Permeability Characteristics of Various Intestinal Regions of Rabbit, Dog, and Monkey

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The *in vitro* permeability of a series of both hydrophilic and lipophilic compounds, as defined by the octanol/water partition coefficient, was measured in four segments of rabbit, monkey, and dog intestine using a side-by-side diffusion cell. A linear relationship was established for tissue resistance to hydrophilic compound diffusion in jejunum and colon among rabbit, monkey, and dog. The results suggest that rabbit jejunum is twice as permeable as monkey and dog jejunum. The colonic tissues of monkey, rabbit, and dog demonstrate similar permeabilities. Measuring the permeabilities of different tissues with compounds of similar physicochemical properties allows comparison of tissue restriction to transport. Thus, *in vitro* permeability measurements may be used to investigate physiological differences of various intestinal tissue segments that influence tissue permeability. Investigating the permeability of different intestinal segments from various species could allow the identification of an appropriate *in vitro* intestinal permeability model that will lead to the prediction of intestinal absorption in humans, eliminating the need for extensive and often misleading *in vivo* animal testing.

KEY WORDS: intestinal permeability; partition coefficient; jejunum; colon; species differences.

INTRODUCTION

It is an early goal of drug development to evaluate the potential oral absorption of a candidate compound in animal studies. The results of these studies depend upon the species selected, and little consistency is observed in the study of specific compounds in different species. No single laboratory species has been defined as a suitable model of human drug absorption. Species selection for early studies often results from such unrelated characteristics as ease of handling and cost.

In vitro methods are a possible alternative to whole-animal studies (1–5). Due to difficulties in obtaining viable human tissues, most studies have focused on the characterization of a single intestinal region from a single animal species.

Transport of compounds across intestinal tissue can occur via paracellular and transcellular pathways. Generally, the paracellular route involves the movement of small hydrophilic compounds, while the transcellular pathway is available to compounds that are lipophilic, capable of binding to a receptor/carrier, or endocytosed. Facilitated absorption via receptors or carriers depends upon the number of receptors/carriers and the affinity of the compound to the receptor/carrier binding site. Passive absorption of small hydrophilic and lipophilic compounds depends upon the physicochemical properties of the compound, such as molecular size, ionic charge, and partition coefficient (6). Intestinal permeability directly measures the interactions of the substrate and the tissue and is influenced by the physiological properties of the tissue and physicochemical properties of the transported compound. Hence permeability measurements should be a more useful parameter to predict absorption than physicochemical data alone. The intrinsic characteristics of intestinal tissue can be compared among various intestinal segments and species and may permit extrapolation to human tissue. Additionally, although substrates are generally absorbed throughout the GI tract, site-specific absorption has been demonstrated in several cases (7,8).

EXPERIMENTAL

Materials

Radiolabeled mannitol, polyethylene glycol (PEG) 900, PEG 4000, hydrocortisone, and progesterone were vacuum-dried immediately before use. Radiolabeled naproxen and ganciclovir were purified by thin-layer chromatography (TLC) and vacuum-dried before use. Naproxen was purified using a silica plate and a mobile phase consisting of hexane: ethyl acetate:acetic acid (66:33:1). Ganciclovir TLC utilized and avicel stationary phase and water as the mobile phase. Radiolabeled methanol and water were used as received. Supplier, specific activity, and type of radiolabel are indicated for each compound in Table I. All other chemicals were either reagent or analytical grade and used as received. Male albino New Zealand rabbits (Hazelton) weighing between 2.5 and 3.5 kg were fasted overnight before use in experiments. Intestinal sections from cynomologus monkeys and beagle dogs, both male and female, were obtained from animals used as controls various in necropsy studies (Syntex Research, Department of Toxicology, Palo Alto, CA).

