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Research Article

STREPTOCOCCAL AGGLUTININS AND ANTISTREPTOLYSINS IN RHEUMATOID (ATROPHIC) ARTHRITIS

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Numerous attempts during the past several years to isolate bacteria of possible etiologic significance from the blood, synovial fluid, or tissues of patients with rheumatoid (atrophic) arthritis have yielded inconsistent results (1). In general, the lack of uniformity in the results of bacteriologic studies in this disease is striking.

Nevertheless, a suggestion of a possible relationship of hemolytic streptococci to rheumatoid arthritis was offered by the demonstration by Nicholls and Stainsby (2) of agglutinins for hemolytic streptococci in high titer in the blood serum of a majority of patients with this disease. Several subsequent reports (3–8) confirm in general the results of Nicholls and Stainsby, and show that the tendency of hemolytic streptococci to be agglutinated by sera from cases of rheumatoid arthritis is not restricted alone to the "typical strain" of Cecil, Nicholls, and Stainsby (9), but that the reaction is characteristic of *beta* (hemolytic) streptococci as a group.

Following infection by hemolytic streptococci, the blood serum contains streptococcal antihemolysin (antistreptolysin) in high titer. This was demonstrated by Todd (10) and later confirmed by Coburn and Pauli (11), working in conjunction with Todd, and by Myers and Keefer (12), in such infections as acute follicular tonsillitis, scarlet fever, erysipelas, and acute rheumatic fever. Wilson, Wheeler, and Leask (13) reported that following streptococcal infections the average antistreptolysin titer was definitely higher than in normal subjects. Seegal and Lyttle (14) found high antistreptolysin titers in a large percentage of a series of cases of acute glomerular nephritis.

Inasmuch as the demonstration of agglutinins for hemolytic streptococci in the serum of patients with rheumatoid arthritis is at least suggestive of some relation of these organisms to this disease, it appeared that an investigation of the antistreptolysin content of sera from patients with rheumatoid arthritis might be of interest. Myers and Keefer (12), whose paper appeared soon after the inception of this work, claimed that "the sera of patients with rheumatoid, as well as other forms of arthritis, fell into a group which resembled that of normal individuals or patients with non-streptococcal infections."

It is the purpose of this communication, first, to add confirmation of the presence of agglutinins for hemolytic streptococci in high titer in the sera of patients with rheumatoid arthritis, and second, to demonstrate that a certain percentage of these sera contain antistreptolysin in titers above the normal range.

AGGLUTINATION TESTS

Cases studied. Agglutination tests were performed on 69 sera from 62 cases of typical rheumatoid arthritis. The patients in this group presented the typical syndrome of a chronic polyarthritis which tended to progress to ankylosis and deformity. All degrees of severity of the disease were included in the series. The duration of the disease ranged from 3 months to 36 years; the great majority of the cases were of one year's duration or over. The age of the patients, including two children with Still's disease, ranged from 6 to 68 years; three-quarters of the group were under 50 years of age, and about half of the group were in the fourth and fifth decades.

As controls, 129 sera from 125 persons presenting a variety of infectious and non-infectious, orthopedic, medical, and surgical conditions were employed. These included hypertrophic arthritis, spondylitis ankylopoietica (of the Marie-Struempell or Bechterew type), gonorrheal arthritis, osteomyelitis, rheumatic fever, proven infections by hemolytic streptococci, and miscellaneous other conditions. The age distribution of the control series was similar to that of the patients with rheumatoid arthritis.

