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WITH RHEUMATIC FEVER, STREPTOCOCCAL INFECTIONS,
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Robert W. Quinn

J Clin Invest. 1948;27(4):471-475. <https://doi.org/10.1172/JCI101993>.

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ANTIHYALURONIDASE STUDIES OF SERA FROM PATIENTS WITH RHEUMATIC FEVER, STREPTOCOCCAL INFECTIONS, AND MISCELLANEOUS NON-STREPTOCOCCAL DISEASES¹

By ROBERT W. QUINN²

(From the Section of Preventive Medicine, Yale University School of Medicine, New Haven)

(Received for publication December 17, 1947)

INTRODUCTION

In the previous paper the technical aspects of the mucin-clot prevention test which was standardized by McClean (1) have been described. Friou and Wenner (2) using the mucin-clot prevention test have demonstrated the clinical significance of an inhibitory substance in human serum capable of neutralizing an enzyme elaborated by a strain of hemolytic streptococcus. The amount of inhibitory substance was shown by them to be greater in the sera of patients with rheumatic fever than in patients early in the course of uncomplicated hemolytic streptococcal infections or normal individuals. A brief historical background of the work preceding that of Friou and Wenner has been presented in the previous paper.

The purpose of this paper is to report the clinical application of the mucin-clot prevention test in the determination of the antihyaluronidase titre of sera of patients with rheumatic fever, hemolytic streptococcal infections, miscellaneous other diseases, and normal individuals.

MATERIALS AND METHODS

The materials and methods used in these studies were exactly as described in the previous paper.

Enzyme: The enzyme used throughout in these tests was that produced by a strain of Group A, type 4 beta hemolytic streptococcus. Each serum was tested against a constant amount (16 units) of hyaluronidase.

Substrate: The substrate was composed of a 0.15% solution of potassium hyaluronate in distilled water, normal horse serum in a dilution of 1:10 in physiological saline, and distilled water in a ratio of 1:1:2, respectively. Potassium hyaluronate was prepared according to the method of McClean *et al.* (3).

Serum: The blood used in the study of normal individuals and patients was drawn as aseptically as possible.

The serum was separated from the clot within 24 hours in most instances and stored in lusteroid tubes at -70°C .³

Methods of determining the mean antihyaluronidase titre:

An arbitrary code was devised and numbers were assigned to each titre, *e.g.*:

Serum dilution	Code
0	-.2
1:16	1
1:32	2
1:64	3
etc.	4

An equation for determining the code was devised.⁴

$$\text{Code} = \frac{\log \text{reciprocal titre} - .9013}{-.3010}$$

and

$$\log \text{reciprocal titre} = (-.3010 \times \text{code}) - .9031$$

Example: For a serum with the antihyaluronidase titre 1:4096 the code would be:

$$\begin{aligned} \text{Code} &= \frac{\log 4096 - .9031}{-.3010} \\ &= \frac{3.6124 - .9031}{-.3010} \\ &= \frac{-2.7093}{-.3010} = 9 \end{aligned}$$

The mean antihyaluronidase titre for a group of sera was determined by the formula below:

$$\frac{\text{codes of sera}}{\text{number of sera}} = \text{mean code.}$$

The titre to which the code number was arbitrarily assigned was then determined.

³ Blood specimens were collected from the wards of the New Haven Hospital and Dispensary Clinic. They also were obtained from patients at the U. S. Naval Hospital at St. Albans, Long Island, through the courtesy of Capt. H. L. Weaver and Capt. W. D. Small, from patients in the St. Francis Sanatorium for Cardiac Children at Roselyn, L. I., through the courtesy of Dr. Leo Taran and the Rev. Mother Superior, and from patients in the Children's Center, New Haven, Conn., through the assistance of Dr. C. W. Woodruff.

⁴ Dr. John H. Watkins, Assoc. Prof. of Public Health devised this formula and his advise was followed in determining the statistical significance of the results.

