Passive Permeation of Organic Compounds through Biological Tissue: a Non-Steady-State Theory

JOHN T. PENNISTON,[1] LAUREL BECKETT, DONALD L. BENTLEY, AND CORWIN HANSCH

Departments of Chemistry and Mathematics, Pomona College, Claremont, California 91711

(Received March 8, 1969)

SUMMARY

A theoretical analysis of the penetration of drug molecules to their sites of action in terms of their lipophilic character is presented. This analysis justifies the parabolic equations which have been used empirically to describe the relationship between effectiveness of drug and its lipophilic character.

INTRODUCTION

The recent literature on membrane permeability contains extensive discussions of mediated and active transport involving sugars, amino acids, and inorganic ions (1–4). Passive movement of these normally quite polar compounds through a cellular membrane is assumed to be negligible in the presence of special transporting mechanisms. Many other types of molecules which act on cellular systems are not transported by specific systems; for these molecules, passive movement governs the speed with which they reach their sites of action.

Passive permeation is particularly important for the action of drugs which are not native to the living system and for which no special transporting mechanisms exist. The factors which determine the rate of passive penetration of a given compound are quite different from the factors which obtain when a special mechanism for penetration exists. In the case of active or mediated transport, the structure, stereochemistry, size, and charge of the penetrating molecule are factors determining its ease of penetration, and great specificity often exists in such systems. The passive permeation of molecules depends more on general physical properties of the molecule and much less on the small differences which are crucial in active transport.

The property upon which passive permeation depends is the ease with which the molecule passes into and through a membrane; i.e., the solubility of the molecule in the membrane continuum. In considering the penetration of a molecule to its site of action, not only must passage through membranes be considered, but also absorption to and desorption from macromolecules. We assume that these two processes are mathematically similar, but we shall consider only the former process in the model of permeation presented below.

Since it is impossible to ascertain the solubility of the compounds in question in all of the membranes through which they must pass, it is important to have a model reference system which can predict the general behavior of a molecule as it passes into and out of a membrane. The easily measured partitioning of a compound between water and a relatively nonpolar solvent provides a good measure of the

This work was supported by Research Grant GM 07492 from the National Institutes of Health.

[1] Present address, Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin 53706.

ease with which a molecule will pass between a membrane of macromolecules and an aqueous phase. This correlation was discussed by Davson and Danielli (5), and recent studies have confirmed that this partition coefficient does indeed serve as a useful indicator for the behavior of a compound in a living system. We have chosen octanol and water as our reference solvents, and will use P to represent the partition coefficient in this system except where another system is explicitly indicated.

Direct measurements have been made on the binding of compounds to membrane and macromolecules. Such measurements are made at equilibrium, but are otherwise closely related to the situation of passive penetration. Equation 1 correlates

$$\text{Lo g}\frac{1}{C} = 0.751 \log P + 2.301, \quad n = 42, r = 0.960, s = 0.159 \quad (1)$$

partition coefficient with the binding of a wide variety of organic compounds to bovine serum albumin (6). In Eq. 1, C represents the molar concentration of organic compound necessary to produce a 1:1 complex with BSA[2] in equilibrium dialysis; that is, various concentrations of small organic compound were placed in equilibrium with BSA, and from the binding results the concentration necessary to affix 1 small molecule per molecule of BSA was calculated. The equilibrium constant so expressed is analogous to log $1/C$, used to express relative activity of drugs. In Eq. 1, P represents the partition coefficient, n is the number of compounds considered, r is the correlation coefficient, and s is the standard deviation. This equation is essentially the same as the earlier reported result using only phenols (7). The compounds used in formulating Eq. 1 had P values of about 10–10,000 and were quite diverse in structure. Among the compounds included were weakly basic aromatic amines, weakly acidic phenols, neutral compounds such as naphthalene and azobenzene, bulky molecules such as 1-hydroxyadamantane and camphorquinone, and aliphatic compounds such as neopentanol and 2-nonanone. We have found similar equations with slopes between 0.5 and 0.7 for the binding of aliphatic alcohols by ribonuclease (6), of miscellaneous molecules by bovine hemoglobin (8), of fatty acids by BSA (6), of anilines and acetanilides by nylon and rayon (9), of barbiturates by a variety of homogenized rabbit tissues (10), as well as various other examples (11). Recently Scholtan (12) showed that linear relations of the form of Eq. 1, based on an isobutyl alcohol–water reference system, gave very high correlations for the binding of sulfonamide drugs, penicillins, tetracyclines, cardenolides, acridines, and steroid guanylhydrazones to human albumin. He found similar equations for the binding of many of the same drugs to RNA (12). Thus, as far as we are able to ascertain, the above work shows that the partition coefficient can be used to describe *quantitatively* the binding, and hence the localization, of organic compounds by the first biomacromolecular structures (protein, lipid, nucleic acid, or membrane) with which they come in contact.

