A COMPARATIVE STUDY OF ANTIHYALURONIDASE, ANTISTREPTOLYSIN "O," ANTISTREPTOKINASE, AND STREPTOCOCCAL AGGLUTINATION TITERS IN PATIENTS WITH RHEUMATIC FEVER, ACUTE HEMOLYTIC STREPTOCOCCAL INFECTIONS, RHEUMATOID ARTHRITIS AND NON-RHEUMATOID FORMS OF ARTHRITIS [1]

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INTRODUCTION

The antibody response of patients with rheumatic fever and group A hemolytic streptococcal infections to certain extracellular and intracellular substances elaborated by the hemolytic streptococcus has been studied by numerous workers. The four substances which will be considered in this study are antistreptolysin "O," antistreptokinase, antihyaluronidase, and agglutinins to the autoclaved streptococcus.

HISTORICAL

Antistreptolysin O. Present conceptions of the streptolysins are based on investigations of Todd (1–3) who reported in 1928 the presence of streptolysins elaborated by the hemolytic streptococcus. These were: *i*) streptolysin "O" which is oxygen labile, reversibly oxidized or reduced, and is antigenic; *ii*) streptolysin "S" which is oxygen stable, extremely labile to acid and heat and is not by itself antigenic. Antibodies against streptolysin "O" are formed in response to infection with the hemolytic streptococcus and for this reason measurement of antistreptolysin "O" has proved a useful test for indicating recent infection with the hemolytic streptococcus. Among the earlier important investigations of the antistreptolysin "O" titer in patients with hemolytic streptococcal infection, rheumatic fever and glomerulonephritis were those of Todd (1932) (3), Myers and Keefer (1934) (4), Blair and Hallman (1935) (5), Wilson, Wheeler, and Leask (1935) (6), Coburn and Pauli (1935) (7), Longcope (1936) (8), Bunim and McEwen (1940) (9), Windblad (1941) (10), and Mote and Jones (1941) (11). More recent studies have confirmed these earlier observations that the antistreptolysin "O" titer increases significantly following hemolytic streptococcal infections of the upper respiratory tract and during rheumatic fever. These studies include the work of Kalbak (12, 13) Rantz, Boisvert, and Spink (14), Windblad, Malmros, and Wilander (15), and Anderson, Kunkel, and McCarthy (16). A rise in antistreptolysin titer usually can be demonstrated during the second and third week following an acute hemolytic streptococcal infection and a titer above 200 units generally has been considered as serological evidence of a recent infection with the hemolytic streptococcus.

Antistreptokinase. In 1933 Tillett and Garner (17) discovered that culture filtrates of beta hemolytic streptococci were capable of lysing normal human fibrin clots. This agent was given the name of fibrinolysin. However, the more recent studies of Milstone (18), Christensen (19) and Kaplan (20) suggest that fibrinolysin is a kinase and the fibrinolysin reaction may be regarded as analogous to the transformation of the trypsinogen into trypsin by enterokinase. A new name has been suggested for fibrinolysin (21, 22)—streptokinase. Antistreptokinase is the new name suggested for the old term antifibrinolysin. This substance which inhibits streptokinase may increase in patients following acute infections with the hemolytic streptococcus (22). The studies of Mote and Jones (11) and Anderson, Kunkel and McCarthy (16) furnished further evidence of the specificity of streptococcal streptokinase; they also demonstrated a rise in antistreptokinase titer following hemolytic streptococcal infections.

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Antihyaluronidase. The presence in human serum of an inhibitory substance which is capable of neutralizing an enzyme elaborated by a strain of hemolytic streptococcus has been demonstrated by Friou and Wenner (23). This enzyme has been termed hyaluronidase, and the inhibitory substance in human serum, antihyaluronidase. Haas (24) has shown the presence in serum of heat labile inhibitors of hyaluronidase. Thompson and Moses (25) have investigated the inhibitors of streptococcal and other hyaluronidases and have found that the inhibitor of streptococcal hyaluronidase may be either thermostable or thermolabile. The thermolabile inhibitors may be destroyed by heating at 56° C. for 30 minutes and the work of Marcus and Fulton (26) shows that the thermolabile inhibitors can be completely or partially restored following the addition of complement. Friou (27) however, was unable to show any measurable decrease in serum inhibitor of streptococcal hyaluronidase after heating sera at 56° C. for 30 minutes. Only after heating the sera for two hours did any measurable decrease in titer occur. Friou and Wenner using the mucin-clot prevention test further showed that the amount of inhibitory substance in the sera of patients with rheumatic fever was greater than in sera of patients early in convalescence from uncomplicated streptococcal infection or in sera from normal individuals. Recently their work has been confirmed by Quinn (28) and by Harris and Harris (29). A thermostable inhibitory substance in human serum of bull's testicle hyaluronidase has not been demonstrated by use of the mucin-clot prevention test.

