Limitations of the 'Direct Linear Plot' in Evaluation of Drug-Protein Binding Parameters

N. KOLASSA and K. TURNHEIM

Department of Pharmacology, University of Vienna, Währingerstrasse 13a, A-1090 Wien IX (Austria),
18 February 1976.

Summary. The applicability of the 'direct linear plot' is compared with that of the 'Scatchard plot' for the estimation of protein binding parameters. Only, if one class of binding sites exists in the system tested, binding parameters may be estimated by use of the 'direct linear plot'. On the other hand the 'SCATCHARD plot' also provides estimates in systems with more that one class of binding sites.

Non-cooperative drug-protein binding may be described in analogy to the law of mass action[1,2] by the equation

$$r = \sum_i \frac{n_i K_i c}{1 + K_i c} \qquad (1)$$

where r denotes that fraction of available binding sites on a protein molecule occupied by the drug, i.e. moles of drug bound per mole of binding protein; n_i represents the number of binding sites in the i'th class with the intrinsic association constant K_i, and c is the concentration of unbound drug.

A plot of r/c as a function of r (SCATCHARD[3]) allows the estimation of Σn_i and $\Sigma n_i K_i$ by extrapolation to the x- and y-axis. The individual parameters of each class of binding sites can be obtained by graphic or algebraic procedures[4-6]. Since, in practice, the amount of free and bound drug is measured with some inaccuracy, the 'SCATCHARD plot' heavily weights experimental points at low drug concentration and leads to biased parameter estimates, unless a great number of observations is used. PERRIN et al.[7] described a statistically unbiased method of estimating binding constants by means of a computer program, which allows for the inclusion of appropriate weights compensating for varying precision in the data. While this procedure necessitates a great deal of calculating facilities, a graphical analysis providing unbiased estimates of the binding parameters would be helpful at least for preliminary evaluations.

EISENTHAL and CORNISH-BOWDEN[8] introduced the 'direct linear plot' as a new graphical method for the unbiased estimation of enzyme kinetic parameters. It was also recommended for application to binding experiments, since the analysis of binding data is also derived from mass-law considerations like the equilibrium treatment of enzyme kinetics. The 'direct linear plot' transforms equation (1) – assuming one class of binding sites – into the form:

$$\frac{n}{r} - \frac{1/K}{c} = 1 \qquad (2)$$

which is of the general form:

$$\frac{x}{a} + \frac{y}{b} = 1 \qquad (3)$$

i.e. the equation of a straight line in xy-space with intercept a on the x-axis and b on the y-axis. Correspondingly equation (2) represents a straight line for each observation in the parameter space with the observed values of r as intercepts on the n-axis and $-c$ on the $1/K$-axis. The co-ordinates of the point where the lines intersect provide the only values of n and $1/K$ that satisfy equation (2) for every observation. Because of the experimental error in measuring r and c, there are $^1/_2\ m\ (m\text{-}1)$ intersections for

[1] A. GOLDSTEIN, Pharmac. Rev. *1*, 102 (1949).
[2] M. C. MEYER and D. E. GUTTMAN, J. Pharm. Sci. *57*, 895 (1968).
[3] G. SCATCHARD, Ann. N.Y. Acad. Sci. *51*, 660 (1949).
[4] H. E. HART, Bull. Math. Biophys. *27*, 87 (1965).
[5] H. E. ROSENTHAL, Analyt. Biochem. *20*, 525 (1967).
[6] I. M. KLOTZ and D. L. HUNSTON, Biochemistry *10*, 3065 (1971).
[7] J. H. PERRIN, J. J. VALLNER and S. WOLD, Biochim. biophys. Acta *371*, 482 (1974).
[8] R. EISENTHAL and A. CORNISH-BOWDEN, Biochem. J. *139*, 715 (1974).

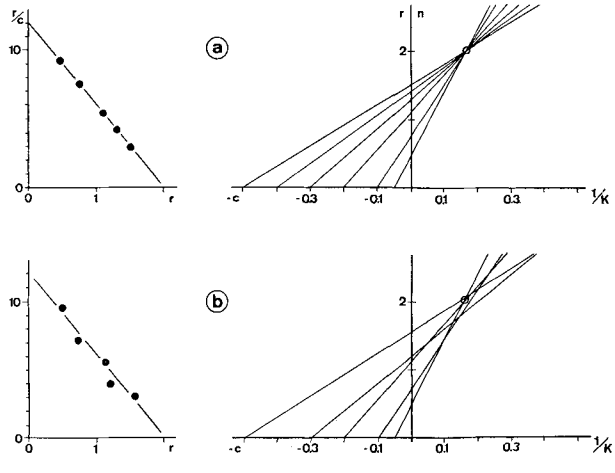

Fig. 1. Graphical analysis – 'SCATCHARD plot' (left side) and 'direct linear plot' (right side) – of drug-protein binding by one class of binding sites. Values (●) ate assumed without (a) or with (b) experimental error in the measurement of r; the medians of the intersections in the 'direct linear plot' are marked by empty circles (○).