Methods

Preparation of Buffers

Mucosal and serosal solutions were prepared as described previously (1,2). In brief, oxygenated (O_2/CO_2, 95/5) Kreb's ringer bicarbonate buffer, pH 7.4, was used to prepare all solutions. The serosal solution included unlabeled D-glucose (40 m*M*) to maintain tissue viability. Trace amounts of radiolabeled compounds were added to the mucosal solution. To equalize osmotic pressure between mucosal and serosal chamber, 40 m*M* mannitol was added to the mucosal solution in all cases with the exception of D-glucose transport studies, where 10 m*M* D-glucose and 30 m*M* mannitol were used instead.

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Table I. Radiolabel, Supplier, Specific Activity, and Concentration Applied to the Tissue for Compounds Used in *in Vitro* Permeability Determinations

Drug name	Label	Supplier	Specific activity (Ci/mmol)	Applied concentration ($\times 10^{-5}$ M)
Methanol	^{14}C	NEN[a]	0.058	0.15
Mannitol	^{14}C	Amersham[b]	0.055	4000
D-Glucose	^{3}H	Amersham	6.44	1000
Ganciclovir	^{3}H	Syntex[c]	18.63	0.002
Naproxen	^{3}H	Syntex	18	0.002
PEG 900	^{3}H	NEN	8.55	0.003
PEG 4000	^{14}C	Amersham	0.006	1500
Hydrocortisone	^{3}H	NEN	99.8	0.001
Progesterone	^{3}H	Amersham	56	0.0008

[a] New England Nuclear, Boston, MA.
[b] Amersham, Arlington Heights, IL.
[c] Syntex Research, Palo Alto, CA.

In Vitro *Experiments*

Rabbits were sacrificed by rapid injections of sodium pentobarbital through a marginal ear vein. Following a midline incision, the intestinal tract was removed and individual segments were separated. Monkeys and dogs were sacrificed by an overdose of sodium pentobarbital and exsanguinated before surgery. The intestinal segments were removed within 10 min of death. Tissues were placed immediately in ice-cold oxygenated Kreb's ringer bicarbonate buffer.

Tissues were mounted in diffusion cells according to an established procedure (1,2). Briefly, the tissue section was opened along the mesenteric border, placed on a preheated (37°C) acrylic half-cell (Precision Instrument Design, Tahoe City, CA), and stripped of its muscle layers. The matching half-cell was joined to seal the diffusion apparatus. The cells were then immediately placed in an aluminum block heater (37°C) and the reservoirs filled with warmed (37°C) mucosal and serosal solutions, which were circulated by gas lift (O_2/CO_2, 95/5) at a flow rate between 15 and 20 ml/min (9).

Samples (0.1 ml) of the mucosal solution were taken before addition to the diffusion cell and from the mucosal chamber at the conclusion of the experiment. Samples (1.0 ml) from the serosal chamber were taken at appropriate time points, with replacement of the sampled volume with fresh serosal solution. Samples were placed in scintillation vials, mixed with scintillation cocktail (Ready Safe, Beckman Instruments, Inc., Fullerton, CA), and counted in a scintillation counter using an external standardization method. Unless noted otherwise permeability was measured over a 2-hr period.

Tissue Thickness Measurements

Samples of duodenum, jejunum, ileum, and proximal colon from rabbit, monkey, and dog were stripped of their muscle layers, fixed in 10% formalin, embedded in paraffin, sectioned, mounted, and stained with hemotoxylin and eosin. Sections were viewed by light microscope and photographed. Using a micrometer, measurements of tissue thickness were taken from the serosal edge to the tips of the villi.

Statistics

Results are presented as means ± SE. Unless otherwise noted, statistical comparisons were made with analysis of variance (ANOVA) at a 95% confidence level, using Fisher's protected least significant difference (PLSD) as the test statistic.

RESULTS

The permeabilities of both hydrophilic and lipophilic compounds, and an active transport marker, D-glucose, were measured in the duodenum, jejunum, ileum, and proximal colon of rabbit, monkey, and dog (Fig. 1, Tables II and III). In selected cases, the inhibitors ouabain and phlorizin were used in conjunction with D-glucose (Table III). Mannitol, ganciclovir, naproxen, and PEG are known to be absorbed intact (10–13), however, metabolism of probe compounds was not determined in these studies.

