

ELECTROPHORETIC STUDIES OF THE EXTRACELLULAR PRODUCTS OF GROUP A STREPTOCOCCI

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During growth group A streptococci elaborate a variety of products which can be identified in the supernatant broth by their respective biological activities. Among the better known of these extracellular products are the erythrogenic toxins, streptolysins O and S, streptokinase, and streptococcal hyaluronidase, desoxyribonuclease, ribonuclease, proteinase, amylase, and diphosphopyridine nucleotidase (1, 2). Investigation of the individual properties of these products and of their possible significance in the pathogenesis of streptococcal diseases and their complications has been hampered by the nature of the preparations available for study. Crude concentrates of the extracellular products contain a mixture of the known streptococcal enzymes,¹ additional streptococcal substances of uncertain nature, and considerable amounts of constituents from the growth medium. Although some of the extracellular substances have been obtained in a more purified state, only streptococcal proteinase and its precursor have been crystallized (3).

In the present investigation, concentrates of the extracellular products of group A streptococci were prepared which were essentially free of constituents of the medium. These concentrates were studied by zone electrophoresis. Separation of several of the known enzymes was achieved, and certain strain variations in the electrophoretic patterns were demonstrated.

Materials and Methods

Strains of Group A Streptococci.—Sixty-six different strains of group A streptococci were studied for their ability to grow in the dialysate medium used for preparation of concentrates of extracellular products. Forty serological types were represented.

Concentrates of the extracellular products were prepared from the following strains:

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.

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¹ Although some of the extracellular products, *e.g.* streptolysin O, have not been clearly identified as enzymes, for purposes of convenience this term is used to embrace them as well as those whose enzymatic nature is more definitely established.

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

Strain Scheuring, No. 3, obtained from a family epidemic of acute glomerulonephritis in Minneapolis, type "Red Lake" (4).

Strain Jiminez, No. 1, obtained from a patient with acute glomerulonephritis in Chile, supplied by Dr. Richard Krause, type "Red Lake" (4).

Dialysate Medium.—Group A streptococci were grown in a dialysate medium, which was a modification of that described by Dole (5). It consisted of combined dialysates of beef heart infusion, casamino acids, and Pfanstiehl peptone, R.I., plus dextrose, sodium bicarbonate, and buffer salts.

The dialysate of beef heart infusion could be prepared in advance, concentrated, and stored in the frozen state. Fresh beef hearts were stripped of fat and ground. Ten pounds of the ground meat was infused overnight at 4°C. in 2 liters of cold tap water. The next day the infusion was heated to 85°C. for 30 minutes, allowed to cool, and filtered through filter paper (Eaton and Dikeman No. 195). The infusion was concentrated *in vacuo* to approximately 300 ml. and dialyzed at 4°C. against three changes of distilled water for 12 hours each. *Thick wall* seamless cellulose casings, $2\frac{1}{2}$ inch inflated diameter (the Visking Corporation) were used for dialysis. The three dialysates were combined, concentrated to about 400 ml. *in vacuo*, and stored in the frozen state. One-twentieth of a lot was used per liter of complete medium.

For preparing 5 liters of complete broth, 208 gm. of Pfanstiehl peptone, R.I., 416 gm. of casamino acids (Difco "certified"), and 25 gm. of charcoal (decolorizing carbon, General Chemical Co., New York City) were heated in 1 liter of distilled water to 80°C. for 15 minutes and filtered while hot through filter cel. Upon cooling a precipitate formed which subsequently disappeared with dialysis. The suspension was dialyzed against 2 liters of distilled water at 4°C. After 18 hours, the first dialysate was removed, and the dialysis was repeated against 2 liters of fresh distilled water. The combined dialysates were reabsorbed at 80°C. with 105 gm. of charcoal, and filtered through filter cel. The volume was brought to 5 liters by addition of distilled water; and 7.5 gm. of Na_2HPO_4 , 6.4 gm. of KH_2PO_4 , 25 gm. of dextrose, 10 gm. of NaHCO_3 , and one-fourth lot of concentrated dialysate of beef heart infusion were added. The pH was adjusted to 7.8 with 5 N NaOH. The completed broth was sterilized by filtration through Coors P3 filters and immediately inoculated. For starting cultures, smaller amounts could be stored in the frozen state. In frozen portions, it was preferable to defer addition of the NaHCO_3 , adjustment of the pH, and filtration until thawing just prior to use.

