ANTIBODY RESPONSE TO INFECTIONS WITH TYPE III AND THE RELATED TYPE VIII PNEUMOCOCCUS [1]

By MAXWELL FINLAND and ALEXANDER W. WINKLER

(From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston)

(Received for publication September 19, 1933)

Artificial immunity to the Type III pneumococcus varies with different animal species and differs from that obtainable with Types I and II (1). The antibody response, in man, to lobar pneumonia due to Type III is less constant and of lower grade than that following infection with the latter types. The Type VIII pneumococcus (2), which is immunologically related to but not identical with Type III (3), has been found frequently in association with human disease (4). In the present communication are presented the results of tests for pneumococcus antibodies in patients with infections associated with Type III and with Type VIII pneumococci.

EXPERIMENTAL

Subjects, materials and methods

The sera of 71 patients with infections associated with Type III or Type VIII pneumococci were studied. Patients with lobar pneumonia, bronchopneumonia or other infections without pneumonia were included. The pneumococcus type was usually obtained from a culture of the heart's blood of a mouse inoculated with sputum. All cultures were agglutinated both macroscopically and microscopically in Type III and Type VIII antisera, progressive dilutions of the sera being used where cross-agglutination was encountered. In many instances subcultures of colonies from the surface of blood agar plate cultures were used for typing. Blood cultures were made by inoculating, at the bedside, 5 to 10 cc. of blood into beef infusion broth at pH 7.8 and pneumococci thus obtained were similarly typed. Typing sera for Types I to XXXII (5) were obtained from the Laboratories of the New York City Department of Health through the kindness of Miss Georgia Cooper and Dr. William H. Park. Additional sera for Types I, II and III were furnished by Dr. Benjamin White of the Antitoxin and Vaccine Laboratory of the Massachusetts Department of Public Health and by Dr. Augustus B. Wadsworth of the Laboratories of the New York State Department of Health.

The materials and methods used in testing for agglutinins and mouse protective antibodies were similar to those employed in other studies (6). Further antigens were obtained from single colony cultures of strains encountered during this study. The tests for cross-agglutination of strains of pneumococci

[1] This investigation was aided, in part, by a grant given in honor of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

were made with fresh, live, fully grown (10 to 14 hours), plain broth, single-colony cultures incubated with serial dilutions of typing sera for 1 hour at 56° C. and the readings made after overnight icebox storage. Only floccular agglutinations were considered positive.

Absorption experiments were carried out with freshly prepared, heat-killed, saline suspensions of pneumococci, the packed sediment of 50 to 150 cc. of a fully grown culture being used for each cubic centimeter of serum. The mixture was incubated at 37° C. for 2 hours, with frequent shaking, then stored in the icebox overnight and the cleared supernatant used for agglutination and protection tests.

RESULTS

Agglutination of Types III and VIII strains in anti-pneumococcus horse sera

Tests for cross-agglutination were carried out with 6 Type III and 21 Type VIII strains of pneumococci recently isolated from the sputum, blood or lungs of pneumonia patients. One Type VIII and 3 Type III horse antisera from different laboratories were used. Two of the Type III antisera agglutinated homologous strains in dilutions up to 1:40 or 1:80, and the third up to 1:160 or 1:640. One of the first 2 sera failed to agglutinate 5 Type VIII strains and agglutinated the rest only when undiluted or in dilutions up to 1:4; the other agglutinated all Type VIII strains, usually in dilutions up to 1:20 or 1:40. The third Type III serum failed to agglutinate most Type VIII strains. The Type VIII antiserum agglutinated homologous strains in dilutions up to 1:80 or 1:160. This serum failed to agglutinate 4 Type III strains and agglutinated two others only in 1:2 dilutions. Microscopic agglutinations carried out in each instance with 1:5 dilution of the different antisera, showed corresponding differences in the occurrence and character of the agglutination observed.

The "typing" sera were thus found to vary considerably in the degree to which they cross-agglutinated strains of pneumococci of the related type. This was not dependent on the titers of homologous agglutinins.

Antibody response to infections associated with Types III and VIII pneumococci

The results of the agglutination and protection tests with both Types III and VIII pneumococci in the sera of patients with Type III infections are shown in Table I. Except as indicated in this table, each of these patients had lobar pneumonia clinically and by x-ray, and Type III pneumococci were obtained from the sputum on one or more occasions. The blood cultures were sterile in all the recovered patients and in one-half of the fatal patients. Similar data for the Type VIII patients are given in Table II. The sputum of each of these patients had Type VIII pneumococci on one or more examinations. The results of the blood cultures are indicated in each instance. The data in Tables I and II are summarized in Table III.

