THE FURTHER SEPARATION OF TYPES AMONG THE PNEUMOCOCCI HITHERTO INCLUDED IN GROUP IV AND THE DEVELOPMENT OF THERAPEUTIC ANTISERA FOR THESE TYPES*

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An investigation of the possibility of treating cases of pneumonia with specific antisera other than Type I and Type II antisera was undertaken because clinical results indicated that pneumonia cases were benefited slightly, if at all, by treatment with heterologous antisera. For this purpose it was necessary to classify further the infecting types of pneumococci, to prepare diagnostic antisera for their identification and specific therapeutic antisera for treatment.

The results given in an earlier report (1) are as follows: 58 per cent of pneumococcus strains isolated from lobar pneumonia cases in adults which did not fall into Types I, II and III were classified in ten types which were termed Types IV to XIII. The cross-protective power of potent antisera for Types I, II and III against these types was tested by protection tests in mice and was found to be very low. Monovalent antisera of high agglutinative and high protective value were prepared for these types by injecting rabbits. Also, monovalent antisera suitable for clinical trial were prepared for five types by immunizing horses. A study of the possibility of preparing suitable polyvalent antisera was begun and there was an indication that the potency of the polyvalent antiserum for the separate types injected as compared with good monovalent antisera was lowered somewhat in proportion to the number of type strains injected.

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The results obtained and the problems encountered in the further development of the investigation with the exception of the possibility of preparing suitable polyvalent antisera are discussed in this report. The findings in regard to production of polyvalent antisera will be given in a separate report.

Further Separation of Types and Correlation of Types with Severity of Disease

As 42 per cent of the Group IV strains from the lobar pneumonia cases of adults were left unclassified and several of the strains were isolated from the blood stream or from the sputa of severe or fatal cases it seemed worth while to carry further the classification of strains. Antisera were prepared for strains isolated from the more severe cases. The strains from the first series of cases which were not used for serum production were discarded on account of the difficulty of maintaining a large number of stock cultures intact through the summer months. A classification of strains freshly isolated from cases in the season 1928 and 1929 was carried out. Strains isolated from cases of pneumonia of children were also studied. In the season 1929 and 1930 a new series of cultures was studied. In 1931 only strains which were not classifiable or gave doubtful reactions or were wanted for special work were investigated. The majority of strains as in the earlier work were isolated from cases at the Harlem and Bellevue Hospitals.[1]

In this study nineteen additional types were identified which were termed Types XIV to XXXII, continuing the numbering from the Types I, II and III of the early investigators and the types called IV to XIII by us.

These types were correlated as far as we were able with the types described by others. The similarities of the whole classification are as follows:—

Type IV—Pn. 10, Griffith (2); Group IV B, Robinson (3).
Type V—Sub. II A, Avery (4); Group IV E, Robinson (3).

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[1] We are indebted to Dr. J. G. M. Bullowa, Miss C. Wilcox and Miss E. Greenbaum at the Harlem Hospital and to Dr. R. L. Cecil, Dr. N. Plummer, Dr. A. Raia and Miss S. Schultz at the Bellevue Hospital for the case histories and the strains and wish to express our appreciation of their generous cooperation.

Type VI—Sub. II B, Avery (4).
Type VIII—Group IV A, Robinson (3); Atypical III, Sugg, Gaspari, Fleming and Neill (5).
Type XV—Pn. 98, Griffith (2).
Type XXI—Pn. 160, Griffith (2).
Type XXII—Pn. 41, Griffith (2).

The cross-agglutination reactions of representative strains of each type with antisera for each of the types were studied. The antisera of rabbits or horses or of both were used for these tests.

The cross-agglutination and cross-protection reactions of Types II and V and of Types III and VIII have been discussed in the earlier report (1).

A number of strains which were agglutinated slightly to moderately in Type VI (Sub. II B of Avery) antisera, were found to be agglutinated to titer in an antiserum prepared for one of the strains. This antiserum agglutinated Type VI cultures to a marked degree. The strains corresponding to the original Type VI strains, we have termed Type VI *a*. The strains agglutinating to titer in the new antiserum and slightly to moderately in the original Type VI antiserum we have termed Type VI *b*. At first we called the latter strains Type XXVI; but later changed the terminology; because, on account of their cross-reactions with Type VI, division of the strains into two types did not seem to be justified. After further investigation, it may be found that the differences are negligible from a practical point of view and that by the use of a dominant strain for serum production, they can be disregarded. Then the strains can be classified simply as Type VI.

Cross-protection tests were carried out with Type VI *a* and Type VI *b* antisera. Two Type VI *b* antisera, one having 200 and the other 500 units, each had 100 units protection against Type VI *a*; Type VI *a* antisera having 500 units had 200 to 500 units against Type VI *b*. On account of these strong cross-reactions it would seem to be feasible to group the strains of VI *a* and VI *b* together for serum treatment.

Also, there were cross-agglutination reactions between Types VII and XVIII, especially strong with the horse antisera and with the Type VII strains which had been selected for immunizing horses. The cross-reactions with rabbit antisera prepared for representatives

of both types were less. Cross-protection tests were carried out with horse and rabbit antisera for these types. The Type XVIII horse antiserum had 500 protection units both against a Type VII culture and against the homologous strain. One Type XVIII rabbit antiserum had 20 units of protection against VII and 50 units against Type XVIII; another had 5 units against VII and 1000 units against Type XVIII. A Type VII horse antiserum had 5 protection units against XVIII and 200 units against Type VII. Type VII antisera from rabbits had less than 1 unit of protection against Type XVIII and 200 units against Type VII. The fact that the Type VII strain used for protection tests was considerably less virulent for mice than the Type XVIII strain may account for the discrepancy in the degree of cross-agglutination and cross-protection. We have tested many strains of Type VII and Type XVIII for their cross-agglutination reactions and found them to differ considerably.

