Diffusion of Sulfonamides in Aqueous Buffers and into Red Cells

LARRY B. HOLDER AND SHERRARD L. HAYES

Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida

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SUMMARY

Diffusion characteristics of eight sulfonamides including five carbonic anhydrase inhibitors and three antibacterial drugs were studied in detail. There was very litttle variation in magnitudes of aqueous diffusion coefficients among the drugs.

The diffusivities into red cells were 10^{-4} to 10^{-8} those in aqueous medium. Drugs showed a range of 4000-fold in rates of red cell penetration. Lipid solubilities of the various drugs were approached by means of the $CHCl_3$:H_2O partition coefficients which covered a range of 2.5×10^5. A diffusion constant for drugs into red cells was calculated using the square root of the lipid solubility; the range of penetration rates was thereby reduced from 4000 to 100.

All these drugs appeared to diffuse passively down a concentration gradient into the red cells. It was proposed that the rate-limiting step in the passage of these drugs into the red cell is at the cell membrane and that aqueous diffusion normally is of little significance in affecting the overall rates of penetration.

From penetration kinetics of sulfonamides into dog and human red cells, it was concluded that the two most important characteristics which influence these rates are plasma drug binding and lipid solubility, as measured at physiological pH.

INTRODUCTION

Despite a considerable accumulation of data regarding the permeability of various biologic membranes to organic and inorganic substances, unified efforts to compare basic diffusion properties with rates of permeability through a viable membrane are lacking.

Some of the more pertinent studies of permeability rates include those concerned with differential penetration of sulfonamides into CSF (1, 2), aqueous humor (2, 3, 4), sweat (5), saliva (6), and passage of some organic bases and anions into human red cells (7, 8). Although Maren *et al.* (9) have shown that there are considerable differences among the rates with which various sulfonamides enter canine red cells, no quantitation of these permeability rates was attempted.

With regard to simple diffusion, i.e., diffusion of a substance through an aqueous medium, even the most fundamental values for sulfonamides, the aqueous diffusion coefficients, are heretofore nonexistent in the literature. The capillary cell method of determining diffusion coefficients offered itself as a satisfactory means for doing so with the sulfonamides used for this study.

In the present paper we compare aqueous diffusion coefficients of eight sulfonamides (including antibacterial drugs and carbonic anhydrase inhibitors) with their rates of penetration into red cells.

MATERIALS AND METHODS

Drugs. Drugs were obtained as pure crystals or as powder, in their acid forms from the American Cyanamid Company, with the following exceptions: ethoxzol-

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amide was obtained from Upjohn; sulfisoxazole from Hoffman-LaRoche. Primary solutions were made by the addition of 1–1.6 moles of NaOH per mole of drug. For red cell diffusion subsequent dilutions were made in buffered 0.9% NaCl. For aqueous diffusion studies subsequent dilutions were made with distilled water to the final concentrations: sulfanilamide 200 μg/ml; sulfamethoxypyridazine, sulfadiazine, and sulfisoxazole 100 μg/ml; acetazolamide, methazolamide, ethoxzolamide, and CL 11,366 50 μg/ml. Structures and properties of these drugs are given in Table 1.

Analysis of chemicals and drugs. Analysis for acetazolamide, CL 11,366, methazolamide, sulfanilamide, and ethoxzolamide was done by the method of Maren (10) which utilizes the conversion of CO_2 to H^+ and HCO_3^- by carbonic anhydrase. Analyses for sulfadiazine, sulfamethoxypyridazine, and sulfisoxazole were done by the method of Bratton and Marshall (11). Optical densities were measured by Bausch and Lomb Spectronic 20 colorimeter at 540 mμ. NaCl and KCl standards were analyzed for Cl^- by $HgNO_3$ titration.

Aqueous diffusion procedure. A modification of the capillary cell method of measuring diffusion coefficients, described by Wang (12), and modified by Saraf *et al.* (13) was employed. The method allows solute to diffuse from the open end of a capillary tube into a surrounding bath of pure solvent. Instead of a sealed-end capillary as originally used by Wang, the modified method makes use of a 50-μl or 100-μl glass syringe with stainless steel plunger (kindly provided by Hamilton Company, Inc. of Whittier, California). Set screws permitted fixation of the plunger at desired depths. The syringes were filled to the selected capillary length, 3.0 cm, with primary drug solutions and immersed open-end up in 7.0 liters of distilled water adjusted to either pH 5.0 or 7.4. Drug and bath solutions were adjusted to the above pH conditions by the addition of 0.1 N HCl and 0.077 M phosphate buffer (pH 7.4) respectively. For each diffusion run intracapillary and bath solutions were brought to within 0.2 pH units of each other as determined by Beckman pH meter.

The solutions were allowed to diffuse for 36–48 hr in the 7.0-liter bath which was slowly stirred with a magnetic stirring device. To minimize convection currents the magnetic stirring rod was placed inside a small beaker which was covered in the bath by a polyethylene rack adapted for holding the syringes. Currents were permitted escape through several small apertures punched into the side of the rack. Visual observation of the currents as indicated by drops of ink placed in the bath in both trial and actual runs gave sufficient evidence that the convection currents were minimal. At the end of the diffusion period the syringes were removed and the contents emptied into a known volume of water. Aliquots of this solution were used for analysis. To obtain initial concentration values the syringes were then refilled to their previous depths with primary solution and replaced in the bath for a period of 15 min. The syringes were again removed from the bath, and the solutions were extracted for analysis. All diffusions were conducted at room temperature (24°).

