VARIETIES OF PNEUMOCOCCUS AND THEIR RELATION TO LOBAR PNEUMONIA.*

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In a previous paper Dochez and Gillespie[1] have shown that pneumococci derived from lobar pneumonia may be divided according to their immunological reactions into certain well defined groups. There developed from this study the fact that pneumococci fall into two general groups. The larger of these, consisting of about 80 per cent. of the strains encountered, can be further subdivided into smaller groups. The latter have been arbitrarily numbered groups I, II, and III, and it has been found that a single member of either group I or group II is characterized by the possession of immunity reactions identical with those of other strains of the homologous group. Group III consists of the type known as *Pneumococcus mucosus,* and the first classification of organisms into this group depended upon differences of morphology and cultural reaction. A subsequent study by Hanes[2] has shown that members of the *mucosus* group manifest cross agglutination, so that it has been possible to relate further the members of this group by means of at least one immunological test. The immunity reactions of these three groups apparently do not change on artificial cultivation or on animal passage.

The smaller of the two main groups, which has been named group IV, is peculiar in that it seems to consist of a series of independent varieties which do not cross in their immune reactions with members of groups I, II, or III, or with each other. This group represents about 20 per cent. of the strains obtained from cases of lobar pneumonia, and this frequency seems to be fairly constant from year to year.

In view of our ability to recognize by means of specific reactions

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[1] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.,* 1913, lxi, 727.

[2] Hanes, F. M., *Jour. Exper. Med.,* 1914, xix, 38.

definite types of disease-producing pneumococci, we have deemed it important, in addition to confirming the constancy of these types, to study also the character of the pneumococci present in the normal human mouth, and on the mucous membranes of patients recovered from pneumonia, and to determine whether these strains differ in any way from those encountered during disease. If the type of organism that lives as a saprophyte on normal mucous membranes could be differentiated by certain fundamental and constant reactions from the ordinary disease-producing type, a reconstruction of our ideas concerning the epidemiology of lobar pneumonia would become necessary. Owing to the apparent lack of contact infection in pneumonia, investigators have been led to assume that the disease is due in most instances to infection with the pneumococcus that has been previously harbored in the mouth of the infected individual. If the strains present in normal mouths should show constant differences from the types found during actual disease, and if the latter types should be absent from the normal mouth flora, the likelihood would become great that infection in pneumonia takes place either by contact with infected individuals or apparently healthy carriers, however occult the steps which lead to infection may be.

During the past year we have been able to make a more extended study of the serological relationships of strains of pneumococci obtained from lobar pneumonia. The methods of classification have been the same as those previously employed; namely, agglutination and specific protection of animals against infection. Up to the present time no new group relationships have been discovered, and the recurrence of the types previously described has been constant.

In table I is shown the relative frequency of occurrence of organisms of the different types during the year 1912–13.

TABLE I.

	No.	Per cent.
Group I	35	47
Group II	13	18
Group III (*mucosus*)	10	13
Group IV (heterogeneous)	16	22
Total typical	58	78
Total heterogeneous	16	22
Total	74	

Table II shows a like classification of the strains obtained during 1913–14.

TABLE II.

	No.	Per cent.
Group I	21	30
Group II	28	39
Group III (*mucosus*)	6	8
Group IV (heterogeneous)	16	23
Total typical	55	77
Total heterogeneous	16	23
Total	71	

Tables I and II show the constancy with which the four groups described occur in two successive years. The percentage incidence of infection with members of the typical groups and with strains belonging to the heterogeneous group IV has been practically the same in both years. In 1912–13 the dominant type was group I. During 1913–14 the incidence of group I has diminished somewhat and there has been a corresponding rise in the number of infections with organisms belonging to group II.

The study of the various groups has brought out the interesting fact that the four types described differ in their degree of virulence for human beings. It has been impossible for us to obtain absolute figures of the mortality due to the different groups, because most of the individuals infected with groups I and II have been treated with specific antisera. In spite of the alteration in mortality brought about by this method of treatment, it has become clear that certain of the groups are more likely to cause a fatal infection than others. In general the severest forms of pneumonia result from infection with organisms belonging to groups II and III. The average virulence of group I seems to be somewhat lower, and, though infection with this type is usually severe in character, the mortality is definitely lower than in corresponding individuals infected with organisms belonging to groups II and III. The lowest grade of virulence is manifested by organisms of the heterogeneous group IV. Although infection with this group may run a severe course, it is unusual for it to terminate fatally.

