



The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and examples

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Received 17 September 1999; Accepted 3 October 1999

Summary. In this review, we first examine the contextual background of structure–pharmacokinetic relationships. Some concepts in drug disposition are briefly recalled, and inherent difficulties in structure–pharmacokinetic relationships are outlined. Lipophilicity is then investigated in the light of the intermolecular and intramolecular interactions it encodes. In the main body of the review, a number of pharmacokinetic processes are examined for their relations with lipophilicity. These processes are taken in a logical sequence of permeation, absorption (intestine, skin, cornea, brain), plasma protein binding, tissue distribution, volume of distribution and renal clearance. Relations between metabolism and lipophilicity are more complex, since biotransformation involves both low-energy (enzyme binding) and high-energy (catalysis) processes. Only the former may be related to lipophilicity. The conclusion argues against faulty statistics and over-interpretation.

Key words: adipose tissue storage, blood–brain barrier permeation, gastrointestinal absorption, lipophilicity, metabolism, permeation, plasma protein binding, renal clearance

Abbreviations: 3D-QSAR: three-dimensional QSAR; AAG: α_1 -acid glycoprotein, orosomucoid; alk: alkane; ASI: adipose storage index; BBB: blood–brain barrier; CL_R : renal clearance; CNS: central nervous system; CoMFA: comparative molecular field analysis; cyc: cyclohexane; CYP: cytochrome P450; dce: 1,2-dichloroethane; f_u : unbound fraction in plasma; GI: gastrointestinal; H_B : total H-bonding capacity; IAM: immobilized artificial membranes; hep: heptane; K_a : association constant; k_{cat} : turnover number; K_m : Michaelis constant; K_s : spectral dissociation constant; $\log D_{oct}^{7.4}$: log of octanol/water distribution coefficient at pH 7.4; $\log P$: log of partition coefficient; MW: molecular weight; oct: octanol; PC: permeability coefficient; (Q)SARs: (quantitative) structure–activity relationships; (Q)SMRs: (quantitative) structure–metabolism relationships; (Q)SPkRs: (quantitative) structure–pharmacokinetic relationships; RP-HPLC: reversed-phase HPLC; V_{max} : maximal velocity; V_{ss} : volume of distribution at steady state.

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Background

Pharmacodynamic and pharmacokinetic responses

A daily challenge faced by medicinal chemists is that of uncovering and applying quantitative structure–activity relationships (QSARs). During decades, biological activity was equated almost exclusively with a *pharmacodynamic response*, i.e. the effects elicited in a biological system by a drug or any other xenobiotic. These effects include, among others, perturbation of membrane function, activation (agonism) or blockade (antagonism) of receptors, inhibition or induction of enzymes, binding to nucleic acids, stimulation or blockade of a functional biological response, and all manifestations of toxicity.

Progressively, a paradigm shift has taken place such that the notion of biological response has been expanded to include also the *pharmacokinetic response*, i.e. all effects and influences a biological system exerts on a xenobiotic [1,2]. For many years, these effects have remained a lesser explored territory of drug design. Recently however, the new armamentarium of drug discovery (combinatorial chemistry, high-throughput screening, molecular modeling, rational drug design, etc.) has shifted the bottleneck of drug research from hit and lead discovery to lead optimization, and more specifically to pharmacokinetic lead optimization.

As a result of this evolution, medicinal chemists now find themselves trying to relate molecular structure to the various components of the pharmacokinetic response, just to discover that what the body does to a drug can be as varied and complex as what a drug does to the body:

- At the *molecular level*, the pharmacokinetic response includes (a) low-energy processes such as membrane permeation, reversible binding to circulating and tissular macromolecules, and uptake by transporters, and (b) high-energy processes involving the cleavage and formation of covalent bonds, in other words biotransformation (metabolism) to form reactive or stable metabolites.
- At the *organismic level*, the pharmacokinetic response comprises all processes of drug disposition, namely absorption, distribution, storage, metabolism (chemical elimination) and excretion (physical elimination) [3,4]. In this review, we focus on some of the important low-energy processes of drug disposition, giving only limited attention to biotransformation.

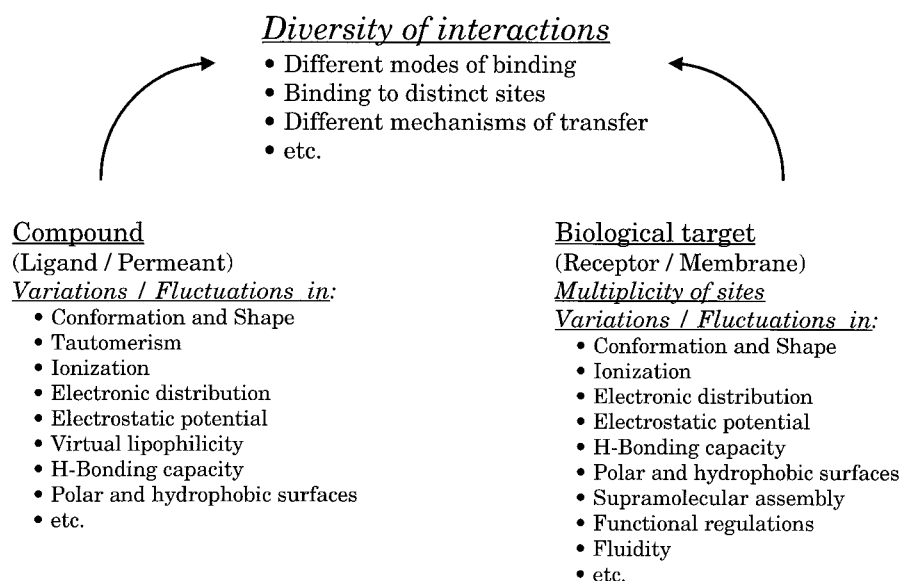


Figure 1. Ambiguities in structure–pharmacokinetic relationships due to (a) the multiplicity of molecular states in the drug, (b) fluctuations of the biological site, and (c) the diversity of their interactions (adapted from Reference 13).

Inherent difficulties in deriving structure–pharmacokinetic relationships

Relating the various (and interconnected) pharmacokinetic processes to molecular properties implies uncovering structure–pharmacokinetic relations (SPkRs) and deriving quantitative SPkRs (QSPkRs). This, however, has never been an easy task, and seldom if ever have *global relations* of high statistical significance and broad predictive capacity been published. In contrast, numerous *local QSPkRs* are known, as discussed below, i.e. correlations derived for and valid within well-defined chemical series. It is also revealing that the literature contains far more reviews and chapters on QSARs than on QSPkRs [5–12]. The reasons for this state of affairs are not well understood and deserve more insight. Two possible reasons come to mind.

First, and with the exception of in vitro metabolic and binding data, pharmacokinetic responses result from a multiplicity of interactions with a variety of biological constituents (macromolecules, membranes, enzymes, transporters, etc.). These constituents can be seen as *fuzzy targets* due to their diversity and fluctuating property space. As a consequence, different xenobiotics may interact differently with various components [13,14], resulting in highly composite responses which will not fit well in statistical models (Figure 1).

Second, the difficulty of deriving QSPkRs may also be due to the *nature of pharmacokinetic parameters*. Indeed, the vast majority of pharmacodynamic

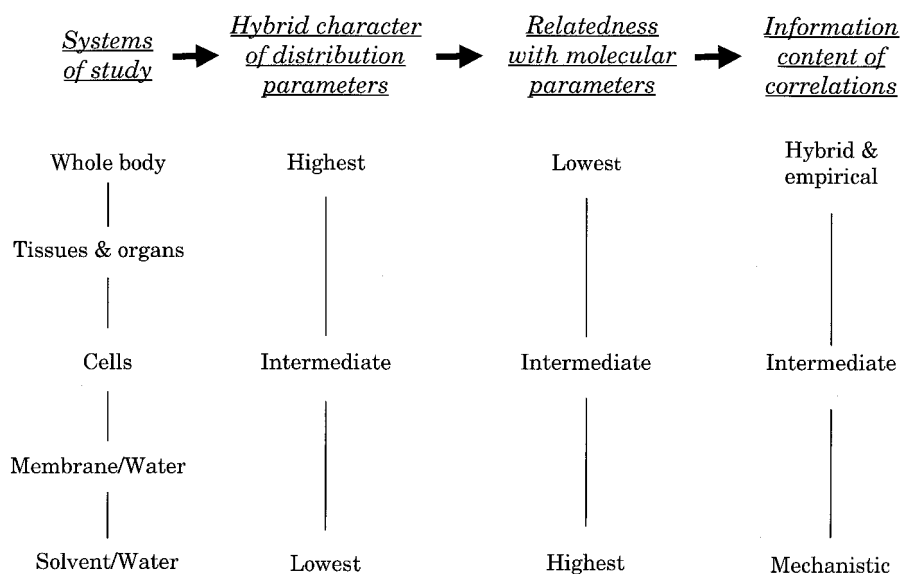


Figure 2. Level discrepancies in structure-activity relationships.

parameters used as dependent variables in QSAR analyses are obtained in simple in vitro biological systems such as receptor or enzyme preparations (e.g., IC_{50} or ED_{50} values). In contrast, a number of pharmacokinetic parameters are obtained in complex biological systems, e.g. in living organisms. Significantly, the hybrid character of pharmacological parameters (both pharmacodynamic and pharmacokinetic) increases dramatically with increasing complexity of the biological systems in which they were obtained [15,16]. In turn, this increase in the hybrid character of pharmacological parameters is inevitably accompanied by a decrease in their categorical relatedness to the molecular parameters used as independent variables.

