THE OCCURRENCE OF BACTERIOSTATIC PROPERTIES IN THE BLOOD OF PATIENTS AFTER RECOVERY FROM STREPTOCOCCAL PHARYNGITIS¹

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Although no definite proof is available, our observations covering 6 years suggest that repeated attacks of streptococcal pharyngitis occurring in the same individual are usually due to Group A streptococci of different types. Reinfection with the same type was never observed, suggesting that type-specific immunity may develop. At the present time, however, serological evidence of such immunity is meager.

REVIEW OF LITERATURE

Fothergill and Lium studied the bactericidal activity of the blood of 4 convalescent scarlet fever patients (1). Two of these patients recovered without developing complications; the other 2 developed otitis media, in one instance accompanied by mastoiditis, and in the other by adenitis. Only the 2 patients with purulent sequelae showed an increase in the bactericidal activity of the blood for the homologous streptococcus.

Hare reported the development of specific bactericidal properties in the blood of patients during the course of puerperal sepsis (2).

Spink and Keefer studied the streptococidal power of defibrinated blood obtained from 30 erysipelas patients (3). An increase in bactericidal power for the homologous organism during the course of the disease was observed in one-third of the cases.

Chandler and Taussig, in a recent article, reported that the blood of 2 rheumatic children, who were carriers of Group A streptococci, were bactericidal for the homologous strains (4). The blood of 2 carriers of Group C streptococci was also found to contain homologous bactericidal antibodies.

Tillett (5) showed that sera obtained from patients acutely ill with a variety of infectious

diseases, such as pneumonia, meningococcus meningitis, tuberculous pleurisy, as well as diseases due to streptococci, was highly bactericidal for certain strains of streptococci. This property disappeared as the patients recovered and was not considered to represent a specific immune response.

Swift and Hodge described the occurrence of type-specific anti-M precipitins in the sera of patients following streptococcal infections (6). Positive reactions were obtained, both in patients who developed rheumatic sequelae and in those who did not.

Walker (7) has reported the presence of type-specific agglutinins for the homologous streptococcus, demonstrable by the slide agglutination technique of Griffith, in the sera of 6 of 22 patients. The 6 positive reactions were obtained with sera of individuals recovering from purulent streptococcal infections (otitis media, empyema, and paronychia accompanied by axillary adenitis).

Rantz *et al.* reported the development of agglutinins for the homologous types, demonstrable by the slide agglutination technique, in 13 of 22 cases of scarlet fever (8).

Diefendorf (9) was able to demonstrate mouse protective antibodies in the sera of 10 of 14 patients. Six of these 10 sera were obtained from patients recovering from scarlet fever and 4 from rheumatic subjects during the fourth week of the rheumatic attack.

MATERIAL

Outbreak of streptococcal pharyngitis. As previously reported, an outbreak of pharyngitis, due to a Group A streptococcus of a single type, type 36, occurred in this institution during the winter of 1941-1942 (10, 11). Six cases occurred in October, 9 in November, followed by 2 in December and one in January (making a total of 18 cases). Sera for the determination of antistreptolysin O titer, slide agglutination, and anti-M precipitin reactions were collected at frequent intervals, following the upper

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respiratory infection, from 16 of these 18 patients. Bacteriostatic tests with whole blood were not started until March 1942 when the outbreak had subsided and all the children were afebrile.

EXPERIMENTAL

Slide agglutination reactions. The sera of 10 children were tested by the slide agglutination method (Griffith) with the homologous as well as with heterologous types of Group A streptococcus (12). Five to 15 sera from each individual, obtained at intervals ranging from 1 day to 5 months after infection, were used. Slight agglutination occurred with a few sera from 7 children when tested with a strain of the homologous type. In every instance, however, equally strong or stronger reactions were observed with heterologous types. The results obtained by the slide agglutination test were therefore considered to be non-specific.

Anti-M precipitin reactions. The sera of 14 of these 18 children were tested for type specific anti-M precipitins (Lancefield (13)) for the homologous type 36 streptococcus. In 8 instances, 2 or more sera from the same child were used. The M extract was used undiluted and diluted 1 : 2, 1 : 4, and 1 : 8. The results were uniformly negative.³

Protection tests. Protection tests were not performed because type 36 streptococcus was only slightly virulent for mice.

