
 OCCASIONAL SURVEY

THE HISTORY OF THE ZIEHL-NEELSEN STAIN

By P. J. BISHOP

from the Library, Institute of Diseases of the Chest, London

and

G. NEUMANN

Städt, Gesundheitsamt, Abt. Tuberkulosefürsorge, 7 Stuttgart 1, Schickhardtstrasse, 35, Germany

Although there are some good accounts in the literature of the history of staining the tubercle bacillus, in particular those by the German historian of tuberculosis Predöhl (Predöhl, 1888) and Calmette (Calmette, 1923), many others are incomplete, contradictory, or even inaccurate. For example William Bulloch in his history of bacteriology wrote—'The so-called Ziehl-Neelsen method was in reality Neelsen's' (p. 385) (Bulloch, 1938). This paper merely tries to trace the chronology of events leading up the recognition of what is now known as the 'Ziehl-Neelsen method', while at the same time reference is made to a few important or interesting early references to the tubercle bacillus and its staining in some British journals of the period 1882-1887.

First of all it is necessary to say something about the two men eponymously commemorated in this way. ^{Fig 2} Friedrich Ziehl was born on the 13th April, 1857 at Wismar in Mecklenburg-Schwerin, the son of a civil servant who later became an advocate. He studied and qualified at Heidelberg in 1881. From 1882 until 1886 he was assistant in the Medical Clinic at Heidelberg, but he had to leave for economic reasons (the death of his father). From 1887 until his death on the 7th of April, 1926, he worked as a neurologist at Lübeck, where he published several papers on neurological subjects. The above details are taken partly from Fischer (Fischer, 1933) and partly from an obituary published in a local Lübeck journal. Fischer's account would seem to be the only readily accessible one in the literature. It stated that Ziehl's name is known through his stain and gives the reference to his 1882 paper. The Lübeck obituary makes it clear that Ziehl was well-known in his home town as the first to stain the tubercle bacillus with carbol fuchsin (*Lübeckische Blätter*, 1926). No obituary of Ziehl appeared in the medical press, and as far as we have been able to find out the only time he has been commemorated in recent years was in the paper by the late Dr. Walter R. Bett (Bett, 1957).

Friedrich Karl Adolph Neelsen was born on the 29th March, 1854, at Uetersen, Holstein, the son of a clergyman. He studied and qualified at Leipzig in 1876, where he was a pupil of Ernest Wagner. From 1876 until 1878 he was an assistant at the Pathological Institute at Leipzig, and from 1878 until 1885 he occupied a similar position at Rostock, where in 1884 he was made professor. In 1885 he moved to Dresden, where he followed Birch-Hirschfeld as prosector at the Städtischem Krankenhaus. He died on the 11th of April, 1894. The above is again taken from Fischer (Fischer, 1933) who stated that he is remembered for the Ziehl-Neelsen stain, and also referred to his books: his '*Grundriss der pathologisch-anatomischen Technik*', Stuttgart, 1892, and a '*Beiträge zur Pathologie*', Vienna, 1888, written with F. M. Oberländer. Pagel's account differs little from that of Hirsch (Pagel, 1901). He gave a list of Neelsen's writing, but did not mention his stain. He wrote 'Neelsen war ein tüchtiger Anatom und Patholog'. Apart from the above-mentioned accounts we have been able to find only one informative obituary notice of Neelsen. This was written by a Dr. Grenser and tells us that Neelsen published papers on disposition to disease in general; syphilis; carcinoma; the role of bacteria within the body; methods of staining bacteria; spontaneous rupture of the

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heart; haemorrhagic infarction of the heart; and myocarditis (Grenser, 1893-94). It seems certain that the cause of Neelsen's death was tuberculosis. The symptoms appeared in the autumn of 1893, and from the end of the year he suffered from emaciation, hoarseness, cough, and a general deterioration. In the winter of 1893/94 he had intended to speak about carcinoma of the lung (Dresden was the centre of cigarette manufacturing, and the increase in carcinoma of the lung was first observed in Dresden), but he could not do this because of progressive hoarseness. He died on the 11th of April, 1894, leaving a wife and a three year old son. *Virchows Archiv* contained a note of Neelsen's death in a calendar of obituaries published at the end of the year, but it does not tell us anything new. It gives as sources of information: Hirsch; Leopoldina; and Vossische Zeitung Berlin. The first of these has already been mentioned. The Leopoldina reference merely records Neelsen's death. The third reference is to a newspaper, which we have been unable to see, but which we strongly suspect only records Neelsen's death.

