technique. The sensitivity of the GC–EC (signal to noise ratio >5:1) was demonstrated to be <10 ng/mL for I and II. Assuming an initial serum volume of 1.0 mL and a final sample volume of 0.1 mL, the analytical technique can detect and quantify 1 ng/mL of serum for I and II.

The sample preparation procedure for the extraction of I and II from serum was developed through a series of experiments. The choice of toluene as the extraction solvent was made because of its low EC response and the high solubility of the compounds in this solvent. The serum samples were made alkaline to ensure a un-ionized species for extraction, and the addition of the salt to increase the ionic strength improved the extractability of the compounds. Initial studies indicated that stable emulsions formed when serum was extracted with toluene alone. By adding a small amount of methanol to the first toluene extraction, the emulsion problem was eliminated and the extraction efficiency was not affected. When only the first toluene extractions were employed to remove I and II from the serum, acceptable results were obtained for high levels, *i.e.*, >150 ng/mL, of I or II. However, interfering serum components at the GC elution positions of I and II prevented the single extraction technique from providing acceptable results below 150-ng/mL levels. Acidification of the toluene extracts from the serum removed I and II from the organic phase. The aqueous phase was alkalinized, and I and II were extracted with toluene. The double extraction gave very clean GC–EC chromatograms, and the recovery of I and II from the serum was high considering the extensive cleanup procedure employed. Figure 2 presents representative chromatograms of a reference solution, a blank serum sample, and a serum sample fortified with 64 ng/mL of I and 58 ng/mL of II.

The final sample preparation procedure was validated by preparing and analyzing replicate serum samples with added I and II at 16 concentration levels. The concentration ranges were 8–1600 and 7–1440 ng/mL for I and II, respectively. The results for these analyses are summarized in Table I. The average amount found for I and II at each concentration level and the standard deviation and relative standard deviation of the average are presented. Linear regression of the average amount found *versus* the amount added gave the following equations and correlation coefficients: $y = 0.947x + 8.5$, $r = 0.999$ for I and $y = 0.981x - 0.6$, $r = 0.999$ for II.

A small interfering serum component was observed at the elution position of I and gave a y-intercept of 8.5 ng/mL for I. The level of this unknown component in the blank serum was uniform and, thus, the level of I can be obtained by substracting the amount of the interfering component in blank serum from the level of I in the samples. However, serum levels of I <10 ng/mL must be considered questionable due to the possible presence of the unknown serum component. The percent recovery (%R) of I and II added to the serum gave average recoveries of 93.5 ± 7.9 for I and 97.7 ± 8.5 for II, indicating that the extraction efficiencies for I, II, and the internal standard were very similar and that no corrections for recovery of I and II from serum were necessary. These data demonstrate that the sample preparation procedure and analytical technique can provide reliable quantitative data over a large concentration range and that the recovery of I and II extracted from serum is quantitative.

The analytical method was utilized to determine the serum levels of I and II in two dogs administered a single oral 10-mg/kg dose of I. Blood samples were obtained before dosing and at 0.25, 0.5, 1, 2, 4, and 8 h after dosing. The serum from each dog was assayed for I and II, and the results are presented graphically in Fig. 3. Little or no compound was detected until 2 h after dosing. The level of the parent drug (I) in the serum was considerably lower than the *N*-demethyl metabolite (II), indicating possible extensive first-pass metabolism in the liver. Since both I and II have been reported to have similar therapeutic effects (3), the combination of the serum levels of I and II must be used to determine the concentration of active drug substance.

The method was also employed to assess if the serum levels of I and II correlated with the oral dose for dogs considered to be under steady-state conditions. Four dogs at each of three dose levels (10, 3, and 1 mg/kg) and four control dogs were evaluated; blood samples were obtained at 1 and 4 h after dosing. The data indicate that the serum levels of the parent drug and its *N*-demethyl metabolite may increase with increasing dose for the 10- and 3-mg/kg levels (Table II). At the 1-mg/kg dose, the levels of I and II in the serum approached the sensitivity limit of the method. Figure 4 presents representative chromatograms of serum samples from dogs at each dose level. The limited number of serum samples analyzed precludes statistical evaluation of the correlation of serum levels of I and II to the administered dose of I.

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Effect of pH on Theophylline Transfer Across the Everted Rat Jejunum

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Received July 7, 1980, from the *Departments of *Pharmacology and Toxicology, ‡Pediatrics, and §Pharmaceutical Sciences, Colleges of Pharmacy and Medicine, University of Arizona, Tucson, AZ 85721.* Accepted for publication January 10, 1983. ¶Present address: Departments of Pediatrics and Pharmacology, Columbus Children's Hospital, Columbus, OH 43205.

