The Transport Barrier of Epithelia: A Comparative Study on Membrane Permeability and Charge Selectivity in the Rabbit

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The transport barrier of the epithelia presents one of the major problems limiting the effective use of these tissues as alternate delivery routes for macromolecules such as peptides and proteins. In the present study, two membrane transport properties, namely, the *permeability* and *permselectivity* of the shunt pathway, were investigated and compared in various tissues including the nasal, tracheal, bronchial, buccal, rectal, vaginal, corneal, epidermal, duodenal, jejunal, ileal, and colonic epithelia. Membrane permeability was evaluated using a combined method based on electrical conductance and flux measurements of a hydrophilic fluorescent probe, 6-carboxy fluorescein (CF). Membrane permselectivity or the charge discriminating ability of the membrane was evaluated by KCl diffusion potential measurements. The results indicate that all epithelia under investigation possess a relatively high degree of permeation barrier and are highly selective for the absorption of positively charged solutes. Shunt path permeability was found to vary greatly among tissues from different epithelia, whereas membrane charge selectivity was relatively constant in these tissues. A good correlation was observed between membrane electrical conductance and steady-state flux of CF, indicating a paracellular transport of the compound. The rank order of the intrinsic membrane permeability was as follows: intestinal ≈ nasal ≥ bronchial ≥ tracheal > vaginal ≥ rectal > corneal > buccal > skin. Membrane permselectivity, expressed as the ratio of transport number (positive over negative), ranges from 1.78 for the buccal to 1.33 for the rectal epithelium. These results suggest that, for effective delivery purposes, permeation enhancing methods, by either increasing tissue permeability or modifying drug-membrane charge selectivity, are generally required. The permeation data also suggest that the respiratory epithelia represent good alternate routes for drug delivery, particularly for those that are orally ineffective, i.e., due to extensive gastrointestinal tract degradation or first-pass metabolism.

KEY WORDS: epithelial transport; permeability; permselectivity; absorption; electrical resistance; electrical conductance; diffusion potential.

INTRODUCTION

In recent years, there has been an increasing interest in the use of epithelial membranes, i.e., nasal, rectal, buccal, and transdermal, as delivery sites for the therapeutic administration of peptide and protein drugs. These alternate routes offer a number of potential advantages over traditional routes, e.g., improved patient compliance and avoidance of health hazards as compared to the injectable route and the reduction of enzymatic degradation and first-pass metabolism as compared to the oral route. While these advantages seem appealing and a great deal of research has been devoted to alternate drug delivery, the actual clinical use of the epithelial membranes as delivery sites thus far has been limited, mainly because of low drug bioavailability. The unfavorable properties of the epithelia to drug absorption, i.e., high degree of hydrophobicity, charge selectivity, and tight junctions, as well as those of the peptide and protein molecules, i.e., large molecular size and high charge density, make delivery of these compounds difficult. For most drugs, it is generally accepted that transport through the cellular epithelial barriers occurs via a nonspecific diffusion process governed primarily by a concentration gradient. Under these conditions, the physicochemical properties of the diffusing solute and the physiologic function of the cell layer involved are the important factors dictating the transport rate. For hydrophilic drugs which do not partition well into the cell membrane, as is the case for most peptides and proteins, leakage through the intercellular lateral space (paracellular) and/or endocytic fluid-phase pinocytosis (transcellular) should be the major transport pathways. The transport barrier of the paracellular pathway has two properties that influence the absorption rates of drugs, particularly those with charges: first, the general *permeability* or magnitude of the barrier, which is controlled mainly by the tight junctions; and second, the *permselectivity* of the barrier, which is a quantitative measure of the ability of the epithelia to discriminate or show preference for the transport of molecules of different charges. These two membrane properties have been studied by several investigators, but only a few (1,2) have compared their relative magnitude and their subsequent effect on drug absorption, particularly in the epithelia relevant to drug delivery. Thus, it remains unclear which of these membranes may represent the best alternate route for drug delivery purposes. Furthermore, the differences in experimental design and conditions used in previous studies, i.e., type of drugs, species of animals, and analytical techniques used, make it impossible to compare the transport data. Therefore, it is the intent of this work to systematically determine and compare membrane permeability and permselectivity of various epithelia in light of the search for appropriate alternate delivery routes. The epithelial membranes under investigation in this study are the nasal, tracheal, bronchial, buccal, rectal, vaginal, corneal, skin, and intestinal including the duodenal, jejunal, ileal, and colonic epithelia.

