# Book Review

**Targeting of Drugs 2, Optimization Strategies,** NATO ASI Series, Series A: Life Sciences, Vol. 199, Edited by Gregory Gregoriadis, Anthony C. Allison, and George Poste. Plenum Press, New York, 1990 vii + 185 pp. ISBN 0-306-43739-2.

This well-written text comprises the proceedings of the 5th NATO ASI "Targeting of Drugs: Optimization Strategies" held in Cape Sounion, Greece, between June 24 and July 5, 1989.

In keeping up with its great tradition, this volume in this outstanding series provides excellent coverage of a wide range of topics where significant advances in targeting of drugs have been made in recent years. As stated in the Preface, the text especially deals with "strategies by which milieu interference curtailing the function of drug carriers is circumvented or removed." The book comprises 16 chapters by 38 contributors who are well-qualified individuals from academia and the pharmaceutical industry.

The topics covered in reviews and research papers are diverse and include chapters on the design of site-specific therapeutic systems, modeling of cell membrane targeting, various ways of targeting liposomes, drug delivery to the brain, and polymeric delivery systems. Each of the 16 chapters is well introduced and the reader is carefully guided through a substantial amount of literature. In each case the reference is current through 1989, with some 1990 references included, and draws on work from the authors' laboratory and other laboratories. The papers on modeling of cell membrane targeting, antitumor effects of Six Ricin A-chain, tissue-specific serum opsonins, phagocytosis of liposomes, and stabilization of lipid microstructure are well organized and thoughtfully presented, with relevant references and a concise conclusion, thus providing the reader with a sense of future direction in the subject areas. Similarly the papers on targeting of liposomes, therapeutic systems, enhancement of hormone activity by monoclonal antibodies, drug delivery—industrial view, drug delivery to brain, and polymeric drug delivery systems are well written, with pertinent references. However, most of these topics have been extensively reviewed elsewhere.

I found this to be an excellent book which will be of interest to both students and research workers in several disciplines including pharmaceutics, pharmacology and experimental medicine and would be a welcome addition to libraries of colleges of pharmacy, the pharmaceutical industry, and organizations interested in targeted drug delivery.

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# Letters to the Editor

## The Influence of Molecular Volume and Hydrogen-Bonding on Peptide Transport Across Epithelial Membranes

Recent studies (1–3) conclude that peptide transport across Caco-2 cell monolayers is dictated primarily by the number of hydrogen bonds ($N_H$) which the peptide can form in aqueous solution. We suggest that this interpretation should also include the molecular volume (MV) in a model which is generally consistent with transcellular transport through diverse biomembranes.

We consider the permeability coefficient ($P$) through lipid lamellae in terms of the solutes's physicochemical properties (4,5),

$$P = K \cdot D/\delta \quad (1)$$

where $K$ is the membrane–water partition coefficient, $D$ is the diffusion coefficient in the membrane, and $\delta$ is the diffusion pathlength. The diffusivity is related to MV,

$$D = D_o \cdot \exp(-\beta \cdot MV) \quad (2)$$

where the constant $\beta$ is inversely proportional to the average free-volume available for diffusion, and $D_o$ is the diffusivity of a molecule with vanishingly small MV (6).

$K$ can be estimated (7) from the solute's octanol–water partition coefficient ($K_{oct}$):

$$\log K = \alpha \cdot \log K_{oct} \quad (3)$$

where $\alpha$ is another constant. Combining these equations gives

$$\log P = \alpha \cdot \log K_{oct} - (\beta/2.3) \cdot MV + \log D_o/\delta \quad (4)$$

It has also been demonstrated that $K$ is a function of the solute's MV and hydrogen-bond donor/acceptor activity (8) and can be related to the solute's MV and $N_H$ by

$$\log K = a_1 \cdot MV + a_2 \cdot N_H \quad (5)$$

where all hydrogen bonds are considered equivalent. Combining Eqs. (1), (2), and (5) gives $P$ in terms of MV and $N_H$,

$$\log P = (a_1 - \beta/2.3) \cdot MV + a_2 \cdot N_H + \log D_o/\delta \quad (6)$$

Conradi and co-workers (1–3) analyzed the permeability of a series of peptide analogues and found no correlation ($r^2 = 0.03$) between $\log P$ and $\log K_{oct}$ for compounds I–VI



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(numbering from Ref. 1) whose partition coefficients were known. This analysis, however, assumes a constant $D$ for all peptides, regardless of MV. If however, the same data are analyzed using Eq. (4), the regression is excellent ($r^2 = 0.87$, $f = 10.3$). Moreover, the regression coefficient for MV is negative, consistent with $D$ being inversely related to MV [Eq. (2)]. Thus, a simple lipid-based model, which recognizes both partitioning- and volume-dependent transport, accounts for 87% of the variation in the data.