There were moderate cross-agglutination reactions between Types XV and XXX. We have not been able to carry out satisfactory cross-protection tests because of a lack of fully virulent test strains for these types.

The strains examined in this study were isolated from sputa, throat cultures, blood, spinal fluid and postmortem cultures. All the spinal fluid and blood cultures examined yielded only one serological type. A few of the mouse heart cultures were found to contain two types. The findings in such cases with the type identification made at the hospital is as follows: "Type III?" contained Types III and XVIII; "Type XIX," Types III and XIX; "Type IV," Types IV and XVIII; "unclassified," Types XIII and XVIII; "unclassified," Types IV and XXII; and "unclassified," Types XI and XIV. In only one of these cases was it clearly evident which type was the more infective, *i.e.* the case in which Types IV and XVIII were found in the culture made from the heart blood of the mouse inoculated with sputum; Type XVIII was isolated from the blood, pleural fluid and spinal fluid. It is probable that in cases in which more than one type of pneumococcus is found, examination of blood taken during convalescence for antibodies for the different types would give helpful information.

A classification of 181 strains isolated from lobar pneumonia in adults in the season 1928 and 1929 was carried out by agglutination

tests with Antisera IV to XX and of 97 strains isolated in the season of 1929 and 1930 with Antisera IV to XXIX. In the 1929–30 cases special attention was given to the presence of more than one type of pneumococcus. The separation of the strains into types, the percentage of all strains in each type and the number and percentage of deaths are given in Table I. Types IV, V, VII and VIII were most prevalent, constituting 50 per cent of the total. There was an increase in Type VI *b* cases in the late winter and spring of 1931. This occurred after we had discontinued examination of all strains of the different types and therefore we have no figures as to the relative number of cases caused by this type in that period. In this series of cases pneumococci and *B. friedlaenderi* were found in three cases, pneumococci and tubercle bacilli in one, and two types of pneumococci in eight. The organisms were found early in the disease and it is impossible to tell in the majority of the cases, from the data available, whether one or both organisms were responsible for the infections.

Sixty-eight strains isolated from lobar pneumonia in children in the season of 1928 and 1929 and sixty-eight strains isolated in the season 1929 and 1930 were studied (Table II). Types V, VI *a* and XIV were found most often and amounted to 39 per cent of the total. Two or more types were found in thirteen of the lobar pneumonia cases. Types VI *a* and XXIII occurred most often in such combinations. The rôle of the different types when more than one are present needs investigating. The case mortality of 12 per cent (19 per cent in children under 2 years and 6 per cent in the older group) was low in comparison with the adult series.

In their studies of the bacteriology of pneumonia in children, Plummer, Raia and Schultz (6), using our antisera reported that 50 per cent of the strains were classifiable in Types I to XIII; Types I and VI were found most frequently, Type I occurring in 9.5 per cent of the cases and Type VI in 12.9 per cent. In the later study (7), about 85 per cent of the strains were classifiable in Types I to XXIV and Types I, IV, VI, VII, XIV and XVIII were most prevalent. Also, in order to test the reliability of the original type determination, strains isolated from different sources in the same child, as throat (swab), chest fluid, blood, sputum, ear and organs postmortem, were examined. There were a few disagreements; but the findings, in general, confirmed the validity of the original typing.

TABLE I

Grouping of Strains of Pneumococci of So Called Group IV from Lobar Pneumonia in Adults into Specific Types
(Seasons 1928–29, 1929–30)

Types	Total No. of strains	Percentage of all strains in each type	No. of deaths	Percentage of deaths in each type	13–40 years		Over 40 years		Age unknown	
					No. of strains	No. of deaths	No. of strains	No. of deaths	No. of strains	No. of deaths
IV	38*[1, 2, 3, 4, 5]	14	12[1, 5]	32	27[1, 2, 3, 4, 5]	6[1, 5]	6	4	5	2
V	39	14	14	36	26[4]	8	13	6		
VI *a*	11	4	2	18	7	0	4	2		
VI *b*	5[6]	2	2	40	3[6]	1	2	1		
VII	36[6]	13	10	28	29[6]	5	7	5		
VIII	27[7]	10	7[7]	26	18[7]	4[7]	8	3	1	0
IX	10[8]	4	6[8]	60	7	3	3[8]	3[8]		
X	8[9]	3	2[9]	25	3	0	4[9]	2[9]	1	0
XI	6	2	2	33	2	0	2	0	2	2
XII	8[3]	3	2	25	5[3]	1	1	1	2	0
XIII	10[10]	4	3	30	5	2	3	1	2[10]	0
XIV	5	2	2	40	2	0	2	1	1	1
XV	4[2]	1	1		4[2]	1	0			
XVI	2	1	1		2	1	0			
XVII	5	2	2	40	2	1	1	1	2	0
XVIII	12[5, 10]	4	6[5]	50	7[5]	4[5]	3	1	2[10]	1
XIX	6[7]	2	2[7]	33	3[7]	1[7]	3	1		
XX	7	3	2	29	5	1	0		2	1
XXI	2	1	1		1	0	1	1		

XXII	2[4]	1	0		2[4]	0	0			
XXIII	2[11]	1	1[11]		1	0	1[11]	1[11]		
XXIV	1[12]	0.5	1[12]		0		1[12]	1[12]		
XXV	3	1	1		1	0	2	1		
XXVII	0	0			0		0			
XXVIII	1	0.5	0		1	0	0			
XXIX	0	0			0		0			
Negative	28†	10	6	21	22	5	3	1	3	0
Total.....	278 (271 cases)		86	32	185 (179 cases)	42	70 (70 cases)	37	23 (22 cases)	7

* The small figures indicate cases in which two or more organisms were found which might have been the cause of the pneumonias. Cases in which two types of pneumococci were isolated are listed under both types.

