Cells grown to a density of 1.3 mg. dry wt./ml. of medium in shallow stationary culture with peptone-yeast extract were washed once in 5 mM-MgCl₂, crushed with a modified Hughes press (Sutton & King, 1966), and then ground with alumina in 50 mM-tris-HCl buffer, pH 7.5, containing 5 mM-MgCl₂ and 13 mM-2-mercaptoethanol and centrifuged at 30 000g. Supernatant containing 16 mg. of protein was added to buffer, MgCl₂, ATP, 1 μ mole each of L-asparagine, L-glutamine, L-ornithine, L-proline, L-tyrosine and L-valine, and 0.2 μ mole of DL-[1-¹⁴C]phenylalanine in a final volume of 3 ml. After 1 hr., trichloroacetic acid to a final concentration of 5% (w/v) and 0.5 mg. of tyrocidine were added, the mixture was centrifuged and the sediment washed successively with 5% (w/v) trichloroacetic acid, 2% (w/v) NaCl and ethanol-0.2 M-HCl (9:1, v/v). Tyrocidine was separated from the ethanol-soluble fraction on acid alumina (Fujikawa, Suzuki & Kurahashi, 1968) and its purity checked by chromatography on Sephadex LH-20 with the solvent *NN*-dimethylformamide-water (9:1, v/v) (I. Bartley & B. Hodgson, unpublished work).

With 0.1 M-tris-HCl buffer, pH 7.5, 2 mM-ATP and 10 mM-MgCl₂, 1.29 and 0.86 nmole of [¹⁴C]-phenylalanine were incorporated into ethanol-soluble material and tyrocidine respectively. Freezing and thawing the extract decreased the incorporation into the respective fractions to 0.41 and 0.14 nmole after 24 hr. at -15°, and to 0.24 and 0.03 nmole after a second freezing. Extracts from cells harvested at either 0.9 and 1.6 mg. dry wt./ml. of medium gave less incorporation.

With 2 mM-ATP and 10 mM-MgCl₂ and either 0.1 M-tris-HCl buffers in the pH range 7.2-8.5 or 0.1 M-KH₂PO₄-KOH buffers in the pH range 6.2-7.8, the extract catalysed a maximum incorporation of [¹⁴C]phenylalanine into tyrocidine at pH 7.5 of 0.91 and 1.32 nmole respectively; 1 mM-ATP with the KH₂PO₄-KOH buffer, pH 7.5, allowed an incorporation of 0.60 nmole of [¹⁴C]-phenylalanine into tyrocidine, and either 10 mM-ATP or 1 mM-ATP + 10 mM-phosphoenolpyruvate + pyruvate kinase allowed a maximum incorporation of 2.04 nmole. Added MgCl₂ was not required. Under optimum conditions more than 90% of the counts in the ethanol-soluble material were recovered as tyrocidine.

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A Comparison of Dehydrogenase Activities in Tissue Homogenates and Tissue Sections

By F. P. ALTMANN. (*Division of Cellular Biology, The Mathilda and Terence Kennedy Institute of Rheumatology, London W.6*)

Pentose-shunt dehydrogenation can be demonstrated in the supernatant fraction of tissue homogenates by following the production of NADPH at 340 nm. (Glock & McLean, 1953). It can also be demonstrated in intact tissue sections by following the reduction of certain tetrazolium salts to insoluble coloured formazans (Altmann & Chayen, 1965). These formazans can be eluted from the sections and measured quantitatively (Altmann, 1969; F. P. Altmann, unpublished work). 6-Phosphogluconate dehydrogenase has been tested, under optimum conditions (Altmann, 1968), in homogenates and intact sections of rat liver taken from the same animal. Activities from the homogenate and section experiments were converted into the common unit of μ moles of hydrogen formed/cm.³ of tissue/30 min. at 37°.

The homogenate gave 212 units of activity for 6-phosphogluconate dehydrogenase. The sections were tested with seven different tetrazolium salts: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; INT, 2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride; TNBT, 2,2',5,5'-tetra-*p*-nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; NBT, 2,2'-di-*p*-nitrophenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; BT, 2,2',5,5'-tetraphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene)ditetrazolium chloride; NT, 2,2'-(*p*-diphenylene)-bis-(3,5-diphenyl)ditetrazolium chloride; TT, 2,3,5-triphenyltetrazolium chloride. The most active of these (MTT), in the presence of phenazine methosulphate (PMS), gave 250 units of activity. Within experimental error, therefore, the activity recorded was the same in both systems. The homogenate system measures the total amount of NADPH produced by the dehydrogenation (NADPH-oxidizing systems were absent). With MTT and PMS the sections gave the same activity. In the absence of PMS, the activity was decreased to 77 units. This fall may represent NADPH hydrogen that is being used for fat biosynthesis; adipose tissue shows a far greater increase in activity with PMS (about ten times). The activities recorded with some of the other tetrazolium salts were lower; for example, NT gave 220 and 87 units with and without PMS respectively, and TT gave 200 and 60 units respectively. These lower activities obtained with tetrazolium salts that accept the NADPH hydrogen at points further along the

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

By R. G. BUTCHER. (*Division of Cellular Biology, The Mathilda and Terence Kennedy Institute of Rheumatology, London W.6*)

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

By P. JEWESS and N. R. MCFARLANE (introduced by R. B. BEECHEY). (*'Shell' Research Ltd., Woodstock Agricultural Research Centre, Sittingbourne, Kent*)

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,