It is also argued that $N_H$ affects $P$ (1–3). Regression of the log$P$ values on MV and $N_H$ for neutral peptides IV–IX (from Ref. 1) using Eq. (6) produces a very good fit ($r^2 = 0.98, f = 57.9$). However, there is strong cross-correlation between MV and $N_H$ ($r = 0.93$) for these peptides. As a consequence, simple regression of log$P$ on $N_H$ ($r^2 = 0.97, f = 147$) or log$P$ upon MV ($r^2 = 0.81, f = 16.9$) also provides reasonable correlations. Thus, while $N_H$ is indeed an important determinant of $P$, this deduction was reached using data where MV and $N_H$ are highly correlated.

Burton *et al.* published similar results for peptides I–X (from Ref. 3). The correlation between log$P$ and log$K_{oct}$ for seven neutral peptides (IV–X) (Table I, Model 1) is poor. However, log$P$ is reasonably correlated with $N_H$ (Table I, Model 3); the addition of a MV term significantly improves the fit (Table 1, Model 4). Interestingly, MV and $N_H$ are weakly correlated ($r = 0.28$) for this series of compounds. These results imply, therefore, that the peptide permeability data are well described by a model encompassing $N_H$ and MV-dependent transport.

The regressions (Table I) demonstrate that $P$ decreases with increasing $N_H$, due to the energy required to desolvate the peptide as it transfers from aqueous solution into the lipid bilayer's hydrocarbon interior. The regression coefficients for MV have different signs for the analyses based upon Eqs. (4) and (6). In Eq. (4) (Table I, Model 2), the coefficient is negative, consistent with decreased $D$ as MV increases [Eq. (2)] [a phenomenon common to a number of lipid membranes (4–6)]. In Eq. (6) (Table I, Model 4), though, the MV term contains both partitioning ($a_1$) and diffusive ($\beta$) dependencies and is positive, reflecting increased partitioning with increasing MV (i.e., $a_1 > |\beta| > 0$) (7).

Table I. Analysis of Data from Burton *et al.* (3), Peptides IV–X: MV Values Were Determined by the Method of Bondi (9)

| Model | Regression coefficient (SE) | $r^{2a}$ | $f^b$ |
|---|---|---|---|
| 1 | $\alpha = 0.12\ (0.22)$<br>$C_1 = -5.78\ (0.19)$ | 0.06 | 0.3 |
| 2 | $\alpha = 2.8\ (1.5)$<br>$\beta/2.3 = -3.93\ (2.15) \times 10^{-2}$<br>$C_2 = -0.76\ (2.60)$ | 0.49 | 1.9 |
| 3 | $a_2 = -0.34\ (0.05)$<br>$C_3 = -2.98\ (0.52)$ | 0.80 | 20.0 |
| 4 | $a_2 = -0.39\ (0.03)$<br>$(a_1 - \beta/2.3) = 3.26\ (0.50) \times 10^{-2}$<br>$C_4 = -3.52\ (0.19)$ | 0.98 | 113 |

Model 1: $\log P = \alpha \cdot \log K_{oct} + C_1$

Model 2: $\log P = \alpha \cdot \log K_{oct} + \beta' \cdot MV + C_2$ (4)

Model 3: $\log P = a_2 \cdot N_H + C_3$

Model 4: $\log P = a_2 \cdot N_H + (a_1 - \beta') \cdot MV + C_4$ (6)

[a] $r$, correlation coefficient of the regression.
[b] $f$ ratio for the regression.

