

This Week's Citation Classic®

Chamness G C & McGuire W L. Scatchard plots—common errors in correction and interpretation. *Steroids* 26:538-42, 1975.

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This paper discussed some common errors in the calculation of binding affinities and numbers of binding sites using the ubiquitous Scatchard plot. It offered a simple method of correcting for "nonspecific" binding sites of much lower affinity. It also showed how too high a concentration of unlabeled competitor can produce erroneous values of nonspecific binding and how the stabilization of a protein by its ligand can lead to a plot that looks like cooperative binding. [The SCI® indicates that this paper has been cited in more than 445 publications.]

Interpreting Ligand Binding Data

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When I became a postdoctoral fellow with Bill L. McGuire, the use of estrogen receptor assays to predict the response of breast tumors to hormone therapy was still being established.¹ I helped on some improvements in the receptor assay, which then most commonly involved adding several concentrations of ³H-estradiol to a tumor cytosol, removing unbound ligand with dextran-coated charcoal, counting the remaining bound ligand, and analyzing the results with a Scatchard plot. At the same time, I had the privilege (though I probably didn't think it was a "privilege" at the time) of reviewing for McGuire many of the manuscripts on this subject that various journals sent him to referee.

Eventually, I must have complained about how often people "calculated" their Scatchard plots simply by drawing a line through the steeper part, which can give a very significant error when lower affinity binding components are present, resulting in their papers being sent back for revision or even rejected. The next thing I knew, McGuire had called Al

Segaloff, who was then editor of *Steroids*, for which many of our reviews had been prepared, and together they decided that we should write an editorial comment on the subject. The intention was not to break new ground but rather to help people like ourselves avoid a common mistake. I was at first reluctant, since there were already much more sophisticated analyses available by far more qualified experts like David Rodbard.² But McGuire convinced me that a sophisticated approach was not what we needed, and together we set out to draft a commentary that was brief and straightforward.

In addition to pointing out the original problem and offering an easily applied correction for the simplest case of nonspecific binding, the final editorial illustrated two other common difficulties: the error that can arise if too much competing ligand is used to determine "nonspecific" binding, and the way that receptor instability, in the absence of ligand, can sometimes yield a Scatchard plot that looks very much like cooperative binding.

None of this material was really new, as we had admitted in the text itself, and all of it has since been treated more extensively in various ways [e.g., references 3-6]. Nevertheless, people apparently found its appearance in simple terms, in one place, to be useful. Certainly this little editorial demonstrates again that a relatively small methodological contribution, that is nevertheless usable by people outside one's own microspecialty, may receive more attention in the literature than more weighty but specialized contributions. Perhaps, too, our final sentence appealed to a goal we all share as scientists: "We have therefore felt it worthwhile to discuss these problems, in hopes of avoiding errors and speeding publication of otherwise useful and significant work."

[We regret to report Dr. McGuire's untimely death on March 25, 1992. He will be greatly missed, both personally and professionally.]

1. McGuire W L, Carbone P P, Sears M E & Escher G C. Estrogen receptors in human breast cancer: an overview. (McGuire W L, Carbone P P & Vollmer E P. eds.) *Estrogen receptors in human breast cancer*. New York: Raven Press, 1975. p. 1-7. (Cited 450 times.)

2. Rodbard D & Feldman H A. Theory of protein-ligand interaction. *Meth. Enzymology* 36:3-16, 1975.

3. Norby J G, Ottolenghi P & Jensen J. Scatchard plots: common misinterpretation of binding experiments. *Anal. Biochem.* 102:318-20, 1980.

4. Goldenberg K & Carroll R E. Estrogen receptor in breast cancer: a Scatchard analysis of plasma protein interference. *J. Steroid Biochem.* 13:1265-8, 1980.

5. Rodbard D, Munson P J & Thakur A K. Quantitative characterization of hormone receptors. *Cancer* 46:2907-18, 1980.

6. Morgan D D & Grossman C J. Analysis and properties of the cytosolic estrogen receptor from rat thymus. *Endocrine Res.* 10:193-207, 1984.

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