THE GRAM STAIN AND THE ETIOLOGY OF LOBAR PNEUMONIA, AN HISTORICAL NOTE

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Although the historical facts concerning the etiology of lobar pneumonia and Gram's original work on the widely used stain which now bears his name are well documented, an examination of the original reports of these two topics has brought to light an unusual facet of the story pertaining to them. It is a tale which re-emphasizes the widely quoted aphorism of Pasteur that chance favors only the prepared mind. The polemic waged over the cause of bacterial pneumonia, which took place in the 1880's, was one of unusual bitterness, at least on the part of one of the participants engaged therein. Had the arguments been less heated and analysis of the data then at hand more perceptive, events might have followed a course other than that recorded in the ensuing paragraphs.

In 1881, Pasteur (13) and Sternberg (15) recovered from rabbits injected with human saliva what were probably the first strains of pneumococcus to be isolated in the laboratory. At the time, however, the relation of these organisms to lobar pneumonia was far from clear. Despite the fact that bacteria had been seen in the bronchial contents of patients dying of pneumonia by Klebs (12) as early as 1875, their significance remained largely obscure. In the ensuing several years, little significant progress was made aside from the observations of Pasteur and Sternberg.

The first contribution of Carl Friedländer (5) to the etiology of pneumonia appeared in Virchow's Archiv in 1882. Beginning in September, 1881, he had examined the fibrinous exudate of the bronchi and histologic sections of the lungs of eight patients dying of lobar pneumonia. His material was stained with aniline dyes by the Weigert-Koch technique. From his descriptions of the organisms in the pulmonary tissues, it appears altogether probable that Friedländer was looking at the pneumococcus.

Friedländer's second communication (6) on the micrococi of pneumonia appeared on November 15, 1883, and touched off a controversy over the causative agent of pneumonia that was to continue for the next three years. In this paper he reported the study of more than 50 additional cases of pneumonia, in the tissues of nearly all of which bacteria were seen. Those sections from which they were absent were from the lungs of patients dying late in the course of the disease. Friedländer remarked also that the difficulty others had had in recognizing organisms in sections resulted from their being obscured by nuclei and fibrin which stained like bacteria with the techniques usually employed. It is here that he makes the first published reference to the stain of Gram, which facilitated to a hitherto unparalleled degree the recognition of bacteria in histologic sections. Friedländer goes on to describe some of the morphologic properties of the micrococcus of pneumonia placing especial emphasis on its capsule. Of greater interest, however, is the section devoted to the isolation of the "micrococcus." The bacteriologic work was carried out in collaboration with Dr. Frobenius, who had received his technical training in this field from Koch.

For the growth of organisms, in addition to blood serum, Frobenius prepared a nutrient gelatin which combined meat infusion, peptone and sodium chloride.

The first attempted isolation with this medium was successful. "We isolated from a case of acute pneumonia of the right upper lobe in the stage of grey hepatization (a case with which cirrhosis of the liver was combined but, however, without any complications whatever, even swelling of the spleen was not present) a large number of completely identical cultures, the material for the inoculation of which was taken from three different places of the hepatization."

The cultural properties of the organism isolated are noteworthy. At room temperature, it gave rise in 24 hr to visible growth at the surface of
the nutrient gelatin resembling the rounded head of a nail. Similar growth was observed on blood serum, and the organism grew well on potato. Preparations of the micrococci stained with gentian violet in aniline water were examined and several illustrations accompany the report. In all, the organism is capsulated. Two of the figures show single bacillary forms.

Further attempts to isolate a micrococcus with the properties set forth above were, in the main, unsuccessful. From two additional cases of pneumonia, however, in which both encapsulated and nonencapsulated micrococci were found, organisms were cultivated which showed a type of colonial growth resembling a flat headed nail with a central depression rather than a round headed nail. This description of colonial morphology suggests that the latter strains were pneumococcus. These two strains were found to be pathogenic in mice but were not subjected, apparently, to extensive study.

