from first-pass hepatic drug removal when quinidine is administered orally (21).

The two procedures used in this work measure the total fluorescence of either the unchanged quinidine and hydroquinidine or the two unchanged alkaloids and their metabolites (5). As a result, the contamination by hydroquinidine would be expected to produce a certain error in the evaluation of quinidine pharmacokinetic parameters. However, the corresponding percentages of hydroquinidine in Tablets A, B, and C were only 8.23, 4.33, and 6.71, respectively. Furthermore, a study on the pharmacokinetics of quinidine and hydroquinidine by Ueda *et al.* (22) indicated that the differences in the distribution and elimination characteristics of the two alkaloids are not significant. In view of these facts, the bias introduced in the value of quinidine pharmacokinetic parameters estimated in this work (Table II) appears to be negligible.

Several parameters measured in this study demonstrated significant intersubject variability. This observation is in agreement with previously published works and demonstrates the need to individualize the dosage regimens of this drug (18). Drug monitoring with a given dosage form is usually based on the range of plasma concentrations known to produce the desired therapeutic response in most patients. However, better drug monitoring could be obtained if the adjustment of the dose is also based on therapeutic and toxic effects observed in individual patients.

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Ditheophylline Succinate: Transfer of Theophylline across Everted Rat Intestinal Sacs

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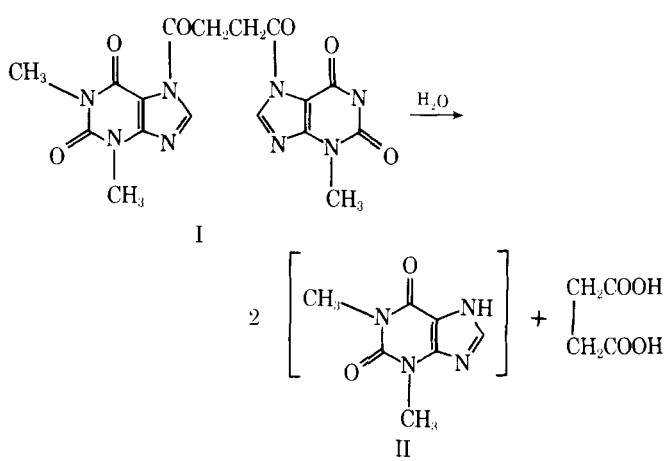
Abstract □ The cumulative theophylline transfer rate across 10-cm everted rat intestinal sacs incubated at 37° in pH 7.4 Krebs phosphate buffer was determined. A suspension of ditheophylline succinate (a potential prodrug of theophylline) and a solution of theophylline at equimolar concentration were evaluated to determine the magnitude of the difference between the cumulative theophylline transfer rates from the two preparations. A linear concentration dependency for the rate across the intestinal wall was evidenced. The theophylline formation rate from ditheophylline succinate suspended in pH 7.4 Krebs buffer at 37° followed apparent zero-order kinetics. The observed difference (fourfold) between the cumulative transfer rates estimated for the theophylline solution and the ditheophylline succinate suspension was attributed to the prevailing theophylline concentration in the mucosal solutions. The biopharmaceutical implications of these observations are discussed.

Keyphrases □ Ditheophylline succinate—theophylline transfer rate across everted rat intestinal sacs □ Theophylline—transfer rate across everted rat intestinal sacs □ Transfer rate—theophylline from solution and ditheophylline succinate suspension across everted rat intestinal sacs □ Prodrugs—ditheophylline succinate, theophylline transfer rate across everted rat intestinal sacs □ Relaxants, smooth muscle—theophylline, transfer rate across everted rat intestinal sacs

7,7′-Ditheophylline succinate[1] (I) is a potential theophylline prodrug. In the presence of moisture or water, it hydrolyzes rapidly to yield two molecules of theophylline (II) and one molecule of succinic acid[2] (Scheme I).



Scheme I

The aqueous solubility of I is less than 0.1 mg/ml; however, the dissolved material undergoes ultrafast hydrolysis

[1] SC-30163.

[2] H. K. Lee and H. Lambert, Internal Report DVR7611023, Searle Laboratories, Chicago, IL 60680.

