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Research Article



# HETEROPHILE ANTIBODIES IN PNEUMONIA[1]

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Special significance has been ascribed recently to heterophile antibody in relation to pneumococcus infections. It has been suggested that the variable amounts of heterophile antibody in human sera may have some relationship to lobar pneumonia in man. The purpose of this communication is to record the results of tests for sheep cell hemolysin in the sera of patients with pneumococcus lobar pneumonia and to correlate these results with the course of the disease. The results of similar tests in normal human subjects and in subjects receiving injections of various pneumococcus antigens are included for comparison. It was hoped to shed some light on the relationship, if any, of heterophile antibody to the course of lobar pneumonia in man.

Bailey and Shorb (1, 2, 3,) showed that rabbits injected with a large number of cultures of pneumococci of different types develop a potent antisheep hemolysin in their sera. This antibody could be removed from these sera by absorption with homologous or heterologous boiled pneumococci or with boiled sheep red blood corpuscles. All the strains of the different types of pneumococci which they tested, with the exception of some Type III strains, possessed this property of combining with the heterophile antibodies from antisera for homologous and heterologous pneumococci and other heterophile antigens. Rabbits immunized with sheep red blood corpuscles were shown to be relatively resistant to intravenous infection with Type I pneumococci. In experiments with the dermal pneumococcus infection of Goodner in rabbits (4), Powell, Jamieson, Bailey and Hyde (5) found that the usual mouse protective antibody is very much more effective therapeutically when fortified with heterophile antibody. This was particularly true when the heterophile antibody was incited by injections of pneumococci in rabbits. For example, they obtained equal curative effects in Type I infections in rabbits with the following: (1) 500 mouse protective units of Felton's antipneumococcus horse serum, (2) less than 5 mouse protective units and 500 heterophile units in rabbits' antipneumococcus serum, and (3) a pooled serum containing 100 mouse protective units of Type I antibody from horse serum and 500 heterophile units of rabbit antisheep hemolysin.

It was argued by these investigators that since pneumococci have a marked affinity for heterophile antibodies, and sensitization of pneumococci with this antibody, either *in vivo* or *in vitro,* influences the course of infection of rabbits with these organisms, it would seem reasonable to suppose that the variable amounts of heterophile hemolysin in human sera influence the course of lobar pneumonia in man. The larger the amount of such antibody present, the greater should be the resistance to primary invasion and growth in the tissues of the host.

In their earlier work, Bailey and Shorb (1) reported the finding of a definite increase in sheep-cell hemolysin in the sera of cases of lobar pneumonia which recovered. They considered this to be a specific reaction to pneumococcus infection. Although these latter findings are considered highly significant and are frequently referred to, the data in only one case are mentioned as an example. In this "one case infected with an organism reported as a Type IV pneumococcus, the titer of the serum on the fifth day of the disease was 16 units per cc. and at the end of the twelfth day (sixth afebrile) the serum contained 100 units of anti-sheep hemolysin per cc." Data on the titer of sheep cell hemolysin in the sera of a considerable number of patients with lobar pneumonia are not available in the literature.

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## METHOD AND MATERIALS

*Titrations of sheep cell hemolysin* were carried out as follows: serum was incubated in a water bath at 56° C. for 20 minutes; to 1 cc. of serum dilutions 1:10, 1:20 etc. to 1:5120 was added 1 cc. of a mixture of equal parts of a 2.5 per cent suspension of washed sheep cells and a 1:10 dilution of fresh guinea pig serum; the mixture was incubated at 37.5° C. for 1 hour and read directly. The titer of hemolysin was read as the greatest dilution of serum showing almost complete (more than 50 per cent) hemolysis. Many sera were tested simultaneously, known hemolytic and non-hemolytic sera being included each time. While the true "heterophile" character of the antibody thus measured has not been proven here, it may be considered as such for practical purposes. Furthermore, the results are considered only for purposes of comparison and not for their absolute values. All the sera of pneumococcus pneumonia patients were tested for agglutinins for the homologous type of pneumococcus and for several or all of the other 31 Cooper types (6).

*Subjects.* Sera were obtained during the acute disease and during convalescence from patients over 16 years of age, who had definite clinical and roentgenographic evidences of lobar pneumonia and from whom pneumococci were isolated and typed. A total of 227 sera from 120 patients were tested. These included 51 sera from 24 patients treated with concentrated antibody from anti-pneumococcus horse serum. The distribution of the cases and sera in relation to pneumococcus types and to the outcome of the disease is shown in Table I.