Stated differently, dependent and independent variables in QSPkR analyses both contain information of a molecular nature when simple biological systems were used, but their logical levels diverge for complex biological systems. This has profound implications for the value and interpretation of QSPkR correlations. As shown in Figure 2, a mechanistic interpretation is allowed and meaningful only for pharmacokinetic parameters of low hybrid character, whereas parameters with a high hybrid character can lead at best to empirical correlations and 'rules of thumb'.

The example of β -blockers

As an early example of what follows, we present here the case of β -blockers. This class of drugs is indeed of marked interest in lipophilicity-pharmacoki-

netic relationships, for two reasons. First, there are clear trends, and even some plausible QSPkRs, that lipophilicity is one of the predominant factors influencing the pharmacokinetic behavior of β -blockers. And second, the common β -blockers span a very broad range of log P_{oct} values: sotalol (–0.79), atenolol (0.23), nadolol (0.71), practolol (0.76), pindolol (1.75), acebutolol (1.87), timolol (2.10), metoprolol (2.15), oxprenolol (2.18), alprenolol (2.61) and propranolol (3.65) [17].

The relations between the pharmacokinetic behavior of β -blockers and their lipophilicity can be summarized as follows:

- *Oral absorption*: The four most hydrophilic drugs above are characterized by incomplete oral absorption with a mechanism dominated by aqueous phase transport, possibly via membrane pores [17]. In contrast, the more lipophilic drugs are well absorbed, with absorption rate constants roughly related to lipophilicity.
- *Penetration in the central nervous system (CNS)*: The most lipophilic β -blockers (e.g. propranolol) penetrate readily into the CNS and elicit central effects (e.g. somnolence), whereas the more hydrophilic drugs have a low CNS penetration and negligible central effects [18].
- *Renal clearance*: As discussed separately in a later section, β -blockers show an inverse relation between log D and renal clearance.
- *Degree of biotransformation and plasma half-life*: Propranolol and other lipophilic β -blockers are extensively metabolized in humans and show short plasma half-lives of a few hours. In contrast, the more hydrophilic β -blockers undergo little or no metabolism and are slowly excreted by the kidney, with long plasma half-lives of approximately 10–20 h. In this class of drugs, elimination is thus by metabolism rather than renal excretion, and a clear trend exists between lipophilicity and extent of metabolism.
- *Cardioselectivity*: This property is both pharmacokinetic and pharmacodynamic. As a trend, polar β -blockers are cardioselective (they block preferentially the cardiac β_1 -adrenoceptors, leaving peripheral β_2 -adrenoceptors relatively unaffected [19]). The more lipophilic β -blockers are non-selective.
- The *corneal penetration* of β -blockers used in ophthalmology is discussed separately in a later section.

The meaning and significance of lipophilicity

Lipophilicity is since long recognized as a meaningful parameter in structure–activity relationships. It is also the single most informative and successful

physicochemical property in medicinal chemistry [20–22]. Not only has lipophilicity found innumerable applications in QSARs and QSPkRs, but its study has revealed a wealth of information on intermolecular forces, intramolecular interactions, and molecular structure in the broadest sense [13,23–25].

Lipophilicity is determined experimentally as *partition coefficients* (log P, valid only for a single chemical species) or as *distribution coefficients* (log D, referring to a pH-dependent mixture of ionic forms, see Equation 6 below) [26]. As such, lipophilicity expresses a balance of intermolecular forces and intramolecular interactions whose understanding sheds light on biochemical recognition forces [27].

Intermolecular forces expressed in lipophilicity

First, lipophilicity is the net result of all intermolecular forces involving a solute and the two phases between which it partitions. A highly informative interpretation of lipophilicity is based on its factorization into a number of parameters [26–28], e.g.:

- π^* , a measure of the solute's *dipolarity/polarizability* and thus of its capacity to elicit orientation and induction forces.
- α and β , the solute's *H-bond donor acidity* and *H-bond acceptor basicity*, respectively.
- V, the molar or molecular volume which assesses the solute's capacity to elicit non-polar interactions, mainly *hydrophobic and dispersion forces*.

Thus, the octanol/water and the heptane/water partition coefficient can be expressed as [29]:

$$\begin{aligned} \log P_{\text{octanol}} = & 5.83 (\pm 0.53) \cdot V/100 - 0.74 (\pm 0.31) \cdot \pi^* \\ & - 3.51 (\pm 0.38) \cdot \beta - 0.15 (\pm 0.23) \cdot \alpha \\ & - 0.02 (\pm 0.34) \end{aligned} \quad (1)$$

$$n = 78; \quad r^2 = 0.92; \quad s = 0.30; \quad F = 248$$

$$\begin{aligned} \log P_{\text{heptane}} = & 6.78 (\pm 0.69) \cdot V/100 - 1.02 (\pm 0.39) \cdot \pi^* \\ & - 5.35 (\pm 0.50) \cdot \beta - 3.54 (\pm 0.30) \cdot \alpha \\ & - 0.06 (\pm 0.43) \end{aligned} \quad (2)$$

$$n = 75; \quad r^2 = 0.96; \quad s = 0.36; \quad F = 438$$

As a result of such equations, it is now common to *factorize lipophilicity* into two sets of terms, namely hydrophobicity which accounts for hydrophobic

<u>Recognition Forces</u>	<u>Lipophilicity</u>	
	Liquid/liquid; RP- HPLC	Liposomes; IAM
<i>Electrostatic interactions</i>		
Charge transfer and aryl/aryl stacking interactions		
Ionic bonds		
Ion - dipole bonds (permanent, induced)		
Reinforced H-bonds		
Normal H-bonds		
Van der Waals forces		
Orientation forces (permanent dipole - permanent dipole)		
Induction forces (permanent dipole - induced dipole)		
Dispersion forces (instantaneous dipole - induced dipole)		
<i>Hydrophobic interactions</i>		

Figure 3. A comparison between the intermolecular forces that govern molecular recognition in biochemical and pharmacological processes, and those expressed in lipophilicity (modified from Reference 25).

and dispersion forces, and polar terms which account for hydrogen bonds, and orientation and induction forces [26]:

$$\text{Lipophilicity} = \text{Hydrophobicity} - \text{Polarity} \quad (3)$$

The major recognition forces of significance in molecular pharmacology and biology bear a clear analogy with the intermolecular forces expressed in lipophilicity (Figure 3). Even ionic bonds now begin to be taken into account by modern methods to measure lipophilicity, namely liposomes/water distribution coefficients [30,31] and capacity factors measured by reversed-phase HPLC (RP-HPLC) on immobilized artificial membrane (IAM) columns [32–35].

Intramolecular interactions influencing lipophilicity

Intramolecular interactions have a major influence on lipophilicity, and they are themselves influenced by structural factors [25]. These aspects are mentioned only briefly here. *Intramolecular interactions* considered for them-

selves can be classified as follows [25]:

- Electronic conjugations in aromatic systems and across aliphatic segments.
- Interactions involving polar groups, e.g. proximity effects between two neutral polar groups [21,36], internal H-bonds and dipole–dipole interactions, internal ionic bonds, hydrophilic folding, and proximity effects between polar and non-polar groups.
- Steric/hydrophobic effects, such as shielding of polar groups, hydrophobic interactions [37], and hydrophobic collapse [38,39].

