THE LYSIS OF GROUP A HEMOLYTIC STREPTOCOCCI BY EXTRACELLULAR ENZYMES OF STREPTOMYCES ALBUS

II. Nature of the Cellular Substrate Attacked by the Lytic Enzymes

By MACLYN McCARTY, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

Plate 26

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In the preceding paper (1) evidence is presented for the view that lysis of group A streptococci by filtrates of *Streptomyces albus* is initiated by a non-proteolytic enzyme of undetermined nature. The potent proetolytic enzyme present in the filtrates is by itself incapable of dissolving streptococci and it appears to play a secondary role in lysis. The present investigation is concerned with the identity of the substrate in the streptococcal cell which is attacked by the non-proteolytic enzyme in the first stages of lysis. Identification of this substrate should not only provide an indication of the type of enzyme involved but in addition should lead to a better understanding of the structure of the streptococcal cell.

The experiments herein described indicate that the bacterial cell wall is the structure attacked in enzymatic lysis of streptococci. Studies on the chemical composition of the streptococcal cell wall are presented, and the data are related to previously known facts concerning the serological constituents of group A streptococci.

Materials and Methods

Group A Streptococci.—Group A strains of several serological types were employed. The organisms were grown in Todd-Hewitt broth, collected by centrifugation and washed three times with 0.85 per cent sodium chloride. The washed cells were treated with several volumes of acetone, washed with acetone and finally dried *in vacuo*.

Streptomyces albus Enzyme Preparations.—The concentrates of the lytic enzymes were prepared by the methods described in the preceding paper (1).

Analytical Methods.—Total nitrogen was determined by the direct nesslerization micro-Kjeldahl procedure of Koch and McMeekin (2). Total phosphorus determinations were made by the method of Allen (3). Quantitative hexosamine analyses were made by a modification (4) of the Elson and Morgan procedure (5) after hydrolysis with 2 N HCl at 100°C. in sealed ampoules. Reducing sugars were determined after hydrolysis by the Hanes modification (6) of the Hagedorn-Jensen method.

Methylpentose was determined without prior hydrolysis of the sample by the cysteine sulfuric acid method of Dische and Shettles (7).

EXPERIMENTAL

Preparation and Properties of Streptococcal Cell Walls

Preparation of Insoluble Residue of Mechanically Disrupted Cells.—In order to obtain mechanically disrupted cells in reasonably large quantities the procedure of grinding in a ball-mill was adopted.

The mill consists of a thick walled, round pyrex flask of one liter capacity about one-half full of stainless steel balls ¼ inch in diameter. Grinding is accomplished by rotating the flask at a 45° angle at a rate of 50 to 60 revolutions per minute. 1 gm. portions of acetone-dried streptococcal cells are ground for a period of 1 to 2 hours at 4°C. After this period of grinding, 95 to 99 per cent of the cells have been broken up so that they no longer possess their characteristic morphology, and Gram-stained preparations reveal that the material is largely in the form of Gram-negative debris. The ground cells are washed from the mill with 0.85 per cent NaCl.

Despite the apparent complete disruption of the cells, only about one-fourth to one-third of the total dry weight is extracted into solution in saline, and suspensions of ground cells have about the same appearance as heavy suspensions of intact bacterial cells. Much of the protein and nucleic acid of the streptococci remains in the insoluble residue. However, in contrast to the lack of effect of proteolytic enzymes on intact streptococci, the bulk of this insoluble residue can be put into solution by digestion with pancreatic enzymes. Trypsin alone removes more than two-thirds of the material remaining in the cellular debris. The following procedure has been employed for obtaining an insoluble streptococcal fraction of constant composition:

The saline suspension of ground streptococcal cells is centrifuged and the residue washed twice with saline. The residue is then resuspended in M/15 phosphate buffer pH 8 (about 20 cc. for each gram of original dried cells) and treated with crystalline pancreatic trypsin at a final concentration of 0.1 mg. per cc. After incubation at 37°C. for 2 to 3 hours, the residue, now greatly diminished in bulk, is thrown down by centrifugation. The sediment is resuspended in fresh phosphate buffer, crystalline chymotrypsin and ribonuclease are added at a final concentration of 0.05 mg. per cc. each, and incubation is continued for an additional 2 hours. In some instances, it has been found useful to use intestinal phosphatase in order to obtain preparations free of nucleic acid products. It is generally unnecessary to use desoxyribonuclease, since the desoxyribonucleic acid goes into solution without this treatment.