Agglutinins to autoclaved streptococci. Streptococcal agglutination has long been used in studies of rheumatoid arthritis, but much less in rheumatic fever (30, 31). The majority of workers found that this agglutination titer was usually low in cases of rheumatic fever but high in rheumatoid arthritis (32, 33). The bacterial suspension employed was either live organisms or heat-killed organisms (at 56° C.). The difficulties inherent in this test were the spontaneous agglutination of the cells and the non-homogeneity of the suspension. Thulin (34) introduced a new element when he used autoclaved hemolytic streptococci and found an increased agglutination titer in the sera from patients with rheumatic fever as well as those with rheumatoid arthritis. His results have been confirmed in this laboratory (35).

The agglutination test employing autoclaved bacteria is apparently quite different from the procedure in which live organisms are used. The group specific polysaccharide apparently plays an important role in rendering the former reaction specific for hemolytic streptococci (36) whereas according to Boots and associates (37) a substance on the surface of the bacterial cells seems to be responsible for agglutination of live bacteria.

It is the purpose of this paper to report the results of the above four tests in groups of patients convalescent from acute beta hemolytic streptococcal infections, and in particular patients with rheumatic fever, patients with rheumatoid arthritis, patients with non-rheumatoid forms of arthritis and normal subjects, as well as to compare and correlate the results of the two relatively *new* tests, (the agglutination test employing autoclaved beta hemolytic streptococci and the antihyaluronidase test) simultaneously with those obtained by the better known antistreptolysin "O" and antistreptokinase tests. An attempt was made to show antibody patterns for these specific antibodies in the groups of patients studied. A few representative cases are presented to demonstrate the rise and fall of the various antibodies in patients during the course of a beta hemolytic streptococcal infection of the upper respiratory tract and of rheumatic fever.

MATERIAL AND METHODS

Serum specimens [3]

When blood was obtained, the serum was separated within 24 hours and then stored in lusteroid tubes at − 10° C. Serum was heated at 56° C. for 30 minutes before testing. In previous studies by the authors (35, 38) serum was not heated before testing.

Description of patients and normal subjects

The six different groups of individuals studied will be described briefly.

[3] The serum specimens were obtained from patients on the medical wards and in the arthritic clinic of the New Haven Hospital, from the Children's Center, New Haven, and from Southbury Training School, Southbury, Conn., through the courtesy of Dr. Herman Yannet and Miss Rose Liebermann; and the U. S. Naval Hospital, St. Albans, Long Island, through the courtesy of Captain H. L. Weaver and Captain W. D. Small.

1. Streptococcal disease (33 patients)

This group was composed of children and young adults who were convalescent from an acute beta hemolytic streptococcal infection (upper respiratory in the form of pharyngitis, tonsillitis or scarlet fever) which had occurred within the previous month but not within two weeks of the time serum was obtained. Seventeen of these individuals had received one of the sulfonamides or penicillin, but in no case did treatment extend beyond five or six days nor was it begun sooner than three days after the onset of the infection. Doses of either drug were relatively small. No patient received a total of more than 15 grams of sulfonamide or 500,000 units of penicillin during the period of treatment.

2. Rheumatic fever, active (86 patients)

This group of patients was composed of young adults and a few children with both clinical and laboratory evidence of active rheumatic fever. Many degrees of activity were represented in these patients. All of those classified as having rheumatic fever, active, acute were receiving salicylates in therapeutic doses. About half of those with active, subsiding rheumatic fever were receiving salicylates and but a negligible percentage of those with chronic rheumatic fever received salicylates.

3. Rheumatic fever, inactive (64 patients)

In this group of patients were those who had rheumatic heart disease or a past history of rheumatic fever, but in whom there was no evidence of rheumatic activity.

4. Rheumatoid arthritis (56 patients)

Four-fifths of these patients had both clinical and laboratory evidence of active rheumatoid arthritis and about one-fifth were judged to have inactive disease. The arthritic process had been recognized a minimum of four months and a maximum of 19 years in these patients. The majority with active disease were taking salicylates in small doses.

5. Non-rheumatoid arthritis (32 patients)

Under this classification were grouped patients with osteoarthritis, gout, Reiter's syndrome, and tuberculous arthritis.

6. Normal subjects (53 subjects)

Sera were obtained from so-called normal, healthy young adults in whom there had been no historical evidence of a hemolytic streptococcal infection or severe upper respiratory infection within the previous four months.

Antistreptolysin "O"

The source of the streptolysin "O" was a strain of beta hemolytic streptococcus known as 089.[4] The method used to determine the antistreptolysin "O" content of sera was that described by Todd (2) and modified by Hodge and Swift (39).

[4] This organism was furnished through the courtesy of Dr. Benedict Massell, House of the Good Samaritan.

Antistreptokinase

The procedure for the quantitative estimation of serum antistreptokinase was that described by Kaplan(20). Sources of materials were as follows:

Fibrinogen: Lyophilized fraction I (Human).[5]

Thrombin: A commercial preparation of hemostatic globulin (clotting globulin) was used.[6]

Streptokinase

The source of streptokinase was a strain of beta hemolytic streptococcus known as No. 98 secured from Melvin H. Kaplan.

