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Transport of Alkyl Homologs across Synthetic and Biological Membranes: A New Model for Chain Length-Activity Relationships

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Abstract ☐ The ability of each member of a biologically active homologous series to reach the receptor site can often be equated with its relative ability to permeate biological barriers. This paper presents a structure–activity model based entirely on firmly established diffusional theories as well as solubility relationships generally applicable to aqueous systems. The equations derived and presented here were previously experimentally verified using synthetic membranes. These equations are generally applicable for transport across membranes and can be of use in describing a variety of passive absorption or permeation phenomena. Since true equilibrium is rarely attained in biological systems, a kinetic model provides an appropriate description of the physiological situation. Furthermore, a kinetic model offers considerable practical utility, because it can readily be incorporated into the overall pharmacokinetic treatment.

Keyphrases ☐ Membrane permeability, alkyl homologs—model for chain length–activity relationships, equations ☐ Transport across synthetic and biological membranes—alkyl homologs, model for chain length–activity relationships, equations ☐ Structure–activity relationships—model for transport of alkyl homologs across synthetic and biological membranes based on diffusion theories ☐ Chain length–activity relationships—alkyl homolog transport across membranes

Many theories have been advanced to explain relative biological activity of the members of a series of structurally related compounds (1–5). These theories usually relate the affinity of a particular congener for the receptor site to some property such as its molecular weight, its solubility, its surface tension, its partition coefficient between water and an organic solvent, or some other related physicochemical parameter. The structure–activity models can be categorized operationally as being either of kinetic or equilibrium nature. Current mathematical models for the former are generally of empirical design; quantitative relationships for the latter are complex and difficult to apply to actual data.

The present paper is concerned with a new kinetic approach applicable to many structure–activity profiles. The ability of each member of a homologous series to reach the receptor site is equated with its ability to permeate biological barriers, including the biomembrane(s) and contiguous aqueous layers. This is accomplished by modification of firmly established diffusional theories. Virtually all aspects of the theory and equations derived and presented here were experimentally verified (6–11) using synthetic membranes. It will be shown that much of the current biological data are interpretable based on these simple diffusional relationships.

These equations are general for transport across any membrane and can be of use in describing a variety of passive absorption or permeation phenomena. Furthermore, since true equilibrium is rarely attained in biological systems, a kinetic model may provide a more appropriate description for the general physiological situation, facilitating incorporation of passive transport processes into the total pharmacokinetic picture.

THEORETICAL

Importance of Diffusion Layers in Membrane Transport—The resistance to the transport of a solute across a membrane, which separates two aqueous compartments, lies not only within the membrane but also within the regions of unstirred water adjacent to its surfaces (6–11). The resistances of these aqueous regions of diffusion layers can be treated mathematically as additive resistances in series with the membrane (8–10). The flux of a solute across a membrane system (membrane and diffusion layers) is proportional to the concentration difference, C, across the barrier and is inversely proportional to the sum of the resistances of the laminates. Thus:

$$F^c = \frac{C}{R_{aq} + (R_m/PC)} \quad \text{(Eq. 1)}$$

where F^e is the steady-state flux across the barrier at a concentration gradient of C; R_m and R_{aq} are the resistances of the membrane and aqueous regions, respectively; and PC is the membrane-water partition coefficient of the diffusant. For a single membrane of unit area, R_m and R_{aq} are equal to h_m/D_m and $(h_{aq}{}^d + h_{aq}{}^r)/D_{aq}$, respectively, where h is the laminate thickness. The superscripts d and r designate the high (donor) and low (receptor) concentration sites of the membrane, and D_m and D_{aq} are the membrane and aqueous diffusion coefficients of the solute, respectively. For a sequential arrangement of j membranes, the resistances can be defined by:

$$R_m = \sum_{i=1}^{j} \frac{h_m{}^i}{D_m} \qquad \text{(Eq. 2a)}$$

$$R_{aq} = \sum_{k=2}^{2j} \frac{h_{aq}{}^k}{D_{aq}} \qquad \text{(Eq. 2b)}$$

where $h_{aq}{}^k$ and $h_m{}^i$ are the thicknesses of the individual diffusion layers and membranes that are traversed; *i.e.*, the resistance of thick tissues can be viewed in terms of the sum of the resistances of the tissue's component strata.

Equation 1 shows that for diffusants of low partition coefficient the flux is approximated by $(C)(PC)/R_m$; *i.e.*, the membrane resistance controls the transport rate of the permeant. On the other hand, when the permeant's partition coefficient becomes large, $R_m/(PC)$ becomes negligible and, thus, the flux is determined primarily by the diffusion layer resistance, R_{aq}. These conditions are referred to as membrane control of flux and diffusion layer control of flux, respectively.

Transport of Homologs at Equimolar Concentration—In a homologous series the respective diffusivities, D_m and D_{aq}, for all practical purposes, remain constant as the series is ascended. Some workers (12, 13) argued that the change in molecular weight (MW) or, more realistically, molecular volume with increasing chain length should be accounted for by multiplying the flux $(MW)^{1/2}$ for small molecules and $(MW)^{1/3}$ for larger molecules. This refinement is unnecessary for alkyl derivatives of large molecules, because the factor $(MW)^{1/3}$ is essentially constant over a reasonable alteration of chain length. Furthermore, other flux-determining factors will be shown to be exponentially dependent on chain length, and these changes completely mask any small changes in D_m or D_{aq}. A relevant literature example illustrative of the lack of dependency of D on alkyl chain length is found in the work of Scheuplein and Blank (14), who showed that there is less than a 30% decrease in diffusion coefficient for normal alcohols (methanol through octanol) within the stratum corneum. Thus, for a homologous series of compounds the values of R_{aq} and R_m can be treated as being essentially invarient.