[1] Type IV and *B. friedlaenderi*. [2] Types IV and XV. [3] Types IV and XII. [4] Types IV and XXII. [5] Types IV and XVIII. Type XVIII in blood, spinal fluid and postmortem lung culture. IV and XVIII in sputum. [6] Types VII and VI *b*. [7] Types VIII and XIX. Type XIX in blood and postmortem lung. [8] Types IX and III. [9] Type X and *B. friedlaenderi*. *B. friedlaenderi* in blood, X and *B. friedlaenderi* in sputum. [10] Types XIII and XVIII. [11] Type XXIII and tubercle bacillus. [12] Type XXIV and *B. friedlaenderi*. *B. friedlaenderi* in blood. XXIV and *B. friedlaenderi* in sputum.

† Twenty cultures isolated season 1928–29 were negative in Antisera I to XX. Antisera for the other types were not available. One culture was negative in Antisera I to XXIII, one negative I to XXIV, two negative I to XXVII and four negative I to XXIX. The cultures which were not completely examined were discarded or were lost before the work was completed.

TABLE II

Grouping of Strains of Pneumococci of So Called Group IV from Lobar and Bronchial Pneumonia in Children into Specific Types (Seasons 1928–29, 1929–30)

Types	Lobar pneumonia								Bronchial pneumonia			
	Total No. of strains	Percentage of all strains in each type	No. of deaths	Percentage of deaths in each type	Less than 2 yrs.		2–13 yrs.		Less than 2 yrs.		2–13 yrs.	
					No. of strains	No. of deaths	No. of strains	No. of deaths	No. of strains	No. of deaths	No. of strains	No. of deaths
IV	6	4	1	17	3	1	3	0	0		2^{14}	0
V	16	12	2	13	1	0	15	2	1	0	0	
VI *a*	$20^{*1,2,3,4,5,6}$	15	2^{6}	10	$11^{2,3,6}$	2^{6}	$9^{1,4,5}$	0	6^{15}	1^{15}	3^{14}	0
VI *b*	1	1	0		1	0	0		1	1	0	
VII	$6^{4,7}$	4	0	0	1^{7}	0	5^{4}	0	1	1	2	1
VIII	3^{5}	2	1		1	1	2^{5}	0	0		0	
IX	5^{6}	4	1^{6}	20	3^{6}	1^{6}	1	0	0		0	
X	2^{8}	1	1		1^{8}	0	1	1	0		0	
XI	5	4	0	0	1	0	4	0	0		1^{16}	0
XII	2^{9}	1	0		2^{9}	0	0		1	0	0	
XIII	1^{5}	1	0		0		1^{5}	0	1	0	0	
XIV	17^{10}	13	3	18	11	3	6^{10}	0	0		2^{16}	0
XV	7^{11}	5	0	0	1	0	6^{11}	0	1	1	0	
XVI	2	1	0		1	0	1	0	0		0	
XVII	4	3	0		1	0	3	0	0		0	
XVIII	$5^{2,12}$	4	0	0	$4^{2,12}$	0	1	0	2	1	2^{17}	1
XIX	7	5	0	0	4	0	3	0	4	1	0	
XX	3^{3}	2	1		2^{3}	1	1	0	1	0	0	
XXI	1	1	0		1	0	0		1	1	0	

XXII	2[12]	1	0		1[12]	0	1	0	0		1	0
XXIII	5[7, 9, 10, 13]	4	1	20	2[7, 9]	0	3[10, 13]	1	1	1	1	1
XXIV	1	1	0		0		1	0	0		0	
XXV	0				0		0		0		0	
XXVII	0				0		0		0		0	
XXVIII	0				0		0		0		0	
XXIX	2	1	0		1	0	1	0	0		0	
Negative	13†	10	3	23	9	3	4	0	2	1	0	
Totals.....	136 (126 cases)		15	12	63 (57 cases)	17	73 (69 cases)	4	23	9	14 (12 cases)	3

* The small figures indicate cases in which two or more pneumococcus types were found which might have been the cause of the pneumonias. Such cases are listed under each type.

[1] Types VI *a* and I. [2] Types VI *a* and XVIII. [3] Types III, VI *a* and XX. Type III in blood. [4] Types III, VI *a* and VII. [5] Types VI *a*, VIII and XIII. [6] Types VI *a* and IX. [7] Types VII and XXIII. [8] Types I, III and X. [9] Types XII and XXIII. [10] Types I, XIV and XXIII. [11] Types II and XV. [12] Types XVIII and XXII. [13] Types I and XXIII. [14] Types IV and VI *a*. [15] Types VI *a* and III. [16] Types XI and XIV. [17] Types XVIII and III.

† Eleven cultures isolated season 1928–29 were negative in Antisera I to XX. Antisera for the other types were not available.

In the course of our work, examinations were made of a few strains from normal individuals and from respiratory conditions other than pneumonia. The distribution of those which could be classified is

TABLE III

Grouping of Strains of Pneumococci of So Called Group IV from Normal Individuals, from Respiratory Cases Other than Pneumonia and from Pneumococcus Meningitis

Types	From normal individuals	From respiratory cases other than pneumonia	From pneumococcus meningitis
IV	2	1	1
V	4	1	0
VI *a*	9	4	0
VI *b*	0	1	1
VII	1	1	2
VIII	5	1	2
IX	1	1	0
X	1	0	1
XI	3	1	1
XII	0	0	2
XIII	2	2	0
XIV	0	1	1
XV	1	1	0
XVI	1	2	0
XVII	1	0	0
XVIII	2	1	4
XIX	8	4	0
XX	0	3	2
XXI	0	3	1
XXII	0	0	0
XXIII	1	3	1
XXIV	1	3	1
XXV	0	1	0
XXVII	1	2	1
XXVIII	0	0	0
XXIX	0	1	0
XXX	0	1	0
XXXI	0	1	0
XXXII	0	0	0

given in Table III. Types VI *a* and XIX were most prevalent. About 30 per cent of the strains were unclassifiable in the antisera which were available.

