STUDIES OF DIPHTHERIA ANTITOXIN IN RHEUMATIC FEVER SUBJECTS: ANALYSIS OF REACTIONS TO THE SCHICK TEST AND OF ANTITOXIN RESPONSES FOLLOWING HYPERIMMUNIZATION WITH DIPHTHERIA TOXOID

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The importance of group A hemolytic streptococcal infections in the pathogenesis of rheumatic fever is widely recognized, but little is known of the mechanism by which the streptococcal infection gives rise to the disease. The concept that an antigen-antibody interaction may be involved in the origin of the pathologic processes has received increasing attention, and recent antibody studies (1, 2) provide suggestive evidence in support of this point of view. For example, studies in several laboratories dealing with a variety of streptococcal antigens have demonstrated that the mean antibody response of a group of rheumatic fever patients to these antigens is greater than that of a comparable group of patients with uncomplicated streptococcal disease (3–6). In view of these findings, it is of importance to determine whether the hyperreactivity of rheumatic subjects is limited to antigens derived from the streptococcus or is a reflection of a fundamental difference which would be demonstrable in an enhanced response to a variety of different antigens.

Because it has been impossible in natural infections with the hemolytic streptococcus to obtain accurate quantitative information concerning the antigenic stimulus, one of the possible explanations of the relative enhancement of the immune response in rheumatic subjects is that these individuals may have been exposed to comparatively greater quantities of antigen. At the present time it is difficult to approach this problem experimentally using streptococcal antigens, but other antigens are available, some in highly purified form, for use in a comparative study of the antibody response in rheumatic and control subjects. By this approach it should be possible to determine whether the exaggerated response to an antigenic stimulus is a general characteristic of rheumatic subjects. However, a negative result would not invalidate the hypothesis that rheumatic fever is related to hypersensitivity to the hemolytic streptococcus or its products. On the other hand, a discrepancy in the responses of rheumatic and control individuals would support the thesis that rheumatic subjects possess an innate difference in the antibody forming mechanism.

In the present study, diphtheria toxoid was selected as the immunizing antigen because it was considered to possess a number of advantages over other possible materials. It is available as a highly purified substance, and sensitive procedures have been devised for the measurement of antitoxin. Furthermore, quantitative studies have shown that highly purified diphtheria toxoid and human antitoxin behave as a single antigen-antibody system. In addition, the use of this system makes it possible to obtain qualitative as well as quantitative data concerning antibody formation, since techniques are available to study both precipitating and nonprecipitating antitoxin (7–9).

The investigations to be reported are based upon skin reactions observed in 245 Schick tested rheumatic and control subjects and upon the antitoxin responses occurring in 121 of these individuals following a single booster dose of purified diphtheria toxoid.

MATERIALS AND METHODS

Clinical material. The total study group consisted of 245 individuals; 132 members gave a history of rheumatic fever or rheumatic heart disease and this group was comprised of (a) 104 adult males ranging from 18

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to 26 years of age, and (b) 28 male and female children ranging from 9 to 15 years of age. The older subjects were United States Air Force personnel stationed at the Fort Francis E. Warren Air Force Base, Wyoming.[2] The younger subjects were patients at Irvington House, Irvington-on-Hudson, New York, a convalescent rheumatic fever home.[3] Fifteen of the former and fifteen of the latter group gave a definite or suggestive history of more than one previous rheumatic fever attack. Many had had repeated demonstrable throat infections associated with the presence of group A hemolytic streptococci. However, at the time this study began the rheumatic subjects with few exceptions showed no clinical or laboratory evidence of rheumatic activity. Most of the rheumatic children had some evidence of rheumatic valvular heart disease, as did a lesser proportion of the rheumatic adults. Treatment in one form or another had been given to all individuals during the period of activity, and included administration of corticotrophin, cortisone, aspirin and hydroxyphenyl-cinchoninic acid, or a combination of these. However, the time between treatment and the administration of diphtheria toxoid ranged from 4 to 18 months in the adult group and from 1 to 11 months in the younger age group. Each of the two groups of rheumatic patients was compared with a group of control individuals of the same average age with no history of rheumatic fever or chorea and no evidence of rheumatic valvular heart disease.

