

## PERSISTENCE OF STREPTOCOCCAL GROUP A ANTIBODY IN PATIENTS WITH RHEUMATIC VALVULAR DISEASE\*

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Certain aspects of the relationship of Group A streptococcal infection to rheumatic fever and rheumatic heart disease suggest that the pathogenesis of these nonpurulent complications are of an immunological nature. The proposition that the rheumatic individual is a hyperimmune responder has given way recently to the concept of an "autoimmune" process initiated by Group A streptococcal infection. This concept is supported by finding antigens common to both the Group A streptococcus and heart tissues as well as cross-reacting antibodies in the sera of patients with rheumatic fever and rheumatic heart disease (1, 2). Recently, Goldstein et al. demonstrated a specific immunological relationship between the Group A polysaccharide and structural glycoproteins in human and bovine heart valves (3). Several authors had previously shown the occurrence of circulating antibodies to the streptococcal Group A polysaccharide in the sera of patients following streptococcal infections and their sequelae (4-8). Furthermore, Karakawa et al. demonstrated the presence in human sera of antibodies to the A polysaccharide as well as to a degradation product of this antigen, the A-variant polysaccharide (8).

The purpose of this study was to determine the antibody levels to both the A and A-variant antigens as well as the frequency of the occurrence of these antibodies in normal individuals and in patients with nonpurulent sequelae of Group A streptococcal infection. Antibody levels to these two antigens were assayed in patients with the nonpurulent sequelae as a possible means of determining whether an abnormality in the in vivo processing of the Group A polysaccharide existed in some of the patients. Because of the reported cross-reactivity of antibody to the A polysaccharide with heart valve glycoprotein, the streptococcal Group A polysaccharide antibody levels were determined in sera

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of patients with rheumatic valvulitis. The following report presents our findings on the distribution of antibodies to the A and A-variant antigens in patients with rheumatic and nonrheumatic sequelae of Group A streptococcal infection together with data showing prolonged persistence of the antibody reacting with the Group A polysaccharide in patients with rheumatic valvulitis.

#### *Materials and Methods*

*Sera.*—The majority of patients studied with acute rheumatic fever, Sydenham's chorea, and acute glomerulonephritis had been previously evaluated for the antibody response to the streptococcal extracellular antigens (9, 10). The diagnostic criteria for these diseases were previously defined (9, 10). Follow-up sera for antibody studies during the convalescent and inactive stages of these diseases were obtained on these patients as well as additional patients whose initial manifestation fulfilled the required diagnostic criteria. Control sera were obtained from individuals who had no clinical evidence of streptococcal infections or their sequelae or a history of an undiagnosed pharyngitis in the 2 months prior to bleeding. The controls were age matched with the patients in each category studied.

Sera were processed with sterile precautions and stored at  $-10^{\circ}\text{C}$  until used.

*Strains of Streptococci.*—The Group A streptococcal strain, B-140, and Group A-variant strain 6108, were kindly supplied by Dr. Rebecca Lancefield.

*Hyperimmune Rabbit Antisera.*—A and A-variant rabbit antisera were kindly supplied by Dr. Rebecca Lancefield.

*Chemical Analyses.*—Rhamnose determinations were performed using the method of Dische and Shettles (11). Glucosamines were determined by the method of Rondle and Morgan (12) after hydrolysis of the carbohydrate in 2 N HCl for 4 hr at  $100^{\circ}\text{C}$ .

*A and A-Variant Antibody Determinations.*—The antibody levels were determined by a modification of the radioimmune precipitation technique described by Halpern and Goldstein (6).

*Preparation of  $^{14}\text{C}$ -labeled Group A and A-variant carbohydrate antigens:* Group A streptococci were grown in Todd-Hewitt broth to which  $^{14}\text{C}$ -labeled acetate had been added to a final concentration of  $2.5\ \mu\text{C}$  of  $^{14}\text{C}$ -acetate/1 ml of broth. Group A-variant organisms were labeled with  $^{14}\text{C}$ -glucose using  $0.6\ \mu\text{C}$ /1 ml of broth. After overnight incubation, the cells were collected by centrifugation and washed. Cell walls were prepared by Mickle disintegration and differential centrifugation according to the method of Salton and Horne (13). Group-specific carbohydrates were extracted from lyophilized cell walls by the hot formamide method of Fuller as modified by Heymann et al. (14). The solution of the formamide-extracted labeled carbohydrate was dialyzed for 48 hr against two changes of distilled water. Because of the low yield expected from the small amount of cell walls extracted, the labeled carbohydrate was not lyophilized. The dialyzed solution was used as a stock from which appropriate dilutions were made for the tests. In order to determine the concentration of carbohydrate antigen in solution, 0.1 ml aliquots were assayed for rhamnose and glucosamine content. The A carbohydrate solution was found to contain  $600\ \mu\text{g}$  rhamnose/ml and  $280\ \mu\text{g}$  glucosamine/ml while the A-variant preparation contained  $210\ \mu\text{g}$  rhamnose/ml and  $10\ \mu\text{g}$  glucosamine/ml. The relative percentages of rhamnose and glucosamine in these preparations are similar to those obtained by Krause and McCarty (15) for their preparation of formamide-extracted A and A-variant carbohydrates.

Based on these results, it was considered that the A carbohydrate solution contained the equivalent of 1 mg of antigen/ml and the A-variant solution contained 0.233 mg of antigen/ml. The validity of the correlation of these antigen concentrations with the rhamnose-glucosamine ratios obtained on the solutions was verified by using the labeled A and A-variant carbohydrate

preparations in a quantitative precipitation technique with specific rabbit A and A-variant antisera. The curves obtained in these tests were similar to those obtained using corresponding amounts of unlabeled A and A-variant antigens with known rhamnose content.

