Rate-Limiting Barriers in Intestinal Absorption (36823)

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(Introduced by G. Levy)

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Absorption rates of certain poorly lipid-soluble compounds across the isolated rat intestine increase substantially and continually with time (1), apparently due to progressive loss of structural integrity of the epithelium (2). However, loss of structural integrity has little effect on the absorption rates of certain nonpolar, lipid-soluble compounds suggesting that the epithelium is not a critical barrier to the intestinal absorption of such compounds (1). In the present study, this hypothesis was tested directly by comparing the *in vitro* and *in situ* flux of various solutes across the normal intestine of the rat and across the rat intestine in which the epithelium was substantially disrupted. The epithelial border was found to be a critical anatomical barrier to the *in vitro* and *in situ* intestinal absorption of both charged and uncharged poorly lipid-soluble compounds. However, contrary to the prevailing view (2–4), our studies also showed that the intestinal epithelium is not the rate-limiting barrier to the passive diffusion of rapidly absorbed, freely lipid-soluble compounds.

Methods. Intestinal transfer rates were determined *in vitro* with everted jeunal segments (10 cm in length) from fasted male Sprague-Dawley rats (weighing about 250 g) using a modification (5) of the method of Crane and Wilson (6), (condition I). Two milliliters of a modified physiologic Krebs bicarbonate buffer (pH 7.4) were placed inside the everted sac (serosal solution) and the entire preparation was placed in 100 ml of buffer solution containing a test compound (mucosal solution) which was continually gassed with O_2:CO_2 (95:5; v/v) and maintained at 37°. The serosal fluid was quantitatively sampled every 10 min for 30 min and each sample was assayed for the test compound. Aniline, salicylamide, salicylate, sulfanilamide, pyridine aldoxime methiodide, riboflavin and methyl orange were assayed as previously described (7). ^{14}C-Butanol (New England Nuclear; sp act, 3.7 mCi/mmole) was determined by liquid scintillation spectrometry. Methylene blue was determined spectrophotometrically at 663 nm. Bretylium tosylate was determined by ion-pair extraction with bromothymol blue; the resulting complex was assayed spectrophotometrically at 410 nm. The concentration of a given compound in the mucosal solution remained essentially constant throughout the experiment, due to the large volume of that solution.

In other experiments, the everted intestinal sac was incubated for 30 min in buffer at 37°. The mucosal surface was then scraped with forceps to separate the epithelial layer from the muscularis. The resulting preparation (condition III) was then transferred to mucosal solution containing the test compound and transfer rates determined in the usual manner. For comparison, permeability studies were also carried out using jejunal segments which were incubated at 37° in buffer for 30 min but not scraped (condition II).

In situ absorption rates were determined using a previously described intestinal preparation with an intact blood supply (8). The disappearance of several test compounds from the entire small intestine of fasted, male Sprague-Dawley rats (weighing about 250 g), anesthesitized with ethyl carbamate (1.3 mg/g, ip), was followed under control conditions and experimental conditions wherein the mucosal epithelium was substantially disrupted by pretreatment with sodium deoxycholate. The entire intestine was first

washed with saline and 7 ml of either isotonic buffer or 50 m*M* sodium deoxycholate in modified Krebs buffer was placed in the intestine for 5 min. At the end of this period the intestine was thoroughly rinsed with saline and 7 ml of buffer containing the test compound(s) was placed in the intestine and sampled at convenient intervals for 20 or 30 min. All samples were diluted and centrifuged to remove tissue. Riboflavin (9) and salicylamide (10) concentrations were determined by fluorimetry. ^{3}H-QX-572 [*N*, *N*-bis (phenylcarbamoylmethyl) dimethyl ammonium chloride, Astra Pharmaceuticals, 15.4 μCi/mg] was determined by liquid scintillation spectrometry. Benzocaine concentrations were determined by means of the Bratton–Marshall procedure (11). The volume of luminal solution was maintained constant by adding buffer immediately prior to sample removal.

Results and Discussion. Histological examination of sections of jejunal tissue prepared immediately after eversion indicated little morphological change (see Fig. 1A). Accordingly, absorption data obtained with these segments (condition I) were considered representative of the permeability of the isolated intact jejunum. As seen in Fig. 1B, jejunal segments subjected to incubation and scraping (condition III) showed total disruption of the epithelial border. Tissue sections prepared immediately after the everted jejunal segment was incubated for 30 min in buffer (condition II) revealed rupture of some of the epithelial cells and significant loss of cellular detail.

Histological studies were also carried out during the course of the *in situ* absorption procedure. Examination of intestinal sections prepared immediately after the 5 min incubation with buffer reveals essentially normal villi (see Fig. 1C). However, as shown in Fig. 1D, sections prepared after incubation with sodium deoxycholate indicate almost total rupture of the epithelial border and a virtually complete loss of cellular detail.