Structural factors influencing intramolecular interactions can be schematized as follows [25]:

- Positional isomerism, including regioisomerism and tautomerism.
- Stereoisomerism, which comprises diastereomerism and conformational isomerism.
- Ionization [26].
- Molecular size and chameleonic behavior, exemplified by self-coiling [40] resulting from hydrophobic collapse and/or hydrophilic folding [41].

This brief introduction should serve to convince the reader that many physico-chemical and structural factors influence lipophilicity. However, this richness is also a weakness, since it may confuse or hinder a mechanistic interpretation of lipophilicity-permeation relations. This is easily understood considering that such relations will not reveal which molecular attribute(s) and resulting intermolecular force(s) have the largest influence on the permeation response under investigation. For this reason, medicinal chemists have come to derive novel parameters from partition coefficients. Such lipophilicity-derived parameters aim at expressing a single intermolecular force and/or a single structural attribute, e.g. the capacity to donate hydrogen bonds.

Lipophilicity-derived parameters

Schematically, it is convenient to distinguish between lipophilicity descriptors obtained in different or the same solvent systems. The former cases are exemplified by the well-known $\Delta \log P_{\text{oct-alk}}$ parameter. The latter cases are more recent and are exemplified by descriptors designated as *diff*(log P), e.g. *diff*(log $P^{\text{N-I}}$) which is the difference in log P between the neutral (N) and ionized (I) form of a given solute in the same solvent system [42].

The $\Delta \log P$ parameters

Lipophilicity descriptors obtained in *different solvent systems* can only be compared to derive a $\Delta \log P$ when the two $\log P$ values do not contain identical structural information. For example, the $\Delta \log P$ derived from Equations 1 and 2 above is [29]:

$$\log P_{\text{oct-hep}} = 0.12 (\pm 0.30) \cdot \pi^* + 1.96 (\pm 0.42) \cdot \beta + 3.40 (\pm 0.25) \cdot \alpha - 0.43 (\pm 0.27) \quad (4)$$

$$n = 75; \quad r^2 = 0.92; \quad s = 0.31; \quad F = 288$$

or, after removal of the terms with no or low significance:

$$\log P_{\text{oct-hep}} = 3.54 (\pm 0.36) \cdot \alpha - 0.37 (\pm 0.15) \quad (5)$$

$$n = 75; \quad r^2 = 0.84; \quad s = 0.45; \quad F = 325$$

In other words, and because $\log P$ values in octanol/water and in alkane/water do not express the same balance of polar forces, their difference is physico-chemically meaningful and expresses mainly the H-bond donor capacity of solutes.

Four types of isotropic solvent systems (the *critical quartet* octanol/water, alkane/water, chloroform/water and dibutylether/water) express in partly overlapping and partly complementary ways the recognition forces which account for membrane partitioning and biological selectivity [43,44]. To overcome some experimental problems caused by the low alkane solubility of many compounds, 1,2-dichloroethane/water (dce/water) has become the system of reference to replace the alkane/water system and to characterize H-bonding properties of solutes [45]. In other words, $\Delta \log P_{\text{oct-dce}}$ is now proposed instead of $\Delta \log P_{\text{oct-alk}}$ [29,46–54].

The diff parameters

The second approach to search for intramolecular interactions is based on $\log P$ or $\log D$ values obtained in the *same solvent system*. The resulting *diff* parameters proposed by us usually refer to two distinct electrical states of a single solute, although other possibilities exist, e.g. *diff*($\log P^{\text{exp-calc}}$) when comparing calculated and experimental $\log P$ values [55]. These parameters are influenced by intramolecular interactions, and it is indeed this insight which renders them so valuable.

The lipophilicity of *ionizable compounds* is described by their $\log D$ at a given pH (D = distribution coefficient), $\log D$ expressing the contributions of all electrical species (neutral and ionized) present at that pH (Equation 6).

Log D is a general notation requiring a superscript for the pH and a subscript for the biphasic system *aaa*:

$$D_{aaa}^{pH} = f_N \cdot P^N + f_I \cdot P^I \quad (6)$$

where f_N and f_I are the molar fractions of the neutral and ionized form(s) and P^N and P^I are their respective partition coefficients.

Log D is often the best way to obtain the experimental log P values of the neutral and ionized species of an ionizable solute. The difference between these two parameters, $\text{diff}(\log P^{N-I})$, is itself a parameter containing important structural information, since it expresses the influence of ionization on the intermolecular forces and intramolecular interactions of a solute [56]. However, this parameter is also influenced by the nature of the solvent system.

As already shown by Kubinyi [57], the $\text{diff}(\log P^{N-I})$ of a monoprotic substance in octanol/water ranges from 3 to 4 depending on charge delocalization. This value differs in other solvent systems, being for example around 5 in dce/water [58,59]. This is in agreement with Born's solvation model for which the larger $\text{diff}(\log P^{N-I})$ obtained in the dce/water system implies that the molecular radius of ions is larger in octanol than in dichloroethane. In fact, localized ions retain more water molecules when they transfer into octanol than in a less polar solvent, due to the greater solubility of water in *n*-octanol and to the large H-bonding capacity of *n*-octanol. In other words, the more similar to water the organic solvent, the smaller $\text{diff}(\log P^{N-I})$.

For a given system, $\text{diff}(\log P^{N-I})$ is rather constant for compounds with related chemical structures. Thus, an anomalous value of $\text{diff}(\log P^{N-I})$ corresponds to solutes characterized by specific intramolecular interactions affecting either their neutral or cationic species. The parameter $\text{diff}(\log P^{N-I})$ is also of interest in the study of *zwitterions*, which are receiving much interest due to their peculiar partitioning and pharmacokinetic behavior [52,60–63]. As demonstrated, the pH-partitioning profiles of ampholites [64] can be bell-shaped or U-shaped. Zwitterions with large K_Z (tautomeric equilibrium constant, defined as the ratio of concentrations of the zwitterionic and neutral tautomers) can display either behavior. Intramolecular effects play a marked role in enhancing the lipophilicity of the zwitterionic species and thus in determining the shape of the lipophilicity profile. The difference between the log P of the neutral and zwitterionic forms ($\text{diff}(\log P^{N-Z})$) has been found to be useful in pointing to intramolecular effects [64]. As previously outlined, in octanol/water the difference in lipophilicity between the neutral and ionized form of a given solute ranges from 3 to 4 and thus a $\text{diff}(\log P^{N-Z})$ of about 6 is expected for zwitterions for which internal neutralization is totally absent.

The Λ parameter

Besides the $\Delta \log P$ and *diff* parameters, the Λ term is another parameter accounting for the H-bonding capacity of a solute [27,65]. Factorizing lipophilicity into a cavity or volume term and a polarity term yields Equation 7:

$$\log P = a \cdot V - \Lambda \quad (7)$$

where V is the molar volume and Λ the *global polarity* of a given solute in a given solvent system (e.g., *n*-octanol/water or alkane/water). The coefficient a depends on the solvent system and on the mode of calculation of molar volumes. The Λ term is mostly related to H-bonding capacity, but its information content is different depending on the solvent system in which the original $\log P$ values were obtained:

$$\Lambda_{\text{oct}} = -4.17 (\pm 0.25) \cdot \beta - 0.49 (\pm 0.11) \quad (8)$$

$$n = 168; \quad r^2 = 0.87; \quad s = 0.32$$

$$\Lambda_{\text{alk}} = -6.81 (\pm 0.62) \cdot \beta - 3.82 (\pm 0.45) \cdot \alpha - 0.49 (\pm 0.115) \quad (9)$$

$$n = 168; \quad r^2 = 0.87; \quad s = 0.32$$

Pharmacokinetic processes

We now present some examples of relations between lipophilicity and various low-energy processes of drug disposition. The sequence of pharmacokinetic processes begins with permeation/absorption, continues with reversible binding and distribution, and ends with excretion. As illustrated below, a good number of statistical regressions (linear, multilinear, parabolic, etc.) have been calculated between pharmacokinetic processes and various molecular descriptors, but they pertain to rather limited numbers of drugs and/or well-defined chemical series. Published regressions offer good or partial correlations, and sometimes misleading ones containing statistical defects (e.g. overfitting or cross-correlations).

When it comes to very large (several hundreds or more) and varied series of drugs, modern statistical tools are needed, such as cluster analysis and principal components analysis. The models they yield may only allow yes/no or other scaled classifications, and their results are no longer straightforward to interpret in physicochemical and biological terms.

