receiving probenecid suggest that the turnover of brain 5-hydroxytryptamine might be diminished in cases of depression (Van Praag & Korf, 1970).

Thus studies so far suggest that 5-hydroxytryptamine might be decreased in depressed patients, and clinically it has been found that feeding with tryptophan will potentiate the antidepressant properties of monoamine oxidase inhibitors (Coppen, Shaw & Farrell, 1963; Pare, 1963) and possibly even have an antidepressant effect when given by itself (Coppen, Shaw, Herzberg & Maggs, 1967), whereas phenylalanine seems to be ineffective either alone or with monoamine oxidase inhibitors (C. M. B. Pare, unpublished work). In this respect 3,4-dihydroxyphenylalanine (dopa) is of interest, as, although it causes excitement when given in acute experiments with animals, it has been reported to cause depression when given to patients with Parkinson's syndrome (Jenkins & Groh, 1970). A possible explanation lies in the report by Kopin (1970) that 3,4-dihydroxyphenylalanine may result in the formation of dopamine in 5-hydroxytryptamine-producing cells and then act as a false transmitter in place of the 5-hydroxytryptamine.

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COMMUNICATIONS

Inhibition of Rat and Mouse Brain Monoamine Oxidases By (+)-Amphetamine

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The molecular basis of amphetamine's central stimulant action is still disputed, but inhibition of monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating), EC 1.4.3.4] is nowadays almost invariably discounted as a contributory factor, partly on the grounds that amphetamine is too weak an inhibitor (Zirkle & Kaiser, 1964), and partly on the grounds that the stimulation produced by amphetamine differs qualitatively from that displayed by irreversible inhibitors of the hydrazine type (Stein, 1964).

Monoamine oxidase inhibition by amphetamine *in vitro* has previously been studied mainly at substrate concentrations well above the K_m value for this enzyme; such experiments would grossly underrate the degree of inhibition produced by a competitive reversible inhibitor *in vivo*, where the endogenous substrate concentrations are low. K_m

values for rat and mouse brain monoamine oxidase determined at 25°C and pH 7.4 in an air atmosphere with ^{14}C -labelled substrates were about 70 μM , 250 μM and 100 μM for 5-hydroxytryptamine, (-)-noradrenaline and tyramine respectively. At substrate concentrations of 11–20 μM (all well below K_m but still higher than the probable endogenous concentration of free 5-hydroxytryptamine or noradrenaline) oxidation of 5-hydroxytryptamine or noradrenaline by either mitochondrial or microsomal brain fractions was inhibited by 50% by about 6 μM (+)-amphetamine. Slightly higher concentrations (8 μM for rat fractions, 20 μM for mouse fractions) were required to inhibit tyramine oxidation by 50%. These concentrations are well within the range (3–100 μM) reached in the brain by (+)-amphetamine given at doses (0.25–10 mg/kg) sufficient to elicit measurable behavioural changes in either species (Fuller & Hines, 1967; Maikel, Cox, Miller, Segal & Russell, 1969).

Kinetically inhibition is competitive but is not consistent with the enzyme being homogeneous. This is particularly so with tyramine as substrate,

for which a much higher concentration of (+)-amphetamine (1mM for mouse brain) is needed to inhibit oxidation by 90% than would be expected from the concentration inhibiting by 50%. This apparent heterogeneity appeared alike in both mitochondrial and microsomal fractions.

Thus, although amphetamine unquestionably affects brain function in other ways besides monoamine oxidase inhibition, any interpretation of the stimulant action that ignores monoamine oxidase inhibition may be incomplete. For example, the ability of amphetamine to cause motor stimulation in reserpine-treated animals (Smith, 1963) could depend on such inhibition being present.

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substrate for 15min. Amphetamine (0.1mM), a substrate analogue that cannot be oxidized (Blaschko, 1952), was therefore preincubated with clorgyline (concentration above) and monoamine oxidase. Samples were removed over a period of 1 h for assay with the oxygen electrode.

Under these conditions a time-dependent inactivation was observed. The enzyme was finally inhibited 48% at 1 μ M-clorgyline and 67% at 0.1 mM-clorgyline. Amphetamine is therefore able to increase clorgyline inhibition of monoamine oxidase to a value approaching that obtained in the presence of substrate. The possibility that this is a result of amphetamine binding at the active site requires further investigation.

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The Inactivation of Monoamine Oxidase by Clorgyline (M&B 9302)

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Rat brain mitochondrial monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating) EC 1.4.3.4] has been assayed by using the oxygen electrode (Tipton, 1968; Sweetman & Weetman, 1969). Mitochondria were prepared from the brains of ten rats and were made up to a final volume of 4ml with 0.1M-triethanolamine buffer, pH 7.4, containing 0.1 mM-EDTA and 0.1 mM-dithiothreitol. A 0.1 ml portion of this suspension was assayed with 2mM-substrate in 0.1M-triethanolamine buffer, pH 7.4, in the presence of 2mM-KCN in a total volume of 1ml. The assay was carried out in an oxygen-electrode assembly (Rank Bros., Cambridge, U.K.) and the rate of oxygen uptake was recorded on a Honeywell 10mV strip-chart recorder.

This initial rate assay has revealed an interesting feature of the reaction of clorgyline (M&B 9302) with monoamine oxidase. The inhibition of tyramine oxidation observed at clorgyline concentrations of 1 μ M and 0.1 mM, with or without preincubation of enzyme and inhibitor, was 25 and 42% respectively. Previous work (Hall, Logan & Parsons, 1969) has shown that the equivalent values for inhibition obtained with radioactive tyramine are 60 and 95% respectively.

This latter assay involves incubation of monoamine oxidase and clorgyline in the presence of

A Monoamine Oxidase-Tetrazolium Reaction for Locating Aromatic Amino Acid Decarboxylases in Electrophoretic Media

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The involvement of aromatic amino acids and aromatic amines in the biochemistry of neurological conditions such as Parkinsonism (Calne & Sandler, 1970) and migraine (Smith, Kellow & Hanington, 1969) suggested that a study be made of the enzymes involved in the decarboxylation of aromatic amino acids. The most sensitive procedures presently available for the detection and assay of aromatic amino acid decarboxylases depend on the use of 14 C-labelled substrates (see, e.g., Murali & Radnakrishnan, 1969). However, such procedures are too time-consuming for locating decarboxylases in electrophoretic media, and so we have devised the following colour localization of the enzyme(s) that can be applied to whole electrophoretic media (e.g. a micro-gel) without sectioning.

The basis of the reaction is the enzymic decarboxylation of an appropriate amino acid to the corresponding amine, which is then oxidized by added monoamine oxidase, the hydrogen acceptor being a tetrazolium salt that forms an insoluble coloured product as it is reduced. Because the customary substrate 3,4-dihydroxyphenylalanine (dopa) is very susceptible to atmospheric oxidation,