Apparent Directional Permeability Coefficients for Drug Ions: *In Vitro* Intestinal Perfusion Studies

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Abstract □ The *in vitro* absorption kinetics for nine drugs were followed using a perfusion apparatus. Identical perfusion runs were made on everted and noneverted segments of the same rat intestine so that the ratio of directional permeability constants (reported as everted rate to noneverted rate) could be calculated. Both negatively and positively charged drug ions (including the quaternary ammonium compound pralidoxime chloride) exhibited permeability coefficient ratios around 1.3, while completely unionized drugs showed the expected ratio of 1.0. In light of the similarity in the ratio of permeability constants for drug ions and sodium ions, salicylate was tested in a sodium-free buffer resulting in a ratio of 1.08. It appears that the difference in directional permeability constants observed with ionized drugs in the intestine may be explained in relation to sodium transport. It is pointed out that *in vitro* intestinal transport studies could lead to erroneous conclusions concerning the degree of absorption of ionizable drugs *in vivo*.

Keyphrases □ Absorption kinetics—*in vitro* intestinal perfusion □ Perfused intestine—drug transport □ Everted, noneverted intestine—directional permeability constants □ Permeability constants, directional—ratios □ Ionization effect—drug permeability coefficient ratios

Considerable work is being and has been carried out on the transport of various drugs across intestinal membranes. This work has been adequately reviewed by Wilson (1) and more recently by Benson and Rampone (2). The forces causing movement across the intestinal membrane can be divided into five classes: cellular metabolic energy, activity gradients, electrical gradients, hydrogen-ion gradients, and solvent drag. One of the first areas studied extensively was that of hydrogen-ion gradients from which a theory evolved to explain the absorption of ionized and unionized drugs. This theory, called the "pH partition hypothesis," was developed by Brodie *et al.* (3–7). These authors concluded from their investigations that most drugs are absorbed from the gastrointestinal tract by a process of passive diffusion of the unionized drug species across a lipoidal membrane. Thus, in line with this hypothesis, Hogben *et al.* (4) suggested that the unequal distribution of weak organic acids or bases across the gut wall is due to: (*a*) much greater permeability of the gut wall to the unionized form of the compound than to its ionized form, and (*b*) a difference in pH on the two sides of the intestinal wall. They further postulated that the distribution of weak acid or base is dependent on the "virtual" pH of the mucosal solution (the pH of a narrow microclimate adjacent to the mucosal intestinal surface) rather than the pH of the bulk mucosal solution. They pointed out that such a microclimate of fluid with a low pH calculated to be 5.3 would lead to a relatively high concentration of unionized acid next to the mucosa (as compared to the concentration of unionized acid in the bulk mucosal solution at a higher pH, normally 6.6) and consequently lead to increased mucosal to serosal movement of the acid by means of nonionic diffusion. By this mechanism, values greater than one for the steady-state concentration ratio, C_{plasma}/C_{gut}, of a weak acid could be explained without postulating a specific active-transport mechanism.

This hypothesis seems to be consistent with the *in vivo* data of Schanker *et al.* (5) who found that the lowest pKa of an acidic drug showing rapid absorption was about 3, while the corresponding highest pKa for a basic drug was 8. However, there seems to be a great discrepancy between the results obtained *in vitro* and those obtained *in vivo*. It would appear that ionized drug species are much more easily absorbed when membrane permeability is measured using one of the three commonly employed *in vitro* techniques: the everted sac method of Wilson and Wiseman (8), the Crane and Wilson method (9), and use of a perfusion appartus (10, 11). In 1966, Benson and Rampone (2) stated: "This is the era of the everted sac, developed by Wilson and Wiseman, and much of the investigation reported in this review was based upon this *in vitro* technique." Most intestinal absorption studies in the pharmaceutical sciences (see *Reference 12* for review) are still run using the *in vitro* techniques already mentioned. Therefore, this paper is directed toward identifying the discrepancy between *in vivo* and *in vitro* results and to point out the possible unapplicability of *in vitro* intestinal studies, especially when the transport of ionized drugs is observed.