Membrane permeation

Some studies have used *biological membranes* to assess the permeation of a large series of solutes and look for structure–permeation relations. One work of this type used the permeability coefficients (PC) in human red blood cell (RBC) membranes and toad urinary bladder (TUB) of 36 model solutes (mostly alcohols, diols, polyols, amides and carboxylic acids) [66]. For a subset of 21 nonelectrolyte compounds, fair relations were found:

$$\begin{aligned}\log P(\text{RBC}) = & 1.32 (\pm 0.29) \cdot \log P_{\text{oil/water}} \\ & - 3.22 (\pm 1.11) \cdot \log \text{MW} \\ & + 0.21 (\pm 0.08) \cdot H_B + 9.83 (\pm 0.32)\end{aligned}\quad (10)$$

$$n = 21; \quad r^2 = 0.88; \quad s = 0.45; \quad F = 42$$

$$\begin{aligned}\log P(\text{TUB}) = & 0.66 (\pm 0.19) \cdot \log P_{\text{ether/water}} \\ & - 2.71 (\pm 1.41) \cdot \log \text{MW} \\ & + 0.06 (\pm 0.32) \cdot H_B + 7.30 (\pm 0.73)\end{aligned}\quad (11)$$

$$n = 21; \quad r^2 = 0.85; \quad s = 0.39; \quad F = 32$$

These equations indicate a direct relation between permeability and lipophilicity (the oil/water and ether/water systems express a comparable balance of intermolecular forces). In contrast, the molecular weight (MW) appears unfavorable to permeability. Unexpectedly, the total H-bonding capacity (H_B) was deduced to contribute favorably to permeability. However, its contribution in Equation 10 appears low, whereas it is devoid of statistical significance in Equation 11.

From the 36 solutes in the full set, 15 were not included in Equations 10 and 11. These were a few organic acids, plus a number of nonelectrolytes which had to be eliminated to improve the statistics. As a consequence, and also because of the chemically unbalanced nature of the set of solutes, Equations 10 and 11 have a limited predictive capacity. Yet they suggest some mechanistic trends, and as such are of interest.

Gastrointestinal absorption

Gastrointestinal (GI) absorption is a property of major significance in the pharmacokinetic profiling of clinical candidates. Many determinations of GI absorption have therefore been published, based on in vitro or in vivo approaches.

Caco-2 cell monolayers have become an established tool to screen for GI absorption [67,68]. For example, the monolayer permeability of 10 phenylalanine oligomers was examined for its relation with molecular properties.

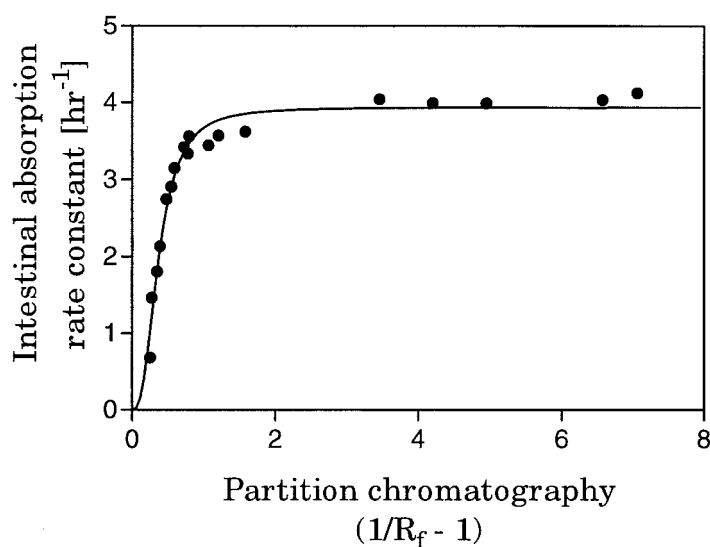


Figure 4. Plot of intestinal absorption rate constants versus chromatographic partition constants for antibacterial sulfonamides [73].

For 7 of the 10 peptides, good correlations were found with measures of H-bond capacity, such that the lower the H-bond donor capacity, the higher the permeability [69]. The parameters assessing the H-bond donor capacity were $\Delta \log P_{\text{oct-alk}}$, and the log of the heptane/ethyleneglycol partition coefficient. The latter is a promising and little used molecular parameter.

Due to its high hydrophilicity, the antitumor agent 5-fluorouracil (5FU) displays low GI absorption. A series of 13 N-acylated derivatives were prepared as potential *prodrugs* and their permeability was compared in Caco-2 cell monolayers [70]. Some significant increases in permeability coefficients were seen. The relation with $D_{\text{oct}}^{7.4}$ was not calculated statistically but was visibly sigmoidal. Permeability was low for the hydrophilic solutes ($\log D < -1.0$), increased in the range $-1 < \log D < 0$, and had reached a maximum for $\log D > 0$. Interestingly, sigmoidal relations are frequently observed between permeability and lipophilicity.

In vitro models of GI absorption can only be of value as screens if they show high correlations with in vivo absorption in humans [71], as indeed seen in a number of cases. Thus, the permeability in Caco-2 cells of 17 drugs showed a good sigmoidal relation with their intestinal absorption in humans, which ranged from ca. 1 to 100% [72].

The *in situ rat gut technique* is an informative tool yielding realistic absorption rates. In a study of 18 sulfonamides, the absorption rate constant k_a was correlated with the lipophilicity parameter $[(1/R_f) - 1]$ obtained by

reversed-phase TLC [73]. The best equation was a hyperbola with asymptotic value k_m , where k_m is the rate constant for transfer out of the membrane ($k_a \rightarrow k_m$ when lipophilicity $\rightarrow \infty$), and n and Q are constants for each given system (Equation 12 and Figure 4):

$$k_a = \frac{k_m \cdot [(1/R_f) - 1]^n}{Q + [(1/R_f) - 1]^n} \quad (12)$$

In this example, $k_m = 3.940$, $n = 2.636$, $Q = 0.0719$, with good statistics ($r^2 = 0.97$).

Good gastrointestinal absorption was for many years a difficult objective in the development of penicillins. A systematic study in mice and rats showed that the two major molecular properties influencing the GI absorption of penicillins are their stability in acidic solutions and their lipophilicity [74]. Resistance to proton-catalyzed hydrolysis was shown to be related to the Taft's inductive substituent constant of the side chain at the 6-amino group, with a $\sigma_1 > 0.34$ being necessary for orally active penicillins. The second prerequisite was lipophilicity, with $1.9 < \log P < 3.3$ ensuring the highest plasma levels or relative bioavailability.

Novel approaches based on molecular surface properties, in particular polar surface area, are of interest in quantitative relations with in vitro and in vivo absorption data [72,75–80]. The polar surface area encodes information similar to that of the polar components of lipophilicity. The resulting relations are usually found to have a sigmoidal shape, such that high absorption/permeability is associated with low polarity. Such approaches appear capable of relating polar properties to absorption for large series of compounds.

With very large series of compounds (10^3 – 10^4 and more), *qualitative relation* models can be useful to guide medicinal chemists. A recently published alert procedure, called the 'rule of 5' [81], has generated considerable interest. This rule is based on a distribution of calculated properties among several thousands of drugs belonging to different therapeutic classes. It predicts that a candidate is more likely to show poor absorption or permeation when it has > 5 H-bond donor groups, > 10 H-bond acceptor groups, a molecular weight > 500 , and a calculated $\log P$ (CLOGP) > 5 . Substrates of biological transporters are exceptions to the rule. When two of these criteria are met, poor absorption or permeability may be expected. The rule of 5 can be considered as a 'wide spectrum' qualitative model to guide the medicinal chemist.

Skin permeability

Skin permeability is another active front of pharmaceutical research, transdermal delivery being investigated as an alternative route of administration. There are indeed a number of drugs and therapeutic situations for which non-invasive parenteral administration can afford some ready benefits. In an opposite perspective, skin absorption of industrial and environmental toxins can be highly detrimental and should be prevented. There is therefore a great need to reach a better understanding of the molecular and structural factors that facilitate or hinder the cutaneous penetration of drugs and other xenobiotics [82,83].

An extensive review published a few years ago [84] concluded that qualitative relations between structure and cutaneous penetration can be deduced from our current knowledge, but that quantitative structure–penetration relationships remain of limited scope and predictive value. Thus, the permeability coefficient through excised human skin or nude mouse skin showed fair linear correlations with $\log P$ (r^2 ranging approximately from 0.7 to 0.9), but only within well-defined chemical series of compounds (e.g., *n*-alkanols, steroids) [48,84–86]. In all cases did skin permeability increase with lipophilicity, although the slope was strongly series-dependent.

