Foundations of Molecular Pharmacology

Volume 2

The Chemical Basis of Drug Action

J. B. STENLAKE

Foundations of Molecular Pharmacology

Volume 2

The Chemical Basis of Drug Action

J. B. STENLAKE
PH.D., D.SC., F.P.S., C. CHEM., F.R.I.C., F.R.S.E.
Professor of Pharmacy and Pharmaceutical Chemistry
University of Strathclyde, Glasgow

THE ATHLONE PRESS *of the University of London* 1979

Preface

This text has emerged from some thirty years of teaching undergraduate courses and conducting research in medicinal and pharmaceutical chemistry. It is conceived essentially as a foundation course in the basic principles of organic chemistry applied to the study of medicinal agents and the formulations in which they are used. It is intended primarily to cater for the needs of undergraduate students of pharmacy and medicinal chemistry up to Honours level. References to original papers, however, should extend its use to postgraduate students and others engaged in the search for new drugs.

My intention was to contain the text within the covers of a single volume, concentrating essentially on the fundamental groundwork chemistry which must of necessity be taught in any undergraduate course. Experience, however, has shown the value of more general discussion of certain selected topics of wide general applicability in the study of drug action, and it was always my objective to conclude the book in this way. In the event, I have been defeated, partly by the ramifications of the subject, but mainly by my enthusiasm and attempts to achieve a realistic degree of coverage. Publishing costs, too, have risen enormously in the ten years of writing. Attempts to overcome the twin difficulties of coverage and cost, therefore, left no alternative other than to divide the book between two volumes.

It is just possible that some readers may find virtue in the necessity which has forced this publication of the Foundations of Molecular Pharmacology in two separate volumes. I hope, nonetheless, that serious students will not be deterred by this somewhat artificial division from pursuing the broader approach to the subject contained in Volume 2. In order, therefore, to reinforce the continuity of the subject, I have provided a system of cross-referencing between chapters, both within and between the two volumes. Such cross-references are denoted by two numbers, the first indicating volume, and the second chapter; thus, for example, (1, 13) indicates Volume 1, Chapter 13, and (2, 5) Volume 2, Chapter 5.

The basic philosophy underlying the text is that those concerned with the design and use of drugs and medicines are interested fundamentally in properties rather than in methods of manufacture. Accordingly, the chemistry in this book almost entirely ignores the synthesis of medicinal agents. Instead, attention is focused, in Volume 1, on the physical and chemical properties of medicinal agents, pharmaceutical additives and cellular components, that determine the way in which they interact with each other. To achieve this end, substantial accounts of relevant intermediary tissue metabolism, drug transport and metabolism, and other factors affecting both stability and availability of drugs from

dosage forms have been brought together in the general body of the text. This approach emphasises the close similarity between chemical and biochemical transformations, and should help to give students and others engaged in the design of new drugs a better understanding of the fundamental mechanisms which control interactions between drugs and body chemistry.

The more general, but essentially similar approach to the Chemical Basis of Drug Action adopted in Volume 2, which reinforces the basic principles for the specialist, should also appeal in its own right to clinical pharmacologists and others whose interests lie rather more in the action and use of drugs than in their design.

Since this book is designed to assist in the education of students, many of whom will be engaged in later life in the handling and use of drugs in practice, I have deliberately chosen to draw my examples from drugs in current use in western medicine. My text, however, is essentially British, and British Approved Names, denoted by italics, are used throughout, notwithstanding the difficulties that this may make for North American readers. Fortunately, British and American drug nomenclature is convergent, but where important and confusing differences still exist, I have endeavoured to overcome them by also giving the United States Adopted Name.

It is an unfortunate fact of life that the vast majority of modern drugs have chemical structures which are infinitely more complex than those of the simple examples commonly used in most textbooks of organic chemistry. Indeed, their very complexity frequently presents an educational hurdle, so that students of medicinal and pharmaceutical chemistry often fail to grasp the essential simplicity of drug action mechanisms and transformations. I am, therefore, most grateful to the publishers for their help and co-operation in the use of printing devices involving bold type and colour to focus attention on the simple stepwise transformations of otherwise complex compounds.

