STUDIES ON THE RELATIONSHIP OF STREPTOCOCCUS HEMOLYTICUS TO THE RHEUMATIC PROCESS

III. Observations on the Immunological Responses of Rheumatic Subjects to Hemolytic Streptococcus

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There are three phases to the rheumatic attack: first, a brief illness of respiratory infection with hemolytic streptococcus; second, a quiescent interval of approximately 10 days; third, the period of clinical manifestations of rheumatic fever. The relationship of a throat infection to the development of the rheumatic attack was described in the first paper of this series. In each of the three groups being studied, nurses, convalescent rheumatic patients and ambulatory rheumatic subjects, there was a well defined quiescent interval between the subsidence of the local infection and the onset of the rheumatic attack. The observations in 1930–31 illustrate these findings.[1] In the case of each student nurse, the quiescent period was at least 3 weeks; in the case of convalescent rheumatic patients at The Pelham Home, it varied from 5 to 27 days, with an average of 12 days; among the ambulatory rheumatic subjects there was usually a similar lag; however, in a few instances severe rheumatic phenomena followed immediately after a "sore throat" subsided.

The precise date of onset of rheumatism could not be judged with accuracy, and for this reason it has not been possible to determine the exact length of the quiescent interval. In the case of certain severe local infections and of a few tonsillectomies, almost immediate rheumatic attacks were observed. The usual finding was a definite period

[1] Haig-Brown (1) in 1886 and more recently Hector (2), Campbell and Warner (3) and Schlesinger (4) have also observed the constant occurrence of this quiescent interval.

of quiescence, which varied from a few days to a few weeks. The observations in this study are adequately described by the words of Haig-Brown (1) written nearly half a century ago: "perhaps a month, more commonly 10 days or simultaneously."

Observations in The Pelham Home

Throat infections	Interval	Onset of rheumatic attack	Throat infections	Interval	Onset of rheumatic attack
	days			*days*	
Apr. 21–22	14	May 6	Feb. 22–25	8	Mar. 6
Mar. 1– 3	9	Mar. 12	June 17–20	22	July 12
Sept. 23–25	5	Oct. 1	Jan. 24–26	10	Feb. 6
Jan. 25–27	5	Feb. 2	Mar. 23–24	27	Apr. 21

Observations on Ambulatory Rheumatic Subjects

Throat infections	Interval	Rheumatic attack	Throat infections	Interval	Rheumatic attack
	days			*days*	
Feb. 21, 22, 23	10	Mar. 7	Apr. 16–18	8	Apr. 27
May 1	10	May 10	Jan. 25	3	Jan. 28
Mar. 1, 2, 3, 4	8	Mar. 12	Feb. 21–25	12	Mar. 9
Feb. 4	3	Feb. 7	Nov. 29	5	Dec. 3
Jan. 27, 28	7	" 5	" 1	14	Nov. 14
" 15	5	Jan. 21	Jan. 18	20	Feb. 7
Feb. 12–16	17	Mar. 5	Feb. 22	10	Mar. 2
" 14–16	17	" 3	" 13	7	Feb. 20

The occurrence of a 10 day period of quiescence between the subsidence of infection and the onset of rheumatism suggests that the rheumatic attack begins when the immune response is at its height. This observation has led to the conception that the development of rheumatic fever may be associated with the immunity mechanism of certain individuals for handling products of hemolytic streptococcus. In order to investigate the immunological changes occurring in the rheumatic subject between the subsidence of the local infection and the development of rheumatism, sera have been examined for the presence of antibodies to hemolytic streptococcus. Four types of reactions were selected for investigation: agglutination; fixation of complement; occurrence of precipitins; development of antistreptolysin. The

methods employed and the findings for the 4 year period 1928–32 are as follows:

A. Agglutination Reactions

During the early part of this study the blood sera of a number of acutely ill rheumatic patients were tested for the presence of agglutinins to *Streptococcus viridans*. Although agglutinins were present in high titer in patients with bacterial endocarditis, there was no instance in which this was true of the sera of patients with acute rheumatism.

The sera of five groups of individuals were tested for the presence of agglutinins to hemolytic streptococcus against strains of hemolytic streptococcus: No. C17 (rheumatic fever throat strain), hemolytic streptococcus Nos. NY5 and 273 (scarlet fever strains). The sera tested were obtained from the following groups: individuals with proven hemolytic streptococcus infections (scarlet fever, tonsillitis, erysipelas); acutely ill rheumatic patients; quiescent rheumatic subjects; patients with lobar pneumonia; normal, healthy individuals as controls.

The sera[2] of the patients in the first two groups in most instances agglutinated one or all of the organisms to a titer 1:10 to 1:40. In a few instances agglutination occurred in titer 1:80. In only one instance was agglutination observed in titer 1:160. The sera of the pneumonia patients, quiescent rheumatic subjects and controls usually failed to agglutinate entirely or agglutinated to a titer no higher than 1:40. These tests demonstrated that the sera of acutely ill rheumatic subjects resemble in their agglutination titer the sera of patients recovering from streptococcus infection. Because of their low titer this method of study was regarded as unsuitable.

B. Complement Fixation Reactions

Complement fixation tests were performed with three antigens—hemolytic streptococcus carbohydrate, scarlet fever streptococcus toxin, scarlet fever streptococcus nucleoprotein. With the carbohydrate fractions and toxins no positive reactions were obtained, and these procedures were discontinued. The protein fractions, however,

[2] Some of these tests were done through the kindness of Dr. M. H. Dawson.

gave reactions and were made up in the following dilution of milligrams of protein:

1. No. 273 —1:1,000; 1:10,000; 1:15,000; 1:20,000; 1:25,000; 1:50,000
2. " C15DIII—1:1,000; 1:10,000; 1:15,000; 1:20,000; 1:25,000; 1:50,000
3. " C17D —1:1,000; 1:10,000; 1:15,000; 1:20,000; 1:25,000; 1:50,000

The fractions tested showed great differences in their antigenic strength. Fraction C8D gave only negative reactions and Fraction C15DIII gave reactions only about one-fifth as strong as Fraction 273, of the same dilution. This is illustrated in the following table. The reactions with Protein 273 were consistently more intense than those with C15D—illustrated by two rheumatic patients, S and D.