For controls, 276 sera from as many normal adults were tested. These sera were obtained from individuals who later received injections of various pneumococcus antigens. A second group of 168 sera was obtained from 122 of the latter individuals at intervals after immunization. All of these sera were tested and found to have pneumococcus mouse protective antibody. They were also tested for hemolysin content and the results included for comparison.

TABLE I

*Sources of sera tested*

| Pneumococcus Type | Number of patients | Number of sera |
|---|---|---|
| Pneumonia patients | | |
| I | 42 | 79 |
| II | 4 | 7 |
| III | 13 | 21 |
| IV | 8 | 14 |
| V | 3 | 5 |
| VI | 2 | 3 |
| VII | 5 | 12 |
| VIII | 14 | 36 |
| IX | 2 | 2 |
| X | 1 | 1 |
| XI | 1 | 1 |
| XII | 3 | 6 |
| XIV | 5 | 10 |
| XVIII | 1 | 1 |
| XIX | 4 | 9 |
| XX | 3 | 7 |
| XXI | 5 | 6 |
| XXIV | 1 | 1 |
| XXIX | 1 | 2 |
| XXXI | 1 | 2 |
| XXXII | 1 | 2 |
| All types | 120 | 227 |
| Fatal cases (average age 42.2 years) | 26 | 38 |
| Recovered cases (average age 36.9 years): | | |
| Tested in febrile period only | 9 | 9 |
| Tested only in postfebrile period | 44 | 58 |
| Tested before and after crisis | 41 | 122 |
| | 120 | 227 |
| Non-pneumonic individuals | | |
| Controls (average age 30.4 years) | 276 | 276 |
| Normal subjects after immunization | 122 | 168 |
| Total number of sera tested | | 671 |

## ANALYSIS OF RESULTS

*Comparison of hemolytic titers in pneumonia patients and in controls.* The frequency with which various titers of sheep cell hemolysin were encountered in the pneumonia patients, in the immunized individuals and in the normal controls is shown in Table II. The percentage incidence of the various hemolytic titers among the sera of pneumonia patients is essentially similar to that found among the normal controls. This is par-

TABLE II

*Comparison of titers of sheep cell hemolysin in pneumonia patients, in immunized individuals and in normal controls*

| Titer of sheep cell hemolysin (serum dilution) | Patients with pneumococcic pneumonia | | Individuals immunized with pneumococcic antigens | | Normal controls | |
|---|---|---|---|---|---|---|
| | Number of sera | Per cent | Number of sera | Per cent | Number of sera | Per cent |
| 0† | $18^{2*}$ | 7.9 | 8 | 4.8 | 23 | 8.3 |
| 1:10 | $14^{2}$ | 6.3 | 24 | 14.3 | 34 | 12.3 |
| 1:20 | $11^{2}$ | 4.8 | 24 | 14.3 | 30 | 10.9 |
| 1:40 | $53^{3}$ | 23.3 | 33 | 19.6 | 49 | 17.7 |
| 1:80 | $67^{6}$ | 29.5 | 42 | 25.0 | 70 | 25.3 |
| 1:160 | $33^{6}$ | 14.6 | 29 | 17.3 | 49 | 17.7 |
| 1:320 | $20^{3}$ | 8.8 | 6 | 3.6 | 15 | 5.4 |
| 1:640 | $7^{2}$ | 3.1 | 2 | 1.2 | 5 | 1.9 |
| 1:1280 | $4^{3}$‡ | 1.8 | 0 | 0 | 1 | 0.4 |
| Number of sera | $227^{30}$ | | 168 | | 276 | |

* Raised numerals represent numbers of sera obtained after horse serum administration.

† No hemolysis in 1:10 dilution.

‡ Including 1 serum with titer 1:5120.

