BACILLUS CAPSULATUS (BACILLUS PNEUMONIÆ OF FRIEDLAENDER?) WITH ESPECIAL REFERENCE TO ITS CONNECTION WITH ACUTE LOBAR PNEUMONIA.

A Report of Twelve Cases in which Bacillus Capsulatus Occurred in the Medical and Surgical Wards of the Boston City Hospital.

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Method. The method of investigation will be further described with the reports of cases. Löffler's blood serum slants were used as a routine procedure. Descriptions of growth, appearances of colonies, etc., refer to this medium unless otherwise mentioned. In addition to the blood serum the various solid and fluid media were used, as well as animal inoculations and various special staining methods.

REPORTS OF CASES.

The twelve cases in which the Bacillus capsulatus occurred were as follows: (These cases, with the one reported by Wright and Mallory (1) in 1895, represent all the cases in which the capsulated bacillus was observed in the Boston City Hospital during the past three years.)

Case I. Acute lobar pneumonia. The patient entered the hospital March 15, 1896, in the service of Dr. V. Y. Bowditch, with considerable dyspnœa and cyanosis and with signs of consolidation in the middle lobe of the right lung. Death occurred within 24 hours after admission.

Autopsy (Dr. Mallory), March 16. *Anatomical diagnosis: Acute fibrinous pneumonia of middle lobe of right lung, with extension into upper and lower lobes. Cloudy swelling of liver, kidneys and heart, and acute splenic tumor.*

Coverslips from consolidated lung at autopsy showed numerous large and small bacilli with rounded ends surrounded by a wide capsule, also many small lanceolate, encapsulated diplococci. The bacilli were completely decolorized by Gram, whereas the lanceolate cocci retained the stain.

Cultures from the hepatized right lung showed a profuse, confluent, colorless, stringy, mucoid growth with a few small, slightly opaque, pinhead colonies at the top of the culture medium. The profuse growth contained mostly large bacilli with rounded ends, surrounded by wide capsules containing from one to four rods, decolorizing slowly by Gram. The small colonies consisted of irregular groups of small cocci. The water of condensation contained both the large capsulated bacilli and many lanceolate diplococci.

From the liver there grew a number of elevated viscid colonies, varying in size from a pin's head to 5 mm. in diameter. These showed the same capsulated bacillus as in the lung.

The culture from the kidney contained 8 to 10 colonies similar to those from the liver. Cultures from the heart and spleen were negative.

Microscopical sections of the middle lobe showed distended alveoli filled with fibrin, red corpuscles, leucocytes and a moderate number of desquamated cells. By the Gram-Weigert stain many lancet-shaped diplococci were found in the alveolar and pleural exudates. In sections stained by Unna's alkaline methylene-blue were seen a few large and medium-sized bacilli in the alveolar exudate, and large numbers in the bronchi. In sections stained by the Gram-Weigert method no bacilli were observed.

The capsulated bacillus was completely decolorized in smears from the lung, in preparations from cultures, and in sections stained by the Gram-Weigert method, the decolorization in smears being considerably slower than in preparations from cultures.

Case II. Gangrene of the lung. Patient aged 68 years, a roofer by trade, was brought to the hospital in a moribund condition. From patient's brother it was learned that he had been sick for four weeks; there was considerable expectoration emitting a foul odor, and of a purulent character, sometimes containing blood. Physical examination showed evidences of a large cavity in an area about 2½ inches in diameter just below the right clavicle. Patient died on the day following admission.

Autopsy by Dr. Mallory. Anatomical diagnosis: *General arteriosclerosis; gangrene of upper lobe of right lung, and acute vegetative endocarditis.*

Upon the mitral valve were fresh vegetations. In the right lung was a large cavity occupying almost the entire upper lobe, filled with a foul-smelling fluid, which contained small pieces of detached gangrenous lung. Other parts of this lung and the entire left lung were normal.

