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HEART-REACTIVE ANTIBODY ASSOCIATED WITH RHEUMATIC FEVER: CHARACTERIZATION AND DIAGNOSTIC SIGNIFICANCE

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SUMMARY

The sera of patients with acute rheumatic fever, uncomplicated streptococcal infections and post-streptococcal glomerulonephritis contain a heart-reactive antibody which binds to the sarcolemmal membrane of myofibrils of heart muscle as well as to other mammalian muscle tissue. The sera of patients with acute rheumatic fever have about four times as much of this antibody as is found in sera of patients convalescent from uncomplicated streptococcal infections or acute post-streptococcal glomerulonephritis. This antibody is not present in a number of other diseases, including idiopathic myocardiopathies, lupus erythematosus, rheumatoid arthritis, multiple myeloma, and sarcoidosis. The titre of heart-reactive antibody declines slowly over a period of 2–3 years following acute rheumatic fever but re-appears with subsequent rheumatic recrudescences. Following cardiotomy a heart-reactive antibody also is demonstrable in the serum but is differentiated from that antibody which follows acute rheumatic fever. While streptococcal-induced antibody is absorbed with streptococcal membrane, heart-reactive antibody following cardiotomy is absorbed only with cardiac tissue. The presence in the sera of patients with acute rheumatic fever of high titres of heart-reactive antibody, absorbable by streptococcal membrane, provides an additional laboratory tool for the differential diagnosis of rheumatic fever and may be of value in the long-term management of this disease.

INTRODUCTION

Following Cavelti's (1945) observation that extracts of human heart tissue reacted with sera of patients with acute rheumatic fever and rheumatic heart disease, a number of investigators have confirmed the presence of antibodies in human sera which are reactive against constituents of human heart tissue. The literature concerning these heart-reactive

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antibodies has been recently reviewed by Kaplan (1964), and, in addition, he demonstrated that both IgG and IgM classes of γ-globulins were involved in this serological reaction.

Answers concerning the specific nature of these antibodies have been, however, difficult to obtain. Kaplan's observation that bound γ-globulin was present in a significant number of rheumatic auricular appendages suggested a direct relationship between these heart-reactive antibodies and the fixed globulin in rheumatic tissues. Yet attempts to correlate the amount of circulating heart-reactive antibodies in these patients with the bound globulin present in the tissues were unsuccessful (Kaplan & Dallenbach, 1961). In addition, heart-reactive antibodies were observed by several investigators (Hess *et al.*, 1964; Gery, Davies & Ehrenfeld, 1960; Van der Geld, 1964), to occur following procedures such as cardiotomy *per se* or acute myocardial infarctions.

The publications (Kaplan & Meyeserian, 1962; Kaplan, 1963; Zabriskie & Freimer, 1966) describing the presence of an antibody in group A streptococcal antisera capable of binding to cardiac tissue have reopened the question of the origin of these heart-reactive antibodies in the sera of patients with recent streptococcal infections and their sequelae. In the work reported below, serial dilutions of these sera have revealed that patients with acute rheumatic fever contain approximately four times the amount of heart-reactive antibody as that observed in the sera of patients with uncomplicated streptococcal infections, glomerulonephritis, and arthritic disorders. The significance of this antibody in relation to the onset of rheumatic fever, as well as its use as a diagnostic tool in the differential diagnosis of rheumatic fever, will be discussed.

MATERIALS

Serum specimens

The sera used in these studies were obtained from patients who were admitted to one of the following hospitals: Columbia-Presbyterian Medical Center, Great Lakes Naval Station, New York Hospital-Cornell Medical Center, and The Rockefeller University. In each case both clinical and laboratory diagnosis of the disease process were established.

Laboratory tests

The C-reactive protein (CRP) determination was carried out by the capillary precipitin method of Anderson & McCarty (1950). The determination of anti-streptolysin 'O' titres was based on a method of Todd as modified by Hodge & Swift (1933). The erythrocyte sedimentation rate (ESR) was determined by the Westergren method using oxalated blood (Wintrobe, 1961).

Tissue specimens

The tissues used for the immunofluorescent staining procedures were obtained from the following sources: (1) Auricular biopsies either from patients with rheumatic heart disease undergoing valvular repair or from patients undergoing repair of congenital heart defects; (2) Autopsy tissues obtained within 4 hr of death from patients dying of diseases unrelated to cardiac disorders.

