CERTAIN ASPECTS OF MOUSE PROTECTION TESTS FOR ANTIBODY IN PNEUMOCOCCUS PNEUMONIA*

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In continuation of the study of antibody in lobar pneumonia 342 mouse protection tests have been done on 63 patients† as follows: 17 Type I, 4 Type II and 5 Type III pneumococcus infections which had no specific therapy and 29 Type I and 8 Type II cases treated with Felton's antibody. The method has already been described (1). Our findings in the series are, on the whole, similar to those obtained by Neufeld and Haendel (2), Dochez (3), Clough (4), Baldwin and Rhoades (5) and Trask, O'Donovan, Moore and Beebe (6) and serve to amplify and sustain the results presented in a previous communication dealing with a part of this material (1).

In view of substantial agreement as to the usual results of mouse protection tests in pneumonia, it seems unnecessary to present our findings in detail, but certain aspects of the relation of antibody to pneumonia deserve further brief discussion.

Early Appearance of Antibody and Its Relation to the Time of Recovery

In general in our series, as noted by Dochez (3) in his investigation, the time of appearance of antibody coincides rather sharply with the

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period of critical fall in the temperature. Our opportunity to investigate the early period of the disease has been limited. The serum from eight patients tested during the 1st or 2nd day showed no protective power. Protection was demonstrated as early as the 3rd day in three (one Type I, one Type II and one Type III) of eleven cases, and on the 4th day in five (four Type I and one Type III) of fourteen cases.

As shown in the accompanying chart (Chart 1) in one patient (Case 1, Type I) with protection on the 3rd day, the temperature fell to

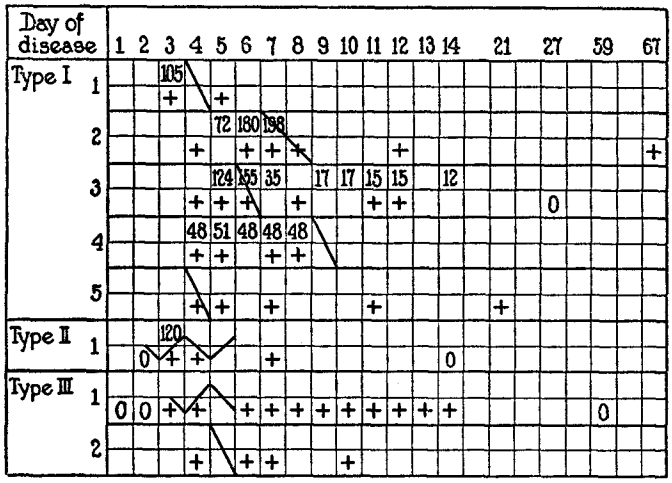

CHART 1. Early appearance of antibody and its relation to time of recovery.

O = no mouse survivals. + = one or more surviving mice. \ = fall in temperature. ∧∧ = irregular temperature fall. The figures in the upper space show the number of thousands of Felton units of the corresponding type which were administered after the serum sample was taken.

normal on the 4th day and in another (Case 5, Type I) with protection on the 4th day the patient became afebrile the same day. The early appearance of protection is not, however, always followed by a speedy termination of the disease, as the two other patients (Case 1, Type II, and Case 1, Type III) with protection on the 3rd day remained febrile until the 6th while three (Case 3, Type I, Case 2, Type I, and Case 4 Type I) with protection on the 4th day had fever until the 7th, 9th and 10th day respectively, in spite of considerable amounts of Felton's antibody after the collection of the first blood sample.

Recovery without Demonstrable Antibody

Recovery without demonstrable protection during or immediately after the fall in temperature was noted in two of ten cases by Dochez (3). One of these (Experiment 9) showed protective substance 16 days after crisis, on the 22nd day of the disease. Clough (4) failed to demonstrate protective power after crisis or lysis in three of twelve cases, but in these three only one specimen of serum was tested. One (Case 9) of Baldwin and Rhoades' cases (5) had a crisis on the 15th day without protective substance in the serum, but later developed a fatal bacteriemia. Two of our seventeen untreated Type I cases had no demonstrable antibody at or near the time of crisis. In one of these, with crisis on the 8th day, the blood was negative when tested on the 8th, 9th, 10th, 12th, 14th and 20th days and was not tested thereafter. In the other, with crisis on the 6th day, the blood was negative on the 4th, 5th, 6th, 8th, 11th and 17th days but showed protection on the 25th, 32nd and 39th days after onset. A third Type I case, which received 60,000 units of Felton's Type I antibody near the time of his crisis on the 3rd day, showed no protection on the 3rd day (before serum), the irregular survival of only one of fourteen mice on the 5th day, and no protection on the 6th, 8th or 11th days, but by the 29th day after onset showed a relatively high degree of protection. The late appearance of protection in the last two cases is an assurance that the disease was due to a Type I infection. These three cases illustrate the importance of frequent repetition of tests to avoid erroneous negative results.