Bacteriostatic tests. In each test, the bacteriostatic activity of blood of children who had recovered from pharyngitis due to type 36 streptococcus was compared with that of children living in the institution who had escaped this streptococcal upper respiratory infection, and who were considered to be controls. Different children served as controls in experiments done at different times.

BACTERIOSTATIC METHOD

A modification of the technique described by Todd (15) was used. Most of the tests were done with the epidemic-inducing strain, type 36 streptococcus, but in some experiments, recently isolated cultures of other types were included. All the cultures were preserved by drying on small pieces of filter paper *in vacuo*, according to the technique described by Brown (16). Numerous cultures of each strain were prepared. On the day before the test, one of these cultures was opened and the filter paper transferred to broth which was incubated for 18 to 20 hours. On the morning of the experiment, dilutions of the cultures were made in broth.

The blood was collected the morning of the test; 3 cc. of blood were placed in test tubes containing 1 mgm. of

heparin (Hynson, Westcott, and Dunning). Nine-tenths cc. of heparinized blood was pipetted into test tubes of soft glass (12 cm. in length with an outside diameter of 7 mm.). One-tenth cc. of a 10^{-8} or 10^{-6} dilution of culture was added. In some instances, 0.1 cc. of a 1 : 2,000,000 dilution was used. The number of bacteria inoculated varied considerably in different experiments. The best results were obtained when from 10 to 30 microorganisms were added to 0.9 cc. of blood. One-tenth cc. of the infected blood was transferred immediately to 15 cc. of melted agar containing 0.5 cc. of horse blood and a "pour" plate made. The test tube containing the blood was then sealed by heat, placed in a shaking machine, and shaken for 3 hours, at moderate speed, while incubating at 37° C. At the end of this period, 0.1 cc. was again transferred to 15 cc. of melted agar containing 0.5 cc. horse blood and a second "pour" plate made.

The number of colonies in the plates poured before and after incubation were counted. The horse blood was added to facilitate the counting of the colonies and exerted no demonstrable bacteriostatic effect.

The results of these bacteriostatic tests with the whole blood of 16 patients, 3½ to 17 months following pharyngitis due to type 36 streptococcus, are shown in Table I.

The tests recorded in Table I show a well marked difference between the bacteriostatic activity of the blood of children who had recovered from pharyngitis due to type 36 streptococcus, and that of children who had escaped this infection and who served as controls.

Children in whom the clinical symptoms due to this upper respiratory infection were mild or lacking, as well as those in whom they were moderately severe, developed this property. It was not correlated with the rise in antistreptolysin O titer or the development of rheumatic sequelae. The bacteriostatic activity persisted for many months and in some instances could be demonstrated more than a year after the upper respiratory infection. In 3 patients, however, Cases 5, 6, and 14, who were tested after intervals of 16 to 17 months, a decrease in the bacteriostatic action of the blood was apparent.

Although the uniformity of the results obtained suggested that the bacteriostatic activity of these patients was definitely related to the type 36 streptococcal pharyngitis, it was thought of interest to determine whether this property was absent before infection. In 6 instances, the bacteriostatic activity of sera obtained before and after the upper respiratory infection was compared. Heparinized blood obtained from

³ In an outbreak of pharyngitis due to type 32 streptococcus (14), a few sera obtained after the subsidence of the upper respiratory infection were tested for type specific anti-M precipitins. It was found that sera which gave positive precipitin reactions with homologous M extracts also reacted with M extracts prepared from heterologous types.

