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Floyd W. Denny Jr., ... , William D. Perry, Lewis W. Wannamaker

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TYPE-SPECIFIC STREPTOCOCCAL ANTIBODY¹

By FLOYD W. DENNY, JR.,² WILLIAM D. PERRY,³ AND LEWIS W. WANNAMAKER⁴

(From the Streptococcal Disease Laboratory, Francis E. Warren Air Force Base, Wyoming, and the Department of Preventive Medicine, Western Reserve University, Cleveland, O.)

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Immunity to group A streptococcal infections has been shown to be associated with the formation of antibody to the type-specific component of the streptococcus, M-protein (1). In spite of its apparent importance, little is known concerning this antibody. A variety of techniques has been employed in the study of type-specific antibody but for practical purposes the bacteriostatic test is the only method which has proved to be satisfactory. Using this technique, Kuttner and Lenert (2) reported that, following infection with a Type 36 streptococcus, all patients developed type-specific antibody, and that the antibody persisted for long periods of time and was unrelated to the development of antistreptolysin O. These authors, however, were unable to demonstrate significant type-specific antibody in patients following infection with Types 15 and 19 streptococci. In extensive studies of the bacteriostatic test in three patients, Rothbard (3) found that type-specific antibody appeared 3 to 5 weeks following infection and persisted for at least 37 weeks. Rothbard, Watson, Swift, and Wilson (4) observed that bacteriostatic antibody appeared in 76 per cent of 82 streptococcal infections (71 patients) 2 to 10 weeks following onset of the disease; patients who developed rheumatic fever exhibited rises more frequently than did those patients who did not suffer this complication. In addition, these authors reported that bacteriostatic antibody appeared later than antistreptolysin O and that the

rise in bacteriostatic antibody was slightly delayed in patients who developed rheumatic fever. In a study of patients with scarlet fever treated with penicillin, Daikos and Weinstein (5) reported that the administration of penicillin suppressed formation of type-specific bacteriostatic antibody and that there was a relation between the dose and route of administration of penicillin and the degree of suppression.

In the studies to be described, the time of development of type-specific bacteriostatic antibody in patients with untreated infections due to several types of group A streptococcus was determined; the effect of penicillin and Aureomycin® treatment on the inhibition of type-specific antibody as related to the persistence of the streptococcus was studied; and the time of development of type-specific antibody in patients with rheumatic fever and uncomplicated streptococcal infections was compared. In most instances, the development of this antibody was compared with the development of the more widely studied streptococcal antibody, antistreptolysin O.

DESCRIPTION OF STUDY

This study was conducted at Francis E. Warren Air Force Base, Wyoming, on airmen whose ages were between 19 and 21 years. The following criteria for inclusion in the study were employed: 1) The presence of exudative pharyngitis or tonsillitis, 2) a peripheral leucocyte count of 10,000 or greater, 3) the isolation of typable group A streptococci from the throat culture, and 4) availability for follow-up examinations for at least three months after the acute infection. In addition, a small group of patients with streptococcal infections who did not meet all the above criteria but who subsequently developed acute rheumatic fever was included.

Ninety-four patients fulfilled the above criteria and were admitted to the study. Forty-nine patients had uncomplicated streptococcal infections and were given no specific treatment; classification of the streptococci isolated from the throat cultures of these patients showed that 9 were Type 5, 9 were Type 6, 12 were Type 24, and 19 were Type 14. Thirteen patients with rheumatic fever were included; 8 had previously had Type 14 infec-

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² Present address: Western Reserve University School of Medicine, Cleveland 6, O.

³ Present address: Barnes and Wohl Hospitals, St. Louis 10, Mo.

⁴ Present address: Hospital of the Rockefeller Institute, New York 21, N. Y.

tions and 5 had had Type 24 infections. Twenty-two patients with Type 14 streptococcal infections were treated with Aureomycin®, and 10 patients with Type 14 streptococcal infections were treated with penicillin. These patients all had uncomplicated infections and were treated at random as part of another study. Aureomycin® therapy consisted of the oral administration of a total dose of 10.5 Gm. over a period of five days. Procaine penicillin G in sesame oil with 2 per cent aluminum monostearate was administered by intramuscular injection in doses of 600,000 units on admission to the study, and again in 72 hours.