Abstract □ The effect of pH on the cumulative transfer of theophylline across the everted rat jejunum *in vitro* was ihvestigated. Intestinal integrity was assessed by light and scanning electron microscopy, while the biochemical viability of the intestine was evaluated using glucose transfer measurements. The initial (0–30 min) clearance of theophylline was directly proportional to the fraction un-ionized at pH 5.5, 7.4, 8.0, and 10.0. Plots of cumulative theophylline transfer *versus* time over 60 min were nonlinear, but could be subdivided into two linear segments of 30-min duration. Due to this nonlinearity, differences in theophylline transfer with pH were significant only over the first 30 min of the experiment. Intestinal tissue integrity and viability correlated with the time at which the clearance (slope) increased, while the magnitude of the increase in clearance was proportional to the degree of ionization of theophylline.

Keyphrases □ Theophylline—transfer across the everted-jejunum, rats, effect of pH □ Jejunum—everted, effect of pH on theophylline transfer, rats, □ pH—effect on the theophylline transfer across the everted jejunum, rats

Theophylline, a bronchodilator widely used for treating chronic obstructive pulmonary diseases such as asthma or emphysema, may be given orally or parenterally in the form of various salts. Although theophylline is usually given orally, its absorption from the GI tract can be erratic. Nausea and gastric irritation often accompany the oral

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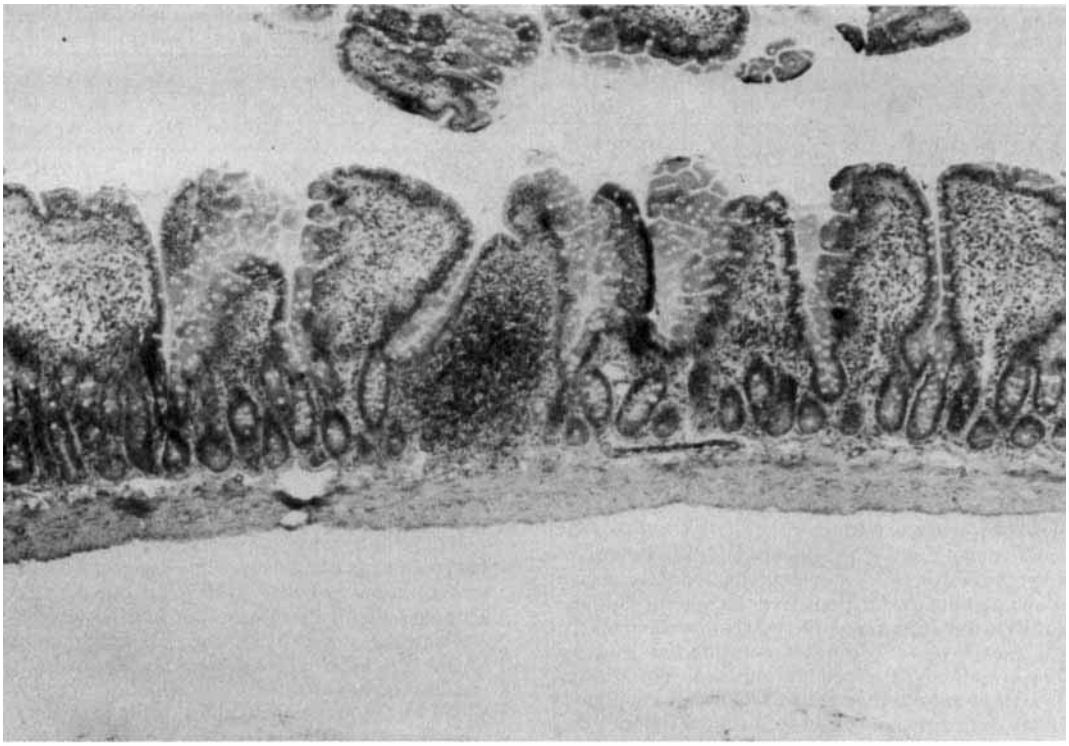

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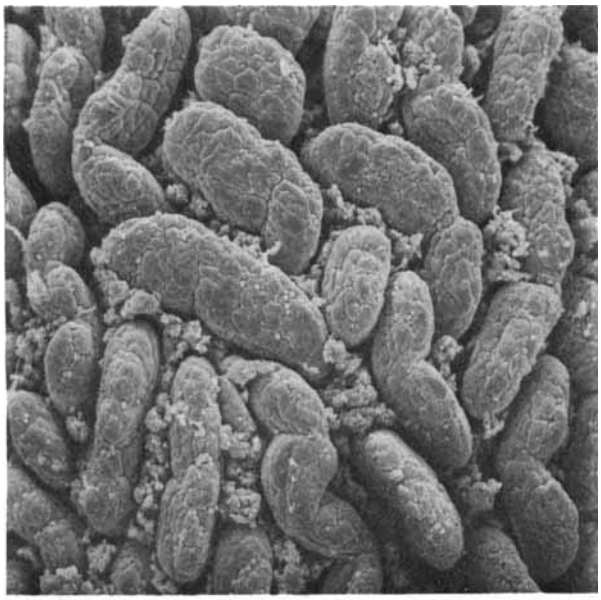