A 50 ml. sample of the completed medium was brought to 0.8 saturation by the addition of 23 gm. of ammonium sulfate. In suitable preparations, no precipitate formed at this salt concentration. Since some variation was found among lots of Pfanstiehl peptone, R.I., lots were selected which gave no precipitate.

Estimation of Growth in Dialysate Medium.—Growth of group A streptococci in the dialysate medium was estimated turbidimetrically. The optical density was read at 620 μ in a Coleman junior spectrophotometer. Uninoculated dialysate medium from the same lot was used as a blank.

In the survey of 66 strains for their ability to grow in the dialysate broth the strains were first grown overnight in blood broth. One loopful of this overnight growth was transferred to 5 ml. of dialysate medium, and the mixture was incubated at 37°C. for 18 hours.

Preparation of Concentrate of Extracellular Products.—Strains of group A streptococci were grown overnight in blood broth, and 0.1 ml. of the fresh culture was inoculated into 40 ml. of dialysate broth. After 18 hours at 37°C., the culture was centrifuged, the supernatant fluid was discarded, and the streptococcal cells were washed twice with sterile physiological saline. The washed cells were resuspended in 5 ml. of sterile physiological saline and inoculated into 250 ml. of dialysate broth. After 6 hours at 37°C., this culture was inoculated in 50 ml. amounts into flasks containing 1.5 liters of dialysate broth. The inoculated flasks were incubated at

37°C. for 16 hours. The cultures were chilled in an ice bath, and all further preparative procedures were done at 4°C. The bulk of the streptococcal cells was removed by centrifugation at 2000 R.P.M. for 1 hour. The supernatant fluid was decanted, and 0.7 per cent chloroform added. After mixing, excess chloroform was removed by decanting. The volume was measured, and the fluid was brought to 0.8 saturation by the addition of 560 gm. of ammonium sulfate per liter. After standing for 1 to 12 hours, the resulting precipitate was removed by suction filtration through a pad of washed potato starch. Suction was discontinued while the precipitate on the starch pad was still moist. The precipitate was redissolved by washing the starch pad three times with glycine buffer pH 9, $\mu = 0.01$, in a total volume approximately one-hundredth that of the original culture supernate. The solution of extracellular products was placed in a collodion bag (Schleicher and Schuell Co.) and dialyzed under vacuum (40 to 50 cm. Hg) against glycine buffer pH 9, $\mu = 0.01$. Further concentration to a final volume of approximately 1 ml. was achieved by vacuum filtration through the collodion bag (6). The concentrate was either immediately examined by zone electrophoresis or frozen and dried *in vacuo*. When dried in the presence of buffer salt, the product was more readily redissolved than when dried after dialyzing salt-free.

Blank concentrates of uninoculated dialysate medium were prepared by the same methods used for concentrating culture supernates. The pH of the uninoculated broth was lowered to 6.5 by the addition of lactic acid in order to make this comparable to the pH of the culture supernatant.

Electrophoresis.—Zone electrophoresis with starch as a supporting medium was done by the method of Kunkel and Slater (7). Veronal buffer, $\mu = 0.1$, pH 7.6 and 8.4, and glycine buffer, $\mu = 0.1$, pH 9, were used. The starch was washed twice in distilled water and once in the appropriate buffer prior to pouring the block.

The concentrates were prepared for placing on the starch block by dialysis against the buffer to be used. *Thin wall* dialysis casings, $2\frac{1}{2}$ inch inflated diameter (The Visking Corporation), were soaked in sodium versenate, pH 7.6, to remove heavy metals and rinsed in distilled water and buffer. The concentrate was placed in the casing and dialyzed against buffer on a mechanical rocker for 6 hours at 4°C. The concentrate was centrifuged in the cold at 2500 R.P.M. for 30 minutes to remove any insoluble residue, and placed on a 45 cm. starch block. A direct current with a potential difference of 400 volts was used in all experiments. Electrophoresis was done in a cold room with an ambient temperature of 2–4°C. The time used for separation varied from 13 to 24 hours as indicated.

