transport system (F. P. Altmann, unpublished work) may be due to losses of the hydrogen into other NADPH-dependent processes, and hence not available to the tetrazolium salts that act at points beyond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

I, may be represented by the equations (Wilson & Harrison, 1961; Wilson, Harrison & Ginsburg, 1961):

The solution of these equations, with some assumptions, can be put in a form suitable for experimental determination of some of the kinetic constants. This has been applied to a series of oxime carbamates in which both the 'leaving group' and the carbamoyl moiety have been substituted in a regular manner. In this way we have been able to measure K_I/k_1^1 and k_2^1 for each compound, where K_I is the Michaelis-Menten constant $(k_-^1 + k_1^1)/k_+$.

The results can be interpreted on a model for the active site of acetylcholinesterase similar to that suggested by Krupka (1964, 1966a,b).

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Comparison of various Alkylboronic Acids for the Characterization of Corticosteroids by Gas-Liquid Chromatography-Mass Spectrometry

By C. J. W. BROOKS and D. J. HARVEY. (*Department of Chemistry, University of Glasgow, Glasgow W.2*)

The characterization of corticosteroids by combined gas-liquid chromatography and mass spectrometry may be achieved by the use of suitable derivatives that decrease polarity and also stabilize the C-17 side chains. We are extending earlier studies (Brooks & Watson, 1968; Anthony, Brooks, Maclean & Sangster, 1969) of the cyclic esters formed by the reaction of alkylboronic acids with corticosteroids. The equilibrium reactions favour ester

formation, and may be performed by mixing equimolar proportions of the reactants in a neutral solvent. Regeneration of the steroids may, in many instances, be accomplished by addition of an excess of a diol, such as propane-1,3-diol, that forms more stable cyclic boronates.

Previous work has been devoted mainly to derivatives formed from phenyl- and *n*-butylboronic acid. The phenylboronates give well-defined g.l.c. peaks, but have long retention times, even at 250°, because of the polarity of the phenyl group, coupled with the molecular-weight increment of 86 accompanying derivative formation. The *n*-butylboronates show shorter retention times, in accordance with their lower polarity and molecular weight.

We now report results for corresponding derivatives of methylboronic and *tert*.-butylboronic acid. It was considered that the *tert*.-butyl group might improve the stability of the boronate group towards hydrolysis. Both acids were comparatively difficult to synthesize and handle, but the corticosteroid boronates, once formed, gave satisfactory peaks on 1% OV-17, with conveniently short retention times. Typical retention indices are those of the cortisone alkylboronates, e.g. methyl-, 3450; *n*-butyl-, 3660; *tert*.-butyl-, 3490; cyclohexyl-, 4065; phenyl-, 4230.

The mass spectra of the different boronates of any one particular steroid were generally similar. Fragment ions containing the boronate ring system, though occasionally observed, were not usually abundant. In most instances, however, abundant molecular ions were produced; their intensities in the series of cortisone alkylboronates, expressed as percentages of the base peak (*m/e* 258 throughout) were: methyl-, 73; *n*-butyl-, 47; *tert*.-butyl-, 74; cyclohexyl-, 50; phenyl-, 55. Methylboronates appear slightly superior to *tert*.-butylboronates for general separation of corticosteroids, while their lower molecular weights facilitate mass-spectrometric characterization.

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