¹ Aided by a grant from the Life Insurance Medical Research Fund.

² Milbank Memorial Fund Fellow 1946-47.

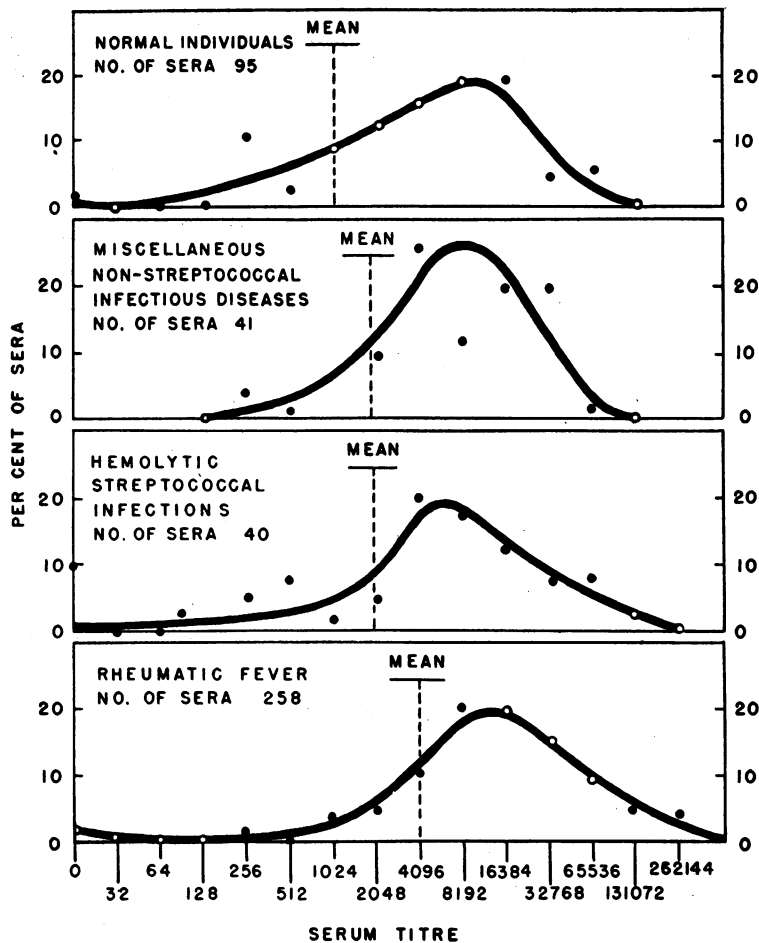


FIG. 1. FREQUENCY DISTRIBUTION OF ANTIHYALURONIDASE TITRES FOR SERA FROM PATIENTS WITH RHEUMATIC FEVER, HEMOLYTIC STREPTOCOCCAL INFECTIONS, MISCELLANEOUS NON-STREPTOCOCCAL INFECTIOUS DISEASES, AND NORMAL INDIVIDUALS

RESULTS

A total of 495 sera from 387 individuals was tested. As a base line the mean antihyaluronidase titre of 95 normal adult sera was first determined as 1:1024. The mean antihyaluronidase titre of all groups of sera are recorded in Table I and Figure 1. Subsequently the mean of 40 sera from patients convalescent from scarlet fever and other acute beta hemolytic streptococcal infections was determined to be slightly less than 1:2048. That of ten patients with active rheumatoid arthritis was 1:1024. Forty-one sera from patients with non-streptococcal infectious diseases including tuberculosis, syphilis, bacterial and "virus" pneumonia, leprosy, Vincent's angina, gonococcus urethritis,

etc. had a mean titre slightly less than 1:2048. The mean antihyaluronidase titre for 41 sera from the patients with non-streptococcal infectious diseases combined with the ten sera from patients with rheumatoid arthritis was slightly less than 1:2048. Sera from all the 258 patients with rheumatic fever had a mean antihyaluronidase titre of 1:4096. The sera from patients with rheumatic fever were analyzed further according to the state of activity of the rheumatic process at the time the blood was collected. The mean antihyaluronidase titres for these different groups of sera were as follows: For rheumatic fever, active, acute, it was higher than 1:16,384 being slightly nearer to 1:16,384 than the next highest dilution which would be 1:32,768. In this group of patients