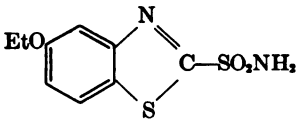
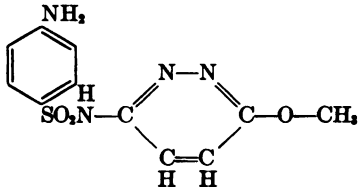
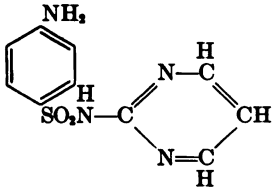
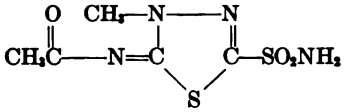
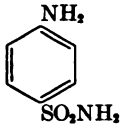
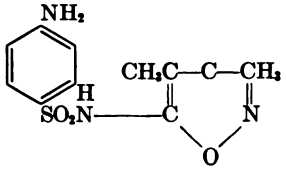
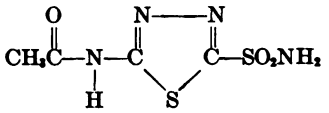
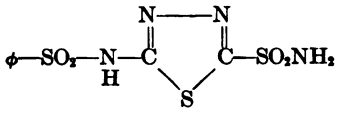
Fick's equation. Solution of Fick's diffusion equation for the conditions of this method yields the following expression (Eq. 1) (14):

$$\frac{C_f}{C_o} = \frac{8}{\pi^2} \sum_{n=0}^{n=\infty} \frac{1}{(2n+1)^2} \cdot e^{[-(2n+1)^2\pi^2 Dt/4L^2]} \quad (1)$$

Where: C_f = concentration of solute within the capillary at the completion of diffusion
C_o = concentration of solute within the capillary at the beginning of diffusion
e = base of natural logarithms
D = diffusion coefficient of solute in cm^2/sec
t = time allowed for diffusion in sec
L = length of capillary in cm

By substituting various values for the expression Dt/L^2 it may be seen that if

TABLE 1

Structure	Name and mol. wt.	$CHCl_3$ / H_2O	pK_a[a]	% ionized pH 5	% ionized pH 7.4	% bound to plasma Dog	% bound to plasma Man
	Ethoxzolamide 258	25.0[b]	8.1	0.1	17	96[b]	96[b]
	Sulfamethoxy-pyridazine 280	1.6[f]	6.7	2.0	83	60[c]	83[c]
	Sulfadiazine 250	0.050[f]	6.5	3.0	89	17[a]	33[c]
	Methazolamide 236	0.035[b]	7.2	0.6	61	55[e]	55[e]
	Sulfanilamide 172	0.020[b]	10.4	0.0004	0.1	11[d]	11[d]
	Sulfisoxazole 268	0.005[b]	5.8	16.0	98.5	68[c]	84[c]
	Acetazolamide 222	0.001[b]	7.4 9.1	0.4	50	50[e]	95[e]
	Cl 11,366 320	0.0001[b]	3.2 9.0	98.0	99.994	92[e]	95[e]

Respective references as follows:
[a] (9).
[b] C. E. Wiley, unpublished data from this laboratory. Binding value for 10 μg/ml plasma.
[c] (25) at 100 μg/ml plasma. See references cited for values at other concentrations.
[d] (26).
[e] (27) at 10 μg/ml plasma. See references cited for values at other concentrations.
[f] (23).

$Dt/L^2 \geqq 0.2$ all terms of the series may be neglected except the first with an error of no more than 0.5%. For values of $Dt/L^2 < 0.2$ more terms of the series must be included to attain similar accuracy. To expedite calculations a graph was constructed (Fig. 1) by assigning values to

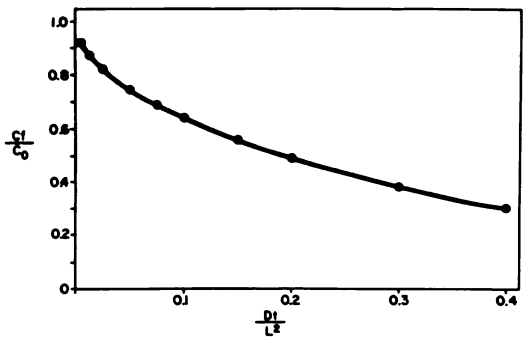


FIG. 1. *Graphic solution for Eq. (1)*

Dt/L^2 and solving Eq. 1 for corresponding values of C_f/C_o, using as many terms of the infinite series as were necessary to claim an accuracy of 99.5% or greater.

