An Analysis of Biological Linear Free-Energy Relationships

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Further development of equations based on a quantitative form of the principle of hard and soft acids and bases leads directly to a physical interpretation of the linear free-energy expressions developed by Hansch and coworkers for biological systems.

In an earlier report[1] an initial attempt was made to place many of the current physicochemical approaches to the study of drug action into a general frame of reference. The approach taken was restricted to a consideration of drug–receptor interactions, as is the present one, and drug response was postulated as being directly related to the free energy for the formation of a pharmacologically important drug–receptor complex. Following a procedure outlined by Leffler and Grunwald,[2] the essential free-energy change was, in first approximation, separated into independent electronic ΔG^e, desolvation ΔG^d, and steric ΔG^s components.

$$A_n = \Delta G^e + \Delta G^d + \Delta G^s + k \qquad (1)$$

In this expression, the free-energy component due to possible conformational changes of a given receptor substance is included in the constant k along with all interaction terms. The subscript n designates that for a series of N compounds there will be a set of n equations, *i.e.*, $N = 1, 2, \ldots, n$.

Based on recent theories of chemical reactivity[3,4] and desolvation,[5] each interacting pair of atoms s and r associated with drug and receptor, respectively, could be said to contribute independently to the electronic and desolvation free-energy components. By analogy with the Madelung constant used in crystallography,[6] and because of the assumption of constant receptor conformation, each atom of a drug was assumed capable of being assigned a constant characteristic of its location in the essential drug–receptor complex. Thus, the respective terms in eq 1 become

$$A_n = \sum_r \sum_s (E_{rs} + L_r + V_s) + k \qquad (2a)$$

The electronic component in eq 2a then can be further separated into ionic E_{rs}^I and nonionic E_{rs}^C contributions. Similarly, the desolvation component can be separated into two parts identified with the desolvation of drug atoms L_s and receptor atoms L_r. An alternative form of eq 2a is then

$$A_n = \sum_r \sum_s (E_{rs}^I + E_{rs}^C + L_s + L_r + V_s) + k \qquad (2b)$$

Employing the definitions of Klopman and Hudson,[3,4] charge-controlled interactions may be identified with the condition $E_{rs}^C = 0$ and $L_{rs} = L_s = L_r = 0$, and eq 2 can be written

$$A_n = \sum_r \sum_s (E_{rs}^I + V_{rs}) + k \qquad (3)$$

At the other extreme, frontier-controlled interactions may be identified with the condition $E_{rs}^I = 0$, and eq 2 can be written

$$A_n = \sum_r \sum_s (E_{rs}^C + L_s + V_{rs}) + k' \qquad (4)$$

where for a common receptor substance the desolvation contributions due to the interacting receptor atoms are contained in k'.

Since the ionic, nonionic, and desolvation components contained in eq 2 can be estimated using molecular orbital (MO) methods in the linear combination of atomic orbital (LCAO) approximation,[3-5] it will be found convenient, for the purposes of this analysis, to convert eq 2 into these terms and to neglect steric factors.

Three forms of the general LCAO–MO equivalent to eq 2 are convenient to work with when considering drug–receptor interactions.[1] If the receptor substance can be considered similar to a semiconductor, at least with regard to the narrowness of the energy band widths for its filled and empty MO's (*cf.* ref 7 and 8), then $E_{rs}^I \neq 0$ and $E_{rs}^C \neq 0$ and for this case eq 2 becomes

$$A_n = \sum_s (aq_s + \xi S_s^{(E)} - \xi' S_s^{(N)} + \lambda q_s \pm \lambda' q_s^2) + k' \qquad (5)$$

In this equation, the first term may be identified with E_{rs}^I, the next two terms with E_{rs}^C, and the last two terms with L_s.[9] The variables for each atom are the net charge q and the electrophilic and nucleophilic superdelocalizability[10] $S^{(E)}$ and $S^{(N)}$.

For the charge-controlled case, $E_{rs}^C = 0$ and $L_{rs} =$

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(2) J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1963.
(3) G. Klopman and R. F. Hudson, *Theoret. Chim. Acta*, **8**, 165 (1967).
(4) G. Klopman, *J. Am. Chem. Soc.*, **90**, 223 (1968).
(5) G. Klopman, *Chem. Phys. Letters*, **1**, 5 (1967).
(6) G. M. Barrow, "Physical Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1966.
(7) O. Chalvet, *J. Theoret. Biol.*, **3**, 51 (1962).
(8) B. Pullman and A. Pullman, "Quantum Biochemistry," Interscience Division of John Wiley and Sons, Inc., New York, N. Y., 1963, p 312.
(9) In practice, eq 5 is evaluated in the form

$$A_n = \sum_s (\alpha q_s + \xi S_s^{(E)} - \xi' S_s^{(N)} \pm \lambda' q_s^2) + k'$$

where

$$\alpha = a + \lambda$$

It is thus seen that in the absence of a q^2 term desolvation effects are implicitly taken into account, since the coefficient α consists of terms characterizing the interacting receptor atoms a and the desolvation of the drug atoms λ with which they interact.
(10) K. Fukui, T. Yonezawa, and C. Nagata, *J. Chem. Phys.*, **26**, 831 (1957).

