Chapter 34. The Use of Substituent Constants in Drug Design

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The subject of structure-activity relations in medicinal chemistry is almost boundless. Few papers are now published without some discussion of this problem. The work considered in this review is limited to that concerned with mathematical correlations between structure and activity.

The use of physical-chemical reference systems to serve as scales with which one could discuss in quantitative terms the relation between biological activity and chemical structure excited great interest at the turn of the century. C. Richet, J. Traube, H. Führner and especially H. Meyer and E. Overton gave thrust to this movement. It was observed, almost entirely in aliphatic systems, that as one increased the chain length in a homologous series a regular increase in a standard biological response occurred. Linear relations between a standard response and the number of carbon atoms in the chain, water solubility, surface tension lowering ability, vapor pressure and oil-water partition coefficients were often found in many different tests. An example of this type of correlation using partition coefficients coming from the work of Overton is:

$$\log 1/C = 0.858 \log P + 0.837 \qquad n = 28 \qquad r = 0.978 \tag{1}$$

In eq 1, C represents the molar concentration of various alcohols, ketones and esters producing isonarcosis in tadpoles, P is the octanol-water partition coefficient, n is the number of data used in finding the constants via the method of least squares, and r is the correlation coefficient. The constants in eq 1 are slightly different from those published[1] and the correlation is a little better because of the use of improved P values.

An important advance was made by Ferguson[2,3] who showed that the following generalization holds.

$$C_i = kA_i^{1/n} \tag{2}$$

In eq 2, C_i is the concentration of the i^{th} member of a series producing an equivalent response, k and n are the constants, and A_i is a physical-chemical distribution constant of the above mentioned kind. Ferguson pointed out that for nonspecific narcotics in states of equilibrium, biological activity is related to thermodynamic activity. Ferguson's approach was extended by Brink and Posternak[4] and it is now evident that there are many instances where equal degrees of narcosis are caused by molecules having equal thermodynamic activities. Other approaches to explaining the effect of narcotic activity in terms of physical-chemical parameters are those of McGowan[5-8] and Mullins[9]. McGowan using parachor and Mullins using Hildebrand's solubility parameter and molal volume, have

assembled evidence to support the view that the molecular volume of the narcotic is of critical importance in determining narcosis.

Pauling[10] and Miller[11] have suggested that anesthetic potency is related to the ability of anesthetics to form hydrates. Miller, Paton and Smith[12] and others[13] have criticized the Pauling-Miller view.

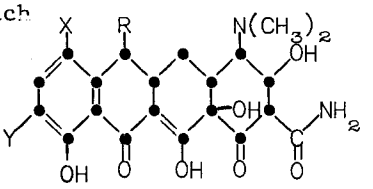
Agin, Hersh and Holtzman[14] have shown that eq 3 gives an excellent correlation for the relation between minimum blocking concentration (MBC) of 39 local anesthetics in frog sartorius muscle with polarizability (α) and the ionization potential (I) of the drugs.

$$\log (MBC) \propto \alpha I \qquad (3)$$

Zahradnik[15], in a systematic study using mice and carefully controlled conditions, developed a set of constants for alkyl groups from the linear Hammett-like postulate that $\log (\tau_i/\tau_{Et}) = \alpha\beta$ where τ_i represents the molar concentration of the i^{th} member of a set of aliphatic congeners producing a standard biological response and τ_{Et} is the concentration of the ethyl derivative. β is a constant characteristic of the substituent (R of RX) and α is a constant characteristic of the system. The constant β was determined for 25 R groups and α was evaluated for 39 different biological systems. While this single parameter approach works well for inhibitory studies of homologous groups of aliphatic compounds where highly specific electronic and steric effects are not critical, it has not been extended to more complex systems.