As previously stated, the membrane-water partition coefficient (PC) changes exponentially with chain length and can be related to the number of methylene and methyl groups in excess of those in a reference homolog, n, by:

$$\log (PC)_n = \log (PC)_o + \pi n \qquad \text{(Eq. 3)}$$

where $(PC)_o$ and $(PC)_n$ are the partition coefficients of the reference homolog and the higher homolog, respectively; π is a constant which is characteristic of the membrane and its bathing solvent, which for biological systems is essentially aqueous. For a simple homologous series, it is convenient to define $(PC)_o$ as the partition coefficient of the hypothetical zero chain length congener (the Y intercept of a plot of $\log PC$ versus n); n, in this circumstance, becomes the alkyl chain length. The relevance of this partitioning relationship with respect to biological transport can be found by examining Eq. 1; for any given concentration differential, C, the steady-state flux of a particular homolog, $F_n{}^e$, can be related to alkyl chain length by substituting Eq. 3 into the logarithmic form of Eq. 1:

$$\log F_n{}^e = \log C + \log (PC)_o +$$
$$\pi n - \log [R_{aq}(PC)_o 10^{\pi n} + R_m] \qquad \text{(Eq. 4)}$$

For small values of n, R_m is usually much greater than $R_{aq}(PC)_o 10^{\pi n}$. This is the condition for membrane control of flux and is

described by reducing Eq. 4 to:

$$\log F_n{}^e = \log \left(\frac{C(PC)_o}{R_m} \right) + \pi n \qquad \text{(Eq. 5)}$$

Thus, for the shorter homologs the log [flux] at fixed concentration differential grows by the value π per methylene unit added. On the other hand, for large values of n, where $R_{aq}(PC)_o 10^{\pi n}$ dominates the last term of Eq. 4, the system is under diffusion layer control of flux and:

$$\log F_n{}^e = \log \left(\frac{C}{R_{aq}} \right) \qquad \text{(Eq. 6)}$$

In other words, a plot of the logarithm of the flux across a given membrane for equimolar concentrations of a series of homologs usually has an initial slope of π and ultimately levels off as n becomes large. Since π is a property of the membrane and the contiguous solvent and the solvent is assumed to be water, all series of homologs should have the same initial slope for a given membrane.

Obviously, the critical parameter determining flux and mechanism is not alkyl chain length *per se* but the membrane-water partition coefficient. Unfortunately, in biological systems partition coefficients are not easily measurable. Because of the relationship between chain length and partition ceofficient (Eq. 3), chain length is not only a convenient reference parameter but also of equivalent utility to partition coefficients obtained in arbitrary partitioning systems such as ether-water and octanol-water. The parallelism between *in vitro* partitioning and partitioning in biological systems is not clearly known. Since the present discussion is primarily limited to homologs, n is a parameter chosen for the mathematical model.

Limitations Based upon Solubility—For any given diffusant and membrane system, R_m, R_{aq}, and (PC) are presumed constant; therefore, the transport rate is dependent only upon the effective concentration gradient. Regardless of the distribution of the gradient in the various laminae, the gradient can be maximized by saturating the donor solution and maintaining the receptor compartment at zero concentration, *i.e.*, in a sink condition. The steady-state flux from a maximized gradient system, F^s, is described by Eq. 1 in which C has been replaced by the solubility of the permeant, S:

$$F^s = \frac{S}{R_{aq} + (R_m/PC)} \qquad \text{(Eq. 7)}$$

Several workers (15-18) demonstrated that the aqueous solubility of each member of a homologous series can usually be related to alkyl chain length by:

$$\log S_n = \log S_o - \delta n \qquad \text{(Eq. 8)}$$

where S_o and S_n are the solubilities of the reference congener and the nth linear alkyl homolog of the series, respectively, and δ is a constant. The value of δ varies somewhat from series to series but usually lies between 0.5 and 0.7. These values represent a decrease in solubility by a factor of about 3.2–5.0 for each methylene unit.

By substituting Eq. 8 into Eq. 4, an expression for the saturation-limited flux as a function of chain length is obtained:

$$\log F_n{}^s = \log S_o + \log (PC)_o + (\pi - \delta)n -$$
$$\log[R_{aq}(PC)_o 10^{\pi n} + R_m] \qquad \text{(Eq. 9)}$$

For small values of n, Eq. 9 is approximated by:

$$\log F_n{}^s = \log \frac{S_o(PC)_o}{R_m} + (\pi - \delta)n \qquad \text{(Eq. 10)}$$

For large values of n, Eq. 9 becomes:

$$\log F_n{}^s = \log \frac{S_o}{R_{aq}} - \delta n \qquad \text{(Eq. 11)}$$

Therefore, a semilogarithmic plot of flux obtained from saturated donor solutions (into a receptor sink) against alkyl chain length has an initial slope of $(\pi - \delta)$. This slope can be positive, negative, or zero, depending upon the relative magnitudes of π and δ. As chain length is increased, the flux from saturated solutions becomes a direct function of the solubility (Eq. 11) and decreases by
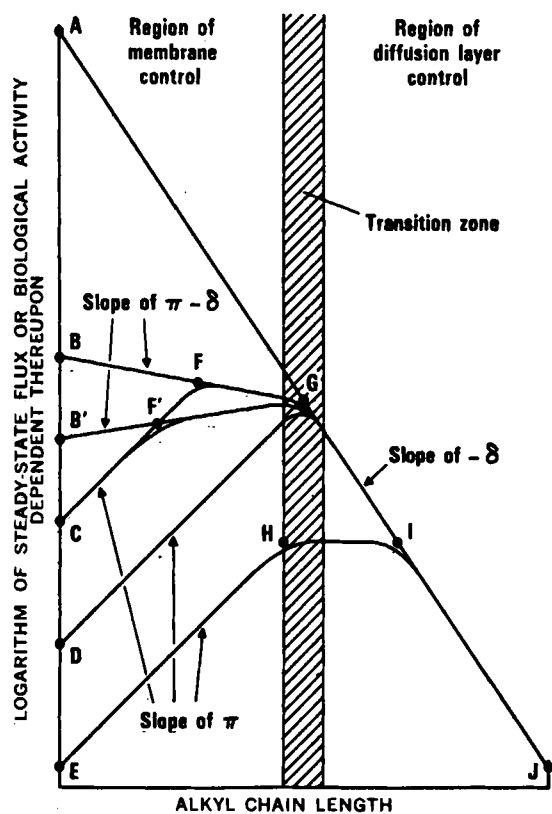
Figure 1—*Hypothetical plot illustrating the various possible flux dependencies for a homologous series as a function of concentration in a system fixed with regard to diffusional parameters. Where biological activity parallels flux, the plot can be regarded as a series of structure-activity profiles.*

higher than the corresponding member of the reference series. If the two series have similar δ values, the solubilities of corresponding homologs will differ by a constant. Therefore, all curves in Fig. 1 dependent upon solubility will show a parallel or nearly parallel upward displacement. Secondly, the membrane-water partition coefficient of each member of the new series will be lower than the corresponding homolog of the reference series. Consequently, the curves of Fig. 1 that are dependent upon the partition coefficient will be parallel to, but lower than, those of the reference series.

Figure 2 illustrates this point for curve DGJ of Fig. 1. Other curves found in Fig. 1 are omitted from Fig. 2 for clarity only; they would experience similar shifts. When the increase in log [solubility] is less than the decrease in log [partition coefficient], curve D'G'J' is formed. The maximum flux occurs at a longer chain length and is less in magnitude than the maximum of the original series. When the factor by which solubility increases is greater than the factor for the partition coefficient decrease (D'G" J"), the maximum flux exceeds that of the parent series.

Applications to Biological Systems—Stehle and Higuchi (19, 20) and Barrie *et al.* (21) showed the *in vitro* applicability of the equimolar expressions (Eqs. 4–6), and Flynn and Yalkowsky (6) experimentally verified the equations for both equimolar and saturated cases using synthetic membranes. Of the utmost significance is the fact that this approach to membrane transport has broad applicability; it is equally suited to biological membranes and to synthetics. This means the π value for biological membranes can be determined by studying the relationship between alkyl chain length and membrane permeability or a directly related response under the condition of membrane control of flux. Differences between biological π values, π^B, reflect differences in polarities of barriers. All of this can be accomplished even when the values of R_m, R_{aq}, PC_o, and S_o are unknown.

The inclusion of diffusion layers or, more generally, aqueous resistances as part of the total barrier is likely of great importance in biological systems, because agitation *in vivo* would be expected to be inefficient and because biological membranes, particularly cell membranes, are much thinner than synthetic barriers commonly chosen to mimic biological systems. It should also be realized that cytoplasm contributes to the "unstirred" regions. In characterizing
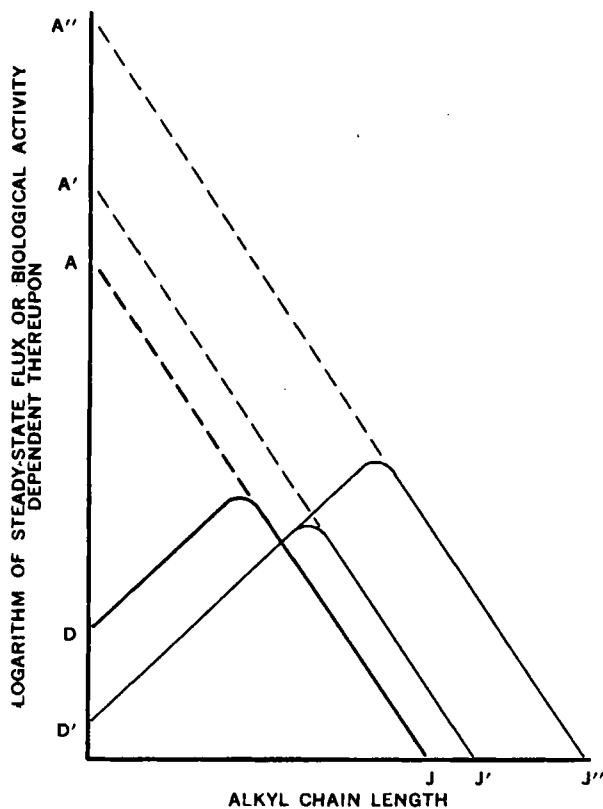
the antilogarithm of δ for each methylene unit. Even if the members of a series are studied at equimolar concentrations, the solubility becomes determinative, because any arbitrary concentration must eventually exceed $S_o 10^{-\delta n}$ for some value of n, *i.e.*, for some long chain derivative.

The importance of this solubility restriction on the transport rate profile of a homologous series can be seen in Fig. 1, which is a pictorial summary of the equations presented. The curves drawn place the steady-state flux on an arbitrary logarithmic scale and represent some of the expected relationships between transport rate, concentration, and solubility. Line AJ is the solubility restriction under diffusion layer control as described by Eq. 11. It has a slope of $-\delta$ and an intercept at A of log (S_o/R_{aq}). It can be thought of as representing the flux from a saturated solution into a sink receptor solution across an infinitely thin membrane, where the resistance to transport lies only within the aqueous diffusion layers.

