

Da Prada M & Zürcher G. Simultaneous radioenzymatic determination of plasma and tissue adrenaline, noradrenaline and dopamine within the femtomole range.

Life Sci. 19:1161-74, 1976.

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The paper describes a radioenzymatic assay for measuring simultaneously femtomole quantities of adrenaline, noradrenaline, and dopamine. [The *SCI*® indicates that this paper has been cited in over 1,150 publications.]

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July 9, 1987

Twelve years ago I took charge of the biochemical section within pharmaceutical research on the central nervous system (CNS) at Hoffmann-La Roche in Basle, Switzerland. In the process I inherited some new collaborators, among them Gerhard Zürcher, who proved to be the *deus ex machina* for the implementation of our highly sensitive radioenzymatic method.

At that time, measuring the extremely low quantities of catecholamines (CA) present in human and animal blood (a few picograms/ml plasma) was virtually impossible for most pharmacologists and clinicians. Our belief that the development of a single-isotope, catechol-O-methyltransferase (COMT)-derived radioenzymatic assay would be essential for preclinical and clinical research was kindled by lively discussions with an enthusiastic clinical investigator at Basle University, Fritz U. Bühler, who was urging the development of a reliable assay to measure CAs in human plasma for his clinical investigations of hypertension. The method was soon elaborated in our laboratories by Zürcher. His tenacity and profound experience in radioenzymology proved essential in solving the main methodological steps, namely, defining optimal transmethylation conditions and realizing extensive extraction by complexing the [³H]-methoxylated products with sodium tetraphenylborate, as well as achieving a reliable and rapid thin-layer chromatographic separation of the labeled methoxylated CA derivatives.

While developing this assay was an exciting and relatively brief endeavour, getting the paper that described our method accepted for publication by *Life Sciences* required a time-consuming correspondence to rebut criticisms from the referees and the performance of additional experiments. This frustrating de-

bate, lasting more than seven months, became a kind of nightmare for us. Indeed, the manuscript was revised and resubmitted five times! Eventually the paper was accepted and appeared in the December 1976 issue of the journal. The assay soon became the method of reference and the most frequently used technique for the measurement of CAs in plasma.

Unlike previous investigators who kept "religiously secret" the details of their methodology, we described ours with the utmost transparency. Additionally, we opened the doors of our laboratory to numerous investigators from several countries who were interested in acquiring and practicing the technique. Due to its high sensitivity, our radioenzymatic assay permitted a precise and simultaneous measurement of noradrenaline, adrenaline, and dopamine that could be made in 50 microliters of plasma. This made it possible for the first time to perform physiological and pharmacological experiments on small laboratory animals such as rats. An accurate study that critically investigated the best method to collect blood under nearly physiological conditions provided the basis for a meaningful interpretation of the changes in plasma CA concentrations following physiological and pharmacological manipulations.¹

Our COMT-derived radioenzymatic assay became seminal in demonstrating that dopamine is virtually the only CA present in the retinas of mammals, amphibians, and fishes. It allowed us to accurately measure the rapid changes in the dopamine content that occurred in the retinal neurons upon exposure to light.² Using this method we were able to demonstrate that reserpine, as well as dopaminergic agonists and antagonists, alters the level of dopamine and/or its synthesis rate by closely similar mechanisms both in the retina and in the CNS.² Our technique also became instrumental in assessing the concentration gradient of CAs between blood platelets and plasma in humans and animals.³ In addition, our expertise with the COMT-based radioenzymatic methodology was essential for devising an extremely sensitive assay for the measurement of levodopa in plasma and tissues⁴ as well as for a rapid and sensitive single-step radiochemical assay for measuring the COMT activity in tissue homogenates.⁵

For more than a decade our single-isotope radioenzymatic assay has been recognized as the method of choice for the measurement of minute amounts of CAs in plasma. It is also useful in studying the physiological dynamics of CAs in plasma.⁶ In a few years plasma CA may be measured advantageously by electrochemical detection, using sophisticated high-pressure liquid chromatography systems for the direct purification of crude plasma and tissue extracts.

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