Segmental Permeability Differences

For all three species, an increase in tissue permeability was noted from proximal to distal intestine (Fig. 1, Table II). Exceptions to this trend include hydrocortisone and progesterone permeability in monkey intestine and hydrocortisone permeability in rabbit small intestine, where there was no statistical difference in permeability between segments.

Differences in permeability between the small intestine and the proximal colon were apparent (Fig. 1). For all species, the proximal colon was more permeable than the proximal and mid small intestine ($P \leq 0.01$). For monkey and rabbit, hydrophilic compounds ($\log PC < 0$) demonstrated only a relatively modest increase in permeability, ex. jejunum, and colon. In contrast, lipophilic compounds, especially progesterone, were subject to large increases in permeability when comparing jejunum to colon. For dog, however, permeability increased significantly in colonic tissue compared to that of small intestine, relatively independent of partition coefficient.

Net water flux was also determined in both jejunum and colon of rabbit. For both segments, a small but statistically

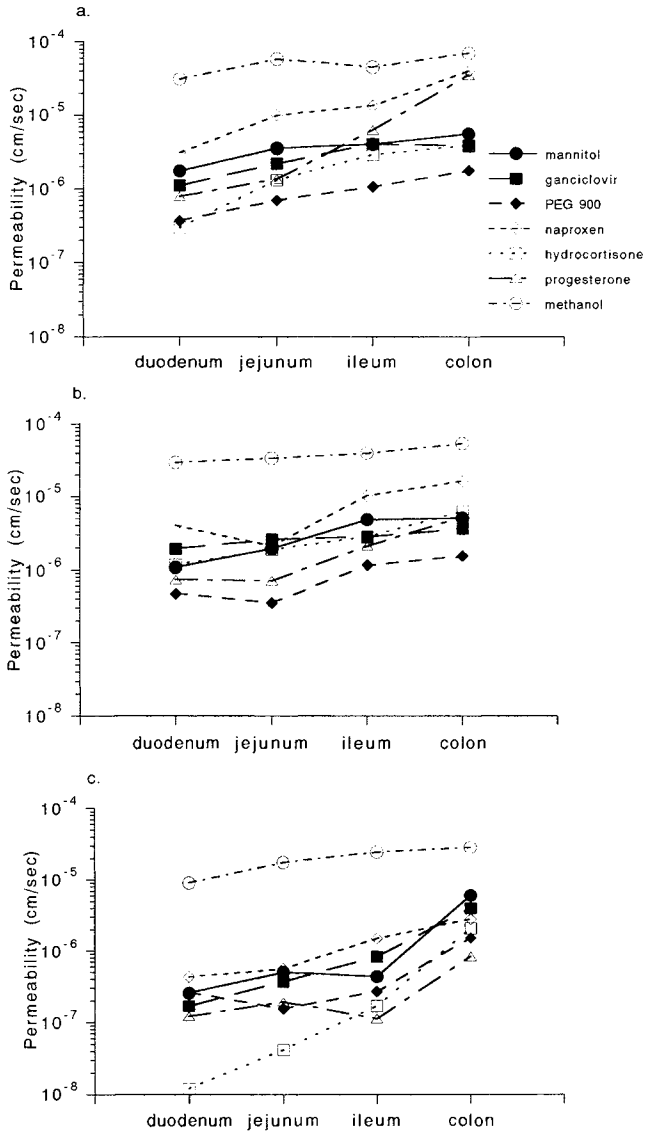

Fig. 1. Segmental permeability of (a) rabbit, (b) monkey, and (c) dog intestine.

insignificant difference was noted in the transport of tritiated water in both the mucosal-to-serosal and the opposite directions (Table II).