KOCH'S DISCOVERY OF THE TUBERCLE BACILLUS

Koch's formal announcement of his discovery of the tubercle bacillus was made on the 24th of March, 1882. His first publication on the subject appeared in the *Berliner Klinische Wochenschrift* of April 10th, and bore the title—'Die Aetiologie der Tuberculose' (Koch, 1882). This first paper included an account of the method he had used to stain the bacillus—

'The material to be examined is prepared in the usual manner for examining for pathogenic bacteria, and either spread on the cover slip, dried and heated or cut into sections after fixation in alcohol. The cover slips or sections are placed in a staining solution of the following constitution: 200cc. of distilled water are mixed with 1cc. of a concentrated alcoholic solution of methylene blue, shaken up, and then 0.2cc. of a 10% solution of potassium hydroxide is added with repeated shaking. This mixture must show no precipitate after standing for several days. The materials to be stained remain in this solution for 20 to 24 hours. By heating the solution to 40°C. in a water bath this time can be shortened to a half to one hour. Following this the cover slips are covered with a concentrated aqueous solution of vesuvin (Bismarck brown) which is filtered each time before using, and after one to two minutes rinsed with distilled water. When the cover slips come from the methylene blue, the attached layer appears dark blue and is markedly (p. 222) overstained. During the treatment with vesuvin this blue colour is lost and it appears stained a faint brown. Under the microscope all the constituents of animal tissue, that is, the cell nuclei and their products of disintegration appear brown, while the tubercle bacilli, on the other hand, stain a beautiful blue. Moreover, all other bacteria which I have investigated to date, with the exception of the lepra bacilli, take on a brown color with this staining method. The color contrast between the brown stained tissue and the blue tubercle bacilli is so striking that the latter, which are present often only in very small number, nevertheless, are to be found and identified with the greatest certainty' (Pp. 227-28 in the original) (Koch, 1882) (the translation is that of William de Rouville, 1938, Pp. 854-55.).

It is interesting to note that the three important factors in staining the tubercle bacillus: i.e. the use of mordant, heat, and decolorizing all but the mycobacteria, are all included in Koch's method, recorded in his first paper of 1882. The present Ziehl-Neelsen stain has evolved from attempts to improve on Koch's method. The significant contributions to this would appear to be those of:—

Ehrlich (1882) who improved it by decolorization with a mineral acid—he also used a red stain, fuchsin, and changed the mordant to aniline oil.

Ziehl (1882) who changed the mordant to carbolic acid.

Rindfleisch (1882) who heated the slide instead of putting it into hot water.

Neelsen (1883) who combined Ziehl's mordant with Ehrlich's red stain.

It is also interesting to relate these developments to others occurring during the same period, and to examine chronologically some of the post-Koch (1882) literature.

EHRlich

As we have said, the first suggestions for the improvement of Koch's method came from Paul Ehrlich, who was one of those present at Koch's sensational announcement in Berlin. Ehrlich's communication was made to the Berlin Society of Internal Medicine on May 1st, 1882. He recommended staining the bacillus with aniline methyl violet instead of alkaline methylene blue:—

'After these remarks I wish to briefly report the method. I have worked almost exclusively with dry preparations, i.e. of sputa, but I have done controlled trials which have proved that the method is also applicable to section preparations. I proceed by taking out a small particle with a dissecting needle from the sputum and squeezing it flat between two cover-glasses, and I have found that those of 0·10 to 0·12mm. are best. Under these conditions it is easy to obtain uniformly thin layers from the small plug of sputum. Then both glasses are torn from each other and one then gets two thin layers, which can easily be air-dried. These preparations are not yet suitable. It is necessary to fix the protein. Usually I have done this by keeping the preparations for one hour at 100 to 110°C.; however there exists a more convenient method which I have seen used in the Kaiserlichen Gesundheitsamt and which consists in passing the air-dried preparations three times through the flame of a Bunsen burner. For staining I use water, saturated by aniline oil, which may be prepared in a few minutes by shaking water with surplus aniline oil and filtering it through a moistened filter. To the water-clear fluid obtained in this way one adds drop by drop a saturated alcoholic solution of fuchsin or methylene violet until a distinct opalescence indicates saturation with the dye-stuff. Upon this fluid the preparations are made to float and staining is adequate within a quarter to half an hour. The colour of the tubercle bacilli does not disappear under the influence of vesuvin (Bismarck brown) or only very slowly. It is necessary to use an acid and I soon turned to the use of strong, I may even say heroic, ones. A mixture consisting of 1 volume of official nitric acid and 2 volumes of water can be recommended. Under its influence one sees the preparation grow pale within a few seconds, yellow clouds rise and the preparation becomes white.