TABLE III

*Distribution of titers of sheep cell hemolysin in the sera of patients with pneumococcic pneumonia*

| Titer of hemolysin (serum dilution) | Fatal cases | | Recovered cases | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Febrile period | | Postfebrile period | | Postfebrile (Excluding serum recipients) | |
| | Number of sera | Per cent | Number of sera | Per cent | Number of sera | Per cent | Number of sera | Per cent |
| 0† | 6 | 15.3 | 7 | 9.9 | 5 | 4.2 | 3 | 3.2 |
| 1 : 10 | 4 | 10.5 | 4‡ | 5.6 | 6 | 5.1 | 5 | 5.4 |
| 1 : 20 | 2 | 5.3 | 2 | 2.8 | 7 | 5.9 | 5 | 5.4 |
| 1 : 40 | 14 | 36.5 | 18* | 25.4 | 21 | 17.8 | 20 | 21.5 |
| 1 : 80 | 5 | 13.2 | 27 | 38.0 | 35 | 29.7 | 30 | 32.3 |
| 1 : 160 | 3 | 7.9 | 8 | 11.3 | 22 | 18.6 | 14 | 15.1 |
| 1 : 320 | 3‡ | 7.9 | 3 | 4.2 | 14 | 11.9 | 12 | 12.9 |
| 1 : 640 | 0 | 0 | 2 | 2.8 | 5 | 4.2 | 3 | 3.2 |
| 1 : 1280 | 1‡ | 2.6 | 0 | 0 | 3 | 2.6 | 1 | 1.1 |
| Total number of cases | 38 | | 71 | | 118 | | 93 | |

* Including 2 sera obtained after horse serum administration.

† No hemolysis in 1 : 10 dilution.

‡ Including 1 serum obtained after horse serum administration.

ticularly true when those sera are excluded which were obtained after pneumococcus antibody treatment, thus excluding the possible heterogenetic activity of the horse serum injected (7, 8). The titers of the sera of the individuals immunized with various antigenic pneumococcus substances were also very similar in distribution to those of the controls.

The results of the tests for sheep cell hemolysin in the sera of the pneumonia patients are analyzed in further detail in Tables III, IV and V. The numbers involved in these tables are too few to be statistically significant. There seems to be a slightly higher incidence of lower titers among the sera of the fatal cases than among those that recovered (Table III). Similar differences may be noted between the sera with and those without demonstrable pneumococcus agglutinins (Table IV) and also between the sera obtained early and those obtained later (Table V). These differences are less evident if the sera obtained after horse serum administration are excluded. Differences between groups of patients, however, are difficult to evaluate because of the many factors which may come into play. The average age of the patients, for example, was greater among those who died than among those who recovered (*cf.* Table I). This may account, at least in part, for the seemingly lower titers among the former (9). A better possibility of evaluating the relationship of heterophile antibody to the course of pneumonia is offered by a study of the changes in titer of hemolysin observed in individual cases.

*Titers of sheep cell hemolysin in individual patients and changes in these titers in the course of*

TABLE IV

*Correlation of pneumococcus agglutinin titer and titer of sheep cell hemolysin in sera of patients with pneumococcic pneumonia*

| Titer of homologous pneumococcus agglutinin (Serum dilution) | Titer of sheep cell hemolysin (Serum dilution) | | | | | | | | | Total number of sera |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 : 10 | 1 : 20 | 1 : 40 | 1 : 80 | 1 : 160 | 1 : 320 | 1 : 640 | 1 : 1280 | |
| 0 Fatal | 6 | 3 | 2 | 11 | 5 | 3 | 1 | 0 | 1 | 32 |
| 0 Febrile, lived | 4 | 2 | 2 | 7 | 21 | 7 | 2 | 2 | 0 | 47 |
| 0 Postfebrile | 1 | 4 | 3 | 6 | 8 | 6 | 0 | 0 | 1 | 29 |
| 1 : 2 | 0 | 0 | 0 | 3 | 2 | 0 | 2 | 0 | 2† | 9 |
| 1 : 4 | 4 | 2 | 1 | 7* | 9 | 8 | 6 | 1 | 0 | 38 |
| 1 : 8 | 2 | 1* | 2 | 8* | 10 | 3 | 3* | 1 | 0 | 30 |
| 1 : 16 | 0 | 1 | 0 | 5 | 7 | 3 | 4 | 0 | 0 | 20 |
| 1 : 32 | 1 | 1 | 1 | 4 | 3 | 3 | 1* | 2 | 0 | 16 |
| 1 : 64 | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 1 | 0 | 6 |
| 0 | 11 | 9 | 7 | 24 | 34 | 16 | 3 | 2 | 2 | 108‡ |
| 1 : 2 to 1 : 64 | 7 | 5 | 4 | 29 | 33 | 17 | 17 | 5 | 2 | 119§ |
| All sera | 18 | 14 | 11 | 53 | 67 | 33 | 20 | 7 | 4 | 227 |

* Including 1 serum from a fatal case.