These studies demonstrate the constancy with which the groups of pneumococcus described occur in New York City, and have now

been carried on for a sufficiently long period of time to render it unlikely that new groups closely related by immunity reactions will be encountered. In addition to these observations, investigations carried on by Dr. I. C. Walker,[3] in the Peter Bent Brigham Hospital, Boston, and by Dr. Paul Lewis,[4] in the Pennsylvania Hospital, Philadelphia, have shown that in these two cities the same groups of pneumococci are concerned in the causation of lobar pneumonia. Recently, by the use of immune sera prepared in this country, Professor Neufeld[5] has demonstrated the existence in Germany of groups of pneumococci having immune reactions identical with organisms belonging to our groups I and II. Previous study by Schottmüller[6] has already demonstrated the association of *Pneumococcus mucosus* with lobar pneumonia in Germany. Through the kindness of the South African Institute for Medical Research we have recently been able to test strains of pneumococcus isolated from cases of lobar pneumonia among natives in the Rand. The interesting observation has been made that even in this remote region of the world typical representatives of our groups I, II, and III are the causative agents in the production of lobar pneumonia. Lister[7] has described five groups of pneumococcus among the strains studied by him in South Africa. Three of these groups are identical with the groups met with in North America and in Germany. The other two groups, one of which appears to be dominant in South Africa, have not as yet been found in the cases of pneumonia studied by the writers. The possible significance of these two new races of pneumococcus will be discussed later in this paper.

STUDY OF STRAINS OF PNEUMOCOCCUS ISOLATED FROM THE MOUTHS OF NORMAL INDIVIDUALS.

Although there are in the literature instances of the apparent contagiousness of lobar pneumonia, it has been commonly assumed that most cases of the disease represent probable infection with a pneu-

[3] Personal communication.

[4] Personal communication.

[5] Personal communication.

[6] Schottmüller, H., *München. med. Wchnschr.*, 1903, 1, 909.

[7] Lister, F. S., *South African Institute for Medical Research* [*Publications*], Dec. 22, 1913.

mococcus dwelling during health on the buccal mucous membrane of normal human beings. Owing to a sudden accession of virulence of the pneumococcus, or unusual depression of resistance of the individual, this organism is supposed to be able to penetrate the lungs and set up disease. In view of the constant relationship of certain definite groups of pneumococci to lobar pneumonia, an opportunity has been afforded to test the validity of this assumption.

Isolation of pneumococcus from the sputum of a large proportion of healthy individuals has been fairly easy. Care was taken to avoid persons in direct contact with cases of lobar pneumonia, and the organisms were obtained at a season when the incidence of the disease is at a low ebb. Organisms which did not fulfill the requirements necessary for identification of the pneumococcus were discarded unless retained for special reasons. Owing to the large number of tests necessary for sufficient comparison of the organisms, the study was limited to fifteen different strains obtained from separate sources. One of these belonged to the group of *Streptococcus viridans* and was carried along with the rest for purposes of comparison, and in order to see if any important change in character occurred in this organism during the period of manipulation. All organisms were first tested for agglutination and protection with sera developed from immunization of animals with standard members of pneumococcus groups I and II. Subsequently rabbits were immunized with the strains from normal sputum, and cross relationships between these strains were tested. The general result of the investigation has been to demonstrate that strains of pneumococcus isolated from normal sputum do not belong to any of the fixed groups of pneumococcus, *i. e.*, groups I, II, or III, but resemble, as far as we can determine, the heterogeneous group IV, which has been previously described. Of the fifteen strains examined, in thirteen the morphology was that of a typical lance-shaped diplococcus. Of the latter, eleven strains were typical pneumococci in every way, showing the characteristic encapsulation, bile solubility, cultural and fermentative reactions, and two showed certain variations such as the absence of capsules even after repeated observation under suitable conditions and a tendency to precipitate in broth media. Both of these strains were bile-soluble; one fermented, and the other failed

to ferment inulin. Of the two strains showing morphological variations one was *Streptococcus viridans,* and the other a bile-soluble, inulin-fermenting organism which morphologically and culturally resembled *Pneumococcus mucosus.* It produced a characteristic sticky exudate in the peritoneal cavity of infected animals, but repeated attempts failed to demonstrate clearly defined capsules. The virulence of the different strains varied from a relatively high degree to practical non-pathogenicity for white mice. On the whole, the virulence of the most typical organisms was greater than that of those presenting variations. Of the fifteen strains eleven were typical pneumococci, three were closely enough related to be included in the pneumococcus group, and one was *Streptococcus viridans.* The serum reactions of all the strains were tested in the manner described in a previous paper on the serological reactions of *Pneumococcus.* A study was first made of the power of immune sera derived from stock cultures of groups I and II to agglutinate and to protect against infection with the sputum strains. In preparing the agglutination reactions serum and twenty-four hour broth cultures were mixed in equal quantities in the tubes. Readings were made after two hours at 37° C. and twenty-four hours on ice. The results of the agglutination tests are shown in table III.

