

## CHANGES IN THE TITER OF ANTIPNEUMOCOCCAL HUMORAL IMMUNITY IN ADULT HUMAN BEINGS

By JAMES B. GRAESER, M.D., AND M. CORNWELL HARRISON

(From the Douglas Smith Foundation for Medical Research and the Department of Medicine of The University of Chicago, Chicago)

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Adult human beings, as a group, possess a relatively high degree of humoral immunity against the pneumococcus. This property of blood is a specific one, since it has been shown that individuals may vary greatly in their pneumococidal activity against the different types (1-4). Little is known, however, concerning the constancy of this reaction of the blood of individuals of any particular age group. Ward<sup>1</sup> (2), who has made a few observations on this point, states that he noted no change in the titer of humoral immune substances in nine normal persons on whom pneumococidal tests were repeated. No details were given in these instances as to the actual time interval elapsing between tests. That changes in the titer of the immune substances do occur under normal circumstances is suggested by the studies of Sutliff and Finland (4), who found that the incidence of pneumococidal power in human beings varies with age. In rabbits Woo (5) has shown that the titer of antipneumococcal substances increases with the age of the animal.

The present investigation was undertaken to study the relative constancy of the degree of humoral immunity found in the individuals of the adult age group.

### *Materials and Methods*

*Pneumococidal Tests.*—These were carried out according to the technique described by Robertson and his coworkers (6, 7), employing quantitated mixtures of serum, washed leucocytes, and pneumococci in sealed tubes, which were agitated

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<sup>1</sup> Ward (2) retested one individual, from whom 40 to 50 cc. of blood had been withdrawn at frequent intervals over a period of 2 to 3 months, and found a diminution in amount of humoral immunity against Types I, II and III pneumococci.

during incubation. Human leucocytes were obtained from Group I (Jansky) donors.

The organisms used in the tests were laboratory strains of Type I and Type II pneumococci which had been kept at maximum mouse virulence by frequent animal passage since originally isolated. Preceding each group of tests animal passage was repeated. 0.1 cc. of a  $10^{-7}$  dilution of a broth culture of each organism killed mice in repeated tests in from 24 to 36 hours.

Five dilutions of a standard suspension of these organisms ( $10^{-3}$  to  $10^{-7}$  dilutions of a suspension containing approximately one billion pairs of pneumococci) were added to the tubes containing the mixture of leucocytes and test serum. The actual number of organisms in each dilution was estimated from the colony count of plates seeded with 0.1 cc. of the  $10^{-7}$  dilution (theoretically to contain 100 organisms). Actually, the number of organisms found varied between 21 and 200 per 0.1 cc. of the suspension. This divergence of colony counts is taken into consideration in the following manner.

The counts in the  $10^{-7}$  dilution of the Type I series varied between 75 and 200, and in the Type II series between 13 and 220. It so happened that all of the Type II series with colony counts below 68 showed pneumococidal activity in the tube containing the  $10^{-7}$  dilution. For this reason all cases in both Type I and Type II series could conveniently be grouped within the range of colony counts of 68 to 220, which would thus include the range of colony counts in the Type I series. The difference between the lowest and highest colony counts in the  $10^{-7}$  dilutions in which growth occurred in the two series is thus equivalent to a 3.2 dilution.

It has been found that the pneumococidal tests as carried out in this study are accurate only within one 10-fold dilution. Consequently, a difference in pneumococidal power in repeated tests equivalent to a single 10-fold dilution is disregarded as within the limits of error of the method. To facilitate comparison of the tests with divergent colony counts a 3-fold dilution is also disregarded. Consequently, to exceed what we have thus established as experimental variation, there must occur a difference in pneumococidal power, expressed in terms of the number of organisms killed, equivalent to a 300-fold dilution.

#### *Cases Selected for Study<sup>2</sup>*

Thirty-four individuals, 22 to 40 years of age, in good health, who were employed in various capacities in the Albert Merritt Billings Hospital, were first tested in 1931 in the latter half of the month of September and throughout the month of October. During this time nine members of the group of thirty-four developed a common cold, and serum specimens obtained at the time of maximum symptoms

<sup>2</sup> Four of these cases gave a history of pneumonia 5 or more years previous to the time of this study. None of them had had pneumonia within the last 5 years.

were retested for comparison with their pneumococidal power during the normal period. All of the group of thirty-four individuals were retested during March and April of 1932.