Webster and Hughes (8), employing antisera for Types I to XXV in a study of the incidence and spread of pneumococcus types in normal persons, found Types III, XIII and XIV most prevalent.

Plummer, Raia and Schultz (6), testing with antisera for Types I to XIII, found Type VI most prevalent and Type III next in number, in children who did not have pneumonia.

Dr. Griffith[2] in England, found representatives of the majority of the types in the healthy population of a boys' school.

Dr. Wilson G. Smillie[2] studied the incidence of pneumococcus types in St. John, Virgin Islands, and with antisera for Types I to XVIII found the majority of these types present there.

These results show that the pneumococcus types found in lobar pneumonia cases may be found in normal individuals and that the types found in New York are also present in distant localities. Types III, VI *a*, XIII, XIV and XIX, during the period of these studies, apparently were more prevalent in normal individuals in this locality.

The classification of strains from pneumococcus meningitis also is given in Table III. Fourteen of the recently separated types were found. Type XVIII, found in four cases, outnumbered the others.

Preparation of Diagnostic Antisera

Antisera for determination of types have been prepared in quantity for nearly all the representative strains by injection of horses.[3] It is our plan to have sufficient antisera available for carrying out the identification of types in hospital laboratories by ordinary routine methods. Also, we plan to preserve representative strains of all types over a considerable period of time and to furnish these strains and small amounts of homologous antisera to investigators who are studying the incidence of pneumococcus types in other localities, in order that the general distribution of the types may be determined and the classifications of other workers may be correlated with ours. As we pointed out in our earlier paper, Group IV strains isolated from various conditions had been studied by different workers; but, with few exceptions, strains of the types separated by them could not be ob-

[2] Personal communication.

[3] The immunization of horses for pneumococcus antisera was carried out by Dr. G. W. Welton and Dr. E. M. Schryver at the New York City Antitoxin Laboratories at Orisville, New York.

tained. We believe it is very desirable to preserve a set of representative type strains.

Preparation of Therapeutic Antisera

Therapeutic antisera for fourteen types have been prepared for clinical trial.

The potency of these was calculated in units per cc. on the basis that a unit is ten times the smallest amount of antiserum that protects a majority of mice against approximately 100,000 fatal doses of culture. As soon as expedient, a lot of antiserum for each type was carefully titrated and adopted as a standard. All preparations were then compared with the standard antiserum which was included as a control in each set of protection titrations. It is apparent that antisera for different types, having equal values in units as calculated by this method, are not necessarily of equal therapeutic value in human cases; because the test cultures for the types differ in their virulence for mice and the virulence of pneumococci of different types for mice does not correspond to their virulence for human beings. Estimations in units are of value because they permit comparison of the relative potency of different antisera for the same type. The comparative value of the antisera for different types for treatment of pneumonias of human beings can only be found by clinical trial.

Laboratory tests made in an earlier study (1) indicated that therapeutic antisera for Types I and II had very little value in infections caused by heterologous types. These antisera, having 1000 to 2000 protective units for the types injected, usually had less than 1 unit against heterologous types as calculated by tests in mice. The highest cross-protection was by a Type II serum against Type V, amounting to about 20 units. In our recent work, with antisera for some of the new types, we have encountered instances of greater cross-protection; for example, Types XVIII and VII discussed above. The early work with Type I indicated that therapeutic antisera for that type should have at least a certain minimum potency (0.2 cc. of antiserum should protect against 0.1 cc. of highly virulent culture), which is equivalent to about 50 to 100 units per cc. as we now calculate the strength. We have found that antisera for Type I having 1000 units or more can be obtained in certain horses after immunization for 8 months or longer. It is probable that many of the antiserum preparations which were used in the early clinical trial were several times, perhaps even ten to twenty times, the minimum strength. It is our belief that the

stronger antisera are more efficacious than the weaker product because the necessary protective units can be given in smaller volume and consequently less foreign proteins are injected. Also, in severe cases more antibodies can be given in a dose than would be possible with

TABLE IV

The Development of Protecting Antibodies in Horses in Response to Injections of Types IV, V, VI a, VII, VIII, XVIII and XXII, and Types I and II

Types	Horse	Protection in units per cc. after immunization for the following mos.														
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
IV	174*		100	100	100	100										
	297*, †	5	20	50	100	500	500	1000		100	50					
	321*		100	100	200	100	100	100	200	500	1000	1000				
V	188*		75	200	500	1000	500	500								
VI *a*	258			100	200	200	200	200	200	500	1000	1000				
VII	241*		500	500	200											
	282*	100	200	500	200											
	485	100	200	200	200											
VIII	292*	40	100	200	500											
	373	−20	100	100	200	200	100	100	100	100						
XVIII	235	20	50	200	500	200	500	500	750	500	500	500	500	500	500	
XXII	372*		200	500	500	1000	1000									
I	121*	−50	500	500	1000	1000	1000	1000	1000	1000	750	500	300	300	500	
	170*	100	200	200	500	500	100	500	500	200						
	265*	−20	100	200	500	500	500	500								
	54	20	50	200	500	1000	500	500	500	500	500	500	1000			
II	50*	50	100	100	100	100	200	200	200	500	500	500	500	200	500	1000
	99‡		50	50	50	50	75	75	75	200	200	200	200	200	500	750
	222*		50	100	100	100	200	200	500							
	223*		100	100	100	200	200	200	200	200						

* Horse died within 2 months of the last bleeding reported.

