poor microscopes with which he worked. He described one organism as follows: "The whole top of its body is covered by a fine well formed mask of a

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[22]L. L. Woodruff, "Microscopy Before the Nineteenth Century," American Naturalist, 73 (1939), p. 490.

human face, perfectly well made in which one sees six legs and one tail, emerging from below this mask, which is crowned by a peculiar head dress."[23]

By 1773, the compound microscope had developed somewhat more and the Danish scientist Muller was able to examine tiny forms of life more satisfactorily. He published illustrations and a detailed classification with scientific names. Like Leeuwenhoek, he regarded practically all the forms he saw as animals. Yet, from his studies, it is obvious that some of these "animals" were bacteria. He first introduced the terms Bacillus, Vibrio, and Spirillum which are now used as generic names in bacteriology.[24]

During the next half century still better microscopes were developed. The chief difficulty before had been in chromatic aberrations. As lenses of shorter focal distance and more spherical are used, the angle of the rays greatly increases the aberration and false colors appear. Limiting the size of the opening to keep down the chromatic aberration also shuts out the light and makes the field too dark for the observer. In 1759 Dolland made a telescope with lenses of different kinds of glass with respect to difference in color. These glasses gave almost complete correction of the chromatic aberration. Not until the early part of the nineteenth century however, was the principle

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[23]Robert E. Buchanan, Bacteriology (New York, 1951), p. 7.
[24]Ibid.

applied to the microscope. In 1812, Amici, corrected the chromatic aberration in microscope objectives by a combination of plano-convex lenses of flint glass with biconvex lenses of crown glass.

In 1830 Lister, the father of Lord Lister, and Tulley constructed a device for the combination of lenses so that the errors of one were corrected by the other. With this instrument one could get a highly magnified image free from distortion and color. Due to their efforts, the compound microscope became a practical working instrument. By 1837 excellent microscopes were available, giving a high magnification and a clear image.

With the aid of such good instruments, the German scientist Ehrenberg (1838) made a very outstanding and accurate survey of microscopic organisms. Two large volumes, containing his results, were published. Ehrenberg, like Muller, regarded all of these organisms as animalcules, but several species of bacteria were included for the first time. He first proposed the name Bacterium to signify a genus.[25]

Improvements in the microscope still continued. Amici discovered the principle of water immersion lenses and Andrew Ross in 1839 invented a collar adjustment for them. An immersion lens works under the principle that when a high powered objective is immersed at its end in water (or

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[25]Hans Zinsser and Philip Hanson Hiss, <u>Microbiology</u> (New York, 1960), p. 3.

some clear fluid) that has a higher refractive index than air, small objects can be seen more clearly. The first microscopist to use oil instead of water was Wenham who demonstrated this with cedar oil in 1870. About this time Abbe furthur corrected chromatic and spherical aberrations and introduced the substage condensor which is named after him. This improved the lighting of microscopic objects and allowed the use of lenses which gave still higher magnification.

Every increase in clearness and magnification aided the studies of the microscopic world. Thus, by the middle of the nineteenth century there was finally a suggestion and acceptance to the differentiation of two groups of microscopic organisms: plants and animals. Bacteria were placed with the plants, although their right to be there is still disputed.

With these greatly improved microscopes, more detailed studies of the fine characteristics of bacterial structure were possible. This stimulated interest in attempting to classify these mysterious plants. A German botanist, Ferdinand Cohn, worked out the first system for classifying bacteria as plants rather than animals between 1872 and 1876. His was the first usable classification.[26]

With the exception of the electron microscope, the microscopes used by Cohn were very similar to the ones

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[26] S. E. Wedberg, Microbes and You (New York, 1954), p. 19.

used today. The major microscopic improvements needed to give bacteriology its start had been accomplished. Thus the dependence of this field on the development of the microscope is quite apparent from observation of the close correlation between the date bacteriology began its progress (1860) and the date well-developed high power microscopes were available (1840).

CHAPTER II

THE THEORY OF SPONTANEOUS GENERATION

After Leeuwenhoek had revealed to man the vast new world of microorganisms present in water and organic fusions, scientists began to wonder about the origin of these forms. Some believed the microorganisms formed spontaneously from non-living materials present in the infusion while others believed that the "seeds" of these microscopic creatures are always in the air and will grow when they light on such infusions. It is obvious that before the science of bacteriology could be established it was not only necessary to observe these microorganisms, but the dispute as to their origin needed to be settled.

