4000, inulin, and insulin) dramatically decrease the uptake, especially when extreme care is not taken to remove these compounds from the external environment. The latter compounds, although they do not form an integral part of the bilayer, apparently adsorb to some extent.

It is not clear at this point whether the adsorption of these markers blocks adsorption of the liposomes to the gut wall or blocks penetration into deeper layers. The important point is that "inert" markers can profoundly affect the uptake of liposomes as determined by this *in situ* procedure. This study also raises questions as to how materials normally found in the gastrointestinal environment, e.g., food, protein, etc., affects liposomal uptake. These observations may also offer some explanation for the divergent results reported in the literature on *in vivo* absorption of liposomally entrapped drugs.

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Relationship of Octanol/Water Partition Coefficient and Molecular Weight to Cellular Permeability and Partitioning in S49 Lymphoma Cells

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Abstract: We have used modified standard methods and derived new formulae to quantitate cell permeability (P), cell/media partitioning (λ), and intracellular sequestration or binding rate constants (m) for cultured S49 murine lymphoma cells in suspension. Using 15 standard compounds and anticancer drugs, we found quantitative relationships among log P, log P_o (octanol/pH 7.4 buffer partition coefficient), and molecular weight (MW) such that $\log P = -4.5 + 0.56\log(P_o(MW)^{-1/2})$. A good correlation among P, λ, and MW was also determined with $\lambda = 0.67 + 5890\,gm^{1/2}\,cm^{-1}\,sec\,(P\,(MW)^{1/2})$. These studies show that there is a strong partitioning (λ) dependence to molecular weight and permeability that can be predicted even for known carrier-transported and biotransformable compounds. Furthermore, results of this study show that the slope of the plot of permeability and lipophilicity is not necessarily unity as has been postulated from the results of other studies.

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The therapeutic efficacy of chemotherapy for solid tumors generally has been disappointing; this ineffectiveness is a reflection of the fact that frequently drugs cannot be delivered to cells at high enough levels to achieve a cytotoxic effect. We have modeled the effects of various physical factors on drug delivery to the extracellular environment around tumor cells that are various distances from tumor capillaries (1). We could not extend these observations to intracellular drug levels because, despite their importance, data on the permeability of chemotherapeutic agents and the rates of binding and sequestration in normal and transformed eukaryotic cells were not available. Therefore, we have measured the permeability and intracellular compartmental distribution and sequestration or binding of compounds with a wide range of molecular weights and lipophilicities, and have derived equations that correlate these data with the partitioning of a drug and the lipophilic nature of the cell membrane and cytoplasm. Measurements were made in the S49 murine lymphoma cell line using a modification of standard methods (2).

Materials and Methods

Radioisotopes and Chemicals

^{14}C-Labelled D-glucose, 2-deoxy-D-glucose, inulin, putrescine, and urea, and ^{3}H-labelled inulin and mannitol were

purchased from New England Nuclear; ^{3}H-3-O-methyl-D-glucose and ^{14}C-creatinine from Amersham-Searle; ^{3}H-glycerol from ICN Corporation; and ^{3}H-vincristine from Moravek Biochemicals. ^{14}C-Labelled daunomycin, ellipticine, adriamycin, PCNU, CCNU, and misonidazole were supplied by Dr. Robert Engle, Chemical Resources Section, National Cancer Institute. Specific activities for these compounds ranged from 12 mCi/mmole to 22.4 Ci/mmole. Other reagent grade chemicals were purchased from standard suppliers.

Octanol/Water Partition Coefficients

The values cited for log P_o (octanol/water partition coefficient) in Table I were determined either by Hansch and Leo (3) or were determined in our laboratory using their technique (4). Studies were performed at pH 7.3–7.4.

Cell Culture Conditions

S49 Murine lymphoma cells of the "wild" type were grown at 37°C in a humidified 5–12 % CO_2/95–88 % air atmosphere (pH 7.28 to 7.42) in Dulbecco's modified Eagle's medium containing 10 % heat-inactivated horse serum (5). For uptake studies, exponentially growing S49 cells were harvested at a density of 1×10^6 cells/ml by centrifugation. For a preliminary study of the effects on permeability of position in the cell cycle, G_1-, S-, and G_2/M-phase cells were fractionated by centrifugal elutriation (6, 7).

Determination of Cell Number and Cell Volume

Suspensions of single cells were counted and sized in a Coulter ZB1 counter and channelyzer calibrated with polystyrene spheres of known diameter. For the measurement of permeability to be valid, cells must be reasonably spherical at the time of study. The median volume of unswollen cells is approximately 540 μm^3. Cells in suspension were swollen with a 159 mOsm hypotonic saline solution; because the increase in cell volume was less than 8 %, S49 cells were considered to be spherical; therefore, the use of volume measurements to calculate cell surface area is appropriate.