Species Permeability Differences

Differences in the permeability of these compounds through tissues of different species were evident (Fig. 1, Table II). For segments of small intestine, rabbit and monkey tissues were more permeable than corresponding segments of dog intestine ($P \leq 0.01$). However, specific exceptions included PEG 900 permeability, which was equivalent in the duodenum of all species, hydrocortisone permeability, which was similar in rabbit and dog duodenum, and progesterone permeability, which was equal in monkey and dog ileum. No consistent difference between rabbit and monkey duodenal and ileal permeability was noted.

D-Glucose Permeability

The permeability of D-glucose was measured with and without the inhibitors ouabain and phlorizin (Table III). In monkey and rabbit, significant inhibition of D-glucose transport was noted with both inhibitors, although phlorizin inhibition was greater at the concentration used. In dog, however, significant inhibition of D-glucose transport was noted only in duodenum.

Mannitol has physicochemical characteristics similar to those of D-glucose but is passively transported. Comparisons were made between the permeabilities of these compounds. Consistent with the decrease in permeability with inhibitors, D-glucose transport was a factor of two to three times that of mannitol. Notable exceptions include monkey jejunum, where glucose transport was over six times that of mannitol, and this result is consistent with the large decreases observed with inhibitors. In contrast, dog colon demonstrated no significant difference in permeability between mannitol and D-glucose.

Tissue Thickness

Intestinal tissue, stripped of its serosal muscle layers, was measured to estimate the thickness of the *in vitro* permeability barrier (Fig. 2). Little difference in thickness was noted between intestinal segments of rabbit or monkey. However, dog intestine was considerably thicker than corresponding segments of rabbit or monkey.

DISCUSSION

D-Glucose Transport

Active transport of D-glucose by rabbit and monkey jejunum *in vitro* has been previously demonstrated with the use of the inhibitors ouabain and phlorizin (1,2). Surprisingly, active transport of D-glucose could not be determined in dog jejunum using similar methods. Given the relatively greater thickness of dog jejunum compared to that of the other species, it is possible that the serosally administered ouabain does not reach the absorptive cell at a high enough concentration to inhibit a significant amount of ATP production and, hence, does not effectively inhibit D-glucose transport (14). However, D-glucose transport was significantly greater than that of the passive transport marker mannitol, suggesting that dog jejunum does actively transport D-glucose *in vitro*.

Transport of Hydrophilic Compounds

Transport of polar solutes such as mannitol and PEG occurs by passive diffusion since these compounds are known to be restricted to the extracellular space. Although the exact mechanism of transport of ganciclovir is not known, it is similar to mannitol in physicochemical characteristics and measured permeability (Table II). Ganciclovir is also structurally similar to acyclovir, a compound passively absorbed by rat jejunum (10,15). Ganciclovir permeability in rabbit jejunum was concentration independent and was not inhibited by 0.2 m*M* ouabain (unpublished data).

Restriction of the paracellular space is determined primarily by the characteristics of the tight junctions between cells. The epithelial junctions become tighter progressing

Table II. Measured Permeability ($\times 10^{-6}$ cm/sec) of Various Compounds in Segments of Rabbit, Monkey, and Dog Intestine