Throat cultures and sera were obtained from all patients at the time of admission to the hospital, at approximately 2-week intervals for 2 months, and then at monthly intervals. Cultures were taken with dry sterile cotton swabs and streaked directly onto sheep-blood agar. In most instances another swab was also incubated in Pike's selective broth (6) for 18 hours and then subcultured on sheep-blood agar. Beta-hemolytic streptococci were classified according to the method of Swift, Wilson, and Lancefield (7). Antistreptolysin O titers of all sera from each patient were determined concomitantly by a modification of the technique of Hodge and Swift (8). Differences of two dilution increments (0.2 log) were considered indicative of infection.

Type-specific antibody was measured by a modification of the technique as reported by Rothbard (3). Details of this method are as follows:

Streptococcal strains. Strains of streptococci freshly isolated from throat cultures of patients acutely ill with streptococcal infections were used in all experiments.

Preparation of cultures. Stock strains of streptococci were kept at -20° C. These were prepared by growing the streptococcus in fresh beef-heart infusion broth for 6 to 8 hours, adding 2 per cent sterile rabbit or sheep serum, and rapidly freezing in small aliquots in a carbon dioxide ice-alcohol bath. The day before each experiment, 0.25 cc. of the stock culture was added to 5 cc. of beef-heart infusion broth to which had been added a drop of sheep blood. This culture was incubated for eight hours at 37° C. with frequent shaking. One-tenth cubic centimeter of this rapidly growing culture was added to 5 cc. of beef-heart infusion broth and incubated at 37° C. for 15 hours. Tenfold serial dilutions of this culture were made in dextrose phosphate broth and were the dilutions used in the bacteriostatic test. The number of chains of streptococci per cubic centimeter was calculated by pouring sheep blood agar plates containing 0.5 cc. of the 10^{-4} dilution and 1.0 cc. of the 10^{-7} dilution. The 10^{-4} dilution usually contained 100 to 200 colonies per cc., and the 10^{-7} dilution, 10 to 20 colonies per cc.

Source of blood. Blood from normal adults which showed no bacteriostatic activity for the streptococci under investigation was used in all experiments. Heparin in a final dilution of 1:30,000 was used as an anticoagulant. Leucocyte counts were performed on all specimens and were always above 4500 per cubic millimeter.

Bacteriostatic test. All sera from each patient were

TABLE I

The development of type-specific antibody and antistreptolysin O in patients with untreated infections due to different types of Group A streptococci

Streptococcus type	No. cases	Patients developing significant antibody increase*			
		Type-specific antibody		Antistreptolysin	
		No.	Per cent	No.	Per cent
5	9	8	89	9	100
6	9	9	100	9	100
14	19	17	89	19	100
24	12	11	92	11	92
Total	49	45	92	48	98

* Type-specific antibody—2 logs or greater; antistreptolysin O—0.2 log or greater.

tested concomitantly against homologous and heterologous types of streptococci. Five-hundredths of a cubic centimeter of each serum was added to a series of six tubes (100 by 7 mm.). To these tubes was added 0.05 cc. of the appropriate dilution of culture, 10^{-1} through 10^{-6} . Freshly drawn heparinized blood, 0.25 cc., was then added to each tube. The tubes were sealed in an oxygen-gas flame and incubated at 37° C., in an electrically driven machine which rotated the tubes on the long axis at 8 R.P.M. Gross readings for the presence of hemolysis of the blood were made after 14 hours; the presence of hemolysis indicated growth of the streptococcus and absence of bacteriostatic antibody; the absence of hemolysis indicated failure of the streptococcus to grow and the presence of bacteriostatic antibody. Differences in readings of 2 tubes (2 logs) or greater were considered significant.