Friedländer’s investigations of the virulence of his organism in several species of animals are of considerable interest. Rabbits proved to be completely refractory to infection following trans-thoracic inoculation of the lungs. On the other hand, of 11 guinea pigs, 6 succumbed following this type of infection, and mice proved even more susceptible when treated in the same way. Five dogs were inoculated, one of which died, the remainder showing clinical evidence of a mild and transient infection. The findings, in retrospect, resemble more closely those which would be expected to follow inoculation with Friedländer’s bacillus, for rabbits are highly susceptible to infection with most types of pneumococcus. Guinea pigs, on the other hand, are often resistant to pneumococci other than type 19 (2) but are known to be susceptible to infection with klebsiellas (1). Friedländer was aware that others had recovered from patients with pneumonia organisms that were virulent for rabbits, but he stated at the time that the materials with which these investigators had worked were not above objection and concluded that the organism he had isolated differed from theirs by virtue of its harmlessness in this laboratory animal.

On April 21, 1884, Fraenkel (3) presented at the Third Congress for Internal Medicine in Berlin the first of a series of communications which was to include important observations establishing the role of pneumococcus in human pneumonia and which contained throughout comments critical of the work of Friedländer. In his initial presentation, Fraenkel described an organism he had isolated from the lung of a 30-year-old man who had succumbed to pneumonia of the right lung. This organism, unlike the one isolated by Friedländer, caused the prompt death of rabbits when inoculated by the same route employed by the latter. Guinea pigs varied in their response, some surviving, others developing pleural and peritoneal effusions from which the injected organism could be recovered. In culture, Fraenkel’s cocci failed to produce the nail-head type of growth described by Friedländer. Of two other strains isolated by Fraenkel, however, one differed but little from that described by Friedländer. Fraenkel was unable to decide whether or not the discrepancies observed were the result of differences in the organisms existing prior to their invasion of the human host or of alterations resulting from their residence in the lungs. He took exception, however, to Friedländer’s emphasis on the capsule as a unique property of the pneumonia cocci and stated also that the nail-head form of growth was not an essential character of the causative agent of pneumonia. Friedländer responded without rancor and pointed out that, just as he was willing to accept causes of roseola other than typhoid, so was he willing to consider the possibility of more than one agent’s ability to cause pneumonia.

Thus the polemic was joined. It is apparent now that at least two bacterial species were probably under study, separable by their cultural characteristics and virulence in laboratory animals. It may be inferred that one was pneumococcus, the other Klebsiella pneumoniae or Friedländer’s bacillus. Confusion of these two bacterial species today is inconceivable, for they may be distinguished readily by a variety of techniques, none more convenient, perhaps, than by the staining technique of Gram.

And where was Dr. Gram? He was in Friedländer’s laboratory where he had devised a technique of staining described briefly by Friedländer in his publication of 1883. Gram published his own report on March 15, 1884 (8), four months after the initial reference to his work. As is already well known to many, Gram devised his technique of staining not for the purpose of distinguishing one group of bacteria from another but to enable bacteria to be seen more readily in stained sections of mammalian tissues. Previously available techniques of staining had made the
recognition of bacteria very difficult because nuclei, fibrin, and bacteria manifested similar properties of coloration. By applying a solution of iodine and potassium iodide to sections stained previously with aniline-gentian violet solution and then immersing the sections in alcohol, Gram succeeded in decolorizing the cells of the tissue slices without altering in many instances the blue color of the bacteria therein. He found useful also counterstaining of the tissue with a weak solution of Bismarck brown or Vesuvin.