† Including 1 with titer 1 : 5120.

‡ Average titer = 1 : 104.

§ Average titer = 1 : 182.

TABLE V

*Correlation of stage of the pneumonia and titer of sheep-cell hemolysin*

| Days after onset of pneumonia | Titer of sheep-cell hemolysin (serum dilution) | | | | | | | | | Total number of sera tested |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 : 10 | 1 : 20 | 1 : 40 | 1 : 80 | 1 : 160 | 1 : 320 | 1 : 640 | 1 : 1280 | |
| 1–3 | 4(2)* | 2(2) | 1 | 4(1) | 9(1) | 4(1) | 2(1) | 0 | 0 | 26 |
| 4–6 | 4 | 5(1) | 2 | 11(6) | 18(2) | 5(2) | 2 | 2 | 0 | 49 |
| 7–9 | 4(2) | 1(1) | 3(1) | 14(2) | 11 | 6 | 1 | 0 | 0 | 40 |
| 10–12 | 5(2) | 1 | 1 | 7 | 12(1) | 5 | 4 | 1 | 2 | 38 |
| 13–15 | 0 | 4 | 0 | 6 | 8(1) | 5 | 1 | 2 | 0 | 26 |
| 16–18 | 0 | 1 | 1 | 5(1) | 2 | 2 | 5(1) | 0 | 0 | 16 |
| 19–21 | 1 | 0 | 2(1) | 1 | 0 | 3 | 2 | 0 | 0 | 9 |
| 22+ | 0 | 0 | 1 | 5(4) | 7 | 3 | 3(1) | 2 | 2†(1) | 23 |
| All sera | 18(6) | 14(4) | 11(2) | 53(14) | 67(5) | 33(3) | 20(3) | 7 | 4(1) | 227 |
| 1–9 | $12^{2}$‡ | $8^{3}$ | $6^{2}$ | $29^{2}$ | $38^{4}$ | $15^{1}$ | 5 | 2 | 0 | $115^{14}$§ |
| 10 or more | $6^{1}$ | $6^{1}$ | 5 | $24^{5}$ | $29^{3}$ | $18^{4}$ | $15^{3}$ | $5^{2}$ | $4^{3}$† | $112^{22}$‖ |

* Parentheses enclose numbers of sera from fatal cases which are included.
† Includes 1 with titer of 1 : 5120.
‡ Superscripts represent sera obtained after specific serum treatment.
§ Average titer is 1 : 85; excluding those obtained after serum therapy = 1 : 90.
‖ Average titer 1 : 208; excluding those obtained after serum therapy = 1 : 136.

*pneumonia.* In the preceding sections, the sera of pneumonia patients have been considered as a group without reference to the hemolytic titers of individual patients and to changes in these titers observed in successive sera obtained from the same individual. In Table VI are indicated the numbers of patients in whom the higher and the lower titers of hemolysin were encountered at any time. Here again, the numbers are too few to be statistically significant. It is worth noting, however, that both the high and the low titers were frequent in each of the groups of patients. An appreciable number of the recovered patients had received injections of horse serum, thus accounting for the predominance of high titers among these patients.

It is of interest to note that 5 of the 7 fatal cases with sera which hemolyzed sheep's red

TABLE VI

*Occurrence of high and low hemolytic titers and of changes in these titers among patients with pneumococcic pneumonia*

| | | | Fatal cases | Recovered cases | | |
|---|---|---|---|---|---|---|
| | | | | Tested only in febrile period | Tested only during convalescence | Tested in febrile and in post-febrile period |
| Number of patients | | | 26 | 9 | 44 | 41 |
| Patients whose serum, at any time, had sheep-cell hemolysin in titers of: | (*a*) 1 : 20 or lower | Number of patients | 9 | 2 | 10 | 10 |
| | | Per cent | 34.6 | 22.2 | 22.7 | 24.4 |
| | (*b*) 1 : 160 or higher | Number of patients | 7 | 1 | 17* | 22† |
| | | Per cent | 26.9 | 11.1 | 38.6 | 53.6 |
| Patients in whom the hemolytic titer in successive sera showed: | (*a*) No change | | 6 | — | 6 | 14‡ |
| | (*b*) Twofold increase | | 2 | — | 4 | 4‖ |
| | (*c*) Twofold decrease | | 2 | — | 3 | 9 |
| | (*d*) Fourfold or greater increase | | 1 | — | 1§ | 11¶ |
| | (*e*) Fourfold or greater decrease | | 1 | — | 0 | 3 |