The widely cited experimental studies of Collander (13) indicated that the logarithm of the rate of membrane penetration (log Pen) by a great variety of compounds increased linearly with log P. However, a recent and more thorough statistical evaluation of Collander's results by Milborrow and Williams (14) shows that Collander's results are not strictly linear, and that in view of the fact that Collander's study was limited to molecules with quite low partition coefficients, his results are not inconsistent with the postulate of parabolic dependence (15) of penetration rate on the partition coefficient. The theory presented here predicts that the rate of penetration will increase, pass through a maximum, and then decrease as P increases. Figure 1 shows the type of behavior predicted by this theory.

In a survey of the literature on the penetration of sets of organic compounds

[2] The abbreviation used is: BSA, bovine serum albumin.

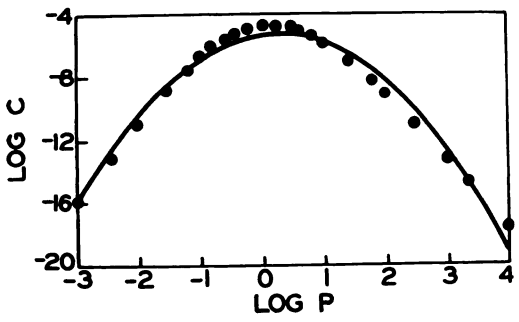

FIG. 1. *Concentration in 20th compartment as a function of log P when t = 10, m = 1*

The curve is a parabola fitted to the calculated points by the method of least squares.

through tissue, we have found that Collander's evidence of the direct linear relation between log Pen and log P holds only within certain limits of log P values. In fact, instances are cited below where an *inverse* relationship between log Pen and log P gives a much better correlation. Insufficient attention has been paid to these exceptional examples. Equations 2 and 3 are derived from the data of Marzulli *et al.* in Table 1. Equations 2 and 3 correlate

$$\text{Log } k = -0.261\ (\pm 0.12) \log P - 0.013\ (\pm 0.27), \quad n = 5,\ r = 0.971,\ s = 0.124 \quad (2)$$

$$\text{Log } k = -0.040\ (\pm 0.04)(\log P)^2 - 0.125\ (\pm 0.15) \log P - 0.040\ (\pm 0.15), \quad n = 5,\ r = 0.997,\ s = 0.048 \quad (3)$$

the rate of penetration of radioactive alkyl phosphates through human skin (17). Most interesting is the fact of the negative slope of Eq. 2. This indicates that rate of penetration actually slows down with increasing values of the partition coefficient for these molecules, which are much more lipophilic than those studied by Collander. While Eq. 2 gives a rather good correlation with negative slope, indicating an approximate inverse relation between log k and log P, Eq. 3 gives an improved correlation, indicating the curvilinear relation between log k and log P. Because of the relatively narrow range of log P values of the compounds studied, the degree of curvature is small but statistically significant. One would expect that, including molecules with much lower log P values, a more sharply defined parabolic relation between log k and log P could be established. In effect, Collander was studying molecules falling on the left side of the parabola of Fig. 1, while Marzulli *et al.* were studying a set falling primarily on the right side. The lowest log P value of Marzulli and co-workers was —0.51, while Collander's highest value of log $P_{\text{olive oil}}$ was 0.4. Although only five data points are available, Eq. 3 is a statistically significant improvement over Eq. 2; $F_{1,2} = 18$; $F_{1,2\ \alpha.95} = 18.5$.

The relationship becomes more significant if one includes two very lipophilic aromatic phosphates whose rates were also studied (17). Because of their great insolubility we have not been able to obtain accurate partition coefficients for these, and hence have excluded them.

