BACTERIOSTATIC EFFECT OF HUMAN SERA ON GROUP A STREPTOCOCCI

I. Type-Specific Antibodies in Sera of Patients Convalescing from Group A Streptococcal Pharyngitis

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Plate 4

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Although the presence in convalescent human sera of type-specific antibodies to group A streptococci has been reported by several workers (1–10), the specificity has not always been supported by unequivocal evidence. The newer knowledge of the different types of group A streptococci provided by Lancefield (11, 12) and Griffith (13), and of the antigenic structure by the former, has made possible a new approach to the problem.

A reliable and sensitive method of determining type-specific circulating antibodies would help greatly in solving the question of type-specific immunity; it would also provide a technique for investigating epidemiological and clinical problems resulting from streptococcal infections and their sequelae. Type-specific antibodies in the blood of patients recovering from group A streptococcal infections have been investigated by a variety of methods: agglutination, precipitation, complement fixation, mouse protection, opsonic, and bacteriostatic techniques. Both slide and macroscopic agglutination reactions have proved unsatisfactory because of non-type-specific reactions and also because of the difficulty in obtaining uniformly stable bacterial suspensions. Precipitin and complement fixation tests with human sera and M extracts reveal many cross-reactions with extracts of heterologous types. The mouse protection test requires strains of high mouse virulence which are infrequent in streptococci freshly isolated from human sources; this test apparently requires a higher antibody content than that usually found in convalescent human sera; moreover, large amounts of sera are necessary. The opsonic index technique is frequently difficult to interpret. The bacteriostatic test, although dependent upon the opsonization by the serum and phagocytosis of the streptococcal cell by leukocytes, has yielded reliable and easily interpreted information concerning the presence of type-specific antibodies. In conformity with previous investigators, the term bacteriostasis used in this report implies not merely the inhibition of bacterial growth but also the destruction of microorganisms by sensitization with convalescent serum and their subsequent phagocytosis by leukocytes.

Recently, Kuttner and Lenert (14), using both the direct and indirect methods for bacteriostasis, were able to show the presence of type-specific antibodies in the blood of children convalescent from group A streptococcal pharyngitis. In the direct technique, which yielded better results in their studies, whole blood from the infected patient is used; and in the indirect method, whole blood, with no bacteriostatic activity against the streptococci under consideration, is obtained from a control and added to the serum being tested.

The object of the present study was to determine whether, by the indirect method, type-specific antibodies could be demonstrated in the sera of adult patients convalescent from group A streptococcal infections.

Materials and Methods

Source of Sera.—Samples of sera were obtained at weekly intervals from 3 patients during periods of 38, 7½, and 37 weeks respectively, and stored without preservative at 4° C. for one-half to two years before testing. Sera taken at varying intervals were selected for detailed study.

The first patient (H. A., No. 11,387), a man aged 20, was admitted on the 3rd day of scarlet fever. Group A, type 6 streptococci, strain 1RSC86, were cultured from his nose and throat. Because he developed a severe primary attack of rheumatic fever, observation continued for 38 weeks.

The second patient (E. B., No. 11,302), a man aged 37, entered the hospital on the 7th day of scarlet fever. Group A, type 19 streptococci, strain 1RSC42, were cultured from the nose and throat. He was discharged 7½ weeks after onset of his illness.

The third patient (C. DeM., No. 7500), a woman aged 20, known to have rheumatic heart disease, entered the hospital on the 3rd day of the fourth definite attack of rheumatic fever. This had been preceded by a sore throat 15 days before. Group A, type 26 streptococci, strain C 118, were cultured from the throat. She was observed for 37 weeks.

Streptococcal Strains Employed.—The hemolytic streptococci recovered from the nasopharyngeal cultures of the patients on admission were dried from the frozen state (15). Other strains were selected because of their antigenic composition (12) from laboratory stock cultures which had been preserved in the dried state, often for many years. All strains were in the matt phase, belonged to group A, and were typed by the precipitin method (16, 17).

