THE NATURE OF THE CELL WALL OF *CORYNEBACTERIUM DIPHTHERIAE*. ISOLATION OF AN OLIGOSACCHARIDE

by

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The empirical nature of the staining reactions used in microbiology is steadily becoming replaced by knowledge of the chemical nature of the structure which is being stained. The most striking example is the recognition that the Gram stain is associated with the possession by the organism of a magnesium ribonucleoproteinate complex, considered to be present in the cell surface (HENRY AND STACEY[1]). In a recent publication BARTHOLOMEW AND MITTWER[2] have shown that exterior to the Gram-staining substance lies the less readily stainable cell wall. This cell wall can be stained if it is first mordanted by tannic acid (KNAYSI[3]), or by cetyl pyridinium chloride (DYARS[4]). There is little published work on the composition of this cell wall, earlier claims that it consists of cellulose or chitin have not been substantiated (LEWIS[5]). Lack of affinity for dyes and resistance to chemical treatment (except strong acid or alkali) suggest that its function is chiefly concerned in the mechanical protection of the cell constituents.

Corynebacterium diphtheriae has been referred to as a variable Gram-positive organism (STEARN AND STEARN[6]) and can be obtained free from Gram-staining material by washing with dilute salt solutions. Since this organism has been shown, by staining reactions and by observation in the phase-contrast microscope (HEWITT[7]), to have a well-defined cell wall, it is suitable for the separation of the cell wall free from the Gram complex. By shaking washed cells of *C. diphtheriae* with glass beads and washing the cell-debris with 2% sodium acetate solution and with 90% phenol, an insoluble protein-carbohydrate complex has been prepared, which is probably the material forming the cell wall. The complex has been dissociated into a protein, and an oligosaccharide. This protein differs in amino-acid pattern from the intra-cellular proteins. The composition and properties of the oligosaccharide already described in a preliminary report (HOLDSWORTH[8]), have been further investigated.

EXPERIMENTAL METHODS

The Organism and Method of Culture

The strain of *C. diphtheriae* used was the Park Williams No. 8 (Toronto) strain. The organism was grown in a medium containing 2% proteose-peptone (Difco), 1% yeast extract (Difco), 1% maltose and 0.5% sodium chloride. Roux bottles containing 100 ml of the medium were inoculated from a 24 hr broth culture of the organism and incubated at 35° C for 4 days. The cells were harvested, washed by centrifugation three times with physiological saline and finally with distilled water.

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Separation of C. diphtheriae into Fractions

A freshly harvested washed suspension of *C. diphtheriae* was shaken with glass beads (Chance Bros. Ballotine No. 12) in a high speed shaker for 3 hours and the suspension centrifuged. The supernatant was poured off and freeze-dried. The residue was suspended in 2% sodium acetate, frozen at −5° C and allowed to thaw whilst being shaken on the high speed shaker. This process of freezing and thawing, and shaking with glass beads was repeated until no further material could be extracted by sodium acetate solution. The residue of disrupted cells was washed with distilled water, filtering the suspension through glass wool to remove the beads, then freeze-dried. The sodium acetate extracts were dialysed against running water for 24 hours, concentrated to small volume, freeze-dried and combined with the first supernatant.

90% phenol is a solvent for some proteins (MORGAN AND PARTRIDGE[9]), and it was found that a small amount of material could be removed from the cell-residues by repeated extraction at 37° C with this reagent. The material insoluble in 90% phenol was washed free from phenol by alcohol and ether, then dried *in vacuo* over P_2O_5. The phenol solution was poured into acetone and the precipitate washed with acetone and ether.

The phenol-insoluble residue appeared to be a fairly stable entity. It was unaffected by extraction at 37° C with formamide, diethylene glycol, 50% aqueous pyridine, saturated aqueous urea, or by boiling with 10% acetic acid. Crystaline pepsin (Armour) at pH 2 and crystalline trypsin (Armour) at pH 8 did not digest the protein present in this material. From the manner of the preparation of this insoluble fraction of the bacterial cell and its stability, it is assumed that this material is the substance forming the cell wall.

Total nitrogen determination

The micro-Kjeldahl method described by BELCHER AND GODBERT[10] was used.