Dilutions of antigen	1:10,000	1:20,000	1:40,000	1:50,000	1:100,000
S (C15D)	++++	++±	−	−	−
S (273)	++++	++++	+++	++±	++
D (C15D)	++++	+	−	−	−
D (273)	++++	+++	++	−	−

The technique employed for these tests was the method customarily employed in the Wassermann reactions. The tubes together with a positive and negative serum control as well as antigen controls consisting of 0.4, 0.3, 0.2, 0.1 cc. amounts were water bathed at 37.5°C. for 1 hour, after which each tube received 0.2 cc. of sensitized 5 per cent sheep cells and was again incubated at 37.5°C. Readings were made as soon as the antigen and serum controls cleared and were interpreted as follows:

Human serum reactions	0.02 cc.	0.01 cc.
++++	No hemolysis	No hemolysis
+++	" "	Slight "
++	" "	Complete "
+	Slight "	" "
Negative	Complete "	" "

The patients whose sera were tested included a control group believed to be non-rheumatic; a control group of student nurses, some known to be in good health, others known to have acute hemolytic streptococcus pharyngitis; rheumatic subjects believed to be quiescent, and patients with acute rheumatic fever. The determinations are recorded in Table I.

TABLE I

Complement Fixation Reactions
Antigen Nucleoprotein C15DIII

Dilutions of antigen	1:1,000	1:10,000	1:15,000	1:20,000	1:25,000	1:30,000	1:35,000	1:40,000
Control group, non-rheumatic; spring months								
T.	−	−	−	−	−	−	−	−
Be.		+	−	−	−	−	−	−
Bo.		±	−	−	−	−	−	−
G.	−	−	−	−	−	−	−	−
N.	−	−	−	−	−	−	−	−
P.	−	−	−	−				
Nurses, normal and recovering from hemolytic streptococcus pharyngitis; spring months								
Br., normal	−							
L. "	++							
S. "	+++							
C.,* pharyngitis	+++	++++	++	++	+	−	−	−
T.* "	−	−						
F. "	++	+						
H. "	+	−						
Rheumatic subjects clinically quiescent; spring months†								
Fi.	−	−	−					
C.	−	−	−	−				
Fa†	++++	++++	++++	+++	+++	+	−	−
W. H.	−	−	−					
Wa.	−	−						
Ma.		++						
W.	−	−						
La.	−	−						
Ku.		+						
Active rheumatism; spring months								
Ma.		+±	+	±	−	−	−	−
Gi.		+	−	−	−	−	−	−
Ga.		+++	+	±	−	−	−	−
Fe.		++++						
Fa.		+	−	−	−	−	−	−
Pa.		++++						
T.		+	−	−	−	−	−	−
Br.		+	−	−	−	−	−	−
H.		+	−	−	−	−	−	−

* C and T had scarlet fever. † Serum examined during the fall showed no strong reactions.

As is indicated in Table I, the complement fixation reaction appeared extremely sensitive for the determination of antibody. Because of the variability in hemolytic systems the method was not found satisfactory for sharp interpretation. In certain instances, the strongest reactions were detected among rheumatic patients clinically quiescent; while in fulminating attacks the reaction was frequently less striking. The sera of non-rheumatic individuals infected by hemolytic streptococcus gave a positive fixation at a dilution 1:1,000 in most instances, and the serum of each acutely ill rheumatic subject gave a positive complement fixation in 1:10,000 dilution of the antigen. This type of immunity response suggests a recent infection with *Streptococcus hemolyticus*.

The results of the complement fixation tests are as follows: The majority of the control sera were negative. The sera of individuals convalescing from sore throat or scarlet fever were positive. The sera of some rheumatic subjects without clinical symptoms showed strongly positive reactions in the spring. The sera of all rheumatic subjects during the attack showed a positive reaction, but in some instances this reaction was not so strong as in the sera of individuals who were symptom-free.

C. Precipitin Reactions

To investigate further the possible relationship of the rheumatic attack to infection with *Streptococcus hemolyticus*, the occurrence of precipitins to hemolytic streptococcus fractions has been studied in detail over a long period of time. That these reactions are not entirely specific has been demonstrated by the work of Lancefield (5) which indicates an antigenic relationship of the proteins of streptococcus to those of pneumococcus, staphylococcus and other organisms.

The materials used as precipitinogen consisted of the following hemolytic streptococcus substances: toxins, carbohydrates and proteins. The toxins were tested for precipitation in several dilutions and the carbohydrate fraction in concentrations 1:25,000, 1:100,000, and 1:500,000. Reactions with the former were uniformly negative and with the latter rarely positive. Their use was discontinued. The protein fractions of Streptococcus 273 previously used in skin testing (6)[3] and protein fractions recently prepared by Dr. Michael Heidelberger (7) have been employed throughout the study. The D fractions were obtained after acidification of the cells at pH 6.5. The K fractions and 273 were obtained at pH 11 to 13 after removal of the fractions between pH 6.5 and pH 11. In general the dilution used was milligrams of protein 1:2,000.

The technique of precipitin tests was as follows: To 0.2 cc. each of fresh human sera, was added an equal quantity of nucleoprotein fraction. (It is essential to have both clear sera and protein solutions.) After mixture each tube was immediately and thoroughly shaken. Readings were taken at various intervals, the first after 20 minutes at room temperature, the second after 2 hours in 37.5°C. water and the third after overnight refrigeration.

The development of precipitins was first studied among a class of 50 student nurses. The antigen used was hemolytic streptococcus Frac-

[3] Coburn (6), p. 226.

tion C15D, dilution 1:2,000. In September, 1930, all of the individuals were tested for the presence of precipitins in their blood and none were found. During the spring months the sera of the same individuals were again tested, and the results are summarized in Table II.

TABLE II

A Study of Precipitin Reactions to Hemolytic Streptococcus Fractions among a Class of 50 Nurses

Patient	Condition	Fall, 1930	Spring, 1931
Group A			
B.	Normal health		− − −
L.	" "	− − −	− − −
M.	" "	− − −	− − −
S.	" "	− − −	− − −
Group B		− − −	
S.	Normal health		
	*Hemolytic *Streptococcus pharyngitis*	− − −	− − −
W.	Normal health		
	Hemolytic *Streptococcus pharyngitis*	− − −	+ +± +++
H.*	" " "		− − −
Bix.	" " "		− − +±
Bil.	" " "		− + +
F.	" " "		− − ±
Group C			
T.	Normal health		
	Scarlet fever	− − −	− − −
C.	Normal health		
	Scarlet fever	− − −	− ± ±
M.	" "		− − −
Group D			
Cr.	Normal health		
	Acute hemolytic streptococcus infection followed by rheumatic fever	− − −	+ +± +++
Me.	Normal health		
	†Acute hemolytic streptococcus infection followed by rheumatic fever	− − −	− − ±
Sm.	Normal health		
	Acute hemolytic streptococcus infection followed by rheumatic fever	− − −	+ +± +++

These readings are expressed: one, immediately, second, at 2 hours at 37°C. and the last, overnight in ice box. They represent uncentrifuged findings. ++++ = complete disc at bottom of tube with clear supernatant. +++ = flocculated sediment. ++ = no sediment; granules or floccules in suspension. + = clouding with whirl. ± = clouding with no whirl.