To evaluate membrane permeability, two analytical methods based on electrical resistance and flux measurements were used. The electrical resistance is a measure of charge flow across the membrane and thus reflects the permeability of the paracellular shunt pathway (3). Flux measurements were conducted using a hydrophilic fluorescent probe, 6-carboxyfluorescein (CF; MW = 376, pK_a = 6.4). Membrane permselectivity was determined by means of KCl-diffusion potential measurements. All experiments were conducted *in vitro* in Ussing chambers using freshly isolated rabbit epithelial tissues.

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EXPERIMENTAL

Animals

Female albino New Zealand rabbits (Greenmeadows Rabbitry, PA) weighing 2.5 and 3.0 kg were used throughout the studies. Lighting was maintained on a 12-hr cycle on/off basis in the caging facility, and the animals were fed a regular diet, with no restriction on the amount of food or water consumed.

In Vitro Perfusion Studies

Rabbits were sacrificed with an intravenous injection of an overdose of sodium pentobarbital given via the marginal ear vein. Epithelial tissues mentioned above were removed (≈1-cm^2 size) with fine scissors and placed in ice-cold Krebs–Ringer buffer (KRB), pH 7.4, containing 7.0 g/L NaCl, 0.34 g/L KCl, 0.1 g/L Na_2HPO_4, 0.18 g/L NaH_2PO_4, 0.1 g/L $MgCl_2 \cdot 6H_2O$, 0.11 g/L $CaCl_2$, and 1.8 g/L glucose. Great care was taken to avoid any direct trauma and damage to the tissues. In some tissues including rectal, vaginal, nasal, and skin, the underlying connective tissue and muscle were also removed. These tissues were then immediately mounted on supporting rings with the epithelial side facing the donor half-cell and clamped in Ussing chambers described previously (4). Silicone grease was applied to the contact surfaces to prevent possible leakage of the bathing fluid. Tissue preparation and the mounting process for each tissue were normally completed within 15 min after removal. After the tissues were mounted, the chambers were filled with bathing solutions described below. Aeration and circulation in the tissue bath were provided by means of bubbling with a mixture of 95% O_2 and 5% CO_2. Tissue viability and integrity during the experiments were checked by measuring transepithelial electrical potential and resistance. Only tissues with a high membrane potential and resistance were used in this study (see results in Table I). All experiments were conducted at 37°C using a constant-temperature bath connecting through a water jacket of the Ussing chambers. A 0.67-cm^2 area of tissue was exposed to the donor and receptor compartments, each having a volume of 7 ml.

Solution Preparation

All solutions were made with analytical-grade reagents using deionized, distilled water. In experiments involving electrical resistance and flux measurements, KRB solution with the addition of 1 μg/ml CF on the donor (epithelial) chamber was used. Since this amount of CF was found to have no significant effect on the membrane electrical resistance, the two measurements were conducted simultaneously to provide a better comparison between the two membrane parameters and to minimize animal use. In studies involving diffusion potential measurements, KCl solutions with a 10:1 KCl concentration gradient were used. The more diluted solution (solution 1 with C_1 = 16 m*M*) was applied to the receptor (serosal) side and the concentrated solution (solution 2 with C_2 = 160 m*M*) was applied to the donor side. The two solutions were kept isoosmotic with the tissues by the addition of a nonelectrolyte, sucrose, to prevent convective flow resulting from the osmotic gradient.

