From: T.D. Brock, Robert Koch: A Life in Medicine & Bacteriology (Springer, 1988)

7

Koch's Role in the Microscope Revolution

As long as the makers of microscopes do not offer us equipment of higher powers . . . we will find ourselves, when studying bacteria, like a traveler who wanders into an unknown country at the hour of twilight, at the moment when the light of day no longer suffices to enable him clearly to distinguish objects, and when he is conscious that, notwithstanding all precautions, he is liable to lose his way.

—FERDINAND COHN[1]

One of Robert Koch's main contributions was the successful adaptation of the light microscope to the study of bacteria, especially those found in diseased tissues. He was the first to use oil immersion lenses and the Abbe condenser, and he was the first to publish photomicrographs of bacteria. His research on the staining of bacteria for microscopy provided the foundation for this important topic. These remarkable accomplishments were made with equipment and supplies that Koch had to purchase with his own money.

Koch's initial work on photomicroscopy

After his anthrax work, Koch worked hard to obtain better images of bacteria, realizing that his microscope was the limiting factor (Figure 7.1). Much of his work was motivated by a desire to photograph bacteria through the microscope, as he realized that hand drawings were unsatisfactory for communicating the results of bacteriological investigations. His work on photomicroscopy not only forced him to improve

drawings

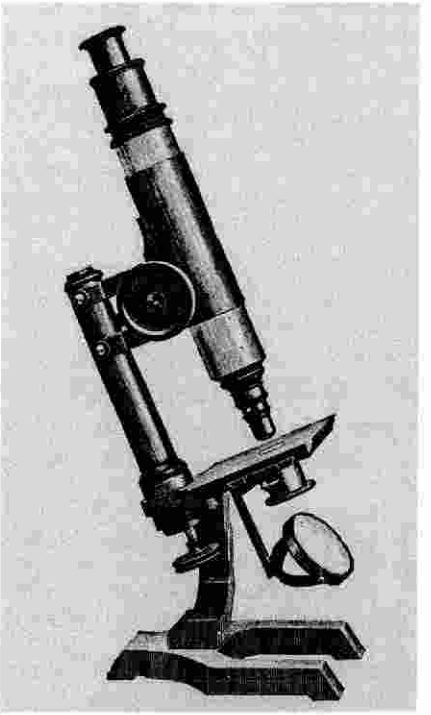

Figure 7.1 *A Seibert microscope, the type Robert Koch used in his first work.*

his microscopy but also to perfect better methods of preparing specimens for microscopy. The slide techniques that have served bacteriology for over 100 years stem directly from Koch's work of 1876–1877. As we noted in Chapter 2, Koch's Uncle Eduard had caused him to become interested in photography as a child. Now, he would use his photographic skills on something really important.

In attempting to obtain better images, Koch had extensive correspondence with Seibert and Krafft of Wetzlar, one of the leading microscope builders of the day.

photography

July 14, 1876

I have encountered great difficulty executing proper drawings of bacteria and hope to use photomicroscopy to get around these problems. For years my work on photomicroscopy has been based on the book by Reichardt and Stürenburg.[2] I have been informed by the Institute of Plant

> Physiology in Breslau that your optical firm supplies the best apparatus for photomicroscopy. I have obtained your catalog and I do not find among your many models an apparatus that is suitable for my work. I need an apparatus that provides the highest magnification, at least 1200 or even more, but I do not require a large picture size, no larger than 10 cm diameter. . . . I would be exceedingly grateful if you would inform me as to whether it would be possible to obtain good photographs of tiny transparent objects such as bacteria, and if you could advise me which photomicroscopic apparatus would be the best for my purposes.[3]

Koch received an answer and quickly (24 July 1876) ordered the recommended apparatus. He waited impatiently to receive it, firing off a series of letters to Seibert & Krafft inquiring as to the delivery date. Finally, on 2 October he wrote an ultimatum, and a few days later he received his equipment.

