et al., 1962; Terry & Linker, 1964; Thompson & Castor, 1966; Manley *et al.*, 1968; Manley & Williams, 1969; Pennock *et al.*, 1971), and complexing with 9-aminoacridine hydrochloride (Muir *et al.*, 1963), but the quantitative reliability of both methods has been questioned (Muir *et al.*, 1963; Di Ferrante, 1967; Dean *et al.*, 1971).

The hexuronic acid content of the precipitated material is subsequently determined, but this does not measure keratan sulphate.

Scott & Dorling (1965) and Scott & Willett (1966) studied the interaction of polyanions and Alcian Blue 8GX under controlled conditions and extended their findings to the field of histochemistry. The method presented here depends on the formation of an insoluble complex between acid glycosaminoglycans and Alcian Blue 8GX (Imperial Chemical Industries Ltd.) under similar controlled conditions. It has been adapted to determine directly the glycosaminoglycan content of the urine of patients with a suspected diagnosis of mucopolysaccharidosis.

Maximum interaction of glycosaminoglycans in standard solutions and in urine was found to occur with 0.05% Alcian Blue 8GX in 50mm-sodium acetate (adjusted to pH 5.8 with acetic acid) containing 50mm-MgCl₂; in the standard procedure 20 μ l of glycosaminoglycan solution or centrifuged urine was mixed with 1ml of Alcian Blue reagent. After equilibration for 2h at room temperature, the glycosaminoglycan-Alcian Blue complex was centrifuged at 2000g for 15min. After the precipitate had been washed with ethanol (2ml) it was dissociated with 1ml of 40% (w/v) Manoxol-IB (dibutyl ester of sodium sulphosuccinic acid; BDH Chemicals Ltd.) in 50mm-sodium acetate buffer, pH 5.8. The extinction of the resulting clear blue solution was measured at 620nm. Blank values were low, and the whole procedure can be carried out in small disposable polystyrene tubes.

It was found convenient to standardize the method with chondroitin 4-sulphate. Calibration curves were almost linear over the range 50–500 μ g of glycosaminoglycan/ml. Sulphated glycosaminoglycans bearing two anionic groups/disaccharide unit gave similar standard curves. Hyaluronic acid and keratan sulphate gave slightly lower readings.

The procedure is relatively specific, although Tamm-Horsfall glycoprotein does react with Alcian Blue 8GX under these conditions. However, the colour development due to this glycoprotein is less than one-quarter of that from a comparable concentration (w/v) of glycosaminoglycan.

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Investigation of the Polysaccharides Produced by Extracellular Glycosyltransferases from *Streptococcus mutans*

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Large amounts of dental plaque are formed as a result of feeding on diets containing sucrose (Carlsson & Egelberg, 1965). Streptococci isolated from the human oral cavity can synthesize extracellular polysaccharides from sucrose, and the water-soluble polysaccharides have been characterized as 'dextrans' and 'levans'. A water-insoluble glucan produced from sucrose by culture filtrates of *Streptococcus mutans* strain OMZ 176 has been shown to contain almost exclusively α -(1 $\rightarrow$ 3)-linkages (Guggenheim & Schroeder, 1967).

Streptococcus mutans strain Ingbritt A was grown in batch culture (4% glucose), and the culture supernatant was incubated with sucrose. The high-molecular-weight products, which represent less than 10% by weight of the sucrose hydrolysed, were fractionated on the basis of their insolubility in water and in 55 and 75% ethanol solutions.

The water-insoluble glucan is soluble in 1 M-NaOH and has $[\alpha]_D^{21} + 203^\circ$ (c 1). The material was largely resistant to dextranase (8% hydrolysis on incubation with a commercial dextranase from Koch-Light Laboratories Ltd.).

The glucan was methylated with sodium hydride and methyl iodide, and the methylated polysaccharide was extracted with chloroform and methanolysed with methanolic 3% HCl. After neutralization with Ag₂CO₃, the methyl *O*-methylglycosides were separated by g.l.c. The only methyl ethers of methyl α -D-glucopyranoside present were identified as 2,3,4,6-tetra- (10%), 2,3,4-tri- (27%), 2,4,6-tri- (51%) and two di-*O*-methylglycosides (12%) at present un-

identified. The identity of the tri-*O*-methylglucosides has been confirmed by mass spectroscopy.