The residue after enzymatic treatment does not centrifuge readily at low speeds and consequently it is possible by differential centrifugation to remove any intact or partially fragmented cells. This is carried out with microscopic control until the preparation is essentially free of formed elements. The final insoluble residue is thoroughly washed with distilled water and is kept in distilled water suspension or dried *in vacuo* from the frozen state. Material so prepared represents 10 to 15 per cent of the dry weight of the original bacterial cells.

Properties of the Insoluble Residue.—Cell residues prepared by this procedure proved to be highly resistant to a variety of procedures employed in attempting to bring them into solution. Enzymes such as pepsin, papain, and lysozyme

have no observable effect. A variety of organic solvents appear neither to dissolve the material nor to extract any components, and this is true of reagents frequently employed in bacteriological studies such as 90 per cent phenol, strong solutions of glycine and urea, and various detergents. Indeed, with the exception of hydrolysis with strong alkali or acid, the material appears to be little affected by any chemical treatment employed.

In contrast to this resistance to chemical treatment, practically complete dissolution of the cell residues results when the material is treated with filtrates of *Streptomyces albus*. Tests of fractions of the filtrates show that the cell residue reacts in the same way as the intact streptococcal cells. Thus, the proteolytic enzyme of *Streptomyces albus* is incapable of dissolving the residue and fractions containing the non-proteolytic enzyme concerned in lysis of the cell are highly active in solubilizing the residue. It is likely, therefore, that the cell residue material contains the substrate involved in the lysis of group A streptococci by *Streptomyces albus* preparations.

On general grounds, and on the basis of work by others with different bacterial species (8), it appeared probable that the insoluble residue obtained by the procedure described might represent the cell walls or cytoskeletons of the bacteria. Since the material has little affinity for bacterial stains and shows no characteristic structure on examination with the light microscope, its nature was further explored by means of electron microscopy. Fig. 1 shows the characteristic appearance of chromium-shadowed preparations of the cell residue, and it is clear that the material is not made up merely of amorphous debris. It consists of flat, relatively empty structures which retain a recognizable coccoid appearance despite the considerable fragmentation resulting from mechanical grinding. The short chain of incompletely disrupted cells was included for comparison, and the outline of the cell wall is visible around the margin of the electron-dense cytoplasmic material. The similarity of the appearance of this cell wall to the remainder of the material is obvious. The micrographs appear to provide clear evidence that the streptococcal residue consists of cell walls. It is likely that the preparative methods have removed some of the components of the cell wall as it functions in the living cell, but from the point of view of the present investigation the significant fact is that the insoluble skeleton of the cell wall is susceptible to lysis by the enzymes of *Streptomyces albus*. Accordingly, one is led to the view that the action of the lytic filtrates on intact cells is one of dissolution of the bacterial cell wall with the resultant release of cellular materials into solution.

Chemical Analysis of the Cell Wall Material.—The results of chemical analyses of the various cell wall preparations are summarized in Table I, and it is evident from the range of values that the cell walls of different strains of group A streptococci have a relatively uniform composition. The values for reducing sugars indicate that as much as two-thirds of the cell wall is carbohydrate in

character, and a portion of the total nitrogen is attributable to hexosamine. Up to the present time it has not been possible to demonstrate the presence of a lipid component. The nature of the phosphorus-containing constituent is unknown, but it is not nucleic acid or nucleotide. Qualitative chemical tests indicate that the major portion of the nitrogen is protein nitrogen. On the basis of these analyses, the cell wall material appears to consist primarily of a carbohydrate-protein complex.