> 10 October 1876
>
> I hasten to write that the photomicrographic apparatus I ordered has arrived in good condition. It does not exactly fit my microscope but I hope that I will be able to adapt it by making several minor alterations. . . . It would please me greatly if you would give me some advice about how the illumination system should be used. How is it used with sunlight? How is the fourth set screw on the shutter used? Is it perhaps necessary to use a piece of ground glass or something similar? In my order I also requested a stage micrometer. Would you please send this to me (1 mm divided into 100 equal parts) as soon as possible? I will then pay you immediately for the micrometer and the apparatus.[4]

Koch's work on photomicroscopy ultimately led to his second paper, also published in Cohn's journal, which contained the first photomicrographs ever published of bacteria (see below). As he continually improved his photographic technique, Koch came to realize that the photographic plate was often better for examining the bacteria in a preparation than direct observation through the microscope. This was because (he said) the light-sensitive plate was not dazzled by bright light, as was the eye, so that small differences in intensity could be seen better on the negative plate than through the microscope. "Often I have easily found fine objects on the negative that I could see only with difficulty through the microscope."[5] At this time he wrote to Cohn:

> During the summer I have worked first with anthrax bacilli and later with other Schizophytes, using special methods of preparation to study these organisms precisely, with the goal of distinguishing various species and perhaps accurately telling them apart. My results have exceeded my fondest expectation. I now have a rather large collection of slides of a variety of bacterial forms: Spirillum, several species of spirochetes, bacilli, micrococci, and bacteria. Since it hasn't been possible to show by hand drawings the characteristic arrangement and size relationships, I have decided to prepare photographs. At first I ran into many problems but I believe I have solved the worst of them and I hope to send you in several weeks some photographs of Schizophytes.[6]

However, Koch underestimated the difficulties of getting good photographs. It took him over a year more before this work was ready for publication.

Initially, Koch used a vertical camera-microscope arrangement, as had been described by Reichardt and Stürenburg[7] (Figure 7.2), but this arrangement only permitted a magnification through the microscope of 300 X. Further magnification had to be made by enlargement of the

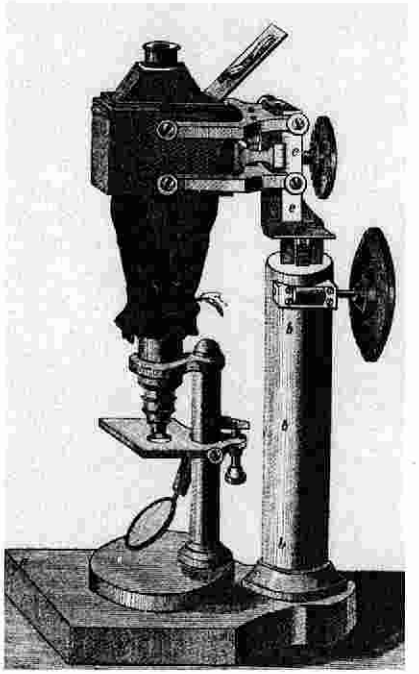

Figure 7.2 *Vertical photomicroscopic apparatus of the type Robert Koch used in his first work, as illustrated by Reichardt and Stürenburg.*[2]

negative, an unsatisfactory procedure. Later, Koch acquired a horizontal microscope-camera setup (Figure 7.3) in which the camera, microscope, and mirror lighting arrangement were carefully aligned on an optical bench. Sunlight was directed onto the microscope mirror by means of a *heliostat*, a device which followed the sun. On the window through which the sunlight was directed, Koch arranged a shutter so that the time of exposure could be controlled by opening and closing this shutter rather than by pulling the dark slide of the photographic plate. In this way, no movement of the microscope occurred when the shutter was opened.

Photography in Koch's time

It is hard for us to appreciate today how primitive the photographic possibilities were in Koch's time (Figure 7.4). Photographic film did not exist and all pictures were taken with emulsion-coated glass plates which the photographer prepared at the time of use. The best images were

Figure 7.3 *Reconstruction of Robert Koch's work room in Wollstein, as shown at a Robert Koch exhibition in Berlin in 1935. The horizontal photomicroscopic apparatus Koch used in his later work is on the left. Note the string-operated shutter that controls the admission of sunlight into the room.*

