THE DIFFERENTIATION OF THREE DISTINCT DESOXYRIBONUCLEASES OF GROUP A STREPTOCOCCI

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The occurrence of desoxyribonuclease activity in the supernatant fluid of cultures of group A streptococci has been independently reported by Tillett, Sherry, and Christensen (1), and by McCarty (2). This activity has been found regularly and abundantly in the extracellular fluid from a wide variety of strains of group A streptococci (1–4). Production begins early in the active growth phase and increases logarithmically throughout the logarithmic growth phase of the organism (2). Bernheimer and Ruffier (4) have reported that washed cocci of certain strains elaborate desoxyribonuclease activity extracellularly when suspended in a simple solution which is incapable of supporting growth. The addition of specific substrate or split products of the substrate does not influence the formation of enzymatic activity (2).

Desoxyribonuclease activity is occasionally encountered in the supernatant fluid from cultures of other groups of streptococci, notably groups C, B, and D (1, 3, 4). Activity has also been demonstrated in association with other microorganisms (5–9), and in various plant (10, 11) and animal (12–18) tissues. The best known and most widely studied desoxyribonuclease is that derived from pancreatic tissue (12). This enzyme has been crystallized by Kunitz (13) and has been referred to as pancreatic desoxyribonuclease or desoxyribonuclease I to distinguish it from other animal tissue desoxyribonucleases, particularly that one derived from thymus (desoxyribonuclease II) (14, 15). In contrast to the pancreatic enzyme, the thymic enzyme does not appear to require magnesium ions for activation, has a lower pH optimum, and hydrolyzes desoxyribonucleate without the formation of undialyzable residue.

In studies of certain inhibitors, differences have been demonstrated in the inhibition of desoxyribonucleases derived from various sources. Inhibitors of pancreatic desoxyribonuclease have been identified in a number of tissues, including growing pigeon crop glands (18), malignant tumors (16), normal animal tissues (16), and mature white blood cells (19). The inhibitor from the crop glands of brooding pigeons appears to be protein in nature and does not inhibit thymus desoxyribonuclease. Zamenhof

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and Chargaff (7) have described a protein in yeast extracts which specificially inhibits yeast desoxyribonuclease but not pancreatic, thymus, neurospora, barley, or streptococcal (4) desoxyribonucleases. Bernheimer and Ruffier (4) have prepared extracts of streptococci which partially inhibit the desoxyribonuclease activity of group A streptococci. It appears that this inhibitor is a ribonucleic acid since it is destroyed by ribonuclease. Preparations of ribonucleic acid from several bacterial species cause similar inhibition, but preparations of ribonucleic acid from mammalian pancreas and liver, tobacco leaves, wheat germ, and yeast fail to inhibit (20). This inhibitor is specific in that it does not inhibit desoxyribonuclease preparations from pancreas, yeast and barley or even those produced by several strains of streptococci belonging to groups B and C (4).

McCarty (21) has shown that the desoxyribonuclease activity of streptococcal supernates is inhibited by antisera against streptococcal preparations but not by antisera against pancreatic desoxyribonuclease. Likewise, pancreatic desoxyribonuclease is inhibited by its specific antibody but not by antibody against streptococcal preparations. It has, therefore, been concluded that the two sources represent similar but independent enzymes attacking the same substrate.

In the immunologic response following natural infections with group A streptococci, a paradox has been noted (21). Virtually all strains of group A streptococci produce desoxyribonuclease activity in appreciable amounts but the number of patients developing neutralizing antibody for this enzyme has been fewer than the number developing neutralizing antibody for other common streptococcal antigens.

The present report presents evidence that group A streptococci can produce three different enzymes, each independently capable of depolymerizing intact desoxyribonucleic acid. The three streptococcal desoxyribonucleases migrate separately on starch zone electrophoresis and are serologically distinct. In addition, they show certain differences in the degree of inhibition by citrate and in optimal pH of activity.

Materials and Methods

Strains of Group A Streptococci.—Five strains have been used in these studies:

Strain H105 OP, an opaque variant of Tillett's "CO." This strain is an undesignated new type and produces high yields of streptokinase.

Strain D58, Colebrook's strain "Richards," type 3.

Strain Dematteo, isolated from a patient with acute glomerulonephritis, supplied by Dr. Charles H. Rammelkamp, Jr., type 12.

Strain Jiminez, No. 1, obtained from a patient with acute glomerulonephritis in Chile, supplied by Dr. Richard Krause, type "Red Lake" (22).

Strain T1, originally obtained from Dr. F. Griffith.

Preparation of Concentrates of Extracellular Products.—Group A streptococci were grown in a dialysate medium of Pfanstiehl peptone, R.I., casamino acids, and beef heart infusion (23). Concentrates of the extracellular products in the supernatant fluid were prepared by precipitation with ammonium sulfate and filtration through a collodion bag (23).