Nuclei for the determination of nuclear volume were obtained by both hypotonic lyses and detergent treatment with Triton-X to remove the cell membrane; nuclei were sized on the Coulter counter. Nuclei obtained with both methods had a median nuclear volume of approximately 302 μm^3.

Cell Permeability and Uptake Studies

Cells were equilibrated for 1 h in the cell chamber, and cell size and number were determined before and after the uptake study. Trace amounts of radiolabeled compounds (200,000 cpm ^{14}C or 400,000 cpm ^{3}H/ml cell suspension) were mixed with trace amounts of radiolabeled inulin (200,000 cpm ^{14}C/ml or 400,000 cpm ^{3}H/ml cell suspension); radiolabeled inulin was repurified by Sephadex G-25 chromatography the day of the study. Inulin was used as a marker to determine trapped water, and was always labelled with a different radioisotope than the study compound.

At various times, 1 ml samples of cell suspension were removed from the chamber, added to 1.5 ml polypropylene Eppendorf microtest tubes that contained 0.25 ml of a silicone: mineral oil mixture (Dow-Corning 550 fluid and Robinson laboratory mineral oil, 5:1), and centrifuged in an Eppendorf 3200 centrifuge that reached a speed of 12,000 g in less than 5 seconds. In this procedure, the oil mixture separated cells from the suspending medium and effectively terminated drug uptake into the cell. Because of the lipophilicity of vincristine, CCNU, and ellipticine, no oil mixture was used with these drugs.

After 2 min of centrifugation, 50 μl aliquots of supernatant were removed in duplicate, transfered to scintillation vials, and 0.5 ml of NCS and 15 ml of Permablend were added. The remaining supernatant was aspirated and the mineral oil: silicone phase was rinsed three times with fresh medium. The tip of the test tube containing the cell pellet was then cut off with a razor blade and digested in 0.5 ml of NCS at 55°C for at least 2 h before adding 15 ml of Permablend. All samples were then counted in a Beckman LS250 scintillation counter.

Counts were corrected for background and quench and were standardized for volume. Cell counts (C_c) and media counts (C_m) were used to calculate ratios of the amount of study compound in the cell pellet to the amount of compound in the media. The ratio of inulin dpm/g in the cell pellet to the dpm/g in medium represented the medium trapped in the cell pellet. This ratio was substracted from the cell/medium ratio (C_c/C_m) for the study compound to obtain the value of the distribution ratio (R). A plot of R vs. time was obtained for each compound on a Data General Nova 830 computer using the equations derived in Appendix A.

Results

Because temperature, pH, osmolality, and oxygen tension of cells and their environment change membrane fluidity, rate of diffusion, and the amount of hydrogen bonding and thereby affect the penetration of molecules through a cell membrane, these factors were controlled carefully in these studies. Borghetti et al. (8) reported that variations in cell density can affect membrane transport mechanisms; therefore, all experiments were performed at the same cell density.

For 51 experiments, the average cell radius was 5.1 $\mu m \pm 0.1$ (SD). Because the total cell volume (V_c) was in the range of 1.31 to 1.83 μl/ml of medium, the medium volume/cell volume was always large enough that radioactivity in the medium did not vary appreciably during an experiment as a result of cell uptake.

In most experiments, measurements with labeled inulin showed that less than 10 % of the cell pellet volume contained trapped medium. If the inulin space was larger than 10 % by volume, the result was discarded from analysis because we assumed this large value indicated the presence of damaged cells. The mineral oil : silicone oil phase did not contain measurable amounts of radioactivity, which indicated that the cell pellet separated completely from the supernatant and that no drug was transferred from the cell to the mixed oil phase. Permeability was unaffected by either the presence or absence of horse serum in the medium.

Data for the 15 compounds studied are summarized in Table I. Values for the distribution ratio of a drug at equilibrium (R) and for permeability (P) were calculated with equations derived in Appendix A. Examples of typical plots of $R(C_c/C_m)$ vs t, from which the data in Table I were generated, are shown in Figures 1–3. Figure 1 is a plot for the uptake of urea, a compound that distributes in a single compartment and is not metabolized (fit by eq. A3); Figure 2 is an uptake plot for vincristine, a drug that distributes in the cell and binds intracellularly (fit by eq. A6). The rate of intracellular binding was calculated to be 0.078 min^{-1} using eq. A6.