There are a number of studies in the literature which show large differences between *in vitro* results and those expected on the basis of the *in vivo* hypotheses. For example, Hogben *et al.* (4) calculated the ratio of permeability coefficients for unionized and ionized (P_u/P_i) salicylic acid to be 4500; but Nogami and Matsuzawa (13), using absorption kinetics with *in vitro* perfused segments, found this ratio to be 6. Likewise for the drug aminopyrine, Hogben *et al.* (4) calculated a permeability coefficient ratio approaching infinity, but Nogami and Matsuzawa (14) found P_u/P_i equal to 11 in their *in vitro* studies. Nogami *et al.* (15) also found that the *in vitro* permeability coefficients for sulfathia-

zole in ionized forms exceeded the permeability coefficient for the unionized drug.

More recently, Kakemi *et al.* (16) studied the absorption of barbituric acid derivatives through the *in vitro* rat small intestine. Here they found that the pH partition hypothesis was only partially operative, in that absorption rate constants increased in the pH range 6.5 to 7.5 over those rate constants determined at pH 5.5 where barbituric acid derivatives are almost completely unionized. These authors also found that plots of percent barbiturate bound to intestinal mucosa against initial pH of the perfusate, all had maxima in the pH 6.5 to 7.5 region. They speculated that the complexation of barbituric acid derivatives to proteins of the mucosal surface raised the concentration of drug at the membrane surface, which in turn favored absorption in the serosal direction. This proposal is similar to that suggested by Singh *et al.* (17) and Levy and Matsuzawa (18) where drugs complexed to inert ingredients still show measurable absorption rates.

It became apparent to the present investigators that in these *in vitro* studies, the assumption was made that drugs were transported through the intestine by an identical process in each direction. Permeability constants for mucosal (gut) to serosal (plasma) transport were assumed to be the same as for serosal to mucosal transport. However, there were some interesting aspects of the reported data which might lead to the assumption that there was a difference in directional permeability constants. For instance, Nogami and Matsuzawa (13) found that the permeability coefficients for ionized salicylate and unionized salicylic acid increased with the lapse of time. They attributed this increase to the fact that salicylic acid is a toxic substance for living cells and might cause a physiological change in the intestinal tissue. However, this increase in calculated permeability coefficients could also be explained by the fact that the permeability coefficients for mucosal to serosal transport are greater than those for serosal to mucosal transport. The Nogami and Matsuzawa *in vitro* data for aminopyrine (14) would also be consistent with a difference in directional permeability coefficients; and the hypothesis of Kakemi *et al.* (16) implies that in a certain pH range, mucosal to serosal transport of barbituric acid derivatives is favored. Therefore, in this study, the authors have undertaken to determine experimentally whether there is a basis for assuming a difference in directional permeability coefficients and, if such a difference exists, to identify its mode of action.

EXPERIMENTAL

Materials—The following reagent, USP, or NF grade drugs and chemicals were tested: salicylic acid, salicylamide, aminopyrine, aniline, antipyrine, acetanilide, quinine sulfate, pralidoxime chloride (2-PAM chloride), and 5-nitrosalicylic acid. Monobasic sodium phosphate with one water of hydration, dibasic sodium phosphate anhydrous, monobasic potassium phosphate anhydrous, and dibasic potassium phosphate anhydrous were used in preparing the isotonic buffers.

Buffer Solutions—Three considerations entered into the selection of the buffer solutions to be used in these experiments. First, the authors wished to prepare an isotonic buffer solution having the greatest possible buffer capacity, since it is known that the jejunal epithelium of the rat maintains a slightly acidic pH within its lumen by hydrogen-ion secretion (4) and since there is the possibility of an acidic zone adjacent to the mucosal surface as was proposed by Hogben *et al.* (4). A buffer with a very high buffering capacity should eliminate the possibility of this acidic layer influencing drug absorption since Hogben *et al.* (4) have shown *in vivo* that strong buffers decrease the effect of the virtual pH. Second, the authors wanted to use only buffers having pH values which corresponded to the physiological values found either within the intestine or in its intact blood supply. Therefore, the buffers utilized were only those at pH 6.6 and pH 7.4. In light of the unusual results described in the Kakemi *et al.* (16) study of barbituric acid derivatives, it was felt that working at pH's other than 6.6 and 7.4 could possibly introduce effects not normally seen in the intestine. Third, since the authors wish to identify a possible cause for the difference in directional permeability coefficients, they wanted to use buffer solutions containing a minimum number of compounds. Therefore, they chose to make the isotonic buffers using dibasic and monobasic sodium phosphate exclusively.