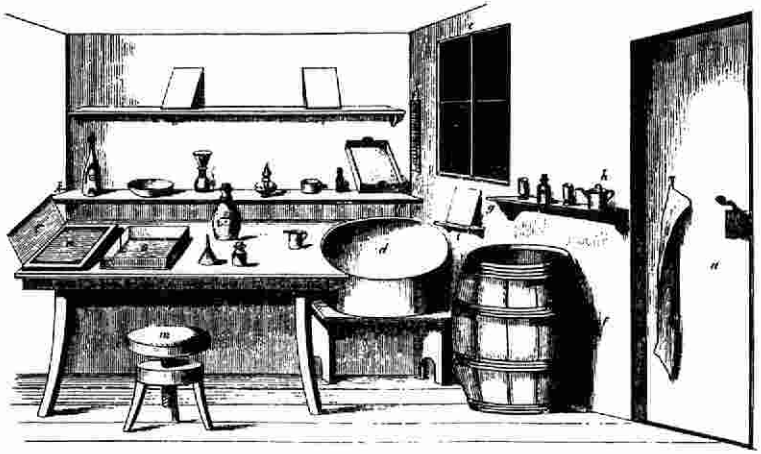

Figure 7.4 *Arrangement of a photographic darkroom in Robert Koch's time. (a) Door; (b) work table; (c) and (d) arrangement for waste water; (e) window covered with yellow paper to make the room light-safe for photographic work; (f) water storage; (i) shelves holding glass plates being dried; (n) open cassette; (o) tray for the silver bath.*[9]

obtained with wet plates, as dry plates had insufficient sensitivity at the high magnifications needed. We can obtain an idea of how Koch must have worked from the descriptions given in Reichardt and Stürenburg[8] and Gerlach[9].

The glass plates used had to first be carefully cleaned. The photographic emulsion was made from collodion, a solution of cellulose nitrate in alcohol-ether. When spread in a thin film, the solvents evaporated leaving a tough, colorless film. The light-sensitive agent consisted of silver iodide, which had to be formed *in situ* by the photographer. The collodion could be purchased commercially already saturated with iodine.[10] First, the iodized collodion was poured over the glass plate in a thin film, the plate being held vertically to allow the liquid to drain (Figure 7.5*a*). Then, in a dark room or dark chamber (Koch had a large box built for him by a local carpenter) the glass plate containing the collodion-iodine film was immersed in a silver bath (Figure 7.5*b*). The silver bath consisted of highly purified silver nitrate dissolved in either distilled water or rainwater. The silver ions reacted with the iodine of the collodion film and tiny crystals of light-sensitive silver iodide were formed. It took several minutes to prepare each plate, and the plate had to be used immediately. For the actual photography, the glass plate

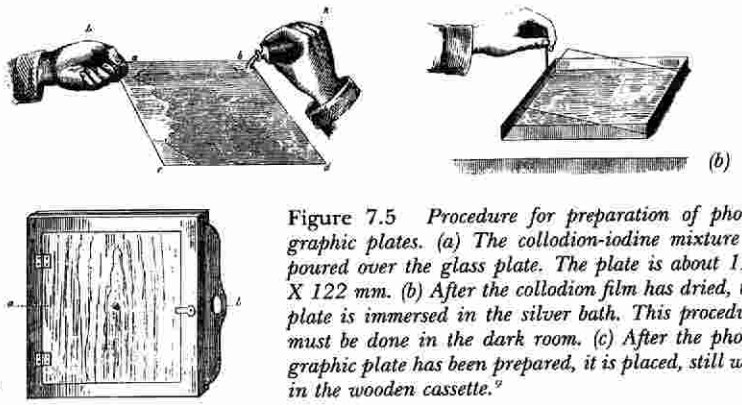

Figure 7.5 *Procedure for preparation of photographic plates. (a) The collodion-iodine mixture is poured over the glass plate. The plate is about 130 X 122 mm. (b) After the collodion film has dried, the plate is immersed in the silver bath. This procedure must be done in the dark room. (c) After the photographic plate has been prepared, it is placed, still wet, in the wooden cassette.*[9]

was placed into a wooden photographic plate holder (Figure 7.5*c*). Once the cassette was closed one could then remove it from the dark chamber and take it to the photomicroscopic apparatus.

How was the actual photomicrograph made? *Before* preparing the wet collodion plate, the microscope slide had to be placed on the stage and the desired specimen put into focus. Then the photographic plate was prepared in the darkroom. Back at the microscope, the cassette was placed on the photomicroscopic apparatus, the dark slide of the cassette opened, and the exposure made (exposure was generally four to five minutes with ordinary daylight). After the exposure, the dark slide was reinserted and the cassette taken back to the darkroom for developing. In the dark, the plate was removed from the cassette and developed, washed, and fixed. Only then could one examine the plate to see if the exposure was correct and if the specimen had remained in focus.