Table I. Values of Log P_o, P, λ and m for S49 Cells

Compound no.	Compound	N	MW	Log P_o	P × 10^{-6} cm/s	λ	m min^{-1}
1	^{3}H-mannitol	9	182	−3.10	0.074	0.28	—
2	^{3}H-3-O-methyl-glucose	2	194	−2.28	2.8(0.32)	0.64	—
3	^{14}C-creatinine	9	113	−1.77	0.022	0.79	—
4	^{14}C-urea	9	60	−1.66*	0.330	0.81	—
5	^{3}H-putrescine	4	130	−2.66	6.4(0.63)	(0.82)	0.091
6	^{14}C-2-deoxyglucose	3	164	−2.28	3.2(0.40)	0.66	0.012
7	^{14}-C-D-glucose	3	180	−2.28	3.8(0.47)	0.67	0.007
8	^{3}H-glycerol	3	92	−1.75	0.34	0.79	0.050
9	misonidazole	3	185	−0.37	13	1.50	0.083
10	^{14}C-adriamycin	2	543	−0.10	370	45	0.015
11	^{14}C-PCNU	3	263	+0.37	42	1.50	0.033
12	^{14}C-daunomycin	2	528	+1.80	530	107	0.040
13	^{3}H-vincristine	2	825	+2.80	27	12	0.078
14	^{14}C-CCNU	1	234	+2.80	205	7.6	0.022
15	^{14}C-ellipticine	1	246	+4.80	900	220	0.520

*The value for ^{14}C-urea was erroreously given as −2.80 in an earlier report (4).
N = number of experiments; MW = molecular weight; P_o = octanol/buffer (pH 7.4) partition coefficient; P = cell permeability coefficient; λ = cell/medium partition value. The number in parentheses are values at 4°C; facilitated diffusion at 22°C produces values approximately 8–12 times higher. Values of m were based on studies at 22°C.

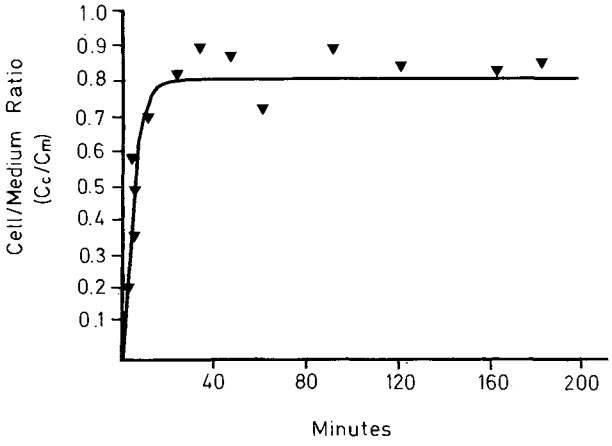

Fig. 1 Representative experiment of ^{14}C-urea uptake into asynchronously growing S49 cells at 22°C. P = 3.2 × 10^{-7} cm/sec and λ = 0.81.

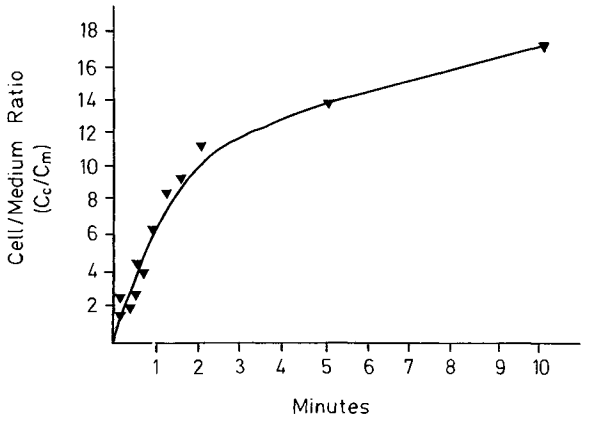

Fig. 2 Representative experiment of ^{3}H-vincristine uptake into asynchronously growing S49 cells at 22°C. P = 2.8 × 10^{-5} cm/sec, λ = 12.3, and m = 1.3 × 10^{-3} sec^{-1}, which corresponds to a half-time of 9 min.

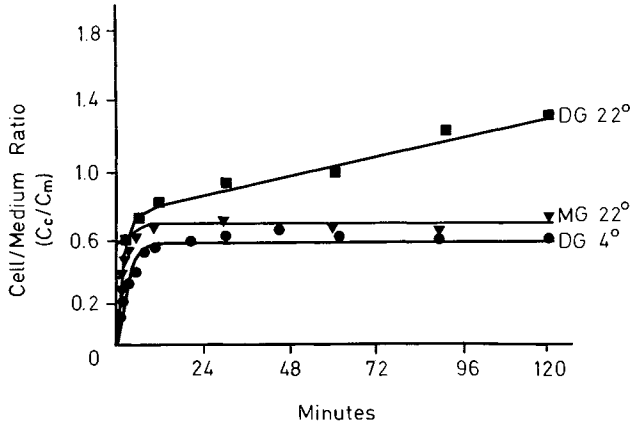

Fig. 3 Representative experiments of ^{14}C-2-deoxy-D-glucose (DG) uptake at 22°C (■) and 4°C (●) and ^{14}C-3-O-methyl-D-glucose (MG) uptake at 22°C (▼) into asynchronously growing S49 cells. DG studies at 4°C to those at 22°C reduce P from 2.8 × 10^{-6} cm/sec to 4 × 10^{-7} cm/sec. The MG P = 3.2 × 10^{-6} cm/sec. All experiments were carried out at a total glucose concentration of 5.56 mM, a value well above the saturation level.

