The influence of substrates and buffer solutions on the estimation of monoamine oxidase activity

Monoamine oxidase (MAO) behaves as if it were a family of related enzymes since activity found in different species and in different tissues varies in its properties towards enzyme inhibitors (Squires, 1972), substrate specificity (Alles & Heegaard, 1943), sensitivity to heat (Youdim & Sourkes, 1965) and incubation pH (Barbato & Abood, 1963). With pH, many studies have reported that maximum MAO activity occurs at pH values below 9·0 (Alles & Heegaard, 1943; Youdim & Sourkes, 1965; Youdim, Collins & Sandler, 1969; Tipton, Youdim & Spires, 1972). However, in recent experiments (Callingham & Laverty, 1973) we found that MAO activity towards tyramine did not fall when the pH of the phosphate buffer was raised to 9·1 and that the activity towards benzylamine was still increasing rapidly at pH 9·1; this confirmed the early observations of Hare (1928) on tyramine oxidase. Because of this discrepancy, it was decided to study the variation in rat MAO activity with pH using a number of buffers, several substrates and with enzyme from two tissues, to see whether the change in MAO activity at high pH was a function of the source of enzyme, the substrate, the assay technique, or the buffer solution used.

Hearts and livers were removed from male adult albino rats and homogenized in 0·001 M potassium phosphate solution (pH 7·8) at a tissue dilution of 1:20. Buffer solutions used were potassium phosphate (0·2M), tris-HCl (0·1M), borate-HCl (0·025M) and barbitone-HCl (0·04M) adjusted to the appropriate pH. MAO activity using tyramine and benzylamine as substrates was measured by a radiochemical technique (McCaman, McCaman & others, 1965; Callingham & Laverty, 1973), using 10 μl homogenate in 90 μl buffer containing the appropriate substrate (1 mM final concentration). With heart MAO estimation using benzylamine as substrate, it was necessary to use 25 μl homogenate to ensure sufficient metabolite production. The homogenate substrate mixture was incubated at 37° in an atmosphere of oxygen for 10 min in the tyramine experiments or 30 min in the benzylamine experiments. Kynuramine oxidase activity was measured fluorimetrically (Kraml, 1965; Squires, 1968) incubating 25 μl homogenate in 225 μl buffer with kynuramine (50 μl 0·15M in water) in air at 37° for 30 min since incubation in oxygen did not alter the apparent activity. In all cases an equivalent homogenate sample was incubated in buffer for 30 min at 37° before the addition of the substrate to test for the effect of incubation at various pH's on MAO activity.

The results summarized in Table 1 show that there is a complex interaction between the buffer, the substrate and the source of enzyme activity. For example, in tris buffer, the tyramine oxidase activity of both heart and liver homogenates is very much less than in phosphate buffer, whereas the kynuramine and benzylamine oxidase activities are, if anything, greater than in phosphate buffer. Barbitone buffer shows a similar but less marked inhibition of tyramine oxidase activity than tris, whereas borate increases the activity of both heart and liver kynuramine oxidase but only that of liver tyramine oxidase.

The effect of changing the pH of the medium is also complex. The activity of the enzyme in these experiments was measured using a technique which involves the amount of metabolite produced at the end of the incubation period. This is the resultant of the combined effects of rate of reaction of the enzymatic oxidation and the rate of destruction or inactivation of the enzyme during the incubation. This latter effect was examined by pre-incubation of the homogenate sample in the appropriate buffer for 30 min before assay of MAO activity. The degree of pH inactivation appears to depend on the nature of the buffer used. In phosphate, barbitone and borate buffers, considerable reduction of oxidase activity occurs, especially at pH 9·1, but

Table 1. *Relative activities in rat heart and liver homogenates in four buffer solutions in the pH range 7·8–9·1; using tyramine, kynuramine or benzylamine as substrates.* Mean activities (of 5–6 observations) are expressed as percentages of the activity in phosphate buffer at pH 7·8. The standard error of the ratio did not exceed ±10%. Pre-incubation was carried out at 37° in the absence of substrate.