After analyzing solutions to determine C_f and C_o, measuring capillary lengths with Vernier calipers and recording elapsed time in seconds, C_f/C_o was computed, and the corresponding values for Dt/L^2 were read from the graph. Known values for t and L were then introduced into the resulting equation, which was solved for D. Table 2 gives an example of these calculations.

freshly drawn, heparinized human or canine blood was used. Ten milliliters of whole blood was placed in a 25-ml Erlenmeyer flask to which was added 0.1 ml of drug solution in the appropriate concentration. The flask was then shaken in horizontal motion on a Burrell shaker at 37°. At periodic time intervals approximately 2–3 ml of sample was withdrawn and immediately subjected to centrifugation at 850 *g* for 10 min. The hematocrit was noted, and plasma and packed red cell aliquots were taken for analysis. Because of plasma trapping among the packed red cells, an error found by ultracentrifugation, at 20,000 *g*, to be 9%, an appropriate correction factor was applied in all red cell determinations.

For determination of rates of penetration of drugs from buffered saline media into red cells the procedure was the same except that prior to addition of drug, the blood was centrifuged, plasma was discarded, and packed red cells were washed once with buffered saline and then suspended in an equal volume of phosphate-buffered saline made from 0.15 M NaCl and 0.01 M phosphate at pH 7.4.

Several of the drugs, namely ethoxzolamide, and methazolamide, sulfamethoxypyridazine penetrated the red cells from saline media so rapidly that entry into red cells was complete within less than 1 min of incubation; hence, the limiting values for P_s in Table 5. Penetration rates of these same three drugs from whole plasma was

TABLE 2
Diffusion of sulfanilamide at pH 7.4 in aqueous buffers

Syringe	C_o (μg/ml)	C_f (μg/ml)	C_f/C_o	Dt/L^2	sec × 10^{-5}	$D \times 10^5$ (cm²/sec)
1	200	112	0.560	0.150	1.69	0.778
2	200	109	0.545	0.160	1.69	0.830
3	200	104	0.520	0.180	1.69	0.960
4	200	110	0.550	0.158	1.69	0.846

Red cell penetration procedure. Penetration rates of drugs into red cells were measured both from whole plasma and from buffered saline media. To determine penetration rates from plasma into red cells, rapid enough to necessitate sampling after 60 sec of incubation. For these drugs it was possible to get a crude estimate of rate of penetration by incubation of drug with red cells for 15 sec, then centrifugation for 45

sec, followed by immediate sampling of cells from the bottom of the centrifuge tube with a pipette. It was shown by ultracentrifugation that the plasma trapping error in this case was only 12%. Drug recovery from cells and media ranged from 90 to 100% in all experiments.

Fick's equation. When red cells are incubated with plasma or saline which contains drug, and if each drug penetrates the red cell only by simple passive diffusion, the rate of penetration of drug into red cells will be proportional to the concentration gradient across the cell membrane. The rates of entry of drug into red cells will coincide with the decay in concentration of drug from the incubation media as a logarithmic function of time, until equilibrium is reached as illustrated in Figs. 2a and 2b. If one considers only the portion of the diffusion curve before diffusion equilibrium is established and the time intervals for sampling are appropriately short in duration, the following form of Fick's equation can be applied:

$$\text{Vel} = \frac{KA(C_o - C_i)}{\text{Vol}\,\Delta x} \qquad (2)$$

Where: Vel = velocity of diffusion in moles/liter·sec
K = membrane diffusion coefficient in cm²/sec
A = surface area of red cell in cm²
C_o = average concentration outside red cell in moles/liter
C_i = average concentration inside red cell in moles/liter
Δx = thickness of red cell membrane in cm
Vol = volume of red cell in cm³

Permeability constant. For practical purposes $KA/\text{Vol}\Delta x$ is usually combined into a single constant P, called the permeability constant for a single system so that

$$\text{Vel} = P(C_o - C_i) \qquad (3)$$

Where: P = permeability constant in sec⁻¹

Should the drug be bound to proteins either inside or outside the red cell, that portion of bound drug would be unavailable for diffusion and values of C_i and/or C_o in the above expression would be altered. Maren *et al.* (9) have shown that ethoxzolamide, methazolamide, acetazolamide, and CL 11,366 are bound to red cell receptors inside the red cell with a dissociation constant in the order of 10^{-8} M. It has also been shown that these receptors become saturated at intracellular concentration of 140 μM in man and 38–92 μM in dog. In the

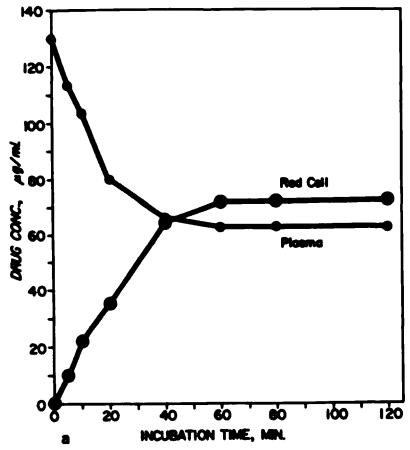


FIG. 2a. *Penetration of CL 11,366 into human red cells from plasma*

Concentrations in red cells calculated for cell water (66% of volume); that for plasma is total concentration in the fluid.

