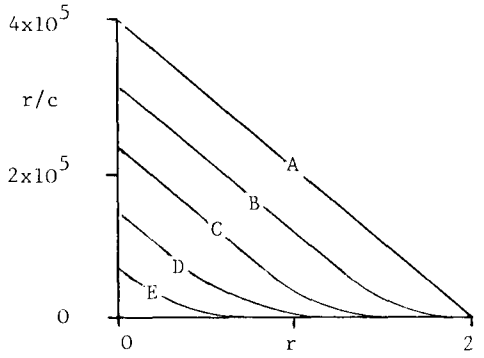
ON REPRESENTATION AND INTERPRETATION OF DRUG DISPLACEMENT INTERACTIONS

L.J. Aarons, W.L. Schary, M. Rowland, Department of Pharmacy, University of Manchester, M13 9PL

Plots such as the Scatchard plot and the double reciprocal plot, enjoy wide use in the analysis of drug-protein binding. The reason for this is that they give a convenient graphical representation of the data and, if linear, aid in interpretation. There is a potential for misinterpretation when these transformations are used to analyse drug displacement interactions.

As an example consider the case of a one drug - one site interaction which gives rise to a linear Scatchard plot, as shown in the figure (A). However, when a second drug is present which competes for the same binding site the resultant plots are hyperbolic (B,C,D,E) and interpretation becomes more difficult.



Scatchard plots for competitive binding.

The appropriate Scatchard parameters used were:-

$n_A = n_B = 2$

$K_A = K_B = 2 \times 10^5 M^{-1}$

protein concentration = $5.8 \times 10^{-4} M$

A. Drug A alone
B. Drug A + 2.5×10^{-3}M drug B
C. Drug A + 5.0×10^{-3}M drug B
D. Drug A + 7.5×10^{-3}M drug B
E. Drug A + 1.0×10^{-3}M drug B

Curvature occurs because, although the total concentration of the displacing drug is constant within an experiment, its unbound concentration varies being a complex function of both its total concentration and the total concentration of the original drug.

In practice the situation is vastly more complicated than depicted in this simple example in that both competitive and non-competitive interactions together with the possibility of multiple binding sites must be catered for. We have tried to accommodate these complexities in a method based upon multiple stepwise equilibria. In this method the interaction between protein P, and the two drugs, A and B, is described by stepwise equilibria of the form:-

$$A + P \underset{}{\overset{K_1}{\rightleftharpoons}} AP \qquad B + BP \overset{K_4}{\rightleftharpoons} B_2P$$

$$B + P \overset{K_2}{\rightleftharpoons} BP \qquad B + AP \overset{K_5}{\rightleftharpoons} BAP$$

$$A + AP \overset{K_3}{\rightleftharpoons} A_2P \qquad A + BP \overset{K_6}{\rightleftharpoons} ABP$$

etc.

where K_i is the i^{th} equilibrium constant. The resulting binding isotherm is a function of both the total and unbound concentrations of the two drugs, as well as the protein concentration and consequently in any experiment all these quantities have to be measured. A convenient method of representing the binding interaction is in the form of a three-dimensional plot of the unbound concentration of drug A, for example, versus the total concentration of drug A and the total concentration of drug B. The protein concentration would add a fourth dimension but in most situations it remains relatively constant. It is stressed that this approach is one of representation and not of interpretation, in that no significance is attached to the model adopted or to the parameters.