The above ideas have provided insight into nonspecific biological inhibitions; however, this knowledge has not been of great help in the design and modification of drugs of high specificity. Two approaches have been emerging in recent years to supplement the medicinal chemists' intuition on the effect of substituent variation on drug activity. In one aproach de novo substituent constants have been derived by simply finding the "best numbers" to go with given substituents on a particular drug acting in a standard test. The other approach has used substituent constants derived from model nonbiological systems. The first work on the former of these two tracks appears to be that of Bruice, Kharasch and Winzler[16] working with thyroxine derivatives. Their approach has been stated in more general terms by Free and Wilson[17]. The method can be illustrated with their example for a set of tetracycline (I) analogs. The relative biological activity for each derivative can be formulated as the linear combination of the contribution of each of the groups represented by X, Y and R:

$$\text{Biological activity} = \mu + a[X_i] + b[Y_i] + c[R_i] \qquad (I)$$



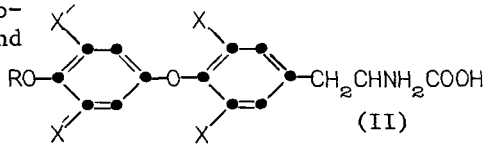
One can write a set of simultaneous equations, one for each compound tested, the solution of which gives the contribution to the activity for each $a[X_i]$, etc. Table I lists the results for the tetracyclines.

Table I

R		X		Y		
a[H]	75	b[Cl]	84	c[NH_2]	123	
a[CH_3]	-112	b[Br]	-16	c[CH_3CONH]	18	μ = 161
		b[NO_2]	-26	c[NO_2]	-218	

Of the total of 18 possible derivatives resulting from different combinations of R, X and Y, 10 had been tested. Presumably, activities for the other eight can be calculated from the substituent constants in Table I. The method is of greatest value when a large group of derivatives and functions is involved. There are of course many objections that come to mind in considering the above technique. Modern chemistry has long recognized the great importance of steric and electronic effects of substituents on rate and equilibrium processes[18]. From the Meyer-Overton work and from more recent studies[19-25] the hydrophobic bonding power of functional groups is seen to be a very important substituent effect. These three effects are lumped into each of the constants under R, X and Y of Table I. Hence it would seem that in general, constants derived for one set of congeners will not be useful for another set causing a different biological response. Nevertheless, this empirical method of deriving constants for the relative effects of chemical groups on biological activity is bound to be more helpful in the long run than unguided intuition. Purcell[34] has been studying the Free-Wilson method.

Some information is already in hand to show that such empirical constants are related to more fundamental parameters. Cilento and Berenholc have shown[26] that there is a good linear correlation between f(X) obtained for[16] thyroxine analogs (II) and the negative logarithm of the lifetime of the phosphorescent state (equivalent to the triplet to singlet transition in naphthalene analogs) for the five functions where X = H, CH_3, Cl, Br, I. They conclude that the strong $T \leftrightarrow S$ transition in diiodotyrosine and thyroxine derivatives makes these compounds very efficient in the transfer of triplet-state energy. Using the substituent σ for the electronic effect and π for the hydrophobic effect[27] of substituents, it is seen from eq 4 that f(X′) derived by Bruice et al. is in fact related to these more fundamental constants.



(II)

$$f(X') = 0.308\pi - 0.564\sigma - 1.672 \qquad n = 7 \qquad r = 0.934 \qquad (4)$$

It has been shown[28] that π and σ can be used to rationalize the SAR of thyroxine analogs and Jorgensen[29] has tested the predictive value of the use of σ and π with a tert-butyl analog of thyroxine. It has also been shown[30] that Zahradnik's β constants are linearly related to π.

Kopecký and Boček[31,32] have also been studying the utility of empirical substituent constants in an investigation of the toxicity of disubstituted benzenes. In their equation (5) they have used an interaction

term of the type discussed by Miller[33] for the linear combination of terms in the formulation of mathematical models.

$$\log \frac{[LD_{50}]HH}{[LD_{50}]XY} = bX + bY + eXeY \qquad (5)$$

In eq. 5, HH represents benzene and XY a disubstituted derivative. Quite good correlations were obtained using eq 5 for nonspecific toxicity. The constant bX is stated to be linearly related to f(X′) of Bruice et al.

Purcell[36], following this general approach, has studied amides which are inhibitors of cholinesterase. Purcell[35,36] and co-workers have also investigated a variety of physical chemical parameters to rationalize the SAR for these inhibitors.