The doctrine of spontaneous generation, or the belief that non-living material can give rise to living organisms, has had a long existence and was never seriously doubted until fairly late in history. It is easy to understand how the prescientific thinker found support for his belief that living creatures were generated in non-living substances. He saw earthworms materialize from manure piles, maggots appear in meat, and flies emerge from the slime of wells. Almost all scientists from Aristotle's time to the middle of the nineteenth century supported the theory of spontaneous generation. The teachings of Aristotle were given such a great amount of authority that for centuries no one questioned his views.[1] During the years that followed such outstanding men as St. Augustine, Basilius, Parcels us, Van Helmont, Harvey, Descartes, and Newton were protagonists of this theory.[2]

There were some very unusual and highly imaginative ideas and recipes that were used for creating human beings and other living forms. In the middle ages, there was the widely accepted tale that geese and ducks came from sea shells, which themselves had come from the fruit of trees! Birds could also be produced directly from these fruits. This theory of the plant source of geese and ducks was so widely accepted that, for some time Catholics could eat this meat on their fast days.[3] Theophrastus Parcelsus (1493-1541), a Swiss medical philosopher, gave a recipe for the creation of human beings:

> Place certain substances in a bottle, stopper it, and bury it in a dung heap. Every day certain incantations must be uttered over the submerged bottle.[4]

Jean Baptiste van Helmont (1577-1644), a physician and alchemist, offered this recipe for producing mice:

> Place a dirty shirt in a vessel containing wheat, and after twenty-one days storage in a dark place, to allow

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[1]A. I. Oparin, Origin of Life (New York, 1953), p. 7.

[2]Ibid., pp. 7-12.

[3]Ibid., p. 8.

[4]S. E. Wedberg, Microbes and You (New York, 1954), p. 23.

> fermentation to be completed, the vapors of the seeds and the germinating principle in human sweat contained in the dirty shirt will generate live mice.[5]

These are just a few of the many examples that could be cited to illustrate some of the fantastic views held by scientists. As knowledge of living organisms gradually accumulated, careful observation showed that the spontaneous generation of plants and animals simply does not occur. The first scientific experiments to disprove this theory as applied to animals were performed by an Italian physician, Francesco Redi (1668). He placed meat in a vessel under a frame covered with Neapolitan muslin. Although flies swarmed around the muslin, he found no maggots on the meat which was even allowed to decay. In his paper "Esperienze intorno alla generazione degl' insette" he described these experiments and concluded that decaying meat only gives a place for insects to develop but that eggs must be deposited before maggots can appear. Yet his work was not greatly acclaimed and many still believed in the possibility of spontaneous generation from other matter. Even Redi still believed that many organisms, such as the worms enclosed in oak gulls, were spontaneously generated.[6]

Yet by exact studies of the development and life cycles of plants and animals, this doctrine as applied to visible organisms, had already been weakened at the time

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[5] Ibid., p. 24.

[6] A. I. Oparin, Origin of Life (New York, 1953), pp. 12, 13..

Leeuwenhoek discovered the world of microorganisms. The immediate effect of his revelation was that it greatly strengthened the belief in spontaneous generation and many thought that the riddle of life had finally been solved.[7] Anyone with a microscope could see that bacteria emerged from decaying matter or any nutrient material which was kept warm. Even newly prepared material that showed no signs of life would be crawling with these creatures within a few days.[8]

Leeuwenhoek did not agree that these organisms were spontaneously generated. He believed that they developed from something which entered the infusions from the air. Although he did not perform any experiments to show this, a follower of his did. Louis Joblot (1711) boiled hay infusions swarming with microorganisms for fifteen minutes and poured the infusions into separate bottles. Tiny creatures developed in the ones that were left open. The others were sealed with parchment before they cooled and no life appeared. When he uncovered them, the bottles were soon populated with microorganisms.[9]

Joblot's experiments did not convince the people of his time and the theory of spontaneous generation gained new support when the famous French biologist Buffon (1707-1788) and the Scotch priest Needham (1713-1781) favored it.

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[7] J. E. Greaves and E. O. Greaves, Elementary Bacteriology (Philadelphia, 1946), p. 15.

