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Effect of Initial Conditions and Drug–Protein Binding on the Time to Equilibrium in Dialysis Systems

Keyphrases □ Drug–protein binding—effect of initial conditions on the time to equilibrium in dialysis systems □ Equilibrium dialysis—effect of initial conditions and drug–protein binding

To The Editor:

A major disadvantage of using equilibrium dialysis methods for determining *in vitro* plasma protein binding of drugs is the time needed to reach equilibrium. Recently, Øie and Guentert (1) mathematically showed that equilibration is more rapid when the drug is initially added to the plasma side as opposed to the buffer side. The approach to equilibrium was described by integrated equations for the concentration of drug on the buffer side as a function of time. The relative time to equilibrium for the two initial configurations was found to be:

$$R = \frac{t_B}{t_P} = \frac{\ln \delta + \ln \alpha}{\ln \delta} = 1 + \frac{\ln \alpha}{\ln \delta} \qquad \text{(Eq. 1)}$$

where t_B and t_P are the times to reach some fraction (δ) from the equilibrium concentration when the drug is initially added to the buffer or plasma side, respectively, and α is the unbound fraction in plasma which is assumed to be constant during the dialysis. For example, a δ value of 0.05 indicates a deviation of 5% from the true equilibrium value.

The authors indicated that the closer to the true equilibrium value one wants to be, the closer the ratio is to unity. Furthermore, a smaller α (stronger binding) or a larger δ increases the advantage of spiking the plasma side. While we are in complete agreement with these conclusions, some interesting and practical information may be lost if the examination of this system is limited only to the ratio of the times to equilibrium. In this communication the concept of approach to equilibrium in dialysis systems will be further developed and factors affecting comparative equilibration times will be discussed.

When drug is initially added to the plasma side, the concentration on the buffer side (C_B) at any time (t) is:

$$C_B = \frac{C_0\alpha}{1+\alpha}(1 - e^{-K_T(1+\alpha)t}) \qquad \text{(Eq. 2)}$$

where C_0 is the initial concentration, and K_T is the rate constant governing the transfer of drug across the membrane (1).

When drug is initially placed in the buffer side, a similar equation is obtained:

$$C_B = \frac{C_0}{1+\alpha}(\alpha + e^{-K_T(1+\alpha)t}) \qquad \text{(Eq. 3)}$$

Although the actual concentration on the buffer side is the variable of interest, a more useful relationship for examining the influence of α on equilibrium times would be an expression of C_B in relative terms. A fraction away from the equilibrium concentration in the buffer side (δ) is defined as:

$$\delta = \frac{\text{Absolute Value } (C_B^\infty - C_B)}{C_B^\infty} \qquad \text{(Eq. 4)}$$

where C_B^∞ is the concentration of C_B as $t \to \infty$ and $C_B^\infty = C_0\alpha/(1+\alpha)$ for both cases (buffer or plasma spiked).

In a form analogous to Eq. 2, the time course of this fraction when drug is initially on the plasma side is then described by:

$$\delta_P = e^{-K_T(1+\alpha)t} \qquad \text{(Eq. 5)}$$

When drug is initially placed on the buffer side, the time course of δ is described by:

$$\delta_B = \frac{e^{-K_T(1+\alpha)t}}{\alpha} \qquad \text{(Eq. 6)}$$

A hypothetical semilog plot for the time course of these fractions from equilibrium concentration is shown in Fig. 1. As dictated by Eqs. 5 and 6, the δ values decline exponentially with time. For a given value of α, $\ln \delta_P$ and $\ln \delta_B$ decrease at the same rate with slope $= -K_T(1+\alpha)$. As $\alpha \to 0$, the slopes become $-K_T$ and as $\alpha \to 1$, the slopes become $-2K_T$. At $t = 0$, $\delta_P = 1$, whereas the $\delta_B = 1/\alpha$. Thus, δ_P values are always ≤ 1 whereas δ_B has no limit. Although the actual rate constants for approach to equilibrium are the same for both buffer and plasma spiked systems, the buffer spiked system requires more time to reach comparable δ values, because a greater amount of drug must be

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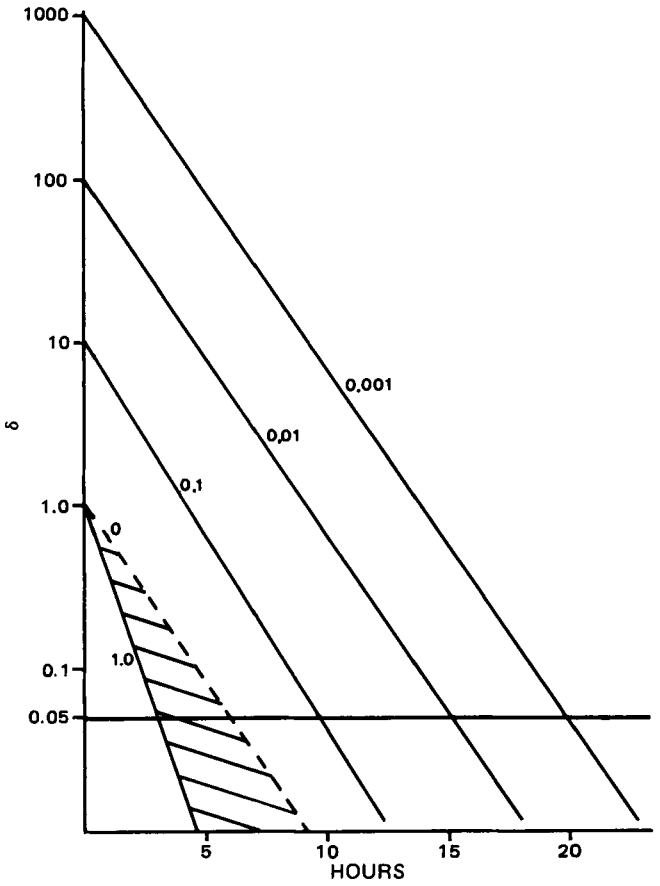