After the rather intense work on the correlation of biological activity with partition coefficients of the early years of this century had reached a standstill, new hope for rationalizing the biological activity of organic compounds appeared in the work of Hammett[37]. The Hammett equation and other variations of it[18,38] have proved to be very successful in correlating chemical reactivity with the electronic or steric effects of substituents for the reactions of organic compounds in homogeneous solutions. Although a good many attempts have been made[39-47] to apply the Hammett equation to biochemical systems, the results have, with few exceptions, been disappointing. Such an exception is seen in the enzymatic hydrolysis of phenyl sulfates[39] from which eq 6 results[24].

$$\log 1/K_m = 0.930\sigma + 2.522 \qquad n = 10 \qquad r = 0.931 \qquad (6)$$

In eq 6, K_m is the Michaelis constant. Good correlation with σ was also obtained with V_{max}. The best linear relations with σ have been found using more or less pure enzymes. The lack of success with σ in biochemical systems has generally been attributed[48] to steric interactions of substituents with the enzyme or lipoprotein membranes. Recent work[21-25] would indicate that while steric interactions are extremely important, the concept of lock-and-key fit of enzyme and substrate has been over-emphasized at the expense of hydrophobic bonding. The importance to the medicinal chemist of the more flexible character of enzymes which is emerging from the work of Koshland[49] and others[50] has been analyzed by Belleau[51].

A most important new concept for the designer of drugs is that of the hydrophobic bond[19,20,52]. The view of Hansch and co-workers is that if a suitable parameter can be formulated for this, then by means of the well known constants[18] σ, σ^+, σ^-, σ^*, and E_s, many of the powerful tools of physical organic chemistry might be brought to bear on medicinal chemical problems. To this end octanol-water partition coefficients (P) have been studied as a reference standard[1,24,27]. The additive-constitutive nature of log P and π ($\pi = \log P_X - \log P_H$ where P_H refers to a parent molecule and P_X to a derivative) means that from a relatively few values of log P, many others can be calculated[1,24,27,53,54]. The usefulness of these parameters for measuring binding of neutral molecules with proteins is illustrated in eq 7-9.

$$\log 1/C = 0.71\ \log P + 1.51 \qquad n = 17 \qquad r = 0.950 \qquad (7)$$

$$\log 1/C = 0.58\ \log P + 2.40 \qquad n = 4 \qquad r = 0.961 \qquad (8)$$

$$\log 1/C = 0.68\pi + 3.48 \qquad n = 19 \qquad r = 0.962 \qquad (9)$$

In eq 7-9, C is the molar concentration of compound necessary to produce a 1-to-1 complex with bovine hemoglobin[21] (eq 7) and bovine serum albumin (eq 8 and 9). Equation 7 correlates the binding of 17 miscellaneous compounds (e.g., phenols, anilines, naphthalene) and eq 8 correlates the binding of 4 barbiturates[24]. In eq 9, the comparative constant π is used[55] to correlate the binding of phenols by serum albumin. The slopes of the three equations are surprisingly close, indicating that the binding of neutral molecules to two different proteins can be quantitatively defined using log P or π as hydrophobic bonding constants. For these situations no highly specific steric or electronic parameters are necessary to rationalize the results.

Going to the next more complex situation, that of enzymic reactions, it can be shown that the linear combination of π and σ can account for the substituent effects in the hydrolysis of phenyl glucosides by emulsin[56]. Using regression analysis, eq 10-13 are derived to illustrate how one can factor substituent effects on a biochemical reaction.