A typical session of photomicroscopy

The following, taken from Gerlach[11], shows us what Koch had to contend with:

> Before beginning, assess the weather. Only a clear day with a high barometer reading and good sunlight is suitable for taking pictures. . . . Start early in the day, making fresh plates and getting everything ready. It often takes three hours or more to obtain four to six good pictures.
>
> It is best to take the whole microscope apparatus outdoors, rather than try to shoot through a window, since you will get a lot more shooting time outside.
>
> Make sure the apparatus is firmly mounted so that it does not move when pulling the dark slide in and out. I use a specially made four-legged table of 55 cm height. Clean all the lenses, screw them in completely, and place the illuminating mirror on the sunny side of the microscope. With a dark cloth over your head, look through the ground glass and adjust the light and focus the specimen. . . . Once the image is in focus . . . go inside to prepare the photographic plates. In the darkroom . . . remove a clean glass plate with forceps and pour over its surface the iodized collodion solution, making sure the film spreads evenly and completely. Once the collodion film is ready, close the darkroom door and carefully lower the plate into the silver bath. . . . [After it is ready] allow it to drain and put it in the cassette. Close the cassette and go back outdoors to the photomicrographic apparatus. Remove the black cloth . . . and check to be certain that the proper image is still in focus. . . . Then carefully place the cassette on the apparatus and slowly remove the dark slide from the cassette, being careful not to move anything. After the exposure . . . push the slide back in the cassette, remove the cassette from the microscope, and cover the microscope again with the black cloth. This whole procedure must be done quickly! Run back to the darkroom with the closed cassette, close the darkroom door tightly, take the glass plate out of the cassette, develop the plate, and fix the negative. If the photographic image is not completely sharp, or if there are imperfections in the emulsion . . . it is necessary to repeat the whole process, since nothing is more disheartening in the photographic technique than to try to make prints from unsatisfactory negatives.

We can imagine Robert Koch carrying out the above procedures in between patients! To avoid the problem of running outside, Koch used a clock-operated heliostat which followed the sun and directed the light into his window through a shutter (see Figure 7.3). Then, only Emmy Koch, his little "Wolkenschieber", had to stay outside and warn him when a cloud was about to block the sunlight.

Koch's efforts to perfect photomicroscopy of bacteria

Koch spent the latter half of 1876 and the first part of 1877 attempting to perfect his technique of photomicroscopy. At this time, he viewed photomicroscopy not only as a tool for communication, but as a procedure to aid in the classification of bacteria. He wrote to Ferdinand Cohn on 15 November 1876:

> I am working hard to develop a technique that will make it possible to

> distinguish the various species of Schizophytes, even the smallest and least characteristic. . . . [By making photomicrographs, I can reveal the bacteria] true to nature and free of subjective misinterpretation. . . . Frisch's paper [see Chapter 6] has convinced me even more so of the necessity of improved methods.[12]

However, things did not go well. On 4 December 1876 he wrote:

> In order to obtain good negatives at the needed magnifications, one must have very expensive apparatus and bright sunlight. The first is beyond my means and the latter is also unfortunately missing this time of year.[13]

During this period, Koch made contact with several other individuals who were having success with photomicroscopy, including an industrialist named Janisch and Gustav T. Fritsch, a Professor of Physiology at the University of Berlin. They had used a horizontal photomicrographic apparatus, also made by Seibert and Krafft, rather than the vertical one that Koch had obtained the previous summer. Koch then ordered one of these horizontal cameras for himself:

> Please don't make me wait as long as I had to last summer before you send me the instrument.[14]

When he finally received the instrument, he worked it over extensively, modifying it for his own purposes (Figure 7.3). Finally, he obtained some negatives which he was satisfied with and sent them off to Cohn. Cohn was extremely excited with the photomicrographs and asked Koch to prepare a paper for his journal. The preparation of this paper occupied Koch throughout the winter, spring, and summer of 1877. The paper not only contained the first photomicrographs of bacteria ever published, but also described in detail all of Koch's procedures, including slide preparation, staining, and preservation of specimens.

