amounts of up to 10,000 mcg. of the polypeptide I. To each tube was added the equivalent point amount of the homologous antigen (30 mcg.), and the tubes were then incubated at 37° for 1 hr. After standing at 4° for 48 hr., the precipitates were collected, washed twice with buffer (0.05 M K_2HPO_4–NaOH, pH 7.0), and collected by centrifugation. The total amount of protein precipitated was estimated by nitrogen analysis (Kjeldahl). No inhibition of the precipitin reaction was observed using the polypeptide I.

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Correlations between *In Vitro* Transport in a Three-Phase Model Cell and *In Vivo* Absorption of a Series of Sulfanilamides

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Abstract □ A three-phase model cell, employing a liquid lipid barrier, was used to establish correlations between reported *in vivo* absorption data and experimentally determined *in vitro* transport rates for a series of N^1-substituted heterocyclic sulfanilamides. These studies, using isopentyl acetate as the lipid phase, involved the effect of pH on the rate constants for three derivatives and the effect of the N^1-substituent on the overall transport for unionized species. Correlations were made between the *in vitro* data and *in vivo* gastric, intestinal, and rectal absorption data, indicating the utility of this model to simulate passive drug absorption. Transport rates and partition coefficients were also found to be related.

Keyphrases □ Sulfanilamides—correlations between *in vitro* transport in three-phase model cell and *in vivo* absorption □ Cell, three-phase model—*in vitro* transport–*in vivo* absorption correlations, sulfanilamides □ Absorption, *in vivo*—correlated to *in vitro* transport, three-phase model cell, sulfanilamides, liquid lipid barrier

There are several types of three-compartment kinetic models in use to simulate the transport of drugs across biological membranes, especially the GI tract. These systems were classified and reviewed recently by Herzog and Swarbrick (1). Those *in vitro* systems utilizing a solid membrane have an obvious advantage in that they more closely resemble the *in vivo* situation. However, one advantage of using a liquid lipid to form the membrane phase is the ease with which the composition of the latter can be varied. This readily permits investigation of such factors as lipid polarity (2) and solubility parameter (3). Three-compartment model cells employing a liquid lipid membrane phase have been used in several attempts to establish correlations between *in vitro* and *in vivo* transport processes. Agostini and Schulman (4) used the so-called "Schulman cell" to investigate ion flux and water migration across biological membranes. Perrin (5) and Augustine and Swarbrick (2) reported using this cell in studies designed to simulate the GI absorption of drugs. In addition to the lipid phase composition, it was shown that stirring rate, the surface to volume ratio of the phases, and temperature also affect the transport rate constants obtained (2). To date, however, no work has been reported using this cell to study a homologous series of compounds.

The present study reports the *in vitro* transport rates of a series of N^1-substituted heterocyclic sulfonamides determined using a Schulman-type liquid lipid transport model. Correlations between the *in vitro* transport rates, partition coefficients, and *in vivo* absorption data, taken from the literature for the same compounds, are described.

EXPERIMENTAL

Materials—The following chemicals were used as received (the figures in parentheses give the determined melting points which, in all cases, corresponded to literature values): sulfanilamide (164.5–166.5°), sulfathiazole (200–204°), sulfamerazine (234–238°), sulfamethazine (198–199°), sulfapyridine (191–193°), and sulfisoxazole (194°). The buffer ingredients were reagent grade and were used as

Table I—Effect of pH of Compartment 1 on Sulfonamide Transport *In Vitro* and Apparent Partition Coefficient

pH	*In Vitro* Rate Constant K_{12}', cm./hr.[a] (Apparent Partition Coefficient in Parentheses)[b]					
	Sulfanilamide	Sulfamerazine	Sulfisoxazole	Sulfamethazine	Sulfathiazole	Sulfapyridine
1.5	0.32 (nil)	0.85 (0.15)	1.76 (5.8)	—	—	—
2.3	0.61 (0.16)	1.29 (0.92)	2.01 (12.9)	—	—	—
3.3	0.73 (0.31)	1.69 (1.45)	2.57[c] (20.7)	—	—	—
4.7	0.99 (0.26)	1.67[c] (2.01)	1.98 (11.9)	—	1.27[c] (0.40)	—
4.9	—	—	—	1.82[c] (3.17)	—	—
5.5	0.91 (0.31)	1.46 (1.75)	1.96 (3.9)	—	—	2.11[c] (2.16)
6.4	1.15[c] (0.37)	1.48 (1.00)	0.98 (0.6)	—	—	—

[a] $K_{12}' = k_{12}/(S/V)$. [b] Between isopentyl acetate and water at pH shown. [c] pH_i.

