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RESEARCH ARTICLES

Drug Transfer across Intact Rat Intestinal Mucosa following Surgical Removal of Serosa and Muscularis Externa

DONALD L. WOLFE*, STEVEN C. FORLAND*, and LESLIE Z. BENET▲

Abstract ☐ The *in vitro* absorption kinetics for salicylate ion were followed through rat intestinal membranes from which the serosa and longitudinal and transverse layers of the muscularis externa had been removed. Techniques are described where up to 15 cm. of intestine may be stripped of musculature so that absorption studies may be carried out utilizing commonly employed *in vitro* methods such as the everted sac, the Crane–Wilson technique, and a perfusion apparatus. A histological examination of the stripped intestinal mucosa showed that the section consisted of epithelium, underlying lamina propria, muscularis mucosa, and submucosa. The latter two layers do not remain when mucosa is isolated by scraping off the mucosal surface with a glass slide, but they are necessary for maintaining an intact epithelial membrane during *in vitro* transport studies. Transfer rates were measured using 10 cm. of everted intestine in a perfusion apparatus and 5 cm. of everted intestine made into a Crane–Wilson sac. Absorption rates for salicylate through stripped intestinal segments were found to be 1.5–1.8 times greater than those found with nonstripped segments.

Keyphrases ☐ Drug transfer—*in vitro–in vivo* correlations improved, serosa and muscularis externa removed from intact rat intestinal mucosa ☐ Transfer rates—procedure for removal of serosa and muscularis externa from intact rat intestinal mucosa preparations, improved *in vitro–in vivo* drug transfer correlations ☐ Absorption kinetics, *in vitro*—salicylate ion through rat intestinal membrane stripped of serosa and muscularis externa, improved correlation with *in vivo* data ☐ Intestinal mucosa preparation, rat—procedure for stripping serosa and muscularis externa to improve *in vitro–in vivo* drug transfer correlations

Since many intestinal absorption studies in the pharmaceutical sciences are done employing *in vitro* techniques, the obvious question arises: What correlation can be attached to observations made from *in vitro* studies and events occurring *in vivo*? Ideally, one would hope that the information gained from studying absorption mechanisms can be applied ultimately to the design of more predictable and more effective oral drug dosage forms.

As pointed out by Turner *et al.* (1), a number of studies in the literature showed large differences between *in vitro* results and those expected upon the basis of *in vivo* hypotheses. While one cannot expect *in vitro* preparations to provide a direct reflection of *in vivo* events, one can hope to gain insight into mechanisms that are difficult or impossible to elucidate *in vivo*. However, the reliability of *in vitro* observations in providing meaningful information depends upon experimental conditions conducive to optimum physiological activity. Up to the present time, three commonly employed *in vitro* techniques have been utilized in studying drug absorption: the everted sac method of Wilson and Wiseman (2), the Crane–Wilson method (3), and a perfusion apparatus (4, 5) where transmembrane potentials are neither measured nor short circuited. Some of the problems inherent to these *in vitro* methods that may be contributory to *in vitro versus in vivo* absorption discrepancies are: (*a*) maintenance of biological viability, (*b*) maintenance of structural integrity, and (*c*) artifactual influences due to unnatural absorption barriers.

A number of workers have investigated the extent of viability of excised gut preparations. Under controlled conditions, rather constant metabolic activity for 2–3 hr. was demonstrated with the rat intestine by Bramford (6) and Jordana and Ponz (7) through oxygen consumption studies and by Robinson and Felber (8) with L-methionine and L-phenylalanine uptake. Duration of viability during drug absorption studies would be affected by drugs that are toxic to, or interfere with, metabolic mechanisms and/or the use of bathing solutions (especially buffered solutions) that are not of optimum physiological compatibility.

The problem of structural integrity has been considered by some (9, 10) to be even more crucial to the absorption processes than viability, especially where passive mechanisms seem to predominate. Alteration in structural relationships certainly would have a major influence upon the passive absorption of drugs and, consequently, the ques-

tion of discriminating between absorption mechanisms becomes a moot point. Levine *et al.* (9) made histological studies of everted rat gut preparations and found that although the intestinal sacs were morphologically intact after eversion, they began to lose structural integrity rapidly after 10–15 min. of incubation under conditions normally employed (oxygenated Krebs–Henseleit buffer at 37°), even in the absence of drug. This phenomenon has been attributed to fluid uptake into the tissue (water follows mucosal to serosal sodium flux), with the resultant distension due to the loss of a functional vascular system into which the accumulated water and solutes are normally dissipated.

