THE TYPES OF PNEUMOCOCCI IN TUBERCULOUS SPUTUM.*

By HAROLD W. LYALL, Ph.D.

(From the Laboratory of the New York State Hospital for Incipient Tuberculosis, Ray Brook.)

The success of Cole[1] and his associates[2] in establishing four groups among the pneumococci affords a new basis for the study of the transmissibility and pathology of acute lobar pneumonia. The most important result of this work is, perhaps, its bearing on the epidemiology of the disease. It has been generally accepted that the pneumococci of the normal mouth may, under favorable conditions, become implanted in the lung and are there able to set up the condition known as lobar pneumonia. The work of Rosenow[3] has been considered as favoring this theory by supposing that the pneumococci of the mouth, under changed environmental conditions, undergo a mutation into a virulent type. On the other hand, the work indicating that different types of pneumococci may operate in producing pneumonia, raises the question as to whether pneumonia may not be a transmissible or epidemic disease caused by distinct, virulent types of this organism, passed from individual to individual. To prove the first theory, it must be shown either that the organism of acute pneumonia and that ordinarily present in the healthy mouth are the same, or, postulating a mutation, that the comparatively avirulent mouth strain, as a result of the changed lung condition, assumes the characteristics of one of the virulent groups associated with the acute disease.

It was with this problem in mind that the present work was undertaken. In pulmonary tuberculosis and the other lung conditions

* Received for publication, November 27, 1914.

[1] Cole, R., Pneumococcus Infection and Lobar Pneumonia, *Arch. Int. Med.*, 1914, xiv, 56.

[2] Dochez, A. R., and Gillespie, L. J., A Biologic Classification of Pneumococci by Means of Immunity Reactions, *Jour. Am. Med. Assn.*, 1913, lxi, 727.

[3] Rosenow, E. C., Transmutations within the Streptococcus-Pneumococcus Group, *Jour. Infect. Dis.*, 1914, xiv, 1.

represented in the present series of cases there is to be found, perhaps, the nearest approach to the pathological condition found in the pneumonic lung. If the changed condition of the lung tissue is to be regarded as the important factor in the transformation of the pneumococci ordinarily present in the mouth into the virulent organisms of acute pneumonia, it might be reasonably expected that the similarly changed conditions in tuberculosis would bring about the same result. If, in a study of such cases, it were to be found that the virulent groups I and II predominated, this would be strong evidence that the access to pathological lung tissue called forth a definite change in the biological characteristics of the pneumococcus.

In the present series fifty cases were studied with a view to determining the type of pneumococcus present in the sputum. These cases were, with one exception, patients at the New York State Hospital for Incipient Tuberculosis. The one exception, case 32, upon examination proved to be acute bronchial asthma. Of the remaining forty-nine, forty-three were cases of frank pulmonary tuberculosis, five were diagnosed as bronchiectasis (probably non-tuberculous), and one was a case of chronic asthma in which the diagnosis of tuberculosis had not been definitely made. The fact that all patients had received sanatorium treatment for varying lengths of time might conceivably have had some influence on the bacterial flora of the mouth or sputum.

In the selection of patients the more advanced cases of tuberculosis were purposely chosen. As judged by the Turban scale, the Gaffky count, and the general physical findings in the monthly examinations, the forty-three cases were distributed as follows: stage 1, three; stage 2, nineteen; and stage 3, twenty-one. Of these, eighteen were cavity cases, and eleven were subject to recurrent hemoptysis. Whenever possible, these latter cases were examined during the height of the hemorrhage. The five cases of bronchiectasis were of long duration, with copious expectoration, and all were probably non-tuberculous. One of the two cases of asthma was of the chronic type, complicated with some symptoms which were suspicious of tuberculosis. The other was acute bronchial asthma. Only three gave a history of a previous pneumonia, two of which were recent. It is interesting to note that from case 43, with a

history of pneumonia in January, 1914, an organism of group I was isolated.