Total reducing sugar estimation

The materials were hydrolysed with $2NH_2SO_4$ at 100° C for 2 hours, neutralised with 2 *N* NaOH, filtered, and diluted to suitable volumes. Portions of this solution were taken for reducing sugar estimation using the alkaline copper reagent of SOMOGYI[11]. All results are expressed as % reducing sugar calculated as glucose.

Estimation of pentose

Pentose was estimated in the hydrolysed material by the orcinol method of BARRENSCHEEN AND PEHAM[12], using D-*arabinose* as the standard.

Estimation of amino-sugars

A modification of the MORGAN AND ELSON method was used (JOHNSTON, OGSTON AND STANIER[13]).

Chromatography

1. *Amino acids.* The two-dimensional technique of CONSDEN, GORDON, AND MARTIN[14] was used, employing phenol/water/NH_3 and butanol/acetic acid/water (PARTRIDGE[15]), as the two solvents. The material for analysis was hydrolysed with 6 *N* HCl for 18 hours at 110° C and the HCl removed by repeated evaporation *in vacuo*. The residue was dissolved in a small volume of 50% ethanol and 5 μl used for each chromatogram.

2. *Carbohydrates.* For the qualitative detection of sugars the samples were hydrolysed with 2 *N* H_2SO_4 at 100° C for 2 hours, then adjusted to pH 4.6 with barium hydroxide, the barium sulphate was removed and the hydrolysate concentrated to small volume. Two-dimensional chromatograms were prepared using the previously mentioned solvents. After development the sugars were revealed by spraying the paper with 3% *p*-anisidine hydrochloride in butanol and heating for 15 min at 110° C (HOUGH, JONES, AND WADMAN[16]). With this reagent aldohexoses yield orange spots, which fluoresce yellow-green in ultraviolet light, whereas aldopentoses yield a cherry-red colour. Amino-sugars were detected by the MORGAN AND ELSON reaction as applied to paper chromatography by PARTRIDGE[15]. It must be emphasised that adequate separation of the sugars galactose mannose and arabinose can only be achieved by the two-dimensional technique, *e.g.*, in butanol acetic acid mannose has a similar R_F to arabinose, in phenol mannose has an R_F similar to galactose.

RESULTS

The various fractions prepared from *C. diphtheriae* were dried over P_4O_5 and anal-

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ysed. The results are shown in Table I, together with the proportions in which the fractions occur in the cell. These proportions vary during growth (HOLDSWORTH[17]) and the figures quoted here are for cells harvested from a culture which had just reached the stationary phase.

TABLE I

ANALYSIS OF *C. diphtheriae* FRACTIONS

Fraction	*Amount present in whole cells* %	*Total* N_2 %	*Total reducing sugar* %	*Pentose* %	*Glucosamine* %	*Ash* %
Original		11.8	13.6	6.3	2.0	6.9
Intracellular material	43.0	13.7	2.8	2.0	none	
Cell debris	57.0	10.2	24.9	9.7	3.5	
Phenol soluble	12.0	13.5	2.0	1.6	none	
Phenol insoluble	45.0	9.5	25.4	9.8	4.1	10.1

The analytical results show that the main carbohydrate of *C. diphtheriae* is concentrated in the insoluble cell wall. This applies also to the amino-sugars present in the bacterium. Qualitative examination for amino acids and sugars after hydrolysis of the fractions led to the following conclusions.

a) The carbohydrate of the intracellular and phenol-soluble portions of *C. diphtheriae* consisted of ribose and desoxyribose (trace). These sugars presumably arose from the nucleoproteins of the organism. Examination of the various fractions suspended in 90% glycerol for specific absorption at 260 mμ (MITCHELL[18]) showed that the nucleic acids occurred in those portions of the cell soluble in sodium acetate solution and 90% phenol.

b) Two-dimensional chromatograms for amino acids showed that diamino-pimelic acid, discovered by WORK[10], in *C. diphtheriae* was almost exclusively present in the cell wall. A trace of diamino-pimelic acid occurs in the phenol soluble fraction (private communication — E. WORK). This may be due to the fractions not being pure, the phenol soluble fraction probably containing a small amount of the insoluble fraction as an impurity. From the intensity of the ninhydrin colours at least 95% of the diamino-pimelic acid present in *C. diphtheriae* occurs in the phenol insoluble fraction.