Skin testing. Highly purified diphtheria toxin diluted in buffered[4] heated human serum albumin to 0.2 guinea pig MLD (0.004 Lf) per cc. was used in the Shick test[5] which was performed on all subjects in this study. Highly purified toxoid containing 0.08 Lf per cc. was used as the control. The volume injected intradermally was 0.1 cc. Earlier work (8) has shown a correlation between immediate skin reactivity of the wheal and erythema type to Schick toxoid and the presence of non-precipitating skin sensitizing antitoxin; therefore, skin tests in rheumatic and control groups were evaluated, together with other tests to be described, as an indication of possible differences in antibody reactivity. Immediate reactions were read at the Schick control site 15 to 30 minutes after performing the Schick test. The diameter of the area of wheal and of erythema were both measured and the presence of pseudopodia, pruritis, and color intensity noted. On the basis of these criteria, reactions were rated as negative, ±, +, ++, +++, ++++ (8). In the adult groups additional skin sites were injected with 0.1 cc. buffered human albumin (Schick diluent) as a control and equally strong immediate reactions against both toxoid and albumin diluent were arbitrarily classified as negative. The Schick test was read at 48 hours.

Immunization. Immunization was carried out in 121 individuals who were Schick negative and who did not show a delayed reaction to Schick control toxoid at 48 hours. After the Schick test was read, a single injection of purified diphtheria toxoid was given subcutaneously. The dose was 37.5 Lf fluid toxoid or 200 Lf of purified toxoid absorbed on alumina cream[6] (11). Forty-four adult male Schick negative rheumatic subjects and an equal number of non-rheumatic male controls received 37.5 Lf fluid toxoid (1 cc.). Twenty-two Schick negative rheumatic children (17 male and 5 female) and 11 male controls received 200 Lf alum toxoid (1 cc.). Local reactions subsequent to immunization were mild or non-existent except in three instances where a febrile response was accompanied by considerable local swelling and discomfort.

Pre-immunization bleedings were taken at the time the Schick tests were given. Following the booster dose of toxoid given 48 hours later, post-immunization bleedings were drawn between the first and second, and second and third weeks, usually the ninth and nineteenth days, and in certain cases at intervals thereafter. Serum was removed using sterile precautions and stored in small glass tubes at 4° C. until used.

Antibody determinations. The *in vivo* antitoxin titer was determined using the rabbit intracutaneous test, following the technic of Fraser (12). Unknown specimens were compared with a simultaneous titration carried out with standard antitoxin obtained from the National Institutes of Health. Dilutions were spaced in such a manner that the titrations were accurate to within 15 to 20 per cent.

Tests for specific precipitation of antitoxin with toxin were carried out by a capillary tube precipitation method (13). A single concentration of serum containing an appropriate amount of antitoxin was mixed with varying amounts of toxin. On the basis of titers obtained using the rabbit skin test, sera of sufficiently high titer were diluted to contain about 30 units per cc. Other specimens containing between 10 and 30 units per cc. were used undiluted. The amount of toxin varied from 5 Lf to 40 Lf per cc. The purified toxin used contained 2480 Lf per cc. and 1 Lf was equivalent to 0.46 μg. specifically pre-

[2] It is a pleasure to acknowledge assistance provided by Dr. Charles Rammelkamp, Director of the Streptococcal Disease Laboratory, Fort Francis E. Warren Air Force Base, Wyoming, for his many courtesies and for permission to use the facilities of his laboratories during various phases of this study. The staff of the Streptococcal Disease Laboratory assisted in the immunization of the adult subjects.

[3] The authors wish to thank Dr. Gene Stollerman, Medical Director of Irvington House, Irvington-on-Hudson, New York, for providing facilities used at the time subjects of the younger age group were given booster toxoid. Staff members of Irvington House assisted in the immunization of these subjects.

[4] Schick toxin and control were diluted in Glenny's borate buffer containing 0.4 per cent of heated human serum albumin and 0.02 per cent merthiolate (10).

[5] The authors are grateful to Dr. J. A. McComb and Mr. L. Levine of the Massachusetts Antitoxin Laboratory, Jamaica Plains, for this material.