Electrophoresis.—Extracellular concentrates were examined by starch zone electropho-

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resis according to the method of Kunkel and Slater (24). Glycine buffer, $\mu = 0.1$, pH 9, was used; a direct current with a potential difference of 400 volts was applied for 16 hours at 2–4°C. (23). The protein was eluted from sections of the starch block with glycine buffer. Quantitative recovery was facilitated by suction and rinsing through a sintered glass filter. Protein was determined in each electrophoretic fraction by a modification of the Folin-Ciocalteu method (25). Bovine serum albumin was used as a standard.

Determination of Desoxyribonuclease Activity.—Desoxyribonuclease activity was measured by adaptations (23) of either the alcohol precipitation (21) or viscosimetric (12) methods. For both tests, unless otherwise indicated, the substrate solution contained 0.1 per cent sodium desoxyribonucleate prepared from calf thymus (21) and 0.01 M $MgSO_4$ in 0.025 M veronal buffer, pH 7.7. Dilutions of enzyme were made in neopeptone dialysate broth, pH 7.6 (23).

The alcohol precipitation test was used for locating desoxyribonuclease activity in eluates from sections of the starch block (23). 0.1 ml. of appropriate dilutions of each eluate in neopeptone broth was mixed with 0.5 ml. of substrate solution. The mixture was incubated at 37°C. for 30 minutes in a water bath. One ml. of ethyl alcohol was added and the resulting mixture was agitated. The end point was read as the highest dilution of enzyme which prevented the appearance of a fibrous precipitate upon the addition of alcohol.

For more precise measurement of desoxyribonuclease activity, the viscosimetric method was used. 0.5 ml. of the appropriate enzyme dilution in neopeptone broth was added to 4.5 ml. of substrate solution in an Ostwald viscosimeter with a flow time of 60 to 70 seconds. Enzymatic activity was determined by monitoring the change in relative viscosity at 5 minute intervals during incubation at 37°C. for a 30 minute period. A unit was defined as that amount of enzyme resulting in a decrease of 1.0 in relative viscosity during a 20 minute period in which the rate of change of viscosity is relatively constant (12).

Preparation of Specific Rabbit Antisera.—Rabbits were immunized with electrophoretic fractions from the starch block. An emulsion was prepared containing two parts eluate from a section of the starch block, two parts heavy mineral oil, and one part aquaphor. The adjuvant mixture was chilled in an ice bath and injected subcutaneously in a volume of 1 to 8 ml. Four to 8 injections were given at 5 to 30 day intervals over a 2 to 4 month period. A total of 1 to 3 mg. of protein was administered to each rabbit.

Rabbit sera showing cross-reactions were absorbed with appropriate amounts of heterologous antigen, obtained by electrophoretic separation. The cross-reacting serum and heterologous antigen preparation were incubated at 37°C. for 1 to 2 hours and then refrigerated at 4°C. for 2 to 7 days. Chloroform (0.7 per cent) was used to control bacterial contamination. The precipitate which formed was removed by centrifugation. The absorbed serum was tested for specific inhibition, residual inhibition of the heterologous enzyme, and excess heterologous enzyme activity. Repeat absorptions were done when indicated. Excess heterologous enzyme activity could be destroyed by heating the absorbed sera at 65°C. for 30 minutes. Immunization with antigen preparations which had been recycled on the starch block minimized or eliminated cross-reactions.

Measurement of Inhibition of Desoxyribonuclease Activity by Antisera.—Antisera were tested for neutralizing antibody either by the alcohol precipitation test or by the viscosimetric test (21). All sera were heated at 65°C. for 30 minutes to destroy or reduce intrinsic serum nuclease activity.

For the alcohol precipitation test, the appropriate dilution of the enzyme preparation to be used was determined as follows: Serial dilutions of the enzyme preparation were made in neopeptone dialysate broth, and 0.25 ml. of the dilutions was mixed with 0.25 ml. of neopeptone dialysate broth and 0.5 ml. of substrate solution. After 30 minutes at 37°C., 1 ml. of ethyl alcohol was added. The highest dilution of enzyme degrading the desoxyribonucleate

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to the point at which no fibrous alcohol precipitate formed was determined, and four times this amount was used in the test for antibody neutralization. For measuring antibody neutralization, serial twofold dilutions of antisera, beginning at 1:10, were prepared in neopeptone dialysate broth. 0.25 ml. of each dilution was mixed with 0.25 ml. of the appropriate dilution of enzyme, and then the mixture was incubated at 37°C. for 30 minutes. 0.5 ml. of substrate solution was added and the incubation was continued for another 30 minutes. One ml. of ethyl alcohol was added. The end point was defined as the highest serum dilution which inhibited enzyme activity so that a definite fibrous precipitate appeared in the presence of alcohol.

