Antigenic Dissimilarity of Streptokinases. (20460)

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The demonstration of an increase in the capacity of serum to inhibit streptokinase has been assumed to indicate recent experience with the beta hemolytic *Streptococcus*(1-4). Anti-fibrinolytic activity, however, may not be due entirely to the presence of specific antibody. Since the dissolution of clotted plasma is actually carried out by proteolytic enzymes in the plasma, an increase in the quantity of non-specific antiprotease may be responsible, in part, for an antifibrinolytic effect(5,6). A deficiency of the lytic factor in serum may produce the same effect(7,8). Most of the studies of antifibrinolysis have until recently been complicated, therefore, by these non-specific factors. Kaplan(9), however, has devised a quantitative serological method which minimizes the effects of antiprotease and deficiency of lytic factor.

In carrying out determinations of antifibrinolytic activity of serum, it has been customary to use the streptokinase produced by one strain of beta hemolytic *Streptococcus*. This has been based on the assumption that all streptokinases of Group A *Streptococcus* are antigenically similar. Van Deventer(10), on the basis of an investigation of 40 strains of streptococci, concluded that the fibrinolysins elaborated by all of them were of one immunological type. These observations were confirmed recently by Kaplan(11) who demonstrated the similarity of streptokinases of Group A, C, and G streptococci. In contrast, Mote, Massell, and Jones(12) found that the resistance of plasma clots varied with the fibrinolysins derived from different strains. They attributed these results to immunological differences in antifibrinolysin.

The purpose of the present study was to reinvestigate the problem of the antigenic similarity of streptokinase, using the quantitative technic of Kaplan(9). It was found that the streptokinases produced by different strains of the beta hemolytic *Streptococcus* were antigenically dissimilar.

Methods. The 10 strains of beta hemolytic streptococci used for the preparation of streptokinase were isolated from patients with scarlet fever, with the exception of strain CO which was obtained from Dr. Tillett; all belonged to group A. The organisms were grown in heart infusion-yeast-blood broth for 24 hours and the fibrinolysin prepared by alcoholic precipitation, according to the method of Garner and Tillett(13). Titrations of fibrinolytic activity of each strain were repeated before each group of sera was examined for antistreptokinase activity. The procedure of Kaplan(9) was employed for estimation of antifibrinolytic activity and all determinations were carried out at least twice.

The serums used in this study were obtained from patients with untreated scarlet fever. Group A *Streptococcus pyogenes* was isolated from the pharynges of all these individuals and all developed high antistreptolysin titers. The sera were first assayed for antifibrinolysin using streptokinase prepared from strain CO, the organism with which all antistreptokinase titrations had been carried out in this laboratory for several years. On the basis of these results, the cases were divided into 2 groups; those showing a "normal" titer (14 strains) and those with an "elevated" titer of antistreptokinase (11 strains). Although it is difficult to establish the exact point beyond which the antistreptokinase activity may be considered to be increased, levels over 125 (that is, 179 units or greater), were considered by the Commission on Acute Respiratory Diseases to be elevated (14).

Results. In Table I are presented the results of the titrations of sera which appeared to contain a "normal" antistreptokinase content when examined with the fibrinolysin of Strain CO. As can be seen from the data, only 2 (No. 5 and 8) reacted with the 10 streptokinases so that all the determinations were less than 2 tubes apart. The remaining

TABLE I. Antistreptokinase Titers of Serums Using Different Streptokinases. Low-titer serums.

Serum No.	Antistreptokinase titer — Strain of strep. from which streptokinase was prepared: CO	12	23	18	5	15	17	8	6	9	No. of tubes difference
1	83	<25	<25	83	25	36	<25	<25	36	<25	4
2	56	<25	<25	56	179	<25	36	56	56	<25	6
3	83	<25	<25	25	<25	36	56	<25	36	<25	3
4	56	83	417	83	<25	278	36	36	179	<25	8
5	<25	<25	<25	<25	<25	<25	<25	<25	<25	<25	0
6	25	36	<25	278	36	<25	278	36	<25	<25	7
7	36	25	<25	36	<25	36	25	83	<25	36	3
8	<25	<25	<25	25	<25	<25	36	<25	36	<25	1
9	<25	25	<25	<25	56	<25	56	<25	83	<25	3
10	56	278	179	83	<25	278	83	56	179	278	7
11	36	125	83	83	36	36	36	179	179	56	4
12	25	36	125	125	36	179	36	56	56	56	5
13	125	<25	<25	179	83	<25	56	<25	36	36	6
14	125	125	125	125	125	125	83	125	125	25	4

TABLE II. Antistreptokinase Titers of Serums Using Different Streptokinases. High-titer serums.

