THE USE OF THE SKIN TEST WITH THE TYPE SPECIFIC POLYSACCHARIDES IN THE CONTROL OF SERUM DOSAGE IN PNEUMOCOCCAL PNEUMONIA

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The use of the skin test with the specific capsular polysaccharide in determining the amount of serum necessary for the treatment of Type I pneumococcal pneumonia was described by Francis in 1933 (1). A series of 53 cases was reported, 48 of whom were treated with Type I unconcentrated antipneumococcal horse serum. In all but 1 of the 46 recovered cases, a positive reaction was obtained at about the time of recovery, and in 7 fatal cases the skin tests were consistently negative. Francis pointed out that a positive skin test was invariably associated with the presence of circulating type specific antibody but that, in addition, reactive tissues are necessary, since in the cases who died, even though antibody was present in the blood, the skin test remained negative.

In the treatment of Type I and Type II pneumonia with concentrated antipneumococcal horse serum Finland and Sutliff (2) reported that 20 of the 23 patients who recovered gave positive skin reactions. Of 5 treated patients who died, the reaction remained negative in all but one.

Abernethy (3) discussed the value of the skin test as a guide in the control of dosage of concentrated antipneumococcal horse serum in his report on 25 cases of Type I pneumonia, and stressed its importance in determining the minimum amount of serum necessary for the treatment of a given case.

The present communication deals with the use of the skin test in the control of dosage of Types I and II concentrated antipneumococcal horse serum, and of unconcentrated antipneumococcal rabbit serum Types I, II, III, V, VII, and VIII.[1]

[1] Throughout this paper concentrated antipneumococcal horse serum and unconcentrated antipneumococcal rabbit serum will be referred to respectively as horse serum and rabbit serum.

MATERIALS AND METHODS

The study was carried out on patients with pneumococcal pneumonia admitted to the Hospital of The Rockefeller Institute. The Type I and Type II concentrated antipneumococcal horse serum employed in therapy was obtained through the courtesy of Dr. Augustus B. Wadsworth of the New York State Department of Health. The unconcentrated antipneumococcal rabbit serum was prepared in this laboratory according to the method of Goodner, Horsfall, and Dubos (4). All serum used was monovalent, and was given intravenously.

Specific polysaccharides. Protein-free specific polysaccharides of Pneumococcus Types I, II, III, and VIII were prepared by the methods employed in this laboratory (5), and were free of the species specific "C substance." The Type V polysaccharide was prepared by a modification of the Sevag method (6). The Type VII polysaccharide was a commercial preparation in which the species specific "C substance" was present as an impurity.

The polysaccharides were dissolved in physiological salt solution. The saline was freshly prepared from water doubly distilled in glass and immediately sterilized. It was known not to produce an erythematous reaction in the normal skin. Sterile stock solutions of the various polysaccharides containing 1.0 mgm. per cc. were stored without preservative in rubber stoppered glass vials in the ice box. In this form the preparations have been found to retain their activity for as long as 2 years. Immediately before use the stock solution was diluted tenfold with saline to make a final concentration of 1:10,000 of the specific substance.

Skin tests. Five-hundredths to one-tenth cc. of the 1:10,000 solution (0.005 to 0.01 mgm.) was used for intradermal injection on the volar sur-

face of the forearm. A corresponding control injection of physiological saline was always made.

Tests were done before administering serum in order to assess the reactivity of the patient's skin, since false positive reactions are occasionally encountered. In patients to whom serum was given in divided doses, the tests were repeated frequently during the course of treatment, and in the other cases in which the total amount of serum given was administered at a single injection, the skin tests were done at short intervals following therapy. If false positive reactions were obtained with one preparation, other preparations of both homologous and heterologous polysaccharides were generally used to check the reaction.

Skin tests were read after 15 minutes, and again after 30 minutes. A positive reaction was defined as consisting of a firm, edematous wheal almost invariably showing pseudopodia extending outward from its border and surrounded by an erythema. If there was any doubt as to whether or not a reaction was positive, more serum was given. This has proved to be a good practical rule, since an unequivocally positive and specific reaction was almost always obtained after the administration of more serum.

Immediately following the administration of serum, a commonly observed phenomenon was the transient "lighting-up" of a reaction at the site of previously negative tests. In these instances the earlier test had been done usually within two hours before the provocative dose of serum had been given. Generally the occurrence of this phenomenon indicated that sufficient serum had been given, but not always, since tests performed subsequently would occasionally be negative and more serum would then have to be given.