The following routine procedure was adopted. The first sample examined was that of the unwashed sputum. This consisted of deep morning sputum, expectorated directly into a sterile Petri dish. This was carried to the laboratory and carried through as quickly as possible, as follows:

A small piece of sputum, about the size of a pea, was rubbed up in a sterile mortar with a little nutrient bouillon. 0.5 to 1 c.c. of this mixture was injected into the abdominal cavity of a white mouse weighing 15 to 20 gm. Upon the death of the animal, which usually occurred inside of forty-eight hours, an aseptic autopsy was performed and cultures were made from the peritoneal exudate and heart's blood on plain blood agar and plain beef broth (reaction +0.3 to +0.6 per cent. to phenolphthalein). In practically all cases, a pure culture of the infecting organism was obtained. In addition to the cultures, the spleens of all animals were saved and preserved by desiccation. In this way a stock culture was retained in case of an accident to the broth or agar culture. Capsule and Gram stains were done on the peritoneal exudates and heart's blood of all animals. The washed specimen was treated in the same manner, except that it was first thoroughly washed through several changes of sterile saline, according to the Kitasato method as modified by the Saranac Lake Laboratory.

All organisms, when finally isolated in pure culture, were tested for their power to ferment inulin, to coagulate litmus milk, and for their solubility in bile. If these three tests were positive, and the organism showed a capsule, agglutination tests with sera I and II were done, according to the technique used at The Rockefeller Institute Hospital. Finally, pathogenicity and protection tests were carried out with each organism. However, before this was done, the virulence of the strains was increased by mouse passage. This is necessary with the majority of sputum strains in order to insure reasonably uniform results in the protection experiments. The protection was carried out in accordance with the routine technique used at The Rockefeller Institute Hospital, and was as follows:

Dilutions, from 0.1 to 0.00001, of a twenty-four hour plain broth culture, were made, so that 0.5 c.c. of dilution contained the required amount of culture. The protective sera were also diluted so that one protective unit (0.2 c.c.) was contained in 0.5 c.c. All injections were made intraperitoneally. Three series of mice were treated as follows: series 1 received varying dilutions of the broth culture, called the pathogenicity control; series 2, the same dilutions of culture plus one unit of serum I; series 3, the same dilutions of culture plus one unit of serum II. The animals were kept under observation for a period of five days. On account of its greater delicacy, the protection test is considered more definite than the agglutination.

Table I presents the findings together with a brief description of each case.

TABLE I.

Case No.	Stage, Turban.	Gaffky sputum count.	Description of case.	Prognosis.	Pneumococcus in sputum unwashed.	Pneumococcus in sputum washed.	Virulence.	Remarks.
1	III	IV	Active lesion; recent hemorrhages	Unfavorable	0	0	.....	2 specimens examined. Patient hemorrhaging at time of collection.
2	III	IV	Progressive; toxic; hydrothorax	Died	IV	IV	0.1 D. 96 0.01 D. 96 0.001 D. 120 0.0001 S. 120 0.00001 D. 96	Patient died 1 mo. after examination.
3	III	VI	Large cavity; no hemoptysis; lesion stationary at present	No improvement to be expected	0	0	.....	2 specimens examined.
4	II	IV	Active lesion; toxic	Not good	I	I	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 48 0.00001 D. 48	.....
5	II	V	Recurrent hemoptysis; no cavity; chronic type	Unfavorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 48 0.00001 D. 48	.....
6	III	IV	Large cavity; lesion progressive	Bad	0	0	.....	2 specimens examined.
7	III	V	Small cavity (old); lesion stationary	Fair	0	0	.....	2 specimens examined.
8	II	Neg.	Arrested hemorrhage case	Favorable	0	0	.....	Friedländer's bacillus recovered.
9	....	Neg.	Bronchiectasis	Unfavorable	0	0	.....	.....
10	II	Neg.	Hemorrhage; bronchiectasis	Favorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 72 0.00001 S. 120	.....
11	III	VIII	Cavity; active	Unfavorable	0	0	.....	2 specimens examined.
12	II	Neg.	Pneumothorax; lesion quiescent	Favorable	0	0	.....	.....

D. = died; S. = survived. The figures represent the number of hours before the death of the animal.

Inulin, bile, and litmus milk were positive on all except strain 47, which was insoluble in bile.