In his report, Gram made several additional observations, among them the decolorization of the typhoid bacillus when treated by the method outlined. Of greater interest, however, are the findings resulting from the examination of sections of the lungs from fatal cases of lobar pneumonia. In all, Gram examined sections stained by his method from 20 such cases. In 19, the organisms retained the gentian violet (i.e., were gram-positive). In the twentieth case, however, the organisms were decolorized! Of this case, Gram wrote as follows: “One case of croupous pneumonia with capsule-coccus. Here one finds very many cocci which do not all lie in the cells of the exudate. They decolorize very easily in alcohol and, what is more, with and without treatment with iodine. From this case stem a great part of the cultures of Dr. Friedländer. Most of those from animals injected and exposed to infection behave in this fashion (mice, guinea pigs and a dog). Of these, I have investigated some 25 cases. Now and then, the cocci in the experimental animals remain colored after treatment with iodine but then they show no capsule formation; as everyone knows, capsules are always very difficult to demonstrate in cut preparations.”

Here then was the key to the problem, but its significance was unappreciated. Although the tinctorial properties of the organism isolated by Friedländer were unique among those of the twenty cases of fatal pneumonia studied by Gram, neither he nor Friedländer gave any sign of having attached any importance to this observation at the time when Gram’s paper appeared. Nor, for that matter, did any of the other workers in the field. The potential value of Gram’s technique to bacteriology as well as to pathology was to remain unperceived for at least another year.

In the meantime, additional publications relating to the etiology of pneumonia appeared. Of these, perhaps the most extensive in its scope was that of Weichselbaum (18), who described in 1886 his findings in 120 cases of fatal pneumonia of which 102 were “primary” in character. On the basis of cultural characteristics and of pathogenic properties, he differentiated clearly pneumococcus, to which he gave the name Diplococcus pneumoniae, from Friedländer’s bacillus, which he designated Bacillus pneumoniae. He recognized that both organisms were capsulated and that the latter could give rise to morphologic variants which resembled cocci. In 94 of the 129 cases studied, Weichselbaum demonstrated pneumococcus microscopically, and he isolated the organism on 54 occasions. Bacillus pneumoniae was demonstrated alone or in association with other organisms in 9 instances and was cultivated from 6 of these, from one case together with pneumococcus.

On November 1 of the same year, the following remarks of Friedländer (7) appeared. “Weichselbaum found it (Diplococcus pneumoniae) 54 times in the pneumatic lung fluid of 83 cases in which cultural techniques were employed; i.e., approximately two thirds of the cases; he holds this coccus identical with the organism found by Fraenkel in seven cases of pneumonia, in lung fluid and in pleural exudate, the description of which appeared several months earlier. From the microscopic state of affairs it is to be added that the coccus is intensely stained by the Gram procedure whereas the organism studied by me is decolorized by the Gram method. . . . From the foregoing investigations it emerges, therefore, that the most frequent organism in pneumonia is a capsulated coccus which was first found by me by microscopic examination (with the attribute of the capsule and the reaction toward the Gram stain), the cultural properties of which meanwhile were established first by Fraenkel and by Weichselbaum. The capsulated bacterium cultivated by me (bacillus of the authors) occurs only in a minority of cases; that it has in fact caused pneumonia in these cases emerges with greater certainty from its presence alone in the lungs and especially from the results of experiments. For it is still, until now, the only known microorganism which evoke, through inhalation, in animals an affection analogous to human lobar pneumonia. The diplococcus studied by the other authors is apparently, for a great number, perhaps for the majority of cases, to be viewed as pathogenic. . . . That an apparently so typical affection as acute pneumonia should be produced
by different causes is, as I have already remarked several years ago, to be considered analogous to the causes of acute suppuration. On the other hand, that the same schizomyecete should cause two so different affections as rhinoscleroma and pneumonia is likewise most noteworthy."

In this fashion did Friedländer acknowledge the views now held regarding the etiology of pneumonia and at the same time reveal his recognition of the apparent value of the Gram stain. His patience with Fraenkel, however, had begun to wear a little thin for he added in a footnote: "Of the manifold personal attacks and remonstrances which Fraenkel has directed against me in different places of his work, let them cease. I do not hold them fitting."

