ANTIPNEUMOCOCCIC IMMUNITY REACTIONS IN INDIVIDUALS OF DIFFERENT AGES*

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It is probable that certain characteristics of the patient, as well as the inherent qualities of the organism, determine differences in the degree of immunity to attack, in the clinical course, in the pathological lesions, and in the outcome of pneumococcic pneumonia. Little direct information has been obtained of variations in individuals or population groups which might influence the incidence or nature of the disease. Clinical observation (1–3) has indicated, however, that there are groups, notably those of different ages, in which the disease picture and outcome of pneumonia has characteristics which differ from those in other groups. It appears, also from clinical data, that pneumonia due to the Type I pneumococcus is consistently more frequent than pneumonia due to other types, suggesting the possible existence of differences in susceptibility to attack by the individual types of pneumococci. The study presented below was undertaken in order to secure information that might be compared with these clinical observations. Groups of normal persons of different ages were examined by means of several of the methods of immunity. The possibility was entertained that certain differences in immune reactions with age would suggest the existence of relationships between these immune reactions and the age variations in incidence, character, or outcome of pneumococcic pneumonia.

Methods were selected which involved the use of pneumococcus type-specific antigens and pneumococcus species-specific antigens. Of the former were the whole blood pneumococcidal test, the mouse pro-

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tection test, the agglutinin test, and the skin reaction to the pneumococcus polysaccharide. Of the latter were the skin test to the pneumococcus protein and the skin test to the pneumococcus autolysate. These methods have occasionally been used separately in studies of susceptibility to infection with the pneumococcus or related organisms.

A method similar to the whole blood pneumococcidal test has been used by Robertson and Sia (4) in studying the resistance to pneumococcus infection in common laboratory animals. In the rabbit, guinea pig, dog, and cat, the growth-inhibitory action of serum, when mixed with a standard suspension of leucocytes, varies as the natural resistance of the animal to infection by the pneumococcus. Determinations of the ability of the blood of normal adult human beings to kill pneumococci have been made by Sutliff and Rhoades (5) for Type I pneumococci and by Ward (6) for pneumococci of Types I, II, and III. Marked variability was found among individuals with respect to the number and type of pneumococci killed.

Mouse-protective antibodies for the pneumococcus were noted in the blood serum of normal individuals by Neufeld (7), and have been studied by Clough (8), by Cecil and Austin (9), and by Gundel and Schäfer (10). They are not so frequently present as whole blood bactericidal power and are usually of low titer.

Type-specific agglutinins are thought not to be present in normal human sera (8). A test for the presence of species agglutinins for an avirulent strain of pneumococcus was recently employed by Blake (12). The sera from normal subjects and those with a variety of diseases of various ages were examined using the living culture of a degraded avirulent pneumococcus as the antigen. By charting the incidence of subjects of different ages who had an agglutinin titer greater than 1:320, a curve was obtained which is similar to that shown below for whole blood pneumococcidal power.

A considerable incidence of positive skin reactions to the specific soluble substance of the pneumococcus among normal adults has been reported by Finland and Sutliff (11).

Clinical Material and Methods

The subjects were patients in the wards and outpatient department of the hospital and were chosen because they gave no history of having had pneumonia or a recent acute upper respiratory infection. For the most part those from 2 to 83 years of age were in the hospital for the following conditions: fractures, non-septic wounds, convalescence from surgical operations, functional disorders, and chronic degenerative disease, notably essential hypertension, chronic hypertrophic arthritis, and generalized arteriosclerosis. About one-half of the children from 2 to 6 years of age were studied late in convalescence from diphtheria or scarlet fever. The infants less than 10 days of age were normal, and the infants from 3 weeks to 2 years of age were brought to the out-patient department for regulation of feeding.