$L_s = L_r = 0$, and eq 2 becomes

$$A_n = \sum_s aq_s + k \quad (6)$$

while for the frontier-controlled case $E_{rs}^{I} = 0$ and eq 2 becomes

$$A_n = \sum_s (bc_s^{(E)} + b'c_s^{(N)} + \lambda q_s \pm \lambda' q_s^2) + k' \quad (7)$$

where $c^{(E)}$ and $c^{(N)}$ are the frontier orbital coefficients for an atom of a drug.

In this report, we will investigate certain of the implications provided by eq 5–7 with regard to the linear free-energy equation developed by Hansch and coworkers.[11,12]

Results and Discussion

In operator form,[2] eq 1 is given by

$$\delta A_n = \delta(\Delta G^e) + \delta(\Delta G^d) + \delta(\Delta G^s) + \delta k \quad (8)$$

Following Hansch,[13] the drug–receptor interactions for a congeneric or homologous series of drugs could be investigated using the equation obtained upon performing the indicated operation on eq 8. In eq 9, σ, π, and E^s are the Hammett,[14] Hansch,[11] and Taft[15]

$$\delta A_n = \bar{a}\sigma + \bar{b}\pi + \bar{c}E^s + d \quad (9)$$

constants defined, respectively, to measure electronic, hydrophobic, and steric effects associated with a substituent when taken relative to a standard substituent.

Because of the equivalence of eq 1 and 2, a similar operation performed on eq 2 should enable eq 9 to be analyzed in greater detail. The merits and faults of the quantities σ and π are well known,[2,13,16–18] and the prediction of certain of these is offered as partial justification for the present approach. Steric effects will be neglected, however, in the subsequent discussion.

In operator form, the three special cases of eq 2 can be written. The equations are given in the manner

$$\delta A_n = \sum_s [\delta(aq_s) + \delta(\xi S_s^{(E)}) - \delta(\xi' S_s^{(N)}) + \delta(\lambda q_s) \pm \delta(\lambda' q_s^2)] + \delta k' \quad (10)$$

$$\delta A_n = \sum_s \delta(aq_s) + \delta k \quad (11)$$

$$\delta A_n = \sum_s [\delta(bc_s^{(E)}) - \delta(b'c_s^{(N)}) + \delta(\lambda q_s) \pm \delta(\lambda' q_s^2)] + \delta k' \quad (12)$$

shown to facilitate an identification of terms (see below).

If the coefficients in these expressions can be considered constant throughout a congeneric or homologous series of drugs, or at least if some average value can be used for the series, they will not be affected by the indicated operation. On the other hand, the MO quantities associated with the drugs are affected by the operation, and those quantities which refer to the compound chosen as standard can be incorporated into the terminal constant. Equations 10–12 thus afford relations which differ from eq 5–7 only in the value of the terminal constant.

$$\delta A_n = \sum_s (aq_s + \xi S_s^{(E)} - \xi' S_s^{(N)} + \lambda q_s \pm \lambda' q_s^2) + d'' \quad (13)$$

$$\delta A_n = \sum_s (aq_s) + d' \quad (14)$$

$$\delta A_n = \sum_s (bc_s^{(E)} + b'c_s^{(N)} + \lambda q_s \pm \lambda' q_s^2) + d'' \quad (15)$$

Within a congeneric series of drugs, if the values for the electronic properties of atoms common to each drug could be assumed constant, then those terms in eq 13–15 (or 5–7) which refer to the common atoms could be included in the terminal constant. The criterion required by this assumption should be most closely followed when considering aliphatic substances or the σ framework of conjugated compounds, but for π systems the electronic properties of certain atoms of the drug nucleus should be subject to some variation depending on the conjugative behavior of the substituents (*cf.* ref 19). To maintain the form of eq 13–15 (and 5–7), it will therefore be assumed that *average* values may be used in place of "real" values when considering the electronic properties of a π system common to each drug. Now, for σ and π systems, the summation in eq 13–15 (and 5–7) is taken only over the atoms of the substituent and the atom of the drug nucleus to which it is attached.