Intermediate readings were also made, such as, +++± = incomplete disc. ++± = flocculated sediment with some large floccules still in suspension. +± = few granules or floccules in suspension with slight clouding of supernatant.

* At onset. † Erythema marginatum—no arthritis.

From Table II, it is seen that Group A escaped infection and their sera remained negative for precipitins; Group B developed hemolytic streptococcus pharyngitis and precipitins appeared in some instances during convalescence; Group C contracted scarlet fever and precipitins were not detected during the acute stage of the illness; Group D contracted hemolytic streptococcus pharyngitis and developed rheumatic fever. The appearance of precipitins to hemolytic streptococcus protein fractions in their sera was striking.

A second study was made to determine the relationship of the development of precipitins for hemolytic streptococcus fractions to activity of the process in rheumatic subjects. At the end of the summer, the rheumatic process appeared quiescent in nearly every individual. During the early fall, two of the group contracted pharyngitis and developed severe rheumatic attacks. In the late winter months, most of the patients were probably exposed to infection. Some escaped symptoms of upper respiratory disease and the rheumatic process appeared quiescent. Others contracted throat infections. Most of these developed severe rheumatic attacks; a few appeared to escape. With the exception of these few individuals, the results of the precipitin tests are presented in Table III.

From Table III it is seen that in the sera of quiescent rheumatic subjects precipitins were not detected in the fall. Sera from the same individuals, clinically quiescent in the spring, gave in most instances negative or weakly positive reactions. In contrast, the sera of rheumatic patients acutely ill in the fall or in the spring months in almost each instance gave a strong precipitin reaction to protein fractions of hemolytic streptococcus. This occurred only in the individuals who developed acute rheumatism. It was pointed out in the first paper of this series that in certain instances following hemolytic streptococcus infection the rheumatic subject escapes a definite attack. The sera of these patients have been studied at frequent intervals during the 4 weeks following infection. In none were precipitins detectable.

In order to correlate the clinical signs of activity of the disease process with the content of precipitins to fractions of hemolytic streptococcus in the sera of rheumatic subjects, a few individuals have been studied closely. Patient Ker. during a severe rheumatic attack in December, 1930, had strong precipitins in her serum. These dis-

appeared with clinical improvement and reappeared again during another intense recrudescence. Individual Kor. with a fulminating rheumatic attack in November, 1930, likewise showed a high concentration of precipitins. Signs of mild rheumatic activity persisted in

TABLE III

A Study of the Relationship of the Precipitin Reaction to Quiescence and Activity of the Rheumatic Process

Patient	Fall, 1930		Spring, 1931	
	273	C15D	273	C15D
Group A—apparently quiescent				
C	− − −	− − −	− − −	− − −
H	− − −	− − −	− − +	− + +±±
Ki	− − −	− − −	− − −	− − ++
La	− − −	− − −	− − −	− − −
Lu	− − −	− − −	− − −	− − +++
Ma	− − −	− − −	− − −	− − +
Ri	− − −	− − −	− − −	− ± ++
Ru	− − −	− − −	− − −	− − ±
Wa	− − −	− − −	− ± +	− ± +
Wi	− − −	− − −	− − ±	− − −
Fa	− − −	− − −	± + +++	± + +++
Group B—active rheumatism				
Ker	+ ++ +++	± + +		
Kor	+ + +	+ + ±		
Kel.*			− ± +	± + ++
Cl.†			± ± +	± + +++
Fa				− − −
Br				− + +
T				± + +++
O			− + ++	
P				+ + +++
S			± ± ±	+ ± ±
F				− + +±
G			− ± +	− − −
Ga				+ +± +++
Gi			+ + ++	− − ±

* Negative 1 month before attack. † Negative 4 months before attack.

this patient for 6 months. The precipitins did not disappear during this period, increased during a slight recrudescence in March, 1931, and finally were no longer detectable when the disease seemed quiescent. These findings are presented in Table IV, *a*.

In addition to the patients in Table IV, *a*, showing the presence of

TABLE IV, *a*

Changes in the Precipitin Reaction with Fluctuations in Activity of the Rheumatic Process

Patient	Protein fraction, dilution 1:2,000			
	C7L	C8D	273	C15D
Ker.				
During attack, Nov., 1930	+ + +	++ + ++	− + +++	
During convalescence, Jan., 1931		− − −	− − −	
Feb., 1931		− − −	− − −	
During recrudescence, Apr., 1931				− − ±
During attack, May, 1931				± + +
During quiescence, Feb., 1932		− − −(−)*	− − −(−)	− − −(+)
Kor.				
During attack, Nov. 1, 1930	++ + −	++ + −	+ + +++	
Nov. 30, 1930	− − −	− − −		− ± ±
During convalescence, Dec., 1930	− − −	− − ±	± − −	
During slight flare up, Mar., 1931		− − +	− − +	− + +±
During quiescence, Feb., 1932		− − −(−)	− − −(−)	− − −(−)

* In addition to the customary readings made immediately, after 2 hours' incubation and after standing overnight in the ice box, a fourth determination has been recorded when indicated. This is expressed in parentheses and represents the findings after 10 minutes' centrifugalization at moderate speed.

TABLE IV, *b*

Changes in the Precipitin Reaction in Relation to the Clinical Course of the Rheumatic Attack

Patient	Date						
	Dec. 3	Dec. 24	Dec. 28	Jan. 4	Jan. 11	Jan. 19	Feb. 9
Maz.							
Condition	Sick	Sick	Very sick	Sick	Recovering		
Reactions to 1:2,000 dilution of Fraction C17D	+	+	+±	++	+	±	±
Fraction C17K	+	+	+±	++	+	±	±
	Jan. 3	Jan. 6	Jan. 10	Jan. 15	Jan. 27		
Man.							
Condition	Sick	Sick	Very sick		Moribund		
Reactions to 1:2,000 dilution of Fraction C17D	±(+)*	−(+)	+(++)	+	+±		
Fraction C17E	+(++)	+(+)	+(+++)	+	+±		

* Parentheses indicate readings after centrifugation, single determinations represent 18 hour uncentrifuged readings.

precipitins during acute rheumatism, absence during recovery and reappearance during recrudescence, the sera of other individuals were examined at frequent intervals during a single, severe attack of rheumatic fever. The findings in two of these are presented in Table IV, *b*. Patient Maz. experienced a fulminating rheumatic attack and recovered; Patient Man. died 3 weeks after the onset of intense rheumatism.