TABLE I

Antibody response to infections associated with Type III pneumococci *

Case number	Patient	Age	Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years								
1	J. H.	22	Lysis	10	8	0	0	0	0	
					22	8	0	10	0	
2	E. C.	44	Lysis	22	23	0	0	0	0	
					32	32	0	10^2	0	
3	A. S.	49	Lysis	7	3	0	0	0	0	Bronchopneumonia, bronchial asthma. Readmitted for asthmatic attack after 3 months. Agglutinins for Pn. VII (1 : 4) in last serum
					10	64	0	10^6	0	
					67	4	0	10^5	0	
					74	4	0	10^2	0	
					124	4	0	—	—	
4	M. I.	52	Crisis	8	8	0	0	0	10^2	
					19	64	0	10^6	10^4	
5	A. G.	31	Crisis	4	2	0	0	0	0	
					5	0	0	—	—	
					8	4	0–2	0	10	
					13	2	0–2	10^2	10	
					19	2	0	10^3	0	
6	D. VanF.	39	Lysis. Recrudescence	23? 26–34	25	8	8	—	—	Pn. III in sputum 22nd day, Pn. VII on 30th day. Agglutinins and protection for Pn. VII absent 31st day and present on the 40th day. (Agglutinins 1 : 4, protection 10^6)
					31	8	4–8	10^3	10	
					40	4	2	—	—	
7	T. C.	54	Lysis	9	12	4	0	10^4	0	
					33	4	0	10^4	0	
8	E. E.	26	Crisis	8	7	16	0	—	—	
					8	32	0	—	—	
					10	32	0	—	—	
9	W. L.	37	Lysis	7	10	4	0	10^4	0	Readmitted 82nd day with acute upper respiratory infection. Lungs clear. Only Pn. X and Pn. XVII recovered from sputum on repeated examination on 2nd entry. No agglutinins for the latter types in any of the sera.
					19	4	0	10^3	10	
					83	0	0	0	10^4	
					94	0	0	0	10^4	
10	P. O'B.	44	Lysis	14	14	8	0	—	—	Bronchopneumonia
					22	4	0	—	—	

TABLE I—(*continued*)

Case number	Patient	Age	Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years								
11	D. R.	18	Crisis	6	6	4	0	10^3	10	Postoperative lobar pneumonia
					11	2	0	10^2	0	
12	S. S.	72	Crisis	6	13	8	0	10^3	0	
13	F. G.	65	Crisis	5	7	8	0	10^4	0	
14	T. A.	42	Crisis	9	6	0	0	—	—	Agglutinins (1 : 8) and protection for Pn. V (10^5) in this serum
15	E. H.	42	Crisis	10	20	0	0	0	0	
16	J. S.	52	Lysis	14	12	0	4	0	10^3	Bronchopneumonia. No pneumococci recovered from sputum 12th day, Pn. III obtained on 14th day
					18	0	2–4	0	10^5	
17	M. W.	69	Lysis	10	8	0	0	0	—	
					11	0	0	0	0	
18	M. K.	41	Crisis	12	8	0	0	—	—	
					15	0	16	0	10^3	
					19	0	4	0	10^2	
19	F. L.	48	Crisis	5	5	0	0	—	—	Bronchopneumonia and pulmonary tuberculosis
					10	0	0	0	0	
					18	0	0	—	—	
					30	0	0	0	0	
20	M. D.	62	Lysis	12	6	0	0	0	0	
					11	0	0	—	—	
					18	0	0	0	0	
					24	0	0	0	0	
					31	0	0	0	0	
21	P. Ci.	36	Pseudo-crisis	11	12	0	0	0	0	Recrudescence 15th to 20th day. Pn. III from sputum 10th day. Only Pn. V in sputum 12th and 15th day. Agglutinins (to 1 : 32) and protection (to 10^4) for Pn. V
				20	17	0	0	0	0	
					24	0	0	—	—	
					31	0	0	0	0	
22	W. W.	58	Lysis	8	20	0	0	0	0	Postoperative bronchopneumonia
23	R. W.	50	Crisis	5	9	0	0	0	0	
24	J. O'B.	36	Lysis	8	8	0	0	0	0	Pn. III and Pn. VIII (no Pn. II) in sputum on 3rd day. Pn. II (no Pn. III or Pn. VIII) in sputum on 6th day. Agglutinins (to 1 : 8) and protection for Pn. II (10^4) found 10th day and later
					10	0	0	0	0	
					12	0	0	0	0	
					23	0	0	0	0	