The pieces of auricular biopsy and of autopsied tissues were quick-frozen in an alcohol and dry ice bath and stored at $-70°C$ in a CO_2 cold box. Companion pieces were fixed and stained with haemotoxylin and eosin for histological examination.

Human tissue homogenates

Pieces of human heart, kidney and skeletal muscle weighing 10–20 g were obtained within 6 hr of death from individuals dying of diseases unrelated to cardiac damage. These tissues were immediately trimmed of excess fat and connective tissue, washed several times in a cold 0·05 M phosphate buffered saline and homogenized in a Waring blender. The suspensions were strained through a coarse gauze mesh to remove the remaining pieces of fat and connective tissue and washed again with cold buffered saline until the supernatant fluid was clear. The tissues were then dialysed overnight in the cold against frequent changes of distilled water, lyophilized and stored in a desiccator.

Preparation of immune sera

Rabbit antisera containing antibodies to human γ-globulins were prepared in the following manner. Five mg of commercial human Cohn Fraction II (Pentex, Inc., Kankakee, Ill.) in 5 ml of saline were emulsified in 5 ml of Freund's complete adjuvant and intracutaneously injected in four or five separate sites in rabbits. This was repeated every 10 days for a total of four injections and 10 days after the last injection, the animals were bled and the sera tested. High-titred antisera were selected by testing the antiserum against a 1 mg/ml solution of human γ-globulin using the capillary precipitin test. Serum reactions at dilutions greater than 1:32 were considered adequate and the animals were exsanguinated by cardiac puncture. The serum fraction was separated under sterile conditions and stored at 4°C.

Rabbit antisera to highly purified human γ-globulin (IgG) was kindly supplied by Dr Arthur Strauss (1964) and used as a control in a number of critical experiments.

Immunofluorescent staining techniques

The preparation of fluorescein-labelled antibodies was carried out as previously described (Zabriskie & Freimer, 1966). Staining of the cardiac and skeletal tissues was done as follows: frozen sections 4 mμ thick were cut in a cryostate at -20°C, placed on glass slides (previously washed with 95% alcohol and dried), and allowed to dry for 18 hr at room temperature in a desiccator under vacuum. Following 1-min fixation in acetone, dilutions of human serum were layered over the sections and allowed to incubate at room temperature in a moist chamber for 30 min. The sections were then washed with several rinses of phosphate buffered saline pH 7·5 for 5 min and then stained with the appropriate fluorescein-labelled anti-IgG serum for another 30 min at room temperature. Following a final wash of 5 min in phosphate buffered saline, the sections were dried and mounted in glycerol and buffered saline at pH 7·0. The microscopic examination of the tissues, the ultra-violet source and filters used were as previously described (Zabriskie & Freimer, 1966).

All sections were read by at least two observers and graded independently. In the absorption experiments the intensity of staining was recorded without prior knowledge of the sections. In addition, in crucial experiments, each section was photographed under identical exposure conditions, and contact prints of strips of these serial photomicrographs were used to evaluate intensity of immunofluorescent staining.

Absorption techniques

The strains of streptococci, culture media, preparation of cell walls and membranes and absorption techniques were all as previously described for rabbit antisera to streptococcal structures (Zabriskie & Freimer, 1966).

EXPERIMENTAL

Immunofluorescent staining reactions with human sera

When 1:5 dilutions of the sera of patients with acute rheumatic fever or uncomplicated streptococcal infections or acute nephritis were layered over sections of human cardiac tissue, and then counter-stained with fluorescein-labelled rabbit anti-human γ-globulin, the pattern of immunofluorescent staining illustrated by Fig. 1 was found. The staining was primarily limited to the sarcolemmal membrane of myofibres, while the connective tissue