The 1877 paper

In his 1877 paper, Koch laid out clearly the precise procedures that he followed when preparing, staining, observing, and photographing bacteria. His methods were described in such detail that others could easily follow them. Koch began by describing how a microscope slide is prepared, beginning with a suspension of bacteria in liquid. Koch emphasized the importance of drying the bacteria-containing fluid in a very

thin layer on the cover glass, so that the bacteria were fixed in a single plane. This not only stopped motility and Brownian motion, but also stabilized the sample. (The slide technique for examining cultures still used today differs very little from that first described in Koch's paper.) The preparation was allowed to dry in the air, and such dried preparations could be kept for weeks or months. Koch especially emphasized the importance of this "conservation" technique, since it made it possible for one to keep a bacterial sample for later comparative microscopic study. He noted that drying a preparation prevented the development of contaminating bacteria ("fremder Bakterienarten"). He also described in this paper how he took fresh cover glasses with him to the patient's bedside, so that the patient's fluids could be sampled. (He would later use this technique extensively when searching for the causal agent of cholera, see Chapter 15.) Koch commented on the possible objection one might have to studying dried preparations:

> I saw to my astonishment that bacteria do not collapse and become deformed upon drying, as do infusoria, monads, or algae, but retain their shape, becoming fixed firmly to the glass by way of their outer slime layers without changing either their length or width.[15]

Once dried on a cover glass, the preparation could be later rehydrated with water and stained. The best stains were the aniline dyes. The use of aniline dyes for staining fluids and tissues had been discovered by Cohnheim's assistant Carl Weigert[16], who recommended the procedure to Koch. Important improvements in staining were also made by Paul Ehrlich (1854–1915), Weigert's cousin (see Chapters 14 and 19). Koch noted that these aniline dyes stained bacteria specifically and permitted distinction of bacteria from nonliving precipitates, fat droplets, or other tiny bodies. Koch tried a number of aniline dyes, including methyl violet, fuchsin, safranin, eosin, and methyl green. In some cases, fuchsin worked best but in most cases methyl violet was preferable. For photographic work, where color sensitivity of the emulsion was a consideration, aniline brown was used. Koch described his staining procedures in detail, emphasizing the importance of choosing a proper concentration of dye and the necessity of rinsing well. Once the bacteria were stained, the preparation could be conserved with Canada Balsam or another suitable suspending fluid. Koch's detailed experiments on the staining of bacteria provided a solid foundation for his most important work, the discovery of the tubercle bacillus (see Chapter 14).

Throughout 1877, Koch and Cohn had extensive correspondence, as Koch tried to get his photographs ready for the paper, and Cohn tried to arrange for good positive prints. Koch also travelled occasionally to Breslau, to show his photographs to Cohn and to keep up on the latest news of the bacteriological world. We have a fascinating account of this stage in Koch's life from Carl J. Salomonsen's diary. Salomonsen (1847–1924), a Dane, spent the months from April to August 1877 in Cohnheim's institute and met Koch.[17]

> I must also mention another guest whose visit to Breslau was considered important by both Ferdinand Cohn and Cohnheim: Robert Koch of Wollstein. Koch had achieved world fame as a result of his paper last year that described his research on anthrax. This time he brought with him a series of photomicrographic negatives. According to Koch, all other methods of illustrating bacteria were obsolete, now that photomicroscopy of stained bacterial preparations had been perfected.[18] I was invited to midday dinner with Koch and Cohn; Eidam was also there. We talked about almost nothing but bacteria. Later, we all went to the commercial photographer who was making marvelous prints of Koch's negatives for publication in Cohn's *Beiträge*. Although Koch's visit was brief, he and I remained in friendly contact as "bacteriologists", which was a great aid to me in my later research.[19]

Throughout the spring and summer, Koch and Cohn corresponded frequently about the prints for the paper, arranging the plates, deciding on which pictures to use, commenting on what each photomicrograph showed. At one time, Koch queried Cohn:

> Do you believe, Herr Professor, that my photomicrographs would be suitable for distinguishing the various species/differences of bacteria? The longer I study the Schizophytes, the more convinced I am of my ignorance of these lowest forms of plant life.[20]

Koch also spent a lot of time trying to obtain photomicrographs of what he was calling bacterial flagella, without great success. Finally, he gave up this particular line of work:

> It would be very interesting to follow this line of work further, but it would take me too far from the field of medicine.[21]

Later he wrote:

> I am especially proud of the photomicrographs of Bacillus anthracis, since it is rare that one finds conditions appropriate to photograph living bacteria by sunlight.[22]

Over this year he worked feverishly on his research, never taking a vacation, seeing patients only when absolutely necessary. It was not until November 1877 that the prints were finished to Koch's satisfaction and the paper could be published. Finally, after one and one-half years of intense effort, the manuscript went to press. Koch had the reprints just before Christmas. He sent reprints to a number of people with botanical and medical interests, including Professor Fritsch in Berlin, to whom he wrote:

