was optimum at pH 4.5 with globin as substrate. Glycyltyrosine amide, hippurylphenylalanine, benzoyloxycarbonylglutamylphenylalanine and to a smaller extent leucine amide were readily hydrolysed by the proteinase, but hippurylarginine and benzoylarginine amide were not. The partially purified protease was very active in releasing NADH-cytochrome *b₅* oxidoreductase from microsomes.

The oxidoreductase released from microsomes was isolated from the high-speed supernatant after digestion by a procedure involving ultrafiltration through a Diaflo XM 50 membrane, chromatography on Sephadex G-100 and isoelectric fractionation in the pH ranges 3–10 or 5–8. The final preparation was enriched 140-fold in oxidoreductase activity over the starting microsomes. The preparation behaved as a single molecular species on polyacrylamide gel and had an isoelectric point of pH 6.5. The enzyme transfers electrons from NADH to cytochrome *c* only in the presence of cytochrome *b₅*.

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Distribution of Arylsulphatase C in Subcellular Membranes of Rat Liver

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Earlier studies from these laboratories have established that arylsulphatase C, one of the arylsulphatase enzymes (arylsulphate sulphohydrolases, EC 3.1.6.1) found in mammalian tissues, is localized in the endoplasmic reticulum in the rat liver cell (Dodgson, Spencer & Thomas, 1954; Dodgson, Rose & Spencer, 1957). Further, the enzyme was found to be firmly bound to the insoluble membrane component of this fraction, and it has been suggested that it might be useful as a microsomal marker enzyme (Milsom, Rose & Dodgson, 1968). However, in routine cell-fractionation studies the nuclear, mitochondrial and lysosomal fraction were always found to possess some arylsulphatase C activity, and it was decided to determine whether this was due solely to contamination with microsomal material.

Nuclei could be readily freed from the enzyme by sedimentation through 2.2M-sucrose by the method of Widnell & Tata (1964). Purification of the mitochondrial and lysosomal fractions by washing five

times with 0.25M-sucrose or by density-gradient centrifugation removed up to 90% of their original activity. That which remained in washed mitochondria was mainly concentrated in the outer-membrane component obtained by the 'shrink' method (Sottocasa, Kuylensstierna, Ernster & Bergstrand, 1967), although the specific activity was only 20% of that of microsomal membranes. The activity of the inner-membrane component was too low to measure accurately.

Lysosomal-membrane material that has been repeatedly washed in iso-osmotic sodium chloride still retained some activity. However, the enzyme was not found in association with tritosomes obtained from rats receiving Triton WR-1339 (Wattiaux, Wibo & Baudhuin, 1963), although the activity of the remainder of the subcellular components was normal.

In all these experiments the distribution of glucose 6-phosphatase closely paralleled that of arylsulphatase C. It is concluded from these studies that both enzymes are most probably localized only in the endoplasmic reticulum of the rat liver cell and that the presence of activity in other membranes represents contamination with this fraction. A more precise determination of the intracellular localization of arylsulphatase C will not be possible until a specific histochemical method for its detection has been developed.

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Further Evidence for the Role of the Essential Thiol Groups in Adenosine Triphosphate-Creatine Phosphotransferase from a Comparison of the Human and Rabbit Enzymes

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Human muscle creatine kinase has two reactive thiol groups and a total of 7–8 thiol groups/molecule just like the rabbit enzyme. It also has two sub-

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