Twelve cases of a severe influenza-like infection occurring in epidemic form among the students of the University of Chicago were chosen for another series. Their ages varied between 20 and 35 years. The clinical course of this infection was similar in all cases. There were 3 to 4 days of temperature ranging from 100–104°F., marked malaise, generalized aching of muscles, mild sore throat, headache, and often a harsh, unproductive cough. There were no abnormal physical signs in the chest. The white blood cell counts ranged between 3,900 and 6,800. Blood samples for the pneumococidal tests were obtained in most instances at the time of the highest temperature, or a day or so thereafter. The first series of determinations were made in February, 1932. 6 to 8 weeks later, after complete convalescence, a second blood sample was similarly tested. All pneumococidal determinations were made within 1 to 2 days after procuring the blood samples.

#### *Changes in Humoral Immunity in Normal Individuals*

The results of the pneumococidal tests of the thirty-four normal individuals are given in Table I. It is seen that about one-third of the individuals possessed no humoral immunity against the Type I pneumococcus, while only one case lacked this property against the Type II. A majority of the members of the series showed relatively high immunity against Pneumococcus Type II. With a similar technique Sutliff and Finland (4) and Ward (2) have demonstrated this same relative difference in pneumococidal activity against these two types.

Considering a change of 300-fold dilution as significant in evaluating this data we found seven individuals who showed a decrease in pneumococidal activity against Type I pneumococcus. Only two cases of diminished humoral immunity occurred against Type II. No increase in pneumococidal power was found in this series. Of the seven individuals showing decreased pneumococidal power toward Type I, two also had a decrease in activity toward Type II. The other five exhibited no significant change in their reaction to the Type

TABLE I  
*Pneumococcal Promoting Activity of the Serum of Normal Individuals*

Individuals	Type I pneumococcus		Change in titer of serum immune substances	Type II pneumococcus		Change in titer of serum immune substances
	No. killed			No. killed		
	Fall, 1931	Spring, 1932		Fall, 1931	Spring, 1932	
1. Po	0	0	0	220,000	28,000	0
2. Ed	0	0	0	144,000	44,000	0
3. Wa	0	0	0	14,400	52,000	0
4. Ma	0	0	0	70,000	5,200	0
5. Sm	0	0	0	7,000	28,000	0
6. Ca	0	0	0	74,000	52,000	0
7. Sa	0	0	0	680	186	0
8. Ha	0	0	0	680,000	130,000	0
9. Or	0	0	0	0	0	0
10. Re	0	0	0	220	1,860	0
11. Ke	200	0	0	23,000	28,000	0
12. Ch	91	0	0	74,000	13,000	0
13. Ro	126	0	0	68,000	130,000	0
14. Hi	1,530	0	Dim.	140,000	540	Dim.
15. Fr	1,530	680	0	1,400	520	0
16. Gi	1,530	680	0	140,000	52,000	0
17. Ri	19,800	0	Dim.	1,440,000	130,000	0
18. Da	19,800	8,100	0	1,440,000	44,000	0
19. He	19,800	8,100	0	144,000	28,000	0
20. Pa	126,000	0	Dim.	68,000	44,000	0
21. Se	126,000	0	Dim.	68,000	44,000	0
22. Sa	15,300	14,500	0	140,000	186,000	0
23. Ha	15,300	80,000	0	140,000	280,000	0
24. Ha	20,000	76,000	0	7,000	130,000	0
25. Br	91,000	81,000	0	74,000	44,000	0
26. St	1,980,000	6,800	Dim.	1,440,000	520,000	0
27. Ro	78,000	8,000	0	7,400	2,800	0
28. Ki	2,000,000	8,000	Dim.	70	0	0
29. J. H.	1,980,000	760,000	0	1,440,000	130,000	0
30. Re	15,300	80,000	0	14,000	54,000	0
31. Fr	91,000	81,000	0	74,000	44,000	0
32. Ma	1,260,000	800,000	0	680,000	28,000	0
33. V. S.	15,300	0	Dim.	1,400	0	Dim.
34. M. H.	780,000	680,000	0	7,400	5,200	0

In the tables, dim. indicates diminished; incr., increased.