*Radioimmune precipitation technique:* 0.2 ml of patient sera and the appropriate amount of  $^{14}\text{C}$ -labeled carbohydrate antigen (*vide infra*) in 0.8 ml of normal saline were mixed in thick-walled glass tubes, incubated for 60–90 min at 37°C and then allowed to stand for 24 hr at 4°C with occasional shaking. An equal amount (1 ml) of saturated ammonium sulfate solution was then added with shaking to each sample. After allowing the samples to stand for 1 hr in the cold, the tubes were centrifuged at 4°C at 6000 g for 15 min. The supernatant was removed and the precipitate was solubilized in 1 ml of NCS reagent,<sup>1</sup> 5 ml of scintillation fluid<sup>2</sup> added to the clear solution, the contents were mixed and transferred to a counting vial. The tube was rinsed with an additional 5 ml of scintillation fluid which was combined with the solution in the counting vial. Samples were counted at 4°C for 20 min in a Packard Tri-Carb liquid scintillation counter.

Preliminary experiments using longer periods of incubation and precipitation at 4°C or additional washings did not significantly alter the amount of radioactivity obtained for the samples.

To determine the total radioactivity (100%) of the antigen added to each serum, an equivalent amount of the labeled carbohydrate contained in 0.1 ml of distilled water was added to a vial containing precipitated protein from 0.2 ml of normal serum solubilized in NCS reagent followed by the addition of 10 ml of scintillation fluid.

The antibody level in the test serum was calculated by dividing the counts per minute in the test serum by the cpm obtained for the total activity (100%) of labeled antigen added.

*Determination of antigen-antibody ratio:* According to Farr (16), optimal antibody determinations are obtained in this system in slight antigen excess or when approximately 80% of the antigen is precipitated. Because Halpern and Goldstein (6) had shown that the highest antibody levels were present in patients with acute rheumatic fever, different concentrations of the  $^{14}\text{C}$ -labeled Group A carbohydrate antigen were tested with varying amounts of sera from several patients with acute rheumatic fever in order to establish optimal amounts of antigen and antibody to be used in the determinations. The results showed that in most patients in this group approximately 80% of the radioactivity was precipitated when 1  $\mu\text{g}$  of the labeled carbohydrate and 0.2 ml of serum were used. These amounts are approximately proportional to the amounts of antigen and sera used by Halpern and Goldstein (1.5–2.0  $\mu\text{g}$  of antigen/0.5 ml sera) (6).

Initial tests with the A-variant antigen revealed that 0.2 ml of serum from patients with acute rheumatic fever precipitated approximately 40–50% of the 1  $\mu\text{g}$  of labeled A-variant antigen. Although these values were in the antigen excess range, the same amount of total carbohydrate antigen (1  $\mu\text{g}$ ) was used for both the A and A-variant antibody determinations in order to measure the relative magnitude of the response to these two antigens.

*Antistreptolysin O (ASO) and Anti-Desoxyribonuclease (Anti-DNase B) Determinations.*—ASO and anti-DNase B titers were measured by either the macro method or micro methods previously described (9, 17, 18).

## RESULTS

*A and A-Variant Antibodies in Acute Rheumatic Fever and Acute Glomerulonephritis.*—A and A-variant antibody levels as well as ASO and anti-DNase B

<sup>1</sup> Quarternary ammonium compound, Nuclear-Chicago Corp. Des Plaines, Ill.

<sup>2</sup> 4 g PPO, 300 ml absolute ethanol, 700 ml toluene.

titers were determined on sera from 48 patients with acute rheumatic fever and 33 patients with acute poststreptococcal glomerulonephritis. Sera were obtained within 2 months after the onset of symptoms in each disease. 32 of the patients with rheumatic fever had experienced an initial attack while 16 patients had a recurrent attack. All patients with rheumatic fever had clinical evidence of carditis during the acute stages of the illness. Antibody determinations obtained

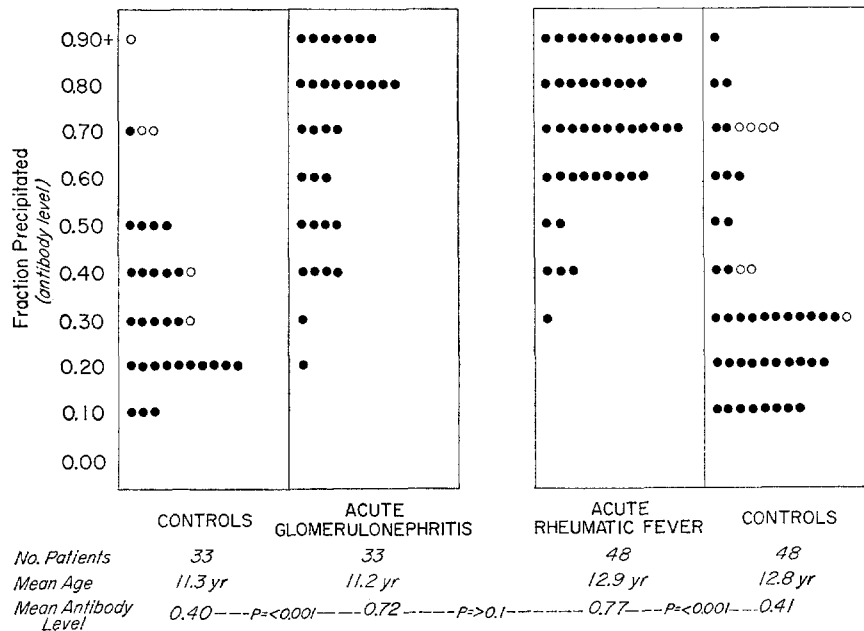


FIG. 1. Distribution of streptococcal Group A carbohydrate antibody levels (fraction precipitated) in acute glomerulonephritis, acute rheumatic fever, and in age-matched normal controls. Open circles, used for controls only, indicate individuals with elevated anti-streptolysin O (ASO) and/or anti-deoxyribonuclease (anti-DNase B) antibody titers. The geometric means of the antibody levels for each category are shown at the bottom of the figure, together with the *P* value for the statistical significance of the difference between the means.