* In 3 of these cases the sera were obtained after serum therapy.
† Including 9 patients with serum sickness.
‡ In 1 there was an increase and in another a decrease (twofold in each instance) before returning to original level.
§ A serum treated case.
‖ In 2 instances there was a twofold drop in titer before the rise.
¶ In 5 instances there was a drop in titer preceding the rise; this drop was twofold in one, fourfold in another and twofold in the remaining 3. In 8 of these 11 patients, the increase in hemolysin was associated with serum sickness.

TABLE VII

*Summary of observations in 5 cases referred to in the text*

| Name, sex and age | Pneumococcus Type | Termination of pneumonia | | Patient's sera | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | Mode | Day | Day of disease obtained | Homologous type pneumococcus agglutinins (Titer = serum dilution) | Sheep cell hemolysin | |
| J.G. ♂ 49 years | VIII | Lysis | 11–14 | 4 | 0 | 1 : 160 | Blood culture positive 2nd to 6th day |
| | | | | 5 | 0 | 0 | |
| | | | | 6 | 0 | 1 : 10 | Irregular fever 20th to 39th day |
| | | | | 12 | 1 : 4 | 1 : 80 | |
| | | | | 18 | 1 : 2 | 1 : 80 | Subcutaneous abscess drained |
| | | | | 25 | 1 : 4 | 1 : 80 | |
| | | | | 34 | 1 : 4 | 1 : 80 | Well after 40th day |
| | | | | 39 | 1 : 4 | 1 : 80 | |
| | | | | 52 | 0 | 1 : 1280 | |
| W.G. ♂ 59 years | I | Crisis | 8 | 7 | 0 | 1 : 40 | Blood culture negative |
| | | | | 11 | 0 | 1 : 160 | |
| | | | | 15 | 0 | 1 : 160 | |
| E.M. ♀ 34 years | XX | Crisis | 10 | 7 | 0 | 1 : 80 | Blood culture negative |
| | | | | 11 | 1 : 16 | 1 : 80 | |
| | | | | 17 | 1 : 4 | 1 : 320 | |
| J.W. ♂ 78 years | XIV | Died | 17 | 9 | 0 | 1 : 40 | Blood cultures: 9th day positive; 17th day positive, 700 colonies per cc. |
| | | | | 17 | 0 | 1 : 320 | |
| J.E.S. ♂ 15 years | II | Crisis | 5 | 4 | 0 | 1 : 80 | Blood culture negative. Concentrated antibody (antipneumococcus horse serum) 100 cc. given on 4th and 5th day |
| | | | | 6 | 1 : 8 | 1 : 20 | |
| | | | | 15 | 1 : 16 | 1 : 320 | |

blood corpuscles in a dilution of 1 : 160 or higher had a pneumococcus bacteremia which was demonstrated in the same blood. The serum of one of these patients had a hemolytic titer of 1 : 1280 on the day before death. This patient had Type II pneumococcus pneumonia and received serum 7 days previously or 3 days after the onset. Bacteremia persisted throughout this period. The sera of two fatal cases of pneumococcus meningitis following one month after pneumonia each had a hemolytic titer of 1 : 320. The meningitis was preceded in one case by mastoiditis and in the other by empyema and recurrent bacteremia. The former had a Type III pneumococcus, and the latter had Type I and received antipneumococcus serum.

Table VI also shows the number of patients in whom changes in hemolytic titer were found in successive sera. It is seen that among the fatal cases, the 12 in which multiple determinations were obtained showed no consistent changes in hemolytic titer. In 4 cases with unchanged titers and in one with an eightfold increase (J. W., see Table VII), pneumococcus bacteremia was present and persisted until the time of death. The one patient who showed a fourfold decrease in hemolytic titer had a negative blood culture and had received injections of horse serum on the day before the second serum was obtained. The patients from whom multiple sera were obtained only during convalescence also showed no significant changes in the titers of hemolysin.