Serum No.	Antistreptokinase titer — Strain of strep. from which streptokinase was prepared: CO	12	23	18	5	15	17	8	6	9	No. of tubes difference
15	278	278	179	278	625	278	278	83	179	56	6
16	179	56	125	179	179	83	125	56	56	25	5
17	179	<25	<25	83	36	<25	25	<25	36	<25	6
18	417	278	417	36	<25	417	83	179	83	56	8
19	179	417	417	179	83	278	83	179	125	83	4
20	179	125	278	278	278	179	278	83	125	83	3
21	179	125	83	417	125	56	179	179	83	179	5
22	278	56	<25	56	25	56	25	125	56	25	7
23	278	278	83	625	278	83	179	179	83	<25	9
24	417	278	278	179	83	278	125	278	179	83	4
25	417	83	56	56	125	83	83	36	83	36	6

12 gave variable results with the different kinases, the titrations ranging from 3 to 8 tubes apart. Although all of the serums had been classified as showing no elevation of antistreptokinse with CO, several of them (No. 2, 4, 6, 10, 11, 12, and 13) appeared to contain an increased quantity of antibody when tested with other fibrinolysins. While the remaining 5 sera showed variations in titer, all of them were within the range of what is considered to be normal.

The discrepancies between antistreptokinase titers determined by using different streptococcal fibrinolysins were even more pronounced when sera originally considered to contain an increased quantity of antibody against the CO kinase were examined for their ability to inactivate lysins prepared from other strains. The smallest difference in titers of any single serum was 3 and the largest 9 tubes.

Although all of the sera had been considered to have an elevated quantity of antibody when first examined, using the CO streptokinase, all also revealed only a "normal" content of anti-fibrinolysin when tested with the enzyme elaborated by other strains of streptococci. In most instances the differences in titer for each serum fell well outside the limit of experimental error of 2 tubes. It is striking that with no other lysin than that derived from strain CO did the sera give consistently elevated anti-streptokinase levels (Table II).

Discussion and summary. The results obtained in this study indicate that streptokinases derived from different strains of Group A *Streptococcus pyogenes* are antigenically dissimilar. Since Kaplan's method of determining antifibrinolysin(9) has been claimed to minimize the non-specific effects

of antiprotease and inadequacy of lytic substance in the blood, the discrepancies in quantity of antifibrinolysin observed in the present investigation probably cannot be attributed to these factors. It is a well known fact that an increase in antistreptokinase occurs much less frequently than a rise in antistreptolysin in streptococcal infections. Rantz, Boisvert, and Spink(15) and the Commission on Acute Respiratory Diseases and Kaplan(16) have attributed this to the marked variations in the ability of different strains of streptococci to produce fibrinolysin. On the basis of the observations made in the present study it would appear likely also that the relative infrequency of increase in antistreptokinase after streptococcal infection may be related to the use of a fibrinolysin antigenically different from that elaborated by the strain of organism responsible for the disease.

The reason for the difference in the results obtained by Kaplan(11) and those presented in this paper are not readily apparent. They may be related, however, to the fact that, by chance, the 3 lysins which Kaplan used happened to be antigenically the same. With an increase in the number of kinases examined, the possibility of detecting immunologic differences probably was increased.

If, as the data presented in this paper indicate, streptococcal fibrinolysins are antigenically dissimilar, the determination of antistreptokinase titers probably is of little value in the diagnosis of streptococcal infection. A low antibody content may be related not to lack of immune response but to the fact that the fibrinolysin used in carrying out the test was immunologically different from that produced by the organism which infected the patient. It is evident that elevation of antistreptolysin is of much greater significance in the recognition of recent invasion by the beta-hemolytic *Streptococcus*.

Conclusions. 1. Streptokinases produced by different strains of Group A beta-hemolytic *Streptococcus* are antigenically different. 2. Determination of antistreptokinase titers is probably of little value in the diagnosis of streptococcal infection.

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