CC/NUMBER 24
JUNE 14, 1993

This Week's Citation Classic®

Beaven M A, Jacobsen S & Horáková Z. Modification of the enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum and urine. *Clin. Chim. Acta* 37:91-103. 1972.
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The paper describes an assay in which histamine was converted to its radiolabeled metabolite, W-[^{14}C]methylhistamine, with histamine-W-methyl transferase and the methyl donor, S-adenosyl-methionine-[^{14}C]methyl ([^{14}C]SAMe). The precision of the assay was increased by inclusion of [p(side chain label)-^{3}H]histamine as an internal standard and unlabeled methylhistamine as a carrier. The utility of the assay was demonstrated for a wide range of animal and human tissues. [The *SCI*® indicates that this paper has been cited in more than 380 publications.]

Histamine Revisited

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While a postdoctoral fellow in the Laboratory of Chemical Pharmacology, the Laboratory Chief, Bernard (Steve) B. Brodie, sparked my interest in histamine and the use of isotopes in research. In those days (1966) histamine was known to be localized in granules of tissue mast cells and blood basophils and to be released by chemicals such as compound 48/80 as well as by antigens. There was, however, a 48/80-resistant pool of histamine in most tissues. My task was to measure and determine the role of this "nonmast cell" histamine, which is now known to be located in 48/80-resistant mucosal mast cells and neurones. The limitations of available fluorimetric assays for histamine were becoming evident but a radioenzymatic assay of histamine[1] held promise for the task at hand. This was one of several assays for biogenic amines, devised by Julie Axelrod and his colleagues, which took advantage of the substrate specificity of methyltransferases and the availability of [^{14}C]SAMeas methyl donortoconvertthe amine to its [^{14}C]methylated product which was then extracted into chloroform, dried, and counted. Small amounts of [^{3}H]histamine were added to correct for incomplete methylation of substrate.

Although the assay appeared feasible, it failed to work; recoveries of the methylhistamine were much too low. I examined in detail each step of the assay, altered conditions to optimize the reaction and minimize extraction of extraneous ^{14}C-containing material, substituted our own side chain-labeled [^{3}H]histamine[2] for [ring labeled ^{3}H]histamine because of problems with the ring label, and prepared a year's supply of histamine- W-methyl transferase from 300guinea-pig brains. The recoveries were still too low. Some months later it dawned on me that the small quantities of radioactive product might be lost in the final extraction/evaporation step. The fix was simple—addition of excess, newly synthesized unlabeled methylhistamine before extraction. Over the course of several years, I and my two colleagues gained extensive experience with the assay and we decided to convey this experience by including detailed commentaries for each step in the final publication. We continued to refine the assay and use it and other isotopic assays[2] in clinical studies which indicated a role for histamine in some forms of urticaria and the ectopic production of histamine-metabolizing enzymes in certain tumors (see citations in reference 3). These refinements, including the discovery that kidneys from three rats provided sufficient enzyme for one year, were incorporated in an updated version of the assay that was now sufficiently sensitive to measure histamine directly in 10 µl plasma.[4]

As the focus of our work shifted to studies of mechanisms of histamine release in vitro, we initially used the assay,[5,6] but have since turned to simpler alternatives (e.g., measurement of hexosaminidase release) for monitoring degranulation of mast cells. So the incentive for further refinement passed to others as discussed in a recent review.[7] The reason for the high citation of this and the update[4] was, I suspect, because the assay was needed. In fact both enzyme and [^{14}C]SAMe are now sold as a kit by New England Nuclear Corp. Also, the detailed commentaries in the paper ensured that others could adapt the assay for their own needs.

1.. **Snyder S H, Baldessarini R J & Axelrod J.** A sensitive and specific enzymatic isotopic assay for tissue histamine. *J. Pharmacol. Exp Ther.* 153:544-9, 1966. (Cited 210 times.)
2. **Beaven M A & Jacobsen S**. A new assay for histaminase activity: measurement of tritiated water from β(side chain label)-^{3}H-histamine. *J. Pharmacol Exp. Ther.* 176:52-64. 1971.
3. **Beaven M A.** Histamine: its role in physiological and pathological processes. *Mcmogr. Allergy* 13:1-113, 1978.
4. **Shaff R E & Beaven M A.** Increased sensitivity of the enzymatic isotopic assay of histamine: measurement of histamine in plasma and serum. *Anal. Biochem.* 94:425-30. 1979. (Cited 325 times.)
5. **Beaven M A, Moore J P, Smith G A, Hesketh T R & Metcalfe J C**. The calcium signal and phosphatidylinositol breakdown in 2H3 cells. *J. Biol. Chem.* 259:7137-42. 1984. (Cited 180 times.)
6. **Beaven M A, Rogers J, Moore J P. Hesketh T R, Smith G A & Metcalfe J** C. The mechanism of the calcium signal and correlation with histamine release in 2H3 cells. *J. Biol. Chem.* 259:7129-36. 1984. (Cited 190 times.)
7. **Beaven M A.** Current techniques of histamine determination: radioenzymatic assays of biological fluids.
(Uvnas B, ed.) *Handbook of experimental pharmacology . Volume 97 Hislamine and hislamine antagonists.*
Berlin, Germany: Springer-Verlag, 1991. p. 39-48 .
Received February 24. 1993