[8] J. H. Rush, The Dawn of Life (New York, 1957), p. 92.

[9] A. I. Oparin, Origin of Life (New York, 1953), p. 15.

Buffon believed that all living matter consisted of particles or organic molecules that did not change. After death these particles were released and became active enough to unite and form new living organisms. In other words, when something dies the materials from it recombine to form a new organism. According to Buffon, this is how microorganisms were formed.[10]

Needham believed that a "productive" or "vital force" was necessary for the creation of living things. This life-giving force was present in every tiny particle of organic matter. In his experiments published in 1749, Needham tried to justify the possibility of spontaneous generation. He reported:

> I took a quantity of mutton gravy hot from the fire and shut it up in a phial closed with a cork so well masticated that my precautions amounted to as much as if I had sealed my phial hermetically.

He also heated the vessel on hot ashes. Even so, after a few days his phial was full of microorganisms. Studies of this organic substance and others revealed the same results. He concluded that spontaneous generation from organic material did occur.[11]

Scientists now know that Needham didn't take the proper precautions to destroy the organisms in his infusions and to protect them from the air. Fifteen years later the

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[10] J. E. Greaves and E. O. Greaves, Elementary Bacteriology (Philadelphia, 1946), p. 15.

[11] A. I. Oparin, Origin of Life (New York, 1953), p. 16.

Italian scientist, Abbe Spallanzani, was successful in showing Needham's errors. Spallanzani showed in hundreds of experiments that heating can prevent microorganisms from appearing in infusions, although the length of heating necessary sometimes varies. He also decided that animalcules can be carried by the air even when an infusion is well heated. Needham had closed his flasks with corks, but Spallanzani used hermetically sealed flasks to be absolutely certain no air could enter. He said:

> I used hermetically sealed vessels. I kept them for one hour in boiling water, and after opening and examining their contents, after a reasonable interval I found not the slightest trace of animalcules, though I had examined the infusion from nineteen different vessels.[12]

Needham and others criticized Spallanzani saying that during such long heating of the liquids the air became devitalized and this prevented the growth of microorganisms. He had destroyed the creative force of the infusions. Spallanzani performed some more tests to meet these criticisms, such as cracking the flasks to show how decay set in when air entered. He was not able to convince them for they maintained that sealing the flasks excluded the air that was necessary for life.[13]

To meet this criticism Franz Schultze (1836) set up

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[12]S. E. Wedberg, Microbes and You (New York, 1954), p. 25.

[13]James Bryant Conant, Pasteur's and Tyndall's Study of Spontaneous Generation (Mass., 1953), p. 19.

an experiment where an infusion in a flask was sterilized by heat and the air passing through the infusion was filtered from organisms by sulfuric acid.

> The flask containing the medium was boiled over a sand bath and, while steam was streaming from the vessel, an absorption bulb was attached to each end. One bulb contained sulfuric acid and the other a solution of potassium hydroxide. Schulze applied his mouth to the open end of the potash bulb and aspirated fresh air into the vessel containing the medium. The air, in passing through the sulfuric acid, was freed from bacteria. The medium remained sterile for more than two months. Control flasks showed growth in two days.[14]

This experiment showed that the air in the flask was not the cause of growth in the liquid for "pure air" did not allow growth.

Two other ingenious experiments were performed to show that neither growth or decomposition will occur in an infusion that has been properly heated and exposed to air that has been treated for the removal of germs. In 1837 Theodore Schwann passed the air through heated tubes before it came in contact with the sterile meat bouillion. His flask had no bulbs but two tubes were connected to it. One tube went to a jar containing mercury and the other had spiral twists and an open end. When the contents of the flask were heated, the spiral tube was also so that no germs could enter the infusion. The flask remained sterile for six weeks and there was no spontaneous generation.[15]

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[14] A. J. Salle, Fundamental Principles of Bacteriology (New York, 1948), pp. 698, 699.

[15] James Bryant Conant, Pasteur's and Tyndall's Study of Spontaneous Generation, (Mass., 1953), pp. 20,21.