II pneumococcus. The majority of cases showed no particular change in their titer of immune substances in the period between the two

series of tests. It is to be noted that the number of microorganisms killed in the spring tests in individual instances tended to be less than in the fall, although the difference did not exceed the 300-fold dilution. One possible reason for this may have been that the colony counts in the respective dilutions happened to be lower in the spring tests.

Examination of the data in the Type I series on the basis of the dilution at which growth occurred rather than the number of microorganisms killed gives the same contrast between the two series of tests. No change occurred in twenty-eight cases. Four showed a drop in titer of three dilutions, three of two dilutions, and five of one dilution. Three cases showed an increase in pneumococcal activity equivalent to one dilution.

Another point to note is the fact that decrease in titer of pneumococcal power for one type was not necessarily associated with a change in reaction toward the other type.

#### *Effect of Colds*

In the series of nine individuals tested while suffering from colds, five exhibited a definite change in pneumococcal power of their blood (Table II). One showed a decrease against Type I but no alteration in reaction toward Type II. Of the four cases with altered reaction toward Type II pneumococci, two showed an increase, and two a decrease. In none of these instances was there any change in their reaction toward the other organism.

At least fourteen of the thirty-four normal persons mentioned above had colds in the interval between the two sets of pneumococcal tests. Three of these were among those showing diminution in pneumococcal titer. Three other cases in the normal series with a similar alteration in their degree of immunity were free of colds throughout the period covered by these tests.

There was thus no uniform response to colds, and we are inclined to interpret these findings as having no relation to the cold itself but to represent fluctuations due to some other cause. It is seen, for instance, in Cases 5, 6, and 8, that the change was a gradual one and not an abrupt reaction to the cold.<sup>3</sup>

<sup>3</sup> The first two tests in eight of the cases were made at intervals of 1 to 3 weeks. In the ninth case 7 weeks elapsed between the normal test and the "cold" test. The last determinations were made from 5 to 6 months after the initial ones.

TABLE II  
*Pneumococcal Promoting Activity of the Serum of Subjects Having Colds*

Individuals	Type I pneumococcus			Change in titer of serum immune substances	Type II pneumococcus			Change in titer of serum immune substances
	No. killed				No. killed			
	Normal	Cold	Normal		Normal	Cold	Normal	
	Fall, 1931	Fall, 1931	Spring, 1932		Fall, 1931	Fall, 1931	Spring, 1932	
1. Fe	1,980	0	76	Dim.	1,440	2,200	13,000	0
2. Gr	780,000	2,000,000	81,000	0	74,000	7,000	44,000	0
3. Sc	200	0	0	0	70,000	25,000	54,000	0
4. St	168	0	0	0	0	2,500	4,400	Inc.
5. Co	0	0*	0	0	2,500	70,000*	280	Dim.
6. Mi	0	0	0	0	220,000	25,000	186	Dim.
7. Co	780,000	1,740,000	680,000	0	74,000	2,200,000	520,000	0
8. Ei	9,100	1,740	1,300	0	740	20,000	520,000	Inc.

\* In this case the test during the cold preceded the first test when patient was normal.

TABLE III  
*Pneumococcal Promoting Activity of the Serum of Patients with Influenza-Like Infection*

Individuals	Type I pneumococcus		Change in titer of serum immune substances	Type II pneumococcus		Change in titer of serum immune substances
	No. killed			No. killed		
	Influenza-like infection*	Normal*		Influenza-like-infection*	Normal*	
1. Ha	0	0	0	0	5,400	Inc.
2. Sc	0	0	0	0	0	0
3. Cl	0	0	0	4,900	5,200	0
4. Be	0	0	0	6,300	1,860	0
5. Bu	0	80	0	4,900	54,000	0
6. Sh	0	0	0	63,000	54,000	0
7. Jo	97,000	80	Dim.	0	0	0
8. Mo	97,000	130,000	0	4,900	52,000	0
9. As	0	0	0	4,900	18,600	0
10. Re	600	0	Dim.	6,300	52,000	0
11. Ba	600,000	1,450,000	0	6,300	18,600	0
12. Fl	60,000	14,500	0	630,000	18,600	0

\* 6 to 8 weeks elapsed between the two series of tests.