RESULTS

The development of type-specific antibody and antistreptolysin O in patients with untreated and uncomplicated infections due to Types 5, 6, 14 and 24 group A streptococci is shown in Table I. Type-specific antibody developed in 45 of 49 patients, or 92 per cent, while a diagnostic increase in antistreptolysin was demonstrated in 48, or 98 per cent, of the patients. The difference in the percentage of patients who developed these two antibodies according to the type of the infecting streptococcus was insignificant.

Figure 1 shows the time of development of antistreptolysin O in the same group of patients. This figure shows, by 10-day periods following the onset of the streptococcal infection, the cumulative per cent of patients who developed a 2-tube or significant rise of antistreptolysin. By the end of 30 days the majority of patients had developed in-

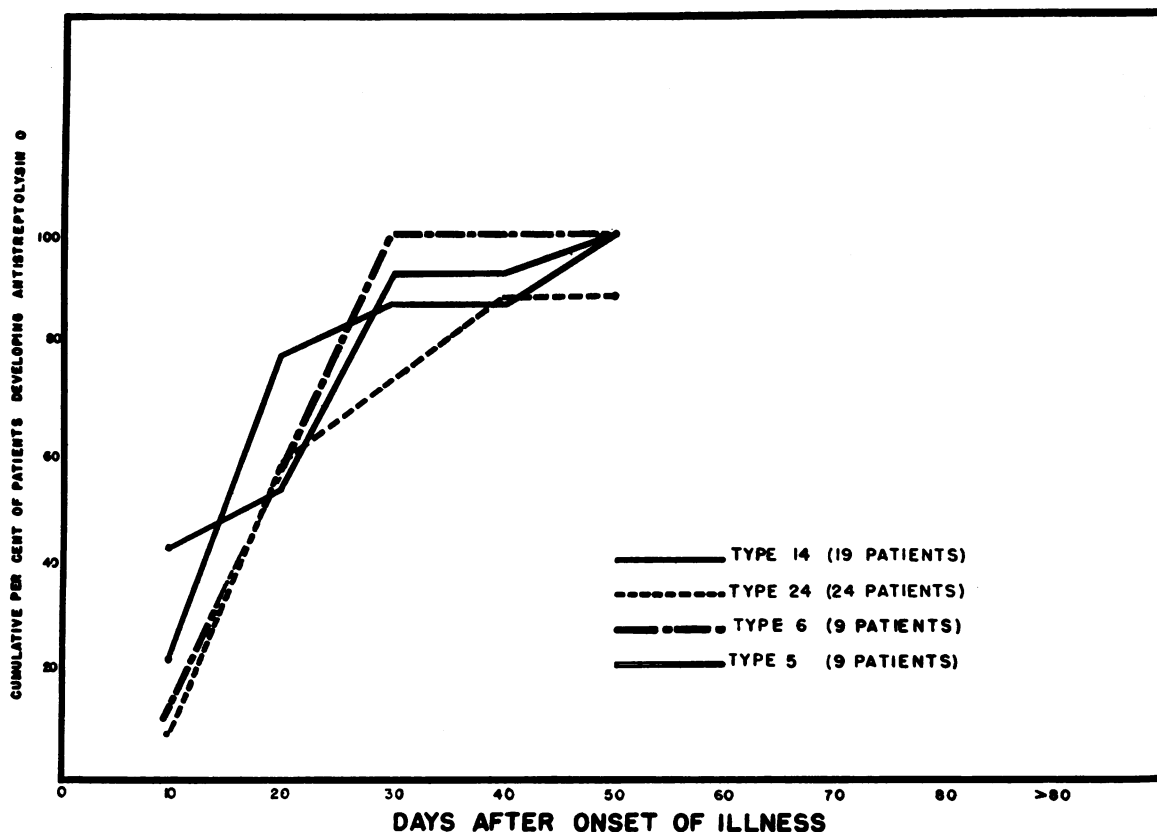


FIG. 1. TIME OF ANTISTREPTOLYSIN O RESPONSE IN PATIENTS WITH INFECTIONS DUE TO DIFFERENT TYPES OF GROUP A STREPTOCOCCI

Serum samples were not obtained from all individuals in every 10-day period in the studies presented in Figures 1 through 4. The values for each period, however, were calculated using the total number of individuals in the group as the denominator; patients were included in the numerator at the time of the demonstrated antibody increase, regardless of the time that had elapsed since the previous antibody determination. This was considered justifiable after it had been demonstrated that the shapes and relative positions of the curves were almost identical when patients were included in the numerator in the period immediately following the time of the last serum that showed no antibody increase, regardless of the time that elapsed until the antibody increase was exhibited.

creases in antistreptolysin and there was no significant difference among patients with infections due to different types of streptococci.

