- Ellis, R. J. & McDonald, I. R. (1967). *Pl. Physiol., Lancaster*, **42**, 1297.
- Loening, U. E. & Ingle, J. (1967). *Nature, Lond.*, **215**, 363.
- Lyttleton, J. W. (1962). *Expl Cell Res.* **26**, 312.
- Parenti, F. & Margulies, M. M. (1967). *Pl. Physiol., Lancaster*, **42**, 1179.
- Sagan, L. (1967). *J. theor. Biol.* **14**, 225.
- Schwartz, J. H., Meyer, R., Eisenstadt, J. M. & Brawerman, G. (1967). *J. molec. Biol.* **25**, 571.
- Spencer, D. & Wildman, S. G. (1964). *Biochemistry, Easton*, **3**, 954.
- Vazquez, D. & Monro, R. E. (1967). *Biochim. biophys. Acta*, **142**, 155.

A New Procedure for the Separation of Mitochondrial Membrane Proteins

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Studies on mitochondrial membrane proteins so far have been in aqueous solutions. The hydrophobic nature of the membrane protein suggests that a large part of the protein may exist in the non-polar section of the membrane. Therefore organic solvents might be more suitable and there have been reports of membrane protein dissolved in such solvents (Lowden, Moscarello & Morecki, 1966; Zahler & Wallach, 1967; Green, Haard, Lenaz & Silman, 1968). Preliminary work by Curtis (1969) showed that rat liver mitochondrial membrane protein dissolved completely in acidic chloroform-methanol (2:1, v/v) in the absence of phospholipids. Further use of chloroform-methanol as a solvent for membrane proteins has been investigated.

Exhaustive methylation of Sephadex G-75, G-100 and G-200 (Nyström & Sjövall, 1965) gave products that swelled in chloroform-methanol (2:1, v/v) approximately as much as in water. Chromatography of polystyrene fractions of narrow molecular-weight range in chloroform-methanol (3:1, v/v) showed that separation was according to size. Gel filtration of the mitochondrial membrane protein dissolved in chloroform-methanol (2:1, v/v) was achieved most successfully on methylated Sephadex G-100 equilibrated with chloroform-methanol (2:1, v/v) containing acetic acid (10mm). Polyacrylamide-gel electrophoresis (Neville, 1967) at pH 2.7 in 8M-urea showed that the major component of membrane protein was largely separated from the other components, and further purification was possible by rechromatography on methylated Sephadex G-100.

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Curtis, P. J. (1969). *Biochim. biophys. Acta*, **183**, 239.

- Green, D. E., Haard, N. F., Lenaz, G. & Silman, H. I. (1968). *Proc. natn. Acad. Sci. U.S.A.* **60**, 277.
- Lowden, J. A., Moscarello, M. A. & Morecki, R. (1966). *Can. J. Biochem.* **44**, 567.
- Neville, D., jun. (1967). *Biochim. biophys. Acta*, **133**, 168.
- Nyström, E. & Sjövall, J. (1965). *Analyt. Biochem.* **12**, 235.
- Zahler, P. & Wallach, D. F. H. (1967). *Biochim. biophys. Acta*, **135**, 371.

Presence and Spatial Localization of Carbonic Anhydrase in Rat Liver Mitochondria

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About 2–10% of the carbonic anhydrase activity in homogenates of rat liver, kidney and cerebral cortex is recovered in the mitochondrial fraction (Datta & Shepard, 1959; Karler & Woodbury, 1960). Datta & Shepard (1959) believed these low recoveries indicated that the enzyme was confined to the cytoplasm, but Karler & Woodbury (1960) concluded that carbonic anhydrase was present in mitochondria and was tightly bound to membranes.

We recently reported that choline oxidation in liver mitochondria was accelerated under conditions enabling carbon dioxide to pass into the mitochondria and suggested that carbonic anhydrase in the matrix space accelerated the production of internal H^+ ions, which then facilitated choline entry by ionic exchange (Holton & Tyler, 1969). This hypothesis has now been tested by making electrometric measurements of carbonic anhydrase activity in mitochondria. Reaction mixtures containing carbon dioxide were rapidly stirred in a closed cell at 0.5–2°C. Changes of pH were measured by recording the e.m.f. of a glass electrode-calomel electrode system, with a strip-chart recorder running at 4 in/min. Rapid addition of acid or alkali yielded biphasic traces, which were analysed to yield a first-order velocity constant for the rate of approach to pH equilibrium. Between pH 6 and pH 7 mitochondria in distilled water increased the rate constant for the approach to equilibrium from the alkaline side (CO_2 hydration predominating). At pH 6.4 the rate constant was doubled by 1.2mg of mitochondrial protein/ml. In contrast, mitochondria had no effect on the rate constant for approach to equilibrium from the acid side (HCO_3^- and H_2CO_3 dehydration predominating).

These properties suggest the presence of a membrane-bound enzyme accessible to substrate by a diffusion path traversed more rapidly by CO_2 than by HCO_3^- ion. They are interpreted as showing that liver mitochondria contain carbonic anhydrase located on the matrix side of the inner membrane, which is known to be permeable to CO_2 but impermeable to