Preparation of Cultures.—Cultures of different dissociative states of the same strain of streptococci showed varying susceptibility to bacteriostasis by normal human whole blood: the glossy variant was the most susceptible; the mouse virulent matt variant, the most resistant; and the matt variant which was avirulent for mice was intermediate in its resistance to phagocytosis. When strains freshly isolated from patients were available, they were used with as few subcultures as possible from the original strain and without respect to their virulence for mice. When old stock cultures were used, the mouse virulent strain derived from the original mouse passage was selected as being most suitable. To maintain the matt phase, each strain was taken from the frozen dried stock, seeded into 5 cc. of blood broth, and incubated at 37° C. in a water bath for 4 hours. One cc. of this young culture was inoculated into 500 cc. of Todd-Hewitt broth and incubated for 12 hours, then centrifuged; the streptococci were resuspended in 2 cc. of broth and distributed in 0.2 cc. portions into each of 10 glass tubes, which were kept in the CO_2 ice chest at −72° C.

The day before each experiment the contents of one tube were warmed and pipetted into 5 cc. of blood broth, which was incubated in a water bath at 37° C. for 4 hours; 0.5 cc. was inoculated into 45 cc. of Todd-Hewitt broth and grown for 10 to 12 hours. To avoid any

antiphagocytic effect of the metabolic products of growth in the culture broth, the streptococci were centrifuged and resuspended to original volume in fresh broth. Tenfold serial dilutions of the culture were made in broth, and the number of streptococci per cubic centimeter calculated by pouring rabbit blood agar plates containing 0.5 cc. of 10^{-7} or 10^{-8} dilutions. In all the experiments, from 20 to 30 colonies per cc. grew from the 10^{-7} and 2 to 3 from the 10^{-8} dilutions.

Source of Blood.—In preliminary observations, it was noted that both adult and fetal human blood often possessed bacteriostatic activity for the matt variants of group A streptococci, but that the blood of afebrile children from 3 to 9 years of age, and free of any infection, did not have this bacteriostatic property. These children had usually been hospitalized for an elective orthopedic or plastic operation.[1] When necessary, the blood from several children was pooled to provide a sufficient quantity. Heparin (Connaught Laboratory, Toronto University) in a final dilution of 1:16,000 was used as an anticoagulant.

Bacteriostatic Test.—Since old, stored sera were employed in this study, it was necessary to use the indirect method, in which fresh whole blood was derived from a source other than the patient whose serum was to be tested. Heat inactivation of such stored sera was not required. Para-aminobenzoic acid to make a concentration of 5 mg. per cent was added to sera taken during sulfonamide therapy. The most sensitive test zone for bacteriostasis of the cultures used was found to be 10^{-3} through 10^{-6} dilutions. The technique was that of Todd (18) as modified by Ward (19). Because of a possible prozone effect as reported by Thomas and Dingle (20) in studies on meningococcal infections, various dilutions of sera were also employed. The test consisted of determining the specific property of convalescent serum for sensitizing group A streptococci of homologous or heterologous types to the phagocytic action of the leukocytes contained in whole blood. To each tube (100 × 7 mm.) were added 0.25 cc. of human heparinized blood obtained within the previous 2 hours, 0.05 cc. of a culture dilution, and 0.05 cc. of varying concentrations of serum. Undiluted serum and serum diluted 1:10 and 1:100 were used. Each concentration of serum was tested against tenfold serial dilutions of the culture ranging from 10^{-3} through 10^{-6}. To find out whether the whole blood used inhibited the growth of streptococci, control tubes containing saline solution in place of convalescent serum were always included. The tubes were sealed with sterile, tightly-fitting rubber stoppers with a smooth lower surface and incubated at 37° C. in an electrically driven mixing machine which rotated the tubes on the long axis at 8 R.P.M. After 3 hours, a sample of each mixture was removed with a platinum loop 3 mm. across, and streaked evenly over one quadrant of a rabbit blood agar plate which was incubated for 18 to 24 hours. The resulting growth was recorded as follows: growth covering the quadrant, ++++; three-fourths, +++; one-half, ++; and one-quarter, +; fewer than 10 colonies were represented in arabic numerals; 0 indicated no growth (Fig. 1).