The Carbohydrate Constituent of the Cell Wall

The carbohydrate component of the cell wall is of special interest since it has been identified as the well known group-specific C carbohydrate of the group A streptococcus. Soluble preparations of the carbohydrate fraction were obtained by the following procedure:—

Dried cell wall material is suspended in M/30 phosphate buffer pH 8 (approximately 10 cc. buffer for each 100 mg. dried material) and is mixed with a concentrate of the *Streptomyces albus* enzymes. The suspension is incubated for 16 hours at 37°C. with chloroform as a preservative, during which time it becomes almost completely clear. The solution is then shaken with successive portions of chloroform (one-third volume) and amyl alcohol (one-tenth volume) until no further gel appears at the interface after centrifugation. Usually only 2 or 3 such treatments with chloroform are required. The partially deproteinized solution is brought to 0.85 per cent with respect to sodium chloride by the addition of solid salt and then mixed with two volumes of ethanol. A light precipitate forms which is discarded. The carbohydrate is precipitated from the alcohol supernate by the addition of five volumes of acetone. The precipitate is collected by centrifugation, dissolved in water, dialyzed against distilled water, and finally dried from the frozen state.

TABLE I

Chemical Analysis of Cell Wall Preparations of Group A Streptococci

	per cent
Total nitrogen	7.1–7.7
Total phosphorus	0.6–0.7
Reducing sugars after hydrolysis (as glucose)	55–62
Hexosamine	18–22

The dried carbohydrate preparations are highly soluble in water and give solutions which show no evidence of appreciable viscosity even at concentrations up to 5 per cent. The serological reactivity of the carbohydrate when tested with group A antistreptococcal rabbit sera is comparable to that of the C carbohydrate prepared by Lancefield's standard method of heating streptococcal cells at pH 2 (9) or by Fuller's formamide method (10). At comparable carbohydrate concentration, material prepared by the three different methods removes the same amount of antibody from antisera, and cross-adsorption experiments give no evidence of differing specificities. Thus, there

is nothing to suggest that the C carbohydrate prepared from the cell walls is either more or less degraded than that prepared by the standard methods.

Chemical Analysis of the Cell Wall Carbohydrate.—Chemical data on several preparations of cell wall carbohydrate from different strains of group A streptococci are summarized in Table II. As suggested by the nitrogen values, these carbohydrates are not free of protein or protein-split products, and this is confirmed by the fact that solutions containing more than 1 per cent of the material give positive biuret reactions. Up to the present time, no preparation has been obtained which is entirely free of protein or peptide nitrogen, although in a number of instances two-thirds or more of the total nitrogen is represented by hexosamine nitrogen. Although the figures are not included in Table II, most of the phosphorus of the insoluble cell wall material is present in the soluble carbohydrate fraction and the concentration of total phos-

TABLE II

Chemical Analysis of Group A Carbohydrate Preparations from Streptococcal Cell Walls

Strain	Serological type	Total N	Reducing sugars (as glucose)	Hexosamine	Methylpentose
		per cent	*per cent*	*per cent*	*per cent*
K43	1	4.85	82.5	25.4	42.2
S43	6	3.15	83.0	28.4	44.0
T27	27	3.14	85.3	27.6	47.1
C510	28	—	78.8	22.8	49.6
C649A	28	4.30	—	25.6	43.1
C121	32	4.02	—	24.6	44.7
H105	—	4.95	81.0	27.4	44.2

phorus is approximately 0.8 per cent. The ultraviolet adsorption spectrum of concentrated solutions of the purified material shows no maximum in the region of 260 mμ, confirming the impression that the phosphorus is not referable to nucleic acid or nucleotides. Similarly, there is little evidence of a maximum in the region of 280 mμ, suggesting that aromatic amino acids do not form an appreciable component of the peptide fraction.

