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This Week's Citation Classic

Shore P A, Burkhalter A & Cohn V H, Jr. A method for the fluorometric assay of histamine in tissues. *J. Pharmacol. Exp. Ther.* **127**:182-6, 1959. [National Heart Institute, National Institutes of Health, Bethesda, MD]

Histamine reacts with o-phthalaldehyde (OPT) in basic solution to form a fluorophore which, on acidification, rearranges to a stable, highly fluorescent product. Extraction of histamine from tissues or body fluid and reaction with OPT allows the estimation of sub-microgram amounts of the biogenic amine. [The *SCI*® indicates that this paper has been cited over 1,345 times since 1961.]

P.A. Shore
Department of Pharmacology
University of Texas
Health Science Center
Dallas, TX 75235

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"In the late 1950s, there was a rapidly expanding interest in the biogenic amines, and methods were being developed for their estimation in tissues. Thanks to the interest and drive of Bernard Brodie, Sidney Udenfriend, and Robert Bowman at the National Heart Institute (NHI), the first working spectrofluorometer was developed and new or improved fluorescent methods were developed for catecholamines and serotonin. I was fortunate to be at the NHI at the time, and thought that a sensitive fluorometric method for histamine would be a boon for better understanding the functions of this still mysterious amine.

"Histamine is not a natural fluorophore, but couples readily with various aldehydes to form a Schiff base product. This was not much help since such a coupling with a fluorescent aldehyde occurs with many amines, and one had also to cope with the presence of the excess fluorescent aldehyde. Scrutiny of the chemical literature revealed that histamine and pyridoxal form an acid stable condensation product,[1] and while this product was not fluorescent, it prompted my colleagues and me to search for a non-fluorescent aldehyde which might condense with histamine to form a fluorophore. We felt we needed to work with aromatic rather than aliphatic aldehydes and tried several, to no avail. After almost giving up, we attempted to obtain o-phthalaldehyde (OPT) for a final effort, but were unsuccessful for some time until we did obtain a small sample of a tarry mess that was reputed to be OPT. After consulting Beilstein we managed to purify it. To our joy, the reaction of OPT with histamine produced an immensely fluorescent product which, although apparently proceeding initially via a Schiff base, rearranged in acid to a stable fluorophore. Working out the details of a method was then simple.

"Subsequent work by us and others showed that OPT reacts with a variety of amines, each under quite specific conditions.[2] At the present time, there are specific conditions for fluorophore formation with histamine, catecholamines, serotonin, and m-hydroxyphenylethyl amines.

"The paper has been cited frequently because it was an innovation in the estimation of this biogenic amine and also because quantitative measurement of histamine release is frequently used in research on immediate hyper-sensitivity reactions. Many adaptations have been made of the initial procedure and, needless to say, highly purified OPT is now available from numerous sources. W. Lovenberg and K. Engel man have recently published a review in this field."[3]

1. **Heyl D, Luz E, Harris S A & Folkers K**. Chemistry of vitamin B_6. VII. Pyridoxylidene- and pyridoxylamines. *J Amer. Chem. Soc* **70**:3669-71, 1948.
2. **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.]
3. **Lovenberg W & Engelman K**. Assay of serotonin, related metabolites, and enrymes (Glick D, ed.) *Analysis of biogenic amines and their related enzymes* New York: Wiley, 1971. p. 1-34