Para Groups

$$\log K_e = 0.52\sigma + 2.03 \qquad n = 8 \qquad r = 0.753 \qquad (10)$$

$$\log K_e = 0.33\pi + 0.620 + 1.80 \qquad n = 8 \qquad r = 0.921 \qquad (11)$$

Meta Groups

$$\log K_e = 0.95\sigma + 1.63 \qquad n = 6 \qquad r = 0.949 \qquad (12)$$

$$\log K_e = 0.12\pi + 0.96 + 1.59 \qquad n = 6 \qquad r = 0.963 \qquad (13)$$

K_e represents the equilibrium constant for the enzyme substrate complex. Comparison of eq 10 and 11 for the para derivatives indicates that complex formation depends on both electronic and hydrophobic factors. The correlation coefficient is much better and statistically quite significant for eq 11. The positive signs of the coefficients for π and σ indicate that lipophilic and electron-attracting groups promote complex formation. Comparing eq 12 and 13, one finds that for meta isomers hydrophobic bonding is apparently not possible. No significant improvement in correlation results on the introduction of the π term. A similar set of equations was derived[56] to show the substituent effect on the hydrolysis rate constant k_3. Equally good correlations were obtained except in this step; as one might expect, the coefficient with π has a negative sign. This indicates that hydrophobic bonding slows down desorption of the cleaved products. Since good correlations were obtained in eq 11 and 13 without the use of steric constants, it is assumed that these are unimportant, at least for functions as large as those studied.

The meaning of the words steric and electronic in these discussions is somewhat ambiguous. For example, the linear relations between parachor and toxicity found by McGowan or the use of the solubility parameter δ by Mullins could be viewed as indicating a direct relation between the size of the substituent and its ability to produce a given biological response. The affinity of an apolar group for a lipid phase will also be a function

of its size and, to a lesser extent, its shape. Thus it is not always easy, even in the abstract, to separate the binding role of hydrophobic bonding by an apolar function from its role of distorting a hydrophobic region by its size. We are attempting to use hydrophobic bonding as defined by partition coefficients as simple holding of the drug to the active site, realizing that often this role cannot be separated from the conformational change the apolar group will, in the binding process, produce in an enzyme or membrane. Steric effects, then, are those due to size or arrangement in space of substituents which cannot be accounted for by the hydrophobic constants log P and π. These will be both intra- and intermolecular in nature. In the same way, electronic effects will overlap with hydrophobic bonding since the position of equilibrium in the distribution of a drug between phases will be a function of its electronic structure. Indeed, it has been shown[27] that π varies with σ. However, this variance is not great if the substituents are separated by one or more atoms. The term electronic effects means highly specific effects in general not associated with the partitioning process. These would be effects involved in a chemical reaction or charge transfer process where a change in electron density too small to make a significant difference in log P could cause a large change in a rate or equilibrium constant. Using these somewhat arbitrary divisions of substituent effects and regression analysis, a start can be made in separating the effects of groups of atoms on the biological activity of a set of congeners. Hansch and co-workers have not, for example, attempted to factor out hydrogen bonding. In general they have worked with systems where this could be accounted for in terms of σ and π. Purcell et al. have suggested a way of dealing specifically with this term[36].

In addition to many early examples[15], biological response has been found to be quantitatively linearly dependent on log P or π in the inhibition of the Hill reaction[57,58] activity of penicillins[59], toxicity of benzoic acids to mosquito larvae[28], phenol coefficients[28], cholinesterase inhibitors[35], and catechol-amine activity[60].

Hemker[61] was one of the first to attempt the quantitative correlation of biochemical response using both partition coefficients and ionization constants to account for the uncoupling action of phenols. The linear combination of π and σ has been found to hold for several enzymic reactions[22,56] as well as the binding of phenols by protein[55], the toxicity of phenols[62], the uncoupling action of phenols[62], and the relative sweetness of nitroanilines[23]. An interesting application is that of McMahon[63]. Equation 14 was formulated for the enzymic reduction of aromatic ketones to alcohols.

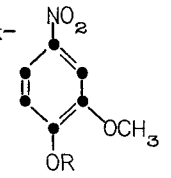
$$\log V_{max} = 0.334\pi + 1.239\sigma + 0.824 \qquad n = 10 \qquad r = 0.89 \tag{14}$$

Of course the most interesting and difficult drug studies are those in which whole organisms are involved. It has been postulated[64] that in general, for these studies one would expect a parabolic relation between log 1/C and log P. This has led to the development[28,65] of eq 15.