Table I—Isotonic Buffer Formulas per Liter

	$NaH_2PO_4 \cdot H_2O$, g.	Na_2HPO_4, g.	KH_2PO_4, g.	K_2HPO_4, g.
Sodium phosphate buffer, pH 6.6	11.44	8.73		
Sodium phosphate buffer, pH 7.4	3.06	14.70		
Potassium phosphate buffer, pH 6.6			11.03	10.46
Potassium phosphate buffer, pH 7.4			3.07	18.36

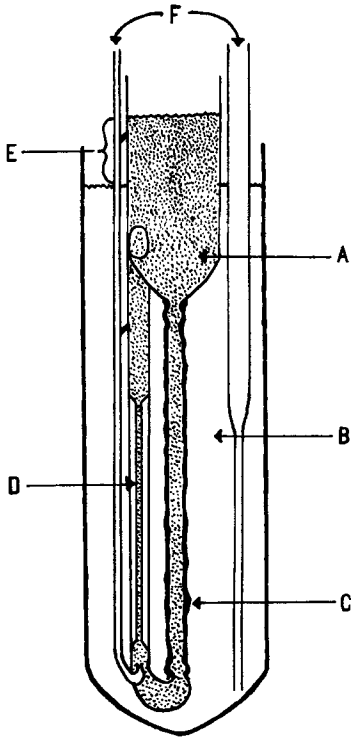

Figure 1—*Diagrammatic representation of the* in vitro *perfusion apparatus. Key: A, inside compartment, 15 ml.; B, outside compartment, approximately 140 ml.; C, intestinal segment, 10-cm. length available for absorption; D, capillary bubble pump; E, difference between heights of inside and outside compartments, 2 cm.; and F, 100% oxygen.*

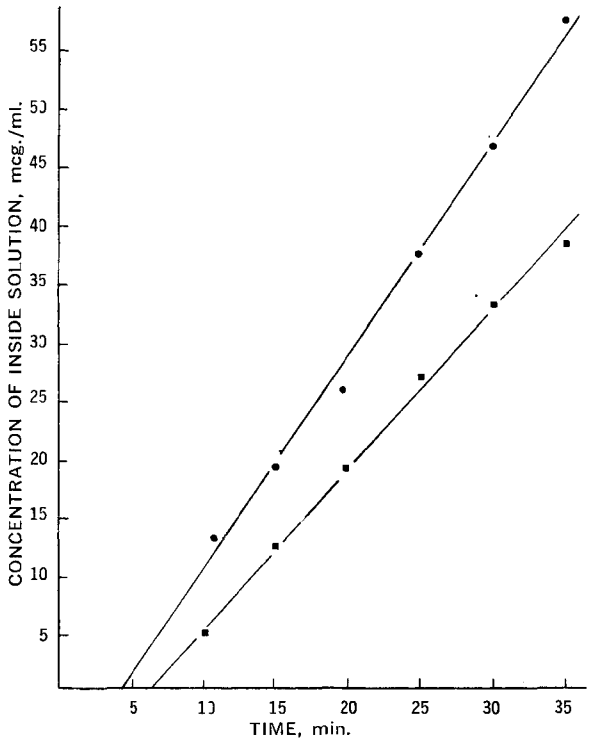

Figure 2—*Sample data plot for the absorption of salicylate through the rat intestine. Outside solution, 1000 mcg./ml. drug in isotonic pH 7.4 sodium phosphate buffer. Inside solution, isotonic pH 7.4 sodium phosphate buffer. Initial absorption rate through: everted segment* (●) = *1.76 mcg./ml./min.; noneverted segment* (■) = *1.39 mcg./ml./min. Permeability coefficient ratio* P_E/P_{NE} = *1.27.*

Using the proper sodium chloride equivalents, a reasonable activity coefficient value for hydrogen ions in an isotonic solution, and the Henderson-Hasselbalch equation, it is possible to calculate (19) the required amount of sodium phosphates in each buffer, as presented in Table I. Using the Van Slyke equation, the pH 6.6 sodium buffer was found to have a buffer capacity of 0.078 and the pH 7.4 buffer had a capacity of 0.048. The buffer capacities of these solutions are approximately twice those for the isotonic buffer solutions at similar pH's listed by Martin (19). During the course of a 40–60-min. perfusion run, the buffering solutions were found to vary by no more than 0.03 pH units, a value well within the accuracy expected in making pH measurements (20). In addition, some perfusion studies were run in a sodium-free buffer, where monobasic potassium phosphate and dibasic potassium phosphate were utilized in preparing the buffers. The formulas for these isotonic buffers, pH 7.4 and 6.6, are presented in Table I.