Benet *et al.* (11), Mayersohn *et al.* (12), Mayersohn and Gibaldi (13), and Morishita *et al.* (14) demonstrated that the presence of various cations and solutes in the bathing buffer solution can affect the transfer rates of certain drugs across intestinal membranes. The degree of inhibition is believed to be correlated to the degree of fluid uptake by the tissue under the influence of the presence or absence of these cations and solutes. Benet *et al.* (11) suggested that the determination of the constancy of transfer rate as a function of time might be a valid approach to assessing the functional integrity (*i.e.*, both biological and structural) of the everted gut preparation. These workers found that a Krebs–Henseleit, pH 7.4 buffer maintained constant transport rates over 2 hr. Although Mayersohn *et al.* (12) demonstrated decreased transfer rates for polar compounds in the presence of glucose, Taraszka (15) made a gross observation (without histological examination) that inclusion of 10 m*M* glucose in the buffer appeared to enhance the structural durability of her cannulated rat gut preparations.

Finally, there is the consideration of overcoming the artifactual influences due to unnatural absorption barriers. One difficulty in extrapolating *in vitro* flux data to the *in vivo* transport of drugs is the fact that the *in vitro* fluxes are measured through the columnar epithelium plus the underlying connective and muscle tissue. Therefore, the rate-limiting step for *in vitro* transport might well be the intestinal musculature, a barrier that would not be encountered during *in vivo* transport. Nayak and Benet (16) attempted to measure the transport rate through rat intestinal musculature after the columnar epithelium was removed by edetic acid treatment. Their findings—high transfer rates through the muscle layer and no difference in directional transport—were in accord with earlier observations (17, 18) that the selective and absorptive elements in the absorption of various drugs are epithelial. Currently, drug transport across the short-circuited epithelial membrane is being followed using radioactive tracers, following the removal of the intestinal musculature by surgical techniques. Field *et al.* (19) observed that the transmural potential and sodium transport capacity of the intestinal mucosa are better maintained *in vitro* if the muscularis is removed. A possible explanation for this observation may be that the unstripped intestine is relatively hypoxic due to an interference by the muscularis externa with oxygen diffusion from the serosal bathing solution to the basal surface of the epithelium.

Considering this information, it would be advantageous to carry out *in vitro* mucosal absorption studies across gut preparations stripped of musculature. The purpose of this study was to develop techniques for the removal of the muscularis externa from large segments of rat intestine so that transport studies across the mucosa could be carried out using any of the three commonly employed *in vitro* techniques. Since the problems of biological and structural integrity involved with these methods have been investigated and defined, greater control over those parameters is possible.

EXPERIMENTAL

Materials—The following USP, NF, or reagent grade chemicals were employed: sodium salicylate, monobasic sodium phosphate with one water of hydration, dibasic sodium phosphate anhydrous, sodium chloride, calcium chloride, magnesium sulfate with seven waters of hydration, sodium bicarbonate, oxygen–carbon dioxide gas (95:5), and air–carbon dioxide gas (95:5).

Surgical Procedures—Male Sprague–Dawley rats, weighing 250–300 g., were allowed free access to both food and water prior to the experiment. The rats were sacrificed by a sharp blow at the base of the skull. The small intestine was exposed *via* a midline abdominal incision, and the gut was wetted with a pH 7.4 Krebs bicarbonate buffer (11), which was used throughout the experiment. The first 25 or 65 cm. of gut distal to the pylorus was discarded, depending upon which section of intestine was to be examined. The next 30 cm. of the intestine was flushed with approximately 20 ml. of the pH 7.4 buffer at room temperature. The 30-cm. segment was then cut into appropriate lengths, depending on the procedure to be followed as discussed below. Sections of intestine were then slid onto glass rods with tapered points that had been prewetted in the pH 7.4 buffer at room temperature, and then the intestine and rod were placed in buffer chilled on an ice bath (3–4°).

The following procedures were carried out on only one-half of the segments placed onto the rods. The fat and mesentery were pulled loose along the mesentery reflection with a pair of tweezers. The serosa and longitudinal and transverse layers of the muscularis externa were loosened, starting at the point of the mesentery reflection and working around the intestine in both directions. This was done until about 2.54 cm. (1 in.) of the intestine was free of these layers at the mesentery reflection and the reverse side had about 0.32 cm. (0.12 in.) free. The remaining length of intestine was stripped by loosening the layers of the mesentery reflection and progressively moving the stripped front down the length of the section. (Tissue layers are diagrammatically shown in Fig. 1.)

