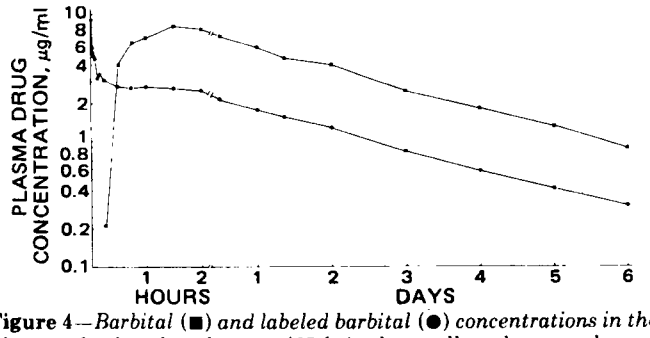

Figure 4—*Barbital (■) and labeled barbital (●) concentrations in the plasma of a female volunteer (65 kg) who swallowed a capsule containing 4.5 mg of barbital sodium/kg and simultaneously received 1.5 mg of [$^{15}N_{1,3}$, $^{13}C_2$]barbital sodium/kg iv (within 20 sec).*

sodium/kg. Blood (7 ml) was withdrawn at intervals over 6 days, and aliquots of plasma (1.0 ml) were assayed for barbital. Concentration–time curves of both isotopes in plasma are illustrated in Fig. 4, and the parallelism observed between their elimination phases strongly supports the evidence that both isotopes behave similarly in the organism.

In conclusion, the described GLC–mass spectrometric procedure is sensitive and specific and should be applicable to studies on the effect of congestive heart failure on barbital disposition.

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Permeability of Everted Rat Small Intestine to Lidocaine and Derivatives

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Received June 11, 1979, from the **College of Pharmacy, South Dakota State University, Brookings, SD 57006*, and the ‡*Department of Pharmaceutics, University of Houston, Houston, TX 77004.* Accepted for publication January 8, 1980.

Abstract □ The transfer rates of lidocaine and five derivatives were measured across the everted rat intestine. No obvious relationship was observed between the first-order rate constants for transfer and the lipophilicity of the compounds as measured by their apparent partition coefficients. The intestinal mucosal epithelium also did not appear to be the rate-limiting barrier for the passage of these agents across the intestinal membrane regardless of the respective clearance of the compound. It is suggested that the ionized form of these agents may be capable of crossing the intestinal barrier at substantial rates, although it is not known if a similar situation exists *in vivo*. The transfer rate is believed to be due to the passage of intact drug and not the metabolite.

Keyphrases □ Lidocaine—determination of transfer and clearance rates of parent drug and analogs across everted rat intestinal mucosa, mucosal concentrations and pH, *in vitro* absorption □ Absorption—lidocaine and derivatives, determination of transfer rates across everted rat intestinal mucosa, *in vitro* □ Permeability—lidocaine and derivatives across everted rat intestinal mucosa, *in vitro* absorption

The *in vitro* everted rat small intestine is a technique that has been utilized as a biological screen for the assessment of drug permeability and may be indicative of *in vivo* absorption problems. Feldman *et al.* (1) found a rank-order agreement between the *in vitro* transfer of prednisolone across the everted rat small intestine in the presence of various substituted propionamides with that reported for *in situ* experiments in the rat. Kaplan and Cotler (2) used the everted rat intestine as a biological screen to assess permeability characteristics of numerous compounds and concluded that this technique, employed along with a dissolution screen, allows evaluation of potential problems in the initial stages of product development.

The nature of the mechanical separation of the intestine from the intact animal imparts inherent advantages and disadvantages to the technique. Separation enables the investigator to control the physical environment of the intestinal preparation and to study some effects of the physicochemical properties of the drugs on the transfer of these agents across the intestinal preparation. However, separation of the intestinal preparation may alter or obliterate some important influencing factors present in the absorption of a compound *in vivo*. Concern has been expressed over viability (2–5), structural integrity (6), and the presence of unnatural absorption barriers.