The pneumococcidal power of the whole defibrinated blood was determined for pneumococci of Types I, II, and III by a method similar to that of Ward (6). Measured quantities of culture were secured, uncontaminated by the blood cells used for enrichment of the medium, by removing 0.5 cc. of the upper portion of the culture and diluting, successively, 0.5 cc. with 4.5 cc. quantities of meat infusion broth. 0.1 cc. of each dilution containing amounts of culture ranging from 10^{-2} to 10^{-8} cc. were added to 0.5 cc. amounts of defibrinated blood in Pyrex tubes, sealed by means of a gas-oxygen flame and incubated while rotating slowly end for end during 18 to 24 hours. At the end of this time the color change in the tubes was noted, and 1 loopful was planted on blood agar plates to confirm the presence or absence of growth of organisms. The dilution method of measuring the number of organisms was controlled by culturing 1 cc. of the dilution containing 10^{-7} cc. of the original culture. A variation of from 30 to 170 organisms was found. The results in the blood tubes that received less than 30 to 170 organisms were discarded, and only persons 0.5 cc. of whose blood killed from 30 to 170 or more organisms were listed as having pneumococcidal power.

The mouse protection tests were done by the usual technique.

Agglutination tests of the sera against Type I, Type II, and Type III pneumococci were performed, as described by Tillett and Francis (13).

Three materials derived from the pneumococcus were used for skin tests, namely, soluble specific substances, acetic acid-precipitable proteins, and autolysates.

The soluble specific substances for Types I, II, and III were made by the method of Heidelberger and Avery, (14, 15) and supplied through the courtesy of Dr. W. S. Tillett of the Hospital of The Rockefeller Institute. They were injected intradermally in 0.1 cc. amounts of a dilution in normal saline containing 0.01 mg., together with a normal saline control. The wheal and erythematous skin reactions elicited by these materials have been described by Tillett and Francis (13). A wheal 0.8 cm. in diameter with erythema 1.5 cm. in diameter was considered positive.

The acetic acid-precipitable proteins of the pneumococcus were made according to Woolridge's method, as used by Avery and Morgan (16) and as adapted for the hemolytic streptococcus by Lancefield (17). Filtering the streptococcic protein solution through a Berkefeld V candle before sealing in vials was the only departure from the chemical procedures outlined by the above authors. Five different protein substances were prepared at one time from four strains of pneumococci and one strain of hemolytic streptococcus and used in the course of 5 months. One virulent Type I pneumococcus, one avirulent pneumococcus derived from a Type I pneumococcus, one virulent Type II pneumococcus, one avirulent pneumococcus derived from a Type II strain, and one Dick strain of *Streptococcus scarlatinae* were used. The preparations were standardized on the basis of their protein content, as measured by total nitrogen determinations. Concentrated solutions in physiological saline were made neutral to phenolphthalein by means of the addition of a small amount of NaOH, and were kept in rubber-capped vials. These solutions were diluted for use so that the amount injected,

0.1 cc., contained 0.01 mg. or 0.1 mg. The protein of the scarlatinal streptococcus was used in a dilution such that 0.1 cc. contained 0.02 mg. The skin reactions elicited by the pneumococcic proteins injected intradermally were characteristic at the end of 24 hours and appeared very much like the skin response to tuberculin. The reaction has been described by Tillett and Francis (13). An erythema 1.0 cm. in diameter was considered positive. The protein of the scarlatinal streptococcus elicited more marked and more frequent reactions, but of quite a similar character.

Autolysates were prepared from three different strains of pneumococci, namely, from a virulent Type I, from an avirulent organism derived from a Type I pneumococcus, and from a virulent Type II. The method of Zinsser and Grinnell (18) was followed with the addition of subsequent heating, as done by Sharp and Blake (19). These preparations were also standardized by means of the protein content, and were used in dilutions such that 0.1 cc. contained 0.1 or 0.01 mg. The skin reactions to the autolysates were similar to those to the pneumococcus proteins both in form and occurrence; except that, in addition to the rather frequent occurrence of a diffuse non-specific erythema within the 1st hour after the administration of the test, occasional definite wheal and erythema reactions, which faded within the first 30 to 60 minutes, occurred with one or another autolysate.