TABLE III.

Agglutination of Sputum Strains with Stock Sera of Groups I and II.

Culture of pneumococcus.	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
Hours.	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24	2	24
Group I.	0	0	+	++	0	0	0	0	0	+	0	0	0	+	0	+	0	+	0	0	0	0	0	0	0	0	0	+	0	±
Group II	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	++	0	+	0	0
Normal horse serum.	0	0	0	+	0	0	0	0	0	+	0	0	0	±	0	±	0	0	0	0	0	0	0	0	0	0	0	+	0	0

Of the fifteen strains presented in this table, only two showed agglutination with the type sera at the end of two hours. In all, eight strains showed variable degrees of agglutination at the end of twenty-four hours. Of these eight strains, five agglutinated in more than one of the sera employed in the tests, and in most instances the heterologous agglutination occurred in normal horse serum, thus showing a tendency of the sputum strains to undergo

spontaneous agglutination. Such a variation practically never occurs in strains of pneumococcus isolated from lobar pneumonia, all of which either show specific agglutination or, when this does not occur, remain uniformly suspended for twenty-four hours. Of the fifteen cultures tested, No. 13 was the only one that showed a characteristic specific agglutination in either of the type sera.

In the original study which led to a serological classification of the disease-producing pneumococci, protection of animals against infection was adopted as probably the most specific of the immunological tests which could be applied, and it was only later, when agglutination was found to correspond very closely in specificity with the protection tests, that it was also accepted as a satisfactory method of differentiation. In table IV are presented the results obtained by testing the protective power of type sera against sputum pneumococci. White mice were given intraperitoneally varying doses of pneumococci, and at the same time a fixed quantity of immune serum. Animals surviving for five days were considered effectively protected. All animals except the virulence controls received 0.2 of a cubic centimeter of immune serum intraperitoneally. Serum of type I, in quantities of 0.2 of a cubic centimeter, uniformly protects against 0.1 of a cubic centimeter of a broth culture of the homologous organism, which kills mice regularly in doses of 0.000001 of a cubic centimeter. The same amount of serum of type II protects against 0.01 of a cubic centimeter of the homologous organism of similar virulence. The specimen of type II serum used in the following tests had a somewhat lower protective value than usual, but was sufficiently high to show dependable differences.

The results of the protective tests with thirteen of the fifteen strains of cocci studied are presented in table IV. The two organisms not tested were of such low virulence that it was impossible to kill mice with the moderate doses necessary for the successful carrying out of the test. In no instance has the protective value of the type sera been sufficiently high to justify placing any of the organisms studied in either of the fixed groups I or II. The irregular survivals occurring with the smallest doses of culture are somewhat difficult to explain. As is observed, these survivals sometimes occur among the control animals, with one or both of the specific immune

TABLE IV.

Protective Power of Type Sera for Pneumococci from Normal Sputum.

Dose of culture.	Strains of pneumococcus.									
	1			2			3 (*Pneumococcus mucosus*).			
	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Normal horse serum.
0.1 c.c.	.....	D. 14	D. 14	.....	D. 14	D. 14	.....	D. 20	D. 20	.....
0.01 c.c.	D. 18	D. 18	D. 18	D. 14	D. 18	D. 18	D. 20	D. 20	D. 20	S.
0.001 c.c.	.....	.....	.....	.....	.....	.....	D. 20	D. 20	D.20(?)	S.
0.0001 c.c.	D. 42	D. 18	D. 28	D. 28	S.	S.	S.	D. 48	D. 20	S.
0.00001 c.c.	D. 96	D. 28	S.	D. 36	S.	S.	S.	S.	D. 84	S.

	4			6				7			
	Controls.	Serum I.	Serum II.	Normal horse serum.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Normal horse serum.
0.1 c.c.	.....	D. 20	D. 20	.....	.....	D. 14	D. 18	.....	D. 20	D. 20	.....
0.01 c.c.	D. 20	D. 20	D. 20	D. 20	D. 18	D. 14	D. 18	D. 20	D. 20	D. 20	D. 20
0.001 c.c.	D. 20	D. 20	D. 20	D. 20	.....	.....	.....	D. 20	D. 20	D. 30	D. 20
0.0001 c.c.	D. 30	D. 20	D. 20	D. 20	D. 42	D. 24	D. 36	S.	D. 42	D. 20	D. 30
0.00001 c.c.	D. 42	D. 24	D. 30	D. 42	S.	D. 36	D. 42	S.	S.	D. 24	S.