Table I. Electrophysiological Properties of Various Epithelia in Rabbit[a]

	R ($\Omega \cdot cm^2$)[b]		pd (mV)[c]	
Tissue	Mean	SD	Mean	SD
Nasal	261	55	−13.8	−4.4
Tracheal	291	65	−13.9	−5.3
Bronchial	266	97	−12.4	−3.7
Rectal	406	70	−18.1	−7.6
Vaginal	372	85	−16.5	−6.8
Buccal	1803	175	−28.4	−12.1
Corneal	1012	106	−24.1	−6.5
Skin[d]	9703	175	−32.0	−21.3
Duodenal	211	91	−3.9	−1.8
Jejunal	224	104	−3.8	−2.2
Ileal	266	95	−4.4	−1.5
Colonic	288	72	−5.3	−2.6

[a] The resistance was measured by applying alternating current pulses of variable duration (1–10 sec) and intensity (up to ±10 $\mu A \cdot cm^{-2}$, at 1 Hz) and recording the voltage drop across the membrane. Experiments were conducted in Krebs–Ringer buffer, pH 7.4, using a four-electrode Ag/AgCl system at 37°C; n = 6.
[b] Steady-state electrical resistance.
[c] Steady-state transepithelial potential difference (epithelial with respect to serosal).
[d] Abdominal skin.

The final osmolarity of all test solutions was ≈290 mOsm as determined by the Wescor 5500 vapor pressure osmometer. The effect of sucrose on electrode standard potentials and ionic activity at this concentration was found to be negligible (5).

Electrical and Flux Measurements

Membrane resistance was measured using a four-electrode Ag/AgCl system according to the method described previously (4). Two electrodes (located 1 cm from each side of the membrane) were connected to a high input-impedance microvoltmeter (Keithley 197) and used as potential sensing electrodes. The other two (positioned 2 cm from each side) were used to inject current pulses. To avoid problems associated with membrane polarization, which may interfere with the true reading of the resistance (this is due to surface charge accumulation caused by direct current), alternating sinusoidal currents (current density = 1–10 $\mu A \cdot cm^{-2}$ and frequency = 1 Hz), generated from a function generator (Simpson 422), were used. In all experiments, the anode was placed on the donor side and the cathode on the receptor side. Measurements were conducted every 0.5 hr for a total of 6 hr. To correct for the potential drop due to solution resistance between the sensing electrodes and the membrane, measurements were carried out before each membrane resistance determination using the same bathing solution but without the membrane in the perfusion chamber. The actual membrane resistance was then calculated by subtracting the resistance obtained without the membrane from that determined with the membrane. For flux measurements, 1-ml samples were taken from the receptor chamber every 0.5 hr for a period of 6 hr and replaced with an equal volume of drug-free buffer solution. The samples were ana-

lyzed spectrofluorometrically, with the excitation and emission set at 492 and 524 nm, respectively. For KCl diffusion potential measurements, only the sensing electrodes were used and no current was applied through the membrane. Steady-state potentials (normally obtained within 0.5 hr after initiation) were used to calculated membrane permselectivity according to the Planck and Henderson equations:

$$\Delta\lambda_m = \Delta\lambda_{obs} - \Delta\lambda_e \quad (1)$$

$$\Delta\lambda_e = -(RT/F) \ln (a_2^{KCl}/a_1^{KCl}) \quad (2)$$

$$\Delta\lambda_m = \lambda_2 - \lambda_1 = -RT/F\,(t^K + t^{Cl}) \ln (a_2^{KCl}/a_1^{KCl}) \quad (3)$$

where $\Delta\lambda_m$ is the transmembrane potential difference, $\Delta\lambda_{obs}$ is the observed potential difference, $\Delta\lambda_e$ is the electrode potential difference, a^{KCl} is the mean KCl ionic activity, subscripts 1 and 2 refer to the two bathing solutions, R is the gas constant, F is the Faraday constant, and t is the transport number. Ionic nonideality was corrected according to Robinson and Stokes (6), i.e., $a^{KCl} = g_{\pm} \cdot m$, where $g_{\pm}$ is the mean ionic activity coefficient and m is the molality. Detailed derivation and application of the equations to membrane transport study were described previously (7).

Data Treatment

A minimum of five tissue samples from different animals was used in each determination. Data values are expressed as the mean ± standard deviation. Statistical differences between the means of the membrane parameters were determined using the Student t test of unpaired samples. Steady-state flux of CF in each tissue was calculated based on Fick's first law of diffusion and is expressed as the amount of drug in the receptor chamber per unit surface area per unit time ($\mu g/cm^2 \cdot hr$). The values were obtained from the linear portion of the concentration–time profile using the method of least squares.