In the viscosimetric test for neutralizing antibody, equal volumes of the appropriate enzyme and serum dilutions were mixed. After incubation at 37°C. for 30 minutes, 0.5 ml. of the mixture was added to 4.5 ml. of the substrate solution in an Ostwald viscosimeter with a flow time of 60 to 70 seconds. Residual desoxyribonuclease activity was measured by determining relative viscosity at intervals during incubation at 37°C.

Measurement of Inhibition of Desoxyribonuclease Activity by Citrate and by Versenate.—The inhibition of desoxyribonuclease activity by citrate and by versenate was measured viscosimetrically. The substrate solution was prepared so as to contain, in addition to the usual components, 0.03 M sodium citrate or 0.03 M sodium versenate. The versenate solution was prepared by mixing ethylenediaminetetraacetic acid with veronal buffer. The resulting suspension dissolved when the pH was readjusted to 7.7 by the addition of 5 N NaOH.

Determination of Optimal pH of Desoxyribonuclease Activity.—The optimal pH of activity of the several desoxyribonuclease preparations was determined viscosimetrically. Substrate solutions were prepared in 0.025 M buffers as follows: phosphate pH 5.5, phosphate pH 6.5, veronal pH 7.5, veronal pH 8.5, and veronal pH 9.5. The activities of a constant dilution of each enzyme preparation were compared at these five levels of hydrogen ion concentration.

RESULTS

Analysis of the electrophoretic distribution of various known extracellular enzymes of group A streptococci (23) revealed a separation of desoxyribonuclease activity into three distinct components, which, in Fig. 1, are designated A, B, and C. Under the conditions of this study, the peak activities of the three components were separated on the starch block by intervals of approximately 10 cm.: B remained at or near the origin, usually moving only 1 or 2 cm. toward the anode; A migrated approximately 11 cm. and C migrated approximately 21 cm. in the same direction.

In the preparation shown in Fig. 1, component A predominated, but further experiments indicated considerable strain variation in the relative proportions. For example, as can be seen in Fig. 2, strain H105 produced primarily component A, whereas strain Dematteo produced a large bulk of component B with only a shoulder representing A and a small peak of C.

Fig. 3 shows that the variations in enzymatic activity correlated with fluctuations in the protein pattern. In the Dematteo strain high titers of enzymatic activity were found in the same location as a large peak in the protein curve. In the H105 strain the major desoxyribonuclease activity was located in a shoulder of a major peak in the protein patterns. However, it should be noted that the relative values of the scales for protein and for enzymatic activity in Fig. 3 have no meaning since it was not possible to determine what portion

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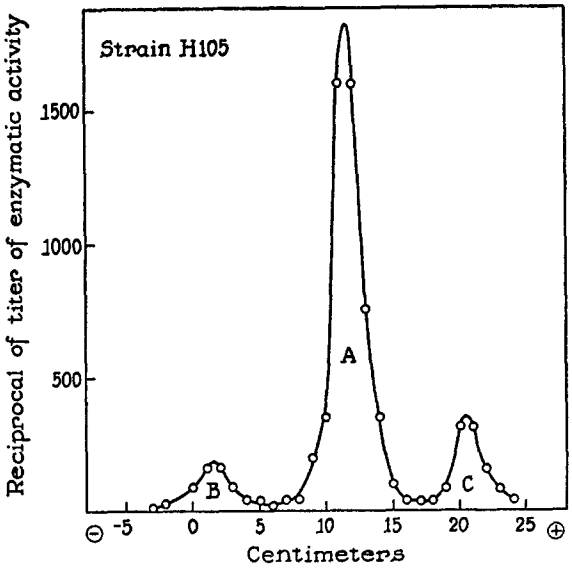

FIG. 1. The electrophoretic distribution of desoxyribonuclease activity (strain H105, glycine buffer pH 9, $\mu = 0.1$, 16 hours at 400 volts. The original point of inoculation of the concentrate of extracellular products is at zero).