for both groups of patients were compared with those obtained from a similar number of age-matched normal controls. Fig. 1 shows the distribution and means of the A antibody levels, expressed as fractions of radioactivity precipitated, in sera of patients with acute rheumatic fever and acute glomerulonephritis, and of age-matched normal controls. Although the A antibody levels of patients with acute rheumatic fever appear slightly higher than those of patients with acute nephritis, the means of these levels were not significantly different in these two groups of patients. However, the mean A antibody levels for both the rheumatic fever and nephritis groups differed significantly from those obtained

on control groups ( $P = < 0.001$ ). The occurrence of high A antibody levels in some normal controls in the absence of elevated ASO and/or anti-DNase B titers is probably due to a relatively slower decline of the A carbohydrate antibody than either the ASO or anti-DNase B antibodies.

Fig. 2 shows the distribution of A-variant antibody levels in sera from these same patients and controls. The A-variant antibody levels were consistently

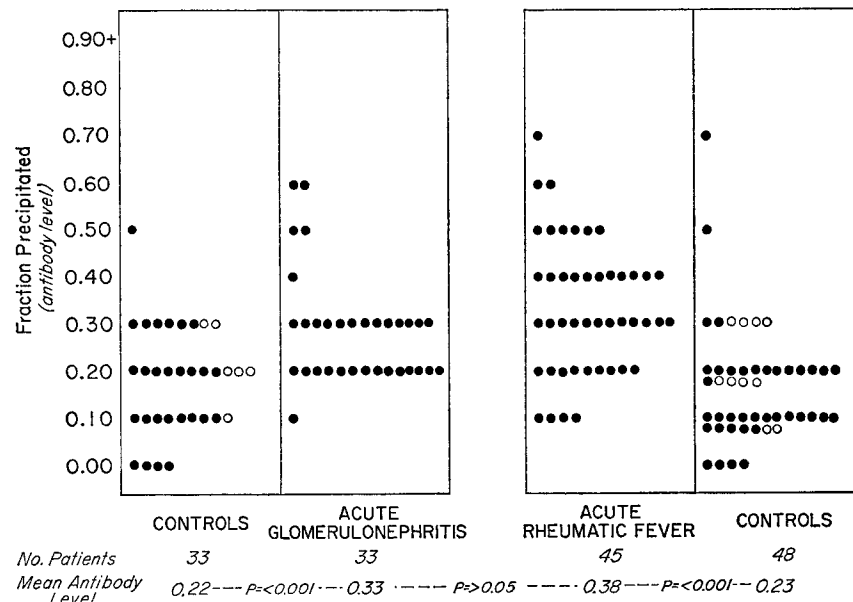


FIG. 2. Distribution and means of Group A-variant carbohydrate antibody levels (fraction precipitated) in acute glomerulonephritis, acute rheumatic fever, and controls. Open circles, used for controls only, indicate individuals with elevated anti-streptolysin O (ASO) and/or desoxyribonuclease (anti-DNase B) antibody titers. The geometric means of the antibody levels for each category are shown at the bottom of the figure, together with the  $P$  value for the statistical significance of the difference between the means.

lower than those for the A antibody but the mean ratio of A-variant to A levels was similar for patients with acute rheumatic fever (0.49) and acute nephritis (0.48). There was again no significant difference in the mean A-variant antibody level among patients with rheumatic fever and acute nephritis, whereas mean A-variant levels for both diseases were significantly greater than control levels. Table I summarizes the results of the mean A and A-variant levels as well as the geometric means of the ASO and anti-DNase B titers for patients and their controls.

In the study of Karakawa et al. (8), a positive correlation was found between the ASO titers and both the indirect anti-Group A and the direct anti-Group

A-variant agglutination titers. An evaluation was carried out to determine whether a similar correlation would be obtained for the ASO or anti-DNase B titers and the Group A antibody levels assayed by the radioimmune precipitin technique. Fig. 3 illustrates the correlation of the A antibody levels with ASO and anti-DNase B titers in patients with acute rheumatic fever and acute nephritis, and in control patients. The values for the degree of correlation of the A

TABLE I  
*Mean Antibody Determinations in Acute Rheumatic Fever and Acute Glomerulonephritis and Controls*

Antibody test	Acute rheumatic fever		Acute glomerulonephritis	
	Patients	Controls	Patients	Controls
A antibody	0.77	0.41	0.70	0.40
A-variant antibody	0.39	0.23	0.33	0.22
Ratio, A-variant to A antibody	0.49	0.56	0.48	0.55
Geometric mean, ASO	400	120	500	100
Geometric mean, anti-DNase B	400	80	500	65

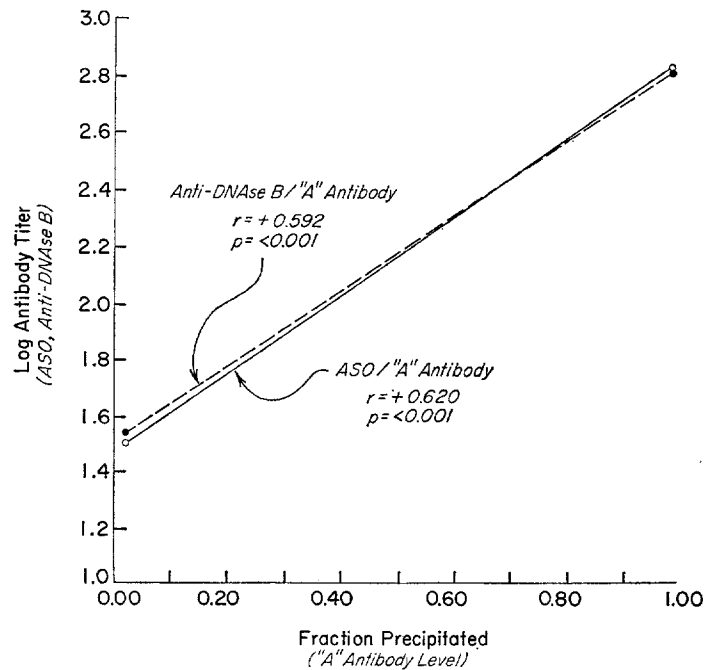


FIG. 3. Correlation of Group A carbohydrate antibody levels with the log of ASO and anti-DNase B antibody titers for 48 patients with acute glomerulonephritis, 33 patients with acute rheumatic fever, and 63 age-matched controls.

