Measurements of monoamine oxidase activity using [^{14}C]tryptamine

Wurtman & Axelrod (1963) described a method for the assay of monoamine oxidase (MAO) using [^{14}C]tryptamine as the substrate whereby the deamination products were extracted into acidified toluene. This method has been criticized because of the binding of the deamination products to the denatured proteins (Southgate & Collins, 1969), so that inaccurate results may be obtained if significant quantities of both indoleacetaldehyde and indoleacetic acid are produced. The mitochondrial fraction of rat liver is frequently used as a source of MAO in screening tests for MAO inhibitors. The products resulting from the enzymic oxidation of [^{14}C]tryptamine have now been examined in order to prove the validity of studies with this enzyme system.

The washed mitochondrial fraction of rat liver used as the source of MAO was prepared by the method of Hogeboom (1955). The oxidation products of [^{14}C]-tryptamine in the toluene extracts were examined by gas chromatography and paper electrophoresis.

In the gas chromatographic studies, 1 mM[^{14}C]tryptamine (10·7 mCi/mmol) was incubated with the mitochondrial fraction (0·62 mg protein) in a total volume of 0·3 ml, protein content being estimated by the method of Lowry, Rosebrough & others (1951). After incubation for 20 min, the mixture was extracted with toluene (Wurtman & Axelrod, 1963). An aliquot of the extract (50 μl) was mixed with hexamethyldisilazane (40 μl), non-radioactive indoleacetic acid (10 μg) and indoleacetaldehyde (10 μg) in toluene (10 μl), and allowed to stand at room temperature (20°) for 30 min. Reaction between indoleacetic acid and hexamethyldisilazane was complete within this time. Aliquots of the reaction mixture (30 μl) were chromatographed in a Packard series 7800 gas chromatograph. In duplicate runs, the column was connected to the flame detector and the effluent was collected on *p*-terphenyl in a Packard gas chromatography fraction collector. Radioactivity in the fractions was estimated in 0·4% 2,5-diphenyloxazole and 0·02% 1,4-bis-2-(5-phenyloxazolyl)-benzene in toluene (15 μl) in a Packard Tri-Carb liquid scintillation spectrometer.

In the electrophoresis studies, 5×10^{-5}M [^{14}C]tryptamine (1·6 mCi mmol) was incubated with the mitochondrial fraction (0·16 mg protein) in a total volume of 1·5 ml. Labelled components of the toluene extract (0·5 ml) together with non-radioactive indoleacetic acid (20 μg) were separated on Whatman 3 MM paper in an EC electrophoresis apparatus (Parker Instrument Co.).

Examination of the toluene extract by paper electrophoresis showed that approximately 10% of the labelled compounds had the same mobility as indoleactic acid while the bulk of the radioactivity was present as neutral compounds (Fig. 1). Gas chromatographic analysis indicated that the major neutral compound was indoleacetaldehyde with 10 to 15% of the radioactivity present as indoleacetic acid (Fig. 1). The presence of some tryptophol cannot be excluded as the separation factor between tryptophol and indoleacetaldehyde was only 1·2. However, little tryptamine should be converted to tryptophol. Tryptamine is metabolized to tryptophol in the intact rat, but only after pre-treatment with disulphiram (Smith & Wortis, 1960). In liver homogenates, the related compound, 5-hydroxytryptamine is metabolized principally to 5-hydroxyindoleacetic acid and the corresponding aldehyde while 5-hydroxytryptophol is only a minor metabolite (Feldstein & Williamson, 1968).

The enzymic behaviour of the mitochondrial fraction was examined also by estimating the levels of radioactivity in the toluene extracts after incubation with varying levels of mitochondrial protein. There was a linear relation between radioactivity levels in the toluene extracts and the concentrations of protein, although the regression

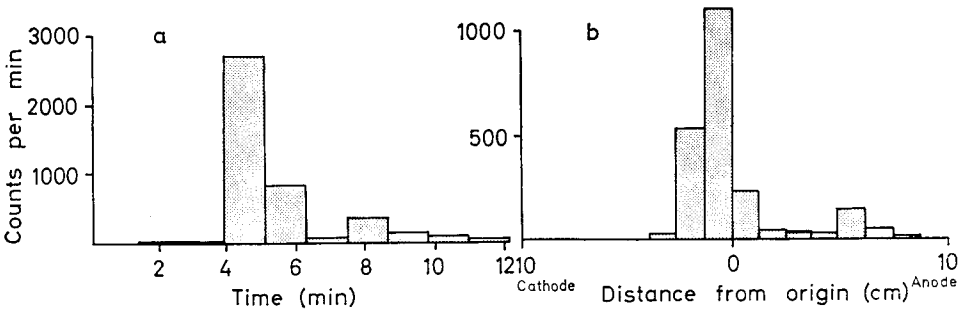

FIG. 1. Fractionation of the toluene extract of an incubation mixture of [^{14}C]tryptamine with the mitochondrial fraction of rat liver.

(a) Separation by gas chromatography on 10% silicone DC 550 on Gas Chrom Q, 80–100 mesh at 200° on glass columns (200 × 0·2 cm i.d.) with carrier gas (nitrogen) flow rate of 50 ml/min. Retention times of standards: indoleacetaldehyde, 4·4 min; indoleacetic acid, 8·6 min.