Table II—Comparison of *In Vivo* and *In Vitro* Transport Rate Constants of Sulfonamides

Sulfonamide	pH_i	Transport Rates				
		In Vitro		*In Vivo*, hr.$^{-1}$		
		K_{12}', cm./hr.	K_u[a], hr.$^{-1}$	$K_{u(gastric)}$[b]	$K_{u(intestinal)}$[c]	$K_{12(rectal)}$[d]
Sulfanilamide	6.4	1.15	0.058	0.075	0.88	0.044
Sulfathiazole	4.7	1.27	0.072	0.061	0.33	0.0007
Sulfamerazine	4.7	1.67	0.120	0.070	1.27	—
Sulfamethazine	4.9	1.82	0.114	0.14	1.25	—
Sulfapyridine	5.5	2.11	0.123	0.087	1.19	0.0696
Sulfisoxazole	3.3	2.57	0.477	0.21	1.96	0.248

[a] Calculated from $K_u = \frac{k_2(k_1/k_{-1})}{1 + (k_1/k_{-1})}$. [b] From Koizumi *et al.* (6). [c] From Koizumi *et al.* (11). [d] From Kakemi *et al.* (7).

received. Isopentyl acetate was distilled before and after each run, the absence of sulfonamide in the distillate being verified spectrophotometrically.

Determination of Partition Coefficients—Apparent partition coefficients for the six sulfonamides under investigation were determined between aqueous phases of varying pH (including pH_i, the pH intermediate between the two pKa values for each sulfonamide) and isopentyl acetate. At the pH_i, the amphoteric sulfonamides exist mainly in the unionized state (6). All determinations were carried out at 37°, and only the aqueous phase was assayed for drug concentration. The initial concentration of each sulfonamide was 0.5 m*M*. The buffers used were similar in composition to those reported by Koizumi *et al.* (6). The data obtained are shown in Table I. The apparent partition coefficients are comparable to values in the literature for similar lipid–water conditions (6, 7).

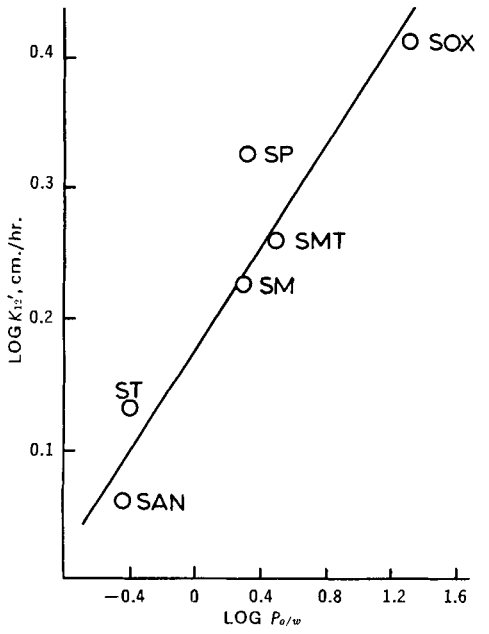

Figure 1—*Logarithmic relationship between partition coefficient and rate constant* (K_{12}') *for sulfonamides. Key: SAN, sulfanilamide; ST, sulfathiazole; SM, sulfamerazine; SMT, sulfamethazine; SP, sulfapyridine; and SOX, sulfisoxazole.*

***In Vitro* Transport Studies**—The effect of pH on the transport rates of the sulfonamides was studied. Cylindrical glass transport cells were employed in these investigations, and the experimental procedure was similar to that described previously (2). Isopentyl acetate was used as the lipid phase (Compartment 2), and the distal aqueous compartment (Compartment 3) was maintained at pH 7.4. Compartments 1 and 3 were stirred at 580 r.p.m. using 1.27-cm. (0.5-in.) circular magnets with fins on the top surface. Compartment 2 was stirred at 60 r.p.m. Transport rates are reported as the rate constant (hr.$^{-1}$) divided by the surface to volume ratio (cm.$^{-1}$) for each particular cell used. The volume of each phase in the three compartments was 225 ml. The initial concentration of drug in Compartment 1 was 0.5 m*M*, and the cells were operated at a temperature of 37°. The transport rates, averaged from duplicate runs, are presented in Tables I and II.

Analysis of Data—Both analog[1] and digital[2] computer curve-fitting programs were utilized in calculating the rate constants governing the *in vitro* transport. The analog computer program was similar to that reported previously (2). A digital curve-fitting program was devised to facilitate the determination of the rate constants. This program was found to be especially useful when drug transport from Compartment 1 was rapid.

RESULTS AND DISCUSSION

Partition Coefficients and *In Vitro* Transport—The apparent partition coefficients as a function of pH (Table I) suggest that it is the unionized species that is predominantly partitioned into the lipid isopentyl acetate phase. The maximum partition coefficient was observed at the pH_i, where the maximum concentration of unionized drug exists. Conversion of the data for sulfanilamide, sulfamerazine, and sulfisoxazole to true partition coefficients, expressed in terms of the fraction of unionized drug at each pH, supports this view, since the calculated partition coefficients are now independent of pH (sulfanilamide, 0.33 ± 0.02 *SE;* sulfamerazine, 1.58 ± 0.15 *SE;* and sulfisoxazole, 15.2 ± 1.24 *SE*).