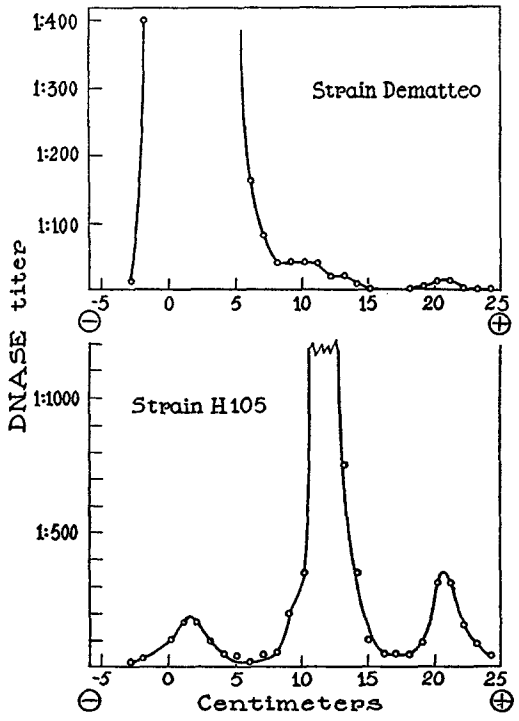

FIG. 2. Comparison of the electrophoretic distributions of desoxyribonuclease activity of two different strains (Dematteo and H105).

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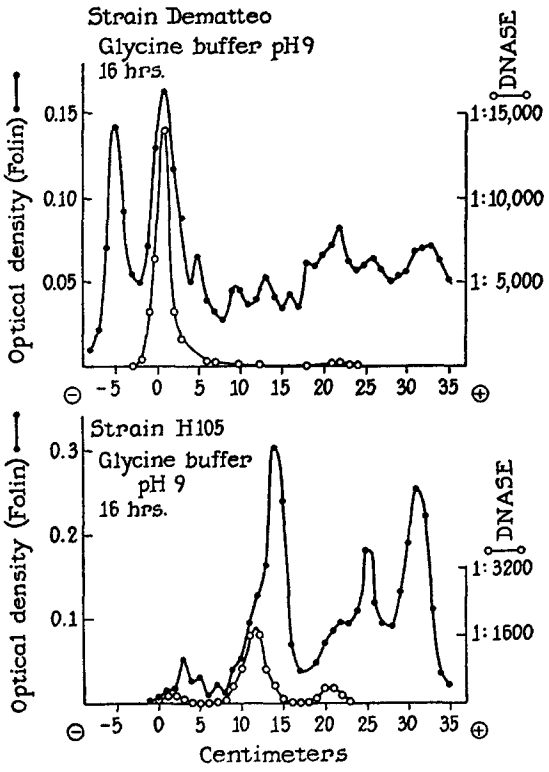

FIG. 3. The relationship of the distribution of protein to the distribution of desoxyribonuclease activity for strains Dematteo and H105.

TABLE I

Inhibition of the Three Desoxyribonuclease Components by Serum from Patient, RP66

Enzyme component	Dilutions of serum										
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10,240
A	0	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
B	0	0	0	0	0	0	0	0	0	++	++++
C	+	0	0	0	0	++	++++	++++	++++	++++	++++

The enzymatic activity in the 1:10 dilution of this and subsequent tables represents residual serum nuclease not destroyed by heating (see discussion of Table II in text).

0, intact fibrous precipitate (complete inhibition of enzymatic activity).

++++, no fibrous precipitate (no inhibition of enzymatic activity).

of the protein in a given area represented a particular enzyme. Electrophoretic analyses of strains Dematteo, D58, T1, and Jiminez No. 1 showed B as the major component, while in strains H105 and H46A[1] (a group C streptococcus),

[1] We are indebted to Dr. Alan Bernheimer for providing a concentrate of the extracellular products of this strain.

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A predominated. None of the six strains examined showed C as the major component.

The possibility that the three electrophoretic components of desoxyribonuclease activity represented three distinct enzymes was next investigated. Preliminary examinations of human sera suggested that there were considerable

TABLE II

*Specific Inhibition of the Three Enzyme Components by Homologous Rabbit Antisera**

Antiserum	Enzyme	Dilutions of antiserum						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
Anti-A	A	+	0	0	0	0	+++	++++
	B	++++	++++	++++	++++	++++	++++	++++
	C	++++	++++	++++	++++	++++	++++	++++
Anti-B	A	++++	++++	++++	++++	++++	++++	++++
	B	0	0	0	0	0	0	++++
	C	++++	++++	++++	++++	++++	++++	++++
Anti-C	A	++++	++++	++++	++++	++++	++++	++++
	B	++++	++++	++++	++++	++++	++++	++++
	C	++++	0	0	0	++++	++++	++++

* Unabsorbed sera.

TABLE III

Absorption of a B Cross-Reaction from an Anti-A Serum

Antiserum	Enzyme	Dilutions of antiserum						
		1:10	1:20	1:40	1:80	1:160	1:320	1:640
Unabsorbed	A	0	0	0	0	0	0	++++
	B	0	0	0	0	++	++++	++++
	C	+++	++++	++++	++++	++++	++++	++++
Absorbed	A	+	0	0	0	0	+++	++++
	B	++++	++++	++++	++++	++++	++++	++++
	C	++++	++++	++++	++++	++++	++++	++++

differences in the capacity of the sera to neutralize the enzymatic activity of the three components. Table I illustrates the differences in titer shown by a single human serum when tested with A, B, and C components. Subsequent studies of large numbers of human sera have indicated that the ability of a serum to inhibit one component is independent of its ability to inhibit the other two components (26).