Species	Compound	MW	log *PC*	Duodenum	*N*[a]	Jejunum	*N*	Ileum	*N*	Colon	*N*
Rabbit											
	Water (m → s)	18	−1.73	—[b]		44.74 (2.93)[c]	17/4	—		66.26 (2.05)	9/2
	Water (s → m)			—		41.68 (3.86)	5/2	—		68.89 (2.86)	7/2
	Methanol	32	−0.74	30.79 (1.46)	15/3	57.41 (5.29)[d]	25/5	44.90 (1.56)	14/3	68.98 (7.14)	10/3
	Mannitol	182	−3.10	1.73 (0.24)	11/3	3.54 (0.19)[e]	20/5	4.02 (0.19)	7/3	5.53 (0.52)	12/4
	Ganciclovir	255	−1.65	1.10 (0.18)	8/3	2.19 (0.19)	24/4	4.06 (0.47)	8/3	3.80 (0.64)	7/3
	PEG 900	900	—	0.37 (0.07)	14/4	0.70 (0.07)[e]	15/4	1.06 (0.18)	18/5	1.76 (0.27)	10/4
	PEG 4000	4000	−5.10	—		0.24 (0.37)[e]	15/5	—		—	
	Naproxen	250	0.42	3.06 (0.49)	12/4	9.86 (1.08)[d]	18/5	13.31 (2.09)	20/5	39.37 (5.02)	15/5
	Hydrocortisone	362	1.20	0.30 (0.07)	8/3	1.31 (0.12)	12/4	2.91 (0.45)	11/3	3.85 (0.65)	7/3
	Progesterone	315	3.87	0.78 (0.09)	18/4	1.36 (0.18)[e]	22/4	6.23 (1.11)	17/4	34.79 (5.18)	13/4
Monkey											
	Methanol			29.41 (1.81)	9/1	33.65 (2.44)[e]	13/3	39.33 (2.24)	13/3	53.58 (3.16)	13/3
	Mannitol			1.08 (0.21)	13/4	1.94 (0.17)[e]	24/6	4.84 (0.59)	7/3	5.03 (0.67)	13/3
	Ganciclovir			1.94 (0.56)	10/3	2.59 (0.24)	14/5	2.79 (0.57)	9/3	3.65 (0.58)	9/3
	PEG 900			0.47 (0.64)	10/3	0.35 (0.04)[e]	24/7	1.15 (0.11)	9/3	1.55 (0.12)	8/3
	PEG 4000			—		0.20 (0.05)[e]	9/3	—		—	
	Naproxen			3.99 (0.88)	5/2	2.12 (0.38)[e]	12/4	10.22 (4.47)	4/2	16.23 (3.16)	6/2
	Hydrocortisone			1.17 (1.41)	9/3	1.90 (0.75)	7/3	2.81 (0.74)	8/3	6.22 (1.74)	7/3
	Progesterone			0.73 (0.22)	8/3	0.70 (0.13)[e]	15/5	2.11 (0.46)	6/2	5.24 (1.02)	9/3
Dog											
	Methanol			16.08 (4.28)	13/3	17.60 (1.35)	11/3	23.16 (1.16)	7/2	30.77 (1.86)	12/3
	Mannitol			0.26 (0.07)	13/4	0.51 (0.08)	10/3	0.44 (0.20)	10/4	6.01 (0.70)	10/3
	Ganciclovir			0.17 (0.06)	8/4	0.37 (0.07)	17/5	0.83 (0.14)	18/5	4.01 (0.44)	16/4
	PEG 900			0.26 (0.07)	6/4	0.16 (0.06)	14/5	0.27 (0.06)	10/3	1.53 (0.30)	10/3
	PEG 4000			—		—		—		—	
	Naproxen			0.43 (0.11)	26/6	0.56 (0.13)	21/6	1.50 (0.45)	19/7	2.83 (0.61)	19/7
	Hydrocortisone			0.01 (0.003)	6/3	0.42 (0.01)	9/4	0.11 (0.05)	9/3	2.08 (1.02)	7/3
	Progesterone			0.12 (0.02)	14/5	0.19 (0.03)	22/6	0.11 (0.02)	10/3	0.84 (0.18)	10/4

[a] Number of measurements/number of animals.
[b] Not determined.
[c] Standard error of the mean in parentheses.
[d] Additional data added to value previously reported by this laboratory.
[e] Previously reported by this laboratory.

from the small intestine to the colon. Transepithelial electrical resistance of the rabbit ileum and colon, which were stripped of their muscle layers, has been determined as 21 and 385 $\Omega \cdot cm^2$. This suggests a restriction of the paracellular space in the colon and, therefore, a decreased permeability to polar solutes. Consistent with this observation, a decreasing PEG 400 permeability was determined from proximal to distal segments in humans (11).