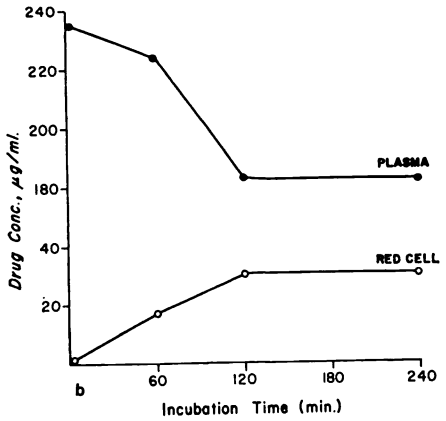


FIG. 2b. *Penetration of sulfisoxazole into human red cells from plasma*

Concentrations in red cells calculated for cell water (66% of volume); that for plasma is total concentration in the fluid.

present paper these values are adjusted to the volume of water in red cells (66%). The range noted for dog indicates differences among the several drugs. The dissociation constants of these four drugs for carbonic anhydrase and possible other red cell receptors are so low ($<10^{-7}$ M) that when drug concentration inside the red cell is less than the drug receptor saturation level, there is essentially no free drug intracellularly and C_i assumes the value of zero in Eq. 3. For these four drugs a drug level was chosen which allowed samples to be taken before the intracellular drug receptors became saturated so that C_i could be neglected. Table 3 is presented

TABLE 3
Diffusion of CL 11,366 into human red cells from plasma

ΔT (min)	Red cell flux (μg/ml ΔT)	Velocity (μg/ml · hr)	Average plasma drug conc. (μg/ml)	P_p (hr^{-1})
0–5	10.3	123	122	1.01
5–10	11.9	144	109	1.32
10–20	13.0	78	92	1.07
20–40	29.0	88	73	1.20

to illustrate the manner in which Eq. 3 was applied to the diffusion data. The data in Table 3 are taken from the representative curves of Figs. 2a and 2b.

Since sulfadiazine, sulfamethoxypyridazine, and sulfisoxazole are not bound intracellularly (9), C_i was included in the calculations. Figure 2b exemplifies the data obtained for these three drugs, and it should be noted that intracellular drug concentration never equals extracellular. Since sulfisoxazole is moderately bound by protein in dog plasma there is a rather marked difference between intracellular and total plasma concentration at equilibrium. In Fig. 2b the unbound concentration in plasma water at 2–4 hours is about 50 μg/ml, while in red cell water it is 30 μg/ml. This follows the passive distribution of an anion across a membrane whose potential is about 10 mV, and agrees with ratios for halides.[1]

Since sulfanilamide is bound intracellulary with a dissociation constant of 10^{-6} M, the total drug inside the red cell is not free, but neither does C_i equal zero. It was therefore necessary to study sulfanilamide penetration in a different fashion. An outside concentration range was used which resulted in a total intracellular concentration (>500 μM) greatly exceeding the drug receptor saturation level, thereby permitting the amount of drug bound intracellularly to be neglected.

Since the drugs studied are bound to plasma albumin and only the free, *i.e.*, unbound, molecules are at liberty to traverse the red cell membrane, we assume that velocity of penetration is dependent upon the concentration of unbound drug rather than total concentration of drug in plasma. On this assumption two permeability constants were determined in the case of each drug and defined as follows:

$$P_p = V/Pl_t \tag{4a}$$

and

$$P_{ub} = \frac{P_p \cdot 100}{\%\text{ of drug unbound in plasma}} \tag{4b}$$

Where: P_p = rate constant measured from whole plasma in sec^{-1}
V = velocity of diffusion in moles/liter sec
Pl_t = total plasma drug concentration in moles/liter
P_{ub} = rate constant considering only free drug in plasma, in sec^{-1}

In some of the tables and figures P_p and P_{ub} are given in hr^{-1}.

Rate constants were also determined from buffered saline (P_s) into red cells to eliminate completely the factor of plasma binding and thus clarify the significance of P_p and P_{ub}. The plasma binding values re-

[1] There are some interesting parallels between the present data and the classical studies of Tosteson on halide transport in red cells. Chloride flux across red cells is passive, and very rapid; however the resistance in the membrane was calculated as about 10^5 that in free solution (28).

ported in Table 1 were done at plasma concentration close to those used in red cell diffusion experiments, as illustrated in Figs. 2a and 2b.

Chloroform:water partition coefficient. The chloroform:water partition coefficient of each drug was determined in this laboratory as follows: Mallinckrodt chloroform analytical reagent was washed with an equal volume of pH 7.4 phosphate aqueous buffer for 1 hour and separated. Phosphate-buffered drug solution at pH 7.4 was mixed with an equal volume of washed $CHCl_3$ and placed on a shaker for 1 hour. The aqueous and chloroform layers were separated and aliquots were taken for drug analysis. The aqueous aliquot was analyzed directly: 0.5 ml of the chloroform aliquot was placed in a glass-stoppered test tube and allowed to evaporate down in a water bath at 60°. The residue was then dissolved in water, using 1 ml of 0.05 N NaOH, and analyzed for drug.

Diffusion constant. In order to compare the aqueous diffusion coefficients of the eight sulfonamides with the rates of penetration into red cells, all components of Eq. 2 were used. The following geometrical measurements of the red cell as reported in the literature (15) were substituted into Eq. 2: surface area $163\,\mu^2 = 1.63 \times 10^{-6}$ cm^2, volume $87\,\mu^3 = 8.7 \times 10^{-11}$ cm^3, thickness of red cell membrane $100\,A = 10^{-6}$ cm. Thus, $A/\text{Vol}\Delta x$ (in units of cm^{-2}) remains a constant in this system and Eq. 2 reduces to:

$$\text{Vel} = (1.9 \times 10^{10})K(C_o - C_i) \quad (5a)$$

or

$$K = \frac{(5.3 \times 10^{-11})\ \text{Vel}}{(C_o - C_i)} \quad (5b)$$

Where: K = diffusivity of drug in the red cell plasma membrane in cm^2/sec

It should be noted that K has the same units as D from Eq. 1.