Confirmation of the serological specificity of the three components was obtained by immunizing rabbits with electrophoretically separated fractions.

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Antibody formation was followed by the alcohol precipitation test. Table II demonstrates the specific inhibition of each component by its homologous

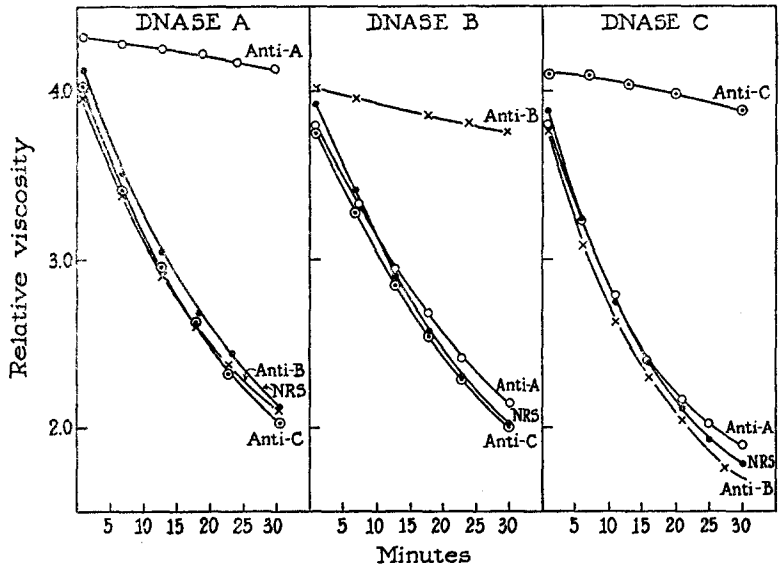

FIG. 4. Specific inhibition of desoxyribonuclease activity by rabbit antisera, as demonstrated in the viscosimetric test (DNASE = desoxyribonuclease).

TABLE IV

Neutralizing Effect of Antibody to B Component from Two Different Strain Sources

Antiserum	Enzyme		Dilutions of antiserum								
	Component	Strain source	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Anti-B (H105)	A	H105	++++	++++	++++	++++	++++	++++	++++	++++	++++
	B	H105	++++	0	0	0	0	0	+++	++++	++++
	B	Dematteo	++++	0	0	0	0	+	++++	++++	++++
	C	H105	++++	++++	++++	++++	++++	++++	++++	++++	++++
Anti-B (Dematteo)	A	H105	++++	++++	++++	++++	++++	++++	++++	++++	++++
	B	H105	++	0	0	0	0	0	0	0	++++
	B	Dematteo	+	0	0	0	0	0	0	+++	++++
	C	H105	++++	++++	++++	++++	++++	++++	++++	++++	++++

antiserum. It should be noted that the enzymatic activity recorded in the lowest dilution of homologous antiserum (particularly anti-C) did not represent unneutralized streptococcal desoxyribonuclease, but rather a zone phenomenon due to the presence of residual intrinsic serum nuclease which was not destroyed by heating. In general, serum nuclease is present in larger amounts in rabbit sera than in human sera (21).

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TABLE V

Neutralizing Effect of Antibody to A Component on A Component from Two Different Sources

Antiserum	Enzyme		Dilutions of antiserum					
	Component	Strain source	1:10	1:20	1:40	1:80	1:160	1:320
Anti-A (H105)	A	H105	+++	0	0	0	++	++++
	A	H46A*	+++	0	0	0	+	+++
	B	H105	+++	++++	++++	++++	++++	++++
	C	H105	+++	++++	++++	++++	++++	++++

* Extracellular concentrate provided by Dr. Alan Bernheimer.