In summary, we have considered peptide permeation to be dependent upon solubility in, and diffusion through, a lipophilic environment. In contrast, transport via an aqueous pore must be independent of partitioning and $N_H$. These results suggest, therefore, that the route of peptide transport through Caco-2 monolayers is rate limited by lipid lamellae. A similar conclusion, based upon permeability measurements for nonpeptide drugs (4,5), has been reached for other membranes.

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# Reply to the Comments by Drs. Potts and Guy

Linear regression models, which apparently account for the interplay of physicochemical factors responsible for membrane transport of drugs, are most often empirically derived and oversimplified. However, because of this simplicity and reasonable statistical fit to the data at hand, they are attractive. Outliers are, more often than not, incorporated within the statistical error and therefore are dismissed even though they may provide important clues or basic insights into more precise physicochemical interactions. As more pa-

rameters are included in the models, statistical fits will improve. However, this does not make the model correct from a fundamental point of view. In the course of our research in delineating the rate-determining steps and factors involved in the transepithelial diffusion of model peptides, including selected nonpeptides, we have deliberately attempted to move beyond empirical relationships.

On the other hand, by means of such empirical regression techniques, Potts and Guy maintain that we have overlooked the important contribution of molecular volume to transport in our work. Using our results, they propose an alternate model which seems to better fit the data if a volume term is included. We, of course, agree that molecular volume is a determinant of diffusion in general, and have stated such in the past. However, by specifically designing a series of peptides in which chain length (and volume) was kept essentially constant, we found, instead, that the contribution of volume to permeability in our studies to be minimal.

In developing their model, Potts and Guy find an apparently excellent fit of the data for our peptides I–IV (their Ref. 1), to Eq. (4). It should be pointed out that three of these peptides are zwitterionic at physiological pH and cross Caco-2 cell monolayers via the paracellular pathway, i.e., restricted pore diffusion within an electrostatic field of force (1). Thus, they have arrived at a conclusion unsupported by the experimental results.

Continuing, model 4 is derived by adding a molecular volume term to our hydrogen bond numbers. We find this to be a questionable elaboration of a very imprecise parameter in order to develop a quantitative model. As was pointed out several times, assigning unit hydrogen bond numbers to the functional groups present in these solutes makes the naive assumption that all hydrogen bonds are energetically equivalent. Thus both the amide NH and the carbonyl are assigned hydrogen bond numbers of one. In reality, it is well appreciated that these groups do not form equivalent bonds. Of the 6 kcal/mol present in the amide, 4 is associated with the NH and 2 with the carbonyl (2). To attempt to attach any more quantitative significance to this simple bookkeeping method is, we feel, unjustified. The purpose of the exercise was simply to demonstrate the concept that desolvation is an important determinant of transport for peptides.

We have in fact recently addressed the problem of nonequivalent hydrogen bond energies in our earlier studies. The partition coefficient difference method ($\Delta\log PC$) has been shown to approximate the actual desolvation potential of a solute incorporating different polar functional groups (3). It is also sensitive to steric and proximity considerations, which the hydrogen bond number method is not. Using octanol and isooctane as the reference phases, we showed an excellent correlation between Caco-2 cell permeability and $\Delta\log PC$ ($r^2 = 0.95$) for these peptides (4). This relationship is particularly significant in the present discussion since the $\Delta\log PC$ parameter has also been shown to have essentially no molecular volume dependence (5).

Clearly, this last result shows that transport of these peptides across Caco-2 cell monolayers is overwhelmingly dominated by hydrogen-bonding interactions. These findings are consistent with a model of transport which involves movement of the solute through a series of microdomains, any one of which can be rate limiting. In the present case, the barrier to transport of these peptides is movement from the membrane interfacial region to the membrane interior (4). Since it is expected that a substantial hydrophobic component will have already been largely satisfied at the interface, this will not be a major driving force for transfer to the interior. However, since the peptide can remain fully solvated at the interface (2), the desolvation of functional groups will be the principal factor involved in this step.

In conclusion, it seems clear that in attempting to correct our simplistic correlations of transport with hydrogen bond number by adding other parameters, Potts and Guy are actually defending a different simplistic dogma which misrepresents the interactions of highly functionalized solutes with real cell membranes. It is these deviations from expected behavior that lead to new insights into the mechanisms of these processes. Consequently, we believe that attempts to incorporate all transport data into a single comprehensive model which assumes a common rate-controlling step will ultimately prove incorrect.

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