TABLE I

Bacteriostatic tests comparing the whole blood of children after recovery from streptococcus type 36 pharyngitis with those who escaped infection

Case No.	Name Hosp. No.	Age	Pharyngitis		Rise in anti-streptolysin O titer	Interval between infection and tests	Dates of tests	Results		
			Date	Severity				Patient after recovery	Children who escaped type 36 pharyngitis	
									Control No. 1	Control No. 2
9 PATIENTS WHO DEVELOPED RHEUMATIC MANIFESTATIONS										
1	J. W. 3692	15	October 27, 1941	+	units 250 to 1000	months 5	March 27, 1942	$\frac{7^*}{6}$	$\frac{6}{137}$	$\frac{4}{92}$
						5½	April 7, 1942	$\frac{7}{23}$	$\frac{9}{\infty}$	$\frac{8}{\infty}$
2	H. T. 3649	10	October 27, 1941	++	50 to 250	5½	April 9, 1942	$\frac{4}{36}$	$\frac{6}{\text{many}^\dagger}$	$\frac{0}{\text{many}}$
						7	May 28, 1942	$\frac{2}{17}$	$\frac{2}{416}$	$\frac{5}{275}$
3	D. B. 3668	14	October 28, 1941	++	80 to 165	5	March 31, 1942	$\frac{5}{0}$	$\frac{2}{110}$	$\frac{7}{299}$
						5½	April 16, 1942	$\frac{3}{4}$	$\frac{7}{258}$	$\frac{16}{262}$
4	J. C. 3687	8	November 2, 1941	++	32 to 400	5½	April 16, 1942	$\frac{1}{33}$	$\frac{7}{258}$	$\frac{16}{262}$
						6	May 3, 1942	$\frac{2}{0}$	$\frac{5}{\infty}$	
5	A. F. 3663	9	November 4, 1941	++	50 to 80	5	April 9, 1942	$\frac{1}{8}$	$\frac{6}{\text{many}}$	$\frac{0}{\text{many}}$
						6½	May 18, 1942	$\frac{2}{8}$	$\frac{1}{190}$	$\frac{1}{189}$
						8½	July 14, 1942	$\frac{5}{17}$	$\frac{10}{\infty}$	$\frac{5}{\infty}$
						14	January 6, 1943	$\frac{2}{1}$	$\frac{2}{\text{many}}$	$\frac{3}{\text{many}}$
						16	March 25, 1943	$\frac{3}{92}$	$\frac{5}{\infty}$	$\frac{2}{\infty}$
						17	April 28, 1943	$\frac{2}{141}$	$\frac{4}{\text{many}}$	$\frac{5}{\infty}$

* The numerator indicates the number of colonies in the plate poured before and the denominator the number in the plate poured after 3 hours' incubation.

† Many = 500 to 1000 colonies.

TABLE I—*Continued*

Case No.	Name Hosp. No.	Age	Pharyngitis		Rise in anti-streptolysin O titer	Interval between infection and tests	Dates of tests	Results		
			Date	Severity				Patient after recovery	Children who escaped type 36 pharyngitis	
									Control No. 1	Control No. 2
9 PATIENTS WHO DEVELOPED RHEUMATIC MANIFESTATIONS—Continued										
6	C. D. 3671	11 years	November 4, 1941	0 (Throat culture positive)	125 to 500	5 months	April 7, 1942	$\frac{5}{14}$	$\frac{9}{\infty}$	
						6	May 7, 1942	$\frac{5}{11}$	$\frac{5}{\infty}$	
						17	April 28, 1943	$\frac{3}{243}$	$\frac{5}{\infty}$	$\frac{2}{\text{many}}$
7	H. L. 3703	7	November 6, 1941	++	125 to 400	5	April 16, 1942	$\frac{0}{1}$	$\frac{7}{258}$	
						6½	May 28, 1942	$\frac{2}{0}$	$\frac{5}{275}$	$\frac{2}{416}$
8	M. M. 3596	11	December 7, 1941	+	200 to 600	3½	March 23, 1942	$\frac{24}{13}$	$\frac{36}{\infty}$	$\frac{33}{\infty}$
						5	May 3, 1942	$\frac{5}{0}$	$\frac{5}{\infty}$	
						6	June 4, 1942	$\frac{0}{2}$	$\frac{2}{166}$	$\frac{0}{60}$
9	E. J. 3655	12	November 16, 1941	±	125 to 165	4½	March 31, 1942	$\frac{3}{31}$	$\frac{7}{299}$	$\frac{2}{110}$
						5	April 21, 1942	$\frac{6}{11}$	$\frac{4}{246}$	$\frac{4}{204}$
						6	May 14, 1942	$\frac{1}{6}$	$\frac{0}{135}$	$\frac{2}{121}$
7 PATIENTS WHO ESCAPED RHEUMATIC SEQUELAE										
10	A. H. 3673	8	October 26, 1941	+++ (adenitis)	13 to 165	5½	April 10, 1942	$\frac{61}{95}$	$\frac{60}{\infty}$	$\frac{67}{\infty}$
						7	May 20, 1942	$\frac{4}{8}$	$\frac{3}{\infty}$	$\frac{2}{\text{many}}$
						10	August 21, 1942	$\frac{7}{23}$	$\frac{7}{\infty}$	$\frac{4}{\infty}$