This study concerned the transfer rates of lidocaine (I) and five experimental derivatives across the everted rat intestine. These compounds were chosen because they represent minor molecular modifications of the parent

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compound lidocaine and have some similar physicochemical properties, such as molecular weight and size, but a relatively wide degree of difference in their lipophilicity. Previous studies (7) in these laboratories showed that the effect of lidocaine and these derivatives on the pharmacological end-points of overturn and death in goldfish was related to the lipophilicity of these compounds and the effect on the absorption rate.

EXPERIMENTAL

Determination of Transfer Rates of Drugs across Everted Rat Intestine—Sprague–Dawley rats[1], 200–300 g, were fasted 24 hr prior to the experiment. Water was allowed *ad libitum*. The animals were anesthetized with ether, a midline abdominal incision was made, and the intestine was cut at the ileo–cecal junction. The intestine also was cut at the pyloric junction and removed from the animal. The first 15 cm proximal to the pyloric junction was discarded, and the remaining intestinal segment was rinsed inside and out with normal saline.

The proximal portion of the intestine was fastened to a glass rod, and the intestine was everted. Two consecutive 10-cm everted intestinal segments were attached to cannulas, placed in 60 ml of mucosal solution in a test tube, suspended in a constant-temperature water bath[2] at 37°, and oxygenated with 95% oxygen–5% carbon dioxide. The mucosal solution was modified Krebs bicarbonate buffer (8) of either pH 6.4 or 7.4 containing various concentrations of lidocaine or its derivatives[3]. Two milliliters of serosal solution consisting of the modified Krebs bicarbonate buffer without any drug present was introduced into the everted segment *via* a syringe and polyethylene tubing. At the end of the predetermined sampling time, the serosal solution was removed and the serosa was rinsed with 2 ml of the modified Krebs bicarbonate buffer. This rinse was combined with the initial sample and was retained for analysis. Two milliliters of fresh serosal solution then was added to the everted segment for the next sampling interval.

Stripping Experiments—The procedure for the preparation of the segments was modified as follows. Either the proximal or distal segment was stripped of its epithelial layer after eversion by running forceps up and down the length of the segment until the epithelial tissue was sloughed off about the tips of the forceps. The experiment then was performed as described previously.

Assay—The samples were alkalinized by the addition of 1 ml of 2 *N* NaOH and extracted into 10 ml of carbon tetrachloride by shaking[4] for 5 min. The aqueous layer was aspirated, and 8 ml of the organic phase was shaken with 10 ml of 0.004% bromthymol blue in pH 6.0, 0.05 *M* phosphate buffer for 5 min. Following centrifugation[5] for 6 min at 4000 rpm, the bromthymol blue layer was removed by aspiration. Five milliliters of the organic layer and 5 ml of 0.1 *N* NaOH solution were shaken for 5 min, and the samples were centrifuged[6] at 2000 rpm for 5 min. The aqueous supernate was read at 615 nm on a double-beam spectrophotometer[7].

The drug concentration in each sample was determined using absorbance *versus* concentration plots obtained in the described manner which were linear over the concentration range studied.

Qualitative Assay for Metabolites—Everted sacs were attached to cannulas and placed in 60 ml of bathing mucosal solutions consisting of pH 7.4 modified Krebs bicarbonate buffer containing 400 μg of lidocaine/ml. Two milliliters of the buffer was placed on the serosal side of the membrane. A 10-cm long segment of intestine was used as a blank and was placed in an erlenmeyer flask containing the pH 7.4 buffer. All solutions were placed in a constant-temperature water bath at 37° and oxygenated with 95% oxygen–5% carbon dioxide.

At the end of 1 hr, the sacs were removed from the bath and serosal samples were placed in glass bottles. Sacs were washed with 2 ml of the pH 7.4 buffer, which was added to serosal samples. Two milliliters of mucosal solution was taken and added to bottles containing 2 ml of the buffer. To the serosal and mucosal samples, 1 ml of 2 *N* NaOH was added. The treated and untreated sacs were rinsed with distilled water and added to bottles containing 4 ml of the buffer and 1 ml of 2 *N* NaOH.

[1] TIMCO Breeding Laboratories, Houston, Tex.
[2] Thermomix 1420, B Braun Melsungen AG.
[3] Generously supplied by Astra Pharmaceuticals, Worcester, Mass.
[4] Eberbach shaker power unit, No. 6000.
[5] Damon/IEC Division model HN-S centrifuge.
[6] Clay Adams Dynac centrifuge.
[7] Perkin-Elmer model 124.

