THE SPECIFIC POLYSACCHARIDE CONTENT OF PNEUMONIC SPUTA*

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The microscopic examination of sputum from patients with pneumonia has revealed that Type III infections are distinctly different from those due to other pneumococci. The evidence obtained supported the hypothesis that the prognosis was primarily dependent upon the amount of Type III capsular carbohydrate produced and secondarily upon the number of organisms present in the pneumonic exudate (1). The character of the visualized background in Wright stained smears of rusty sputum was the first means of classifying patients with Type III pneumonia into two major groups. In one group called "non-reticulated" the usual granular pink background was present on the slides as contrasted with a second group called "reticulated" in which the background was composed of a branching, fibrin-like network which appeared to originate from the similarly stained capsules of the interdispersed pneumococci. In the non-reticulated instances the bacteremic incidence was 7 per cent and the fatality rate was 9 per cent; whereas in the reticulated group the bacteremic incidence was 65 per cent and the fatality rate was 83 per cent (2). It was then found that by the addition of as little as 0.02 mg. of Type III S to 0.1 cc. of any pneumonic sputum an artificial reticulation could be produced which closely resembled that seen in the naturally occurring pneumonia. Further evidence that the reticulation was due to an unusually large amount of carbohydrate was obtained by means of the "quellung" reaction. Neufeld preparations of reticulated specimens of sputum revealed a meshwork of excess capsular material either in the form of swollen free masses, or in the form of strands connecting one pneumococcus to another (2). The striking differences in the amount of S observed in the "quellung" reaction suggested that quantitative methods could be utilized. Therefore, in further test of the above hypothesis, specimens of sputum from patients with Type I, II, III, VII, and VIII pneumonia were analyzed for their capsular polysaccharide content by a method based on the work of Avery and Goebel (3), Heidelberger, Kendall, and Scherp (4), and Wadsworth and Brown (5).

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Methods

Samples of sputum were obtained prior to and within 48 hours from the beginning of therapy. To insure that the samples originated from the pneumonic areas in the lung, only rusty or bloody specimens were analyzed. Each specimen was made homogeneous with the aid of glass beads and a mechanical shaker. To an accurately measured aliquot (10 ml. if available) was added an equal volume of acetate buffer solution,[1] glass beads, and a drop of caprylic alcohol to prevent foaming. This mixture was then shaken vigorously for 30 minutes. Two volumes of 95 per cent alcohol were added slowly and the sample placed in the cold room overnight. The next morning the samples were centrifuged, the supernate discarded, and the residue was extracted with 10 ml. of acetate buffer in the manner described above. The mixture was centrifuged, the supernate decanted, and the insoluble fraction was extracted with acetate buffer until the washings no longer gave a positive ring test with homologous antiserum. The acetate buffer washings were then combined, precipitated with two volumes of 95 per cent alcohol, and the centrifuged precipitate reextracted and reprecipitated twice more for purification. The last alcohol precipitate was dissolved in slightly acidified, warm saline which was subsequently neutralized to litmus with dilute NaOH. The purified solution of S was then adjusted to the original volume of the sputum aliquot.

A modification of the Heidelberger and Kendall procedure was employed to measure the amount of S in the sputum extracts (6, 7). A given antiserum for each type being studied was standardized with known amounts of a highly purified polysaccharide. The amount of nitrogen precipitated was plotted against the corresponding amounts of S. The analysis of an unknown solution of S must be conducted in the region of antibody excess (7). The quantity of S in the unknown purified sputum aliquots was adjusted to fall within this range. This was accomplished by comparing the relative precipitin titers of the unknown with a standard solution of S. In general the samples with the highest titers contained the most carbohydrate, but the well known limitations of the precipitin test prohibited its use for detecting small differences. Triplicate 1 ml. aliquots of the unknown S solutions were added to 1 ml. quantities of standard antiserum and the precipitable nitrogen determined by the micro Kjeldahl technique. On the basis of these values the quantity of S in the sample was read from the standard curve.