The idea of relating diffusivity across biological membranes to lipid partition coefficients has been developed in mathematical form by Danielli (16). In this context we have used an additional diffusion constant K' to test the equation of Riggs (17) which relates diffusivity across an homogeneous lipid barrier to R, the distribution ratio of drug between the lipid and water phases. $CHCl_3:H_2O$ partition coefficients were used as values for R. In this circumstance Eq. 2 is modified as follows:

$$\text{Vel} = \frac{K'AR(C_o - C_i)}{\text{Vol}\ \Delta x} \quad (6a)$$

Or using the form of Eq. 5b:

$$K' = \frac{(5.3 \times 10^{-11})\ \text{Vel}}{R(C_o - C_i)} \quad (6b)$$

Examination of the data suggested a further modification of these relations in which the square root of R was used in the type of equation as 6b, then:

$$K'' = \frac{(5.3 \times 10^{-11})\ \text{Vel}}{\sqrt{R}(C_o - C_i)} \quad (7)$$

Resultant values for each drug appear in Table 6 showing diffusion constants calculated according to Eqs. 5b, 6b, and 7.

RESULTS

Aqueous diffusion: standardization of the method. Prior to the diffusion runs with sulfonamides, several calibration determinations were performed using 0.5 N NaCl and 0.5 N KCl solutions. The mean diffusion coefficient (in $cm^2\ sec^{-1} \times 10^5$) and standard error of the mean for NaCl and KCl, respectively, were 1.33 ± 0.11 ($n = 4$) and 1.74 ± 0.29 ($n = 4$). The diffusion coefficients of NaCl and KCl determined by various early methods as recorded in the International Critical Tables are 1.40 ± 0.10 and 1.78 ± 0.10, respectively. It was felt that the similarity of mean values by the capillary-cell method with those from previous methods sufficiently justified further utilization of the method for delimiting sulfonamide diffusion coefficients.

Sulfonamide diffusion coefficients. Table 2 for sulfanilamide is an example of the measurements and calculations performed on all the drugs. Values for Dt/L^2 are taken from Fig. 1 and correspond to respective C_f/C_o ratios. Values for D expressed in

Table 2 reappear in Table 4 along with values for the remaining drugs. Table 1 shows the fraction of each sulfonamide

TABLE 4
Aqueous diffusion coefficients, $D = cm^2/sec \times 10^5$

Drug	pH 5.0			pH 7.4		
	Mean	S.E.	*n*	Mean	S.E.	*n*
Ethoxzolamide	0.59	0.06	3	0.93	0.10	4
Sulfamethoxy-pyridazine	0.58	0.03	4	0.62	0.05	4
Sulfadiazine	0.55	0.05	5	0.56	0.05	4
Methazolamide	0.71	0.11	4	0.44	0.07	4
Sulfanilamide	0.98	0.22	4	0.85	0.08	4
Sulfisoxazole	0.47	0.04	4	0.47	0.04	5
Acetazolamide	0.63	0.15	4	0.38	0.11	4
CL 11,366	0.52	0.04	4	0.71	0.06	4

existing in ionized form at the different pH conditions of the diffusion experiments. It should be noted that at pH 7.4 the lowest value for D, that of acetazolamide, differs by a factor of less than three from the highest value, that for ethoxzolamide.

Red cell penetration. Three penetration rate constants were determined for each of the eight sulfonamides. P_p represents penetration rates from whole plasma; P_{ub}, penetration rate from plasma considering only the unbound or free drug; and P_s, penetration rate from saline. Exemplary data showing measurements and calculations are shown in Table 3, and rates for all the drugs, in Table 5. In general, all eight sulfonamides diffuse more rapidly into human than into dog red cells as evidenced by the values for P_p and P_s. This small but consistent difference is inexplicable at this time since it has been shown that the type, quantity, and structural interrelationships of the lipid components in the red cells of the two species are not dissimilar (18, 19).

P_p. The relative rates of penetration of the sulfonamides into red cells from whole plasma are similar in dog and human. In order from least to most rapid penetration, they rank as follows: sulfisoxazole, CL 11,366, acetazolamide, sulfamethoxypyridazine, sulfadiazine, sulfanilamide, methazolamide, and ethoxzolamide. The range is 10^3. The P_p values of each drug in man and dog are generally the same except for acetazolamide and sulfamethoxypyridazine. The P_p of acetazolamide in dog is about 10 times that in man, while the P_p for sulfamethoxypyridazine in dog is approximately half that in man. These differences are entirely accounted for by respective differences in plasma binding of these drugs.