Within the limitations imposed by the above assumptions, eq 13–15 (and 5–7) may be regarded as presenting relationships that should exist between the biological activities of congeneric series of drugs and certain electronic properties of the substituents attached to the drug nucleus. Certain compounds may deviate from the behavior expected of them, but the present analysis suggests that deviations are most likely to occur when considering conjugated compounds. In particular, when the conjugative behavior of a substituent is sufficiently different from that of other substituents so as to require the electronic properties of the atoms making up the drug nucleus to be given in terms other than what might be considered as average, then significant deviations are expected. In more familiar terms, when multiple substituents undergo a mutual interaction, as, for example, in *p*-nitrophenol, the substituent properties are no longer additive.[2,13,16–18]

Since eq 13–15 are expressed with regard to properties of substituents, a comparison of the terms in these equations with corresponding terms in eq 9 may now be made. While eq 13 may be considered representative of the form a general relation must take under the conditions $E_{rs}^{I} \neq 0$ and $E_{rs}^{C} \neq 0$, it is plain that for practical purposes desolvation effects cannot be treated explicitly (see ref 9). There is, however, an alternative form that might be considered in which the

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(13) C. Hansch, "Annual Review of Medicinal Chemistry," C. K. Cain, Ed., Academic Press, New York, N. Y., 1967.
(14) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940; for a compilation of values see H. H. Jaffé, *Chem. Rev.*, **53**, 191 (1953).
(15) R. W. Taft, "Steric Effects in Organic Chemistry," M. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956.
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conditions associated with a general relationship are at least partially met and for which desolvation effects may be considered explicitly.

A charge-controlled interaction ($E_{rs}^{1} \neq 0$, $E_{rs}^{C} = 0$) may be considered in terms of the identity

$$a\sigma = \sum_{s}(aq_s) \tag{16}$$

and a frontier-controlled interaction ($E_{rs}^{1} = 0$, $E_{rs}^{C} \neq 0$) may be considered in terms of the identity

$$b\pi = \sum_{s}(bc_s^{(E)} + b'c_s^{(N)} + \lambda q_s \pm \lambda' q_s^2) \tag{17}$$

Equation 9 could then be interpreted as a combination of the charge-controlled and frontier-controlled cases and would then represent another manifestation of the principle of hard and soft acids and bases.[20,21]

In an absolute sense, eq 16 and 17 may be inadequate for a complete description of substituent effects, but they do provide a practical guide to the interpretation of substituent effects. For example, if it can be assumed that the net charge on a substituent experiences an average field due to the receptor atoms, then the coefficients in eq 16 may be removed from the summation and replaced by an average value ρ. Since ρ

$$a\sigma = \rho\sum_{s} q_s \tag{18}$$

is a function of the charge on the receptor atoms,[1] it is expected to vary from one drug–receptor system to another, but to remain constant for a given system. Equation 18 may also be used for the interpretation of the additivity of substituent effects by grouping the charges in the summation so that each group refers to a substituent.

An alternative form of eq 16 may also be considered. The summation may be grouped into two parts representing the charge on a substituent due to inductive effects and the charge on a substituent due to conjugative (resonance) effects. Assuming that each charge associated with the substituent experiences an average field due to the receptor atoms, and dividing both sides of the equation by a, the "extended Taft" relation developed by Charton[22] (the generality of which has been demonstrated by Swain[23]) is obtained.

$$\sigma = h\sigma_I + k\sigma_R \tag{19}$$

Similar interpretations may be made using eq 17 as a basis. Hansch has defined π as a hydrophobic index, and eq 17 may be interpreted readily in these terms. If $c^{(E)}$ is thought of as providing a measure of the polarizability of an atom in a molecule, the summation over the atoms of a substituent corresponds to the polarizability of a substituent. According to this interpretation all terms involving $c^{(N)}$ must be neglected since the polarizability of a material is a measure of the "looseness" of an electron cloud and the terms containing $c^{(N)}$ do not refer to an orbital containing electrons. Also, because polarizability may be considered primarily a surface phenomenon, the terms in eq 17 that refer to atoms which are "buried" within a substituent may be neglected. A similar neglect of terms cannot be made for the charge-containing terms since these give the net charge experienced by the surrounding solvent. Thus, assuming that each atom of a substituent experiences an average effect due to its surroundings, eq 17 may be written

$$\pi = pP_E + m\sigma \pm m'\sigma^2 \tag{20}$$

where P_E is the electronic polarizability of a substituent, and the identity provided by eq 18 has been used with $a = \rho = 1$.

By the classical definition of a hydrophobic bond, a hydrophobic bond is formed when two or more nonpolar groups come into contact, thus decreasing the extent of interaction with the surrounding water, and results in the liberation of water originally bound by the molecules[24,25] (desolvation). Since P_E represents a measure of the ability of a substituent to participate in an induced dipole–induced dipole interaction and σ and σ^2 are identified with desolvation, eq 20 is in accord with the definition of π as a hydrophobic index. Further, since all terms in eq 20 are additive, it follows that π should also be additive.