However, the results were quite different when he used liquids containing sugar for his experiments. Using the same technique, microorganisms appeared in this case.[16]

The other ingenious experiment was conducted by two Heidelberg professors, H. Schroder and von Dusch, in 1853. They simplified the experiment by using wool stoppers in the flasks which allowed air to enter without "devitalizing" it in any way. This wool, as long as it was kept dry, worked as a mechanical filter to keep out the microorganisms.[17] These experimentors found this technique to work for keeping heated meat decoction or beer wort free from life, but meat without water or milk became filled with microorganisms.[18] Today we know these occassional failures were due to technique, and that some materials are more difficult to sterilize. Schroeder and von Dusch incorrectly concluded that milk and meat can decay spontaneously when air is present. Nevertheless, their discovery was important for today the use of cotton wool to plug culture flasks is used in laboratories around the world.

None of these results were sufficient to alternate the prevailing belief in spontaneous generation. The argument over this theory reached its highest peak when F. Pouchet,

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[16] A. I. Oparin, Origin of Life (New York, 1957), p. 18.

[17] S. E. Wedberg, Microbes and You (New York, 1954), p. 27.

[18] James Bryant Conant, Pasteur's and Tyndall's Study of Spontaneous Generation (Mass., 1953), p. 24.

in 1859, published a 700 page book where he tried to prove that spontaneous generation does occur.

> He heaped experiment upon experiment and argument upon argument spiced with logic and sarcasm in favor of spontaneous generation.[19]

Pouchet believed in a vitalistic theory of auto generation (self generation) that was very similar to those of Buffon and Needham. He maintained that before life can be created, fermentation and decomposition of living substances must take place. Only this material from once living organisms can produce new life and therefore life can not arise de novo from inorganic substances. These free, decomposed organic particles wander until they associate due to their natural tendency to combine. Thus, new life is formed. Pouchet repeated many of the experiments that had been performed by others, and his results always revealed an occurrence of spontaneous generation.[20]

The French Acadamy of Sciences had made an offer in 1860 to anyone who could prove, by scientific methods, whether spontaneous generation did or did not occur. When Pouchet presented his work, the great French scientist Louis Pasteur felt compelled to reveal his opposite point of view. In 1862 Pasteur published his investigations on spontaneous generation and the results of his thorough and

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[19] J. E. Greaves and E. O. Greaves, Elementary Bacteriology (Philadelphia, 1946), p. 16.

[20] A. I. Oparin, Origin of Life (New York, 1953), p. 20.

accurate experiments that left no room for doubt.[21]

First of all, he clarified the problem concerning the presence of microorganisms in the air. Pouchet, among others, had refused to accept this, for he said:

> How could germs contained in the air be numerous enough to develop in every organic infusion? Such a crowd of them would produce a thick mist as dense as iron.[22]

Pasteur showed by a series of experiments that the air does contain microscopic bodies. First he drew large amounts of air through a tube that contained a plug of guncotton to serve as a filter. He received this idea from Schroeder and Dusch. The guncotton was then removed and dissolved in alcohol and ether and the sediment was examined under a microscope. He found it contained numbers of small round bodies like the spores of plants as well as thousands of organisms. The presence of large numbers of organisms in the surrounding air was finally proven.[23]

Next, he repeated the experiments of Schwann and verified the fact that heated air can be supplied to a boiled infusion without the occurrence of microbial growth. He boiled organic liquids in flasks whose necks were drawn out and fused to a platinum tube. The platinum was heated until red hot so that after boiling ceased, the air entering the flask was freed from germs as it passed through the

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[21] S. E. Wedberg, Microbes and You (New York, 1954), p. 28.

[22] Ibid., p. 29.

[23] James Bryant Conant, Pasteur's and Tyndall's Study of Spontaneous Generation (Mass., 1953), pp. 30-34.

hot platinum. Yet before the air entered the flask it was cooled by a stream of water. When the flask was filled with air and the neck sealed off; it could be kept unspoiled and free from microorganisms for any amount of time. To prove that the boiled infusion hadn't lost its ability to sustain microorganisms, the seal was removed and the germ filled gun-cotton was placed in it. It was then quickly resealed. The fact that the infusions became alive with microbial growth not only showed that the infusion hadn't been harmed by boiling, but also that microorganisms can grow in a closed system without air.[24]

These experiments showed Pasteur how germs can enter infusions and that they must be carried in the air. This next demonstration showed how boiled infusions will remain sterile or free from life indefinitely in open flasks, if the neck is bent in such a way that the germs can't ascend it. He softened the neck of the flask in a torch flame and pulled it out in the form of a letter S. The contents of the flask remained unchanged even though they were in direct contact with the air for the germs were trapped in the bend of the tube. However, when the bent section was cut off, the flask quickly became contaminated. This type of experiment finally disposed of all criticisms concerning the necessity of air for the development of life