EXPERIMENTAL RESULTS

The bacteriostatic activity of sera obtained in the acute and convalescent phases against each patient's own strain of streptococcus is recorded in Table I. These sera included those obtained when the patients were admitted to the hospital, when antibodies first appeared, when the antibody level was highest, and when the patients were last seen. A distinct difference between acute and convalescent phase sera is apparent. The bacteriostatic antibodies first ap-

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peared in the blood of the patients between 3 and 5 weeks following infection, did not attain their maximal concentration until convalescence was well advanced, and persisted in the sera of 2 of these patients for at least 37 weeks.

In many tests done in duplicate and on different days with various samples of children's whole blood, the findings were reliable and reproducible. In several instances, after a loopful of the mixture was removed for streaking on the blood agar plate, the remaining contents of the tubes were subcultured in blood broth, which uniformly remained sterile when no growth appeared on the plate. This indicated that the streptococci had been killed and not temporarily inhibited.

In the subsequent experiments, samples of sera were taken at various intervals during convalescence and not necessarily when the antibodies were at the highest level, since sufficient serum was not always obtained at that period.

The tests were repeated with strains other than that with which each patient was infected, although of the same serological type; the results of these tests are summarized in Tables II*a*, II*b*, and II*c*. These experiments show that each convalescent serum tested possessed antibodies for at least 7 strains of the same type as that which caused the patient's disease. Within each series the susceptibility of the streptococci to bacteriostasis was strikingly uniform, except for those recorded in Table II*c* where slight variability occurred.

Lancefield (12) has found that many group A streptococci in the matt phase have at least two type-specific antigens, designated M and T, but some strains have only M, as reported by Elliott (21) and Lancefield and Stewart (22). In the strains used in this experiment, M and T antigens were present in all except the following: strain D6C (type 19) in Table II*b*; strain C118 (type 26), which caused the infection of the patient from whom the serum was obtained, and strain 11RS50 (type 26) in Table II*c*; these contained only the M component. Despite differences in antigenic composition, the degree of bacteriostasis was essentially the same. This observation suggests that M and its corresponding antibody rather than T and its antibody are concerned in this test. Evidence has also been presented by Lyons and Ward (23) that the anti-M precipitin is probably identical with the specific opsonin which brings about opsonization of hemolytic streptococci of human origin.

In order to test this hypothesis, a series of strains with serologically related T and different M antigens (24) was employed. Serum from a patient infected with a type 19 strain which contained both M and T antigens was tested with types 15, 17, 19, 23, and 30 streptococci, as illustrated in Table III. The type 19 strains, J17D and 1RSC42, with the same M antigen, had almost identical susceptibility to the type 19 serum; but the remaining strains with different M antigens, even though all contained at least one serologically identical T component, grew equally well in the presence of this patient's serum. Strains with only the T antigen cannot be studied under these test conditions; such

TABLE I

Time of Appearance and Duration of Bacteriostatic Antibodies

Convalescent sera from patients with:		First reading				Start of bacteriostasis				Highest level				Last reading			
		1st wk.				5th wk.				35th wk.				38th wk.			
		Dilution of culture of group A type 6 streptococci, strain 1RSC86															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 6 infection strain 1RSC86	Undiluted serum	++++	+++	++±	++±	++	++	+	8	6	0	0	0	1	1	0	0
	1:10 dilution	++++	+++±	++±	++	++±	++±	++±	+±	+	4	0	0	++	++	0	0
	1:100 "	+++±	+++±	++±	++	+++	+++	++±	+±	++±	+	5	4	++++	++++	++±	+
	Control: no serum	++++	++++	+++	++	++++	++++	+++	++±	++++	++++	+++	++±	++++	++++	+++	++±
		1st wk.				3rd wk.				5th wk.				8th wk.			
		Dilution of culture of group A type 19 streptococci, strain 1RSC42															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 19 infection strain 1RSC42	Undiluted serum	++++	++++	+++	++	+±	+	0	0	1	0	0	0	2	0	0	0
	1:10 dilution	++++	++++	+++	++	++++	+++	+±	3	+	0	0	0	+	0	0	0
	1:100 "	++++	++++	+++	++±	++++	+++	++	+	++	9	0	0	+±	3	1	0
	Control: no serum	++++	++++	+++	++±	++++	++++±	+++	++	++++	++++	+++	++±	++++	++++	+++	++±
		2nd wk.				4th wk.				17th wk.				37th wk.			
		Dilution of culture of group A type 26 streptococci, strain C 118															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 26 infection strain C 118	Undiluted serum	++++	++++	+++	++	++±	++	+	0	0	0	0	0	+++	2	1	1
	1:10 dilution	++++	++++	+++	++	+++	+++	+++	+±	++	9	+	0	++++	+++	5	0
	1:100 "	++++	+++	+++	++	+++	+++	+++	+±	+++	+++	++	1	+++±	+++	++±	0
	Control: no serum	++++	+++	+++	++	++++	+++	+++	++	++++	+++	+++	++	++++	+++	+++	++