[6] The alum toxoid used in this study was kindly provided by Dr. John Osborne, New York University College of Medicine, New York, New York.

cipitable Nitrogen.[7] The approximate point of equivalence was taken as the tube in which maximal precipitation resulted following two hours of incubation at 37° C. and overnight incubation at 4° C. Quantitative precipitin titers were performed in the case of some sera by methods described elsewhere (7) and *in vivo/in vitro* ratios were determined in these instances. This ratio indicates the proportion of total antitoxin that is precipitable and thus provides an index of the amount of non-precipitating antibody.

Criteria used to determine the presence of human non-precipitating antitoxin have been described in detail in another publication (9): the criteria used in this study included immediate skin reactivity to Schick toxoid, the relative ability of antitoxin containing sera to form specific precipitates in the presence of purified toxin or toxoid, and the ability to fix guinea pig complement. Individuals showing immediate skin reactivity to Schick toxoid possess non-precipitating, skin sensitizing diphtheria antitoxin. Sera containing this type of antitoxin demonstrate poor or no fixation of guinea pig complement in contrast to precipitating antitoxin which is usually able to fix relatively large amounts of complement. The technique employed in comparing the complement fixing ability of various antitoxin-containing sera has been described elsewhere (9). Evaluation of rheumatic and control sera in regard to the presence of non-precipitating antitoxin was made on the basis of whether more or less than one unit of antitoxin in sera containing 10 units or more per cc. was capable of binding two units of guinea pig complement. Past experience has shown this to be a convenient level of demarcation in judging relative amounts of precipitating and non-precipitating antitoxin in a given serum.

Because it was thought of interest to determine the possible non-specific anamnestic rise of antibodies to a product of the streptococcus following administration of diphtheria toxoid, antistreptolysin O determinations were performed on pre- and post-immunization sera of the young-age-group rheumatic and control subjects who received alum toxoid. The method used was that described by Todd as modified by Hodge and Swift (14).

RESULTS

Reactions to the Schick test

Table Ia shows the results of the immediate wheal and erythema reactions which were produced following the injection of Schick test (toxin) and control (toxoid) materials in 195 adult rheumatic and control subjects. Of 104 rheumatic subjects, 45 or 43.3 per cent showed positive immediate reactions, and 59 or 56.7 per cent showed negative reactions to Schick toxoid. A slightly higher incidence of positives (52 or 57.1 per cent) occurred in 91 control subjects. However, most of these immediate reactions were not severe, and 75.5 per cent of the rheumatic subjects and 77 per cent of the controls were graded as ± or 1 +. Only 11 of 45 rheumatic subjects (24 per cent) and 12 of 52 controls (23 per cent) demonstrated reactions of 2 + or greater. Immediate wheal and erythema reactions were not analyzed in rheumatic subjects comprising the younger age category. Analysis of delayed skin reactions to Schick toxin and toxoid (Table Ib) in the older subjects showed that in the rheumatic group 39 were Schick positive (37

[7] We wish to thank Dr. A. M. Pappenheimer, Jr. who made available the highly purified diphtheria toxoid used for the precipitation tests.

TABLE I

Skin reactions to Schick toxin and toxoid in 104 adult rheumatic and 91 adult control subjects

	Rheumatic	Control
a. Immediate wheal and erythema reactions to Schick toxoid		
Positive	45 (43.3%)	52 (57.1%)
±	16	8
1+	18	32
2+	4	8
3+	5	3
4+	2	1
Negative	59 (56.7%)	39 (42.9%)
b. Schick test reactions		
Schick positive	39 (37.5%)	42 (46.2%)
Schick negative	53 (51.0%)	35 (38.5%)
Pseudo reaction	12 (11.5%)	14 (15.4%)

TABLE II

Comparison of immediate wheal and erythema reactions to Schick toxoid before and after hyperimmunization of Schick negative adult rheumatic and control subjects

		Rheumatic	Control
Before immunization	Positive	19 (45.2%)	18 (54.5%)
	±	7	4
	1+	7	9
	2+	1	3
	3+	2	1
	4+	2	1
	Negative	23 (54.8%)	15 (45.5%)
		42	33
After immunization	Positive	32 (78%)	29 (87.9%)
	±	1	1
	1+	8	13
	2+	9	7
	3+	7	5
	4+	7	3
	Negative	9 (22%)	4 (12.1%)
		41	33

per cent), 53 Schick negative (51 per cent) and 12 pseudoreactors (11 per cent). In the control group, 42 were Schick positive (46 per cent), 35 Schick negative (38 per cent) and 14 pseudoreactors (15 per cent).

