CC/NUMBER 50
DECEMBER 13, 1982

This Week's Citation Classic

Thompson W J & Appleman M M. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry—USA* **10**:311-16, 1971.
[Depts. Biological Sciences and Biochemistry, Univ. Southern California, Los Angeles, CA]

Physical and kinetic criteria defined the multiplicity of cyclic nucleotide phosphodiesterase(s), the enzyme system solely responsible for cyclic AMP and cyclic GMP catabolism. A theoretical basis for the anomalous kinetic behavior of the enzyme was derived and a new assay procedure described in this paper. [The SCI® indicates that this paper has been cited in over 700 publications since 1971.]

W. Joseph Thompson
Department of Pharmacology
University of Texas Medical School
Houston, TX 77025

September 20, 1982

"As a graduate student in the late-1960s interested in the hormonal regulation of carbohydrate or lipid metabolism, I was led inexorably to cAMP (this despite the fact that one of my thesis advisers swore that cAMP was an artifact). Ultimately, this translated in the laboratory to having to watch Dowex columns drip. I was okay with big columns, but little Pasteur pipettes or glass columns of Dowex resin dripping seemed like the world's biggest waste of time and drove me up the wall. I was struggling with my columns in Mike Appleman's laboratory at the University of Southern California in order to use ^{3}H-cAMP (just then commercially available) as a substrate for PDE as a way to increase the sensitivity of the published procedure. This work was based on that of Bob Kemp and Mike published a year earlier.[1] Their studies also prompted Gary Brooker to develop an isotope dilution assay for tissue cAMP[2] during his graduate studies with Mike. Gary's cAMP assay was never used for much because the radioimmunoassay for cAMP came out about the same time. However, his work provided us with the basis for a PDE assay because the success of the isotope dilution assay required the existence of high affinity PDE. Mercifully, that assay required no columns since the exchange resin quenches bound tritium which allowed the whole thing to be done in a scintillation vial or centrifuged and an aliquot measured. It is ironic that several years later we discovered Dow had changed its resin processing procedure such that too much nonspecific reaction product binding occurred and we ultimately had to go back to little columns again. This time we designed a vacuum approach for a hundred columns so separation on the little monsters only takes about two minutes and is thus tolerable.[3]

"It is sort of remarkable to me that this assay is still being used by so many labs 11 years after it was published. I think the paper is cited because the method is cheap, easy to do, and works. It is also cited as a reference to the initial characterization of the multiple forms of the enzyme and the logic of why a mixture of two separate catalytic sites of varying affinity may display apparent negative cooperativity. I was surprised to learn that only 25 percent of the *Citation Classics* are methods papers.[4] This paper does not fit that category solely, but its method component is certainly a major cause of its popularity. I plead guilty to standing in the right place at the right time.

"I suggest to graduate students that in addition to dedication and hard work, anticipation of where fields are going is a useful practical attribute for a scientist.

"I believe that this paper is successful because my thesis adviser, Mike Appleman, is a good scientist for which there is no substitute for any graduate student. Its significance is that we are able to study hormonally regulated PDE forms, but it represents only a small step in understanding hormone regulatory mechanisms and their pharmacological or genetic control. As for honors, what better for such an egotistical lot as we than to be recognized and mimicked by our colleagues?"

1. **Appleman M M & Kemp R G.** Puromycin: a potent metabolic effect independent of protein synthesis. *Biochem. Biophys. Res. Commun.* **24**:564-8, 1966.
2. **Brooker G, Thomas L J, Jr. & Appleman M M.** The assay of adenosine 3',5'-cyclic monophosphate and guanosine 3',5'-cyclic monophosphate in biological materials by enzymatic radioisotopic displacement. *Biochemistry—USA* **7**:4177-81, 1968.
3. **Thompson W J, Terasaki W L, Epstein P M & Strada S J.** Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. *Advan. Cyclic Nucl. Res.* **10**:69-92, 1979.
4. **Garfield E.** *Citation Classics*—four years of the human side of science. *Current Contents* (22):5-16, 1 June 1981.