inhibited the adenosine triphosphatase more effectively than ouabain. Oligomycin has an ouabain-like action on cell membranes (Whittam, Wheeler & Blake, 1964) and may therefore be considered as an alternative inhibitor for the characterization of $\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase, particularly in the rat, which has a low sensitivity to cardiac glycosides (Allen & Schwartz, 1969).

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The Preparation of Acetylcholinesterase from Human Erythrocytes

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Acetylcholinesterase is firmly bound to the erythrocyte membrane (Augustinsson, 1948) so that it is desirable in any investigation of its molecular properties to bring the enzyme into solution. Gordon & Rutland (1967) used ultrasonic vibration to 'solubilize' the acetylcholinesterase of ox erythrocytes, but the adaptation of this method to a large-scale preparation might prove difficult; also, treatment with ultrasound could change the electrophoretic properties of the enzyme (Dubbs, 1966). We now report a method using a mixture of KCl and the non-ionic detergent Triton X-100 to prepare soluble acetylcholinesterase.

Human erythrocytes were washed three times in saline, lysed in 6 vol. of water and after three washings in distilled water, stroma were suspended in 8 vol. of KCl-Triton X-100. A range of concentrations of KCl and Triton X-100 was examined to determine the optimum conditions for solubilization. The enzyme was considered to be soluble if it did not sediment when subjected to 100 000g for 1 h. Over 90% of the enzyme was soluble in a mixture containing 1.2M-KCl and 1% Triton X-100.

Addition of ammonium sulphate to 60% saturation precipitated about 70% of the soluble enzyme activity. The precipitate was soluble in saline and had a specific activity of 0.5 $\mu\text{mol}/\text{min}$ per mg of protein, representing about a 50-fold purification with respect to the haemolysate. The Michaelis constant of the enzyme at this stage was 75 μM compared with 69 μM for the crude haemolysate.

Electrophoresis of the partially purified preparation on 6% polyacrylamide gel at pH 8.1 (Smith, 1968) showed between one and three bands of esterase activity with α -naphthyl acetate as substrate (Paul & Fottrell, 1961): all the esterase bands were sensitive to eserine. Whether the occurrence of more than one band represents several forms of the enzyme is under investigation.

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Isolation of Nicotinamide-Adenine Dinucleotide-Cytochrome b_5 Oxidoreductase from Rat Liver Microsomes

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Strittmatter & Velick (1957) isolated NADH-cytochrome b_5 oxidoreductase (EC 1.6.2.2) from calf liver microsomes after digestion with snake venom at pH 5.7. Takesue & Omura (1968), however, have reported that release of the oxidoreductase is due not to snake venom but to lysosomes contaminating the microsomes. The present communication confirms the latter result and presents evidence that the active lysosomal principle is an acid proteinase. A simple method for isolating the oxidoreductase in a pure state is also presented.

Treatment of rat liver microsomes with snake venom, steapsin, pepsin, trypsin or chymotrypsin failed to release appreciably active oxidoreductase. A lysosomal fraction of rat liver sedimenting between 5×10^4 and 8×10^4 g-min was active in releasing the microsomal oxidoreductase into the supernatant phase after centrifuging at 9.45×10^6 g-min. Essentially quantitative release of the oxidoreductase occurred with equal weights of microsomes and lysosomes on a protein basis were incubated at 37°C for 120 min in 0.1M-tris-maleate buffer, pH 5.7. A three- to four-fold activation of the enzyme was consistently observed after its release.

The lysosomal fraction was fractionated with Triton X-100 to yield a high-speed supernatant, which, when extracted with pentanol and chromatographed on Sephadex G-200, yielded an acid proteinase fraction 20–25 times enriched over the starting lysosome fraction. The proteinase activity

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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