et al., 1963; Drummond *et al.*, 1969, 1971; Wallenfels *et al.*, 1969; Tung & Nordin, 1969; Marshall & Whelan, 1971). Some of these methods involve chemical modification of the substrate and are, in general, time-consuming. We have now developed a rapid and universal method for such investigations by using an ultrafiltration technique.

The actions of several enzymes were examined on their respective substrates, such as pullulanase on pullulan, α -amylase on amylose and β -amylase on amylose. All the substrates were washed before enzymic reaction, by ultrafiltration with an Amicon cell with PM 10 membrane (Amicon Corp., Lexington, Mass., U.S.A.). This resulted in exclusion of polysaccharides with molecular weights less than 10000. The enzymic reactions were carried out in the ultrafiltration cells at 20°C with a flow rate of 0.4 ml/min, and the initial products were fractionated as they were formed.

Analysis by t.l.c. of the fractions from the reactions of pullulanase and α -amylase showed the appearance of a dark spot at the base-line. The intensity of this immobile spot increased with increasing fraction number along with the appearance of faster-moving spots. The latter were the homologues of maltotriose and maltose for the two enzymes respectively. The later fractions contained only maltotriose and maltose for these enzymes. The spot on the base-line in the first fraction would presumably consist of carbohydrate units with molecular weights below 10000. This therefore must be the result of the endo action of the two enzymes. If the reaction is not carried out in the ultrafiltration cell and the enzymic digest as such is analysed, one would not be sure whether the spot at the base-line is a product or the unchanged substrate. The exo action of an enzyme, on the other hand, would produce a single fast-moving spot on t.l.c. This was confirmed by the experiment with β -amylase and amylose.

It is expected that the ultrafiltration technique could also be applied for the proteases and the nucleases.

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The Presence of a Sulphated Polysaccharide in the Cell Envelopes of *Halobacterium halobium*

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Media containing at least 4M-NaCl are required for optimal growth of the obligate halophilic bacterium *Halobacterium halobium*. This Gram-negative rod-shaped organism lacks the peptidoglycan component typically found at the surface of most other bacteria. The anionic nature of envelope proteins and lipids in this organism (Stoeckenius & Kunau, 1968; Kates *et al.*, 1967) suggested that acidic groups might be involved in its survival in high-salt media. In this context studies by Doyle & Birdsell (1972) indicated that charged glucose-containing polymers may change conformation with varying salt concentrations. No uronic acids were detected in envelope polysaccharides of this organism. The possibility of bound sulphate or phosphate rendering these polysaccharides acidic was the basis for the present studies.

Polysaccharides and glycoproteins were extracted from cell envelopes of *H. halobium* with aqueous phenol and fractionated on a column of Sephadex G-100–Sephadex G-200 (2:1, w/w). One carbohydrate fraction containing mainly mannose but no protein could not be precipitated from aqueous solution by ethanol in the absence of bivalent cations. The presence of sulphate residues in this fraction was initially demonstrated by studying the incorporation of [³⁵S]sulphate. The organism was grown in sulphate-free medium for ten generations, and 0.5mCi of [³⁵S]sulphate was added to give a final concentration of 1 μ M. The envelope polysaccharides were isolated and chromatographed under normal conditions on Sephadex G-100–Sephadex G-200. The bulk of the radioactivity was eluted with the polysaccharide peak. Evidence for the presence of sulphate was obtained after acid hydrolysis for 24h, when 92% of the recovered radioactivity could be precipitated from acidic solutions by BaCl₂ plus carrier sulphate.

Confirmation of the presence of sulphate hemi-ester groups in this material was obtained by i.r. spectroscopy. A strong absorption at 1240cm⁻¹ characteristic of S=O in sulphate esters was observed together with other peaks characteristic of hexopyranoses. Phosphate was not detected.

The chemical properties of this polysaccharide support the contention that it is sulphated. Cetyltrimethylammonium bromide precipitates the polysaccharide even after acidification to pH 2 with acetic acid, indicating that the acidic group is still ionized at pH 2. This material is also strongly adsorbed on DEAE-cellulose and requires 1M-salt at pH 6 for elution, providing further evidence for the anionic nature of the polymer.

This report appears to be the first indication of sulphated polysaccharides in bacteria. Such polymers are more typically found in the mucopolysaccharides of higher organisms and seaweeds.

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Peptidoglycan Lytic System associated with Mesosomal Membranes from *Micrococcus lysodeikticus*

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Previous studies (Ellar, 1969; Ellar & Freer, 1969; Ellar *et al.*, 1971) have described the purification and properties of mesosomal membranes from *Micrococcus lysodeikticus* and *Bacillus megaterium* KM. Although mesosomal membranes were seen to differ from plasma membranes in their morphology and chemical composition and in the activities of certain enzymes, pulse-labelling experiments (Ellar *et al.*, 1971) revealed no preferential synthesis of membrane lipid in the mesosome.

Several reports (Rogers, 1970; Ellar *et al.*, 1967) have observed that mesosomes are invariably associated with the growing transverse septum during division of Gram-positive bacteria. Studies with *Streptococcus faecalis*, which showed that peptidoglycan-hydrolysing enzymes are located in the region of the septum and the newly synthesized cell wall (Shockman *et al.*, 1967a), suggested that the mesosome in this region might represent the site for the enzymic remodelling of the peptidoglycan that is thought to occur during division and cell separation (Rogers, 1970). The present report describes a peptidoglycan-hydrolytic enzyme system associated with mesosomal membranes isolated from *M. lysodeikticus*.

Cytoplasmic and mesosomal membranes were isolated and washed five times as described by Ellar &

Freer (1969). Purified *M. lysodeikticus* cell walls used as substrate in the assay of lytic enzyme were prepared from exponentially growing cells by mechanical disruption (Shockman *et al.*, 1967b) and treated with sodium dodecyl sulphate to inactivate any endogenous autolytic activity. Lytic assays were carried out at 23°C with 2ml of the substrate (150 µg/ml) suspended in 50mM-tris-HCl buffer, pH 7.5, or 50mM-sodium phosphate buffer, pH 6.7. Cytoplasmic or mesosomal membrane contained in the same buffer was added and the total volume was adjusted to 3.0ml. The reaction was followed by observing the change in the absorbance at 450nm. One unit of enzyme was defined as the amount giving a decrease of 0.001 E_{450} unit/h (Shockman *et al.*, 1967b). Assays revealed a high content of peptidoglycan-lytic activity associated with mesosomal membranes (15000 units/mg of protein) whereas the plasma membrane showed a 30-fold less specific activity (430 units/mg of protein). Since no lytic activity could be detected in cell cytoplasm, these results indicate a concentration of over 70% of the total lytic activity of the cell in the mesosomal membranes. Preliminary investigation of the properties of the enzyme system including sensitivity to inhibitors and reaction kinetics have been carried out.

The possibility that this lytic activity might represent contaminating residual lysozyme employed in the isolation of membranes and mesosomes was eliminated by control experiments that included the use of 125 I-labelled lysozyme and lysozyme-specific antisera. The relationship between cell and nuclear division and this apparent concentration of peptidoglycan-lytic activity in mesosomal membranes will be discussed.

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Endotoxins in a Lipopolysaccharide Preparation and Cell Envelope of *Escherichia coli*

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Certain groupings detectable in some endotoxins by shifts in the spectrum of a dye (Janda, 1971) were investigated in extracellular materials released by