If one examines the preparation at this stage, it is seen to be decolorized and only the bacillus has maintained its intensive staining. It might be possible to examine such a preparation, but the technical difficulties of focussing the bacillus are extremely great. It is recommended to stain the background yellow if the bacilli are violet, and blue if they are red.

I have succeeded in staining the bacillus with all basic aniline dyes, even with Bismarck brown, and hence it follows that the substance of the bacillus itself does not differ from that of other bacilli in its staining properties. But the discrimination of the tubercle bacillus by staining depends on the presence of a surface layer with characteristic and specific properties. The first of these to which Koch's technique is pointing is that the covering layer is permeable only to dyes under the influence of alkalis' (Pp. 269-70) (Ehrlich, 1882).

One of the earliest references in the British literature can be found in an editorial in the *British Medical Journal* of May 13th, 1882. It begins:—

'Experiments which have been carried on during the last few years have demonstrated beyond a doubt that tuberculosis belongs to the group of infective diseases, although various questions have arisen as to the origin of a similar disease from inoculation with non-tubercular matter. Many of these difficulties have been cleared up, and a great advance has been made in the pathology of this subject, by the beautiful experiments recently published by Dr. Koch' (Editorial, 1882).

This editorial includes a description of Koch's method of staining. The same issue of the journal refers to a meeting at the Physiological Laboratory at King's College, London, on May 8th, at which Mr. Watson Cheyne and Mr. E. M. Nelson had exhibited some specimens of the tubercle bacillus, prepared by Koch, and brought over to London by a Dr. Goldammer (British Medical

Journal, 1882a). The *British Medical Journal* of June 17th, 1882, has a note entitled—'Ehrlich's method of staining tubercle bacilli' and we read:—

'This method is a great improvement over Koch's original method, Ehrlich has employed it chiefly in the examination of sputa; and it has now been adopted by Dr. Koch, in preference to his own method' (*British Medical Journal*, 1882b).

Professor P. Baumgarten published a paper on June 24th, 1882, in which he described a staining method using potash lye (Baumgarten, 1882). The *British Medical Journal* of July 22nd, 1882, published a note under the heading 'The bacillus of tubercle':—

'In reply to many questions which have been addressed to us on this subject, we may say the bacilli of tubercle discovered by Dr. Koch are observed with difficulty, if his method of

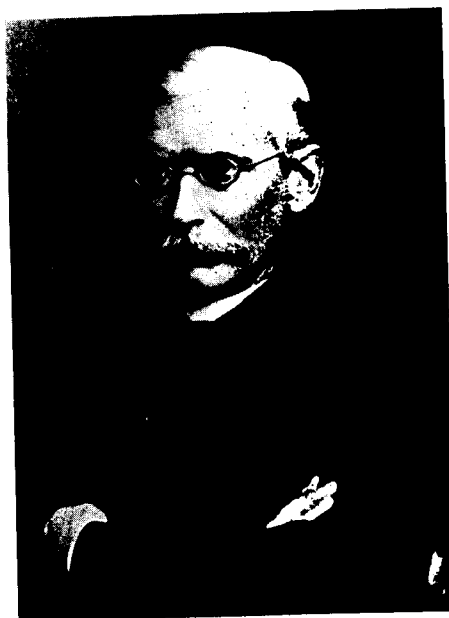


FIG. 1

Franz Ziehl (1857-1926). A photograph taken between 1904 and 1908.

preparation be adopted. Dr. Ehrlich has devised a mode of preparation (published in this Journal, June 17th, 1882), recently modified by Professor Rindfleisch of Würzburg, by which their presence is more easily detected than by any other hitherto made known' (*British Medical Journal*, 1882c).