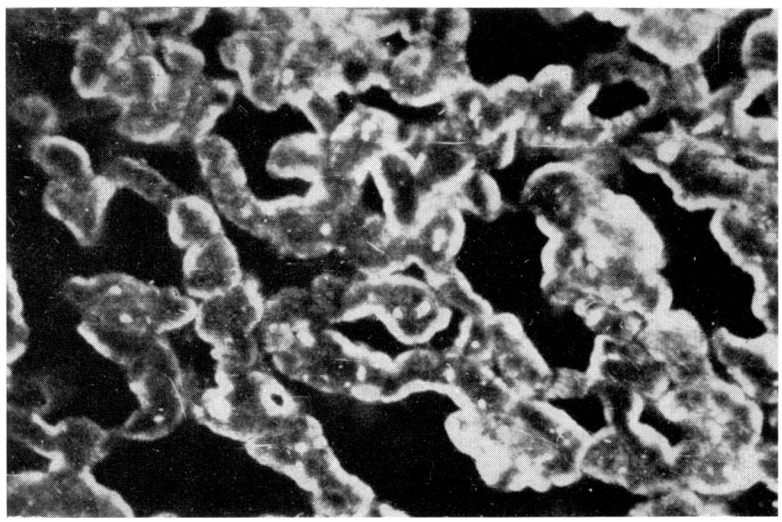

FIG. 1. Section of human myocardium which has been treated with the serum from a patient with acute rheumatic fever and then has been stained with fluorescein-labelled antibody to human γ-globulin. The serum of the patient has bound to the cardiac myofibres. The most intense staining is in the region of the sarcolemmal membranes. ×480.

elements remained unstained. Occasionally, sera also exhibited the diffuse sarcoplasmic and intermyofibrillar patterns described by Kaplan, Meyeserian & Kushner (1961).

Heart sections from both rheumatic and non-rheumatic patients were used in the staining procedures, and the pattern and intensity of the immunofluorescent reaction were similar in all sections. A number of different human skeletal muscle specimens also exhibited bright sarcolemmal staining patterns, indicating that the serological reactivity of these sera was not confined exclusively to cardiac muscle. In contrast, sections of human uterus, liver, kidney, spleen and synovium similarly tested were consistently negative.

Whereas rabbit antibody prepared against group A streptococcal membranes consistently bound to the smooth muscle layer of arteriorlar walls (Zabriskie & Freimer, 1966), only 10% of sera from patients with either streptococcal infections or rheumatic fever demonstrated a similar pattern of staining.

Distribution of heart-reactive antibody in human sera

The presence of an antibody in human sera which bound to mammalian cardiac tissue prompted an investigation into the relative frequency with which this antibody was present

in the sera of patients with streptococcal infections and their sequelae as compared to unrelated arthritic and immunological disorders. As undiluted sera obtained from 'healthy' individuals occasionally exhibited a mild degree of non-specific binding to cardiac tissue, all sera to be tested were first diluted 1:5 with phosphate buffered saline. The intensity of the staining reaction of each serum was then calculated. The results are summarized in Table 1.

TABLE 1. Incidence of heart-reactive antibody in sera from individuals with streptococcal infections, their sequelae, and other diseases

Clinical disorder	Total number of patients	Per cent with heart-reactive antibody	Intensity of staining*
		%	
Streptococcal infection	38	81	+
Post-streptococcal nephritis	12	80	+
Acute rheumatic fever	80	87	4+
Rheumatic heart disease	50	47	1+/2+
Post-cardiotomy	12	100	4+
Idiopathic myocardiopathies	7	0	0
Lupus erythematosus	20	0	0
Rheumatoid arthritis	15	0	0
Multiple myeloma	10	0	0
Sarcoidosis	10	0	0

* These results represent the average of the individual staining reactions for each clinical disorder. The staining reaction is graded from 0 (no staining) to 4+ (maximum brightness).

More than 80% of sera from individuals with either uncomplicated streptococcal infections, acute glomerulonephritis or acute rheumatic fever contain an antibody which reacts with human or rabbit heart tissue. Heart-reactive antibodies were also observed in the sera of patients with rheumatic heart disease, but the frequency with which the antibody was detected was less than that observed during the acute disease. Sera from patients who had undergone cardiac surgery for valvular disease or congenital defect repair also produced a similar pattern of staining. However, as will be shown later, this serological reactivity appeared to differ from that observed with acute rheumatic fever sera. In contrast, not one serum from fifty-five individuals with unrelated arthritis and hyper-γ-globulinaemia disorders exhibited serological binding to cardiac tissue. Of interest was the fact that patients with various myocardiopathies were also negative.