(c) The insoluble residue, which contains most of the carbohydrate of the cell, contained the following sugars: galactose, mannose, arabinose and an amino-sugar which had an R_F corresponding to either glucosamine or chondrosamine. When the amino-sugar was examined on a one-dimensional chromatogram using water-saturated collidine as the solvent, and allowed to develop for 50 hours, it could be separated from chondrosamine but had the same R_F as glucosamine.

Thus the analytical results and chromatographic examination show that the insolu-

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ble cell wall substance contains protein and a high proportion of carbohydrate, this latter containing four sugars.

PREPARATION OF AN OLIGOSACCHARIDE FROM THE INSOLUBLE PORTION OF C. DIPHTHERIAE

When investigating the somatic antigen of *shigella paradysenteriae* (Flexner) GOEBEL, BINKLEY AND PERLMAN[20] dissociated the protein-polysaccharide-lipoid complex by heating with saturated picric acid solution. This reagent was equally effective with the insoluble portion of *C. diphtheriae*.

12 g of the insoluble fraction were treated with 100 ml saturated aqueous picric acid and placed in a boiling water bath for 10 mins. The mixture was centrifuged and the residue treated with a further quantity of picric acid. Four extractions served to remove all extractable material. The combined supernatants were rapidly concentrated *in vacuo* to 40 ml and poured into 400 ml acetone. The precipitated polysaccharide was filtered on a sintered glass filter and washed free from picric acid with acetone containing 0.5% HCl. Yield 2.8 g crude oligosaccharide.

The insoluble residue of protein picrate was treated with acetone containing 1% HCl, until free from picric acid, washed with ether and dried *in vacuo*. The protein was now almost entirely (98%) soluble in 90% phenol and contained 14.2% N_2. This material still contained 5.1% reducing sugar but all this could be accounted for as amino-sugar by the modified MORGAN AND ELSON method.

Purification of the oligosaccharide

2 g of the crude oligosaccharide was dissolved in 20 ml distilled water and centrifuged to remove a small amount of insoluble material. The clear supernatant was placed in a 100 ml centrifuge cup, cooled in ice, and ethanol containing 1% sodium acetate was added dropwise to the stirred solution until some precipitation occurred. This fraction was separated and more ethanol cautiously added to give a further precipitation. Fractions obtained at 15% and 40% alcohol contained little carbohydrate, gave a positive biuret test and were rejected. The bulk of the material precipitated between 60–80% alcohol, was biuret-negative and yielded 80% reducing sugars after hydrolysis. This fraction was treated with 50% alcohol, centrifuged and shaken with acid-washed decolorising charcoal. The solution was then dialysed against frequent changes of distilled water for 24 hours at 0° C. The oligosaccharide was again fractionated using *iso*propyl alcohol containing 0.2% concentrated hydrochloric acid as the precipitant, collecting the fraction precipitating at 66–70% of *iso*propyl alcohol. The dialysis and fractionation was repeated once more to give a final yield of 0.7 g oligosaccharide. The low yield was due to the loss sustained when this low molecular weight material was dialysed.

EXAMINATION OF THE OLIGOSACCHARIDE

Electrophoresis and the diffusion coefficient

These experiments are described in an addendum to this paper. It will be sufficient to point out here that the oligosaccharide appears to be homogeneous and of low molecular weight.

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Absorption spectrum

A 0.5% aqueous solution was examined in the range 200–400 mμ There was no definite absorption peak, although the curve had a point of inflexion in the region 270–280 mμ, which may indicate a trace of protein impurity. Repeated fractionation from alcohol failed to change the absorption curve.

Chemical analysis

The oligosaccharide had an ash content of 1.9%, phosphorus 0.3%, nitrogen 0.2%. Qualitative examination for uronic acids with naphthoresorcinol gave a negative result. Amino-sugars could not be detected. The unhydrolysed oligosaccharide showed no reduction of the SOMOGYI alkaline-copper reagent, even after 1 hour at 100° C. Hydrolysis with 2*N* H_2SO_4 showed a maximum yield of reducing sugar in 2 hours, 89% calculated as glucose. The pentose content was 41% as arabinose.

Optical rotation and hydrolysis of the oligosaccharide

A 2% solution of the oligosaccharide had a specific rotation of $[\alpha]_D^{20} + 109°$. After hydrolysis the mixture of sugars was laevorotatory $[\alpha]_D^{20} - 29°$.