For a homogeneous set of phenols, human skin permeability (K_p) was correlated with $\log P_{\text{oct}}$, but in this case the relation was a parabolic one [48]:

$$\log K_p = -0.37 (\pm 0.16) \cdot (\log P)^2 + 2.39 (\pm 0.76) \cdot \log P - 8.71 (\pm 0.88) \quad (13)$$

$$n = 18; \quad r^2 = 0.86; \quad s = 0.21; \quad \text{optimal } \log P = 3.2$$

A reportedly parabolic relation was also found for non-steroidal antiinflammatory agents between their permeability across human epidermis and $\log P_{\text{oct}}$, but the limited number of compounds (six!) weakens the correlation [87].

No correlation between skin permeability and $\log P$ existed when *n*-alkanols and steroids were examined together. In contrast, $\Delta \log P_{\text{oct-hep}}$ yielded a fair correlation ($r^2 = 0.81$) for the two series taken together [48]. The correlation was further improved in a multiple linear relation with $\log P$ added [48]:

$$\log K_p = -1.46 (\pm 0.23) \cdot \Delta \log P + 0.29 (\pm 0.14) \cdot \log P - 3.75 (\pm 0.61) \quad (14)$$

$$n = 21; \quad r^2 = 0.91; \quad s = 0.35$$

Here, there is a clear indication that skin permeation is (a) markedly favored by a low H-bond donor capacity, and (b) modestly improved by lipophilicity.

Corneal penetration

Corneal penetration is a critical condition for the therapeutic success of ocularly administered drugs such as β -blockers used as anti-glaucoma agents. Several years ago, an important study showed that lipophilicity indeed plays a major role in penetration through intact cornea [88]. In a series of 12 β -blockers, the log PC (permeability coefficient) showed a parabolic relation with lipophilicity (octanol/water):

$$\log PC = -0.108 \cdot (\log D)^2 + 0.623 \cdot \log D - 5.03 \quad (15)$$

$$n = 11; \quad r^2 = 0.95; \quad \log D_{\text{optimal}} = 2.9$$

In all equations reported in this study, acebutolol proved to be an outlier for unknown reasons, hence its exclusion from Equation 15. Using log P instead of the distribution coefficient log D (pH 7.65) yielded an equation of similar statistical significance. It is also of interest to note that corneal penetration appeared to be negatively influenced by increasing MW, but the reliability of overfitted equations (3 or 4 independent variables for 12 observations) is doubtful at best.

Blood–brain barrier permeation

For drugs whose site of action is the CNS, permeation through the blood–brain barrier (BBB) is a condition of success, whereas for other drugs brain penetration may elicit unwanted side effects and be the cause of low patient compliance. It is thus clear that BBB permeation is a major issue in drug research, to be favored or prevented by molecular design depending on prerequisites [89].

The influence of lipophilicity

Over the years, many QSAR studies have uncovered relations between central activity and lipophilicity. Thus, Hansch and co-workers found that the ideal lipophilicity of neutral compounds for passive penetration into the brain is around 2 (log P_{oct} scale) [90]. As a consequence, Corwin Hansch put forward the principle of minimal lipophilicity [91], in which he advocated that peripherally acting drugs should be made as hydrophilic as possible without loss of efficacy, so as to minimize unwanted central effects.

In this context, an illustrative study was carried out in adult baboons [92]. Following bolus injections of 14 [^{11}C]-labeled model compounds into the carotid artery, their brain extraction could be calculated. The results (Figure 5) clearly show that the more hydrophilic and lipophilic compounds had

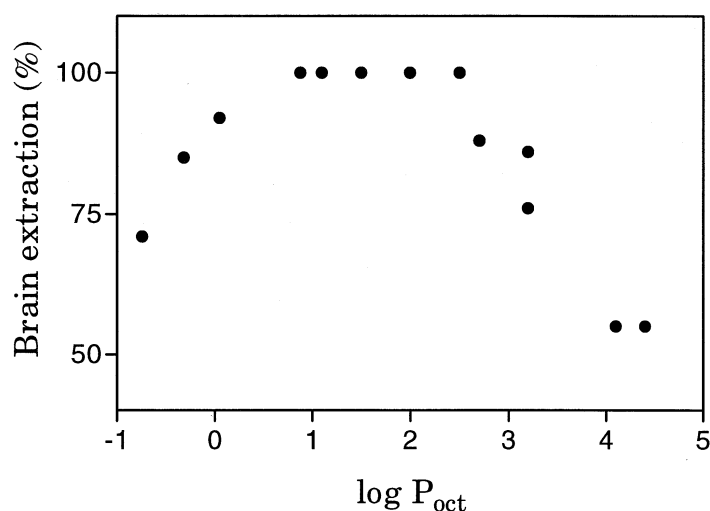


Figure 5. Plot of brain extraction of [^{11}C]-labeled model compounds in adult baboons versus $\log P_{\text{oct}}$ [90]. The compounds (listed by increasing lipophilicity) were methanol, ethanol, isopropanol, *n*-butanol, benzyl alcohol, benzyl methyl ether, *n*-hexanol, pentyl methyl ether, hexyl methyl ether, diphenylmethanol, *n*-octanol, benzophenone, benzyl hexyl ether and triphenylmethanol.

an incomplete extraction, whereas complete extraction was the rule for the compounds of $\log P$ in the range 0.9 to 2.5. The results in Figure 5 thus draw a bell-shaped curve and confirm the existence of an optimal $\log P$, or rather a range of optimal lipophilicity for BBB permeation. However, not all known quantitative relations between structure and BBB permeation assume a bell-shaped form. Thus, the permeation of various β -blockers and anti-inflammatory drugs in cerebrovascular endothelial cell cultures showed a sigmoidal relation with $\log D$ [93]. In the case of quinolone antibacterials, the drugs with $\log D < 0$ all had the same and very low permeability, which increased linearly for the more lipophilic drugs ($\log D$ between 0 and 2) [94]. At least two reasons can explain the absence of a bell-shaped curve in these examples. First and quite likely, the explored lipophilicity range may have been too narrow due to the absence in the set of highly lipophilic drugs. A second reason may be the influence of other molecular factors.

The influence of other molecular factors

That other molecular properties beside lipophilicity may influence BBB permeation is well known. One such factor is the *molecular weight* (MW), since the larger a molecule the more restricted its BBB permeation. For a variety

of drugs, the rat brain capillary permeability coefficient P_c increased with lipophilicity and decreased with MW [95]:

$$\log P_c = 0.41 (\pm 0.04) \cdot \log \left[\frac{P}{\sqrt{MW}} \right] - 4.60 \quad (16)$$

$$n = 22; \quad r^2 = 0.83; \quad s = 0.04$$

A similar relation was found for a smaller but also very varied series of solutes [96].

Another molecular property often found to influence permeation through the BBB (and other barriers) is the *H-bonding capacity*, more precisely the H-bond donor acidity. This was revealed quite clearly by a series of 20 H₂-receptor antagonists, whose brain penetration in vivo (ratio at steady state of brain/plasma concentration ratio) was negatively correlated with the $\Delta \log P_{\text{oct-cyc}}$ parameter (cyc = cyclohexane) [47], which contains the same structural information as other $\Delta \log P_{\text{oct-alk}}$ parameters (see Equations 4 and 5 above):

$$\log (C_{\text{brain}}/C_{\text{plasma}}) = -0.48 (\pm 0.16) \cdot \Delta \log P_{\text{oct-cyc}} + 0.89 (\pm 0.50) \quad (17)$$

$$n = 20; \quad r^2 = 0.69; \quad s = 0.44$$

In contrast, poor or unsatisfactory correlations were found with $\log P$ values in either solvent system.

These brain penetration data were re-analyzed using the Λ_{alk} parameter (see Equation 9 above) or the polar surface area [65]. Fair correlations with a negative slope were found ($r^2 = 0.61$), indicating again that H-bonding capacity and polarity were unfavorable for BBB permeation. Inclusion of the molar volume improved the correlation, but the fact that its contribution was positive or negative depending on the first independent variable casts doubts on the robustness of the results.