	9				10			11		
	Controls.	Serum I.	Serum II.	Normal horse serum.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.
0.1 c.c.	.....	D. 20	D. 20	.....	.....	D. 18	D. 18	.....	D. 18	D. 18
0.01 c.c.	D. 20	D. 20	D. 30	D. 20	D. 18	D. 18	D. 18	D. 18	D. 18	D. 18
0.001 c.c.	D. 20	D. 20	D. 30	D. 20	.....	.....	.....	.....	.....	.....
0.0001 c.c.	S.	D. 36	D. 20	D. 30	D. 28	D. 18	D. 120	D. 72	D. 20	D. 20
0.00001 c.c.	S.	D. 42	S.	D. 30	D. 36	D. 36	S.	D. 66	D. 28	D. 120

	12			13			
	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Normal horse serum.
0.1 c.c.	.....	D. 18	D. 18	.....	D. 20	D. 20	.....
0.01 c.c.	D. 18	D. 18	D. 18	D. 20	D. 20	D. 20	D. 20
0.001 c.c.	.....	.....	.....	D. 20	D. 20	D. 20	D. 20
0.0001 c.c.	D. 18	S.	D. 18	D. 20	D. 3(?)	D. 24	D. 25
0.00001 c.c.	D. 120	S.	S.	D. 72	D. 34	S.	D. 20

	14				15			
	Controls.	Serum I.	Serum II.	Normal horse serum.	Controls.	Serum I.	Serum II.	Normal horse serum.
0.1 c.c.	.....	S.	D. 20	.....	.....	D. 20	D. 20	.....
0.01 c.c.	S.	S.	S.	D. 20	D. 20	D. 20	D. 48	D. 20
0.001 c.c.	S.	S.	S.	S.	D. 20	D. 20	D. 20	D. 20
0.0001 c.c.	S.	S.	S.	S.	D. 30	D. 34	D. 20	D. 48
0.00001 c.c.	S.	S.	D. 72	S.	D. 34	D. 34	D. 24	D. 132

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

sera, and sometimes among the animals treated with normal horse serum. The average virulence of the sputum pneumococci for white mice is generally somewhat lower than that of pneumococci obtained from cases of lobar pneumonia. Our experience has been that among pneumococci of relatively low virulence variations in the effect of serum are often observed. In some instances the administration of serum seems to increase the infective power of the pneumococcus, and in other instances there may be a variable degree of protection that is apparently non-specific in character. Such effects are not manifest when highly virulent pneumococci from lobar pneumonia are used. For purposes of comparison a small number of such tests are shown in table V.

Table III shows that pneumococci 2, 9, 13, and 15 showed the nearest approach to specific agglutination, and we should expect some evidence of protective power with the corresponding sera, if the organisms in question belong definitely to the group in the sera of which agglutination occurred. Pneumococcus 2, which agglutinates slightly in serum I and strongly in serum II, shows a slight grade of protection with both sera; pneumococcus 9, which agglutinates slightly with serum I, shows no protection in either serum; pneumococcus 13, which develops strong agglutination only in serum II, shows a minimal degree of protection with the corresponding serum; pneumococcus 15 agglutinating slightly with serum I shows no protection with either of the type sera employed. These irregular crossings are not at present explainable, but they do not seem to indicate a close degree of specific relationship, such as is observed in the highly parasitic fixed types of pneumococcus. Raising the virulence when possible by animal passage does not change these organisms into typical representatives of any of the fixed types.

TABLE V.

Agglutination of Fifteen Strains of Pneumococcus from Lobar Pneumonia with Stock Sera of Groups I and II.

Culture of pneumococcus.	Group I.					Group II.					Group IV (heterogeneous).				
	A1	67	75	78	88	85	A3	A5	A10	A69	A38	A67	A71	A75	A78
Serum I...	++	++	++	++	++	....	....	....	....	....	....	....	....	....	....
Serum II...	....	....	....	....	....	++	++	++	+	++	....	....	....	....	....

Tables V and VI are presented to show how closely related are the strains of pneumococci obtained from lobar pneumonia to other members of the homologous group.

TABLE VI.

Protective Power of Type Sera for Pneumococci from Lobar Pneumonia.