antibody with ASO and anti-DNase B antibodies were  $r \pm 0.620$  and  $r \pm 0.590$  respectively. In each instance, a  $P$  value of  $< 0.001$  was obtained indicating a correlation of high significance.

*A and A-Variant Antibody in Chronic Inactive Rheumatic Heart Disease.*—The possibility of a prolonged persistence of the A antibody suggested by the findings in some patients of elevated A antibody levels with normal ASO and anti-

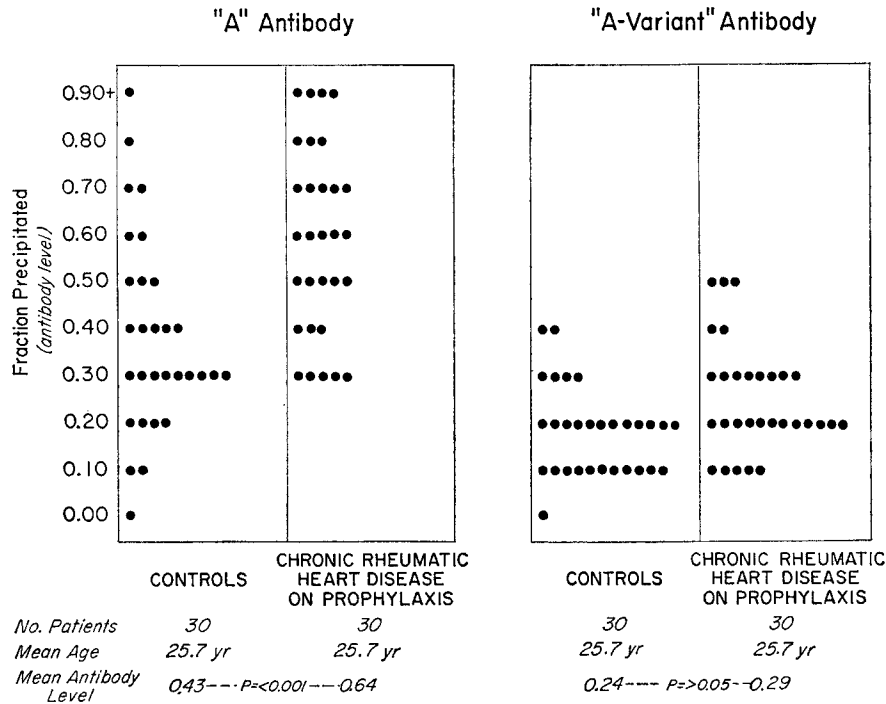


FIG. 4. Distribution and means of Group A and A-variant carbohydrate antibody levels (fraction precipitated) in patients with chronic inactive rheumatic heart disease on penicillin prophylaxis and in normal controls.

DNase B titers was investigated by determining A and A-variant antibody levels on sera from 45 patients with inactive rheumatic heart disease, obtained 1-25 yr after the last acute episode. Although the mean A-variant level was similar to the control value, the mean A antibody level was 0.58, a value intermediate between that obtained for patients with acute rheumatic fever (0.77) and their controls (0.40). In order to exclude the possibility that recent streptococcal infections contributed to this elevated A antibody level, 30 patients who had been on adequate secondary prophylaxis, and whose date of most recent active disease could be established, were selected for further analysis.

A and A-variant antibody determinations as well as ASO and anti-DNase B

titers performed on sera from these 30 patients with chronic inactive rheumatic heart disease were compared with those obtained from 30 age-matched controls. The mean A antibody level (0.64) for patients with chronic inactive rheumatic heart disease was significantly higher than the mean antibody level for the control group (0.43), whereas there was no significant difference in mean A-

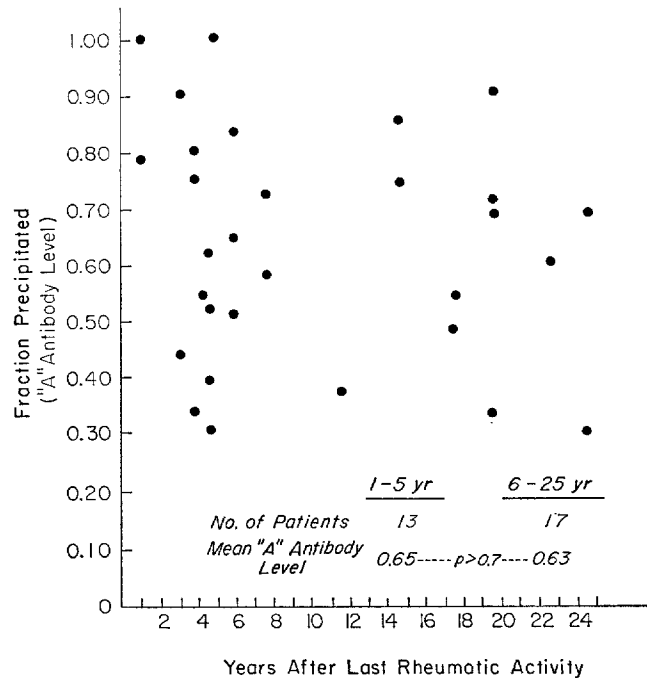


FIG. 5. Distribution of Group A carbohydrate antibody levels (fraction precipitated) in patients with chronic inactive rheumatic heart disease on penicillin prophylaxis shown in relation to the number of years elapsed after the patient's last acute rheumatic attack. The geometric means for titers obtained on patients seen within 5 yr after last active attack are compared with the mean of the titers obtained on patients tested 6-25 yr after their last active episode. The difference in the means shows no statistical significance ( $P$  value  $> 0.70$ ).