Table II—Analytical Procedures

Drug	pH of Perfusion Study	Diluent	Dilution, ml.[a]	Apparatus and Wavelengths[b]
Acetanilide	6.6	pH 6.6 buffer	1.0	UV at 237
Aminopyrine	7.4	pH 7.4 buffer	1.0	UV at 260
Aniline	7.4	pH 7.4 buffer	3.0	SPF: *Ex* 285, *Em* 354
Antipyrine	6.6	pH 6.6 buffer	1.0	UV at 255
5-Nitrosalicylic acid	7.4	0.1 *N* HCl	2.0	UV at 308
Quinine sulfate	6.6	0.1 *N* H_2SO_4	3.0	SPF: *Ex* 352, *Em* 454
2-PAM chloride	7.4	0.1 *N* NaOH	1.0	UV at 335
Salicylic acid	7.4	0.1*N* NaOH	5.0	SPF: *Ex* 300, *Em* 408
Salicylamide	6.6	0.1 *N* NaOH	4.0	SPF: *Ex* 332 *Em* 421

[a] Added to the 0.1-ml. samples. [b] UV, ultraviolet measurement at wavelength in mμ; SPF, spectrophotofluorometric measurement; *Ex*, excitation wavelength in mμ; *Em*, emission wavelength in mμ.

Perfusion Studies—Male Sprague-Dawley rats weighing about 250 g. were starved 24 hr. prior to the experiment but allowed free access to water. The rats were sacrificed with a sharp blow; the intestines (jejunum and ileum) were immediately removed and flushed with 40 ml. of the buffer solution to be used in the absorption run. The upper portion of the intestine was cut into two segments of 12 cm. each, and one of the two segments was everted. (In a series of experiments, the first segment of the intestine was everted alternatively.) The *in vitro* absorption kinetics for various drugs were followed using a perfusion apparatus (see Fig. 1) modified slightly from that described by Dietschy *et al.* (21). Each of the two intestinal segments (one everted and one noneverted) was tied securely onto a perfusion apparatus, being certain to maintain the same relative tautness in the stretched segments. The perfusion apparatuses were so designed that only a 10-cm. segment of the intestine was available for absorption. The inside compartment of each apparatus was then filled with 15 ml. of the appropriate isotonic phosphate buffer solution. The inside compartments were placed in large test tube shaped vessels that were suspended in a controlled-temperature water bath, which was maintained at 37.0 ± 0.5°. The outside compartment was filled initially with 130 ml. of a solution containing 1000 mcg./ml. of the drug dissolved in the same isotonic buffer as was used in the inside compartment. Additional drug solution was then added to the outside compartment until a 2-cm. difference in height resulted between the solution in the inside compartment and that in the outside compartment (see Fig. 1). Oxygen was bubbled through the drug solution by means of a capillary tube, and through the solution in the inside compartment by means of a built-in capillary bubble pump which circulated the buffer through the intestinal segment. The rates of bubbling in the two separate apparatuses were adjusted to approximately the same rate, one bubble/second. At regular intervals, 0.1-ml. samples were removed from the inside solution of each apparatus. For each drug, the time intervals were adjusted so that the sixth and final sample was removed from the inside compartment before the concentration in that compartment reached 100 mcg./ml. (*i.e.*, less than 10% of the outside concentration).

Assay Procedures—The 0.1-ml. samples taken from the inside compartments were diluted and assayed by either a UV measurement on the Cary 15 spectrophotometer or by a spectrofluorometric procedure on the Aminco-Bowman spectrofluorometer. Table II lists the various drugs studied, the pH of the buffer in which they were run, the diluent used in the analytical procedure, the amount of diluent added to the 0.1-ml. sample, and the apparatus and wavelengths at which the drug was measured. Standard plots for each drug were made using five known concentrations of the drug in the appropriate diluent.

RESULTS

After assaying each of the drug samples, plots were made of concentration of the inside solution *versus* time for both absorption through the everted and through the noneverted intestinal segments. Figure 2 contains a sample plot of data for the absorption of salicylate ions through two adjacent segments of rat intestine.