Dose of culture.	Strains of pneumococci of group I.														
	A1			67			75			78			88		
	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.
0.1 c.c.	D. 72	S.	D. 72	D. 17	S.	D. 41	.....	D.120	.....	D. 18	D. 18	D. 18	.....	D. 18	.....
0.01 c.c.	D.144	S.	D. 96	D. 17	S.	D. 25	.....	S.	.....	D. 36	D. 20	D. 18	.....	S.	.....
0.001 c.c.	S.	S.	S.	D. 41	S.	D. 41	.....	S.	.....	D. 23	S.	D. 18	.....	S.	.....
0.0001 c.c.	D. 72	S.	D. 72	D. 41	S.	D. 41	D. 19	S.	D. 19	D. 19	S.	D. 20	D. 24	S.	D. 20
0.00001 c.c.	.....	.....	.....	D. 96	S.	D. 41	D. 30	S.	D. 24	D. 24	S.	D. 18	D. 20	S.	D. 22
0.000001 c.c.	.....	.....	.....	D. 48	S.	D. 72	D. 30	S.	D. 40	D. 36	S.	D. 36	D. 40	S.	D. 40

	Strains of pneumococci of group II.														
	85			A3			A5			A10			A69		
	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.
0.1 c.c.	.....	.....	D. 18	.....	.....	.....	.....	.....	.....	.....	D. 18	D. 30	.....	D. 18	D. 18
0.01 c.c.	.....	.....	S.	.....	.....	.....	.....	D. 18	S.	D. 19	D. 48	S.	.....	D. 18	S.
0.001 c.c.	D. 18	D. 18	S.	D. 18	D. 18	D. 24	.....	D. 18	S.	D. 30	D. 36	S.	.....	D. 18	S.
0.0001 c.c.	D. 18	D. 20	S.	D. 18	D. 18	S.	D. 30	D. 36	S.	D. 36	D. 48	S.	D. 18	.....	S.
0.00001 c.c.	D. 40	D. 22	S.	D. 18	D. 18	S.	D. 26	D. 18	S.	D. 36	D. 36	S.	D. 18	.....	S.
0.000001 c.c.	D. 40	S.	S.	D. 18	D. 18	S.	D. 26	.....	S.	.....	.....	.....	D. 18	.....	S.

	Strains of pneumococci of group IV (heterogeneous).														
	A38			A67			A71			A75			A78		
	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.	Controls.	Serum I.	Serum II.
0.1 c.c.	.....	D. 18	.....	.....	D. 18	.....	.....	D. 18	.....	.....	D. 18	.....	.....	D. 18	D. 18
0.01 c.c.	.....	D. 24	.....	.....	D. 18	D. 24	.....	D. 18	D. 36	.....	D. 20	.....	.....	D. 20	D. 20
0.001 c.c.	D. 20	D. 22	D. 20	D. 22	D. 30	D. 18	D. 20	D. 18	D. 18	D. 18	D. 20	D. 18	D. 18	D. 24	D. 24
0.0001 c.c.	D. 48	D. 22	D. 48	D. 18	D. 36	D. 36	D. 25	D. 36	D. 25	D. 22	D. 21	D. 22	D. 22	D. 22	D. 22
0.00001 c.c.	D. 48	.....	D. 48	D. 36	.....	D. 36	D. 24	.....	D. 36	.....	.....	.....	.....	.....	.....
0.000001 c.c.	D. 48	.....	D. 48	D. 36	.....	.....	D. 36	.....	.....	.....	.....	.....	.....	.....	.....

Strains of pneumococcus isolated from patients with lobar pneumonia, as has been previously demonstrated, can be classified into

definite groups by means of their serological reactions. In tables V and VI are presented the agglutination and protection reactions of a small number of these strains belonging to groups I, II, and IV. The reactions of group III, the *Pneumococcus mucosus* group, are not given, because they are developed with some difficulty and have been discussed elsewhere. It is seen that groups I and II correspond exactly in their agglutination and protection reactions, and show practically no crossing. In group IV have been placed those organisms which do not react with serum I and II, and which by their growth characters are differentiated from *Pneumococcus mucosus.* Previous study has shown that individual members of this group show very little crossing in their serological reactions, and in this they resemble closely the pneumococci isolated from normal sputum. In addition, they are, as a rule, less pathogenic than members of the fixed groups.

Comparison of immunological reactions of pneumococci from normal sputum and of those from lobar pneumonia reveals certain facts which may have an important bearing on the epidemiology of this disease. In about 80 per cent. of cases of pneumonia, pneumococci are found which fall into well defined serological groups, and these organisms do not occur in normal mouths except in exceptional cases, limited, as far as our experience goes, to intimate contacts. Some evidence is therefore offered in support of the assumption that infection with one of the fixed types of pneumococcus represents contact infection, either direct or indirect, from some previous case of pneumonia, and not infection with a pneumococcus which habitually dwells upon the mucous membranes. On the other hand, about 20 per cent. of cases of pneumonia are due to heterogeneous pneumococci which cannot be differentiated from the strains occurring in normal sputum. It is possible that these infections are autogenic in character.