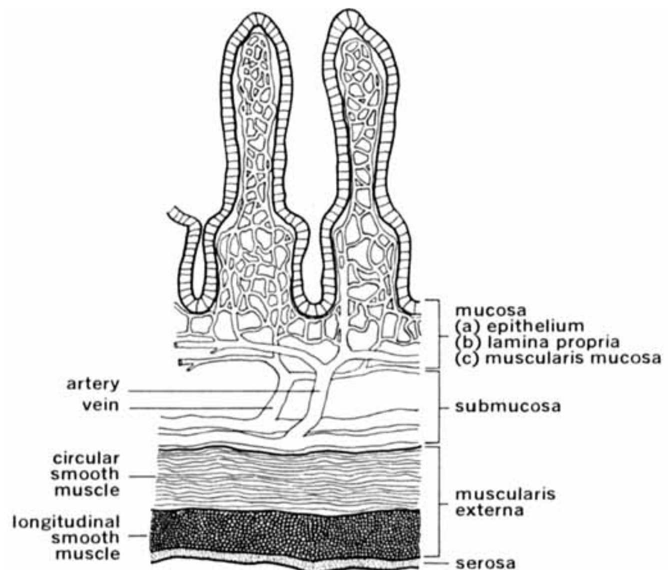


Figure 1—*Diagrammatic representation of the small intestine in cross section.*

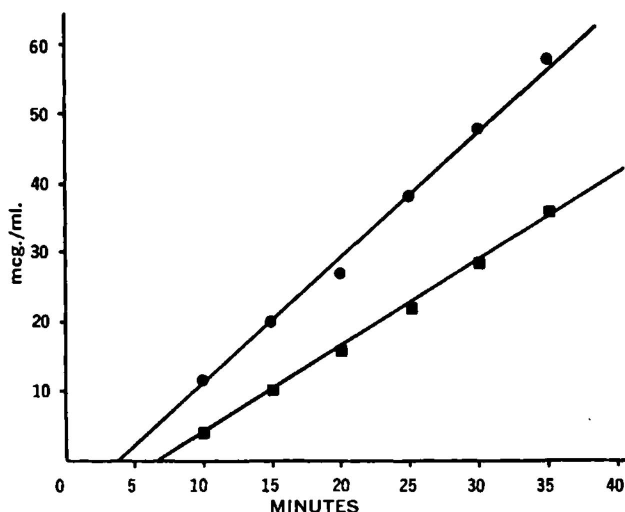


Figure 2—*Sample data plot for the absorption of salicylate through stripped (●) and nonstripped (■) everted rat intestine 25 cm. distal to the pyloric junction, using a perfusion apparatus. Outside solution = 1 mg./ml. drug in isotonic pH 7.4 Krebs bicarbonate buffer. Initial absorption rates through stripped segment = 1.77 mcg./ml./min. and through nonstripped segment = 1.20 mcg./ml./min. Ratio of rates stripped/nonstripped = 1.48.*

All sections of the intestine (stripped and nonstripped) were then slipped off the glass rods into the bathing solution at 3–4°. The sections were placed into pH 7.4 buffer at room temperature and then everted.

Perfusion Experiments—Adjacent 15-cm. everted intestinal segments, one from which the muscularis externa had been removed and one intact, were placed on perfusion devices and absorption studies were carried out following the techniques previously described (1). The inside compartment of each apparatus was filled with pH 7.4 buffer, and the outside compartments were filled with pH 7.4 buffer containing 1000 mcg./ml. salicylate as the sodium salt. Six 0.1-ml. samples were removed from the inner chamber of each apparatus at the following times after the perfusion experiment was begun: 10, 15, 20, 25, 30, and 35 min.

Crane–Wilson Studies—Four successive 6.5-cm. sections of intestine were obtained; two were stripped of the external musculature, and the remaining two were reserved as nonstripped controls. In successive runs, the order of the segments used for stripping

Table I—Salicylate Transfer across Stripped and Nonstripped Segments of Everted Rat Intestine with Perfusion Apparatus

	Rat Number				
	1	2	3	4	5
			25 cm.		
Stripped rate, mcg./ml./min.	1.69	2.21	2.73	1.77	2.24
Nonstripped rate	1.06	1.25	1.96	1.20	1.53
Ratio of rates stripped/nonstripped	1.59	1.77	1.39	1.48	1.46
Stripped clearance, ml./hr./cm.	0.152	0.199	0.246	0.159	0.201
Nonstripped clearance	0.096	0.112	0.176	0.108	0.137
Lag time, min.					
Stripped	6.2	5.7	4.0	3.7	2.9
Nonstripped	7.5	7.4	5.4	6.6	5.7
			65 cm.		
Stripped rate, mcg./ml./min.	1.94	1.84	1.96	2.13	2.09
Nonstripped rate	1.30	1.22	1.31	1.32	1.23
Ratio	1.49	1.51	1.50	1.61	1.70
Stripped clearance, ml./hr./cm.	0.175	0.166	0.176	0.192	0.188
Nonstripped clearance	0.117	0.110	0.118	0.119	0.111
Lag time, min.					
Stripped	2.8	2.4	2.7	4.2	3.7
Nonstripped	4.2	4.7	6.2	6.0	6.3