Heart-reactive antibodies in acute rheumatic fever patients

The intensity of the reactions observed indicated that patients with acute rheumatic fever had higher titres of heart-reactive antibody when compared to other streptococcal infections or other types of disease. The sera from a carefully selected patient population were therefore examined for the relative titres of heart-reactive antibodies. The sera, the streptococcal strains, and the clinical records of these patients have been preserved in The Rockefeller University Rheumatic Fever Service, and from this series it has been

possible to select two comparable groups of patients. Group I was composed of individuals who developed typical rheumatic fever. Group II contained individuals who had uncomplicated streptococcal infections, including scarlet fever. Serum samples were chosen from each patient's file: in Group I a serum at the onset of rheumatic fever and in Group II a serum from a comparable time during the convalescence phase of the streptococcal infection. In addition, a number of sera obtained from patients with active rheumatoid arthritis or systemic lupus erythematosus were included for comparison. Serial dilutions of each serum were prepared and the binding demonstrable at 1:5, 1:10, and 1:20 dilutions of these sera was recorded. The intensity of staining for all sera in each group was then calculated. The results are recorded in Table 2, where it can be seen that, at the onset of rheumatic fever, the

TABLE 2. Heart-reactive antibody titres in the sera of patients with acute rheumatic fever as compared to uncomplicated streptococcal infections and other arthritic disorders

Clinical disorder	Number of patients	Serum dilutions			Average ASO titre
		1:5	1:10	1:20	
Acute rheumatic fever (Gr. I)	34	4+	2+	+*	700
Uncomplicated streptococcal infections (Gr. II)	40	+	0	0	560
Rheumatoid arthritis	10	0	0	0†	ND
Lupus erythematosus	10	0	0	0	ND

* Serum samples obtained at onset of rheumatic fever and at a comparable time in the group with uncomplicated scarlet fever.

† Sera obtained during active disease. ND = Not determined.

titres of heart-reactive antibody were four times greater than that observed in patients with uncomplicated streptococcal disease. Furthermore, strongly positive reactions were still obtained at a 1:10 dilution of the serum in Group I whereas sera from patients with uncomplicated streptococcal infections did not exhibit heart-staining antibody at the same dilution. In contrast, sera of patients with active rheumatoid arthritis or lupus erythematosus were consistently negative even at 1:5 dilutions of serum. Although the average antistreptolysin-o (ASO) response is different for both groups in Table 2, many patients in the uncomplicated streptococcal infections group had higher ASO titres and more C-reactive protein than did Group I; yet they did not develop rheumatic fever or heart-reactive antibody.

Heart-reactive antibody titres in the sera of patients with acute rheumatic fever and rheumatic recurrences

The persistence of heart-reactive antibodies in rheumatic subjects was investigated next. Serial dilutions of representative samples obtained from thirty-nine rheumatic patients during the acute and follow-up stages of an attack of rheumatic fever were tested for the presence of heart-reactive antibodies. These patients had been followed by The Rockefeller University Rheumatic Fever Service for periods of time ranging from 6 months up to 10

years after the initial attack of rheumatic fever. The average intensity of staining for each group of serum samples was calculated and the results plotted against the time following the onset of the acute attack. In Fig. 2 it can be seen that the highest titres of heart-reactive antibody occur during the first 2 months, and that after the 6th month following the acute disease, the level of heart-reactive antibody gradually declines. At the end of 3 years, most sera were negative at a 1:5 dilution. Unless a streptococcal infection or a recurrence of rheumatic fever occurred during this period, the vast majority of these patients had little or no detectable heart-reactive antibody at the end of 4–5 years. Examination of a number of sera drawn 10 years after the initial attack of rheumatic fever also failed to show heart-reactive antibodies.

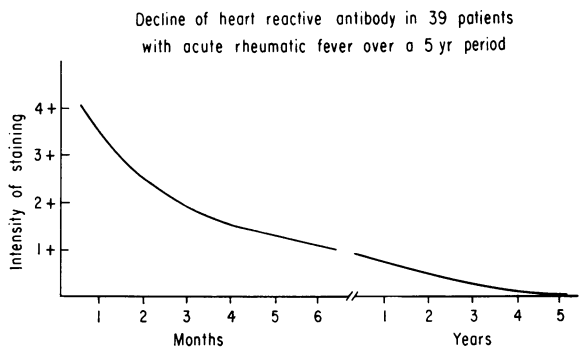

FIG. 2. Heart-reactive antibody titres plotted against time in the sera of thirty-nine patients with acute rheumatic fever. Serum dilutions from each patient were graded from 0 to 4+ intensity. The average intensity of staining found in each month or year for all patients was then plotted against time following the acute attack.