TABLE I—(*continued*)

25	F. Co.	39	Died	9	7	0	0	—	—	Pn. III from blood culture on 7th day
					9	0	0	—	—	
26	M. L.	50	Died	10	7	0	0	0	0	Blood culture sterile 6th day, showed Pn. III on 7th day
27	D. McC.	49	Died	10	5	0	0	0	10^2	Blood culture sterile 4th and 5th days, showed Pn. III on 9th day.
					9	0	2	0	—	Agglutinins for Pn. II (1 : 2) and Pn. V (1 : 4) without protectection on 7th day, none on 9th day
28	S. T.	60	Died	9	9	16	0	10^3	0	Bronchopneumonia complicating carcinoma of lung. Blood cultures: 7th day, negative; 9th day Pn. III; at autopsy, negative
29	M. G.	57	Died	9	7	32	0	10^5	0	Blood culture sterile on 7th day, bronchopneumonia
30	S. J.	54	Died	22	19	0	0	0	0	Bronchopneumonia. Blood culture sterile on 19th day
31	H. P.	24	Lysis	3	7	4	0	10^2	0	"Grippe." No pneumonia. Pn. III and Pn. VIII in sputum
32	C. W.	33	Lysis	3	8	0	0	—	—	"Grippe." No pneumonia
					11	0	0	0	0	
33	F. Ce.	38	Lysis	7	9	0	0	10^3	0	Influenza. Lungs clear
34	M. D.	30	——	—	—	0	0	0	0	Pulmonary tuberculosis, febrile, positive sputum
35	M. H.	41	Lysis?	6	8†	0	0	0	0	Fractured ribs, bloody sputum with Pn. III, 8 days later had
					4	0	0	0	0	fever for 6 days. No evidence of pneumonia.
					11	0	0	0	0	

TABLE I—(*continued*)

Case number	Patient	Age	Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years								
36	J. McD.	24	——	—	—	0	0	—	—	Bronchiectasis, afebrile
37	R. V.	52	——	—	—	0	0	—	—	Bronchial asthma, afebrile
38	M. McL.	44	Lysis	4	4	0	0	0	10^2	Postoperative fever, lungs clear. Pn. III from 1 of 4 throat cultures
					17	0	0	—	—	
39	T. P.	58	Lysis	26	25	0	0	0	0	Acute bronchitis with fever. No pulmonary consolidation
					34	0	0	0	0	
					41	0	0	—	—	
					47	0	0	0	0	
40	M. P.	54	——	—	—	0	0	0	0	Pn. III from abscessed foot 9 days before serum taken

Explanation of Tables I–IV

* The following abbreviations and notations apply to this and subsequent tables of this paper:

Pn. III, Pn. VIII, etc. = *Pneumococcus Type III, Pneumococcus Type VIII,* etc.

"Day" = The numbers represent the number of days after the onset of the disease.

"Agglutinins" = The numbers represent the highest dilution of serum in which floccular agglutination was observed. More than one number in these columns are recorded when different titers were obtained with different Type VIII antigens.

"Mouse protection" = The figures represent the highest number of lethal doses against which mice were protected.

— = Indeterminate, or test not done.

"*Strep. hem.*" = *Streptococcus hemolyticus.*

"*Staph. aureus*" = *Staphylococcus aureus.*

† Days after onset of fever.

