may be differences in the specificity of the sulpho-transferases involved.

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Haslewood, G. A. D. (1967). *J. Lipid Res.* **8**, 535.

Spencer, B. (1960). *Biochem. J.* **77**, 294.

Production of Sulphated Intermediates by *Pseudomonas fluorescens* during Growth on Potassium D-Glucose 6-O-Sulphate

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Pseudomonas fluorescens N.C.I.B. 8248 was cultured at 37°C in a phosphate-ammonium salts medium containing 60 mM-potassium D-glucose 6-O-[³⁵S]-sulphate (mixture of 55 mM unlabelled and 5 mM labelled) as sole carbon and sulphur source. Paper electrophoresis of samples of the cell-free culture fluid taken during growth showed the progressive production of three ³⁵S-labelled intermediates. At the termination of growth approx. 13% of the radioactivity had disappeared from the medium, 50% was present as starting material and the three metabolites made up the remainder.

One of the metabolites was present in traces only (1%) and has not yet been identified. A clue to the identity of a second metabolite (4% yield) came from previous work (Lloyd *et al.* 1965; Lloyd, Large, Tudball & Dodgson, 1965; Lloyd, Large, Tudball, Wusteman & Dodgson, 1965) on the production of D-gluconate 6-O-[³⁵S]-sulphate from D-glucose 6-O-[³⁵S]-sulphate by *Trichoderma viride*. A sample of the former compound was synthesized by controlled oxidation of the latter, and its chromatographic (six solvent systems) and electrophoretic mobilities were identical with those of the second *Pseudomonas* metabolite.

The remaining metabolite was present in quantity

(approx. 32%) and its mobility on electrophoresis (close to that of SO₄²⁻ ions), together with a consideration of known metabolic pathways of D-glucose in *Pseudomonas* sp., pointed to its possible identity as D-glycerate 3-O-[³⁵S]-sulphate. An unlabelled sample of this ester was prepared by definitive synthesis (N. Tudball & P. Thomas, unpublished work), and a labelled sample was prepared by direct sulphation of glycerate with chloro[³⁵S]sulphonic acid in the presence of dimethylformamide. The chromatographic (six solvent systems) and electrophoretic mobilities of these samples were identical with those of the major *Pseudomonas* product. Isotope-dilution experiments confirmed the identity of the product.

When *P. fluorescens* was cultured at 30°C in phosphate-ammonium salts medium containing 32 mM-potassium glucose 6-O-[³⁵S]-sulphate (mixture of 27 mM unlabelled and 5 mM labelled) as sulphur source and 2% glycerol as principal carbon source, 12% of the initial radioactivity had disappeared from the medium at the termination of growth. Approx. 56% of the remainder was present as starting material and 32% as a metabolite that was identified as D-gluconate 6-O-[³⁵S]-sulphate. D-Glycerate 3-O-[³⁵S]-sulphate was not produced under these circumstances.

Neither D-gluconate 6-O-[³⁵S]-sulphate nor D-glycerate 3-O-[³⁵S]-sulphate appear to be end products of sulphur metabolism in *P. fluorescens*, as both esters can serve as sole sulphur sources when the organism is grown with glycerol as carbon source.

The implications of the various findings will be discussed.

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Lloyd, A. G., Large, P. J., Tudball, N., Davies, M., Olavesen, A. H. & Dodgson, K. S. (1965). *Biochem. J.* **97**, 41 p.

Lloyd, A. G., Large, P. J., Tudball, N. & Dodgson, K. S. (1965). *Biochem. J.* **97**, 42 p.

Lloyd, A. G., Large, P. J., Tudball, N., Wusteman, F. S. & Dodgson, K. S. (1965). *Biochem. J.* **97**, 43 p.