Table II—Salicylate Transfer across Stripped and Nonstripped Segments of Everted Rat Intestine with Crane–Wilson Apparatus

	Rat Number				
	1	2	3	4	5
			25 cm.		
Stripped rate, mcg./ml./min.	4.37	4.55	4.35	4.61	4.50
Nonstripped rate	2.24	2.68	2.39	2.56	2.22
Ratio of rates stripped/nonstripped	1.95	1.70	1.82	1.80	2.03
Stripped clearance, ml./hr./cm.	0.158	0.164	0.157	0.166	0.162
Nonstripped clearance	0.081	0.096	0.087	0.092	0.080
Lag time, min.					
Stripped	5.5	7.1	4.6	2.9	4.7
Nonstripped	6.8	8.5	7.2	6.8	6.4
			65 cm.		
Stripped rate, mcg./ml./min.	5.22	3.73	4.44	5.71	4.66
Nonstripped rate	3.24	2.74	3.35	3.43	3.23
Ratio	1.61	1.36	1.33	1.66	1.44
Stripped clearance, ml./hr./cm.	0.188	0.134	0.162	0.206	0.168
Nonstripped clearance	0.118	0.099	0.121	0.123	0.117
Lag time, min.					
Stripped	5.4	4.1	6.0	5.3	2.7
Nonstripped	5.6	7.0	5.1	5.9	3.7

was alternated. Following eversion, cannulated sac preparations were made from the gut segments in the manner described by Crane and Wilson (3). The reservoir of each gut segment was filled with 3 ml. of pH 7.4 buffer, and the gut was immersed in 15 ml. of pH 7.4 buffer containing 1000 mcg./ml. salicylate as the sodium salt. Six 0.1-ml. samples were withdrawn at 5-min. intervals beginning with t_1 = 10 min.

Assay Procedures—The 0.1-ml. samples were diluted with 5.0 ml. of 0.1 *N* sodium hydroxide, and drug concentrations were determined spectrophotofluorometrically as described previously (1). Blank values were sufficiently low at all times during the sampling period and could be ignored. Plots were made of the concentration in the inner chamber *versus* time, as shown in Fig. 2 for a typical perfusion study. Rates of transfer, intercepts, and ratios were obtained by the use of a weighted least-squares CPS program[1]. Weights were assigned as the inverse of the concentration squared; thus, theoretically, all data points are assumed to be equally accurate. All statistical comparisons were tested at 95% or greater confidence levels using Student's *t* test.

Histological Examination—A histological examination was also made to compare an untreated, uneverted intestinal segment with stripped and control everted preparations to confirm which anatomical portions of the membrane were removed and to assess the extent of mucosal trauma induced by the procedures. The tissues were fixed in formalin solution and stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

The rate data were determined as described previously (1) and are reported in Tables I and II. Clearance values, in units of milliliters per hour per centimeter of intestinal length, were calculated by dividing the rate of drug transport across the intestine by the product of the drug concentration in the outside compartment and the length of the intestinal segment. Equation 1 defines clearance in terms of Fick's law parameters:

$$\text{clearance} = \frac{dQ_i/dt}{(C_o)(\text{length})} = \frac{D_m R_{m/s} A_m}{(\Delta x_m)(\text{length})} \quad \text{(Eq. 1)}$$

where:

Q_i = amount of salicylate in the inside compartment at any time, t

[1] Executed on an IBM 360/50 computer through a 2741 remote terminal.

Table III—Summary of Data Presented in Tables I and II (Averages and Standard Deviations)

	Perfusion		Crane–Wilson	
	25 cm. (±*SD*)	65 cm. (±*SD*)	25 cm. (±*SD*)	65 cm. (±*SD*)
Stripped rate, mcg./ml./min.	2.13 (0.42)	1.99 (0.18)	4.48 (0.11)	4.75 (0.76)
Stripped clearance, ml./hr./cm.	0.191 (0.037)	0.179 (0.011)	1.161 (0.004)	0.172 (0.027)
Nonstripped rate	1.40 (0.36)	1.28 (0.05)	2.42 (0.20)	3.20 (0.27)
Nonstripped clearance	0.126 (0.032)	0.115 (0.004)	0.087 (0.007)	0.116 (0.010)
Ratio of rates (or clearances), stripped/nonstripped:	1.54 (0.15)	1.56 (0.09)	1.86 (0.13)	1.48 (0.15)
Lag time, min.				
Stripped	4.5 (1.4)	3.2 (0.8)	5 (1.5)	4.7 (1.3)
Nonstripped	6.5 (1.0)	5.5 (1.0)	7.1 (0.8)	5.5 (1.2)