In all tables the degree of growth of streptococci is indicated on a ++++ to + scale; fewer than 10 colonies are represented by arabic numerals; 0 indicates no growth.

strains are in the glossy phase and are therefore susceptible to bacteriostasis by normal whole blood without the addition of convalescent serum. The possibility that the T antigen might have been masked by the M in these cultures grown at 37°C. is ruled out because T substance was demonstrable in cultures at this temperature in 4 of the 6 strains. The remaining 2 could not be tested for T because of the granularity of the culture suspension. Thus, the T antigen apparently had no importance in the bacteriostasis of the streptococci tested.

TABLE II *a*

Bacteriostasis of Type 6 Strains from Different Sources in Serum from a Patient Infected with Type 6 Strain 1RSC86

Group A type 6 streptococci		Convalescent serum from patient with type 6 infection strain 1RSC86	Dilution of culture			
Strain*	Source		10^{-3}	10^{-4}	10^{-5}	10^{-6}
1RSC86	Scarlet fever and rheumatic fever Rockefeller Hospital (1943)	Undiluted serum Control: no serum	++ ++++	+ ++++	0 +++±	0 +++
D 206	Scarlet fever Roumanian strain No. 6121 Dr. Schwentker (1938)	Undiluted serum Control: no serum	++± ++++	+ ++++	2 +++±	0 +++
C 166	Acute pharyngitis Rockefeller Hospital (1942)	Undiluted serum Control: no serum	++ ++++	3 ++++	3 +++	0 +++
C 108	Rheumatic fever Newport Naval Station No. 12946 (1942)	Undiluted serum Control: no serum	++± ++++	3 ++++	1 +++	0 ++±
F 96	Scarlet fever Roumanian strain Dr. Gordon (1935)	Undiluted serum Control: no serum	++± ++++	5 ++++	3 +++	0 +++
10RS28	Rheumatic fever Rockefeller Hospital (1941)	Undiluted serum Control: no serum	++ ++++	9 ++++	2 +++	0 ++±
C 209	Rheumatic fever Bellevue Hospital (1942)	Undiluted serum Control: no serum	++ ++++	+ +++	0 +++	0 +++
D 182A	Rheumatic fever Rockefeller Hospital (1939)	Undiluted serum Control: no serum	++ ++++	+ +++±	0 +++±	0 +++

* All strains have both M and T antigens.

The type-specificity of the antibodies in these convalescent sera was further tested with 2 strains of heterologous types having both M and T antigens different from those in the patient's own strain. The results shown in Table IV indicate definite type-specificity because no significant cross-reactions with the heterologous types were observed, although in serum from the patient infected with type 19, a slight degree of stasis occurred in the 10^{-6} dilution of type 26 streptococci. Sera from all 3 patients, in dilutions as high as 1:100 inhibited the growth of the homologous types; in addition, data not recorded in this table showed that bacteriostasis occurred in 1:1,000 dilution of serum from

TABLE II *b*

Bacteriostasis of Type 19 Strains from Different Sources in Serum from a Patient Infected with Type 19 Strain 1RSC42