The role of hyperimmunization with toxoid in altering immediate skin reactivity to Schick toxoid was demonstrated in 75 Schick negative adult subjects, 42 of them rheumatic and 33 control. Table II indicates that there was a large increase in the percentage of subjects showing immediate skin reactions in both the rheumatic and control groups, although there was no significant numerical difference between the groups. Similar data are not available for Schick tested subjects in the younger age category.

It was concluded from these results that rheumatic and control subjects reacted similarly to intradermal Schick toxin and toxoid.

Amounts of antitoxin produced following hyperimmunization with diphtheria toxoid

The titers determined by rabbit skin test on two different dates following immunization were compared in the rheumatic and control groups and showed that the mean antitoxin responses were the same, as shown in Table III. It was also demonstrated that the titers varied over a wide range in both groups at nine and nineteen days following immunization.

A comparison of the amounts of antitoxin formed showed that a majority of the post-immunization responses were at levels of 50 units per cc. or less, comprising 78 per cent and 66 per cent at nine and nineteen days in rheumatic subjects, and 68 per cent and 67 per cent at corresponding times in the controls. Only 11 per cent of persons in both groups formed more than 100 units per cc. at nine days, and 24 per cent of rheumatic and 19 per cent of control subjects developed titers higher than 100 units per cc. at nineteen days.

TABLE III

Comparison of antitoxin titers in 44 adult rheumatic and 44 adult control subjects hyperimmunized with diphtheria toxoid

	9 Days following immunization		19 Days following immunization	
	Rheumatic	Control	Rheumatic	Control
Lowest titer (*units/cc.*)	1	1	1	1
Highest titer (*units/cc.*)	400	140	300	250
Mean titer (*units/cc.*)	44.3	41.4	57.6	55.1

TABLE IV

Comparison of antitoxin titers in 22 rheumatic and 11 control children hyperimmunized with diphtheria toxoid

	9 Days following immunization		19 Days following immunization	
	Rheumatic	Control	Rheumatic	Control
Lowest titer (*units/cc.*)	1	10	4	3
Highest titer (*units/cc.*)	240	440	100	320
Mean titer (*units/cc.*)	45.9	115.0	38.2	75.4

The mean titer response in young rheumatic children (Table IV) was the same as that of the adult series at nine days, but was considerably less than the mean adult response at nineteen days. As in the adult group, a wide range in titer responses was demonstrated. Greater quantities of antitoxin were formed in the young control subjects. This is a factor of doubtful significance because the small group (11 subjects) included two persons who formed particularly large amounts of antitoxin. If these two members are eliminated from the series, mean titers are obtained which approximate those of the rheumatic patients.

There seems to be no apparent difference in the post-immunization antitoxin response between subjects giving a history of a single attack of rheumatic fever and patients with a history of more than one attack. Comparison was made between maximum titers observed in 49 rheumatic children or adults who gave a history of but one attack of rheumatic fever, and in 17 subjects who gave a history of more than one rheumatic fever attack. The range of titer in the former group was from 1 to 300 units per cc. with a mean titer of 46 units per cc.; in the latter group the titer also ranged from 1 to 300 units per cc. with a mean titer of 45 units per cc.

The foregoing evidence suggests that the antitoxin response is quantitatively similar in rheumatic and control subjects.

Nature of the antitoxin produced following hyperimmunization with diphtheria toxoid

In order to determine whether rheumatic subjects possessed a superior ability to form nonprecipitating antitoxin compared with the con-

TABLE V

Complement fixation by antitoxic sera from 26 adult rheumatic and 25 adult control subjects hyperimmunized with diphtheria toxoid*

	Amount of antitoxin bound by two units of guinea pig complement		
	Under 1 unit	Over 1 unit	Totals
Rheumatic	16 (61.5%)	10 (38.5%)	26 (100%)
Control	16 (64%)	9 (36%)	25 (100%)

* All sera used in this experiment contained 10 units/cc. or more.

trols, both groups initially were compared on the basis of immediate skin reactivity to Schick toxoid. The results in 195 subjects, previously analyzed under *Reactions to the Schick test* (see Tables I and II), showed no significant differences between the groups. It has been possible to obtain additional data concerning the nature of antitoxin in a lesser number of persons whose sera were tested for antitoxin by the qualitative and quantitative precipitin tests and by the guinea pig complement fixation test. The sera used for these experiments contained 10 or more units of antitoxin per cc.

