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A rapid microfluorimetric determination of monoamine oxidase*

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AMONG the many methods available to assay monoamine oxidase (MAO) activity, the simplest and most rapid appears to be the spectrophotometric method of Weissbach *et al.*[1] Kynuramine is used as substrate and its disappearance can be followed directly in a cuvette by monitoring the decrease in optical density at 360 mμ in a recording u.v. spectrophotometer. In our laboratory this method has been found to be very convenient in testing for the effect of inhibitors of MAO, both *in vitro* and *in vivo*, on liver and brain MAO enzymes.[2] That this method has not found a more widespread application is probably due to its lack of sensitivity. Highly active tissues such as rabbit or guinea pig liver are readily assayed, but in other species or in other less active tissues this technique is often difficult to apply. Increasing the amount of tissue in the incubation medium renders it too opaque for direct spectrophotometry. In other studies, such as those on enzyme topography, the tissue samples available are simply too small to be assayed. Lovenberg *et al.*[3] have described a sensitive micro-MAO assay, using tryptamine as the substrate and measuring the indoleacetic acid formed, fluorimetrically. This method was applied to MAO estimation in single ganglia. More sensitive, but more complex, are the assays of MAO that depend on estimating, by liquid scintillation counting, the indoleacetic acids formed from ^{14}C-tryptamine[4] or ^{14}C-serotonin.[5] All of these highly sensitive

TABLE 1. MAO ACTIVITY OF SELECTED TISSUES

Tissue	Activity (μmoles 4HOQ/g/hr*)	
	Range	Avg. ± S.E.
Rat brain	1·25–1·64 (10)†	1·45 ± 0·04
Guinea pig atrium	1·26–3·98 (10)	2·86 ± 0·36
Cat ganglia, vagal	1·18–2·05 (7)	1·38 ± 0·16
Cat ganglia, sympathetic	1·73–5·22 (8)	3·11 ± 0·46

* Activity expressed as μmoles 4HOQ formed per gram of wet weight of tissue per hour at 37°, with air as the gas phase.

† Values in parentheses indicate number of tissue samples analyzed.

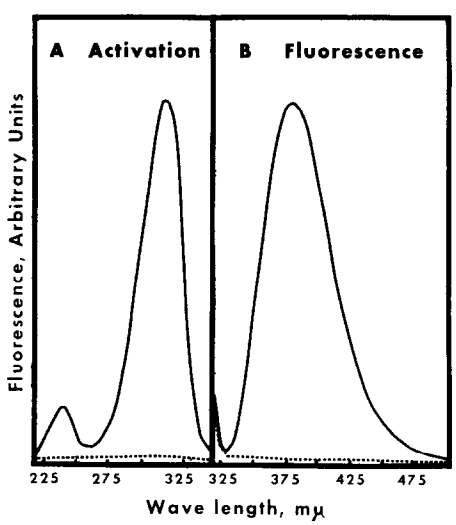

FIG. 1. Activation and fluorescence spectra of kynuramine (— — —) and 4HOQ (———) in 1 N NaOH. 4HOQ has an activation maximum of 315 mμ and a fluorescence maximum of 380 mμ. At tenfold higher concentrations kynuramine does not fluoresce.

methods involve a fair amount of sample manipulation, i.e. extraction, washing, etc., before fluorimetry or scintillation counting can be applied.

A fluorimetric adaptation of the MAO assay of Weissbach *et al.* has now been developed which provides a highly sensitive and, I believe, rapid and convenient assay of MAO activity. Instead of determining the disappearance of kynuramine one can readily monitor fluorimetrically the appearance of 4-hydroxyquinoline (4HOQ) which arises from the spontaneous cyclization of the intermediate aldehyde formed by the oxidative deamination of kynuramine.[1] The activation and fluorescence spectra obtained for kynuramine and 4HOQ, in 1 N NaOH, are shown in Fig. 1. On activation at 315 mμ, 4HOQ exhibits an intense fluorescence maximum at 380 mμ*. Under these conditions a tenfold excess of kynuramine has no appreciable fluorescence. At the sensitivity settings employed, slit system 3, meter multiplier 0·03, sensitivity 50,[6] full-scale galvanometer deflection is obtained with between 0·1 and 0·2 μg of 4HOQ per milliliter. At the highest sensitivity settings, as little as 1 mμg of 4HOQ could be detected. Fluorimetric estimation of 4HOQ can thus form the basis of an extremely sensitive MAO assay.