TABLE I—Continued

Case No.	Name Hosp. No.	Age	Pharyngitis		Rise in anti-streptolysin O titer	Interval between infection and tests	Dates of tests	Results		
			Date	Severity				Patient after recovery	Children who escaped type 36 pharyngitis	
									Control No. 1	Control No. 2
7 PATIENTS WHO ESCAPED RHEUMATIC SEQUELAE—Continued										
11	D. G. 3624	7	October 31, 1941	++	50 to 100	5	March 27, 1942	$\frac{4}{0}$	$\frac{6}{137}$	$\frac{4}{92}$
						7	May 26, 1942	$\frac{1}{2}$	$\frac{2}{127}$	$\frac{2}{98}$
						12	October 30, 1942	$\frac{5}{0}$	$\frac{3}{\text{many}}$	$\frac{1}{277}$
12	J. O'L. 3626	11	November 4, 1941	++	32 to 80	5	April 3, 1942	$\frac{5}{4}$	$\frac{3}{107}$	$\frac{3}{106}$
						7½	June 25, 1942	$\frac{0}{3}$	$\frac{0}{\infty}$	$\frac{1}{\text{many}}$
13	M. C. 3635	8	November 5, 1941	++	60 to 100	5½	April 21, 1942	$\frac{1}{1}$	$\frac{4}{246}$	$\frac{4}{204}$
						7	June 12, 1942	$\frac{3}{0}$	$\frac{0}{\text{many}}$	$\frac{1}{\text{many}}$
						13	December 15, 1942	$\frac{3}{39}$	$\frac{2}{\text{many}}$	$\frac{6}{\text{many}}$
14	M. N. 3682	8	November 9, 1941	++	no change	5	April 7, 1942	$\frac{7}{0}$	$\frac{8}{\infty}$	$\frac{9}{\infty}$
						6½	May 28, 1942	$\frac{4}{3}$	$\frac{1}{216}$	$\frac{1}{190}$
						8½	August 27, 1942	$\frac{0}{16}$	$\frac{0}{383}$	$\frac{0}{407}$
						14	January 26, 1943	$\frac{8}{11}$	$\frac{6}{\text{many}}$	
						16	March 25, 1943	$\frac{7}{\text{many}}$	$\frac{2}{\infty}$	$\frac{5}{\infty}$
15	K. W. 3734	11	November 14, 1941	0 (Throat culture positive)	100 to 165	5½	April 23, 1942	$\frac{0}{10}$	$\frac{0}{258}$	$\frac{0}{70}$
						6½	May 28, 1942	$\frac{2}{8}$	$\frac{2}{416}$	$\frac{5}{275}$
16	C. B. 3634	13	December 31, 1941	0 (Throat culture positive)	60 to 100	4	April 21, 1942	$\frac{2}{23}$	$\frac{4}{246}$	$\frac{4}{204}$
						5	May 24, 1942	$\frac{2}{6}$	$\frac{0}{\text{many}}$	$\frac{0}{\text{many}}$

one child known to have no bacteriostatic activity against type 36 streptococcus was used throughout the experiment. Five-tenths cc. of the serum to be tested was added to 0.9 cc. of this boy's blood. The results are presented in Table II.

Unfortunately, only small amounts of sera obtained before the attack of pharyngitis were available and the tests therefore could not be repeated. Although the bacteriostatic activity of the sera was weak, in 4 of the 6 cases, a definite difference between the sera taken before and after infection was apparent.