Line BFG is for saturated solution flux under membrane control and is drawn for the case where δ exceeds π, which would be the general situation. Since the line is described by Eq. 10, the intercept at B is equal to log $[S_o(PC_o/R_m)]$. Curve EHI is the equimolar case described by Eq. 4. The initial portion EH has a slope of π and an intercept at E of log $[C_E(PC)_o/R_m]$ (see Eq. 5). The plateau occurring at subsaturation conditions is described by Eq. 6; but as chain length is increased, the concentration C_E becomes greater than the solubility and curve EHIJ is formed. Line DG is also initially equimolar, but at a higher concentration than the previously described case. There is no plateau because of its early intersection with line AJ to form curve DGJ. Line CF is also for the equimolar case but at a still higher concentration. The solubility is exceeded while the system is still under membrane control to form curve CFG. Its ultimate intersection with diffusion layer control produces curve CFGJ. This type of dependence is likely to occur when R_{aq} is small compared to $R_m(PC)$.

Molecular Modification other than Increasing Chain Length— The effects of adding a polar substituent to each member of a homologous series to make a new series will be twofold. In the first place, the aqueous solubility of each member of the new series will be

Figure 2—*Expected effect of increasing polarity on the flux profile of a homologous series. See text for explanation of curves.*

Table I—Values of π_{CH_2} for Alcohols

Source[a]	π_{CH_2}	Correlation Coefficient	Number of Alcohols	Biological System Studied
V7	0.436	0.993	5	10-mv. change in rest potential, lobster axon
V8	0.419	0.996	5	5-mv. change in rest potential, lobster axon
V9	0.652	0.999	7	Stabilization against hypotonic hemolysis, red cell
V12	0.522	0.994	4	Narcosis, A. faetida
V13	0.503	0.999	5	I_{1s0}, rabbit gut
V14	0.588	0.987	4	Narcosis, goldfish, 37°
V15	0.470	0.965	4	I_{50}, tortoise heart
V16	0.478	0.990	4	I_{50}, paramecium mobility
V17	0.558	0.998	8	Narcosis, larvae barnacle
V18	0.653	0.997	7	I_{100}, movement, tadpoles, 18°, 2.5 days old
V19	0.617	0.998	7	I_{100}, movement, tadpoles, 1°, 12 days old
V20	0.496	0.997	8	I_{50}, ileum, guinea pig
V21	0.598	0.998	7	I_{100}, movement, tadpoles, −18°, 85 days old
V22	0.505	0.998	6	MIC, fish
V23	0.460	0.985	5	MLD, fish, carp
V25	0.471	0.993	5	MLD, fish, goby
V26	0.471	0.993	5	MLD, fish, roach
V27	0.488	0.986	7	T_{50}, pancreatic lipase, rat
V28	0.543	0.980	5	LD_{100}, cat
V29	0.418	0.997	4	Inhibition oxygen consumption, kidney, rabbit
V33	0.421	0.994	7	I_{50} oxygen consumption, lung, guinea pig
V34	0.403	0.998	4	Toxicity, paramecium
V35	0.381	0.999	5	I_{50}, generation time, L. aerogenes
V37	0.397	0.970	5	MLD, eel
V38	0.403	0.998	4	MLD, fish
V44	0.428	0.996	4	I_{50}, indophenol oxidation, kidney, rabbit
V45	0.510	0.999	8	MLD, S. typhosa
V46	0.498	0.998	7	Cytolysis, yeast cells
V55	0.497	0.999	6	Narcosis, larvae arenicola
V98	0.454	0.996	4	MLD, South African toad

[a] Table and column number of Reference 24 from which data were abstracted.

diffusional resistance within whole tissues or isolated cells, cognizance must be given to the thickness and diffusivity in cytoplasmic regions. Nothing is sacrificed by the inclusion of the aqueous resistance term, R_{aq}, as part of the overall resistance because, if aqueous resistance is negligible, the term vanishes from the equation.

In applying this type of treatment to biological systems, it is important that the biological end-point be a reflection of permeability only. If a biological parameter other than permeability is measured, it must be assumed that the variances in the intrinsic activity of the homologs are negligible and overshadowed by the expected variations in permeability. Care must be taken not to apply these equations to studies in which intrinsic activities span a range as wide as that produced in permeability by the molecular modifications. (The activity of a series of homologs in an isolated enzyme system is an example of the kind of data that cannot be treated by the above equations.)

With the above-mentioned limitations in mind, the authors attempted to organize some literature data for diverse biological systems and homologous series and to interpret these data in terms of transport concepts. By not including modifications other than chain length, the introduction of new specific interactions with biological receptors is avoided, as are the needs to define and to assign additional substituent constants. Also, since the effect of polar substituents on diffusion coefficients in biological membranes can be quite large (22, 23), inclusion of polar moieties in the treatments would undermine our basic premise: that the values of R_m and R_{aq} are constant.

Equimolar Studies—Two very different types of end-points are generally chosen for structure–activity studies, namely the relative activity of a substrate at a fixed concentration and the reciprocal of the substrate concentration required to produce a given response. In either case, the data can be reported in terms of activity per mole. It has already been shown in this presentation that, at constant concentration and under membrane control, the slope of log (response) versus n is equal to the biological π value of the organism or tissue studied. Data from a large number of equimolar or concentration-normalized structure–activity studies on homologous series are given in Tables I–III. These data were excerpted largely from the existing compilations of Hansch and his coworkers (24–28), who analyzed them in terms of the octanol–water partition coefficient. To keep these tables representative and reasonably concise, only

systems containing four or more homologs and having correlation coefficients greater than 0.980 were treated. These arbitrary restrictions were chosen for convenience and in no way affect the conclusions to be drawn. The biological π values reported in the tables give the increase in activity with respect to chain length rather than with respect to the octanol–water partition coefficient. Since the change in log (PC octanol–water) per methylene unit is about 0.50, the π value of the biological systems studied by Hansch and his coworkers can be approximated simply by dividing the slopes reported by 0.50.