(b) Separation by paper electrophoresis after 4 h at 10 V/cm in 0·1 M Sorenson phosphate buffer, pH 7·4. Mobility of indoleacetic acid, 1·4 cm/h.

line did not pass through the origin. Although not studied in detail, the intercept of the abscissa appeared to vary with the time of pre-incubation before the addition of substrate. However, this phenomenon is probably of little practical importance. The intercept, even after pre-incubation for half an hour at 37°, was much lower than the concentration of mitochondrial protein which has been used routinely in screens for MAO inhibitors (0·1 mg/ml).

The fractionation studies were made on the toluene extracts of incubation mixtures of [^{14}C]tryptamine with the mitochondrial fraction. With the same extraction procedure, Southgate & Collins (1969) found that the relative extraction of indoleacetaldehyde and indoleacetic acid varied with the concentration of protein. In the present studies, both protein and substrate concentrations were varied over a twenty-fold range. However, the relative amounts of indoleacetic acid and indoleacetaldehyde in the toluene extracts were independent of protein or substrate concentrations. Assuming that the low levels of indoleacetaldehyde produced do not saturate any enzymes converting this first deaminated product to indoleacetic acid, the relative amounts of the two products formed in the incubation medium should likewise be independent of the protein or substrate concentrations. Thus, if both the toluene extract and the incubation medium contain constant, although possibly different, ratios of the deaminated products, the total amounts of the tryptamine metabolites in the toluene extracts should always be proportional to the total levels in the incubation medium. This conclusion is confirmed by the linear relation between the mitochondrial protein concentration and radioactivity in the toluene extracts. The overall conclusion is that the level of radioactivity in the toluene extract is proportional to the rate of oxidative deamination of [^{14}C]-tryptamine. Consequently, the method of Wurtman & Axelrod (1963) will yield valid kinetic constants such as apparent K_m and K_i values. However, it is advisable not to use very low concentrations of mitochondrial protein.

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REFERENCES

FELDSTEIN, A. & WILLIAMSON, O. (1968). *Life Sci.*, **7**, II, 777–783.
HOGEBOOM, G. H. (1955), *Methods in Enzymology*, Vol. I, pp. 17–19, Editor: Colowick, S. P. & Kaplan, N.O. New York: Academic Press.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). *J. biol. Chem.*, **193**, 263–275.
SMITH, A. A. & WORTIS, S. B. (1960). *Biochem. biophys. Acta*, **40**, 569–570.
SOUTHGATE, J. & COLLINS, G. G. S. (1969). *Biochem. Pharmac.*, **18**, 2285–2287.
WURTMAN, R. J. & AXELROD, J. (1963). *Ibid.*, **12**, 1439–1440.

Metabolism of [^{14}C]griseofulvin in the mouse

The metabolism of griseofulvin has been studied in man, rats, rabbits and dogs. A single metabolite of griseofulvin, the 6-desmethyl derivative was demonstrated in rabbit dog and human urine. (Barnes & Boothfoyd, 1961; Kaplan, Riegelman & Lee, 1960; Harris & Riegelman, 1969; Riegelman, Epstein & Dayan, 1962). In rat, two major metabolites, the 4- and 6-desmethylated derivatives, were identified (Symchowicz & Wong, 1966a,b). McNall (1960) reported that in mice following a single oral dose of griseofulvin, the blood and serum drug levels reached a maximum 6–8 h later but he did not study the excretion pattern and metabolic fate of this drug.

Prolonged intake of large doses of griseofulvin in mice lead to the ultimate formation of multiple hepatomas in the enlarged liver (Barich, Schwartz & others, 1961: DeMatteis, Donnelly & Runge, 1966; DeMatteis & Rimington, 1963; Hurst & Page, 1963). We have therefore investigated the fate of griseofulvin in mice.

[^{14}C]griseofulvin (368·7 μCi/mM) was prepared biosynthetically (Symchowicz & Wong 1966b). The authentic 6-desmethylgriseofulvin (6-DM) and 4-desmethylgriseofulvin (4-DM) were supplied by Glaxo Group Ltd., England.

Charles River male mice, about 20 g, were given the drug (10 mg/kg, i.v.) in a solution of 75% *NN'*-dimethylformamide, or by mouth (25 mg/kg suspended in 0·5% Tween-80). The radioactivity in urine was measured directly by liquid scintillation spectrometry. The radioactivity in plasma, faeces, intestines and liver samples were combusted by Schoniger technique (Kelly, Peets & others, 1961; Woeller, 1961), before counting.

The urine was acidified to pH 1·0 with concentrated hydrochloric acid and extracted with ether. The metabolic pattern of ether extractable material in urine was then studied using ascending paper chromatography with benzene–cyclohexane–methanol–water (5 : 5 : 6 : 4, v/v). Glacial acetic acid (0·5%) was added to the organic phase after equilibrium. The radioactivity patterns on the paper strips were analysed by scanning with a Vanguard 4π automatic strip counter attached to an automatic integrator.

The plasma drug disappearance curve after an intravenous dose shows two components, one with a 10 fold fall from 6000 to 600 d/min over the first 2 h, with a half life of 30 min, the other with a fall from 600 to 200 d/min over the next 2 h with a half life of 75 min.

Over 96 h, about 74% of the dose was excreted in the urine while some 64% of the oral dose was excreted in the same period (Fig. 1). Almost 50% of the total radioactivity in the urine was excreted during the first 4 h after intravenous or 8 h after oral administration of the drug.