$$\log 1/C = -k(\log P)^2 + k' \log P + \rho\sigma + k'' \tag{15}$$

In the development of this model a probabilistic view is taken for movement of drug from the exobiophase or point of injection to the sites of action. It is assumed that as $P \to 0$ drugs become more and more isolated in the water phase and eventually become unable to cross lipid barriers. As $P \to \infty$ the reverse is true. Somewhere between $P=0$ and $P=\infty$ there will be an ideal value (P_o) for a given set of congeners in a given biological system such that those members having this value will find the sites of action via a random walk process in the minimum time. This assumes steric and electronic (pK_a, etc.) factors are constant. In other words, a greater number of molecules of drug with P_o would reach the sites of action in the test interval than drugs having other P values. In effect, one expects a change in the mechanism of movement in a set of drugs having a sufficient spread in P values. The movements of the lower members of the series will be mostly determined by interactions with water, while those of the higher members will be determined by hydrophobic interactions with lipids. Such parabolic relations may even be found in closed systems of the type used in narcosis studies. Ferguson[66] has stated that probably in most experiments on narcosis a complete equilibrium is never reached in the period of exposure and the results are time dependent. Results will be even more time dependent in open systems such as whole animals where elimination and biotransformation are continuous processes. Such results can even be expected in tissue experiments or work with partially purified enzymes. Since activity is usually expressed as log 1/C, the most active compounds tested are often in very low concentration, sometimes less than 10^{-6} M. While log 1/C may be linear with respect to log P at higher concentrations eq 6-8 indicate that highly lipophilic molecules will be very tightly bound to proteins so that true equilibrium is not reached in test time. The lower the test concentrations become, the more likely the departure from linearity through localization of molecules in particularly lipophilic material.

Of course there are reasons other than binding by lipids or proteins which might cause a departure from linearity in the relationship of log 1/C and log P or π. Metabolic or elimination reactions not significant at low values of log P could, with increasing log P, become very important. A good example of this is in the metabolism of alkylaryl ether in rabbits [67]. In (III) when R is ethyl or methyl, dealkylation is the main reaction. When R is propyl or butyl, ω-1 hydroxylation becomes more important. Since the rate of metabolism of drugs may be linearly related[68] to log P, this may be an important contributing factor for the parabolic dependence of activity on lipophilic character. Another unknown is the lipophilic space at the site of action available for the hydrophobic moiety of the drug. If this is quite limited, then a point is soon reached where log 1/C and log P are no longer linearly associated. The probabilistic nature of the log P terms in eq 15 does a good deal to insure a reasonable fit of a set of data as long as individual congeners in the set do not depart radically from the behavior of those having similar lipophilic character. In principle, loss through metabolism or elimination is not different than loss through binding if these losses depend only on log P. Good results have

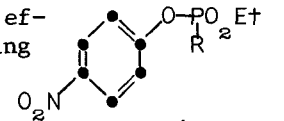


(III)

been obtained with eq 15 for plant-growth regulators[65], chloromycetin analogs[65] (Garrett[69] and colleagues have found a more linear dependence of chloromycetin activity under different test conditions), thyroxine analogs[28], phenol coefficients[28], carcinogenicity of aromatic compounds[28], and the localization of benzeneboronic acids in brain and tumor tissue[70].

A constant which may prove to be very useful in drug design is log P_o (or π_o). This figure can be found by taking the partial derivative of eq 15 and setting this equal to zero. Once this has been established for a set of drugs, it becomes a useful bench mark from which to start the design of a completely new drug to act on the same sites. For example, it was found that log P_o for phenoxyacetic acids acting as plant-growth regulators is 2.03. Log P_o for phenylacetic acids acting in the same system is 2.47. If one wished to design a new acid to act in this system, one of the features which one would design into the first test molecule would be log P of about 2.2. By means of the additive-constitutive character of log P one could design such compounds on paper without carrying out extensive partition coefficient studies.

While suitable techniques to handle steric effects between substrate and the material comprising the sites of action are at present out of reach, intramolecular steric interactions can be handled quantitatively, at least in some instances using Taft's E_s parameter[22,56]. Equations 16 and 17, formulated from the work of Metcalf and Fukuto correlate[22] substituent effects of R in (IV) on the inhibition of cholinesterase by alkylphosphonic acid esters.