Riggs (22) has shown that the transfer of substances by simple diffusion across thin membranes may be described by a useful simplification of Fick's law as presented in Eq. 1:

$$\frac{dQ_i}{dt} = D_m A_m R_{m/s} (C_o - C_i)/\Delta x_m \qquad \text{(Eq. 1)}$$

When this equation is specifically applied to this experiment:

Q_i = the amount of drug in the inside compartment at any time, t
D_m = the effective diffusivity of the drug in the intestinal membrane
A_m = the area of the membrane available for free diffusion
$R_{m/s}$ = partition coefficient between membrane and solvent

Table III—Absorption Rates and Permeability Coefficient Ratios for Nine Drugs Using Buffers Containing Sodium Ions

Drug, pKa	pH of Perfusion Study	Number of Paired Runs	Average Rates ± *SD* in mcg./ml./min. Everted	Average Rates ± *SD* in mcg./ml./min. Noneverted	Permeability Coefficient Ratio ± *SD* P_E/P_{NE}	Level of Significance Ratio *vs.* 1.00
Acetanilide (0.6)	6.6	5	6.60 ± 0.82	6.59 ± 0.93	1.00 ± 0.04	N.S.[a]
Aminopyrine (5.0)	7.4	8	4.23 ± 0.90	3.21 ± 0.61	1.31 ± 0.12	$p < 0.001$
Aniline (4.6)	7.4	8	11.67 ± 1.95	10.67 ± 2.10	1.12 ± 0.22	N.S.
Antipyrine (1.4)	6.6	9	3.10 ± 0.39	3.19 ± 0.52	0.98 ± 0.11	N.S.
5-Nitrosalicylic acid (2.3)	7.4	3	1.94 ± 0.62	1.50 ± 0.41	1.29 ± 0.10	$p < 0.05$
Quinine sulfate (4.1, 8.4)	6.6	5	1.34 ± 0.31	1.11 ± 0.17	1.21 ± 0.13	$p < 0.02$
2-PAM chloride (quaternary ammonium compound)	7.4	8	1.36 ± 0.12	1.15 ± 0.15	1.27 ± 0.11	$p < 0.0001$
Salicylic acid (3.0)	7.4	7	1.78 ± 0.22	1.33 ± 0.21	1.35 ± 0.17	$p < 0.002$
Salicylamide (8.4)	6.6	6	5.82 ± 0.61	5.19 ± 0.66	1.13 ± 0.09	$p < 0.02$
Salicylic acid	7.4 Potassium phosphate buffer	10	1.35 ± 0.31	1.27 ± 0.31	1.08 ± 0.16	N.S.

[a] Not significant.

Δx_m = the thickness of the membrane
C_o = concentration of drug in the outside compartment at any time, t
C_i = concentration of drug in the inside compartment at any time, t

For the experiments run in this work, the last sample of the inside compartment of the perfusion apparatus was always taken at a time when the concentration of that compartment could be considered negligible compared to the concentration of the outside compartment. For example, in Fig. 2 the highest concentration reached in the inside compartment was 57.6 mcg./ml. as compared to an outside concentration of 1000 mcg./ml. Therefore, it may safely be assumed that the backward flow of drug from the inside compartment to the outside compartment is negligible during the time of the experiment and, therefore, C_i can be eliminated from the equation. In addition, since the outside solutions were of such a high concentration and of such a large volume (approximately 140 ml.), it is safe to assume that C_o is a constant throughout the absorption run. Therefore, for these experiments, Eq. 1 may be written as Eq. 2 when absorption is studied through the everted gut and as Eq. 3 when absorption is studied through the noneverted gut:

$$V_i \frac{dC_{iE}}{dt} = P_E C_o \qquad \text{(Eq. 2)}$$

$$V_i \frac{dC_{iNE}}{dt} = P_{NE} C_o \qquad \text{(Eq. 3)}$$

where P_E is defined as the permeability coefficient for absorption through the everted gut—this permeability coefficient contains the diffusion constant, D, the area of the membrane, A, the partition coefficient, $R_{m/s}$, and the thickness of the membrane Δx. The left-hand sides of Eqs. 2 and 3 have been converted into concentration units to correspond with the method of assay where C_{iE} refers to the concentration of the inside compartment when absorption is studied through the everted gut, and NE refers to measurements taken when the gut is not everted. Thus, it may be seen from Eq. 2 that as per the design of the experiment, the absorption rates through the everted and noneverted gut should appear to follow zero-order kinetics as is shown by the straight-line relationships in Fig. 2. Since the concentrations of drug in the outside solutions were always the same (1000 mcg./ml.), and since the volumes of the inside compartments (V_i) were always the same, dividing Eq. 2 by Eq. 3 shows that the ratio of the absorption rates will equal the ratio of apparent permeability coefficients. Table III contains the absorption rates and permeability coefficient ratios for the nine drugs followed in this study. The table contains the pKa of each drug, the pH of the buffer solutions used in the tests, the number of paired trials (each trial refers to one everted and one noneverted absorption run through intestinal segments of the same rat), the average everted and noneverted absorption rates plus or minus the standard deviations for these average rates, and the average of the ratios of permeability coefficients for individual paired runs, plus or minus the standard deviation. As will be noted from the data in Table III, the standard deviations for the average rates of absorption are relatively much higher than the standard deviations for the averages of the permeability coefficient ratios. This would be expected since the absorption rates are a function of the biological variability of the absorption of a drug through the intestine of a number of different rats. However, each of the ratios of permeability coefficients was determined from absorption studies through intestinal segments from the same rat. Therefore, the biological variability of differences in absorption rates due to the individual physiological characteristics of the intestine should be cancelled out to a large extent in the permeability coefficient ratios. Table III also compares the level of significance between the average ratio of permeability coefficients for each drug and the value 1.00 (the value expected if drug passage through the intestinal membrane was identical in each direction). Student's t test was used and 0.05 was taken as the minimum level of significance.

Table IV presents the data for the individual paired runs for salicylic acid in pH 7.4 sodium phosphate buffer. The regression coefficient for each set of data points is also presented in parentheses after the rate determined from the slope of the line drawn through these points. Figure 2 is the plot for the data listed as Run 6 in Table IV.

At the end of the perfusion runs, the difference in heights between the solution in the inside compartment and that in the outside tube was measured and found to have decreased by 0.5 cm. Measurements show that this decrease corresponds to a loss of approximately 1.4 ml. from the inside compartment. Since six 0.1-ml. samples were taken out of the inside compartment during the course of the experiment, there was an overall diffusional loss of 0.8 ml. from the inside compartment to the outside during the course of an absorption run. Since solvent movement was always against the drug flux, it may safely be assumed that solvent drag was not a significant factor in the permeability coefficient ratio.

DISCUSSION

The resulting permeability ratios presented in Table III may be classified into three areas: those very close to a ratio of 1.0, those close to a ratio of 1.3, and an indeterminate group with a ratio midway between 1 and 1.3. The two compounds with ratios very close to 1.0, acetanilide and antipyrine, can be considered to be completely unionized at the pH of the perfusion study. However, a compound such as 2-PAM chloride, which must be completely ionized because it is a quaternary ammonium compound, exhibits a permeability ratio that significantly differs from 1.0. The two other compounds in the study, which may be assumed to be completely ionized at the pH of the perfusion run, salicylic acid and 5-nitrosalicylic acid, also show permeability coefficient ratios very close to 1.3. Quinine sulfate, which is also ionized to a large extent (at least 97%, the majority of which will be the singly positively charged species) exhibits a ratio closer to those of the other ionized drugs than to the ratios around 1.0 for the unionized drugs. Aniline and salicylamide, two compounds which would be expected

Table IV—Individual Paired Data for Salicylic Acid in pH 7.4 Sodium Phosphate Buffer

Run	Everted Rate, mcg./ml./min.	(Regression Coefficient)	Noneverted Rate, mcg./ml./min.	(Regression Coefficient)	Ratio
1	1.790	(0.9935)	1.311	(0.9924)	1.365
2	1.467	(0.9990)	1.143	(0.9945)	1.283
3	2.054	(0.9992)	1.594	(0.9971)	1.289
4	1.835	(0.9908)	1.055	(0.9873)	1.739
5	2.010	(0.9996)	1.598	(0.9931)	1.258
6	1.762	(0.9932)	1.391	(0.9977)	1.267
7	1.542	(0.9956)	1.222	(0.9987)	1.262
Av.	1.780		1.331		1.352