The intercepts at $n = 0$ of these plots are equal to the product of the intrinsic activity (α_{int}) of the members of the series (which is assumed to be invarient) and $[C(PC)_o/R_m]$ (Eq. 5). Because the intercept is dependent upon three parameters (R_m, PC_o, and α_{int}) that cannot be evaluated independently, as well as on the concentration C which differed from study to study, no attempt will be made to interpret intercept data.

In Table I, biological π values obtained by least-squares analysis of data for linear aliphatic alcohols are presented. As mentioned previously, the validity of the biological π values rests upon the assumption that all of the alkanols have at least similar intrinsic activities, an assumption that is probably valid for most of the nonspecific activities cited. Data collected and processed by Hansch and his coworkers that involve isolated enzymes were deliberately excluded in compiling Table I. Such data are essentially independent of a transport step. The π^B values of Table I are all around 0.45 ± 0.1 and appear to be independent of the nature of the system studied.

In contrast to Table I, Table II contains data for a fixed biological response, the hemolysis of red blood cells. As can be seen, hemolysis data are available for many different types of homologous series. This response was selected not only because it has been studied extensively but also because the phenomenon is clearly physically produced and involves no specific cellular interactions. Thus, the activities observed are, of necessity, directly dependent upon permeabilities.

Because of the general interest that exists in the relative antibacterial activities of homologs on Gram-positive and Gram-negative organisms, it was decided to determine and compare π values obtained with differing homologous series tested on a representative organism of each type. These data are given in Table III.

About half of the π^B values in Tables II and III were determined

Table II—Values of $\pi^s_{CH_2}$ Determined from Hemolysis of Erythrocytes

Source[a]	π_{CH_2}	Correlation Coefficient	Number of Homologs	Homologous Series	Type of Erythrocyte
1A	0.429	0.985	5[b]	n-Alcohols	Rabbit
1B	0.477	0.999	7[b]	n-Alcohols	Bovine
1C	0.464	0.998	4[b]	Alkyl acetates	Bovine
1D	0.473	0.999	6	n-Alcohols	Rabbit
1G	0.528	0.989	6	n-Alcohols	Rabbit
1H	0.330	0.990	4[c]	α-Monoglycerides	Dove
1L	0.485	0.997	6[c]	N-Alkyl pyridinium bromides	Dove
1M	0.445	0.997	7[c]	N-Alkyl piperidine hydrochlorides	Dove
1N	0.439	0.988	4[c]	Alkyl dimethylbenzyl-ammonium chlorides	Sheep
1O	0.487	0.978	5[c]	Alkyl trimethylammonium bromides	Sheep
1P	0.585	0.979	4	Alkyl trimethylammonium bromides	Human
1Q	0.386	0.995	4	Alkyl trimethylammonium bromides	Bovine
1S	0.477	0.984	5	Alkyl amine hydrochlorides	Dog
1T	0.440	0.988	5	N-Alkyl pyridinium iodides	Dog
1V	0.536	0.997	4	Sodium alkyl sulfates	Human
1W	0.458[d]	0.990	6[b]	Sodium alkyl sulfates	Dog
1X	0.272	0.988	5[c]	n-Carboxylic acids	Sheep
1Y	0.447	0.992	5[c]	n-Carboxylic acids	Dove

[a] Table and column number of *Reference 26* from which data were abstracted. [b] Only data for straight-chain compounds used. [c] Only data for the ascending portion of the parabola used. [d] Two data points were assumed to be high by a factor of 10.0.

Table III—Values of $\pi^s_{CH_2}$ for Gram-Positive and Gram-Negative Organisms[a]

Homologous Series	Gram-Positive, *M. pyrogines* var. *aureus* π_{CH_2}	Correlation Coefficient	Number of Homologs	Gram-Negative, *S. typhosa* π_{CH_2}	Correlation Coefficient	Number of Homologs
3-Alkyloxy phenols	0.434	0.997	7	0.409	0.997	5
4-Alkyloxy phenols	0.455	0.997	7	0.431	0.992	5
4-Alkyl phenols	0.456	0.999	7	0.486	1.00	4
2-Alkyl 4-chlorophenols	0.432	0.998	6	0.403	0.987	4
4-Alkyl 2-chlorophenols	0.438	0.997	6	0.412	0.998	4
4-Thioalkyl phenols	0.423	0.997	4	0.432	0.997	4
2-Alkyl 4-bromophenols	0.479	0.991	5	0.440	0.993	5
Alkyl amines	0.486	0.997	4	0.228	1.00	5
Alkyl diguanidines	0.478	0.997	5	0.331	0.985	7
Alcohols	0.450	0.993	5	0.511	0.999	8

[a] Data abstracted from *Reference 25*.

from the linear ascending portion of so-called "parabolic"[1] structure–activity curves. These data are indicated by the superscript *c*. From the data, it can be seen that the π^B values obtained from linear and parabolic structure–activity data are equivalent and that these values are independent of the nature of the series studied. Furthermore, they all fall within the range of the values given in Table I. This independence of π^B values on the nature of the homologous series and the biological response studied has been repeatedly noted in the literature (1–6) and can be explained on the basis of both equilibrium and kinetic structure–activity theories, including the one presented here.