The absorption data presented in Tables I and II demonstrate that the intestinal epithelium is indeed a rate-determining barrier to the transport of a number of the test solutes. *In vitro* (see Table I), the flux of relatively polar, poorly lipid-soluble molecules with clearance values of less than 0.02 ml/min across the intact intestine (condition I) increases markedly upon disruption of the epithelium by incubation or incubation and scraping. Similarly, extensive disruption of the intestinal epithelium *in situ* (see Table II and Fig. 2) increases substantially the ab-

TABLE I. Mucosal-to-Serosal Clearance of Various Solutes Across the Everted Isolated Rat Jejunum.[a]

Test compound	Mucosal soln concn (μg/ml)	Clearance (ml/min $\times$ 10^4) $\pm$ SD[b]			Condition III/ condition I
		Condition I	Condition II	Condition III	
Aniline	100	648 $\pm$ 71	632 $\pm$ 31	717 $\pm$ 100	1.11
^{14}C-Butanol	1000	560 $\pm$ 30	557 $\pm$ 90	610 $\pm$ 53	1.09
Salicylamide	1000	480 $\pm$ 38	453 $\pm$ 41	495 $\pm$ 50	1.03
Salicylate	2000	145 $\pm$ 14	195 $\pm$ 14	250 $\pm$ 20	1.72
Sulfanilamide	100	118 $\pm$ 12	143 $\pm$ 11	220 $\pm$ 22	1.86
Bretylium tosylate	2000	109 $\pm$ 21	116 $\pm$ 23	197 $\pm$ 16	1.81
Pyridine aldoxime methiodide	400	98 $\pm$ 29	150 $\pm$ 26	191 $\pm$ 15	1.95
Riboflavin	20	43 $\pm$ 6	77 $\pm$ 8	136 $\pm$ 30	3.16
Methyl orange	250	12 $\pm$ 2	33 $\pm$ 13	103 $\pm$ 7	8.58
Methylene blue	3150	1.1 $\pm$ .6	.7 $\pm$ .4	17 $\pm$ 6	15.45

[a] Transfer rates were determined with freshly prepared segments with an intact epithelial border (condition I), segments which were incubated in buffer at 37° for 30 min (condition II) and segments which were incubated and scraped free of epithelium (condition III).

[b] Clearance values represent the mean of 5 segments (1 segment/rat) and were calculated by dividing the mean transfer rate over a 30 min period by the mucosal concentration.