It is not altogether clear who was the first to suggest the value of Gram's method of staining in the identification of bacteria. It may have been Ferdinand Hueppe (11) whose book entitled "The Methods of Investigation of Bacteria" was published in 1885. In his discussion of Gram's method he wrote, "A provisional differential diagnosis by means of the microscope becomes possible thereby, for with the Gram method these capsulated cocci2 are decolorized whereas the remaining cocci (all?) retain the dye." Similar use of the Gram stain is implied in the text of Huber and Becker (10) published in 1886. Fraenkel was aware of both these texts, as well as of the work of Weichselbaum, when his second paper on the subject of pneumonia that year appeared (4). He is still berating Friedländer: "Worthy of further mention is the status of microscopic sections of the organs of the different animals with regard to the handling of them by the Gram method of staining. While genuine pneumonia micrococci in sections retain tightly the stain in this procedure, Friedländer's bacillus under identical conditions; i.e., iodine impregnation followed by treatment with alcohol, will be decolorized. In the same way, the rod-shaped organism isolated from the lungs by me behaved." In a footnote he adds: "Herr Weigert had the friendliness to bring first to my attention this peculiar property of Friedländer's bacillus in relation to the Gram method of staining. Thus Gram himself alleged in the original work by him on the method of staining discovered by him that the cocci (one must really call them bacilli) from the case of pneumonia from which a great part of the cultures of Dr. Friedländer stemmed decolorized very easily in alcohol and in fact, with or without treatment with iodine (see Fortschritte der Medicin, 1884, No. 6 S. 189). Hueppe (Die Methoden der Bakterienforschung, Wiesbaden 1885, 1. Aufl. S. 66) says directly that a provisional differential diagnosis of Friedländer's organism from other coccal-like forms becomes possible thereby, that the capsulated cocci stained by the Gram method decolorize and, likewise, in accord with this is the statement in the just published "pathologisch-histologischen und bakteriologischen Untersuchungsmethoden" by Huber and Becker (Leipsig 1886), conf. daselbst S. 89. Herefrom is also to be drawn the conclusion that the Gram method of staining affords us in fact with the potentiality of deciding very directly whether or not in a concrete case of genuine fibrinous pneumonia the organism of Friedländer is present in the exudate."

By 1886, it had been recognized that the typhoid bacillus, cholaer vibrio and gonococcus were decolorized by the staining technique of Gram. Of the latter Roux (14) wrote in 1886: "The procedure of Gram employed for the recognition of microorganisms gives no result whatever in gonorrhoeal pus if the gonococcus is present there alone. One can always recognize their true nature in doubtful cases, however, after having established their presence by staining with gentian violet used alone, by adding successively the liquid of Gram and alcohol. If there is complete disappearance of the cocci, they are indeed those of Neisser; on the other hand, if they retain their violet coloration, one should have doubts as to the gonorrheal nature of the affection and look elsewhere for its true nature."

I am unable to ascertain with certainty when a description of the Gram stain first found its way into a text of bacteriology with intent that it be used for the purpose for which it is employed today. In 1888, Unna (17) published a series of articles on the development of bacterial staining and devoted much space to several aspects of Gram's method. It was recognized at that time that the cocci of pneumonia, pyemia, osteomyelitis, several types of suppuration, and erysipelas retained the aniline dye whereas the organisms of typhoid, glanders, cholera, and relapsing fever failed to do so. In Thoinot and Masselin's Précis de Microbie (16) published in Paris in 1889, the following statement appears: "The method of

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2 He is referring to Friedländer's organism.
Gram should always be tried with each given microbe for it furnishes an important diagnostic element according to whether the microbe takes the Gram or not" (italics are the authors’).

Gram (9), himself, published only one short note on his method of staining after his initial description of it. It appeared in the proceedings of the 8th Session of the “Congrès Périodique International des Sciences Médicales,” which took place in Copenhagen from August 10 to 16, 1884, and contains nothing of additional interest. His subsequent work, until his death in 1935, dealt with other topics.

REFERENCES

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