† Horse 297 was ill for several months.

‡ Horse 99 produced antisera having an average of 500 units per cc. in the period from 30 to 48 months after immunization.

low grade antiserum. At present we have selected 500 units as the minimum strength for our Type I and II preparations. We are trying to produce antisera for the other pneumococcus types which by laboratory tests will be equal to high grade Type I antiserum.

Moderate amounts of therapeutic antisera have been prepared for

Types IV, V, VI *a* and *b*, VII, VIII, IX, X, XIII, XIV, XV, XVII, XVIII, XX and XXII. These antisera were usually concentrated[4] in order that as potent products as possible could be made from the moderate number of bleedings available.

The stimulation of antibodies in horses inoculated with Types IV to XXII was similar in general to that with Types I and II as far as could be judged by agglutination, precipitation and protection tests (Table IV). For some types, antisera were as easily prepared as for Type I; for others, there was more difficulty as for Type II; but none of those studied apparently offer as great difficulty as Type III. Where we have sufficient data, the potency of the antisera produced for each type is given in the summary of results with each type.

Summary of Results with Each Type

The data collected for each type as to prevalence, severity of cases and the production of antisera during the whole period of the study are summarized below.

We have described the virulence of the strains as fully, very highly, highly, moderately, slightly virulent and non-virulent. "Fully" indicates that 1 to 5 diplococci, as determined by colony count in poured plates, killed mice in 3 days or less; "very highly" indicates that 1 to 50 diplococci killed in 4 days or less; "highly" indicates that 50 to 500 diplococci killed in 4 days or less; "moderately" that 500 to 500,000 killed in less than 4 days; "slightly" that 500,000 to 5,000,000 were required to kill.

Type IV (Pn. 10, Griffith; Group IV B, Robinson).—Fifty-seven strains from lobar pneumonia of adults were studied. Thirty-four cases from which this type was isolated were rated as severe, twenty-three as moderate or mild. Nineteen patients died, of whom nine were shown to have positive blood cultures. Three patients shown to have positive blood cultures recovered. One patient developed a Type IV meningitis. This type was found in six cases of lobar pneumonia in children. Five cases were moderate in severity. One child who was shown to have a positive blood culture died. Type IV was one of the more prevalent types in adults and less prevalent in children.

Examination of further Type IV strains supported our earlier observation that they are generally fully virulent for mice. There was no difficulty in maintaining

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[4] The concentration of the antisera was carried out under the direction of Dr. L. D. Felton at the Harvard Medical School or Dr. E. J. Banzhaf at the New York City Research Laboratory.

the virulence of the strain used in determining the potency of antisera by mouse protection tests.

Antisera for Type IV prepared in horses averaged between 100 and 200 units per cc.; after immunization for 1½ years, the antiserum of one horse had 500 units. Concentrated preparations made from early bleedings had 800 to 1200 units per cc. It is possible that the potency of these antisera, as estimated by protection tests in mice, is uniformly low because of the high virulence of the test strains for mice. The agglutination and precipitation titers, however, were not especially high.

The polysaccharides of Type IV have been studied by Heidelberger and Kendall (9). They found a type-specific carbohydrate which was different from those of Types I, II and III, a carbohydrate which was chemically similar but was inactive in specific reactions and a species-specific polysaccharide "C" substance found also in Types I, II and III by Tillett, Goebel and Avery (10).

Type V (Sub. II A, Avery; Group IV E, Robinson).—Forty-five strains from lobar pneumonia of adults were studied. Thirty-one cases were severe and fourteen were mild. Nineteen patients died, including fourteen who were shown to have positive blood cultures. Five patients from whom positive blood cultures were obtained, recovered. This type was found in sixteen cases of lobar pneumonia of children. Thirteen cases were moderate in severity; three were severe. Two children died of whom one was shown to have a positive blood culture. Type V was one of the more prevalent types in the pneumonias of both adults and children.

This type was found to be the probable causative agent of an epidemic of colds, bronchitis and pneumonia in a children's home (11).

Type V strains when grown in blood broth had a greater tendency to hemolyze blood cells than the other types studied. A special avidity for blood cells may account for the fact that there was a large percentage of positive blood cultures in Type V cases.

After immunization of horses for 8 months antisera were obtained which had 500 to 1000 units per cc. The agglutination and precipitation titers of the sera also were high. When the antiserum was concentrated ten times using methods which were successful with the antisera of the majority of the other types, the loss was great; the finished preparation having only 500 to 1000 units. The development of a method for concentrating this type antisera which will ensure a good recovery is needed.

Strains of this type were moderately virulent for mice. The maintenance of the test strain in a fully virulent condition required very frequent mouse passage.

Because of the marked cross-reactions of many Type V strains with Type II antisera and *vice versa*, it would be desirable, in order to avoid mistakes, where serum treatment is to be given to cases of either type or a careful study is to be made of their prevalence and prognosis, to titrate all strains reacting with either antiserum with antisera for both types. Perhaps it will be advisable to have the

antiserum preparations for treatment potent for both types. We are undertaking the preparation of such antiserum. It will take a long while to collect sufficient statistics to make a comparison of the relative therapeutic value of any of these new antisera and Type I antiserum. We have received favorable case reports however, that indicate Type V antiserum is probably of value.

Type VI a (*Sub. II B, Avery*).—This type was found in eighteen cases of lobar pneumonia of adults. Five were rated as severe, thirteen as mild. Three of four patients who died were shown to have positive blood cultures. This type was found in twenty cases of lobar pneumonia in children. Five cases were severe; fifteen were moderate. Two children died. Type VI *a* was frequently found also in normal individuals and in respiratory conditions other than pneumonia.