Many workers (1–5, 12–14) have noted (although in different terminologies) that the π values for a methylene unit in several common organic solvents (Table IV) are not appreciably different from the biological π^B values given above. Therefore, if, in lieu of plotting *n*, one plots the logarithms of the partition coefficients of the compounds obtained in a solvent for which all the substituent π values are the same as the biological system against the logarithms of their respective biological responses, the slope will be equal to unity. This method has the convenience of enabling substituents other than methylene units to be analyzed. However, because of the scatter and nonlinearity frequently introduced into such plots

[1] The term parabolic is used in a general sense to describe any curve that has an ascending portion and either a descending or plateauing region rather than in its strict mathematical sense which describes a curve of constantly changing slope.

by nonhydrocarbon substituents, this type of data must be analyzed cautiously, especially when the range of the data is less than one to two orders of magnitude. The presumed scatter introduced by hetero groups is an expected consequence of predictable differences in *in vitro* and *in vivo* π values for the hetero moieties. To illustrate, consider the π and π_{OH} values obtained for partitioning between water and red blood cell ghosts on one hand and several commonly used reference solvents on the other. The π values in Table IV for most of the solvents listed are fairly close to the value for erythrocyte ghosts. There is a marked lack of agreement in the π_{OH} values between the solvents and the biophase as well as among the solvents themselves. This shows that a particular organic solvent may be a good model for the red blood cell ghosts as far as methylene groups are concerned and, at the same time, not accurately reflect the partitioning influence of polar substituents. In view of the fact that it is necessary to use separate equations to relate octanol–water and ether–water partition coefficients when dealing with hydrogen donor and hydrogen acceptor solutes (29), it is unlikely that a single *in vitro* partitioning system will accurately mimic biological membranes, which themselves may differ significantly. Thus, if truly meaningful substituent constants are to be acquired, *they must be obtained for the biological systems themselves*. Seeman *et al.* (30) already demonstrated the feasibility of the approach using erythrocyte ghosts. Substituent constants could also be obtained from experimental transport studies using biological membranes and the equations described in the first portion of this report.

Solvent	π_{CH_2}	π_{OH}	Reference
Ether	0.573	−1.51	45
Ether	0.612	−1.86	46
Octanol	0.500	−1.18	29
Chloroform	0.609	−2.01	46
Olive oil	0.525	−0.530	47
Castor oil	0.545	−2.48	48
Red blood cell ghosts	0.526	−2.08	30

The process of analyzing certain biological data has led to some conclusions in conflict with previously reported biological trends suggested by Hansch and his coworkers (24–28). Using the octanol–water partition coefficient as a reference parameter, they found the slopes obtained in both Gram-positive and Gram-negative bacterial systems to be significantly lower than the slopes found for hemolysis, narcosis, and most other parameters. The authors believe that this discrepancy has several explanations. Since Hansch and his coworkers were primarily interested in overall structure–activity relationships, they analyzed a much wider variety of data, including many sets of data for which it is questionable whether the activities were primarily dependent upon transport to the receptor site. Some of their slopes, particularly those obtained with isolated enzymes, reflect changes in intrinsic activity rather than transportability with chain length, or log (PC). Moreover, since Hansch and his coworkers did not restrict themselves only to homologs and since the biological π values for some hetero substituents found in some analogs are not the same as those for octanol–water partitioning, the slopes would be affected. Finally, if there is a slight plateauing of activity at high partition coefficients (see next section) and all of the data are fitted to a linear equation, the slopes obtained will be artifactually low.

The average π values obtained from the analysis of structure–activity studies of homologous series on several biological endpoints, including those already discussed, are shown in Table V. For all studies involving simple membranes, the value of π does not differ significantly from 0.45. However, the π values for studies involving skin or mucosa of mammals are consistently lower than 0.45. The average value determined from the data of seven series, in which homologs were tested on mammalian epithelial tissue, is 0.25. The reason for this low value is not entirely clear but is probably related to the high degree of hydration of these tissues.

Many equimolar structure–activity curves show, in addition to an initial slope of π, a leveling off of activity with increasing chain length beyond some critical value. This plateauing effect was observed by several workers for both steady-state flux (19, 20) and biological activity (31–35) and can be explained as being a consequence of the transition from membrane control to diffusion layer control of transport (see Eqs. 4–6 and curve EHI of Fig. 1). A plateau is only to be expected in systems where considerations dictate the existence of a significant diffusion layer. It is not generally observed in the analysis of antimicrobial or hemolysis data because small particles (i.e., the cells) have extremely thin diffusion layers associated with them.

An apparent case demonstrating the plateauing effect is found in the work of Rosen et al. (31) on the permeability of the toad bladder to nonionic aliphatic acids at pH 4 and 6. Their data (plotted semi-logarithmically in Fig. 3) indicate that there is a linear increase in

Figure 3—Permeation rate of unionized aliphatic acids across toad bladder (31). Key: O, pH 4; and ●, pH 6.

transport rate constant from propanoic to hexanoic acid and a plateau at higher chain lengths. The differences in the initial slopes are explained by Rosen et al. The results of Buchi and Perlia (35) on local anesthetics applied to rabbit cornea likely provide an additional example of a transition from membrane to diffusion layer control of transport. These workers determined the minimum effective concentration and the duration of activity (Fig. 4) of several alkyl derivatives of cinchocaine. The reciprocal of the minimum effective concentration of each homolog is a measure of activity and is a direct reflection of its ability to penetrate the skin and reach the site of action. The duration of activity is a measure of the time it takes for all of the drug applied to pass through the region in which it is active (where the receptor sites are located)