TABLE 1

Penetration of human skin by $(RO)_3PO$

R	log P[a]	Observed log k[b]	Calculated log k[c]	$\lvert\Delta \log k\rvert$
Methyl	−0.52	0.020	0.014	0.01
Ethyl	0.98	−0.206	−0.200	0.01
Propyl	2.48	−0.541	−0.593	0.05
Isopropyl	1.88	−0.456	−0.415	0.04
Butyl	3.98	−1.174	−1.163	0.01

[a] Unless otherwise indicated, P in this report refers to the octanol/water partition coefficient. The value of trimethyl phosphate was determined experimentally; the other values were obtained by the additivity principle (16).

[b] k is equivalent to the maximum steady-state penetration rate constant (μmoles/cm^2/min $\times 10^2$) as determined by Marzulli *et al.* (17).

[c] Calculated using Eq. 3.

TABLE 2
Penetration of cockroach cuticle by insecticides

Molecule	log $P_{\text{olive oil}}$[a]	Observed log $t_{1/2}$[a]	Calculated log $t_{1/2}$[b]	$\lvert\Delta \log t_{1/2}\rvert$
Phosphoric acid	−1.00	1.204	1.256	0.05
Dimethoate	−0.469	1.431	1.350	0.08
Paraoxon[c]	0.609	1.740	1.757	0.02
Dieldrin[d]	1.806	2.505	2.544	0.04
DDT[e]	2.500	3.190	3.163	0.03

[a] From Olson and O'Brien (18).
[b] Calculated using Eq. 5.
[c] Diethyl *p*-nitrophenyl phosphate.
[d] 1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-*endo*-*exo*-1,4:5,8-dimethanonaphthalene.
[e] 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane.

Setting the derivative (d log k/d log P) equal to zero, we can calculate an ideal lipophilic character for rate of permeation of human skin. This value of log P is −1.58.

Turning to the data of Table 2 on the penetration of cockroach cuticle by various insecticides, we derive Eqs. 4 and 5.

$$\text{Log } t_{1/2} = 0.543\ (\pm 0.21) \log P_{\text{olive oil}} + 1.639\ (\pm 0.32), \quad n = 5,\ r = 0.978,\ s = 0.198 \quad (4)$$

$$\text{Log } t_{1/2} = 0.124\ (\pm 0.13)(\log P)^2_{\text{olive oil}} + 0.360\ (\pm 0.22) \log P_{\text{olive oil}} + 1.492\ (\pm 0.22), \quad n = 5,\ r = 0.998,\ s = 0.077 \quad (5)$$

Again we find a high significance for Eq. 5 ($F_{1,2} = 18$). In Eqs. 4 and 5, $t_{1/2}$ is the half-time of disappearance of radioactive insecticides from the surface into the interior of the cuticle. Olson and O'Brien (18) recognized that their results were not in agreement with Collander's hypothesis, but were unable to suggest a satisfactory explanation. Again we see in Eq. 4 that the half-time of disappearance increases with increase in log P; that is, this set of insecticides falls on the right side of the parabola of Fig. 1. The ideal lipophilic character for the set ($\log P_{o,\ \text{olive oil}}$) is −1.46.

Still another example of the nonlinear relationship between permeation and partition coefficient stems from the work of Ross (19), who measured the rate of penetration of a variety of organic compounds (Table 3) into the aqueous humor of the eye of the living rabbit. We have derived Eqs. 6 and 7 from his data in Table 3.

$$\text{Log } k = 0.280\ (\pm 0.09) \log P_{\text{ether}} + 0.202\ (\pm 0.19), \quad n = 7,\ r = 0.960,\ s = 0.116 \quad (6)$$

$$\text{Log } k = -0.067\ (\pm 0.05)(\log P)^2_{\text{ether}} + 0.073\ (\pm 0.16) \log P_{\text{ether}} + 0.140\ (\pm 0.11), \quad n = 7,\ r = 0.992,\ s = 0.059 \quad (7)$$

For Eq. 7, $F_{1,4} = 15.4$; $F_{1,4\ \alpha.95} = 7.7$. Log P_o from Eq. 7 is 0.55. Unfortunately, Ross' set of compounds, like Collander's, is quite polar, with the highest log P_{ether} for ethanol of 0.28. The data of Collander and Ross constitute two sets with moderately good correlations of positive slope for the log P term in the simple linear equation. The much more lipophilic sets of compounds (Tables 1 and 2) yield reasonable linear equations with negative slopes of the log P term. Strangely, none of the four independent investigations covers a proper set of molecules so that log P_o can be established with much confidence. Nevertheless, these four examples constitute the strongest evidence for an over-all parabolic dependence of log k on log P as illustrated in Fig. 1.