(IV)

$$\log K = 3.74\ E_s + 7.54 \qquad n = 13 \quad r = 0.901 \qquad (16)$$

$$\log K = -1.68\sigma^* + 0.15\pi + 4.05\ E_s + 7.21 \qquad n = 13 \quad r = 0.907 \qquad (17)$$

Comparison of eq 16 and 17 indicates that neither electronic nor hydrophobic factors play an important part in the inhibition. Thus it appears that the phosphonates interact with the enzyme in such a way that they cannot come in contact with a hydrophobic region of the protein. The range of electronic forces covered by the R groups was small so that σ^* may be more important than eq 17 would indicate. Some overlap between E_s and σ^* constants tends to obscure the role of σ^*. The fact that E_s, a constant derived from hydrolysis studies under homogeneous conditions, should apply to heterogeneous catalysis is surprising and indicates much more use for this parameter than one would have had reason to expect.

Another method for obtaining biochemical substituent constants is available in the various forms of chromatography. Considerable effort[71-73] has been made to gather R_M and ΔR_M values for the effect of substituents on R_F.

$$R_M = \log(1-R_F)/R_F \qquad \Delta R_M = R_{M_X} - R_{M_H} \qquad (18$$

In eq 18, R_{M_H} is calculated from the R_F value of a parent compound and R_M from that of a derivative having substituent X. ΔRM is a constant analogous to π and in fact a close correlation exists between the two as is

shown[1] in eq 19.

$$\pi = -1.103\Delta R_M + 0.647 \qquad n = 12 \qquad r = 0.970 \tag{19}$$

In measuring the ΔR_M values in eq 19, Green and Marcinkiewicz[71] used trigol and diisopropyl ether in reversed-phased, tankless flat-bed chromatography. Boyce and Milborrow[74] have shown that R_M values correlate the molluscicidal activity of a series of N-n-alkyltritylamines assuming a parabolic reaction exists between log LD_{50} and R_M. Thus R_M or ΔR_M values can be used in eq 15 in place of log P or π.

Considerable effort[35,75-80] has been made to understand biochemical and pharmacological problems using quantum mechanical calculations of electron densities with molecules. While this has been useful in explaining how a particular bond is broken or made[75], linear relations between calculated electron densities and biological activity are notably lacking. An exception coming from the work of Fukui is illustrated in eq 20 and 21 which correlate the nicotine-like activity of meta derivatives of $C_6H_5OCH_2CH_2\overset{+}{N}(CH_3)_3$ with the superdelocalizability ($S_o^{(N)}$) of electrons at the ortho position and the frontier electron density ($f_{oxy}^{(E)}$) on the ether oxygen.

$$\log A = 13.742\ S_o^{(N)} - 10.465 \qquad n = 6 \qquad r = 0.994 \tag{20}$$

$$\log A = 30.392\ f_{oxy}^{(E)} - 20.924 \qquad n = 6 \qquad r = 0.949 \tag{21}$$

The combination of such electronic densities with a hydrophobic bonding constant does yield good results[28,56] as illustrated[56] by eq 22 and 23.

$$\log A_X = 22.91\varepsilon - 42.49 \qquad n = 6 \qquad r = 0.685 \tag{22}$$

$$\log A_X = 0.29\pi + 18.16\varepsilon - 33.82 \qquad n = 6 \qquad r = 0.995 \tag{23}$$

In the above equations A_X, from the work of Jacobson, represents the relative rates of acylation of aromatic amines having substituents X by means of pigeon liver acetyl transferase. ε represents the calculated electron densities on the nitrogen atom made by Perault and Pullman. Equation 22 accounts for only 47% of the variance in the data while eq 23 accounts for 99%. Thus it appears that quantum mechanically obtained electron densities can be extremely useful to the medicinal chemist. In this way the relative electron density on each atom in the molecule can be found and through regression analysis the relative importance of this density at one or more points in the molecule can be evaluated. This approach offers, in principle, a great advantage over the use of σ.