On oxidation with 15mM-NaIO₄ the periodate uptake was 0.68mol/mol of anhydroglucose units. The polyaldehyde was reduced with NaBH₄ to give a water-insoluble polyol. The products of hydrolysis with dilute acid (0.5M-HCl at 37°C) were glycerol and a series of glycerol glycosides. Hydrolysis with 2M-HCl at 100°C gave glycerol and glucose in the ratio 3:5, determined enzymically and by g.l.c. of their alditol acetates. Methylation analysis of the Smith degradation product gave the following methyl ethers of methyl α -D-glucopyranoside: 2,3,4,6-tetra- (19%), 2,3,4-tri- (9%) and 2,4,6-tri- (72%).

On the basis of these results it is proposed that the (water-)insoluble glucan is a heteroglucan containing both α -(1 $\rightarrow$ 3)- and α -(1 $\rightarrow$ 6)-linkages, and the high proportion of α -(1 $\rightarrow$ 3)-linkages may explain the insolubility of the glucan in water and the resistance to hydrolysis by dextranase.

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Particulate Malate Oxidation in Strictly Aerobic Bacteria: the Respiratory System of *Moraxella lwoffii*

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Moraxella lwoffii has two malate dehydrogenases (Jones, 1969; Jones & King, 1970). One is a very active soluble NAD-linked dehydrogenase, and the other is very firmly bound to the membranes and associated with the electron-transport system. On passage of a sample of disintegrated cells through a Sephadex G-200 column the two malate dehydrogenases are well separated.

More than 90% of the cytochromes are located in the particulate fraction obtained by centrifugation at 140000g, as shown by analysis. The room-temperature-oxidized versus Na₂S₂O₄-reduced difference spectra of samples of whole and disintegrated cells indicate absorbance maxima at 427, 524 and 558nm and an increase in absorbance at about 625nm. Liquid-N₂ (77°K) spectra of the same samples carried out with a Perkin-Elmer 356 double-beam dual-wavelength spectrophotometer showed an absorbance maximum at 428nm, shoulders at 440 and 521nm and maxima at 528, 535, 556, 561 and 625.5nm, indicating the presence of an *a*-type cytochrome (625.5 and 440nm), a *b*-type cytochrome (561, 535 and 428nm), a *c*-type cytochrome (554, 521 and 428nm) and a *b*- or *c*-type cytochrome (556, 528 and 428nm). Results so far show only one

type of cytochrome *a*. The absorbance maxima suggest it to be an *a*₂-type cytochrome, although further work is necessary to confirm this.

Reduction of oxidized samples by malate also reduces this cytochrome. Experiments are continuing to confirm the idea that in *Moraxella lwoffii* the particulate malate dehydrogenase, succinate dehydrogenase and NADH dehydrogenase have common or similar flavoprotein prosthetic groups linking into the cytochrome chain via ubiquinone.

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Defluorination of Fluoroacetate by Lettuce

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Although fluoroacetate is toxic to most animals, it is relatively non-toxic to plants. It has been isolated from forage crops grown in a fluoride-rich environment (Yu & Miller, 1970), and it seemed worth while to investigate how the compound is metabolized by plants.

A lettuce (*Latua sativa* L. var. *capitata*) was placed in a closed desiccator with its roots immersed in water. Air was bubbled through the water, and the effluent gas from the desiccator was dried with CaCl₂ and bubbled through 2-methoxyethanol-ethanolamine (2:1, v/v) to trap CO₂. The desiccator was constantly illuminated. Plants were incubated in this apparatus in one of three ways: (1) for 43h with sodium [1-¹⁴C]acetate (1mg/kg wet wt.); (2) for 24h with sodium fluoroacetate (50mg/kg wet wt.) followed by 43h with sodium [1-¹⁴C]acetate (1mg/kg wet wt.); (3) for 43h with sodium fluoro[1-¹⁴C]acetate (50mg/kg wet wt.) (Ward & Huskisson, 1966).

In the [¹⁴C]acetate-treated lettuces 15–20% of the radioactivity was expired as CO₂, and this was unaffected by the presence of non-radioactive fluoroacetate. In the fluoro[¹⁴C]acetate-treated plants only 0.5% of the radioactivity appeared in the CO₂. Citrate concentrations in fluoroacetate-treated plants were about 28mol/g dry wt., almost double those of untreated plants.

The distribution of radioactivity was examined in the fluoro[¹⁴C]acetate-treated plants. It could all be extracted with ethanol-water mixtures (Canvin & Beevers, 1961). Up to 50% of it was associated with unchanged fluoroacetate, about 2% with fluorocitrate and the remainder with two low-molecular-weight acidic compounds. These compounds were purified by ion-exchange and paper chromatography and found to be devoid of fluorine, but contained nitrogen and sulphur. Hydrolysis of one of the compounds