TABLE I
Mean antihyaluronidase titre of sera from groups of patients according to diagnosis

Diagnosis	Number of sera	Mean antihyaluronidase titre
Normal Individuals	95	1:1024
Miscellaneous Non-streptococcal Infectious Diseases	41	slightly less than 1:2048
Miscellaneous Non-streptococcal Infectious Diseases plus Rheumatoid Arthritis	51	slightly less than 1:2048
Rheumatoid Arthritis, Active	10	slightly higher than 1:1024
Scarlet Fever plus other Beta Hemolytic Streptococcal Infections	40	slightly less than 1:2048
Rheumatic Fever	258	1:4096
	495	
Rheumatic Fever, Active, Acute	15	higher than 1:16,384
Rheumatic Fever, Active	83	midway between 1:4096 and 1:8192
Rheumatic Fever, Active, Subsiding	31	slightly higher than 1:8192
Rheumatic Fever, Active, Chronic	21	midway between 1:2048 and 1:4096
Rheumatic Fever, Inactive	81	slightly higher than 1:2048

were those whose illness had begun within three weeks of the time the serum was collected and who still had high fever, acute arthritis, tachycardia, carditis, and other clinical and laboratory evidence of acute rheumatic fever. These patients were all receiving salicylates by mouth in therapeutic doses. The highest individual antihyaluronidase titres were observed in this group of patients. The titre of some sera in this group was as high as 1:262,144 and furthermore this high titre was not observed in sera from any individuals other than those with rheumatic fever, active, acute. The mean titre for the group with rheumatic fever, active, *i.e.*, those whose illness was more than three weeks old but who still had evidence of ac-

tivity as manifested by fever, tachycardia, carditis, rapid sedimentation rate, electrocardiographic changes, etc., was midway between 1:4096 and 1:8192. The group with active but subsiding rheumatic fever had a mean antienzyme titre slightly higher than 1:8192. About one fourth of the patients with active, or active, subsiding rheumatic fever were receiving salicylates. The mean titre of the sera from patients whose illness was classified as rheumatic fever, active, chronic, was midway between 1:2048 and 1:4096. These patients had been ill with low-grade, active rheumatic fever for many months. Two were receiving salicylates. The mean antihyaluronidase titre for sera from patients with inactive rheumatic fever was slightly higher than 1:2048.

In the statistical analysis of the differences between the mean antihyaluronidase titres of groups of sera, the *t* test (4) was employed. Values of *t* of 1.96 or greater were interpreted to mean that the differences between the mean titres of the two groups of sera under comparison were statistically significant.

The mean antihyaluronidase titre of sera from rheumatic fever patients of 1:4096 was significantly higher than the mean titre of sera from any other group of patients or from normal individuals. These comparisons are recorded in Table II along with the computed standard difference, the standard error of difference and the value of *t*. The frequency distribution of the antihyaluronidase titres of sera from each group of patients is plotted in Figure 1. This figure shows graphically the

TABLE II
Statistical analysis of the differences between the mean titres of groups of sera