There was a marked similarity in reactions demonstrated by sera in both groups (total of 49 subjects tested) as judged by the ability to fix a standard amount of guinea pig complement (see Table V). Analogous results were also observed when the sera of 50 subjects were tested for the ability to form precipitate when mixed with an approximately equivalent amount of toxin using the qualitative technique (Table VI). In order to demonstrate possible quantitative differences, quantitative precipitin titers were carried out using sera of sixteen rheumatic patients in the younger age category. All of these sera contained 15 units per cc. or more as judged by the rabbit skin test. The *in vitro* titer was taken as the number of micrograms of antitoxic nitrogen precipitable following the addition of equivalent amounts of toxin. The *in vivo/in vitro* ratio was the figure obtained upon division of the titer as judged by the rabbit neutralization test (*in vivo*) by the *in vitro* titer. Cohn and Pappenheimer (7) found that the ratio varied from 1.0 to 1.8 in a study of 12 apparently normal subjects. The ratios calculated for the sixteen rheumatic sera studied quantitatively showed a similar range of distribution

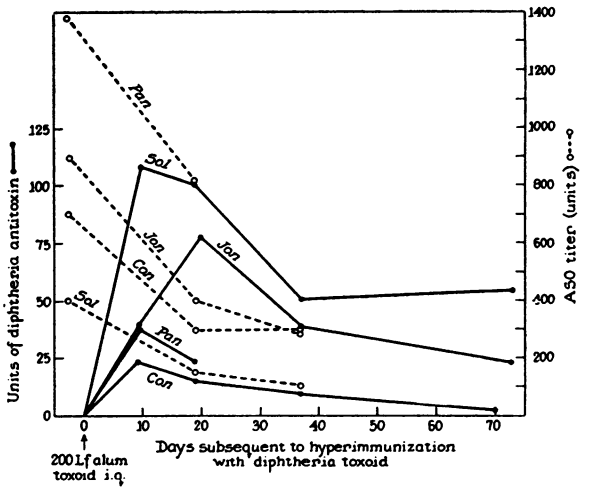

FIG. 1. ANTISTREPTOLYSIN O AND DIPHTHERIA ANTITOXIN RESPONSE IN HYPERIMMUNIZED RHEUMATIC CHILDREN WITH RECENT STREPTOCOCCAL INFECTIONS

with the exception of one subject who formed 40 units per cc. of antitoxin as judged by the rabbit skin test in the absence of any precipitating antitoxin.

The above tests revealed no apparent significant differences between rheumatic and control groups in regard to the nature of the antitoxin formed subsequent to booster toxoid.

The course of immune responses subsequent to booster toxoid

In many instances it was not possible to obtain follow-up serological studies beyond 30 to 40 days. However, in a small number of persons sera were studied up to 80 days following immunization. The antitoxin responses of four rheumatic and five control subjects in the 9 to 15 year age group who received alum toxoid are shown in Figures 1 and 2. Comparison of these data in both groups shows that the period of time during which the

TABLE VI

Incidence of precipitating and non-precipitating antitoxic sera in 25 adult rheumatic and 25 control subjects hyperimmunized with diphtheria toxoid*

	Precipitating	Non-precipitating	Totals
Rheumatic	20 (80%)	5 (20%)	25 (100%)
Control	18 (72%)	7 (28%)	25 (100%)

* All sera used in this experiment contained 10 units/cc. or more.