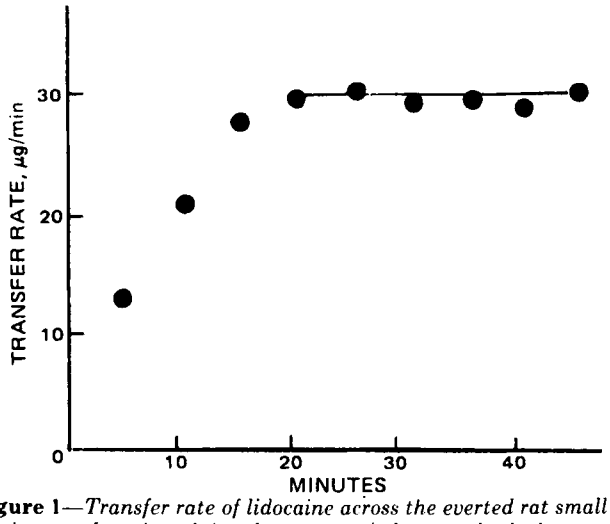


Figure 1—*Transfer rate of lidocaine across the everted rat small intestine as a function of time for a 400-μg/ml mucosal solution.*

Sacs were homogenized[8] and centrifuged[9] at 10,000 rpm for 10 min. The supernate was added to 5 ml of benzene. The serosal and mucosal samples also were added to 5 ml of benzene. All samples then were shaken and centrifuged[6] at 2000 rpm for 5 min. The supernatant benzene then was drawn off and placed in test tubes immersed in a constant-temperature water bath[2], which was maintained at 50° under a continuous-flow air hood until the benzene was evaporated completely.

The evaporated samples were reconstituted with 100 μl of benzene of which 5 μl was injected into a gas–liquid chromatograph[10] equipped with a flame-ionization detector. A 198-cm × 3.2-mm column, packed with 3% OV-17 coated onto 100–120-mesh Gas Chrom Q, was utilized. The

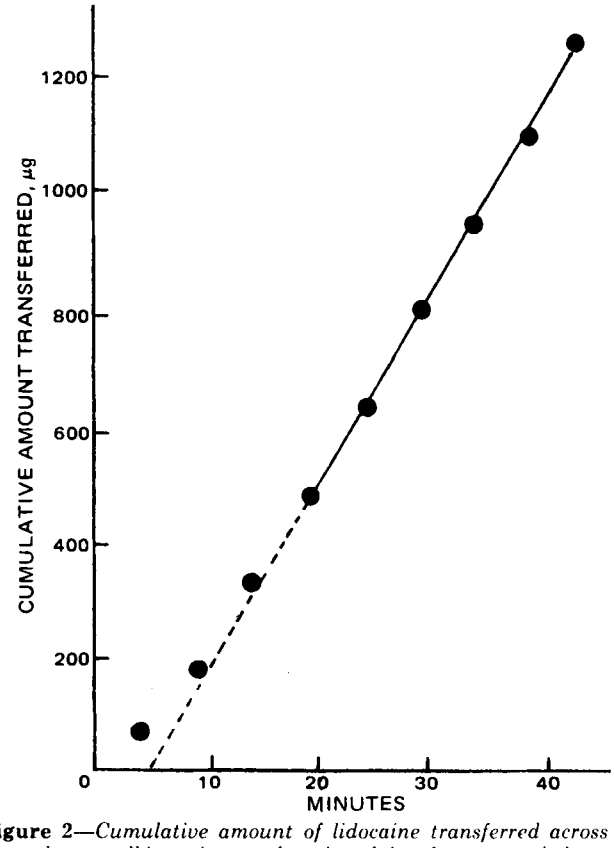


Figure 2—*Cumulative amount of lidocaine transferred across the everted rat small intestine as a function of time for a 400-μg/ml mucosal solution.*

[8] Brinkmann Polytron type PT 10 10 20 3500.
[9] Sorvall RC-5 superspeed refrigerated centrifuge.
[10] Shimadzu GC-4BPTF.