TABLE I

Agglutination by sera from cases of rheumatoid arthritis

Case number	Sex and age	Hemolytic streptococci					*Streptococcus viridans*	Staphylococci		Anti-streptolysin titer	Sedimentation rate	Duration of arthritis
		"Typical strain"	Rheumatic fever	Erysipelas	Erysipelas	Scarlet fever		*Micrococcus deformans*	*Staphylococcus aureus*			
		AB66	Q33	21	B3	C203	208			units per cc.	mm. in 45 minutes	
1	F38	2560	320		320	640	—			190	81	3 months
		2560	320		640	640	—			190		
2	M26	1280	1280		—	40	—			571	43	6 months
		1280	1280		—	40	10			380		
3	F40	1280	1280		80	640	—			190		3 years
4	F48	1280	1280		80	40	—			190		8 years
5	F50	1280	640	160			—	320	640		90	5 years
6	F27	1280	640	80			20	160	—			3 years
7	F55	1280	640	40			—	80	160			8 years
8	F25	1280	640		160		—			95	33	5 years
9	M25	1280	640		10	160	—			95	50	2 years
		320	640		—	—	—			47	45	
10	F57	1280	640		320	1280	—			95	93	3 years
11	F38	1280	320		160	160	—			380		5 years
12	F36	1280	320		80	80	—			95		22 years
13	M42	1280	320		320	320	—			47	40	3 years
14	M34	1280	160		20	—	—			95	42	18 months
15	F40	1280	40		—	320	—			190	11	1 year
16	M46	1280	320		320		—			95	79	6 years
17	F 7	1280	—		—	—	—			95		4 years
18	F26	1280	160		—		—			<47		7 months
19	M51	640	640	—			—	—	—		33	4 years
20	F 6	640	320		40	20	—			95	20	3 years
21	M54	640	160		640		—			47	54	4 years
22	F27	640	320	40			—	10	40		40	6 years
23	M39	640	320		320		—			47	72	1 year
24	F30	640	320	—			10	—	—			5 years
		320	320		320	—	—			95		
25	M37	640	320		—	40	—			190	50	1 year
26	F50	640	320	20			—	40	40		56	2 years
27	M21	640	320		160	320	—			380	10	2 years
		640	640		320	320	—			285	13	
		640	320		40	160	—			95	15	
		1280	640		160		—			95		
28	M54	640	320	—			—	—	40			5 months
29	F42	640	320		—	80	—			95	17	2 years
30	F45	640	320		40	640	—			47		1 year
31	M24	640	80		—	—	—			380	75	3 years
32	M51	640	80	—			20	160	—			1 year
33	M44	640	—		40	—	—			47	65	3 months
34	F28	320	640		80	80	—			47	10	3 months
35	F56	320	640	—			—	—	640			4 years
36	M35	320	320		80		—			95	18	12 years
37	F60	320	320	80			—					36 years
38	F52	320	160		160		—			47	35	19 years
39	M41	320	320		40	10	—			47	45	4 years
40	F44	320	160		160		—			190	70	3 years
41	F49	320	320	80			—	—	—			12 years
42	F20	160	640		—	10	20			210	47	6 months
43	F40	160	640		20	1280	—			190	19	3 months
44	M49	160	320	—			—	—	—		24	5 years
45	F62	160	320		—	—	—			47		20 years
46	F38	160	320		20	—	80			952	20	2 years
47	F68	160	160	40			—	—	—			10 years
48	F39	160	80		40	40	—			<47	90	5 months
49	F19	160	80		—	—	—			47	25	4 years
50	F49	160	80		20	—	—			95	33	1 year
51	M39	160	80		40	40	—			47		6 months
52	F42	80	—	—			—	—	—			4 years
53	F61	40	640		40	320	—			28	82	2 years

TABLE I (*Continued*)

Case number	Sex and age	Hemolytic streptococci					*Streptococcus viridans*	Staphylococci		Antistreptolysin titer	Sedimentation rate	Duration of arthritis
		"Typical strain" AB66	Rheumatic fever Q33	Erysipelas 21	Erysipelas B3	Scarlet fever C203	208	*Micrococcus deformans*	*Staphylococcus aureus*	units per cc.	mm. in 45 minutes	
54	F57	40	80		20	20	—			47		7 years
55	F54	40	80		160	40	—			0		3 years
56	M47	—	160		—	—	—			133	65	10 years
57	F67	—	—		—	—	—			190		9 months
58	M50	—	—	—			—				20	3 months
59	M18	—	—	—			—	—	1280			3 months
60	F47	—	—		—	—	—			<47	8	7 years
61	F34	—	—		—	640	—			95		4 years
62	F22	—	—	—			—	80	320		38	5 years

The clinical diagnosis of Cases Number 17 and 20 is Still's disease. In the instances where more than one sample of serum was obtained, the time intervals between taking of the samples was as follows: Number 1—20 days; Number 2—2½ months; Number 9—4½ months; Number 24—12 months; Number 27—3 months, 5½ months, and 4½ months.

Technic of agglutination tests

The organisms employed in the agglutination tests were:

Hemolytic streptococci:

"AB66"—Cecil's "typical strain," isolated from the blood of a patient with rheumatoid arthritis.

"Q33"—a rheumatic fever strain, "from the tonsillar exudate of a patient who had suffered for months from severe polyarthritis and carditis with congestive failure" (15).

"21"—from a case of erysipelas.

"B3"—from a case of erysipelas.

"C203"—from a case of scarlet fever.

Streptococcus viridans:

"208"—isolated in this laboratory from the blood in a typical case of subacute bacterial endocarditis.

