This Week's Citation Classic

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Wurtman R J & Axelrod J. A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmacol.* **12**:1439-41, 1963.
[Lab. Clinical Science, Natl. Inst. Mental Health, NIH, Bethesda, MD]

An assay for monoamine oxidase (MAO) is described using isotopically labeled tryptamine as substrate and a toluene:dilute HCl system for separating products from unreacted substrate. One worker could assay 60 or more samples in a morning. [The *SCI*® indicates that this paper has been cited in over 660 publications since 1963.]

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"I studied philosophy in college, and decided to work on the 'mind-body problem.' Hence I enrolled in medical school and sought opportunities to do research on brain mechanisms underlying behavior. Having heard that infusions of epinephrine caused some people to become anxious, I approached Peter Dews, a Harvard pharmacology professor, about using his operant conditioning techniques to characterize epinephrine's behavioral effects: pigeons in Skinner boxes would be allowed access to food if they pecked at a key 15 or more minutes after their last feeding; they quickly developed a regular behavior pattern, wasting no energy on fruitless pecking for ten minutes, then pecking vigorously. Various drugs characteristically altered this pattern. With a classmate, Michael Frank, I found that epinephrine caused a dose-dependent change in the animals' behavior. We presented our findings at a FASEB meeting, and they were well received. Thirty minutes later, Julius Axelrod—then unknown to me—gave his classic paper[1] showing that exogenous catecholamines are unable to cross the blood-brain barrier (!). (To this day I have no idea how the epinephrine worked; perhaps it gave the birds a headache.) When I described what had transpired to Dews, he suggested that I learn neurochemistry under Axelrod. In 1962, I entered Axelrod's laboratory at the National Institute of Mental Health and began exploring catecholamine metabolism.

"Several years earlier Axelrod had shown that catecholamines are inactivated not primarily by an enzymatic mechanism but by re-uptake into nerve terminals.[2] Two enzymes also could initiate catecholamine metabolism: catechol-O-methyltransferase and monoamine oxidase (MAO); the latter apparently functioned to set catecholamine levels within nerve terminals. That MAO could be involved in human behavior had been suggested by the antidepressant activity of MAO inhibitors.

"Axelrod proposed that I examine the effects of hormones on the fate of circulating catecholamines in rats. For this I would need also to measure MAO. Existing assays, based on manometric or fluorimetric procedures, were cumbersome and insensitive, so Axelrod suggested that we develop our own, using an isotopically labeled substrate and an appropriate organic solvent to separate deaminated metabolites from the unutilized substrate. The substrate used, tryptamine, was highly charged at an acidic pH, and couldn't pass from the aqueous phase into toluene, the organic solvent. However, once deaminated it passed into the toluene. In a day or two we worked out an MAO assay based on 14C-tryptamine's deamination, and used it to characterize the effects of hormones on MAO activity[3] and the extent to which tissue MAO activity had to be inhibited before catecholamine metabolism actually was affected.[4] At first we planned only to describe our MAO assay in the methods sections of these reports. However, we subsequently decided that its speed and sensitivity might make it useful to other people, so we wrote the paper cited here. Apparently, this has been the case.

"The intellectual godfathers of our assay were Lyman Craig, who pioneered the use of liquid-liquid chromatographic systems, and Bernard Brodie, who, with Axelrod,[5] applied such systems for separating biologically active compounds (like amphetamine) from their metabolites. MAO is now known to be a family of enzymes, acting on different substrates.[6,7] Fortunately, MAO assays using the single substrate tryptamine often provide adequate information about the behavior of the family in general.

"The mind-body problem remains unsolved."

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2. **Hertting G, Axelrod J, Kopin I J & Whitby L G.** Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature* **189**:66, 1961.
3. **Wurtman R J, Kopin I J & Axelrod J.** Thyroid function and the cardiac disposition of catecholamines. *Endocrinology* **73**:63-4, 1963.
4. **Wurtman R J & Axelrod J.** Sex steroids, cardiac 3H-norepinephrine, and tissue monoamine oxidase levels in the rat. *Biochem. Pharmacol.* **12**:1417-19, 1963.
5. **Axelrod J.** Studies on sympathomimetic amines. II. The biotransformation and physiological disposition of d-amphetamine, d-p-hydroxyamphetamine and d-methamphetamine. *J. Pharmacol. Exp. Ther.* **110**:315-26, 1954.
6. **Haenick D, Boehme D H & Vogel W H.** Monoamine oxidase in four rat and human tissues. *Biochem. Med.* **26**:451-4, 1981.
7. **Giambalvo C T & Becker R E.** Modulators of monoamine oxidase in plasma. *Life Sci.* **29**:2017-24, 1981.