Group A type 19 streptococci		Antigenic components	Convalescent serum from patient with type 19 infection strain 1RSC42	Dilution of culture			
Strain	Source			10^{-3}	10^{-4}	10^{-5}	10^{-6}
1RSC42	Scarlet fever Rockefeller Hospital (1943)	M and T	Undiluted serum Control: no serum	+ ++++	1 ++++	0 +++	0 ++±
16RS6	Acute pharyngitis Rockefeller Hospital (1941)	M and T	Undiluted serum Control: no serum	+ ++++	1 ++++	0 +++±	0 +++
C 23A	Scarlet fever Halifax epidemic (1941)	M and T	Undiluted serum Control: no serum	6 ++++	1 ++++	0 +++	0 +++
C 272	New Haven epidemic (1942)	M and T	Undiluted serum Control: no serum	+ ++++	0 +++	0 +++	0 +++
C 296	Carrier strain Dr. Kuttner (1942)	M and T	Undiluted serum Control: no serum	+ ++++	3 ++++	0 +++	0 +
D 6C	Acute tonsillitis Dr. Griffith (1936)	M	Undiluted serum Control: no serum	4 ++++	0 ++++	0 +++±	0 +++±
H31/0/4	Rheumatic fever Rockefeller Hospital (1933)	M and T	Undiluted serum Control: no serum	+ ++++	0 +++	0 +++	0 +++
H 106A	Scarlet fever Dr. Tillett (1934)	M and T	Undiluted serum Control: no serum	+± ++++	0 +++	0 +++	0 +++

TABLE II *c*

Bacteriostasis of Type 26 Strains from Different Sources in Serum from a Patient Infected with Type 26 Strain C 118

Group A type 26 streptococci		Antigenic components	Convalescent serum from patient with type 26 infection strain C 118	Dilution of culture			
Strain	Source			10^{-3}	10^{-4}	10^{-5}	10^{-6}
C 118	Rheumatic fever Rockefeller Hospital (1942)	M	Undiluted serum Control: no serum	+ ++++	0 ++++	0 +++	0 ++±
C 560	Acute tonsillitis Camp Borden Hospital Canada (1943)	M and T	Undiluted serum Control: no serum	2 ++++	2 ++++	0 +++	0 +++
C 383	Scarlet fever Japan (1939)	M and T	Undiluted serum Control: no serum	+++ ++++	++ +++±	2 +++	0 ++±
C 508	Toe infection New Haven Hospital (1943)	M and T	Undiluted serum Control: no serum	+± ++++	+ +++	2 +++	0 +++
11RS50	Rheumatic fever Rockefeller Hospital (1941)	M	Undiluted serum Control: no serum	++± ++++	+ ++++	2 +++	0 +++
1RSC108	Scarlet fever Rockefeller Hospital (1943)	M and T	Undiluted serum Control: no serum	++± ++++	+± ++++	0 +++	0 +++
24RS60	Rheumatic fever Rockefeller Hospital (1942)	M and T	Undiluted serum Control: no serum	+± ++++	6 +++	3 +++	0 ++±
14RS15	Acute pharyngitis Rockefeller Hospital (1941)	M and T	Undiluted serum Control: no serum	++± ++++	+ +++±	0 +++	0 +++

the patient infected with type 6 streptococci. Serum from the patient convalescent from type 26 infection was tested with strains of 5 other heterologous types: types 4 (strain T 4), 24 (strain C 115), 28 (strain D140A), 29 (strain D 23), and 46 (strain C 105); no bacteriostasis of these streptococci was observed.

TABLE III

Bacteriostasis of a Series of Strains of Group A Streptococci with Related T but Distinct M Antigens

Type and strain of group A streptococci	Convalescent serum from patient with type 19 infection strain 1RSC42	Dilution of culture			
		10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 15 (strain T 15)	Undiluted serum	++++	++++	+++±	++±
	1:10 dilution	++++	++++	+++±	++±
	Control: no serum	++++	++++	++++	++±
Type 17 (strain J 17E)	Undiluted serum	++++	++++	++++	+++
	1:10 dilution	++++	++++	+++±	+++
	Control: no serum	++++	++++	++++	++
Type 19 (strain J 17D)	Undiluted serum	8	0	0	0
	1:10 dilution	5	0	0	0
	Control: no serum	++++	++++	+++	++±
Type 19 (strain 1RSC42)	Undiluted serum	9	0	0	0
	1:10 dilution	8	0	0	0
	Control: no serum	++++	+++	+++	++±
Type 23 (strain T 23)	Undiluted serum	+++	++	++±	+
	1:10 dilution	+++±	+++	++±	+
	Control: no serum	++++	+++	++±	+
Type 30 (strain D 11)	Undiluted serum	++++	++++	+++	+++
	1:10 dilution	++++	++++	+++	+++
	Control: no serum	++++	++++	+++	++±