The activities of the S preparations used in standardizing the serums were as follows:—

		Precipitin titer	Antibody N precipitated by 0.1 mg. S	Potency of homologous antiserum
			mg./ml.	*mg. N/ml.*
S I	Lot 2	1:10,000,000	1.0	2.0*
S II	Lot 3	1: 5,000,000	1.2	2.1*
S III	Lot 5	1: 5,000,000	1.1	2.1‡
S VII	Lot 3	1: 2,000,000	0.7	2.3*
S VIII	Lot 2	1: 5,000,000	1.4	2.1*

* Horse serum.

‡ Rabbit serum.

[1] Sodium chloride 0.9 per cent, sodium acetate 4 per cent, glacial acetic acid 2 per cent.

The final results of the sputum analyses are reported in terms of mg. per cent. The practice of calculating the results to the basis of dry weight of the sample was abandoned because in 17 such analyses there were no significant differences between the two sets of data.

RESULTS

In Table I are presented the results of the analyses of 19 samples of sputum from 14 patients with Type I pneumonia. It can be noted that the cases are relatively mild as judged from the degree of involvement, incidence of bacteremia, and leukopenia. The sputum counts represent the highest number

TABLE I

Specific Polysaccharide Content of Type I Sputa

Patient No.	Age	Bacteremia	Leukopenia*	Multiple lobe	Sputum count‡	S	Outcome
	yrs.					*mg. per cent*	
1	39	−	+	+	13	0.8	L
2	24	−	−	−	1	0.8	L
3	30	+	−	−	14	1.1	L
4	38	−	−	+	15	1.2	L
5	35	−	−	−	12	1.2	L
6	60	−	−	−	5	1.3	L
7	48	−	−	−	26	1.3	L
8	39	+	−	+	57	1.3	L
9	16	+	−	−	3	1.8	L
10	40	−	+	−	25	1.8	L
11	32	−	−	+	8	2.0	L
12	60	−	−	−	14	2.5	L
13	67	+	−	+	55	2.6	D
14	29	−	−	−	23	5.3	L

* The term leukopenia refers to those patients with total leukocyte counts of less than 10,000 during the acute stages of the pneumonia.

‡ Refers to the highest number of pneumococci per oil immersion field during the period when sputum specimens were collected.

of pneumococci per oil immersion field in Wright stained smears from the same specimens which were analyzed. The single death in the series was due to cardiac failure. The amount of Type I pneumococcus polysaccharide in the sputum varied from 0.8 to 5.3 mg. per cent with an average of 1.8. The maximum concentration of Type I S encountered was 10.4 mg. per cent in lung exudate obtained at autopsy.[2] Sufficient sputum could not be obtained from this patient to make an analysis.

Table II presents the results of the analyses of 20 specimens of sputum from 10 patients with Type II pneumonia. It can be noted that the cases were more severe in character as judged from the clinical and sputum data. The amounts of specific carbohydrate varied from 1.0 to 94 mg. per cent with

[2] The results of the autopsy analyses will be reported in a separate communication.

an average of 16.1. The sputum from patient 24 contained numerous pneumococci and the value of 94 mg. was only exceeded by a value of 112 mg. per cent in the lung exudate from the same patient at autopsy.

TABLE II

Specific Polysaccharide Content of Type II Sputa

Patient No.	Age	Bacteremia	Leuko-penia	Multiple lobe	Sputum count	S	Outcome
	yrs.					*mg. per cent*	
15	43	−	−	−	5	1.0	L
16	56	+	+	+	12	1.1	L
17	43	−	+	+	48	3.9	L
18	61	+	+	+	23	8.3	D
19	30	+	−	+	48	8.6	L
20	66	+	+	+	18	8.6	L
21	21	+	−	+	40	9.8	L
22	35	+	−	+	16	12.2	L
23	59	+	+	+	100±	13.2	D
24	33	+	+	+	200±	94.0	D

TABLE III

Specific Polysaccharide Content of Type VII Sputa

Patient No.	Age	Bacteremia	Leukopenia	Multiple lobe	Sputum count	S	Outcome
	yrs.					*mg. per cent*	
25	38	+	−	+	10	1.9	L
26	25	−	−	−	8	2.3	L
27	26	+	−	+	14	3.0	L
28	39	−	−	+	4	4.2	L
29	23	+	+	+	30	5.5	L
30	64	−	−	−	15	7.0	L
31	38	+	−	+	60	7.0	L
32	23	+	−	+	5	9.0	L
33	20	−	−	−	3	10.5	L
34	46	−	−	+	4	18.5	L
35	44	+	+	+	200±	20.0	D
36	56	+	+	+	200±	76.0	D