variant antibody levels (0.29, 0.23) (Fig. 4). The geometric mean ASO and anti-DNase B titers for the patients with chronic rheumatic heart disease were 100 and 83, respectively; values which were identical with those for the same antibodies obtained in the control group. The finding of similar A-variant levels, ASO and anti-DNase B titers in these patients with chronic rheumatic disease who had been on secondary prophylaxis, makes unlikely the possibility that the elevated A antibody levels were the result of intercurrent streptococcal infections.



An estimate of the duration of the persistence of the Group A antibody in these 30 patients was obtained by comparing each patient's antibody level with the number of years that had elapsed since the patient's last rheumatic activity (Fig. 5). No significant difference was observed when the mean A antibody level (0.65) obtained for 13 patients who had evidence of active disease within the past 5 yr was compared to the mean level (0.63) for 17 patients who had active disease 6–25 yr prior to the date the serum specimen examined was obtained.

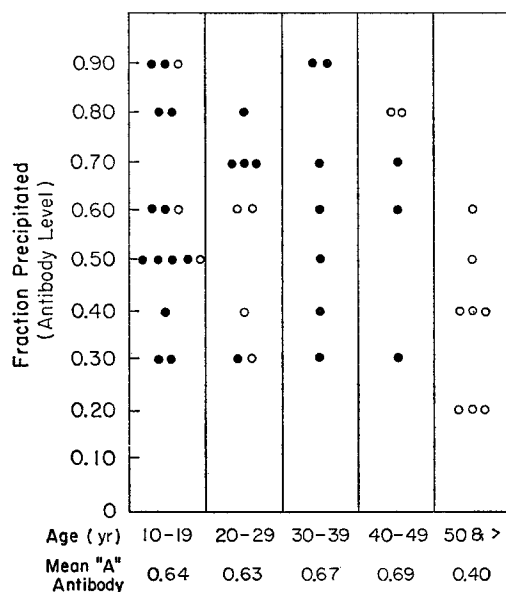


Fig. 6. Distribution of Group A carbohydrate antibody levels (fraction precipitated) in 45 patients with chronic inactive rheumatic heart disease shown in relation to the age of the patients. Open circles indicate those patients who had not been on penicillin prophylaxis within 1 yr prior to the time the blood specimen was obtained.

The relation of age to A antibody levels in patients with inactive rheumatic valvulitis was examined and the results are shown in Fig. 6. Group A antibody levels on all 45 patients with chronic inactive rheumatic heart disease were included in this analysis and were correlated with the age of each patient at the time the blood specimen was obtained. The results suggest that the mean A antibody levels in patients with chronic inactive rheumatic heart disease are elevated in the 10–40 yr age range but decline to normal levels after 50 yr of age.

*A and A-Variant Antibody Levels in Patients with Nonpurulent Sequelae of Streptococcal Infection, with or without Valvular Disease.*—The finding of elevated A antibody levels in patients with chronic inactive rheumatic heart disease which could not be explained by a recent streptococcal infection suggested

the possibility that persistence of these antibodies might be a specific immunologic phenomenon found only in patients with rheumatic carditis and residual valvular disease. This possibility was studied by comparing the pattern of decline in the A, A-variant, ASO, and anti-DNase B antibodies in three groups of patients with various sequelae of Group A streptococcal infections: (a) post-streptococcal glomerulonephritis, (b) Sydenham's chorea (without evidence of carditis), and (c) acute rheumatic fever with carditis and residual valvular disease.

The sera obtained on these patients were examined at three periods following the suspected precipitating streptococcal infection: up to 4 months, 5–12 months and 1–5 yr. All available paired sera were used and, in the absence of paired sera, single sera obtained from patients seen during the time interval being studied were used. A total of 21 sera were available for this evaluation from 12 patients with glomerulonephritis (9 single sera, 12 paired); 21 sera from 15 patients with rheumatic valvulitis (15 single sera, 6 paired sera); 18 sera from 16 patients with Sydenham's chorea who exhibited no rheumatic heart disease (14 single sera, 4 paired sera). Thus, seven sera for each patient category were available for study at each of the three time intervals except for the period of 1–5 yr in patients with Sydenham's chorea, where only four sera were available.

In patients with no history of an antecedent streptococcal infection, the interval from the infection to the date of the bleeding was based on the expected mean period of latency for the various sequelae, i.e., 10 days for acute glomerulonephritis, 20 days for acute rheumatic fever (19), and 3–4 months for Sydenham's chorea (20). It should be pointed out that because the initial period extended up to 4 months after the onset of the clinical manifestation, variations of about 2 wk in the latency period for rheumatic fever and glomerulonephritis were not considered a factor that would affect significantly these comparative evaluations. For similar reasons, and because of the longer latency period, all patients with Sydenham's chorea from whom sera were obtained within 1 month following the onset of clinical symptoms were arbitrarily included in the initial period.

Mean A antibody levels during these three intervals are shown in Fig. 7. During the initial period of 4 months, the mean A antibody levels were elevated to a similar level in patients with acute nephritis, chorea, and rheumatic carditis. During the following 8 months, the mean A antibody level in rheumatic patients with valvular disease showed a minimal decline (0.87–0.83) while patients without valvular disease (Sydenham's chorea) and acute nephritis exhibited a marked decrease in their mean A antibody levels (0.82–0.53 and 0.82–0.48). The mean A antibody decreased further during the following 4 yr period in these latter two groups of patients while the rheumatic patients with heart disease showed only a minimal decline (0.83–0.80).