ZIEHL

It is now that Franz Ziehl appears on the scene (Fig. 1). In his paper published on August 12th, 1882, he described how he had modified Ehrlich's procedure by using carbolic acid instead of aniline; and also by using a weaker acid for decolorization. The significant part of his paper would seem to be:—

'Up to the present we have not been able to obtain the pure constituents of aniline oil. As it was necessary to use a substance belonging to the aromatic series we tested such a one, the reaction of which was known to us from that second aniline oil, namely carbolic acid' (p.451) (Ziehl, 1882).

The *British Medical Journal* of October 14th, 1882, carried two papers about staining the bacillus.

The first was by G. A. Heron, from the City of London Hospital for Diseases of the Chest, and described in detail Ehrlich's method. This was an eminently practical paper with special reference to the author's own experience (Heron, 1882). The second paper was by Heneage Gibbes, Curator of the Anatomical Museum at King's College. This paper was entitled—'A new method for the detection of the tubercle bacillus'. The author wrote that:—

'Koch's original method has utterly failed in the many cases in which I have tried it' (Gibbes, 1882).

He also criticised Ehrlich's modification, and suggested using two staining fluids: one, magenta, to stain the bacillus; the other, chrysoïdin, which stains the surrounding substance, but not the bacillus.

The *British Medical Journal* of October 28th, 1882, published a paper by a William Vignal, Collège de France, with the title—'Some remarks on Ehrlich and Gibbes's methods for the detection of bacillus tuberculosis' (Vignal, 1882). This paper is an indication of the interest that the subject had already aroused. In the same journal of December 16th, 1882, we find a report of a meeting of the Aberdeen, Banff, and Kincardine Branch, on November 22nd, at which Professor Alexander Ogston gave a demonstration of the tubercle bacillus. Dr. Mackenzie Davidson, who had seen the slides prepared by Dr. Heron of London, described the process. The remarks of a Dr. Simpson (Medical Officer of Health) are of interest, as they reveal something of how some people still thought:—

'Dr. Simpson . . . questioned the connection between these bacilli and the diseases with which they were associated. To him we seemed to be looking into a new world yet unknown, and our connecting bacilli and disease as cause and effect seemed to have come too soon. Bacilli were found everywhere, and it was a question unsettled in his mind whether or not they really had any connection with disease' (Simpson, 1882).

Dr. Schill's paper of January 10th, 1883, on the identification of tubercle bacilli in sputum, described Ehrlich's method with Rindfleisch's modification of heating the slide. Mention was also made of Ziehl's modification (Schill, 1883). Early in 1883, L. Lichtheim, of Berne, published a paper which summarized the importance of finding tubercle bacilli in the sputum and in the faeces (Lichtheim 1883). Ziehl's second paper appeared on January 31st, 1883, and bore the title—'The importance of finding tubercle bacilli especially with regard to diagnosis and prognosis'. In this paper Ziehl reported that he had found the bacilli in 73 cases of pulmonary tuberculosis, and he finished his paper with the following conclusions:—

- (1) In most cases of pulmonary tuberculosis tubercle bacilli may be found; exceptions do occur.
- (2) The finding of tubercle bacilli may confirm the diagnosis of tuberculosis. In some cases the differential diagnosis is possible by this finding.
- (3) If tubercle bacilli cannot be found, it is not always possible to exclude tuberculosis.
- (4) The number and kind of tubercle bacilli do not allow any prognostic conclusions (Ziehl, 1883a).

The *Lancet* of February 24th, 1883, referred to a description by Professor Rosenstein, of Leyden, of a case in which the bacilli were found in the urine in considerable numbers, and went on:—

'The observations is of much interest, but at the same time rather by way of suggestion than of proof. The question of the diagnostic value of tubercle bacilli is still in the region of probable rather than of certain knowledge' (Lancet, 1883a).

De Giacomi, of Berne, published a paper in March, 1883, in which he differentiated between tubercle bacilli and other bacilli in the faeces (De Giacomi, 1883). The *Lancet* of March 17th, 1883, had another note about tubercle bacilli in urine, and referred to a Dr. Babesiu of Pesth, who had followed in parts the methods of Ehrlich and Koch, but instead of nitric acid had used strong acetic acid (Lancet, 1883b). Ehrlich took part in a discussion of Koch's discovery published in the *Deutsche Medizinische Wochenschrift* of March 14th, 1883. He criticized a technique described by

Spina as giving unfavourable results (Ehrlich, 1883). The *Lancet* under the heading 'The tubercle controversy', referred in its issue of April 21st, 1883, to Dr. Spina's monograph in which he sought to minimize the importance of Koch's discovery, and also mentioned the *Vienna Allgemeine Medizinische Zeitung*, which apparently published a series of articles or reports under the heading—'The tubercle-bacillus war' (*Lancet*, 1883c).