As many as 20 skin tests were done simultaneously in a number of cases, in which both 0.1 and 0.01 mg. of each protein and each autolysate were used. The usual number of skin tests done simultaneously was 8 to 16. Readings were made 20 to 30 minutes after the injections and again at the end of 18 to 24 hours.

FINDINGS

Pneumococcidal Action of Whole Blood

The number of organisms killed by 0.5 cc. of defibrinated blood varied in different individuals from approximately 10 to approximately 1,000,000. There was little difference between the three pneumococcus types or between the subjects of different age groups in the number of organisms disposed of. Of the subjects whose tests were positive against pneumococci of Type I, Type II, or Type III, 83 per cent, 78 per cent, and 90 per cent killed, respectively, 10,000 organisms or less; 17 per cent, 18 per cent, and 8 per cent killed, respectively, 100,000 organisms; and none, 3.2 per cent, and 1.6 per cent killed, respectively, 1,000,000 organisms. The data presented below contain only the incidence of pneumococcidal power without relation to the number of organisms killed.

The incidence of whole blood pneumococcidal power was determined for Type I, Type II, and Type III pneumococci in 112 individuals of va-

rying ages, and for the mothers of the 22 infants less than 10 days of age. The distribution of these individuals in eight different groups and the percentage of each group, whose blood was pneumococcidal for Type I, Type II, and Type III, are shown in Fig. 1. The mothers, whose ages ranged from 19 to 33, showed an incidence of bactericidal power similar to that of normal adults. Among the new-born infants and

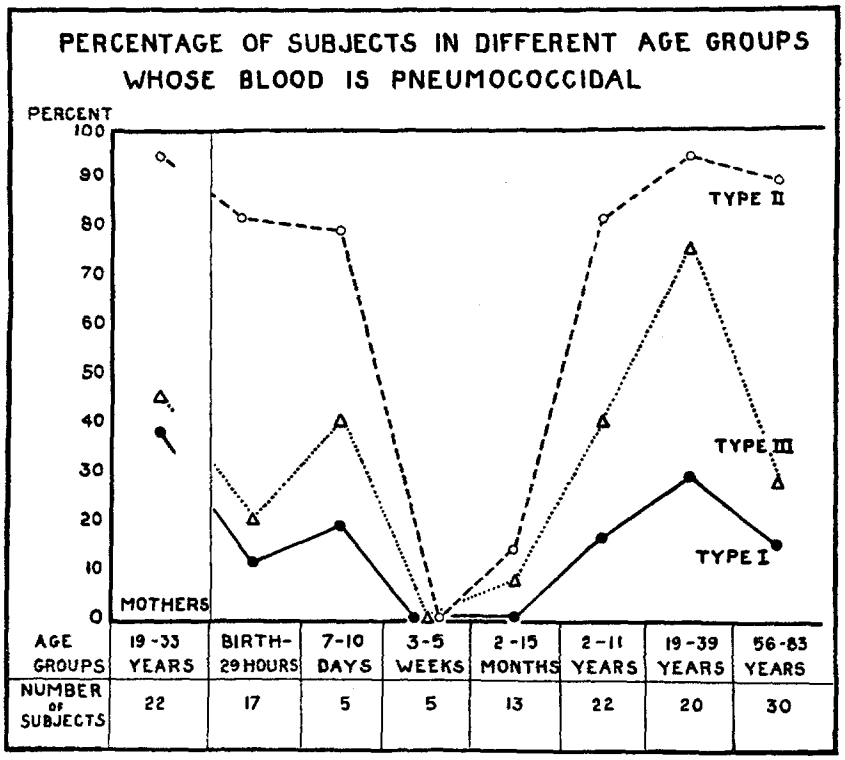

FIG. 1

those in the first 10 days of life, bactericidal power was frequently present. In later infancy, from the age of 3 to 5 weeks and from 2 to 15 months, it was absent or quite rare. In childhood from 2 to 11 years pneumococcidal power was again as frequent as among new-born infants. The highest incidence for all three types of pneumococci was reached among adults. There was moderate drop in old age.