I am very much indebted to my colleagues, past and present, and friends, who between them provided the stimulus to write this book, and all those who, once I was embarked upon it, so patiently answered my questions, and helped to resolve the many problems I inevitably encountered. I am especially grateful to Dr G. A. Smail and Dr R. E. Bowman, both of whom read the entire original draft and commented so helpfully upon it. I am sure others will still find errors, oversights and misconceptions, but there would have been many more without the help of these two colleagues. For similar reasons, I am also grateful for all the many valuable comments and criticisms I received from the Athlone Press's own anonymous referees. My most grateful thanks are also due to Tom Moody for help with the preparation of diagrams, to Dr N. C. Dhar for assistance in locating and checking references, and especially to my ever willing Secretary, Mrs Sylvia Cohen, for her invaluable help in typing the manuscript, for countless hours devoted to the dull routine of checking text and references at every stage right through to the final proofs, and for her help in compiling the index.

The time I have taken to write this book has been taken away from many things I might otherwise have done, and most of all, taken from my wife, Anne, and our family. Their tolerance and support made it possible. I have tried to make this book one that they, too, can be proud of, and worthy of the hours of pleasure in their company which I have sacrificed.

1978 John B. Stenlake

Contents

1 The Characteristics of Drug–Receptor Interaction

SPECIFIC AND NON-SPECIFIC DRUG ACTION

Introduction

The action of drugs at the molecular level may be broadly classified in one of two ways depending upon whether it is determined essentially by the presence and arrangement of specific chemical groupings, or not. Drugs may, therefore, be classified according to their mode of action as either **structurally specific** or **structurally non-specific**.

Structurally specific action depends, as it implies, on the presence in the drug molecule of specific chemical groupings usually arranged in some specific spatial relationship with respect to each other. Structurally non-specific drug action, on the other hand, is a manifestation of a specific but purely physical property, which may arise in compounds of quite diverse chemical properties.

Structurally Non-specific Action

The majority of drugs have structurally specific actions, but a few groups of chemically-unrelated compounds are capable of inducing identical and sometimes intense pharmacological reactions, due to the possession of a common physical property unrelated to any one particular chemical structure. The action is therefore **structurally non-specific**. In contrast to the structurally specific compounds, small changes of chemical structure have little or no effect on the nature and intensity of the pharmacological response which they elicit. Also, their action is usually directly related to their **thermodynamic activity**, which is high, implying that their action is largely a function of concentration.

Ferguson's Principle and the Non-specific Action of General Anaesthetics

Ferguson (1939) reasoned that toxic concentrations of drugs are reached by a series of distributions between unspecified heterogeneous biophases, linking the external circumambient phase and the particular biophase which is the site of drug action. We thus have a series of compartments, unspecified in nature and number, interpolated between the external circumambient phase and the biophase, each one in thermodynamic equilibrium with the next. Since all the compartments are in equilibrium, the thermodynamic activity of the drug is the same in each compartment. Measurement of the thermodynamic activity of a drug in the external phase, therefore, provides a measure of its thermodynamic activity in the biophase. Thus, measurement of the concentration of a gaseous anaesthetic in alveolar air or of the solubility of a non-volatile hypnotic in blood plasma will

provide a measure of its thermodynamic activity in the tissues of the central nervous system.