Ziehl published another paper on April 25th, 1883, entitled 'On the staining of tubercle bacilli'. This dealt with the acid-fastness of the bacilli, and his controversy with Ehrlich (Ziehl, 1883b). Shortly afterwards, Heneage Gibbes, whom we have already mentioned, published a paper in the *Lancet* of May 5th, 1883, in which he described—'A rapid method of demonstrating the tubercle



FIG. 2

Friedrich Carl Adolf Neelsen (1854-94). A photograph taken between 1885 and 1894.

bacillus without the use of nitric acid' (Gibbes, 1883). Carl Weigert, who was a cousin of Ehrlich published on June 13th, 1883, a paper—'New notes concerning the pathogenesis of acute generalized miliary tuberculosis'. In this he stated that he had given up using aniline oil:—

'As it is not always possible to rely upon the aniline oil, as Ziehl has found, I have given up its use completely, and I make use of a 2% filtered aqueous solution of gentian violet to which I add half per cent liquor ammonii caustici (It is possible to raise the concentration up to 1½%. It is known from the experience of potash dyers that lye is harmful to methyl violet). This solution becomes stable for weeks by adding 10% alcohol, but it may be better to prepare it new every time. This is simple even without special laboratory equipment if one has a pipette graded in cubic centimeters and a graduated cylinder. It is best to stain for 20 to 30 minutes at incubator temperature, or over a longer time at room temperature, and one then continues as described by Koch and Ehrlich' (p. 351) (Weigert, 1883).

NEELEN

Friedrich Neelsen now comes into the story (Fig. 2). His first publication on the subject would appear to have been in the German abstracting journal—*Centralblatt für die medicinischen*

Wissenschaften—of July 14th, 1883, which at that time published a considerable number of original papers. Neelsen's article had the rather general title—'A casuistic note on the theory of tuberculosis', but included a footnote:—

'In this case I used first as a staining fluid a three-quarter per cent solution of fuchsin in 5% carbolic acid with admixture of some alcohol and decolorised with 25% sulphuric acid. This method, which I have used almost exclusively for a long time, gives in my hands better results than the other methods (perhaps only because I am most accustomed to it)' (p. 500 (Neelsen, 1883).

Prior, of Bonn, published a paper on staining the bacillus on August 13th, 1883, which discussed the acid fastness (Prior, 1883). The *Lancet* of November 10th, 1883, reported that:—

'Opinion is still divided in Vienna as to the significance of the bacillus tuberculosis, but the record of the last meeting of the Medical Society there shows that not every observer has arrived at the same negative conclusions as Spina. For on that occasion a paper was read by Dr. Heitler on the diagnostic and prognostic importance of the tubercle bacillus, in which he maintained that those only could claim to speak with authority who had repeated with every precaution the experiments of Koch' (*Lancet*, 1883d).

On November 26th, 1883, Dr. Petri, writing from Dr. Brehmer's sanatorium at Görbersdorf, referred to staining by fuchsin and malachite green, using glacial acetic acid (Petri, 1883). In 1884 Koch published his important, second article, or rather monograph on 'Die Aetiologie der Tuberculose' (Koch, 1884). In the words of Dr. Arthur L. Bloomfield:—

'In this important article of eighty-six folio pages Koch makes his definitive presentation of the discovery and isolation of the tubercle bacillus and the proof of its causal relation to the disease. It is really a masterful elaboration of his preliminary communication' (Bloomfield, 1954).

Copies of the *Berliner Klinische Wochenschrift* of 1882 and the '*Mittheilungen aus dem Kaiserlichen Gesundheitsamte*' of 1884 are to be found only in the larger, older medical libraries, so we have indicated in our bibliography those more accessible sources where readers may find them reprinted.

