This Week's Citation Classic

Snyder S H, Axelrod J & Zweig M. A sensitive and specific fluorescence assay for tissue serotonin. *Biochem. Pharmacol.* **14**:831-5, 1965.
[Lab. Clinical Science, Natl. Inst. Mental Health, Natl. Insts. Health, Bethesda, MD]

A sensitive and specific method for estimating serotonin in biological materials was developed based on the reaction between serotonin and ninhydrin when the mixture is heated. The resultant fluorescence is eight times more intense than the native fluorescence of serotonin in strong acid. [The SCI® indicates that this paper has been cited in over 520 publications since 1965.]

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June 23, 1983

"My scientific career began as a research associate with Julius Axelrod a year after Richard Wurtman had joined the laboratory. Wurtman and Axelrod had just completed epochal studies establishing melatonin as a hormone of the pineal gland. Axelrod suggested I think about doing some research involving the pineal gland. I was impressed with the report of Wilbur Quay[1] of an extraordinary diurnal rhythm in the serotonin content of the rat pineal gland with levels ten times higher at noon than midnight, clearly the most dramatic biochemical diurnal variation I had ever seen. Quay measured serotonin using the native acid fluorescence assay reported by Udenfriend and colleagues[2] in their pioneering development of the spectrophotofluorometer as a powerful tool for measuring biogenic amines and numerous other body chemicals. Unfortunately, the rat pineal gland weighs only 1 mg. Though its serotonin content is rather high, Quay still required dozens of pineal glands for a single determination making detailed studies of the rhythm almost hopeless.

"About this time I noticed a publication by Vanable[3] showing that when serotonin and ninhydrin are heated together in H_2O solution, an intense fluorescent product results. However, the paper did not indicate whether the reaction was specific for serotonin and no attempts had been made to assess the feasibility of using the fluorescence to assay tissue serotonin.

"Axelrod and I, with the assistance of Mark Zweig, a summer medical student, modified the technique to produce maximal fluorescence and then found that we could extract serotonin from tissues into organic solvents, return the serotonin to an aqueous phase, heat with ninhydrin, and measure the resultant fluorescence. We found that the reaction was quite specific for serotonin and that tissue levels monitored by the ninhydrin technique were essentially the same as those measured by the method of Bogdanski *et al.*[2] Most exciting was that the ninhydrin technique gave fluorescence eight to ten times more intense than that of serotonin in strong acid solution. We could detect serotonin in tissues such as the adrenal gland and heart where it was not apparent by previous methods.

"More importantly, with the ninhydrin technique we could measure serotonin in one or two rat pineal glands. This permitted a series of studies showing that the pineal serotonin rhythm behaves like a biological clock, remaining intact even in complete darkness or in blinded rats. However, exposing the rat to light would abruptly block the nocturnal decline in serotonin levels.[4] We then found that light regulated pineal gland serotonin in neonatal rats directly through the skull even in blinded neonates, suggesting that in the rat, a mammal, the pineal still can function, as it does in reptiles, as a third eye.[5]

"A large number of citations to the paper is no mystery. Any methods paper which describes an improved technique is likely to be well cited. However, no technique remains indispensable for long. Three years after our paper appeared, Roger Maickel and colleagues[6] reported another method for measuring serotonin based on its condensation with o-phthalaldehyde, providing yet a further improvement in the assay of tissue serotonin."

1. **Quay W B.** Circadian rhythm in rat pineal serotonin and its modification by estrous cycle and photoperiod. *Gen. Comp. Endocrinol.* 3:473-9, 1963.
2. **Bogdanski D F, Pletscher A, Brodie B B & Udenfriend S.** Identification and assay of serotonin in brain. *J. Pharmacol. Exp. Ther.* **117**:82-8, 1956. (Cited 1,005 times.)
3. **Vanable J W.** A ninhydrin reaction giving a sensitive quantitative fluorescence assay for 5-hydroxytryptamine. *Anal. Biochem.* 6:393-403, 1963.
4. **Snyder S H, Zweig M, Axelrod J & Fischer J E.** Control of the circadian rhythm in the serotonin content of the rat pineal gland. *Proc. Nat. Acad. Sci. US* **53**:301-5, 1965.
5. **Zweig M, Snyder S H & Axelrod J.** Evidence for a nonretinal pathway of light to the pineal gland of newborn rats. *Proc. Nat. Acad. Sci. US* **56**:515-20, 1966.
6. **Maickel R P, Cox R H, Jr., Saillant J & Miller F P.** A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. *Int. J. Neuropharmacol.* 7:275-82, 1968.
[Citation Classic. *Current Contents/Life Sciences* **24**(27):18, 6 July 1981.]