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Diffusion coefficient in native mucus gel of rat small intestine

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Abstract—The diffusion coefficients of [^{3}H] water, urea, benzoic acid, antipyrine, aminopyrine, α-methyl-glucoside, L-phenylalanine and of hydrogen ions were measured at 38°C in native mucus gel from rat small intestine. The diffusion in the gel was reduced to 37–53% (for hydrogen ions to 7%) compared with buffer solution. In addition, the buffering capacity of the gel retarded the permeation of hydrogen ions before a steady state flux was attained. A model calculation revealed that in the preparation a gel layer of 80 μm thickness represents 23% of the total permeation resistance for substances with high epithelial permeability. The aqueous part of the pre-epithelial diffusion resistance amounts to 77% of the total resistance.

The intestinal epithelium and its covering layers represent a barrier to the absorption of substances in the gastrointestinal tract. The mucous part of the "pre-epithelial diffusion resistance" (Winne 1984) consists of mucus gel adhering to the mucosa. The thickness of this layer is approximately 80 μm in rat duodenum (Allen et al 1983) and 180 μm in mouse colon (Sakata & von Engelhardt 1981) although it varies considerably. The aqueous part is represented by the fluid layer between the mucus and the well-mixed bulk phase in the lumen (unstirred layer) or—in the closed or perfused intestinal segment with laminar flow in the latter—by the radial diffusion from the axis to the mucus layer. Gastric and intestinal mucus gel and soluble mucin reduce the diffusion or permeation of hydrogen ions (Williams & Turnberg 1980; Pfeiffer 1981; Lucas 1984; Sarosiek et al 1984; Piasek et al 1985; Turner et al 1985), [^{3}H]water, benzylpenicillin (Cheema et al 1984), sodium ions (Lucas 1984), pindolol, ergot alkaloids (Nimmerfall & Rosenthaler 1980), butyrate (Smith et al 1986), tetracycline (Braybrooks et al 1975), phenylbutazone (Barry & Braybrooks 1975), sucrose and peroxidase (Piasek et al 1985). To calculate the diffusion resistance in the mucus gel layer it is necessary to know the diffusion coefficient of the permeating substance in this layer. Therefore, in view of previous absorption experiments in rat jejunum (Winne 1978; Winne et al 1987), the diffusion coefficients of [^{3}H]water, urea, benzoic acid, antipyrine, aminopyrine, α-methyl-glucoside, L-phenylalanine and, for comparison, of hydrogen ions were determined in native mucus gel from rat small intestine.

Materials and methods

Substances and solutions. The substances obtained from Radiochemical Centre, Amersham, UK were: [dimethylamine-^{14}C]aminopyrine, 1·11 GBq mmol^{-1}; [*N*-methyl-^{14}C]antipyrine, 2·18 GBq mmol^{-1}; [carboxyl-^{14}C]benzoic acid, 2·07 GBq mmol^{-1}; methyl(α-D-[U-^{14}C]gluco)pyranoside, 10·32 GBq mmol^{-1}; [^{14}C]urea, 2·18 GBq mmol^{-1}; [^{3}H]water, 185 MBq mL^{-1}. L-[U-^{14}C]Phenylalanine, 1·67 GBq mmol^{-1} was purchased from Centre d'Etudes Nucleaires de Saclay, Gif-sur-Yvette, France.

L-Phenylalanine, urea, D-(+)-glactose and *N*-acetylneuraminic acid for biochemical use were obtained from Merck, Darmstadt, FRG. Antipyrine purum, sodium benzoate purum, methyl-α-D-glucopyranoside purissimum, cyclohexanesulphamic acid sodium salt purum were obtained from Fluka, Buchs, Switzerland. Aminopyrine DAB 7 was from Pharma-Zentrale, Herdecke, FRG, DNA from calf thymus research grade was from Serva, Heidelberg, FRG, and bovine serum albumin pure and mucin from porcine stomach crude type II were from Sigma, Deisenhofen, FRG.

Labelled and unlabelled substances were dissolved in buffer, so that 1 mL of this solution added to 90 mL buffer resulted in 1 mmol L^{-1} substrate. The buffer solution (pH 6·8) was identical to the perfusion solution used previously in intestinal absorption experiments (Winne et al 1987) and contained 5·03 mmol L^{-1} KH_2PO_4, 4·97 mmol L^{-1} Na_2HPO_4, 16·8 mmol L^{-1} urethane, 109·5 mmol L^{-1} NaCl, and 39·8 mmol L^{-1} cyclohexanesulphamic acid sodium salt (to maintain the constancy of the luminal volume in the jejunal segment as much as possible). The osmolality of the solution was 320 mosmol kg^{-1} and corresponded to the osmolality of plasma from rats anaesthetized with urethane. The unbuffered isotonic (300 mosmol kg^{-1}) saline contained 161·6 mmol L^{-1} NaCl.