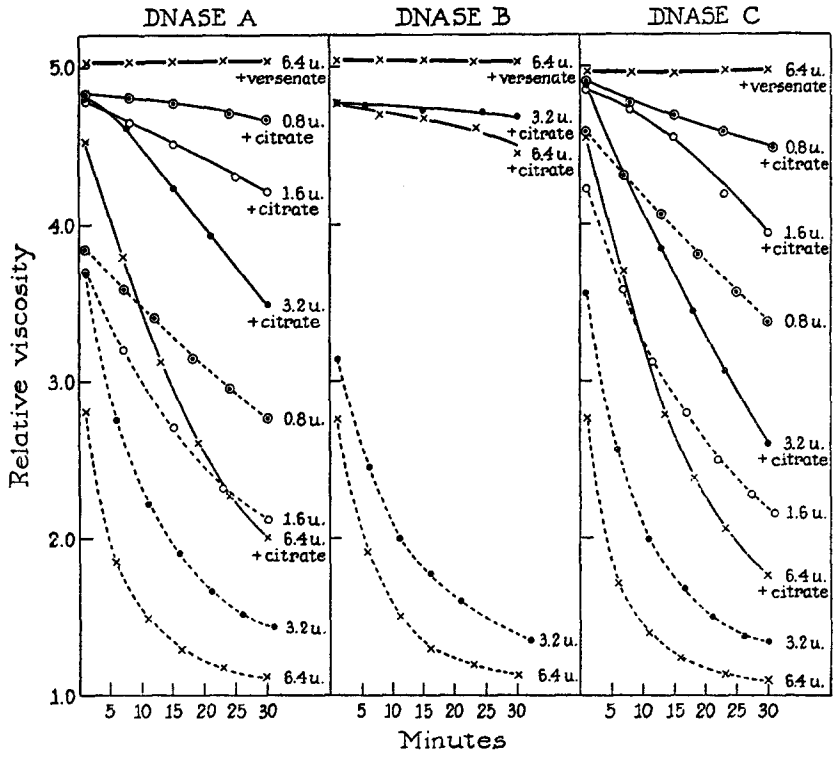

FIG. 5. Inhibition of enzymatic activity by citrate and versenate, as demonstrated in the viscosimetric test (DNASE = desoxyribonuclease). The broken lines indicate activity without inhibition and the solid lines indicate activity in the presence of inhibitor (0.03 M sodium citrate or 0.03 M sodium versenate as indicated).

Cross-reacting sera were encountered in some rabbits following immunization. Cross-reactions could be removed by absorption with appropriate antigen preparations. An example of the absorption of a B cross-reaction from an anti-A serum is given in Table III. Cross-reacting sera were apparently the

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result of contamination of antigen preparations with small amounts of heterologous antigen, since increased specificity (minimal or no cross-reactions) were obtained when immunization was performed with antigen preparations which had been recycled on the starch block.

The serological specificity of the three components could also be demonstrated by the more sensitive viscosimetric test. As shown in Fig. 4, each of

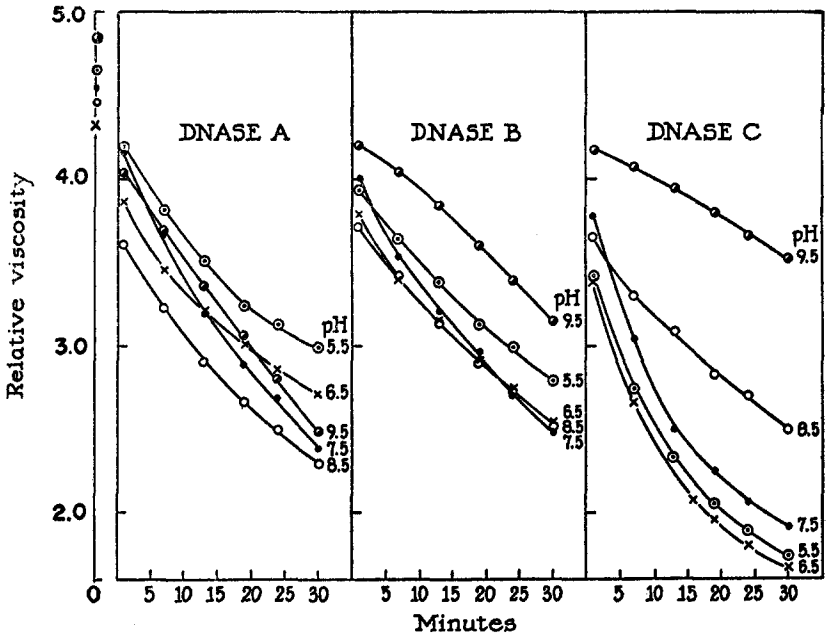

FIG. 6. Variation of enzymatic activity with pH (DNASE = desoxyribonuclease). Determinations at pH 5.5 and 6.5 were in 0.025 M phosphate buffer; determinations at pH 7.5, 8.5, and 9.5 were in 0.025 M veronal buffer. The initial viscosity (at zero time) is an average of three determinations.

the three enzymes was specifically neutralized by homologous antiserum but was unaffected by normal rabbit serum or heterologous antiserum.