Ferguson (1939) postulated that the same degree of biological activity would be produced by the non-specific action of different compounds at the same level of thermodynamic activity, i.e. when the same relative saturation of the biophase is reached. He tested the toxicity of a variety of substances in wireworms and mice (1, 9), and showed that although the actual lethal concentrations varied by a factor of 10^4, the index of thermodynamic activity (P_t/P_s, where P_t is the partial vapour pressure at the toxic concentration, and P_s is the saturation vapour pressure) at lethal concentrations in the biophase approximated more closely to a constant figure. The same applies to the activity of general anaesthetics in man, taking the ratio of the anaesthetic vapour pressure in the inhaled gas mixture to the saturation vapour pressure as the measurement of thermodynamic activity. Thus, Eger and his collaborators (1965) have shown that measurement of equipotent alveolar concentrations of the anaesthetics, *Methoxyfluorane*, *Chloroform*, *Halothane*, *Ether*, *Cyclopropane*, xenon and *Nitrous Oxide*, correspond more closely to the oil/gas partition coefficient than any other physical constant. They have also shown (Eger, Saidman and Brandstater, 1965) that the minimum alveolar concentration of halothane and cyclopropane to prevent movement in dogs in response to painful stimulation varies linearly with temperature. Enthalpies of absorption calculated from the experimental results correlate well with enthalpies for the absorption of these anaesthetics by lipoprotein surface films. The results, therefore, are in agreement with Ferguson's principle.

The discovery that inert gases, such as nitrogen and helium, under pressure produced anaesthesia (Behnke and Yarbrough, 1938, 1939) led to the examination of the more highly fat-soluble inert gases, krypton and xenon, as potentially useful anaesthetics in mice (Lawrence, Loomis, Tobias and Turpin, 1946), and eventually to the demonstration of the anaesthetic activity of xenon in man by Cullen and Gross (1951). Administered with oxygen (20%), xenon gives rapid induction of anaesthesia, good muscle relaxation and rapid recovery, demonstrating as an inert gas the purely physical nature of the drug–biophase interaction.

A number of theories have been proposed concerning the mechanism of anaesthetic action. Wulf and Featherstone (1957) suggested that anaesthetic activity was due to displacement of the bimolecular phospholipid leaflets, which are thought to form the basic structure of cellular membranes (Danielli and Davson, 1935). Thus, it was suggested that any substance of molecular volume greater than that of oxygen or water, which normally separate the protein and phospholipid layers, is capable of physically disorientating the membrane structure, and in this way initiating anaesthetic action. The constant, b, which derives from the van der Waals equation

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT$$

Table 1. Van der Waals Molecular Volume Constants (b) and Anaesthetic Activity

	Van der Waals Constant (b)[1] ($l\ mol^{-1} \times 10^2$)	Minimum Alveolar[2,3] Conc. (MAC) (Atmospheres)	Oil gas partition[3] coefficient	MAC × Oil gas[3]
Nitrous Oxide	4.4	1.88	1.4	2.63
Xenon	5.1	1.19	1.9	2.26
Cyclopropane	7.5	0.175	11.8	2.06
Chloroform	10.2	0.0077	265	2.08
Ether	13.2	0.030	65	1.95
Halothane		0.0087	224	1.95
Methoxyflurane		0.0023	970	2.23

[1] Wulf and Featherstone (1957)

[2] Minimum alveolar concentration (MAC) in atmospheres to prevent movement in response to painful stimulation (Eger, Brandstater, Saidman, Regan, Severinghaus and Munson, 1965)

[3] Eger, Lundgren, Miller and Stevens (1969)

gives a measure of molecular volume, and is greater in the case of most common anaesthetics (Table 1) than that of water ($3.047 \times 10^{-2}\ l\ mol^{-1}$) or oxygen ($3.183 \times 10^{-2}\ l\ mol^{-1}$), increasing approximately in order of the anaesthetic activity, as determined by Eger and his collaborators.