TABLE V

A Study of the Precipitin Reaction to Streptococcus Protein Fractions

Patient	273	C15D
Scarlet fever		
Mc.*	+ + +	+ + +
Br.	− + ±	− − −
R.	− ± −	− − −
H.	± + +	− − −
I.	− ± −	− − −
R.	± − −	− − −
Lobar pneumonia (pneumococcus)		
W.–Pn. IV	− + +	− − −
B.–Pn. III	− − ±	− − ±
Bo.–Pn. II	− + +	− − +
Gi.–Pn. IV	− + +	− − +
Sh.–Pn. IV	− − −	− − −
Erysipelas and mastoiditis		
T.	− − ±	− − ±

* Mc. developed rheumatic heart disease.

From Table IV, *b*, it is seen that with recovery, the precipitin content diminished in the serum of Patient Maz. In contrast, the content of precipitins to hemolytic streptococcus protein fraction increased in the serum of Patient Man. to the day of death. These were both examples of intense rheumatism. In the less severe attacks, the precipitin content of the sera has been found to change more rapidly, remaining at a high level for only a few days. There is a close parallelism between intensity of the rheumatic process and the strength of the precipitin reactions.

For purposes of comparison, a study was made of the content of precipitins in the sera of patients with a variety of respiratory infec-

tions. Determinations were made during the course of lobar pneumonia, during the 3rd week of scarlet fever and during recovery from erysipelas. The findings are presented in Table V.

From Table V it is seen that weak precipitin reactions occurred in the sera of patients with respiratory infections. The only strong per-

TABLE VI, *a*

Precipitin Reactions to Hemolytic Streptococcus Fractions in Quiescent Rheumatic Subjects Inoculated with T.A.B. Vaccine

Patient	Fraction 273	Fraction 17D	Fraction 17K	Control
Before vaccination with *B. typhosus* and *paratyphosus* A and B				
Br.	− − − (−)	− − − (+++)	− − − (+)	− − − (−)
By.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
C.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
H.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
M.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
Mo.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
1 and 3 wks. after vaccination				
Br.	− − − (−)	− − − (+±)	− − ± (+)	− − − (−)
By.	− − − (−)	− − − (+±)	− − − (−)	− − − (−)
C.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
H.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
M.	− − − (−)	− − − (−)	− − + (−)	− − − (−)
Mo.	− − − (−)	− − − (−)	− − − (−)	− − − (−)
R.	− − − (−)	− − − (−)	− − − (−)	− − − (−)

TABLE VI, *b*

Precipitin Reactions in a Rheumatic Subject with Pneumococcus Type II Pneumonia

Patient Cr.	Fraction 17D	Fraction 17K	Control
1. Before Nov. 1, 1930	− − − (±)	− − − (±)	
2. Onset Jan. 22, 1932	− − ± (+)	− − − (±)	− − − (±)
3. During Jan. 27, 1932	− − + (+±)	− − + (±)	− − − (±)
4. Convalescence Feb. 8, 1932	− − ±	− − ±	− − ±
5. Recovery Feb. 22, 1932, 4 wks. after onset	− − − (±)	− − − (−)	− − − (−)

sistent precipitin reaction among these patients was in an individual with scarlet fever which terminated in rheumatic heart disease.

As a control measure, studies were made to determine the influence of a reaction to heterologous bacterial proteins on the formation of precipitins to hemolytic streptococcus products. First, ten quiescent

rheumatic patients, who were under close observation at The Pelham Home, were given one subcutaneous dose of T.A.B. vaccine (*B. typhosus* 1 billion, Para A and Para B $\frac{1}{2}$ billion each). This was followed by a sharp reaction in almost each instance. The sera were tested before inoculation, and at the end of 1 and 3 weeks. Second, a few rheumatic subjects contracted pneumococcus lobar pneumonia. Their sera were tested during the disease and in convalescence. The findings are presented in Tables VI, *a*, and VI, *b*.

As shown in Tables VI, *a*, and V, *b*, the precipitin content of the sera was not influenced by a non-specific reaction. A few rheumatic subjects with pneumonia showed the presence of weak precipitins during pneumococcus infection and their disappearance in convalescence.

Summary of Precipitin Tests

The results may be summarized as follows:

In the sera of 150 apparently healthy rheumatic subjects in the fall of 1930, only two individuals were found to have precipitins for hemolytic streptococcus protein fractions. In the sera of 50 healthy student nurses entering training in the fall of 1930, no precipitins were detected. In the sera of patients with lobar pneumonia, the formation of precipitins to hemolytic streptococcus fractions was slight, definite but not persistent. In the sera of twenty patients during the acute stages and convalescence from scarlet fever or erysipelas, marked precipitin formation was detected in one individual. She was the member of a rheumatic family and developed rheumatic carditis. In the sera of twenty student nurses who contracted hemolytic streptococcus throat infections in the spring of 1931, precipitins were not detected during the acute illness but appeared 4 weeks later in slight concentration in most individuals. In four instances the appearance of precipitins was marked; three of these individuals developed rheumatic fever. In the sera of ten rheumatic subjects given prophylactic vaccination with typhoid and paratyphoid vaccine, there was no development of precipitins to the protein fractions of hemolytic streptococcus. One rheumatic subject who contracted Pneumococcus Type II pneumonia developed precipitins to protein fractions of a streptococcus but they disappeared with complete resolution in the lung and did not reappear within 4 weeks. In the sera of ten patients with

acute rheumatism in the fall months of 1930 and 1931, precipitins were detected in each serum.[4] In the sera of 50 patients with fulminating rheumatism in the spring months of 1928–31, precipitins were detected in all except two instances. In the sera of six rheumatic subjects who appeared to escape attacks following hemolytic streptococcus infection, precipitins were not detected. In the sera of rheumatic subjects without demonstrable precipitins in the fall of 1930, fourteen manifested rheumatic attacks in the spring and all developed precipitins. Of thirty whose disease appeared to remain quiescent in the spring, five developed precipitins. This was marked in only one instance. In the sera of ten individuals with acute rheumatism, the concentration of precipitins became more marked as the activity of the process heightened. With subsidence, precipitins disappeared. They returned during recrudescence. In one fatal, fulminating attack which perhaps represented the onset of the disease in a rheumatic baby, the precipitin titer rose during the fortnight's illness and was marked on the day of death.

