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.

We are grateful to the Medical Research Council for financial support.

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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

Escherichia coli A.T.C.C. 12408 by using a spectrophotometer (air blank). Other experimental conditions were similar to those published by Janda & Work (1971) or to those used by Janda *et al.* (1971).

Determination of the groupings in non-fractionated culture supernatants was accurate and reproducible. The standard deviation was $0.6\mu\text{g}$ of a relevant lipopolysaccharide preparation. This preparation was similar to others (S, 0.2–0.4%; glucose, 8.5–9.6%, w/w). Use of the same standard in a phenol- H_2SO_4 test (Knox *et al.*, 1966) for the determination of lipopolysaccharide allowed the comparison of these procedures.

The results agreed if the samples were taken from later stages of lysine-limited fermentations (within 5% relative), but significant discrepancies (up to 25%) were found in samples from earlier stages. The discrepancies between the methods applied to fractions of the fluid samples and of the preparation were up to 95%. Their trend was continuous and followed an elution pattern (the fractions were obtained by molecular sieving on a Bio-Gel 0.5m column, with water as eluent).

Certain fractions of the preparation caused variations in wavelengths of the shift. These could be decreased by urea treatment of the preparation before it was subjected to sieving (no effect on the total grouping content was observed). The remaining variations resembled spectra previously demonstrated (Janda & Work, 1971) for endotoxins representing various stages in biosynthesis of the lipopolysaccharide of *Salmonella*.

Similar spectral variations were detected in certain fractions of all fluids. The variations appeared to be related to fermentation stages, e.g. a fraction causing a secondary shift at 650nm (like glycolipid from *Salmonella minnesota* mR 595) was relatively increased at the end of a lysine-limited fermentation.

The total final grouping content in fluids from exponentially growing cultures (in non-limited fermentations) was about 2% of the content found in the limited ones. There the groupings were accumulated into fluids by cells in an unbalanced growth. Penicillin G (100mg/l added at the start of a stationary growth phase) prevented a secondary growth (see discussion in Work, 1971), but the accumulation continued and reached normal production.

Solvolysis of the preparation (99% dioxan for 60min at 100°C) resulted in loss of the detectable groupings and its toxicity for mice.

This work was supported by a grant from Beecham Research Laboratories Ltd.

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The Interpretation of Abrupt Transitions in Lineweaver-Burk Plots

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Lineweaver-Burk plots for glutamate dehydrogenase (Dalziel & Engel, 1968) and glucose 6-phosphate dehydrogenase (Muto & Uritani, 1970) show abrupt transitions between two or more linear sections. With decreasing values of $1/s$, at each transition the slope increases and the intercept decreases, both approximately twofold. The enzymes appear to pass to forms with successively higher V_{max} and higher K_m . Dalziel & Engel (1968) explained the data for glutamate dehydrogenase by invoking negatively interacting NAD^+ -binding sites. Schwert (1970) doubted whether such a model would give abrupt transitions between sections differing so little in slope and intercept. This prompted reinvestigation of the conditions governing such transitions.

Engel (1970) suggested that eqn. (1) might yield abrupt transitions:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s + n_2 s^2 \dots n_q s^q}{d_1 s + d_2 s^2 \dots d_q s^q} \quad (1)$$

if the values of s giving $n_2 s^2 = n_0$ and $d_2 s^2 = d_1 s$ approximately coincided, and similarly the values giving $n_3 s^3 = n_1$ and $d_3 s^3 = d_2 s^2$ etc. Successive sections would approximately obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_1 + n_2 s}{d_2 s} \quad \text{etc.}$$

This situation, in which each term is significant over some range of s , is designated Case 1.

In Case 2 d_2, d_4, d_6 etc. are chosen to render the corresponding terms always small relative to at least one adjacent term. Linear sections obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_2 + n_3 s}{d_3 s} \quad \text{etc.}$$

In Case 3 coefficients are chosen to effectively eliminate $d_2 s^2$ and $d_3 s^3$, $d_5 s^5$ and $d_6 s^6$, $d_8 s^8$ and $d_9 s^9$ etc. and also $n_2 s^2$, $n_5 s^5$, $n_8 s^8$ etc. Linear sections obey:

$$\frac{e}{V_0} = \frac{n_0 + n_1 s}{d_1 s}, \quad \frac{e}{V_0} = \frac{n_3 + n_4 s}{d_4 s} \quad \text{etc.}$$

Three conditions for abrupt transitions may be stated: (i) corresponding switches in dominance of terms within numerator and denominator must