Coverslips from material in the pulmonary cavity showed many large and small capsulated bacilli, some cocci and a few slightly staining rods. Coverslips from the mitral vegetations showed a few medium-sized capsulated bacilli which decolorized by Gram's method.

Cultures from the upper lobe of the right lung presented a diffuse, translucent, stringy, mucoid growth of numerous small and large bacilli, some faintly staining rods without capsules, and a small number of cocci.

From the heart grew 10 to 12 viscid colonies, varying in size from a pin's head to 4 mm. in diameter, similar to those described in Case I.

The tube from the spleen showed 5 colonies, similar to those from the heart, and containing the same capsulated bacillus. Cultures from the liver and kidneys were sterile.

All the large colonies were composed of the capsulated bacillus. A guinea-pig, weighing 410 grms., inoculated in the peritoneal cavity with a pure culture on blood serum, died in 17 hours with general peritonitis and marked congestion of the liver and spleen. Cultures from the animal gave a profuse growth of the same organism as that from Case I.

Case III. Acute croupous pneumonia complicated with acute otitis media. The Bacillus capsulatus and the diphtheria bacillus were found in cultures made from the middle ear. The Micrococcus lanceolatus was found in the lung, heart's blood and kidney, but not in the middle ear in which only the capsulated bacillus and the diphtheria bacillus were demonstrated. There was no extension of inflammation from the ear into the mastoid sinuses or the cranial cavity.

Case IV. Fracture of base of skull accompanied by acute otitis media. The capsulated bacillus was found in pure culture in the middle ear, from which there was no extension of inflammation.

Case V. Diphtheria. In a young man, about 21, convalescing from diphtheria, the capsulated bacillus was found, with the diphtheria bacillus, in cultures from the throat. The capsulated bacillus persisted in daily cultures from the throat, until the patient was discharged nearly five weeks later. Diphtheria bacilli were present in small numbers for four weeks. Pure cultures of the diphtheria bacillus, isolated during four weeks, failed to kill guinea-pigs, even when injected in large quantities.

Case VI. After diphtheria. Culture from the throat showed diffuse growth of capsulated bacilli with a few streptococci, but no diphtheria bacilli.

Case VII. Diphtheria. Capsulated bacilli and diphtheria bacilli isolated in pure cultures. In the nose there were no capsulated bacilli

but many diphtheria bacilli. In the culture from the throat the capsulated bacilli grew so profusely that the diphtheria colonies could not be made out. The diphtheria bacilli in small numbers were seen only after smearing from the general surface of the serum tube in the water of condensation. The growth of the capsulated bacilli disappeared in three or four days, after which the diphtheria colonies grew luxuriantly.

Case VIII. Diphtheria. A few colonies of diphtheria bacilli and many large colonies of the capsulated bacillus.

Case IX. Tonsillitis and pharyngitis. Cultures showed a profuse growth of the capsulated bacillus and in the water of condensation a moderate number of streptococci. Patient ill three days.

Case X. Diphtheria. Cultures from the throat gave a profuse growth of capsulated bacilli and a few colonies of diphtheria bacilli. The cultures were examined daily for two weeks. The capsulated bacillus persisted up to the time of discharge. The diphtheria bacillus disappeared in a little over one week. The attack was very mild. The mucous membrane of the pharynx was congested, granular, and covered with a thick, glairy, slightly opaque, sticky material, resembling a diffuse growth of the capsulated bacillus on Löffler's blood serum. This material when touched with a platinum loop would draw out in long threads.

Case XI. Tonsillitis. Cultures from the throat gave a profuse growth of capsulated bacilli with a few chains of streptococci. The symptoms cleared up in a few days.

Case XII. Diphtheria. Cultures from the throat showed many capsulated bacilli and a few diphtheria bacilli.

Three other of the throat cases were inspected clinically by the writer and the throats showed the presence of a mucoid, glairy material similar to that in Case X. Of the foregoing twelve cases, the first four were seen by the writer only at the post-mortem examination. The other cases were observed clinically as well as studied bacteriologically. The six diphtheria cases were mild. The two instances of tonsillitis presented no unusual symptoms and recovered after a few days. In these eight cases, therefore, the severity of the disease was not increased by the presence of the capsulated bacillus.