Collection of native mucus gel. Conventionally bred male Wistar rats, 450–550 g, were anaesthetized with urethane (4·5 mL kg^{-1} i.p., 25% solution). After laparotomy the small intestine was excised and stored in ice cold saline. Segments of about 10 cm length were opened at the mesenteric border, unfolded and excess fluid was blotted. The superficial mucus layer was gently scraped off with a glass slide and collected in a small beaker. Sufficient for one diffusion experiment, 0·5–0·7 g mucus gel, was obtained from one small intestine.

Analyses. Fifty or 20 μL solution was mixed with 4·5 mL Lumagel and the radioactivity was measured in a liquid scintillation counter without quench correction. The chemical analysis (DNA, protein, hexose, glycoprotein, free sialic acids) of the mucus gel was performed according to Croft & Lubran (1965), Lowry et al (1951), Winzler (1955), Mantle & Allen (1978), and Warren (1959), respectively.

Determination of the diffusion coefficient. Samples of fresh mucus were sandwiched between two Millipore filters (HWPA, pore size 0·45 μm, Smith et al 1986) separated by a stainless steel spacer 0·6, 1 or 1·6 mm thick with three rounded rectangular (2·5 × 0·3 cm) slits of 2·254 cm^2 total cross sectional area. The sandwiched mucus layer was clamped between two outer stainless steel supports (1 mm with corresponding slits) and two plexiglass chambers. The whole apparatus was suspended in a water bath so that the temperature in the chambers was maintained at 38°C. The donor chamber (chamber A) was filled with 90 mL and the receptor chamber (chamber B) with 4·7 mL buffer. Chamber A was stirred by a paddle (1 × 1 cm, 1200 rev min^{-1}) and chamber B by a magnetic bar (1200 rev min^{-1}). After 30 min equilibration a 50 μL and a 20 μL sample were taken from chamber A and B, respectively, for the determination of the background activity. At zero time 1 mL substrate solution was added to chamber A. The substrates were labelled with ^{14}C or ^{3}H and their initial concentration in the donor chamber amounted to 1 mmol L^{-1}. Subsequently, three 50 μL samples and six 20 μL samples were taken from chamber A and B, respectively, at equal intervals so that the last sample was taken when the

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Table 1. Diffusion coefficient in buffer solution and native mucus gel of rat small intestine. Temperature 38°C, number of measurements in parentheses, [a] = data from Winne et al (1987), [b] = correct value (misprint in original reference), [c] = hydrogen ion in isotonic saline. The difference between results from the diffusion cell method and the capillary method were not significant ($P < 0.05$).

	Diffusion coefficient × 10^5 ($cm^2\ s^{-1}$)				
	Diffusion cell method				Capillary method[a]
Substance	Buffer[c]	Mucus gel	Ratio		Buffer
L-Phenylalanine	0·90 ± 0·06	0·41 ± 0·02	0·45 ± 0·02	(17)	0·97 ± 0·03
α-Methyl-glucoside	1·09 ± 0·22	0·40 ± 0·07	0·37 ± 0·06	(18)	1·06[b] ± 0·01
Aminopyrine	1·00 ± 0·22	0·38 ± 0·04	0·38 ± 0·05	(14)	1·08 ± 0·02
Antipyrine	1·10 ± 0·20	0·44 ± 0·05	0·40 ± 0·04	(14)	1·13 ± 0·02
Benzoic acid	1·23 ± 0·19	0·56 ± 0·07	0·46 ± 0·05	(18)	1·36 ± 0·02
Urea	1·66 ± 0·14	0·84 ± 0·08	0·51 ± 0·03	(18)	1·76 ± 0·03
[^{3}H]Water	3·14 ± 0·32	1·67 ± 0·17	0·53 ± 0·03	(17)	3·33 ± 0·03
Hydrogen ion	5·31 ± 0·54	0·35 ± 0·02	0·066 ± 0·007	(33)	

activity in chamber B amounted to about 30% of the activity in chamber A. The binding of the substrate to the Millipore filter was 3% for aminopyrine, antipyrine, and L-phenylalanine and less than 0·2% for the other substrates.

The H^+-ion diffusion coefficient was determined in the same manner except that saline was used instead of buffer and pH was recorded in both chambers at 5 min intervals. At zero time 1 mL of 0·1 or 0·01 M HCl was added to chamber A resulting in a pH value of 3 or 4, respectively.

The overall permeability coefficient P ($cm\ s^{-1}$) of the layers between the well-mixed bulk phases of the chambers was determined for each experiment from the measured radioactivity in the samples using a closed two compartment model. The overall permeation resistance 1/P was regarded as the sum of the partial resistances:

$$1/P = R + \delta/D \qquad (1)$$

R = permeation resistance ($s\ cm^{-1}$) of the two Millipore filters and the adjacent unstirred fluid layers, δ = thickness (cm) of buffer solution or mucus gel (i.e. the thickness of the spacer), D = diffusion coefficient ($cm^2\ s^{-1}$) of substrate in buffer solution (D_w) or apparent diffusion coefficient in mucus gel (D_m). The diffusion coefficients D_w and D_m and their ratio D_m/D_w were determined by weighted non-linear regression analysis with P as dependent variable, δ as independent variable and R, D_w, D_m as unknown parameters using equation 1. The reciprocal variance of P was used as a weighting factor.

