

SOME BIOLOGICAL PROPERTIES OF PNEUMOCOCCUS TYPE 37  
AND THE CHEMISTRY OF ITS CAPSULAR POLYSACCHARIDE\*

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Pneumococcus Type 37 was isolated initially in 1934 and published descriptions of it appeared in 1940 (1) and 1941 (2). The former report cited the striking resemblance of the newly recognized type to pneumococcus Type 3 in that both produce very large capsules and give rise to large mucoid colonies on the surface of solid media. In contrast to the highly virulent Type 3, however, Type 37 was only slightly virulent for mice. The first American account of pneumococcus Type 37 (2) was in essential agreement with that from Denmark. The organism was found with a frequency of 0.6% among 2,591 pneumococcal isolates recovered in 1940. Of 23 strains tested for virulence in mice, 1 was described as avirulent, 21 as slightly virulent, and 1 as moderately virulent. One fatal infection in man was recorded. More recently, in the Clinical Research Laboratory at the Philadelphia General Hospital, two strains of pneumococcus Type 37 have been recovered among 1,620 pneumococcal isolates over a period of 4 yr. Both strains were derived from cultures of sputum and both were thought initially to be strains of Type 3 from the appearance of their large mucoid colonies. Both strains were found in 1964, and no additional ones have been recovered subsequently. Among 1572 bacteremic pneumococcal infections occurring during the past 3 yr in hospitals in 10 American cities, only one strain of pneumococcus Type 37 has been identified, that in the blood of a patient in Chicago. These observations indicate that, although pneumococcus Type 37 resembles pneumococcus Type 3 in several ways, it differs from the latter by virtue of its infrequent recovery from man and its very low virulence.

It has been held that the virulence of pneumococcus Type 3 results in part from its production of a large capsule (3) and it has been shown that its capsule strikingly inhibits phagocytosis in the absence of antibody (4). Type 3 capsular

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polysaccharide is a polymer of glucose and glucuronic acid linked in repeating units of cellobiuronic acid. Because of their differing virulence, it was thought to be of interest to study the behavior of pneumococcus Type 37 in systems comparable to those used in the study of pneumococcus Type 3 and to examine the chemical structure of the capsule of the former type.

#### *Materials and Methods*

*Strains of Pneumococcus.*—Two strains of pneumococcus Type 37 isolated from the respiratory secretions of patients at the Philadelphia General Hospital were available. Both had been lyophilized shortly after their initial recovery. A third strain was received in the dried state from Dr. Erna Lund, Statens Seruminstitut, Copenhagen, Denmark. All three strains behaved in comparable fashion. A strain isolated from a patient at the Philadelphia General Hospital, strain 103, was employed in these experiments.

In comparative tests of virulence and phagocytosis, the Type 3 pneumococcal strain, A66, isolated initially at The Rockefeller Institute many years ago was employed.

*Media.*—All strains were grown in fresh beef heart infusion broth with Neopeptone (Difco Laboratories, Inc., Detroit, Mich.).

*Tests of Virulence.*—Mice or rats were injected intraperitoneally with 1 ml of an 18 hr culture of the designated strain or a serial tenfold dilution of it and were observed for survival or death over a period of 7 days. The injected organism was recovered from all animals succumbing to infection.

*Phagocytic Studies.*—Phagocytosis of pneumococcus Types 37 and 3 by rat polymorphonuclear leukocytes in the absence of anticapsular antibody was studied by the technique of surface phagocytosis described by Wood et al. (4). The experiments were carried out in Dr. Wood's laboratory in the Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Md. by Mary Ruth Smith to whom grateful acknowledgement is made.