Number of sera being compared	Standard difference	Standard error of difference	<i>t</i>	Statistically significant
Rheumatic Fever (258) vs. Normal Individuals (95)	2.135	0.256	8.04	Yes
Rheumatic Fever (258) vs. Scarlet Fever and other Beta Hemolytic Streptococcal Infections (40)	2.330	0.396	5.95	Yes
Rheumatic Fever (258) vs. Miscellaneous Diseases plus Rheumatoid Arthritis (51)	2.042	0.343	4.19	Yes
Scarlet Fever plus other Streptococcal Infections (40) vs. Miscellaneous Diseases plus Rheumatoid Arthritis (51)	2.773	0.582	1.89	No
Scarlet Fever plus other Streptococcal Infections (40) vs. Normal Individuals (95)	2.609	0.492	0.803	No
Miscellaneous Diseases plus Rheumatoid Arthritis (51) vs. Normal Individuals (95)	2.093	0.363	1.94	No
Rheumatic Fever, Active, Acute (15) vs. Normal Individuals (95)	3.088	0.8579	5.042	Yes
Rheumatic Fever, Active, Acute (15) vs. Miscellaneous Non-streptococcal Diseases (41)	0.2849	0.8597	3.998	Yes
Rheumatic Fever, Active, Acute (15) vs. Scarlet Fever and other Beta Hemolytic Streptococcal Infections (40)	2.436	0.7377	6.264	Yes
Rheumatic Fever, Active, Acute (15) vs. Rheumatic Fever, Active (83)	2.626	0.736	2.35	Yes
Rheumatic Fever, Active, Acute (15) vs. Rheumatic Fever, Active, Subsiding (31)	2.1659	0.6812	1.67	No
Rheumatic Fever, Active, Acute (15) vs. Rheumatic Fever, Active, Chronic (21)	1.911	0.2042	13.426	Yes
Rheumatic Fever, Active, Acute (15) vs. Rheumatic Fever, Inactive (81)	1.941	0.5454	5.423	Yes