D. The Development of Antistreptolysin

Through the kindness of Dr. E. W. Todd of The Belmont Laboratories, Sutton, England, the development of another antibody has been studied in the patients described in the first paper of this series. While working with streptococcal hemolysin, Todd (9) observed that antihemolysin was not formed in animals immunized to Klebs-Löffler bacillus, pneumococcus, hemolytic staphylococcus, non-hemolytic streptococcus or other infectious agents, but only in animals immunized to *Streptococcus hemolyticus*. In conjunction with Dr. Todd, the antihemolysin titers of the sera previously examined for precipitins have been determined. Some of these findings are being reported elsewhere (10) in units of antihemolysin. In the present paper, the antistreptolysin titers are recorded as the volume of the patient's serum required to neutralize $2\frac{1}{2}$ minimal hemolytic doses of yeast extract streptolysin (M.H.D.). The titers are therefore expressed as fractions of 1.0 cc., and N.D. is used to designate neutralizing

[4] These sera were tested with pneumococcus "C" substance. Positive reactions were obtained only during the febrile period. This phenomenon was identical with that previously described by Tillett and Francis (8).

dose. The serum of Patient Ker. is used as a control throughout the present study.

The sera of three groups of non-rheumatic subjects were tested first.[5] These consisted of normal individuals in good health; patients convalescing from hemolytic staphylococcus or tuberculous bone infections, and individuals recovering from hemolytic streptococcus infection. The findings are presented in Table VII.

TABLE VII

A Study of the Antistreptolysin Content of Sera from Rheumatic Subjects

		cc.			cc.
A. Normal individuals					
Wh....	Good health	N.D. = 0.05	Wa....	Good health	N.D. = 0.02
Wo.....	" "	" = 0.05	Do.....	" "	" = 0.02
Ba.....	" "	" = 0.02	Mo.....	" "	" = 0.02
Ha.....	" "	" = 0.1	Io.....	" "	" = 0.02
To.....	" "	" = 0.3	Wi.....	" "	" = 0.02
Co.....	" "	" = 0.01			
B. Patients convalescing from infections other than hemolytic streptococcus					
Gr.....	Osteomyelitis	N.D. = 0.03	Ga.....	Tuberculosis of hip	N.D. = 0.02
Sh.....	"	" = 0.02	Ba.....	Poliomyelitis	" = 0.07
Go.....	Hemolytic staphylococcus abscess	" = 0.02	Ta.....	Psoas abscess	" = 0.02
			Kl.....	Tuberculous spine	" = 0.02
Bn.....	" "	" = 0.02	Pe.....	" "	" = 0.009
Ca.....	Tuberculosis of hip	" = 0.02			
C. Patients convalescing from hemolytic streptococcus infections					
Ca.....	Scarlet fever,* 1st wk.	N.D. = 0.01	In.....	Scarlet fever, 3rd wk.	N.D. = 0.004
Ro.....	" " 1st "	" = 0.006	†Mc....	" " 4th "	" = 0.005
Br.....	" " 3rd "	" = 0.006	He.....	" " 8th "	" = 0.003
Hi.....	" " 3rd "	" = 0.006	Tu.....	Erysipelas, 3rd "	" = 0.0008

* These sera were obtained through the kindness of the Willard Parker Hospital.

† Mc. developed rheumatic fever.

Table VII shows that the sera of individuals and patients with tuberculous or hemolytic staphylococcus infection had a low content of antistreptolysin. In contrast, the serum of patients with hemolytic streptococcus infection was high in almost each instance.

[5] All of these antistreptolysin determinations were made by Dr. E. W. Todd, Belmont Laboratories, Sutton, Surrey, England. Through his generosity they are presented here.

A second series of observations was made on a group of normal student nurses to observe the effect of streptococcus infections on the antistreptolysin titer. On admission to the Training School in September, 1930, while apparently free of hemolytic streptococcus and in good health, their sera showed a normal titer (except in two instances). During the month of April, 1931, sera were again obtained. The individuals were divided into three groups: those exposed to infection but in good health; those acutely ill with scarlet fever; those

TABLE VIII

The Influence of Hemolytic Streptococcus Infection on the Antistreptolysin Titer of Student Nurses under Close Observation Clinically and Bacteriologically

	Apr., 1931
Group A. In good health but exposed to infection	
	cc.
Patient L.	N.D. = 0.02
" B.	" = 0.03
" S.	" = 0.02
Group B. At onset of scarlatina	
	cc.
Patient T.	N.D. = 0.07
" C.	" = 0.02
Group C. During convalescence from hemolytic streptococcus tonsillitis	
	cc.
Patient Fan.	N.D. = 0.007
" B.	" = 0.003
" H.	" = 0.005
" S.	" = 0.007

convalescing from hemolytic streptococcus pharyngitis. The results are summarized in Table VIII.

Group A, the individuals who remained in good health, although exposed to infection, showed no change in titer; Group B, the individuals acutely ill with hemolytic streptococcus throat infections, showed no change in titer; Group C, the individuals convalescing from hemolytic streptococcus throat infections, showed a marked rise in antistreptolysin content. These determinations indicate that there occurs normally a rise in antistreptolysin content of the serum during convalescence from hemolytic streptococcus infection.

For purposes of control, two studies were made to test the effect of non-specific stimulation with a heterologous antigen. First, the sera of ten children convalescing from rheumatic fever were tested before and after T.A.B. subcutaneous inoculation. Second, the serum of a patient with rheumatic heart disease was tested before, during and after Pneumococcus Type II pneumonia. The results are presented in Tables IX, *a*, and IX, *b*.

TABLE IX, *a*

Antistreptolysin Titers of Convalescent Rheumatic Patients before and 1 and 3 Weeks after Typhoid Vaccination

Patient		Before	1 wk. after	3 wks. after	Patient		Before	1 wk. after	3 wks. after
		cc.	*cc.*	*cc.*			*cc.*	*cc.*	*cc.*
Br.	N.D. =	0.009	0.009	0.02	Kr.	N.D. =	0.006	0.006	
By.	" =	0.02	0.02		Me.	" =	0.009	0.009	
Cu.	" =	0.02	0.02		Mo.	" =	0.004	0.02	0.03
Ga.	" =	0.02	0.02		Po.	" =	0.02	0.02	0.02
Ha.*	" =	0.01	0.01		Ra.	" =	0.008	0.008	0.01

*Ha. contracted hemolytic streptococcus pharyngitis 8 weeks later and in the 2nd week the N.D. rose to 0.008 cc.

