CC/NUMBER 21
MAY 21, 1984

This Week's Citation Classic ™

Murphy B E P & Pattee C J. Determination of thyroxine utilizing the property of protein-binding. *J. Clin. Endocrinol. Metab.* **24**:187-96, 1964.
[Clin. Invest. Unit, Queen Mary Veterans' Hosp., and Dept. Investigative Med.. McGill Univ., Montreal, Canada]

This article described the first practical assay for serum thyroxine (T4), with data demonstrating its diagnostic validity in hyper- and hypothyroid patients and its freedom from the effects of iodine [The *SCI*® indicates that this paper has been cited in over 545 publications since 1964]

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

April 20, 1984

"The work in this paper formed part of my PhD thesis[1] done under the supervision of Chauncey Pattee. Since the gestation of this paper paralleled that of my second child, conception of both occurring in the fall of 1962, my memories of them remain associated.

"I had recently (June 1962) presented a paper describing a new method for the determination of serum corticoids based on their binding to human corticosteroid-binding globulin[2,3] and I had a hunch that a similar technique would be applicable to the specific determination of thyroxine since a comparable sort of protein –thyroxine-binding globulin (TBG)– existed for thyroxine.

"In the fall of 1962, I began doing preliminary experiments–I obtained thyroxine and its analogues and then attempted to determine thyroxine in precisely the same way used for serum corticoids. The necessary steps were to precipitate the proteins to free the hormone, then to measure it according to its competition with ^{131}I-thyroxine for sites on a fixed amount of TBG. The second step involved the separation of the protein-bound and unbound fractions; for this I used Sephadex gel filtration, a method we had recently substituted for dialysis in the determination of corticoids. Development of the thyroxine method was a matter of working backward, i.e., first establishing the separation technique, then getting a satisfactory standard curve, and finally measuring the hormone in serum.

"The initial experiments went well, with the setting-up of 12 small Sephadex columns which successfully separated the bound and free hormone. However, the standard curve was unsatisfactory, not behaving at all as I had expected, and quite useless for assay purposes. One day in January 1963, feeling ill with a cold, nauseated due to my pregnancy, and discouraged with my results, I decided to go home to bed. On my way out, I picked up Antoniades's *Hormones in Human Plasma* to read. Between trips to the bathroom, I made my way through the chapter on thyroid hormones by Ingbar and Freinkel[4] and suddenly realized what was wrong. I had neglected to consider the effects of prealbumin, another, but weaker, T4 binder in blood. My reading that afternoon also provided the remedy –simply alter the buffer from phosphate to barbital. The following day, I obtained an excellent standard curve and from then on all went smoothly (with both method and pregnancy). Ably assisted by technician Sorel Cohen, we rapidly accumulated data and in March we were able to submit an abstract; in May, a manuscript. In June, I presented our findings in London, Ontario. Three weeks later, two professors from Ottawa visited the laboratory to learn the technique and invited me to lunch (dining out is one of Montreal's delights). Declining reluctantly, I instead made my way over to the maternity hospital and reported back to the lab a little later that it was a girl. During that summer, we revised the paper, which was accepted in October.

"This paper owes its popularity to describing the first validated method for the specific determination of thyroxine, demonstrating a rapid means of diagnosing with considerable accuracy the two common diseases, hypo- and hyperthyroidism.

"Currently, serum thyroxine is often determined by radioimmunoassay, a technique which is similar in principle but replaces TBG with an antibody to thyroxine."[5]

1. **Murphy B E P**. *Some aspects of the protein-binding of corticosteroids and thyroxine in human blood.* PhD thesis. McGill University. 1964. 205 p.
2. ---------- Some sludies of.lhe 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. Endocrnol Metab* 27:973-90, 1967.
3. ----------Citation Classic. Commentary on *J. Clin. Endocrinol. Metab.* 27:973-90, 1967.. *Current Contents/Life Sciences* **24**(3):17. 19 January 1981.
4. **Ingbar S H & Freinkel N**. Thyroid hormones. (Anloniades H N. ed.) *Hormones in human plasma.* Boston: Little, Brown. 1960. p. 515-74.
5. **Reieloff S.** Thyroid function tests. (De Groot LH. Cahill G F. Odell W D. Martini L. Potts J T . Jr.. Nelson D. H. Steinberger E & Winegrad A I. eds.) E*ndocrinology.* New York: Grune and Stratton. 1979. Vol. 1 p.387-428.