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Figure 1—(*A*) *Details of intestinal villus immediately* (t = *0*) *following eversion (original magnification 800×). (B) Scanning electron micrograph showing surface detail of everted intestine* (t = *0*).

administration of theophylline, a condition which is frequently treated with antacids. A recent study by Shargel *et al.* (1) indicated that the concurrent administration of 15 mL of magnesium aluminum hydroxide antacid did not significantly affect the pharmacokinetics of theophylline. This result contrasts with the work of Arnold *et al.* (2) who reported that 30 mL of magnesium aluminum hydroxide suspension significantly decreased the absorption rate constant for theophylline. Furthermore, a study by Rohr *et al.* (3) indicated that theophylline may be adsorbed onto some antacid materials but not others. Since antacids can possibly alter the GI absorption (4) of drugs, it was of interest to determine how changes in the pH might alter the absorption of theophylline.

Several studies (5–7) have previously demonstrated the utility of everted intestinal preparations for evaluating the *in vitro* absorbability of drugs and nutrients and how various factors influence this process. In this report, cannulated everted jejunal segments of rat intestine (8) were used to evaluate the influence of pH on theophylline transfer. The structural integrity and biochemical viability of the intestinal segments were monitored by means of light and scanning electron microscopy and by measuring the rates of active glucose transfer, respectively.

EXPERIMENTAL

Reagents—The following reagent-grade chemicals were employed: theophylline[1], 1-[2-(β-D-glucopyranosyloxy)-4,6-dihydroxyphenyl]-3-

[1] Sigma Chemical Co., St. Louis, MO 63178.

Table I—Effect of pH on Theophylline Transfer Across the Everted Rat Jejunum

Time, min	pH 5.5	pH 7.4	pH 8.0	pH 10.0
10	0.117 ± 0.30[a]	0.099 ± .047	0.071 ± .016	0.052 ± .016
20	0.278 ± .030	0.256 ± .065	0.187 ± .034	0.137 ± .056
30	0.476 ± .074	0.443 ± .097	0.335 ± .062	0.264 ± .076
40	0.671 ± .118	0.653 ± .128	0.502 ± .083	0.446 ± .084
50	0.861 ± .166	0.879 ± .160	0.695 ± .102	0.668 ± 0.93
60	1.05 ± .217	1.10 ± .199	0.898 ± .130	0.910 ± .107

[a] Values represent the cumulative micrograms of theophylline transferred per unit mucosal concentration ±*SD*. All values represent the mean of six segments (proximal and distal combined) except at pH 7.4 and 8.0 where eight segments were used. A one-way analysis of variance was performed on the data to test for significant differences between means at a given time point. Bars enclosing values indicate means are not significantly different ($p > 0.05$). Means not connected are significantly different ($p < 0.05$) from each other.

(4-hydroxyphenyl)-1-propanone (phlorizin)[1], 3-*O*-methyl-D-[1-^{3}H]-glucose (I)[2], glucose[3], and acetonitrile[4]. Monobasic sodium phosphate monohydrate, anhydrous dibasic sodium phosphate, sodium carbonate, and sodium bicarbonate were used in preparing the isotonic buffers.

Preparation of Everted Jejunum—Everted jejunal segments from nonfasted Sprague–Dawley rats of both sexes, weighing 150–300 g, were prepared according to methods described previously (8). The intestines were removed from ether-anesthetized animals following a midline abdominal incision and transection of the intestine at the pyloric and ileocecal junctions. The proximal 30 cm of unstretched intestine was discarded to prevent inclusion of the bile duct. The next 30 cm was separated, rinsed in physiological saline at 37°C, then cut into two 15-cm segments designated as proximal and distal in reference to their original position relative to the pyloric junction.

The everted segments were positioned on the perfusion apparatus as described previously (8), and the total assembly time following removal was 7–10 min. The serosal surfaces were rinsed with 25 mL of serosal buffer solution prior to the first collection. Buffer pH was measured[5] before and after each experiment. Oxygen was bubbled through the mucosal solutions continually. Serosal and mucosal buffer solutions were warmed to 37°C prior to coming in contact with the tissue. Serosal buffer samples (5 mL) were collected at 10-min intervals for 1 h and replaced with fresh buffer within 20 s.

Buffer Composition—A Modified Krebs–Henseleit phosphate buffer was used for all experiments conducted at pH 5.5, 7.4, and 8.0 (9). This buffer was chosen instead of the Krebs bicarbonate, which reportedly provides the best environment for maintaining the viability of the intestine (10), because bicarbonate buffer does not function optimally over the pH range of 5.5–8.0. Experiments conducted at pH 10 used the same Krebs–Henseleit electrolyte solution except that the phosphate buffer was replaced by an equimolar amount of carbonate–bicarbonate buffer (9). Calcium ion was deleted from the buffer solution due to its tendency to precipitate in the presence of phosphate at pH >7.4. The absence of calcium ion was observed to have no effect on drug transfer. Glucose at a concentration of 130 mg% (7 mM) was added to the mucosal buffer only.