The time of development of type-specific antibody in the same patients is shown in Figure 2. Those patients with Type 24 infections developed antibody most rapidly, so that by 40 days after the onset of the acute illness 83 per cent of patients had shown significant rises. This is in contrast to the development of type-specific antibody in those patients with Type 5 infections. By 40 days only 11 per cent had developed antibody, and it was not until after 80 days that 88 per cent had shown significant rises. Patients with Type

6 and Type 14 infections developed type-specific antibody at rates that were between these two extremes. It is apparent that antistreptolysin O developed rapidly following streptococcal infections and the time of development was not altered by the infecting type of streptococcus. In contrast, type-specific antibody developed slowly and the rate at which this antibody appeared was dependent on the serological type of the infecting organism.

The time of development of type-specific antibody in the eight patients with Type 14 infections, and in five patients with Type 24 infections who subsequently developed rheumatic fever, was the

same as in the patients with uncomplicated infections due to these same types.

Figure 3 shows the development of antistreptolysin in patients with Type 14 streptococcal infections as it is affected by treatment with Aureomycin® or penicillin. The group of patients who received no treatment is the same as that shown in Figures 1 and 2. Aureomycin® therapy caused only slight inhibition to the development of antistreptolysin, while penicillin therapy resulted in marked suppression. By 30 days only 40 per cent of patients treated with penicillin had shown a significant increase in titer, and it was not until 60 days that 80 per cent of the patients had a 2-tube rise.

Figure 4 shows the development of type-specific antibody in the same group of patients. Aureomycin® treatment resulted in partial inhibition of this antibody; only one patient had developed

antibody before 50 days, and only a little over 60 per cent of patients developed antibody by the time the study was terminated. In contrast to this, no patients in the penicillin treated group developed type-specific antibodies, even though they were followed for periods of up to 115 days.

Because of the marked differences that were seen between the group treated with Aureomycin® and that treated with penicillin in the development of both kinds of antibody, an attempt was made to explain these differences. The effect of treatment on the persistence of the infecting type of streptococcus in the follow-up cultures is shown in Table II. All patients who received no therapy continued to harbor the infecting type of streptococcus; of the 10 patients treated with penicillin, 1 patient had one positive culture following treatment; of the 22 patients treated with Aureomycin®, 19 showed a positive culture at some time