In Figure 3, uptake of 3-O-methyl-D-glucose, a compound that distributes in a single compartment and is not metabolized, is compared to 2-deoxy-D-glucose , a compound that should distribute in the same compartment but that is phosphorylated to 2-deoxy-D-glucose-6-phosphate, which remains in the cell. At 22°C, P for the two compounds are similar (3.2 × 10^{-6} and 2.8 × 10^{-6} cm/sec); values for λ were also similar (0.64 and 0.66). At 4°C, however, P for 2-deoxy-D-glucose is reduced to 4 × 10^{-7} cm/sec and 3-O-methyl-D-glucose to 3.2 × 10^{-7} cm/sec; no intracellular phosphorylation appears to occur because the uptake curve is flat between 12 and 120 min. Under these conditions, λ = 0.62 and is similar to the 22°C experimental values.

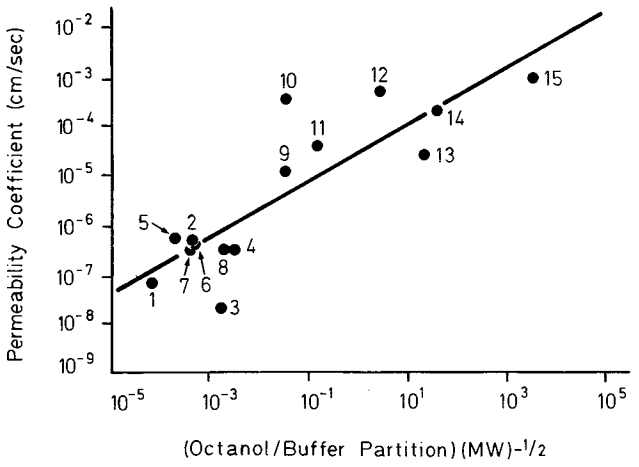

Fig. 4 Relationship of P to P_o and MW in S49 cells at 22°C. The line is the fit by the method of least squares giving the equation $\log_{10} P = -4.50 + 0.56 \log_{10}(P_o(MW)^{-1/2})$; SE EST = 0.85 and the correlation coefficient = 0.85. Values were taken from Table I; for points 2, 5, 6, and 7, the 4°C values were used because at 22°C facilitated diffusion of these drugs occurs. If log $(P(MW)^{1/2})$ is plotted against log P_o, the slope is 0.58 and the correlation coefficient is 0.86. If only 22°C values are used, the slope is 0.50 instead of 0.56.

Figure 4 is a log-log plot of P vs. the product of P_o and the reciprocal of the square root of the molecular weight for all compounds listed in Table I. Values for D-glucose , 2-deoxy-D-glucose, 3-O-methyl-D-glucose, and putrescine were lowered to the 4°C values to correct for the increase in cell uptake that is the result of faciliated transport of these compounds into the cell. We conducted studies with labeled 3-O-methyl-D-glucose and putrescine between 4° and 25°C, and found that permeability was asymptotic at 4°C. Therefore, even though the 4°C values may not be completely free of carrier transport effects, the influence of the carrier on these values should be minimal.

The fit for three other molecules deviated more than expected. The value for creatinine is below the line; there is no obvious reason that will explain this result. Values for adriamycin and daunomycin, both of which bind extensively to the external membrane wall, were significantly above the line, and may reflect some external and possible internal membrane binding.

In Figures 4 and 6, we use the relationship $MW^{-1/2}$ to account for the molecular size in the diffusion coefficient. While it can be assumed that the diffusion coefficient is proportial to the reciprocal of the molecular radius (or approximately $MW^{-1/3}$) as in the Einstein formula, $MW^{-1/2}$ was used in our analysis because the fit to the experimental data on diffusion has been found to correlate better in some biological situations (4, 9–11).

As a practical point, we found that over the ranges of molecular weights of compounds used in this study, molecular weight had little effect on the slope because the range in the square root of molecular weight, 3.7, is negligible compared to that of P_o, which is in the range 8×10^7. We evaluated three relationships of P to P_o, $P_oMW^{-1/3}$, and $P_oMW^{-1/2}$. When values for all 15 compounds studied were used for the least squares fit of the data, slopes of the plots were virtually identical (0.55–0.56). Even if the four compounds that are highly ionized at physiologic pH (Cmpds **5, 10, 12,** and **13**) were excluded from analysis, slopes were lower but essentially the same (0.51–0.53), although the correlation coefficient and standard error of the estimate were improved compared to computations that included all 15 compounds. If the amphoteric compound creatinine (Cmpd **3**) also is dropped, the slope is reduced further (0.48–0.50) and the correlation coefficient and standard error of the estimate are improved compared to the two other cases. Thus, because this method of plotting has been used frequently, we have plotted P against P_o divided by $MW^{1/2}$.