*Isolation of Capsular Polysaccharide.*—Capsular polysaccharide was recovered from the supernatant fluid of centrifuged fully grown cultures of pneumococcus Type 37 by precipitation with 1 volume of 95% ethanol. The precipitate was washed with ethanol, dried over  $P_2O_5$ , redissolved in water, and reprecipitated with 1 volume of ethanol in the presence of 0.1 M sodium acetate. The latter two steps were repeated twice. The yield was 150 mg/liter of culture. The crude polysaccharide was deproteinized by shaking with chloroform-octanol, and the negatively charged polymers were removed by precipitation with a 1% aqueous solution of cetylpyridinium chloride in the presence of 0.1 M NaCl (5). The supernatant fluid was clarified and the polysaccharide recovered from it by precipitation with 3 volumes of 95% ethanol. It was redissolved, dialyzed, reprecipitated with ethanol, and dried *in vacuo*. The dried preparation was taken up in water and fractionated in the presence and absence of sodium acetate. To an aqueous solution of polysaccharide (3 mg/ml) was added one volume of 95% ethanol. The solution was allowed to stand overnight in the cold. The slight precipitate which formed was removed by centrifugation. Addition of sodium acetate to the supernatant solution to a final concentration of 0.1 M resulted in the immediate precipitation of the polysaccharide. The precipitate was washed and dried in the usual manner. The polysaccharide was resolubilized in 0.1 M sodium acetate and 0.8 volume of 95% ethanol was added. The solution was allowed to stand overnight in the cold. Following centrifugation at 20,000 rpm, an additional 0.2 volume of ethanol was added to the supernatant liquid. After the precipitate was allowed to form overnight in the cold, it was collected by centrifugation, washed, and dried. The final yield of purified polysaccharide was 70 mg/liter of the original culture fluid.

*Preparation of Pneumococcal Type 37  $^{14}C$ -Capsular Polysaccharide.*— $^{14}C$ -labeled capsular

polysaccharide was prepared in a 100 ml culture containing 0.05 mCi of uniformly labeled  $^{14}\text{C}$ -glucose. The capsular polysaccharide was precipitated from the supernatant culture fluid with ethanol, taken up in 40 ml of water to which 100 mg of unlabeled polysaccharide was added as carrier, and the polysaccharide purified further by chromatography on Sephadex G 200 (Pharmacia Fine Chemicals, Uppsala, Sweden), Dowex 1,  $\text{Cl}^-$ , and Dowex 50,  $\text{H}^+$ . The final preparation yielded 63 mg of capsular polysaccharide with a specific activity of  $5 \times 10^4$  cpm/mg, representing 0.9% incorporation of the original  $^{14}\text{C}$ -glucose. Samples were assayed either directly in counting vials or on 1 cm discs of Schleicher and Schuell No. 581 paper (Schleicher and Schuell, Inc., Keene, N.H.) in a Packard Tri-Carb liquid scintillation counter. The fluor used contained 0.43 g PPO (2,5-diphenyloxazole) and 0.24 g dimethyl POPOP (1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene) in 800 ml toluene and 200 ml ethanol.

*Analytical Methods.*—Total carbohydrate was determined by the phenol-sulfuric acid method (6), hexose by the primary cysteine reaction (7), pentose by the orcinol method (8), glucose by the glucose oxidase technique ("glucostat assay"), hexosamine by a modification of the Boas method (9), O-acetyl by the procedure of Hestrin (10), uronic acid by the carbazole method (11), nitrogen by a modified ninhydrin method (12), and phosphorous by the ascorbic acid technique (13). Amino acids were measured in the automatic amino acid analyzer following hydrolysis of the polysaccharide for 22 hr in 6 N HCl at 100°C. N-acetylglucosamine and N-acetylgalactosamine were included in the standard amino acid mixture. Reducing sugar was measured by the method of Park and Johnson (14) and by the triphenyl tetrazolium assay (15); procedures for formaldehyde, borohydride reduction, and periodate oxidation were performed as described by Kabat and Mayer (16). Formic acid was determined by titration against 0.01 N NaOH in a pH meter as well as by an iodometric technique (17). In all analytical methods involving intact polysaccharide, glucose, calculated as anhydroglucose, was used as a standard.

