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 100 000 *g* for 1 h. Over 90% of the enzyme was soluble in a mixture containing 1.2 M-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 0.1 M-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