Diffusion of hydrophilic compounds is influenced by both the size and the number of paracellular channels (16). The number of extracellular channels is determined by the mucosal surface area and cell density; i.e., the greater the number of cells, the greater the number of intercellular spaces. The number and shape of villi gradually change from the proximal to the distal intestine, with the more distal section having fewer and smaller villi. In humans, the mucosal area per centimeter of serosal length in the lower ileum is five times less than that of the jejunum. A decreasing gradient of villus surface area from proximal to distal intestinal segments has also been observed in rabbit and dog (17,18). If it is assumed that the epithelial cell size is similar throughout the gut, the number of available extracellular channels, i.e., the functional absorptive area, should decrease from proximal to distal intestine. However, the results of the present studies indicate an increased *in vitro* permeability in colon when compared to the small intestine.

In vivo molecular movement via extracellular channels consists of both convective and diffusive forces. Segmental differences in the *in vivo* drug movement from rat and rabbit intestine have previously been correlated with segmental differences in net water flux (13,19). In these previously reported studies, compound and net water transport increased in distal intestinal segments. Mucosal-to-serosal and serosal-to-mucosal permeabilities of water were measured in rabbit jejunum and colon to determine if net absorption of water occurred in this present study. However, no statistically significant net water movement was observed in these studies (Table II). Although *in vitro* permeability increased in proximal segments, solvent drag does not appear to influence the *in vitro* passive movement via extracellular channels.

Absorption occurs predominately at the villus tips and may occur along the intervillus space if a sufficient concentration gradient is developed. Villus height and density, in addition to the solute permeability and diffusion coefficients, influence the solute concentration along the intervillus space (20). Differences in villus density, height, and width, rather

Table III. Measured Permeability ($\times 10^{-6}$ cm/sec) of Markers of Active and Passive Transport in Segments of Rabbit, Monkey, and Dog Intestine

Species	Treatment	Duodenum	N^a	Jejunum	N	Ileum	N	Colon	N
Rabbit	D-Glucose	4.55 (0.61)[b]	15/4	10.17 (0.75)[c]	42/9	14.50 (2.35)	15/4	9.28 (0.64)	10/4
	D-Glucose + 0.2 m*M* ouabain	—[d]		7.50 (0.52)[c]	15/5	—		—	
	D-Glucose + 1.0 m*M* phlorizin	—		5.33 (0.43)	10/3	—		—	
	Ratio glucose/mannitol	2.63		2.87		3.61		1.68	
Monkey	D-Glucose	—		12.12 (1.28)[c]	19/5	—		—	
	D-Glucose + 0.2 m*M* ouabain	—		4.48 (0.54)[c]	17/5	—		—	
	D-Glucose + 1.0 m*M* phlorizin	—		1.92 (0.23)	14/4	—		—	
	Ratio glucose/mannitol			6.24					
Dog	D-Glucose	0.69 (0.16)	11/4	1.27 (0.20)	22/6	1.93 (0.51)	12/5	5.53 (0.79)	13/4
	D-Glucose + 0.2 m*M* ouabain	0.30 (0.09)	7/3	1.52 (0.29)	23/6	2.86 (0.83)	11/3	8.39 (0.82)	3/1
	D-Glucose + 1.0 m*M* phlorizin	—		0.95 (0.08)	9/3	—		—	
	Ratio glucose/mannitol	2.65		2.49		4.38		0.83	

[a] Number of measurements/number of animals.
[b] Standard error of the mean in parentheses.
[c] Values previously reported by this laboratory.
[d] Not determined.

than a gross measurement of surface area, may be a better representation of the absorptive surface area, and segmental permeability differences to hydrophilic compounds may reflect differences in the absorptive area. Therefore, although the macro surface area was consistent in these experiments, differences in transport, especially for hydrophilic compounds, may be due to differences in micro surface area. The discrepancy between the increased permeability of colon compared to small intestine and the decreased absorption of paracellular markers distally *in vivo* suggests that permeability measurements alone do not accurately describe this aspect of the *in vivo* situation.