Table III presents the analytical results of 16 specimens from 12 patients with Type VII pneumonia. The amounts of specific polysaccharide varied from 1.9 to 76 mg. per cent with an average of 13.7. The greatest amounts of Type VII S were obtained from the sputa of cases 35 and 36 and the maximum value of 76 mg. was only exceeded by a concentration of 96 mg. per cent in the lung exudate of patient 36 at autopsy.

Table IV presents the analyses of 16 specimens of sputum from 11 patients

TABLE IV

Specific Polysaccharide Content of Type VIII Sputa

Patient No.	Age	Bacteremia	Leukopenia	Multiple lobe	Sputum count	S	Outcome
	yrs.					*mg. per cent*	
37	39	+	−	+	23	0.5	L
38	29	−	+	+	12	1.0	L
39	36	−	−	−	3	1.0	L
40	38	−	−	−	25	1.4	L
41	42	+	+	+	18	1.7	L
42	40	−	−	−	6	2.1	L
43	33	−	+	−	13	3.2	L
44	43	+	+	−	26	3.8	L
45	66	+	−	+	22	3.9	D
46	53	−	+	+	15	6.2	L
47	58	−	−	−	3	6.8	L

TABLE V

Specific Polysaccharide Content of Non-Reticulated Type III Sputa

Patient No.	Age	Bacteremia	Leukopenia	Multiple lobe	Sputum count	Excess S*	Reticulation‡	S	Outcome
	yrs.							*mg. per cent*	
48	52	−	−	−	5	−	−	4.0	L
49	37	−	+	+	15	+	−	4.8	L
50	33	−	−	−	<1	−	−	6.0	L
51	39	−	−	−	7	−	−	6.0	D
52	30	−	−	+	13	−	−	8.0	L
53	55	−	−	−	9	−	−	8.4	L
54	57	−	−	−	<1	−	−	14.0	L
55	46	−	−	−	<1	−	−	20.0	L
56	48	−	−	−	4	−	−	24.0	L
57	23	−	−	−	<1	−	−	25.0	L
58	38	−	−	−	4	−	−	26.0	L
59	36	−	−	−	1	−	−	26.0	L
60	40	−	−	−	13	−	−	30.0	L
61	10	−	−	−	17	−	−	32.0	L
62	43	−	−	−	<1	+	−	44.0	L
63	57	−	−	−	24	+	−	48.0	L
64	38	−	−	−	7	−	−	68.0	L
65	23	−	−	−	5	+	−	92.0	L
66	42	−	−	−	6	−	−	92.0	L
67	49	−	−	+	51	+	−	300.0	L

* Excess S refers to the amount of capsular carbohydrate observed during typing.

‡ Classification on the basis of Wright stained sputum smears (1, 2).

− signifies that all S present is in the capsules of the pneumococci.

+ or ++ signifies that S present is in capsules and in strands connecting two or more pneumococci.

+++ or ++++ signifies that large amounts of S in the form of strands and free masses are present.

with Type VIII pneumonia. The patients in this group were only mildly ill as judged from the clinical data and sputum counts. The single death was attributable to pericarditis and empyema. The amounts of specific polysaccharide varied from 0.5 to 6.8 mg. per cent with an average of 2.9. The maximum value of Type VIII S was 25.2 mg. per cent obtained at autopsy from a patient whose sputum was not analyzed.