TABLE II

Antibody response to infections associated with Type VIII pneumococci

Case number	Patient	Age	Blood culture		Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Result	Day	Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years										
41	H. B.	32	Pn. VIII	4	Crisis	8	6	0	0	10	0	
			Negative	8			10	0	4	—	—	
							13	0	4	10	10^3	
							18	0	4–8	10^2	10^2	
42	E. J.	40	Pn. VIII	4	Crisis	9	4	0	0	—	—	
			Negative	9			8	0	0	0	0	
							12	0	4–8	—	10^4	
							18	0	8–16	0	10	
43	R. S.	37	Negative	7	Crisis	7	7	4	0	10	0	Bronchopneumonia and rheumatic heart disease
							19	2	2–4	10	0	
							37	2	2–4	10^2	10^3	
44	J. W.	37	Negative	6	Lysis	0	6	0	—	0	10	Recurrence 12–17th day. Sterile pleural effusion 24th
			Pn. VIII	13	Recurrence	17	10	0	4–8	—	10^2	day. Thrombophlebitis 24–29th day
			Negative	25			20	0	4–8	0	10^3	
							41	0	2	0	10^4	
45	C. F.	36	Negative	13	Lysis	18	13	0	0	10	0	
							20	0	4	10^3	0	
							30	0	0	10^2	0	
							38	0	2	—	—	
							46	0	0	10^2	0	
46	L. F.	18	Negative	8	Crisis	10	10	0	0	0	10^3	
			Negative	10			16	0	16–32	10	10^6	

TABLE II—(*continued*)

Case number	Patient	Age	Blood culture		Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Result	Day	Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years										
47	C. McC.	32	Negative	3	Lysis	12	4	0	0	0	0	
			Pn. VIII	4			9	0	0	—	—	
			Negative	7			16	0	0	—	—	
			Negative	12			23	0	2	0	10^4	
							25	0	4–8	—	—	
48	E. F.	36	Negative	3	Lysis	4	3	0	0	0	0	
							10	0	4–16	10	10^5	
							17	0	4–8	0	10^5	
							22	0	8	—	—	
49	J. A.	43	Pn. VIII	2	Crisis	6	5	0	0	0	0	
			Pn. VIII	5			8	0	2	10	10^3	Diffuse bronchopneumonia
							19	0	2	0	10^4	
50	C. L.	45	Negative	8	Crisis	8	12	2	16–32	10^2	—	Bronchopneumonia
							16	4	32	10^4	10^4	
51	J. McLe.	42	Negative	11	Lysis	12	22	0	8–16	0	10^5	
52	S. H.	58	Negative	6	Lysis	8	9	2	4–8	10^2	10^3	
							14	2	8–16	10^3	10^4	
							20	0	2–4	10^3	10^5	
53	E. S.	18	—	—	Crisis	6	12	4	4	10^4	10^5	
							15	4	4–8	10^4	10^4	
54	F. H.	52	Negative	—	—	—	—	16	0–2	10^5	10^3	

TABLE II—(*continued*)

Case number	Patient	Age	Blood culture		Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Result	Day	Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years										
55	J. C.	40	—	—	Crisis	5	7	0	0	0	0	Bronchopneumonia
							11	0	0	—	—	
							11	0	0	0	10	
56	W. B.	60	Pn. VIII	4	Lysis	9	4	0	0	0	0	
							8	0	2	—	—	
							18	0	0	0	0	
57	F. DeB.	48	Negative	5	Crisis	3	7	0	0	—	—	Extended after pseudocrisis
			Negative	7	Recurrence	9	13	0	0	0	0	
58	R. J.	42	Pn. VIII	6	Lysis	12	7	0	0	0	0	Pn. XVIII recovered from 1 of 4 sputa. No other pneumococci found. Thrombophlebitis 13-27th day
							9	0	0	—	—	
							15	0	0	0	0	
							27	0	0	0	0	
59	W. J.	40	Negative	14	Crisis	16	13	0	0	0	0	
							18	0	0	0	0	
60	W. T.	45	Negative	6	Lysis	8	11	0	0	0	0	
							16	0	0	0	0	
							22	0	0	0	0	
61	H. T.	34	Negative	5	Crisis	5	5	0	0	0	0	
24	J. O'B.	36	Negative		Lysis	8		(see Table I)				Also has Pn. III in sputum (see previous table)

TABLE II—(*continued*)