The number of subjects who had no pneumococcidal power for any of the three types varied as would be expected with the general incidence of pneumococcidal action. 4 of 22 infants less than 10 days of age had no pneumococcidal power, 15 of the 18 infants from 3 weeks to 15 months of age had none, and 1 child among the 22 from 2 to 11 years had none. Among 20 adults 19 to 39 years of age, all the subjects killed pneumococci of at least one type. 4 of the 30 persons over 56 years of age had no pneumococcidal power.

A striking characteristic of the bactericidal power of the 22 infants, ranging from birth to 10 days of age, and their mothers, examined simultaneously, was that in every case the bactericidal power of the infant, although slightly less in degree, was a qualitative reproduction of its own mother's bactericidal power. The infants sometimes lacked bactericidal power for one or more pneumococcus types, as is indicated by the lower incidences in Fig. 1, but this occurred only when the blood of the mother killed few pneumococci.

The relative frequency of bactericidal power against the three types of pneumococci can be studied in Fig. 1. The relationship is the same in each age group. Bactericidal power is least frequent for Type I, is most frequent for Type II, and is intermediate in frequency for Type III.

In the individual blood samples each of the eight possible combinations of type-specific pneumococcidal power appeared. Some combinations, such as Type I negative with Types II and III positive, occurred frequently, while others, such as Types I and III positive with Type II negative, were rare.

The frequency, with which each of these combinations could be expected, was calculated, by means of the mathematical law of probability, from the frequency with which killing was found for each type individually. The result for each of the possible combinations was almost identical with the frequencies actually observed, indicating that these combinations arose according to the laws of chance. It would appear likely that the pneumococcidal power for any one type originates independently of the pneumococcidal action against other types.

In summary, bactericidal power of the blood for pneumococci of Types I, II, and III may be described for five age groups with differing incidences. (1) It is nearly as frequent in the first 10 days after birth

as in adults. Each individual infant resembles its own mother in the pneumococcidal power it possesses. (2) Infants from 3 weeks to 15 months of age have little or no blood pneumococcidal power. (3) The incidence among children from 2 to 11 years of age is somewhat less than among adults. (4) The highest incidence is found between the ages 19 to 39 in adult life. (5) The incidence among persons from 56 to 83 years of age is somewhat less than among younger adults.

The relative incidence for the three common immunologically specific varieties of the organism is similar in all the age groups studied; Type I is least often killed, Type II is most frequently killed, and Type III occupies an intermediate position. The associations of killing power specific for type in individuals appear to be subject to the laws of chance.

Mouse Protection

Serum antibodies were determined by the mouse protection test for 9 infants at birth, 15 infants 3 weeks to 15 months of age, 5 children from 2 to 11 years, 20 adults from 19 to 39 years, and 30 persons from 56 to 83 years of age. The incidence of positive tests was low, varying from 0 to 3 per cent for Type I, from 0 to 22 per cent for Type II, and from 0 to 22 per cent for Type III. None of the 15 infants aged 3 weeks to 15 months had mouse-protective antibodies in their serum against pneumococci of Type I, II, or III. No other definite trend with age could be made out. When the tests for all age groups are added together, the relative incidence of mouse protection for the three pneumococcus types is the same as the total relative incidence of pneumococcidal power for the three types. Type I protection is the least frequent, Type II is the most frequent, and Type III protection occupies the middle position.

Agglutination

The number of positive type-specific agglutinations was quite small. Among 142 patients tested, 2 showed agglutination for Type I pneumococci, 3 showed agglutination for Type II pneumococci, and 12 showed agglutination for Type III pneumococci. The titers ranged from 1:4 to 1:16. Here, again, none of the infants from 3 weeks to 15 months of age showed specific agglutinins.