With an incubation medium very similar to that of Weissbach *et al.* the fluorimetric method has been applied to a variety of tissues, e.g. rat brain, guinea pig atria, and cat ganglia† (Table 1). The incubations were carried out as follows: to 1·0 ml of enzyme (an aqueous homogenate of tissue containing the desired amount of wet weight of tissue) were added 0·5 ml kynuramine (100 μg kynuramine dihydrobromide), 0·5 ml phosphate buffer (0·5 M, pH 7·4), and water to 3·0 ml. After incubation at 37° for 30 min with air as the gas phase, 2·0 ml of 10% trichloroacetic acid (TCA) were added. The precipitated proteins were spun down and 1·0 ml of supernatant was added to 2·0 ml of 1 N NaOH in a quartz cuvette. The solution was activated at 315 mμ and the fluorescence measured at 380 mμ or recorded by scanning from 320 to 450 mμ. Suitable blanks and 4 HOQ standards are carried through the entire procedure. A 4HOQ standard of 10 mμM (1·99 μg) carried through the entire procedure gives an arbitrary fluorescence value of 77 ± 2 units and under these conditions the blank values are 5 ± 1 units; 4HOQ carried through the entire procedure gives only one quarter the fluorescence obtainable in 1 N NaOH. This is not due to absorption losses on precipitated proteins or due to quenching by tissue extracts or kynuramine, these effects being minimal, but to quenching by TCA. In the concentration range employed, a 10% change in TCA concentration causes a 5% change in 4HOQ fluorescence. As the 4HOQ standard has never varied more than 2 units from its average value of 77, TCA fluctuations in practice are minimal. TCA has been retained as deproteinizing agent because it is convenient to use and because, despite quenching, the fluorescence yield is sufficient to permit MAO assay in all tissues so far tested.

The agreement between duplicate analyses is very good, the average error being less than 2%. The manipulations involved are obviously simpler and less time consuming than those of the previously reported methods.[3–5] Finally, the assay does not depend on the tissue sample containing aldehyde dehydrogenase. Whereas MAO is responsible for the oxidative deamination of a given amine to the corresponding aldehyde, many methods are based on the assay of the acid produced by further oxidation of the aldehyde by a second enzyme, an aldehyde dehydrogenase. While liver contains sufficient aldehyde dehydrogenase to make MAO rate limiting, this is not true in all tissues;[4, 5] for ganglia, in order to obtain true MAO levels, Lovenberg *et al.*[3] were obliged to add exogenous aldehyde dehydrogenase. In the present assay, the aldehyde produced by the oxidative deamination of kynuramine undergoes spontaneous and complete cyclization,[1] and thus 4HOQ production depends only on MAO activity.

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* An Aminco-Bowman spectrophotofluorometer coupled to an Electro Instruments model 101 X-Y recorder was employed. All wavelengths are reported as uncorrected values.

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Inhibition of histamine methylation by antimalarial drugs*

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In studies designed to assess the physiologic role of biogenic amines such as serotonin and norepinephrine, one approach has been to inhibit either their biogenesis or their catabolism and note the corresponding changes in function.[1] A similar approach to the study of histamine's physiologic role should likewise be useful. Histamine is metabolized by two principal pathways: oxidation of the side chain amino group by diamine oxidase (DAO) and methylation of an imidazole nitrogen by imidazole N-methyl transferase (IMT).[2] Aminoguanidine has been known for many years to be a potent and selective inhibitor of DAO, both *in vitro* and *in vivo*.[3, 4] Although chlorpromazine has been shown to inhibit IMT both *in vitro*[5, 6] and *in vivo*,[7] it appears to be only a weakly effective inhibitor.[6, 7] The desirability for finding an inhibitor of IMT with greater effectiveness and specificity is apparent.

Attempts to inhibit catechol O-methyl transferase by quinacrine† led to the study of the effect of this compound on IMT. Quinacrine proved to be an effective inhibitor of IMT, and compounds structurally related to quinacrine were tested for similar activity. Since the most active of these compounds were antimalarial drugs, other compounds strucutrally unrelated but possessing antimalarial activity were also tested. Many of these were found to be potent inhibitors of IMT. The results of the studies are reported here.

METHODS

Imidazole N-methyl transferase activity was assayed by the procedure utilized but not described in detail by Netter *et al.*[8] The enzyme was prepared from kidney homegenates of male Sprague-Dawley rats. Kidneys were removed immediately after the rat was killed by cervical dislocation, and chilled on ice. After weighing, the kidneys were homogenized in 9 volumes of ice-cold 0·25 M sucrose and centrifuged at 30,000 *g* for 20 min. The 30,000-*g* supernatant fluid was used as the enzyme source. A representative incubation vessel contained 1 ml enzyme, 200 μmoles phosphate buffer (pH 7·2), 0·5 μmole aminoguanidine (to inhibit metabolism by DAO), 0·5 μmole S-adenosyl methionine (to serve as methyl donor), 0·09 μmole histamine, and 0·4 mμmole to 4 μmoles inhibitor in a final volume of 4 ml. Incubation vessels were preincubated in a Dubnoff metabolic shaker for 10 min at 37° in an air atmosphere to ensure temperature equilibrium and to provide a standard time period for the inhibitor to interact with the enzyme before the substrate (histamine) was added. In control experiments aliquots of the incubation mixture were removed at various times, and the rate of histamine disappearance was shown to be linear for at least 30 min. In subsequent experiments, rates were determined from aliquots removed at 0 and 30 min. In the absence of added S-adenosyl methionine, no histamine disappearance could be detected, thus indicating the specificity of the assay procedure. The enzymatic reaction was stopped by adding a 0·5 ml aliquot of the incubation mixture to an extraction tube containing 4 ml of 0·4 N $HClO_4$. The tube contents were then made alkaline by the addition of 0·5 ml of 5 N NaOH, and any fluorescent inhibitors were extracted from the aqueous phase with 5 ml chloroform. After this extraction no interference in the histamine assay was produced by any of the compounds studied. Histamine was then extracted from a portion of the alkaline