The values for reducing sugar reflect a substantial increase over those of the intact cell wall material and confirm the removal of part of the non-carbohydrate constituents. The hexosamine and methylpentose content is similar in the various preparations examined, with the hexosamine concentration ranging from 22 to 28 per cent and the methylpentose from 42 to 49 per cent. The ratio of methylpentose to hexosamine is approximately 1.6.

Hydrolysates of the carbohydrate were subjected to paper chromatography by the method of Partridge (11) using butanol acetic acid and collidine as solvents. The spraying reagents used were ammonia silver nitrate and the

hexosamine reagents. Completely hydrolyzed samples revealed only two components reacting with these reagents—one corresponding to glucosamine and the other to the methylpentose, rhamnose. Partially hydrolyzed material gave in addition a strongly reacting spot corresponding to *N*-acetyl-glucosamine, and consequently it is likely that the glucosamine occurs in the acetylated form.

The results indicate that the group-specific carbohydrate prepared from the cell wall consists primarily of acetyl-glucosamine and rhamnose. Schmidt (12) has recently published chemical data on the C carbohydrate of group A streptococcus prepared by an entirely different method, and he has concluded that his material also consists of glucosamine and rhamnose. The quantitative data reported by Schmidt are similar to those obtained in the present study except that the rhamnose content of his preparations is consistently somewhat higher. The difference is not attributable to the fact that he carried out his rhamnose determinations on hydrolyzed material, since following this procedure did not alter the results obtained with the cell wall carbohydrates.

Significance of the Group-Specific Carbohydrate in the Streptococcal Cell.—The finding that the group-specific carbohydrate is a major component of the cell wall suggests that the cell wall may be its primary site of localization in the streptococcal cell. In terms of weight, the carbohydrate of the cell wall represents a significant portion of the total cell material. As mentioned previously, the cell wall preparations make up at least 10 to 15 per cent of the dry weight of the cells, and since two-thirds of this is carbohydrate, the latter must represent 6 to 10 per cent of the cell weight. Actually, preparative yields of 6 to 8 per cent are frequently obtained, thus confirming this estimate and suggesting that the maximum amount may be even higher than 10 per cent.

The possibility that the group-specific carbohydrate might occur in other sites in the cell in addition to the cell wall was subjected to direct test by analyzing the various fractions obtained in the course of preparing the cell wall material from ground streptococcal cells. The most accurate estimates of the carbohydrate in the fractions depended on the use of the quantitative methylpentose reaction, on the assumption that all of the rhamnose of the cell occurs in the carbohydrate. Table III gives the results of rhamnose determinations on the fractions obtained in two different preparations of cell wall material. As will be seen, there was a small percentage of the total rhamnose in the saline washings of the ground cells, and only very small amounts in the supernatant fluid after enzymatic treatment, despite the fact that large amounts of material passed into solution during this treatment. Over 90 per cent of the rhamnose occurred in the insoluble residues—consisting of the cell wall preparation and the low speed residues made up of cell walls mixed with undisrupted cells. Similar results were obtained when quantitative serological techniques were used to estimate the amount of group-specific polysaccharide in the

various fractions. These determinations were undoubtedly less accurate than the estimates based on the rhamnose content, however, since the available antisera are not specific for the polysaccharide and give high results with crude fractions such as the saline washings.