To evaluate the relationship of cell partitioning to hydrophobicity, we generated Figure 5, a plot of λ vs. P_o using Equation B6. On the assumption that there is negligible

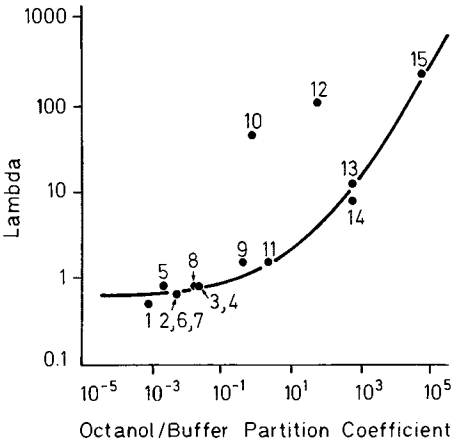

Fig. 5 Relationship between λ and the P_o in S49 cells. The curve is the fit to Eq. B6 using a nonlinear method of least squares with values taken from Table I, except points 10 and 12, where $V_w = 0.72$, $V_L = 0.28$, $\gamma = 0.58$, the root mean square deviation per the degrees of freedom being 0.11 in $\log_{10}$ units (corresponding to a factor of 1.3).

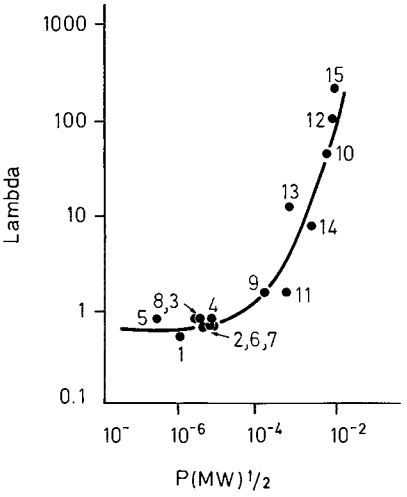

Fig. 6 Relationship between λ and P and MW in S49 cells. The curve is the fit to Eq. B7 by a nonlinear method of least squares with data from Table I where $V_w = 0.67$ and $a = 5890\,gm^{-1/2}\,cm^{-1}$ sec, the root mean square deviation per degrees of freedom was 0.24 in $\log_{10}$ units (corresponding to a factor of 1.75).

penetration into the nucleus, the value of λ for mannitol has been increased by the factor cell volume/cytosol volume = 540/302. Note that $V_W + V_L = 1.0$ (from eq. B6), which leaves 0 % of the cell inert; this latter value can be made to vary from 0 to 20 % without greatly affecting the curve in Figure 5. However, compared to the fitted data, the values for adriamycin and daunomycin are discrepant.

We considered the case in which λ is more a function of P than of P_o, and derived Equation B7 on this assumption. Figure 6 is a plot of λ vs. the log of the product of P and $MW^{1/2}$. For compounds that cross cell membranes by facilitated transport, the corresponding free diffusion permeability values were taken to be equivalent to their P values at 4°C (approximately 0.1 of the measured values at 22°C); this correction has no significant effect on the degree of fit, and reductions of P by factors of 0.05 to 0.2 for these compounds would also yield satisfactory fits. While the fit in Figure 6 is not as good as the fit in Figure 5, values for all compounds studied were used for the plot in Figure 6. On the other hand, the root mean square deviation for the line in Figure 6 is only about 0.3 as large as that in Figure 4, suggesting that the assumption involved in deriving Equation A3, that the lipid property of the membrane is similar to that of the lipid component of the cytosol, is valid. Daunomycin (Cmpd **12**) and adriamycin (Cmpd **10**) are probably much more soluble in both membrane and cytosol lipid than would predicted by their respective P_o values. Note that the cell water volume, $V_w = 0.72$, in Figure 5 is quite comparable to $V_w = 0.67$ in Figure 6.

Discussion

While our initial purpose was to establish permeability and intracellular binding or sequestration rates for anticancer agents in mammalian cells to allow us to extend the mathematical models we developed (1), the results of the current study provide additional insights into permeability-lipophilicity relationships. The early literature in this field, some of which dates to the turn of this century, has been reviewed by Davson and Danielli (9). Table II summarizes some of the data reviewed by them and data from later studies of the relationship of permeability to lipophilicity.

Table II. Comparison of Slopes of Solvent Partitioning Vs. Permeability Coefficient Measurements

	Slope	Solvent	Reference
Chara	1.29 (0.90)*	Olive oil	9,12
Arbacia	1.13(0.48)	Olive oil	9
Beggiatoa	0.55(0.66)	Olive oil	9
Erythrocytes	0.97(0.98)	Octanol	13
Stratum corneum	0.51(0.97)	Octanol	17, 18
Liver cells (K_s)	0.41(0.73)	Octanol	16
Brain capillaries	0.41(0.91)	Octanol	4
Intestine	0.24(0.93)	Octanol	19
	0.28(0.80)	Octanol	20
S49 cells	0.56(0.85)	Octanol	Current study

*Correlation coefficient.