A relationship between the persistence of the A antibody and the presence of

valvular heart disease in rheumatic patients was again suggested by the results of antibody determinations performed on four additional sera obtained at 1–5 yr following the onset of disease from patients with Sydenham's chorea who had evidence of valvular heart disease. The mean A antibody level in four such patients' sera was 0.72, a value which more closely approaches the mean of 0.80 for

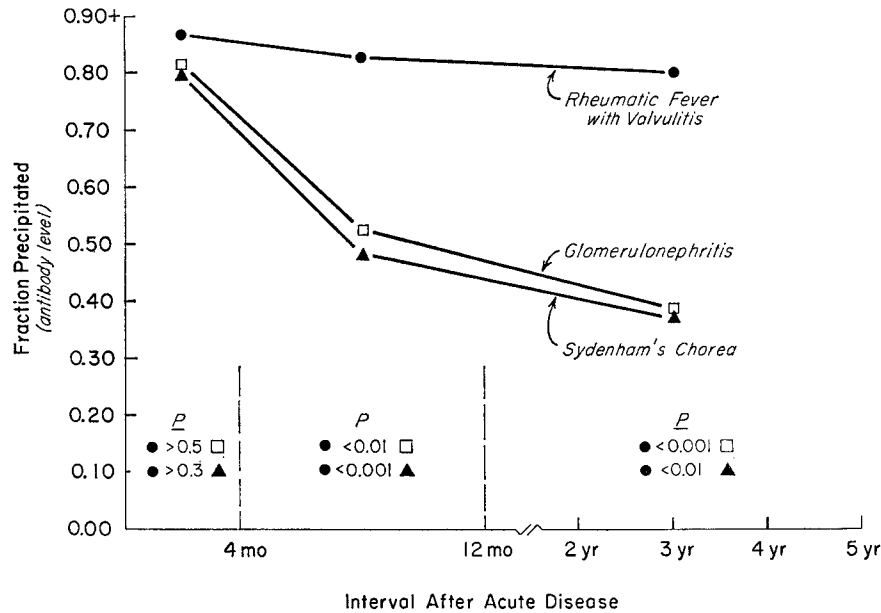


FIG. 7. Mean Group A carbohydrate antibody levels in patients with rheumatic fever with valvular heart disease, poststreptococcal glomerulonephritis, and Sydenham's chorea determined at various time intervals after the onset of the acute episode. All of the patients with rheumatic valvulitis had been placed on penicillin prophylaxis after the acute attack.

rheumatic patients with valvulitis in the follow-up period than the mean of 0.38 obtained in the four patients with "pure" chorea.

The results of all antibody determinations (A, A-variant, ASO, and anti-DNase B) on these sera from rheumatic patients with valvulitis and patients with acute glomerulonephritis are shown in Figs. 8 and 9. During convalescence and follow-up, the means of the ASO and anti-DNase B titers decline in both groups of patients, while only in those rheumatic patients with valvulitis does the mean A antibody remain elevated.

In patients with rheumatic valvulitis, the decline in the A-variant antibody appears to parallel the decline of the A antibody. However, the pattern of decline for the A-variant antibody in these patterns is similar to that seen in patients with glomerulonephritis and Sydenham's chorea. Statistical analysis of

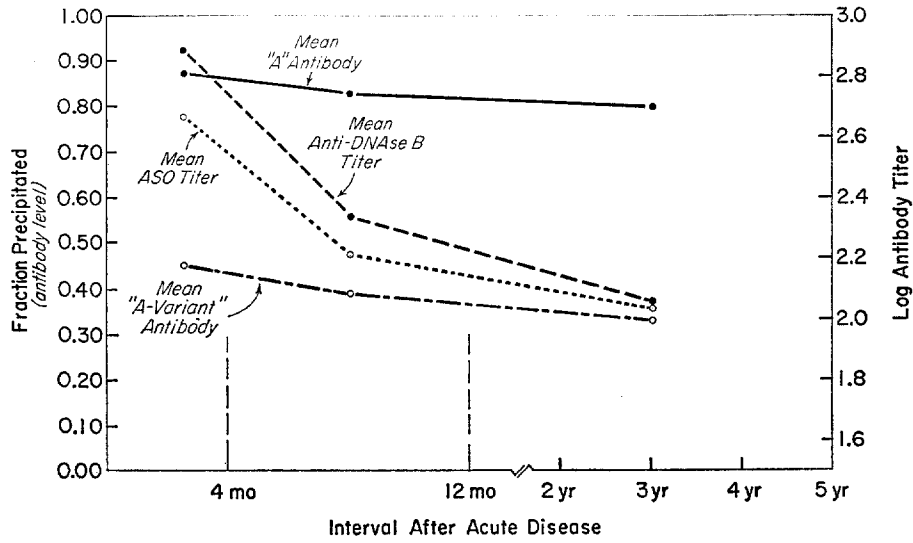


FIG. 8. Mean Group A and A-variant antibody levels and mean log ASO and anti-DNase B antibody titers in patients with rheumatic fever with valvulitis determined at various time intervals after the onset of the last acute episode.