Type VI *a* strains were generally slightly to moderately virulent for mice. Frequent mouse passage was required to keep the test culture fully virulent.

The antiserum produced from a horse which was immunized during a period of nearly 2 years was comparatively low in value having only 100 to 200 units. The agglutination and precipitation titers were correspondingly low. Concentrated serum preparations were obtained which had 800 to 1000 units per cc. The immunization of another horse has been more successful, the antiserum of this horse having 500 units after immunization for 8 months and 1000 units after a year and a half. The difference in the response of these two horses to immunization with Type VI is noteworthy. We have encountered similar difference, however, in horses immunized with Types I and II. The reason is not apparent. It is not because of an inherent inability to produce antibodies, for several of the horses which have not responded well to immunization with pneumococci have produced potent antiserum when inoculated with other organisms.

Type VI b.—Data were obtained on eight cases of lobar pneumonia in adults. Five cases were rated as severe and three as moderate. Four patients died, two of whom were shown to have positive blood cultures. This type was found in the spinal fluid of a case of meningitis following a fractured skull. A case in a baby 9 months old was moderate in severity.

The strains were generally moderately virulent for mice.

On account of the strong cross-reactions of Types VI *a* and VI *b*, a Type VI *a* horse was given injections of both types. This horse whose serum had a potency of 500 units against Type VI *a* and 100 units against Type VI *b*, after 3 months immunization with both types had 500 units per cc. and after 6 months had 1000 units per cc. against each type.

Type VII.—Type VII was found in forty-seven cases of lobar pneumonia in adults. Twenty-six cases were rated as severe, nineteen as moderate. Twelve patients died of whom five were shown to have the organism in the blood stream. Two patients shown to have positive blood cultures recovered. Type VII was isolated from two spinal fluids sent to us for examination. This type was found in six lobar pneumonias of children; one case was severe, the others moderate or mild. This type was prevalent in the pneumonias of adults but rare in those of children.

Antisera having 500 to 1000 units were obtained from horses after 6 to 8 months immunization. We are not sure whether it was by chance or because of a susceptibility of horses to inoculation with this strain that there were many deaths of horses being immunized with this type. The method of concentrating antisera of this type as of Type V requires further investigation. Only 500 to 1000 units per cc. of protective antibody were recovered in preparations in which the serum was concentrated ten times.

Strains of this type usually were moderately virulent for mice. The test strain required very frequent passages to maintain full virulence.

Reports of the serum treatment of cases caused by this type have been encouraging.

Type VIII (Group IV A, Robinson; Atypical III, Sugg, Gaspari, Fleming and Neill).—This type was isolated from thirty-one cases of lobar pneumonia in adults. Twelve cases were rated as severe, sixteen as moderate; eight patients died. Five of seven patients which were shown to have positive blood cultures died. Two pneumonia cases developed meningitis and Type VIII was found in the spinal fluids. This type was found in three cases of lobar pneumonia in children; two were mild; one child who was shown to have a positive blood culture, died.

Long periods of immunization were required to obtain antiserum of suitable potency for this type. Antisera having 100 to 300 units per cc. were obtained from two horses after they had been immunized for 8 months. Concentrated antisera were prepared having 800 to 1500 units per cc.

The strains of this type generally were fully virulent for mice. No special care was necessary to maintain the virulence of the test strain.

There are marked cross-reactions between this type and Type III. Differences in colony morphology, however, clearly differentiate strains of this type from freshly isolated Type III strains. We have never found Type VIII to have the large mucoid colonies characteristic of Type III. If agglutination and precipitation reactions are chiefly relied upon for identification of Type III strains, Type VIII may be confused with it. We believe that titrations of strains reacting with either type should be made with both antisera.

Type IX.—Type IX was isolated from sixteen cases of lobar pneumonia in adults. Seven were rated as severe, nine as moderate. Seven patients died including two which were shown to have positive blood cultures. One patient shown to have a positive blood culture recovered. This type was isolated from three cases of lobar pneumonia in children; two cases were moderate in severity; one child died.

Antisera having 200 units per cc. were prepared in one horse. Concentrated antisera having 1000 units per cc. were prepared from this antisera.

Strains of this type generally were only slightly virulent for mice.

A suitable test strain was discovered only after prolonged search.

Type X.—This type was isolated from thirteen cases of lobar pneumonia of adults of which two were rated as severe, three as moderate. Six patients died,

two of these were shown to have positive blood cultures. One patient having a positive blood culture recovered. Type X was isolated from the spinal fluid of one meningitis case sent to us for examination. This type was found in two cases of lobar pneumonia in children; one case was mild and the other terminated fatally.

The strains were of low virulence for mice.

Type XI.—This type was isolated from ten adult cases, of which four were severe and six moderate. Three patients died. One case from which a positive blood culture was obtained was rated as moderate in severity. This type was isolated from five lobar pneumonia cases in children; three cases were moderate in severity; two were severe; of the latter, one terminated fatally.

Antisera were prepared having 200 to 500 units per cc.

The strains were moderately to fully virulent for mice.

Type XII.—This type was found in twelve cases of lobar pneumonia in adults; six were rated as severe and six as moderate. Four patients died; of whom three were shown to have positive blood cultures. Type XII was found in two spinal fluids sent for examination. This type was isolated from two severe cases of lobar pneumonia in children.

The strains were generally of low virulence for mice.

Type XIII.—This type was isolated from fourteen cases of lobar pneumonia of adults. Seven were rated as severe and seven as moderate. Three patients died; two of these were shown to have positive blood cultures. This type was isolated from one child having a mild lobar pneumonia.

Type XIII was found by Webster (8) to be one of the most prevalent types in the normal individuals which he studied.