D_m = effective diffusivity of salicylate in intestinal membrane
A_m = total area of the membrane available for salicylate diffusion
$R_{m/s}$ = partition coefficient for salicylate between the membrane and solvent
Δx_m = thickness of the membrane
C_o = concentration of salicylate in the outside compartment at any time, t

Since salicylate was used in all runs at the same outside concentration (1000 mcg./ml.) and at the same pH, it seems reasonable to conclude that D_m and $R_{m/s}$ are constant throughout all experiments. Assuming that there is no significant difference in the circumference (A_m/length) of the intestinal segments used, clearance should equal a constant divided by the thickness of the intestinal strip. In Tables I and II the ratios reported are the rates through the stripped segment divided by the rates through the nonstripped segment, both from the same rat. This ratio should exhibit an inverse relation with respect to the thickness of the intestine, *i.e.*, the greater the ratio the thinner the intestine.

Lag times (Tables I and II) should be directly related to the thickness of the intestinal wall. That is, the thinner the intestinal wall, the shorter is the time required for a steady state to be reached in the membrane. This is reflected in the shorter lag times seen for the stripped intestine as compared to those for the nonstripped intestine (Table III).

The average ratios of the clearance through the stripped segment to clearance through the nonstripped segment, as well as average lag times, are given in Table III. The inverse relation of the ratios and the direct relation of lag time to thickness were not found to be related by an absolute proportionality constant. This result is certainly due to the heterogeneity of the cell types and tissues densities encountered in transport. For example, the cellular composition of the submucosa is less dense than that of the muscularis externa, which is surgically removed from the stripped segment. Although an absolute proportionality cannot be made between lag time or rate of absorption and thickness, it should be noted that the average ratios for perfusion at 65 cm. (Table III) and for perfusion at 25 cm. have values close to the ratios of the average lag times (*i.e.*, at 65 cm., the lag time ratio is 1.72; at 25 cm., the lag time ratio is 1.44).

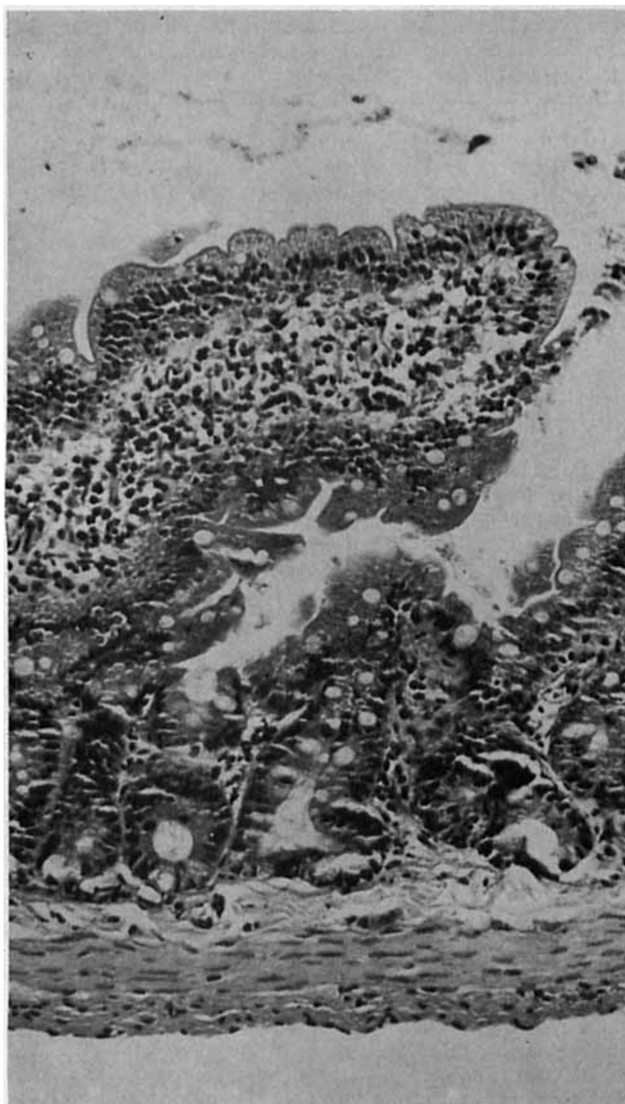
During this work, several other experiments were undertaken to determine the significance of: (*a*) the effect of using 95% air with 5% CO_2 *versus* the effect of using 95% O_2 with 5% CO_2 to oxygenate the preparations during each run, (*b*) the effect on the rate of transport of aerating the inside compartment in the Crane–Wilson studies *versus* nonaeration, and (*c*) differences between rates of absorption 25 and 65 cm. distal to the pylorus. In the trials undertaken to determine the effect of 95% O_2 *versus* 95% air, no significant difference was found either in the rates of absorption or in lag times. In the studies to determine the effect of aerating the inside compartment in the Crane–Wilson method, no advantage in total oxygenation was found over the method in which only the outside of the compartment was oxygenated. However, problems arose when the inside compartment was oxygenated, since air bubbles occasionally became lodged in the intestine, thereby separating the fluid at the bottom of the cannulated sac from that at the top and, thus, preventing uniform mixing of the inside fluids. For this reason, oxygenation of the inside compartment was discontinued and all reported Crane–Wilson data were obtained with outside aeration only. Experiments were also run to determine if any significant difference in absorption rates would be obtained through intestinal segments 25 and 65 cm. distal to the pylorus. In Table III it can be seen that the ratios of rates or clearances (stripped to nonstripped) for perfusion studies at 25 and 65 cm. and for Crane–Wilson studies at 65 cm. are not significantly different. The nonstripped Crane–Wilson studies at 25 cm. are significantly different ($p < 0.05$) than the other three ratios, but no explanation for this difference can be offered at this time.