Case number	Patient	Age	Blood culture		Termination		Day of serum	Agglutinins		Mouse protection		Remarks
			Result	Day	Mode	Day		Pn. III	Pn. VIII	Pn. III	Pn. VIII	
		years										
62	E. Ha.	59	Negative Negative	5 9	Died	11	5 9	0 0	0 32–64	0 0	0 10^5	Autopsy: Bronchopneumonia. Cultures: Heart's blood = *Strep. hem.* Lungs = *Strep. hem.* and *Staph. aureus*
63	J. R.	36	Negative	5	Died	5	5	0	0	0	10^4	
64	W. W.	40	Negative Negative	3 5	Died	9	6	0	0	—	—	Bronchopneumonia
65	G. T.	35	Pn. VIII	3	Died	4	4	0	0	—	—	
66	F. H.	52	Pn. VIII	24	Died	30	29	0	0	0	0	
67	J. P.	48	—	—	Died	25	10	0	0	10^2	0	
68	F. D.	38	—	—	Crisis	8	11	0	0	0	10^4	Acute laryngitis; no pneumonia
31	H. P.	24	Negative	4	Lysis	3	7	4	0	10^2	0	"Grippe," no pneumonia. Also had Pn. III in sputum
69	W. D.	25	—	—	Crisis	4	6	0	0	0	0	"Grippe," no pneumonia
70	C. S.	50	—	—	Crisis	5	13	0	0	0	0	Postoperative fever; no pneumonia. Pn. VIII in one of 4 throat cultures (Pn. X, XXIII and XXXI in others). Agglutinins for each of these types absent
71	A. Y.	55	—	—	Improved	—	3 times	0	0	0	0	Pulmonary infarct (Pn. III in sputum 3 months previously)

TABLE III

Summary of Tables I and II: Immunity and cross-immunity resulting from infections associated with Types III and VIII pneumococci

	Patients' type	Number tested	Only homologous positive*	Only heterologous positive*	Both positive	Both negative	Agglutination			Mouse protection		
							Number tested	Agglutinins demonstrated		Number tested	Protection demonstrated	
								Pn. III	Pn. VIII		Pn. III	Pn. VIII
Pneumonias recovered	III	24	8	2	5	9†	24	13	4	21	11	7
	VIII	22	6	0	9	7†	22	5	14	22	9	13
Pneumonias fatal	III	6	2	1	0	3	6	2	1	5	2	1
	VIII	6	2	1	0	3	6	0	1	4	1	2
Infections without pneumonia	III	10	2†	1	0	7	10	1	0	8	2	1
	VIII	5	1	1†	0	3	5	1	0	5	1	1

* Homologous and heterologous refer only to Types III and VIII tests in relation to the type obtained from the patient.

† Cases 24 and 31 had both Type III and Type VIII pneumococci and are listed twice.

It will be seen from these tables that the serum of one-half of the Type III and two-thirds of the Type VIII patients with pneumonia who recovered and one-third of those who died had agglutinins and protective antibodies for the homologous type pneumococcus late in the disease, or during convalescence. Sera taken early in the disease showed no such antibodies. Cross-agglutination and cross-protection between the Types III and VIII were frequent in patients who had either of these types. With some exceptions, the patients with antibodies for the related type also had antibodies for the homologous type, and the titer of the latter was usually higher than that for the heterologous but related types.

Additional agglutinations were carried out in each serum with from 2 to 8 different strains of Type VIII, with the stock Types I, II and V strains, and with strains of about 15 other types of pneumococci. The results obtained with the various Type VIII strains were remarkably uniform; those with the remaining types were usually negative, even with undiluted sera. Exceptions are noted in the tables.

Among the pneumonia patients were 14 with clinical and x-ray or anatomical evidence of patchy consolidation, which may be termed "atypical" or bronchopneumonia. The findings in these patients were very similar to those obtained in the patients with typical lobar pneumonia.

Of the 14 patients without pneumonia, two had antibodies for the homologous, and one for the related type only. All three of these patients had acute infections of the upper respiratory tract without clinical or

roentgenological evidence of pulmonary consolidation. The titer of antibodies in each of these patients was low.

For each type of pneumococcus, the relationship between the findings of agglutinins and the findings of protective antibodies was similar to that found among cases of Types I and II (7). They are consistent with the concept that, in general, mouse protection is more sensitive than agglutination as an index to type-specific immunity following infection or immunization.

Mixed infections

It was pointed out elsewhere (4) that pneumococci of other types and other significant organisms are found in patients with Types III and VIII infections, particularly the former, more frequently than in pneumonia due to any other of the pneumococcus types. Some of these cases represent concomitant or consecutive infection, but in most of them one or the other organism has no relation to the disease. Antibody studies may aid in determining the possible etiological relationship.

In the present series, 9 cases of mixed infection were studied. Two of these (Cases 6 and 62) represent consecutive infections. The former developed antibodies for 2 types of pneumococcus, in turn, and the latter succumbed to hemolytic streptococcus sepsis after antibodies against Type VIII had developed. In 2 patients (Cases 9 and 58), the Types III and VIII were the significant invaders and the other pneumococci were probably incidental. In the remaining 5 patients (Cases 14, 21, 24, 31 and 70), the Type III or VIII pneumococci or both were probably incidental, as judged by antibody formation. In Case 14, the Type V pneumococcus, against which antibodies developed, could not be isolated from the patient.