Qualitative examination for sugars

The hydrolysed oligosaccharide was examined chromatographically on paper as previously described. The mixture contained three reducing sugars, glactose, mannose and arabinose. A diagram of the chromatogram is shown in Figure 1.

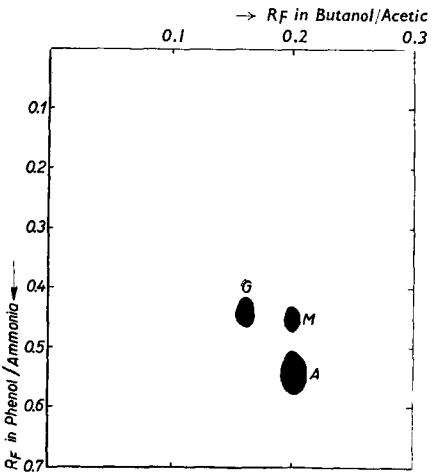

Fig. 1. Chromatography of the hydrolysed oligosaccharide. G = Galactose; M = Mannose; A = Arabinose.

Estimation of the proportions of the sugars

Whatman No. 4 paper was washed before use with 1% ammonia and dried. The solvents were carefully purified before use; butanol by treatment with decolorising charcoal and redistillation, phenol by steam distillation. Quantities of the solution to be analysed were pipetted on to the paper with a micro-syringe and developed for two days in butanol/acetic acid/water. Alternate strips were taken and sprayed with *p*-anisidine hydrochloride. The positions of the spots in the unsprayed strips were then deduced, rectangles containing these spots cut out and the carbohydrate extracted with 5 ml distilled water, shaking vigorously to disintegrate the paper. The suspension was centrifuged and the residue washed with two more portions of distilled water. The eluted carbohydrate was made up to 15 ml in a graduated flask. Duplicate reducing sugar estimations with 5 ml of this solution were performed using the SOMOGYI reagent. Blank determinations on similar areas of paper cut from the chromatogram, were extremely small and could be neglected. This procedure gave the proportion of galactose to mannose plus arabinose. By a similar method using phenol/water/NH_3 as the solvent the proportions of galactose plus mannose to arabinose were determined. The blank deter-

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minations with the chromatogram developed with phenol could not be neglected and the value of reducing sugar was calculated by including a known amount of reference sugar (*d*-ribose) on the chromatogram (HIRST, FLOOD AND JONES[21]). From the results obtained the proportions of the three sugars were calculated to be 2 of galactose 1 of mannose and 3 of arabinose. Thus the minimum molecular weight of the oligosaccharide would be 990.

Separation and characterisation of the sugars

The hydrolysate from 250 mg of oligosaccharide was placed on a column of powdered cellulose (Solkafloc) 50 × 2 cm, and developed with butanol/ethanol/water (HOUGH, JONES AND WADMAN[16]). 5 ml portions of the eluate were collected and examined by one dimensional paper chromatography. Three main fractions were obtained:

Fraction 1 contained arabinose contaminated with mannose and had $[\alpha]_D^{20} - 84°$. The whole of this material was treated with a well washed suspension of *Saccharomyces carlsbergensis* (N.C.T.C. 7248) for 30 mins at 30° C. The arabinose was thus obtained free from mannose and gave only one spot on a paper chromatogram corresponding in R_F to arabinose. The rotation of the sugar was $[\alpha]_D^{20} - 98°$, *i.e.* it was D(—) arabinose. It formed a methyl phenyl hydrazone m.pt. 163° C alone and when mixed with authentic specimen of D-arabinose methyl phenyl hydrazone.

Fraction 2 consisted of a mixture of arabinose and mannose. The mixture was concentrated to 0.5 ml and mannose phenyl hydrazone prepared (RENFREW[22]). The product even after recrystallisation melted over a range of temperature, therefore the phenyl hydrazone was decomposed with benzaldehyde and the sugar examined chromatographically. On a two-dimensional chromatogram one main spot was found when the substance was examined either alone or when mixed with a sample of pure mannose. Two faint spots showed that traces of arabinose and galactose had been precipitated as phenyl hydrazones.