Antibody to a specific component from one strain of streptococcus neutralized the corresponding component from a different strain of streptococcus. This is illustrated in Tables IV and V. As indicated in Table IV, antibody prepared against component B of strain H105 neutralized the B components of both strain H105 and strain Dematteo. Similarly, antibody prepared against component B of strain Dematteo neutralized the B components of both strains. As demonstrated in Table V, antibody prepared against component A of strain H 105 neutralized the A component of this strain and also the A component of strain H46A, a group C streptococcus. Therefore, it would

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appear that serological specificity pertains to the particular component or enzyme tested and not necessarily to the strain, although strains usually produce more of one enzyme than another.

Since it had been previously demonstrated that both pancreatic desoxyribo-

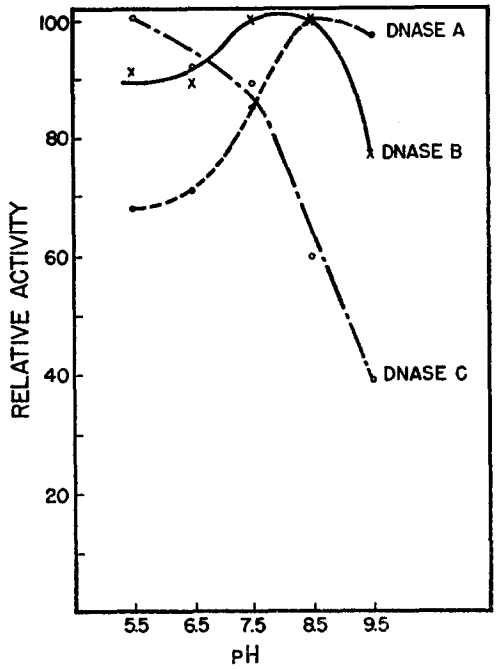

FIG. 7. Relative enzymatic activities at various hydrogen ion concentrations (DNASE = desoxyribonuclease). For each enzyme, the activity at each pH level is expressed as per cent of the maximal activity observed for that enzyme. Relative activities are based upon the decrease in viscosity observed during the first 13 minutes of incubation (Fig. 6). Because of the variation in initial viscosity of the substrate with pH, activities have been calculated as the decrease relative to the initial viscosity at that pH. When a uniform initial viscosity (*e.g.* the average value for all pH levels) is used for calculations, similar curves are obtained except that the decreases indicated at the two extremes of pH are somewhat greater.

nuclease and streptococcal desoxyribonuclease required magnesium ions for activation (12, 1), the effects of chelating agents on the three enzymes were studied. In the experiment illustrated in Fig. 5, comparable amounts of the three enzymes were tested at various levels with and without the addition of a chelating agent. Versenate appeared to inhibit all three enzymes completely even when relatively large amounts of enzyme were used. On the other hand, there appeared to be certain quantitative differences in the inhibition of the three enzymes by citrate. The B component was strongly inhibited by citrate

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whereas both the A and C components were only partially inhibited. The inhibition of the latter enzymes was only approximately 50 per cent, and the degree of inhibition was remarkably constant at several levels of enzyme concentration.

The effects of variation in pH on the enzymatic activity of the three electrophoretic components were compared. The viscosimetric test was used. As demonstrated in Fig. 6, there was some variation in the initial viscosity of the substrate with pH. The A component appeared to be more active in the alkaline range than in the acid range. Conversely, the C component was more active in the acid range and less active in the alkaline range. The B component appeared to be intermediate and was comparatively active over a rather broad neutral range. In Fig. 7, these data are presented in a different form. The activities of the three enzymes at the various pH levels were calculated and the relative activities determined. It is apparent from this figure that the activity of component A was about 30 per cent less at pH 5.5 than at the optimal pH of 8.5. The activity of component C was appreciably inhibited at pH 8.5 and markedly inhibited at pH 9.5 with an optimum at pH 5.5. The broad range of the B component extended from the nearly optimal activities at pH 5.5 and 6.5 to the maximal activities at pH 7.5 and 8.5, with some drop off in activity at pH 9.5.

DISCUSSION

In highly polymerized desoxyribonucleic acid, the only bonds known to be susceptible to the action of enzymes are the phosphoric ester linkages between nucleotide groups, and, so far as is known at present, these are attacked only by specific desoxyribonucleases. Phosphatases, deaminases, and nucleosidases will not attack desoxyribonucleic acid unless it has been previously degraded or enzymatically digested. Sherry and Goeller (27) have reported the liberation of free purine bases and pyrimidine desoxyribonucleosides by prolonged digestion of desoxyribonucleic acid with large amounts of streptococcal preparations. However, these preparations were unfractionated concentrates of extracellular products, containing a mixture of enzymes (23), and presumably included some of those identified here which could have initiated digestion.