The starch block was sectioned into 1 cm. strips. The segments were eluted with buffer and examined for protein and enzymatic activity. Quantitative recovery was facilitated by the use of a sintered glass filter of medium porosity (Corning Glass Works).

Protein and Enzyme Determinations.—Protein determinations were performed by the modified Folin-Ciocalteu phenol reaction (8). A preparation of bovine plasma albumin was used as reference standard. The carbonate reagent was prepared by mixing equal parts of a 1 per cent copper sulfate solution and a 2 per cent sodium tartrate solution and adding one part of this fresh mixture to 50 parts of 2 per cent sodium carbonate in 0.1 N NaOH solution. The phenol reagent was diluted with an equal volume of distilled water. To a 0.1 or 0.2 ml. aliquot of each eluate from the starch block, 2 ml. of the carbonate reagent and 0.2 ml. of the diluted phenol reagent were added. The mixture was allowed to stand for 30 minutes at room temperature and the optical density read at 650 $m\mu$ in a Coleman junior spectrophotometer. Since a small amount of color resulted from the starch alone, either the eluate of the first segment of the starch block or the eluate from the final washing of the starch in buffer was used as a blank.

For measuring streptolysin O, desoxyribonuclease, and ribonuclease activity, dilutions were made in neopeptone dialysate broth, pH 7.6 (5).

Streptolysin O was determined by incubating 0.1 ml. samples of the dilution mixtures

with 0.2 ml. of 0.1 M cysteine in neopeptone dialysate broth, pH 7.6, and 0.2 ml. of a 5 per cent suspension of washed rabbit red cells. Hemolysis was read after 45 minutes at 37°C. The highest dilution resulting in detectable hemolysis was taken as the end point.

Desoxyribonuclease activity was measured by the alcohol precipitation test of McCarty (9). Sodium desoxyribonucleate was extracted from calf thymus (9). Substrate solution was prepared containing 0.1 per cent sodium desoxyribonucleate and 0.01 M $MgSO_4$ in 0.025 M veronal buffer, pH 7.7. 0.1 ml. samples of appropriate dilutions of the starch block eluates were added to 0.5 ml. portions of the substrate solution and incubated at 37°C. for 30 minutes. One ml. of absolute alcohol was added, and the end point was read as the highest dilution in which no floating fibrous mass appeared. For monitoring the amount of enzymatic recovery during the preparation of concentrates, desoxyribonuclease activity was measured by the more precise viscosimetric test (9). 0.5 ml. of the appropriate enzyme dilution in neopeptone dialysate broth was added to 4.5 ml. of the substrate solution in an Ostwald viscosimeter. The change in viscosity was determined at approximately 5 minute intervals for 30 minutes during the course of enzymatic degradation at 37°C. A unit has been defined as the amount of enzyme which causes a fall of 1.0 in relative viscosity in a 20 minute period (10).

Ribonuclease activity was determined turbidimetrically (11). The substrate solution contained 0.2 per cent sodium yeast ribonucleate (Schwarz Laboratories, Inc.). 0.5 ml. of the dilutions to be tested was incubated with 0.5 ml. of substrate solution for 30 minutes at 37°C. One ml. of 1 N HCl was added, and after 10 minutes, the turbidity was read in a Coleman junior spectrophotometer at 425 $m\mu$. The highest dilution which resulted in a 50 per cent reduction in optical density was taken as the end point.

Streptokinase activity was estimated by a modification of the method of Kaplan (12). Dilutions of the eluates from the starch block were made in buffered saline solution (0.05 M veronal buffer, pH 7.7, diluted to 0.01 M with 0.85 per cent NaCl solution). The substrate solution contained 0.4 per cent fraction I human plasma (E. R. Squibb & Sons) in buffered saline. 0.5 ml. of substrate solution was added to 0.1 ml. of appropriate dilution of the starch block eluate. This mixture was clotted by adding 0.1 ml. thrombin solution (10 units/ml.). The clots were incubated for 30 minutes at 37°C. The end point was read as the highest dilution resulting in lysis of the standard clot.