*Preparation of Monosaccharides.*—Maximal yield of monosaccharides was obtained after hydrolysis of polysaccharide for 2½–3 hr in 1.8 N  $\text{H}_2\text{SO}_4$  at 100°C. The hydrolysate was neutralized with  $\text{Ba}(\text{OH})_2$ , the  $\text{BaSO}_4$  removed by centrifugation, and the supernatant fluid lyophilized. Hydrolysis was performed also for 2½–3 hr in 2 N HCl at 100°C. The HCl was removed in a rotary evaporator with several additions of water. Chromatography of the material so obtained was carried out on Whatman No. 1 paper in three solvent systems: (a) N-propanol:ethylacetate:water, 7:1:2; (b) ethyl acetate:pyridine:water, 8:2:1, and (c) butanol:pyridine:water, 10:3:3. Spots were located with  $\text{AgNO}_3$  or acid aniline phthalate reagent. For quantitative work, Schleicher and Schuell No. 581 Green Ribbon sheets were used after washing with solvent 1 and carbohydrates were located by staining guide strips with  $\text{AgNO}_3$ . Corresponding areas of the unstained chromatogram were cut, eluted with water, filtered through sintered glass, lyophilized, resolubilized, and analyzed.

*Preparation of Oligosaccharides.*—Oligosaccharides were recovered optimally by hydrolysis of a 10% solution of the polysaccharide for 4½ hr in 1 N  $\text{H}_2\text{SO}_4$  at 85°C. Areas on the chromatogram corresponding to lactose and maltose were eluted and rechromatographed on paper and on Sephadex G 25. The phenol-sulfuric acid positive fractions eluting in the position corresponding to maltose were pooled and lyophilized.

*Immunochemical Methods.*—Antiserum to pneumococcus Type 37 was prepared in rabbits by a standard technique (18). Quantitative precipitin analyses were performed by the modified ninhydrin method (12), and immunodiffusion studies by the Ouchterlony technique.

## RESULTS

*Virulence of Pneumococcus Type 37.*—Studies of the virulence of pneumococcus Type 37 in the CFW strain of white mice were entirely in accord with the

earlier observations of others on the low level of virulence of this pneumococcal type for the laboratory mouse (2). Whereas one colony-forming center of pneumococcus Type 3 usually caused fatal infection,  $10^7$ – $10^8$  colony-forming centers of pneumococcus Type 37 were necessary to induce a fatal outcome following intraperitoneal injection.

Because studies of the phagocytosis of pneumococcus Types 37 and 3 were carried out with rat polymorphonuclear leukocytes, the virulence of each of the two strains employed was assayed in the rat. The marked difference in virulence of the two pneumococcal types for the laboratory white rat is apparent from the data in Table I.

*Phagocytosis of Pneumococcus Type 37.*—The phagocytosis of pneumococcus Type 37 by rat polymorphonuclear leukocytes was studied in the absence of anticapsular antibodies and compared with that of pneumococcus Type 3.

TABLE I  
*Virulence of Pneumococcal Types 3 and 37 for the White Rat*

Type	Inoculum*	D/T†
37	$7 \times 10^7$	3/3
	$7 \times 10^6$	0/3
	$7 \times 10^5$	0/3
3	$3 \times 10^2$	3/3
	$3 \times 10^1$	3/3
	$3 \times 10^0$	1/3

\* Colony-forming units/ml.

† D/T = Died/Total.

Fully capsulated cells of both types were employed and were mixed in a ratio of 10:1 with rat polymorphonuclear leukocytes harvested from the peritoneal cavity following induction of chemical peritonitis with a starch-aleuronat mixture. The dense suspensions of pneumococci and leukocytes were incubated together on filter paper in a closed Petri dish at 37°C for 30 min. The cells were then washed off the filter paper, spread on slides, and stained. 400 polymorphonuclear leukocytes from each experiment were examined for the presence of ingested pneumococci.