> I am well aware of how imperfect my photographic efforts have been but I am absolutely certain that a bad photograph of a living organism is a hundred times better than a misleading or possibly inaccurate drawing.[23]

An examination of a copy of Koch's paper today fills us with admiration. The photomicrographs are, for the most part, outstanding, and would not be out of place even in a modern publication (Figure 7.6). The photos in the journal are not halftones, as are used today, but individually prepared prints. Each photographic print had to be hand-fixed to its place on the plate for each copy of the journal. Examination of these prints with a lens reveals an amazing amount of detail. However, we should note that except for *Bacillus anthracis* (whose large size makes it very favorable for microscopy), all of the samples that had been photographed had been suspended in culture fluid or blood. No tissue slices or pathological preparations were used. Koch was to find out later, when he began to study bacteria that grew in diseased tissue, that the Seibert and Krafft microscope which he used here was not suitable for examination of bacteria in tissue and that photomicroscopy of bacteria in tissues was virtually impossible by the methods available to him.

Ernst Abbe and the development of microscopy

The history of microscopy has been well covered by Bradbury.[24] By Koch's time, achromatic lenses were available, and the principle of immersion had been discovered, but only water-immersion lenses were used, unsuitable for examining such tiny objects as bacteria. The further developments in microscopy, so crucial for the field of bacteriology, are closely linked with the name of Ernst Abbe (Figure 7.7). Abbe (1840–

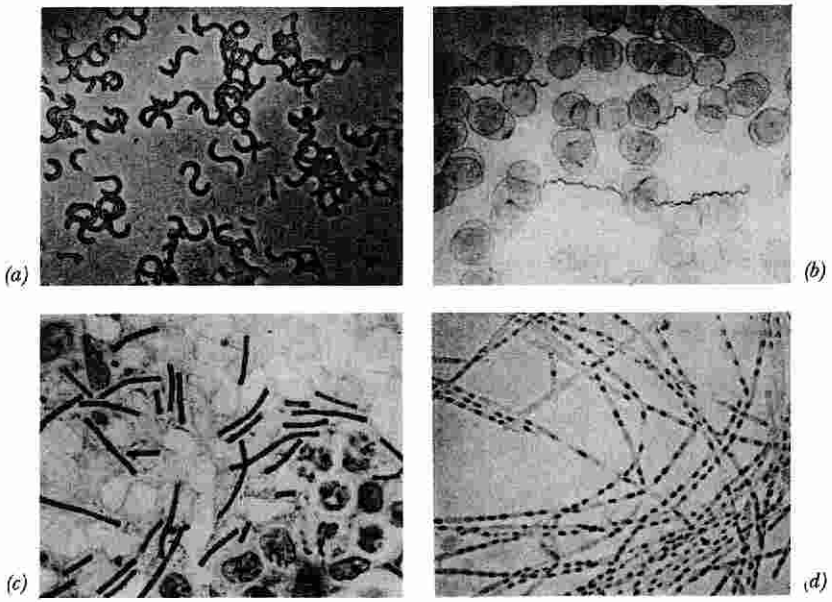

Figure 7.6 *A few of Koch's photomicrographs from his 1877 paper. (a)* Spirillum Undula *with "flagella", photographed from a dried unstained preparation. (b)* Spirochaete Obermeieri, *the causal agent of recurrent fever, stained with aniline brown and immersed in glycerol. (c)* Bacillus anthracis, *from infected spleen. A thin layer of tissue was allowed to dry on a cover slip, then the preparation stained with aniline brown and immersed in glycerol. This technique causes the red blood cells to lose their color. (d) Anthrax bacilli which had formed spores after having been cultured in aqueous humor. The preparation was dried on the cover slip, then rehydrated in potassium acetate and photographed without staining.*

1905), a Lecturer in Mathematics, Physics, and Astronomy at the University of Jena, became an optical consultant for the Carl Zeiss Microscope Company. He subsequently (1871) left the University to become a partner and later (1888) the sole proprietor of the firm. Abbe carried out numerous experiments on lens design and acquired a sound grasp of both the theoretical and practical aspects of optics. As a result of his work, Abbe realized the important distinction between *magnification* and *resolution*. He understood that magnification could be readily increased but that this did not necessarily make it possible to *see* anything better. The key requirement was to *resolve* into separate images tiny objects that lay close together.