0022-3549/78/1000-1459$01.00/0

Table I—Cumulative Transfer of Theophylline (Milligrams ± *SD*) across 10-cm Everted Rat Intestinal Sacs ($n = 4$) Immersed in a Solution of Theophylline (10^{-3} *M*) or a Suspension (0.045% w/v) of Ditheophylline Succinate (I) in pH 7.4 Krebs Phosphate Buffer at 37°

Minutes	From Theophylline Solution	From I Suspension
15	0.079 ± 0.009	0.034 ± 0.007
30	0.166 ± 0.003	0.063 ± 0.032
45	0.252 ± 0.052	0.088 ± 0.032
60	0.348 ± 0.053	0.109 ± 0.035
75	0.455 ± 0.085	0.139 ± 0.042
90	0.548 ± 0.099	0.163 ± 0.051
Cumulative transfer rate	0.0063 mg/min	0.0017 mg/min

in an aqueous medium, thus providing a continuous and steady supply of theophylline in solution[2]. The advantage of this approach to theophylline therapy is that a dissolution-controlled theophylline formation is established and, consequently, its absorption through the GI wall can be controlled at a pseudo-zero-order rate.

The usefulness of everted rat intestinal sacs for evaluating the *in vitro* transfer of drugs and other organic compounds is well documented (1–5). This study was initiated to determine the theophylline transfer rate across everted rat intestinal sacs. Experiments were conducted with theophylline solutions and I suspensions to ascertain the difference between the cumulative theophylline transfer rates.

EXPERIMENTAL

Materials—All reagents used for the preparation of buffer solutions were analytical reagent grade. Theophylline[3] was used as supplied. 7,7′-Ditheophylline succinate[4] had a surface area of 1.4 m^2/g.

Methods—In Vitro *Transfer Rate Studies*—The general procedure adopted to determine the cumulative theophylline transfer rate across everted rat intestinal sacs was similar to that reported previously (4, 5). Briefly, everted intestinal segments of approximately 11-cm length were ligated at one end with surgical grade silk thread. The other (open) end was mounted and tied on a 3.5-cm long polytef tube, which protruded inward through a neoprene rubber stopper of suitable dimensions. The ligated end was then tied to the end of an L-shaped, blunt-edged, hypodermic needle[5].

The modified needle, which was fitted into the rubber stopper, was used to deliver gaseous 95% O_2–5% CO_2. A small hole drilled into the rubber stopper served as the outlet for escaping gas. The everted rat intestinal sac was restrained parallel to the needle, thus facilitating easy access to fill and empty it as desired. Four such intestinal preparations were immersed in 85 ml of a solution or a suspension (0.045% w/v) of the test compound in pH 7.4 Krebs phosphate buffer (6) without calcium and magnesium chlorides. A 12 × 3.3-cm glass test tube was used to hold each preparation. The rubber stoppers were fitted on the test tube mouths, and the gas was bubbled through the gas outlet port. The tube was agitated mechanically up and down by a disintegration test shaker[6] to maintain a coarse suspension of the compound in the test medium. Four assemblies of the intestinal preparation were immersed in a constant-temperature bath at 37 ± 0.5°.

At the start of the experiment, approximately 0.8 ml of Krebs buffer was placed in the intestinal sac, withdrawn, and discarded. This step was repeated a second time, and the withdrawn sample was collected to serve as a blank to quantify the theophylline by UV spectrophotometry. The intestinal sac was filled again with blank buffer, and the contents were withdrawn 15 min later, followed by a quick rinse of the sac with buffer. This procedure was repeated every 15 min for 90 min of incubation of the intestinal sacs in the corresponding mucosal medium. The sample and the rinse were mixed and diluted to 10 ml with blank buffer. For studies with the I suspension, a large excess of I was maintained in the dissolution (mucosal) medium during the entire incubation period.

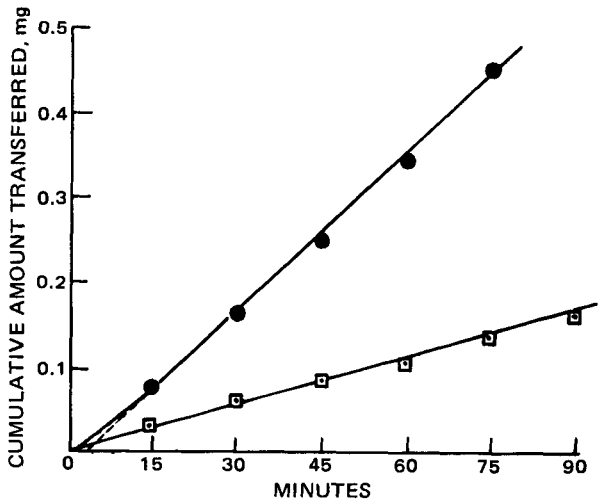


Figure 1—*Plot illustrating the cumulative transfer of theophylline across everted rat intestinal sacs immersed in pH 7.4 Krebs phosphate buffer at 37°. Key: ⊡, I suspension (10^{-3} M), slope = 0.0017 mg/min; and ●, theophylline solution (10^{-3} M), slope = 0.0063 mg/min.*