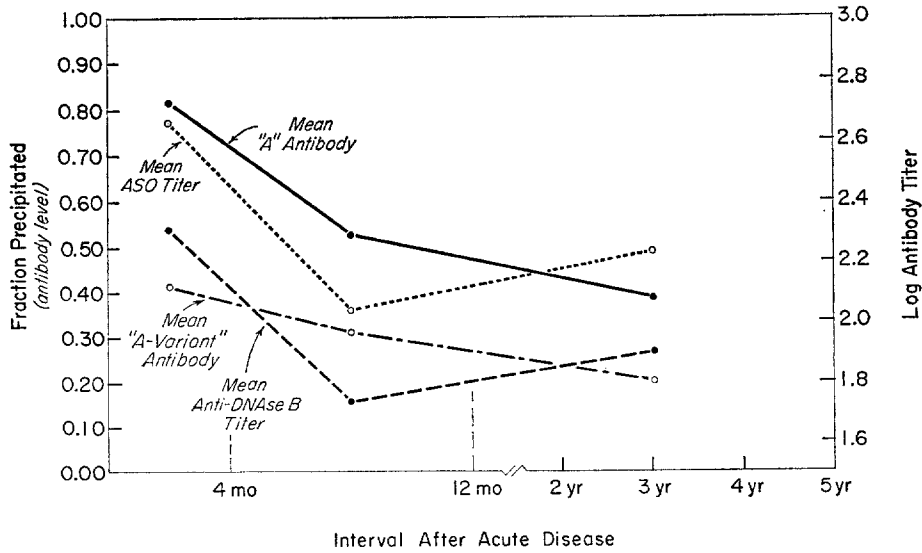


FIG. 9. Mean Group A and A-variant antibody levels and mean log ASO and anti-DNase B antibody titers in patients with poststreptococcal glomerulonephritis determined at various time intervals after the onset of the acute episode.

the mean A-variant antibody levels for the three time intervals studied in the three patient groups revealed differences that were either of borderline or no significant differences (Fig. 10).

#### DISCUSSION

The occurrence of antibodies to streptococcal Group A carbohydrate in man has been described by several authors (4-8). Halpern and Goldstein first utilized the radioimmune precipitin technique to quantitate the levels of this antibody in

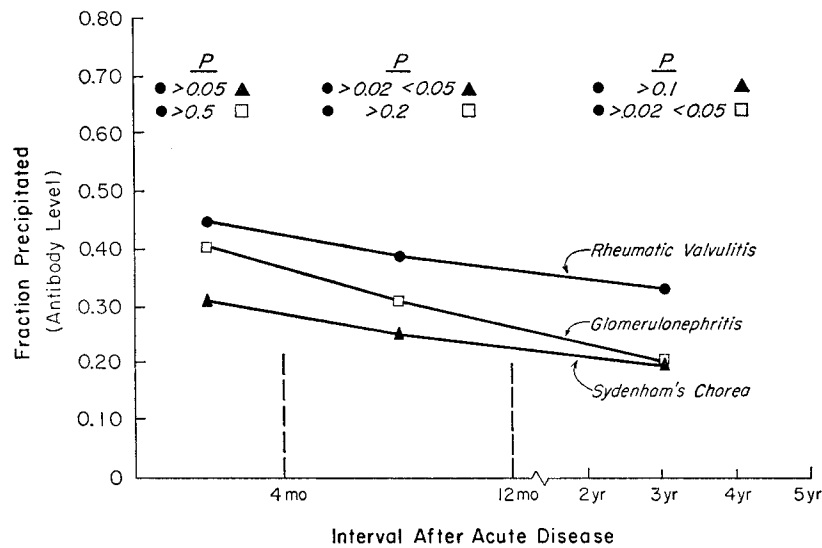


FIG. 10. Mean Group A-variant antibody levels in patients with rheumatic fever with valvular heart disease, poststreptococcal glomerulonephritis, and Sydenham's chorea determined at various time intervals after the onset of the last acute episode.

human sera (6). Their study showed the presence of higher levels of this antibody in patients with rheumatic fever than in a group of controls. Using a tanned red cell hemagglutination technique, Schmidt and Moore (7) demonstrated low but measurable A antibody titers in most normal children and adults, and high titers in patients with streptococcal infections and their complications. Antibodies to both A and A-variant carbohydrates were detected in human sera by Karakawa et al. (8) using direct and indirect agglutination techniques with cell walls. The A and A-variant antibody titers measured by this method showed correlation with the ASO titers suggesting that the occurrence of the carbohydrate antibodies was another feature of the host response to streptococcal infection. Karakawa et al. attributed the occurrence of the A-variant antibodies in human sera (8) and in experimental animals (21) to an in vivo degradation of

the group A antigen. This was supported by the evidence obtained by Ayoub and McCarty (22) for a  $\beta$ -*N*-acetylglucosaminidase in phagocytic cells that can degrade the group A carbohydrate to the A-variant carbohydrate, and the findings of Schwab and Ohanian (23) of an *in vivo* degradation of the A antigen in experimental animals.

The radioimmune precipitation technique, unlike other methods for antibody determinations, is a semiquantitative method for assaying antibody. For this reason, the word level was substituted for titer in our reporting of the results. Nevertheless, the correlation ( $r$  values) (Fig. 3) for the ASO antibody titers versus the A antibody levels using this method was similar to that reported by Karakawa et al. (8) for the A antibody titers assayed by the hemagglutination technique. Given the above limitation, the results obtained in this study indicate the usefulness of this technique in comparative evaluations as shown by the significant differences obtained for the antibody levels in the patient and control sera. As pointed out by Halpern and Goldstein (6), the optimal amount of antigen to be used in this system is that amount which will yield antibody levels of 0.80, which corresponds to a slight antigen excess. In this study, the mean A antibody level in patients with acute rheumatic fever was 0.77, indicating that optimum proportions of antigen to antibody were used. However, the mean A-variant antibody level for patients with acute rheumatic fever was 0.38 reflecting an excess of antigen in our assay for this antibody. The use of this amount of antigen was dictated by the limited amount of serum available on the patients and the low specific radioactivity of the labeled A-variant carbohydrate antigen.