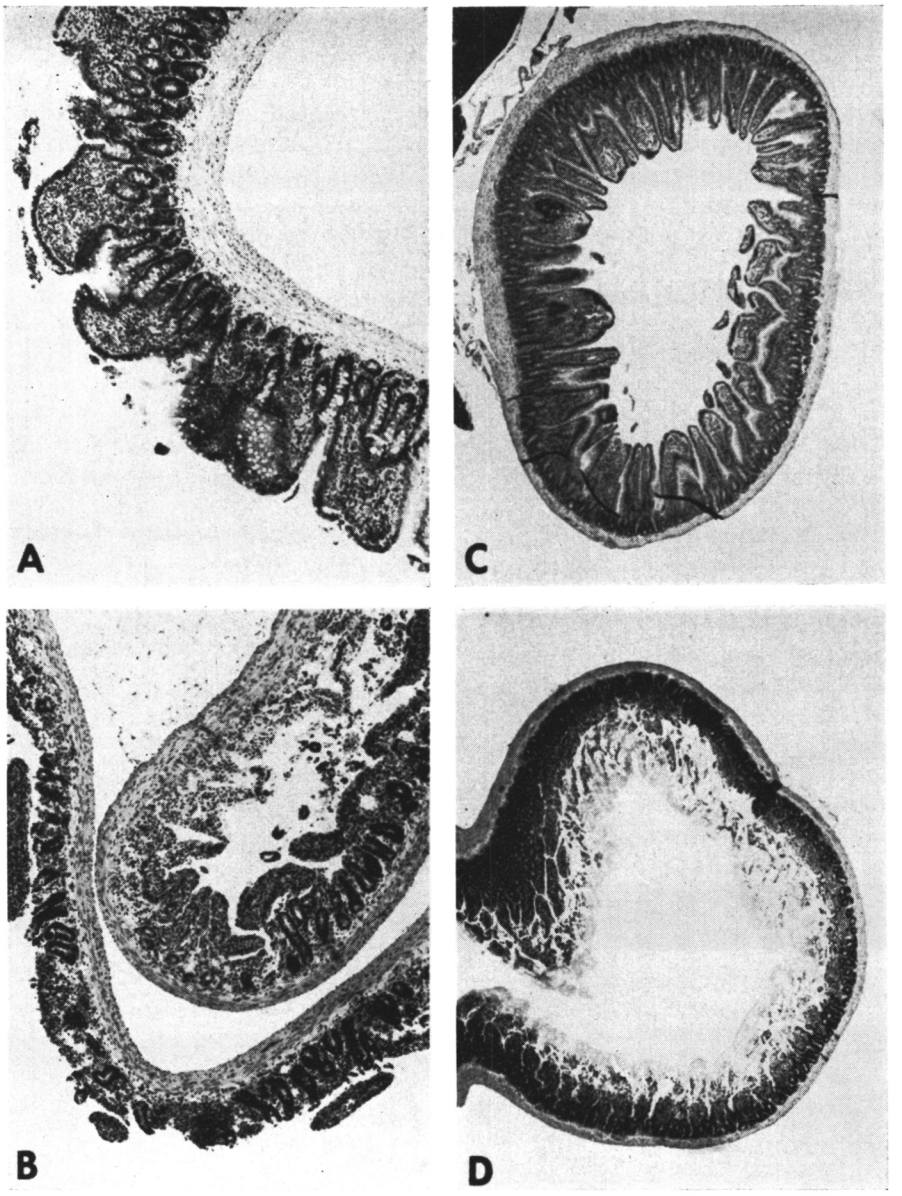

FIG. 1. Rat jejunal sections stained with haemotoxylin and eosin. (A) Freshly prepared everted segment. (B) Everted segment in which the mucosal epithelium was removed by scraping. (C) Cross-section of jejunal segment after *in situ* pretreatment with buffer. (D) Cross-section of jejunal segment after *in situ* pretreatment with sodium deoxycholate. The high magnifications are ×68 and the low magnifications are ×25.

sorption rate of the poorly lipid-soluble compounds, riboflavin and QX-572.

On the other hand, the intestinal epithelium represents an insignificant barrier to the absorption of freely lipid-soluble compounds. *In vitro*, the three most lipid-soluble compounds (aniline, butanol and salicylamide) show virtually the same clearance across intact intestine and the intestine scraped free of the epithelial border. Moreover, disruption

TABLE II. Effect of Epithelial Disruption by Sodium Deoxycholate on the *in Situ* Absorption of Several Test Compounds from the Small Intestine of Rats.[a]

Test compound	Initial concn (μg/ml)	Percentage absorbed $\pm$ SD[b] at 10 min		Percentage absorbed $\pm$ SD[b] at 20 min	
		Control	SDC	Control	SDC
Riboflavin	62	15 $\pm$ 11	35 $\pm$ 5	13 $\pm$ 10	47 $\pm$ 3
^{3}H-QX-572	400	22 $\pm$ 5	43 $\pm$ 3	27 $\pm$ 4	60 $\pm$ 4
Benzocaine	390	67 $\pm$ 6	51 $\pm$ 5	87 $\pm$ 4	73 $\pm$ 6
Salicylamide	1760	78 $\pm$ 3	68 $\pm$ 3	91 $\pm$ 2	82 $\pm$ 7

[a] Each value represents the mean of five animals. "Control" indicates absorption studies conducted after pretreatment with isotonic buffer and "SDC" denotes absorption studies conducted after pretreatment with 50 m*M* sodium deoxycholate in modified Krebs buffer.

[b] Each value represents the mean of 5 determinations (1 determination/animal). The absorption of riboflavin and salicylamide was studied separately while the absorption of benzocaine and ^{3}H-QX-572 was determined simultaneously.

of the epithelial border *in situ* had no enhancing effect on the absorption of benzocaine and salicylamide. In fact, pretreatment of the intestine produced a modest but statistically significant inhibitory effect on the initial absorption rate of these compounds.

The rate-limiting step in the intestinal absorption of lipid-soluble compounds may be diffusion across unstirred water layers adjacent to the biologic membranes (12). If this were rate-determining, then reduction in the thickness of these water layers should enhance the transfer rate. However, vigorous stirring of the mucosal solution (sufficient to produce a distinct vortex) during permeability studies under condition I, had no effect on the transfer rate of either aniline or salicylamide. Therefore, since neither a reduction in the thickness of unstirred layers nor a disruption of the epithelium affected the *in vitro* permeation rate of lipid-soluble compounds, one must conclude that an anatomical barrier distinct from the epithelial cell surface is rate-limiting with respect to passive diffusion of these compounds across the isolated intestine. The importance of unstirred water layers (12) as well as blood flow (13), as rate-controlling factors in the *in vivo* intestinal absorption of lipid soluble compounds must be assessed since it is evident that the epithelial cell surface is of little importance in this process.

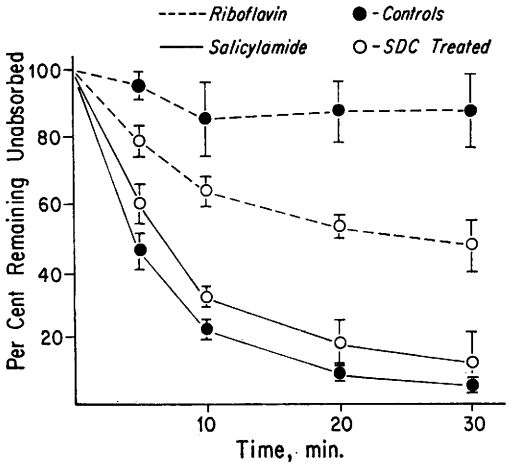

FIG. 2. Effect of epithelial disruption on the *in situ* intestinal absorption of a poorly lipid-soluble compound (riboflavin) and freely lipid-soluble compound (salicylamide).

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