Figure 1—*The time course of the fraction away from equilibrium buffer concentration (δ) when* $K_T = 0.5\ hr^{-1}$. *The α values are given on the continuous lines representing* δ_B. *The dashed line is* δ_P *as* $\alpha \to 0$. *All plasma spiked systems fall within the shaded area bounded by the α = 1.0 (*$\delta_B = \delta_P$*) and dashed lines. The horizontal line at δ = 0.05 indicates a 5% deviation from the true equilibrium value for all conditions.*

transported across the membrane. For example, 99% of the initial mass present in a buffer spiked system ($\alpha = 0.01$) must cross the membrane to reach equilibrium. In a comparable plasma spiked system, only 1% of the initial mass must diffuse to the buffer side at equilibrium.

These comparisons can be shown more clearly by considering the times required to reach a given δ level. Rearrangement of Eq. 5 for the plasma spiked system gives:

$$t_p = \frac{-\ln \delta_P}{K_T(1 + \alpha)} \qquad \text{(Eq. 7)}$$

Thus, as α becomes smaller, t_p reaches a limiting value [$\lim_{\alpha \to 0} t_P = -(\ln \delta_P / K_T)$].

Conversely, when the buffer is spiked, the value of t_B is written as:

$$t_B = \frac{-(\ln \delta + \ln \alpha)}{K_T(1 + \alpha)} \qquad \text{(Eq. 8)}$$

Instead of reaching a limiting value as α decreases, t_B continues to increase in proportion to the negative logarithm of α ($\lim_{\alpha \to 0} t_B = \infty$).

Table I compares the time to reach concentrations 5 and 1% from the true equilibrium concentration. These levels were chosen since they are similar to differences which would be experimentally acceptable. The practical advantage of initially placing drug in the plasma side is obvious throughout the range of α, but becomes most striking below $\alpha \simeq 0.05$. Although t_P becomes essentially constant, t_B continues to increase with decreasing α. If only the ratio of t_B/t_P were used, the important observation of an essentially constant equilibration time (t_P) below $\alpha \simeq 0.05$ would be overlooked. Table I also illustrates a second point brought out by Øie and Guentert (1): the closer to the true equilibrium value one wishes to be, the closer the ratio is to unity. Thus, at $\delta = 0.05$ spiking of plasma would be 78% faster, whereas at $\delta = 0.01$, the advantage is 51%, assuming a constant α value of 0.1.

Table 1—Influence of Initial Conditions and Fraction Unbound in Plasma (α) on the Time to Reach a Fraction (δ) away from Equilibrium Concentration [a]

	α	t_B	t_P	t_B/t_P
$\delta = 0.05$				
	1.0	3.0	3.0	1.00
	0.5	4.9	4.0	1.22
	0.1	9.6	5.4	1.78
	0.05	11.4	5.7	2.00
	0.01	15.0	5.9	2.54
	0.005	16.5	6.0	2.75
	0.001	19.8	6.0	3.30
$\delta = 0.01$				
	1.0	4.6	4.6	1.00
	0.5	7.1	6.1	1.15
	0.1	12.6	8.4	1.51
	0.05	14.5	8.8	1.65
	0.01	18.2	9.1	2.00
	0.005	19.7	9.2	2.15
	0.001	23.0	9.2	2.50

[a] $K_T = 0.5\ hr^{-1}$.

Most clinically important drug–protein interactions occur with compounds having unbound fractions (α) of ≤0.2. Experimentally, the time to equilibrium is usually established (and fixed) in preliminary studies using plasma obtained from healthy subjects, and it is rarely re-examined, even if the extent of binding varies in subsequent samples. To avoid potentially serious errors in binding data, the following points should be considered when conducting equilibrium dialysis experiments:

1. Preliminary studies should include establishment of the equilibration time in a buffer *versus* buffer system. If the logarithm of δ is plotted *versus* time (Eq. 5 or 6 with $\alpha = 1$), the slope = $2K_T$. Changes in the membrane, drug, cell volume, or agitation method may require that K_T be redetermined. With a value for K_T and an estimate for α in plasma for a particular drug, the time to equilibrium and time difference between plasma- and buffer-spiked configurations can be estimated.