In order to demonstrate still further the differences between pneumococci occurring in disease and in the normal mouth, we have studied the disappearance of the fixed types from the sputum during convalescence from pneumonia, and the character of the pneumococcus which then becomes manifest. This study is being extended at the present time, and the results of only a few observations are

here presented. The pneumococci studied during recovery were obtained from the sputum and differentiated by the tests previously described. Observations made at varying intervals after the onset of the disease are presented in table VII.

TABLE VII.

Case.	Type of pneumococcus during height of disease.	Type of pneumococcus after recovery.
W.R.	Type I	60 dys. Type I.
		65 dys. Type IV.
A.W.	Type II	30 dys. Streptococcus.
		48 dys. Type IV.
		108 dys. Type IV.
A.	Type II	60 dys. Type IV.
S.H.	Type II	34 dys. Streptococcus.
		40 dys. Type IV.
K.W.	Type III	13 dys. Type III.
		73 dys. Type IV.
S.H.	Type II	47 dys. Streptococcus.
		78 dys. Type IV.
S.	Type I	59 dys. Streptococcus.
		73 dys. Streptococcus.
A.I.	Type II	53 dys. Type II.
McG.	Type I	30 dys. Type IV.
A.	Type I	90 dys. Type I.
U.	Type II	20 dys. Type IV.
		25 dys. Type IV.
B.	Type I	33 dys. Type IV.
S.	Type I	30 dys. Type IV.
M.S	Type II	30 dys. Type II.
C.	Type II	14 dys. Type IV.
B.	Type II	63 dys. Type II.
D.	Type II	21 dys. Type IV.
S.	Type II	24 dys. Type IV.
F.	Type I	12 dys. Type IV.
H.	Type II	15 dys. Streptococcus.

Study of the above table reveals the fact that only in exceptional instances does one find in the sputum, any considerable period of time after recovery, the type of pneumococcus with which the individual was infected during the disease. In four instances type organisms were still present at the time the patient was lost to observation, a period varying from thirty to ninety days after the onset of the disease. In two of these the signs of pneumonia persisted for a

long time and in one the type organism was obtained by lung puncture some three weeks after the onset of pneumonia. The pneumococcus can be isolated from the sputum of a large percentage of recovered cases, and this organism, except when the type strain has persisted, corresponds to the type of pneumococcus found in the mouths of normal individuals. The shortest period in which the type strain has been replaced by an organism of the sputum type has been twelve days after onset. Undoubtedly in a number of cases the first observations were made at too late a period to determine the exact time of disappearance of the type strain, and more carefully conducted studies are now showing that the type organisms disappear at an earlier period than table VII would lead one to suppose. The sputum type of pneumococcus obtained after recovery has been placed in what we term group IV, which consists, as has been stated previously, of a heterogeneous series of independent varieties according to our methods of classification. A systematic search for new fixed groups among these varieties is being carried on, but up to the present time no new group relationships have been discovered. From this study it is seen that the type organisms which are readily distinguishable during pneumonia are, in general, fairly rapidly supplanted by a pneumococcus which, as far as we can determine, corresponds to the type found in the sputum of normal individuals.

DISCUSSION.

As a result of the work described in this paper certain questions in regard to the etiology and epidemiology of lobar pneumonia present themselves for discussion. In order to produce disease, pathogenic microörganisms must gain entrance to the body through one of its exposed surfaces. Probably the commonest portal of entry is one of the mucous surfaces. It is well known that the mucous membranes of the body are constantly inhabited by a large variety of bacteria and that some of these microörganisms are closely related to certain strictly pathogenic types. Examples of such a condition are the Gram-negative, non-pathogenic diplococci, and the pathogenic and strictly parasitic gonococcus and meningococcus, also various bacilli of the gastro-intestinal group, and the typhoid bacillus. As a rule, the pathogenic members of these groups are readily dis-