Skin Reactions to the Soluble Specific Substances

Skin tests with the soluble specific substances of the three pneumococcus types were done in all five groups of patients and the results are shown in Table I. In infants less than 15 months of age, an erythema was invariably produced which obscured any immediate specific erythematous reaction. No wheal formation was observed. There is no significant difference in the incidence of positive reactions in children, adults, and old persons. The relative incidence of positive skin reaction to the three type-specific carbohydrates is of interest. Type I carbohydrate caused skin reactions least frequently, Type II carbohydrate caused skin reactions most frequently, and reactions to Type III carbohydrate occurred with intermediate frequency.

TABLE I

The Age Distribution of Positive Skin Reactions to Pneumococcic Type-Specific Carbohydrates among Hospital Patients with No History of Pneumonia

Skin test substance	No. of subjects with positive reactions				
	Birth to 10 days	3 wks. to 15 mos.	2 to 11 yrs.	19 to 39 yrs.	56 to 83 yrs.
Type I S.S.S. 0.01 mg.	?	?	1	1	1
" II " 0.01 "	?	?	5	3	5
" III " 0.01 "	?	?	2	0	3
No. of subjects tested	11	14	19	9	19

? = erythema occurred regularly. No wheals were observed.

Skin Reactions to Acetic Acid-Precipitable Protein and to Autolysate

Among the acetic acid-precipitable proteins derived from 4 strains of pneumococci and used in two different concentrations, all showed an increasing percentage of positive reactions with age. Variations in the incidence of positive reactions were observed with the protein from different strains and still more marked variations in incidence were seen when the amount of protein was varied from 0.1 to 0.01 mg. Individual subjects who did not react to all these pneumococcic proteins, gave positive reactions to different preparations. The results with the autolysates of three strains of pneumococci were similar, except that a slightly higher proportion of the subjects gave positive

reactions. The positive reactions to the streptococcic protein were in turn, more numerous than those to the pneumococcic autolysates. Marked differences in the incidence of positive reactions resulting from small changes in the concentration of the same protein or autolysate indicate that the concentration must be carefully controlled. It is further possible that chemical determinations of the nitrogen content of such complex solutions may not indicate the concentration of other substances, which can alter the incidence of positive reactions. On account of such factors and also on account of the small number of observations, the actual incidences reported here are not of great significance. The general trend with age, however, is quite clear and is similar for

TABLE II

The Age Distribution of Positive Skin Reactions to Pneumococcic Protein and Autolysate among Hospital Patients with No History of Pneumonia

Skin test substance	No. of subjects with positive reactions				
	Birth to 10 days	3 wks. to 15 mos.	2 to 11 yrs.	19 to 39 yrs.	56 to 83 yrs.
Pneumococcic protein 0.1 mg. Type I (avirulent)	0	0	7	—	5
Pneumococcic autolysate 0.1 mg. Type II (virulent)	0	2	10	8	7
Streptococcic protein 0.1 mg. Dick *Streptococcus scarlatinae*	1	3	10	7	—
No. of subjects tested	10	9	12	8	9

all three types of substances. An increase occurs from no reactions, or few reactions, at birth to a rather high incidence of reactions in the age group 2 to 11 years as is shown in Table II.

Correlations between the Tests

Type-specific antibodies, indicated by the mouse protection test and the agglutination test, were always confirmed by the presence of type-specific whole blood pneumococcidal power in the same blood sample. The whole blood pneumococcidal test was positive frequently in the absence of demonstrable serum antibodies, as would be expected from its greater sensitivity, noted by Sutliff and Rhoades (5), and from the

greater sensitivity of the growth-inhibitory action of serum-leucocyte mixtures, noted by Robertson and Sia (4). The skin reactions to the soluble specific substance were not correlated with other specific antibodies. This is surprising in view of the strict association with type-specific antibodies in the course of lobar pneumonia (11, 13). Skin reactions to the pneumococcic protein and streptococcic protein and pneumococcic autolysate were not correlated with any other tests.

DISCUSSION

Four of the methods described above, namely whole blood bactericidal tests, mouse-protective tests, skin tests performed with protein solutions, and skin tests performed with autolysates, showed an incidence of positive reactions that varied with age. Skin tests with autolysates and proteins were quite similar in their incidence and will be discussed together. Agglutinin reactions were so seldom positive that comparisons of their incidence in different age groups are not significant. Type-specific skin tests were not successfully interpreted in infants and showed no variations in incidence in other age groups.