It is evident from these data that the great bulk of the group-specific carbohydrate of group A streptococci occurs as a component of the bacterial cell wall. It is possible that even the small amount found in the other fractions has a similar localization and has been converted into a soluble form as a result of the rather drastic procedure of dry grinding. These observations lead to the conclusion that the major role of the group-specific carbohydrate of

TABLE III

Distribution of Group A Carbohydrate in Fractions of Ground Streptococci

Fraction	Rhamnose—per cent of total	
	Type 28 cells	Type 6 cells
Saline washings of ground cells	5.5	5.8
Supernate after trypsin treatment	2.4	4.0
Supernate after chymotrypsin and ribonuclease treatment	1.7	0.8
Residue of low speed centrifugation (mixture of intact cells and cell walls)	18.8	12.4
Cell wall preparation	71.6	77.0

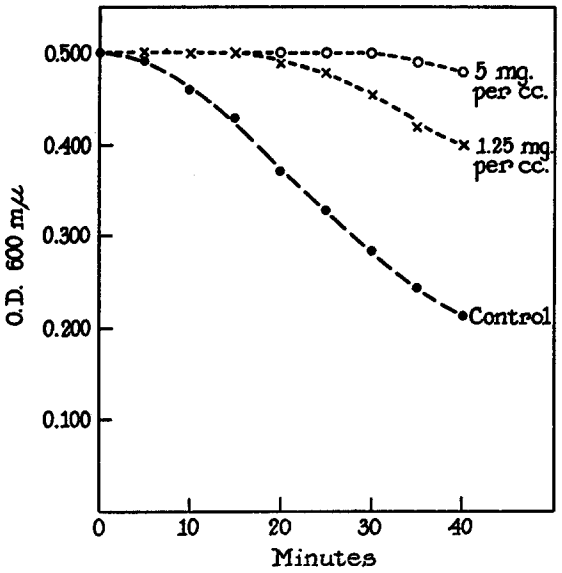
group A streptococci is a structural one, and that in combination with the appropriate protein it forms the rigid and resistant skeleton of the cell.

Evidence for Participation of the Carbohydrate in Enzymatic Lysis

In view of the findings discussed above, the question naturally arises whether the carbohydrate portion of the cell wall is the site of attack of the component of the *Streptomyces albus* enzyme system which brings about dissolution of the cell. This is suggested not only by the fact that the carbohydrate is a major component of the cell wall but also by the finding that the enzyme responsible for initiating lysis is apparently not a proteolytic enzyme. There are, however, certain theoretical objections to the concept that the group A carbohydrate is the site of action of the lytic enzyme. For example, the streptomyces enzymes, as shown by Welsch (13) and others, are capable of lysing a wide variety of Gram-positive bacteria; and even if all of them have a carbohydrate as an integral part of the cell wall, it is obvious that the carbohydrates of the different species are chemically and serologically distinct from one another. Consequently, if the same enzyme is to be considered as attacking all these carbohydrates, a common chemical linkage must be present. The possibility of such a common linkage is provided by the fact that glucosamine ap-

pears to be a constituent of all the known bacterial carbohydrates which can be considered analogous to the group A carbohydrate. It is conceivable, therefore, that lysis of the bacterial cell wall could be achieved by enzymatic hydrolysis of a specific linkage between glucosamine and some other component of the complex.

A certain amount of experimental evidence has been obtained that suggests that group A carbohydrate is indeed attacked in the course of enzymatic lysis



TEXT-FIG. 1. Inhibition of lysis of group A streptococci by carbohydrate prepared from streptococcal cell walls.

of the streptococcal cell wall. Three different experiments will be described, all of which support this possibility.

Inhibition of Enzymatic Lysis of Streptococci by Carbohydrate Preparations.—The carbohydrate preparations made by enzymatic treatment of the cell wall as described above can be considered as one of the products of enzyme action, and, if intimately concerned in the reaction, should behave as a split product in inhibiting the enzyme. In the following experiment, intact, heat-killed streptococcal cells were used as substrate as described in the preceding paper and the enzyme employed was a dilution of a concentrated preparation of the streptomyces enzymes. To 0.5 cc. of the bacterial suspension was added 0.25 cc. of a solution of carbohydrate prepared from a cell wall preparation of strain S43, type 6. Finally, 0.25 cc. of the enzyme dilution was added and readings of the optical density at 600 mμ were made at 5 minute intervals during incubation at 37°C. The results are presented in Text-fig. 1. At a final concentration of 5 mg./cc. of the carbohydrate the fall in turbidity was completely inhibited for 30 minutes, and even at 1.25 mg./cc. lysis was markedly inhibited in comparison with the control. This type of result was obtained with several lots of carbohydrate de-