Specificity of the bacteriostatic activity of the blood of patients, following recovery from streptococcal pharyngitis

Strains of heterologous types (types 6, 15, 19, 26, and 39), isolated from patients with acute pharyngitis, were included in many of the tests. It was shown by Todd (17) that the virulence of streptococci can be judged by their ability to grow in the blood of normal individuals. Although the heterologous types were recently isolated, most of these strains appeared to be of low virulence and failed to grow in human blood unless a fairly large number of bacteria were inoculated. With the homologous strain, type 36, a considerable variation in the size of the inoculum did not alter the results. With the heterologous types, the bacteriostatic activity of the blood of many of the children varied with the number of bacteria inoculated. In many instances, when a small inoculum was used, the blood appeared to be bacteriostatic, whereas, with a larger inoculum, the bacteria were able to multiply. Furthermore, the findings with heterologous types were often equivocal because no definite contrast between the child who had recovered from the type 36 streptococcal pharyngitis and the children chosen to serve as controls was obtained. In most instances, however, the bacteriostatic effect was more marked against the homologous type 36 than against heterologous types.

Absorption. It was thought of interest to determine whether the bacteriostatic activity of sera obtained from children whose whole blood inhibited the growth of type 36 streptococcus could be reduced by absorption with the homologous strain. The inactivated serum in each in-

stance was divided into 3 parts: one part was not absorbed, one was absorbed with the homologous strain, and one with a streptococcus of a heterologous type. Two parts of serum were mixed with one part of heat-killed bacterial sediment, and incubated at 37° C. for 30 minutes. The mixture was allowed to stand overnight in the ice box, centrifuged the following morning, and the clear serum pipetted off. Five-tenths cc. of each of these sera was added to 0.9 cc. of whole heparinized blood, obtained from a child known to have no bacteriostatic action against type 36 streptococcus.

The indirect method of doing bacteriostatic tests, using serum and blood from a control, usually gave less definite results than those obtained with whole blood of children following recovery from type 36 pharyngitis. In most instances, however, the bacteriostatic action of serum absorbed with the homologous type was reduced, as compared with the unabsorbed serum or that absorbed with a heterologous type. A protocol illustrating these findings is presented in Table III. The bacteriostatic effect as shown in this table is most marked with serum obtained from Case 5 although this child was bled 16 months after the attack of pharyngitis. In all 3 of these cases, the bacteriostatic action of the serum was definitely reduced after absorption with the homologous type.

Comparison of the bacteriostatic activity of the blood of children receiving prophylactic doses of sulfanilamide with that of children after recovery from pharyngitis

In a number of experiments, the blood of patients receiving daily prophylactic doses of sulfanilamide was included. No para-aminobenzoic acid was added. It was found that, in most instances, the blood of patients containing concentrations of free sulfanilamide, varying from 1.2 to 2 mgm. per cent, was less bacteriostatic for type 36 streptococcus than that of children who had recovered from pharyngitis due to this micro-organism.

Is type 36 streptococcus peculiarly susceptible to the bactericidal action of serum described by Tillett?

It was noted by Tillett that different strains of streptococci varied greatly in their suscepti-

TABLE II

Comparison of the bacteriostatic activity of serum obtained before and after infection with type 36 streptococcus

Case No.	Date of pharyngitis	Inactivated serum		Blood from control, C.B.	Result
		Date	Amount		
1	October 27, 1941	October 16, 1941	cc. 0.5	cc. 0.9	<u>6</u> many
		March 27, 1942	0.5	0.9	<u>7</u> 26
7	November 6, 1941	July 2, 1941 October 25, 1941	0.5	0.9	<u>8</u> many
		March 20, 1942	0.5	0.9	<u>7</u> 55
10	October 26, 1941	July 30, 1941	0.5	0.9	<u>5</u> many
		January 12, 1942	0.5	0.9	<u>7</u> 83
12	November 4, 1941	August 27, 1941	0.5	0.9	<u>8</u> many
		January 6, 1942	0.5	0.9	<u>13</u> 304
14	November 9, 1941	October 6, 1941	0.5	0.9	<u>8</u> many
		December 16, 1941	0.5	0.9	<u>4</u> many
16	December 31, 1941	December 27, 1941	0.5	0.9	<u>5</u> many
		February 12, 1942	0.5	0.9	<u>7</u> 83
Control C. B.			(0.5 saline)		<u>5</u> ∞