Effect of Tissue Thickness

The permeability coefficient of passively transported compounds can be related to tissue resistance ($1/D$) by the following equation:

$$1/D = 1/(P * h) \quad (1)$$

which defines the relationship between the diffusion coefficient of a compound (D) and the permeability coefficient (P) of that compound across the permeability barrier of thickness (h) (6). The reciprocal of the calculated diffusion coefficient approximates the tissue resistance.

Using permeability measurements of hydrophilic compounds, linear relationships between species tissue resistance were established for rabbit vs monkey jejunum (R^2 = 0.996) and colon (R^2 = 0.998) as well as rabbit vs dog jejunum (R^2 = 1.00) and colon (R^2 = 0.980) (Figs. 3 and 4). A slope of unity indicates that tissue resistance to compound diffusion was similar in each species. The established relationship between rabbit and monkey jejunum (slope = 2.1) suggests that monkey jejunum is twice as resistant to hydrophilic compound diffusion than rabbit jejunum. Rabbit jejunum has been shown to be 1.8 times more permeable than monkey jejunum (2). A similar relationship exists between rabbit and dog jejunum (slope = 2.1). In contrast, rabbit and monkey colons appear to offer a similar resistance to hydro-

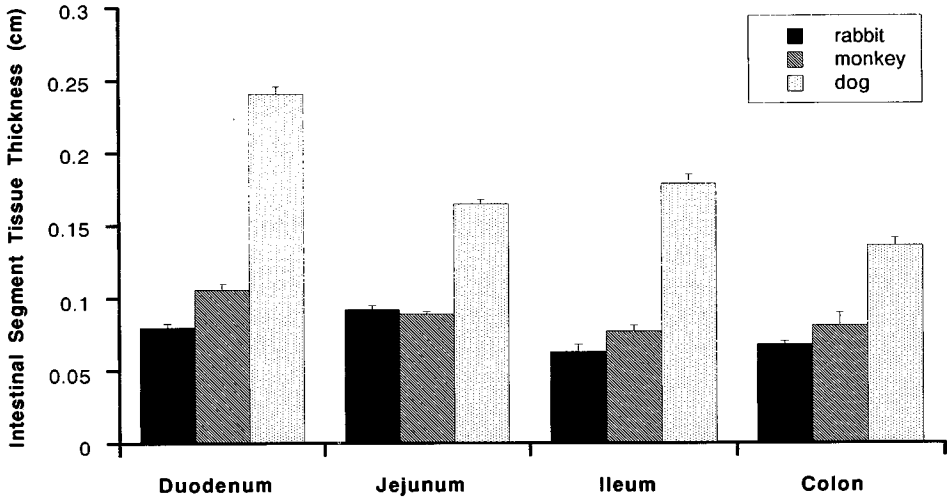

Fig. 2. Stripped tissue thickness of rabbit, monkey, and dog intestine. Measurements taken from serosal edge to villus tip.