Theophylline Formation Rate in pH 7.4 Krebs Buffer at 37°—To determine the theophylline formation rate from I, the same assemblies without the rat intestinal sacs were used. A 38-mg portion of I was added to 85 ml of Krebs buffer. The suspension was shaken once vigorously, and then the loop-type stirrer was placed inside the test tube to provide the identical conditions of pH, temperature, and agitation adopted for the transfer rate studies.

Approximately 2 ml of sample from each tube was withdrawn in a syringe[5] using a hypodermic, 35.5-cm long needle[5]. It was filtered quickly through a membrane filter fitted in accompanying filtration units[7]. The samples thus collected every 15 min for 90 min after preparation of I suspensions were diluted appropriately and assayed for theophylline by the adopted procedure.

Quantitation of Theophylline—The samples from transfer rate studies with everted rat intestinal sacs were filtered through sintered-glass funnels and diluted with pH 7.4 Krebs buffer as required. UV absorption spectra were recorded on a dual-beam UV–visible recording spectrophotometer[8]. The absorbance of blank samples was subtracted from the absorbance of the samples at 270 nm (λ_{max} for theophylline) to quantitate the theophylline in the serosal solution. A calibration curve of absorbance (at 270 nm) *versus* theophylline concentration (in milligrams per milliliter) in Krebs phosphate buffer was prepared to assist in the quantitation of theophylline transfer as a function of time. The amount of theophylline transferred was calculated with:

$$\text{amount transferred per unit time} = \left(\text{corrected absorbance at 270 nm}\right)\left(\text{dilution factor}\right)\left(\frac{1}{m}\right) \quad \text{(Eq. 1)}$$

where m is the slope of the calibration curve for theophylline in Krebs buffer. All other samples were diluted appropriately with Krebs buffer and assayed by the adopted UV spectrophotometric procedure.

RESULTS AND DISCUSSION

Table I shows results of transfer rate studies conducted with four everted rat intestinal sac preparations. A plot of the cumulative amount of theophylline transferred from the mucosal to the serosal side is illustrated in Fig. 1. The slope of the line gives an estimated value for the cumulative theophylline transfer rate from the I suspension and theophylline solution at equimolar concentration. The cumulative transfer rate of theophylline from the I suspension was less than that observed for the theophylline solution.

With the I suspensions, one would expect that the theophylline transfer rate during the early incubation period (<15 min) would be concentration dependent. Since an adequate build-up of the concentration gradient

[3] Lot 13C-2810, Sigma Chemical Co., St. Louis, Mo.
[4] Lot A13177, synthesized by the Searle Chemical Development Department and released by Quality Control for investigational use.
[5] Becton-Dickinson Co., Rutherford, N.J.
[6] Model 39-133, Hansen Research Corp., New York, N.Y.
[7] Swinney type, Millipore Corp., Bedford, Mass.
[8] Coleman model 124 D, Perkin-Elmer Corp., Maywood, Ill.

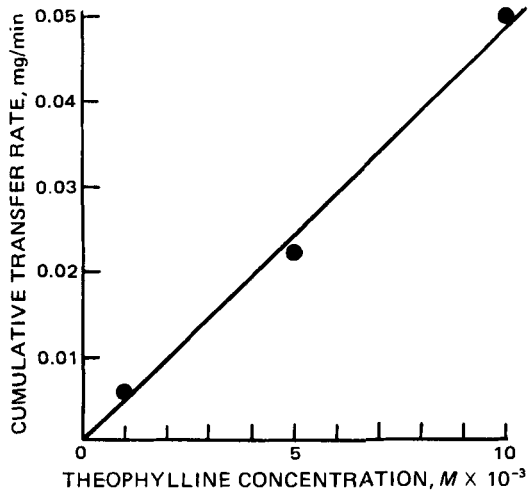


Figure 2—*Concentration dependency of theophylline transfer rate across everted 10-cm rat intestinal sacs immersed in theophylline solutions prepared with pH 7.4 Krebs buffer at 37°.*

was lacking during this period, a lag time for the appearance of theophylline in the serosal side is to be expected. Such a lag time of over 2 min (Fig. 1), as estimated from back-extrapolation (dotted line) of the amount transferred *versus* time plot, was evidenced for theophylline transfer from the I suspension. The cumulative transfer rate of theophylline formed from I in the mucosal side was retarded by as much as fourfold.