Table I—Average Rates of Transfer and Clearances for Lidocaine at Different Mucosal Concentrations at pH 7.4

Mucosal Concentation, μg/ml	Number of Segments	Slope, μg/min	Clearance, ml/min
200	7	11.64 ± 1.00	0.0582 ± 0.0050
400	12	27.34 ± 2.84	0.0684 ± 0.0071
700	3	40.73 ± 4.84	0.0582 ± 0.0069

Table II—Transfer Rates of Lidocaine for a 200-μg/ml Mucosal Solution

	Transfer Rate, μg/min		
	Proximal Segment		Distal Segment
	11.85		13.30
	11.11		10.16
	11.27		12.39
	11.41		—[a]
Mean	11.41		11.64
SD	0.31		1.61
t		−0.6726	
		$p > 0.05$	

[a] Intestinal preparation was torn

operating temperatures were: injection port, 250°; detector, 260°; and column, 180°. Nitrogen was used as the carrier gas at a flow rate of 42 ml/min.

RESULTS AND DISCUSSION

Drug concentrations in each sample were converted into amounts of the drug, which then were divided by the sampling time to give the average transfer rate (micrograms per minute). The time at which the transfer rate plateaus can be considered as the start of steady-state transfer of drug. A typical plot of the transfer rate as a function of time is illustrated in Fig. 1 for lidocaine. In this example, the time to reach steady state is ~20 min. The cumulative amount of drug transferred as a function of time for each drug was plotted, and a representative plot is illustrated in Fig. 2 for lidocaine. The slope of the line calculated from the values obtained after steady state is achieved represents the transfer rate (micrograms per minute) for the respective compound and was obtained by linear least-squares analysis.

The average transfer rates for lidocaine were determined for 200-, 400-, and 700-μg/ml mucosal concentrations of the drug at pH 7.4. The slopes (transfer rate) of the cumulative amount *versus* time plots as determined by linear least-squares analyses are listed in Table I. The drug clearance is defined as the milliliters of drug solution completely cleared of drug per unit time and can be obtained from:

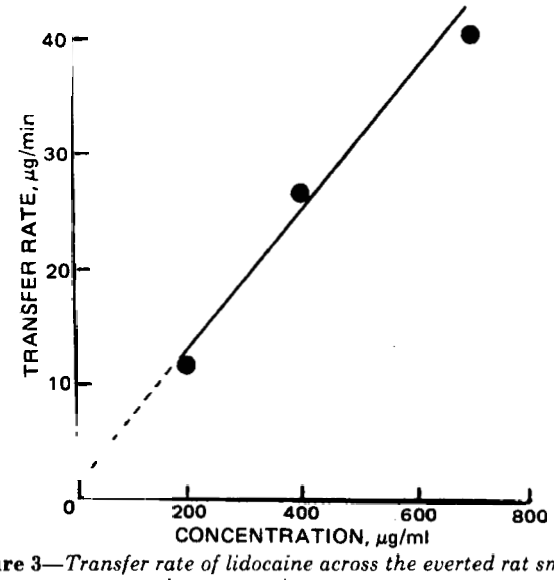
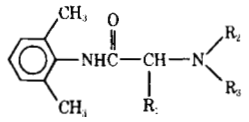


Figure 3—*Transfer rate of lidocaine across the everted rat small intestine* versus *mucosal concentration.*

Table III—Chemical Structure and Physicochemical Parameters



	I	II[a]	III[a]	IV[a]	V[a]	VI[a]
R_1	H	H	CH_3	H	CH_3	CH_3
R_2	C_2H_5	CH_3	CH_3	CH_3	C_2H_5	C_2H_5
R_3	C_2H_5	CH_3	CH_3	C_2H_5	C_2H_5	C_3H_7
pKa	7.72	7.22	7.26	7.53	7.98	7.88
$PC_{hept-buf}$[b]	3.59	0.27	0.79	1.55	9.60	54.56
$PC_{oct-buf}$[c]	68.66	8.75	16.99	19.35	125.86	244.90

[a] Compounds II–VI have the following Astra Pharmaceuticals code numbers: II, W36017; III, W36024; IV, W36004; V, W36023; and VI, W36032. [b] Partition coefficient between *n*-heptane–pH 7.4 buffer. [c] Partition coefficient between *n*-octanol–pH 7.4 buffer.