rived from different strains, but it is significant that carbohydrates prepared by the formamide or acid methods showed no inhibitory effect whatever on the enzyme at comparable concentrations. It appears, therefore, that the streptomyces enzymes must be used in preparation of the material in order to obtain an inhibiting carbohydrate. However, since the inhibitory preparations are not made up solely of carbohydrate but also contain protein or peptide nitrogen and conceivably small amounts of other substances, it is not certain that the material is inhibiting by virtue of its carbohydrate component.

Alterations in the Carbohydrate Component Occurring during Enzymatic Lysis of the Cell Wall.—In the course of experiments concerned with the method of preparing soluble carbohydrate from cell walls, it was observed that the carbohydrate fraction did not behave as a homogeneous unit during enzyme treatment. This is illustrated in the following experiment.

TABLE IV

Alterations in Carbohydrate Component on Enzymatic Digestion of Cell Wall Material

		Total hexosamine	Total rhamnose
		mg.	*mg.*
Cell wall preparation S43, Type 6	Reaction mixture before dialysis	22.6	26.9
+	Reaction mixture after dialysis	16.8	27.1
concentrate of streptomyces enzymes	Dialysate	3.14	00.0

TABLE V

Heterogeneity of Group A Carbohydrate Prepared by Enzymatic Digestion of Cell Walls

	Rhamnose	Hexosamine	$\frac{\text{Rhamnose}}{\text{Hexosamine}}$
	per cent	*per cent*	
Fraction precipitable at ethanol concentration of 83 per cent	40.2	24.2	1.66
Fraction recovered from 83 per cent ethanol supernatant	25.9	23.1	1.12

A suspension of purified cell wall material was suspended in M/30 phosphate buffer pH 8 and mixed with a concentrate of the streptomyces enzymes. After complete clearing of the suspension at 37°C., the reaction mixture was dialyzed against phosphate buffer. The results of glucosamine and rhamnose determinations, recorded in Table IV, show that the total glucosamine of the reaction mixture fell on dialysis while the rhamnose content remained unchanged. The fact that a portion of glucosamine was rendered dialyzable is confirmed by the occurrence of glucosamine in the dialysate. Despite the high sensitivity of the test for rhamnose, no more than traces of this component were detected in the dialysate. The results of chemical and paper chromatographic tests showed that the dialyzable glucosamine is not free glucosamine and that it is not made up entirely of acetyl glucosamine, but the nature of its combination has not been determined. The difference between the two monosaccharide constituents of the carbohydrate under these conditions suggests that the molecule as it exists in the cell wall may be split by the enzyme.

Chemical Heterogeneity of the Carbohydrate Preparations.—In common with carbohydrates prepared by acid heating or formamide extraction of streptococcal cells, the carbohydrates released enzymatically from the cell walls have the property of requiring rather high concentrations of alcohol or acetone to precipitate them from solution. In the case of preparations made from the cell wall, for example, a portion of the carbohydrate remains in solution in 90 per cent ethanol. It has been found that the fractions obtained by precipitation at different ethanol concentrations are not identical chemically, although they appear to have similar serological reactivity. This is illustrated in Table V which shows the comparative rhamnose and glucosamine analyses on two fractions of the same preparation. It will be noted that the fraction precipitable at an ethanol concentration of 83 per cent gives values comparable to those of the preparations given in Table II. On the other hand, the material soluble in 83 per cent ethanol has a much higher relative glucosamine concentration so that the rhamnose-hexosamine ratio approaches 1.0. The serological activity of the two fractions is approximately the same when tested by the precipitin reaction with serial dilutions of the antigens. These results indicate that the carbohydrate may be split enzymatically into fragments of unequal composition. An alternative interpretation would be that it does not occur as a homogeneous substance in the cell wall.