2. The time to equilibrium when spiking buffer increases with decreasing α values, whereas when spiking plasma it is essentially independent of α. The maximum equilibration time in a plasma spiked system should not be more than twice that of a buffer *versus* buffer system. No such maximum exists when drug is initially added to the buffer side (Table I).

3. Unbound (buffer) drug concentrations or fraction unbound should be used to monitor the approach to equilibrium, since total (plasma) concentrations and the fraction bound are relatively insensitive parameters at low α values.

4. If there are no significant advantages in sample preparation, the drug should be added on the plasma side. This minimizes equilibration time and reduces potential

problems such as drug degradation, microbiological growth (2), protein dilution (3), and lipolysis (4).

5. When spiking the buffer side is desirable, establish the time to equilibrium for the system with the smallest expected α value. The smallest α values do not always occur with healthy adult plasma. For example, the interaction of cationic drugs with α_1-acid glycoprotein increased in certain disease states and under various stress conditions (5, 6). When spiking plasma under these conditions, the apparent α value would be smaller than the true equilibrium value, whereas the opposite would occur when spiking the buffer side.

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BOOKS

REVIEWS

The Peptides. Analysis, Synthesis, Biology. Vol. 4. Edited by ERHARD GROSS and JOHANNES MEIENHOFFER. Academic, 111 Fifth Ave., New York, NY 10017. 1981. 309 pp. 15 × 23 cm.

The first three volumes of *The Peptides* were devoted to the methodology concerning the synthesis of peptides. The fourth volume is the first of several volumes planned, according to the editors, dealing with the analytical aspects of peptides. The fourth volume eminently succeeds in reaching the high standards set for it by its predecessors.

The six chapters are divided evenly among the physical and chemical methods for peptide–protein-structure determination. Of those concerned with the physical methods, the first two focus on the crystal structure analysis by X-ray studies. In the first chapter, I. L. Karle discusses the crystal structures of linear and cyclic peptides containing 2 to 15 peptide units. Several useful generalizations are mentioned; for example, "4 → 1 H-bonds begin to appear in cyclic hexapeptides" and "the possibility for several different conformations assumed by the same compound arises starting with the cyclic heptapeptides." The author has made liberal use of tables and figures, which also list pertinent references.

J. Gunning and T. Blundell present in Chapter 2 a crystal structure analysis of the larger peptide hormones. The crystal structures of insulin (A-chain, 21 residues; B-chain, 30 residues), glucagon (29 residues), and the pancreatic polypeptide (36 residues) have been determined. On the basis of the known homology with the amino acid sequence of insulin, the structures of proinsulin and relaxin have been proposed and are discussed.

The chiroptical method for the determination of the absolute configuration of α-amino acids and small peptides is the topic of Chapter 3 by V. Toome and M. Weigle. The chiroptical properties of both the free α-amino acids and of the free oligopeptides, as well as of their metal complexes and chromophoric derivatives, are discussed.

In the fourth chapter, S. Stein describes the technique of peptide and protein-analysis at the picomole level employing HPLC and fluorescence spectrophotometry. The combination of HPLC and fluorescence detection raises the possibility of determination of peptides and proteins in tissues and organs of individual animals. This combination of techniques has also been employed for the determination of the amino acid sequence.

Chapter 5, by J. R. Benson, P. C. Louie, and R. A. Bradshaw, deals with the single-column amino acid analysis of peptides. For the purpose of discussion, the authors have divided the amino acids into four categories according to whether they are (*a*) normally found in proteins, (*b*) formed *in vivo* from the first group by post- or cotranslation, (*c*) formed by chemical modification from Group 1, or (*d*) nonprotein amino acids. Several protocols are given for the separation of these amino acids.

R. A. Laursen in Chapter 6 probes in exquisite detail the solid-phase sequencing technique, which would help overcome problems (such as overlap, increased-background, amino-terminal blocking) experienced with the Edman method.

Both the editors and the authors are to be congratulated for the excellence of this volume, which is a must for those concerned with any and all aspects of proteins and peptides, and for those contemplating a start in this area of research.

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Medicinal Chemistry VI (Proceedings of the 6th International Symposium on Medicinal Chemistry). Edited by M. A. SIMKINS. Wiley, 605 Third Ave., New York, NY 10016. 1979. 477 pp. 16 × 24 cm. Price $94.00.

Medicinal Chemistry VI is a collection of papers presented at the 6th Internation Symposium on Medicinal Chemistry held in Brighton, England in 1978. They were chosen for this volume by the members of the Society for Drug Research and cover a wide range of interests, many being a blend of chemistry, biology, biochemistry, and medicine. The majority of the papers are based on disease states while others discuss theoretical concepts relating to substrate–receptor interactions or predicting activity of molecules based solely on structure.

This volume is divided into plenary lectures and symposium papers. The plenary lectures are given by Dr. Linus Pauling, who speaks of "orthomolecular medicine," a new concept in treating diseases, which he defines as the achievement and preservation of the best of health and the prevention and treatment of disease by using substances (right molecules in the right amounts) that are normally present in the body; professor Sir John Cornforth provides the reader with a greater awareness of