Antihyaluronidase

The mucin-clot prevention test used in the determination of antihyaluronidase was exactly as described in a previous study (38) having been modified from the method described by McClean (40) and Friou and Wenner (23).

The enzyme used in these tests was that elaborated by a strain of group A, type 4, beta hemolytic streptococcus prepared as described in previous work (38). Since all sera were heated at 56° C. for 30 minutes presumably the heat labile inhibitor was inactivated. In this study it is the heat stable and not the heat labile inhibitor of streptococcal hyaluronidase which is being considered.

Agglutination test with autoclaved streptococci

The technique was exactly the same as described by Liao (35) except that all sera were "inactivated" at 56° C. for 30 minutes in the present study. This procedure did not change the agglutination titer of sera previously tested before "inactivation." The organism was a strain of group A, type 4, beta hemolytic streptococcus.

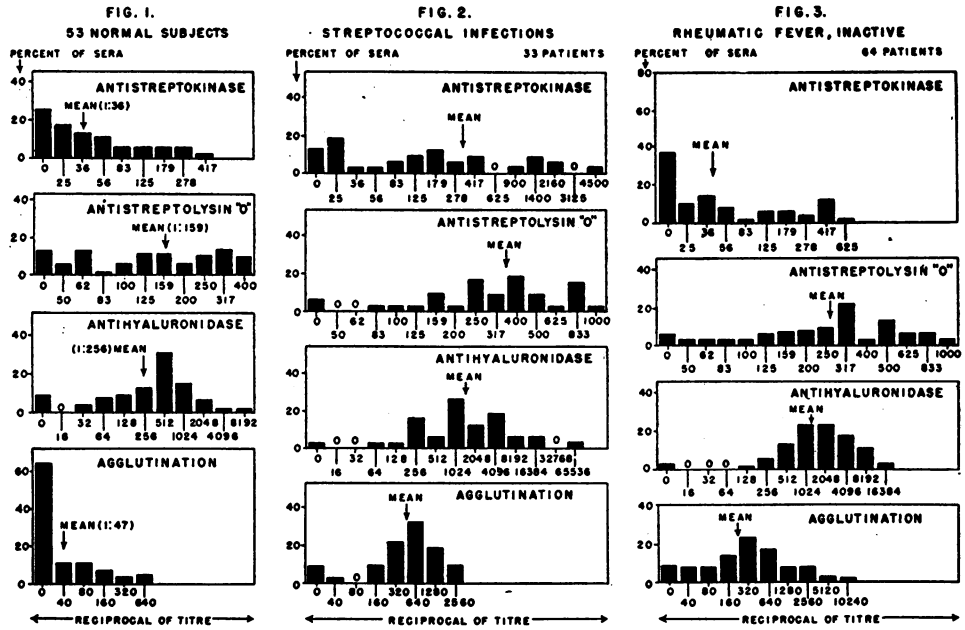
For the sake of brevity, the antihyaluronidase, antistreptolysin "O," and antistreptokinase will be referred to as the anti-enzyme tests.

RESULTS

To provide a baseline, the frequency distribution and mean serum titers were determined for normal subjects and were found to correspond with those for normal individuals reported from this laboratory and others with the exception of the mean antihyaluronidase titer which was 1:256. The mean antihyaluronidase titer for normal young adults in previous studies from this laboratory was 1:1024 (28, 41), (Figure 1). The mean titers for these normals were as follows: streptococcal agglutination 1:47, antihyaluronidase 1:256, antistreptolysin "O" 1:159, and antistreptokinase 1:36. The frequency distribution and mean anti-

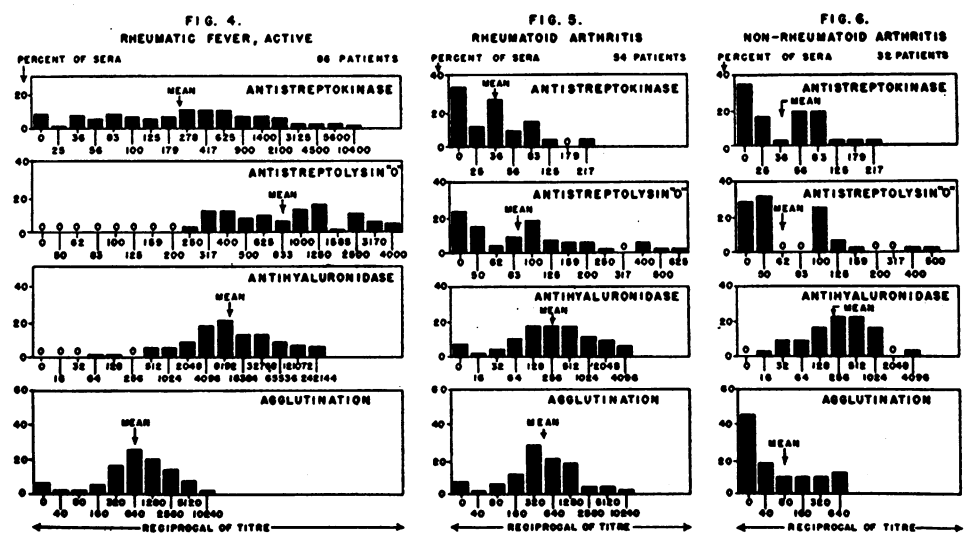
[5] Kindly donated by Cutter Laboratories, Berkeley, Calif.