Staphylococci:

"*Micrococcus deformans*"—isolated by Crowe from the urine of a patient with rheumatoid arthritis.

Staphylococcus aureus "182"—isolated in this laboratory from the blood stream in a fatal case of infected sinus thrombosis.

For their courtesy in supplying certain of these strains, our thanks are due to Dr. W. J. Stainsby, for "AB66"; to Dr. H. F. Swift, for "Q33"; to Dr. K. E. Birkhaug, for "21"; to Dr. M. H. Dawson, for "B3" and "C203"; and to Dr. H. W. Crowe, for *Micrococcus deformans.*

The sera used for the tests were obtained under aseptic precautions and kept in sterile tubes at 4° C. All of the sera were tested with Cecil's "typical strain," Q33, one of the erysipelas strains, and *Streptococcus viridans.* The scarlet fever strain, C203, was used in agglutination tests with 114 sera, and the two staphylococcus strains with 80 sera.

In about half of the agglutination tests, living 24-hour cultures in bacto-heart-infusion broth were employed. Subsequently, younger cultures (16 to 20 hours old) in "streptolysin broth" were used. This medium was made according to the formula described by Swift and Hodge (16) and was found to give excellent diffuse growth. A small series of tests was also made with heat-killed suspensions in "streptolysin broth." Equally good results were obtained with antigens prepared by all three methods. The suspensions were diluted with physiological salt solution to the desired turbidity.

Serial dilutions of the sera were made in serological tubes with physiological salt solution, and an equal volume of bacterial suspension was added to each tube, to give final dilutions ranging from 1:10 to 1:1280. The tubes were incubated in a water bath at 56° C. for two hours, then transferred to the refrigerator, at 4° C. The titer was read after holding the tubes in the refrigerator overnight. The last dilution showing definite clumping of the organisms was taken as representing the agglutination titer.

Results of agglutination tests

Rheumatoid arthritis. The results of agglutination tests with sera from cases of rheumatoid arthritis are shown in Table I. Included in the table, for comparison, are the antistreptolysin

titers and sedimentation rates.[1] Sixty-nine sera from 62 patients were employed in the tests. Of these sera, 59 or 84 per cent (representing 82 per cent of the patients) caused agglutination of Cecil's "typical strain" streptococcus (AB66) in a titer of 1:160 or higher. With few exceptions, agglutination of AB66 was accompanied by agglutination of the streptococcus from rheumatic fever (Q33), and nearly one-fifth of the sera gave a higher titer with Q33 than with AB66. A total of 60 (87 per cent) sera (representing 85 per cent of the patients) caused agglutination of AB66 or Q33, or both, in a titer of 1:160 or higher. The trend of the agglutination titers obtained with the erysipelas and scarlet fever strains was generally appreciably lower than the titers obtained with the "typical strain" or with Q33.

Six of the sera caused agglutination of the strain of *Streptococcus viridans* used, the highest titer obtained being 1:80.

In contrast to the large number of high agglutination titers obtained in the group of rheumatoid arthritis, are the many negative tests obtained with the sera of these "control" groups. When agglutination occurred, the titers obtained with Q33 usually ran parallel to the titers obtained with AB66, and the scattered agglutination titers obtained with the other hemolytic streptococci were generally appreciably lower.

It may be noted that included among the controls are sera from six cases of spondylitis ankylopoietica (of the Marie-Struempell or Bechterew type) and from sixteen cases of hypertrophic arthritis or arthritis of the sacro-iliac articulations. Sera from three cases of spondylitis ankylopoietica and from three cases of hypertrophic arthritis agglutinated AB66 or Q33, or both, in dilutions of from 1:160 to 1:1280. It is recognized that while the joints of the spinal column are sometimes affected in rheumatoid arthritis, all cases of spondylitis ankylopoietica need not have a rheumatoid basis. Furthermore, while cases of the hypertrophic type are generally of a non-infectious origin (idiopathic hypertrophic arthritis), it is known that damage to the tissues of a joint by a previous infection may occasionally be the basis for the development of hypertrophic joint changes.