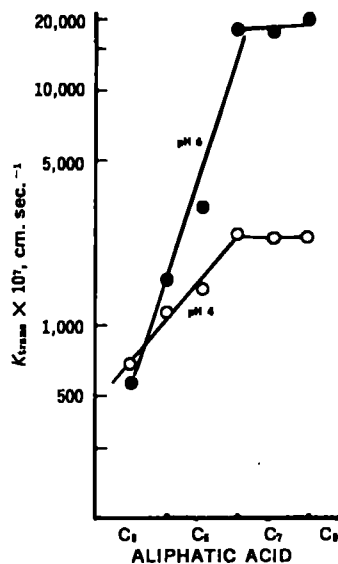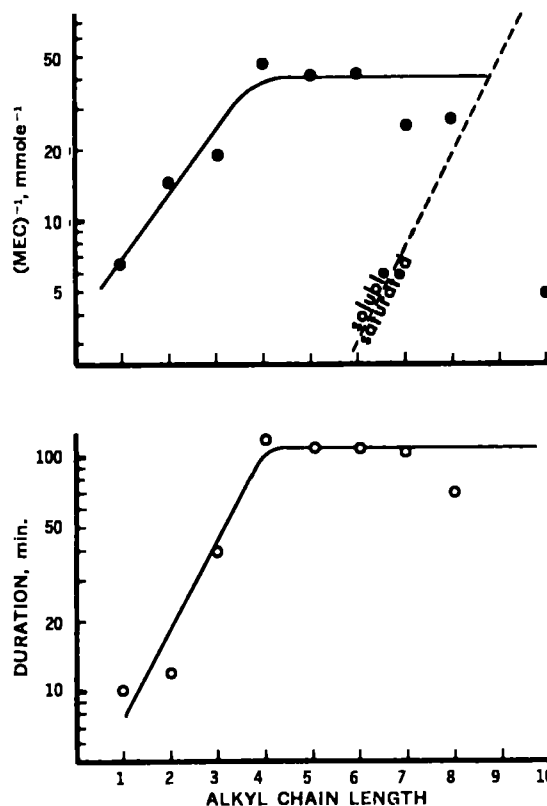| Series	End-Point	Subject	Number of Studies	Mean	SD
Alcohols	Many	Many	32	0.483	0.079
Many	Hemolysis	Erythrocyte	17	0.465	0.057
Many	Antibacterial	Gram positive	10	0.453	0.021
Many	Antibacterial	Gram negative	9	0.428	0.051
Many	Permeation	Mammalian epidermal	7	0.250	0.047

Figure 4—Reciprocal minimum effective concentration (●) and duration of action (O) of alkyl cinchocaines for corneal anesthesia in the rabbit as a function of chain length (35).
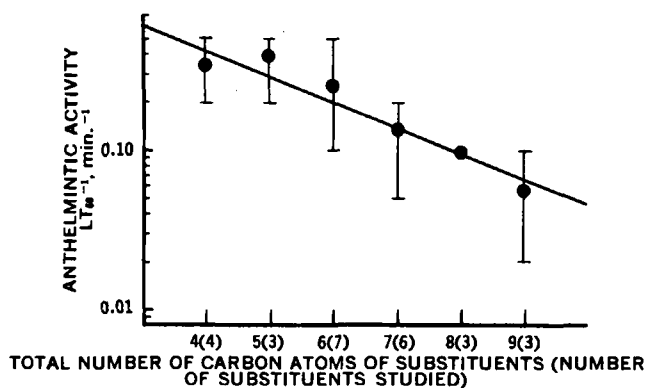
Figure 5—*Anthelmintic activity of 26 mono- and disubstituted alkylphenols from saturated solution (36).*

and be carried away by the circulating blood. If a substance is transported rapidly, it has a rapid onset of activity but a short duration. Conversely, if it is transported more slowly, the onset time is longer but so is the time required for all of the applied drug to pass through the receptor-containing region.

Since both curves of Fig. 4 are dependent upon transport across the same membranes and diffusion layers, it is not surprising that both showed the transition to diffusion layer control for the same chain length. As chain length is increased beyond six carbons, the activity begins to diminish and falls precipitously for the decyl derivative. This decline in activity is probably due to the fact that the concentrations of the higher homologs used in the study are very close to the solubility limit. In fact, the concentration reported for the decyl derivative greatly exceeds the solubility of that compound. The dotted line in Fig. 4 is an extrapolation of solubility data (35) at pH 7.4 for the first six derivatives. The π values ob-
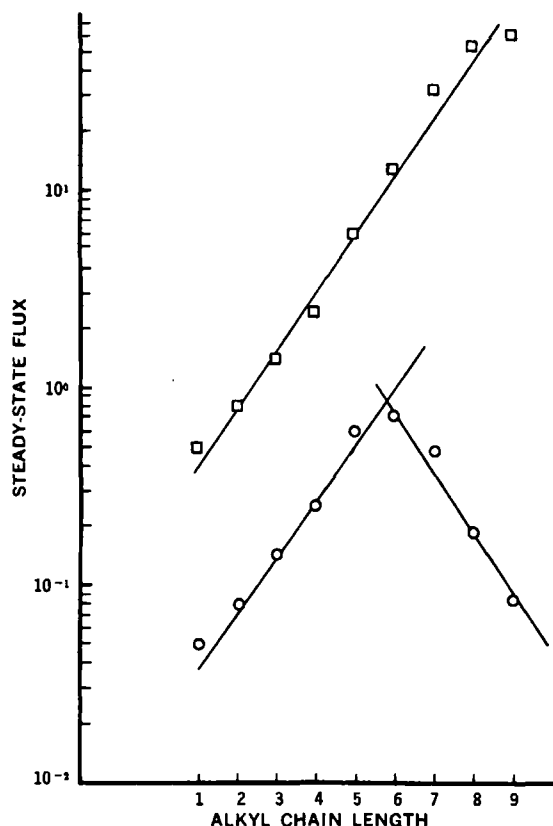

Figure 6—*Data of Scheuplein and Blank (49) for permeation of normal aliphatic alcohols across human stratum corneum in vitro. Key: □, concentration-normalized flux; and ○, flux from 0.10 M solution (last four points represent saturated solutions).*

tained from the two sets of data appear to differ significantly (0.28 for duration data and 0.385 for minimum effective concentration data). There are many possible causes of this discrepancy, including differences in the ability to reach and leave the receptor region and differences in intrinsic activity of the substrates. In any case, these data should not be used to determine π because of their degree of scatter and their proximity to the transition region.