Whereas only 18% of the polymorphonuclear cells were found to have ingested pneumococcus Type 3, 50% of comparable cells suspended with pneumococcus Type 37 had phagocytized this organism. The results are concordant with the observed difference in virulence of these two pneumococcal types in intact rats.

*Analyses of Purified Type 37 Capsular Polysaccharide.*—Analytic values for purified Type 37 pneumococcal capsular polysaccharide are recorded in Table II and show it to consist almost exclusively of repeating units of hexose. Assays for methyl pentose, uronic acid, and O-acetyl were negative. A color yield of

3.1% was obtained in the pentose determination and a hexosamine value of 2% was obtained with the Boas reaction. Examination of 10 mg of the polysaccharide in the amino acid analyzer, however, showed a series of small peaks, none of which corresponded to known amino acids or amino sugars.

The polysaccharide displayed a high degree of asymmetry manifested by extreme viscosity at concentrations in excess of 3 mg/ml water. A comparison of the intrinsic viscosity of Type 3 capsular polysaccharide with that of Type 37 is presented in Table III. As expected for a neutral molecule, the intrinsic viscosity of Type 37 capsular polysaccharide was not affected significantly by solubilization in saline or in plasma. In contrast, the intrinsic viscosity of Type 3 capsular polysaccharide was markedly dependent on the salt content. In

TABLE II  
*Analyses of Purified Pneumococcal Type 37 Capsular Polysaccharide*

N	P	Total carbohydrate	Hexose	Reducing* value	Ash weight	$[\alpha]_D^{20}$
%	%	%	%	%	%	degrees
0.6	0.2	100	95	2.0	0.6	0

\* This value is unchanged after treatment with sodium borohydride.

TABLE III  
*Intrinsic Viscosity of Type 3 and Type 37 Pneumococcal Capsular Polysaccharides*

Polysaccharide type	$[\eta]$		
	Water	Saline	Plasma
37	5.6	5.0	5.0
3	52	8.4	6.3

saline, Type 3 capsular polysaccharide displayed a viscosity almost twice that of Type 37 but, in plasma, the viscosities were more similar. In water, however, Type 3 polysaccharide had a viscosity more than 10 times that of Type 37.

An average molecular weight of  $1.6 \times 10^7$  for Type 37 capsular polysaccharide was obtained in the analytical ultracentrifuge by the Archibald method (19), but the calculations are rendered somewhat uncertain by the hypersharp boundaries displayed by the polysaccharide.

Immunodiffusion studies with the purified Type 37 capsular polysaccharide showed only a single band with homologous anticapsular serum. With the radioimmunoassay technique developed in this laboratory,  $^{14}\text{C}$ -labeled Type 37 capsular polysaccharide was allowed to react with specific antiserum at room temperature and the resulting complex was then precipitated with 1.0 volume of an aqueous solution of  $(\text{NH}_4)_2\text{SO}_4$  which had been saturated at  $37^\circ\text{C}$ . The

precipitate was dissolved in 0.05 ml water and counted in 10 ml fluor. Under these conditions, no counts were precipitated in the absence of antiserum, whereas 100% of the radioactivity was precipitated by Type 37 antiserum. The antigen was not precipitated by antisera to pneumococcal C substance or Type 3 capsular polysaccharide. It was demonstrated also that precipitation of the  $^{14}\text{C}$ -counts could be inhibited completely by the addition of unlabeled Type 37 polysaccharide.

Inhibition of binding of radiolabeled capsular polysaccharide was used also to compare the relative amounts of capsular material remaining on the cells with those which diffused into the medium during growth of both Type 37 and Type 3 pneumococci. After overnight growth, three times as much capsular polysaccharide was found in the culture supernatant as was found associated with the cells of pneumococcus Type 37. A Type 3 pneumococcal culture was observed to have the reverse relationship, twice as much capsular polysaccharide being associated with the cells as was found in the supernatant fluid. Similar results were obtained when each of the two pneumococcal types was grown in an aqueous medium, in media containing 25% human plasma or in 100% human plasma.