Table IV—Average Rates of Transfer and Clearances for Local Anesthetic Agents at a 400-μg/ml Mucosal Concentration at pH 7.4

Compound	Number of Segments	Slope, μg/min	Clearance, ml/min	K, min^{-1}
I	12	27.34 ± 2.84	0.0684 ± 0.0071	0.00114
II	7	17.59 ± 1.76	0.0440 ± 0.0044	0.00073
III	4	15.03 ± 0.70	0.0376 ± 0.0018	0.00063
IV	4	23.42 ± 3.49	0.0585 ± 0.0087	0.00098
V	4	22.83 ± 1.48	0.0571 ± 0.0037	0.00095
VI	4	19.60 ± 1.99	0.0490 ± 0.0050	0.00082

$$Cl = \frac{\Delta x/\Delta t}{C} \qquad \text{(Eq. 1)}$$

where Cl is the clearance of the respective compound, $\Delta x/\Delta t$ is the rate of drug transferred from the driving-force compartment (mucosal solution), and C is the drug concentration in the driving-force compartment. If one assumes that the transfer of drug occurs *via* a passive first-order process, then the drug clearance would be expected to be identical at the different concentrations since the clearances are reflective of a rate of transfer per unit concentration. Inspection of Table I shows no obvious trend of clearance with respect to drug concentration in the mucosal solution. A linear relationship is observed between the average transfer rates and the drug concentration in the mucosal solution (Fig. 3). The average clearance of lidocaine obtained from the slope of the transfer rate *versus* concentration plot was 0.0571 ml/min.

Since the experiments used two segments from the same intestinal preparation of each rat, a statistical analysis was performed to determine whether selection of one segment over another led to significant differences in the observed rates. In general, the selection of one segment over another probably is of greater concern for a drug transported by an active process due to site specificity of the carrier. However, if the size or composition of the intestinal barrier were to change dramatically from one segment to another, changes in the transfer rates reflecting these alterations might be observed.

A *t* test was utilized to determine if any significant differences existed between the proximal and distal segments of the intestinal preparations in terms of transfer rates for the 200-μg/ml mucosal solution (Table II). No differences were found for transfer rates with regard to the segment of the intestinal preparation used. Therefore, no distinction will be made between the proximal and distal segments employed in this study.

Table III includes the structural formulas for lidocaine and the five derivatives. Molecular modification of the lidocaine molecule involves the substitution of methyl, ethyl, and propyl groups at the R_1, R_2, and R_3 positions of the lidocaine molecule. The pKa values of the compounds ranged from 7.22 for II to 7.98 for V. The partition coefficients between *n*-heptane–pH 7.4 buffer[11] and *n*-octanol–pH 7.4 buffer[12] also are listed in Table III.

The transfer rates and clearances for the lidocaine derivatives also were determined utilizing a 400-μg/ml mucosal concentration (Table IV). The

[11] Reported by Astra Pharmaceuticals.
[12] Determined in this laboratory.

Table V—Rank-Order Relationship for Lidocaine and Five Derivatives

Rank	Partition Coefficient (*n*-heptane–pH 7.4 buffer)	Partition Coefficient (*n*-octanol–pH 7.4 buffer)	Rate Constant
1[a]	VI	VI	I
2	V	V	IV
3	I	I	V
4	IV	IV	VI
5	III	III	II
6	II	II	III

[a] The largest value.

apparent rates ranged from 15.03 ± 0.70 μg/min for III (clearance of 0.0380 ml/min) to 27.34 ± 2.84 μg/min for lidocaine (I) (clearance of 0.68 ml/min). Dividing the rate (micrograms per minute) by the total amount of drug (micrograms) in the mucosal solution or dividing the clearance by the volume of mucosal solution yields the apparent first-order rate constant (Table IV).