[6] Lederle Laboratories Inc., New York, N. Y.



FIGS. 1, 2, and 3. THE AVERAGE DISTRIBUTION OF TITERS IN FOUR TESTS ON NORMAL SUBJECTS, STREPTOCOCCAL INFECTIONS, AND RHEUMATIC FEVER—INACTIVE

body titers for all of the groups of patients studied are shown in Figures 1 through 6. The mean agglutination titer and the two mean anti-enzyme titers, antihyaluronidase and antistreptolysin "O," were significantly higher in patients with hemolytic streptococcal infections and inactive rheumatic fever than in normal subjects. In the former group there was no significant difference in any of the antibody titers for the 17 patients treated with sulfonamides or penicillin and the remaining 16 who were not treated with sulfonamides or penicillin. The mean antistreptokinase titers were identical in patients with *inactive* rheumatic fever and normal subjects but significantly higher in patients con-



FIGS. 4, 5, and 6. THE AVERAGE DISTRIBUTION OF TITERS IN FOUR TESTS ON PATIENTS WITH RHEUMATIC FEVER, ACTIVE; RHEUMATOID ARTHRITIS, AND NON-RHEUMATOID ARTHRITIS

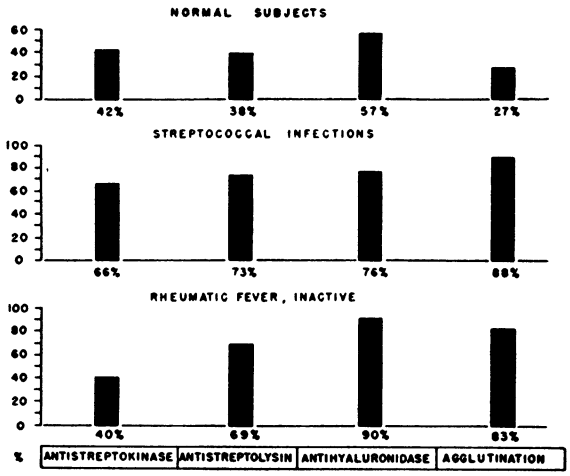


FIG. 7. PER CENT OF NORMAL SUBJECTS, PATIENTS CONVALESCENT FROM B HEMOLYTIC STREPTOCOCCAL INFECTIONS AND PATIENTS WITH RHEUMATIC FEVER, INACTIVE, WHO HAD SERUM ANTIBODY TITERS HIGHER THAN THE MEAN TITERS FOR NORMAL SUBJECTS

valescent from beta hemolytic streptococcal infections. There were no significant differences in any of the antibody titers for the groups of patients with inactive rheumatic fever and streptococcal infections with the exception of the mean antistreptokinase titer which was much higher in the latter group of patients. There was some overlapping of the distribution graphs for the four antibodies in patients with inactive rheumatic fever, hemolytic streptococcal infections and normal subjects, so that to demonstrate more graphically the antibody patterns in these three groups of patients, Figure 7 has been prepared to show the per cent of patients who had antibody titers above the observed mean for normal subjects. The antibody titers were slightly higher in the patients convalescent from hemolytic streptococcal infections than patients with inactive rheumatic fever with the exception of antihyaluronidase.

The frequency distribution and mean titers of the four antibodies for the groups of patients with active rheumatic fever, rheumatoid arthritis, and non-rheumatoid arthritis are shown in Figures 4–6. In patients with active rheumatic fever the mean anti-enzyme (AH, AS–O, ASK) titers were significantly higher than those for any other groups of patients studied. The agglutination titer although higher, was not significantly greater than that found for patients with rheumatoid arthritis, rheumatic fever, inactive, or streptococcal infections, but was significantly higher than in normal subjects or patients with non-rheumatoid arthritis. The mean agglutination titer for patients with rheumatoid arthritis was significantly higher than that for patients with non-rheumatoid arthritis and normal subjects, but the anti-enzyme titers were similar.

Figure 8 has been prepared to show the per cent of patients with active rheumatic fever, rheumatoid arthritis and non-rheumatoid arthritis who had antibody titers above the observed mean for normal subjects. All of the antibody titers were definitely elevated in over 83% of the patients with active rheumatic fever. In the group of patients with rheumatoid arthritis the agglutination titer was elevated in 91%, but the per cent with elevated anti-enzyme titers was even less than those for normal subjects. The per cent of patients with non-rheumatoid forms of arthritis who had antibody titers higher than normal was negligible.