Abbe showed that in order to resolve closely positioned points, the

Figure 7.7 *Ernst Abbe, the force behind the Carl Zeiss microscope company.*

microscope lens must accept not only the rays coming directly up the axis of the objective, but also at least one of the diffracted beams of light. The more diffracted light which entered the objective, the more faithful would be the representation of the structure. Microscopists of the mid-nineteenth century had discovered that one could increase resolution by using oblique light, light that was directed at an angle up through the specimen. Abbe showed that the reason oblique light increased resolution was because with the axial beam directed into one side of the lens there was a good chance that at least one of the diffracted beams would enter the aperture of the lens. He concluded that by the use of immersion lenses, a much larger aperture could be obtained and therefore a larger amount of diffracted light could be admitted to the lens. Abbe developed the concept of *numerical aperture*, which expressed the light-accepting power of a lens.

Building on his theory, Abbe constructed the first oil-immersion lens. The advantage of oil over water for an immersion lens is that an oil can be used that has the same refractive index as the glass, so that complete homogeneity is obtained, and all of the diffracted light is collected by the lens. Although the concept of *homogeneous immersion* had preceded Abbe, its practical importance had not been realized. Abbe published

the first description of the oil-immersion lens in 1879, but his work was known to Koch earlier.[25]

Ever pursuing the better image, in July 1878 Koch traveled to Jena and visited Abbe and the Carl Zeiss Company. On this trip he was accompanied by his Breslau colleague Carl Weigert, and they had the chance to see the Zeiss factory and talk with Ernst Abbe. Koch subsequently obtained one of the first oil-immersion lenses available and used it in his studies on bacteria in wound infections (see earlier in this chapter and in Chapter 8). In Abbe's paper on the oil-immersion lens, Koch's successful use of his lens is mentioned:

> . . . as a proof of excellence of definition which, though indirect, is of special weight, may be mentioned the favourable results which Dr. Koch, of Wollstein, obtained when examining bacteria . . .

These first oil-immersion lenses, supplied by the Carl Zeiss Company, were very successful, and soon they were widely used. They were mainly responsible for the great international success which the Carl Zeiss Company achieved in the microscope field.

However, the oil-immersion lens alone was not enough to ensure superior microscopy of bacteria; one had to provide proper illumination of the microscope field. Abbe's other major contribution was the development of an effective condenser, which came to be known as the Abbe Condenser. In order to provide optimum illumination along the axis of the microscope, Abbe developed a condenser which contained a lens system, so that a full cone of light rays filled the entire aperture of the objective:

> With this illumination, which can only be effected by the aid of a condenser of large aperture, the preparation is simultaneously penetrated in all directions by the incident rays. As a result, the delineation of such parts as stand out in mutual contrast through difference in refractive power (tissue structure, etc.) is almost completely suppressed, and there remain visible only those elements which act as absorbants through staining. . . . Very small and closely clustered elements, as in preparations of bacteria, must certainly . . . become capable of a more thorough resolution than with central illumination of the usual kind.

Koch found that Abbe's condenser was especially valuable when examining stained preparations. Koch made a clear distinction between two kinds of images, which he called the "structure image" and the "color image".[26] Without staining, the structure image was obtained as

a result of the diffraction of light, but when staining was used, the color image obtained was much better. Koch recognized that when viewing stained preparations, diffraction of light was undesirable. By the use of the Abbe condenser, it was possible to fully illuminate the field without the attendant problems of diffraction.

It is of interest to note that Abbe and the Carl Zeiss Company provided the important equipment needed for Koch's work, and in turn that it was Koch's success that helped to establish the Carl Zeiss Company as the preeminent microscope builder of the world.

However, even before he obtained the new Zeiss equipment, Koch had returned to work on experiments involving medical problems. Following the lead of Lister's antiseptic surgery, Koch began to study the important problem of wound infections, using animal models. We discuss this important work, so pivotal for Koch's own career, in the next chapter.

3. The background material on Ferdinand Cohn is taken from: Pauline Cohn, *Ferdinand Cohn. Blätter der Erinnerung.* J.U. Kern's Verlag, Breslau, 1901 and Rosen, F., 1922, in Andreae, F., M. Hippe, O. Schwarzer, and H. Wendt, (editors) *Schlesier des 19.Jahrhunderts*, Korn Verlag, Breslau, pages 167–173.