The present paper includes the data obtained in the study of 104 patients who were tested intradermally with various type specific polysaccharides before, during, and after serum treatment. In the present study stress is laid on the results of the initial skin tests done before the initiation of serum therapy since it had been noted that an occasional patient shows a positive reaction to the homologous polysaccharide even though type specific antibody is not demonstrable in the blood, and the disease is advancing. Under these conditions, if the patient's skin was found to be reactive to the polysaccharide before administration of serum, it is obvious that the test could not be used as a guide to therapy and under these circumstances serum dosage had to be judged by the general clinical criteria of recovery.

Patients showing a positive skin test before serum treatment. In Table I it will be seen that

TABLE I

Incidence of positive and negative skin tests before serum therapy

Type of pneumonia	Number of patients	Number showing positive test before serum	Number showing negative test before serum
Type I.....	60	11	49
Type II....	17	2	15
Type III....	16	0	16
Type V.....	1	0	1
Type VII...	3	0	3
Type VIII..	7	0	7
Totals.....	104	13 (12.5 per cent)	91 (87.5 per cent)

of a total of 104 patients, 13 or 12.5 per cent showed a positive skin test before serum had been given, that is, at a time when the disease was still progressive. In 4 of these patients determination of circulating type specific antibody showed that specific agglutinins were not present in the blood before serum treatment. All of these patients responded satisfactorily to serum therapy and all recovered. In each instance the skin test which was initially positive remained so throughout the course of the disease, and in convalescence. None of these cases gave a history of a previous attack of pneumonia or of known infection with pneumococcus, and in only one was there a history of cutaneous hypersensitivity. This patient was allergic to a wide variety of agents, and suffered from severe eczema. In the remaining 12 patients no reason has been found to account for the presence of a positive skin test while the disease was at its height and before the administration of serum.

Patients showing a negative skin test before serum treatment. The results in this group of cases are shown in Table II. In 91 (87.5 per cent) of the 104 patients the skin test was negative before the administration of serum. In one patient with Type I pneumonia the skin test re-

TABLE II

Results of skin tests after serum therapy in patients showing a negative test before treatment

Type of pneumonia	Number of patients	Results of tests in patients who recovered		Results of tests in patients who died	
		Positive	Negative	Positive	Negative
Type I.....	49	47	1		1
Type II.....	15	11		1*	3
Type III.....	16	11		4†	1
Type V.....	1	1			
Type VII.....	3	3			
Type VIII....	7	7			
Totals.....	91	80	1	5	5

* Died of a vascular accident 6 weeks after admission.
† In 3 of these patients the skin test became negative before death.

mained negative throughout the course of the disease and in convalescence, even though dramatic curative effect was obtained from the administration of Type I rabbit serum. The reason for the failure of the skin to react under these favorable circumstances is unknown.

Ten of the patients in this group died. In 5 of the fatal cases the skin test was negative throughout the course of the acute illness, despite the demonstration of antibody in the blood. The failure of the skin to react in these cases supports the view of Francis (1) that tissue reactivity, as well as free type specific antibody, is necessary in order for a positive skin reaction to occur.

Five of the patients who died showed a positive skin test after serum administration. In 3 of these the skin test became negative before death occurred; the other 2 patients died suddenly, and the reactivity of the skin at the time of death was not determined. In 3 of the 5 fatal cases in whom a positive reaction became negative before death, the presence of specific agglutinins in blood which was obtained postmortem showed that adequate serum had been given and that the loss of skin reactivity was not caused by a lack of humoral antibody. Determination of agglutinins was not performed in the other two cases.

From consideration of the results in patients showing a negative reaction before serum therapy, it will be seen that the greatest value of the test is in those cases in which the specific action of the immune serum is rendered effective by an adequate cellular response on the part of the patient, for in these the development of a positive test serves as a measure of the optimum amount of serum to be given. In fatal cases the results are less clear-cut, since in such patients the ability to react may not be present or if present may subsequently disappear, even though an excess of antibody is present in the circulating blood.

In 80 patients (77 per cent) out of a total of 104, the skin test was considered entirely satisfactory and served as an aid in determining when the optimum amount of serum had been administered.