P_{ub}. Assuming that only the unbound or free drug in plasma determines the concentration gradient, P_{ub} values were determined by dividing P_p by the fraction of drug which was unbound in plasma. These latter values are given in Table 1. The calculated value for P_{ub} resembled closely the P_s value determined experimentally in each case, indicating that P_{ub} and P_s are essentially the same, and thus adding impetus to the proposition that plasma binding is a major factor influencing the rates of penetration of sulfonamides into red cells of man and dog.

P_s. In an attempt to eliminate entirely the factor of plasma binding of the drug and its effect on the rate of red cell penetration, rate constants from saline were determined. The constants from saline were considerably larger than those from plasma in each case. Again, relative rates were of the same order of magnitude in both species, in spite of an alteration of the order from least to most rapid penetration. Some of these drugs passed into the red cell faster than could be measured by our methods, hence the limiting values in Table 5.

Relationship between P_{ub} and the chloroform:water partition coefficient. In an effort to delineate diffusion kinetics more thoroughly, the P_{ub} values in man were plotted against the chloroform:water partition coefficients of each drug at physiological pH, and presented in Fig. 3. Included are values for drug penetration rates into human red cells as obtained in a similar study by Schanker *et al.* (7), who investigated the rates of penetration of drugs from Tyrode's solution into red cells in a system in which the concentration of drug in the medium was maintained constant.

TABLE 5

Penetration rate constants of sulfonamides into red blood cells (hr^{-1})

n = 3–6 in all determinations

	P_p				P_{ub}				P_e			
	Human		Dog		Human		Dog		Human		Dog	
Drug	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Ethoxzolamide	180	6.4	103	3.9	4500	110	2550	105	>200	—	>200	—
Sulfamethoxy-pyridazine	36	0.5	75	11	210	3.1	187	28	>170	—	>135	—
Sulfadiazine	51	2.5	65	7.2	76	3.8	78	8.6	100	4.1	92	1.1
Methazolamide	87	4.2	82	15	195	9.6	181	31	>180	—	195	17
Sulfanilamide	122	4.5	86	3.4	136	5.0	96	3.8	153	10	99	5.2
Sulfisoxazole	0.2	0.07	0.25	0.07	1.2	0.4	0.79	0.36	0.54	0.07	0.36	0.1
Acetazolamide	1.4	0.3	11	2.0	27	4.9	21	3.9	31	0.5	13	1.8
CL 11,366	1.2	0.07	0.95	0.22	23	1.6	12	2.7	25	0.9	19	2.0

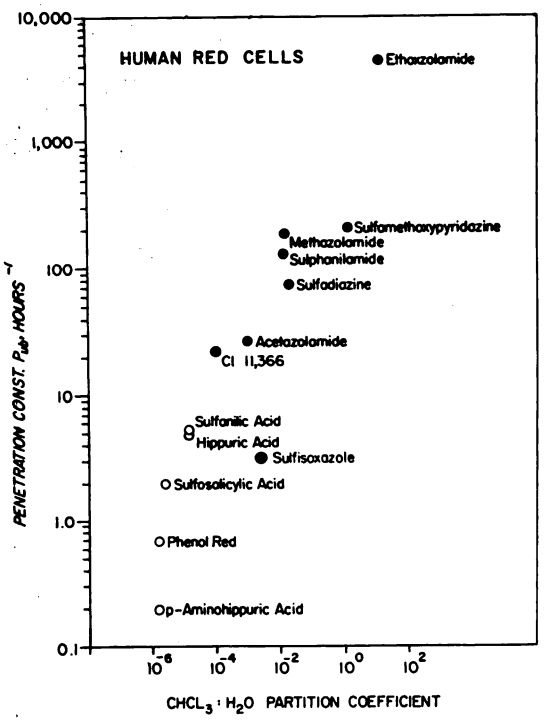


FIG. 3. *Penetration rates of drugs into human red cell vs. chloroform partition coefficients*

Closed points denote values determined in this study, and open points indicate values reported by Schanker *et al.* (7).

His data were handled with the following form of the Fick equation:

$$tp = \ln (1 - C_{rbc}/C_r \times C_{pl}) \qquad (8)$$

Where: t = time
p = penetration constant
C_{rbc} = drug concentration inside the red cell
C_{pl} = drug concentration in plasma medium
$C_r = C_{pl}/C_{rbc}$ at equilibrium

The above form of Fick's equation will not apply to this study because the drug concentration in the medium was not kept constant. However, Schanker's constant, p, is mathematically equivalent to our constants P_{ub} and P_s. In Fig. 3 the combined data of our sulfonamide experiments with those of Schanker *et al.* suggest an almost linear relationship between chloroform: water partition coefficients determined at pH 7.4 and penetration rates. Furthermore, the slope of the data, being nearly ½, suggests that the penetration rates are directly proportional to the square roots of the $CHCl_3$:H_2O distribution coefficients, an observation unlike that of Collander and Bärlund (20), who found a direct relationship between penetration rates of some organic compounds into *Chara ceratophylla* and their respective olive oil:water partition coefficients.

Comparison of D and K. Aqueous diffusion coefficients for these drugs are in the range of 10^{-6} cm²/sec, and values of red cell diffusion coefficients vary from 10^{-10} to 10^{-14} cm²/sec (Table 6). It is manifest that

TABLE 6

Comparison of aqueous diffusion coefficients (D) with human red cell diffusivities (K)

D taken from Table IV; P_{ub} from Table V, converted to units of *K* through Eq. 5b, *K'* through Eq. 6b, and *K"* through Eq. 7.