Pauling (1961) proposed an alternative theory in which it is suggested that the action of general anaesthetics is due to ordering of adjacent water molecules by the anaesthetic in the central nervous system. Anaesthetics are considered either to form hydrates or to promote the formation of clathrates in which the ions responsible for nerve conduction become trapped. Some doubt is cast on the theory by the failure of Eger and Shargel (1969) to demonstrate the formation of hydrates in aqueous anaesthetic mixtures containing *Methoxyfluorane*, *Halothane* or *Ether* at 0 , although cyclopropane forms two distinct hydrates under the same conditions. Furthermore, there is also a lack of correlation between hydrate dissociation pressures and the minimum alveolar concentration (MAC) of anaesthetics required to prevent movement in response to painful stimulation (Eger, Lundgren, Miller and Stevens, 1969). Similar reservations attend the iceberg theory (Miller, 1961), which proposes the formation of microcrystalline water around the anaesthetic molecules at the site of action. Eger, Lundgren, Miller and Stevens (1969), however, did find good agreement between MAC and the oil/gas partition coefficient of anaesthetics (Table 1) in agreement with the Ferguson principle.

Non-specific Bactericidal Activity of Long-chain Cationic Detergents

There is now substantial evidence to show that the bactericidal action of long-chain cationic detergents is structurally non-specific, and purely a function of their surfactant properties. Thus, the bactericidal activity increases with decreased critical micelle concentration (CMC) within a series of long-chain

Table 2. Bactericidal and Thermodynamic Activities of Long-chain Quaternary Ammonium Salts

Structure ($R—\overset{+}{N}R^1R^2R^3$)				CMC (N)	Minimum Inhibitory Conc. ($\times 10^8$)		Thermodynamic Activity	
R	R^1	R^2	R^3		*Staph. aureus*	*E. coli*	*Staph. aureus*	*E. coli*
$C_{12}H_{25}$	Me	Me	Me	0.0228	7.50	7.50	0.033	0.033
$C_{12}H_{25}$	Me	Me	Et	0.0213	7.50	7.50	0.030	0.030
$C_{12}H_{25}$	Me	Me	Et	0.0199	7.50	7.50	0.038	0.038
$C_{12}H_{25}$	Et	Et	Et	0.0193	7.50	7.50	0.039	0.039
$C_{12}H_{27}$	Me	Me	Me	0.0112	2.50	2.50	0.022	0.022
$C_{14}H_{29}$	Me	Me	Me	0.0058	0.75	0.75	0.014	0.014
$C_{16}H_{33}$	Me	Me	Me	0.0015	0.75	0.75	0.050	0.050

quaternary ammonium salts (Cella, Eggenberger, Noel, Harriman and Harwood, 1952). In an extension of the concept that solutions in equilibrium with excess solid have the same thermodynamic activity, to the equilibrium between solutions with micelles, Ecanow and Siegel (1963) concluded that the thermodynamic activity should be equal to a constant fraction of the CMC. They have further shown that the ratio of minimum inhibitory concentrations against *Staphylococcus aureus* and *Escherichia coli* to the CMC is virtually constant (with one exception) for the group of long-chain quaternary compounds studied by Cella and his collaborators (1952), despite the fact that there is a fifteen-fold variation in their respective CMC's (Table 2).

The conclusion that the bactericidal action of long-chain quaternary ammonium salts is structurally non-specific has been confirmed by Weiner, Hart and Zografi (1965), and by Laycock and Mulley (1970).

Structurally Specific Action

Most drugs act by specific intermolecular interaction(s) with bio-receptor molecules. Specificity of action is determined by a precise combination and steric arrangement of chemical groups in the drug molecule which facilitate interaction by chemical bonding and physical interaction with appropriate bio-receptor molecules. The nature and relevance of such interactions is discussed in Chapter 2. Since the forces involved in chemical bonding and physical attraction between molecules are essentially short-range forces, the question of fit between the specific active groups of the drug and the complementary groups on the bio-receptor is highly relevant. Molecular size, overall molecular shape, spacing between essential structural features and the relative orientation of essential groups in a drug molecule, are just as important as criteria of potency as the availability or deficiency of electrons for reaction with the receptor molecule which they complement. The significance of such factors is considered in Chapter 3.