It seemed desirable to ascertain whether the type-specific bacteriostatic antibodies in these sera could be absorbed with the homologous or heterologous type strains used in the preceding experiment. The sera were mixed with heat-killed streptococci, in the proportion 3 parts serum to 1 part packed bacterial cells, and incubated in a water bath at 37° C. for 30 minutes; then stored overnight at 4° C. After centrifugation, the clear supernatant sera were removed. Samples of each serum were tested in the unabsorbed state and after absorption with strains of the homologous and two heterologous types, respectively. Table V demonstrates that in each case the bacteriostatic anti-

TABLE IV

Type-Specificity of Bacteriostasis

Convalescent sera from patients with:		Dilution of culture of group A streptococci											
		Type 6 strain 1RSC86				Type 19 strain 1RSC42				Type 26 strain C 118			
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 6 infection strain 1RSC86	Undiluted serum	1	1	0	0	++++	++++	+++	++	+++±	++++	++±	++
	1:10 dilution	++	5	0	0	++++	++++	+++	+++	++++	+++	+++	++±
	1:100 "	++±	+	1	0	++++	++++	+++	+++±	++++	+++	+++	+++
	Control: no serum	++++	+++	+++	++±	++++	++++	+++	++	++++	++++	+++	+++
Type 19 infection strain 1RSC42	Undiluted serum	++++	++	++	+±	+	0	0	0	+++±	+++	+++	8
	1:10 dilution	++++	+++	+++	++	+	0	0	0	+++	+++	+++	++±
	1:100 "	++++	+++	+++	+±	+±	6	1	0	+++±	+++	+++	+++
	Control: no serum	++++	+++	+++	++±	++++	++++	++	++	++++	++++	+++	+++
Type 26 infection strain C 118	Undiluted serum	++++	+++	++	+	++++	++++	+++	+++	++±	5	1	0
	1:10 dilution	++++	+++	++	+	++++	+++	+++	+++	++±	7	1	0
	1:100 "	++++	+++	++	+±	++++	++++	+++	+++	++±	+	3	0
	Control: no serum	++++	+++	+++	++±	++++	++++	+++	++	++++	++++	+++	+++

TABLE V

Absorption of Human Convalescent Sera with Strains of Homologous or Heterologous Types of Group A Streptococci

Convalescent sera from patients with:		Unabsorbed serum				Serum absorbed with heat-killed group A streptococci											
						Type 6 strain 1RSC86				Type 19 strain 1RSC42				Type 26 strain C 118			
		Dilution of culture of group A type 6 streptococci, strain 1RSC86															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 6 infection strain 1RSC86	Undiluted serum	7	1	0	0	++++±	+++	++	9	+	0	0	0	++±	3	0	0
	1:10 dilution	+±	3	1	0	++++	+++	++	7	++±	++	+	6	+++	+±	8	0
	1:100 "	++++	++	+	0												
	Control: no serum	++++	++++	++±	++												
		Dilution of culture of group A type 19 streptococci, strain 1RSC42															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 19 infection strain 1RSC42	Undiluted serum	++±	1	0	0	++±	0	0	0	++++	++++±	+++	8	++	0	0	0
	1:10 dilution	+++	5	0	0	+++	+	0	0	++++	+++	+++	+	++	0	0	0
	1:100 "	+++±	+++	+	+												
	Control: no serum	++++	+++	+++	++												
		Dilution of culture of group A type 26 streptococci, strain C 118															
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Type 26 infection strain C 118	Undiluted serum	++±	5	0	0	++	+	2	0	+	5	0	0	++++	+++	+++	+++
	1:10 dilution	+++	++±	++	0	++	++	+	0	+++	++	++	5	++++	++++	++++±	+++
	1:100 "	++++	++++	+++	++±												
	Control: no serum	++++	++++	+++	++±												

bodies were almost completely removed from the sera by the homologous streptococcal cells, but that they were relatively unaffected by strains of the heterologous types. Again the uniform results illustrate that the bacteriostasis effected by the convalescent sera is type-specific.

Sera from 48 patients with a total of 54 group A streptococcal infections have been tested by this method for type-specific antibodies and only rarely have cross-reactions been found. A detailed report of this study with a comparison of the clinical findings and the antibody response will be presented later.