Table V gives the rank ordering of lidocaine and the five derivatives in terms of the apparent rate constant and partition coefficient. No apparent relationship appears to exist between the first-order rate constants for transfer and the partition coefficients. Kakemi *et al.* (9) demonstrated a rather good logarithmic relationship between the gastric absorption rate constants for numerous barbiturate derivatives and their corresponding partition coefficients. Plots of the logarithm of the apparent rate constant as a function of the logarithm of the partition coefficient between *n*-heptane–pH 7.4 buffer and *n*-octanol–pH 7.4 buffer are illustrated in Figs. 4 and 5. No relationship appears to exist between the observed transfer rate constants and the partition coefficient regardless of which partition coefficient is used.

To determine if the ionized form of the drug moiety also is capable of being transferred across the everted rat intestine, experiments were performed using lidocaine at pH 6.4 and a mucosal concentration of 400 μg/ml. Figure 6 is a representative plot of the transfer rate of lidocaine as a function of time. The transfer rates were obtained from the cumulative amount *versus* time plots, and the average transfer rates of lidocaine are listed in Table VI. The average transfer rate at pH 7.4 was ~27 μg/min; at pH 6.4, it was ~22 μg/min. A Student *t* test showed that the difference in rates at the two different pH values was significant ($p < 0.001$). It is possible, by using simultaneous equations, to calculate the approximate transfer rates for the unionized and ionized species. The transfer rate is given by:

$$R_t = f_i R_i + f_u R_u \qquad \text{(Eq. 2)}$$

where R_t is the total transfer rate, f is the fraction of drug that exists as a specific species, and R_i and R_u are the respective transfer rates of the ionized and unionized species. Substituting the average transfer rate and the drug fraction that exists as each respective species yields at pH 7.4:

$$27.34 = 0.68R_i + 0.32R_u \qquad \text{(Eq. 3)}$$

and at pH 6.4:

$$22.16 = 0.95R_i + 0.05R_u \qquad \text{(Eq. 4)}$$

Multiplying Eq. 3 by 1.39706 and subtracting from Eq. 4 yield:

$$-16.036 = -0.397R_u \qquad \text{(Eq. 5)}$$

Multiplying both sides of Eq. 5 by −1 and solving for R_u yield:

$$40.39\ \mu g/min = R_u \qquad \text{(Eq. 6)}$$

Substituting 40.39 back into Eq. 3 and solving for R_i yield:

$$21.20\ \mu g/min = R_i \qquad \text{(Eq. 7)}$$

It appears that the ionized species is capable of crossing the everted rat small intestine at a relatively high rate. Whether the same situation exists *in vivo* is not known. The ability of the ionized species to cross the *in vitro* intestinal preparation may be a manifestation of the mechanical process of everting the intestine as well as a loss of structural integrity with time.

Since the *in vitro* everted rat intestine includes the presence of unnatural absorption barriers, evaluation of whether differences in the transfer rates would occur if the intestinal mucosal epithelium was

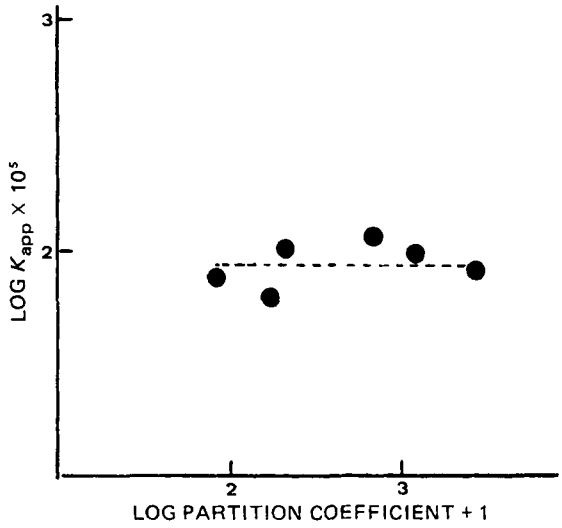


Figure 5—*Log–log plot of apparent transfer rate constants for anesthetic agents across the everted rat small intestine as a function of the partition coefficient* (n-*octanol–pH 7.4 buffer*).