Dr. B. Fränkel's lecture to the Berlin Medical Society on . . . 'The staining of Koch's bacillus and its semiotic importance for diseases of the respiratory tract' was published on April 7th, 1884. It contained a description of the laryngeal swab technique by direct touch, and notes on the prognostic value of bacilli in sputum (Fränkel, 1884). A *Lancet* editorial of March 15th, 1884, referred to Koch's 1884 work as:—

'The very lucid and exhaustive monograph upon the etiology of tuberculosis by Dr. R. Koch, which, although it has been written for some months, has only just now been given to the world, serves to place forcibly before the profession the pathological facts upon which the doctrine of the infectivity of tubercle, and the subsidiary, yet more important, doctrine of the contagiousness of phthisis, are based' (*Lancet*, 1884).

The above editorial is very interesting and informative, but space does not allow us to quote more of it.

The *Lancet* of January 17th, 1885, contains an extremely interesting and illuminating account of a discussion on the tubercle bacillus and the pathology of phthisis held at the Royal Medical and Chirurgical Society on January 13th. Specimens of the bacillus, prepared by Mr. Watson Cheyne and Dr. Percy Kidd were on view. One of the speakers was Dr. Green. This would almost certainly have been T. Henry Green of the Brompton Hospital—a noted pathologist. Referring to scientific criticism of Koch's discovery 'he considered that the question was one for experimental pathologists to investigate, and so far experimental pathology confirmed Koch's and Watson Cheyne's observations' (*Lancet*, 1885). Another speaker was Wilson Fox, one of the greatest authorities of his day on tuberculosis. He is reported as saying that:—

'Although the evidence in favour of Koch's views was so strong, yet, looking to the constant change in our views of phthisis, and seeing how the pathology of tuberculosis

has biennially, triennially, or quinquennially been swayed by histological dogmata, it would be well if we waited before definitely accepting all that the new views implied. Who could tell but that in a few years we should hear expressions in terms which at present we cannot foreshadow? It should be remembered that the bacteria of tubercle depended for their detection on one of the most uncertain of chemical agents, an aniline dye' (Fox, 1885).

Ehrlich's paper 'The biological utilisation by methylene blue' was published on February 21st, 1885, but he had in fact introduced this as early as 1881 (Ehrlich, 1881; 1885). In 1885 Professor Heinrich Albert Johne (1839-1910), remembered for his discovery of Johne's bacillus, and description of Johne's disease, and also for a pioneer German history of tuberculosis, published a paper 'An undoubted case of congenital tuberculosis'. In an interesting footnote he makes mention of Neelsen, and thereby brings him into the story again:—

'This previously unpublished method is one of the modifications of the Ehrlich-Weigert-Koch method described by Ziehl (Deutsche Med. Wochenschr. 1882, No. 33, u 1883, No. 17) by which aniline oil is replaced by carbolic acid. Its preparation and application, for which I am indebted to a courteous personal communication of Herr Neelsen, is as follows: 1.0 fuchsin is dissolved in 100g. of an aqueous 5% solution of carbolic acid and to this solution is added 10g. of alcohol. Staining of the cover-glass preparation with heat up to the rising of steam is very quick and intensive. In microtome sections the bacilli are surprisingly well stained at room temperature within 5 to 10 minutes. Decolorize with a 25% aqueous solution of sulphuric acid, counterstaining with methylene blue. The solution can be stored for several weeks without any change in its excellent staining properties' (footnote 1, Pp. 200-01) (Johne, 1885).

Ehrlich published a long paper in 1886 in which he reported on his studies with aniline and its derivatives, the importance of the waxy envelope of tubercle bacilli, the role of counterstaining, and the importance of repeating the staining of the bacilli (Ehrlich, 1886).

ZIEHL-NEELSEN

The *Lancet* of April 9th, 1887, published a letter from a Dr. Henry S. Gabbett, of Eastbourne, entitled 'Rapid staining of the tubercle bacillus'. This is of interest as it refers to Neelsen's method by name. Gabbett advocated using a stain made from one part of magenta in 100 of a 5% watery solution of carbolic acid, and adding 10 of absolute alcohol. He considered this preferable to any other stains containing solutions of aniline (Gabbett, 1887). In 1892, only two years before his early death, Friedrich Neelsen published a little book—essentials of pathological-anatomical technique for practitioners and students. In it he described his method of staining:—

