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Immunological Similarity of Streptococcal Antifibrinolysins.*

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The development of antifibrinolytic properties in the blood of patients convalescing from streptococcal disease is commonly regarded as an immunological response to infection by the β-hemolytic streptococcus.[1-4] Measurement of the antifibrinolytic capacity of the blood has consequently been employed as a diagnostic aid in the study of infections due to this organism.[2-7]

In general, the capacity of the blood to resist the action of fibrinolysin is measured by the time required for the plasma clot to undergo complete dissolution, such tests being performed either on the patient's plasma clot[1] or on a normal plasma clot to which serum of the patient has been added.[8,9] From the results of studies with fibrinolysins from 40 different strains, Van Deventer[10] suggested that they all probably belong to one immunological type. In contrast, Mote, Massell and Jones,[11] using sharper quantitative methods, found that the resistance of plasma clots varied with fibrinolysins derived from different strains. They attributed their results to immunological differences in antifibrinolysins; the nature of these immunological differences, however, was not investigated further.

Recent investigation has indicated that antifibrinolysin is not the only factor in the blood which may confer antifibrinolytic properties on a plasma clot. Studies of the mechanism of the fibrinolytic reaction have shown that the actual lytic agent in streptococcal fibrinolysis is not fibrinolysin, but a proteolytic enzyme in the plasma (lytic factor) which is activated by fibrinolysin.[12,13] Accordingly, antifibrinolytic effects may result not only from the presence in the plasma of antifibrinolysin, but also from increased amounts of antiprotease.[14,15] In addition, it

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TABLE I.
Determination of Antifibrinolysin Titers in Convalescent Phase Sera by Means of Fibrinolysins from Groups A, C, and G Streptococci.

Group of infecting organism	Convalescent serum No.	Titer with Group A fibrinolysin	Titer with Group C fibrinolysin	Titer with Group G fibrinolyhin
A	100 B	83	83	83
	144 B	625	625	625
	167 B	278	278	278
	234 B	125	179	179
	463 B	179	179	179
C	120 C	83	83	56
	249 B	125	125	125
	258 C	56	56	56
	581 B	<25	25	25
	1070 C	<25	<25	<25
G	8 C	<25	<25	<25
	549 C	25	25	25
	6 C	<25	25	25
	971 D	56	56	83
	694 F	36	56	36

has been suggested that clot resistance may be due, in some instances, to a deficiency of the lytic factor in the plasma.[16,8]

Since the effect of these nonspecific antifibrinolytic factors was not controlled in previous work, the problem of the serological specificity of antifibrinolysins was reinvestigated. Antifibrinolysin was measured quantitatively by a serological method which minimized the effect of antiprotease and deficiency of lytic factor. The sera studied were obtained from patients infected by streptococci from 3 different Lancefield groups: A, C and G. In order to test for possible differences in antifibrinolysins, these sera were titrated with fibrinolysins derived from strains from each of these 3 different groups, and the resulting titers compared. The results of these studies showed that the antifibrinolysins tested in this manner were immunologically similar.

Methods. The sera tested were obtained from patients with acute streptococcal pharyngitis or tonsillitis. Streptococcal etiology was confirmed in each case by the presence of β-hemolytic streptococci in 2 or more cultures of the throat, and by the development of antistreptolysin "O" antibodies during early convalescence.[17] Streptococci were grouped by the Lancefield precipitin-tube technic[18] using bacterial extracts prepared by Fuller's method.[19] An infection was attributed to a strain of a given group if all of the throat cultures taken during the acute illness showed other groups to be entirely absent.

Antifibrinolysin levels were determined by the procedure previously described.[20] The sera were first diluted serially in 1.5-fold dilutions, beginning with a 1/25 dilution, as follows: 1/25, 1/36, 1/56, 1/83, 1/125, 1/179, 1/278, 1/417, 1/625, 1/900, 1/1400. Each dilution was incubated with a standard amount of fibrinolysin. The titer of a given serum was the highest dilution which neutralized the lysis-promoting activity of the standard unit of fibrinolysin. A difference in titer of 2 dilution increments (2 tubes), corresponding to a 2.25-fold antibody change, was considered to be beyond the error of the method.

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[17] Hodge, B. E., and Swift, H. F., *J. Exp. Med.*, 1933, **58**, 277.

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[19] Fuller, A. T., *Br. J. Exp. Path.*, 1938, **19**, 130.

[20] Kaplan, M. H., and Commission on Acute Respiratory Diseases, *J. Clin. Invest.*, 1946, **25**, 347.

TABLE II.
Determination of Antifibrinolysin Titers of Acute and Convalescent Phase Sera by Means of Fibrinolysins of Homologous and Heterologous Groups.

Group of infecting organism	Case No.	Date of serum	Antistreptolysin "O" titer	Titer with Group A fibrinolysin	Titer with Group C fibrinolysin	Titer with Group G fibrinolysin
A	O-463	1-1	159	56	56	56
		2-27	400	179	179	179
A	O-144	12-15	159	125	125	125
		1-12	317	625	625	625
A	O-167	12-21	159	83	125	125
		1-19	500	278	278	278
C	T-101	5-20	50	<25	<25	<25
		6-11	159	36	36	36
G	O-6	10-21	125	<25	<25	<25
		1-26	400	25	25	25
G	O-694	1-1	62.5	25	36	36
		2-23	100	36	56	56

The *fibrinolysins* were prepared from culture filtrates by alcoholic precipitation according to the procedure of Garner and Tillett,[21] and were stored in the frozen state until ready for use.