4. Cohn, F. 1872. *Ueber Bacterien, die kleinsten lebenden Wesen*, C.G. Lüderitz'sche Verlagsbuchhandlung, Berlin. An English translation of this book was published: Dolley, Charles S. 1881. Bacteria: the smallest of living organisms. Rochester, N.Y. Reprinted in 1939 by the Johns Hopkins Press, Baltimore.

5. Pauline Cohn, loc. cit.

6. Heymann, op. cit., page 178.

7. The Koch visit is recorded in the Institute log book in Cohn's own hand. These accounts were published by Heymann, pages 150–154.

8. The information on Cohnheim is taken from the introduction to Cohnheim, Julius F. 1885. *Gesammelte Abhandlungen* edited by E. Wagner. A. Hirschwald Verlag, Berlin.

9. Wagner, loc. cit.

10. Koch, 1876, op. cit.

11. Cohn, F. 1876. Untersuchungen über Bacterien. IV. Beiträge zur Biologie der Bacillen. *Beiträge zur Biologie der Pflanzen*, 2: 249–276.

12. Koch, 1876, op. cit.

13. Tyndall, John. 1877. Further researches on the deportment and vital persistence of putrefactive and infective organisms from a physical point of view. *Philosophical Transactions of the Royal Society of London* 167: 149–206. Tyndall's fractional sterilization technique is sometimes called *Tyndallization.*

14. Heymann, op. cit., page 159.

15. Heymann, op. cit., page 172.

16. Heymann, op. cit., pages 173–175.

Chapter 7 *The Microscope Revolution*

1. Quoted from Cohn (1872), page 15.

2. Reichardt, Oscar and C. Stürenburg. 1868. *Lehrbuch der Mikroskopischen Photographie.* Verlag von Quandt & Händel, Leipzig.

3. Heymann, op. cit., page 167.

4. Heymann, loc. cit.

5. Koch, R. 1877. Verfahren zur Untersuchung, zum Conservieren und Photographiren der Bakterien. *Beiträge zur Biologie der Pflanzen*, 2:399–434.

6. Quoted from Heymann, page 171. Written 14 October 1876.

7. Heymann, loc. cit.

8. Reichert and Stürenburg, loc. cit.

9. Gerlach, J. 1863. *Die Photographie als Hülfsmittel Mikroskopischer Forschung.* Verlag von Wilhelm Engelmann, Leipzig.

10. Sources of photographic collodion in Koch's time were E. Liesegang of Elberfeld and Ferdinand Beyrich of Berlin.

11. Gerlach, loc. cit.

12. Heymann, op. cit., page 173–175.

13. Heymann, op. cit., page 176.

14. Heymann, op. cit., page 179. Note that this letter was written on Christmas day, 1876!

15. Koch, 1877, loc. cit.

16. Weigert, C. 1878. Bismarckbraun als Färbemittel. *Archiv für mikroskopische Anatomie* 15: 258–260.

17. Salomonsen, C.J. 1914. Lebenserinnerungen aus dem Breslauer Sommersemester 1877. *Berl. klin. Wochenschr.* 51:485–490. Another visitor at this same time was William Henry Welch, from America, soon to become Professor of Pathology at the Johns Hopkins University. Welch became one of Koch's main supporters in the United States (see Chapter 21). Because of Welch's enormous influence on American medicine, Koch's work and methods became widely known at an early stage in the United States.

18. Koch later found out that he was overly optimistic about photomicrographs. See the preface to his 1878 book (discussed in Chapter 8).

19. Salomonsen, loc. cit. Salomonsen also took Koch's cholera course in Berlin in the mid-1880's.

20. Heymann, op. cit., page 186.

21. Heymann, op. cit., page 195.

22. Heymann, op. cit., page 201.

23. Heymann, op. cit., page 224.

24. Bradbury, S. 1967. *The Evolution of the Microscope.* Pergamon Press, Oxford.

25. Abbe, Ernst. 1879. On Stephenson's system of homogeneous immersion for microscope objectives. *Transactions of the Royal Microscopical Society* 2: 256–265.

26. Koch, 1877, loc. cit.

Chapter 8 *Studies on Wound Infections*

1. Heymann, op. cit., page 257.

2. Heymann, op. cit., page 197. Koch was later to write very critically of Pasteur's work on anthrax, initiating a running feud with the eminent French scientist that was to last for many years (Chapter 16).

3. Welch, W.H. Collected Works. See also Chapter 21.