TABLE IX, *b*

Antistreptolysin Titers in a Rheumatic Subject with Pneumococcus Type II Pneumonia

Patient Ch.		*cc.*
	Before pneumonia	N.D. = 0.004
	Onset of "	" = 0.006
	During "	" = 0.007
	Convalescing from pneumonia	" = 0.008

Table IX, *a*, shows that inoculation with T.A.B. vaccine did not increase the titer of antistreptolysin. The fall in titer of Patients Mo., Br. and Ra. is unexplained. Table IX, *b*, shows that during Pneumococcus Type II infection the antistreptolysin titer of a rheumatic subject did not rise but tended to fall to normal.

Because of the constant development of a high titer of antistreptolysin in individuals convalescing from infection with hemolytic streptococcus, a study was made of the content of this antibody in the sera of patients who, while under observation, contracted hemolytic strepto-

coccus infection and developed rheumatic fever. The individuals under investigation included children and adults. The sera were obtained during the 1st week of typical attacks of acute rheumatism. The findings are presented in Table X.

TABLE X

A Study of the Antistreptolysin Content of Sera from Patients Developing Acute Rheumatism while Convalescing from Respiratory Infection with Hemolytic Streptococcus

Patient					Patient				
				cc.					cc.
M.	Acute	rheumatic	fever	N.D. = 0.007	Ro.	Acute	rheumatic	fever	N.D. = 0.003
S.	"	"	"	" = 0.003	Ri.	"	"	"	" = 0.003
St.	"	"	"	" = 0.007	N.	"	"	"	" = 0.002
C.	"	"	"	" = 0.007	O.	"	"	"	" = 0.002
W.	"	"	"	" = 0.004	Sh.	"	"	"	" = 0.004

Table X shows the constantly high titer of antistreptolysin in the sera of patients with acute rheumatism following hemolytic streptococcus pharyngitis.

TABLE XI

Antistreptolysin Titer of Patients Admitted to the Presbyterian Hospital Wards with Acute Rheumatic Fever from Spring, 1928, to Fall, 1931, and without Hemolytic Streptococcus in the Throat Flora

Patient					Patient				
				cc.					cc.
K.	Acute	rheumatic	fever	N.D. = 0.002	P.	Acute	rheumatic	fever	N.D. = 0.003
M.	"	"	"	" = 0.004	Co.	"	"	"	" = 0.006
E.	"	"	"	" = 0.004	Co.	"	"	"	" = 0.008
E.	"	"	"	" = 0.003	B.	"	"	"	" = 0.008
S.	"	"	"	" = 0.003	Cr.	"	"	"	" = 0.006
S.	"	"	"	" = 0.002	D.	"	"	"	" = 0.006
L.	"	"	"	" = 0.009	L.	"	"	"	" = 0.004
C.	"	"	"	" = 0.002	R.	"	"	"	" = 0.005
M.	"	"	"	" = 0.003	M.	"	"	"	" = 0.004
Ch.	"	"	"	" = 0.004	Sh.	"	"	"	" = 0.006
Ch.	"	"	"	" = 0.003	G.	"	"	"	" = 0.008
P.	"	"	"	" = 0.003					

Another series of observations were made on patients admitted to the Presbyterian Hospital with acute rheumatic fever and without

hemolytic streptococcus in the throat flora. The group included children and adults, in various seasons, between 1928 and 1931. The findings are presented in Table XI.

The determinations show that the sera of patients admitted to the hospital with acute rheumatic fever following respiratory infection had, irrespective of the bacteriology of the throat flora, a high titer of antistreptolysin.

A third series of determinations were made on the sera of patients with acute rheumatism who denied a preceding respiratory infection. The findings are presented in Table XII.

From Table XII it is seen that the sera of these individuals, like those of the patients known to have a respiratory infection prelimi-

TABLE XII

Antistreptolysin Titer of Patients with Acute Rheumatic Fever Who Denied Preceding Respiratory Infection

Patient				Patient			
			cc.				*cc.*
F.....	Acute rheumatic fever		N.D. = 0.003	Dev.....	Acute rheumatic fever		N.D. = 0.002
K.....	" " "		" = 0.002	R.....	" " "		" = 0.002
H.....	" " "		" = 0.008	N.....	" " "		" = 0.002
Dem.....	" " "		" = 0.002				

nary to the rheumatic attack, contained a high titer of antistreptolysin. This was approximately the same as appears in convalescence from hemolytic streptococcus infection.

For purpose of comparison, a study was made of the antistreptolysin content of the sera of rheumatic subjects whose disease process appeared to be quiescent. The findings are presented in Table XIII.

Table XIII shows that in the majority of sera studied, the antistreptolysin content was normal. In a minority, the titer was elevated, but not to the same degree as observed in most patients with acute rheumatism.

During the acute rheumatic attack, the antistreptolysin titer was found to be high, and during inactivity of the disease process it appeared normal in most instances. To trace the changes in the antistreptolysin titer of rheumatic subjects, sera were examined at inter-

TABLE XIII

Antistreptolysin Titer of Apparently Quiescent Rheumatic Subjects from Spring, 1928, to Fall, 1931

Patient				Patient			
			cc.				*cc.*
Mul....	Jan. 14, 1932	Clinically inactive	N.D. = 0.02	Tre....	Jan. 7, 1931	Clinically inactive	N.D. = 0.03
Gill....	" 14, 1932	" "	" = 0.02	Wa.....	Dec. 18, 1930	" "	" = 0.02
J. Jo....	" 14, 1932	" "	" = 0.007	Mo.....	Feb. 4, 1932	" "	" = 0.02
J. Jo....	Oct. 8, 1930	" "	" = 0.01	Zi.....	Nov. 1, 1930	" "	" = 0.05
Co.....	June 10, 1931	" "	" = 0.01	Ro.....	Jan. 23, 1932	" "	" = 0.03
Cou....	Jan. 14, 1931	" "	" = 0.03	Mah....	" 2, 1932	" "	" = 0.02
Ho.....	Dec. 11, 1931	" "	" = 0.02	Bar....	" 7, 1931	" "	" = 0.03
Rom....	Jan. 7, 1932	" "	" = 0.02	La.....	Oct. 4, 1930	" "	" = 0.006
Fl.....	" 9, 1932	" "	" = 0.02	Mak....	" 4, 1930	" "	" = 0.009
Wy.....	Oct. 1, 1930	" "	" = 0.02	Bus....	" 4, 1930	" "	" = 0.02
Sc.....	June 10, 1931	" "	" = 0.02	Cer....	Jan. 7, 1931	" "	" = 0.02
Ba.....	Oct. 1, 1930	" "	" = 0.03	Tri.....	Dec. 17, 1930	" "	" = 0.04
D. Do..	Jan. 16, 1932	" "	" = 0.02	Len....	Jan. 28, 1932	" "	" = 0.03
Tr.....	Nov. 1, 1930	" "	" = 0.009	Bea....	" 28, 1932	" "	" = 0.03
R. Ha..	Jan. 7, 1931	" "	" = 0.03	Hu.....	" 29, 1932	" "	" = 0.009
Tol.....	Dec. 13, 1930	" "	" = 0.005	Fe.....	" 28, 1932	" "	" = 0.02

vals during health and disease. The findings are presented in Table XIV.