TABLE II

Summary of agglutination of Cecil's "Typical strain" streptococcus (AB66) and of Q33

	Number of individuals	Total number of sera	Number of sera causing agglutination in various dilutions								
			Negative	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280 and over
Rheumatoid arthritis	62	69	6	0	0	0	3	6	10	23	21
Chronic arthritides other than rheumatoid arthritis	31	32	15	1	0	0	2	1	6	4	3
Miscellaneous infectious and non-infectious orthopedic conditions	35	35	29	0	0	0	1	0	3	1	1
Osteomyelitis	14	14	8	1	0	1	0	0	1	0	3
Miscellaneous medical and surgical conditions	11	11	9	0	1	0	1	0	0	0	0
Rheumatic fever	24	25	8	0	0	2	1	2	4	5	3
Infections by hemolytic streptococci	5	7	1	0	0	0	0	0	2	4	0
Normal persons	5	5	2	0	0	0	1	2	0	0	0

Seventeen of the sera were employed in agglutination tests with the two strains of staphylococci. Seven contained no agglutinins for these organisms, and ten caused agglutination of one or both strains, the titers for *Micrococcus deformans* ranging from 1:10 to 1:320, and for *Staphylococcus aureus* from 1:40 to 1:640.

"Control" groups. A summary of the results of agglutination tests with sera from the various disease groups other than rheumatoid arthritis, and from normal individuals, appears in Table II.

We obtained a somewhat larger percentage of high agglutination titers in our series with sera from cases of rheumatic fever than has been reported by others. High titers were obtained with the various hemolytic streptococci and sera from cases of proven infection by hemolytic streptococci.

[1] The sedimentation rate was determined according to the technic described by Weiss (17).

Of the 129 sera from cases other than rheumatoid arthritis, only 7 caused agglutination of *Streptococcus viridans*. These were: 1 bronchial asthma (1:10); 1 gout (1:80); 1 chronic osteomyelitis (1:20); 1 rheumatic fever (1:40); 1 chorea (1:40); 2 proven infections by hemolytic streptococci (1:10 and 1:20).

About half of all the control sera which were tested against the two strains of staphylococci caused agglutination of one or both of the strains. The majority of the titers ranged from 1:10 to 1:80, although occasionally titers ranging up to 1:1280 were obtained.

Agglutination by synovial fluid

Nicholls and Stainsby (2) stated that they performed agglutination tests with "typical strain" streptococci and synovial fluid from three cases of chronic infectious arthritis. The agglutination titers obtained were 1:640, 1:2560, and 1:5120, respectively.

In our series, forty-four specimens of synovial fluid, from a variety of infectious and noninfectious arthropathies, were used in agglutination tests. The technic was the same as that for the sera, except that the final dilutions ranged from 1:5 to 1:160. In the event that agglutination occurred throughout the series, a second test was run, using dilutions up to 1:5120.

Twenty of the synovial fluids were from patients who had also furnished blood serum for agglutination tests. A comparison of the agglutination titers of the fluids and their corresponding sera is found in Table III. The source of the fluids is as follows: rheumatoid arthritis—5; gonorrheal arthritis—3; hypertrophic arthritis—3; chronic synovitis—3; tuberculous synovitis—2; luetic synovitis—1; miscellaneous conditions of joints—3.

The titers of three of the five fluids from rheumatoid arthritis were 1:160, 1:640, and 1:5120, respectively. The homologous sera in all three

TABLE III

Agglutination by serum and synovial fluid from same patient

Serial number	Hemolytic streptococci						*Streptococcus viridans*		Staphylococci				Clinical diagnosis
	AB66		Q33		21		208		*Micrococcus deformans*		*Staphylococcus aureus*		
	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	Serum	Synovial fluid	
15	1280	640	160	80	40	20	—	—					Rheumatoid arthritis
29	640	5120	320	640	—	40	—	—	—	—	40	40	
45	160	160	320	80	—	5	—	—	—	80	—	320	
60	—	—	—	—	—	—	20	40	10	—	1280	80	
63	—	—	—	—	—	—	—	—	80	—	320	80	
67	640	—	640	160	—	—	—	—	20	—	160	160	Chronic arthritides other than rheumatoid arthritis
68	10	—	10	—	—	—	—	—	40	—	—	—	
69	—	—	—	—	320	—	—	—	10	—	—	—	
70	640	160	640	160	40	—	—	—	20	—	1280	160	
71	320	40	320	10	—	—	—	—	10	—	320	80	
72	—	—	—	—	—	—	—	—	—	—	320	80	
73	—	—	—	—	—	—	—	—	—	—	—	—	Chronic synovitis
74	—	—	—	—	—	—	—	—	—	—	—	—	
75	—	—	—	—	—	—	—	—	80	—	—	—	
76	—	—	—	—	—	—	—	—	40	—	—	—	Luetic synovitis
77	—	—	—	—	—	—	—	—	80	—	—	—	Tuberculous synovitis
78	—	—	—	—	40	160	—	—	40	—	—	160	
79	—	—	—	—	—	—	—	—	—	—	—	—	Internal derangement semilunar cartilage
80	—	—	—	—	—	—	—	—	40	—	—	—	Prepatella bursa
81	—	—	—	—	—	—	—	—	80	—	160	160	Lipoma of knee

cases contained agglutinins in significant titer. Two synovial fluids and their corresponding sera contained no agglutinins for hemolytic streptococci.