To assess whether a concentration dependency existed for the transfer rate process, theophylline solutions at three different concentrations in pH 7.4 Krebs buffer were prepared and tested by the described technique (Fig. 2). Consistent with Fick's law of diffusion (7), the concentration dependency of theophylline transfer rates across everted rat intestinal sacs was ascertained. To rationalize the observed differences between theophylline transfer rates from a I suspension and a theophylline solution at equimolar concentration, it became important to determine the theophylline formation rate from I suspension under identical conditions of pH, temperature, and stirring.

Figure 3 illustrates a concentration *versus* time profile for theophylline formation in pH 7.4 Krebs buffer containing a crude suspension (0.045% w/v) of I. After an initial induction period of 10 min, the theophylline formation rate followed zero-order kinetics; that is, the theophylline formation rate was constant in the presence of an excess of suspended I particles. The theophylline concentrations in solution ranged from an initial 2.07×10^{-4} *M* to a final 3.2×10^{-4} *M* during the incubation period. By assuming that a pseudo-steady-state concentration of 2.5×10^{-4} *M* (as estimated on the basis of a midpoint concentration at 45-min intervals) was prevalent during the course of intestinal incubation, a predicted value for the cumulative theophylline transfer rate from I suspensions could be obtained. This predicted value (calculated by multiplying the slope of the line in Fig. 2 by the estimated theophylline concentration at 2.5×10^{-4} *M*) was 0.00124 mg/min. The observed value of 0.0017 mg/min closely agreed with the predicted value. This evidence strongly suggested that only concentration-dependent theophylline transfer occurs from the mucosal to the serosal side of the everted rat intestinal sacs.

The utility of such an *in vitro* animal model for assessing *in vivo* availability of drugs and their metabolites was demonstrated previously (8, 9). The biopharmaceutical implications of these findings are obvious when the advantages of theophylline therapy with a solid oral dosage form

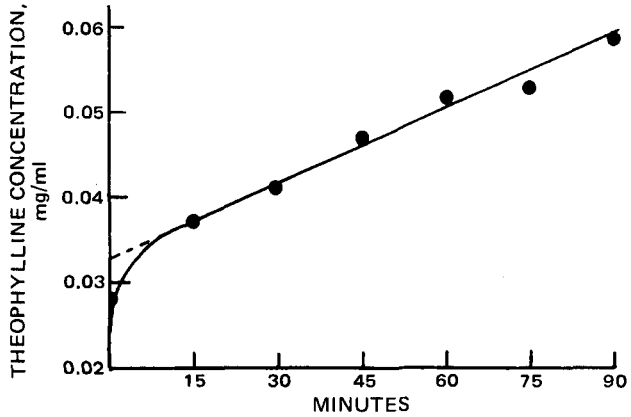


Figure 3—*Formation of theophylline from I suspension (0.045% w/v) in pH 7.4 Krebs phosphate buffer at 37°.*

of I are considered. Since the theophylline formation rate from I is constant at pH 7.4 and 37°, the availability of theophylline for absorption over a prolonged period apparently could be maintained *in vivo*. Consequently, a continuous appearance of theophylline into the vascular compartment would allow the maintenance of a constant plasma theophylline level over a prolonged period after dosing the patient with I. It is conceivable that the toxic manifestations associated with theophylline therapy might be controlled by such a prodrug approach.

In conclusion, this study demonstrated the usefulness of everted rat intestinal sacs for ascertaining the transfer rate of a drug, such as theophylline, from its prodrug entity, namely, ditheophylline succinate. These data provided meaningful biopharmaceutical support indicative of theophylline appearance on the serosal side of the intestinal sacs. The observed and predicted values for the cumulative theophylline transfer rate from a I suspension closely agreed. Evidence presented indicates that, under simulated conditions of physiological pH and temperature, the concentration-dependent transfer rate of theophylline across the intestinal barrier is controlled by the constant (steady) formation of theophylline in solution from a suspension of I in a given physiological medium.

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