bility to the non-specific bactericidal action of sera obtained from febrile patients (5). Although all the children whose blood was used in the bacteriostatic tests were afebrile and had no complaints or symptoms, in a few experiments, the bacteriostatic action of fresh serum was compared with that of whole blood obtained from the same individual. In spite of the fact that the

blood was definitely bacteriostatic, it was found that type 36 streptococcus multiplied freely in the serum. The bactericidal action of fresh serum obtained from one patient with active rheumatic fever, whose temperature ranged between 101.8 to 103° F. on the day of the test, was also tried with type 36 streptococcus. No bactericidal effect was noted. Thus, no evidence

TABLE III

Effect of absorption with the homologous type and a heterologous type of sera, obtained from children after recovery from streptococcus type 36 pharyngitis

Case No.	Date of pharyngitis	Date serum obtained	Inactivated serum		Whole blood from control J.M.	Result
			Absorption	Amount		
5	November 4, 1941	March 25, 1943	Unabsorbed	cc. 0.5	cc. 0.9	$\frac{3}{57}$
			Absorbed with homologous type 36	0.5	0.9	$\frac{3}{\text{many}}$
			Absorbed with heterologous type 11	0.5	0.5	$\frac{3}{59}$
6	November 4, 1941	Pooled sera obtained between December 9, 1941 and July 2, 1942	Unabsorbed	0.5	0.9	$\frac{4}{89}$
			Absorbed with homologous type 36	0.5	0.9	$\frac{0}{\text{many}}$
			Absorbed with heterologous type 11	0.5	0.9	$\frac{1}{117}$
11	October 31, 1941	March 30, 1943	Unabsorbed	0.5	0.9	$\frac{3}{341}$
			Absorbed with homologous type 36	0.5	0.9	$\frac{3}{\text{many}}$
			Absorbed with heterologous type 11	0.5	0.9	$\frac{2}{146}$
		Serum from control S. A.	Unabsorbed	0.5	0.9	$\frac{3}{\text{many}}$
		Serum from control M. H.	Unabsorbed	0.5	0.9	$\frac{0}{\text{many}}$
					1.4	$\frac{3}{\text{many}}$

was obtained to suggest that the phenomenon described by Tillett played a part in our findings.

Is the bacteriostatic action of the blood demonstrable in children following recovery from upper respiratory infections due to other types of Group A streptococci?

Type 15 streptococcus. Blood was obtained from 5 children, who had had pharyngitis, due to type 15 streptococcus, 15 to 18 months after the

upper respiratory infection. No definite bacteriostatic effect against the homologous type was demonstrable.

Type 19 streptococcus. The blood of 2 children, who had had upper respiratory infections associated with type 19 streptococcus during the winter of 1942, was tested with the homologous type and with 2 heterologous types. In one of these patients, the bacteriostatic action of the blood for type 19 streptococcus had been determined be-

fore the infection occurred, and in the other, the first test was done 3 weeks after the attack of pharyngitis. Further tests were performed with the blood of both children, at frequent intervals for periods of 5 to 6 months. In some instances, the blood was set up in duplicate and inoculated with varying numbers of bacteria. In one experiment, the bacteriostatic activity of whole blood was compared with that of freshly obtained serum.

The data presented in Table IV indicate that the bacteriostatic action of the blood in the case of these 2 children became demonstrable only 2 to 3 months after infection. At this time, the serum, in contrast to the whole blood, showed no bactericidal or bacteriostatic effect. The blood of neither of these 2 individuals inhibited the growth of 2 heterologous types, 36 and 37. The bacteriostatic action of J. C.'s blood for the homologous type 19 streptococcus was slightly

TABLE IV

Bacteriostatic tests using the homologous and heterologous types with blood and serum obtained from 2 children who had type 19 streptococcal pharyngitis