TABLE XIV

Antistreptolysin Titer of Rheumatic Subjects before, during and after Attacks of the Disease

Patient		Good health	Acute attack	Recovery	Recrudescence	Patient		Good health	Acute attack	Recovery	Recrudescence
		cc.	*cc.*	*cc.*	*cc.*			*cc.*	*cc.*	*cc.*	*cc.*
R.....	N.D. =		0.005	0.03	0.003	Gi.....	N.D. =		0.002	0.02	
G.....	" =	0.02	0.006		0.008	F.....	" =		0.02	0.02	
C.....	" =	0.03	0.006	0.02		Ker.....	" =		0.002	0.002	0.0008
S.....	" =		0.003	0.005	0.004	H.....	" =	0.02	0.005		
P.....	" =		0.004	0.02		W.....	" =	0.03	0.004		
O.....	" =		0.008	0.02	0.004						

Table XIV shows that in most patients the titer of antistreptolysin became high during the attack of acute rheumatism, returned to a normal level in quiescence and rose again during recrudescence. Patient S. had chronic suppurative otitis during the period of apparent

recovery; his titer changed only slightly. Patient Ker. had three severe rheumatic attacks within 8 months, and during the 3rd the titer was found to be extremely high. Patient F. with rheumatic heart disease developed pericarditis. Hemolytic streptococcus was not recovered from the throat; precipitins to the protein fraction of this organism were not detected in the serum. This was the only normal antistreptolysin titer obtained in this series of patients with frank carditis.

TABLE XV

Antistreptolysin Titer during the Three Phases of the Rheumatic Attack

Patient	Date	Clinical condition	Titer
			cc.
R.	Apr. 13	Hemolytic streptococcus pharyngitis	N.D. = 0.003
	" 18	Quiescent interval	" = 0.003
	May 5	Carditis and nephritis	" = 0.002
A.	Jan. 9	Hemolytic streptococcus pharyngitis	" = 0.04
	Feb. 6	End of quiescent interval	" = 0.02
	" 11	Fever, arthritis, carditis	" = 0.005
H.	Dec. 10	Rheumatism inactive	" = 0.02
	Mar. 12	Hemolytic streptococcus pharyngitis	
	" 17	Quiescent interval	" = 0.02
	" 31	Onset of attack	" = 0.005
	Apr. 5	Severe polyarthritis and carditis	" = 0.004
W.	Oct. 1	Rheumatism inactive	" = 0.02
	June 1	" "	" = 0.03
	Mar. 25	Hemolytic streptococcus pharyngitis	" = 0.04
	" 31	Quiescent interval	" = 0.03
	Apr. 5	" "	" = 0.03
	" 8	Fever, malaise, leucocytosis	" = 0.008
	" 13	Erythema marginatum	" = 0.004
	May 5	Polyarthritis	" = 0.004

Another study was made to determine the exact time relationship between the rise in antistreptolysin and the appearance of rheumatic manifestations. The findings are presented in Table XV.

Table XV illustrates first, the findings in rheumatic patients with a high titer and second, the findings in patients with a normal titer of antistreptolysin at the onset of hemolytic streptococcus infection. In the former, Patient R., there was little change in titer. The slight rise occurred on the day that carditis manifested itself (sudden development of prolonged conduction time P-R interval equal to 0.22

seconds). In the latter, Patients A. and H., the titer rose sharply on the day of onset of the rheumatic attack. In Patient W., under close observation, the quiescent interval ended on April 6. On April 8, vague symptoms and fever with leucocytosis were noted. The titer rose from an N.D. of 0.03 cc. on April 5 to an N.D. of 0.008 cc. on April 8. During the following days the titer of antistreptolysin reached a plateau level of an N.D. of 0.004 cc., and the patient developed skin and joint manifestations of active rheumatism.[6]

Finally, a study was made to compare the relationship of the development of antistreptolysin with the appearance of the precipitins in the rheumatic subject and in the non-rheumatic individual. For this purpose the sera of patients recovering from hemolytic streptococcus infection were tested at frequent intervals. The findings are presented in Table XVI.

From Table XVI it is seen that in the sera of two non-rheumatic subjects, Ho. and O'H., with hemolytic streptococcus infection, precipitins were not detected. Each of these individuals developed antistreptolysin. This titer remained at a high level for a period of weeks. The findings in these two individuals are representative. In the majority of non-rheumatic subjects recovering from hemolytic streptococcus infection, although precipitins were either weak or absent, antistreptolysin appeared in titer which remained at a constantly high level until after recovery. This was shown in the small group of patients convalescing from scarlet fever. Precipitin reactions were strong in only the one individual, who developed rheumatic fever; the antistreptolysin titer was high, however, in each instance.

In contrast, in most patients with acute rheumatism, both of these antibodies were detected. In nearly every instance the antistreptoly-

[6] Two exceptions have been noted. Patient Au., a rheumatic subject, contracted hemolytic streptococcus pharyngitis but failed to develop any clinical evidence of rheumatic fever. The titer of antistreptolysin remained N.D. = 0.02 cc. for 1 month. Patient Le. on the 7th day after hemolytic streptococcus pharyngitis developed acute rheumatism and infectious mononucleosis. The antistreptolysin titer remained constant, N.D. = 0.02 cc. throughout the illness. Dr. John Paul of New Haven studied samples of this patient's serum and detected the development of an heterophile antibody. Agglutinins to sheep red blood cells appeared in titer 1:128 during the 2nd week of the illness.