to be at least 97% unionized, give permeability ratios intermediate between those values expected for unionized drugs and those ratios expected for ionized drugs. The aminopyrine (pKa 5.0) run at pH 7.4 appeared to be an exception, since although the drug would be only slightly ionized at this pH, a ratio of 1.31 was found. Excluding the data for aminopyrine and realizing that, as yet, not enough drugs have been run to substantiate a general theory, the data would suggest the following: drugs that are known to be incapable of ionization show the typical uniform path of distribution of drug molecules through the intestine. That is, the mucosal to serosal transfer was equal to the serosal to mucosal transfer, giving a permeability coefficient ratio of 1. If, however, the drug was capable of ionizing to some degree (salicylamide and aniline), the permeability coefficient ratios increased slightly. However, if the drug was completely ionized, whether positively or negatively charged, the permeability coefficient ratio was found to be very close to 1.3. From the data in Table III, it may be concluded that the passage of ionized drugs through the *in vitro* intestine is not a completely passive process.

In looking for an explanation for the 1.3 value for the permeability ratio of ionized drugs, the authors were struck by the similarity of this ratio to the permeability ratio observed by Curran and Solomon for sodium flux in the rat intestine (23). Recently, there have been a number of studies describing sodium ion dependent transport in the intestine as reviewed by Crane (24) and Curran (25). Therefore, an attempt was made to run a similar absorption rate study for salicylic acid in potassium phosphate buffer where sodium ions were completely excluded. This study (as presented at the bottom of Table III) gave a ratio of 1.08, a value very close to the ratios determined for unionized drugs. Although this result must be considered preliminary, there seems to be good evidence that sodium ion transport may be involved in the difference in apparent directional permeability coefficients which have been found in the *in vitro* rat intestine. Further work in this area is now being carried out.

Another interesting result which is obvious from Table III is that the absorption rates for ionized drugs through the intestine are very similar for all of the compounds studied, whether they are positively or negatively charged drug ions. The rate of 2-PAM chloride is especially significant since this compound obviously exists as an ionized species while it is being transported through the *in vitro* intestine. Previously, on the basis of the virtual pH theory of Hogben *et al.* (4), it might have been assumed that ionized drugs apparently pass through the intestine because a sufficient number of the ionized species become unionized at the virtual pH of the acidic microlayer adjacent to the mucosal surface. However, the similarity in rates between 2-PAM chloride, salicylate ions, and quinine ions seems to indicate that ionized drugs do pass through the *in vitro* intestine.

Another interesting similarity may be seen between the absorption rate of salicylate ion and 5-nitrosalicylate ion. Schanker *et al.* (5) found that there was a wide difference between the absorption of these two acids from the rat intestine *in vivo*. Over a given time interval, 60% of the salicylic acid was absorbed, while only 9% of the 5-nitrosalicylic acid was absorbed. The difference in the absorption of these two compounds was used in the explanation of the virtual pH hypothesis. Hogben *et al.* (4) state: "An absorbing surface with a pH of 5.3 would explain why the lowest pKa of an acidic drug compatible with rapid absorption is about 3, while the highest pKa for bases is about 7.8. In each case, the ratio of unionized to ionized drug is 1:200. This appears to be the minimal proportion of unionized drug necessary to insure rapid absorption under the conditions of our experiments."

Although the validity of these data cannot be questioned with respect to *in vivo* absorption, it would appear that this 1:200 ratio is not significant when considering passage through the *in vitro* rat intestine. Furthermore, it leads the authors to believe that the virtual pH hypothesis should not be applied to passage through the *in vitro* intestine.

It is obvious from the data in Table III that drugs which are assumed to be absorbed as unionized species show absorption rates which are significantly higher than those for ionized drugs. This is especially pertinent when considering the permeability ratios for aniline and salicylamide. It is believed that it is safe to assume that these drugs are being absorbed as unionized species and, therefore, the indeterminate ratios (1.12 and 1.13) are assigned as being related to the experimental error inherent in the methods utilized when the absorption rates become large. It is interesting to note that aniline, the compound absorbed at the highest rate in this study, was only found to have an absorption rate about 9 times higher than that for ionized drugs. This difference in rate between unionized and ionized drugs in the *in vitro* intestine is very similar to that reported by Nogami and Matsuzawa (10, 13), but it is quite different from the relative rates of absorption predicted by Hogben *et al.* in the *in vivo* intestine.