Preparation of Tissue Samples for Histological Evaluation—Histology was performed on jejunal segments immediately after eversion ($t = 0$ min) and after 60 min of incubation in a pH 7.4 phosphate buffer ($t = 60$ min). Everted intestinal segments were also examined using light microscopy after 30 min incubation at pH 5.5, 8, and 10. Specimens for light microscopy were fixed in 10% w/v buffered formalin, paraffin sectioned, and stained with hematoxylin and eosin. Specimens for scanning electron microscopy[6] were fixed in isotonic Karnofsky's solution and stained with 1% w/v osmium tetroxide prior to critical point drying from acetone. These specimens were sputter-coated with gold to a thickness of 300 Å and examined.

Transport of 3-*O*-Methyl-D-[1-^{3}H] glucose—The transport of tritiated 3-*O*-methyl-D-glucose (I), specific activity 2 Ci/mmol, was determined using liquid scintillation counting. Fifty microliters of 3-*O*-methyl-D-glucose (1 mCi/mL) was added to each 100 mL of buffer. This solution was used as the mucosal buffer for each intestinal segment. Two segments also has phlorizin (a specific inhibitor of glucose transport) added to give a final concentration of 1 mM, and two did not. The serosal and mucosal concentrations of I were measured by placing 1-mL aliquots of each solution into vials, adding 10 mL of liquid scintillation cocktail[7], and counting in a liquid scintillation counter[8] for 10 min. Intestinal tissue concentrations of I were measured by weighing accurately ~100 mg of jejunum (~1 cm) after rinsing and blotting with absorbant tissue. Samples were digested in a counting vial by the addition of 0.5 mL of 0.6 M NaOH. Ten milliliters of cocktail and 1 mL of water were then added, and the vial was counted for 10 min. Counting efficiencies were determined by the external standard method. Tissue radioactivity was expressed as disintegrations per min per mg of wet tissue (dpm/mg) divided by the mucosal concentrations of I in dpm/mL. D-glucose levels were determined in duplicate in an automated glucose analyzer[9] using the glucose oxidase method.

Table II—Ionization and Mean Intestinal Clearances (±*SD*) for Theophylline at Various pH Values

pH	Un-ionized[a], %	Clearance (mL/min) × 10^2 Period I (0–30 min)	Clearance (mL/min) × 10^2 Period II (30–60 min)	Clearance Ratio (Period II/Period I)
5.5	99.90	1.794 (0.38)	1.894 (0.50)	1.051 (0.11)
7.4	92.64	1.721 (0.31)	2.236 (0.43)	1.309 (0.18)
8.0	75.97	1.321 (0.25)	1.981 (0.27)	1.526 (0.24)
10.0	3.07	1.058 (0.30)	2.328 (0.36)	2.378 (0.82)

[a] The degree of ionization of theophylline was calculated from the Henderson–Hasselbalch equation, using a pK_a of 8.5 (20).

Theophylline Assay—Theophylline analyses were performed using an HPLC assay. A reverse-phase HPLC column[10], 4 mm i.d. × 30 cm, was used. The mobile phase of acetonitrile–water–acetic acid (10:89:1 v/v/v) was pumped[11] through the column at a flow rate of 3 mL/min. Detection of eluted theophylline was accomplished at 280 nm using a *fixed-wavelength detector*[12].

Aliquots (25 μL) of serosal buffer solution were injected directly onto the column. Quantitation was performed by comparing measured peak heights against a standard curve of theophylline peak height *versus* concentration. Samples were either assayed immediately or frozen at −20°C until analyzed. Theophylline concentrations were stable for 7–10 d under these storage conditions.

Measurement of Cumulative Transfer Rates—Theophylline transfer across the everted intestine was expressed as a cumulative mucosal to serosal clearance (transfer rate constant). In essence:

$$\text{Clearance (mL/min)} = \frac{\text{cumulative amount } (\mu g) \text{ transferred per } t \text{ min}}{\text{mucosal concentration } (\mu g/mL) \times t} \quad \text{(Eq. 1)}$$