Name	Pharyngitis		Rise in antistreptolysin O titer	Interval between infection and tests	Dates of tests	Strains of streptococci	Results				
							Patient		Children who escaped type 19 pharyngitis		
	Whole blood	Fresh serum							Whole blood	Fresh serum	Control 1
J. C.	October 26, 1942	+++	No change	3 weeks	November 18, 1942	Homol.* type 19	0.9 cc. 2 many	0.9 cc.	0.9 cc. 1 many	0.9 cc. 3 267	
				5 weeks	December 4, 1942	Homol. type 19	0 many		0 many	1 327	
				3 months	January 29, 1943	Homol. type 19	3 1	3 many	1 many	3 many	
				3½ months	February 19, 1943	Homol. type 19	4 27		3 many		6 ∞
						Heter.** type 37	1 208		0 many		3 many
H. R.	December 1, 1942	++	No change	Before infection	November 14, 1942	Homol. type 19	1 many		0 113	0 66	
				2 months	January 29, 1943	Homol. type 19	3 50	2 many	1 many	3 many	
				2½ months	February 19, 1943	Homol. type 19	0 46		3 many		6 ∞
						Heter. type 37	5 ∞		0 many		3 many
				5 months	April 28, 1943	Homol. type 19	1 58		1 many		1 ∞
		Heter. type 36	1 many		5 480		2 many				

* Homol. = Homologous.

** Heter. = Heterologous.

more marked than that of H. R. The clinical symptoms were also somewhat more severe in the former patient.

Limitations of bacteriostatic tests

Different observers have used various methods of doing bactericidal tests. Most investigators have emphasized the difficulties and numerous sources of error inherent in this technique.

The modification of the method employed by us differs from that used by other observers in that we obtained merely a bacteriostatic and not a bactericidal effect. The number of bacteria inoculated was small and the period of incubation short. It is only by comparing the results obtained with the blood of suitable controls that our findings are of interest.

DISCUSSION

The development of bacteriostatic properties in the blood of patients following recovery from streptococcal pharyngitis due to a single type of Group A hemolytic streptococcus, type 36, is reported. This property persisted for many months and in some instances was still demonstrable a year after the upper respiratory infection. It developed irrespective of whether the symptoms caused by the pharyngitis were mild or severe and occurred both in patients who escaped and those who developed rheumatic sequelae.

At the present time, the majority of investigators agree that rheumatic recurrences usually follow in the wake of streptococcal pharyngitis. One of the major concerns of physicians who have rheumatic patients under their care is the prevention of this kind of upper respiratory infection. It has been shown by several observers that streptococcal pharyngitis and rheumatic relapses can be prevented by the prophylactic administration of sulfanilamide (18 to 20, 4, 10). There are, however, many objections to prolonged sulfonamide prophylaxis and it seems worthwhile therefore to investigate the possibility that type specific immunity may develop.

It is noteworthy that in outbreaks of streptococcal pharyngitis or scarlet fever due to a single type of Group A hemolytic streptococci, a large proportion of the individuals exposed escape in-

fection. At the present time, the nature of this resistance is not understood. It may indicate a previous infection with the same type or with types so closely related antigenically that an overlapping immunity is produced. If type-specific immunity does develop, it may be cellular and bear no relation to humoral antibodies. On the other hand, it is entirely possible that the failure to contract infection depends on unknown non-specific factors.

During the course of the outbreak of pharyngitis, due to type 36 streptococcus, the failure of certain children to contract this infection, in spite of exposure, was striking. It was thought that if the resistance of these individuals was due to a previous infection with this type of streptococcus, bacteriostatic properties might still be demonstrable in their blood. The results of these tests, however, were entirely negative. It may be that, after a lapse of time, bacteriostatic activity is no longer demonstrable in the blood and that these humoral antibodies represent a temporary expression of a more permanent cellular immunity.

Bacteriostatic tests failed to elucidate the nature of the resistance to streptococcal pharyngitis of individuals who escaped infection. The fact that this property was demonstrable in the blood of patients recovering from pharyngitis due to Group A of a single type, however, gives us another method for studying the development of type-specific immunity following streptococcal upper respiratory infections. By means of this technique, it may be possible to answer the much disputed question: Are so-called "contact" carriers, who during outbreaks of streptococcal pharyngitis acquire the epidemic-inducing strain, really cases of subclinical infection?