Results and discussion

Composition of native mucus gel from rat small intestine. To characterize the mucus barrier in the rat small intestine (the mucous part of the pre-epithelial diffusion resistance) the following components were determined in the mucus gel obtained by gently scraping off the mucus (mg g^{-1} dry weight, mean ± s.e.m., n = 10): protein 374 ± 22 (s.d. = 71), glycoprotein 301 ± 18 (s.d. = 56), hexose 34 ± 3 (s.d. = 9), sialic acid 9 ± 2 (s.d. = 7), DNA 11 ± 1 (s.d. = 3); dry weight 16·2 ± 0·3 (s.d. = 1·1)%. The high dry weight and the low hexose concentration suggest that the native mucus is heavily contaminated with cellular debris (Mantle & Allen 1981).

Diffusion coefficient in buffer solution and native mucus gel. After adding the substrates to chamber A their concentration in chamber B increased linearly, or almost linearly, without significant lag time indicating that pseudo steady-state was reached almost immediately. Mucus gel reduced the steepness of the curves. The only exception was the permeation of H^+-ions through mucus. Due to the buffering capacity of the mucus gel for H^+-ions the entrance of these ions into chamber B was retarded as shown previously by Turner et al (1985). Therefore, only the upper segment of the curves has been used for the calculation of the overall permeability coefficient, since steady state flux can be assumed only in this region.

The diffusion coefficients are listed in Table1. The values measured in buffer solution agree well with the results obtained by the capillary method (Winne et al 1987). The agreement demonstrates that convection in the diffusion cell as observed by Lucas (1984), was absent or insignificant, so that the diffusion coefficients were not overestimated.

Native mucus gel diminished the diffusion coefficient of L-phenylalanine, α-methyl-glucoside, aminopyrine, antipyrine, benzoic acid, urea, and [^{3}H]water by 50–60%. The diffusion of H^+-ions was retarded to a greater extent with the coefficient being reduced to 7%. Thus, compared with free water, the mucus gel represents a considerable hindrance to drugs and nutrients.

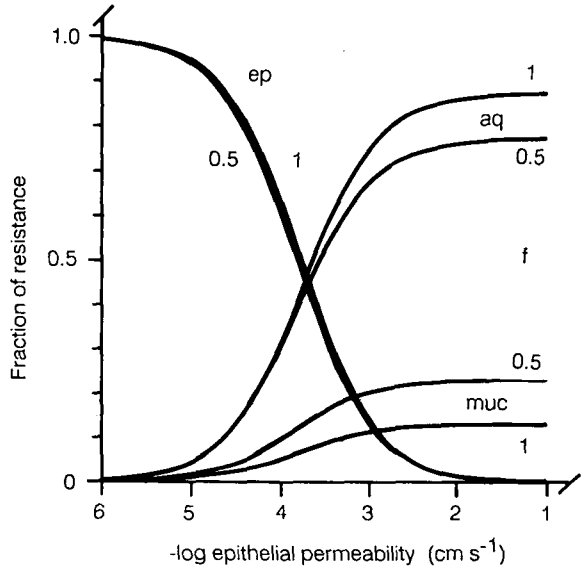

FIG. 1. Partial permeation resistances in perfused rat small intestine. Model calculation with laminar flow, perfusion rate 0·2 mL min^{-1}, segment length 5 cm, intraluminal radius 0·2 cm, free diffusion coefficient of substrate $1 \cdot 10^{-5}\ cm^2\ s^{-1}$, thickness of mucus gel layer 80 μm (mucus layer not stirred), diffusion in mucus gel reduced by 50% (f = 0·5) or not (f = 1), approximative equation of Winne (1989); ep = epithelial resistance (neglecting villous structure), muc = resistance of mucus gel layer, aq = aqueous part of pre-epithelial diffusion resistance = radial diffusion resistance in laminar flow.

Whether the mucus gel layer influences the absorption in the intestine depends on the relative resistance of the mucus gel layer compared with the resistance of the epithelium and the aqueous part of the preepithelial diffusion resistance. Fig. 1 shows the result of a model calculation for a perfused rat jejunum. With increasing epithelial permeability the rate limiting step shifts from the epithelium to the aqueous part of the epithelial diffusion resistance. A mucus gel layer of thickness 80 μm represents maximally 23% of the total permeation resistance, an unstirred water layer of the same thickness only 13%.

Other experimental results demonstrate that mucus can play a role in the intestinal absorption process. The absorption of tetracycline and phenylbutazone from a mucin solution is reduced compared with the absorption from a mucin free solution (Barry & Braybrooks 1975; Braybrooks et al 1975). The absorption of ergot alkaloids runs parallel to their diffusion coefficients in mucus gel (Nimmerfall & Rosenthaler 1980). Mechanical removal of the mucus gel layer increases the absorption rate of tetracycline (Kearney & Mariott 1982).

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