TABLE XVI

A Comparison of Precipitin Formation with the Development of Antistreptolysin

Patient	Time	Clinical condition	Antistreptolysin titer	*Precipitin reaction fraction	
				C17D	C17K
		A. Non-rheumatic subjects			
			cc.		
Ho.	1st wk.	Hemolytic streptococcus pharyngitis	N.D. = 0.01	−	−
	2nd "	" " pansinusitis	" = 0.0008	−	−
	3rd "	Subsiding sinusitis	" = 0.001	−	−
	4th "	Convalescence	" = 0.003	−	−
	5th "	Recovery	" = 0.006	−	−
O'H.	2nd "	Hemolytic streptococcus pneumonia	" = 0.004	−	−
	3rd "	Convalescence	" = 0.003	−	−
	4th "	"	" = 0.003	−	−
	6th "	Recovery	" = 0.003	−	−
		B. Rheumatic subjects			
			cc.		
Maz.	2nd wk.	Acute rheumatic fever	N.D. = 0.003	+	+
	3rd "	" " "	" = 0.003	+	+
	4th "	" " "	" = 0.003	+±	+±
	5th "	" " "	" = 0.003	++	++
	6th "	Subsiding " "	" = 0.003	+	+
	7th "	" " "	" = 0.004	±	±
	8th "	" " "	" = 0.004	±	±
	9th "	" " "	" = 0.004	±	−
	12th "	" " "	" = 0.004	−	−
Wil.	1st "	Acute " "	" = 0.007	−	±
	2nd "	" " "	" = 0.007	±	+
	3rd "	" " "	" = 0.006	±	+
	4th "	" " "	" = 0.007	+	+
	5th "	Subsiding " "	" = 0.008		
	†6th "	Hemolytic streptococcus pharyngitis	" = 0.02	Serum cloudy	
	8th "	Rheumatic recrudescence	" = 0.007		
Man.	1st "	Acute rheumatic fever	" = 0.002	±	+
	2nd "	" " "	" = 0.002	+	+
	3rd "	Death	" = 0.002	+±	+±

* Precipitin determinations represent overnight readings, uncentrifuged.

† The sudden fall in the titer of antistreptolysin has been observed in other patients at the onset of acute hemolytic streptococcus infection.

sin titer remained normal during the infection and the quiescent interval, but rose at the onset of the rheumatic attack. It reached a plateau level during the 1st week of rheumatism and did not increase after this. On the other hand, precipitins which were not detected

during the period of infection nor in the quiescent interval, appeared in weak concentration during the first few days of rheumatism. As the attack increased in severity, the precipitin reaction became more marked. With a certain degree of fluctuation, there was a parallelism between increasing intensity of the disease and the development of strong precipitins. Patient Maz. during the 3rd and 4th weeks appeared extremely ill, and precipitins were most marked in this period, but the antistreptolysin titer did not rise. In the case of Patient Man. with fulminating rheumatism, the antistreptolysin titer remained at a constant level and the precipitins increased in strength until the day of death. In those individuals who recovered, demonstrable precipitins disappeared rapidly, but the titer of antistreptolysin persisted at a high level for a long period of time.

Summary of Antistreptolysin Determinations

In the normal individual in good health, the titer of antistreptolysin is of such a degree that 0.01 cc. of serum neutralizes $2\frac{1}{2}$ minimal hemolytic doses of yeast extract streptolysin. The antistreptolysin titer of patients convalescing from diseases other than hemolytic streptococcus infection is approximately normal. The antistreptolysin titer of patients during the acute stage of illness with hemolytic streptococcus infection is also approximately normal. In patients convalescing from infection with hemolytic streptococcus, the N.D. is approximately 0.005 cc. in most instances. The antistreptolysin titer of rheumatic subjects with inactive disease is usually normal or slightly elevated. The antistreptolysin titer of inactive rheumatic subjects tested after non-specific stimulation with an heterologous antigen either remains normal or falls to normal. In each rheumatic subject during an attack, following hemolytic streptococcus pharyngitis, the N.D. is approximately 0.005 cc. Likewise in each patient with acute rheumatism from whose throat hemolytic streptococcus was not recovered at the onset of the attack, the N.D. is also approximately 0.005 cc. Furthermore, in patients with acute rheumatism denying previous respiratory infection, the N.D. is also approximately 0.005 cc. The antistreptolysin titer falls slowly to normal during recovery and rises in each instance of recrudescence to an N.D. of 0.005 cc. The antistreptolysin titer remains normal in the rheumatic sub-

ject during the first two phases, the period of infection and the quiescent interval, and rises precipitously just before the appearance of rheumatic manifestations. Unlike the development of precipitins, the antistreptolysin titer reaches its high level during the 1st week of the rheumatic attack and maintains this constantly, irrespective of the patient's condition. Finally, the presence of antistreptolysin in titer of such a degree that 0.005 cc. of the patient's serum neutralizes $2\frac{1}{2}$ minimal hemolytic doses of yeast extract streptolysin, is considered a specific indication of infection with hemolytic streptococcus. The constant finding of this high titer of antistreptolysin in the serum of patients with acute rheumatic fever is strong evidence that the rheumatic attack has been initiated by *Streptococcus hemolyticus*. There is a close relationship between the time of appearance of this antibody in the circulation and the manifestations of activity of the rheumatic process.

SUMMARY

In the first two papers findings were presented which point to a close relationship between the incidence of rheumatic fever and the distribution of *Streptococcus hemolyticus*. The fact was emphasized that in the rheumatic subject a recrudescence of the disease process is usually preceded by pharyngeal infection with hemolytic streptococci. These organisms conspicuous in the throat flora during the period of infection preliminary to an attack of acute rheumatism fell into six antigenic groups and produced toxins which in 70 per cent were neutralized by a monovalent streptococcus antiserum. In the present study, four series of observations have been presented, demonstrating the development of immune bodies to hemolytic streptococcus during the course of rheumatic fever. The agglutination and complement fixation reactions of sera from patients with acute rheumatism suggest recent infection with streptococcus. Precipitin tests indicate that at the time of appearance of the rheumatic attack, individuals develop, in their blood, precipitins to the protein fractions of hemolytic streptococcus. That these precipitins may not be entirely specific is recognized from their cross-reactions with antigens of chemically related organisms. The studies made in association with E. W. Todd of England have demonstrated that at the onset of an attack of acute

rheumatism, there occurs in each instance a rise in the antistreptolysin titer of the patient's serum. This titer is much higher than that observed in normal subjects or in patients with bacterial infection other than hemolytic streptococcus. This presence of antistreptolysin with an N.D. of 0.005 cc. is considered strong evidence of recent infection by hemolytic streptococcus. In conclusion, the relationship between the incidence of hemolytic streptococcus and the geographical distribution of rheumatic fever, the relationship between the recrudescence in the rheumatic subject and infection of the throat with hemolytic streptococcus, the development of immune bodies for hemolytic streptococcus at the onset of the rheumatic attack and the apparently specific relationship of antistreptolysin formation to infection with hemolytic streptococcus,—together this combined evidence indicates that the infectious agent initiating the rheumatic process is *Streptococcus hemolyticus*.

These studies have been conducted under the direction of Dr. Alphonse R. Dochez, with the advice of Dr. Michael Heidelberger, with the nursing care of Miss Lucille Miller and Miss Mary Kelly and, finally, with the generous assistance of Dr. E. W. Todd. For their kind help the authors wish to express their appreciation.

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