In a series of seven phenylalanine oligomers with esterified carboxylic groups, brain penetration was again correlated with the hydrogen-bonding capacity [97]. Brain penetration was assessed in vitro (bovine brain microvessel endothelial cell monolayers) and in situ (rat brain perfusion). The H-bonding capacity of the peptides was measured by their $\Delta \log P$ and by their $\log P$ in the heptane/ethyleneglycol system. Both biological responses were well correlated with either lipophilicity parameters, with r^2 in the range 0.88 to 0.96. Consistent with other findings, BBB permeability decreased with increasing H-bonding capacity.

As an interpretation to these results, a limiting step in drug permeation across the BBB may be due to the binding of drugs to the lipid-rich layer of biomembranes by the donation of H-bonds. Indeed, the hydrophilic part of lipids contains H-bonding acceptor groups, which could explain the very slow diffusion of strong H-bond donating solutes.

The above examples should not be interpreted to mean that decent statistical correlations are always found in studies of this type. When one works with great statistical rigor, avoiding parameter values themselves calculated by not-too-robust equations, and also avoiding cross-correlations in multiple regression analysis, failure to find good correlations is no more the exception than the rule. But even in such cases, more modest qualitative approaches can be used to model permeation. For example, the brain-penetration capacity of histamine H_1 -antagonists was modeled with a decision tree containing the two main physicochemical parameters influencing brain penetration, namely $\log D_{\text{oct}}^{7.4}$ and $\Delta \log P_{\text{oct-alk}}$ [98]. In this model the potential for brain penetration of antihistamines was first estimated from their $\log D_{\text{oct}}^{7.4}$. When this value was < 0 or > 3 , penetration was negligible or hindered. For compounds with $\log D_{\text{oct}}^{7.4}$ values within the range 0 to 3, penetration could occur if the compound had a weak H-bond donor capacity ($\Delta \log P_{\text{oct-alk}} < 2$).

Binding to circulating macromolecules

A number of recognition forces (see Figure 3) are involved in the binding of small molecules such as drugs to physiological macromolecules. Depending on the nature and property of the binding groups in both the macromolecule and the ligand, the balance of intermolecular forces can be expected to be quite different. Yet in numerous cases, good relations have been found between lipophilicity and affinity constants, pointing to hydrophobicity as a major binding force.

Thus, the binding of fatty acids to bovine serum albumin was shown to vary with their hydrophobicity [99]. The Gibbs energy of binding (ΔG°) increased from propionic (C_3) to butyric acid (C_4), reached a plateau for valeric (C_5) and caproic acid (C_6), and increased again for heptanoic (C_7) and caprylic acid (C_8). The average increment in ΔG° was 820 cal/mol, confirming the hydrophobic interactions of the alkyl chain. The plateau was explained by the limited extent of the hydrophobic binding area. In another study, a good linear relation ($r^2 = 0.90$) was found for 11 aromatic acids (model compounds and anti-inflammatory drugs) between their binding constant to bovine serum albumin (in logarithmic form) and a hydrophobic index obtained by RP-HPLC [100].

In another study, where the unbound fraction in plasma (f_u) was taken as the biological response, a sigmoidal relation was found between f_u and

$\log P_{\text{oct}}$ [101]. Eleven aromatic acids were examined in human, rabbit, rat and mouse plasma. For human plasma, the statistical model was:

$$\log (1/f_u - 1) = 0.994 \cdot \log P - 1.10 \quad (18)$$

$$n = 11; \quad r^2 = 0.97; \quad s = 0.16$$

The regression and correlation coefficients were comparable in the four species, suggesting great interspecies similarity in the binding sites of albumin.

Interestingly, parabolic relations between protein binding and lipophilicity are also known, confirming the limited dimensions of some binding sites. When large molecules such as cephalosporins were examined for their association constant (K_a) to human serum albumin, a fair parabolic relation was found with lipophilicity measured by RP-HPLC ($\log k_w$) [102]:

$$\begin{aligned} \log (1/K_a) = & -3.91 (\pm 0.62) \cdot \log k_w^2 \\ & + 13.0 (\pm 2.07) \cdot \log k_w - 13.7 \end{aligned} \quad (19)$$

$$n = 11; \quad r^2 = 0.83$$

One of the few 3D QSAR applications in pharmacokinetic studies used comparative molecular field analysis (CoMFA) to rationalize the affinity of 35 chemically very diverse drugs to the A variant of orosomucoid (α_1 -acid glycoprotein, AAG) [103]. Satisfactory statistical models were found which suggested that the steric properties mostly, but also the lipophilic properties of the ligands contributed to affinity.

As mentioned above, most quantitative structure-binding relationships indicate that affinity is correlated with lipophilicity. A thermodynamic basis to linear binding-lipophilicity relations has even been proposed [104]. Furthermore, there are also a few examples to demonstrate that lipophilicity-derived parameters may contain relevant information. For example, the binding parameters of 8 basic, neutral and acidic drugs to AAG were not correlated with $\log P$ or $\log D$ [105]. In contrast, the enthalpy and entropy of binding were negatively and positively correlated with $\Delta \log P$, respectively. This can be taken to mean that the enthalpic drive to AAG binding increased with the H-bonding capacity of the ligands, whereas the entropic drive decreased with it.

Distribution into tissues

The tissue distribution of drugs, which is governed in part by affinity for tissue macromolecules and membranes, is a poorly explored pharmacokinetic

process [106]. However fragmentary the available data, they cover a variety of tissues of pharmacological or toxicological interest. For example, the storage of drugs and many lipophilic xenobiotics in *adipose tissues* poses a clear health problem. Bickel has defined an adipose storage index (ASI) which compares the adipose to 'overall' concentrations of a given xenobiotic [107]. A compilation of the ASI of almost 100 xenobiotics (acidic, basic and neutral drugs, and various polyhalogenated compounds) revealed the importance of lipophilicity ($\log P_{\text{oct}}$). Other factors appear to play a role, but these are less well understood. Thus, practically all neutral and acidic xenobiotics of $\log P > 2$ had an $\text{ASI} > 1$. In contrast, almost all basic drugs, however lipophilic, had low adipose affinity ($\text{ASI} < 1$).

One of the reasons for this low ASI of basic lipophilic drugs is their affinity for lean tissues [107,108]. The isolated perfused rat *lung* appears as a good model of lean tissues and has been investigated with particular care due to the existence of binding sites for basic drugs. For a small set of basic drugs of different lipophilicity, two pulmonary binding sites were seen, one of high affinity and low density, the other of low affinity and high density [109]. The $\log D$ ($\text{CHCl}_3/\text{H}_2\text{O}$ at pH 7.4) were correlated with affinity to both sites, as well as to the capacity to displace a marker ligand. It was also clarified that these pulmonary binding sites are mainly located in the outer membranes of mitochondria [110]. Thus the body distribution of basic, lipophilic drugs appears to be determined in part by their affinity for lean tissues, itself resulting from their binding as cations to macromolecules. This suggests a combination of hydrophobic and polar/ionic interactions.

In a more comprehensive study, the concentration of 10 basic drugs in plasma and 8 non-metabolizing tissues was examined following i.v. administration to rabbits [111]. The drugs were weakly basic benzodiazepines and strongly basic neurological drugs. Good linear relations ($r^2 = 0.92$ to 0.97) were found between the tissue-to-plasma concentration ratios of unbound, non-ionized drugs and their $\log P_{\text{oct}}$. The slope of the linear regressions increased in the series: muscle < skin < bone < brain < gut < heart < lung < adipose. Despite the limitations inherent in such an in vivo/ex vivo study, the results point to a mechanism of physicochemical partitioning different from binding to tissular macromolecules, and which may be related to the chemical composition of the various tissues.

Thus, evidence based on relations with lipophilicity, despite its fragmentary character, suggests the existence of two mechanisms of drug distribution into tissues. One such mechanism is a simple partitioning, with adipose tissue showing the highest avidity for lipophilic xenobiotics. The second mechanism is characteristic of basic drugs and appears to involve binding of their cationic form to some tissular macromolecules.

Volume of distribution

The volume of distribution is an important pharmacokinetic parameter which depends on binding in plasma and on tissue affinity. The best volume of distribution in QSPkRs is the *volume of distribution at steady state* (V_{ss}), a true distribution volume not affected by elimination [11].

When the V_{ss} of 5 β -blockers was compared in obese and normal subjects, it was found to be positively and well correlated ($r^2 = 0.90$) with the $\log D_{oct}$ values at pH 7.4, but not with the $\log P$ values [108]. All available evidence was again consistent with a selectivity for lean over adipose tissues, with the cationic form of the drugs contributing markedly to their tissue distribution.

