sults and by the same procedure no such pigment could be obtained from sarcinae or from *B. coli Communior.*

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Agglutination and Precipitation Between Hemolytic Streptococci of Various Groups and Sera of Rheumatoid Arthritis Patients.

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It has been established[1, 2, 3] that hemolytic streptococci are agglutinated by sera of patients with rheumatoid arthritis. It has also been shown[4, 5] that the C substance of hemolytic streptococci[6] gives precipitation with sera of patients with this and other diseases. Since only strains of hemolytic streptococci isolated from human sources had been used in the investigations quoted, the present study was undertaken to observe the results obtained when antigens for agglutination and precipitation were prepared from hemolytic streptococci of Lancefield's groups A, B, C, D, E, F, and G.[7] Strains of groups B through G were kindly provided by Dr. R. C. Lancefield.

Agglutination tests were done with strains of groups A through E only. Eighteen-hour living broth cultures in 0.5 cc. quantities were mixed with 0.5 cc. of the various dilutions of serum obtained from patients with rheumatoid arthritis. Tests were read after 2 hours at 56°C. and 20 hours in the ice box. The results (Table I) indicate that, while the strongest agglutination usually occurred with strains of Group A, definite reactions were obtained also with those of other groups.

It seemed probable that the cross reactions were due to non-group-specific fractions common to all the strains, and to test this the work

1 Nicholls, E. E., and Stainsby, W. J., *J. Clin. Invest.*, 1931, **10**, 323.

2 Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

3 Keefer, C. S., Myers, W. K., and Appel, T. W., *J. Clin. Invest.*, 1933, **12**, 267.

4 Seegal, D., Heidelberger, M., Jost, E. L., and Lyttle, J. D., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 582.

5 Dawson, M. H., Olmstead, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 355.

6 Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 481.

7 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

TABLE I.

Agglutination of Hemolytic Streptococci of Groups A, B, C, D, and E (Lancefield) by Sera of Patients with Rheumatoid Arthritis.

				Sera of Patients with: Rheumatoid Arthritis				
Group.	Strain	Antigen Control	Rheumatic Fever* Wig.	Gold.	Lev.	Car.	Sil.	John.
Group A	NY5	—	—	640+	640+	640+	640+	640+
	McEv	—	—	640	160	80	320	
	T1	—	40	160	640	640	320	
	Foley	±	20	320	640	—	640+	
	A194	—	—	640+	320	640	640+	
	A216	—	40	320	160	320	320	
	A59	—	—	320	320	160	320	
	N107	—	—	80	80	160	320	
	N71	+	—	160	80	640+	640	
	N96	—	—	640+	640	640+	640+	
	A25A	—	—	160	320	640	640+	
	N110	—	—	20	80	80	640+	
	N56	+	+	320	160	640	640	
	N72	—	160	320	640	320	640+	
	A253	±	—	640+	640+	160	640+	640
	Sylv	++	Spontaneous Agglutination		,,			
	A26B	++	,,		,,			
	N15	++	,,		,,			
Group B	K107	—	—	—		20	80	
	K126	—	—	—		20	160	
	K198	—	—	—			80	80
	V8	±	—	—			40	20
	B116	±	20	80			80	80
	K127	—	20	—			40	—
Group C	P230	—	—	—	80	160	640+	
	K155N	±	—	40	40	40	640	
	K155C	+	—	—	20	20	640	
	K155L	—	—	—		80	640	
	K155K	±	—	20		—	320	
	K155M	—	—	—			80	40
	K132	—	—	—			40	20
	K150A	±	—	—			320	80
Group D	C2	±	—	—			—	—
	C5	—	20	20			40	40
	C6	—	20	—			40	20
	C3	—	—	—			—	—
	C1	+	—	—			—	—
Group E	K131	—	—	—			—	—
	K129	±	40	20			—	—
	K128	+	—				40	—

* Serum of patient with rheumatic fever included as a control.

Figures indicate dilutions through which each strain was agglutinated by the respective sera.

640+ signifies that agglutination at this titer was sufficiently strong to indicate that it would have been positive in higher dilutions had they been tried.

— = negative. Blank = not done.

was repeated using the precipitin reaction against the group-specific C substance. Crude C extracts of strains of groups A through G

were prepared by Lancefield's method.[7] These were tested against sera of 11 rabbits immunized with representative strains of each group. In each instance antigen dilutions were prepared by adding 0.4, 0.1, 0.025, and 0.01 cc. of extract to sufficient physiologic saline to make a final amount of 0.4 cc. Following the addition of 0.2 cc. of serum, the tubes were kept 2 hours at 37°C. and 20 hours in the ice box, and were then read before and after centrifugation. As found by Lancefield,[7] the various groups were specific and showed no significant cross reactions except in the case of serum from one rabbit immunized with a group E strain, which gave distinct crossing with extracts of every group. This apparently was a peculiarity of the animal, for other rabbits immunized with the same strain reacted in the usual manner. When sera of patients with rheumatoid arthritis, rheumatic fever, and convalescent scarlet fever were substituted for those of immune rabbits, however, definite crossing was encountered, occasionally with extracts of all groups and frequently with those of groups A, B, and G. These precipitates were distinct only after centrifugation.

There are at least 3 possible explanations of the unexpected cross reactions: (1) that they are not of true antigen-antibody nature, (2) that they represent responses of the anamnestic type in patients who previously harbored hemolytic streptococci of different groups, or (3) that they are due to the presence of multiple antigens in the crude extracts and to varying capacity of different individuals to form multiple antibodies following infection. The latter seems most probable, especially in view of the wide crossing encountered with one of the immunized rabbits. Work is in progress to test the validity of these possibilities.

Until the mechanism underlying these cross reactions is understood it is believed that they must be taken into account in attributing etiologic significance in rheumatoid arthritis to agglutination of hemolytic streptococci or to precipitation with crude C extracts.