Since heart-reactive antibody titres appeared to be detectable during the period of greatest susceptibility to subsequent rheumatic fever attacks, namely, up to 3 years following the initial episode (Markowitz & Kuttner, 1965), it was of interest to study the relationship of this antibody to recurrent attacks of rheumatic fever. Accordingly, sera obtained from patients with well documented recurrences of rheumatic fever were tested for the presence of heart-reactive antibody during the subsequent attack. The observed titres were then correlated with the clinical course and laboratory data of each patient. Although the number of documented recurrences available for study has been small, the relationship of the levels of heart-reactive antibody to recurrent rheumatic attacks appeared to follow a distinct pattern. This pattern of heart-reactive antibody is best illustrated by comparing a case of a single rheumatic attack (Fig. 3) with two cases of rheumatic recurrences (Figs 4 and 5), differing primarily in the time of their recurrences.

Rheumatic fever without recurrence

Case I (Fig. 3), R.W., a 10-year-old white female was admitted on 17 May 1957 with migratory polyarthralgia, abnormal EKG records, and a grade II apical systolic murmur on physical examination. The throat culture was negative for group A streptococci. On admission her ESR was 120 mm/hr and the CRP was 3+. X-rays showed normal cardiac contour. Following treatment with combination of prednisone and salicylates, all clinical and laboratory data returned to normal and she made an uneventful recovery. Three years later an aortic diastolic

murmur was noted for the first time, but no clinical or laboratory evidence of a recurrence of rheumatic fever has been noted over the past 9 years.

This case illustrates the course of heart-reactive antibody levels in a rheumatic fever patient who had a single attack of rheumatic fever. The titres, originally high during the acute episode, gradually dropped during the 1st and 2nd years and by the 3rd year were no

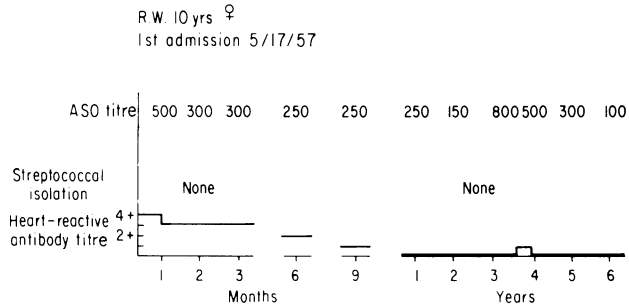

FIG. 3. Heart-reactive antibody titres in patient R.W. following a single episode of rheumatic fever. Heart-reactive antibody titres were negative by the 4th year following the initial episode and have remained negative for over 6 years.

longer demonstrable in the patient's serum. An intercurrent streptococcal infection as evidenced by a substantial rise in the antistreptolysin titre occurred in the 3rd year. This was accompanied by a small rise in the heart-reactive titre which was short-lived and subsided by the 5th year. While only recorded through the 6th year in Fig. 3, heart-reactive antibody titres have been negative up to 9 years after the initial attack in this patient. No evidence of a rheumatic fever recurrence has been noticed in this patient during that time.

Recurrence of rheumatic fever with permanent cardiac damage

Case II (Fig. 4), D.P., a 10-year-old female was admitted twice within 10 months with two attacks of polyarthritis and carditis. Elevated erythrocyte sedimentation rates and strongly

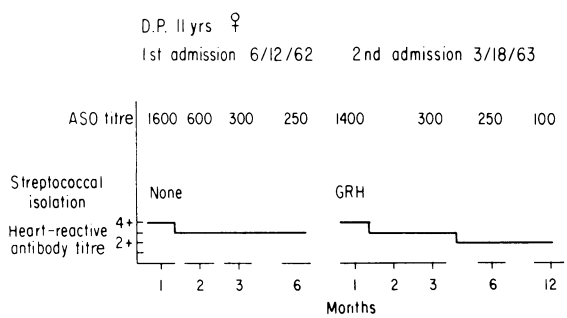

FIG. 4. Heart-reactive antibody titres in patient D.P. observed in two attacks of rheumatic fever within 1 year. Heart-reactive antibody titres decreased only slightly between attacks and returned to their initial value at the time of rheumatic recurrence. GRH indicates that only group H streptococcus was isolated.

positive C-reactive protein in the serum supported the diagnosis of an active inflammatory process. Physical examination on both occasions revealed basal systolic murmurs, and the electrocardiograph was abnormal on both admissions, with premature ventricular contractions and prolonged P. R. intervals noted during the acute stages. Treatment with prednisone and

salicylates was instituted and the recovery from each attack was complicated by mild rebounds following the cessation of medication. Three years after the second attack the basal systolic murmur persisted but there has been no recurrence of rheumatic fever up to the present time.