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[24] Ibid.

in infusions.[25]

Pasteur completed his studies by determining the amount and distribution of microorganisms in the air and showing that these organisms are found quite unevenly in the atmosphere. He prepared a number of sealed flasks and opened them in the streets of Paris. Every flask showed growth of microorganisms when incubated. He opened twenty flasks on the road to Dole and eight showed growth. Of twenty flasks opened on top of a small mountain (2,700 feet), five showed growth but of twenty flasks opened on top of the Mer de Glace (6,000 feet), only one showed growth. It is interesting to note that Pouchet performed similar experiments but even at 9,000 feet every one of his flasks showed growth. Pouchet became so angry with Pasteur's repeated contradictions of his experiments that he challenged him to a duel! The contradiction in this case was probably due to the fact that they used different mediums for growing organisms and Pouchet's was harder to sterilize.[26]

Pasteur obtained uniform results in his own owrk and was able to explain and correct the errors of earlier experiments. He proved that infusions cannot generate microorganisms and that decay of these fluids was due to the results of microorganisms which entered from the air. He became a hero and in 1862 the French Academy awarded him with the prize they had offered for such evidence.

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[25] A. I. Oparin, Origin of Life (New York, 1953), pp. 23, 24

[26] S. E. Wedberg, Microbes and You (New York, 1954), p. 29.

With these experiments, Pasteur won the battle over spontaneous generation as far as the French were concerned, and attempts to criticize his work were no longer taken seriously in France. In England, however, a new and enthusiastic backer of the doctrine of spontaneous generation appeared by the name of Bastian. He showed that microorganisms appear in boiled hay infusions even when the flasks are opened on mountain tops. Even after he heated the air, organisms still remained. For forty years he presented his views and in 1872 he published a large book on the subject.[27] We know today that the spores of the hay bacteria are very persistent and boiling, unless at different time intervals, will not destroy them.

John Tyndall, an English physicist and supporter of Pasteur, took up this challenge presented by Bastian. He not only devised a very ingenious apparatus for determining the presence of visual particles in the air but he also reached the correct conclusion as to why the hay infusion used by Bastian did not remain sterile when boiled.

In his previous experiments, Tyndall had noticed the difficulty of removing particles that float in the air. He found that such particles could be made visible if a strong beam of light was passed through the air in a dark room. Air that was dust-free no longer scattered the light and was "optically empty". This led him to build a special

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[27] A. I. Oparin, Origin of Life, (New York, 1953), pp. 24, 25.

chamber in which experiments on the floating matter of air could be carried out. The front consisted of glass, and the other three walls of wood. Two panes of glass were placed on the sides to allow a beam of light to pass through. At the back was a door for placing material and the bottom contained holes for holding test tubes. Two narrow tubes which were bent up and down were placed on the top to allow dust-free air to enter the chamber. Before he performed an experiment he waited until the beam of light showed that the air was optically empty. The test tubes were then filled with infusions through a pipette which hung from the top. Tyndall found that boiled infusions would remain germ-free in this chamber for months.[28]

However, when working with hay infusions he found that it was more difficult to sterilize them. Tyndall finally realized that dried hay contained spores that were more resistent for heat. He proceeded to test the limits of heat resistence of spores and, even after boiling for five and one half hours, the infusions were not sterile. Tyndall cleverly concluded that bacteria have different phases and therefore developed the famous discontinuous method of sterilization. He repeated boiling at various intervals to insure destroying the bacteria at different stages. This method worked with great success and the error in Bastian's work

[28]James Bryant Conant, Pasteur's and Tyndall's Study of Spontaneous Generation (Mass., 1953), pp. 46-49.

was revealed.[29]

With the publication of Tyndall's findings, the whole scientific world at last denounced the doctrine of spontaneous generation. Thus the final overthrow of this incorrect biological theory was the joint achievement of a chemist (Pasteur) and a physicist (Tyndall).