Drug	D (cm²/sec) × 10^{6}	P_{ub} sec^{-1} × 10^{-4}	K (cm²/sec) × 10^{12}	K' (cm²/sec) × 10^{12}	K'' (cm²/sec) × 10^{12}
Ethoxzolamide	9.3	12,500	66	2.6	13
Sulfamethoxy-pyridazine	6.2	585	3.0	2.0	2.4
Sulfadiazine	5.6	210	1.1	23	5.0
Methazolamide	4.4	542	2.9	82	15
Sulfanilamide	8.5	380	2.0	98	14
Sulfisoxazole	4.7	3.3	0.018	3.6	0.26
Acetazolamide	3.8	75	0.40	410	13
CL 11,366	7.1	64	0.33	3,300	33

the diffusivities of these drugs in water is 10^4–10^8 times greater than their corresponding diffusivities in the red cell plasma membrane.

A significant comparison can be made between aqueous diffusion coefficients and membrane diffusion coefficients according to Eq. 7 (K''), which introduces the $\sqrt{R}$ as a correction factor. When this is done the new membrane diffusion coefficients (K'') are very similar to each other at about 10^{-12}.

DISCUSSION

Aqueous diffusion: Errors of the capillary cell method. A treatment of the errors inherent in the capillary cell diffusion system is given by Mills and Kennedy (21). The two most pertinent sources of error with the method are the convection currents which are generated by the stirring apparatus, and an error introduced when the method is used as it is here for free diffusion determinations rather than self-diffusion of radioactive solute into unlabeled solute of the same physical concentration. The former source of error has been approached in the methods section. Briefly, the latter source is one that is present in many diffusion systems but is magnified here because of the small volume of drug solution (circa 50 μl). As diffusion of the solute into the pure solvent proceeds, there is a concomitant diffusion of free solvent into the capillary cell resulting in a real increase in fluid volume within the cell. This increase in volume displaces solution out of the cell into the enveloping solvent and engenders an apparent high diffusion coefficient. It would seem that this last error could be diminished by using very low initial concentrations which would decrease the solvent concentration gradient and consequently the influx of solvent into the capillary cell. With this source of error in mind primary solutions used for the sulfonamide determinations were as low as the analytical methods permitted.

Even though the capillary cell method seems adequate as applied here, final values of D as obtained are so similar in magnitude and the individual standard errors of the mean are so relatively large, that significant differences among the diffusion coefficients are appreciable only when comparing the extremes of the range of values. Furthermore, our data show no consistent relationship between molecular weight and/or percentage ionization and numerical size of D. What influence such phenomena as hydration and steric hindrance may exert on rapidity of aqueous diffusion are not quantitatively elucidated at this time.

Red cell penetration. Some of the physical, chemical, and physiological factors which have been indicated as influential in affecting passage of any substance from extracellular to intracellular fluid are aqueous diffusion, plasma binding, lipid solubility, molecular size, as well as the structural characteristics and peculiarities of the plasma membrane itself.

Aqueous diffusion as determined for these sulfonamides is apparently of no limiting significance in the overall passage of these drugs into the red cell. When values of D are compared with those of K in Table 6, it is evident that aqueous diffusion is of the order of a million times greater than rates of transit through the cell membrane in question. The relatively small variations in the diffusion rates of these sulfonamides in aqueous medium are negligible compared to those differences observed in rates of penetration into red cells.[1]

The importance of plasma binding is established by the finding that penetration rates of unbound drug from plasma and from saline are very nearly the same. At any given instant only the unbound drug in plasma is free to diffuse into the cell.

Lipid solubility has previously been shown to be of great significance in determining red cell penetration rates. The present data confirm and extend this; Fig. 3 shows that a 2 log unit change in partition coefficients is reflected as a 1 log unit change in penetration rate. It is generally held that the lipid solubility at physiological pH is a function of the innate lipid solubility of the nonionized form of the drug, and the proportion of drug in the nonionized form as defined by the pK_a of

that drug. The factor which is probably of key importance in determining the lipid solubility of the nonionized moiety is the polarity of the molecule as represented by its dipole moment, values which are heretofore unknown for sulfonamides.

As has been stated, pK_a values are important in any consideration of penetration rates in that they define the proportion of drug in nonionized form at physiologic pH. Generally speaking, ionized organic compounds do not readily penetrate plasma membranes because of low lipid solubility and/or large effective size due to hydration. It would be of interest to know the relative lipid solubilities of ionized and nonionized species of the same drug, as well as their relative diffusion rates.

Comparison with other data. It is of interest to compare data procured on the penetration rates of sulfonamides into red cells with data obtained from the literature on passage of these drugs into other tissue compartments. Wistrand, Rawls, and Maren (4) studied rates of penetration of several sulfonamides from plasma to aqueous humor of rabbits. Sorsby (22) performed similar work, utilizing rats. Subjecting their data to the same form of Fick's equation used in this study, a relationship was established between chloroform:water partition coefficients and penetration rates (Fig. 4), suggesting that a 4 log unit change in partition coefficients is reflected as a 1 log unit change in penetration rates.