Among the 41 pneumonia patients whose sera were tested during both the febrile and the postfebrile periods, 27 or 65.9 per cent showed either no change or only a twofold difference after crisis; and decreases of this order were more frequent than increases. The 3 patients whose sera showed a fourfold decrease in titer after recovery all showed agglutinins for the homologous type of pneumococcus in the later sera, and none in the earlier sera which had the higher titers of hemolysin. Of the 11 patients whose postfebrile sera showed more than a twofold increase above the original titer, 8 had serum sickness and 2 others (W. G. and E. M., see Table VII) showed

a fourfold increase above the febrile level without having received serum. Neither of the two latter patients had demonstrable bacteremia, and one failed to exhibit agglutinins for the homologous Type I pneumococcus in the postfebrile serum.

One patient (J. G., see Table VII) who showed a marked increase in the hemolytic action of his serum is of some interest. When first tested the titer of his serum was 1:160. This declined in the next 2 days. Blood cultures during this time yielded Type VIII pneumococcus repeatedly. One week later, after recovery by lysis, the hemolytic titer was 1:80, at which level it remained for 4 weeks. During this interval the patient had low grade fever and several subcutaneous abscesses. These were drained and yielded Type VIII pneumococci in pure culture. Two weeks after all signs of persistent infection had cleared, the hemolytic titer of the serum was 1:1280. This is the only case in which a significant drop in titer occurred during the bacteremic period to be followed by a sharp rise after complete recovery.

Other observations of interest may be noted. In Table VII are noted the relevant data in 4 patients to whom reference has been made in the preceding paragraphs. A fifth patient (J. E. S.) with Type II pneumonia is also included. This patient showed a significant decline in hemolytic titer following serum therapy to be followed later by an increase above the original level associated with the clinical picture of serum sickness. This decrease was the largest observed after serum administration. In 3 other patients twofold decreases were noted within the first day after administration of the therapeutic sera. The average titer of hemolysin in the first 9 days of the disease was 1:90 when horse serum had not been given and 1:45 in those sera obtained after therapeutic antibody injections. After the ninth day the average titer of the sera of cases treated with antipneumococcus horse serum was 1:500 as compared with 1:136 in those not so treated.

The highest titer of hemolysin noted was in a dilution of 1:5120 in serum obtained from a negro of 17 with Type I pneumonia 3 weeks after he had had symptoms of serum sickness. The hemolytic titer during his serum sickness was 1:1280.

Although mild serum sickness was frequent among the antibody treated cases, immediate reactions were not observed. Only one patient, among those studied, had a chill following a second dose of serum (13 cc.). No reaction followed the first dose of 2 cc. in this patient. The hemolytic titer of this patient's serum was 1:320 before antibody treatment was begun.

There was no correlation whatever between hemolytic titer and the type of pneumococcus.

## DISCUSSION

The present study was undertaken to determine whether the pneumococcus, in causing pneumonia in man, also acts as a heterophile antigen and, if so, whether this is of significance in relation to the course of the disease. The possibility that it may do so was suggested by the heterogenetic activity of various pneumococcus strains when they were injected into rabbits or when they produced experimental infections in this species (1, 2, 3, 4). The protective (1) and curative (4) value of heterophile antibody, when actively or passively induced in rabbits, also suggested that such antibody might have therapeutic significance in human cases of pneumonia.

The weight of the evidence adduced from a study of the hemolytic action of sera of pneumonia patients on sheeps' red blood corpuscles indicates that such a heterophilic activity, if present, is low grade, infrequent and probably of no importance in relation to the course and outcome of the disease. 1. During the febrile period in the favorable cases of pneumonia the titers of sheep cell hemolysin in the serum are the same as in normal controls. 2. Moderately high titers of sheep cell hemolysin are frequently encountered in the serum of patients with pneumococcus bacteremia, even shortly before death. 3. Bacteremia may be present without altering the hemolytic titer, and occasionally the hemolytic titer may be found to increase in spite of an increasing bacterial invasion. 4. Low titers are encountered frequently in patients who recover. 5. Successive sera obtained during the febrile and postfebrile period show that the titers of hemolysin may drop just as frequently and to the same degree as they may rise in the course of convalescence from pneumonia. 6. Significant increases in hemolytic titer are en-

countered only rarely, except following the administration of antipneumococcus horse serum.

On the other hand, the slightly higher incidence of lower titers among the sera of fatal cases and the somewhat greater frequency of higher titers during convalescence might possibly be considered to result from the heterogenetic action of pneumococci. The lower titers in the fatal cases might be the result of partial absorption of the "natural" heterophile antibody by the heterophile component of the pneumococcus antigen, and the higher titers would then result from the production of antibody by this antigen. This possibility is not borne out by the consideration of the titers of hemolysin in the sera of individual patients and by the changes noted when successive sera from the same patient are tested.