To illustrate the antibody response of patients following hemolytic streptococcal infections, five cases are shown in Figures 9 through 12.

Case No. 1, R. W. Male, 18½ yrs.

This patient had a sore throat for one month prior to the onset of rheumatic fever on 1/10/47. He may have had rheumatic fever at age 11. By the time the first blood sample was drawn on 2/13/47 all of the antibody titers were markedly elevated. Ten months after the onset of rheumatic fever, when the rheumatic process was clinically inactive, the anti-enzyme titers had fallen but were still

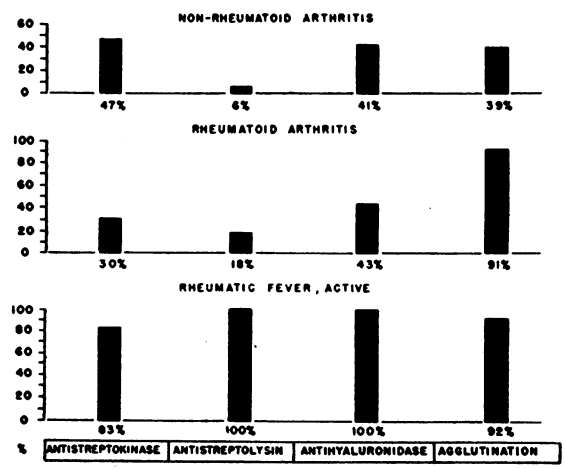


FIG. 8. PER CENT OF PATIENTS WITH NON-RHEUMATOID ARTHRITIS, RHEUMATOID ARTHRITIS, AND RHEUMATIC FEVER, ACTIVE, WHO HAD SERUM ANTIBODY TITERS HIGHER THAN THE MEAN TITERS FOR NORMAL SUBJECTS

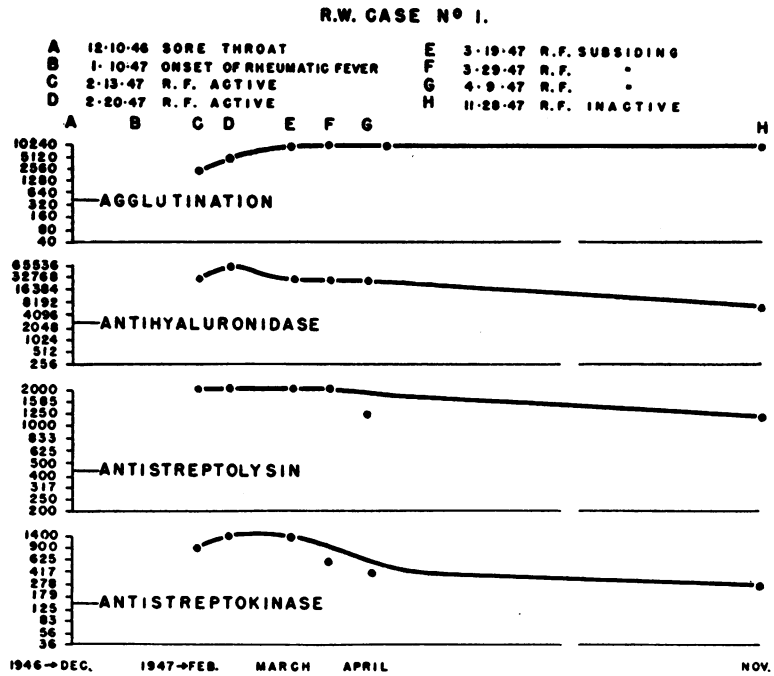


FIG. 9. SERIAL TESTS IN PATIENT R. W.