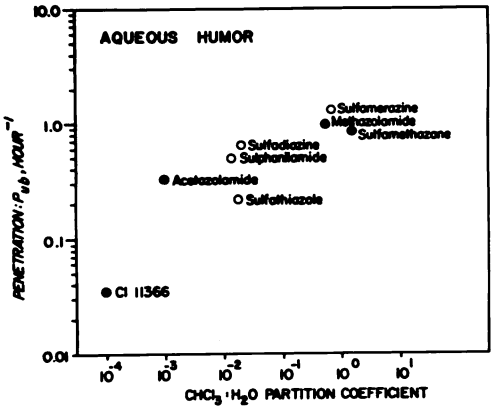


FIG. 4. *Penetration rate of sulfonamides from plasma into aqueous humor for the rabbit (closed points, ref. 4) and for the rat (open points, ref. 22) plotted against $CHCl_3$:H_2O partition coefficients*

Penetration coefficients of sulfonamides into CSF of dogs determined by Rall *et al.* (23) were plotted in Fig. 5 versus chloroform partition coefficients along with data obtained by T. F. Muther (personal communication) for penetration of sulfonamides into rat brain. The same relation between lipid solubility and diffusion rate cited above for aqueous humor was observed.

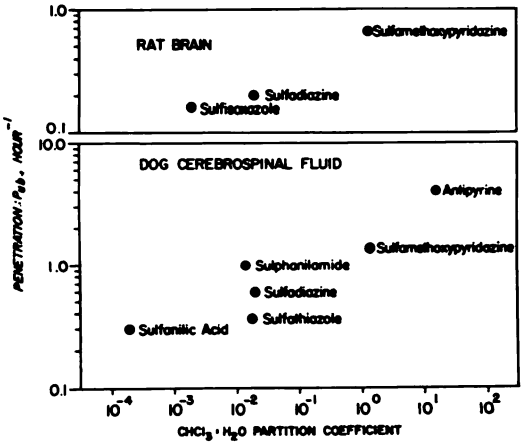


FIG. 5. *Penetration rates of sulfonamides from plasma into rat brain (top, T. F. Muther, personal communication) and into canine cerebrospinal fluid (bottom, ref. 23) plotted against $CHCl_3$:H_2O partition coefficients*

As mentioned earlier, if the passage of some molecules through plasma membranes is dependent on their lipid solubilities and if such membranes behave akin to an homogeneous lipid medium, then discrepancies in penetration rates among different compounds could possibly be minimized by introducing the lipid:water partition coefficients as correction factors into the Fick equation (see Eq. 6a). Using chloroform: water partition coefficients as representatives of lipid solubility such a correction was attempted with our data. From Table 6 it is patently clear that wide variation in penetration rate constants (K') persist de-

spite introduction of R. However, when $\sqrt{R}$ is employed (see Eq. 7) the resultan rate constants are remarkably close to each other. It is possible that the values in Table 6 for K'' would be even closer to each other if we could determine the actual solubilities of the drugs in the membrane rather than use the approximation of $CHCl_3$:H_2O partition coefficients.

Mechanism of diffusion. Currently proposed mechanisms of membrane transport fall into one of two varieties: passive or active. The former requires no expenditure of cellular metabolic energy and is simple diffusion of substance from higher concentration to lower. The latter depends on cellular energy and usually involves transport against a concentration gradient.

At least two significant points suggest that transport of these sulfonamides into red cells is passive. At equilibrium the internal concentrations of free drug never exceeded that outside. In Fig. 2a free drug inside is essentially zero since the drug is all bound to carbonic anhydrase which is present in the human erythrocyte at a concentration of about 140 μM of red cells or 210 μM in cell water (9). In the type experiment of Fig. 2b, inside drug is free, since drugs of this type do not bind to carbonic anhydrase. At equilibrium the intracellular concentration is that predicted by passive diffusion down the electrochemical gradient. The addition of metabolic inhibitors (10^{-4} M iodoacetate) or the elimination of glucose from saline media do not influence the uptake of acetazolamide into the red cells (9).

An additional point involves the subjugation of Maren's (9) data on the passage of acetazolamide into red cells at different temperatures to the Arrhenius equation, with subsequent determination of the energy of activation, ΔH. By that method[2] we have calculated the energy of activation to be 13.7 kcal/mole, a value which is well within the range of values to be expected for simple diffusion of a substance through lipid medium and more than twice the magnitude of values obtained for substances which are diffusing through water (24), supporting passage through lipid rather than through aqueous pores. Finally, as discussed above, rates of penetration of these organic substances are related to their lipid solubilities at physiological pH.

These observations are most simply and consistently explained by the proposal that penetration is the result of dissolution of the compounds into the lipid membrane followed by passive diffusion through it.

[2] The following form of the Arrhenius equation was used:

$$\Delta H = R \cdot \frac{T'' \cdot T'}{T'' - T'} 2.3 \log \frac{k''}{k'}$$

Where R is the gas constant, 2 calories mole^{-1} degree^{-1}, k' and k'' are rate constants at two temperatures, and T' and T'' are the corresponding absolute temperatures. Rate constants were 2.13 hr^{-1}, 6.9 hr^{-1}, and 21.0 hr^{-1} at 9°, 24°, and 37°, respectively.

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