RESULTS AND DISCUSSION

Membrane Resistance

The results of the membrane resistance in various epithelia are shown in Table I. These values represent the steady-state resistance values of the epithelia which were normally obtained during the first 1-hr period of the perfusion. These results indicate that, on a per unit area basis, the shunt path permeability of the epithelia is, as expected, highest in the intestinal ($R \approx 200$–$300\ \Omega\ cm^2$) and lowest in the skin ($R \approx 10{,}000\ \Omega\ cm^2$). These results are in general good agreement with previous findings by other investigators (8,9), although some studies (10) have reported a low resistance value of $\approx 100\ \Omega\ cm^2$ for the tissues from the upper intestinal region. This discrepancy may be attributed to a number of factors including animal variations, tissue preparation, and analytical techniques used. Among the intestinal tissues, the duodenum appears to be the most permeable, followed by the jejunum, ileum, and colon, although these resistance values were found not to be statistically different ($P < 0.05$). Surprisingly, the nasal and pulmonary epithelia are equally or only slightly less permeable than those of the intestine ($R = 261 \pm 55$, 266 ± 97, and $291 \pm 65\ \Omega\ cm^2$ for the nasal, bronchial, and tracheal tissues, respectively). These results are also consistent with previously reported values, i.e., $\approx 200\ \Omega\ cm^2$ for the nasal (11) and ≈ 200–$700\ \Omega\ cm^2$ for the tracheal and bronchial epithelia (12). Following the respiratory epithelia are the vaginal ($372 \pm 85\ \Omega\ cm^2$), rectal ($406 \pm 70\ \Omega\ cm^2$), corneal ($1012 \pm 106\ \Omega\ cm^2$), and buccal ($1803 \pm 175\ \Omega\ cm^2$) epithelia. The resistance value of the alveolar epithelia was not determined in the present study due to the difficulty in isolating intact alveolar tissue, although this value has been suggested to be comparable to, or even lower than, those from the airway (12). The high permeability values of the respiratory tissues are believed to be a result of the presence of numerous aqueous pores through which water-soluble molecules can diffuse. Both large and small aqueous pores were reported in the nasal (13) and pulmonary epithelia (14). The aqueous pores in the nasal epithelium, particularly those of small sizes (0.39–0.84 nm), were found to be more abundant than those observed in the jejunum (0.71–1.60 nm) and the rectum (0.59–1.70 nm) (13). In the pulmonary epithelium, pores of 0.6- to 1.0-nm size and large pores of ≥8 nm were also reported (14). Using metabolically stable peptides as permeability tracers, McMartin *et al.* (15) also found that the nasal epithelium was more leaky to peptide molecules than the intestinal epithelia. In another study, however, Aungst *et al.* (2) demonstrated that insulin administration through nasal tissue was less efficacious than through rectal tissue. Our results, based on both resistance and flux measurements, described subsequently, as well as those reported by Corbo *et al.* (1) using a hydrophilic paracellular tracer, mannitol, indicate otherwise; i.e., the nasal membrane was more permeable than the rectal ($P < 0.05$). These observations suggest that other factors, such as enzymatic stability, absorptive surface area, and perhaps transport mechanism, may also play an important role in determining overall bioavailability. In fact, the same study by Aungst *et al.* (2) also showed that in the presence of the bile salt sodium glycocholate, an absorption-promoting adjuvant known to inhibit some proteolytic enzymes (16), insulin absorption through the nasal tissue was actually greater than that through the rectum. The influence of proteolytic enzymes on insulin absorption was further demonstrated by a five-time reduction in bioavailability when the peptide was administered orally (the administration route with the most dramatic enzymatic activity) as compared to the nasal or the rectal routes (2). Variation in peptidase activity among the epithelial tissues was also reported previously (17). It should also be noted that the above discussion is based on the assumption of paracellular drug transport, potential transcellular transport of insulin, i.e., via endocytosis, which in this case depends on membrane specificity and endocytic activity of the tissue, may also account for the observed discrepancy. Selective endocytosis of insulin was recently demonstrated in the epithelium and endothelium of the cornea, the tissue in which paracellular absorption was found to be the predominant transport pathway of most peptides, i.e., polylysine, thyrotropin releasing hormone, and phalloidin (18,19).