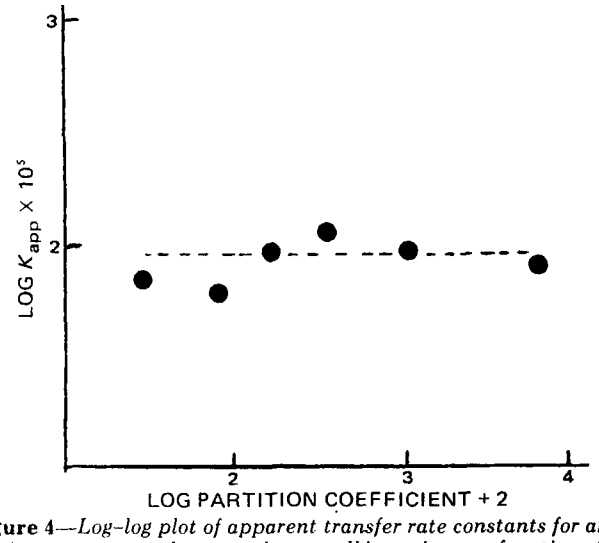


Figure 4—*Log–log plot of apparent transfer rate constants for anesthetic agents across the everted rat small intestine as a function of the partition coefficient* (n-*heptane–pH 7.4 buffer*).

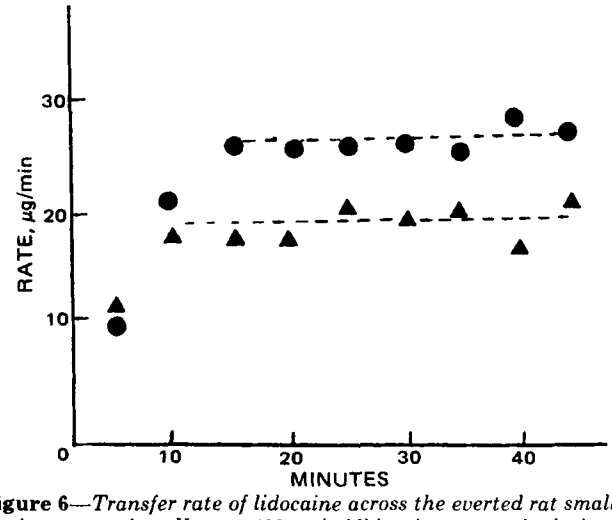


Figure 6—*Transfer rate of lidocaine across the everted rat small intestine* versus *time. Key:* ▲, *400-μg/ml lidocaine mucosal solution, pH 6.4; and* ●, *400-μg/ml lidocaine mucosal solution, pH 7.4.*

Table VI—Average Rates of Transfer and Clearance of Lidocaine (400 μg/ml) at pH 6.4 and 7.4

pH	Number of Segments	Rate, μg/min	Clearance, ml/min
7.4	12	27.34 ± 2.84	0.0684 ± 0.0071
6.4	7	22.15 ± 1.98	0.0554 ± 0.0050

Table VII—Average Transfer Rates for Lidocaine and II across Stripped and Unstripped Intestinal Segments at pH 7.4 and 6.4

Compound	Intestinal Segment[a]	pH	Transfer Rate, μg/min
I	Stripped	7.4	25.70 ± 2.84
I	Unstripped	7.4	26.79 ± 4.22
I	Stripped	6.4	21.27 ± 2.57
I	Unstripped	6.4	22.62 ± 1.36
II	Stripped	7.4	17.64 ± 2.80
II	Unstripped	7.4	16.66 ± 1.72

[a] Three segments were used.

stripped from the intestinal segments was desired. In the *in vitro* model, the intestinal mucosal epithelium represents a distinct barrier that may be rate limiting *in vivo*. Experiments were performed utilizing a 400-μg/ml mucosal concentration of lidocaine at pH 7.4 and 6.4. Stripping was performed alternately on the proximal and distal segments of the intestinal preparations. A representative plot of the cumulative amount transferred as a function of time is illustrated in Fig. 7. The rates for the stripped and unstripped segments at pH 7.4 and 6.4 are listed in Table VII. Statistical analysis using the Student t test showed that the transfer rates of lidocaine were not significantly ($p > 0.05$) different between the stripped and unstripped segments at either pH studied. Since the fraction of unionized and ionized species changed dramatically between the two pH values studied and the rates for the stripped and unstripped were the same, the intestinal mucosal epithelium apparently is not the rate-limiting barrier for either species.

