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Citation Classics

Ellman G L, Courtney K D, Andres V & Featherstone R M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology* 7:88-95, 1961.

A spectrophotometric method for determining acetylcholinesterase activity of tissue extracts, homogenates, cell suspensions, etc., has been described. The activity is measured by following the increase of yellow color produced when the thio anion produced by the enzymatic hydrolysis of the substrate (acetylthiocholine) reacts with DTNB. The method was used to study the activity of human erythrocytes and homogenates of rat brain, kidney, lungs, liver, and muscle tissue. [The *SCI*® indicates that this paper was cited 995 times in the period 1961-1975.]

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"The germ of the idea for using the sulfur analogue of acteylcholine (first used by George Koelle in his famed histochemical procedure) and the sulfhydryl reagent which I invented when I was working at Dow Chemical Company actually occurred many months before I tried it. It was late one Friday afternoon when everyone had gone home and I was alone in the lab that I tried it out. I added the necessary substrate, enzyme, and DTNB to the spectrophotometer while the recorder was going, and, lo and behold, the color began to appear in a fantastically linear way that is the delight of every biochemist's heart. Additions of enzyme and substrate seemed to follow the usual laws of enzymology; all of this I managed to do in about an hour and a half.

"At this point, I went into Robert Featherstone's office with the recordings and said, 'Look here, I can measure cholinesterase.' I was, of course, somewhat dumbfounded to note that his expression was not one of particular interest, but more along the lines of 'Well, that's nice. So, how do you know it is cholinesterase?' He said, 'Is it inhibited by physostigmine?' 'Well I suppose it would be,' I said. 'Have you got any around?' We went to the stock room and got some and went back to the lab and set up the reaction again and put in the physostigmine in the middle of the run and, sure enough, the reaction stopped in about twenty seconds; then Robert Featherstone got all excited. I guess that just proves that the things which make a biochemist happy are a little different than those which make a pharmacologist happy.

"The subsequent work was put together shortly and was sent off to a journal. Some months passed and, much to our chagrin, we received a rejection slip. The referees had many questions which we had not answered in our paper. Then began the serious consideration of whether we should bother with all of those puzzles, when, in fact, we knew it worked.

"Bob Featherstone felt there were things we could do that would make the paper publishable and pressured the people in my lab and myself to go ahead with it. And so we did. Thanks to Diane Courtney and Val Andres, the necessary details of getting a paper published were accomplished. This included such things as studying the activity of various tissues, determining kinetic constants for the red cell enzyme, substrate inhibition, curves, and data on the inhibitory constants from a variety of known cholinesterase inhibitors.

"It is, as Dr. Lowry has pointed out in his Citation Classic commentary of January 3, 1977, not always a great contribution to produce a method, and yet it is, of course, the essence of science upon which all good things are based. So one need not feel too chagrined that a method is produced which is adaptable and has been used by many people. In fact, one of the nicest things is that my technician comes in and says, 'It is still working, just like it was 15 years ago.' It gives one a nice feeling, frankly."