TABLE I.—*Continued.*

Case No.	Stage, Turban.	Gaffky sputum count.	Description of case.	Prognosis.	Pneumococcus in sputum unwashed.	Pneumococcus in sputum washed.	Virulence.	Remarks.
13	....	Neg.	Bronchiectasis; history of previous pneumonia	Unfavorable	III	III	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 36 0.00001 D. 36	2 specimens examined.
14	III	III	Old cavity; no hemoptysis	Bad	0	0	.....	.....
15	....	Neg.	Bronchiectasis; lesion improving	Fair	0	0	.....	2 specimens examined.
16	III	VIII	Large cavity; progressive	Very bad	0	0	.....	.....
17	II	Neg.	Beginning tissue destruction; progressive case	Bad	0	0	.....	.....
18	III	VI	Recent hemorrhage; progressive	Very unfavorable	0	0	.....	2 specimens examined.
19	II	IV	Active lesion	Favorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 24 0.00001 D. 24	.....
20	II	VII	Active; progressive	Unfavorable	0	0	.....	3 specimens examined.
21	II	VI	Chronic	Unfavorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 72 0.00001 S. 120	.....
22	II	IV	Arrested hemorrhage case	Favorable	0	0	.....	.....
23	II	III	Recurrent hemoptysis	Fair	0	0	.....	.....
24	II	IV	Hemorrhage; active lesion	Favorable	IV	IV	0.1 D. 24 0.01 S. 120 0.001 S. 120 0.0001 S. 120 0.00001 S. 120	Patient hemorrhaging at time of examination.
25	II +	VI	Cavity; hemorrhage	Unfavorable	0	0	.....	2 specimens examined, 2 and 4 dys. after hemorrhage.
26	II	IV	Miliary deposits; hemorrhage	Favorable	0	0	.....	2 specimens examined, 1 and 3 dys. after slight hemorrhage.

TABLE I.—*Continued.*

Case No.	Stage, Turban.	Gaffky sputum count.	Description of case.	Prognosis.	Pneumococcus in sputum unwashed.	Pneumococcus in sputum washed.	Virulence.	Remarks.
27	II	VIII	Active lesion; hemorrhage; pneumonia 18 yrs. ago.	Favorable	0	0	.....	.....
28	I	VI	Active lesion	Favorable	0	0	.....	.....
29	III	IV	Lesion stationary; occasional hemorrhage	Unfavorable	0	0	.....	.....
30	III	IX	Cavity; progressive	Unfavorable	IV	IV	0.1 D. 24 0.01 D. 48 0.001 D. 48 0.0001 D. 48 0.00001 D. 72	.....
31	III	IV	Cavity; progressive	Unfavorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 48	.....
32	....	Neg.	Bronchial asthma; non-tuberculous	Improvement marked	III	Not done	0.1 D. 18 0.01 D. 18 0.001 D. 18 0.0001 D. 36 0.00001 D. 36	.....
33	III	III	Base cavity; progressive	Unfavorable	0	0	.....	.....
34	III	III	Progressive	Very bad	0	0	.....	Gaffky count has been higher.
35	III	V	Large cavity; progressive	Very bad	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 48 0.0001 S. 120	Sputum always blood-streaked.
36	III	IV	Cavity; progressive	Bad	0	0	.....	.....
37	III	IV	Cavity; progressive	Bad	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 72 0.0001 D. 96 0.00001 S. 120	Presumptive evidence of intestinal tuberculosis.
38	III	V	Large cavity; progressive	Bad	0	0	.....	.....
39	II	Neg.	Recent large hemorrhage; lesion slowly progressive	Unfavorable	0	0	.....	.....
40	III	III	Cavity; progressive	Unfavorable	0	0	.....	.....
41	III	II	Old cavity; chronic case	Unfavorable	0	0	.....	.....

TABLE I.—*Concluded.*

Case No.	Stage, Turban.	Gaffky sputum count.	Description of case.	Prognosis.	Pneumococcus in sputum unwashed.	Pneumococcus in sputum washed.	Virulence.	Remarks.
42	....	Neg.	Cavity quiescent; possibly bronchiectasis	Very favorable	IV	1st specimen III 2d specimen IV	0.1 D. 24 0.01 D. 24 0.001 D. 48	Virulence done on unwashed strain.
43	II	VI	Chronic case, 15 yrs.' duration; pneumonia, Jan., 1914	Favorable	I	I	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 24 0.00001 D. 24	On protection against serum I, all mice died on 5th dy. Agglutination typical; immediate.
44	II	III	Lesion stationary	Favorable	0	0	.....	.....
45	I	III	Occasional hemorrhage; condition good	Favorable	III	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 24 0.00001 D. 48	Virulence is recorded for unwashed strain. Virulence not done on washed strain.
46	II	V	Condition good	Good	0	0	.....	.....
47	II	III	Lesion stationary; improving	Good	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 72 0.0001 D. 48 0.00001 D. 24	This strain not soluble in bile.
48	....	Neg.	Bronchial asthma; tuberculosis questionable	Good	IV	Not done	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 24 0.00001 D. 24	Washed specimen not done. Patient had gone home.
49	III	III	Old cavity; slowly progressive	Unfavorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 24 0.00001 S. 120	.....
50	....	Neg.	Bronchiectasis	Favorable	IV	IV	0.1 D. 24 0.01 D. 24 0.001 D. 24 0.0001 D. 72 0.00001 D. 96	.....