It would appear that an artifact could be introduced as a function of the design of the apparatus itself. Normally, in carrying out studies using the Crane–Wilson type of sac, a small weight is attached to the bottom end of the intestinal segment so that the segment remains taut during the absorption run. However, weights were not used in this experiment since once the muscularis externa is removed the stripped segments tend to stretch to a length of about 8 cm., even when no weight is attached. This extension is due to the effect of the pressure head of water in the internal reservior. This phenomenon was not noted in the perfusion apparatus where there is a continuous flow of fluid through the tissue segment.

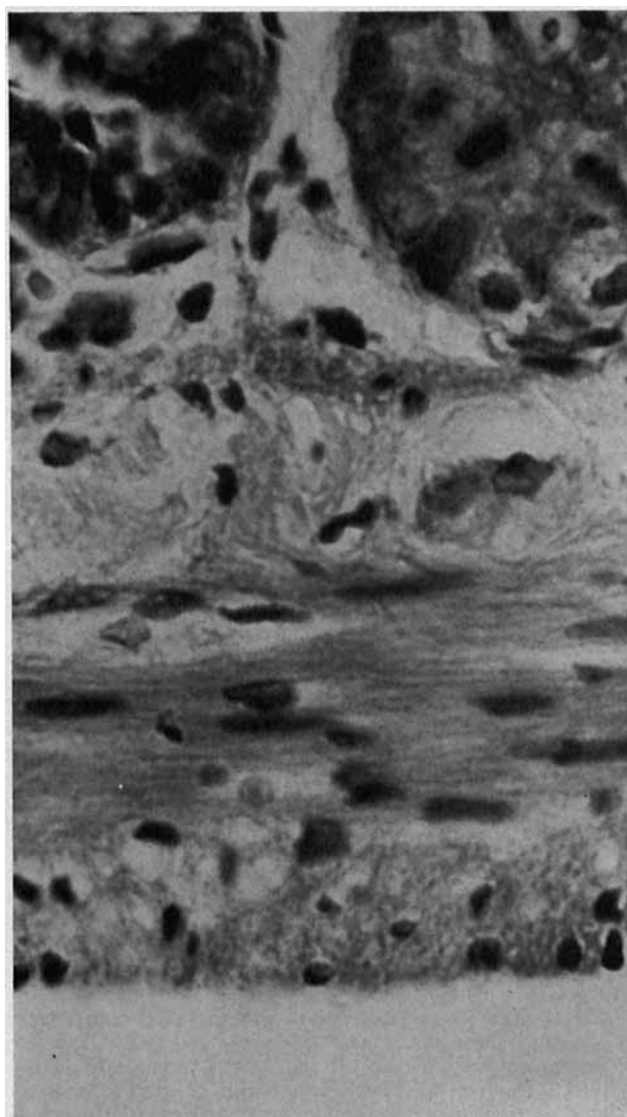
This work was designed to develop a well-founded and easily utilized *in vitro* procedure for studying drug absorption under conditions simulating *in vivo* transport. Therefore, a technique was developed for removing the serosa and the two layers of smooth muscle that form the muscularis externa, so that these anatomical portions of the rat intestine cannot serve as absorption barriers to drug transport. Up to the present time, all *in vitro* intestinal absorption studies with drugs have measured transport across the total thickness of the intestine, including the muscularis externa and serosa as shown in Fig. 1. However, as can be seen in Fig. 1, the capillary supply to the intestine is in the base of the submucosa, at a point in the membrane proximal to the muscularis externa when transport occurs from the intestinal lumen to blood.