The membrane resistance of the vagina was found to be slightly less than that of the rectum but significantly greater than those of the cornea, buccal, and skin ($P < 0.05$). These observations are consistent with previous findings (1) which indicated a slightly higher permeability to the paracellular

tracer, mannitol, for the vaginal over the rectal membranes. Unlike humans or other laboratory animals, the vaginal epithelium of the rabbit which was used in this study was not subject to the time-dependent histology change due to the lack of an estrous cycle (20). This infers that the membrane permeability of rabbit vaginal tissue is expected to be fairly constant and does not fluctuate within the estrous cycle. The high membrane resistance observed in the skin and, to some extent, in the buccal may be attributed to the keratinizing nature of these tissues. In the skin, for example, the outermost layer (stratum corneum) consists of highly compacted, dehydrated, keratinized cells and is generally considered as the rate-limiting barrier for drug absorption through the skin (21).

Flux Measurements

To correlate membrane electrical resistance to transepithelial flux of polar solutes, flux measurements of a model hydrophilic compound, CF, were conducted. These results are given in Table II. The striking relationship between the mean electrical conductance values (inverse resistance; see Table II) and the steady-state fluxes of the compound in all 12 epithelia being studied ($r = 0.9$) is demonstrated in Fig. 1. Since the electrical conductance is a measure of charge flow across the membrane via the aqueous shunt (paracellular) pathway, these results suggest that CF should also be transported via a similar pathway. The flux results also indicate that all the epithelia being studied act as effective barriers against absorption of the test compound, i.e., in all cases, less than 0.1% of the total applied dose is being transported through the membranes. It should be noted that the results reported here are based on a per-unit area (1 cm^2) basis; in reality, the large absorptive surface area and variation among tissues can have a great impact on the actual absorption of the drugs. For drug delivery purposes, tissues with a large absorptive area and high permeability, such as the intestinal and pulmonary epithelia, would be considered advantageous. The latter is particularly attractive as an alternative delivery route for drugs that are orally ineffective due to extensive GI degradation or hepatic first-pass metabolism. In previous studies, Wigley *et al.* (22) demonstrated that the orally ineffective insulin can be sufficiently absorbed to cause a therapeutic effect (reduction of blood glucose level) in humans when administered through the lung. Adjei *et al.* (23) also demonstrated that the peptide leuprolide, a potent luteinizing hormone-releasing hormone (LHRH) agonist, was more effective when delivered through the pulmonary route than through the oral and many noninjectable routes including the nasal and transdermal. Oral absorption of leuprolide was 0.05%, while the absorption following nasal or transdermal delivery was less than 2%. On the other hand, leuprolide bioavailability following inhalation administration was 4–8 and ≈50% after correction for nonabsorptive drug loss. Similarly, Patton *et al.* (24) showed that intratracheal administration of human growth hormone gave a much higher drug bioavailability than the nasal route, i.e., 36 versus <1%.

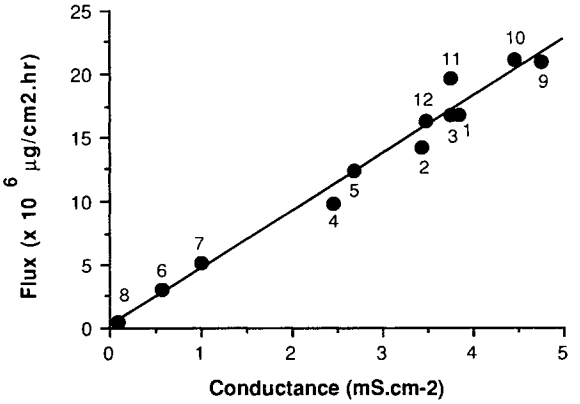

Fig. 1. Relationship between steady-state flux and transmembrane electrical conductance in various epithelia. All data represent mean values and are taken from Table II. The numbers indicate the type of tissue: 1, nasal; 2, tracheal; 3, bronchial; 4, rectal; 5, vaginal; 6, buccal; 7, corneal; 8, skin; 9, duodenal; 10, jejunal; 11, ileal; 12, colonic.