DISCUSSION

The foregoing experiments show that human convalescent sera from 3 adults contained type-specific streptococcal antibodies demonstrable by the indirect bacteriostatic method. This procedure, which required that fresh serum and leukocytes from another human source be added to the test sera, is probably better for testing sera from adults than is the direct method, because whole blood from older individuals without recent infections often has bacteriostatic properties for many strains of streptococci, as observed both in this laboratory and by Lyons and Ward (23). Kuttner and Lenert (14), on the other hand, observed type-specific bacteriostatic properties by both the indirect and direct methods, but analyzed only children's blood which probably does not contain the so called "natural specific opsonins" present in adult blood (23).

To avoid the difficulties inherent in methods involving phagocytosis, and to insure proper standardization of the technique, it is imperative that all strains grow satisfactorily in the whole blood to which the test serum has not been added. Young cultures must also be used, as noted by earlier investigators (23, 25–28) who observed that such cultures are more resistant to phagocytosis than those tested after longer incubation. Another important factor is the prevention of bacterial dissociation, for inconsistent results inevitably occur when different variants of the same strain are studied.

The chief objection to the indirect method is the difficulty of obtaining enough whole blood to study many sera. Unfortunately, as recorded later (29), whole blood from certain laboratory animals could not be substituted for human blood. In spite of the inherent difficulties in the indirect bacteriostatic technique, this method of testing human adult sera is preferable to the precipitin test or to the complement fixation test because it shows no cross-reactions and apparently yields type-specific results. The streptococcidal property of acute phase sera from febrile patients with various diseases, as described by Tillett (30), was not observed. The relative amount of serum used, however, was much less than in Tillett's experiments, and the sera had been stored in the ice box much longer before testing.

The significance of the type-specificity observed in this study is emphasized by the fact that strains of heterologous and homologous types of streptococci

were from many parts of the world and from infections occurring over a 10 year period. Heretofore, experiments of this nature have been limited to the study of strains from localized geographical areas. The absorption experiments provide the most convincing evidence for specificity: the homologous antibodies were always specifically absorbed from the serum. Because absorption was successful in these bacteriostatic tests, it may prove possible to apply similar absorption methods to the precipitin technique which shows many cross-reactions. In our experience thus far, all precipitin reactivity in human sera, which is usually of low titre, has been removed by absorption with heterologous as well as homologous type heat-killed cultures. Perhaps if after absorption the precipitin tests were performed according to the technique of Heidelberger and Anderson (31) with large amounts of absorbed sera and with a longer period in the ice box, cross-reactions might be avoided and homologous reactivity retained.

The type-specificity of human sera in the bacteriostatic test appears to be dependent upon M-anti-M reactions and not upon T-anti-T; and it is probably comparable to the type-specific protection of mice by hyperimmune rabbit sera, in which the antibody to the M protein is one of the factors involved (32).

It is evident, therefore, from these data that type-specific antibodies are demonstrable in human sera. Although sera from only 3 patients were investigated, the fact that these tests were made with numerous samples of sera with a variety of homologous and heterologous streptococci and supported by absorption experiments lends weight to these observations concerning type-specificity.

SUMMARY

1. Type-specific antibodies were demonstrated by the indirect bacteriostatic test in sera from human adults convalescing from group A streptococcal infection of the upper respiratory tract. The time of appearance of the antibodies varied from 3 to 5 weeks; and they persisted in 2 patients for at least 37 weeks after the onset of the infection.

2. The specificity of the antibody response in one serum was tested with strains of 7 heterologous types; in another, with 6; and in the third, with 2; but in no instance were cross-reactions observed. Moreover, each convalescent serum showed approximately equal bacteriostasis for 7 different strains of the same type as that which caused the infection.

3. The antibodies were specifically absorbed from the serum by homologous heat-killed streptococci, but not significantly by strains of heterologous types.

4. The specific M antigen of the streptococcal cell with its respective antibody, and not the T substance, appeared to be concerned in the reaction.

5. In spite of numerous technical difficulties inherent in the method, this bacteriostatic test provides a useful procedure for studying type-specific immunity in streptococcal infections.