RESULTS

The dialysate medium proved satisfactory for the growth of most strains of group A streptococci and for the production of streptolysin O, desoxyribonuclease, ribonuclease, and streptokinase.

Of the 66 strains of group A streptococci, representing 40 different types, 53 multiplied in the dialysate medium. The optical density was greater than 0.30 for 26 strains, 0.20 to 0.29 for 22 strains, 0.10 to 0.19 for 5 strains, and less than 0.10 for 13 strains.

In larger lots, strains such as D58 and H105 grew profusely, yielding about 1 gm. of dried bacterial cells per liter of culture fluid. The activities of the several enzymes in the original broth supernates were measured after removal of the bacterial cells by centrifugation. Centrifugation at 2000 R.P.M. in an International SB2 centrifuge was found to be preferable to Sharples centrifugation, which results in a loss of about half of the streptolysin O activity at this stage. Addition of 0.7 per cent chloroform as a disinfectant did not appreciably lower the enzyme titers in the original supernates.

The original enzyme activities and the per cent recovery at various stages in the preparation of concentrates are illustrated in Table I. In this typical example, the three enzymes desoxyribonuclease, streptolysin O, and ribo-

nuclease were demonstrated in readily measurable titers in the original supernate. After salting out with ammonium sulfate and redissolving the precipitate in one-hundredth the original volume, there was some loss of desoxyribonuclease and streptolysin O activities but an apparent gain in ribonuclease activity. This gain was probably due to the removal of an unidentified inhibitor. The presence of such an inhibitor in the original broth supernate was suggested by the retarded ribonuclease activity of low dilutions of this supernate; activity could be demonstrated by dilution beyond the

TABLE I
Enzymatic Activities at Various Stages of Preparation of Lot 20, Strain D58

Stage of preparation	Volume or weight	Desoxyribonuclease			Streptolysin O		Ribonuclease	
		Total units	Units per ml. or mg.	Recovery	Dilution end point	Recovery	Dilution end point	Recovery
				<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
Original broth supernate...	3000 ml.	161×10^4	537	100	1:16	100	1:16	100
After precipitation with $(\text{NH}_4)_2\text{SO}_4$	30 ml.	150×10^4	502×10^3	93	1:1200	75	1:2500	160
Concentrated with collodion bag.....	3 ml.	103×10^4	345×10^3	64	1:8000	50	1:14,000	90
Dried product.....	104 mg.	78×10^4	7,600	48	5 $\mu\text{g.}/\text{ml.}$	40	2 $\mu\text{g.}/\text{ml.}$	100

TABLE II
Loss of Desoxyribonuclease Activity at 4°C., Lot 15, Strain D58

Stage of preparation	Days at 4°C.	Total units	Recovery
			<i>per cent</i>
Original supernate (10 liters)	0	6.84×10^6	100
1000 \times concentrate	0	2.88×10^6	42
“ “	2	2.08×10^6	30
“ “	4	1.78×10^6	26
“ “	7	1.74×10^6	25

point of inhibition. After one thousandfold concentration by vacuum filtration through a collodion bag, there was some loss in activity of all three enzymes. This loss was not due to passage of enzymes through the collodion membrane since no activity could be detected in the filtrates; apparently it was due to inactivation on standing at 4°C., which occurred in even the most concentrated preparations. An example of the loss of desoxyribonuclease activity under such conditions is given in Table II. The desoxyribonuclease activity of the thousandfold concentrated preparation decreased by almost one-half after 7 days at 4°C.

Assay of the dried product (Table I) indicated a further loss of desoxyribonuclease and streptolysin O activity, with a final recovery of 48 and 40 per cent respectively. The ribonuclease activity of the dried product was approx-

imately equivalent to that of the original, although there was a decrease from the maximal activity observed after removal of the inhibitor. Up to 50 per cent loss of the activity of all three enzymes was incurred in the drying of some preparations. Such large losses were particularly likely to be encountered when the concentrate was dialyzed salt-free or was allowed to stand at 4°C. for several days prior to drying.