*Effect of Influenza-Like Infection*

Nine of the twelve cases tested showed no change in the pneumococcal promoting power of their serum (Table III). In two cases (Nos. 7 and 10) there was a decrease in this serum property for Pneumococcus Type I, and in one (Case 1) an increase for Type II. These tests were done about 2 months apart in the spring of 1932. Here again we note no uniform change in titer as a result of this influenza-like upper respiratory infection.

## DISCUSSION

There are certain limitations to the use of pneumococcal tests for this type of study, although the method is ideally adapted for measuring the relative pneumococcal activity of different blood samples when tested simultaneously. In such experiments the serum-leucocyte mixtures, the state of the microorganism, and the dilutions of the standard pneumococcus suspension are identical for each specimen. In the present experiments the tests in the several serum samples of each individual were done at different times which may have introduced variations in the constituents of the test. The most likely variable would seem to be the possible occurrence of changes in the virulence of the pneumococcus used. While pathogenicity for animals was maintained by frequent passage, we cannot be certain that maximum virulence for human beings is preserved by this means. Thus the possibility cannot be dismissed that some, if not all, of the changes noted in the pneumococcal activity of adult serum were due to biological variations in the pneumococci used in the tests. However, if this were the only explanation of the observed differences we should have expected a uniform reaction of all individuals to this one factor. Actually, the majority of individuals showed no change in the degree of the pneumococcal promoting activity of their serum. On several of the persons in this series we have data from pneumococcal tests done 1 to 2 years previously with precisely the same technique and the same strains of Type I and Type II pneumococci. One individual (No. 23. Ha, Table I), tested in 1929, had no Type I pneumococcal substances in her blood, although in 1931 she had a high degree of immunity against this organism. This particular person had been

working in the laboratory with Type I pneumococci during these intervening years. In the same period no change in pneumococcal activity toward *Pneumococcus* Type II had occurred. Three other individuals tested in 1930 with the same strains have shown no change in their degree of immunity toward these organisms. The data presented in this paper is insufficient to permit any detailed analysis of the possible factors concerned in the changes which we have noted. One might suspect from the studies here reported that seasonal variation was a factor. The tests done in the fall of 1931 were made on individuals in good health who had had the usual advantages of summer outdoor activities. These same subjects in the subsequent months were closely confined indoors during the daylight hours by their duties about the hospital. However, an adequate evaluation of this or any other factor would involve a protracted study over a period of years of a large group of people.

#### SUMMARY

Fifty-five individuals were tested to determine the pneumococcal promoting activity of their serum against Types I and II pneumococci. By repeated tests an attempt was made to study the constancy of the degree of their immunity over intervals of 2 to 6 months. In this group were included nine persons with common colds and twelve cases of a severe influenza-like infection. Fifteen of the fifty-five cases showed a change in titer of their humoral immunity against either Type I or Type II or both. Three of these showed an increase, and twelve a decrease. This reaction in most instances was a specific one in that the altered reaction toward one type was not associated with a similar change toward the other type pneumococcus. Colds and influenza-like infections apparently exerted no effect upon the titer of humoral immune substances.

#### BIBLIOGRAPHY

1. Clough, W. P., *Bull. Johns Hopkins Hosp.*, 1924, **35**, 330.
2. Ward, H. K., *J. Exp. Med.*, 1930, **51**, 675.
3. Robertson, O. H., and Cornwell, M. A., *J. Exp. Med.*, 1930, **52**, 267.
4. Sutliff, W. D., and Finland, M., *J. Exp. Med.*, 1932, **55**, 837.
5. Woo, S. T., *J. Exp. Med.*, 1926, **43**, 623.
6. Robertson, O. H., and Sia, H. P., *J. Exp. Med.*, 1924, **39**, 219.
7. Robertson, O. H., Woo, S. T., and Cheer, S. N., *J. Exp. Med.*, 1924, **40**, 487.