In a recent review, Smith et al. [12] have compiled the volumes of distribution in humans of a large number of drugs. The series were too heterogeneous to allow the derivation of QSPkRs, but the trend is clearly apparent that V_{ss} increased with $\log D$. After correction for $\log D$, V_{ss} was highest for bases, lowest for acids, and intermediate for neutral drugs. Again a marked tissue affinity has been confirmed for basic drugs. Interestingly, the zwitterionic antihistamine cetirizine has an apparent volume of distribution in humans of 0.4 L/kg, i.e. smaller than exchangeable water [54]. With a $\log D$ of 1.5, cetirizine thus exhibits a volume of distribution comparable to that of acids.

Renal clearance

The lungs and skin (via the expired air and sweat, respectively) are minor routes of excretion, whereas biliary excretion is of significance only for drugs and metabolites with good polarity (e.g. an anionic group) and a MW larger than approximately 450–500 (in humans). The most important organs for the excretion of xenobiotics and their metabolites are the kidneys.

Glomerular filtration in the kidneys allows the free passage from plasma of unbound compounds with $MW < 5000$, while macromolecules are not or only minimally filtered. The filtered compounds find themselves in the ultrafiltrate where a very active process of water reabsorption takes place. During this phase, when the ultrafiltrate is transformed into urine by extensive concentration, passive reabsorption of chemicals can occur. In addition to free filtration, some acids and bases are actively secreted [11].

Urinary excretion is assessed by the *renal clearance* (CL_R), which expresses the rate of renal excretion normalized by the free plasma concentration. Renal clearance is thus the net result of the processes of filtration, reabsorption and secretion, and depends on such factors as glomerular filtration rate, urinary pH (which can vary between 5 and 8), and the physicochemical factors influencing reabsorption. The composite nature of CL_R makes it difficult to derive meaningful structure–excretion relationships, except in

some 'well-behaved' series which show that reabsorption depends on the distribution coefficient at the relevant pH. Such studies can yield meaningful results only under conditions of controlled urinary pH. Indeed, the excretion of basic drugs will be largest for hydrophilic compounds and an acidic urine, and smallest for lipophilic compounds and an alkaline urine. For acidic drugs, CL_R will be highest for hydrophilic compounds and an alkaline urine.

In a compilation reported by Smith et al. [12], the renal clearance of β -blockers in humans was seen to be linearly related to their $\log D_{oct}^{7.4}$: for a $\log D$ of -2 , its value was close to 10 mL/min/kg, and it decreased by about one order of magnitude for each increase of one unit in $\log D$. An almost parallel relation has been reported for non-steroidal antiinflammatory acids, except that for a $\log D$ of -2 the renal clearance was close to 2 mL/min/kg [12].

Relations between metabolism and lipophilicity

Global trends

Comparing the overall metabolism of numerous drugs clearly reveals a global relation with lipophilicity. Indeed, there exist some highly polar xenobiotics known to be essentially resistant to any metabolic reaction, e.g. saccharin, disodium cromoglycate and oxaceprol [112–115]. Furthermore, many in vivo metabolic studies have demonstrated a dependence of biotransformation on lipophilicity, suggesting a predominant role for transport and partitioning processes. A particularly illustrative example is offered by antiasthmatic chromone-2-carboxylic acids [116], whose pharmacokinetic behavior in rat revealed the opposite influences of $\log D$ on renal clearance (which decreased with increasing lipophilicity) and metabolic clearance (which increased with increasing lipophilicity). And as mentioned above, the more lipophilic β -blockers are extensively if not completely metabolized (e.g. propranolol), whereas the more hydrophilic ones undergo biotransformation for only a fraction of the dose (e.g. atenolol).

This global trend is in line with the Darwinian rationale for xenobiotic metabolism, which is believed to have evolved in an *animal–plant* 'warfare', with herbivores adaptating to the emergence of protective chemicals (e.g. alkaloids) in plants [117].

The exception to the global and direct relation between extent of metabolism and lipophilicity is offered by the vast number of human-made, highly lipophilic *polyhalogenated xenobiotics* which now pollute our entire biosphere [107,118,119]. Such compounds include polyhalogenated insecticides (e.g. DDT), polyhalogenated biphenyls and dioxins. As mentioned in an earlier section, they have a strong propensity to accumulate in adipose tissues. In addition, these compounds are highly resistant to biotransformation in animals

The three conceptual steps in the interaction of a drug with a biological system

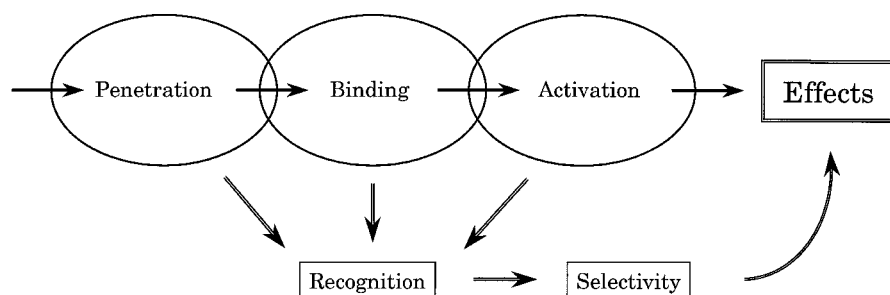


Figure 6. The three conceptual steps in the interaction of a drug with a biological system. These steps underlie any pharmacodynamic or pharmacokinetic event (modified from Reference 120).

due in part to their very high lipophilicity, and in part to the steric shielding against enzymatic attack provided by the halo substituents.

Binding versus catalysis

In schematic terms, three phases can be envisaged in any pharmacological process, be it a pharmacodynamic or a pharmacokinetic one (Figure 6). To elicit an effect, a drug first penetrates into the biological system, be it a membrane, an organelle, a cell, an organ or an organism. Then it must bind to a biological target, usually a molecular ‘machine’ (enzyme, receptor, transporter, etc.). Finally, the biological ‘machine’ with bound ligand produces a biological response which will cascade to, e.g., an observable pharmacodynamic effect or a metabolic reaction [120]. Most significant here are the second and third phases of a biological response, which are considered below in terms of biotransformation reactions.

Indeed, enzyme kinetics allows a metabolic reaction to be readily decomposed into a binding and a catalytic phase. In spite of its limitations, *Michaelis–Menten analysis* offers an informative approach for assessing the binding and catalytic components of a metabolic reaction [121]. Here, the Michaelis constant K_m represents the *affinity*, whereas the *catalytic efficiency* is expressed by V_{max} or the turnover number k_{cat} .

When the results of Michaelis–Menten analyses are examined for quantitative structure–metabolism relationships (QSMRs), it is often found that lipophilicity correlates with K_m but not with V_{max} . The metabolic hydrolysis of esters of nicotinic acid in rat liver and brain subcellular fractions offers an illustrative example of this rule [122]. For 16 esters covering a broad range of structures (alkyl, cycloalkyl, functionalized alkyl or aryl esters), K_m and V_{max} values were determined in rat liver microsomes, mitochondria and cytosol.

No correlation with lipophilicity existed for the $V_{\max}$ response, whereas the K_m parameter was correlated to a lipophilicity parameter measured by RP-HPLC. The relations were parabolic, with r^2 values equal to 0.91, 0.82 and 0.96, respectively. In other words, lipophilicity was shown to have a major influence on the affinity of nicotinate esters for rat liver hydrolases, but to play no role in the subsequent, catalytic step. A comparable conclusion emerged from the QSMRs study of Martin and Hansch on the oxidation of drugs by rat liver microsomes [123,124]. Also, the oxidative deamination of homologous aliphatic amines by rat liver monoamine oxidase and rat aorta amine oxidase revealed bell-shaped relations between K_m and chain length, but no relation for $V_{\max}$ [125,126].

These facts are a clear indication that different molecular properties control binding affinity and susceptibility to catalysis. Because catalysis is characterized by the cleavage and formation of covalent bonds, the *catalytic step* must be controlled mainly, if not exclusively, by stereoelectronic properties of the substrates. An example of a QSMR study correlating electronic properties and catalytic parameters is provided by the glutathione conjugation of para-substituted 1-chloro-2-nitrobenzene derivatives [127]. The values of $\log k_2$ (second order rate constant of the non-enzymatic reaction) and $\log k_{\text{cat}}$ (enzymatic reaction catalyzed by various glutathione transferase preparations) were correlated with the Hammett resonance σ^- value of the substrates, a measure of their electrophilicity. Regression equations with positive slopes and r^2 values in the range 0.88 to 0.98 were obtained. These results quantify the influence of substrate electrophilicity on nucleophilic substitutions mediated by glutathione, be they enzymatic or non-enzymatic.