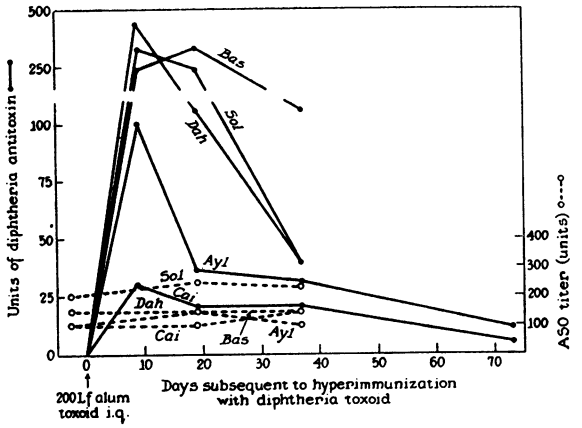

FIG. 2. ANTISTREPTOLYSIN O AND DIPHTHERIA ANTITOXIN RESPONSE IN HYPERIMMUNIZED NON-RHEUMATIC CHILDREN

largest amounts of antitoxin were produced was in the interval between 10 and 20 days after immunization. The antitoxin titer decreased markedly during the next twenty days and much more slowly thereafter. Exceptions to this type of response occurred, as in the sera from rheumatic patients Rod and Dav (Figure 3). Although the follow-up period in the numerically greater adult series is short, the rheumatic and control groups are similar in regard to the course of antitoxin response.

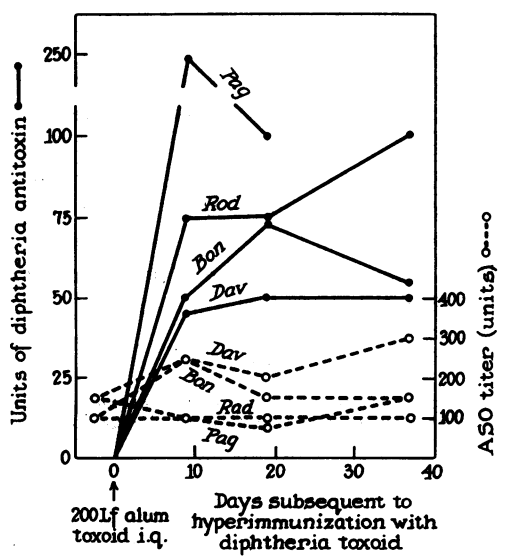

FIG. 3. ANTISTREPTOLYSIN O AND DIPHTHERIA ANTITOXIN RESPONSE IN HYPERIMMUNIZED RHEUMATIC CHILDREN WITH REMOTE STREPTOCOCCAL INFECTIONS

Behavior of antistreptolysin O titer before and after immunization with diphtheria toxoid

Sera from the 33 subjects (ages 9 to 15) who received alum toxoid were tested for antistreptolysin O levels before and after booster toxoid. Because it was thought that rheumatic subjects with very recent attacks might prove refractory to an otherwise demonstrable immune stimulus, it was felt advisable to divide the rheumatic subjects into two categories: (a) 11 patients in whom rheumatic fever had been quiescent for less than six months; (b) 11 patients in whom rheumatic fever had been quiescent longer than six months. Patients in the first group almost without exception showed significant decreases in the antistreptolysin O titer during the period of this study, suggesting relatively recent exposure to group A hemolytic streptococci. Individual titers ranged from 150 to 1400 units prior to immunization with diphtheria toxoid and from 100 to 800 units following booster toxoid, with mean values of 605 and 250 units, respectively. Subjects in group (b) did not show significant variations in the antistreptolysin O titer during the study, suggesting more remote contact with group A hemolytic streptococci. In this series, titers ranged from 100 to 300 units before and after toxoid with mean values of 142 and 182 units, respectively. In neither group was there demonstrable evidence of a significant increase in the antistreptolysin O titer following the injection of diphtheria toxoid during which time a rise and fall in the titer of diphtheria antitoxin occurred. This is shown in Figures 1 and 3. Similar findings were observed in the non-rheumatic control group (Figure 2).

DISCUSSION

The data indicate that rheumatic and control subjects react similarly following a single booster dose of diphtheria toxoid and that the number of previous attacks of rheumatic fever appears to have no bearing upon the ability to form diphtheria antitoxin. In most individuals there occurs a rise in antitoxin titer, oftentimes to high levels, within a matter of days following a booster dose of diphtheria toxoid. A wide spread occurred in both groups with respect to the amounts of antitoxin formed, although the mean values were essentially the same in all groups with the exception

of the numerically small group of control children. These results are in general accordance with the findings of Quinn, Seastone, and Dickie (15) for pneumococcus type I polysaccharide and of Miller, Kibrick, and Massell (16) for influenza vaccine. Similarly Miller, Kibrick, and Massell (16) found considerable overlapping of antibody response to typhoid antigen in rheumatic and control groups. However, they found a small but significant increase in the mean titer of rheumatic subjects as compared with controls. None of the foregoing evidence can preclude the possibility of a limited form of sensitivity restricted to antigens of the group A hemolytic streptococcus or its products.