Since the three enzymes of the present study appear to be independently capable of depolymerizing desoxyribonucleic acid in the absence of other enzymes which might initiate degradation, it can be tentatively assumed that they are true desoxyribonucleases, as defined by Kunitz (13). Certain differences in the mode of action of non-streptococcal desoxyribonucleases isolated from different sources have been reported (9, 28–32). It is possible, therefore, that the three enzymes described here might have distinguishable chemical activities, and studies to determine possible differences in end products of digestion by the three enzymes are in progress.

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Previous investigations with pancreatic desoxyribonuclease (13) have related the concentration of magnesium ion required for optimal effect to the concentration of substrate and not to the concentration of enzyme. It has, therefore, been suggested that activation by magnesium ions is accomplished by combination with substrate rather than by formation of an enzyme-activator complex. This interpretation is in keeping with the present observation that a rather constant degree of citrate inhibition was obtained with various concentrations of a particular streptococcal enzyme. However, the more effective inhibition of desoxyribonuclease B by citrate suggests that the magnesium requirement for this enzyme may be greater than that for the other two. It is somewhat difficult to relate this finding to earlier observations, and further studies will be required to elucidate this point.

In the studies of citrate inhibition, a distinct lag was often observed in the initial rate of depolymerization. A similar phenomenon also occurred with low concentrations of enzyme. An initial lag has been previously noted in measurements of desoxyribonuclease activity by the increase in ultraviolet light absorption (13), and has also been observed in the viscosimetric test when minimal quantities of pancreatic enzyme were used (33). Since the former technique permits more frequent observations during the earlier stages of digestion the lag is more readily demonstrated. On the other hand, viscosimetric readings cannot be conveniently made at frequent intervals; therefore, the initial lag is obscured unless the reaction is slowed down by citrate or by decreased enzyme concentration.

The differences in optimal pH of activity exhibited by the three electrophoretic components provided additional evidence that the enzymes were chemically distinct. However, some caution must be exercised in the interpretation of differences in the viscosimetric test since other factors such as the electrolyte concentration and extremes of pH may have influenced the viscosity of desoxyribonucleate. In order to explore a sufficiently broad hydrogen ion concentration, it was necessary in these studies to use two different buffer systems at identical molar concentrations. Because of variation with pH in the initial viscosity of the substrate solutions prior to the addition of enzyme, relative activity was determined by the fall as related to these initial values.

In the absorption of cross-reacting rabbit sera with heterologous antigen, a precipitate formed slowly over a period of 1 to 7 days. When the precipitate was removed by centrifugation, the supernate showed a decreased or no cross-reaction. By the addition of appropriate antigen, homologous antibody could also be removed coincident with the formation of a precipitate. In the light of these observations, it appears that the question of whether antibodies to these enzymes are of the precipitating or non-precipitating type must be reopened for further examination.

The one group C strain (H46A) examined in these studies showed

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predominantly A enzyme whereas 4 out of 5 group A strains examined showed predominantly B enzyme. These findings may relate to the observations of Bernheimer and Ruffier (4, 20) that bacterial ribonucleic acid partially inhibited the desoxyribonuclease activity of group A streptococci but failed to inhibit the desoxyribonuclease activity of several group C strains. The group C strain (H46A) examined here was also one of the strains whose desoxyribonuclease activity was found by Bernheimer and Ruffier not to be inhibited by bacterial ribonucleic acid. It, therefore, seems possible that their findings are an expression of the difference in response of the three enzymes described here to this inhibitor and in the occurrence of these enzymes among strains of these two groups. Further studies are required to establish or refute this possibility.

The electrophoretic and immunologic similarity of the major desoxyribonuclease components of H105, a group A strain, and H46A, a group C strain, indicates that at least one of the enzymes indentified here is not restricted to group A strains. This latter strain is used in the preparation of varidase[2] and has, therefore, been widely employed in studies of the action of streptococcal desoxyribonuclease (streptodornase). However, the major component of this strain, desoxyribonuclease A, does not appear to be the predominant enzyme of most strains of group A streptococci. From the observations reported here and from more extensive studies of the immunologic response in patients with acute rheumatic fever or following uncomplicated streptococcal infection (26), desoxyribonuclease B appears to be much more commonly encountered than either desoxyribonuclease A or C.

Further studies will be necessary to determine the exact prevalence of these enzymes among strains of group A streptococci. However, the demonstration of three immunologically distinct streptococcal desoxyribonucleases, the occurrence of which is a function of strain variation, appears to provide a logical explanation for the paradox previously noted in the immunological response to this streptococcal enzyme following natural infection (21).

SUMMARY

Group A streptococci were found to produce three different desoxyribonucleases, designated here as A, B, and C. These enzymes migrated separately on analysis with starch zone electrophoresis, were immunologically distinct, and showed certain differences in inhibition by citrate and in optimal pH of activity. The relative amount of each enzyme produced was a function of strain variation.

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[2] Lederle.

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