In Table III the desoxyribonuclease activities per unit weight of the final product are given for several lots. Yields of up to 26,000 units per mg. were

TABLE III
Yield of Total Extracellular Products and Desoxyribonuclease for Various Lots

Strain	Lot No.	Total extracellular products		Desoxyribonuclease	
		Folin	Dry weight	Final concentrate	Dried product
		<i>mg. per liter</i>	<i>mg. per liter</i>	<i>units per mg.</i>	<i>units per mg.</i>
D58	1		7.2		
"	9	11.3	7.7		
"	10		8.3	52,000	26,000
"	17	9.0		27,000	
"	18	13.6		27,000	13,000
"	19	11.6	10.2	22,000	16,000
"	20		35	10,100	7,600
H105 OP	24		11.3*		
"	33	8.4			
Jiminez No. 1	22		6.2*		
Dematteo	23		5.1*		
Scheuring No. 3	26		20*		
Blank preparation	35	<0.02			

* Calculated by deduction of weight of buffer salt.

recovered in the dried product. The yield for lot 20 was somewhat lower than that generally encountered.

Streptokinase was produced by streptococci growing in the dialysate medium and could be readily demonstrated in concentrates, but the per cent recovery of this enzyme was not investigated. The strains used in preparing concentrates did not elaborate hyaluronidase in sufficient quantity for study. Under the conditions employed, proteinase was not produced.

The yield of total extracellular products was determined by several methods. The yield in terms of total protein in the final concentrate was estimated by the modified Folin-Ciocalteu test. In preparations which were subsequently dried, the dry weight was determined. Determinations by the two methods were in fairly close agreement, suggesting that the extracellular products consisted largely of protein material (Table III). Spectrophotometric analysis in the ultraviolet range also gave a pattern typical of protein, with maximal

absorption at 278 and minimal at 250 to 260 at which nucleic acid components are detected (Fig. 1).

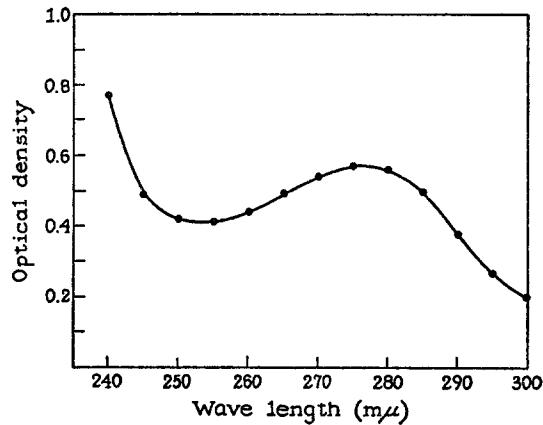


FIG. 1. Spectrophotometric analysis of concentrate of extracellular products of group A streptococci, strain D58.

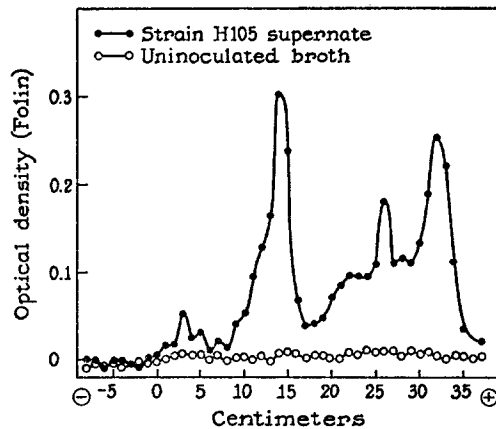


FIG. 2. Electrophoretic patterns of concentrate of extracellular products (culture supernate of strain H105) and of concentrate of uninoculated broth. The blank preparation was equivalent to three times the concentration of the culture supernate preparation. Both preparations were examined under identical conditions of electrophoresis (glycine buffer, $\mu = 0.1$, pH 9, 400 volts for 16 hours). The two patterns illustrate the distribution of total protein as determined by the modified Folin-Ciocalteu technique.

Table III indicates that the weight of the total extracellular products was quite low. Most yields were close to 10 mg. per liter of original culture supernate, with a range from 5 to 35 mg. per liter.

Blank preparations made from uninoculated dialysate medium yielded negligible amounts of protein. For example, Lot 35 (Table III) showed less

than 0.02 mg. per liter of original broth. This indicates that the concentrates of the extracellular products are essentially free from constituents of the growth medium.