The Nature of Drug—Specific Receptor Interactions

The precise target molecule and the ultimate molecular interaction mechanism is known only in the case of a very few drugs. In some cases, it is possible to identify a triggering reaction, which is clearly linked to the observable pharmacological response produced by the drug, even when the subsequent sequence of reactions is not clear. In other cases, there is still only a confusing number of possible and plausible alternative sites and mechanisms of action. Ariens (1964), however, introduced the concepts of **affinity** and **intrinsic activity** to distinguish between molecular interactions which primarily result in binding of the drug to the receptor (affinity), and those which result in triggering a pharmacological response (intrinsic activity). For some drugs, the target molecule is an enzyme, but whether this is so or not, there is a close parallel between drug–receptor, enzyme–substrate, and enzyme–inhibitor reactions. For this reason, the nature and properties of enzymes will be considered (p. 12).

MATHEMATICAL ANALYSIS OF STRUCTURE-ACTION RELATIONSHIPS

Linear Physico-chemical—Activity Relationships

It is well-known that small changes in the chemical structure of structurally specific drugs lead to corresponding, and occasionally, marked changes in the pharmacological response which they elicit. Minor modifications of chemical structure have frequently been used to establish correlations between particular structural features of a drug and its pharmacological action. In this way, it is often possible to delineate the optimum molecular feature to produce a particular type of response. This approach, however, is empirical and fails to distinguish readily the relative influence of electronic, steric, and physical interactions of individual substituents. In order to overcome this difficulty, Hansch and Fujita (1964) adapted the Hammett equation (1, 11), which has been used extensively to establish correlations between structure and reactivity of aromatic compounds, to the analysis of structure–action relationships in molecular pharmacology.

In considering the action of a drug on a living system in which only two parameters can be measured, the dose administered and the response, Hansch and Fujita assume that only one reaction is rate-determining. This will be merely one of several processes which include absorption, protein binding, fat deposition and metabolism, in addition to that at the specific reaction site.

Thus, the rate at the critical reaction site can be expressed as:

$$\text{Rate of biological response} = \frac{\text{d(response)}}{dt} = ACK_X \qquad (1)$$

where A = the probability of a molecule reaching the specific reaction site in the time interval dt

C = the administered dose

K_X = the equilibrium constant for the rate-determining step.

The absorption and transport of drugs is controlled largely by lipid solubility, and transport rates in general correlate with the logarithm of their partition coefficient (log P) (Collander, 1954; Milborrow and Williams, 1968). Hansch and Fujita (1964) therefore expressed the probability term (A) as log P, the logarithm of the partition coefficient of the drug between an organic solvent (octanol) and water. Changes in the probability factor with substitution in a series of compounds can, therefore, be expressed as a **substituent constant**, π, defined by analogy with the Hammett substituent constant as

$$\pi_X = \log\left[\frac{P_X}{P_H}\right] \tag{2}$$

where P_X = the partition coefficient of the substituted compound, and
P_H = the partition coefficient of the unsubstituted compound (i.e. X = H).

On the basis of evidence that the partition coefficients for a group of related compounds, such as those based on *Chloramphenicol* (Hansch, Muir, Fujita, Maloney, Geiger and Streich, 1963), normally exhibit an optimum partition coefficient for activity (P_0), and on the assumption that there is a normal Gaussian distribution of partition coefficients about the optimum, the probability factor A may be expressed as

$$A = f(\pi) = ae^{-(\pi - \pi_0)^2/b} \tag{3}$$

where π_0 is the value of the substituent constant for optimum activity.

Hence, from eq. (1),

$$\frac{\text{d(response)}}{\text{d}t} = ae^{-(\pi - \pi_0)^2/b} CK_X \tag{4}$$

If the applied concentration of drug, C, be measured in terms of the concentration required to elicit a constant response in a fixed time interval, then from eq. (4)

$$\frac{\text{d(response)}}{\text{d}t} = 0$$

hence

$$\log\frac{1}{C} = k'\pi\pi_0 \quad k\pi^2 - k''\pi_0^2 + \log K_X + k''' \tag{5}$$

π_0 at the optimum value (log P_0) of log P is a constant.