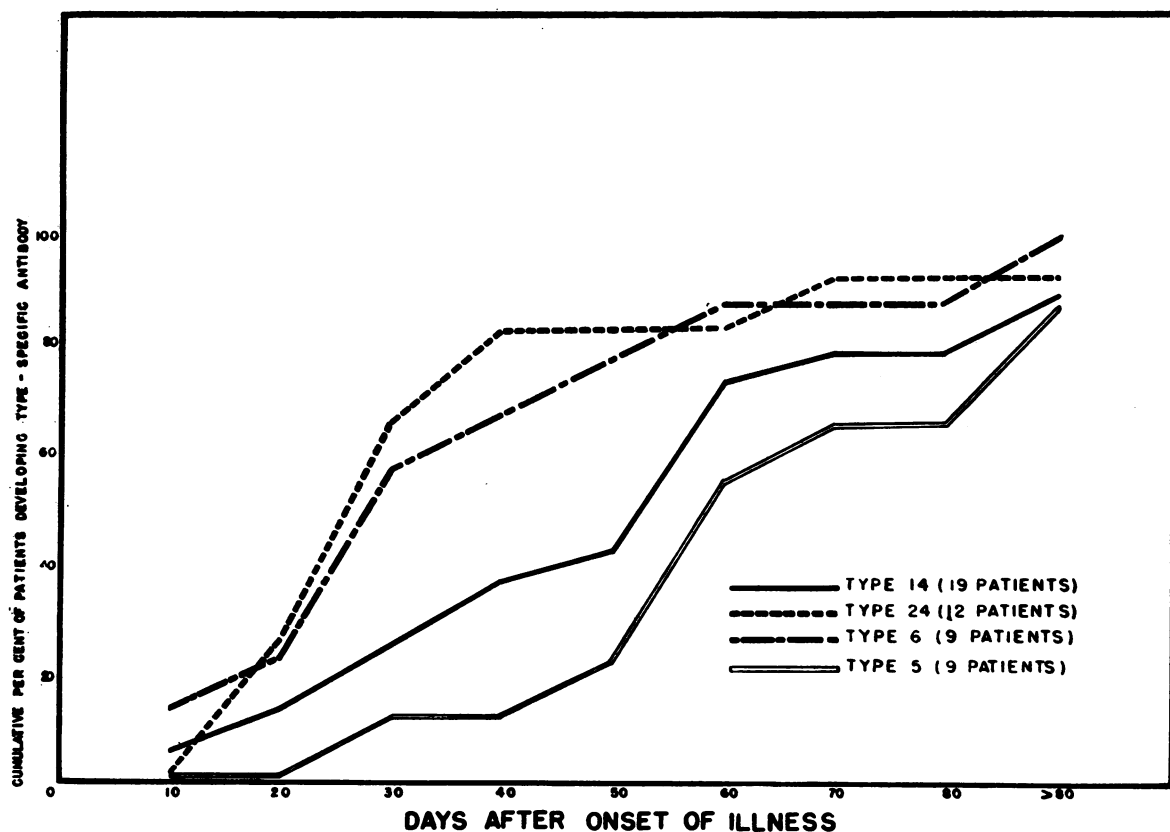


FIG. 2. TIME OF DEVELOPMENT OF TYPE-SPECIFIC ANTIBODY IN PATIENTS WITH INFECTIONS DUE TO DIFFERENT TYPES OF GROUP A STREPTOCOCCI

See explanatory note to Figure 1.