None of these indirect approaches establishes the exact site of action of the enzyme, but the results are compatible with the view that the carbohydrate as it exists in the cell wall is enzymatically attacked in the course of lysis by streptomyces filtrates. Further investigation will be required to obtain more conclusive evidence on this point.

DISCUSSION

The experiments described in the present paper have a bearing on the structure of the streptococcal cell as well as on the nature of the lysis of streptococci by the streptomyces enzymes. The isolated cell walls of group A streptococci have been shown to be similar to intact cells in their resistance to various chemical treatments and in their susceptibility to dissolution by the enzymes of *Streptomyces albus*. The enzyme fractions and experimental conditions required for lysis are the same for both types of substrate. These facts strongly suggest that the mechanism of enzymatic lysis of the streptococcal cell involves dissolution of the bacterial cell wall. Accordingly, the chemical nature of the cell wall assumes importance in the problem of determining the point of attack of the lytic enzymes. Cell wall preparations of group A streptococci obtained by the methods described are composed primarily of protein and carbohydrate, and all of the available evidence supports the view that some linkage involving the carbohydrate component is concerned in enzymatic dissolution of the cell wall.

Chemical data on the cell wall of *Streptococcus faecalis* have recently been published by Salton (14). Although no serological information is given, this organism is presumably a member of Lancefield's group D. Living cells were broken up with glass beads in a Mickle disintegrator (8), and in this instance washing of the disrupted cells with distilled water was sufficient for the removal of cytoplasmic material. Isolated

cell walls of *Streptococcus faecalis* were shown by Salton to consist primarily of carbohydrate and protein with carbohydrate predominating. In this case, however, evidence for the presence of a small amount of lipid was also obtained. Glucosamine and rhamnose, the monosaccharide components found in group A cell walls, were also present in Salton's material, but in addition two other monosaccharides, glucose and galactose, were identified. Salton also studied the amino acid composition of the cell walls and found that aromatic amino acids appeared to be absent. It is obvious that there is some similarity between the chemical composition of the cell walls of group D streptococci and those of group A streptococci. However, assuming that the carbohydrate of the group D cell wall represents the group-specific antigen, there are serological as well as chemical differences between the carbohydrate portions of the two groups of organisms. An additional difference is suggested by the fact that *Streptococcus faecalis* is included by Webb (15) among the Gram-positive organisms which are made susceptible to trypsin lysis by treatment with lysozyme, while in our experience group A streptococci do not have this property.

The probable role played by the cell wall carbohydrate suggests certain analogies between the lysis of streptococci by *Streptomyces albus* enzymes and the lysis of susceptible organisms by lysozyme. Soluble lysozyme substrate prepared from *Micrococcus lysodeikticus* by the methods of Epstein and Chain (16) or of Meyer and Hahnel (17) is a glucosamine-containing polysaccharide, and the method of extraction by prolonged treatment with alkali suggests that it is derived from the cell wall. Thus, it is possible that lysozyme action also involves an attack on a cell wall carbohydrate. This is of interest in view of the finding, previously mentioned, that a variety of Gram-positive organisms are dissolved by trypsin after prior treatment with lysozyme (15). Since group A streptococci appear not to possess this type of susceptibility to lysozyme, one must conclude that the specific linkage hydrolyzed by lysozyme is either not present in the carbohydrate as it exists in the cell wall or is inaccessible to the enzyme. The non-proteolytic component of the streptomyces enzyme system concerned with lysis of streptococci can be visualized as being analogous to lysozyme in its action on a cell wall carbohydrate substrate but differing from it in specificity.