The present study is concerned only with the secondary variety of immune response. All immunized persons were Schick negative and, therefore, possessed a detectable amount of circulating antitoxin prior to booster dose of toxoid. In this respect the present study differs from that of Miller, Kibrick, and Massell (16) in which typhoid vaccine was used. All individuals used in their studies presumably received primary immunization. However, the demonstration of a similarity of immune response in rheumatics and controls to some antigens (15, 16) given as primary, and others as secondary immunization suggests that antigenic conditioning under the present set of experimental circumstances is not one of crucial importance. In view of the possibility that repeated streptococcal infections may be of importance in the pathogenesis of rheumatic fever (17), the secondary type of response may be of greater significance in immunological studies related to the rheumatic state.

Because of the possibility that an unusual type of antibody may play a role in rheumatic fever, it was thought of importance in the present work to determine whether differences occur between rheumatic and non-rheumatic subjects in the type of diphtheria antitoxin produced. Earlier studies (8) have shown that human subjects immunized with purified diphtheria toxoid form either precipitating antitoxin, non-precipitating antitoxin, or a mixture of both. The present investigations confirm the original studies, and in addition, show no significant numerical differences between rheumatic and control groups in the ability to form one or both varieties of antitoxin as measured by the immediate skin test, specific precipitability, the *in vivo/in vitro* ratio, and fixation of guinea pig complement.[8] The possibility that rheumatic patients more readily produce a non-precipitating form of antibody, or a group of such antibodies to certain products of the group A hemolytic streptococcus cannot be ruled out on the basis of the studies described, since an unrelated antigen was used. However, a generalized tendency to this kind of immune response in rheumatic individuals is not suggested by our results.

There is some evidence which indicates that so-called anamnestic antibody responses occur following exposure to, or stimulation with, unrelated materials (18–20), but there is likewise a considerable body of experimental data (21–23) to show that anamnestic responses occur only subsequent to stimulation with antigenically related substances. In the present study, the absence of rises in antistreptolysin O during the period of maximum diphtheria antitoxin response is in accord with the view that non-specific antibody responses do not occur.

SUMMARY

The present study was based upon analysis of skin reactions to Schick toxin and toxoid in 245 rheumatic and control subjects, and upon antitoxin responses in 121 of these individuals following a single booster dose of purified diphtheria toxoid.

The amounts of antitoxin produced in rheumatic and control subjects subsequent to a booster dose of diphtheria toxoid were similar with regard to the range of titer and the mean titer. The course of antitoxin responses in rheumatic patients studied up to 80 days was similar in most instances to that of control subjects.

[8] On the basis of current investigations in this laboratory, it is probable that non-precipitating antitoxin occurs in a form which does not sensitize human skin. This possibility was raised with regard to the original studies (8) because a few sera containing non-precipitating antitoxin possessed relatively high antitoxin titers in the presence of only moderate skin reactivity to toxoid. In the present investigation, several individuals in both rheumatic and control groups who demonstrated no reactivity to Schick toxoid possessed antitoxin which was not precipitable by toxoid. Further related studies using additional immunological techniques are now in progress. The above finding would seem to have no bearing on the present studies, since there was no significant difference in the number of precipitin formers (Table VI) or of immediate skin reactors (Tables I and II) between the rheumatic and control groups.

There was no demonstrable difference in the post-immunization antitoxin response between subjects with a history of one rheumatic fever attack and patients with a history of more than one attack.

Comparison of the relative ability of rheumatic and control subjects to form non-precipitating antitoxin was made on the basis of immediate wheal and erythema reactions to Schick toxoid, specific precipitability with toxoid, analysis of the *in vivo/in vitro* ratio and guinea pig complement fixation. The results of these tests in both groups showed no significant differences.

The above data indicate that, qualitatively and quantitatively, antitoxin responses to diphtheria toxoid are approximately the same in rheumatic and non-rheumatic individuals.

Antistreptolysin O titers, determined in rheumatic and control children during the period of maximal diphtheria antitoxin formation, showed no consistent rises in either group.

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