an accurate and sensitive measure of sodium dodecyl sulphate and some endotoxins. When direct sunlight was avoided and measurements were made within certain standardized times, 1 min for sodium dodecyl sulphate and 5 min for endotoxins, the procedures were usable for the quantitative determination of sodium dodecyl sulphate in the range 0.4–4.0 μ g and of a lipopolysaccharide preparation derived from *Escherichia coli* A.T.C.C. 12408 in the range 2.0–20 μ g.

Apparently certain structural features of the reacting molecules determined the spectral changes that took place. Spectra from model compounds indicated that the reactive groupings possess strong anionic sites with long aliphatic chains attached to them in a certain spatial configuration. The spectral responses of endotoxins were diminished or altered by different treatments of lipopolysaccharide solutions such as mild acid hydrolysis (pH 1, 100°C, 30 min), refluxing in dioxan [aq. 99% (v/v) dioxan, 120 min], cleavage of fatty acids leading to loss of toxic properties (Nowotny, 1964), mild alkaline hydrolysis (1M-sodium hydroxide, room temp., 120 min), urea treatment (4M, 8M), reactions with sodium deoxycholate and with proteins, e.g. with specific antisera.

These effects and additional evidence based on i.r. spectra, sulphate and total sulphur determinations (S was 0.2–0.4%) suggest that the endotoxin anionic sites in question are attached to fatty acids and the sites might contain sulphur atoms. Details of the nature of the reactive endotoxin groupings remain to be established; nevertheless the spectral shifts are useful evidence for the existence of groupings in endotoxins that have not been detected by other means. The spectral shifts could be also used for determination of these endotoxin groupings in fermentation fluids.

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Mutants of *Pseudomonas* AM1 that Require Glycollate or Glyoxylate for Growth on Methanol or Ethanol

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The pathway leading to glycolytic intermediates during the growth of *Pseudomonas* AM1 on C₁ compounds involves condensation of a C₁ unit with

glycine to form serine, which is converted via hydroxypyruvate and D-glycerate into 3-phosphoglycerate (Large & Quayle, 1963; Heptinstall & Quayle, 1970). The net assimilation of C₁ compounds into cell constituents by this 'serine pathway' requires the regeneration of 1 molecule of glycine for each molecule of 3-phosphoglycerate formed. The mechanism of synthesis of the glycine skeleton from C₁ compounds in this organism is unknown. On the basis of this assimilatory scheme, mutants might be isolated containing a lesion in the pathway leading to the regeneration of glycine, leaving the oxidation of C₁ compounds unimpaired. Such mutants would require substrate amounts of glycine for growth with a C₁ compound as energy source, but the toxicity of glycine to *Pseudomonas* AM1 even at low concentrations precludes use of this phenotype for screening purposes. However, Harder & Quayle (1971) showed that glyoxylate (which can be tolerated at substrate concentrations in the growth medium) is a precursor of glycine during growth on C₁ compounds. This fact has permitted preparation of mutants that require substrate quantities of glyoxylate for growth with methanol as energy source. A number of such mutants have been obtained, none of which grows in the absence of either of these substrates. A typical mutant, G5, was used for detailed study. This mutant can use glycollate in place of glyoxylate as supplement in the methanol growth medium. During growth tests with other compounds it was found that mutant G5 also requires substrate amounts of glyoxylate or glycollate for growth with ethanol as energy source. This implies a connexion between the pathways of assimilation of methanol and of ethanol in the wild-type organism, perhaps by way of glyoxylate or a common C₂ precursor of glyoxylate.

Although only a weak enzymic oxidation of glycollate to glyoxylate has been observed in cell-free extracts of *Pseudomonas* AM1, double mutants have been obtained from mutant G5 for which glycollate no longer replaces glyoxylate as a growth substrate with methanol or ethanol as energy source. Further work is needed to establish whether glycollate is an obligatory intermediate between methanol or ethanol and glyoxylate.

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