homogeneous cell-type fractions by isopycnic centrifuging on both sucrose and ficoll gradients. Although fractions were obtained that were considerably enriched in endothelial lining cells or Kupffer cells, homogeneous preparations suitable for precise analysis have not yet been obtained. However, analysis of these fractions indicated that the relative proportion of arylsulphatases A and B in endothelial lining and Kupffer cells was markedly different from that observed for parenchymal cells. Thus, on the basis of the arbitrary method used to express enzyme activity, the ratios of arylsulphatase A to arylsulphatase B in whole liver and in the various fractions were as follows: whole liver, 1:13; parenchymal cells, 1:13; fraction enriched in Kupffer cells, 1:30; fraction enriched in endothelial lining cells, 1:45.

Mehl & Jatzkewitz (1968) have suggested that arylsulphatase A is a component of a more complex cerebroside sulphatase. On the other hand, the natural substrate for arylsulphatase B is quite obscure. A knowledge of the relative distribution and proportions of the two enzymes in mammalian tissues may provide a clue as to the role of the B enzyme and hence to the discovery of its natural substrate.

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Sulphation of Simple Aliphatic Alcohols and Bile Alcohols by the Toad *Xenopus laevis*

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The synthesis of activated forms of sulphate, adenosine 3'-phosphate 5'-sulphatophosphate and adenosine 5'-sulphatophosphate, has been shown to occur in certain micro-organisms and the tissues of plants and animals. In bacteria and other micro-organisms these compounds are principally used for the production of reduced forms of sulphur, whereas

in the higher animals they are used mainly in the synthesis of various types of sulphuric acid ester. The transition in the principal metabolic role of 'active sulphate' between these two extremes is of considerable interest from an evolutionary point of view and, as part of a comparative investigation of sulphate activation and utilization, these processes are currently being studied in the toad *Xenopus laevis*.

Sulphate activation occurs readily in toad liver, although the optimum conditions for the process (pH 8.0, 30mM-ATP and 12mM-Mg²⁺) differ quite markedly from those found for the rat liver system (pH 7.5, 45mM-ATP and 12mM-Mg²⁺). As yet it has not been possible to detect utilization of adenosine 5'-sulphatophosphate and adenosine 3'-phosphate 5'-sulphatophosphate for the production of reduced sulphur compounds in the cytosol of male and female toad livers. However, such preparations readily transferred sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to a wide variety of acceptors, including phenols, primary and secondary aliphatic alcohols, steroids and aromatic amines. Generally speaking this situation is similar to that noted for the rat by Spencer (1960), and suggests the presence of a number of sulphotransferase enzymes of different specificity. However, one striking difference between the two species was apparent in that toad liver preparations were able to sulphate the bile alcohols, β -cyprinol and scymnol, as well as simple aliphatic alcohols such as propan-1-ol and butan-1-ol, whereas rat liver preparations could sulphate only the latter acceptors. A similar lack of ability to sulphate these bile alcohols was also observed in chick liver.

Sulphotransferase activity of toad liver towards simple aliphatic alcohols could be separated from that towards bile alcohols by chromatography on DEAE-cellulose. The activity of the simple alcohol sulphotransferase fraction towards a series of simple aliphatic alcohols was optimum with pentan-1-ol, although, for routine assay purposes, butan-1-ol was a more convenient acceptor (50mM, pH 8.0, in the presence of 75 μ M-adenosine 3'-phosphate 5'-sulphatophosphate). The enzyme showed no requirement for Mg²⁺ but was activated up to 30% by mercaptoethanol and dithiothreitol.

The fraction active towards scymnol and β -cyprinol yielded the C-26/27 sulphates already recognized as being present in certain invertebrate biles (Haslewood, 1967), and was assayed as a routine with 100 μ M- β -cyprinol at pH 8.0 in the presence of 75 μ M-adenosine 3'-phosphate 5'-sulphatophosphate. This enzyme showed a requirement for Mg²⁺ (5mM) but was unaffected by thiols.

Sulphate activation in the toad, as in higher animals, therefore appears to be mainly directed towards sulphate ester biosynthesis, although there

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

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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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