THE NATURE OF THE CELL-WALL OF *CORYNEBACTERIUM DIPHTHERIAE*

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BARTHOLOMEW AND MITTWER[1] have shown that in Grampositive organisms there is an area external to the Gramstaining material which is not readily stainable, and is the cell-wall. There is no reliable information regarding the chemical nature of this structure (*cf.* BISSET[2]). HEWITT[3] by use of the phase-contrast microscope has observed a well-marked cell-wall in unstained preparations of *Corynebacterium diphtheriae*.

A freshly harvested, well-washed suspension of *C. diphtheriae P.W.* 8. was disintegrated by shaking with glass beads and by freezing and thawing, until no further material was extracted by 2% sodium acetate solution. The insoluble residue was then treated with 90% phenol, which removed a further small amount of protein and nucleoprotein. The phenol-insoluble material yielded 25% reducing sugar on hydrolysis with $2NH_2SO_4$ and appeared to be a carbohydrate-protein complex which was unaffected by extraction with saturated aqueous urea, diethylene glycol, formamide or by boiling 10% acetic acid. The protein could not be digested by trypsin or pepsin. The carbohydrate portion was obtained as a white, water-soluble, non-reducing oligosaccharide free from protein, by treating the complex with saturated picric acid solution at the boil, the protein forming an insoluble protein picrate (HOLDSWORTH[4]). After fractional precipitation from alcohol the oligosaccharide gave only one peak of zero mobility in the electrophoresis apparatus, the diffusion constant determined in the same apparatus showed that the molecular weight was approximately 1,200. The component sugars of the hydrolysed oligosaccharide were separated by chromatography and showed that the molecule contained 2 residues of D-galactose, 1 of D-mannose and 3 of D-arabinose, giving a molecular weight of 990. This figure was in agreement with the estimation of chain length by periodate oxidation. Examination of the oxidised polysaccharide showed that the galactose residues had been destroyed and therefore probably occupy the ends of the oligosaccharide. The protein portion of the cell-wall material, freed from picric acid by washing with acetone containing 0.5% HCl, differed in amino-acid composition from the intracellular proteins of the bacterial cell. Glucosamine and the recently discovered diaminopimelic acid (WORK[5]) were present solely in the cell-wall. That the carbohydrate and protein are chemically combined is suggested by the fact that the complex is insoluble in 90% phenol, but after removal of oligosaccharide the protein is soluble in 90% phenol. Until a more gentle procedure is found for dissociating the complex, it is impossible to state that the carbohydrate is present as a small unit of six sugar residues.

SALTER AND HORNE[6] have presented electron-photomicrographs of the cell-walls of *Escherichia coli* and *Streptococcus faecalis* prepared by differential centrifugation of cells disrupted by heat or by shaking with glass beads. In the interest of pure preparations only low yields were obtained. The carbohydrate-protein complex described in this note forms 45% of the dried weight of the *C. diphtheriae* cell.

REFERENCES

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