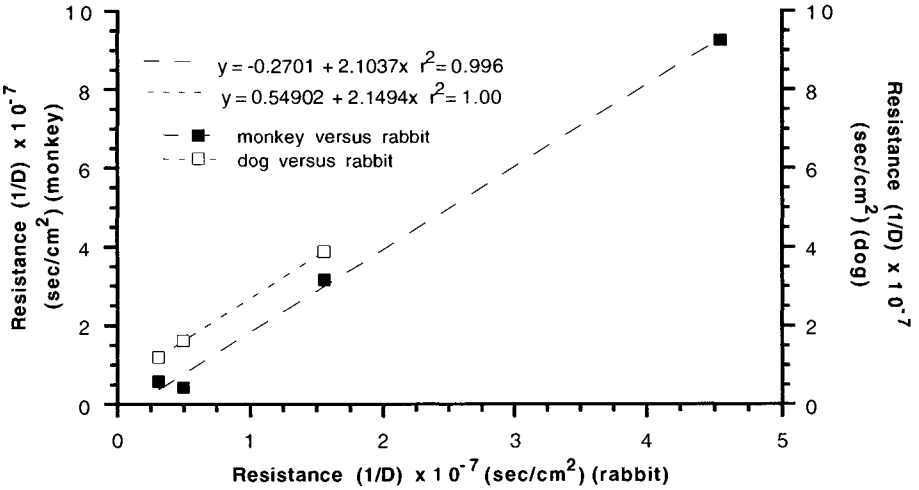

Fig. 3. Calculated resistance of rabbit jejunum versus calculated resistance of monkey and dog jejunum.

philic compound diffusion and both demonstrate a greater resistance than dog colon.

Tissue resistance corrects the measured permeability for tissue thickness. If hydrophilic compound diffusion within the extracellular channels is equivalent across species, differences in tissue resistance between species would indicate differences in intestinal absorptive surface area or cell density between species. Therefore, the relationships established for rabbit versus monkey and rabbit versus dog tissue resistance suggest differences in intestinal absorptive surface area and/or cell density between species.

These correlations are significant in that it may be possible to develop such a relationship between one of these tissue segments and human tissue. Such a relationship will greatly aid the development of predictive models of drug absorption in humans.

Transport of Lipophilic Compounds

Lipophilic compounds, such as hydrocortisone and progesterone, passively diffuse across cell membranes. The permeability of lipophilic compounds is greatly influenced by the thickness of the unstirred water layer adjacent to the epithelium and within the intervillus space (21). Hence, differences in unstirred water layer thickness between regions of the intestine or between species can be responsible for the apparent differences in permeability measurements for the various tissue segments and species.

The log partition coefficient of naproxen is only slightly lipophilic. Under experimental conditions, pH 7.4, naproxen is negatively charged (pK_a = 4.39) in bulk solution. Although ganciclovir is a hydrophilic compound of slightly lower molecular weight, naproxen permeability was greater than ganciclovir permeability in rabbit jejunum, ileum, and colon and monkey ileum and colon. This suggests that naproxen transport is not restricted to the paracellular space but also involves transcellular diffusion. Consistent with these observations, naproxen bioavailability in humans is 100%, while ganciclovir bioavailability is only about 10%.

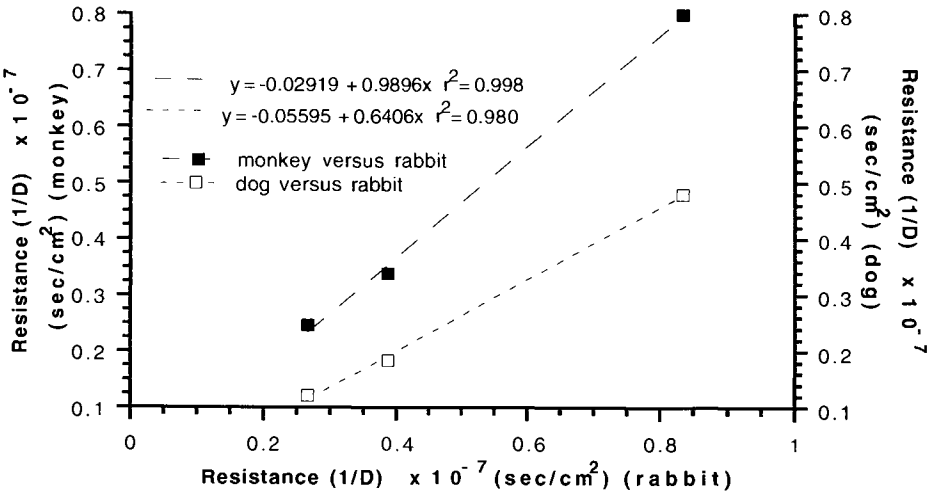

Fig. 4. Calculated resistance of rabbit colon versus calculated resistance of monkey and dog jejunum.

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