Table II. Transport Properties of Various Epithelia in Rabbit[a]

	J_{ss} (10^6 µg/cm^2 · hr)[b]		G (mS · cm^{-2})[c]	
Tissue	Mean	SD	Mean	SD
Nasal	16.8	1.8	3.8	0.8
Tracheal	14.2	5.4	3.4	0.8
Bronchial	16.7	4.5	3.8	1.4
Rectal	9.9	2.3	2.5	0.4
Vaginal	12.4	4.1	2.7	0.6
Buccal	3.0	1.3	0.6	0.1
Corneal	5.1	1.7	1.0	0.1
Skin[d]	0.5	0.4	0.1	0.0
Duodenal	21.0	3.9	4.7	2.0
Jejunal	21.1	6.2	4.5	2.1
Ileal	19.6	3.9	3.8	1.3
Colonic	16.3	6.8	3.5	0.9

[a] Experiments were conducted *in vitro* in Krebs–Ringer buffer, pH 7.4, at 37°C. Steady-state values were calculated from the linear portion of the concentration–time profiles of CF in the receptor chamber; $n = 6$.

[b] Steady-state CF flux (epithelial → serosal).

[c] Electrical conductance (inverse resistance; data calculated from Table I).

[d] Abdominal skin.

Permselectivity Studies

In addition to being affected by the permeability barrier, absorption of charged solutes is also influenced by the permselective properties of the epithelia. This is because the epithelia are known to possess certain charge characteristics and exhibit an electrochemical potential which the solutes must experience during their passage across the membranes. Barrier permselectivity is a complex phenomenon which combines both the passive contribution from electrostatic shunt activity, due to membrane-fixed charges, and the active contribution from cell membrane activity. The latter is generally a reflection of carriers and pumps residing in the membrane, i.e., active sodium and chloride pumps. At physiological conditions, most epithelia are selective to positively charged solutes and discriminate against the opposites, although reverse selectivity was also observed at extreme

acidic conditions (7,25,26). In the present study, the magnitude and polarity of charge selectivity in various epithelia are compared, with the emphasis on the electrostatic shunt path permselectivity. KCl diffusion potentials were measured and their sign and magnitude were taken as an index for membrane permselectivity according to the equations described under Experimental. KCl was used in this study because K^+ and Cl^- have similar mobilities and hydrated radii (6) and are nondegradable; thus, membrane permselectivity can be interpreted directly without the interferring effect of solute size. In addition, the problem of liquid junction potential as a result of unparallel diffusion of solutes in free solution was also minimized. The use of KCl was found to have no significant effect on membrane viability and integrity during the perfusion study period as demonstrated previously (7).

The results of the permselectivity studies are summarized in Table III. It can be seen that the diffusion potential differences in all epithelia studied are negative (epithelial with respect to serosal side), indicating that these epithelia are more permeable to K^+ than to Cl^-. Positive and zero potentials would indicate anion selective and electroneutrality properties of the membranes respectively. The magnitudes of selectivity as indicated by the relative permeability coefficient or transport number ratio [calculated from Eq. (3)] are highest in the buccal ($t^K/t^{Cl} = 1.78$) and lowest in the rectal epithelium ($t^K/t^{Cl} = 1.33$). These results suggest that unlike membrane permeability, which varies greatly among tissues, the selectivities of the epithelia (or the relative permeabilities of two oppositely charged solutes with similar size) are quite similar. The basis for epithelial charge selectivity is not yet clear but has been suggested to be a result of fixed ionizable charged groups arising from protein amino acid residues, i.e., carboxyl and amine groups, which line the transport pathway (27). At physiological pH or pH above the isoelectric point (p*I*), the epithelia are negatively charged and are selective to positively charged solutes [most epithelia have p*I*'s of ≈3–4 (7,25,26)]. Below the p*I*, the reverse was observed (7,25,26). Although not firmly established, the charge discriminating effect of the epithelia is believed to have a significant impact on the absorption of most drugs, particularly those with charges. Absorption of the negatively charged peptide, insulin, was found to be excluded from the aqueous paracellular pathway, whereas positively charged peptides such as polylysine, horseradish peroxidase, and thyrotropin releasing hormone were taken up predominantly via this pathway in the cornea (18,19,28). In the nasal epithelium, Maitani *et al.* (29) demonstrated a greater absorption of the positively charged conjugate of dextran, a hydrophilic macromolecule, than its neutral conjugate of similar size. In the skin, anodal (+) iontophoresis was found to be more effective in enhancing transport of hydrophilic solutes than cathodal (−) iontophoresis, indicating a positively charged selective characteristic of the skin (25). In the present study, the low absorption efficiency of CF observed in various epithelia may also be attributed, at least in part, to the charge characteristic of the compound (CF contains a negatively charged carboxyl group).