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EXPLANATION OF PLATE 4

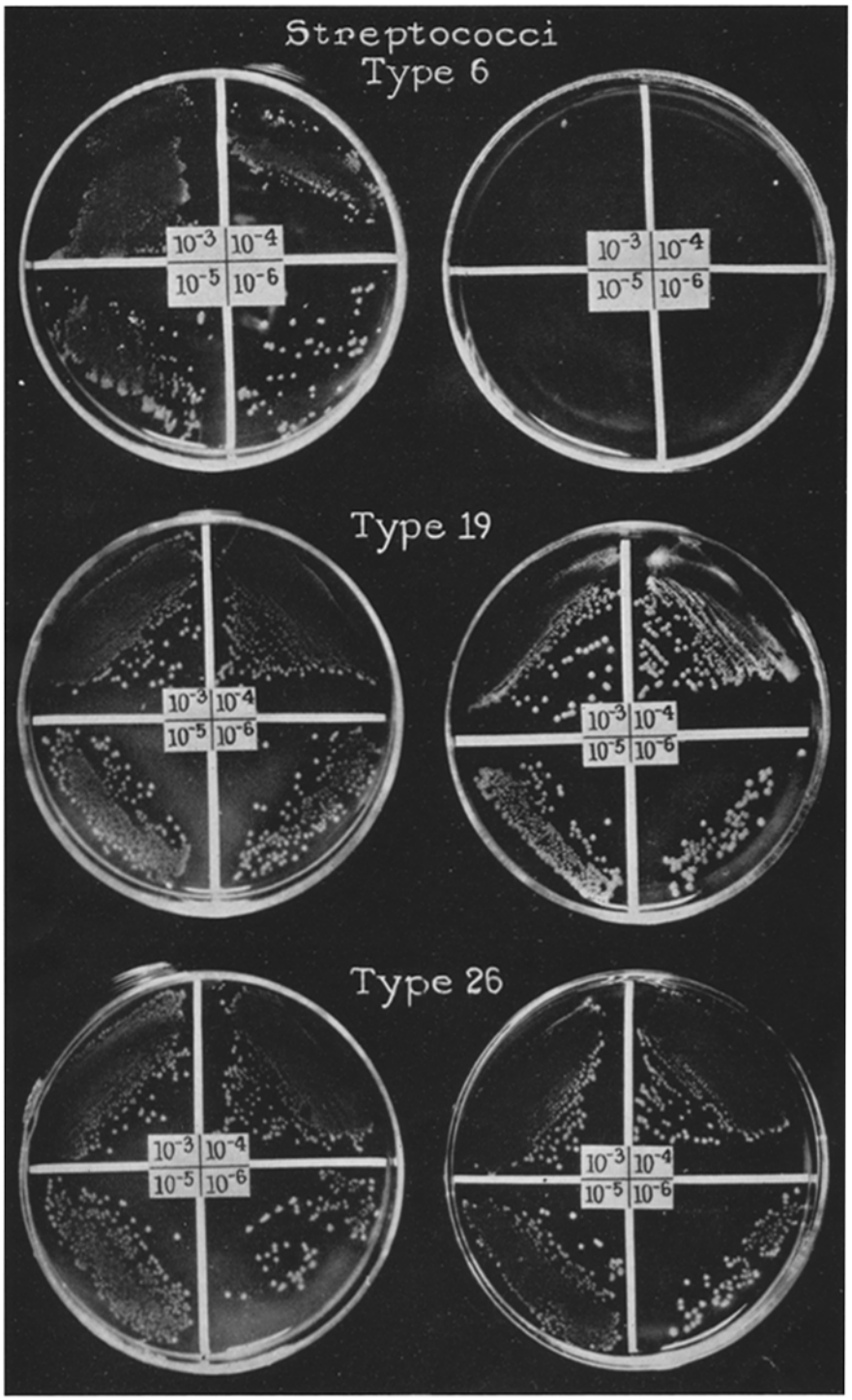
The photographs were made by Mr. Joseph B. Haulenbeek.

FIG. 1. Blood agar plates showing results of an experiment in which convalescent serum from a patient recovering from a group A, type 6 streptococcal infection was tested with strains of the homologous and two heterologous types of streptococci.

Saline control

No serum

Convalescent human serum Type 6



(Rothbard: Effect of human sera on group A streptococci. I)