History has revealed that ideas, like plants, need a favorable climate in which to grow and such an environment was lacking at the time of Redi's experiments in the seventeenth century. By the time Pasteur and Tyndall appeared, other sciences had advanced enough to condition men to the use of a more scientific approach. However, this does not lessen the contributions of these men.[30]

While this controversy was being solved, many new facts about bacterialwere discovered. Such things as how they grew, where they occurred, how they multiplied, and what they looked like, were revealed. The most important thing of all, it was now established that these bacteria must originate from similar organisms. This achievement laid the second firm foundation for the development of the science of bacteriology. It also opened the road for the advancement of the germ theory of disease.

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[29] Ibid., pp. 56-58.

[30] J. H. Rush, The Dawn of Life (New York, 1957), p. 95.

CHAPTER III

THE GERM THEORY OF DISEASE

The germ theory of disease originated long before Leeuwenhoek demonstrated the existence of microorganisms. Although most of the ancient peoples believed that diseases were sent by gods as punishments for their sins, there were some who predicted other origins for disease. The Egyptians evidently had some idea that diseases were transmitted by touch and the Greek, Thucydides (430 B.B.), believed that certain plagues were contagious. Hippocrates thought that the two primary factors in disease were the constitution of the patient and some modification of the air ("miasm"). In about 100 B.C., Marcus Varro stated that invisible animals are carried through the air and enter the body through the nose and throat. Much later, Fracastorus (1546) published a book on contagious diseases where he discussed the transmission of disease by direct contact, by inanimate fomites, and through the air. He even went so far as to call the carriers of a disease "living germs" that passed the disease from one individual to another.[1] Yet

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[1]Hans Zinsser and Philip Hanson Hiss, Microbiology, 12th ed. (New York, 1960), pp. 1,2.

all of these theories which seemed to hint at the present germ theory of disease were pure speculations and never based on demonstration.

Even after Leeuwenhoek's discovery (1676), it was almost 200 years before proof of the relationship of these tiny "seeds" or microorganisms to disease was substantiated. However, during this time there were several scientists whose discoveries helped to pave the path for the tremendous achievements that were to come with such men as Koch and Pasteur.

In 1762 an Austrian physician, Plenciz, made a statement that was quite similar to the idea previously presented by Fracastorus. He ascribed disease to microorganisms or germs that were carried in the air and thereby transmitted from one individual to another.[2]

Although Bretonneau (1778) failed to connect the cause of disease with microorganisms, he stated that diseases of typhoid, malaria, dysentery, and diphtheria were produced by specific elements.[3]

In 1796 Jenner introduced the modern practice of vaccination. He successfully immunized a boy against smallpox by transferring matter from a cowpox lesion taken from a diseased milk maid. This great discovery was due only

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[2]Robert C. Buchanan, Bacteriology (new York, 1951), p. 19.

[3]A. J. Salle, Fundamental Principles of Bacteriology, (New York, 1948), p. 705.

to empirical observation and deduction since Jenner had not observed the microorganism that caused this disease and did not understand the underlying reasons for it.[4]

The first great step forward, approaching the scientific proof of the germ theory, came in 1813 with the discovery that certain fungi cause different specific plant diseases. In 1836, Bassi in Italy clearly showed that the muscardine disease of silkworms was caused by certain microorganisms. He also predicted the discovery that microbes cause small-pox, plague, syphillis, and other human diseases.[5] After this discovery came Schoenlein (1839) who was the first to prove that a human disease could be caused by a microorganism. He showed that a skin disease known as favis was due to a fungus-like growth.[6]

Suggestions were now rapidly being made concerning microorganisms and the disease they could cause. The technique of producing pure cultures was not yet available and there was a lot of confusion resulting from "plausible" guessing and loose speculating. Jacob Henle (1840), the future teacher of Robert Koch, also believed that each disease was caused by a specific organism and that no other microorganism could produce the same disease. Yet he

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[4]Esmond R. Long, A History of Pathology (Baltimore, 1928), pp. 233, 234.

[5]Ibid., p. 235.

[6]Thurman B. Rice, Textbook of Bacteriology (Phil., 1947), p. 4.

thought that some restrictions should be placed on the information that was being gathered. He laid down certain postulates for proving an organism causes a disease that are often attributed to Koch. He insisted that the organism which causes the disease be constantly present in the lesions and absent in other diseases. This organism must also be isolated and then show ability to produce the disease in an undiseased animal.[7] Besides his own investigations on disease, he also collected and judged the work of previous men on this subject. Because of this, he has been accredited with being the real proposer of the germ theory of disease.[8] Henle's work was very influential and stimulated other scientists to isolate and examine the causes of disease.