Although the greater part of the experience has been obtained with Type I and Type II pneumonia, preliminary results in the disease caused by Pneumococcus Types III, V, VII, and VIII indicate that the usefulness of the test applies equally well to the control of dosage in these types.

RESULTS IN VARIOUS TYPES OF PNEUMONIA

Type I pneumonia. Sixty cases have been studied, 32 of whom were treated with Type I horse serum and 28 with Type I rabbit serum. In this series of treated cases only one death occurred. The patient was admitted on the seventh day of disease suffering from Type I meningitis. In this instance, the skin test with the homologous polysaccharide was negative throughout, although the patient's serum contained agglutinins for Type I pneumococcus following serum treatment. Of the recovered cases, only one showed a negative skin test after effective serum treatment.

Eleven patients showed a positive reaction to Type I polysaccharide before the administration of serum and in these the skin test could not be used as a guide to serum dosage. In 4 of this group who were so tested, circulating Type I agglutinins were not present before serum therapy was begun.

In the remaining 47 cases, the skin test which was initially negative became positive during treatment. At the appearance of a positive reaction, specific therapy was discontinued and recovery promptly ensued.

Type II pneumonia. Seventeen cases were studied, of whom 9 were treated with Type II horse serum and 8 with rabbit antiserum. The

skin reaction was positive, before the administration of serum, in 2 patients with advancing pneumonia. For the reasons already stated the skin test was not applicable as a guide to serum therapy in these cases.

Of the remaining 15 patients, 12 developed a positive reaction in the course of serum therapy. One of these patients died of a ruptured aortic aneurysm 6 weeks after admission. In the 3 other fatal cases, consistently negative reactions were obtained throughout the course of the disease.

Type III pneumonia. All of the 16 patients who were studied were treated with Type III rabbit serum. In all cases the skin test was negative before serum treatment. Fifteen patients developed a positive reaction following serum, and in one fatal case the reaction was negative throughout the course of the disease.

Of the 15 patients who developed a positive skin test after serum there were 4 who died. The first of these, a 40-year-old female, developed a severe purpuric reaction at the site of the skin tests, purpura appearing about 12 hours after the positive skin reaction had faded; the skin test became negative before death. The second patient was a female of 68 whose blood became sterile and who developed a positive skin test following serum therapy. Peripheral circulatory collapse supervened, and the skin test became negative and remained so until death. The third patient, a female of 62 years, died of multiple abscesses in the consolidated portions of the lungs and in both kidneys, from all of which lesions, Type III pneumococci were isolated at autopsy. The blood became sterile following serum therapy associated with the development of a positive skin test. The skin test again became negative, however, 48 hours before death. The fourth patient, a female of 64 years who gave a history of intermittent "cardiac irregularity" of 25 years standing, developed a positive skin reaction to the Type III polysaccharide following serum, but died suddenly of pulmonary edema; a skin test was not done within 12 hours before death.

In the first, second, and third patients the presence of circulating Type III agglutinins was demonstrated in association with the positive skin test, and in these cases the blood at the time of death was shown to contain specific agglutinins in high titer. Determination of agglutinins was not made in the fourth case.

The loss of skin reactivity in patients in whom positive reactions occurred following the use of serum has not been observed in pneumonia other than that resulting from Pneumococcus Type III.

Type V and Type VII pneumonia. This group comprises 4 patients only; one case of Type V pneumonia and 3 of Type VII pneumonia, all of whom were treated with the immune rabbit serum of the corresponding type. In all cases the skin test with the homologous polysaccharide was negative before serum therapy, but became positive after an amount of serum sufficient to control the infection had been given. There were no deaths in this group.

The preparation of Type VII polysaccharide used, as previously pointed out, was contaminated by the species specific "C substance" of the pneumococcus, so that skin reactions to this material were obtained also. As has been described by Francis and Abernethy (7, 8) the reaction with the "C substance" shows certain differences from that obtained with the type specific polysaccharides of the pneumococcus. However, since the primary reaction with "C substance" shows only a quantitative difference from the reaction with the specific polysaccharide, the two reactions can be distinguished only with difficulty. It is essential, therefore, that the test substances used should be as free as possible from extraneous impurities, otherwise the issue becomes confused and the reactions difficult of interpretation.

Type VIII pneumonia. The 7 patients in this group were treated with Type VIII rabbit serum. None showed a positive skin test before the administration of serum, and in each instance the test became positive after an amount of serum sufficient to control the infection had been given.