To ascertain which anatomical portions of the intestine had been removed by the surgical procedures and to make sure that the structural integrity of the mucosal border had been maintained, histological studies of the everted rat gut preparations were undertaken. Four photographs, representative of the observations made, were taken of the intestine of a single rat. Figures 3*a* and 3*b* are of a cross section of rat jejunum on which no mechanical manipulations have been performed. That is, this section was neither placed on glass rods nor everted. The portions of the intestine in the lower half of Fig. 3*b* are those layers of muscle removed by the surgical procedure; Fig. 3*c* was made from a section of rat jejunum that would serve as the nonstripped control segment discussed with respect to Tables I and II. This section was placed on the glass rod, subjected to ice bath temperatures, removed from the rod, brought back to room temperature, and everted. Figure 3*d* was made using a section of rat jejunum that had undergone the surgical procedure to remove the serosa and muscularis externa, as described in the *Experimental* section, as well as the procedures listed for the control above.

At the start of this experiment, it was feared that the integrity of the epithelium and underlying submucosa would be damaged by the stripping and eversion process. As can be seen by comparing Fig. 3*a* with Fig. 3*d*, there was no damage to the epithelium or to the integrity of the submucosa of the stripped segment. Figure 3*c*, depicting an intestinal section subjected to control procedures, indicated that the control process and eversion of the intestine cause some disruption of the muscularis externa, in comparison to the untreated section in Figs. 3*a* and 3*b*. The control procedure could account for the fact that the clearance values obtained from the control (unstripped) runs for a 10-cm. segment ranged from 0.87 to 1.16 ml./hr. for salicylate, while the clearance for salicylate reported by Mayersohn *et al.* (12) using the Crane–Wilson method



(a)



(b)

Figure 3—(a) *Cross section of rat jejunum on which no mechanical manipulations have been performed* (×*150*). (b) *High power magnification of the muscularis externa and submucosal portions of Fig.* 3a. *Note cellular composition and density of serosa and muscularis externa* (×*625*).

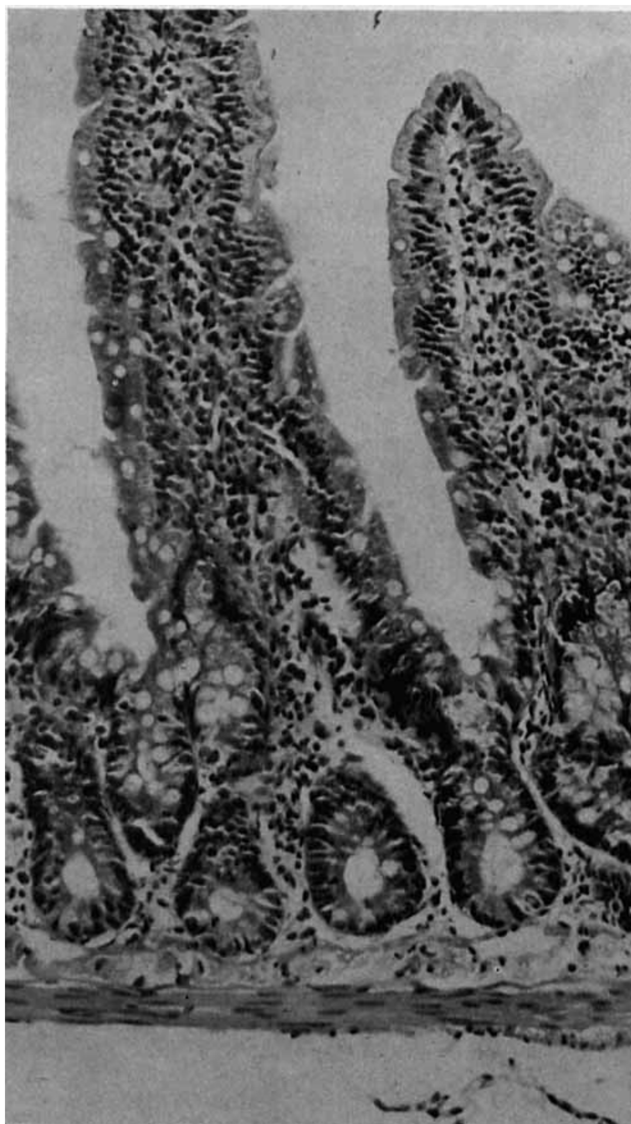
was 0.8 ml./hr. However, statistically there is no significant difference between the average values found in this study and the previously reported studies at the 95% level of confidence.