Simple reflection on the problem leads to the idea of a nonlinear relationship and suggests that permeability must first increase with increasing P, reach a maximum, and then decrease. In the case when

TABLE 3
Penetration of rabbit eye by miscellaneous compounds

Compound	log P_{ether}[a]	Observed log k[a]	Calculated log k[b]	$\|\Delta \log k\|$
Urea	−3.30	−0.86	−0.832	0.03
Glycerol	−2.96	−0.66	−0.665	0.01
Thiourea	−2.14	−0.23	−0.324	0.09
Methylthiourea	−1.64	−0.21	−0.160	0.05
Ethylthiourea	−1.36	−0.10	−0.084	0.02
Propylthiourea	−0.41	0.07	0.099	0.03
Ethanol	0.28	0.18	0.156	0.02

[a] From Ross (19).
[b] Calculated using Eq. 7.

P approaches zero, the compound would be so hydrophilic as to be unable to enter a lipid barrier; but as P approaches infinity, the compound would become so lipophilic as to be unable to return to an aqueous phase from the first lipid barrier it meets. It is evident, then, that there will be an intermediate value of P such that a compound possessing this partition coefficient will penetrate a series of lipid and aqueous barriers most rapidly. We term this optimum value for penetration P_o.

Another source of evidence concerning the nature of the dependence of biological permeability on P is the studies on biological response to series of related compounds. Studies of this point (11, 20, 21) indicate numerous instances in which the biological response (most generally defined as log $1/C$, where C is the molar concentration of drug causing a standard response) is approximately linear with respect to P up to a certain point, after which activity actually falls off with increasing values of P. While there are a number of reasons why one might expect such a fall-off, other than by limitation in rate of penetration (e.g., metabolism, steric hindrance in transport or at the site of action), it has been shown (22) (Eqs. 8 and 9), using the data of Soloway *et al.* (23), not only that the

$$\text{Log } C = 0.64 \log P + 0.31, \quad n = 14, r = 0.802, s = 0.303 \quad (8)$$

$$= -0.54(\log P)^2 + 2.47 \log P - 1.05, \quad n = 14, r = 0.915, s = 0.214 \quad (9)$$

rate of localization of a set of benzeneboronic acids in mouse brain is parabolically dependent on P, but that this dependence is very closely related to that of a wide variety of drugs acting on the central nervous system. The value of 2.3 for log P_o obtained from Eq. 9 is close to the mean value of 2.0 found (24) for 16 different sets of hypnotics acting in the central nervous system. Since no adequate theory has been proposed to account for this parabolic dependence of rate of penetration on P, we shall present here a nonsteady-state model to account for the permeation behavior of molecules.

METHODS

Our model is a simple kinetic theory which is suited to describe cases in which a steady-state theory is not applicable. The model used is closely related to those used in chemical kinetics and is identical in type with the models of multicompartment analysis (25). It does not assume any particular picture of membrane structure, but simply treats the membrane or macromolecule as a single phase of lipid character, ignoring the nature of its structure. The simplest case analyzed here is represented in Fig. 2.

In this model, compartment 1 has a given volume, V_1, and, at zero time, a given concentration of solute, A_1. Corresponding values obtain for the other compartments. The surface area common to each pair of compartments is assumed to be the same for all and is symbolized by

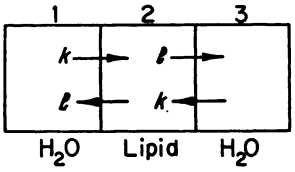

FIG. 2. *A single lipid barrier between two aqueous compartments*

The rate constant for passage from aqueous to lipid phase is k, and that for the reverse passage is l.