Given its spectral characteristics, cytochrome P450 (CYP) is of particular interest to investigate the *binding step* and the molecular factors that influence it. Indeed, substrate binding to CYP can be detected as a type I difference UV spectrum (peak at 385–390 nm, trough at ca. 420 nm) and quantified as K_s (in M units). This productive mode of binding is to be contrasted with unproductive ligand binding (peak at 425–430 nm, trough at about 390 or 410 nm) whereby a nitrogen or oxygen atom in the ligand interacts with the Fe(III) atom in the heme [128]. Of relevance here is the fact that the substrate binding mode to microsomal cytochrome P450 has repeatedly been shown to be related to lipophilicity. This is exemplified by an extensive study of the type I binding affinity of 50 model compounds to hamster liver microsomes. Linear relations were found between K_s and $\log D_{\text{oct}}^{7.4}$ [129]. These relations incorporated monocyclic hydrocarbons, bicyclic hydrocarbons, higher hydrocarbons, homologous carbamates, fatty acids, and fatty acyl methyl esters, respectively, with r^2 values in the range 0.90 to 0.99. The fact that all com-

pounds could not be fitted into a single regression indicates that size and shape influenced affinity in addition to lipophilicity.

Hydrophobic substrate binding of hydrocarbons to CYP has also been described by a partition model incorporating kinetic and thermodynamic aspects [130]. Using this approach, it was found that the type I binding of benzene, toluene, ethylbenzene, *n*-propylbenzene and *n*-butylbenzene to CYP was size-dependent and increased by -0.63 and -0.77 kcal/mol/ CH_2 group (liver microsomes from untreated and phenobarbital-induced rats, respectively) [131]. This value compared very well with the octanol/water partitioning of the substrates (-0.68 kcal/mol/ CH_2 group). In contrast, their microsomal/water partitioning was quite different and increased by -0.27 kcal/mol/ CH_2 group, indicating that the distribution of substrates between water and octanol is a suitable model for complex formation with CYP, but not for membrane partitioning. Further studies by the same group examined which component of the hydrophobic effect accounts for substrate binding to CYP. The results indicated that the predominant force involved in substrate binding is the ability of the active site of CYP (and many other enzymes) to draw the hydrocarbons from the aqueous medium (pull), rather than the compounds being dehydrated and expelled from it (push) [132].

Changes in lipophilicity resulting from metabolism

Another aspect of the relations between structure and metabolism that has received insufficient consideration is the compared lipophilicity of a drug and its metabolite(s). In other words, the focus in this approach is to assess the change in lipophilicity resulting from biotransformation [133].

The Darwinian rationale for the versatility of xenobiotic metabolism is the need for animals to excrete useless or harmful compounds with minimal expenditure of water (see above). In this perspective, all reactions of xenobiotic metabolism should yield metabolites of increased polarity and water solubility relative to the parent compounds. This rule is often, but far from always, verified. Indeed, a marked number of xenobiotics and metabolites fortuitously resemble endogenous compounds and become substrates of endobiotic-metabolizing pathways which yield *products of increased lipophilicity* [134]. Examples include:

- mono-O-methylation of catechols
- N-acetylation of basic primary amines (rare)
- esterification of some alcohols with fatty acids
- esterification of some carboxylic acids with cholesterol (few examples)
- esterification of some carboxylic acids with diacylglycerol to yield mixed triglycerides

- elongation of some carboxylic acids by two-carbon units (very few examples).

These examples pertain to conjugation reactions, and particularly lipid biochemistry.

Glucuronidation reactions produce metabolites whose lipophilicity is sometimes not as low as expected, as seen with the 3-O- and 6-O-glucuronide of morphine [41]. Another possibility (where lipophilicity studies are yet to be conducted) is the formation in humans of zwitterionic N-glucuronides from numerous basic tertiary amines such as (*N,N*-dimethyl)arylalkylamines, particularly CNS drugs [3]. Here, a protonated amine becomes a zwitterion – with an effect on log D that is difficult to predict [64]. This is to be contrasted with the O-glucuronidation of phenols and alcohols, which indeed yields metabolites of high polarity [135].

Reactions of oxidation are known to yield polar metabolites (alcohols, acids, etc.). Since a few years, systematic investigations are carried out to quantitate the changes in lipophilicity resulting from oxygenation, comparing sulfides with sulfoxides and sulfones [56], pyridines with their N-oxides [53], and recently (*N,N*-dimethyl)arylalkylamines with their N-oxides [136]. The conclusion of the latter study was that the protonated N-oxides had log P^C values that were quite comparable to those of the protonated parent amines. Because of the differences in basicity, the difference in distribution coefficients at physiological pH (log $D^{7.4}$) between a tertiary arylalkylamine and its N-oxide was 0.82 ± 0.66 ($n = 9$). The pharmacokinetic implication is that N-oxygenation may have a smaller effect on the urinary excretion of tertiary amines than usually assumed.

Also in reactions of oxidation, transformation of a basic amine into a zwitterion is sometimes possible. This is seen with some antihistamines which form active zwitterionic metabolites. For example, the first-generation antihistamine hydroxyzine yields a zwitterionic metabolite marketed as cetirizine. The lipophilicity profile of the two drugs is vastly different, resulting in major differences in the pharmacokinetic profiles and side effects [54].

Conclusions

This critical review of (mostly recent) studies concludes with some general statements which serve as summary and caveats:

- First and above anything else, there is ample qualitative and quantitative evidence that *lipophilicity is indeed a major determinant* in all pharmacokinetic processes which involve only low-energy interactions. These

processes are membrane and cell permeation (GI tract, skin, BBB, kidneys, etc.), reversible binding to circulating macromolecules and tissue constituents (blood and tissue storage), and productive binding to enzymes and transporters (metabolism and active transport).

- Statistically viable correlations between lipophilicity and pharmacokinetic responses imply that both terms (i.e. lipophilicity and the dependent variable) are mechanistically related. That is to say, that they result from a comparable *balance of intermolecular forces* (mainly hydrophobic and H-bonding interactions). When this balance is notably different, lipophilicity-derived parameters (e.g. $\Delta\log P$) may be more informative and lead to improved correlations (e.g., some cases of skin and BBB permeation).
- Drugs are not rigid, frozen molecular abstractions, but they fluctuate between a number of formal and functional states (e.g. conformational and electrical states) [13]. The preferred or average conformation of a solute appears to be less relevant here than the *amplitude of its conformational space*. Indeed, the larger this amplitude, the greater the propensity of a solute to adapt its lipophilicity to a changing environment (aqueous versus lipidic). Much remains to be understood on the pharmacokinetic significance of a ‘*chameleonic behavior*’ resulting from hydrophobic collapse and/or hydrophilic folding [41]. Also, dealing with such a chameleonic behavior in QSPkRs remains an open challenge.
- Another major challenge is the *fuzziness of biological constituents and their interactions with permeants*. As outlined above, pharmacokinetic responses result from a multiplicity of interactions with many different biological constituents such as macromolecules and membranes. These constituents display fluctuating property spaces. More importantly, they lack binding sites with high ligand specificity. As a result, different permeants in a series may interact differently with these biological constituents [13]. In such situations, global molecular parameters (e.g., lipophilicity, H-bonding capacity and polar surface area) are best apt to lead to decent correlations, provided they were obtained for biologically relevant conformers.
- Our last point is a *statistical warning*. Given the intrinsic fuzziness of pharmacokinetic responses and mainly the unavoidably *large experimental errors* in their determination, it is vain to expect QSPkR equations with standard deviations smaller than the experimental errors. Yet equations of this type sometimes do appear in the literature, with emphasis on their global character and predictive capacity. Closer inspection of such misleading equations usually reveals overfitting due to collinearity in ‘independent’ variables. Another defect is worse because better hidden,

namely that the values of some independent variables were themselves obtained from overfitted structure–property relations.

To close, we stress that pharmacokinetic lead optimization is more than ever a critical issue in drug design. This field, at the interface of physicochemistry, biology and informatics, holds infinite fascination for medicinal chemists. And that is the quintessential message of our review.

Acknowledgements

B.T. and P.A.C. are indebted to the Swiss National Science Foundation for support.

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