Assuming K_X depends on electron availability, substitution for K_X in the Hammett equation gives the general Hansch equation

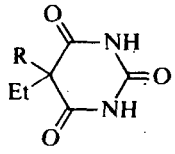
$$\log\frac{1}{C} = k_1\pi - k_2\pi^2 + \rho\sigma + k_3 \tag{6}$$

where k_1, k_2 and k_3 are constants for the system determined by regression analysis, σ is the substituent constant and ρ the reaction constant as in the Hammett equation (**1**, 11).

Since log P and hence π (equation 2) are free energy terms, they are additive, and

$$\log P = \sum_{1}^{n} \pi$$

It is possible, therefore, to calculate log P and π values by addition of the corresponding values for their separate components (Hansch, Quinlan and Lawrence, 1968; Bird and Marshall, 1967). Thus, the π_X for the methylene unit can be calculated from the log P values of a pair of barbiturates, such as *Barbitone* (Barbital; log P 0.65) and *Butobarbitone* (Butethal; log P 1.65), which differ only in the constitution of one of the alkyl substituents.



Barbitone R = $CH_3 \cdot CH_2-$
Butobarbitone R = $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2-$

The difference in log P values (Δ log P) is 1.0 for two methylene units. The methylene (and methyl) group therefore has a π_X value of 0.5, and from this it is readily possible to calculate that the barbiturate ring has a π_X value of -1.35 [i.e. $0.65 - (4 \times 0.5)$].

Measurements of log P values have now been made on large numbers of different compounds from which tables of log P and π_X values have been compiled using methods similar to that illustrated above (Tute, 1971).

Applications of Hansch Analysis

The π^2 term in the general Hansch equation (6) is only essential in complex biological systems, where a large number of compartments (reaction steps) exist between the point of administration and the critical reaction site. In simpler systems, such as drug–protein binding, where transport from a remote site of administration is not involved, the best fit of data is obtained in equations which do not involve a π^2 term. Thus, in the binding of penicillins to human serum albumin (Bird and Marshall, 1967), an excellent fit of data for some 79 penicillins was obtained with equation (7), the correlation coefficient (r) being 0.924 and the variance about the mean value of log B/F, $S^2 = 0.66$

$$\log\left[\frac{\text{Bound penicillin}}{\text{Free penicillin}}\right] = 0.504\Sigma\pi - 0.665 \qquad (7)$$

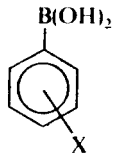
The excellent fit with this equation, with but a single π term implies that whilst the extent of binding is dependent on the lipid–water partition coefficient of the penicillin, it relates to the hydrophobic character of the penicillin side-chain and its interaction with hydrophobic binding sites of the protein.

The significance of the π^2 term in determining the importance of transport processes for the concentration of a drug at its specific reaction site was demonstrated in an analysis of the distribution of a series of benzeneboronic acids in mice (Soloway, Whitman and Messer, 1960). The rates of accumulation of boron in brain tissue (C_b) and tumour tissue (C_t) within 15 min of injection were found to give the best fit in equations (8) and (9) respectively (Hansch, Steward and Iwasa, 1965a).