The results of the tests that varied in their incidence of positive reactions with age may be compared with the scattered results previously obtained by others.

The incidence of adult subjects possessing pneumococcidal power was much less than that found previously by Sutliff and Rhoades (5), who used a somewhat different method, and by Ward (6). These authors found the normal incidence among adults of killing power for the Type I pneumococcus to be 66 and 70 per cent, respectively, as compared with 26 per cent found here. Ward reported an incidence of 100 per cent for pneumococcidal power for Types II and III in 10 subjects, in contrast to 90 per cent and 70 per cent, respectively, reported above. It is possible that the organisms used in the present work had greater virulence, when measured against human blood, than those of the investigators quoted, since they were passed daily through mice, were transferred at 8 hour intervals in media enriched by rabbit blood, and were diluted for use together with the surrounding culture fluid.

Mouse protection tests of the sera showed that the general incidence of these type-specific antibodies is low and that infants from 3 weeks to 15 months of age have such antibodies less often than individuals of

other age groups. Gundel and Schäfer (10) reported similarly that infants do not have mouse-protective antibodies. The incidence of protection for Type I among adults is lower than that found by Sutliff and Rhoades (5) but, since the same cultures were used for pneumococcidal tests and for protection tests in mice in both studies, the possibility is again suggested that the virulence of the cultures differed.

The frequency of skin reactions to the acetic acid-precipitable proteins and the autolysates of the pneumococcus, increases with age in a manner similar to that found by Derrick and Fulton (20) for similar tests with proteins derived from *Streptococcus hemolyticus* and *Streptococcus viridans*.

The nature of the antibodies in normal persons which have given rise to the positive reactions may be described in immunological terms. Positive whole blood bactericidal tests and mouse protection tests depend upon the presence of type-specific opsonins in the serum of the individual. The cells of the whole blood and of the mouse, while essential to the reactions, play a passive part (4). The apparent independence of the reaction against any one pneumococcus type from that against any other suggests that the opsonins in normal persons are type-specific in character, as they are in persons who have recovered from the disease. In case of the skin reactions to the pneumococcic protein, however, the antigen is species-specific in the type of antibodies that its injection produces and in its reactions with antipneumococcic sera. In contradistinction to type-specific antibodies, Tillett and Francis (13) have noted no association of positive skin reactions to protein in patients recovering from lobar pneumonia with the appearance of serum antibodies against the pneumococcic protein. By their occurrence in the same patients and the similar nature of the reaction, the positive tests to injection of the autolysates seem probably due to the same sort of mechanism as the positive tests to the pneumococcic protein.

The incidence of these positive reactions may be discussed with relation to possible modes of development and with relation to a possible correlation with clincal observations.

The mode of origin of the reactions to type-specific and species-specific antigens is suggested by some of the characteristics of their occurrence. The type-specific antibodies at birth were similar to those of

the mother and disappeared before the age period 3 to 5 weeks. This suggests a passive transfer of such antibodies through the placenta from mother to child.

After 2 years of age, the type-specific antibodies appear with considerable frequency and in combination according to the laws of chance. This seems to point toward a chance contact with specific organisms as the cause of their development. A carrier state, usually of short duration for Type I and Type II pneumococci, but longer for Type III pneumococci, was demonstrated in one-half of a group of normal subjects during the course of 7 months by Powell, Atwater, and Felton (21) and has been confirmed by Webster (22). Whether such contact leads to the production of antibodies has not been determined, but the slight specific stimulus needed for antibody production noted in the following study (23) suggests that the presence of a few organisms on a mucous membrane might be effective. In following further the suggestion that contact with organisms leads to the production of the type-specific antibodies, a number of factors that could influence their production must be considered. In the first place, the relative efficiency of the pneumococci in inducing type-specific antibody production may vary. It has been shown (24) that the Type III pneumococcus is relatively much less efficient in rabbits than Type I or Type II. The relative frequency of opportunities for contact of an individual with the three pneumococcus types presented by the work of the authors above is, in the order from least to greatest frequency, I, II, III; but the relative frequency with which type-specific antibodies were demonstrated here is, in the same order, I, III, II. Type III thus has an anomalous position. Although contact with Type III pneumococcus is most often observed, antibodies for Type III are less often seen than antibodies for Type II. The Type III pneumococcus may thus be less efficient than Type I and II in the production of specific antibodies in man. Other factors may further modify the production of antibodies by contact. In the individual subject the site of the organisms, whether in a normal pharynx or a purulent focus, may influence their effectiveness as antigens. The responsiveness of the antibody-producing cells of individuals may vary. It is also possible that antigens derived from organisms other than the pneumococcus may produce type-specific pneumococcic antibodies (25, 26).