Examination of blank concentrates by starch zone electrophoresis also indicated that components of the medium did not contribute appreciably to the electrophoretic pattern obtained.

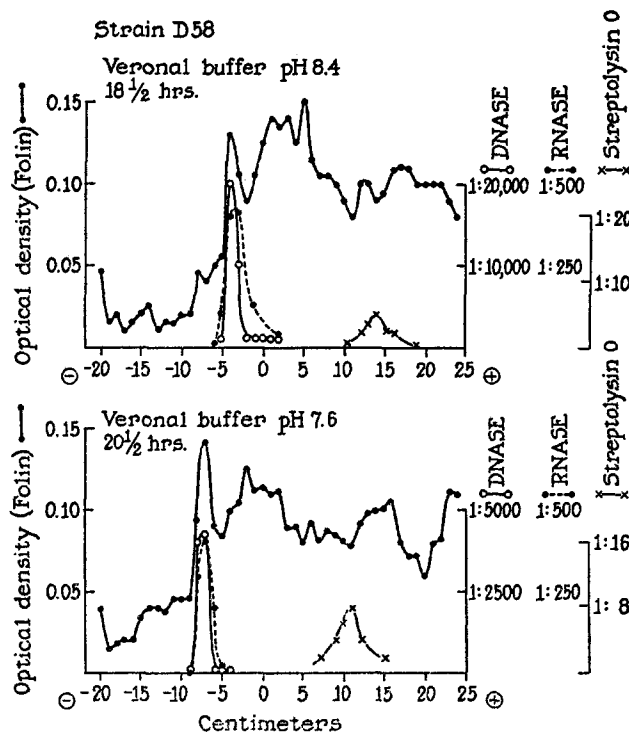


FIG. 3. Electrophoretic patterns of concentrate of extracellular products of strain D58 in veronal buffer, pH 8.4, and 7.6. The uppermost curves illustrate the distribution of total protein. DNASE, desoxyribonuclease. RNASE, ribonuclease.

In Fig. 2, the electrophoretic pattern of a blank preparation (Lot 35) is contrasted with that of a preparation made from a culture supernate. The two concentrates were prepared in a similar manner and the electrophoretic separation was performed in the same way. Although the blank preparation was equivalent to three times the concentration of the culture supernate, it gave a pattern which is essentially flat and which did not rise above the base line. In contrast, the preparation from the culture supernate (strain H105) showed considerable protein with separation into several distinct peaks.

Concentrates of the extracellular products were examined by starch zone electrophoresis under a variety of conditions. The buffer system, the pH, and the length of time were altered in an attempt to find the optimal method of separation. Strain D58 was used for these studies.

Fig. 3 illustrates the types of electrophoretic separation obtained with veronal buffer, $\mu = 0.1$ at pH 8.4 and at pH 7.6. In this and in subsequent illustrations, the concentrate of extracellular products was initially placed at the point indicated as 0 on the abscissa. The distribution of protein as determined by the modified Folin-Ciocalteu method is indicated by the uppermost curve, and the location of several of the known enzymes is shown in the other curves. In Fig. 3, it can be seen that after 18½ and 20½ hours, respectively, there was considerable spreading of the protein in both of the patterns, with the develop-