		Buffer							
		Phosphate		Tris		Barbitone		Borate	
		Preincubation time (min)							
Substrate	pH	0	30	0	30	0	30	0	30
		Heart homogenate							
Tyramine	7·8	100	84	16	18	73	71	90	87
	8·5	102	59	20	16	77	62	93	62
	9·1	95	40	20	19	82	50	79	21
Kynuramine	7·8	100	69	114	86	84	64	127	89
	8·5	143	52	173	107	125	55	157	59
	9·1	129	36	192	96	129	36	166	18
Benzylamine	7·8	100	87	118	111	117	92	93	87
	8·5	134	87	164	128	129	104	107	85
	9·1	136	84	187	135	154	95	110	57
		Liver homogenate							
Tyramine	7·8	100	84	32	28	70	71	125	119
	8·5	115	89	30	26	83	64	127	91
	9·1	111	81	28	23	87	63	123	36
Kynuramine	7·8	100	77	109	105	102	80	126	102
	8·5	114	57	152	118	121	95	159	71
	9·1	116	41	165	102	145	61	157	23
Benzylamine	7·8	100	87	118	111	117	92	93	87
	8·5	134	87	164	128	129	104	107	85
	9·1	137	84	187	135	154	95	110	57

in tris buffer pre-incubation had less effect. Interestingly, pre-incubation in tris buffer of MAO activity had least effect when tyramine was used as substrate, this lack of incubation effect accompanied the suppression by tris buffer of MAO activity using tyramine as substrate suggesting that tris buffer inactivates a heat-labile fraction of the tyramine oxidase activity.

The pH range and buffer molarities used in these experiments are typical of those found in the published methods for the estimation of MAO activity. A further comparison could be made to study the influence of changes in molarity within the same buffer-substrate mixture on the enzyme activity. This has only been done in this study for phosphate buffer. Over the range from 0·025 to 0·2M at pH 7·8 there were no significant differences between the values obtained. This was found to be true both for the series with pre-incubation and for the series without pre-incubation. At pH 8·5 and particularly at pH 9·1, while changing molarity had no effect without pre-incubation, increasing the buffer strength increased the inhibitory effect. For example, pre-incubation in 0·025M phosphate buffer at pH 9·1 reduced the apparent MAO activity to 72% of its control value, with tyramine substrate, while at 0·2 M, the activity was reduced to only 40%. Thus it seems probable that, where a particular buffer has an influence on the apparent MAO activity, increasing its molarity will increase its effect.

The present results would suggest that MAO has an optimal pH between 8·5 and 9·1 when phosphate or borate is used as a buffer, but an optimal pH greater than 9·1 when tris or barbitone is used. However, when allowance is made for the effect of the buffer on the inactivation of the enzyme during the estimation of MAO activity, it is apparent that the optimal pH in all cases is greater than 9·1.

These experiments indicate some of the complexities associated with the estimation of MAO activity and some of the difficulties which may arise when attempting to correlate the results of different workers in different laboratories using a wide variety of techniques.

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Department of Pharmacology,
University of Cambridge,
Medical School, Hills Road,
Cambridge CB2 2QD, U.K.

BARBARA J. BROWNE
R. LAVERTY
B. A. CALLINGHAM

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Substrate specificity of monoamine oxidase activity in various mouse and rat tissues

Adrenalectomy causes a rise in the activity of monoamine oxidase (MAO) in the rat heart (Avakian & Callingham, 1968), but there is no change following adrenalectomy in the activity of mouse heart MAO using either tyramine or benzylamine as substrate (Laverty, unpublished). Rat heart MAO also differs from mouse heart MAO since its activity increases rapidly with age (Horita, 1967). It was therefore decided to compare the substrate specificity of homogenates of mouse and rat hearts and other tissues from these animals in an attempt to see if other differences exist.

Tissues from adult male albino rats (300–350 g) and mice (30–35 g) were homogenized in 0·001M potassium phosphate solution at pH 7·8, and diluted to a 1:20 suspension. MAO activity was measured radiochemically (McCaman, McCaman & others, 1965; Callingham & Laverty, 1973) using [^{3}H]tyramine, [^{3}H]dopamine, [^{3}H]5-hydroxytryptamine or [^{14}C]benzylamine (1 mM in 0·1 M potassium phosphate solution, pH 7·8) as substrates, or fluorimetrically (Kraml, 1965; Squires, 1968) using kynuramine (0·15 mM) as substrate.

The absolute MAO activities of rat and mouse tissues to the five substrates are shown in Table 1. In both species, the maximum activity with all substrates was found in the liver with least in the spleen. The pattern of activity between the substrates was similar in mouse and rat liver, brain and spleen, with the exception that