Because of the unusual nature of the permeability coefficient ratio for aminopyrine, a drug which would be expected to be unionized and therefore to have a ratio close to 1, preliminary paired runs of aminopyrine in a potassium phosphate buffer have been run. These runs give an average ratio of 1.10. Although work is continuing on this compound, it would appear that aminopyrine is being transported by a process which is different than the other compounds in this study. The authors believe that this result might be significant with respect to *in vivo* transport as well as to *in vitro* transport, since aminopyrine was one of the two drugs studied by Hogben *et al.* (4) which yielded results consistent with a calculated virtual pH of 5.3 at the intestinal wall.

Since the permeability coefficient ratios for the completely unionized drugs, acetanilide and antipyrine, are so close to 1.0, it would appear that the difference in surface area between the mucosal and serosal membranes is not important. This leads the authors to believe that the rate-limiting step in intestinal transport is passage through the membrane rather than passage into or out of the membrane. Under these conditions the surface area of the membrane exposed to the drug solution does not affect the rate of passage. This, in fact, is an implicit assumption in Rigg's simplification of Fick's law as was presented in Eq. 1.

CONCLUSIONS AND SUMMARY

The initial absorption rates for nine drugs were determined *in vitro* through both the everted and noneverted rat intestine using a perfusion apparatus. Two of these drugs, acetanilide and antipyrine, may be considered to be completely in the unionized form at the pH of the buffers used in this work, 6.6 and 7.4. Two other compounds, an acid, salicylamide, and a base, aniline, should be at least 97% unionized in the buffers in which they were studied. The other five drugs studied, including a quaternary ammonium compound—pralidoxime chloride—may be considered to be completely ionized in these studies. On the basis of the nine drugs observed in this work, the authors feel that the following conclusions can be made (with full realization that more extensive studies in different buffer solutions must be undertaken to confirm these results):

1. Drug ions do pass through the *in vitro* rat intestine.
2. These ions pass through the *in vitro* rat intestine at a rate which is much faster than that predicted on the basis of *in vivo* results.
3. Drug ions show a difference in directional permeability coefficients in the *in vitro* rat intestine, with mucosal to serosal transfer occurring about 1.3 times faster than serosal to mucosal transfer. This difference is not observed for the completely unionized drugs.
4. It appears that this difference in directional permeability coefficients might be related to a sodium ion coupled transport.

since when sodium was completely replaced by potassium in the bathing buffer solutions, only a 1.08 value was obtained for the ratio of permeability coefficients for salicylate ion.

5. It would appear that the virtual pH hypothesis of Hogben *et al.* (4) is inoperative when considering *in vitro* intestinal transport.

6. If the presently accepted hypotheses concerning the *in vivo* intestinal absorption of ionizable drugs are valid, it would appear that *in vitro* intestinal transport studies could lead to erroneous conclusions concerning the degree of absorption of ionizable drugs *in vivo*.

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Parameters Affecting Absorption of Griseofulvin in a Human Subject Using Urinary Metabolite Excretion Data

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Abstract □ Using the urinary excretion of 6-demethylgriseofulvin as the index of absorption, the effects of three previously studied parameters (high-fat breakfast, particle size, and dissolution rate) on the absorption of griseofulvin in a single subject are compared with published blood level data derived from many subjects. The results of the effects of three new parameters (time of dose administration, low-dose level, and gastrointestinal transit time) are also reported.

Keyphrases □ Physiological availability—griseofulvin, man □ Griseofulvin absorption parameters—urinary excretion data, man □ 6-Demethylgriseofulvin metabolite—griseofulvin absorption parameters, man □ Trimethylsilyl ether derivative—6-demethylgriseofulvin, griseofulvin metabolite □ GLC—analysis

The factors (1–12) affecting the absorption of orally administered griseofulvin in man have been studied by measuring (using a fluorometric chemical analytical procedure) the level of griseofulvin in blood at various times after drug administration. The calculation of the degree of absorption is based on the assumption that the physiological availability of the drug is proportional to the area (usually determined by the trapezoidal rule) under the blood level–time curve (3, 5, 13–15).

The factors affecting absorption were found to be a function of the diet (high-fat meal) (1, 2), the dosage formulation [particle size (3–7), dissolution rate (8, 9), solubility (7, 10), and presence of surfactants (4, 7)], and the dosage regimen [dosage level (3, 11, 12) and repetitive dosage schedule (6, 7, 9, 11)].

The nonlinear (logarithmic) relationship (3, 11, 12) between the dose ingested and the area under the blood level–time curve has raised questions concerning the validity of the model on which this method of calculating absorption is based. Atkinson *et al.* (11) suggested