Results of absorption experiments

A number of sera in which antibodies were demonstrated for the homologous or the related type or for both were absorbed with both Types III and VIII pneumococci. The effects of such absorption on the agglutinin and protective titers are shown in Table IV. The results were similar for the Type III and the Type VIII patients and corresponded to those obtained in immunized rabbits (3). Absorption with organisms of the homologous type removed the antibodies for these organisms and for pneumococci of the related type, whereas the related organisms absorbed only the antibodies for the same type but not for the type with which the patient was infected.

DISCUSSION

Inasmuch as the typing of pneumococci depends largely on the agglutination reaction, the results obtained with different strains in the several horse antisera are significant. It would seem, on the basis of these find-

TABLE IV

Effect of absorption with Types III and VIII pneumococci on the agglutinins and protective antibodies in serum of patients convalescing from Types III and VIII pneumonia

Case number	Patient	Patients' type	Day of serum	Agglutination with Pn. III				Agglutination with Pn. VIII				Protection against Pn. III				Protection against Pn. VIII			
				Unabsorbed	Absorbed with			Unabsorbed	Absorbed with			Unabsorbed	Absorbed with			Unabsorbed	Absorbed with		
					Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II		Pn. III	Pn. VIII	Pn. II
4	M. I.	III	19	64	8	64	64	0	—	—	—	10^6	0	10^4	10^3	10^4	0	0	10^4
3	A. S.	III	10	64	0	64	64	0	—	—	—	10^6	0	10^4	—	0	—	—	—
16	J. S.	III	12	0	—	—	—	2	0	0	—	0	—	—	—	10^3	10^2	—	—
	J. S.	III	18	0	—	—	—	2–4	0	0	—	0	—	—	—	10^5	10^4	0	—
7	T. C.	III	33	4	0	0	0	0	—	—	—	10^4	0	10^2	10^4	0	—	—	—
13	F. G.	III	7	8	0	0	0	0	—	—	—	10^4	0	10^3	10^2	0	—	—	—
18	M. K.	III	15	0	—	—	—	16	0	—	—	0	—	—	—	10^3	0	—	—
52	S. H.	VIII	14	2	0	0	0	8	4	0	—	10^3	0	1	—	10^4	10^5	0	—
	S. H.	VIII	20	0	0	0	0	4	4	0	—	10^3	0	10	—	10^5	10^5	0	—
53	E. S.	VIII	12	4	0	0	—	4	0	0	—	10^4	0	0	—	10^5	10^4	0	—
	E. S.	VIII	15	4	0	0	—	8	4	0	—	10^4	0	10^2	—	10^4	10^4	0	—
51	J. McL.	VIII	22	0	—	—	—	16	32	0	8	0	—	—	—	10^4	10^4	0	10^3
44	J. W.	VIII	41	0	—	—	—	2	0	0	0	0	—	—	—	10^4	10^4	0	—
46	L. F.	VIII	16	0	—	—	—	16	8	0	—	10	—	—	—	10^6	10^6	0	—
48	E. F.	VIII	10	0	—	—	—	16	4	0	—	10	0	0	—	10^5	10^5	0	—
71	F. D.	VIII	10	0	—	—	—	0	—	—	—	0	—	—	—	10^4	10^3	0	10^5
50	C. L.	VIII	16	4	—	0	—	32	—	—	—	10^4	0	0	—	10^4	10^5	0	—

ings, that the choice of a suitable Type III agglutinating serum and additional agglutination, in Type VIII antiserum, of strains reacting with it, should serve to differentiate between these 2 types. Titration in progressive dilutions of both sera are seldom necessary. Prolonged incubation should be avoided. Microscopic agglutination in the same dilution of both antisera gives a rapid and clear differentiation. The precipitin reaction is apparently no more reliable than the agglutination test (3). Type VIII strains, however, do not produce large mucoid colonies on the surface of blood agar plates similar to those characteristic of freshly isolated Type III strains (5). As to the serum, the variations in cross-agglutination observed with different species suggest the possibility that some suitable species will be found in which the Type III immunity is strictly type-specific, as it is in the mouse (3).