A priori, there is no inherent theoretical reason that all plots that relate permeability and lipophilicity (measured in hydrocarbon solvents) should invariably have a slope of unity in biological systems, even though studies with chara (9, 12), arbacia (9), erythrocyte membranes evaluated with phenols and alcohols (13), and artificial planar bilayer membranes (14, 15) demonstrate such a relationship. Slopes of less than unity have been calculated for studies conducted in mammalian cells. Using the data of Yih and Rossum (16), as corrected by Lien, for perfused liver cells, we calculated a slope of 0.41 for log K_s vs. log P_o for 32 compounds, and using data pooled from two studies of stratum corneum (17, 18), we computed a slope of 0.51 for 16 compounds. Lien and Wang (19) computed a slope of 0.24 for permeability of 10 neutral alcohols in rat intestine; Tai and Lien (20) computed a slope of 0.28 for 39 benzene derivatives. In addition, the plot in Figure 4 is surprisingly similar to a plot obtained for the permeability of 27 compounds in normal rat brain capillaries, the slope of which was 0.41 (4).

The fact that we did not find a slope of unity for plots of permeability vs. hydrocarbon partitioning in these studies may be the result of factors such as unstirred layer effects (15) or ion charge factors (21). Unstirred layer effects are more difficult to measure and of potentially less importance in biological systems than in artificial bilayer systems. The unstirred layer in mammalian cells is relatively thinner, on the order of the cell radius (5 microns), than layers in artificial bilayer systems. In our system, an unstirred layer would slow the initial permeation and produce a sigmoidal curve of cell uptake at very short times (less than 1 min). This was never observed; on the contrary, the initial uptake was found on occasion to be higher than expected when measured at 20 to 40 sec.

Compared to unionized forms, ionization of Compounds **5, 10, 12,** and **13** at physiologic pH could reduce membrane permeation in S49 cells. However, because measurements were made at the same pH and temperature, both λ and P_o would accurately reflect the distribution of ionized to nonionized forms. No relevant trend in the data that could be related to the charge of the compounds was observed. In fact, the permeabilities of Compounds **10** and **12** appear to be higher than the values predicted by the least squares fit (Fig. 4); values for Compounds **3** and **13** are lower than predicted; and the value for Compound **5** falls on the line. Thus, the effect of charge on permeability appears to be negligible. If the four ionized compounds and the one amphoteric compound (cmpd **3**) are not included in the least squares fit of data from Table I, slopes are only slightly lower (0.50) than slopes calculated using all 15 compounds (0.56). Furthermore, the least squares fit of λ to P_o was found to be reasonably good even when the four ionized compounds were included (Fig. 5). Discrepancies in the fit of actual data points were found for compounds **10** and **12**; the larger-than-predicted value of λ could be the result of rapid membrane binding that is independent of hydrophobic interactions. Thus, we believe that, depending on the membrane characteristics of the cell being studied and the hydrophobicity of the solvent used for measuring "lipophilicity", the relationship of permeability to lipophilicity will vary over a range of approximately 0.5 to 1.0. Further compounding these relationships in transformed or neoplastic cells will be changes in membrane fluidity and carrier protein integrity.

In view of the rather satisfactory relationship shown in Figure 6, the value of V_L was measured and found to be 8.9 % (unpublished data provided by Dr. C. J. Fielding). Using this value for V_L and a value of $V_W = 0.7$, the average from Figures 5 and 6, we can directly estimate the average cell lipid/buffered medium partition coefficient λ_L from $(\lambda - V_W)/V_L$ for each compound. If we take d_m, the membrane thickness, to be

approximately 75 Å (22) and assume, as above, that the cell membrane lipid has about the same partition coefficient as the rest of the cell lipid, then we can estimate the diffusion coefficient in the membrane lipid from $D = Pd_m/\lambda_L$. Table III summarizes these computations and also the values for log $(D(MW)^{1/2})$. These values are remarkably constant, the average being -10.9 ± 0.42 (cf. 17). From the above values of D we can estimate that the time required to fill the membrane to one-half the maximum value would be about 1 sec under the assumption of a square potential well for the membrane, that is, $\tau = d_m^2/4D$. The contribution to the average cell content during this time would be about 4.5% and hence probably could not be detectable in the method employed. In addition, we found no experimental indication of an appreciable systematic jump in the initial concentration for any of the drugs used.