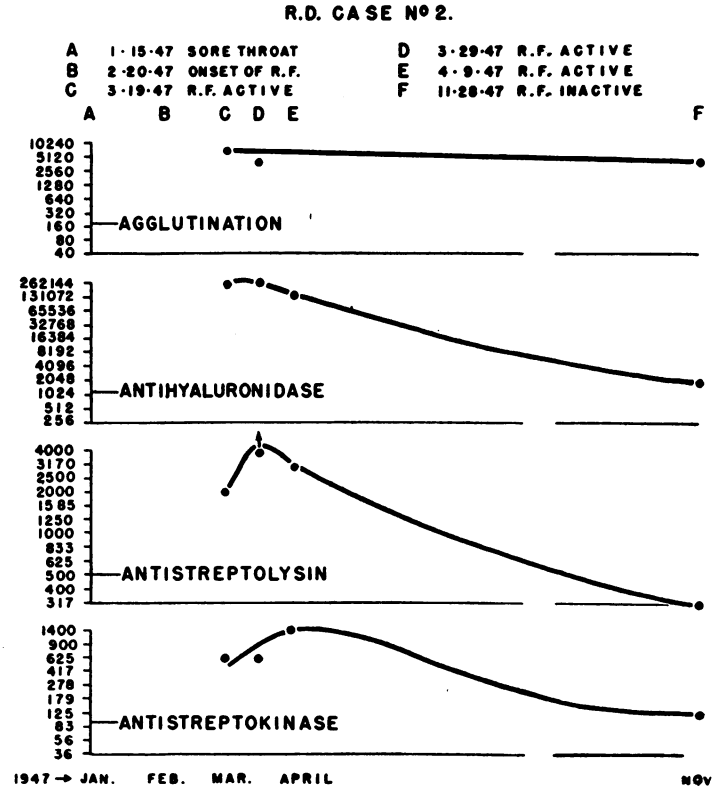


FIG. 10. SERIAL TESTS IN PATIENT R. D.

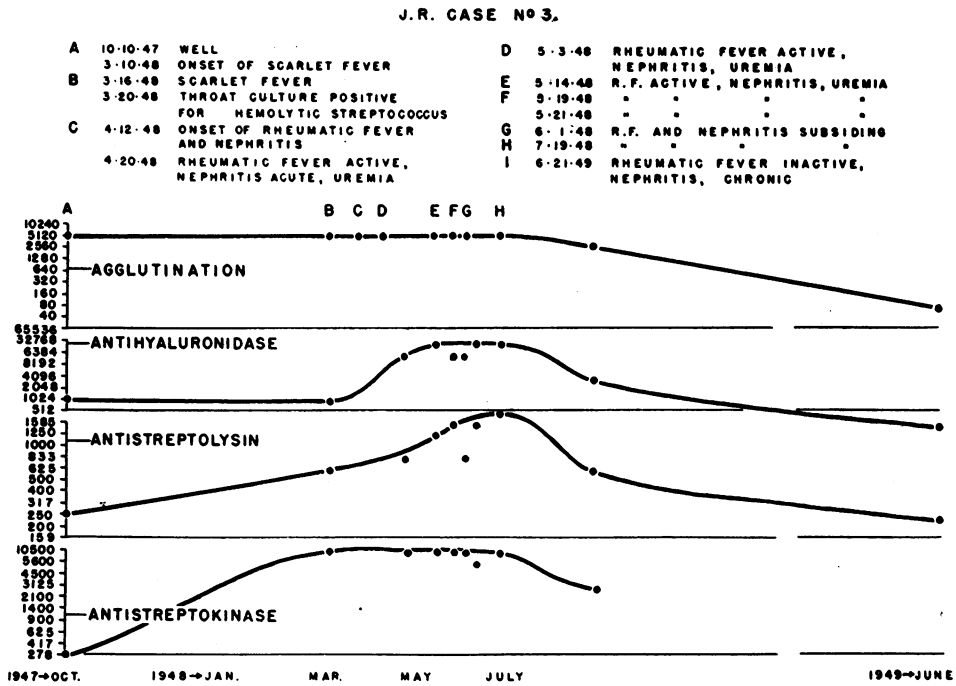


FIG. 11. SERIAL TESTS IN PATIENT J. R.

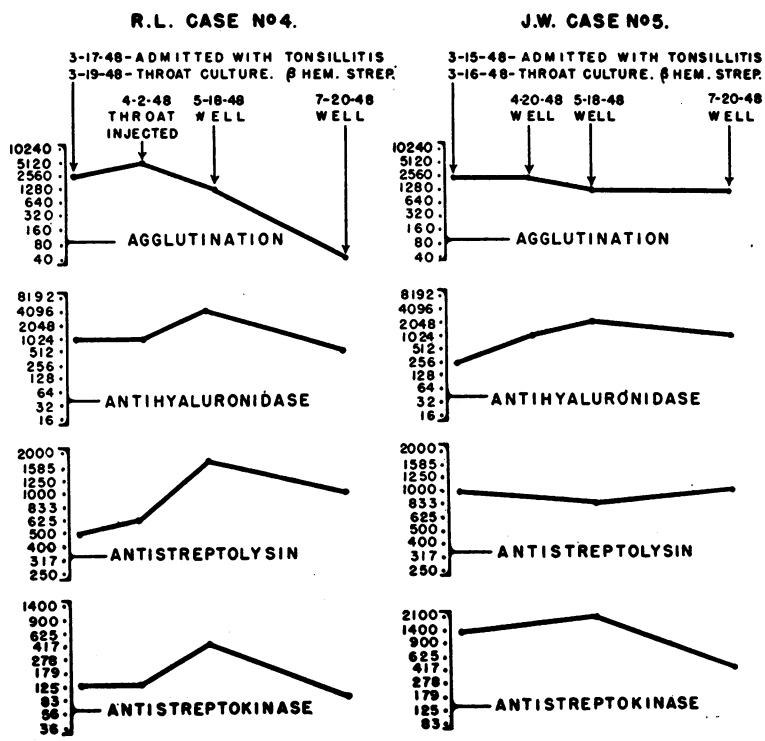


FIG. 12. SERIAL TESTS IN PATIENTS R. L. AND J. W.

considerably above the normal range even for patients with inactive rheumatic fever. The agglutination titer had not changed.