tinguishable from their non-pathogenic relatives by a variety of simple reactions. Evidence is, however, gradually accumulating that the same condition of affairs exists among groups of organisms in which it is not so easy to differentiate the pathogenic from the non-pathogenic forms. It would seem probable that to this latter class belong the pneumococci, and that in this group we have strictly disease-producing types, and others which exist as more or less harmless saprophytes, which cannot be distinguished from the pathogenic members of the group, by the ordinary simple bacteriological methods. The studies reported in this paper show that about 80 per cent. of the pneumococci obtained from cases of lobar pneumonia belong to types which occur only in association with disease, and are not discoverable in the sputum of normal individuals. These seem to form the strictly parasitic types and are comparable to the true pathogens of certain other bacterial groups. In addition to these there are a smaller number of organisms associated with pneumonia which cannot be distinguished from the type of pneumococcus in normal sputum. Their lower virulence and general characters seem to indicate that they may be representatives of the type found in normal sputum. The more highly pathogenic forms are never found in normal sputum except in the case of intimate contacts. Wherever such contacts have been observed the type of pneumococcus in the contact has always corresponded exactly with that of the infected individual with whom he has been associated. These type organisms disappear from the mouths of contacts in the same manner as the same types disappear from the sputum of patients recovering from pneumonia.

It might be urged that the differences which we have demonstrated between various races of pneumococci are transient in character; that change from one type into another occurs with variation in environment. It is not possible at the present time to bring positive proof that such a phenomenon does not take place. Lyall[8] has recently isolated the pneumococci present in the deep sputum of a series of cases of pulmonary tuberculosis. In most instances these resembled the pneumococcus found in normal sputum. If change from the sputum type into the pathogenic types occurs with any

[8] Lyall, H. W., *Jour. Exper. Med.*, 1915, xxi, 146.

degree of readiness, one would expect to observe such changes under the conditions of this study, since in pulmonary tuberculosis the pneumococcus lives in the lung under conditions closely resembling those of lobar pneumonia. As our experience increases, we are more and more impressed with the constancy with which the varieties of pneumococcus retain their differential characters. No amount of animal passage, growth under a great variety of artificial conditions, or storage in a dried state has as yet caused the change of one type of pneumococcus into that of another, or the loss of the special characters by means of which it was originally classified. That the pneumococcus in its natural environment has reached a certain stage of equilibrium is fairly certain. In our experience we always obtain the same type of pneumococcus from normal sputum, and the same variety of groups from cases of lobar pneumonia. The pathogenic groups are now known to occur over widely separated areas of the globe. The status of bacterial mutation is so unsettled that in the absence of positive examples of permanent variations it has not seemed useful to us at the present time to make a study of the possibility of such changes among the pneumococci.

In view of the differences which seem to exist between pneumococci found in normal mouths and the dominant types occurring in lobar pneumonia, there is some interest in seeking an explanation of how they may be related to each other, and how the parasitic type has arisen from the more commonly saprophytic varieties. One might assume that today certain strains of pneumococcus are changing from more or less harmless organisms of the mouth to the disease-producing type found in pneumonia. The fact that a certain percentage of the organisms found in disease correspond more or less closely to those living in the normal mouth is some evidence in favor of this assumption. However, the majority of pneumococci which produce disease differ sharply from the type of organism found in normal sputum, and no new groups consisting of a large number of closely related strains have been found. If, then, the sputum type of pneumococcus rises to a high plane of parasitism, which enables it to produce a typical lobar pneumonia, it would seem to lose this quality after a single instance of infection, and not be able to perpetuate itself as a permanently pathogenic form and thus give rise to a number of instances of the disease.

We have recently received some strains of pneumococcus from South Africa, which seem to throw light on the manner in which new typical groups of pathogenic pneumococci arise, and the conditions that are necessary for these organisms to maintain their newly acquired disease-producing qualities. For a number of years pneumonia has been epidemic among the native mine laborers on the Rand. These men come for the most part from the more tropical regions of Africa where pneumonia is practically unknown, and they may be assumed to have a high degree of susceptibility to pneumococcus infection. The studies of Sir Almroth Wright[9] lend support to this assumption. As soon as the natives are brought to the Rand and come in contact with whites among whom pneumonia is fairly common, a high rate of incidence of pneumonia immediately develops among the blacks. Lister[10] has studied the strains of pneumococci here concerned and finds that they fall into five different groups showing specific immunological reactions. He has sent us five typical representations of these groups for comparison with the groups of pneumococci occurring in this country. Three of the strains give the characteristic reactions of members of the three typical groups found in New York. Two of the strains which belong to the dominant groups in South Africa have not as yet been met with here, and in view of the large number of organisms that have been examined by us, and from Neufeld's experience in Germany, it seems that they must be peculiar to South Africa. In other words, the condition in the Rand has been favorable to the origin of new fixed groups of pneumococcus. The most likely explanation of this phenomenon seems to be that in South Africa, among the whites, as in this country, there are a number of instances of pneumonia due to organisms resembling the sputum pneumococcus, the slightly virulent group IV of our classification. When such a pneumococcus is communicated to the susceptible black its pathogenic history, in spite of its relatively low virulence, does not stop with the production of a single instance of pneumonia, as it seems to among individuals whose racial immunity is relatively high, but the organism is readily passed on to other susceptible blacks, and