Table V lists the results of 32 specimens of sputum from 20 patients with Type III pneumonia. These cases were classified as non-reticulated on the

TABLE VI

Specific Polysaccharide Content of Reticulated Type III Sputa

Patient No.	Age	Bacteremia	Leukopenia	Multiple lobe	Sputum count	Excess S	Reticulation	S	Outcome
	yrs.							*mg. per cent*	
68	61	−	−	−	29	++	+	18.0	L
69	54	+	−	+	16	++++	++++	28.0	D
70	33	+	+	+	12	+	+	72.0	L
71	52	−	+	+	59	+	+	130.0	D
72	63	+	+	+	74	++	+	180.0	L
73	53	−	−	+	36	++	+	240.0	L
74	72	−	+	+	38	++	+	244.0	D
75	67	−	+	+	19	++	++	300.0	D
76	—	+	−	+	4	++	++	340.0	D
77	67	+	+	+	53	++++	++++	400.0	D
78	54	−	−	−	82	+++	+++	640.0	L
79	64	−	+	−	13	++	++	640.0	D
80	48	−	−	−	16	++++	++++	800.0	D
81	42	+	−	+	29	++++	++++	1,200.0	D
82	52	+	−	+	36	++++	++++	1,440.0	D
83	49	+	+	−	100±	++++	++++	2,120.0	D
84	49	−	+	+	73	++++	++++	2,200.0	D
85	68	+	−	+	44	++++	++++	4,800.0	D
86	50	+	+	+	25	++++	++++	10,000.0	D

basis of Wright's stained smears of sputum but they were further subdivided depending upon the amount of excess polysaccharide observed during the Neufeld typing. The clinical data and outcome emphasize the mild character of the disease but the amounts of Type III S recovered from the sputa varied from 4 to 300 mg. per cent with an average of 45.

The above results are to be compared with those in Table VI which represent the analyses of 29 specimens of sputum from 19 patients who were classified as reticulated and in whom the typing reaction revealed an excess of polysaccharide. This group contained the majority of severely ill Type III patients as can be seen from the incidence of bacteremia, leukopenia, multiple lobe involvement, and fatality rate. The amounts of specific polysaccharide varied

from 18 to 10,000 mg. per cent with an average of 1,360. The maximum value of 10,000 mg. per cent was the largest amount of Type III S obtained from all of the specimens examined.

DISCUSSION

To the authors' knowledge the above results represent the first attempt to determine quantitatively the amount of capsular polysaccharide in rusty or bloody sputum. We do not wish to imply that the values obtained are absolute. In addition to errors in sampling, extraction, and those inherent in the method, the final figures could also vary depending upon the activity of the purified S used in the preparation of the standard curves. In general it can be said that the S values obtained both from the reticulated and non-reticulated Type III sputums were much higher than had been anticipated. The average for the reticulated Type III cases roughly exceeded by 170 times the amount of S recoverable from the sputa of cases of Type I, II, VII, and VIII pneumonia. The values in the non-reticulated cases were approximately 5 times those obtained for the other types. The data again emphasize the atypical character of Type III pneumonia.

It can be noted from Tables V and VI that the Neufeld typing reaction is a more delicate indicator of the presence of excess S in the sputum than is the Wright stain. The latter, however, is a more dependable prognostic aid. Strands of polysaccharide connecting pneumococci began to be evident in the sputum when the concentrations of S approached 40 mg. per cent; whereas, between 100 and 200 mg. of S were usually required before reticulation became apparent. A few exceptions to this general rule may have been due to errors in extraction or to inadequate specimens. The number of pneumococci per oil immersion field (sputum count) was generally higher in the reticulated than in the non-reticulated cases and coincided with the amount of S recoverable from the sputum. This finding, however, did not appear to hold for infections due to Types I, II, VII, and VIII pneumococci. No obvious explanation for this discrepancy is available at present. The data on sputum counts and analyses in Type III pneumonia suggest that the amount of S elaborated, together with the number of pneumococci in the sputum, should be considered in order to evaluate individual cases. The relationship between the sputum and autopsy analyses together with additional discussion of the total results will be reported in a further communication.

CONCLUSIONS

1. The average specific polysaccharide content of rusty or bloody sputa in Type III was 91 times greater than the average for Types I, II, VII, and VIII pneumonia.

2. Those Type III sputums which were classified as reticulated contained

an average S concentration of 1,360 mg. per cent or 170 times more than the amount found in other types.

3. Those Type III sputa which were classified as non-reticulated contained an average S concentration of 45 mg. per cent or 5.5 times more than the amount found in other types.

4. The amount of specific polysaccharide in the sputa of patients with Type III pneumonia furnishes an index to the severity of the disease and an aid in prognosis.

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