*Identification of Hydrolytic Products of Type 37 Capsular Polysaccharide.*—Qualitative paper chromatography in three solvent systems of monosaccharides obtained by acid hydrolysis showed one major spot identical with glucose. Extremely faint spots appeared directly ahead of and behind the glucose region. These areas of the chromatogram were eluted and the eluates analyzed. The material so obtained represented 0.5% of the original material as determined by the phenol-sulfuric acid procedure and was negative in both the pentose and glucose assays. The glucose spot was eluted and assayed as 100% glucose with glucose oxidase. It was chromatographed simultaneously with authentic  $^{14}\text{C}$ -glucose in three solvent systems.

The behavior on hydrolysis of 1 mg of Type 37 polysaccharide in 1 ml 1.8 N  $\text{H}_2\text{SO}_4$  at  $100^\circ\text{C}$  was compared to that of dextran. Almost 50% of the glucose is released in 30 min from Type 37 polysaccharide, whereas 14% is yielded by dextran in the same period. Release of glucose from Type 37 polysaccharide is essentially complete in 90 min, reaching a value of 88%. Variations in the conditions of hydrolysis did not increase the yield of glucose and no inhibitor of glucose oxidase could be demonstrated.

In order to determine whether or not hexose components other than glucose were present,  $^{14}\text{C}$ -labeled polysaccharide was hydrolyzed and chromatographed as described above. The components visualized by reduction with  $\text{AgNO}_3$  and by counting were compared. Because 19% of the recovered counts appeared on the chromatogram behind glucose, these regions were eluted, rehydrolyzed, and rechromatographed. At least two-thirds of this 19% of the counts appeared in the glucose region. Elution of the glucose region in five adjacent  $1 \times 1$  cm strips

followed by glucose oxidase analyses and radioactive counting demonstrated that the specific activity of the glucose region was uniform throughout. While these results suggest that glucose comprises essentially all the hexose content of the polysaccharide, the validity of this interpretation depends upon the uniformity of incorporation of  $^{14}\text{C}$ -glucose into the Type 37 capsular polysaccharide.

*Periodate Degradation of Intact Polysaccharide.*—Periodate degradation of the intact polysaccharide, controlled with a periodate blank and a periodate-dextran mixture as well, resulted in the consumption of 0.95 mole of periodate per mole of hexose together with the production of 0.24–0.34 mole of formic acid per mole of hexose. The lower formic acid value was obtained by titration against NaOH, whereas the higher figure was determined iodometrically. In the same experiment, following periodate oxidation, ethylene glycol was added to destroy excess periodate, and the polysaccharide was then reduced with excess sodium borohydride, dialyzed, and lyophilized. The periodate-treated and reduced polysaccharide represented 91% of the starting material and contained 31% glucose.

To this material, HCl was added to a concentration of 0.25 N. After 30 hr at room temperature, the hydrolysate was dialyzed. These mild conditions should cause complete hydrolysis of all linkages except those involving nonoxidized sugar residues (20). In this case, dialysis of the hydrolyzed material resulted in the formation of both soluble and insoluble nondialyzable products. The insoluble material was removed by centrifugation, washed with water and with ethanol, and then dried over  $\text{P}_2\text{O}_5$  in a vacuum desiccator.

This fraction represented 28%, by weight, of the hydrolyzed polysaccharide and exhibited a glucose content of 101%. Glucose was the only apparent product upon chromatography in solvents *a* and *b* of a 4 hr hydrolysate of this material in 2 N HCl at 100°C.

The nondialyzable, soluble fraction represented 26%, by weight, of the 0.25 N HCl hydrolyzed polysaccharide, contained 6.5% glucose, and gave a phenol-sulfuric acid color yield of 17%. This fraction was hydrolyzed in 2 N HCl at 100°C for 2 hr, and the acid was removed by rotary evaporation. Chromatography of the hydrolysate in solvents *a* and *b* revealed a major spot with the mobility of glycerol and 3 minor spots in the hexose region. Of these minor spots, the most intense was that corresponding to glucose. A spot of almost equal intensity migrated slightly ahead of glucose with a mobility similar to that of mannose, while the third and faintest spot migrated just behind glucose.