A few scientists began to investigate animal diseases to determine whether or not such incidents as reported earlier primarily for plants, might apply elsewhere. In 1850, Davaine had observed rod-shaped organisms in the blood of animals dead of anthrax. A few years later, after Pasteur had published his views describing how microorganisms were related to fermentation, Davaine conducted some further investigations. He injected some of the blood from the diseased animal into a well animal. This soon proved the di-

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[7]E. R. Long, <u>A History of Pathology</u>, (Balt., 1928), pp. 235, 236.

[8]Wade W. Oliver, <u>Stalkers of Pestilence</u> (New York, 1930), p. 166.

sease was transferable. He found the disease could only be transmitted from blood where the rods were present.[9] Up to this time fungi were still believed to be the chief organisms responsible for disease and this work greatly influenced the theory that bacteria produced infection.

Obermier (1868-1873) showed that relapsing fever was caused by a spirillum bacteria. This was the first time a disease causing microorganism had been observed in the blood of man.[10] By this discovery he opened up a new opportunity for studying diseases of tropical countries. These diseases are spread by insect bites and the organisms which circulate in the blood are usually tiny animals rather than bacteria.

In spite of all these discoveries, very few scientists at this time applied the germ theory to the diseases of man. The convincing and undisputable work of Pasteur and Koch were necessary to assure all mankind that these germs or microorganisms are the carriers of numerous diseases.

Louis Pasteur, having settled the dispute over spontaneous generation, next turned to the study of fermentation. In 1860 he proved that fermentation, both lactic and alcoholic, is due to the growth of microorganisms. In so doing, he disproved the popular belief that organic nitrogenous matter was necessary for the process of fermenta-

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[9]H. Zinsser and P. H. Hiss, Microbiology (New York, 1960), p. 6.

[10]Ibid., p. 6.

tion. He also showed how there are different ferments or microorganisms for each kind of fermentation.[11]

Having settled still another question, Pasteur began his brilliant and persistent experiments which, along with with Koch's, ended in the final proof that germs cause many diseases. From 1865 to 1870 Pasteur spent his time trying to discover the cause of Pebrine which is a disease of the silkworm. This disease was responsible for the near collapse of the silk industry in several countries, including France. The disease is characterized by tiny black spots located on the diseased silkworm. Pasteur believed that these spots were caused by microorganisms which caused the disease. After all his lengthy studies, he showed how the disease was transferred from diseased worms to healthy ones. They could acquire the pebrine disease from being on the same frame with diseased worms or else be born of a diseased female. Pasteur showed how they could eliminate the disease by placing the female moth ready to lay eggs on a linen cloth and then examining the eggs laid. If "corpuscles" were found then the eggs and the linen were to be burnt.[12,13] Thus the same relationship of microorganisms to disease that Davaine had discovered for anthrax

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[11] Wade W. Oliver, Stalkers of Pestilence (New York, 1930), pp. 179-181.

[12] J. E. Greaves and E. O. Greaves, Elementary Bacteriology (Philadelphia, 1946), pp. 31, 32.

[13] Wade W. Oliver, Stalkers of Pestilence (New York, 1930), p. 185.

in 1863, was again discovered in the case of pebrine.

About this time a Scotch surgeon, Joseph Lister (1827-1912), introduced the idea of aseptic surgery. Having read Pasteur's studies on microorganisms in the air and the cause of fermentation, he decided to devise a method for preventing microbes from entering wounds. This might possibly eliminate "decomposition" in the injured part. He used carbolic acid which he applied to his instruments, the operator's hands, and even the air of the operating room.[14] Although he was ridiculed at first, the advantages of such precautions soon became apparent and necessary. When the germ theory of disease was no longer doubted, his idea was greatly acclaimed.

