

ANTIBODY FORMATION IN MEN FOLLOWING INJECTION OF FOUR TYPE-SPECIFIC POLYSACCHARIDES OF PNEUMOCOCCUS*

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In an earlier paper (1) a study was reported of the antibody content, in weight units, of the sera of human volunteers at varying intervals from 2 weeks to 2 years after injection of pneumococci or of the specific polysaccharides of two or three pneumococcal types. On the basis of this study and of earlier, less precise investigations an experiment was undertaken looking toward immunization against Types I, II, V, and VII pneumococci of incoming trainees at an army camp at which lobar pneumonia was endemic. The epidemiological results of subcutaneous injection of trainees with the specific polysaccharides of Types I, II, V, and VII pneumococci have been published (2). Analyses of sera of randomly selected subjects who had participated in this experiment have now been completed, and the data are submitted herewith.

EXPERIMENTAL

The sera of two groups of subjects were analyzed: Group 1: Randomly selected trainees (roughly 1 out of every 300) were bled to the extent of 30 to 50 ml. (0 bleeding) and then injected subcutaneously with 1.5 ml. of a solution containing about 0.03 mg. each of the specific polysaccharides of Types I, II, V, and VII pneumococci per ml. About 2 months later as many as possible of these men were again bled (bleeding 1). Two bleedings were obtained and analyses were completed in nineteen instances. Group 2: Bleedings were collected similarly, but the subjects were injected with 1.5 ml. of salt solution. The sera of this group were studied in order to determine whether or not such exposure to pneumococcal infection as might have occurred in normal contacts during the 2 months' period was sufficient to induce the formation of measurable antibodies to the four pneumococcal types studied. This group, therefore, served as an analytical control to the other group. Analyses were completed in ten instances in group 2.

All analyses were carried out according to (3) as given in detail in (1). As previously emphasized, it is usually necessary to allow each set of analyses to stand 8 days in the cold to complete the separation of the small amounts of specific precipitate ordinarily formed. If

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TABLE I
*Antibody Nitrogen Content of Sera of Trainees before and after Injection of
 Pneumococcal Polysaccharides*

Antibody to.....	Micrograms N per 4 ml. serum				
	C	I	II	V	VII
<i>Subject</i>					
107 ₀ *	9	2	1	0	3
107 ₁ ‡	8	105	28	0	0
109 ₀	19	1	0	1	12
109 ₁	16	13	21	0	41
111 ₀	35	3	3	1	3
111 ₁	8	13	34	5	21§
113 ₀	22	0	5	0	9
113 ₁	23	7	32	0	22
115 ₀	14	0	1	1	4
115 ₁	12	5	13	1	71
116 ₀	24	1	1	0	1
116 ₁	23	5	15	9	11
121 ₀	20	3	1	0	4
121 ₁ ¶	34	50	82	0	171§
123 ₀	10	0	2	0	2
123 ₁	28	26	12	0	58
124 ₀	18	0	0	1	0
124 ₁	45	19	29	9	57
125 ₀	16	1	0	0	1
125 ₁	22	0	1	0	1
129 ₀	33	0	3	0	0
129 ₁	29	23	12	0	20§
131 ₀	77	6	5	0	14
131 ₁	72	37	25	(3)	(169)

Values in parentheses doubtful.

* Preliminary bleeding.

‡ Approximately 2 months later.

§ Five absorptions.

|| Four absorptions.

¶ 6 weeks after attack of Type I pneumonia beginning 3 days after injection.

TABLE I—*Concluded*

Antibody to.....	Micrograms N per 4 ml. serum				
	C	I	II	V	VII
<i>Subject</i>					
133 ₀	55	1	3	0	8
133 ₁	55	24	18	0	40
134 ₀	20	1	0	1	1
134 ₁	41	14	1	5	61**
136 ₀	42	0	0	1	3
136 ₁	35	7	12	0	13
137 ₀	18	1	0	0	1
137 ₁	10	16	86	0	38
138 ₀	21	0	3	1	6
138 ₁	23	34	8	0	42‡
139 ₀	11	0	0	0	0
139 ₁	11	19	24	1	(117)**
141 ₀	20	0	4	1	0
141 ₁	25	19	68	0	(81)‡‡

** Seven absorptions.

‡‡ Six absorptions.

sufficient antibody is present for the precipitates to form within 24 or 48 hours the analyses may be run after this interval, with addition of further small portions of polysaccharide to the supernatants. Since a series of successive analyses is required, precautions to ensure sterility are essential. Analyses were usually carried out in the order anti-C, anti-I, II, V, and VII, with such repetitions as were found necessary. In many instances several absorptions with C-substance were required, particularly when the amount of anti-C present was relatively large. Numerous absorptions with S VII were found necessary with postimmunization sera, the amount of precipitate often increasing to a maximum and slowly decreasing with successive additions of 0.01 mg. of S VII. The precipitates were sterile and appeared to be the result of interaction between S VII and antibody. Absorption of the preliminary bleedings with S VII, on the contrary, presented no difficulties.

DISCUSSION

In so far as comparable, the limited series of analyses given in Table I resembles those presented in the earlier study (1). A disappointing difference is the poor antibody response to S V, since appreciable amounts occurred in only four of nineteen subjects as opposed to eighteen of twenty in the earlier series. The same lot of polysaccharide was used in both instances, but it remains uncertain whether the antigen had deteriorated or whether the poorer response was

due to the variability of the human material. If the latter alternative be assumed, the relative inability to form antibody did not extend to its elaboration against Types I, II, and VII, for most of the subjects reacted well to the injection of the specific polysaccharides of these types. If the sera of these men were, as believed, representative of the entire group of nearly 9,000 injected with the polysaccharide mixture, the antibody response to these three types was adequate to explain the favorable results of the field test (2).

Since pneumococcal pneumonias of Types I, II, V, and VII were endemic at the camp during the period covered by the analyses in Table I, it was of interest to analyze sera of trainees injected only with salt solution, since it was possible that all subjects might show an increase in antibodies to these pneumococcal types during the 2 months in question. Analyses were completed on the sera of ten subjects and these failed to show any differences in the negative or minimal quantities of type-specific antibodies found at the beginning or end of the period. Details of the analyses (group 2) are on file but are not given here.

As in the earlier study, individual values for anti-C content were usually similar in the two bleedings. The average values of anti-C in group 1 were: bleeding 0, 25 μg . per 4 ml. of serum; bleeding 1, 27 μg . In group 2, (injected with saline) the figures were 47 and 44 μg .

Also as previously noted, the initial sera often contained antibodies to S VII, the average for group 1 being 4 μg . per 4 ml., with a range from 0 or 1 (eight instances) to 14 μg . A relatively high initial content of anti-VII was not necessarily followed by a maximal antibody response on injection of S VII. The analytical values for anti-VII in group 2 were similar to those for group 1, with no change in bleeding 1, 2 months after the injection of physiological saline.

SUMMARY

1. Random selections of the sera of trainees injected 2 months previously with the specific polysaccharides of Types I, II, V, and VII pneumococci contained quantities of antibody to Types I, II, and VII sufficient to account for the favorable results of the immunization procedure.
2. As in earlier studies, the levels of the antibody for the C polysaccharide remained relatively constant before and after the injections.
3. The serum from the preliminary bleedings often contained antibody to the Type VII polysaccharide. This remained relatively constant in a control group injected with saline.

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