The pattern of binding of the serum of D.P. with the cardiac tissue was representative of the type of staining seen in the majority of our patients with rheumatic recurrences. The heart-reactive antibody titres did not significantly diminish between the first and second attacks and both attacks were of approximately the same severity clinically.

Recurrence of rheumatic fever with associated intercurrent streptococcal infection

Case III (Fig. 5), M.P., was first admitted on 25 May 1955 at the age of 3 years with active rheumatic carditis of 3 months duration. Physical examination revealed an enlarged heart with a grade III-IV apical systolic murmur. X-rays confirmed the diffuse cardiac enlargement. Throat culture revealed moderate numbers of group A, type 12 streptococci. The antistreptolysin 'O' titre was 2000 units. The response to meticortin therapy was excellent in this patient, although her recovery was complicated by the fact that attempts to withdraw the drug resulted

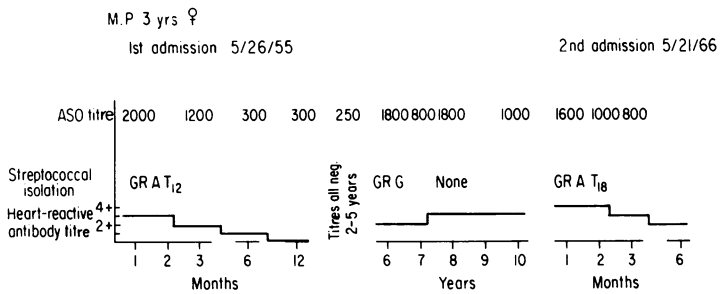

FIG. 5. Heart-reactive antibody titres in patient M.P. with a recurrence of rheumatic fever 11 years following the initial episode. Although heart-reactive antibody titres were negative for 4 years following the initial episode, intercurrent streptococcal infections in the 6th and 8th year were responsible for reappearance of heart-reactive antibody in this patient. A third streptococcal infection in the 11th year following the first attack, complete with elevated heart-reactive antibody titres, was associated with a rheumatic recurrence. Again, only group G streptococci were isolated during one of the intercurrent infections.

in rebounds on two occasions. An enlarged cardiac contour and a persisting grade III apical systolic murmur were present at the time of discharge, and the patient was sent home on oral penicillin prophylaxis 400,000 units daily.

In spite of irregular penicillin prophylaxis, there was no evidence of acute rheumatic activity or intercurrent streptococcal infections from the first through the 5th year following the initial attack. However, in 1961 and again in 1963, while failing to isolate group A streptococci, elevation of the antistreptolysin 'O' titres and a rise in sedimentation rate and C-reactive protein indicated the occurrence of intercurrent streptococcal infections. In spite of this, there was no clinical evidence of rheumatic activity during these episodes.

On 12 May 1966, the patient was readmitted with the history of a week of fever, sore throat, and migratory polyarthritis. Physical examination revealed redness and swelling of the left knee and a loud apical and basal systolic murmurs. The EKG showed changing P. R. intervals, and the throat culture revealed group A, type 18 streptococci. She was again treated with steroids which resulted in a prompt response as indicated by a change in the laboratory data and the disappearance of clinical symptoms. Mild clinical and laboratory rebounds were again noted following attempts to withdraw the medication. The remainder of her hospital stay was uneventful.

In this case an interval of 11 years had elapsed between the two attacks of rheumatic fever, and it was of interest to study the pattern of heart-reactive antibody levels during this time interval. Following the first attack, the decline of heart-reactive antibody titres

occurred in much the same manner as observed in Case 1 (Fig. 3), in which the patient experienced a single rheumatic attack without a recurrence. During a period of 6 years laboratory evidence and clinical criteria of either rheumatic activity or intercurrent streptococcal infections were absent in both patients. Heart-reactive antibody titres were also essentially negative during this same period. The introduction of two intercurrent streptococcal infections in 1961 and 1963 as suggested by the laboratory data in Case III appears to be a crucial point of departure in the two cases. Unlike Case I in which the heart-reactive antibody levels remain consistently negative, Fig. 5 shows a rise in heart-reactive antibody levels during intercurrent streptococcal infection in patient M.P. which was sustained over the next two years. The final streptococcal infection was associated with isolation of group A streptococci from the throat at the time of clinical signs of rheumatic activity and markedly elevated heart-reactive antibody titres.