DISCUSSION

The skin test with the homologous specific polysaccharide has been employed as a guide in controlling the dosage of immune serum in the treatment of pneumonia resulting from pneumococcus Types I, II, III, V, VII, and VIII. The advantages of this test are the ease with which it can be done, the shortness of the time required

for the reaction to appear (15 to 20 minutes), and the accuracy with which the reaction can be read provided adequate criteria are fulfilled.

In 77 per cent of the 104 patients in this series, the skin test was valuable and entirely satisfactory in determining when a sufficient amount of serum had been given. At the appearance of a positive reaction no further serum was given, and the subsequent course of events justified the reliance placed on the test. Antisera prepared in the horse and the rabbit have been used in treatment, and the skin test has been found equally applicable in the control of dosage of both kinds of immune serum.

Thirteen or 12.5 per cent of the patients showed a positive skin test before the administration of specific antiserum, and at a time when the disease was advancing. In this group, therefore, the skin test could not be used as a guide to therapy. In 4 of these individuals, determination was made of circulating antibody before serum was given, and in no instance were agglutinins demonstrable. The occurrence of positive reactions under these conditions in such a proportion of the patients tested, makes it of utmost importance to perform the skin test on each patient before beginning serum therapy since without knowledge of the initial reactivity of the skin, the results of subsequent tests may be entirely misleading. It follows, therefore, that the skin test can be used as a guide to serum dosage only in those patients who show a negative reaction before type specific antibody has been given.

Except in the case of one allergic individual who had extensive eczema, no reason has been discovered to account for positive skin reactions in the 13 patients who were tested early in the disease and before serum was administered. These "false-positive" reactions occurred with preparations of Type I and Type II polysaccharide which were known to be protein-free, and which did not contain any demonstrable "C substance." However, this does not rule out the possibility that the test substances may have contained small amounts of other extraneous materials which could produce nonspecific reactions in the skin of certain individuals. It is of interest in this regard that when, for example, a "false positive" reaction occurred with the homologous polysaccharide, similar reactions were generally observed with preparations of other type specific polysaccharides in the same individual.

The incidence of positive skin reactions in normal individuals tested with Type I and Type II polysaccharides, has been studied by various investigators. In 24 normal persons, Finland and Sutliff (9) reported positive reactions to the Type I polysaccharide in 4, and to the Type II polysaccharide in 10. Rogers and Wagner (10) skin tested 78 normal individuals with the Type I polysaccharide and found 71.8 per cent who reacted positively. Similarly, Alston and Lowdon (11) reported positive skin reactions to the Type II polysaccharide in 63 per cent of 281 persons who had not suffered recently from a pneumococcal infection. In the report of Alston and Lowdon, secondary reactions occurring 5 to 24 hours after intradermal injection were noted, which throws some doubt on the purity of the preparations used, since similar reactions occur in normal individuals following the intradermal injection of pneumococcal protein (12). Delayed reactions of this sort possess none of the specific characteristics of those induced with purified preparations of the type specific capsular polysaccharides.

In individuals acutely ill with pneumococcal pneumonia the incidence of positive skin tests to the homologous polysaccharides is much lower than that reported in the literature for normal individuals who were tested with Type I and Type II specific polysaccharides. In the present series of 104 cases only 12.5 per cent showed a positive skin test before specific antibody had been given.

SUMMARY

Skin tests with the homologous type specific polysaccharides have been performed in 104 patients with pneumonia caused by pneumococcus Types I, II, III, V, VII, and VIII.

The test was found to be applicable to the control of dosage of antipneumococcal horse and rabbit serum.

Thirteen patients (12.5 per cent) showed a positive skin test before the administration of serum, and in this group the test could not be used as a guide to therapy.

The skin test is of greatest value in patients who show a negative reaction before serum administration, and in whom serum is effective in initiating recovery. Eighty-one patients (78 per cent) in the present series fell into this category, and in this group, with but one exception, the skin test proved to be a satisfactory and valuable aid in determining the optimum amount of serum necessary for treatment.

The preparations of specific polysaccharides to be used for skin tests must be as highly purified as possible, otherwise nonspecific reactions occur which are practically indistinguishable from the reaction with the specific polysaccharides. Such impurities make the specific reaction almost impossible to interpret, and destroy the value of the test.

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