'For staining tubercle bacilli on the coverglass I use the ordinary solution of carbolic acid and fuchsin (1.0 fuchsin, 100.0 5% aqueous solution of carbolic acid, 10.0 absolute alcohol) in the following way. The dried cover-glass preparations, heated for 5 minutes to 110°C., are covered by means of a pipette with one large drop of the staining fluid and placed on the copper sheet which serves for heating. (One has to be careful that no fluid runs off the cover-glass, spraying about, and often disturbing the cover-glass). As soon as the fluid has come to the boil which only takes a few seconds, a small drop of staining solution is added to avoid dehydration, and this is repeated so that the preparations remain covered with boiling stain solution for 1½ to 2 minutes (measured by a watch). After this they are put into an aqueous solution of sulphuric acid (25.0 to 75.0 distilled water) where they left for half to two minutes until the dark-red colour has almost completely disappeared. During the succeeding washing with abundant (ordinary) water the red colour is partly restored. If the preparations still appear as dark-red, decolorizing with sulphuric acid must be repeated until they are only a pale red colour. Finally the cover glasses are dipped for counterstaining for half to one minute into a concentrated aqueous solution of methylene

blue, again rinsed in water, dried, and mounted in balsam. The tubercle bacilli appear in the preparation bright red, the nuclei blue, and the cytoplasm pale blue' (Pp. 66-67) (Neelsen, 1892).

This description undoubtedly corresponds to the so-called Ziehl-Neelsen technique of staining tubercle bacilli as used up to now.

In 1888 Predöhl published his history of tuberculosis; one of those invaluable early works to which most later historians are indebted. In it he reported on all the methods of staining described between 1882 and 1888. The modification of Ziehl is mentioned as well as that of Neelsen, but a literal translation of his wording would be—'I must describe the modification of Ziehl's method given by Neelsen' (Predöhl, 1888, p. 371). In other words, the Ziehl-Neelsen method is not actually referred to in those terms.

The phrase 'Ziehl-Neelsen' technique (or method) was not in use before the early 1890's. In 1893 in America, Surgeon-General George M. Sternberg (1838-1915), said to have been the first man in the United States to photograph the tubercle bacillus, published a manual of bacteriology—one of the first works in English on the subject. His book included a list and details of the various methods of staining the tubercle bacillus known at that time, and he actually listed the Ziehl-Neelsen method by name (Sternberg, 1893).

One of the first, if not the first, manuals of bacteriology published in England was written by Edgar March Crookshank (1858-1928), a pupil of Koch who later became Professor of Bacteriology at King's College, London. His first edition of 1886 contained a good list of methods of staining with details, and although his list included both Ziehl's and Neelsen's methods, no mention was made of the Ziehl-Neelsen method as such. His second edition of 1887 and his third edition of 1890 made no change as far as this was concerned; but in his fourth edition of 1896 he wrote that of the numerous methods of examining the tubercle bacillus few will be described as many are only of historical interest. He did, however, say that—'The Ziehl-Neelsen method is preferred by the author for sections and cover-glass preparations' (Pp. 384-87). (Crookshank, 1896).

It would appear then that sometime between 1890 and 1893, the Ziehl-Neelsen method had become known and labelled as such.

In reality the Ziehl-Neelsen technique is based on the work of at least five people: Koch, Ehrlich, Rindfleisch, Ziehl, and Neelsen. So the correct name should be: Koch-Ehrlich-Rindfleisch-Ziehl-Neelsen method, a name which stands little chance of being used in practice. In some ways it seems justified to continue using the well-known name 'Ziehl-Neelsen method'. The publications of these two workers were the last in which an important modification of the original method was recommended, modifications which are still in use. Neelsen's description in particular, is as complete as it is correct, and furthermore is still not obsolete. The participation of other workers means that we cannot be quite satisfied with this eponym. But in reality many such names exist in medical terminology, well established and almost impossible to eradicate; and many are more incorrect than the name for this method of staining tubercle bacilli. This cannot, and indeed should not serve as a source of consolation, but rather as an explanation.

Ziehl died in 1926 and Neelsen in 1894, the latter at the relatively early age of forty.

Now, more than eighty years after the first description of the technique, the names of these two German doctors are known all over the world wherever the tubercle bacillus is sought.

We are much indebted to Dr. E. Ziehl, of Lübeck, for letting us have the photograph of his father, and to Dr. P. Scheid, Direktor des Pathologischen Institutes Dresden-Friedrichstadt, for the photograph of Neelsen, one of his predecessors. Without their co-operation we should have been unable to include photographs of Ziehl and Neelsen.

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