A total of five of the 44 fluids caused agglutination of Cecil's "typical strain" streptococcus. Three of these were the fluids from rheumatoid arthritis described above; the other two were from cases of hypertrophic arthritis. Scattered agglutinations of Q33 and of an erysipelas strain occurred, usually in low titer, although three titers of 1:160, and one of 1:640 were obtained. These titers occurred with fluids from cases of gonorrheal arthritis and tuberculous synovitis (1:160) and from one case of hypertrophic arthritis (1:640). *Streptococcus viridans* was agglutinated only once—by a dilution of 1:40 of a synovial fluid from a case of rheumatoid arthritis; this fluid caused agglutination of no other streptococcus, but agglutinated *Staphylococcus aureus* in a dilution of 1:80.

It is interesting to note that 22 of the 44 fluids caused agglutination of *Staphylococcus aureus*. The titers of seven fluids were 1:40 or 1:80; the titers of fourteen fluids were 1:160, and one had a titer of 1:320. When both fluid and serum from the same patient were tested, agglutination occurred with both, with few exceptions, and usually the titer of the serum was higher than that of the fluid. Agglutination of *Staphylococcus aureus* occurred with some synovial fluids from all of the disease groups represented.

Correlation between agglutination titer and certain phases of rheumatoid arthritis

Several attempts have been made to correlate the agglutination titer with various features of the disease (2, 3, 4, 6), such as its duration, number of joints involved, age of the patient, etc. We were unable to establish a correlation of the agglutination titer with any clinical aspects of the disease. We also found no correlation of the agglutination titer with the sedimentation rate, in confirmation of the reports of Dawson, Olmstead, and Boots (4) and of Keefer, Myers, and Oppel (5).

Comment

It appears to be reasonable to assume that an agglutination titer of 1:160 or higher is "significant," or indicative of infection by streptococci. This titer was considered to be of significance in rheumatoid arthritis by Gray and Gowen (3) and by Dawson (4), and their associates, while a significant titer of 1:320 was adopted by Nicholls and Stainsby (2), and by Cox and Hill (6). A titer of 1:160 is much higher than the titers which are commonly accepted as being diagnostically significant in such infections as typhoid fever, Brucellosis, and typhus fever.

The fact is well established that sera from the majority of patients with rheumatoid arthritis contain agglutinins in high titer for hemolytic streptococci, a finding which can hardly be explained as entirely fortuitous. Furthermore, sera from arthritic conditions other than rheumatoid arthritis do not give such consistently high titers.

Nicholls and Stainsby consider that the agglutination in high titer of their "typical strain" streptococcus by sera from cases of rheumatoid arthritis "appears to be a true immunological response," and that the results they have reported "lend strong support to the theory that the 'typical strain' streptococcus is an important etiologic factor" in this disease. Dawson and his associates concluded that, while many of the features of the reaction are indicative of a true immunological response, the results obtained could be considered only as suggestive of an association of hemolytic streptococci with rheumatoid arthritis. Further suggestive evidence has been adduced, they believe, by their comparative study of agglutination and precipitation reactions, in which "a close approximation, but not an absolute agreement" is obtained in the results of the two tests.

It appears to be satisfactorily demonstrated that *beta* type streptococci, particularly such organisms as Cecil's "typical strain," NY5, or Q33, are characteristically agglutinated by sera from patients with rheumatoid arthritis. Cox and Hill (6) found that no other organism which they used had as great a serologic specificity for sera from cases of atrophic arthritis as did Cecil's "typical strain." However, they employed only one other strain of hemolytic streptococcus (isolated from the stool of a patient with rheumatoid arthritis), and they discarded another strain (NY5) after a few tests, since it always gave parallel agglutinations with Cecil's streptococcus, but in a lesser degree. They felt that definite deductions as to the etiologic

rôle of Cecil's "typical strain" would be premature. Wainwright (7), who used Cecil's "typical strain" and NY5, concludes that a positive agglutination "does not indicate of necessity a causal relationship between hemolytic streptococci and rheumatoid arthritis but it does suggest that streptococci play a rôle in this disease . . . the frequency with which it [agglutination] occurs in rheumatoid arthritis is the most incriminating evidence thus far produced against the streptococcus in this disease and merits some consideration."