Absorption of heart-reactive antibody from the sera of patients with acute rheumatic fever by streptococcal membranes

Although the concept that heart-reactive antibodies played an important role in the pathogenesis of rheumatic fever was attractive, the presence of these antibodies in other disease states (i.e., myocardial infarction, cardiotomy), appeared at first to be paradoxical.

TABLE 3. Absorption of heart-reactive antibody from rheumatic fever and post-cardiotomy sera

Sera	No.	Control staining	Antigens	
			Membranes 1–2 mg	Cardiac tissue 1–2 mg
Acute rheumatic fever	20	4+	0	0
Post-cardiotomy	12	4+	4+*	0

* Same results were obtained using up to 12 mg of streptococcal membranes.

Among the ideas entertained to explain these discrepancies was the hypothesis that the latter sera contained heart-reactive antibodies unrelated to the streptococcal-induced, cross-reactive antibody. To test this hypothesis, sera from patients with acute rheumatic fever, chronic rheumatic heart disease and post-cardiotomy states were absorbed. In order to avoid a non-specific staining pattern, these sera were usually diluted 1:5 or 1:10 prior to the absorption experiment; 0·2 ml of the appropriately diluted serum was then mixed with weighed amounts of both streptococcal membranes and human cardiac tissue (Zabriskie & Freimer, 1966). The results of these experiments are summarized in Table 3. As can be seen, the immunofluorescent staining patterns of nineteen patients with acute rheumatic fever were abolished with as little as 1–2 mg of streptococcal membranes. Similar results were obtained using equivalent amounts of human cardiac tissue. In contrast, the sera of patients undergoing cardiac surgery were abolished by absorption with cardiac tissue but not by streptococcal membranes even when as much as 12 mg of these membranes were used. These results suggest that the sera of patients with acute rheumatic fever contain heart-reactive antibodies which are streptoccocal membrane-induced. In contrast, sera of patients exposed to cardiac tissue damage during the trauma of surgery contain an antibody which binds to cardiac myofibers and is not related to the streptococcal-induced antibody.

DISCUSSION

In agreement with a number of other investigators (Hess *et al.*, 1964; Gery, Davies & Ehrenfeld, 1960; Kaplan & Svec, 1964), this report demonstrates that the sera of patients with uncomplicated streptococcal infections and acute rheumatic fever contain an antibody which binds to a constituent of human heart muscle and other mammalian muscle tissue. However, the frequency with which this antibody was reported was quite variable, ranging from 50% in Hess *et al.* (1964) series to 77% in Kaplan, Meyeserian & Kushner's (1961) series and 81% in our series. While these discrepancies may be real, differences in the methods employed by different investigators might explain some of these variables. For example, Hess used neonatal and foetal tissue exclusively as substrate while the cardiac tissues used in our sections varied considerably in age but were never foetal or neonatal. In our studies the indirect fluorescent antibody technique was used primarily, and only high-titred, fluorescein-labelled, anti-γ-globulin sera, checked against known positive controls, were used. Finally, the precise time at which a serum was drawn during the rheumatic episode was of utmost importance. For example, in some of our patients the titre of antibody fell off quite rapidly and was often negative within 4–6 months following the initial episode.

The presence of higher titres of heart-reactive antibody in patients with acute rheumatic fever was a significant finding and may be an added diagnostic tool in cases of suspected rheumatic fever. It has been possible in each case, without prior knowledge of the clinical history, to determine whether a patient has had an uncomplicated streptococcal infection or acute rheumatic fever. Bright fluorescent staining of cardiac tissue at dilutions of serum greater than 1:10 was not seen in the serum of patients with either uncomplicated streptococcal infections or post-streptococcal glomerulonephritis. Furthermore, the absence of any significant levels of heart-reactive antibody in patients with unrelated arthritic and immunological disorders and the characteristic nuclear staining by sera from patients with lupus erythematosus has been helpful in the differential diagnosis.