Since previous studies (10, 11) suggested that the intestinal mucosal epithelium may be rate limiting for compounds with low clearances and not for compounds with high clearances, it was decided to evaluate whether the intestinal mucosal epithelium was rate limiting for one compound with a lower clearance than lidocaine. Compound III would have been the ideal selection, but lack of a sufficient quantity prohibited its use. Therefore, II was chosen. It has a clearance of 0.0439 ml/min and was utilized with stripping again performed alternately on the proximal and distal segments. The results are listed in Table VII, with the statistical analysis using the Student t test showing no significant difference ($p > 0.05$) between the transfer rates of the stripped and unstripped intestinal segments. Therefore, II, which has the next to lowest clearance value of the drugs investigated, also does not appear to be rate limited in its transfer by the intestinal mucosal membrane.

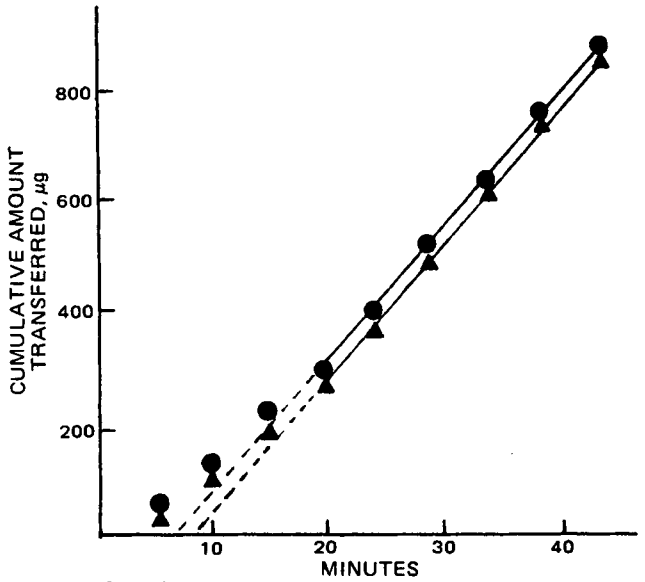


Figure 7—*Cumulative amount of lidocaine transferred across the everted rat small intestine as a function of time from a 400-μg/ml mucosal solution. Key: ●, unstripped intestinal preparation; and ▲, stripped intestinal preparation.*

Since the absorption rate of a drug *in vivo* can be defined as the rate of appearance of free unchanged drug, a qualitative evaluation was performed to determine if the transfer rate of the drug *in vitro* represents the transfer rate of the parent molecule and not the metabolite formed by a first-pass intestinal metabolic phenomenon. Everted sacs were suspended in mucosal solutions containing 400 μg of lidocaine/ml in the pH 7.4 buffer. The inside of the sac (serosa) also was filled with 2 ml of the same solutions. At the end of the predetermined incubation time, the mucosal solution, serosal solution, and tissue homogenates were assayed by GLC.

Only one peak was evident in all of the samples for the mucosal and serosal solutions and for the proximal or distal tissue homogenates. These samples all had the same retention time (10 min) as the control lidocaine samples. No peaks indicative of a lidocaine metabolite were observed[13]. Since amide-linked local anesthetics are metabolized almost completely by the liver in both humans (12) and rats (13), it appears reasonable to conclude that the transfer rates of the compounds across the everted rat small intestine are reflective of the transfer of intact drug.

From these results, it appears that the anesthetic agents are absorbed across the everted rat small intestine by a passive process with no apparent metabolism. The ionized drug molecule also is capable of crossing the membrane at appreciable rates, although it is somewhat lower than the unionized species. No apparent relationship was found between the rate constants and their respective lipophilicity. This result is in contrast to previous studies in goldfish (7). Removal of the epithelial layer by stripping did not appear to affect the transfer rates significantly, regardless of the clearance of the compound or the pH of the bathing solution. Thus, the intestinal mucosal epithelium apparently is not the rate-limiting barrier for the transfer of these compounds across the everted rat small intestine. The relationship between the parameters obtained in the present *in vitro* study and absorption of these agents in an intact animal remains to be evaluated.

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[13] Further work showed that known lidocaine metabolites do not interfere with the assay for lidocaine.