The assumption that the reaction is a true immunological response suggests that it can be used for diagnostic purposes. Gray and Gowen (3) believe that it "is undoubtedly of considerable value in differential diagnosis, particularly in osteoarthritis." Cox and Hill (6), on the other hand, point out that "ordinarily a laboratory procedure is unnecessary in establishing a diagnosis of arthritis," and feel, from the results of their series of tests, that while the test may be used occasionally in doubtful cases and under carefully controlled conditions, it is of doubtful value in prognosis or as an isolated laboratory procedure.

The agglutination reaction with hemolytic streptococci apparently can serve to confirm a clinical diagnosis of rheumatoid arthritis, but its use hardly seems necessary in the majority of cases, where the clinical signs and symptoms render the diagnosis quite apparent. However, in borderline cases which are difficult of clinical diagnosis, the frequency of high titers in rheumatoid arthritis, and the comparative infrequency of similar titers in other types of arthritis, make it possible to indicate a *probable diagnosis,* based upon the results of agglutination tests with hemolytic streptococci, such as Cecil's "typical strain" or a strain giving closely parallel results.

ANTISTREPTOLYSIN TESTS

In testing sera for their antistreptolysin content, Todd (10) titrated against a unit expressed in terms of the minimal hemolytic dose of streptolysin. Inasmuch as streptolysin produced by hemolytic streptococci of human origin is subject to reversible oxidation and reduction, and the filtrate is hemolytic only when in the reduced state, the hemolytic power of a given filtrate may not remain constant.

However, it has been demonstrated by Hodge and Swift (18) that under certain conditions the power of streptolysin to combine with antisera remains constant over a considerable period. Their method of titrating antisera in terms of the "constant combining power" of streptolysin thus assures a reasonable degree of accuracy and obviates the necessity of having to resort to repeated determinations of the hemolytic titer.

Technic of antistreptolysin tests

The streptolysin used throughout the tests was prepared from cultures of the scarlet fever strain of hemolytic streptococcus, C203, which was used in the agglutination tests described above.

The technic used was that described by Swift and Hodge for preparing streptolysin (16), and by Hodge and Swift for titrating the minimal hemolytic dose and combining power of streptolysin, and for determining the antistreptolysin content of serum in terms of the constant combining power of streptolysin (18).

Without going into the details of the technic, a few pertinent facts may be recorded. Streptolysin was reduced *in vacuuo* for 2 to 3 hours with 0.1 per cent of freshly ground sodium hydrosulfite, immediately after the 16 to 17 hour "streptolysin broth" culture had been filtered through a Seitz filter. The reduced streptolysin was immediately dispensed in sterile tubes or flasks, covered with a thick layer of sterile vaseline, and stored in the refrigerator.

Rabbit erythrocytes were used in all of these tests, as the red blood cells of other animal species have been found to give somewhat inconsistent results.

Streptolysin is "standardized" in terms of its *constant combining power* by determining the amount of streptolysin which is just inhibited by one antistreptolysin unit of a previously titrated serum of known antistreptolysin content.

Serum is titrated for its antistreptolysin content by determining that serum dilution which just inhibits hemolysis of 0.5 cc. of a 5 per cent suspension of twice washed rabbit erythrocytes by one combining unit of standardized streptolysin after incubation at 37° C. for one hour. The antistreptolysin titer of a serum is recorded as

the reciprocal of that dilution which just prevents hemolysis, as described above.

Standards

In tests of this type it is obvious that standards should be established for the titration of streptolysin and antistreptolysin, so that the results obtained in various laboratories may all be recorded in similar terms. Only by this means may adequate comparisons be made of published reports from different laboratories. Recognizing this fact, Todd (19) has set aside a quantity of serum of known antistreptolysin potency, to be used as an arbitrary standard in antistreptolysin determinations.

The streptolysin of Hodge and Swift was standardized "with a number of sera furnished by Dr. Coburn . . . duplicates of these sera had been tested by Todd" (18). Through the courtesy of Dr. Hodge, samples of serum and of standardized streptolysin were obtained, and against these our own streptolysin and a few sera were standardized in a preliminary series of titrations. Recently, Dr. Todd kindly sent us some of his standard antistreptolysin serum which was used in a series of comparative tests with sera whose original titrations refer back to the standards supplied by Dr. Hodge. Thus checks on our streptolysin were obtained directly against Todd's standard serum, and indirectly against his standard, by means of serum supplied by Dr. Hodge.