From the conditions imposed in arriving at eq 17 and 20, it is possible to suggest conditions under which the additivity of π breaks down. The additivity of these constants is expected to break down when there is a mutual interaction between substituents, when a given substituent can no longer be desolvated to its maximum potential, as would be the case if additional atoms competed for the solvent molecules about a substituent, or when a combination of the latter circumstances is the case. It is probable that in some instances the desolvation requirement is dominant, and it is indeed found that interactions of two adjacent nonpolar groups and intramolecular hydrophobic bonding resulting from molecular folding cause deviations from π value additivity.[18]

In using π as a substituent parameter, it has been noted that if a value is needed for a *para*-substituted derivative and this value is not available, then the value for the *meta* derivative may be used in its place.[19] This practice follows from the observation that π values derived from *meta*- and *para*-substituted compounds are often of the same order of magnitude.[7] Equation 20 provides a convenient basis for the interpretation of this observation.

At one extreme, a set of substituents may be said to have their lipophilicity determined primarily by their polarizability. It is well known that the polarizability of a substituent is essentially independent of its mode of substitution on an aromatic moiety[26] so that these substituents will have π values which are also independent of their position.

At the other extreme, a set of substituents may be said to have their lipophilicity determined primarily by their charge; that is, the magnitude of the charge on a substituent determines the ease with which water is stripped off of the substituent as it enters a lipophilic phase. This extreme is best represented by strong electron-withdrawing groups like cyano and nitro, and

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(25) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).
(26) Y. K. Syrkin and M. E. Dyatkina, "Structure of Molecules and the Chemical Bond," Dover Press, New York, N. Y., 1964, p 200.

these groups possess closely similar values of σ_m and σ_p. Extending the similarity to other strong electron-withdrawing groups, π should again be independent of the mode of substitution.

A consideration of eq 20 suggests that the dynamic process of forming a hydrophobic bond may be associated with a covariant behavior of the polarizability and desolvation characteristics of a drug or drug substituent. On the other hand, under static, or equilibrium, conditions such covariant behavior may no longer predominate and a drug–receptor interaction may then be considered as either polarizability or desolvation controlled. Under the latter conditions, eq 20 would reduce to

$$\pi = pP_E \quad (21)$$

and

$$\pi = m\sigma \pm m'\sigma^2 \quad (22)$$

In general the σ^2 term may be deleted from eq 22 since for all but strongly electron-withdrawing substituents this term is negligible. The correlations observed between π and σ[11,27] and the linear relationships found between chloramphenicol activity and P_E[28] and between sulfonamide activity and σ[29] may be interpreted on the basis of eq 21 and 22. It must be stressed, however, that interactions with pharmacologically inert substances may also be described in similar terms.

Conclusions.—The possibilities for further study and the interpretations of available data suggested by the present approach are by no means exhausted in the discussion. It is clear, however, that appropriate modifications of current chemical theories are themselves the general frame of reference on which physicochemical approaches to the study of drug action should be based.

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Notes

o,o'-Disubstituted Phenylcyclopropylamines[1a]

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A number of 2-arylcyclopropylamines exhibit potent inhibitory activities on monoamine oxidases[2] from various tissues.[3] Likewise, 2-phenoxycyclopropylamine[4] and several of its derivatives,[5] as well as 2-cyclohexyloxycyclopropylamine,[6] share these properties *in vitro* and *in vivo*. The *o*-toloxy homolog, *o*-$CH_3C_6H_4OC_3H_4NH_2$,[5b] is almost as potent as the parent phenoxy compound, whereas *ortho* substitution of 2-phenylcyclopropylamine decreases the activity.[4] The number of conformations of phenoxycyclopropane compounds is limited and must be restricted further by *ortho* substituents. Even in *o,o'*-disubstituted anisoles[7,8] and sterically hindered 2-aryloxyethylaminoguanidines[9] the oxygen atom does not appear to be conjugated with the aromatic ring, making it impossible for the groups at the ether oxygen to lie in the plane of the phenyl group. The decrease in conjugative effects, the enhanced basicity of the ether oxygen, and the bulk of the *ortho* substituents should lead to alterations in biological profile, and may lead to a block of metabolic reactions attacking the cyclopropoxy side chain. A study of *o,o'*-disubstituted phenoxycyclopropylamines has therefore been made in our laboratories.

The *o,o'*-disubstituted analogs (R = CH_3, Cl) were synthesized according to the outline in Chart I. The addition of carbethoxycarbene to vinyl ethers furnished mixtures of *cis* and *trans* esters, the *trans* isomer prevailing.[5b]

2-(2,6-Xylyloxy)cyclopropylamine was also quaternized to a cyclopropylog (**6**) of xylocholine.[10]

Effects on the Central Nervous System of Mice. Gross Observation.—Compounds **1–3** produced significant behavioral changes while **4–6** were inactive at 400 mg/kg. Compounds **1–3** showed stimulatory effects consisting of increased motor activity, clonic and

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