

in certain tissues differ physically from those in the liver and thus tend to fragment more easily, should also be considered. A recent investigation has in fact demonstrated that liver mitochondria can be fragmented by osmotic lysis, resulting in a mixed population of small vesicles derived from the outer mitochondrial membrane and larger bodies derived from the inner membrane.<sup>13</sup> It is of interest that the MAO activity could be recovered in the small vesicle fraction, indicating that this enzyme is probably associated with the outer membrane of the mitochondrial.

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#### Comment on microfluorometric determination of monoamine oxidase

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A MONOAMINE oxidase (MAO) assay was recently described by Kraml, in which kynuramine is converted to 4-hydroxyquinoline and the end product is assayed fluorometrically.<sup>1</sup> In our laboratory, the use of trichloroacetic acid (TCA) resulted in considerable quenching of the fluorescence, in agreement with Kraml. Some downward drifting of the readings was also observed. The problem of quenching and fluorescence stability can be entirely avoided by stopping the reaction with 2.0 ml of 0.6 M perchloric acid (PCA) instead of with 10% TCA. Examples of the relative fluorescence of standard samples of 4-hydroxyquinoline (4-HOQ) are shown in Table 1. Repeated determinations

fail to show any significant differences between readings from PCA-treated standards and those in water only. Assays of whole tissue homogenates from rats results in the following activities in  $\mu$ moles/g/or brain, 6.5 to 7.0; liver, 35–41; and kidney, 2.5–2.7. Brain activities shown here are higher than those reported by Kraml, but are comparable to those reported by Weissbach *et al.*<sup>2</sup> when corrected for probable differences due to temperature and converted from a wet weight to a protein

TABLE 1. RELATIVE FLUORESCENCE OF 4-HYDROXYQUINOLINE IN THE PRESENCE OF PCA AND TCA\*

4-HOQ ( $m\mu$ moles)	Per cent transmission		
	H <sub>2</sub> O	PCA	TCA
0	0.5	0.5	0.4
0	0.7	0.5	0.4
10	38.8	41.0	11.6
15	58.3	57.2	18.0
20	77.2	77.1	23.8
25	96.0	97.1	29.9

\* Aqueous standards were prepared to a final volume of 5.0 ml and included 2.0 ml of 0.6 M PCA, 10% TCA or water. One ml was added to 3.0 ml of 1 N NaOH and fluorescence was determined at 380  $m\mu$  with activation at 315  $m\mu$ .

basis. No differences were observed in tissue MAO activities between samples precipitated with PCA and those precipitated with TCA, except for the final fluorescence levels and the somewhat better stability of the readings in the PCA samples.

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#### Necessity of considering body temperature in drug–cold stress studies of catecholamines

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WORKERS in a number of laboratories, both in the United States and abroad, have recently reported experiments in which they have administered to rodents drugs that inhibit the biosynthesis of catecholamines and have then stressed the animals by placing them at low temperatures for periods of time