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.

Fuller, R. W. & Hines, C. W. (1967). *J. pharm. Sci.* **56**, 302.

Maikel, R. P., Cox, R. H., Miller, F. P., Segal, D. S. & Russell, R. W. (1969). *J. Pharmac. exp. Ther.* **165**, 216.

Smith, C. B. (1963). *J. Pharmac. exp. Ther.* **142**, 343.

Stein, L. (1964). *Fedn Proc. Fedn Am. Socs exp. Biol.* **23**, 836.

Zirkle, C. L. & Kaiser, C. (1964). In *Psychopharmacological Agents*, vol. 1, p. 445. Ed. by Gordon, M. New York: Academic Press Inc.

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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Blaschko, H. (1952). *Pharmac. Rev.* **4**, 415.

Hall, D. W. R., Logan, B. W. & Parsons, G. H. (1969). *Biochem. Pharmacol.* **18**, 1447.

Sweetman, A. J. & Weetman, D. F. (1969). *Br. J. Pharmacol.* **37**, 550P.

Tipton, K. F. (1968). *Eur. J. Biochem.* **5**, 316.

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 & Radhakrishnan, 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,

alternative substrates had to be employed, with unexpected results.

Supernatant (from centrifugation at 18000g for 10min) from tissue homogenates (in 0.2M-sucrose) were run on 5% polyacrylamide gels (5% cross-linkage) at 2mA/gel in tris-glycine buffer, pH 8.7. The gels were then incubated in 0.05M-phosphate buffer, pH 7.6, containing pyridoxal phosphate (0.1mg/ml), sodium Nitro Blue Tetrazolium (0.4mg/ml), amino acid substrate (0.4mg/ml) and liver mitochondrial monoamine oxidase (Tipton, 1968). Colour development was complete after 16h at 37°C.

When gels obtained from rat liver preparations were incubated with one of the substrates *m*-tyrosine, *o*-tyrosine or 5-hydroxytryptophan a band with high intensity and identical mobility appeared in each case. With *o*-tyrosine and 5-hydroxytryptophan there were at least four additional bands of moderate intensity. On the other hand with *m*-tyrosine only two additional faint bands appeared. These results are consistent with those reported by Coulson, Bender & Jepson (1969), in which four peaks of decarboxylase activity were observed with 5-hydroxytryptophan, but indicate that these peaks have differing substrate specificity. Studies of the distribution of these activities in other tissues are proceeding.

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- Calne, D. B. & Sandler, M. (1970). *Nature, Lond.*, **226**, 21.
Coulson, W. F., Bender, D. A. & Jepson, J. B. (1969). *Biochem. J.* **115**, 63p.
Murali, D. K. & Radnakrishnan, A. N. (1969). *Indian J. Biochem.* **7**, 13.
Smith, I., Kellow, A. H. & Hanington, E. (1969). *Proc. 3rd Migraine Symposium, London*, p. 120. London: W. Heinemann (Medical Books) Ltd.
Tipton, K. F. (1968). *Eur. J. Biochem.* **4**, 103.

Calf *N*-Acetylhexosaminidases

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The β -*N*-acetylhexosaminidases of a number of mammalian tissues have been shown to exhibit two main forms (A and B) distinguishable by their electrophoretic mobility (Robinson & Stirling, 1968; Okada & O'Brien, 1969). These enzymes are thought to be active towards both *N*-acetylglucosaminides and *N*-acetylgalactosaminides. However, Frohwein & Gatt (1967) concluded that three forms of these enzymes existed in calf brain. A particulate enzyme with the above broad specificity was accompanied by two supernatant enzymes showing preferential activity to the *N*-acetylglucosamine and *N*-

acetylgalactosamine configuration respectively and these were selectively isolated by the incorporation of thiol-blocking reagents during the extraction procedure. The particulate enzyme appears to be that responsible for the hydrolysis of gangliosides. So far no enzyme corresponding to the supernatant enzymes has been found in human tissues, and we have therefore examined the cellular distribution and physical forms of the calf enzymes in comparison with human *N*-acetylhexosaminidases A and B.

Not more than 15% of the activity of fresh calf brain was found in the 6×10^6 g-min supernatant of 0.5M-sucrose homogenates containing 1.0mM-EDTA at pH 7.0. During differential centrifugation the total activity of all fractions together was three times that of the original homogenate. Only the most readily sedimentable fractions showed the further liberation of activity by Triton X-100 that is indicative of intact lysosome particles. The increased activity accounted for 20% of the total. The enzyme is clearly readily potentiated during the fractionation procedure while remaining bound to particulate material. Greatly elevated activity was found in the supernatant from samples that had been stored in the frozen state for a few days.

Similar levels of activity and the same proportions of A and B forms were found in all areas of brain tissue examined. DEAE-cellulose chromatography separated the main A and B forms, which could be further resolved by electrofocusing. Sandhoff (1969) has demonstrated at least four different fractions in human brain by this method. Up to five electrophoretic forms have been demonstrated in fresh calf liver samples, organ differences are apparent and kidney and brain also show multiple forms at pH 5.8. The A forms from calf and human brain are not identical.

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- Frohwein, Y. Z. & Gatt, S. (1967). *Biochemistry, Easton*, **6**, 2775.
Okada, S. & O'Brien, J. S. (1969). *Science, N.Y.*, **165**, 698.
Robinson, D. & Stirling, J. L. (1968). *Biochem. J.* **107**, 321.
Sandhoff, K. (1969). *FEBS Lett.* **4**, 351.