[9] Wright, A., *Lancet*, 1914, ii, 1, 87.
[10] Lister, F. S., *loc. cit.*

thus establishes itself in the less immune race as a permanently pathogenic type. If such is in reality the succession of events, then it is probable that permanent parasites arise only under conditions of high racial susceptibility, and that as the immunity of the race develops the ground becomes unfavorable for the occurrence of such a phenomenon, and no more new fixed races appear. Under the latter conditions the organisms of low pathogenicity may produce isolated instances of disease among especially susceptible individuals, but because of their low virulence and the increased racial immunity they can no longer establish themselves as permanently parasitic types. Where relative immunes are brought into contact with non-immunes, the condition, as far as the origin of new disease-producing races is concerned, is much the same as when the whole race is highly susceptible. It is possible that in South Africa the new races arising among the natives may after a number of generations gain sufficient virulence to become highly infectious for the relatively immune white.

SUMMARY.

A study of pneumococci isolated from individuals suffering from lobar pneumonia has shown that the majority of these organisms fall into definite biological groups. These groups have been arbitrarily numbered from I to IV. The first three groups consist of organisms which within the group are closely related to each other by certain immunological reactions; *i. e.*, protection and agglutination. Extensive study has failed to reveal crossing in either of these reactions between members of separate groups. The fourth group is formed of a series of independent varieties which cannot be definitely related to one another by the immune reactions employed. Up to the present time we have observed no tendency of these organisms to lose their specific characters, nor have we observed a change of one type into another. These groups vary in their pathogenicity for human beings, and in the order of their virulence are as follows: group III, group II, group I, group IV. The degree of protective power developed in the sera of animals immunized against members of the different groups varies inversely with the virulence and with the amount of capsular development. This, however, applies only

to tests of passive immunity. The highly virulent groups give as good active immunity as those of lower virulence, if not better.

In view of these constant differential characters of the pneumococcus, it was deemed advisable to study the pneumococci occurring in normal sputum. It has been commonly assumed that infection in pneumonia is autogenic, and occurs from the invasion of the lungs by a pneumococcus habitually carried in the mouth. If this is so, we should find the same types in the normal mouth as occur during the disease. Examination of a series of normal individuals showed this not to be the case. In no instance was an organism found which could be grouped with any of the fixed types of pneumococcus. All exhibited the same characters as those organisms obtained from lobar pneumonia which belong to group IV. Inasmuch as organisms belonging to this group are of low virulence, and are responsible in our experience for only 20 per cent. of the cases of pneumonia, it is at once manifest that the majority and more virulent cases of pneumonia are due to organisms which are not found in normal mouths. To gain further evidence of this difference, a study has been made of convalescents from pneumonia who had been infected by typical organisms. During the period of recovery these typical organisms are supplanted by the type which occurs in normal mouths. The period of disappearance of the typical varieties has varied. The shortest time in which disappearance has occurred has been twelve days, and the longest period in which typical organisms have been carried has been ninety days. In the latter instance the patient was lost sight of, so that he may well have carried the virulent form for a longer period of time. In general, when typical organisms persist for a long time, there is delay in the healing of the lung lesion. If recovery is prompt, as a rule the virulent types disappear rapidly.

We have said that the virulent types do not occur in normal mouths. There are exceptions to this observation. In a number of instances organisms belonging to the typical groups have been isolated from the mouth sputum of healthy individuals. So far this has occurred only in individuals intimately in contact with cases of lobar pneumonia. Wherever typical organisms have been obtained under such circumstances, the type has always corresponded to that with which the case of pneumonia was infected. Such individuals,

therefore, become infected with virulent types of pneumococcus by contact, and may be regarded as healthy carriers of disease-producing types.

This study makes it probable that the majority of cases of pneumonia are dependent upon either direct or indirect contact with a previous case. Mere infection of the mouth by virulent types is by no means sufficient to cause the disease. In order to invade the lungs, these virulent types must find the circumstances favorable, or a suitable condition must arise during the period when they are harbored in the mouth. Comparative study of certain strains of pneumococci received from South Africa suggests that new groups of parasitic organisms develop only during the period of high racial susceptibility. A like condition of affairs is brought about when a group of hitherto unexposed individuals is brought into contact with an infectious microörganism. The development of racial immunity soon limits the number of new types which may arise.

The suggestion is made that strictly parasitic races of microorganisms are pure lines and have established themselves as parasites during a period of high racial susceptibility.