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Assay of a Microsomal Marker Enzyme: Rat Liver Arylsulphatase C

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At least three arylsulphatase enzymes (aryl-sulphate sulphohydrolases, EC 3.1.6.1) distinguishable by their activity towards various substrates, their tissue distribution and intracellular localization have been shown to co-exist in certain mammals (Dodgson, Spencer & Thomas, 1955). In rat liver, two of the enzymes, arylsulphatases A and B, are localized mainly in the lysosome fraction of the cell (Viala & Gianetto, 1955), whereas the third one, arylsulphatase C, is in the microsome fraction, where it is present in a highly insoluble form (Dodgson, Spencer & Thomas, 1954; Dodgson, Rose & Spencer, 1957). The combined activity of arylsulphatases A and B measured under arbitrary conditions is frequently used in cell-fractionation studies as a marker for the lysosome fraction and various studies on arylsulphatase C (e.g. Spencer, Dodgson, Rose & Thomas, 1955; Dodgson *et al.* 1957) have indicated that this enzyme would be useful for the identification of the microsome fraction. This latter possibility has now been investigated fully following the establishment of experimental conditions for the specific assay of the enzyme.

Potassium *p*-nitrophenyl sulphate has frequently been used as a chromogenic substrate for the assay of arylsulphatase C (cf. Dodgson *et al.* 1955), but is unsuitable for use in fractionation studies on liver since the liberated *p*-nitrophenol is not quantitatively recoverable from whole homogenates of this tissue. Furthermore, the activity of the rat liver enzyme towards this substrate is affected by chloride, which is sometimes used as a component of the fractionation and assay media. However, potassium *p*-acetylphenyl sulphate, the substrate chosen originally by Dodgson, Spencer & Thomas (1953) for measuring arylsulphatase activity in rat tissues, shows none of these limitations in that the liberated phenol is completely recoverable from whole homogenates and enzyme activity towards the substrate is not affected by chloride. It has now been shown that this substrate can be used for the assay of arylsulphatase C, provided that the reaction is performed in the presence of a concentration of phosphate ions that is sufficient to inhibit arylsulphatases A and B completely.

The spectrophotometric assay is performed according to the directions of Dodgson & Spencer (1953) with some minor modifications and using a final concentration of potassium *p*-acetylphenyl sulphate of 40 mM in 0.1 M-sodium phosphate buffer, pH 8.0 (optimum conditions).

Under these conditions it has been confirmed that arylsulphatase C sediments in the microsome fraction. Subfractionation of the microsome pellet into rough and smooth endoplasmic reticulum by the method of Dallner, Siekevitz & Palade (1966) has demonstrated that the enzyme is associated with the membrane component of the system (cf. glucose 6-phosphatase).

The intracellular distribution pattern of arylsulphatase C, combined with the relative simplicity of the assay procedure, suggests that the enzyme is a useful marker for the membrane component of the endoplasmic reticulum of the rat liver cell. Whether it can be used with other tissues and species remains to be tested.

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The Formation of Rough Vesicles by the Interaction of Reticulosomes, Phospholipids and Ribosomes

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Reticulosomes as isolated from rat liver are non-membranous granules consisting of protein and phospholipid (protein/phospholipid ratio 8:1), and in the presence of micellar phospholipid they form a lipoprotein complex, which, when viewed with the electron microscope, resembles a membranous reticulum (Pollak, Ward & Shorey, 1966).

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