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Errors in Estimating the Unbound Fraction of Drugs Due to the Volume Shift in Equilibrium Dialysis

Keyphrases □ Equilibrium dialysis—volume shift, unbound fraction of drug □ Unbound fraction of drugs—equilibrium dialysis, volume shift

To the Editor:

Equilibrium dialysis is commonly used to estimate serum protein binding of drugs. Consideration of the influence of the volume shift on the unbound fraction has, however, not been addressed until recently (1). The water flux from the buffer side to the serum side during dialysis causes binding protein dilution as well as an overestimation of the unbound fraction. The overestimation is dependent on the extent of the volume shift, the unbound fraction of drugs, and the concentration dependency of binding. Correction for the volume shift is important when the volume shift is substantial and when the unbound fraction of drugs is small.

The molarity of macromolecules in undiluted serum sample is ~1 m*M*, which gives 0.025 atm (263 mm H_2O) of osmotic pressure at 37°. The pressure causes water to migrate from the buffer side to the serum side (1) and expands the dialysis membrane. Because the serum sample is not completely restrained in the dialysis cells and the dialysis membrane, the hydrostatic pressure due to the volume shift is always less than the osmotic pressure. Osmotic equilibrium is actually never reached in this type of equilibrium dialysis. The extent of the volume shift depends on the time used for dialysis. Tozer *et al.* (1) reported an average volume shift of 31% in 16-hr dialysis. Using the same type of dialysis cells and dialysis membrane, we experienced an average volume shift of 10% in 4–6 hr of dialysis. Undue water flux can be avoided by a judicious choice of equilibration time.

Assuming that binding follows the law of mass action, the unbound fraction (f_u) of a drug that has multiple binding sites on a serum binding protein can be expressed as follows:

$$f_u = 1 \Big/ \left[1 + \mathrm{Pt} \sum_{i=1}^{n} 1/(C_u + K\mathrm{d}_i)\right] \qquad \text{(Eq. 1)}$$

where $K\mathrm{d}_i$ is the dissociation constant for binding site i, Pt is the total concentration of binding sites, and C_u is the measured unbound drug concentration. The extent of the volume shift can be defined as the ratio of serum volume before (V_s) and after ($V_{s'}$) dialysis and expressed as:

$$\mathrm{F} = V_s/V_{s'} = \mathrm{Pt}'/\mathrm{Pt} \qquad \text{(Eq. 2)}$$

where Pt′ is the concentration of binding sites after dialysis. In assessing the importance of the volume shift correction, the unbound fractions, with and without water flux correction, need to be compared. Assuming the unbound concentration to be the same with and without a water flux, the unbound fraction without correction for volume shift ($f_{u'}$) is related to the unbound fraction with volume shift correction by:

$$f_{u'} = f_u/[\mathrm{F} \cdot (1 - f_u) + f_u] \qquad \text{(Eq. 3)}$$

or

$$f_u = f_{u'} \cdot \mathrm{F}/(f_{u'} \cdot \mathrm{F} + 1 - f_{u'}) \qquad \text{(Eq. 4)}$$

(See *Appendix* for derivation.) Neglecting the volume shift, the fractional error [$\mathrm{E} = (f_{u'} - f_u)/f_u$] in calculating the unbound fraction is:

$$\mathrm{E} = (1 - \mathrm{F}) \cdot (1 - f_u)/[\mathrm{F} \cdot (1 - f_u) + f_u] \qquad \text{(Eq. 5)}$$

or

$$\mathrm{E} = (1 - \mathrm{F}) \cdot (1 - f_{u'})/\mathrm{F} \qquad \text{(Eq. 6)}$$

It is apparent from Eqs. 5 and 6 that when the volume shift is <10% (F > 0.9), the error introduced in neglecting volume shift is <11%, which is not critical in comparison with other errors in the protein binding determination. However, if the volume shift is >10% and the unbound fraction calculated without the volume shift correction is <0.9, the volume shift should always be considered in calculating the unbound fraction. Equation 4 can be used for the volume shift correction provided that the binding is not concentration dependent in the measured concentration range.

When equilibrium dialysis is used to determine the unbound fraction of a drug with concentration-dependent binding, the transfer of drug from the serum side to the buffer side causes a decrease in the drug concentration on the serum side with a subsequent decrease in the unbound fraction of the drug (1, 2). The complicated correction

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method suggested by Tozer *et al.*, which corrects for both the volume shift and concentration-dependent binding, becomes necessary in calculating unbound fraction of a concentration-dependent binding drug such as prednisolone (1).

Although Eqs. 3 and 4 were derived under the assumption that drugs bind to a single-binding protein with multiple binding sites, the equations can be used as a good approximation to the correct unbound fractions for drugs that bind to two or more different binding proteins. For example, for a drug with two classes of binding sites, one having high capacity (600 μM) but low affinity (Kd = 100 μM), and the other having low capacity (20 μM) but high affinity (Kd = 1 μM), shows an unbound fraction of 0.04 at 0.1 μM drug concentration (Eq. 3A). A 30% volume shift gives a 40% error in unbound fraction ($f_{u'}$ = 0.056, Eq. 4A). Equation 4 can be used to convert $f_{u'}$ to f_u with good accuracy (f_u calculated = 0.04).