Case No. 2, R. D. Male, 18 yrs.

On 1/15/47, this patient acquired a sore throat. He was on the sick list one week but continued to have general malaise until the onset of rheumatic fever on 2/20/47. On 3/19/47 the antibody titers were all at an extremely high level and both antistreptolysin "O" and antistreptokinase titers continued to rise. Two months after the onset of rheumatic fever the anti-enzyme titers, with the exception of antistreptokinase, had begun to fall, but ten months after the onset when the rheumatic process was clinically inactive the four antibody titers were still somewhat elevated.

Case No. 3, J. R. Male, 14 yrs.

On 3/11/48 this patient became ill with acute tonsillitis and within two days a scarlatiniform rash was present. Throat culture was positive for beta hemolytic streptococci. During the first week of illness he received 32 gm. of sulfadiazine and 600,000 units of penicillin. On 4/12/48 both rheumatic fever and nephritis began. During the next six weeks the patient was extremely ill and was uremic, the N.P.N. being as high as 105 mgm. %. However, two months after the onset of rheumatic fever and nephritis he began to recover. Between 8/20/47 and 10/20/47 he had three furuncles but no cultures were taken. All drained well. Fortunately, five months prior to the onset of scarlet fever a blood specimen had been taken and serum frozen. The anti-enzyme titers were within normal limits with the exception of antistreptokinase which was somewhat above normal and the agglutination titer which was definitely elevated. On 3/16/48, six days after the onset of scarlet fever, the AS–"O" and ASK titers were elevated, the latter to an extremely high level, but it did not rise further. Eight days after the onset of rheumatic fever and nephritis, the antihyaluronidase and antistreptolysin "O" titers had risen to very high levels and subsequently rose even higher. On 7/19, over four months after scarlet fever, the four antibody titers had begun to fall but were still markedly elevated. On 6/21/49, 14 months after the onset of rheumatic fever and nephritis, the patient showed no evidence of active rheumatic fever. The N.P.N. was 42 mgm. % and the urine contained albumin 2 + and both granular and hyaline casts in moderate numbers. At this time the agglutination titer was at the low level of 1 : 80, the antistreptolysin level was 250, and the antihyaluronidase titer was 1 : 512.

Case No. 4, R. L. Male, 10 yrs.

On 3/17/48 this boy was admitted to the hospital with acute tonsillitis. The throat culture was positive for beta hemolytic streptococci. No sulfonamides or antibiotics were administered and the child made an uneventful recovery. One month after the onset of the infection on 4/20/48 none of the anti-enzyme titers had risen significantly, but the agglutination titer was elevated to 2160. Two months later all of the anti-enzyme titers had increased significantly, but the agglutination titer had decreased. Four months after the onset, the four antibody titers had returned to normal levels with the exception of antistreptolysin which was decreasing yet still elevated.

Case No. 5, J. W. Male, 13 yrs.

On 3/15/48 this patient was admitted to the hospital with acute tonsillitis. The throat culture was positive for beta hemolytic streptococci. He received sulfadiazine gm. 1 t.i.d. for six days. On 3/16 the antistreptolysin "O," antistreptokinase and agglutination titers were already at a high level, but the antihyaluronidase was within normal range. During the next two months the antihyaluronidase titer rose significantly. Both antihyaluronidase and antistreptokinase titers had begun to fall within four months after the onset of the hemolytic streptococcal infection, but the antistreptolysin "O" and agglutination titers were still elevated and had not begun to decrease.

DISCUSSION

The rise in titer of streptococcal agglutinins, antihyaluronidase, antistreptolysin "O" and antistreptokinase demonstrated during the course of hemolytic streptococcal infections and the significantly higher mean titers for these antibodies in patients convalescent from streptococcal infections, when compared with the same antibody titers for normal subjects, illustrates that these tests are sensitive indicators of recent beta hemolytic streptococcal infection. The antistreptolysin "O" and antistreptokinase tests have long been used in this regard and the present results suggest that the antihyaluronidase test is an equally good indicator and possesses a high degree of specificity. These findings confirm those of Harris and his associates (42) and those previously reported from this laboratory (23, 28). It will be noted that the mean antihyaluronidase titer for normal subjects was lower than that reported for normal young adults in previous studies from this laboratory (28, 41). The explanation of this difference is not clear but the fact that sera in this study were heated prior to testing may have inactivated heat labile inhibitors of hyaluronidase and allowed only heat stable inhibitors to remain. However, the present study was not designed to clarify this point and differences in the size of the groups of normal subjects in the three studies and the time of year when serum samples were collected would also have to be considered in any explanation of the observed differences. The agglutination titers to autoclaved streptococci did not rise markedly in the cases re-

ported possibly because blood samples were not obtained early enough; however, in other cases not reported here a significant rise in the agglutination titer has been noted following a beta hemolytic streptococcal infection of the upper respiratory tract (43). Invariably the agglutination titers decreased after a few months.