S. It is assumed that in living tissue we are dealing with a "stirred" solution and that the energy required to form a hole for the penetrating molecule to move into is supplied by the system. Time-lapse photomicrography has given abundant evidence of the constant movement of cells and cellular organelles. From this model, differential equations governing the concentration of solute in the three compartments are easily set up.

$$\begin{aligned}\frac{dA_1}{dt} &= \frac{S}{V_1}(lA_2 - kA_1)\\ \frac{dA_2}{dt} &= \frac{S}{V_2}(kA_1 - 2lA_2 + kA_3)\\ \frac{dA_3}{dt} &= \frac{S}{V_3}(lA_2 - kA_3)\end{aligned} \quad (10)$$

Assuming the three volumes to be equal, these equations become equivalent to those governing two consecutive first-order chemical reactions. The solution to this case is well known (26) and may be expressed by an equation involving transcendental functions.

This scheme was expanded by adding more phases, with lipid and aqueous phases in alternation. A drug molecule, in order to reach its site of action, must interact many times with serum proteins and cross several membranes. Thus, a model which involves traversal of many lipid and aqueous phases is more realistic. It was assumed for simplicity that the same k's and l's apply to each barrier and that all the compartment volumes and surface areas are equal to unity.

The relative volumes of the lipid and aqueous spaces in this model will not affect the general form of the results. Variation of the volume of the lipid phase may shift the value of P for which penetration is fastest, but a maximum rate of penetration will appear for some value of P in any case.

An irreversible first-order chemical reaction was assumed to bind solute in the final phase with a rate constant m. Thus m is an arbitrary rate constant for a hypothetical reaction in the last compartment and has no direct relation to k or l. In this way, the last step in the movement of drug from the point of introduction to its transfer onto the receptor site can be treated independently. In simulation studies m was allowed to vary over a large range of values and was found to have negligible effect on the shape of the curve.

While it is possible to obtain an analytical solution for the simple case described by Eq. 10, a more general expression of the problem is susceptible only to computer analysis. The more general set of differential equations to be analyzed is

$$\begin{aligned}\frac{dA_1}{dt} &= -kA_1 + lA_2\\ \frac{dA_{2i}}{dt} &= -2lA_{2i} + k(A_{2i-1} + A_{2i+1})\\ \frac{dA_{2i+1}}{dt} &= -2kA_{2i+1} + l(A_{2i} + A_{2i+2})\\ \frac{dA_{n-1}}{dt} &= -(l+m)A_{n-1} + kA_{n-2}, \quad n \text{ odd}\\ &= -(k+m)A_{n-1} + lA_{n-2}, \quad n \text{ even}\\ \frac{dA_n}{dt} &= mA_{n-1}\end{aligned} \quad (11)$$

where A_i represents the concentration in the ith phase, and k and l are rate constants. Since A_1/A_n does not depend on A_1^0, an arbitrary initial concentration of 1.0 was used in most cases (10.0 or 100.0 was used in examples with large numbers of barriers). The general procedure for a specific n value was to let the partition coefficient, $P = k/l$, vary over an interval and obtain a series of solutions of the set of equations by integrating over time t.

Values of k and l were chosen so that $k \cdot l = 1$; that is, we have assumed a reciprocal relation between hydrophobic and hydrophilic character. An increase in lipophilic character (an increase in k) results in corresponding decrease in hydrophilic character (a decrease in l). Looked at from another point of view, $k \cdot l =$ constant is a boundary condition, but the choice of constant $= 1$ results in a choice of time units. Values of k and l were taken so that P varied from 0.001 to 1000 with equally spaced logarithmic values. More extreme values of P make calculations too time-consuming and add nothing to our understanding. Solution of the n equations was done by numerical integration, using the Runge-Kutta fourth-order combined with Adams-Moulton methods (27, 28) as programmed for computer use by Richardson (29). The Runge-Kutta method uses the idea of a fourth-order power series approximation to $A_i(t + \Delta t)$, without using any previous values of A_i or A_i'. The primary drawback is that no error estimate exists; thus Δt cannot be altered in the course of integration. The accuracy of the Runge-Kutta integration is approximately five places for appropriately chosen Δt. The method of Adams and Moulton uses a table of backward differences in dA_i/dt to obtain a predicted value of $A_i(t + \Delta t)$. A corrected value can then be obtained and the difference used to estimate error. The step size Δt can thus be halved or doubled to increase accuracy or efficiency of integration. Most of the integration for these equations used a series of five backward differences initially generated by Runge-Kutta integration for use with the Adams-Moulton method with variable step size. The error, which can be arbitrarily bounded within the limits of the computer, was set at $<10^{-5}$.