In Case I the capsulated bacillus was found plentifully in the bronchi, while but few were present in the alveolar exudate. It evidently was not concerned in the pneumonic process. An acute endo-

carditis caused by the capsulated bacillus, as in Case II, is rare. The writer has been unable to find a report of a similar case in this country. Weichselbaum (2) in 1888 reported an instance of acute endocarditis due to a similar capsulated bacillus which he called Bacillus capsulatus endocarditidis.

In Case IV, in which the capsulated bacillus was found in pure culture in the middle ear there was no extension into the mastoid cells or the cranial cavity. Reports of cases of acute otitis media due to capsulated bacilli, while not uncommon abroad, especially in Germany, are not very frequent in this country. In Case III the capsulated bacillus was found with the diphtheria bacillus, and it is a question whether the former was responsible for the inflammation, as we have observed several cases of acute otitis media in this hospital due to the Klebs-Löffler bacillus alone.

It is evident from these cases that the Bacillus capsulatus, while not common in this country, is, nevertheless, not an extremely rare organism. Only in two cases (II and IV), did it seem to have any special pathological significance. Of the other two cases in which the bacillus was found at autopsy, in Case I there was a double infection and death was due to the pneumonia which was caused by the Micrococcus lanceolatus, and in Case III, death resulted from pneumonia and general infection with the Micrococcus lanceolatus.

DESCRIPTION OF THE BACILLUS.

The following description of the capsulated bacillus obtained from the lung of Case I answers for each of the twelve cases.

The bacillus is thick, of variable size, with rounded ends, on an average from two to three times as long as broad, enclosed in an oval wide capsule, and often united in rows of two, three or four elements within a single capsule. Sometimes it grows out into rods five or six times as long as broad. It stains with the usual aniline colors, but not by Gram, by which it is slowly decolorized. The capsule is constant both in tissues and in cultures. The capsule can be stained with any of the usual dyes when dilute acetic acid is used for washing out the excess of stain. The capsule is best demonstrated by the methods described under *capsule stain* (p. 175).

The capsulated bacilli isolated from the twelve cases are essentially identical. There are slight differences only in degrees of virulence, when inoculated subcutaneously into guinea-pigs. This bacillus is practically identical with that described by Wright and Mallory (1), the main difference being that Wright and Mallory's bacillus did not kill guinea-pigs by subcutaneous inoculation, whereas subcutaneous inoculation of guinea-pigs with our bacillus was fatal in from five to seven days, and after death the bacilli were found in each case in the heart's blood and the various organs.

Colonies on blood serum after 18 hours appear as slimy drops, transparent, round, elevated, with convex surface. The size varies from a pin's head up to 5 mm. in diameter. A confluent growth covering the entire surface of the medium is often seen. The colonies are thick and stringy, and when touched with a platinum loop draw out in long threads. The water of condensation is thick and of a whitish color. On 1 per cent. glucose agar slants the growth appears as a broad viscid transparent line. The water of condensation is thickened. Stab cultures in 1 per cent. glucose agar show gas formation at the bottom of the tube, and growth along the entire line of inoculation. In gelatine stabs, growth occurs along the entire needle track. At the point of inoculation there is an elevated mound-like knob or nail-head which is like that described by Friedländer (3). Bouillon becomes cloudy after 15 hours. The bouillon is thickened and viscid at the end of 24 hours. On potato there is a profuse, glairy, colorless, viscid growth. Milk is coagulated and acidified.

The organism kills white mice and rabbits when inoculated into the ear vein. In guinea-pigs intra-peritoneal injections kill in 24 hours, subcutaneous inoculation in from 5 to 7 days. At the autopsy there are found enlargement of lymph glands, a large soft spleen, the blood somewhat thickened, but not to the degree described by Pfeiffer (4) in his experiments with the capsulated bacillus. The adrenal glands of the guinea-pigs were hæmorrhagic as in the experiments of Wright and Mallory.