The localization and function of the group-specific antigen of group A streptococci are clarified by its identification as the carbohydrate component of the cell wall. The procedures required for extraction of C carbohydrate are readily explained in the light of this finding, and indeed Fuller, in the course of his experiments on formamide extraction (10), concluded that the C carbohydrate does not exist free in bacteria but is held in combination with other substances. The occurrence of this carbohydrate, which is of such great value in the serological classification of streptococci, appears to be of structural significance in the cell; and there is no indication that it has any role other than that of a component of the rigid cell wall.

SUMMARY

Cell wall preparations of uniform chemical constitution have been obtained from several strains of group A streptococci. The isolated cell walls are dis-

solved by the same fractions of the *Streptomyces albus* enzymes that are effective in the lysis of intact cells, and it is likely that enzymatic lysis of group A streptococci is effected by an attack on the cell wall.

The streptococcal cell wall, as prepared in this study, consists of approximately two-thirds carbohydrate and one-third protein. Small amounts of other components may be present. The carbohydrate component, which is composed primarily of *N*-acetyl-glucosamine and rhamnose, is the group-specific C carbohydrate. The evidence indicates that one of the streptomyces enzymes is directed toward the carbohydrate component of the cell wall.

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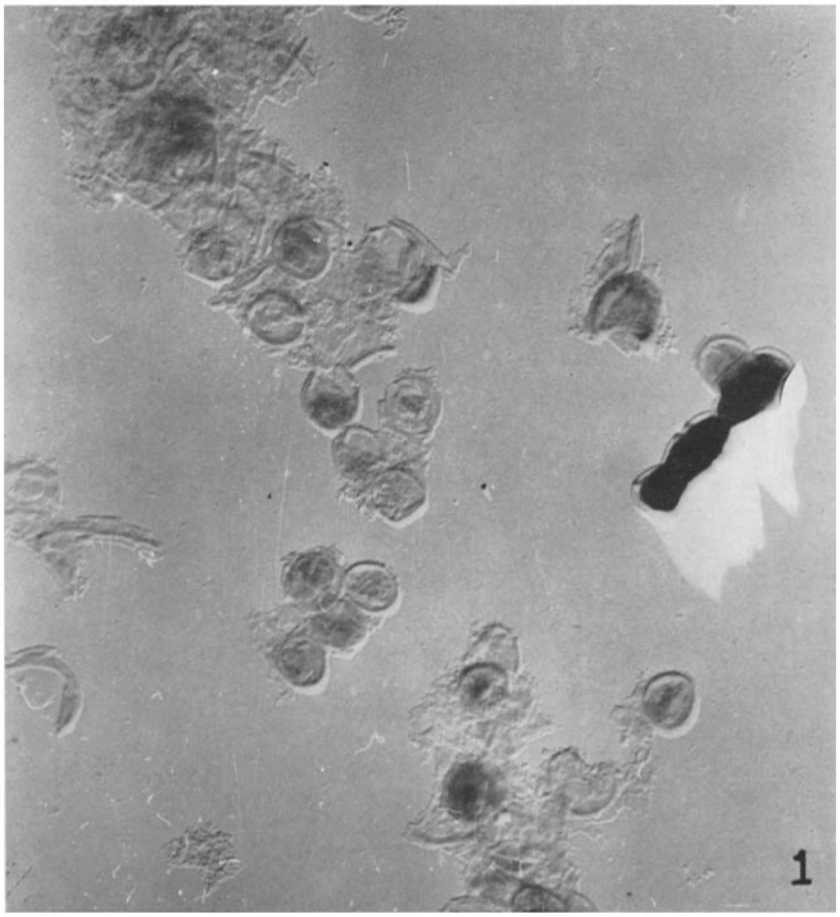
EXPLANATION OF PLATE 26

FIG. 1. Electronmicrograph of insoluble cell residue prepared from group A streptococcus, strain K43, type 1. Preparation shadowed with chromium at an angle of 12° × 9,250.

The micrograph was made through the courtesy of Dr. Keith R. Porter.



(McCarty: Enzymatic lysis of group A streptococci. II)