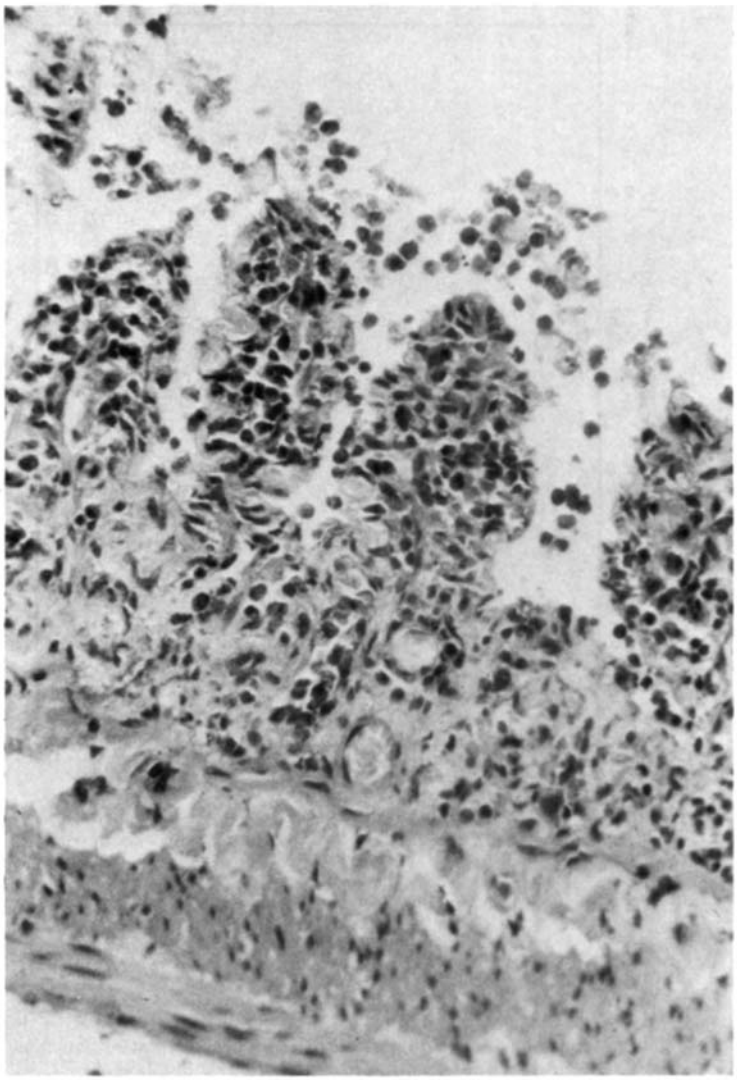
The clearances were actually determined from the slopes of the plots of the cumulative amount of theophylline transferred per unit mucosal concentration *versus* time. The slopes of these plots were calculated using least-squares linear regression.

The average cumulative amount (μg) of theophylline transferred (±*SD*) was calculated at each 10-min interval throughout the course of the 60-min experiments for the various pH values tested, as shown in Table I. These data were subjected to a one-way analysis of variance (ANOVA) to detect any significant differences in clearance between the various pH values over a given time interval. The level of significance used for all analyses was $p < 0.05$. The Tukey test was employed *post hoc* to determine where pair-wise differences between means existed (11).

RESULTS AND DISCUSSION

The viability and structural integrity of an *in vitro* model must be considered when interpreting data from such a system (12, 13). Viability of the rat jejunal model has been characterized by many investigators.

[2] Amersham Radiochemical, Arlington Heights, IL 60006.
[3] Matheson, Coleman and Bell, Norwood, OH 45212.
[4] Burdick and Jackson Laboratories, Muskegon, MI 49422.
[5] pHasar Digital pH Meter, Beckman Instruments, Fullerton, CA 92634.
[6] ETEC Autoscan, ETEC Corp., Hayward, CA 94545.
[7] Beckman GP, Beckman Instruments, Fullerton, CA 92634.
[8] Model LS-3133P, Beckman Instruments, Fullerton, CA 92634.
[9] Glucose Analyzer I, Beckman Instruments, Fullerton, CA 92634.
[10] μBondapak-C_{18}, Waters Associates, Milford, MA 01757.
[11] Model 6000-A pump, Waters Associates, Milford, MA 01757.
[12] Model 400, Waters Associates, Milford, MA 01757.



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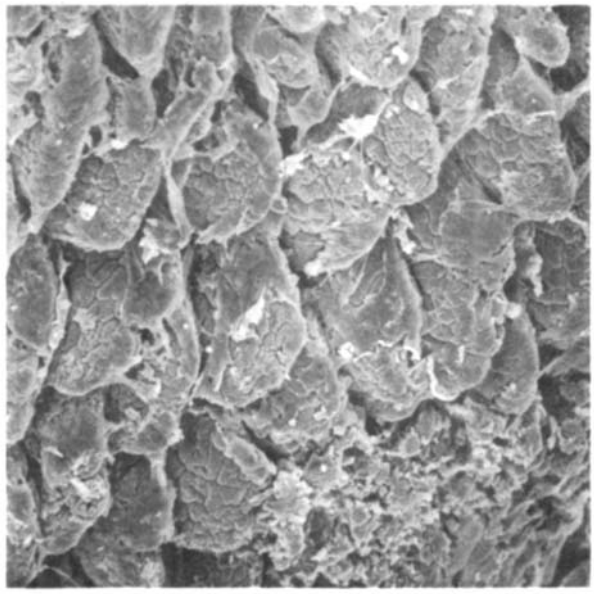

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Figure 2—(*A*) *Appearance of intestinal villus after 60-min incubation at 37°C in pH 7.4 phosphate buffer (original magnification 800×). (B) Scanning electron micrograph of intestinal segment after 60-min incubation at 37°C in pH 7.4 phosphate buffer.*

It has been shown that oxygen uptake is linear for 1–3 h (14, 15), and that active uptake of L-histidine and iodine-131 (14), as well as linear active uptake of D-glucose, occur for up to 1 h (16).