Although it seems likely that contact with pneumococci that does not lead to the production of pneumonia may lead to the production of type-specific antibodies and thus be the determining factor in their development during childhood, the question requires further clarification. There are alternative possible methods for the development of antibodies in normal persons. It has been suggested (27) that normal processes in the growth and maturing of the body or certain organs may underly the development of natural antibodies. A combination of physiological changes and contact with antigens is also possible.

The negative skin tests to protein and autolysate in the new-born infants indicate that reactions to species-specific antigens are not passively transferred from the mother. This is opposed to the observations of Julianelle (28) who found that experimentally induced increased skin reactivity to the protein of the pneumococcus was associated with the presence of circulating species-specific antibodies and was transferable by means of the intravenous administration of serum to another animal. He also found, however, that the increased sensitivity to heat-killed vaccines was not associated with the presence of antibodies and was not transferable. Furthermore, experimental studies of allergic skin reactions to pneumococcus autolysates in animals by Zinsser and Grinnell (18) have shown that the capacity to react is not transferred passively by means of serum. After 2 years of age the skin reactions to protein and autolysate appeared with considerable frequency. The mechanism underlying the development of this capacity may be contact with organisms, changes that are part of physiological growth, or some combination of the two, as in the case of the type-specific reactions.

Immune reactions to type-specific pneumococci occurring in a large proportion of individuals might be thought to exert some influence in protecting individuals from attack, particularly since type-specific antibodies are effective prophylactically in animals, and therapeutically in man. But type-specific whole blood bactericidal power has been shown to be present in patients early in the course of their disease (6, 29–31) and the significance of this type of antibody in protecting individuals from lobar pneumonia is thus questionable. It may be noted, however, that the relative incidence of type-specific bactericidal power and mouse protection for pneumococci of Types I and II in the

serum of normal individuals is inversely proportional to the relative incidence of lobar pneumonia due to these two types of pneumococci. Type III again occupies an anomalous position, with an incidence of pneumococcidal power midway between that of Type I and Type II, but with an incidence in pneumonia less than either Type I or Type II.

Variations in the incidence, the clinical course, the pathological lesions, and in the outcome of pneumococcic pneumonia at different ages may be stated in general terms. The morbidity, as influenced by age, is not known, due to lack of direct studies covering all ages in a sufficiently large community; but the curves of deaths from pneumonia by ages show a preponderance in infancy and extreme old age that suggests a higher morbidity as well as an increased case fatality at these periods. At the extremes of life the disease also tends to manifest fewer of the typical clinical characteristics of lobar pneumonia, as it is seen in youth and middle life. The pathological lesion in pneumonia in infancy and old age frequently differs from that of typical lobar pneumonia, being more often lobular in distribution rather than lobar. The death rate is high at the two extremes of life, is lowest in youth, and gradually increases from youth to old age. The differences in the incidence of whole blood bactericidal power in infancy and old age, the lack of mouse-protective antibodies in the serum of infants, and the lack of capacity of the skin of infants to react to proteins and autolysates of the pneumococcus may be related to these variations in pneumococcic pneumonia with age. Conversely, the possession of type-specific antibodies and the capacity of the skin to react to pneumococcic proteins and autolysates by a relatively high proportion of adults suggests a relationship between the immune mechanism which underlies these reactions and the occurrence of typical lobar pneumonia.

