transport system (F. P. Altmann, unpublished work) may be due to losses of the hydrogen into other NADPH-dependent processes, and hence not available to the tetrazolium salts that act at points beyond the site of these reactions.

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Altmann, F. P. (1968). Ph.D. Thesis: University of London.

Altmann, F. P. (1969). *Histochemie*, (in the Press).

Altmann, F. P. & Chayen, J. (1965). *Nature, Lond.*, **207**, 1205.

Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.

Succinoxidase Activity in Intact Tissue Sections

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It is now possible to use thin unfixed sections of supercooled tissue in biochemical investigations (Chayen, Bitensky, Butcher & Poulter, 1969). However, to ensure that the undenatured protoplasm of such sections is not solubilized during incubation in relatively conventional biochemical media, a sufficient concentration (20%, w/v) of polyvinyl alcohol must be incorporated into the media (Altmann & Chayen, 1965).

Such intact sections have been used to study the oxidation of succinate in two ways. First, the dehydrogenation of succinate has been measured quantitatively by using neotetrazolium as the hydrogen acceptor (Seligman, 1953); the deposit of reduced neotetrazolium (formazan) has been eluted and measured spectrophotometrically, and the results have been expressed per volume of tissue.

The succinoxidase system has also been studied in intact sections by measuring the amount of oxidized cytochrome *c* by microdensitometry (Butcher, Poulter & Chayen, 1968). In the histochemical reaction for cytochrome oxidase (Butcher, Diengdoh & Chayen, 1964), an indophenol dye is produced at the expense of oxidized cytochrome *c*, which becomes reduced in the process. In the presence of succinate oxidation, and hence intramitochondrial hydrogen transport, the cytochrome *c* is maintained in the reduced state, thus inhibiting the production of indophenol.

It has been necessary to show that the system being demonstrated by both these methods was an enzymic system operating via succinate dehydrogenase (EC 1.3.99.1). This has been proved by the fact that increasing concentrations of the competitive inhibitor malonate progressively decreased

the production of neotetrazolium formazan, whereas the amount of demonstrable oxidized cytochrome *c* progressively increased.

To maintain the integrity of the more soluble parts of the cell, even in the presence of polyvinyl alcohol, there is a need for oxidation associated with the pentose shunt (Altmann & Chayen, 1966). The oxidation of succinate has been studied in the presence of glucose 6-phosphate dehydrogenation in the cytoplasm. Under these conditions formazan production is vastly increased. Thus it seems possible that cytoplasmic oxidation may also be essential in maintaining the integrity of the intra-mitochondrial hydrogen-transport pathway.

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Altmann, F. P. & Chayen, J. (1965). *Nature, Lond.*, **207**, 1205.

Altmann, F. P. & Chayen, J. (1966). *J. R. micr. Soc.* **85**, 175.

Butcher, R. G., Diengdoh, J. V. & Chayen, J. (1964). *Quart. J. micr. Sci.* **105**, 497.

Butcher, R. G., Poulter, L. W. & Chayen, J. (1968). *Biochem. J.* **107**, 27P.

Chayen, J., Bitensky, L., Butcher, R. G. & Poulter, L. W. (1969). *A Guide to Practical Histochemistry*. Edinburgh: Oliver and Boyd.

Seligman, A. M. (1953). *Proc. 1st int. Congr. Histochem. Cytochem.* p. 203. Ed. by Wegmann, R. Oxford: Pergamon Press.

The Inhibition of Acetylcholinesterase by some Oxime Carbamates

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Acetylcholinesterase can be isolated from fly heads (C. Donninger & C. W. Kearns, personal communication) (*Musca domestica*) in good yield and can be purified to give material highly active towards acetylcholine hydrolysis (Wilson, Bergman & Nachmanson, 1950). Though the enzyme preparations employed here are not pure, they are soluble fractions with reproducible kinetic characteristics. It is these factors that are of concern to us here.

We have examined acetylcholinesterase with the object of inhibiting its natural reaction by using simple novel molecules as inhibitors. Provided that other features of the molecules do not detract too much from their function as inhibitors of acetylcholinesterase, such molecules can be of importance as insecticides (O'Brien, 1967).

The kinetics of the reaction of acetylcholinesterase, E, with acetylcholine, A, and an inhibitor,