Antistreptolysin titer of normal serum. In order to evaluate the results of antistreptolysin determinations, it is essential to know to what extent antistreptolysin is present in the serum of normal individuals, and to establish a normal value.

Todd (10), and Coburn and Pauli (11) have demonstrated that the maximum antistreptolysin titer of the serum of normal individuals is about 100 units per cubic centimeter. Parallel tests with Todd's serum and with sera which were titrated by the method of Hodge and Swift have shown that the value which we obtained for normal individuals is essentially equivalent to the value obtained by Todd. It would appear that, for the sake of exact comparison with other published results, the titers of antisera should be expressed in terms of some arbitrary standard, such as is represented by the serum which has been made available by Todd. Consequently, the titers recorded in this paper *are given in terms of Todd's standard serum.* Titers above 100 units per cc. are considered to be indicative of infection by hemolytic streptococci.

Results of antistreptolysin tests

Repeated tests have confirmed the assertions of Hodge and Swift (18) that the combining power of streptolysin remains constant over a considerable period of time (provided the streptolysin has been properly prepared, reduced, and stored in the cold), while during the same period the hemolytic titer may vary appreciably. Their statement has been repeatedly confirmed that the antistreptolysin titer of serum remains stable when the serum is kept sterile and cold.

The specificity of the antistreptolysin test (5, 19) has been amply demonstrated by testing for their antistreptolysin content several sera from animals immunized to a variety of organisms. Practically without exception, the antistreptolysin titers of these immune sera were low (averaging 95 to 45 units or less per cc.). In addition, several human sera possessing high antistreptolysin titers were tested for staphylococcal antihemolysin. The low staphylococcal antihemolysin titers which were obtained confirms Todd's assertion that the hemolysins of streptococci and staphylococci are not serologically related (18).

Rheumatoid arthritis. Antistreptolysin determinations were done on 51 sera from 45 patients. The titers obtained are recorded in Table IV. Eighteen of these sera (representing 15 persons) gave titers definitely above the normal range (over 100 units per cc.) and the titers of the remaining 33 sera were within the normal range (100 units or less per cc.). It is interesting to note that, of the 18 sera giving high antistreptolysin titers, 17 also caused agglutination of either AB66 or Q33, or both, in significant titers. On the other hand, 29 sera which gave significant agglutination titers with these two streptococci possessed antistreptolysin titers within the normal range. Of five sera which gave little or no agglutination with AB66 or Q33, four also possessed normal antistreptolysin titers.

"Control" groups. Antistreptolysin determinations were done on 68 sera from 56 cases other

TABLE IV

Antistreptolysin titers

Serial number of serum	Units per cc.	Approximate equivalent in fraction of cc.
Proven infections by hemolytic streptococci		
		cc.
1	95	0.0105
2	285	0.0035
3 (1)	285	0.0035
(2)	437	0.0023
4 (1)	475	0.0021
(2)	952	0.0010
5 (1)	1146	0.0008
(2)	95	0.0105
6	380	0.0026
7 (1)	665	0.0015
(2)	571	0.0017
Rheumatic fever		
8	47	0.0212
9	95	0.0105
10	95	0.0105
11	190	0.0052
12	190	0.0052
13	266	0.0037
14	266	0.0037
15	285	0.0035
16	285	0.0035
17	285	0.0035
18	285	0.0035
19	285	0.0035
20	342	0.0029
21	761	0.0013
22	1332	0.0007
23 (1)	285	0.0035
(2)	437	0.0023
24 (1)	285	0.0035
(2)	285	0.0035
(3)	190	0.0052
25 (1)	380	0.0026
(2)	475	0.0021
(3)	761	0.0013
(4)	665	0.0015
(5)	475	0.0021

Total number of sera giving titers indicated	Units per cc.	Approximate equivalent in fraction of cc.
Rheumatoid arthritis		
		cc.
5	<47	0.0350
13	47	0.0212
15	95	0.0105
1	133	0.0075
9	190	0.0052
1	210	0.0047
1	285	0.0035
4	380	0.0026
1	571	0.0017
1	952	0.0010
Chronic arthritides other than rheumatoid arthritis		
3	47	0.0212
8	95	0.0105
1	114	0.0087
1	190	0.0052
Miscellaneous orthopedic conditions		
1	47	0.0212
1	57	0.0175
1	95	0.0105
Osteomyelitis		
1	47	0.0212
1	95	0.0105
1	195	0.0051
3	285	0.0035
1	380	0.0035
Miscellaneous medical and surgical conditions		
4	47	0.0212
Normal persons		
4	47	0.0212
1	95	0.0105

than rheumatoid arthritis, representing a variety of orthopedic and medical conditions, and normal persons. Included in the control groups are 11 sera from 7 cases of proven infection by hemolytic streptococci; 25 sera from 18 cases of rheumatic fever; 23 sera from 22 miscellaneous orthopedic conditions (including 7 sera from 7 cases of osteomyelitis); 4 sera from 4 miscellaneous medical or surgical conditions; and 5 sera from 5 healthy persons, with no history of preceding infection by hemolytic streptococci. The antistreptolysin titers of these sera are recorded in Table IV.

