from those reported for the enzyme in avian liver (Utter & Keech, 1963; Keech & Utter, 1963) and rat adipose (Ballard & Hanson, 1967), though the pH optimum (8.5 for the enzyme in rat mammary fractions and 7.8 for the rabbit mammary-gland enzyme) was somewhat different. Albumin (fat-free) produced a 3-fold increase in the specific activity of the enzyme in the soluble fraction of rat mammary gland. In all cases pyruvate carboxylase showed a dependence on added acetyl-CoA.

Under optimum assay conditions, cell-fractionation studies in both 0.3M- and 0.88M-sucrose as homogenization medium and using marker enzymes confirmed the subcellular distribution of pyruvate carboxylase in these tissues. Evidence will be presented that the soluble enzyme is not an artifact due to mitochondrial damage, nor is mitochondrial pyruvate carboxylase due to an adsorption of the enzyme from the soluble fraction.

Avidin inhibited the enzyme in the mitochondrial fraction of both tissues, though the inhibition was incomplete. In contrast, the soluble enzyme from rat mammary gland was completely inhibited by avidin. The specific activity of the mitochondrial enzyme from rat mammary gland increased when the fraction was subjected to freeze-drying (2.3-fold), freezing-and-thawing (approx. 5-fold) or ultrasonic treatment (3.6-fold). The mitochondrial enzyme from rabbit mammary gland behaved differently, since little or no change in specific activity was observed under these conditions.

- Ballard, F. J. & Hanson, R. W. (1967). *J. Lipid Res.* **8**, 73.
 Fritz, J. B. (1961). *Physiol. Rev.* **41**, 52.
 Keech, D. B. & Utter, M. F. (1963). *J. biol. Chem.* **238**, 2609.
 Masoro, E. J. (1962). *J. Lipid Res.* **3**, 149.
 Utter, M. F. & Keech, D. B. (1963). *J. biol. Chem.* **138**, 2603.
 Wise, E. M. & Ball, E. G. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 1255.

The Chemical Nature of the Site of Action of Dicyclohexylcarbodi-imide in Mitochondria

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Dicyclohexylcarbodi-imide (DCCD) is a potent inhibitor of oxidative phosphorylation (Beechey, Holloway, Knight & Robertson, 1966; Beechey, Robertson, Holloway & Knight, 1967). Evidence has been adduced that a covalent bond is formed between the di-imide moiety and the site of action. Here we present evidence that proteolipid (Folch & Lees, 1951) is the site of action of DCCD.

Ox heart mitochondria that had been incubated overnight at 0° with 1 μ mole of [14 C]DCCD/g. of protein contained, on reisolation, 0.9–1.0 μ mole of

DCCD/g. of protein. Extraction of these mitochondria with organic solvents (Folch, Lees & Sloane-Stanley, 1957) removed an average of 75% of the radioactivity, which, when analysed by thin-layer, paper, ion-exchange and adsorption chromatography and by gel filtration became irreversibly adsorbed on the support material and thus was neither DCCD nor NN'-dicyclohexylurea.

Phospholipid was removed from these extracts by dialysis against the sequence of solvents described by Tenenbaum & Folch-Pi (1966). Daily, the dialysate was concentrated, analysed by thin-layer chromatography and the 14 C content measured. 15% of the radioactivity was extracted in the first 2 days of dialysis. This proved to be mainly NN'-dicyclohexylurea. A further 15%, most of which was chromatographically similar to the dialysed extract, was removed over 25 days, its release correlated with the aging of the dialysis membrane. The protein contents of the dialysis sac, which became, after drying, insoluble in all but phenol-acetic acid-water (2:1:1, w/v/v), showed two radioactive spots on thin-layer chromatography.

A 350ml. portion of a similar mitochondrial extract was layered overnight under 5l. of water and the accumulated interfacial and chloroform-soluble materials were isolated. They contained 81 and 11.5mg. of protein respectively. Acetone and diethyl ether extractions of these fractions removed much lipid material. However, the greater proportion of the counts remained in the extracted material. Solution of these materials in the phenol-acetic acid-water systems and analysis by thin-layer chromatography suggested that these 'interfacial' and 'chloroform-soluble' protein fractions corresponded to the two radioactive materials found in the sac. Treatment of the chloroform-soluble fraction with Pronase solubilized 25% of the radioactivity, and this soluble fraction ran as a single material on thin-layer chromatography and was eluted as a single peak from Sephadex G-10.

We conclude that DCCD combines with at least two proteolipid fractions in mitochondria. The adherence of radioactivity to the proteolipid fractions throughout these varied physical and chemical procedures supports the theory that a covalent bond is formed between proteolipid and the di-imide moiety.

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- Beechey, R. B., Holloway, C. T., Knight, I. G. & Robertson, A. M. (1966). *Biochem. biophys. Res. Commun.* **23**, 75.
 Beechey, R. B., Robertson, A. M., Holloway, C. T. & Knight, I. G. (1967). *Biochemistry*, **6**, 3867.
 Folch, J. & Lees, M. (1951). *J. biol. Chem.* **191**, 807.
 Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
 Tenenbaum, D. & Folch-Pi, H. (1966). *Biochim. biophys. Acta*, **115**, 141.