The strains which we examined were slightly to moderately virulent for mice.

Type XIV.—This type which was one of the most prevalent types in the pneumonias of children was found in only five pneumonia cases of adults. Three were severe and two mild. Two patients died, of whom one was shown to have a positive blood culture. Type XIV was found in one spinal fluid sent for examination. This type was isolated from nineteen cases of lobar pneumonia in children. Seven cases were rated as severe and twelve as moderate or mild. Four children died; of whom three were shown to have positive blood cultures.

The strains of this type were moderately virulent for mice.

Type XV (Pn. 98, Griffith).—This type was found in only four cases of lobar pneumonia of adults, two were severe and two moderate. One patient who was shown to have a positive blood culture died. This type was found in seven cases of lobar pneumonia in children; six were mild and one was severe.

With one exception the few strains which we had of this type were slightly virulent for mice.

Type XVI.—This type was found very infrequently. It was found in two adult cases; one patient having a positive blood culture died; the other case was mild. It was found in two lobar pneumonias of children.

The few strains studied were slightly virulent for mice.

Type XVII.—Strains were obtained from six cases of which four were severe and two moderate. Two patients died, of whom one was shown to have a positive blood culture. This type was found in four lobar pneumonia cases in children; one was severe, the others were mild.

The strains were slightly to moderately virulent for mice.

Type XVIII.—Strains from twelve cases of lobar pneumonia in adults were studied. Ten cases were severe and two moderate. Six patients, all of whom were shown to have positive blood cultures, died. One of the above patients developed meningitis and Type XVIII was isolated from the spinal fluid. This type was isolated from four spinal fluids sent to us for diagnosis. It was found in five lobar pneumonia cases in children; one was severe; the others were moderate or mild.

Type XVIII strains were moderately to fully virulent for mice. The virulence of our test strains has been maintained without difficulty.

The one horse immunized with this type produced antiserum having 500 to 1000 units per cc. Because of the cross-reactions between Types VII and XVIII this horse was transferred to inoculations of both types and after a few months had 1000 units per cc. against Type VII and 500 units per cc. against Type XVIII. Concentrated refined antiserum having 5000 units against Type XVIII was prepared from the monovalent antiserum.

Type XIX.—Type XIX was isolated from six cases of lobar pneumonia of adults of which five were severe and one moderate. One patient shown to have the organism in the blood died. This type was isolated from seven lobar pneumonia cases in children; three cases were severe; four were moderate or mild. It was found moderately prevalent in normal individuals.

The strains were moderately virulent for mice.

Type XX.—This type was isolated from seven cases of lobar pneumonia in adults. Three were rated as severe and two as moderate. Two patients shown to have positive blood cultures recovered. This type was found in three lobar pneumonia cases in children; two were severe and one was mild. One child died.

The strains of this type differed markedly in their virulence for mice, the majority of the strains being moderately virulent.

Type XXI (Pn. 160, Griffith).—Three strains of this type from lobar pneumonia of adults were studied. Two cases were rated as severe and one as moderate. Two patients died, of these one was shown to have the organisms in the blood and spinal fluid. This type was isolated from one mild lobar pneumonia in a child.

The strains of this type were generally slightly virulent for mice.

Type XXII (Pn. 41, Griffith).—This type was isolated from two mild cases of lobar pneumonia of adults. It was found in two lobar pneumonia cases in children; one was severe and the other mild. Griffith,[2] in England, found this type in a number of normal individuals.

An antiserum having 500 units per cc. was prepared after immunization of a horse for 6 months.

Strains of this type generally were very highly virulent for mice but tended to lose their virulence rapidly.

Type XXIII.—Seven strains of this type from lobar pneumonia of adults were studied. Six of the cases were severe and one moderate. Two patients died, one of whom was shown to have positive blood culture. One patient who had a positive blood culture recovered. This type was isolated from five cases in children; two were mild and three severe; one of the latter terminated fatally. It was found also in the spinal fluid of a child who developed posttraumatic meningitis.

The strains of this type were generally slightly to moderately virulent for mice.

Type XXIV.—This type was isolated from five moderately severe lobar pneumonias of adults and from one fatal case that had *B. friedlaenderi* in the blood. It was isolated also from a spinal fluid sent to us for examination.

Griffith,[2] in England, found this type in a moderate number of normal individuals.

Strains of this type were moderately to fully virulent for mice.

Type XXV.—Five strains from the lobar pneumonia of adults were studied. Two patients died, both of whom were shown to have positive blood cultures. Three cases were rated as moderate.

Strains of this type were generally very highly virulent for mice.

Type XXVII.—This type was isolated from two moderately severe cases of lobar pneumonia in adults. It was also found in a spinal fluid sent for diagnosis and in a case of follicular tonsilitis and a case of measles.

The few strains examined were moderately to very highly virulent for mice.

Type XXVIII.—Four cases of lobar pneumonia in adults were moderate in severity.

The strains examined were very different in their virulence for mice, ranging from slightly virulent to fully virulent.

Type XXIX.—We have data on three adults, one died and two cases were severe. This type was found in three mild cases of lobar pneumonias of children.

The strains were very irregular in their virulence for mice, the majority however being highly virulent.

Type XXX.—We have data on three mild cases in children.

Three strains were examined for their virulence for mice; one was highly virulent, one moderately virulent and the other non-virulent.

Type XXXI.—We have data on two cases in adults from which this type was isolated; one had lobar pneumonia of moderate severity, the other died.

The strains examined were moderately virulent for mice.

Type XXXII.—We have not been able to get data on cases from which this type was isolated.

The strains were moderately virulent for mice.