Our studies on the A and A-variant antibody were carried out to determine whether an abnormality in the processing of the A to A-variant carbohydrate could be detected in patients with rheumatic fever. Such an abnormality could be manifested by finding different levels and ratios of the antibodies to carbohydrate antigens between patients with rheumatic fever and patients with non-rheumatic complications of streptococcal infection. It is felt that the A-variant antibody levels reported in this study do not represent the maximal A-variant levels that can be obtained in the sera of the various populations studied. However the levels obtained were only used to determine the ratio of the A-variant to A antibody levels which could reflect the different capacities of patients with various nonsuppurative complications of Group A streptococcal disease to degrade the A antigen *in vivo*. The similarity of the mean ratios of A-variant to A antibody levels in the patients with acute rheumatic fever and acute glomerulonephritis (Table I) would suggest that degradation of the streptococcal Group A carbohydrate antigen within the host occurs to the same extent in both diseases.

Prolonged persistence of the A antibody was initially suspected when it was noted that several patients and normal controls had elevated levels of this antibody with normal ASO and anti-DNase B titers. The persistence of the A antibody levels was confirmed by finding elevated A antibody levels in patients with

chronic inactive rheumatic heart disease when compared to an age-matched control group. That the elevation of the A antibody in these patients was a result of recent streptococcal infection was made improbable by demonstrating that ASO and anti-DNase B titers as well as A-variant antibody levels in these sera were in the normal range. In some patients' sera high levels of A antibody were found 20–25 yr after their last episode of rheumatic activity. Elevated A antibody levels were found in all ages of patients with chronic inactive rheumatic heart disease with the exception of those individuals past age 50. In these older individuals the mean A antibody level was similar to the mean obtained in control patients' sera.

Because antibodies to the type-specific streptococcal M antigen have been shown to persist for similar periods of time (24) in patients with and without the nonpurulent complications of streptococcal infection, the persistence of the A antibody in patients with poststreptococcal glomerulonephritis was examined. The results revealed a marked difference in the pattern of the decline of the A antibody levels between these patients and those with rheumatic fever, although the decline of antibody titers to the other streptococcal antigens were similar in both patient categories. This finding was interesting in view of the cross-reactivity between the Group A polysaccharide antigen and structural glycoprotein in human heart valves reported by Goldstein et al. (3) and raised the possibility that prolonged persistence of the A antibody may be specifically related to the occurrence of valvulitis in rheumatic disease. The specificity of this relationship was further supported by the data obtained on patients with Sydenham's chorea. Patients with *pure* Sydenham's chorea show a pattern of A antibody decline similar to that seen in poststreptococcal glomerulonephritis while patients with chorea associated with valvular heart disease manifest the pattern of persistence of this antibody seen in patients with rheumatic valvulitis.

As with previous reports of the presence of antibodies common to various components of the Group A streptococcal cell and heart tissues (1–3), the simple occurrence of an antibody that cross-reacts with the Group A polysaccharide and the glycoprotein of heart valves cannot be construed to be of pathogenetic significance. This antibody appears in sera of patients following streptococcal infection and is also present in the acute form of the various nonpurulent complications of this infection. In fact, the highest titers for this antibody obtained in the study reported by Schmidt and Moore (7) occurred in patients with streptococcal sepsis. The presence of elevated levels of this antibody at one point in time can only be interpreted as reflecting an antibody response to this antigen, similar to the response for the other streptococcal antigens. However, the singular phenomenon observed in this study regarding the *persistence* of the Group A carbohydrate antibody compared to antibodies directed to other streptococcal antigens and only in the presence of chronic valvular damage, is of significance in suggesting a pathogenetic relationship.

That genetic differences could be associated with specific manifestations and/or complications of rheumatic fever is suggested by two recent reports. In a study of haptoglobin phenotypes and levels of rheumatic and nonrheumatic individuals, Murray et al. (25) showed an increased frequency of Hp 0 type haptoglobin in rheumatics with heart disease, and an increased frequency of the Hp 1-1 type in patients with pure Sydenham's chorea. The Hp 0 and hypohaptoglobinemic subjects were confined to rheumatics with cardiac sequelae. Recent studies by Spagnuolo and Taranta (26) indicate that siblings with rheumatic fever tend to have the same manifestations and sequelae. Our present findings offer evidence for an immunological peculiarity that suggests a specific relationship with valvular disease. This immunological phenomenon is related to an antigen common to both an organism causally related to the disease, the Group A streptococcus, and to the tissue which is the site of injury in the disease, the heart valve. The mechanism that accounts for this phenomenon is unknown. The specificity of this finding has as yet to be further confirmed by studying patients with valvular heart disease of nonrheumatic etiology. Whether the persistence of this antibody is the result of the valvulitis or is actively involved in the pathogenesis of the disease should be clarified by further studies.

#### SUMMARY

Antibody levels to streptococcal Group A and A-variant carbohydrates were determined using a radioactive immune precipitation technique on patients with rheumatic fever, with and without valvular disease, on patients with post-streptococcal acute glomerulonephritis, and on age-matched controls. During the acute phase of the above illness, the means of the antibody levels to both carbohydrate antigens were equally elevated and were significantly higher than the normal controls. When Group A antibody levels were determined on sera obtained at intervals of 5-12 months and 1-5 yr after the acute illness, it was found that the antibody levels declined within the normal range at the 5-12 month interval in patients with glomerulonephritis as well as in patients with rheumatic fever in whom no valvular involvement had complicated the disease, i.e., patients with pure Sydenham's chorea. However, in patients with rheumatic valvulitis, who had been on penicillin prophylaxis after the last acute episode, the A antibody level showed little decline from the level obtained during the acute illness. The elevated antibody level in patients with rheumatic valvulitis, including patients with Sydenham's chorea with valvulitis, persisted for periods of at least 1 yr and up to 20 yr after the last acute attack. The pattern of the decline of the antibody levels to the A-variant carbohydrate as well as of the antibody titers to the other streptococcal antigens tested, ASO and anti-DNase B, was similar in all patients studied regardless of the presence of valvular disease. These findings suggest that prolonged persistence of the Group A antibody is a phenomenon peculiar to patients with rheumatic valvular disease. Whether



this persistence is involved in the pathogenesis or is an outcome of the valvular disease remains to be determined.

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