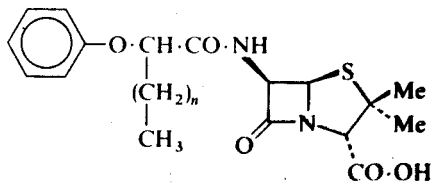
$$\log C_b = -0.540\pi^2 - 0.765\pi + 1.505 \quad (8)$$

$$\log C_t = -0.130\pi^2 - 0.029\pi - 0.405\sigma + 1.342 \quad (9)$$

The ideal partition coefficient (log P_0) for penetration of brain tissue was found from equation (8) to be about 2.3. This compares closely with the value for log P_0 found for optimum hypnotic activity (*ca* 2.0) in a large series of barbiturates and non-barbiturate hypnotics (Hansch, Steward, Anderson and Bently, 1967). In contrast, equation (9) which gives the best fit of data relating to tumour tissue, is significant in both π^2 and σ terms. From this, it was suggested that substituents with π values between -1.0 and -2.0 should favour concentration in tumour tissue. Additionally, dependence on the σ term in the equation indicates a degree of structural specificity which is favoured by the presence of electron-releasing substituents (negative σ values).



Benzeneboronic acids



Phenoxymethylpenicillins

In contrast, an analysis of the minimum inhibitory concentrations of a series of phenoxymethylpenicillins against *Staphylococcus aureus* infections in mice (Gourevitch, Hunt and Lein, 1960), showed a more limited degree of dependence on the π^2 function, despite the complexity of the system. Three equations (10), (11) and (12) were generated by Hansch and Steward (1964) from computations of least squares fit of data.

	r(correlation coefficient)	s	
$\log \frac{1}{C} = 0.053\pi^2 - 0.610\pi + 0.019\sigma + 5.71$	0.918	0.192	(10)
$\log \frac{1}{C} = 0.055\pi^2 - 0.613\pi + 5.756$	0.918	0.187	(11)
$\log \frac{1}{C} = 0.445\pi + 5.673$	0.909	0.191	(12)

Comparison of equation (11) with equation (10) demonstrates that the σ term is unimportant, and hence that electronic effects of substituents in the phenoxy ring contribute little to the activity of the antibiotics except insofar as they influence the partition coefficient. The fact, however, that almost as good correlations are obtained with equation (12), which lacks the π^2 term and attributes an apparently linear relationship to the $\Sigma\pi$ values, and the biological response, must imply that the log P values for the series are relatively remote from that of the optimum (log P_0) and, hence, lie on that part of the distribution parabola which is virtually linear. The negative sign of the coefficient in π in equation (12) suggests that more active compounds would be obtained with substituents having negative values of π.

Other examples of Hansch analysis of linear relationships between lipophilic character and biological response have been summarised by Tute (1971) and Hansch and Dunn (1972).

Theoretical model-based equations have also been developed for the correlation of linear-free energy relationships with biological activity in ionisable substances (Martin and Hackbarth, 1976). The models, which comprise a series of aqueous and non-aqueous compartments, give rise to log $(1/C)$ *vs* log P curves which may be asymptotic, linear, or two-part consisting of two lines of unequal slope. Such equations show:

(a) whether the ion or neutral form of the drug is the active species,
(b) whether there is hydrophobic bonding to the receptor,
(c) the presence of inner compartments.

Parabolic Physico-chemical—Activity Relationships

A number of non-linear, parabolic relationships between lipophilic character and biological properties have also been analysed by Hansch and Clayton (1973). Non-linear effects arise for a variety of reasons, and may be due to kinetic (Penniston, Beckett, Bentley and Hansch, 1969) or thermodynamic factors (Higuchi and Davis, 1970). Other factors contributing to parabolic relationships include bulk tolerance when the active site is unable to accommodate the bulkier substituents of higher molecular weight members of a series; increasingly greater conformational distortions of the active site by successive members of a series due to physico-chemical interactions; micelle formation; limiting solubilities, and inconstant metabolism within a series.