DISCUSSION

The difference in the incidence of cases and in the relative severity of the infection caused by the different types indicates that all the types are not of equal importance, and it is questionable whether it is practical to attempt to produce therapeutic antisera for those which are less prevalent and less virulent. Although there is no immediate benefit to individual patients in determining the type where no therapeutic serum is available, it seems worth while to continue examination for all the types and maintain a full supply of diagnostic antisera until it is learned whether there is a seasonal and periodical variation in the types in this locality and what their distribution in other localities is. The statistics which have been collected by others in regard to the prevalence of Types I, II, III and Group IV indicate that such variation occurs. For example Griffith (12) found the incidence of the pneumococcus types in the Smethwick district in England as follows:—

	Type I	Type II	Type III	Group IV
Apr., 1920–Jan., 1922	30.6	32.6	6.6	30.0
Feb., 1922–Oct., 1924	42.6	21.3	3.2	32.7
Nov., 1924–Mar., 1927	34.3	2.4	4.4	53.7

In the third period there was a decrease in Type II cases and an increase in Group IV cases. An interesting study which was carried out with several antisera other than those generally used is that reported by Ordman (13) where different types of pneumococcus as well as different organisms were found predominating in the pneumonias of the Witwatersrand miners in South Africa during a period of 15 years.

An observation that some of the types, found most frequently in the pneumonias of adults, were found seldom in children and *vice versa* needs study. The theory that children become infected with the types which are most widespread in normal individuals is not supported by the facts observed; for example, although Type VI *a*, one of the types most frequently found in normal individuals, was one of the

most prevalent in the pneumonias of children, Type I, which was comparatively rare in normal individuals, also was very frequently found. The observation that the majority of the types found in normal individuals are not found in the pneumonias of adults may be explained in different ways; as by a lack of virulence or invasive power of the strains; or, that because of the wide distribution of the strains, the majority of individuals become relatively immune to them in early life. Probably different explanations will be necessary for the behavior of different types.

A further study of the invasive powers of pneumococci is needed. The epidemic, among children in an institution, of colds, bronchitis and pneumonias caused by Type V showed that this type has invasive capacity and should be classed as an infectious organism. It seems likely that under ordinary conditions, pneumococci are responsible for a considerable percentage of mild respiratory conditions and that when the resistance of individuals is especially lowered, pneumonia is more apt to occur.

Where more than one type of pneumococcus is found, their importance in the infection needs to be investigated.

The problem of the preparation of potent therapeutic antisera also needs further study. First, there is the selection of suitable strains and methods of immunization. It seems to be the general opinion that strains of the highest virulence are most suitable, an opinion which is probably based on observations that degraded strains stimulate the production of antisera which are often deficient in curative power. Contrary to this opinion we have results as to the relative ability of freshly isolated virulent Type III strains having very large capsules and of less virulent stock Type III cultures having smaller capsules to stimulate antibodies. Antisera more potent for both kinds of strains as determined by laboratory tests were produced by inoculation of the less virulent cultures. If the pneumococcus produces a toxin in significant amount in human pneumonia, then strains of high toxin-producing power and probably the subcutaneous as well as the intravenous method of inoculation should be used. However, at present, we believe convincing evidence of the existence of such toxin has not been presented.

SUMMARY

The unclassified strains known as Group IV have been separated into twenty-nine types which are designated by the Roman numerals IV and XXXII. Only a small percentage of the pneumococcus strains isolated in New York City for this study were left unclassified.

The majority of the types gave very slight cross-reactions, the exceptions being Types II and V, III and VIII, VII and XVIII and XV and XXX.

In the series of cases studied, Types IV, V, VII and VIII were found more prevalent in the lobar pneumonia of adults and Types V, VI *a* and XIV in children.

The majority of the types were also found in normal individuals and in persons having respiratory infections other than pneumonia. Types VI *a* and XIX were most prevalent in the limited number of strains studied by us.

Fourteen of the types were found in pneumococcus meningitis; Type XVIII was found most often.

Antisera suitable for clinical trial have been prepared for fourteen types. From the majority of the horses inoculated for more than a year, antisera having 500 to 1000 units per cc. were obtained. Antisera of lower potency were concentrated and preparations obtained equal to or stronger than high grade unconcentrated serum.

Potent bivalent antisera have been prepared for types which were found to give marked cross-agglutination reactions.

The results with each type as to prevalence, severity of cases, presence in normal individuals, and in spinal meningitis, potency of antisera produced for therapeutic trial and virulence of strains for mice have been considered under the different type headings.

REFERENCES

1. Cooper, G., Edwards, M., and Rosenstein, C., *J. Exp. Med.*, 1929, **49**, 461.
2. Griffith, F., *Rep. Pub. Health and Med. Subj., Ministry of Health, No. 13*, 1922, 20.
3. Robinson, G. H., *J. Infect. Dis.*, 1927, **41**, 417.
4. Avery, O. T., *J. Exp. Med.*, 1915, **22**, 804.
5. Sugg, J. V., Gaspari, E. L., Fleming, W. L., and Neill, J. M., *J. Exp. Med.*, 1928, **47**, 1917.

6. Plummer, N., Raia, A., and Schultz, S., *Am. J. Dis. Child.*, 1930, **40**, 557.
7. Raia, A., Plummer, N., and Schultz, S., *Am. J. Dis. Child.*, 1931, **42**, 57.
8. Webster, L. T., and Hughes, T. P., *J. Exp. Med.*, 1931, **53**, 535.
9. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **53**, 625.
10. Tillett, W. S., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1930, **52**, 895.
11. Schroeder, M. C., and Cooper, G., *J. Infect. Dis.*, 1930, **46**, 384.
12. Griffith, F., *J. Hyg.*, 1928, **27**, 113.
13. Ordman, D., *J. Med. Assn. South Africa*, 1931, **5**, 108.