The data presented in Table I for sulfanilamide, sulfamerazine, and sulfisoxazole indicate that the maximum rate of transport

[1] PACE 261R, Electronic Associates Inc., Long Island, N. Y.
[2] IBM model 360/65.

occurs at the pH_i of each sulfonamide and is obviously related to the fraction of drug unionized. Schulman and Rosano (8) established a relationship between transport and partition coefficient which can be rearranged to a form similar to that demonstrated by Garrett (9), namely:

$$\log K = a \log P_{o/w} + b \qquad \text{(Eq. 1)}$$

where a and b are constants. This relationship holds for the sulfonamides, as shown in Fig. 1, where a linear relationship is observed between the *in vitro* rate constant K_{12}, adjusted for surface to volume ratio, and the isopentyl acetate partition coefficient at the respective pH_i's of the six compounds studied.

The relationship in Fig. 1 is comparable to that used by Fugita and Hansch (10) in relating the hydrophobic binding constant π to the protein binding of 20 N^1-substituted heterocyclic sulfonamides. In this case, π is the difference between the logarithmic values of the partition coefficient of a sulfonamide and the parent compound, sulfanilamide. It is thus apparent that, in the present study, the hydrophobicity of the substituent group is the major factor affecting the *in vitro* transport rate when the compounds are studied at their respective pH_i's.

Correlations between *In Vivo* Absorption and *In Vitro* Transport—Although a correlation exists between the *in vitro* rate constants and the partition coefficients, it is more appropriate to use the *in vitro* transport rate constants as the parameter for correlation with *in vivo* absorption data. This is because the *in vivo* absorption and the *in vitro* transport are both dynamic processes.

Accordingly, an attempt was made to establish a correlation between transport in the model cell and the *in vivo* absorption rate data reported in the literature. Koizumi *et al.* studied the *in vivo* absorption of 17 sulfonamides using a perfusion technique through both gastric (6) and intestinal (11) regions of the rat. Mechanisms of the kinetics of absorption of the sulfonamides through the lipoidal barrier at the absorption site were presented, and a relationship between absorption rates and lipid–water partition coefficients was demonstrated. The absorption rate constant for unionized drug species, K_u, was defined as:

$$K_u = \frac{k_2(k_1/k_{-1})}{1 + (k_1/k_{-1})} \qquad \text{(Eq. 2)}$$

where k_1 and k_{-1} are the forward and reverse rate constants between the stomach (or intestinal) fluid and the biomembrane, respectively, and k_2 is the rate constant for drug transfer from the membrane into blood plasma. K_u is the overall rate constant for removal of unionized drug from the stomach or intestine.

Two aspects of the work of Koizumi *et al.* (6, 11) are open to possible criticism. First, phenol red was used to monitor volume changes of the perfusion fluid during the *in vivo* experimentation. Recent work (12) showed that this dye is absorbed from the intestinal lumen. However, since this occurs only to the extent of approximately 6% in 3 hr. and Koizumi's studies were run for 1 hr., this effect can be ignored. The second factor is that the pH of the perfusion solution changed with time, the magnitude of the change increasing the further the initial pH was from pH 6.5. At the pH_i's used in this study (Table II), the *in vivo* changes observed by Koizumi *et al.* are small and can be reasonably ignored.

The *in vitro* transport rates are reported in Table II as K_u, according to Eq. 2. The rank order of the six sulfonamides' transport in the model cell was independent of whether K_u or K_{12}' was used, even though for two of the sulfonamides there was appreciable back-transport from Compartment 3 to Compartment 2. This was not the mechanism proposed by Koizumi *et al.* (6) for the *in vivo* situation. Both sulfapyridine ($k_{32} = 0.20$) and sulfamethazine ($k_{32} = 0.12$) produced appreciable reverse rate constants, as compared to negligible values for the remaining sulfonamides under investigation. However, correlations were sought between the *in vitro* rate constant and the *in vivo* gastric data and intestinal data. With the *in vivo* gastric data, the correlation coefficient was 0.81 ($p = 0.05$); for the *in vivo* intestinal data, a value of 0.87 ($p = 0.05$) was obtained.

In vivo absorption rates from the rat rectum for four of the sulfonamides studied *in vitro* were reported by Kakemi *et al.* (7) and were used to establish a correlation between *in vitro* transport and *in vivo* rectal absorption. In this instance, k_{12}, corrected for surface to volume ratio, was used as the correlatable parameter, since the *in vivo* rectal absorption data were determined from the slope of a semilogarithmic plot of percent dose remaining in the recirculating solution *versus* time. The *in vitro* transport rates obtained in the model cell correlated reasonably well to the reported rectal absorption rates. Thus, the correlation coefficient was 0.87, although the level of significance was lower ($p = 0.2$) due in part to a smaller number of compounds used in the correlation.

It appears from the *in vivo* absorption results that similar passive transport mechanisms occur across the gastric, intestinal, and rectal membranes in the rat and that those sulfonamides having the highest lipid solubility are more readily absorbed than those that are poorly lipid soluble. This same effect can also be demonstrated in the model cell for the various sulfonamides used in this study.

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