The last fraction to be eluted was dextrorotatory and corresponded on a chromatogram to galactose. The fraction was concentration to dryness dissolved in 0.5 ml water, then 1 ml concentrated nitric acid added, and the mixture kept at 80° C for 30 mins. Mucic acid m.pt. 215° C, was formed, confirming that the sugar was D-galactose.

Thus the oligosaccharide contained D(—) arabinose, mannose and D-galactose. The mannose was not obtained in sufficient quantity to determine the optical rotation, particularly since mannose has a low specific rotation $[\alpha]_D^{20} + 14°$.

Periodate oxidation of the oligosaccharide

HIRST[23] has found that an unbranched polysaccharide on oxidation with periodate yields 1 molecule of formic acid per non-reducing end-group, or 2 molecules of formic acid and 1 molecule of formaldehyde for each reducing end-group. The measurement of the formic acid produced on oxidation is therefore useful in determining chain-length. In a preliminary experiment using raffinose (a trisaccharide) and oxidising with a saturated solution of potassium periodate at room temperature, equilibrium was reached in 200 hours, but a greater amount of formic acid was produced than the theoretical yield. Rapid oxidation with sodium periodate at low temperature (POTTER AND HASSID[24]) was more satisfactory, giving the theoretical yield of acid from raffinose in 30 hours. Three end-group determinations were performed, a typical result is given below.

46 mg oligosaccharide were dissolved in 25 ml of normal physiological saline, cooled

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to 0° C and 5 ml of 0.2 *M* sodium periodate added (0.2 *M* periodic acid was neutralised with caustic soda using methyl red as indicator). The mixture was preserved at 2° C, 3 ml portions were removed at intervals, treated with 0.5 ml ethylene glycol and after standing in the dark for 15 mins, titrated with *N*/100 NaOH using methyl red as indicator:—

15 mins	3 ml = 0.415 ml	*N*/100 NaOH
15 hrs	3 ml = 0.750 ml	,,
24 hrs	3 ml = 0.890 ml	,,
35 hrs	3 ml = 0.898 ml	,,

i.e. 46 mg oligosaccharide = 8.98 ml *N*/100 NaOH

∴ 510 g oligosaccharide = 1 g molecule formic acid.

Formaldehyde could not be detected in the oxidation products on qualitative examination with dimedone. Since the oligosaccharide has no reducing properties, oxidation of the two end groups should yield 2 molecules of formic acid, *i.e.* from the above data the total molecular weight of the sugars in one chain length of the oligosaccharide would be 1020.

Examination of the products of oxidation of the oligosaccharide

The remainder of the solution from the periodate oxidation of 46 mg of oligosaccharide was dialysed against running water for 6 hours, then against frequent changes of distilled water at 0° C for 3 days. The turbid solution was concentrated to 2 ml. *in vacuo* and the sparingly soluble material which was present was centrifuged, washed with a small volume of ice-cold water and dried over P_2O_5, (yield 15 mg). The supernatant contained less than 1 mg of material. On adding SCHIFF's reagent to some of the sparingly soluble substance, a strong bluish-purple colour developed, showing that the oxidation had produced aldehyde groups. 10 mg of oxidised oligosaccharide was hydrolysed with 2*N* H_2SO_4, the sulphuric acid removed with $Ba(OH)_2$ and the reducing sugar and pentose content determined. The alkaline copper reagent could not be used with this hydrolysate and the reducing sugar content was determined by complete oxidation with periodate at 100° C (HIRST AND JONES[25]), giving 81% reducing sugar as glucose. The pentose content was 45%. The remaining hydrolysate was concentrated to 0.1 ml and examined by two dimensional paper chromatography. The mannose and arabinose of the oligosaccharide were apparently unchanged, whereas the galactose spot was missing. A faint spot appeared