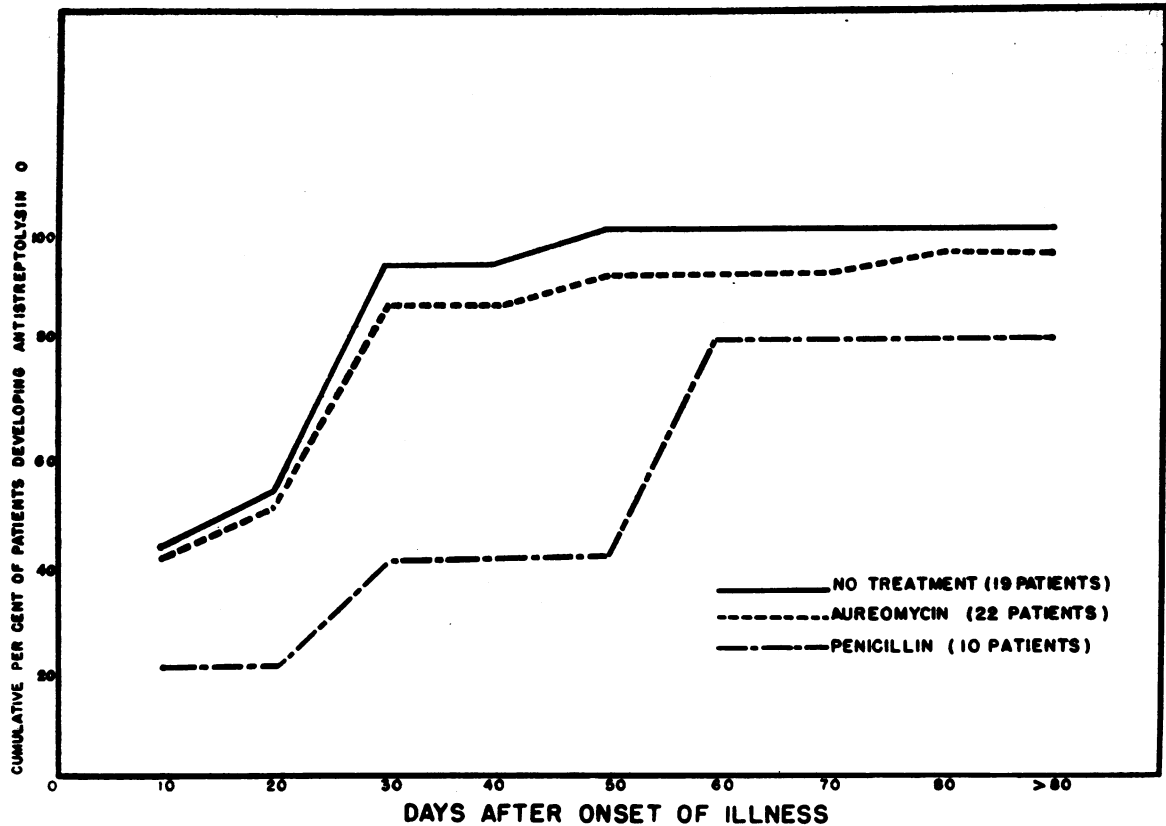


FIG. 3. EFFECT OF THERAPY OF TYPE 14 STREPTOCOCCAL INFECTIONS ON THE ANTISTREPTOLYSIN O RESPONSE
See explanatory note to Figure 1.

following therapy. Table II also shows the relationship of the persistence of streptococci to the development of antibody. None of the patients who had consistently negative follow-up cultures developed type-specific antibody, while 31 of 39 patients with positive follow-up cultures developed this antibody, regardless of the type of treatment. Fourteen of the 19 patients who continued to harbor streptococci in spite of Aureomycin® therapy developed type-specific antibody. All patients with positive follow-up cultures developed antistreptolysin responses; 9 of the 12 patients with negative follow-up cultures developed this antibody. It was concluded, therefore, that in the doses used, penicillin was more effective than Aureomycin® in the eradication of streptococci, that there was close correlation between the development of antibody and the eradication of streptococci, and that this effect was more pronounced in the case of type-specific antibody than in the case of antistreptolysin.

DISCUSSION

The techniques which have been employed in the study of M-protein or type-specific streptococcal antibody include agglutination (9), precipitation (10), mouse protection (11), hemagglutination (12), and bacteriostasis (2, 3). In the study of human infections, all of these methods except the bacteriostatic test have proved to be unsatisfactory because of non-specific reactions, technical difficulties, or difficulty in interpreting results. Rothbard (3) described the bacteriostatic test in detail, and the method used in this study was a modification of his procedure. Preliminary studies had shown that the modified test gave results that were comparable to those obtained with the original test and that it was easier to perform.

The bacteriostatic test has many variables and there is no adequate quantitative control. The differences shown in the present paper in the time of development of type-specific antibody accord-