Table III. Estimates of Diffusion Coefficient and Log $(D(MW)^{1/2})$ in S49 Cell Membrane Lipid

	Compounds from Table I						
	9	**10**	**11**	**12**	**13**	**14**	**15**
λ	(9)	500	(9)	1200	127	80	2500
$D \times 10^{12}$ cm^2/sec	(1.1)	0.6	(3)	0.35	0.2	2	0.3
log $(D(MW)^{1/2})$ + 12	(1.2)	1.1	(1.7)	0.88	0.66	1.5	0.64

Values in parentheses were estimated grossly and are probably unreliable.

As mentioned above, one purpose of these studies was to develop methods to study the kinetics of intracellular binding or sequestration of drugs within cells. These values can be represented by the calculated intracellular rate constant m. For example, the anitumor agents CCNU and PCNU undergo general base-catalyzed decomposition reactions to form reactive intermediates that can alkylate and carbamoylate nucleic acids and proteins. The rate constants of these reactions in buffer at pH 7.4 are $0.013\,min^{-1}$ and $0.027\,min^{-1}$, respectively (23), while the rate constants for their intracellular sequestration or binding, $0.022\,min^{-1}$ and $0.033\,min^{-1}$, respectively (Table I), are somewhat larger. Such accelerated intracellular biotransformation may be caused either by enzymes that accelerate nitrosourea breakdown or by proteins that mediate catalysis. Information obtained with these equations may be useful for the study of intracellular biotransformation of anticancer drugs, activation of mutagens, predicting drug-dose effects on tumor cell populations, and for examining processes such as drug polymerization or absorption on external or internal surfaces of cells that might slow the entry of drugs into certain cell regions.

Although cell permeability may be affected by the position of cells in the cell cycle, preliminary studies of cells in G_1-, S-, and mixed G_2/M-phases of the cell cycle have shown little variability in the passive diffusion across the cell membrane of urea, mannitol, and vincristine; others have reported that membrane fluidity and composition are a function of position in the cell cycle (24).

It is apparent from these studies that in S49 lymphoma cells there is an excellent and predictable relationship between cell permeability and λ or P_o. This study extends the known relationship of lipophilicity to tumor cell membrane permeability by a mathematical approximation that includes the observed drug cell/medium partition coefficient ratio λ, which may be a better biological partition value for biological structure-activity studies than P_o values based on partition into an organic solvents such as octanol.

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Appendix A: Derivation of Equations of Drug Uptake by Cells (R vs. t).

1. Case Without Metabolism or Intracellular Sequestration.

The formula for the permeability coefficient, P, derived from a modified form of Fick's equation for diffusion into a cell where the substance under study can be treated as being approximately uniformly distributed and where internal diffusion is rapid, is:

$$V_c(dC_c/dt) = PA_c(C_m - C_c/\lambda) \quad \text{(A1)}$$

where:

V_c = volume of N cells, ml
V_m = volume of medium, ml
A_c = total surface area of N cells, cm^2
C_c = average concentration of tracer in cells, dpm/V_c
C_m = average concentration of tracer in medium, dpm/V_m
λ = partition coefficient, cell/medium ratio (C_c/C_m) for tracer at equilibrium in the absence of metabolism
t = time, sec.
P = permeability coefficient, cm/sec

Even for the largest molecules used, the time constant for diffusion within the cell, assuming an aqueous medium inside, would be a very small fraction of a second. Thus, unless the structure of the cytosol impedes diffusion several hundred-fold, it is reasonable to ignore limitation that are the result of internal diffusion.

For a short time, t, when $C_c \ll C_m$, eq. A1 can be solved for P to give

$$P = (V_c/A_c)\,(C_c/C_m)\,(1/t).$$

For spherical cells,

$$V_c/A_c = (4/3\,\pi\,r^3) / (4\,\pi\,r^2) = r/3.$$

If we define the distribution ratio (R) at any time as the average concentration of tracer in the cells relative to the average concentration of tracer outside the cell $(R = C_c/C_m)$, then the above equation for P becomes: $P = R(r/3t)$, or

$$R/t = 3P/r. \quad \text{(A2)}$$

Because the early part of the cell uptake curve (R vs. t) is linear, the initial slope of the curve is equal to 3P/r. Thus,

because r can be measured directly, eq. A2 can be used to calculate the value of P.
For larger values of time, eq. A1 can be integrated to give:

$$R = C_c/C_m = \lambda(1 - e^{-(3P/r\lambda)t}). \quad (A3)$$

Fitting this equation to a plot of R vs. t gives values for λ (steady-state partition ratio with R at equilibrium) and for 3P/r (the initial slope as shown in eq. A2).