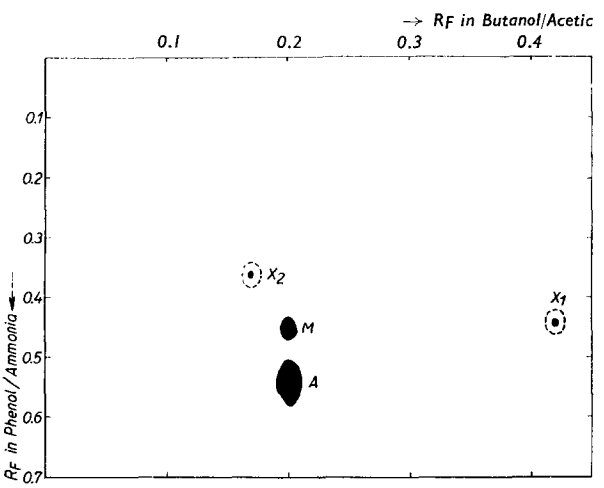

Fig. 2. Chromatography of the hydrolysed oxidised oligosaccharide.
M = Mannose; A = Arabinose.
X_1 unknown in Butanol/Acetic acid/Water
X_2 unknown in Butanol/Ethanol/Water

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on the chromatogram in a position which does not correspond to any known sugar (Fig. 2). The results suggest that the ends of the oligosaccharide molecule are occupied by galactose units, which are destroyed by the oxidation.

DISCUSSION

A fundamental assumption in this work has been that the insoluble fraction obtained from *C. diphtheriae* is the cell-wall material. COOPER, ROWLEY AND DAWSON[26] have shown that 93% of the cells of *Staphylococcus aureous* are broken by shaking with minute glass beads to give a suspension of cell-wall material. In the work reported here the cells were disrupted by freezing and thawing in addition to high speed shaking with glass beads, and the process was continued until no further material could be extracted with 2% sodium acetate solution. The insoluble residue so obtained was tested with various solvents and it was found that 90% phenol would remove a further small amount of protein and nucleoprotein, leaving an insoluble powder, which could not be further altered except by drastic chemical procedures. Since the preliminary report of this (HOLDSWORTH[8]), SALTON AND HORNE[27] have published electron-microscopic studies of *Escherichia coli*, *Salmonella pullorum* and *Streptococcus faescalis* and presented a method for the preparation of pure cell-wall material. They disrupted cells by heating for 5 mins at 75° C and 100° C, removing the undamaged cells by centrifuging for 20 mins at 3,000 r.p.m., then obtained the cell-walls by centrifuging at 10,000 r.p.m. for 10 mins. Electron-photomicrographs show that the material obtained was almost pure cell wall. It is possible that the heat treatment would radically alter the bacterial substance, but similar material was obtained by shaking cells with glass beads and washing the impure preparation with phosphate buffer. Only low yields were obtained since these workers were concerned with preparing a material of high purity. No chemical examination was reported but the material was free from nucleic acids as judged from the ultra-violet absorption spectrum. There seems no doubt from the method of preparation and from its chemical stability that the insoluble residue of *C. diphtheriae* is also cell wall.

The cell-wall substance contained 25% carbohydrate, 10% ash and 60% protein (assuming the factor 6.25 for converting N to bacterial protein). Carbohydrate and protein are probably chemically combined to form an insoluble complex, since after dissociating the material with picric acid, a water-soluble oligosaccharide was obtained and the protein thus released was soluble in 90% phenol. No information was obtained to suggest that this protein was homogeneous but it differed in composition from the intracellular protein in that it contained a high proportion of diamino-pimelic acid and 5% glucosamine in its amino-acid pattern. The method of dissociating the protein-carbohydrate complex with boiling picric acid solution may have resulted in the degradation of the components, although the work of GOEBEL, BINKLEY AND PERLMAN[20] suggests that the degradation would be slight. The oligosaccharide isolated from *C. diphtheriae* was of low molecular weight and until a more gentle means of dissociating the complex is found, it is impossible to state that the carbohydrate occurs as this small unit in the cell wall. LEVENE AND MORI[28] prepared the carbohydrate of ovalbumin by hydrolysis of the protein component with barium hydroxide and subsequent purification of the polysaccaride. This method was applied to the insoluble fraction of *C. diphtheriae* and although the oligosaccharide was not obtained in the same state of purity as that prepared by the picric acid procedure, both oligosaccharides had identical chemical

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properties and the same chain length. Since the different methods gave identical materials, it may be that the carbohydrate is present in the cell wall as a unit of low molecular weight.