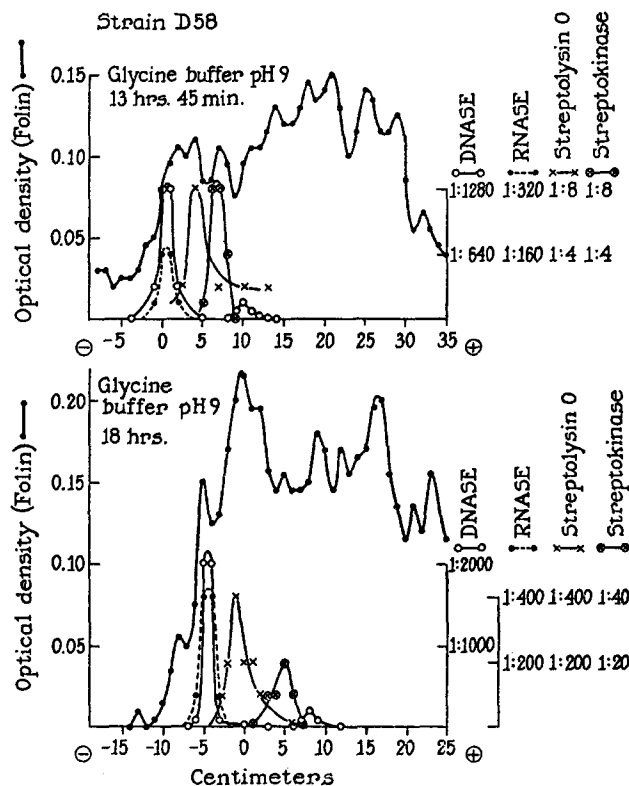


FIG. 4. Electrophoretic patterns of concentrate of extracellular products of strain D58 in glycine buffer, pH 9 at 13 hours, 45 minutes, and at 18 hours. The uppermost curves illustrate the distribution of total protein. DNASE, desoxyribonuclease. RNASE, ribonuclease.

ment of a rather distinct peak moving toward the cathode. Desoxyribonuclease and ribonuclease activities were both located in this peak and do not appear to have separated. Streptolysin O separated clearly from the other two enzymes but did not seem to correspond with any definite peak in the protein pattern.

Glycine buffer, $\mu = 0.1$, pH 9, proved to be preferable to veronal buffers, because of the greater stability of the various enzymes in this buffer. In Fig. 4 two electrophoretic patterns obtained with glycine buffer are shown. The protein curves suggest a number of partially resolved peaks. Streptolysin O and streptokinase showed partial separation at 13 hours, 45 minutes, and better separation after 18 hours. There was perhaps some correspondence of these two enzymes with peaks in the protein curve. The protein peak cor-

responding with the distributions of desoxyribonuclease and ribonuclease had not resolved at 13 hours, 45 minutes, but was more clearly indicated after 18 hours. Again the distribution of activities for these two enzymes appeared to be identical. A smaller secondary peak of desoxyribonuclease activity was detected moving toward the anode. Further investigations indicated that this secondary peak represented another kind of desoxyribonuclease, and it was subsequently shown that group A streptococci can produce three different desoxyribonucleases which migrate separately on electrophoresis and are serologically distinct (13).

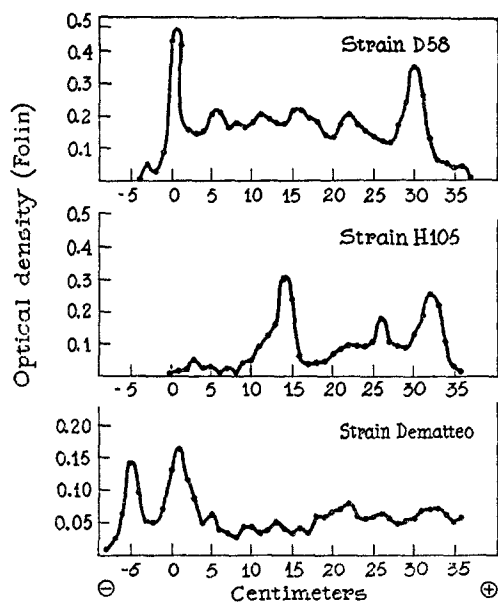


FIG. 5. Electrophoretic patterns of concentrates of extracellular products of three different strains of group A streptococci. All preparations were examined under identical conditions of electrophoresis (glycine buffer, $\mu = 0.1$, pH 9, 400 volts for 16 hours).

It should be noted in Figs. 3 and 4 that the great bulk of the protein mass and many of the distinct peaks did not correspond with any of the extracellular enzymes measured.

Certain variations were observed in the electrophoretic patterns of extracellular products from different strains of group A streptococci.

Fig. 5 illustrates the distributions found when the extracellular proteins of three such strains were fractionated under identical conditions. Both strain D58 and strain Dematteo showed a prominent peak near the origin, whereas this peak appeared to be absent in the pattern for strain H105. Large fast-moving peaks were seen at about 30 centimeters in both of the two upper patterns. However, these may not have been identical, since the one for strain H105 seemed to be moving slightly more rapidly than that for strain D58. The pattern for strain H105 showed a distinct peak at 14 centimeters. This strain is known for its pro-

duction of streptokinase, and it is of interest that high titers for this enzyme could be identified in this peak but nowhere else in the pattern. Clearly defined peaks at this location were not apparent in the other two patterns. In the pattern for strain Dematteo, there was a striking peak migrating toward the cathode, which was absent in the patterns for the other two strains.