In this study, the structural integrity of the everted rat jejunum was evaluated using both light and scanning electron microscopy. Intestinal segments were examined immediately after eversion (t = 0 min) and after 60 min of incubation in isotonic pH 7.4 phosphate buffer (t = 60 min). At t = 0 min, sections examined by light microscopy had good cytoplasmic and nuclear detail and good detail in submucosal structures (Fig. 1A). Examination by scanning electron microscopy also showed normal surface morphology with well-defined and well-separated villi and no evidence of gross damage (Fig. 1B). After a 60-min incubation, light microscopy revealed minimal disruption of submucosal or muscle layers, but a substantial disruption of the villous epithelium and areas where the basement membrane had separated from the lamina propria (Fig. 2A). Electron microscopy also demonstrated changes in villous structure (Fig. 2B). Everted intestinal segments incubated at pH 5.5, 8, and 10 for 30 min exhibited no gross morphological changes when examined using light microscopy.

The functional integrity of the everted rat jejunum was evaluated by determining the active transport of glucose and I. Since the active transport of glucose by the small intestine *in vitro* is known to require intact, functioning intestinal epithelial cells (17), the rate of appearance of glucose on the serosal side of the preparation over the 60-min incubation period was used as a measure of the biochemical and structural state of the epithelium.

The intestinal transport of glucose at pH 7.4 demonstrated that the amount of glucose appearing in the serosal fluid rose from an initial value at t = 0 min to a maximum at 30 min, then declined back to the initial value by 60 min. This coincides with the findings of Porteus and Pritchard (16) who observed that the rates of glucose transport by the intact intestine increased linearly to 30 min, then slowly decreased to zero by 60 min. The concentration gradient present in the direction of transport (*i.e.*, mucosal to serosal) was disregarded since the *in vitro* small intestine actively transports glucose 10–20 times faster than its rate of diffusion (18). Furthermore, at concentrations of ≤500 mg%, the rate of passive glucose transport up or down a concentration gradient is of the same order of magnitude (18).

The biphasic glucose transfer observed here was taken as evidence of a functioning active-transport system. This is supported by the fact that transport was eliminated when phlorizin, a specific inhibitor of active transport, was added to the mucosal solution. The rates of glucose transfer in the presence of phlorizin were considered to be those maximally obtainable by diffusion alone. Since there was no increase in transfer from 0 to 60 min, in the presence of phlorizin, it was concluded that the integrity of the intestinal membrane was not compromised to the extent of becoming "leaky" toward the relatively small (mol. wt. = 180), hydrophilic glucose molecule.

To examine the role of any metabolic utilization and/or transport of endogenous glucose stores by the intestine, experiments were conducted to measure the transport of I. This glucose analogue is actively transported by the same system that transports glucose across the intestine; however, it is not metabolized by the intestine (19). The transport of I showed a biphasic pattern similar to that found for glucose. In addition, the average tissue concentration of I in the presence of phlorizin was about one-seventh (0.5 *versus* 3.4 dpm/mg of tissue per 10^6 dpm in buffer) the control value. These data indicated that the biphasic nature of the transport curves was not due to the metabolism of glucose by the jejunal segments since I, which was not metabolized, exhibited a similar transport pattern. The histological studies and the biphasic transport of glucose

