

This Week's Citation Classic

Bertler Å, Carlsson A & Rosengren E. A method for the fluorimetric determination of adrenaline and noradrenaline in tissues.
Acta Physiol. Scand. 44:273-92, 1958.
[Dept. Pharmacol., Univ. Lund, Lund, Sweden]

This paper describes the first chemical method for the analysis of adrenaline and noradrenaline that proved sufficiently sensitive and specific for permitting accurate quantitative analyses of extracts of animal tissues in general. [The SCP® indicates that this paper has been cited over 975 times since 1961.]

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"Using a colorimetric assay, Hillarp and I had discovered, in 1956, that reserpine treatment resulted in the virtually complete disappearance of catecholamines from the rabbit adrenal medulla. We were eager to find out what happened to the catecholamines in other tissues. At this time, fluorimetry had started to focus general attention owing to its high sensitivity and to the recent development of the spectrophotofluorimeter by Bowman and his colleagues.¹ During a visit in 1955-1956 to the Laboratory of Chemical Pharmacology, National Heart Institute, Bethesda (head B.B. Brodie), I had become acquainted with and enthused by this instrument. Therefore, rather than trying one of the currently used bioassay procedures, we decided to develop a fluorimetric method for our purpose. Ehrlén had shown that adrenaline and noradrenaline can be conveniently converted into strongly fluorescent 'lutines,' but his method could be used only for pharmaceutical purposes.² An isolation technique had to be developed in order to apply the method for tissue extracts. A weak cation exchange resin had previously been used for this purpose but could not be used for tissues other than the adrenal medulla. Essentially, the new thing in our procedure was the introduction of a strong cation exchange resin (Dowex 50) for the column-chromatographic isolation of the catecholamines. When applying Ehrlén's method (somewhat modified) on our eluates, satisfactory results were obtained in terms of tissue blanks, internal standards, recoveries,

and activation and fluorescence spectra. Moreover, the identification of adrenaline and noradrenaline in tissue extracts was secured by quantitative paper chromatography.

"To account for the widespread use of this method two additional points should be emphasized. Firstly, the paper describes a simple apparatus which greatly facilitates the chromatographic procedure. This apparatus consists of an all-glass syringe and a glass reservoir connected to each other and to the cation exchange column via a three-way stopcock. Secondly, the method could be further developed to permit the separation and quantitative analysis of a large number of amines and several of their precursors and metabolites in one single chromatographic procedure. This development has recently been reviewed by Atack.³

"The work reported in our paper was performed at the University of Lund, Sweden, where I held a position as associate professor of pharmacology. My co-authors Åke Bertler and Evald Rosengren were medical students. Their contribution was considerable. They did the main part of the experimental work, together with the technical assistant Carin Larsson, and they took a very active part in planning the work and in the construction of the apparatus. The paper forms part of Rosengren's dissertation in 1960, and both Rosengren's and Bertler's dissertations (in the same year) report results obtained with the new method.^{4,5} At about the same time our paths separated. I became professor of pharmacology at the University of Göteborg. Several years later Bertler moved to Linköping, where he is professor of clinical pharmacology at the Medical School. Rosengren is associate professor of pharmacology at the University of Lund.

"Today our method has several competitors, utilizing not only fluorimetry but also mass fragmentography, isotope techniques based on enzymatic methylations, highpressure liquid chromatography, etc. It is hard to predict how long the method will survive. In our, and presumably several other laboratories, it is still the dominant method for the analysis of catecholamines."

1. **Udenfriend S.** *Fluorescence assay in biology and medicine.* New York: Academic Press. 1962. 505 p.
2. **Ehrlén I.** Fluorimetric determination of adrenaline II. *Farm. Revy* 47:242-50, 1948.
3. **Atack C.** Measurement of biogenic amine using cation exchange chromatography and fluorimetric assay. *Acta Physiol. Scand.* 101 (Suppl. 451):1-99, 1977.
4. **Rosengren E.** Studier över katekolaminer i vävnader. Thesis. Lund, 1960. 21 p.
5. **Bertler Å.** Inverken av reserpin och andra lakemedel på katekolaminomsättningen. Thesis. Lund. 1960. 23 p.