The soluble, dialyzable material obtained following hydrolysis with 0.25 N HCl was lyophilized and the excess acid removed under vacuum in a rotary evaporator with several additions of water. An accurate weight could not be obtained because of the syrupy nature of the material, but this fraction represented 9% of the original glucose content of the oxidized and reduced polysaccharide. This fraction was hydrolyzed in 2 N HCl at 100°C for 2 hr and the acid was removed by rotary evaporation.

Chromatography in solvents *a* and *b* revealed a preponderance of glycerol with trace amounts of three spots in the hexose region similar in mobility to those found in the hydrolysate of the soluble, nondialyzable material.

2.17 mg of the nondialyzable, water-insoluble material obtained following hydrolysis with 0.25 N HCl was suspended in 4 ml water and periodate was added to a concentration of  $8 \times 10^{-3} M$ . Sephadex G 200 (insoluble dextran) was used as a control and the reaction was allowed to proceed in the dark at room temperature.

The insoluble material consumed 0 moles periodate after 24 hr whereas the Sephadex control consumed 1.4 moles periodate per mole of anhydroglucose. These results suggest that the nondialyzable, insoluble segment is composed almost exclusively of periodate-resistant 1  $\rightarrow$  3 glucosyl-glucose repeating units.

An attempt was made to obtain further information on the structure of the Type 37 capsular polysaccharide by a study of its periodate degradation products.

<sup>14</sup>C-labeled polysaccharide was treated with periodate and reduced with sodium borohydride in a manner identical to that used to treat the unlabeled material. The final product was chromatographed on Sephadex G 25 (bead form). 88% of the starting counts were eluted with the void volume. A comparison of this material with unlabeled intact Type 37 polysaccharide was made by means of the radioimmunoassay described above. Although 20% of the counts in the oxidized and reduced <sup>14</sup>C-polysaccharide still precipitated Type 37 antiserum, this material was only one-thousandth as active immunologically as the starting material. The oxidized-and-reduced labeled polysaccharide was hydrolyzed for 1, 2, and 4 hr in 1 N HCl at 100°C. The acid was removed and the residues were chromatographed in solvent *a* on Whatman No. 1 paper. The chromatogram was cut into 1  $\times$  1 cm sections and each section was counted in 5 ml fluor.

Approximately 90% of the counts appeared in the glucose region with no discrete areas of radioactivity in regions of faster mobility. The absence of radioactive small carbon fragments suggests that the Type 37 polysaccharide is resistant to oxidative cleavage by periodate. This possibility is rendered unlikely by the fact that the unlabeled polysaccharide displays a periodate uptake of 0.95 mole periodate under identical conditions and also by the marked decrease in serologic activity of the <sup>14</sup>C-polysaccharide following periodate treatment. One possible explanation of these findings is that the capsular material had not been labeled uniformly with the carbon isotope and that the periodate-sensitive moieties of the polysaccharide had not incorporated the <sup>14</sup>C-glucose.

An alternate approach to the problem of nonuniform incorporation of isotope into the polysaccharide was to label selectively those carbon atoms which were cleaved by periodate.

A 1 mg aliquot was removed from the large-scale, periodate and ethylene glycol-treated unlabeled Type 37 polysaccharide reaction mixture described above. This material was dried over P<sub>2</sub>O<sub>5</sub> in a vacuum desiccator and resolubilized in 0.1 ml water. <sup>3</sup>H-NaBH<sub>4</sub> (5 mCi., 1.3 mg., New England Nuclear Corp., Boston, Mass.) was dissolved in 0.1 ml water and added immediately to the polysaccharide along with a 0.1 ml water wash. The reaction was allowed

to proceed at 60°C for 20 min and then at room temperature overnight. The reaction mixture was applied to a Sepharose 4B column (1.4 × 45 cm.), and the void volume was collected, concentrated in a rotary evaporator, and hydrolyzed in 1 N HCl for 2 hr at 100°C. Following removal of the acid, the hydrolysate was reduced with unlabeled sodium borohydride and chromatographed in solvent *a* on Whatman No. 1 paper. The chromatogram was sectioned and counted as described above. Over 90% of the recovered counts were found in the glycerol region.