The organism here described is closely related to, if not identical with, the bacillus of Friedländer (3), and its description agrees closely

with that of the capsulated bacilli described by Wright and Mallory (1), Pfeiffer (4), Fasching (5), von Dungern (6), Mori (7), Mandry (8), Abel (9), Paulsen (10), Marchand (11). Loeb (12) and others have described capsulated bacilli differing from ours only in minor details and many of them are doubtless identical. Most of the studies of this group of bacilli have been made by foreign investigators. Of the varieties which most closely resemble ours, besides that of Wright and Mallory, may be mentioned those of Friedländer, of Pfeiffer, of Fasching and of Loeb. It is also probable that many described by others are identical and represent varieties of varying virulence. They certainly are all closely related.

Several attempts have been made to classify the various capsulated bacilli but without any great success. Wilde (13) has attempted to divide them into five groups, but his classification can scarcely be recommended. Fricke (14) has made a careful comparative study of the members of this group and has collated the characters of many reported in the literature.

Capsule stain. For staining the capsules both in cultures and in cover-glass preparations made from the organs a modification of Welch's method was used.

Welch's (15) method is as follows:

1. Cover the preparation (prepared without contact with water) with glacial acetic acid for a few seconds.

2. Drain off and replace (without washing in water) with aniline-gentian-violet solution. The staining solution is to be repeatedly added to the surface of the cover-glass until all of the acid is replaced.

3. Wash in aqueous solution of sodium chloride and examine in the same. The strength of the salt solution varies in different cases from 0.5 to 2 per cent.

This method depends upon the precipitation of the mucin-like substance of which the capsule is composed by the acetic acid, the precipitated material being insoluble in a 2 per cent. or sometimes weaker solution of sodium chloride.

I have found that by this method after using the salt solution, the specimen was often covered by a granular deeply staining detritus which often made it difficult to differentiate the capsule. The following modification was used by me and found satisfactory. It gives a much clearer picture and the capsules are stained more deeply.

1. Cover the preparation with glacial acetic acid for a few seconds.
2. Wash off the acetic acid with a 1 per cent. solution of potassium hydroxide.
3. Stain with aniline-gentian-violet for one minute without previously washing off the potassium solution.
4. Wash off excess of stain quickly in water.
5. Dry thoroughly with filter paper and over low flame and mount in balsam.

If the specimen is stained too deeply it may be decolorized by washing lightly in a 0.5 per cent. solution of acetic acid. The specimen should be completely dried before mounting in balsam, otherwise the bacilli will soon decolorize. This method is also well adapted for staining the capsules of the Micrococcus lanceolatus. I have coverslips prepared in this way which have not faded after two years. It may be added that while it is very easy to stain the capsules of the bacillus, it is often very difficult to stain those of the Micrococcus lanceolatus. If the slightest amount of water touches the specimen before the acetic acid is used the capsules are not stained but appear as clear halos around the cocci.

For staining the bacilli in sections Unna's strong alkaline methylene-blue, after the manner described by Mallory and Wright (16), was used. This stain is the most satisfactory to use for bacteria which decolorize by Gram and in connection with the Gram stain. Sections of lung from Case I showed very well the comparative numbers of bacilli and cocci.

RELATION OF THE CAPSULATED BACILLUS TO ACUTE LOBAR PNEUMONIA.

The history of the discoveries concerning the presence of bacteria in croupous pneumonia from the first observations of Klebs (17) in 1875 to the decisive papers of A. Fraenkel (18) and of Weichselbaum (19) in 1886 has been fully given by Welch (15) and need not be here repeated. Fraenkel came to the conclusion that the Micrococcus lanceolatus is the sole cause of genuine acute lobar pneumonia, whereas Weichselbaum, while recognizing this organism as the principal cause, claimed that about 5.5 per cent. of the cases of typical croupous pneumonia are referable to the Bacillus pneumoniæ of Friedländer. While the majority of investigators are probably of Fraenkel's opinion, not a few, especially the writers of text-books, hold Weichselbaum's view that a small percentage of cases of acute lobar pneumonia may be caused by the Friedländer bacillus and even

by other bacteria. Finkler (20), Honl (21) and Ziegler (22) may be cited as advocates of the latter view.