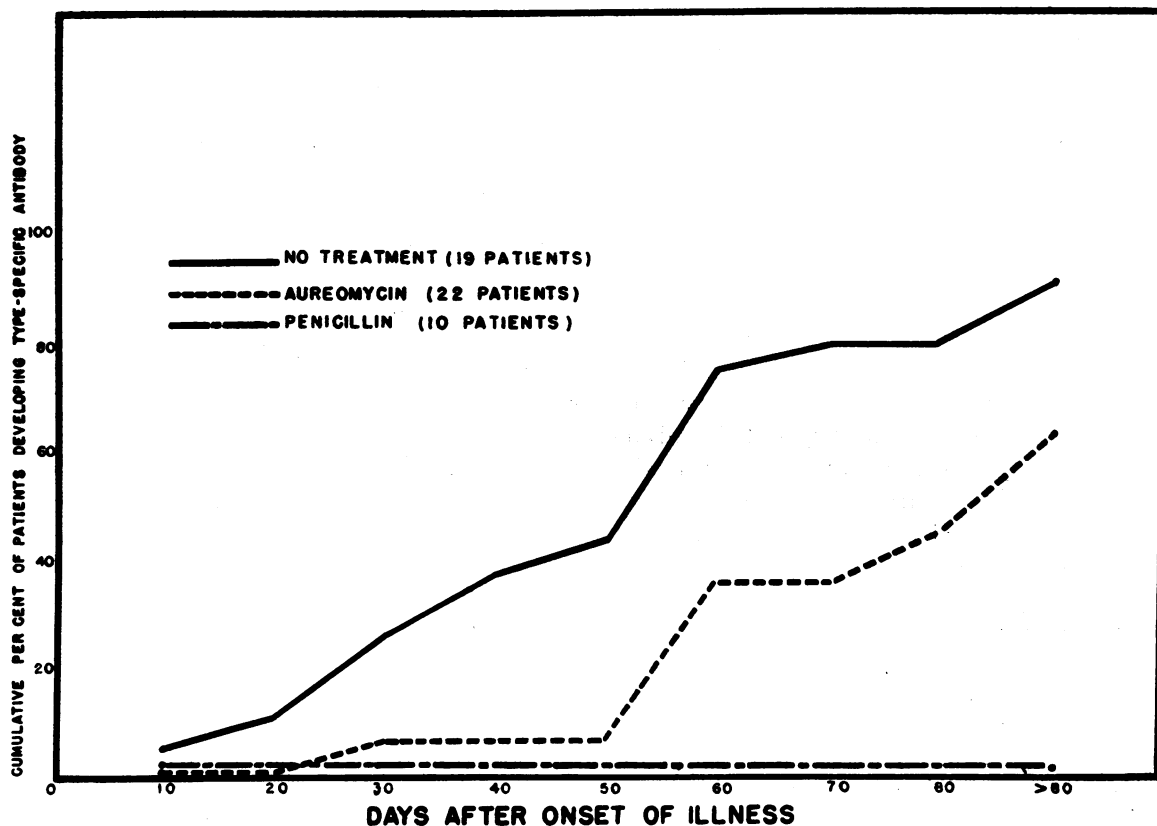


FIG. 4. EFFECT OF THERAPY OF TYPE 14 STREPTOCOCCAL INFECTIONS ON THE TYPE-SPECIFIC ANTIBODY RESPONSE
See explanatory note to Figure 1.

ing to the infecting type of streptococcus, and the effect of therapy on the type-specific antibody response must, therefore, be interpreted with caution. It should be pointed out that there is no assurance, with the present test, that the levels of antibody measured are the effective levels in the

host. It should also be emphasized that there was great variation in the time of development of type-specific antibody among individuals infected with a single type of streptococcus. While there were definite patterns in the time of development with each type, there were overlaps between types at

TABLE II

The relationship of the persistence of streptococci to antibody response in patients receiving various types of treatment

Treatment	Follow-up cultures		Patients developing antibody increase			
			Type-specific antibody		Antistreptolysin	
	Results	No.	No.	Per cent	No.	Per cent
None (19 patients)	Positive	19	17	89	19	100
	Negative	0				
Penicillin (10 patients)	Positive	1	0	0	1	100
	Negative	9	0	0	7	78
Aureomycin® (22 patients)	Positive	19	14	74	19	100
	Negative	3	0	0	2	67
Total	Positive	39	31	79	39	100
	Negative	12	0	0	9	75

each time period. Other facets of the present study which were not ideal, but about which nothing could be done, were the selection of patients by rather rigid criteria, the small numbers of patients available, and the inability to obtain serum samples at exact intervals from all patients.

Rothbard, Watson, Swift and Wilson (4) reported that type-specific antibody begins to rise later than antistreptolysin O in patients with uncomplicated streptococcal infection, the average time of rise being 4.2 and 2.4 weeks, respectively. The results reported in this paper confirm the difference in the time of rise of antistreptolysin and type-specific antibody. According to Rothbard and his associates, type-specific antibody began to rise slightly later and antistreptolysin slightly earlier in patients who developed acute rheumatic fever as compared with those who developed no complications. In the present study, however, no differences were noted in the time of development of Types 14 and 24 antibodies in patients with uncomplicated streptococcal infections and in those who developed rheumatic fever. Since Rothbard and his group did not report differences in the time of development of antibodies according to the serological type of the infecting streptococcus, it is possible that the difference they observed might have been due to a difference in distribution of infecting types between the uncomplicated group and the group developing rheumatic fever. It is also possible that the number of patients observed, particularly in the present study, was too small to demonstrate significant differences of this type.