The news of such discoveries made by Davaine and Pasteur, and the possibilities they presented, intrigued Robert Koch. He began to investigate the disease anthrax which was causing a tremendous loss of domestic animals. Like Davaine, he noticed that the carcass of every diseased animal contained blackened blood with rod-like sticks. He proceeded to inject some of the infected blood with these rods into mice by using a sharpened sterile splinter for lack of a better instrument. The next day all the injected mice were dead and their blood and internal organs contained these rod forms. His next idea was one that is now universally applied in studying bacteria. He decided to

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[14] Ibid., pp. 186-188.

separate some of these rods and cultivate them in order to observe their growth and development. He placed a piece of the victim's infected liver into some fluid drained from the eye of an ox. After several hours he witnessed the elongation of the rods and then the separation of single rods into two new ones until they had multiplied several times within an hour.[15]

Next he decided to determine whether or not the blood from an infected mouse that had been dead for quite some time could produce the disease if injected into healthy sheep. The answer was "yes", for the sheep died. His final experiment was to answer the question concerning the tendency of fields to remain infected even after several months of changing weather. Quite by accident, one of his ox-eye fluid cultures was left out for twenty-four hours. When he examined it under a microscope he found that the rod-like forms had changed into beads. After several months, he saw these beads return to the characteristic rod shape. Thus he now had evidence of the spores that were the resistent forms of bacteria that could remain unharmed in the pastures.[16]

In 1876, with the results of these studies on the life cycle of the anthrax bacillus, Koch presented a demonstrative lecture before a group of imminent scientists.

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[15] S. E. Wedberg, Microbes and You (New York, 1954), pp. 38-39.

[16] Ibid.

These studies proved, in the end to the entire scientific world, that bacteria can be the specific agents which cause disease.

In 1882, Robert Koch presented four postulates to be used in determining whether or not an organism could be said to be the cause of a specific disease. As mentioned earlier, Henle had suggested similar criteria about thirty-six years before Koch. First of all, the suspected organism must be found in every case of the disease as Koch had found true in all the carcasses dead of anthrax. Second, the organisms must be isolated in pure culture from every case of the disease. Koch had succeeded in isolating the anthrax bacillus and had grown it in ox-eye fluid. Third, these isolated cultures must be able to reproduce the original disease in a susceptable animal. The sheep he injected with the diseased blood of the mice had died. Last, the same organism must be re-isolated from the injected test animal. He found he could recover the typical bacillus from the blood of the newly injected animal and repeat this cycle.[17]

Before there could be a complete acceptance of the germ theory there was still one barrier to surmount. This was due to the fact that many scientists, failing to purify the anthrax bacillus taken from diseased animals, were injecting mixed cultures into the animals they wanted to re-

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[17] Ibid., p. 38.

produce the disease. Thus these animals died without showing symptoms of anthrax. This problem was finally cleared up by Pasteur who showed that the workers were injecting two disease organisms at the same time. The other disease organisms, vibrion septique, were causing the death of the animals before the anthrax bacillus had a chance to affect them. By showing that two different organisms present in the blood could definitely cause two separate diseases, Pasteur settled once and for all the question of the validity of the germ theory.[18]

Thus the door was opened and a tremendous flow of bacteriological discoveries in regard to diseases and laboratory techniques followed. They are far too numerous to mention here so let it suffice to say that the general acceptance of the germ theory was the last big hurdle that, once crossed, enabled bacteriology to progress to the successful and useful science it is today. The recognition that bacteria play such a large role in many diseases, and therefore its great practical application, has been the main factor responsible for the intensive studies that have been made in this scientific field.

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[18]Roger Y. Stanier, Michael Doudoroff, and Edward A. Adelberg, General Microbiology (London, 1958), p. 455.

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VITA

Mary Beatrice Webb

Candidate for the Degree of

Master of Science

Report: HIGHLIGHTS IN THE EARLY HISTORY OF BACTERIOLOGY

Major Field: Natural Science

Biographical:

Personal Data: Born at Molene, Illinois, December 14, 1939, the daughter of F. Earle and Shirley Webb.

Education: Attended grade school in La Crosse, Wisconsin, El Dorado, Kansas, and Kansas City, Missouri; graduated from Westport High School in Kansas City, Missouri, in 1957; received the Bachelor of Arts degree from Drury College, Springfield, Missouri, with majors in Biology and English, in May, 1961; did graduate work at Oklahoma State University during the summer of 1961 and the academic year of 1961-1962; completed requirements for the Masters of Science degree in May, 1962.

Professional organizations: Member of Beta Beta Beta National Biological Honorary Fraternity; Alpha Lambda Delta Honorary Scholastic Fraternity; Skiff Honorary Scholastic Fraternity.