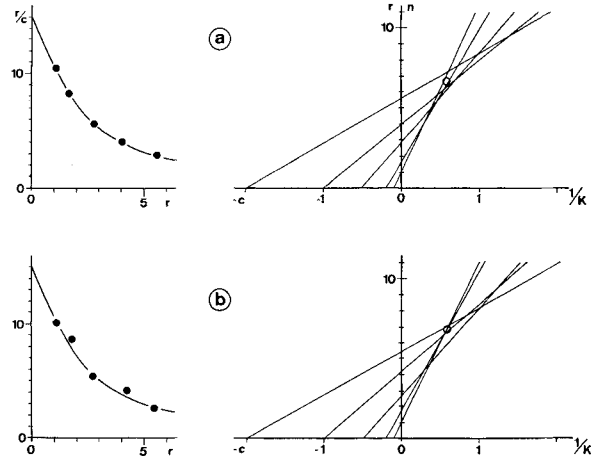

Fig. 2. Graphical analysis – 'SCATCHARD plot' (left side) and 'direct linear plot' (right side) – of drug-protein binding by two classes of binding sites. Values (●) are assumed without (a) or with (b) experimental error in the measurement of r; the medians of the intersections in the 'direct linear plot' are marked by empty circles (○).

m observations. The best estimates for n and $1/K$ are the medians of the individual intersection co-ordinates, since the median as the non-parametric analogue of the arithmetic mean does not require accurate knowledge of the appropriate weights of the experimental deviations from the expectation value[9].

The graphical analysis of binding data by the 'direct linear plot' is compared with the 'SCATCHARD plot': In Figure 1 a single binding class with $n_1 = 2$ and $K_1 = 6$ was assumed. Data without variance due to experimental error (Figure 1a) lie on a straight line in the 'SCATCHARD plot' and give lines with only one common intersection in the 'direct linear plot'. When the observations are subject to error, for instance in the measurement of r (Figure 1b), the points deviate from the straight line in the 'SCATCHARD plot', whereas the 'direct linear plot' yields different intersections for each pair of observation lines and the median of the individual intersection co-ordinates is adopted as an unbiased estimate for the binding parameters.

The assumption of a second class of binding sites ($n_2 = 10$, $K_2 = 0.3$) and data without experimental error (Figure 2a) leads to a curvilinear regression line in the 'SCATCHARD plot' and to a scatter of the intersections in the 'direct linear plot'. The co-ordinates of the intersection median (6.7 for n and 0.58 for $1/K$) are somewhere between the values of n_1 and n_2 and of $1/K_1$ and $1/K_2$, respectively. The individual binding parameters cannot be derived from the intersection co-ordinates, since the binding equation (1) for two classes of binding sites cannot be transformed into the general form (3).

In addition to the disadvantage that the binding parameters of more than one class of binding sites cannot be estimated from the 'direct linear plot', this procedure does not even provide information whether the experimental data may be described by a model consisting of only one or more binding classes, when data with the usual expermimental variance are used (Figure 2b). The scatter of intersections in the case of one class of binding sites (Figure 1b) and in that of two classes of binding sites (Figure 2b) is similar and the two situations cannot be distinguished from each other, while the 'SCATCHARD plot' displays clearly by non-linearity the deviation from the simple case of only one binding class in a non-cooperative system.

Therefore, the 'direct linear plot' may be useful for estimation of binding parameters in the limited number of cases, where only one class of binding sites exists, but it is not suitable to decide whether the scatter of intersections is brought about by poor precision of data or by existence of more than one class of binding sites. In contrast, the 'SCATCHARD plot' differentiates between drug binding to only one and that to more than one class of binding sites and permits the estimation of binding parameters in both cases.

[9] A. CORNISH-BOWDEN and R. EISENTHAL, Biochem. J. *139*, 721 (1974).

IN MEMORIAM

Leopold Ruzicka

(1887–1976)

One of the co-founders and long-time editor of Experientia, the Nobel Prize winner for Chemistry 1939, Professor Dr. Leopold Ruzicka, died on the 26th of September in his 90th year by the Lake of Constance. Inspired by the Paracelsus Medal which he received, Ruzicka gave our journal its name 'Scientia est Experientia'. 'Assumptions in science which extend beyond the field of actual experience are deceptive', he wrote 30 years ago to the redactor in one of his incisive reviews. Leopold Ruzicka propagated, with great decisiveness and passion, a world-wide and supranational treatment of science, and thus formed very definitely the basic concept of Experientia. H.M.