Stetson (13) and Rantz, Boisvert, and Clark (14), have reported that antistreptolysin O response varies with the infecting type of streptococcus. Rantz and his group found differences in the magnitude and time of response, while Stetson reported only on the magnitude of the responses. It is possible that some of the observed differences in the time of the response may be significant, but the present data do not allow this conclusion. In contrast, the differences in the time of development of type-specific antibody according to the serological type in these same patients are quite marked.

Daikos and Weinstein (5), using Rothbard's technique, reported that penicillin treatment of streptococcal infections suppresses the development of type-specific antibody. When penicillin

was given intramuscularly for 10 days, no patients developed this antibody; penicillin given intramuscularly for 7 days, or penicillin administered orally for 7 or 10 days, resulted in significantly higher percentages of patients developing antibody. These authors attributed these differences to the speed with which the various therapeutic schedules eliminated the infecting organism. The development of type-specific antibody in the present study appeared to be correlated with the persistence of streptococci in the throat of the host. No patients in whom the streptococci had been eradicated developed this antibody, while a high percentage of those who continued to harbor the organisms in spite of treatment showed significant rises. Furthermore, in patients in whom the organism was not eliminated, the degree of suppression seemed important. While cultures from 14 of 19 patients treated with Aureomycin® showed streptococci at some time following treatment, the number of positive cultures and the degree of positivity of the cultures were reduced in most instances.

These results are in contrast to those obtained when antistreptolysin was measured. Most patients developed this antibody even though streptococci were eradicated. It is of note that in the penicillin-treated group 80 per cent of patients eventually developed significant increases of this antibody, yet in four patients the increase was not observed until 50 days following the onset of the infection. This failure of penicillin to prevent completely the formation of antistreptolysin, in spite of the apparent eradication of the streptococcus and the complete inhibition of type-specific antibody, suggests that the major antigenic stimulus to the formation of antistreptolysin occurs early in the course of streptococcal infections, while the stimulus to the formation of type-specific antibody occurs over a much longer period. This observation is in agreement with data reported by Brock and Siegel (15). These authors found that treatment of streptococcal pharyngitis with penicillin within 31 hours after the onset of the disease was more effective in the suppression of antistreptolysin formation than therapy delayed for 3 or 5 days. It is also possible that the more frequent antistreptolysin response represents a recall phenomenon, in that the patients probably had had previous contact with streptolysin and a

small amount of antigen might induce detectable antibody; on the other hand, they probably had not had contact with the M-protein of the streptococcus causing the observed infection.

Previously published data have shown that Aureomycin® therapy is as effective as penicillin therapy in relieving the symptoms of acute streptococcal infections (16). The data presented here, however, show that Aureomycin® in the doses employed is not as effective as penicillin in eradicating the streptococcus or inhibiting the formation of type-specific antibody. Theoretically, Aureomycin® would have the advantage of curing the patient but still allowing the formation of antibodies that protect against reinfection. However, the chances of reinfection following penicillin therapy are not great. Furthermore, unless the streptococcus is eradicated, patients may develop recurrences of exudative pharyngitis and the attack rate of rheumatic fever may not be decreased (17). Penicillin, therefore, remains the drug of choice in the treatment of streptococcal infections.

SUMMARY

Type-specific streptococcal antibody developed in the majority of individuals following streptococcal respiratory infections. In contrast to anti-streptolysin, this antibody developed slowly and showed marked variation in the time of development according to the serological type of the infecting streptococcus. Treatment with penicillin or Aureomycin® inhibited the formation of this antibody, penicillin causing the greater degree of inhibition. The degree of inhibition appeared to be related to the successful elimination of the organism by therapy.

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