In the fifty cases tabulated pneumococci were isolated in twenty only, or 40 per cent. With the exception of two cases in which no washed specimen was examined, there were only two differences in the findings on the washed and unwashed specimens. The percentage of positive findings is low compared with the figures given for pneumococci in normal mouths by Longcope and Fox,[4] Buerger,[5] and Wadsworth.[6] This may be due to the favorable influence of sanatorium treatment. On the other hand, the percentage figure is considerably higher than that ordinarily reported for this type of organism as a secondary invader in pulmonary tuberculosis.[7] There are two possible explanations of the higher figure in this instance; first, the application of the more exact technique, and, second, the fact that, as previously mentioned, only cases were selected for examination which showed a progressive lung condition.

Of the forty-three cases of tuberculosis, pneumococci were found in fifteen (34.9 per cent.). The positive cases included six out of the eighteen with definite cavity formation, and four out of the eleven hemorrhage cases. There seems to be no definite correlation between the type of case and the presence of pneumococci in the sputa. Positive results were obtained in three out of five cases of bronchiectasis and in both cases of asthma.

By classifying the pneumococci isolated according to groups, it will be noted that, of the twenty positive cases, group IV organisms were found in fifteen (75 per cent.), group III in three (15 per cent.), and group I in two (10 per cent.), when case 43 is included. No organisms of group II were isolated. Dochez and Avery[8] have

[4] Longcope, W. T., and Fox, W. W., A Comparative Study of Pneumococci and Streptococci from the Mouths of Healthy Individuals and from Pathological Conditions, Report of the Medical Commission for the Investigation of Acute Respiratory Diseases, Department of Health of the City of New York, *Jour. Exper. Med.*, 1905, vii, 430.

[5] Buerger, L., Studies of the Pneumococcus and Allied Organisms with Reference to Their Occurrence in the Human Mouth, Report of the Medical Commission for the Investigation of Acute Respiratory Diseases, Department of Health of the City of New York, *Jour. Exper. Med.*, 1905, vii, 497.

[6] Wadsworth, A., Experimental Studies on the Etiology of Acute Pneumonitis, *Am. Jour. Med. Sc.*, 1904, cxxvii, 851.

[7] Avery, O. T., and Lyall, H. W., Concerning Secondary Infection in Pulmonary Tuberculosis, *Jour. Med. Research*, 1913, xxviii, 111.

[8] Dochez, A. R., and Avery, O. T., Varieties of Pneumococcus and Their Relation to Lobar Pneumonia, *Jour. Exper. Med.*, 1915, xxi, 114.

shown that the organisms usually present in the mouths of normal persons belong in group IV, while pneumococci of the so called fixed types, especially those of groups I and II, occur with great infrequency, and only under such conditions as apparently to justify considering persons who harbor them as carriers of infection. The study of this small number of cases of tuberculosis indicates that the type of pneumococci present is usually that found in the normal mouth, and that the fixed types present in the majority of cases of acute lobar pneumonia occur only infrequently. Further study will be required to show whether the fixed types occur more commonly in the mouth and sputum of patients with tuberculosis than they do in the mouths of normal persons. It must be remembered that the organisms placed in group III are so classified on certain morphological and cultural characters. Whether all the organisms placed in this group are identical from the standpoint of infection and immunity is not yet certain.

It is of significance that, among the twenty organisms studied, only two belonged in groups I and II. One of the patients harboring such an organism had had an attack of pneumonia six months previously. While the evidence obtained does not exclude the possibility that transformation or mutation of type may occur in cases with pathological lung lesions, the occasional occurrence of specialized types in these cases is probably to be explained on other grounds.

The author acknowledges his appreciation of the coöperation of Dr. A. H. Garvin and the members of the Medical Staff of the New York State Hospital for Incipient Tuberculosis. He is also indebted to Mr. M. Morita for valuable assistance.