Table III. Shunt Path Permselectivities of Various Epithelia[a]

Tissue	DP (mV)[b]	t^{Kc}	t^{Cld}	t^K/t^{Cl}
Nasal	−(10.22 ± 2.43)	0.59	0.41	1.44
Tracheal	−(11.36 ± 1.79)	0.60	0.40	1.50
Bronchial	−(10.25 ± 1.96)	0.59	0.41	1.44
Rectal	−(7.95 ± 1.06)	0.57	0.43	1.33
Vaginal	−(11.39 ± 2.44)	0.60	0.40	1.50
Buccal	−(15.90 ± 3.21)	0.64	0.36	1.78
Corneal	−(13.63 ± 2.68)	0.62	0.38	1.63
Skin	−(12.49 ± 2.21)	0.61	0.39	1.56
Duodenal	−(9.08 ± 1.36)	0.58	0.42	1.38
Jejunal	−(9.10 ± 1.71)	0.58	0.42	1.38
Ileal	−(10.20 ± 1.76)	0.59	0.41	1.44
Colonic	−(10.27 ± 2.12)	0.59	0.41	1.44

[a] The transport number is defined as the fraction of charge carried by a given charged species and is calculated from the diffusion potential according to Eq. (3). The diffusion potential resulting from the concentration gradient, being 160 m*M* KCl in the donor side and 16 m*M* KCl in the receptor side, was measured using a Ag/AgCl electrode at 37°C. The data are mean ± SD; $n = 6$.

[b] Steady-state diffusion potential.

[c] Potassium transport number.

[d] Chloride transport number.

CONCLUSIONS

The present study compares the membrane shunt path permeability and charge selectivity of various epithelia and demonstrates their importance on transepithelial transport of charged solutes. The results indicate a wide variation in the magnitude of barrier permeability among different epithelia, with the intestinal epithelium being most permeable, followed very closely by the respiratory and then the vaginal, rectal, corneal, buccal, and skin epithelia. With respect to the permselectivity barrier, all epithelia are selective to positively charged solutes as compared to their oppositely charged counterparts, with the magnitude of selectivity being comparable in most epithelia. The results of the flux measurements indicate a high degree of permeability barrier and paracellular transport of the test solute across the epithelia. The intestinal and pulmonary epithelia, due to their high membrane permeability and large absorptive surface area, are probably two of the most effective epithelia for drug delivery purposes. The latter should be considered more advantageous for the delivery of drugs that undergo extensive GI degradation or first-pass metabolism.

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REFERENCES

1. D. C. Corbo, J. C. Liu, and Y. W. Chien. Characterization of the barrier properties of mucosal membranes. *J. Pharm. Sci.* 79:202–206 (1990).
2. B. J. Aungst, N. J. Rogers, and E. Shefter. Comparison of nasal, rectal, sublingual and intramuscular insulin efficiency and the effects of a bile salt absorption promoter. *J. Pharmacol. Exp. Ther.* 244:23–27 (1987).
3. E. Fromter and J. Diamond. Route of passive ion permeation in epithelia. *Nature New Biol.* 53:9–13 (1972).
4. Y. Rojanasakul and J. R. Robinson. Electrophysiological and