The differentiation of these two types is important because of the clinical and pathological differences between the diseases associated with each of these, particularly the wide divergence in death rates, especially in bacteremic patients (4). It may also become important from the therapeutic point of view, inasmuch as all therapy in human pneumococcic infections has thus far been shown to depend on type-specificity. Both therapeutic antisera and carbohydrate splitting enzymes (8) of value in such infections have been shown to be type-specific in their action.

Immune bodies resulting from Type III infections were encountered less frequently and were of lower grade than homologous antibodies resulting from Types I, II or VIII infections. Low grade or absent immune responses are, however, encountered even with Types I and II infections (7, 10). It is not unlikely that instances of transient appearance of antibodies were missed owing to the small number of sera studied. It is also possible that, owing to the frequent finding of Type III pneumococci in normal throats, some of the patients in whom antibodies for this type were not demonstrated were only carriers and the disease was caused by another organism. Such cases were detected by testing the sera with many different types. No satisfactory explanation was found, however, for the failure of an occasional patient to develop antibodies against organisms recovered from the blood.

The present series offered some opportunity to compare the immunity resulting from lobar pneumonia and that following bronchopneumonia due to the same organism. Such opportunities with Types I and II pneumococci must, of necessity, be quite rare owing to the close association of the latter types with lobar pneumonia and the high fatality in the occasional cases of bronchopneumonia due to these types (11). The antibody response with the different kinds of pulmonary lesion due to the same type were very similar. In the patients with simple respiratory infections without pneumonia, antibodies were usually absent or of low titer.

The results of the absorption tests were similar to those obtaining with major and minor antibodies for other related organisms, notably the typhoid-paratyphoid group. In the present cases, they confirm the etiological relationship to pneumonia of Types III and VIII pneumococci obtained from sputum, especially in recovered patients, in whom the same organism usually cannot be obtained from the blood or lungs (10).

SUMMARY AND CONCLUSIONS

Freshly isolated Types III and VIII pneumococci frequently show significant degrees of cross-agglutination in some horse antisera of the related type. The desirability of further aglutinating in Type VIII antiserum strains of pneumococci which react with Type III antisera was emphasized.

The sera of patients with lobar or bronchopneumonia associated with Type III or Type VIII pneumococci have homologous type-specific antibodies similar to those observed following Types I and II pneumococcus pneumonia. In the Type III patients, antibodies were less frequent and of lower titer. Antibodies for the heterologous but related type were found frequently among both the Type III and the Type VIII patients.

The authors are indebted to Miss Louise N. Batt and Miss Mary S. Carroll for technical assistance.

BIBLIOGRAPHY

1. (*a*) Hanes, F. M., An immunological study of *Pneumococcus mucosus*. J. Exper. Med., 1914, **19**, 38.
(*b*) Cole, R. I., Pneumococcus infection and immunity. New York State J. Med., 1915, **101**, 1.
(*c*) Wadsworth, A. B., and Kirkbride, M. B., A note on the production of antipneumococcus sera. J. Exper. Med., 1917, **25**, 629.
(*d*) Singer, E., and Adler, H., Zur Frage der Gewebsimmunität. Die Immunität gegen Pneumococcus Typus III. Ztschr. f. Immunitätsforsch. u. exp. Therapie, 1924, **41**, 71.
(*e*) Eguchi, Ch., Versuche über Infektion und Immunisierung junger und alter Mäuse und Meerschweinchen mit Pneumokokken und Streptokokken durch Fütterung und Inhalation. Ztschr. f. Hyg., 1925, **105**, 74.
(*f*) Tudoranu, G., Le mécanisme de l'immunité contre le pneumocoque type III. Ann. Inst. Pasteur, 1926, **40**, 606.
(*g*) Tillett, W. S., Studies on immunity to *Pneumococcus mucosus* (Type III). I. Antibody response of rabbitts immunized with Type III pneumococcus. J. Exper. Med., 1927, **45**, 713. II. The infectivity of Type III pneumococcus for rabbits. J. Exper. Med., 1927, **45**, 1093. III. Increased resistance to Type III infection induced in rabbits by immunization with R and S forms of pneumococcus. J. Exper. Med., 1927, **46**, 343. Active and passive immunity to pneumococcus infection induced in rabbits by immunization with R pneumococci. J. Exper. Med., 1928, **48**, 791.