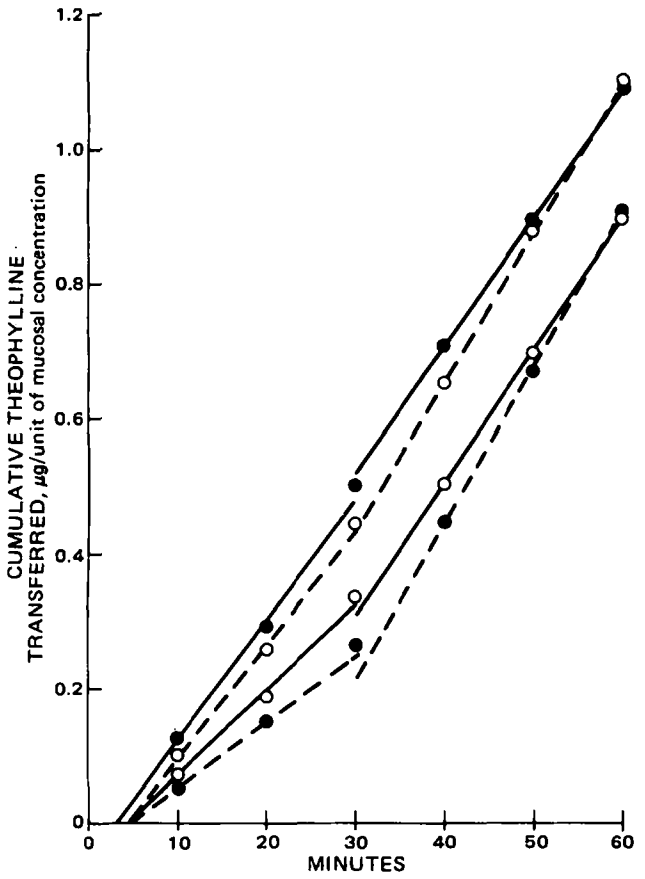

Figure 3—*Cumulative transfer of theophylline across the everted rat jejunum at various pH values as a function of time. All values represent the mean of six segments (proximal and distal combined) except at pH 7.4 and 8.0 where eight segments were used. Error bars are deleted for clarity. Key: (●—●) pH 5.5; (○- - -○) pH 7.4; (○—○) pH 8.0; (●- - -●) pH 10.0.*

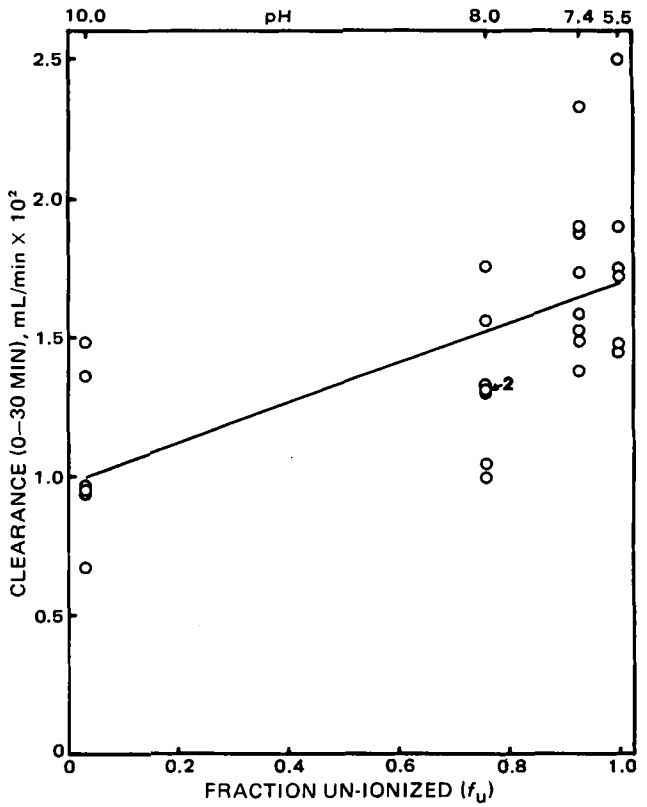

Figure 4—*Relationship between the initial (0–30-min) clearance of theophylline across the everted rat jejunum and its theoretical fraction un-ionized,* f_u *(r = 0.6375; p < 0.0005).*

and I, together with their inhibition by phlorizin, were taken as evidence that the preparation was biochemically intact up to 30 min, but that after 30 min the functional integrity was decreased, and by 60 min there were signs of both functional and structural disruption.

Effects of pH on Theophylline Transfer Rates—The transfer of theophylline appears to follow zero-order kinetics as is shown by the linear segments of the plots shown in Fig. 3. This linear transfer occurred because the concentration of theophylline in the mucosal solution remained essentially constant and was much higher than that in the serosal solution during the course of the experiment. This was achieved by replacing the serosal fluid at 10-min intervals and by using a large volume of mucosal solution relative to serosal solution. The intestinal clearances of theophylline at various pH values are shown in Table II.

Due to the biphasic nature of theophylline clearance over the 60-min time course of the experiments it was not possible to obtain the clearance at each experimental pH using transfer data from all the time points. Examination of Fig. 3 revealed that the slope of the clearance curve began to increase after 30 min for all pH values tested. This change in slope was such that transfer rates at different pH values were statistically nonsignificant ($p > 0.05$) after the first 40 min. Prior to the 30-min time point, the transfer of theophylline at all pH values tested was judged to be sufficiently linear (*i.e.*, all lines had $r^2 \geq 0.95$) to derive a clearance. Therefore, clearance values for all experiments were derived from the cumulative amount of theophylline transferred in the first 30 min. These initial theophylline clearances were directly proportional to the fraction un-ionized (f_u) as shown in Fig. 4.