It has been our experience in the Boston City Hospital to find that true acute lobar pneumonia is invariably due to the Micrococcus lanceolatus. Pearce (23) in his report of 121 cases of acute lobar pneumonia which came to autopsy in this hospital from May, 1894, to May, 1897, found the Micrococcus lanceolatus in 118 or in 97½ per cent. of the entire number. The writer (24) reported in April, 1896, before the Boston City Hospital Medical Society, his investigations of 32 consecutive cases of acute lobar pneumonia. In every one the Micrococcus lanceolatus was found. Welch (15) found it in 10 consecutive cases at the Johns Hopkins Pathological Laboratory. Like results have been reported by many other observers.

It is an easy matter to overlook the Micrococcus lanceolatus, especially in mixed infections, as is illustrated by our Case I. In this case the cultures from the consolidated lung showed apparently on first inspection only the capsulated bacillus, but examination of sections stained by Gram-Weigert and careful examination of cultures with the application of the Gram stain revealed the presence of the Micrococcus lanceolatus. Undoubtedly many of Friedländer's cases were double infections, as he himself described the micrococci in the alveolar exudate and obtained the capsulated bacillus in cultures. It is of interest that Gram (25), working under Friedländer's direction, devised his stain for the purpose of demonstrating organisms in croupous pneumonia which he at the time believed to be identical with the capsulated bacteria obtained by Friedländer in culture, but which we now know to have been the genuine lanceolate diplococcus, this very stain being one of the most valuable means of differentiating these two bacterial species from each other. The occurrence of both these organisms in the same case is not very uncommon. This was so in Friedländer's series and appears to have been true for the cases of acute lobar pneumonia reported by Weichselbaum as due to the capsulated bacillus. The capsulated bacillus grows much more rapidly and profusely than the Micrococcus lanceolatus, thereby inhibiting the growth of the latter. It is well known that it is often difficult to

cultivate the Micrococcus lanceolatus, especially from old pneumonias, and that it will grow only on certain media. Inoculation experiments are often negative, even with pure cultures of the micrococcus. We cannot, therefore, always depend on procuring in cultures the Micrococcus lanceolatus from the solidified lung, especially in older cases, even when it is present. In a large percentage of cases there is a general infection with the lanceolate coccus. We have cultivated it repeatedly from the heart's blood and various organs, even when it did not appear in cultures from the lung.

It is probably due to the careful observance of the following rules that we have found the Micrococcus lanceolatus so regularly in acute lobar pneumonia:

1. Several cultures on blood serum are taken from the solidified lung, both from the older and the fresher areas, also cultures from the heart's blood, kidneys, liver and spleen.

2. Coverslip preparations are made from various parts of the solidified lung, also from the pleural and, if present, the pericardial exudates, at least 3 or 4 preparations being made from each location, and stained for capsules, and in such Cases as No. 1 by the Gram stain.

3. Sections of lung and of other organs, hardened in Zenker's fluid and in alcohol, are stained for histological study, and for bacteriological study both by Gram-Weigert and with methylene-blue.

4. Inoculations of animals are made when the results of the bacteriological examination at the autopsy are not decisive as to the presence of the Micrococcus lanceolatus.

Unless similarly complete examinations are made the absence of the lanceolate coccus cannot be accurately determined in cases of pneumonia. The reports of those investigators who have found instances of acute croupous pneumonia, which they have attributed to the capsulated bacillus, cannot, in my opinion, be accepted, as in none of them, so far as I have been able to determine, have the foregoing requirements been rigidly carried out. We are, therefore, as the result of our investigations, of the opinion that all instances of genuine acute lobar pneumonia are caused by the Micrococcus lanceolatus.

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