In order to obtain satisfactory curves, relatively large amounts of the extracellular products (30 to 100 mg. or the concentrate from 3 to 10 liters of broth supernate) were required. With these amounts, major peaks in the electrophoretic pattern such as those pointed out above were reproducible in repeated patterns, whereas minor fluctuations were often not reproducible. Because of their high biological activities, the location of specific enzymes in the electrophoretic pattern could be identified with smaller amounts of the extracellular concentrate.

DISCUSSION

Most studies of the extracellular products of group A streptococci have been directed toward one specific component of the supernatant fluid. The purpose of the present investigation was to study the electrophoretic pattern of the total mass of extracellular products, to separate some of the known enzymes composing this pattern, and to note differences in pattern among various strains of group A streptococci.

The availability of a suitable medium for the production of the extracellular products of group A streptococci appeared to be essential to the purposes of this investigation. Although certain environmental conditions are known to affect the production of specific enzymes (1), the object here was not to favor the elaboration of any one enzyme, but to devise a medium in which several of the known enzymes were satisfactorily produced. Furthermore, the medium should not contain constituents which would contaminate the concentrates of extracellular products and contribute to the electrophoretic patterns obtained. The advantages of a defined medium for these purposes are obvious, but such media have so far proved unsatisfactory for group A streptococci.² Even semidefined media are suitable for the growth of only a few selected strains (1). The dialysate medium used in the present studies supported the growth of a variety of strains of group A streptococci and the production of a number of the extracellular products. Moreover, when suitable peptone preparations were used, this medium appeared to be free of large molecular materials which might contribute to the final concentrate.

The supernatant fluid of group A streptococci grown in this medium yielded concentrates which were low in weight yet high in specific enzymatic activities.

² Since preparation of this manuscript, a new chemically defined medium for group A streptococci has been described (14). Evaluation of the growth of a wide variety of strains and of the production of the various known enzymes will require further study.

The yield of total extracellular products was of the order of magnitude of only 10 mg. per liter. The weight of each individual enzyme must be only a small fraction of this total; the four enzymes identified in the electrophoretic pattern can at most account for only a small part of the total protein mass. Therefore, it seems likely that there are a number of components of the extracellular products which have not yet been identified. The existence of additional unidentified extracellular components is also suggested by the agar diffusion antigen-antibody studies of Harris, Harris, and Ogburn (15), and of Halbert, Swick, and Sonn (16).

Partial separation of streptokinase and streptolysin O was achieved by zone electrophoresis. On the other hand, desoxyribonuclease and ribonuclease activities appeared to share the same distribution under a variety of electrophoretic conditions. The interpretation of this finding is difficult. The activities may represent two distinct enzymes with similar electrophoretic mobilities. The alternative possibility is that a single protein is capable of depolymerizing both substrates. This latter possibility is supported by the observation of Bernheimer (17) that bacterial ribonucleic acid can partially inhibit the desoxyribonuclease activity of group A streptococci. The significance of these findings will have to be re-evaluated in the light of the recent observation that group A streptococci can produce three distinct desoxyribonucleases (13).

The striking differences in the electrophoretic patterns of several strains suggest that this may be an important aspect of strain variation among group A streptococci and useful in studying additional strains with known biological characteristics and epidemiological associations.

SUMMARY

Concentrates of the extracellular products of group A streptococci were prepared which were essentially free of constituents of the medium. The extracellular products appeared to consist primarily of protein material. The yield of total extracellular products was of the order of magnitude of 10 mg. per liter of supernatant broth. When studied by zone electrophoresis, the concentrates showed protein over a wide distribution with distinct peaks in certain areas. The locations of streptolysin O, streptokinase, desoxyribonuclease, and ribonuclease activities were identified, and partial separation of these enzymes was achieved. Considerable variation in the electrophoretic patterns of the extracellular products from different strains was noted.

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