SUMMARY

The development of bacteriostatic properties in the blood of children, after recovery from upper respiratory infections due to Group A streptococci of a single type, is reported.

BIBLIOGRAPHY

1. Fothergill, L. D., and Lium, R., Value of commercial antibacterial streptococcus sera in hemolytic streptococcus infections. *New England J. Med.*, 1934, 211, 99.

2. Hare, R., Alterations in the bactericidal power of the blood which occur during haemolytic streptococcal infections in the puerperium. *J. Path. and Bact.*, 1935, **41**, 61.
3. Spink, W. W., and Keefer, C. S., Studies of hemolytic streptococcal infection. II. The serological reactions of the blood during erysipelas. *J. Clin. Invest.*, 1936, **15**, 21.
4. Chandler, C. A., and Taussig, H. B., Sulfanilamide as a prophylactic agent in rheumatic fever. *Bull. Johns Hopkins Hosp.*, 1943, **72**, 42.
5. Tillett, W. S., The bactericidal action of human serum on hemolytic streptococci. I. Observations made with serum from patients with acute infections and from normal individuals. *J. Exper. Med.*, 1937, **65**, 147.
6. Swift, H. F., and Hodge, B. E., Type-specific anti-M precipitins in rheumatic and non-rheumatic patients with hemolytic streptococcal infections. *Proc. Soc. Exper. Biol. and Med.*, 1936, **34**, 849.
7. Walker, D. W., Application of the technique of slide agglutination of hemolytic streptococci to human sera. *Proc. Soc. Exper. Biol. and Med.*, 1941, **48**, 338.
8. Rantz, L. A., Kirby, W. M. M., and Jacobs, A. H., Group A hemolytic streptococcus antibodies. I. Griffith type agglutinin and antistreptolysin titers in normal men and in acute infections. *J. Clin. Invest.*, 1943, **22**, 411.
9. Diefendorf, H. W., A method for detecting in human serum protective bodies against hemolytic streptococci. *Proc. Soc. Exper. Biol. and Med.*, 1941, **48**, 56.
10. Kuttner, A. G., and Reyersbach, G., The prevention of streptococcal upper respiratory infections and rheumatic recurrences in rheumatic children by the prophylactic use of sulfanilamide. *J. Clin. Invest.*, 1943, **22**, 77.
11. Krumwiede, E., A comparison of the value of the agglutination and precipitin reactions in the serological typing of Group A streptococci. *J. Bact.*, 1943, **46**, 117.
12. Griffith, F., The serological classification of streptococcus pyogenes. *J. Hyg.*, 1934, **34**, 542.
13. Lancefield, R. C., Antigenic complex of streptococcus haemolyticus; demonstration of type-specific substance in extracts of streptococcus haemolyticus. *J. Exper. Med.*, 1928, **47**, 91.
14. Kuttner, A. G., and Krumwiede, E., Observations on the effect of streptococcal upper respiratory infections on rheumatic children: A three year study. *J. Clin. Invest.*, 1941, **20**, 273.
15. Todd, E. W., A method of measuring the increase or decrease of the population of haemolytic streptococci in blood. *Brit. J. Exper. Path.*, 1927, **8**, 1.
16. Brown, J. H., Vacuum tubes for the storage and shipment of bacteria. *Science*, 1926, **64**, 429.
17. Todd, E. W., Observations on the virulence of haemolytic streptococci. *Brit. J. Exper. Path.*, 1927, **8**, 289.
18. Thomas, C. B., France, R., and Reichsman, F., The prophylactic use of sulfanilamide in patients susceptible to rheumatic fever. *J. A. M. A.*, 1941, **116**, 551.
19. Coburn, A. F., and Moore, L. V., The prophylactic use of sulfanilamide in streptococcal respiratory infections, with special reference to rheumatic fever. *J. Clin. Invest.*, 1939, **18**, 147.
20. Hansen, A. E., Platou, R. V., and Dwan, P. F., Prolonged use of a sulfonamide compound in prevention of rheumatic recurrences in children: An evaluation based on a four year study on sixty-four children. *Am. J. Dis. Child.*, 1942, **64**, 963.