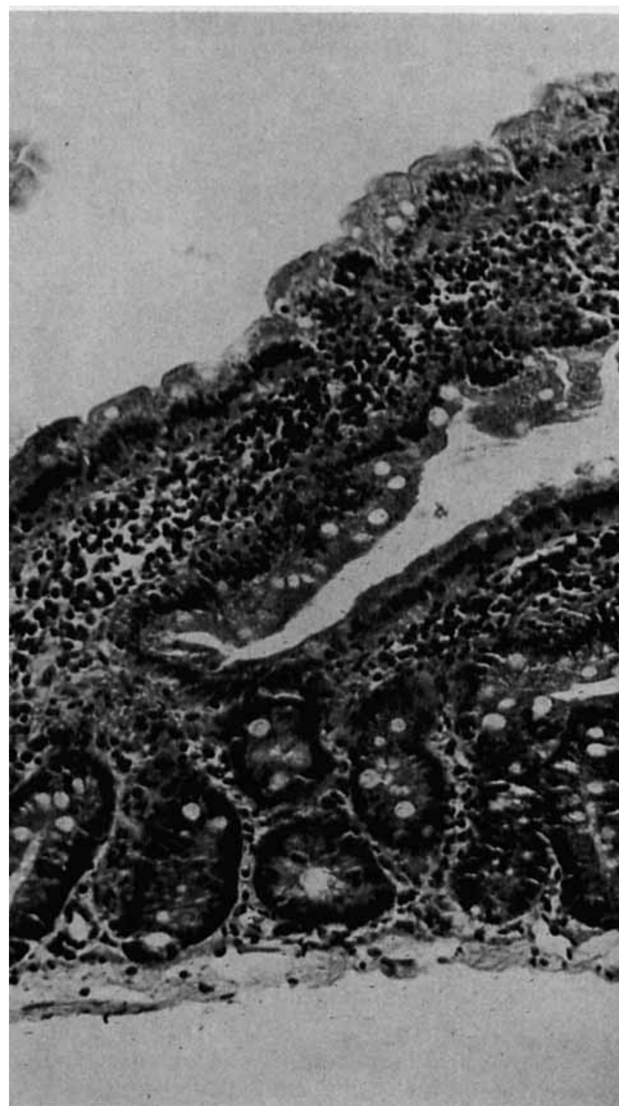
By comparing Figs. 3*a* and 3*c* with Fig. 3*d*, the relative thickness of the muscularis externa barrier to *in vitro* transport can be estimated. The significance of this barrier is clearly shown by the average ratios reported in Table III. The ratios of clearance values (stripped/unstripped) range from 1.54 to 1.86 in the four studies described, indicating that removal of this artifactual barrier to salicylate transport in *in vitro* studies brought about a 50–85% increase in transport rates. With the *in vitro* techniques previously reported in the literature (2–4), drug has to diffuse through several layers of tissue beyond the capillary bed, the point where the drug would enter into the blood circulation during *in vivo* transport. Using the techniques reported in this work, the serosal solution would be in contact with the submucosa of the gut preparation at the bottom of Fig. 3*d*.

SUMMARY AND CONCLUSIONS

The *in vitro* absorption kinetics for salicylate ion were followed through rat intestinal membranes from which the serosa and longitudinal and transverse layers of the muscularis externa had been removed. It was found that the serosa and muscularis externa can be removed from an intestinal segment in one intact sheet and that there is no resulting damage to the absorptive tissue as a consequence of the surgical procedure. Furthermore, it was established that when the stripping procedure is incorporated with the presently used *in vitro* techniques for studying drug absorption, there is a substantial increase in salicylate transport rates and a corresponding decrease in lag times. This is clearly shown by the data in Table III. For example, Crane–Wilson studies at 25 cm. through unstripped intestinal membranes yielded salicylate clearance values of 0.087 ml./hr./cm. [Mayersohn *et al.* (12) reported a value of 0.080 ml./hr./cm.], while salicylate clearance through stripped segments was found to be 0.161 ml./hr./cm. Since the great majority of the capillaries in the small intestine are found in the lamina propria at the base of the epithelial cells, it is highly improbable that *in vivo* drug absorption is retarded by any physical barrier comparable to that of the serosa and muscularis externa.

In summary, the procedure for surgically removing the serosa and muscularis externa did not produce any change in the integrity of the epithelium, lamina propria, or submucosa. The procedure can be easily incorporated into the three commonly employed *in vitro* techniques utilized in studying drug absorption. The procedure allows the removal of a significant artifactual influence to the comparison of *in vitro* and *in vivo* transport studies. In light of these findings, it is the opinion of the authors that the use of stripped intestinal segments for *in vitro* drug transport studies will provide the investigator with a well-controlled, reproducible technique for elucidating the mechanisms involved in the *in vivo* absorption of drugs from the intestinal lumen.





(c) (d)

Figure 3—(c) *Cross section of everted rat jejunum that has been subjected to the control procedure (before incubation)* (×*150*). (d) *Cross section of everted rat jejunum from which the serosa and muscularis externa have been removed. Note the integrity of the epithelium and submucosa* (×*150*).

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