Evidence is given in an addendum to this paper to show that the oligosaccharide obtained by picric acid extraction was a homogeneous substance and the molecular weight calculated from the diffusion coefficient was about 1,200. This agrees with the estimation of the molecular weight from the "chain length" as determined with sodium periodate, which gave the value 1020, and also agrees with the minimum molecular weight deduced from the analytical results. The oligosaccharide contained 2 molecules of D-galactose, 1 molecule of D-mannose and three molecules of D-arabinose. The galactose molecules probably occupy the ends of the chain of sugar residues since they are destroyed by periodate oxidation.

The carbohydrates of the diphtheriae organism have been investigated by GUBAREV[29] who treated a suspension of cells with 40% caustic soda for 2 hours at 100° C, neutralised the resulting solution with acetic acid and fractionated the product with alcohol. One fraction contained galactose, a pentose and gave reactions for an aldobionic acid and an amino-sugar. More recently ORLOVA[30] by the same procedure obtained two fractions, one of which yielded mannose on hydrolysis and the other yielded a mixture of mannose, galactose and arabinose in the proportions 1:1:3. No estimate of the molecular weight of the polysaccharide was given and the material did not act as a hapten towards antisera prepared to *C. diphtheriae*. WONG AND TUNG[31] prepared a polysaccharide, which was group specific when tested with antisera prepared against different types of *C. diphtheriae*. This polysaccharide was prepared in very low yield by extracting defatted cells with dilute alkali at 37° C, but unfortunately no chemical examination was reported. In *C. diphtheriae* P.W.8, harvested under conditions when the cells contain little reserve carbohydrate (HOLDSWORTH[17]), the main carbohydrate of the organism is contained in a proteincarbohydrate complex which is probably the substance which gives the cell wall its mechanical and chemical stability. This complex forms approximately 40% (on ash-free basis) of the dried cell, a proportion which is not surprising in view of the relatively large surface area of the bacterial cell and the highly solvated nature of the intracellular components.

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SUMMARY

An insoluble protein-carbohydrate complex has been prepared from *C. diphtheriae* P.W.8. and is probably the material of the cell wall. The protein portion contains glucosamine and a high proportion of diaminopimelic acid. The oligosaccharide, which is the main carbohydrate of the cell, contains 2 molecules of D-galactose, 1 of D-mannose and 3 of D-arabinose. Periodate oxidation destroys the galactose molecules, which are thus presumed to occupy the terminal positions of the oligosaccharide. Two different methods of preparation gave oligosaccharides of identical properties and thus the carbohydrate may exist in the complex as this small unit of six sugar residues.

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RÉSUMÉ

Un complex insoluble de protéine-hydrate de carbone a été obtenu à partir de *C. diphtheriae*; c'est probablement l'élément de la paroi cellulaire. La partie protéinique contient de la glucosamine et une forte proportion d'acide diaminopimélique.

L'oligosaccharide qui est l'hydrate de carbone principal de la cellule diphthérique, contient deux molécules de D-galactose, une molécule de D-mannose et trois molécules de D-arabinose. L'oxydation par $NaIO_4$ détruit les molécules de galactose, qui sont, par conséquent, présumées occuper les positions terminales de l'oligosaccharide. Deux méthodes de préparation différentes donnèrent des oligosaccharides de propriétés identiques de sorte que l'hydrate de carbone pourrait exister dans le complex sous forme de ce petit élément de six restes de sucre.

ZUSAMMENFASSUNG

Eine unlösliche Kohlenhydrat-Eiweiss Verbindung wurde aus *C. diphtheriae* hergestellt. Es handelt sich wahrscheinlich um die Substanz, aus welcher die Zellwand aufgebaut ist. Der Eiweissanteil enthält Glucosamin und einen hohen Prozentsatz an Diaminopimelinsäure. Das Oligosaccharid, welches den Haupt-Kohlenhydrat-Bestandteil der Zelle von *C. diphtheriae* bildet, enthält 2 Moleküle D-Galaktose, 1 Molekül D-Mannose und 3 Moleküle D-Arabinose. Die Perjodatoxydation zerstört die Galaktose-Moleküle, weshalb angenommen wird, dass Galaktose die Endgruppen des Oligosaccharides bildet. Zwei verschiedene Bereitungsmethoden gaben Oligosaccharide mit identischen Eigenschaften; daher kann das Kohlenhydrat möglicherweise in Form dieser kleinen Einheit von sechs Zuckerresten in Komplex bestehen.

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