(*h*) Lévy-Bruhl, M., Recherches sur le pneumocoque III (*Pneumococcus mucosus*). Origine, caractères généraux et virulence de 20 échantillons. Ann. Inst. Pasteur, 1927, **41**, 458.

(*i*) Bull, C. G., and McKee, C. M., Respiratory immunity in rabbits. VIII. Rabbits immunized with pneumococcus Types II, III and IV resist intranasal infection with Type I. Am. J. Hyg., 1929, **10**, 229.

(*j*) Cotoni, L., and Chambrin, N., Sur l'immunité passive obtenue à l'aide du pneumocoque III. Ann. Inst. Pasteur, 1930, **45**, 706.

2. Cooper, G., Edwards, M., and Rosenstein, C., The separation of types among the pneumococci hitherto called Group IV and the development of therapeutic antiserums for these types. J. Exper. Med., 1929, **49**, 461.
3. (*a*) Sugg, J. Y., Gaspari, E. L., Fleming, W. L., and Neill, J. M., Studies on immunological relationships among the pneumococci. I. A virulent strain of pneumococcus which is immunologically related to, but not identical with typical strains of Type III pneumococci. J. Exper. Med., 1928, 47, 917.

(b) Harris, A. L., Sugg, J. Y., and Neill, J. M., Studies on immunological relationships among the pneumococci. II. A comparison of the antibody responses of mice and of rabbits to immunization with typical Type III pneumococci and to immunization with a related strain. J. Exper. Med., 1928, **47**, 933.

4. Finland, M., and Sutliff, W. D., Pneumococcus Types III and VIII infections. A characterization of pneumococcus Type III pneumonia and that associated with a biologically closely related organism, the Type VIII pneumococcus. Arch. Int. Med. (In press.)
5. Cooper, G., Rosenstein, C., Walter, A., and Peizer, L., The further separation of types among the pneumococci hitherto included in Group IV and the development of therapeutic antisera for these types. J. Exper. Med., 1932, **55**, 531.
6. Finland, M., and Sutliff, W. D., Immunity reactions of human subjects to strains of pneumococci other than Types I, II and III. J. Exper. Med., 1933, **57**, 95.
7. Finland, M., and Sutliff, W. D., Specific cutaneous reactions and circulating antibodies in the course of lobar pneumonia. I. Cases receiving no serum therapy. J. Exper. Med., 1931, **54**, 637.
8. Goodner, K., Dubos, R., and Avery, O. T., The action of a specific enzyme upon the dermal infection of rabbits with Type III pneumococcus. J. Exper. Med., 1932, **55**, 393.
9. Winkler, A. W., and Finland, M., Antibody response to infections with the newly classified types of pneumococci (Cooper). J. Clin. Invest., 1934, **13**, 109.
10. (*a*) Clough, P. W., The development of antibodies in the serum of patients recovering from acute lobar pneumonia. Bull. Johns Hopkins Hosp., 1913, **24**, 295.

(*b*) Trask, J. D., O'Donovan, C., Jr., Moore, D. M., and Beebe, A. R., Studies on pneumonia in children. I. Mortality, blood cultures, and humoral antibodies in pneumococcus pneumonia. J. Clin. Invest., 1930, **8**, 623.

(*c*) Francis, T., Jr., and Tillett, W. S., Cutaneous reactions in pneumonia. The development of antibodies following the intradermal injection of type-specific polysaccharide. J. Exper. Med., 1930, **52**, 573.

(*d*) Lord, F. T., and Persons, E. L., Certain aspects of mouse protection tests for antibody in pneumococcus pneumonia. J. Exper. Med., 1931, **53**, 151.

11. Sutliff, W. D., and Finland, M., The significance of the newly classified types of pneumococci in disease; Types IV to XX inclusive. J. A. M. A., 1933, **101**, 1289.
12. (*a*) Rosenow, E. C., A bacteriological and cellular study of the lung exudate during life in lobar pneumonia. J. Infect. Dis., 1911, **8**, 500.
    (*b*) Thomas, H. M., Jr., and Parker, F., Jr., Results of antemortem lung punctures in lobar pneumonia: Their bearing on the mechanism of crisis. Arch. Int. Med., 1920, **26**, 125.
    (*c*) Glynn, E. E., and Digby, L., Bacteriological and clinical observations on pneumonia and empyemata. Special report Series No. 79; His Majesty's Stationery Office, London, 1923.