The fact that the decline of the heart-reactive antibody parallels the clinical course of recovery in acute rheumatic fever is of interest and suggests more than a casual relationship of this antibody to rheumatic fever. For example, the majority of our patients had only a single attack of rheumatic fever, and by the end of 3–5 years the heart-reactive antibody titres were negative and remained so for as long as 10 years after the initial attack without evidence of a rheumatic recurrence. Heart-reactive antibody titres in the majority of the rheumatic recurrences studied (a total of nine) followed the pattern exemplified by case D.P. (Fig. 4). The titres did not drop off significantly between the attacks and the rheumatic recurrence occurred within 1–2 years following the initial episode. Although the number of recurrences studied was admittedly small, none of the patients had a rheumatic recurrence without evidence of heart-reactive antibody in the serum prior to the second attack. M.P. (Fig. 5) who had no heart-reactive antibody in her serum for a period of years developed heart-reactive antibody following two + intercurrent infections 1–2 years prior to the second attack. The data show that heart-reactive antibody titres are seen during acute rheumatic fever and have to date always been present prior to the rheumatic recurrence, and that, in those cases where the interval between attacks has been long, intercurrent streptococcal infections were responsible for the reappearance of heart-reactive antibody in the serum.

The absorption of acute rheumatic fever sera by streptococcal membranes and cardiac tissue indicates that the heart-reactive antibody in the sera of these patients is streptococcal-

induced. Similar heart-reactive antibodies have also been noted in rabbits immunized with purified streptococcal membranes and were also absorbed by both streptococcal membranes and cardiac tissue (Zabriskie & Freimer, 1966). In contrast, the sera of patients who had undergone cardiac surgery while exhibiting bright fluorescent staining were absorbed only by cardiac tissue. These results would suggest that patients undergoing cardiac surgery develop antibodies against cardiac antigens, while heart-reactive antibodies in acute rheumatic fever are streptococcal-induced.

While these findings in general agree with the observations reported by Kaplan & Svec (1964), certain important differences are present. In Dr Kaplan's studies, heart-reactive antibodies were absorbed by a cross-reactive antigen obtained from group A streptococcal cell walls, while our rheumatic sera were absorbed by material from streptococcal membranes. Although it is possible that rheumatic sera contain heart-reactive antibodies to two distinct streptococcal components, as suggested by Kaplan (1967), it is conceivable that different methods of extraction are responsible for the observed differences.

The failure of Kaplan to absorb all acute rheumatic sera with his streptococcal cross-reactive antigen may be related either to a different configuration of his antigen or to the time when these sera were drawn. In our studies sera drawn from patients during the acute phases of the rheumatic episode (within 1–2 months) were consistently absorbed by streptococcal membranes. However, the immunofluorescent staining patterns observed in sera of patients with long-standing rheumatic heart disease (20 years or more after the attack) were not absorbed with streptococcal membranes. These results suggest that the sera of patients with chronic rheumatic heart disease may contain heart-reactive antibodies related to continuing cardiac damage, while the streptococcal-induced, heart-reactive antibodies may have disappeared from their sera. Thus, the time when the sample was drawn in a patient with acute rheumatic fever, as well as the prior cardiac state of the patient, may be quite important.

While it is still difficult to be certain of the exact role, if any, that heart-reactive antibodies play in the pathogenesis of rheumatic fever, the presence of these antibodies may be a helpful tool in the diagnosis of this disease. High titres of heart-reactive antibody are present in a large majority of patients with acute rheumatic fever; these antibodies decline following the initial episode and reappear with subsequent episodes and, finally, this antibody is not present in other arthritic disorders.

The fact that heart-reactive antibody appears to correlate closely with the time of greatest susceptibility to rheumatic recurrences may also be of value in the long-term management of rheumatic patients. For example, since Markowitz & Gordis (1968) have recently demonstrated the inadequacies of long-term oral penicillin prophylaxis, patients with recently acquired rheumatic disease might be placed on injectable penicillin until heart-reactive antibody titres become negative (i.e. 1–2 years). Oral penicillin might then be instituted and heart-reactive antibody titres checked every 6 months to 1 year. With the reappearance of heart-reactive antibody in a particular patient's serum, injectable penicillin might then be reinstituted until the titres were again negative.

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ABBREVIATION

CRP, C-reactive protein.

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