A regression analysis was performed on the data to obtain an equation of the form

$$\text{Log } A_n = a(\log P)^2 + b \log P + c \quad (12)$$

The resulting curve was then plotted against the points as shown in Fig. 1. Equation 12 is derived (15) from the hypothetical relationship

$$A = \alpha \exp - \left[\frac{(\log P - \log P_o)^2}{\beta}\right] \quad (13)$$

In Eq. 13, A is the probability that a molecule will reach the last compartment in a fixed time under standard conditions.

Figure 1 shows a plot of the points obtained from a 20-barrier model. Log P (log k/l) is plotted along the horizontal axis, and log C, the concentration in the last compartment when $t = 10$ and $m = 1$, is plotted along the vertical axis. The least-squares line, which is obtained by fitting the points to Eq. 12, is drawn through the points. This is the form of our model when highly specific steric and electronic effects can be neglected. The fit is quite good, and the apex of the parabola comes at log $P = 0.20$. Similar curves are obtained for fewer barriers, with, of course, larger values for the concentration in the last compartment.

DISCUSSION

It is evident from the above analysis that the theoretical curve for dependence of penetration on partition coefficient can be very closely approximated by Eq. 12. This equation is identical with that postulated (15) to describe the dependence of biological response on partition coefficient. Equation 14 gives the analogous form of the equation for biological response.

$$\text{Log } \frac{1}{C} = -k (\log P)^2 + k' \log P + k'' \quad (14)$$

The value of A_n is directly proportional to C (the concentration of reagent added to obtain a given biological response). Setting $d(\log 1/C)/d(\log P)$ equal to zero yields log P_o, the value of log P for which the biological response is strongest and penetration most rapid. It has been shown that log P_o is a useful constant in the design of drugs acting on the central nervous system (20) as well as for antibacterial agents (21).

Analysis of a number of theoretical models containing different numbers of lipid/water barriers and different values of m showed that in all cases Eq. 12 gave a good fit to the data obtained. Table 4 shows

TABLE 4
Optimum P value for penetration as a function of number of barriers calculated when m = 1, t = 10

No. of barriers	log P_o
2	+1.39
3	−0.57
4	+0.90
6	−0.30
10	+0.40
11	−0.13
19	−0.07
20	+0.20

that the value of log P_o fluctuated on both sides of zero, depending on the number of barriers in the model. It approaches zero ($P = 1$) as the number of barriers becomes large.

Figure 1 clearly shows that the model which has been used (Eq. 14) to treat experimental data can be justified in terms of this kinetic theory. This is of great importance to those who wish to study the action of organic compounds in biochemical systems. Experimental results, as well as our model, show that for the general case one must expect a nonlinear relationship between log P and log permeability. In practice, there are two kinds of "linear" relations one may encounter in studying limited groups of congeners. The first is the very well-known type, where in equations of the form of Eqs. 1 and 2 we find a positive coefficient with log P. The second, less usual example occurs when one is working with a set of highly lipophilic molecules; if the members of such a set have log P values considerably beyond zero (Fig. 1), one finds a "linear" relation between log P and log C. A good practical illustration of this is found in the study of Gourevitch *et al.* on penicillins [see (30)]; from their work Eq. 15 has been derived.

$$\text{Log } \frac{1}{C} = -0.455 \log \pi + 5.673,$$

$$n = 20, r = 0.909, s = 0.191 \quad (15)$$

In this equation C is the CD_{50} in mice. The interpretation in terms of our model is obvious. The penicillins have a large lipophilic moiety; increasing this means tighter binding to proteins. Tighter binding to the proteins means a lower probability that an effective concentration of the penicillins will reach the reaction sites on *Staphylococcus aureus* in mice. Since adding a squared term to Eq. 15 does not improve the correlation, we assume that we are considering a set of congeners which would fall considerably to the right of zero in Fig. 1. The work on penicillin is, of course, strictly analogous to the study of the penetration of human skin by alkyl phosphates and the penetration of cockroach cuticle by insecticides discussed above. While there are other ways in which one might rationalize the results with the penicillins, we think that the model contained in Eq. 11 is the most reasonable explanation.