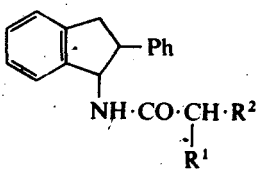
Molecular Connectivity

More precise analysis of both linear and parabolic relationships between physical properties and biological activity has been made possible by invoking the concept of **molecular connectivity** Kier, Hall, Murray and Randic (1975). This employs the branching index devised by Randic (1975) to provide a means of

analysing the relationships between the extent of molecular branching and properties which are critically dependent on molecular size and shape. Thus, this mathematically-derived branching index, renamed as the **molecular connectivity index**, χ, which is in reasonable agreement with the experimentally-derived Kováts index (Kováts, 1961), sums the additive and constitutive properties of any molecule to which it relates. Not only has the molecular connectivity index, χ, been shown to be correlated with a number of physical properties, including partition coefficients (Murray, Hall and Kier, 1975), water solubilities and boiling points (Hall, Kier and Murray, 1975), but it has also been shown to be correlated **linearly** with non-specific local anaesthetic activity (Kier, Hall, Murray and Randic, 1975), antifungal and butyrylcholinesterase activity (Kier, Murray and Hall, 1975). Murray, Kier and Hall (1976) have also examined a number of non-linear relationships using the molecular connectivity index, χ, in place of the physico-chemical parameter, log P, in which P is the octanol–water partition coefficient. Addition of a χ^2 term to the linear equation gave significant correlations at the 0.99 probability level between molecular connectivity and the antibacterial activity of long-chain quaternary ammonium salts, and also the hypnotic activity of barbiturates.

Free–Wilson Analysis

The approach to quantitative structure–action relationships (QSAR) proposed by Free and Wilson (1964) relates the occurrence of particular variable substituents directly to the level of a specified biological response shown by each member of the series. This method of analysis was illustrated by reference to the LD_{50} of the following group of compounds in mice.



Substituent	LD_{50} values (mg/10g in mice)		
R^2	$R^1 = H$	$R^2 = Me$	Mean LD_{50}
NMe_2	2.13	1.64	1.885
NEt_2	1.28	0.85	1.065
Mean LD_{50}	1.705	1.245	1.475

From this, it is evident that the mean LD_{50} for the series is 1.475 and that the

substituent group contributions at the R^1 position, $a[R^1]$, can be derived as follows.

$$\text{Substituent group contribution for H, } a[H] = 1.705 - 1.475 = +0.23$$

$$\text{Substituent group contribution for Me, } a[Me] = 1.245 - 1.475 = -0.23$$

Similarly, substituent group contributions at the R^2 position, $b[R^2]$, are given by

$$b[NMe_2] = 1.885 - 1.475 = +0.41$$

and

$$b[NEt_2] = 1.065 - 1.475 = -0.41$$

Likewise, the biological response for any member of the series can be derived from the expression:

Biological response = mean response (μ) + Σ substituent group contributions

Thus,

$$LD_{50} = \mu + a[R^1] + b[R^2]$$

Substituting, actual LD_{50} values, therefore produces four equations with five unknowns

$$\mu,\ a[H],\ a[Me],\ b[NMe_2], \quad \text{and} \quad b[NEt_2]$$

Since, however, the sum of all individual substituent group contributions at each position is zero,

$$a[H] = -a[Me]$$

$$b[NMe_2] = -b[NEt_2]$$

and the problem then reduces to the solution of four equations with three unknowns.

The value of this type of analysis lies in its ability to predict the minimum number of compounds of a given series which must be prepared and tested biologically to determine the mean response (μ) and each of the individual group substituent contributions. In a series with three variable substituents R^1, R^2 and R^3 with m variations in R^1, n variations in R^2 and p variations in R^3, there are $m \times n \times p$ possible compounds. Calculation of the mean response and individual group substituent contributions is possible with the synthesis and biological examination of $(m + n + p - 1)$ compounds. The number of unknowns is, however, reduced by the restriction that the sum of the substituent contributions at each position is zero. Thus, in an example where $m = 2$, $n = 3$ and $p = 3$, although there are eighteen possible compounds ($2 \times 3 \times 3$), the nine unknowns ($\mu + 2R^1 + 3R^2 + 3R^3$) are reduced by the restrictions to six ($\mu + R^1 + 2R^2 + 2R^3$). Careful selection of six representative compounds