Many sets of biological data that have been treated as parabolic could alternatively be treated by Eq. 9. Similarly, studies for which no chain length dependency has been observed might be examples in which all activities of the compounds studied are on the plateau. Other examples of the plateauing effect are found in *References 6* and *33*.

Saturated Solution Studies—Returning to the arguments built around Fig. 1, one can find an alternative explanation of the plateauing effect which is also built entirely on simple diffusional theory. Since π is usually around 0.45 ± 0.10 and δ is about 0.60 ± 0.05, the value of $\pi - \delta$ is normally between 0.0 and −0.03. This being the case, curve CFG of Fig. 1 can also serve to explain plateauing data. In fact, if $\pi - \delta$ is near zero, it is not possible to distinguish between curves CFG and EHI from biological data unless the extent of saturation of each solution is known.

The data of Lamson *et al.* (36) provide a clearcut example of the relative activity of saturated solutions of alkyl homologs under membrane control of flux (curve BG of Fig. 1). Figure 5, taken from their data, shows the time required to kill at least 50% (LT_{50}) of pig ascaris *in vitro* by saturated solutions of various alkyl phenols. Their LT_{50} data, which represent 26 mono- and disubstituted alkyl phenols, give a slope of −0.16 when plotted semilogarithmically *versus* total chain length. This value falls in the midrange of the expected values for $\pi - \delta$. Significantly, the LT_{50} data of Lamson *et al.* (36) for shorter homologs, which are in true solution, fall below the line, as do the data for $n = 11$ and $n = 12$, suggesting the total curve CFGJ of Fig. 1. These results are clearly contrary to the predictions, based upon the Ferguson principal, *i.e.*, that all saturated solutions of a homologous series have the same biological activity (37, 38).

For almost all examples of "parabolic" structure-antibacterial activity relationships, the concentrations reported on the descending portion of the curve are greater than the solubility. Furthermore, the reproducibility from worker to worker of the data is good on the ascending portion and is usually quite poor above the solubility limit. As pointed out by several authors (2, 37–39), the meaningfulness of the data on this portion of the curve is questionable. If the experiments are performed properly, an exponential increase in activity is observed with increasing chain length until the required concentration for a particular derivative exceeds its solubility and that compound will have little or no activity (37). This is because it is impossible to get sufficient drug in solution to produce the desired biological effect. To get reliable data on the descending portion of the curve, the experiment must be designed to measure the magnitude of the effect produced by a given concentration (saturated or not) of material, as was done in the example just discussed, rather than the concentration required to produce a given effect. The following examples, pointed out by Ferguson (39) in 1939, illustrate the questionable nature of measurements of concentration required to produce a constant effect.

1. The concentrations of a series of alcohols required to produce a particular degree of surface tension lowering appear to go through a maximum, and the position of that maximum with regard to chain length depends upon the degree of surface tension lowering chosen.

2. The adsorption of homologs on charcoal increases with chain length; but as the solubility is exceeded, the adsorption appears to go through a maximum and then decreases with chain length. Thus, the maximum observed is dependent upon the experimental conditions chosen.

The transition from equimolar to saturated solutions under membrane control is nicely illustrated in Fig. 6. The value of π is determined from the concentration-normalized data (open squares) for the permeation of nine aliphatic alcohols across human stratum corneum to be 0.30. This low value is not unusual for epithelial tissue (Table V). The open circles for methyl through pentyl alcohol represent the flux from 0.1 M solution. The solubility of the higher alcohols was below this value, so the last four open circles represent the flux from saturated solutions. The slope of this line (0.30) is in excellent agreement with the expected value of $\pi - \delta$ of 0.30.

SUMMARY

According to this model in its present form, the only explanation for a decrease in activity with chain length is the reduced solubility of the higher alkyl homologs. Other physical or biological phenomena, such as micelle formation (40), incorporation into mixed micelles (41, 42), adsorption onto inert surfaces, competing receptors or absorption into fatty compartments (43), and enzyme specificity, all of which usually show an exponential dependence upon chain length, could be responsible for the descending portion of the curve. Any of these phenomena can be incorporated into the model to extend its range of applicability. Membrane pores have not been discussed in this report. However, the existence of such pores also causes no great difficulty, because they can be readily accounted for by a straightforward extension of this treatment to include parallel pathways (44).

It is interesting (and reassuring) to note that the equimolar curves obtained in this kinetic analysis, which have a slope of $\pi = 0.35$ to 0.55, are in accordance with the predictions of the equilibrium theories of Ferguson (1, 37–39) and others (1–5), even though the physical–chemical foundations differ significantly. The solubility restrictions as described are actually a kinetic facsimile and a quantitation of Ferguson's explanation of the "cutoff" phenomena (37). Nevertheless, unlike other models, this theory predicts, for certain circumstances, a plateau in the activity *versus* chain length profile of a series of drugs under subsaturated conditions. It also predicts a region of slight slope for saturated solutions for reasonably expected conditions. These solubility relationships are presently being investigated in goldfish. These experimental studies strongly support the membrane-diffusion layer model as the operative mechanism in the absorption of homologous anesthetics by the fish.

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