If we assume that both the ionized and un-ionized forms of theophylline are capable of being transported across the everted rat jejunal membrane then the total clearance (CL_t) can be expressed as:

$$CL_t = f_i CL_i + f_u CL_u \qquad \text{(Eq. 2)}$$

wher CL_i and CL_u represent the clearances of the ionized and un-ionized species, respectively, and f_i and f_u are the fractions of the drug present in the ionized and un-ionized forms, respectively. Since:

$$f_i = (1 - f_u) \qquad \text{(Eq. 3)}$$

it follows that:

$$CL_t = CL_i + (CL_u - CL_i) f_u \qquad \text{(Eq. 4)}$$

Thus, from Eq. 4, it is evident that in a plot of CL_t *versus* f_u, such as Fig. 4, the intercepts at $f_u = 0$ and $f_u = 1$ will represent CL_i and CL_u, respectively.

Using this approach, linear regression of the data in Fig. 4 indicated that CL_u and CL_i were equal to 1.693×10^{-2} and 0.978×10^{-2} mL/min, respectively. Thus, the ratio of intrinsic clearances for these two species in the 0–30-min time period was $CL_u/CL_i = 1.732$. It is possible that undetected intestinal tissue damage may have occurred which could have artificially altered some of the clearance data in Fig. 4. However, the excellent agreement of the CL_u/CL_i ratio with the value recently reported by Crommelin *et al.* (21) for theophylline transfer across the rat rectum indicates that if such alterations did occur, their effects on theophylline transfer were minor. These authors reported values for the absorption rate constants of the un-ionized and ionized forms of theophylline which were very similar (*i.e.*, $K_u/K_i = 0.805$ and 1.13) and concluded that the absorption rates of the two forms "do not differ much." Our value is also similar in magnitude to the value (1.905) found for lidocaine at pH 6.4 and 7.4 by Cascella and Feldman (22) and to the values that Crouthamel *et al.* (23) calculated for sulfaethidole (5.56) and barbital (3.08) over the pH range of 5.15–8.52. The results indicate that the ionized forms of these compounds cross the *in vitro* rat intestine at a rate which is comparable with that of their un-ionized forms, a condition which may or may not exist *in vivo*. In fact, Turner *et al.* (24) noted that ionized species are more easily transferred across *in vitro* membrane systems, such as the one utilized here, than they are across *in vivo* membranes.

The use of the early time points to determine clearance values has been proposed by other authors (13) who recognized the phenomenon of increasing clearance with time. The assumption that the initial transfer rates are a more appropriate measure of clearance across the everted intestine than those obtained using the total amount of solute transferred over the course of a typical experiment (*i.e.*, 60 min) was also reached independently by Benet *et al.* (10) and is consistent with the assessment of the everted intestine as a method for studying drug transfer made by Levine *et al.* (12).

In our experiments, we observed that only the initial (0–30 min) transfer rates were pH dependent. Therefore, structural integrity of the everted intestine appeared to be necessary to demonstrate the effect of pH on theophylline transport. In contrast, the biochemical integrity of the intestinal segment was relatively unimportant in affecting the transfer of theophylline, since this compound is believed to be transported passively (25).

The change in theophylline clearance across the intestine was observed to increase as the pH increased, as shown by the data in Table II. There was also a time-dependent increase in the clearance at all pH values. This was believed to be due to a time-dependent degradation of the intestinal membrane, which resulted in an increased permeability of the membrane for theophylline, apparently due to a loss of structural integrity of the membrane.

One possible explanation for these observations is that the epithelial and the underlying connective and muscle tissue barriers are both intact only over the first 30 min of the experiment (26, 27), during which time the epithelial barrier is rate limiting (27). This is substantiated somewhat by Fig. 4, in which the initial clearance values are directly proportional to the fraction of theophylline un-ionized, which would be predicted from the pH-partition hypothesis (28). After 30-min incubation, the epithelial barrier may no longer be intact, with the result that the clearance in the 30–60-min time period (period II) is increased at all pH values relative to the corresponding 0–30-min (period I) clearance. Since the ionized form of the drug is less permeable than the un-ionized form, any disruption of the epithelial barrier would be expected to result in a more pronounced change in the clearance of this species. The relatively small effect of changes in the structural integrity of the intestinal membrane on the transfer rates of the un-ionized species over the 30–60-min period, observed at low pH, is consistent with previous suggestions that the epithelium is not the rate-limiting barrier to the transfer of nonpolar, lipid-soluble molecules (such as un-ionized theophylline) across this membrane model (13).

CONCLUSIONS

It has been shown that pH alters the initial (0–30-min) transfer of theophylline across the everted rat jejunum. The cumulative amount of theophylline transferred over the first 30 min was directly proportional to the calculated un-ionized fraction. A time-dependent change in transfer rate was also demonstrated which correlated with the structural integrity of the preparation. These results indicate that intestinal transfer rates measured during the first 30 min may be more valid than measurements taken over longer time periods. The ionized form of theophylline appears to be transported across the everted rat intestine *in vitro* at a rate comparable with that of the un-ionized form.

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