The significance of the absence of protective substance at the time of recovery and its appearance later is not clear. It is possible that mouse protection tests measure only an excess of antibody over the amount needed for recovery. It is also possible, in view of Goodner's (8) and Tillett's (9) inability to protect mice with immune rabbit serum that mouse tests as an index of available antibody are not wholly reliable and that a part of the human antibodies are undemonstrable with an alien species. Another explanation involves the presence in the blood serum of an antagonistic substance, sufficient in amount to inhibit mouse protection during the course of the infection and its disappearance after recovery.

Irregular Protection Tests in Mice

Irregular protection tests are not uncommon in the investigations of others. Of a total of 379* mouse protection tests, 75 show scattered or irregular survivals of mice, and in 33 tests these irregularities are marked enough to make it impossible to estimate the approximate number of lethal doses of pneumococci against which the individual serum sample protected. We are unable to trace these sporadic survivals to any error in technic, and it seems probable that the survival of even one mouse, in the absence of technical error and in view of the death of the control mice, indicates the presence of some protective substance in the serum injected with the organisms.

Amount of Antibody in Untreated Cases

As noted by Dochez (3) the amount of protection demonstrable at the time of crisis, though variable, is small compared with the potency of sera obtained by active immunization of larger animals. Of 17 untreated cases in our series, tested within 1 day on either side of crisis, 12 (8 Type I, 1 Type II, 3 Type III) had protective power against 100 lethal doses or less of pneumococci of corresponding type and none of the remaining five (3 Type I, 2 Type II) had protective power against more than 10,000 lethal doses.†

Amount of Protection in Treated Cases

In attempting to estimate the effect of late intravenous specific treatment on the protective power of the patient's serum, the problem is complicated by the usual production during the course of the disease of small and varying amounts of protective substances by the infected individual himself.

Twenty-nine surviving cases, treated with Felton's antibody, had protection tests on 1 of the 3 days centering at the critical fall in temperature. Of these, eleven (all Type I) showed protective power

* This figure includes a miscellaneous group of 37 protection tests which has not been included in the series, consisting of cases on which only one test was done, cases in which a reliable history was lacking, Type IV cases which were tested against types for which antibody had been administered and the like.

† The equivalent of only 0.05 Felton units per cubic centimeter of serum.

against every dose of culture used, up to and including one million lethal doses. Of five fatal treated cases, three (2 Type I, 1 Type II) showed the same protective power within 1 day of death. Thus, in 14 out of 34 treated cases it was possible to demonstrate more protective substance in the serum than would have been expected in untreated cases at the time the tests were made.

The Bearing of Protection Tests on Dosage

In attempting to estimate appropriate dosage by mouse protection tests account must be taken of the usual spontaneous production of small amounts of antibody during the course of the disease. The problem is complicated by occasional recovery without evidence of any protective substance and the lack of any definite correlation between the amount of protection and the time of recovery or the apparent severity of the disease.

It would be valuable to know the approximate dosage necessary to produce a large protective balance in the blood stream. In nineteen instances (18 Type I, 1 Type II) where protection tests* followed specific intravenous therapy with less than 200,000 Felton units between the 3rd and 6th days there was protection in amounts not otherwise to be expected in only six (all Type I). Of eight cases (7 Type I, 1 Type II) treated between the 2nd and the 7th days with over 200,000 units only one (Type I) failed to show unusual protective power. In general it may be said that doses of less than 200,000 Felton units are likely to be inadequate for the production of an unusual amount of protection in the blood stream. It must be added, however, that we have found protection against a million lethal doses in the serum after doses as small as 25,000 Felton units and yet this amount of protection has failed to appear after as much as 400,000 units.

The Presence of Antibody with Septicemia

In Baldwin and Rhoades (5) experience, bacteriemia and protective substances occurred simultaneously in the blood in only one instance in a total of 45 specimens of blood. We have been able to demonstrate protective substances late in the disease in blood from which pneumococci were cultivated in three instances.

* These tests were done on blood taken not less than 8 hours after treatment.

One of our cases came to the hospital because of empyema following pneumonia and did not improve after surgical drainage. On the 83rd day of his disease the blood culture was positive for Type I pneumococcus in spite of the presence of mouse protection against 100,000 lethal doses. No specific treatment had been given. Autopsy 3 days later showed a Type I pneumococcus endocarditis.