Proven infections by hemolytic streptococci. Sera were obtained from five patients with acute mastoiditis, one patient with infected varicose ulcer, and one patient convalescing from scarlet

fever. The first specimens of serum were obtained from all but one of the patients from one and one-half to eight weeks after the onset of infection. Hemolytic streptococci were isolated in every instance.

It is obvious that the titers obtained are considerably above the normal range and are confirmatory of the proven infection by hemolytic streptococci. In one case of mastoiditis, an originally high titer of 1146 units (20 days after mastoidectomy) was followed six months later by a normal titer of 95 units, when the patient had clinically recovered from the infection. The one serum from a case of mastoiditis which had a normal titer was obtained only four days after the onset of infection.

Rheumatic fever. Twenty-five sera from 18 patients with rheumatic fever were tested. The three sera which gave normal titers (100 units per cc. or less) were obtained from three patients at a time of inactivity of the disease. All three were in the hospital because of cardiovascular disease which followed an earlier acute rheumatic fever. None gave a history of an acute infection of the throat prior to the time of the antistreptolysin test. The titers of 22 sera from the 15 other patients were decidedly higher than normal. All of these patients had acute rheumatic fever, and all gave histories of acute infections of the upper respiratory tract just prior to the onset of the attack. The gradual rise in titer after an attack of acute rheumatic fever, and the subsequent fall in titer, coincident with clinical improvement are seen in three of the cases.

Miscellaneous other conditions. Of the 32 miscellaneous sera tested for antistreptolysin, only seven gave titers above normal. Two were from cases of spondylitis of the Marie-Struempell type. The other five were from cases of chronic osteomyelitis. The opportunity for secondary infection by streptococci in this type of case is obvious. The titers of five sera from normal healthy persons, with no history of immediately preceding infection by hemolytic streptococci, were well within the normal range.

COMMENT

The appearance of antistreptolysin in cases of proven hemolytic streptococcus etiology and in such diseases as rheumatic fever, scarlet fever, and erysipelas (whose streptococcal etiology is generally accepted) seems to follow a recent infection, or an acute exacerbation of an older process. Coburn and Pauli (11) have shown that in rheumatic fever antistreptolysin rises to a high titer soon after the onset of infection or a recrudescence of the disease, and drops with a return to the quiescent state.

The presence of antistreptolysin in titers above normal in some cases of rheumatoid arthritis is interesting and suggestive, in view of the present general tendency to assume some relationship of streptococci to this disease. The fact that rheumatoid arthritis is a chronic disease obviously precludes the possibility of obtaining a high percentage of antistreptolysin titers above the normal range. However, it may be pointed out that, with one exception, the sera from cases of rheumatoid arthritis which possessed high antistreptolysin titers also caused agglutination of hemolytic streptococci in significant titer. When positive in high titer, antistreptolysin determinations may serve as additional evidence of the recent association of hemolytic streptococci with rheumatoid arthritis.

SUMMARY

Agglutinins for hemolytic streptococci in high titer were demonstrated in the sera of a majority of patients (85 per cent) with rheumatoid arthritis. Agglutination of hemolytic streptococci in high titer by a large percentage of sera was not obtained in other chronic arthritides.

No correlation was found between the agglutination titer and the age of the patient, duration of the disease, number of joints involved, or sedimentation rate, in cases of rheumatoid arthritis.

Antistreptolysin was present in titers above the normal range in the sera of patients with proven infections by hemolytic streptococci, and with acute rheumatic fever. There was a tendency for the antistreptolysin titer to return to within the normal range some time after convalescence.

Antistreptolysin in titers definitely above normal were found in about one-third of the sera from patients with rheumatoid arthritis. With one exception, these high titers accompanied high agglutination titers.

The presence of agglutinins for hemolytic strep-

tococci in sera from patients with rheumatoid arthritis is suggestive of an association of these organisms with this disease. Additional suggestive evidence may be offered by the presence of antistreptolysin, when it is found in titers above normal.

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