CONCLUSIONS

1. The incidence of pneumococcidal power of the whole defibrinated blood in human beings has been shown to vary with age. The age distribution of other type-specific antibodies varies similarly, insofar as they are frequent enough to be compared or technically demonstrable.

2. The incidence of pneumococcidal power of the whole defibrinated

blood for Type I, Type II, and Type III differs. Type I is the rarest, Type II is the most frequent, and Type III is of intermediate frequency. The type-specific antibodies responsible for the other tests employed show a similar relative frequency in regard to Types I and II, but some variation in regard to Type III.

3. The skin reactions to the acetic acid-precipitable proteins and autolysates of the pneumococci are negative or rarely positive in infants, infrequently positive in childhood, and positive in a high percentage of adults.

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REFERENCES

1. Cole, R., DeLamar Lectures, 1927–28, Baltimore, Williams and Wilkins Co., 1928.
2. Cecil, R. L., Baldwin, H. S., and Larsen, N. P., *Arch. Int. Med.*, 1927, **40,** 253.
3. Locke, E. A., personal communication.
4. Robertson, O. H., and Sia, R. H. P., *J. Exp. Med.*, 1927, **46,** 239.
5. Sutliff, W. D., and Rhoades, D. R., *J. Clin. Invest.*, 1930, **9,** 43.
6. Ward, H. K., *J. Exp. Med.*, 1930, **51,** 675.
7. Neufeld, F., and Haendel, L., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, Gustav Fischer, 2nd edition, 1912, **4,** 556.
8. Clough, W. P., *Bull. Johns Hopkins Hosp.*, 1924, **35,** 330.
9. Cecil, R. L., and Austin, J. H., *J. Exp. Med.*, 1918, **28,** 19.
10. Gundel, M., and Schäfer, quoted by Gundel, M., *Ergebn. Hyg., Bakt., Immunitätsforsch., u. exp. Therap.*, 1931, **12,** 207.
11. Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1931, **54,** 637.
12. Blake, F., personal communication.
13. Tillett, W. S., and Francis, T., Jr., *J. Exp. Med.*, 1929, **50,** 687.
14. Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1923, **38,** 73.
15. Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40,** 301.
16. Avery, O. T., and Morgan, H. J., *J. Exp. Med.*, 1925, **42,** 347.
17. Lancefield, R. C., *J. Exp. Med.*, 1925, **42,** 377.
18. Zinsser, H., and Grinnell, F. B., *J. Bact.*, 1927, **14,** 301.
19. Sharp, E. A., and Blake, F. G., *J. Exp. Med.*, 1930, **52,** 501.
20. Derrick, C. L., and Fulton, M. N., *J. Clin. Invest.*, 1931, **10,** 121.
21. Powell, J. P., Atwater, R. M., and Felton, L. D., *Am. J. Hyg.*, 1926, **6,** 570.
22. Webster, L. T., and Hughes, T. P., *J. Exp. Med.*, 1931, **53,** 535.

23. Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1932, **55**, 853.
24. Tillett, W. S., *J. Exp. Med.*, 1927, **45**, 713.
25. Avery, O. T., Heidelberger, M., and Goebel, W. F., *J. Exp. Med.*, 1925, **42**, 709.
26. Sugg, J. Y., and Neill, J. M., *J. Exp. Med.*, 1929, **49**, 183.
27. Friedberger, E., Bock, G., and Fürstenheim, A., *Z. Immunitätsforsch.*, 1929, **64**, 294.
28. Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 643.
29. Sutliff, W. D., and Rhoades, D. R., *J. Clin. Invest.*, 1930, **9**, 55.
30. Robertson, O. H., Terrell, E. E., Graeser, J. B., and Cornwell, M. A., *J. Exp. Med.*, 1930, **52**, 421.
31. Robertson, O. H., *J. Prev. Med.*, 1931, **5**, 221.