These results suggest the absence of significant numbers of 1 → 4 linkages in the polysaccharide and indicate further that <sup>14</sup>C-glucose incorporation into newly synthesized Type 37 polysaccharide may be restricted to regions that are insensitive to attack by periodate.

*Identification of Oligosaccharides.*—Two oligosaccharides were demonstrable on paper chromatograms in solvent *a* of an hydrolysate (1 N H<sub>2</sub>SO<sub>4</sub>, 4½ hr, 85°C) of polysaccharide, one migrating slightly ahead of maltose, the other with a mobility similar to lactose. Both were examined before and after reduction with sodium borohydride.

The fraction migrating slightly ahead of maltose showed a 37% glucose value following reduction, which together with its pattern of migration suggested a disaccharide structure. The low reducing value (15%) indicated a substituent on C2 of the reducing sugar, consistent with the poor staining with AgNO<sub>3</sub> and nonreactivity in the tetrazolium assay in which C2-linked reducing sugars do not react. The low optical rotation of +8° is consistent with a β linkage. Because periodate consumption by disaccharides may be difficult to interpret, the reduced disaccharide was examined by the more specific formaldehyde assay. The production of 1 mole of formaldehyde in this test indicated either a 1 → 6 or a 1 → 2 linkage. This observation, in conjunction with those noted above, suggests that the disaccharide is glucose-β1-2-glucose or sophorose. In addition, its migration on paper in solvents *a* and *b* was identical with that of an authentic sample of sophorose.

Examination of the second oligosaccharide indicated that it was a trisaccharide. It retained 63% glucose upon reduction with sodium borohydride and displayed a reducing value of 36%. It gave a positive reaction in the tetrazolium assay. Oxidation of the reduced trisaccharide with periodate gave 1 mole of formaldehyde, narrowing the choices of trisaccharides to one with a C2- or C6-substituted reducing sugar. The positive tetrazolium assay makes a C2-linked reducing terminus unlikely. Because only a branched 3,6 diglucosyl-glucose would yield pentose upon acid hydrolysis of the periodate-treated molecule, the unreduced trisaccharide and the periodate reaction mixture were analyzed for pentose with the orcinol reagent. With xylose as a standard, a molar ratio of pentose to trisaccharide of 0.7 was obtained. Before periodate treatment, the trisaccharide gave a negative pentose reaction. Assigning a structure on the basis of a pentose color value in the absence of more conclusive evidence would

be premature. It can be stated only that the result of this and the foregoing data are consistent with a branched 3,6 diglucosyl-glucose structure.

#### DISCUSSION

The data presented suggest that the composition of the capsular polysaccharide of pneumococcus Type 37 may be the simplest of all the pneumococcal capsular polysaccharides examined to date. Its basic unit appears to be almost completely, if not exclusively, glucose, differing in composition from the polysaccharide of Type 3, which is 50% glucose and 50% glucuronic acid. Despite the production of capsules of comparable size by these two pneumococcal types, the difference in their behavior as infectious agents is striking and indicative of the fact that the structure of a pneumococcal capsular polysaccharide is of greater importance than the quantity synthesized by the cell. This view is supported by the highly invasive properties of pneumococcus Type 12; it has a small capsule but infections with it are accompanied by a bacteremia rate of approximately 50% in man.