The results of the tests for hemolysin in immunized individuals are also of interest. The antigens used exhibited considerable species activity as indicated by the demonstration, in the recipients, of antibodies for types of pneumococci other than those from which they were prepared (13). The hemolysin titers in the sera of individuals immunized with these antigens were the same as in the normal controls. The heterophile component of the pneumococcus which is active in animals is also species specific, but since its exact composition is not known, beyond its association with the lipoid fraction, the bearing of the present findings on this phase of the problem can not be evaluated.

These observations are not entirely incompatible with the results of the rabbit experiments previously mentioned. The optimal heterogenetic activity of pneumococci was obtained after vigorous treatment of the organisms, for example by boiling, before they were given to the animals. Furthermore, the strain which exhibited the greatest heterogenetic activity was a completely avirulent one originally derived from a Type I pneumococcus. Another very active Type I strain was relatively avirulent as indicated by the marked increase in the fatality rate among rabbits with dermal pneumonia produced by this organism after 2 and especially after 33 rabbit passages. When this latter strain was used to infect rabbits after 33 rabbit passages, no protection was afforded by heterophile antibody actively or passively induced in the animals. It is possible, therefore, that the heterogenetic property of pneumococci is related to changes which occur during cultivation and in the methods of treating the organisms, and has no particular bearing in relation to actual infection. In such infections as pneumonia which are associated with highly type specific and usually virulent strains, the heterogenetic properties of pneumococci probably have no significance.

In contrast to the unconvincing evidence presented by these findings, it is of interest to consider the more positive observations in the patients who received concentrated antipneumococcus horse serum therapeutically. The value of this homologous serum in the treatment of Type I pneumococcus pneumonia is now well established. That horse serum acts as a heterophile antigen in man has been shown by many workers (7, 8, 10, 11, 12). That this refined fraction is potent as a heterophile antigen is indicated by the observations presented, namely, by the decrease in titer of sheep cell hemolysin in the first few days after serum administration and the marked increase in this titer in the same patients later, particularly when serum sickness occurs. There is no indication, however, that this heterophile activity has any influence on the course of the pneumonia. Favorable effects were observed from the use of serum in spite of an average decrease of 50 per cent in the hemolytic titer of the serum. Fatal outcome, on the other hand, has been observed even after the heterophile antibody had increased significantly in patients treated with serum, presumably in response to the horse serum injections and in spite of persistent pneumococcus invasion. Even the immediate reactions to injections of horse serum, such as were observed by Davidsohn (10) with crude therapeutic antisera in patients with heterophile antibody, were not encountered after the intravenous injections of the refined antipneumococcus concentrates. These observations indicate that even known heterophile antigen has no particular relationship to the course of pneumonia in man.

On the basis of all of the findings here presented, it is felt that the heterophile activity of the pneumococcus is probably of no significance in human cases of lobar pneumonia.

## SUMMARY AND CONCLUSIONS

1. The content of hemolysin for sheep red blood corpuscles was measured in 227 sera from 120 patients with lobar pneumonia due to various types of pneumococci. Among these, 51 sera were from 24 patients treated with concentrated antipneumococcus horse serum. Similar tests were made on the sera of 276 normal individuals and in 168 sera from 122 persons who received immunizing injections of pneumococcus antigens.

2. The frequency of various titers of sheep cell hemolysin among the sera of pneumonia cases, particularly when those obtained after administration of therapeutic serum were excluded, was the same as in normal subjects, and this was also true of the sera of the immunized individuals.

3. Successive sera from the same patients only rarely showed significant increases in the titer of sheep cell hemolysin except in relation to horse serum injections.

4. There was no constant relationship between the hemolytic titer of the serum and the outcome of the disease. High titers and increasing titers were found in sera from fatal cases, even in the presence of bacteremia. Low and declining titers were frequently encountered coincident with normal recovery.

5. Concentrated pneumococcus antibody (Felton) is an active heterophile antigen. This is shown by the decrease in hemolytic titer for a few days after its administration and the subsequent marked rise in this titer in a considerable proportion of cases. The heterophile activity of this antibody, however, has no relation either to the occurrence of immediate untoward reactions or to the course and outcome of the pneumonia.

6. Heterophile antibody probably has no significance in human cases of lobar pneumonia.

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