The second case was observed throughout his disease* and was given no specific treatment. The blood was sterile on the 2nd day but from the 8th until death on

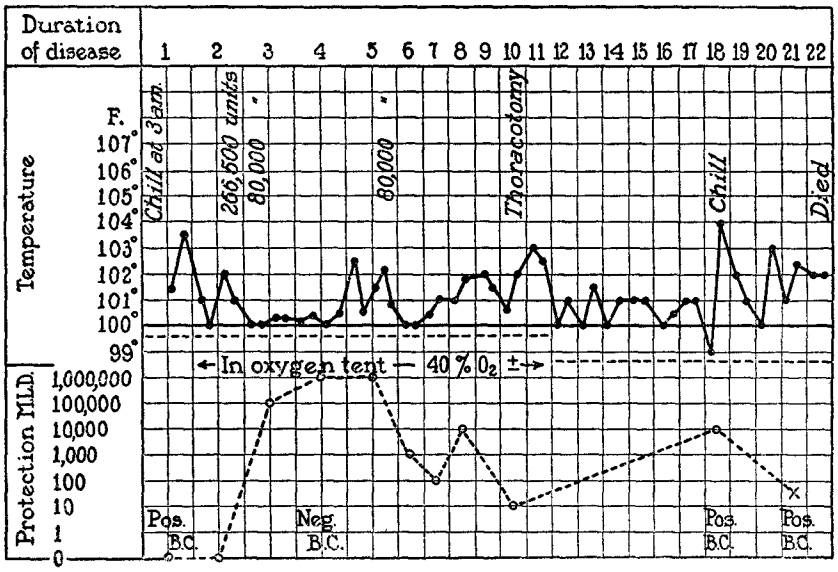

CHART 2. The presence of antibody with septicemia.

A severe case, showing the effect of treatment with antibody on the blood culture and protective substances early in the disease. After the antibody was discontinued the patient developed septicemia in spite of protective power in the blood.

The "normal" base line drops from 99.6° to 98.6°F. on the 12th day when mouth temperatures were substituted for rectal readings. o----o = amount of protection. X = irregular mouse survivals. B. C. = blood culture.

the 23rd day showed Type I pneumococci in each of nine cultures. No protective substance was demonstrated from the 10th to the 13th day by three tests, but on the 17th and 21st days, when blood culture showed a few organisms, his serum protected against 1,000 and 1,000,000 lethal doses respectively, and on the day of death there were numerous irregular mouse survivals. Thoracentesis on the 11th and 12th days produced infected fluid in small amounts but none was obtain-

* At the Boston City Hospital.

able on the 18th day and there were no other signs of localization of the infection. Autopsy was refused.

In a third case (Chart 2), also Type I, there were extensive areas of consolidation involving all but the right middle lobe. In spite of early and intensive treatment a right-sided empyema developed. Respiratory exchange was maintained by the use of an oxygen tent and he did well until the 18th day when he had a chill and showed a positive Type I blood culture with protection in the blood serum against 10,000 lethal doses 13 days after his last treatment with antibody. There were no signs of extension of the lung involvement, but a rough systolic murmur and a pericardial friction rub persisted until death. No autopsy was granted.

These first two cases demonstrate that the formation of some protective substances by the patient himself is no assurance against further progress of the infection to a fatal termination. Although in the third case intensive specific therapy was used, the lapse of 13 days between the last dose of antibody and the time the final serum sample was taken makes it questionable whether the protective substances were due to the treatment or not.

CONCLUSIONS

1. Though in general in pneumococcus pneumonia the appearance of protective substance coincides rather sharply with the fall in the temperature, antibody may appear spontaneously in the blood serum as early as the 3rd or 4th day and crisis and recovery may be delayed until the 6th to the 10th day.

2. Recovery at times occurs without demonstrable protective substance in the blood in patients who later develop protection.

3. The amount of antibody developed in the course of pneumococcus pneumonia is small and in the majority of cases tested was insufficient to protect against more than 100 lethal doses of homologous pneumococci and never against more than 10,000 lethal doses.

4. Treatment with Felton's antibody late in the course of the disease materially increases the amount of protective substances in the blood. A high degree of protection may be established by treatment in fatal cases. After the 3rd day doses of more than 200,000 Felton units are usually necessary to produce a greater degree of protection than might otherwise be expected.

5. The formation of protective substances by the patient himself is not an assurance against progress of the infection to a fatal termination.

6. Protective substance in the blood and pneumococcic septicemia may occur simultaneously.

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