We have presented here a theory for the passive penetration of drugs to their sites of action. This theory differs from those proposed for active transport, because most drugs will not fit the highly specific biological transport mechanisms. It differs from those previously proposed for passive penetration because it recognizes that in drug action, speed is essential and thus steady-state analyses of permeation are inadequate. In the future, knowledge of the optimum value of the partition coefficient for effective penetration in a given organ may vastly shorten the time required to develop an effective drug. Thus we believe that this model has opened exciting new approaches to rational design of drugs.

ACKNOWLEDGMENTS

We wish to thank Roger Smith for extensive advice and help in computer programming, and Dr. E. F. Korman for helpful advice on the manuscript. We are indebted to Miss C. Church for measuring the partition coefficient of trimethyl phosphate.

REFERENCES

1. A. Kleinzeller and A. Kotyk, "Membrane Transport and Metabolism." Academic Press, New York, 1961.
2. H. N. Christensen, "Biological Transport." Benjamin, New York, 1962.

3. W. Wilbrandt, *Annu. Rev. Physiol.* **25**, 601 (1963).
4. H. Davson, "General Physiology," Ed. 2, p. 218. Little, Brown, Boston, 1959.
5. H. Davson and J. F. Danielli, "The Permeability of Natural Membranes," Ed. 2. Cambridge University Press, Cambridge, England, 1952.
6. F. Helmer, K. Kiehs and C. Hansch, *Biochemistry* **7**, 2858 (1968).
7. C. Hansch, K. Kiehs and G. L. Lawrence, *J. Amer. Chem. Soc.* **87**, 5770 (1965).
8. K. Kiehs, C. Hansch and L. Moore, *Biochemistry* **5**, 2602 (1966).
9. C. Hansch and F. Helmer, *J. Polymer Sci.* **6** (A-1), 3295 (1968).
10. C. Hansch and S. M. Anderson, *J. Med. Chem.* **10**, 745 (1967).
11. C. Hansch, in "Drug Design" (E. J. Ariëns, ed.), Academic Press, New York. In press.
12. W. Scholtan, *Arzneimittel-Forsch.* **18**, 505 (1968).
13. R. Collander, *Physiol. Plant.* **7**, 420 (1954).
14. B. V. Milborrow and D. A. Williams, *Physiol. Plant.* **21**, 902 (1968).
15. C. Hansch and T. Fujita, *J. Amer. Chem. Soc.* **86**, 1616 (1964).
16. T. Fujita, J. Iwasa and C. Hansch, *J. Amer. Chem. Soc.* **86**, 5175 (1964).
17. F. N. Marzulli, J. F. Callahan and D. W. C. Brown, *J. Invest. Dermatol.* **44**, 339 (1965).
18. W. P. Olson and R. D. O'Brien, *J. Insect Physiol.* **9**, 777 (1963).
19. E. J. Ross, *J. Physiol. (London)* **112**, 229 (1951).
20. C. Hansch, A. R. Steward, J. Iwasa and E. W. Deutsch, *Mol. Pharmacol.* **1**, 205 (1965).
21. E. J. Lien, C. Hansch and S. M. Anderson, *J. Med. Chem.* **11**, 430 (1968).
22. C. Hansch, A. R. Steward and J. Iwasa, *Mol. Pharmacol.* **1**, 87 (1965).
23. A. H. Soloway, B. Whitman and J. R. Messer, *J. Pharmacol. Exp. Ther.* **129**, 310 (1960).
24. C. Hansch, A. R. Steward, S. M. Anderson and D. Bentley, *J. Med. Chem.* **11**, 1 (1968).
25. H. E. Hart (ed.), *Ann. N. Y. Acad. Sci.* **108**, 1 (1963).
26. A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," p. 160. Wiley, New York, 1963.
27. A. A. Bennett, W. E. Milne and H. Bateman, "Numerical Integration of Differential Equations." Dover, New York, 1956.
28. K. S. Kunz, "Numerical Analyses." McGraw-Hill, New York, 1937.
29. D. Richardson, "Writeup of Mark B." Jet Propulsion Laboratory, Pasadena, 1964.
30. C. Hansch and A. R. Steward, *J. Med. Chem.* **7**, 691 (1964).