Results. The titers of each of 15 convalescent sera were determined with fibrinolysins derived from strains belonging to the 3 groups, A, C and G. Five of these 15 sera were obtained from patients convalescent from a group A infection; 5 were from patients with a group C infection; and the 5 remaining were from cases infected by group G strains.‡ As shown in Table I, the titer of each serum was essentially the same with each of the 3 fibrinolysins employed. Variation of the titer of any one serum did not exceed one tube. The evidence thus indicated that the 3 fibrinolysins reacted with the sera in a quantitatively identical manner.

In a second experiment these 3 different fibrinolysins were used to measure the increase in antifibrinolysin in serum specimens collected during the acute and convalescent phases of proved streptococcal infections. Sera were examined from 3 patients with group A infections, one patient with a group C infection, and 2 patients with group G infections.

As shown in Table II, the antibody rises in the group A and C cases were at least 2 tubes in magnitude; the group G cases showed increases of one tube. The amount of antibody increase in each of these patients was essentially the same with all 3 fibrinolysins employed. It was concluded that the antifibrinolysins produced in human infections due to groups A, C and G streptococci were immunologically similar.

Discussion and Summary. Several studies have indicated that a rise of antifibrinolysin apparently occurs much less frequently in streptococcal disease than a rise in antistreptolysin.[23,24,5,7] One possible explanation for this low frequency of the antifibrinolysin response is that streptococci vary markedly in their ability to produce fibrinolysin, and consequently in the frequency which they may stimulate an antibody response. Evidence has been presented in support of this hypothesis.[25,5]

[21] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239.

‡ The detailed clinical, bacteriological, and serological data on the patients with group C and G infections are to be published elsewhere.[22]

[22] Commission on Acute Respiratory Diseases, *New Eng. J. Med.*, to be published.

[23] Stuart-Harris, C. H., *Brit. J. Exp. Path.*, 1935, **16**, 513.

[24] Winblad, S., *Acta Path. et Microbiol. Scand.*, 1941, Supp. 44, 1.

[25] Commission on Acute Respiratory Diseases and Kaplan, M. H., *Science*, 1945, **101**, 120.

However, a second explanation was suggested by the report[11] that antifibrinolysins differed immunologically. If antibody differences were a significant factor in the determination of antifibrinolysin, it seemed possible that the detection of antibody rises might be limited to the variety of fibrinolysin employed as antigen. The present study was consequently undertaken to test for and, if possible, to determine the nature of the differences in antifibrinolysins.

An attempt was made to obtain evidence of such differences by measuring the antibody level of the sera of patients infected by streptococci belonging to different Lancefield groups. Quantitative antibody titrations were carried out on the sera of patients with infections due to organisms from each of the 3 groups, A, C and G; and the fibrinolysins used for testing for antibody were derived from strains from each of these same groups. The results of the study showed that the titers of the various sera tested were essentially the same with all 3 fibrinolysins.

It would thus appear that the fibrinolysins produced by these different organisms were immunologically identical in their reactivity. This is in agreement with the observations of other workers.[3,10,26] It is not possible to explain the different results reported in the first quantitative studies of the problem.[11] However, as possibly contributing to apparent differences, 2 points may be mentioned: (1) the nonspecific factors in the plasma, such as antiprotease or a deficient lytic factor, may have produced the differences observed, or (2) the variations may have been due to the method of antibody estimation employed. Measurement of antibody was based on the amount of fibrinolysin which permitted clot dissolution. Since fibrinolysin and antifibrinolysin combine in varying multiple proportions,[15] it would appear that a procedure in which the fibrinolysin concentration is varied is not suitable for the measurement of antibody. The evidence thus suggests that the fibrinolysins produced by streptococci pathogenic for man are identical in their immunological behavior.

Conclusion. It was concluded, therefore, that the use of a single fibrinolysin preparation is satisfactory for the serological determination of antifibrinolysin in human infections.

[26] Kirby, W. M., and Rantz, L. A., *Arch. Int. Med.*, 1943, **71**, 620.

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Action of Estrogen on Release of Hypophyseal Luteinizing Hormone.

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The gonad stimulating properties of the pituitary are better understood than is the reciprocal action of the gonadal secretions on the pituitary. In respect to the latter process the terms "suppression" and "inhibition" are frequently used to describe what is obviously a more complicated mechanism than these empirical terms imply. The pituitary gonadotropic complex does not always act as a unit and individual factors should be considered separately in their response to estrogens and androgens. Furthermore, the intracellular synthesis of a pituitary factor is a process distinct from that involving the liberation of the factor from the gland.

We have attempted in the work presented here and in our previous studies,[1,2] to obtain

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[2] Hellbaum, A. A., and Greep, R. O., *Endocrinology*, 1943, **32**, 33.