The extent of volume shift is usually determined by measuring the sample volume before and after equilibrium dialysis. Practically, it is not easy to determine the sample volume accurately after dialysis. It would be advisable instead to measure the binding protein concentration before and after dialysis and apply for correction calculations.

APPENDIX

The unbound fraction of a drug is by definition:

$$f_u = C_u \Big/ \left(C_u + \sum_{i=1}^{n} Cb_i\right) \qquad \text{(Eq. 1A)}$$

where $\sum_{i=1}^{n} Cb_i$ is the sum of concentrations of drugs bound to different binding sites. Based on the law of mass action, Cb_i can be expressed as:

$$Cb_i = C_u \cdot Pt_i/(Kd_i + C_u) \qquad \text{(Eq. 2A)}$$

and Eq. 1A can be written as:

$$f_u = 1 \Big/ \left[1 + \sum_{i=1}^{n} Pt_i/(Kd_i + C_u)\right] \qquad \text{(Eq. 3A)}$$

and

$$f_{u'} = 1 \Big/ \left[1 + \sum_{i=1}^{n} Pt_{i'}/(Kd_i + C_u)\right] \qquad \text{(Eq. 4A)}$$

Assuming a single binding protein with multiple binding sites, Eq. 3A can be simplified to be Eq. 1 and $f_{u'}$ is equal to:

$$f_{u'} = 1 \Big/ \left[1 + Pt' \sum_{i=1}^{n} 1/(C_u + Kd_i)\right] \qquad \text{(Eq. 5A)}$$

Letting

$$S = \sum_{i=1}^{n} 1/(C_u + Kd_i) \qquad \text{(Eq. 6A)}$$

Eq. 1 can be rearranged to:

$$S = (1 - f_u)/(f_u \cdot Pt) \qquad \text{(Eq. 7A)}$$

Substituting Eq. 7A into Eq. 5A, gives:

$$f_{u'} = 1/[1 + Pt' \cdot (1 - f_u)/(f_u \cdot Pt)] \qquad \text{(Eq. 8A)}$$

where Pt'/Pt is equal to F (Eq. 2). Substituting F into Eq. 8A gives Eq. 3. Similarly, Eq. 5A can be rearranged to:

$$S = (1 - f_{u'})/(f_{u'} \cdot Pt') \qquad \text{(Eq. 9A)}$$

Substituting Eqs. 2 and 9A into Eq. 1, Eq. 4 is derived.

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Rate of Recovery from Fazadinium: Relationship to the Rate of Decline of its Plasma Concentration

Keyphrases □ Fazadinium—rate of recovery, relationship to plasma concentration, pharmacokinetics □ Pharmacokinetics—fazadinium, rate of recovery, relationship to plasma concentration

To the Editor:

Fazadinium bromide, introduced into anesthetic practice in 1972, is of clinical interest as a short-acting neuromuscular blocking agent. An approach is presented here which strongly suggests that the differences in the rate of recovery from the neuromuscular blocking effects of fazadinium are solely dependent on the pharmacokinetics of the relaxant. This approach is not new in that it was first presented on theoretical grounds more than a decade ago and utilized with recovery data for succinylcholine in both neonates and adults (1, 2).

If the claim (3) that fazadinium is eliminated by apparent first-order kinetics is true, and if it can be assumed that its metabolite(s) are inactive (4), then the duration (t) of the neuromuscular blocking action of fazadinium and the rate of decline (R) of the effect (paralysis) in the linear (20–80% or 25–75%) range can be related according to the following equations, as derived for succinylcholine (1, 2):

$$t = (2.3/k_{10})(\log A^0 - \log A_{min}) \qquad \text{(Eq. 1)}$$

$$R = m(k_{10}/2.3) \qquad \text{(Eq. 2)}$$

Table I—Pharmacokinetic Analysis of Recovery from the Neuromuscular Blocking Effects of Fazadinium [a]

Patient	Duration (t)[b], min	Rate of Decline (R)[c], % min^{-1}	$t \times R$, %	$k_{app\ 25\text{–}75}$[d], min^{-1}
3	14	3.57	49.98	−0.0382
4	22	2.27	49.94	−0.0286
5	24	2.08	49.92	−0.0210
1	26	1.92	49.92	−0.0251
2	27	1.85	49.95	−0.0219
6	34	1.47	49.98	−0.0149

[a] Based on data from ref. 3. [b] Time interval when the twitch height was depressed between 25 and 75% of its control value: between 75 and 25% muscle paralysis. [c] Rate of recovery in the 75–25% paralysis range. [d] $k_{app\ 25\text{–}75} = (\log C_{25} - \log C_{75}/t_{25} - t_{75})$ where C and t are the plasma concentrations and times respectively at 25 and 75% effect levels.

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