2. Case with Intracellular Sequestration or Biotransformation.

If the concentration of drug in the external medium (C_m) is constant during the uptake study, the rate of increase of the average internal concentration, C_c, is the difference between the net flux rate across the surface (as in eq. A1) and the rate of sequestration, mC_c, where m is the first order rate constant of sequestration, i.e.,

$$dC_c/dt = (PA_c/V_c)(C_m - C_c/\lambda) - mC_c \quad (A4)$$

Eq. A4 can be integrated to give

$$C_c/C_m = (3P/\lambda r)(1 - e^{-\alpha t}) \quad (A5)$$

where $\alpha = m + 3P/r\lambda$.
From eq. A5, we can derive an expression for R by calculating the intracellular sequestration obtained by multiplying the integral of C_c/C_m by m, and adding this to C_c/C_m, the amount in solution, to obtain the total

$$R = (3P/r\alpha^2)[(3P/r\lambda)(1-e^{-\alpha t}) + m\alpha t] \cdot \quad (A6)$$

Note that the initial value of the slope for a plot R vs. t is 3P/r; therefore, P can be calculated using eq. A2. By fitting the entire curve to the data, the values of λ and m can also be estimated, including 3P/r.

The quantity λ, the ratio of the average cell concentration of drug to the external concentration of drug in the absence of m, assumes uniform distribution of drug in the cell. However, λ is more than the simple partition between the exterior and interior of a cell. Its numerical value will be reduced by cell constituents that take up a negligible amount of drug in the time of the longest experiment. On the other hand, rapidly reversible absorption to surfaces of the cell constituents can increase λ. However, if it were assumed that all sequestered drug is retained within the cell when in fact part is rapidly lost to the exterior, the effect would be to reduce the distribution space to a value less than λ. While more complex models can be considered (25), we have considered only the simplest case of uniform distribution within the cell, because this has been found to be satisfactory for the compounds studied for the duration of time used in each instance (between 10 and 300 min).

Appendix B: Derivation of Equations to Relate Partition Coefficient to Octanol/Water Partition and Molecular Weight to Cell Permeability

It is reasonable to suppose that the measured partition coefficient, λ, is a weighted average of 1) the partition coefficient λ_w of the aqueous phase, and hence is approximately one, having volume fraction V_w, and 2) that of the various lipophilic phases. If we lump these latter together, there would be an associated volume fraction V_L and an average partition coefficient, λ_L. Thus we would have

$$\lambda = V_w\lambda_w + V_L\lambda_L \quad (B1)$$

1. λ versus P_o

Consider first relating λ to the octanol-buffer partition coefficient, P_o. We may relate λ_L to P_o in the following way. If $\Delta F_{o,w}$ is the difference between the energy of a given molecule when in octanol (o) and when in buffer or water (w), then the partition between octanol and water, P_o, would be given by the Boltzmann distribution

$$P_o = \frac{\text{Concentration in octanol}}{\text{Concentration in buffer}} = e^{-\Delta F_{o,w}/RT} \quad (B2)$$

Similarly, if $\Delta F_{m,w}$ is the corresponding energy difference between membrane lipid (m) and buffer, the partition between membrane lipid and buffer, λ_L, would be given by

$$L = \frac{\text{Concentration in membrane lipid}}{\text{Concentration in buffer}} = e^{-\Delta F_{m,w}/RT} \quad (B3)$$

where $\Delta F_{m,w}$ depends on the compound being studied. If for a large number of compounds the energy differences for the membrane tend to differ from those of the reference solvent (octanol) values by a constant, on the average, then log λ_L would be approximately a linear function of log P_o, with a slope of unity. This would hold even if λ_L were the average of a set of partitions of different substances provided they each differ from octanol, on the average, by a constant energy difference.

But from Eqs. B2 and B3 we can also write

$$(\log\lambda_L/\log P_o) = \Delta F_{m,w}/\Delta F_{o,w} = \gamma, \quad (B4)$$

or

$$\lambda_L = P_o^{\gamma}, \quad (B5)$$

where γ depends on the compound in question. If the ratio of the energy difference in Eq. B4 tends to be similar for a large number of compounds, γ would be approximately a constant. For this case, if we set $\lambda_w = 1$, and introduce λ_L from B5 into B1, we obtain

$$\lambda = V_w + V_L P_o^{\gamma}, \quad (B6)$$

γ being defined in B4. Conversely, if it is found that data show an approximately linear log-log plot with γ definitely different from 1, then this can be interpreted as being the result of a proportional relationship between energy differences among the compounds.

2. λ versus P

An alternate approach is to relate λ_L to the permeability P directly. It is reasonable to assume that the properties of lipid material inside the cell are more similar to those in the membrane than to those of octanol. If we make the assumption that they are the same, the permeability would be expected to be proportional to the diffusion coefficient in the lipid component of the membrane times the solubility in this component. Again assuming the diffusion coefficient is proportional to $(MW)^{-1/2}$, the permeability, P, would be proportional to $\lambda_L (MW)^{-1/2}$ and hence $\lambda_L = a'P(MW)^{1/2}$, where a' is a propor-

tionality coefficient ($gm^{-1/2}cm^{-1}sec$). From Eq. B1, therefore,

$$\lambda = V_w + aP(MW)^{1/2}, \quad \text{(B7)}$$

where $a = a'V_L$.

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