The findings of this study indicate that the anti-enzyme titers (antihyaluronidase, antistreptolysin "O" and antistreptokinase) of the patients who acquired rheumatic fever following a hemolytic streptococcal infection were significantly higher than for patients with uncomplicated hemolytic streptococcal infections. Similar observations for various streptococcal antibodies have been made by Windblad (10), Kalbak (13), Rothbard and his colleagues (44) and Harris and Harris (29). Furthermore, the antibody titers remained high for a longer period of time and the return to lower levels required a much longer time in patients with rheumatic fever than in those who recovered without complications. The number of cases reported in this study to illustrate this point is small, but this phenomenon has been observed in other patients studied by the authors (43).

It has been reported that early and effective penicillin therapy (16, 45) or occasionally sulfonamide therapy (46) will suppress or entirely inhibit the antibody response to streptolysin or streptokinase. The absence of any significant difference between the penicillin and sulfonamide treated patients in the streptococcal infections and the "non-treated" group with streptococcal infections in relation to streptococcal antibodies is possibly related to the relatively small doses of drugs administered and the comparatively short periods of administration, this method of therapy being inadequate to suppress antigenic stimulation by streptococcal products.

Although the number of patients observed is small and there is some overlapping of the frequency distribution graphs of the four antibodies, a general pattern for the four antibodies studied has been outlined in Table I for the patients with rheumatic fever, active, rheumatoid arthritis, and non-rheumatoid forms of arthritis. In the patients with active rheumatic fever both the anti-enzyme and agglutination tests were consistently elevated; in the patients with rheumatoid arthritis only the agglutination titer was significantly elevated and in those with non-rheumatoid forms of arthritis, none of the antibody titers was regularly elevated. These variations in antibody patterns would suggest fundamental differences between these three diseases.

It is obvious that the agglutination test employing autoclaved streptococci can not be relied upon alone as a differential diagnostic test between rheumatic fever and rheumatoid arthritis. This has been emphasized previously by Liao (35). However, on the basis of differences in antibody patterns outlined above (Table I), the agglutination test used in combination with any one or all three of the anti-enzyme tests can be recommended for diagnostic purposes in the differentiation between rheumatic fever, rheumatoid arthritis and non-rheumatoid arthritis. The agglutination test with *live* organisms usually gives high titers in patients with rheumatoid arthritis but in rheumatic fever the titers are not elevated (33).

One drawback in the use of the agglutination or anti-enzyme tests as aids in the differential diagnosis between rheumatic fever and arthritis is that a recent hemolytic streptococcal infection in a patient may alter the antibody pattern so that it resembles that in a patient convalescent from hemolytic streptococcal infection or even the occasional patient with rheumatic fever. A similar observation in relation to antistreptolysin titers was made by Bunim and McEwen in 1940 (9).

TABLE I

(refers to Figures 4–6)

Tests	Rheumatic fever, active	Rheumatoid arthritis	Non-rheumatoid arthritis
Streptococcal anti-enzyme tests	titers	titers	titers
1. Antihyaluronidase 2. Antistreptolysin "0" 3. Antistreptokinase	high	low	low
Streptococcal agglutination test with autoclaved bacteria	high	high	low

SUMMARY AND CONCLUSIONS

1. A comparative study has been presented of four streptococcal antibody titers, namely, streptococcal agglutinins, antihyaluronidase, antistreptolysin "O," and antistreptokinase in groups of patients with rheumatic fever, active or inactive,

streptococcal infections of the upper respiratory tract, rheumatoid arthritis, non-rheumatoid forms of arthritis and normal subjects.

2. The antihyaluronidase test apparently measures a specific antibody and a rise in this antibody titer was demonstrated following hemolytic streptococcal infections of the upper respiratory tract.

3. The mean antihyaluronidase titer for patients with active rheumatic fever was significantly higher than in any other group of patients studied. This was true also for the mean antistreptolysin "O" and antistreptokinase titers.

4. All three anti-enzyme titers (antihyaluronidase, antistreptolysin "O," and antistreptokinase) appeared to remain high for considerably longer periods of time in patients who acquired rheumatic fever following a hemolytic streptococcal infection, than in patients who recovered from a hemolytic streptococcal infection without complications.

5. Although there was a rise in streptococcal agglutination titers following a hemolytic streptococcal infection of the upper respiratory tract, there were no significant differences between the mean agglutination titers for patients with rheumatic fever, streptococcal infections or rheumatoid arthritis.

6. In active rheumatic fever all four antibodies were found to be high, in rheumatoid arthritis only the agglutination titer was high, and in non-rheumatoid arthritis none of the antibody titers was consistently elevated.

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