ultrastructural characterization of the cornea during *in vitro* perfusion. *Int. J. Pharm.* 63:1–16 (1990).
5. P. H. Barry and J. M. Diamond. Junction potentials, electrode standard potentials and other problems in interpreting electrical properties of membranes. *J. Membr. Biol.* 3:93–122 (1970).
6. R. A. Robinson and R. H. Stokes. *Electrolytic Solutions,* Butterworths, London, 1959.
7. Y. Rojanasakul and J. R. Robinson. Transport mechanisms of the cornea: Characterization of barrier permselectivity. *Int. J. Pharm.* 55:237–246 (1989).
8. L. A. Stoehelin. Structure and function of intercellular junctions. *Int. Rev. Cytol.* 39:210 (1974).
9. S. G. Schultz, R. A. Frizzell, and H. N. Nellans. Active sodium transport and the electrophysiology of rabbit colon. *J. Membr. Biol.* 33:351–384 (1977).
10. D. W. Powell. Barrier function of epithelia. *Am. J. Physiol.* 241:G275 (1981).
11. M. A. Wheatley, J. Dent, E. B. Wheeldon, and P. L. Smith. Nasal drug delivery: An *in vitro* characterization of transepithelial electrical properties and fluxes in the presence or absence of enhancers. *J. Control. Release* 8:167–177 (1988).
12. M. J. Welsh. Electrolyte transport by airway epithelia. *Physiol. Rev.* 67:1143–1184 (1987).
13. M. Hayashi, T. Hirasawa, T. Muraoka, M. Shiga, and S. Awazu. Comparison of water influx and seiving coefficient in rat jejunal, rectal, and nasal absorption of antipyrine. *Chem. Pharm. Bull.* 33:2149–2152 (1985).
14. A. E. Taylor and K. A. Gaar. Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes. *Am. J. Physiol.* 218:1133–1140 (1970).
15. C. McMartin, L. E. F. Huctchinson, R. Hyde, and G. E. Peters. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.* 76:535–540 (1987).
16. S. Hirai, T. Yashiki, and H. Mima. Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.* 9:173–184 (1981).
17. R. E. Stratford and V. H. L. Lee. Aminopeptidase activity in homogenates of various absorptive mucosae in the albino rabbit: Implication in peptide delivery. *Int. J. Pharm.* 30:73–82 (1986).
18. Y. Rojanasakul, S. W. Paddock, and J. R. Robinson. Confocal laser scanning microscopic examination of transport pathways and barriers of some peptides across the cornea. *Int. J. Pharm.* 61:163–172 (1990).
19. Y. Rojanasakul. *Mechanistic Studies of Ocular Peptide Absorption and Its Enhancement by Various Penetration Enhancers,* Ph.D. dissertation, University of Wisconsin—Madison, 1989.
20. R. L. Dickinson. *Human Sex Anatomy,* Williams and Wilkins, Baltimore, MD, 1949, c. 2–3.
21. R. Scheuplein. Mechanisms of percutaneous absorption II: Transient diffusion and the relative importance of various routes of skin penetration. *J. Invest. Dermatol.* 45:334 (1967).
22. F. M. Wigley, J. H. Londono, and S. H. Wood. Insulin across respiratory mucosae by aerosol delivery. *Diabetes* 20:552–556 (1971).
23. A. Adjei and J. Garren. Pulmonary delivery of peptide drugs: Effect of particle size on bioavailability of leuprolide acetate in healthy male volunteers. *Pharm. Res.* 7:565–569 (1990).
24. J. S. Patton, H. J. Fuchs, and J. A. Moore. Pulmonary delivery of peptide and protein drugs. *Proc. 17th Int. Symp. Control. Release Soc.* 1990, p. 214.
25. R. R. Burnette and B. Ongpipattanakul. Characterization of the permselective properties of excised human skin during iontophoresis. *J. Pharm. Sci.* 76:765–773 (1987).
26. D. H. Smyth and E. M. Wright. Streaming potentials in the rat small intestine. *J Physiol. (London)* 182:591–602 (1966).
27. G. Shutten, H. W. Spier, and G. Schwarz. *Handbuch Der Haut und Geschlechtskrankheiten, Vol. I/4B,* Springer, Berlin, 1981, pp. 113–147.
28. A. M. Tonjum. Permeability of horseradish peroxidase in rabbit corneal epithelium. *Acta Ophthalmol.* 52:650–658 (1974).
29. Y. Maitani, M. Yoshiharu, and T. Nagai. Influence of molecular weight and charge on nasal absorption of dextran and DEAE-dextran in rabbits. *Int. J. Pharm.* 49:23–27 (1989).