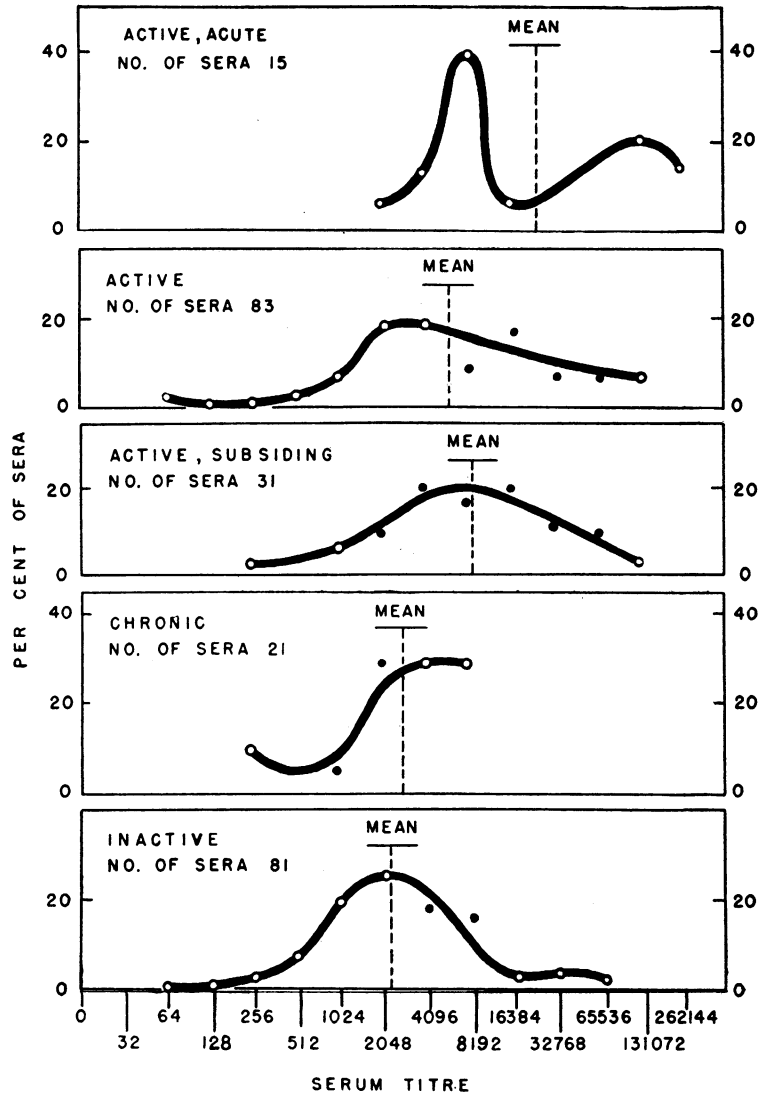


FIG. 2. FREQUENCY DISTRIBUTION OF ANTIHYALURONIDASE TITRES OF SERA FROM PATIENTS WITH RHEUMATIC FEVER IN DIFFERENT STAGES OF ACTIVITY

wider range of titres and higher mean titre of the rheumatic fever sera compared with sera from patients with hemolytic streptococcal infections, non-streptococcal infectious diseases, and normal individuals.

One of the important results of these studies was the finding that the mean antihyaluronidase titre of the sera from patients with rheumatic fever, active, acute, was significantly higher than the mean titre of sera from normal individuals or from any other group of patients except those with rheumatic fever, active, subsiding. The mean titre of

sera from patients with rheumatic fever, active, acute, was actually higher than the mean titre from patients with rheumatic fever active, subsiding, but the difference was not statistically significant. The mean titre of sera from patients with rheumatic fever, active, acute, however, was significantly higher than the mean titre of the sera from patients in any other phase of rheumatic fever. The frequency distribution of antihyaluronidase titres of sera from different groups of rheumatic fever patients was plotted in Table II. Here is shown the higher range of titres of sera from pa-

tients with rheumatic fever, active, acute, and the gradual decrease in mean antihyaluronidase titre from the group, with rheumatic fever, active, acute, to the group with rheumatic fever, inactive.

DISCUSSION

In these studies the contention of Friou and Wenner (2) that the amount of inhibitory substance against streptococcal hyaluronidase in sera from patients with rheumatic fever was greater than in sera from patients early in the course of uncomplicated hemolytic streptococcal infections or from normal individuals has been confirmed. It also has been demonstrated that the serum antihyaluronidase titre in patients early in the course of active rheumatic fever is significantly higher than the antihyaluronidase titres of sera from any other group of patients studied including patients with active, subsiding, or inactive rheumatic fever, hemolytic streptococcal disease, and non-streptococcal infectious diseases. The results do not yield information which indicates that patients with streptococcal disease have a higher mean antihyaluronidase titre than patients with other infectious diseases. However, further studies of the antihyaluronidase titre in patients during the course of streptococcal infection and rheumatic fever along with the antifibrinolysin and antistreptolysin "O" titres are being done and will be reported later.

No attempt has been made to study the effect of salicylates on the antihyaluronidase titre of patients or the effect of salicylates on hyaluronidase *in vivo* and *in vitro*, but from the recent reports by Guerra (5), Pike (6), Dorfman *et al.* (7), and Meyer (8), it would appear that salicylates do inhibit the spreading effect of hyaluronidase in skin but have no inhibitory effect on testicular or bacterial hyaluronidase *in vivo* in concentrations obtained therapeutically.

Obviously much more investigation is necessary

before the meaning of these results will be fully understood or before this test can be proposed as a diagnostic measure.

SUMMARY AND CONCLUSIONS

1. Studies of the determination of antihyaluronidase titres of sera from patients with rheumatic fever, hemolytic streptococcal infections, miscellaneous non-streptococcal infectious diseases, and normal individuals have been presented.

2. The mean antihyaluronidase titre of sera from patients with rheumatic fever was significantly higher than the mean titre of sera from the other groups of patients studied.

3. The mean antihyaluronidase titre of sera from patients with rheumatic fever, active, acute, was significantly higher than the mean antihyaluronidase titre of sera from patients with rheumatic fever in less active forms and from sera from patients in other groups studied.

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