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The estimation of monoamine oxidase using ^{14}C-labelled substrates

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SEVERAL micro-methods have recently been published for the estimation of tissue monoamine oxidase (MAO) activity based on the measurement of labelled metabolites of ^{14}C-tryptamine,[1,2] ^{14}C-tyramine,[1,3,4] ^{14}C-5-hydroxytryptamine,[1] ^{14}C-dopamine[3] and ^{14}C-benzylamine.[1] These methods depend on the isolation of the labelled compounds by either solvent extraction with toluene,[2] ethyl acetate[3] or anisole,[4] or by ion-exchange procedures.[1] In the present work, some of the limitations of these procedures are reported.

The partitioning of ^{14}C-tryptamine between an aqueous solution and ethyl acetate, toluene and anisole was measured at pH values of 1·0, 7·4 and 8·8; extraction was least at acid pH value with each solvent (Table 1). In addition, the solubility of indole-3-acetic acid and indole-3-acetaldehyde in the three solvents was determined at the two lower pH values using the assay procedure described by Larsen and Klungsöyr.[5] It appears that the degree of extraction of the two compounds differs, the acid being more soluble in all three solvents at acid pH (Table 1). In an attempt to verify these results, rat liver aldehyde dehydrogenase was prepared[6] and solubilized MAO was also prepared from rat liver, using the method described by Youdim and Sandler.[7] In some tubes, ^{14}C-tryptamine was incubated with MAO in the presence of phosphate buffer for 20 min, after which 0·4 ml of 2N HC

TABLE 1. EFFECT OF pH ON THE EXTRACTION OF TRYPTAMINE, INDOLE-3-ACETIC ACID AND INDOLE-3-ACETALDEHYDE BY ETHYL ACETATE, TOLUENE AND ANISOLE

	Solvent								
	Ethyl acetate			Toluene			Anisole		
pH	1·0	7·4	8·8	1·0	7·4	8·8	1·0	7·4	8·8
Tryptamine	11·3	25·6	56·3	0·45	3·6	15·6	0·44	9·1	35·4
Indole-3-acetic acid	30·0	13·9	—	74·2	21·0	—	75·0	70·2	—
Indole-3-acetaldehyde	9·3	26·9	—	4·8	37·0	—	17·7	32·7	—

The values given represent percentage recovery.

was added and the mixture extracted with 6 ml toluene. In other experiments, aldehyde dehydrogenase was included in the incubation mixture; in the presence of this enzyme, which oxidises any aldehyde formed to the acid, the total count of the toluene extract was expected to be greater than that when was present, confirming that the acid is more soluble than the aldehyde in acidified toluene. However MAO alone inconsistent results were obtained from nine such experiments in which the protein concentration varied considerably (Table 2).

TABLE 2. EFFECT OF ALDEHYDE DEHYDROGENASE ON A MONOAMINE OXIDASE ASSAY INCUBATION MIXTURE

Expt.	MAO	MAO + aldehyde dehydrogenase	Change (%)
1	366	673	+ 83·9
2	454	649	+ 43·0
3	948	1256	+ 32·5
4	650	755	+ 16·2
5	650	575	− 11·5
6	597	517	− 13·4
7	625	525	− 16·0
8	2080	1610	− 22·6
9	1724	1170	− 33·1

These values represent the counts per minute obtained from an acidified toluene extract of the incubation mixture with and without added aldehyde dehydrogenase.

Incubation mixture—0·1 ml enzyme preparation, 0·1 ml ^{14}C-tryptamine (10,000 cpm, 6·25 mμmoles), 0·2 ml aldehyde dehydrogenase and phosphate buffer (pH 7·4, 0·05M) to give a total volume of 0·7 ml.

These unexpected findings can probably be explained in the following way. In order to exclude the amine, extraction must be carried out at acid pH; however, the protein is denatured at this pH and it appears that non-specific binding of the radioactive metabolites to the denatured protein occurs (Table 3). Radioactive indole-3-acetic acid was isolated by extraction of an incubation mixture of ^{14}C-tryptamine and soluble rat liver MAO with toluene at pH1, evaporating the extract to dryness and dissolving the residue in phosphate buffer (0·05M, pH 7·4); approximately 10 per cent of the counts of this preparation were due to labelled impurities. Extracts containing all the labelled metabolites (acid, aldehyde and alcohol) were prepared by pipetting the incubation mixture directly onto a 30 × 5 mm column of Amberlite CG-50; ^{14}C-tryptamine was retained by the column whereas its metabolites passed through and were collected. The mixtures were incubated for 10 min with various proteins, which were then denatured by the addition of 0·2 ml 10% zinc sulphate and 0·2 ml 0·1M sodium hydroxide and the proportion of bound metabolites determined (Table 3). The binding of

TABLE 3. THE PERCENTAGE BINDING OF ^{14}C INDOLE-3-ACETIC ACID (IAA) AND A MIXTURE OF TOTAL ^{14}C METABOLITES TO DENATURED PROTEINS

Denatured protein	^{14}C-IAA % bound	^{14}C-IAA + ^{14}C-IAAld % bound
Human serum	0·25	7·5
Rat liver homogenate	32·0	22·0
Rat liver Aldehyde dehydrogenase*	32·0	46·0
Purified soluble rat liver MAO*	44·0	60·0

These values are the mean of at least two experiments.
* for preparation see text.

acid and total metabolites is greatest to the solubilized MAO preparation and least to serum protein.

Although the use of ion exchange resins circumvents difficulties specifically associated with solvent extraction, the presence of excess protein may interfere with the recovery of metabolites from the column.[1] Whilst the recovery of aqueous solutions of pure indole-3-acetaldehyde, indole-3-acetic acid and tryptophol from Amberlite CG-50 approached 100 per cent (Table 4), the presence of solubilized MAO (0·5–1·0 mg/0·2 ml of protein, as measured by the method described by Lowry *et al.*)[8] reduced the recovery of aldehyde to 57 per cent and of acid to 76 per cent; recovery of the corresponding alcohol was unimpaired (Table 4). The loss might be due, at least in part, to Schiff's base formation between carbonyl groups of the aldehyde and acid to free amino groups of the protein component.[9]

TABLE 4. RECOVERY OF INDOLE-3-ACETIC ACID, INDOLE-3-ACETALDEHYDE AND TRYPTOPHOL FROM AMBERLITE CG–50 RESIN

	% Recovery of pure compound	% Recovery in the presence of MAO protein (0·5–1·0 mg/0·2 ml) (%)
Indole-3-acetaldehyde (5–50 μg)	95–100	57
Indole-3-acetic acid (1–10 μg)	95–100	76
Tryptophol (5–50 μg)	100	100

Thus, unless the conversion of an aldehyde metabolite to its corresponding acid can either be prevented or driven to completion, the varying aldehyde dehydrogenase activity of tissues will lead to variable counts of an acid solvent extract; the binding of metabolites to the denatured protein introduces a further variable factor into the assay procedure, making comparisons between different enzyme preparations difficult. Similarly, although one of these objections is eliminated with the use of ion-exchange resin, the variable protein content of different incubation mixtures affects the recovery of metabolites from the resin.

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