

This Week's Citation Classic

CC/NUMBER 3
JANUARY 19, 1981

Murphy B E P. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocrinol. Metab.* 27:973-90, 1967. [Dept. Invest. Med., McGill Univ., and Clin. Invest. Unit, Queen Mary Vet. Hosp., Montreal, Canada]

This article extended and refined the methodology employed in our original competitive protein-binding assay for plasma corticoids,¹ increasing the sensitivity 100-fold. It showed how the basic method could be applied to the measurement of a number of different steroids in plasma, urine, and cerebrospinal fluid. [The SC[®] indicates that this paper has been cited over 1,690 times since 1967.]

Beverley E. Pearson Murphy
Montreal General Hospital
Montreal H3G 1A4
Canada

October 17, 1980

"This paper was an outgrowth of my PhD thesis.² The original assay developed accidentally during the final year of my postgraduate training in internal medicine (July 1961-June 1962) as a research fellow under Chauncey Pattee. Since my predecessor, Richard Gillies, had found low 17-hydroxycorticoid levels in a few patients with advanced cirrhosis of the liver and had suggested these might be due to low levels of transcortin, I decided to embark on a study of protein-binding in plasma of cirrhotic subjects. To gain some experience with the technique of dialysis, I repeated a study by W.R. Slaunwhite and A. A. Sandberg in 1959 in which they had looked at the fall in cortisol binding with increasing levels of cortisol in diluted plasma. Plotted as unbound cortisol vs. cortisol added, the results resembled a 'standard curve' for cortisol and our technical assistant William Engelberg and I considered the possibility that this relationship might indeed be used to measure cortisol. Fortunately our knowledge of steroid chemistry was too limited to be discouraging and with the help and blessing of Pattee we had an assay working within a few weeks. In June

1962, I presented my work – my first scientific presentation – to the Quebec Society of Clinical Chemists. Though rejected after four months by *Endocrinology*, the manuscript was accepted promptly by its sister journal. Impressed by the similarity of our assay to those employing antibodies (later radioimmunoassays) I also wrote a general paper suggesting that many proteins, even intracellular ones, could probably be used in this fashion.³ We had already applied the technique to thyroxine.

"From 1963 to 1966 I looked at ways to streamline and extend our assays, and these efforts for steroids culminated in the paper cited. These included the use of various species of transcortin and the investigation of different adsorbents to separate bound and unbound steroid moieties—initially done by trying out every particulate reagent on the shelf from Lloyd's reagent to charcoal, measured out with a cocktail spoon. Sensitivity was also increased by substituting tritium for ¹⁴C as a tracer. With the resulting techniques it was easy to develop methods for a number of different steroids and a large mass of data soon accumulated. Since it was all related I attempted to combine it into one long paper—which took months to review. The reviewers could not agree as to its acceptability and a third reviewer was involved. All the reviewers had many suggestions for revision and all in all it took well over a year to appear in print. This delay was a source of mild embarrassment since some colleagues to whom I had given the methods pre-publication published earlier.

"I suppose the fact that this paper describes a whole group of new methods accounts for its popularity. It intrigues me that, when I do see it quoted, the quoters rarely specify just which method they are referring to, so that one is left to guess. I suspect that it is quoted much more often than read."

1. **Murphy B E P, Engelberg W & Pattee C J.** A simple method for the determination of plasma corticoids. *J. Clin. Endocrinol. Metab.* 23:293-300, 1963.
2. **Murphy B E. P.** Some aspects of the protein-binding of corticosteroids and thyroxine in human blood. Thesis, McGill University. 1964. 205 p.
3. The application of the property of protein-binding to the assay of minute quantities of hormones and other substances. *Nature* 201:679-82, 1964.