Preliminary examination of the properties of Type 37 capsular polysaccharide provides no certain clues to explain the low virulence of this pneumococcal type. No enzymes were found in saliva or blood serum capable of degrading the molecule. Because it is an uncharged molecule, Type 37 capsular polysaccharide is less viscous in aqueous solution than is the negatively charged Type 3 capsular material; but in salt solution the viscosities of the two polysaccharides do not differ strikingly. This fact notwithstanding, there appears to be a more pronounced tendency for the capsular polysaccharide of pneumococcus Type 37 to diffuse away from the cell than is manifested by that of Type 3 in an aqueous menstruum or in plasma. Whether or not these differences *in vitro* relate to the behavior of these two pneumococcal types *in vivo* is not certain. Studies *in vitro* of surface phagocytosis by rat polymorphonuclear leukocytes of the two pneumococcal types are clearly in accord with the behavior of these types in intact rats.

Hydrolysis of Type 37 pneumococcal capsular polysaccharide under a variety of conditions gave a maximum glucose value of 88%. Trace amounts of other monosaccharides were revealed by chromatography, but they represented no more than 1% of the total carbohydrate content of the polysaccharide. The prompt release of 50% of the glucose on acid hydrolysis of the polysaccharide is suggestive of the presence of a significant number of terminal nonreducing glucose residues. This impression is supported by the results of treatment of the intact molecule with periodate, the polysaccharide consuming 0.95 mole of periodate to each 0.23–0.34 mole of formic acid produced. This latter result suggests that approximately  $\frac{1}{4}$ – $\frac{1}{3}$  of the glucose moieties were attacked by 2 moles of periodate with the concomitant release of 1 mole of formic acid. Glucose units containing the three adjacent free hydroxyl groups necessary for the pro-



date from the reduced disaccharide, a negative tetrazolium assay, an optical rotation of  $+8^\circ$ , and the similarity of its chromatographic behavior to that of authentic sophorose. Evidence concerning the structure of the trisaccharide obtained in 4.9% yield suggests that it is 3,6 diglucosyl-glucose.

The low yields of the two oligosaccharides described make it difficult to arrive at a definitive structure of Type 37 pneumococcal capsular polysaccharide in the absence of additional data. One structure consistent with the evidence derived to date (Fig. 1) consists of a backbone of repeating 1  $\rightarrow$  3 glucosyl-glucose units with a disaccharide of glucose- $\beta$ 1  $\rightarrow$  2-glucose attached to C6 of alternate or more frequent units of that backbone. Such a structure does not take into account the 14% of glucose found in the soluble, nondialyzable fraction and in the dialysate following mild acid hydrolysis of the periodate-oxidized and reduced polysaccharide. These residues may represent periodate-resistant segments of the parent molecule which occur either in elongated side chains or at infrequent intervals along the backbone of the molecule. In addition, the presence of trace amounts of sugars other than glucose cannot be excluded completely at the present time.

#### SUMMARY

Pneumococcus Type 37, like pneumococcus Type 3, is characterized by the production of large mucoid colonies on the surface of solid media and by its very large capsule. It differs from the highly virulent pneumococcus Type 3 in that it is only slightly virulent for mice and rats and is isolated infrequently from man. A study of the behavior of pneumococcus Type 37 in systems comparable to those used in the study of pneumococcus Type 3 and an examination of the chemical structure of the capsule of pneumococcus Type 37 are described. The capsular polysaccharide of pneumococcus Type 37 is a viscous, optically inactive polymer composed of 95% hexose. Glucose is obtained in 88% yield upon acid hydrolysis. Periodate oxidation studies and the behavior of the polysaccharide on acid hydrolysis suggest that the molecule consists of a core of repeating units of 1,3 glucosyl-glucose to which short chains of glucose are attached at frequent intervals. Isolation of a disaccharide, the properties of which are identical with those of sophorose ( $\beta$ 1  $\rightarrow$  2 glucosyl-glucose), and of a trisaccharide are described. A tentative structure for the capsular polysaccharide of pneumococcus Type 37 is proposed.

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