What are the guidelines that substituent constant analysis has to offer the designer of drugs? The great success of this method in homogeneous organic reactions[18] and the more modest achievements of this method with heterogeneous biochemical reactions would seem to validate the use of eq 24 and 25 as a reasonable working hypothesis.

$$\Delta F^\circ_{BR} = \Delta F^\circ_{L/H} + \Delta F^\circ_{elect} + \Delta F^\circ_{steric} \propto \log k_{BR} \tag{24}$$

In eq 24 we are assuming[57] that the ultimately measured biological response (toxicity, resistance to a metabolic process, ED_{50}, elimination,

etc.) is governed by one rate-limiting process for which k_{BR} is a rate or equilibrium constant. In eq 24, $\Delta F^{\circ}_{L/H}$ represents that portion of the free energy change which can be attributed to hydrophobic bonding, ΔF°_{elect} represents an electronic component, and $\Delta F^{\circ}_{steric}$ represents highly specific spatial demands of reactants and products on the free energy change. Of course, were sufficient data available, one might profitably factor each of the terms in eq 24 and 25 into several to represent suspected critical parts of a molecule. Substituent effects on log k_{BR} of eq 24 are represented in eq 25.

$$\delta_X F^{\circ}_{BR} = \delta_X F^{\circ}_{L/H} + \delta_X F^{\circ}_{elect} + \delta_X F^{\circ}_{steric} \propto \delta_X \log k_{BR} \qquad (25)$$

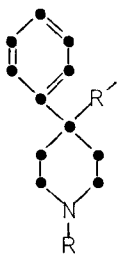
Equations 24 and 25 were formulated for the case where the drug is at the site of action or the situation where true equilibrium with the exobiophase is established as envisaged by Ferguson.

From the present limited work it would appear that $\delta_X F^{\circ}_{L/H}$ might be represented by log P, π, R_M, ΔR_M, β (Zahradnik[15]) and, under certain conditions, parachor. The $\delta_X F^{\circ}_{elect}$ term may be represented using the various forms[18] of σ, quantum mechanically calculated electron densities or chemical shifts[81] obtained via NMR. It should also be possible to formulate suitable constants from IR and UV spectra. Attempts to use polarographically-obtained constants do not seem to be as useful as one might expect[82,83].

The case where substituent changes result in large differences in ionization is one of great importance since so many drugs are either weak acids or weak bases. The degree of ionization has long been recognized as playing a part in drug activity[84]. In a careful analysis, Fujita[62] has shown how substituent effects on ionization should be separated from other electronic effects of substituents.

It does not seem possible to make any general observations about suitable ways of representing $\delta_X F^{\circ}_{steric}$ for the interactions between a drug and its receptors. By considering sets of congeners in which gross steric changes are avoided (e.g., considering D isomers separately from L isomers, etc.) useful correlations can be made. In fact, where the two-parameter equation can be shown to hold over a reasonable range of substituents, one can make deductions about $\delta_X F^{\circ}_{steric}$ by continuing to increase the size of X until the two-parameter equation fails. In this way one can, to a limited extent, map the free space around a receptor site.

Portoghese[85-87], in an extension of the approach of Zahradnik, has shown that linear relationships between different congeneric sets of analgesics can be used to make more firm decisions about whether the sets are acting in the same three-dimensional way on the same receptors. The three sets of congeners in which R of V was held constant (i.e., 1. R′ = CO_2Et, 2. R′ = OCOEt and 3. R′ = $OCOCH_3$) were varied by changes in R. Least square fits of sets 1 vs. 2, 1 vs. 3, and 3 vs. 2 gave



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slopes close to 1 with good correlation coefficients. Such tests can be used to help establish the fact that, for example, R in each series is in the same physicochemical environment on the receptors. By exploring a large enough number of sets of systematically varied congeners one could obtain considerable information about the geometry of the receptor sites.

The fact that simply changing the length of R can lead to a complete change in the mechanism of action (e.g., agonist to antagonist) has been thoroughly documented by Ariëns[88]. A careful case study in which the enthalpic and entropic roles of the substituents in transition from agonist to antagonist have been considered has been made by Belleau, Tani and Lie[89]. Miller and Hansch[25] have presented evidence to show that when two hydrophobic areas are present in a drug and only a limited space for hydrophobic bonding exists in an enzyme, the more hydrophobic of the two groups may determine the configuration of binding.

Thus steric interactions of receptor site and substrate are extremely difficult to evaluate. The conformational perturbations of drug on receptor and receptor on drug will tax to the limit our ability to untangle the mechanism of drug action for many years to come. However, the use of substituent constants and large computers for regression analysis offers us new hope denied workers a few years ago.

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