Evaluation of Viability of Excised Rat Intestinal Segments in the Ussing Chamber: Investigation of Morphology, Electrical Parameters, and Permeability Characteristics

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Purpose. To clarify relations between alterations in electrical and permeability data with time and to elaborate accompanying structural changes of intestinal segments in Ussing chamber experiments.

Methods. Excised intestinal segments from the rat were studied in a modified Ussing chamber. Experiments were run up to 180 minutes during which the electrical parameters, PD, SCC, and R, were measured and the permeability coefficients (P_{app}) of mannitol and propranolol were determined. Each segment was observed under the light microscope for morphological evaluation.

Results. PD and SCC values showed a decrease for most segments while the R values remained steady throughout the experiment. The P_{app} for propranolol increased aborally to the small intestine. For mannitol, the reversed was observed. In some cases, there was a time-dependent change in permeability for these marker molecules. The main morphological changes observed were a decreased nucleo-apical distance, decreased villi amplification factor, initial edema, cell sloughing, and epithelial restitution.

Conclusions. The time-dependent changes in permeability coefficients of mannitol and propranolol are suggested to be related to changes in electrical parameters and morphological alterations. Presented data illustrates the importance of information regarding time-dependent structural changes for correct interpretation of permeability data.

KEY WORDS: rat intestinal segments; Ussing chamber; viability; electrical parameters; permeability; morphology.

INTRODUCTION

Several *in vitro/in situ* methods are used for prediction of oral drug absorption in humans. Excised tissues in the Ussing chamber, Caco-2 cells and perfused intestine are some of the most commonly used (1,2). The Ussing chamber and Caco-2 cell methods allow measurements of apparent permeability coefficients (P_{app}) of drugs over the intestinal epithelia, without interference from liver metabolism and can be used for screening purposes during drug discovery and development. Due to the artificial milieu to which the excised intestine, tissues or cultured cells are exposed in such a system, it is of crucial importance to monitor tissue viability and integrity during the experiment. In addition, variations in permeability values from different laboratories using the same methods could be attributed to variations in the handling of the tissue (1) resulting in various mucosal morphological changes.

In general, tissue integrity is measured by transport of marker molecules, e.g., mannitol, PEG-4000, inulin, and ^{51}Cr-EDTA (3), release of LDH (lactate dehydrogenase) (4), transport of D-glucose, D-glucose induced short-circuit current (SCC), electrical parameters e.g. potential difference (PD), SCC, and resistance (R). In the Ussing chamber system, traditionally, electrical and permeability data are the two main parameters used to characterize the viability and the barrier function of the excised intestinal segments (5). Despite of this, what the changes in these parameters actually represents regarding tissue integrity and viability is not evident from the literature.

In the present study our aim is to clarify the relation between alterations in electrical and permeability data with time and structural changes of excised intestinal segments in Ussing chamber experiments.

MATERIALS AND METHODS

Chemicals and Solutions

Transport studies were carried out in a Krebs-Bicarbonate Ringer's solution (KBR) with the composition reported earlier (5). The fluid had a pH of 7.4 when gassed with a 95% O_2/5% CO_2 gas mixture. ^{14}C-mannitol and ^{3}H-propranolol (NEN™, Boston, USA) were used for studying passive paracellular and transcellular permeability of the segments, respectively.

Animals

Thirty male Sprague-Dawley rats, at least 100 days of age, were used in this study. The rats had free access to food and water prior to sacrifice. All investigations using experimental animals was adhered to the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985).

Preparation of Excised Segments

Rats were anesthetized with isofluran, Forene® (Abbott) to maintain systemic circulation during excision of the intestine. The intestine was rapidly removed from the anesthetized rat and cut into the 4 segments; duodenum, jejunum, ileum and colon, washed with cold KBR solution and put into beakers with KBR solution on ice, which was continuously gassed with a O_2:CO_2 (95:5) gas mixture (5). The intestinal segments were allowed to rest for approximately 30 minutes for lowering of the tissue temperature before preparation of the tissue for the chamber to minimize tissue damage during preparation. Pieces, approximately two cm long, from the mid part of the duodenum, proximal part of the jejunum, the distal to mid part of the ileum, and the descending colon were opened along their mesenteric

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ABBREVIATIONS: PD, potential difference; SCC, short-circuit current; R, resistance; P_{app}, apparent permeability coefficient; KBR, Krebs-Bicarbonate Ringer's solution.

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border. Care was taken to avoid the Peyers patches. In duodenum, jejunum and ileum, the serosa was carefully removed. In the colon, both the serosa and the muscularis externa was removed under the microscope using blunt dissection. During preparation, tissues were submerged in continuously gassed 10°C KBR solution (5).

Ussing Chamber Set-Up

After preparation, tissues were mounted in the Ussing chamber as flat sheets on a segment holder, with a surface area of 1.14 cm^2. Ten ml of KBR was added to each compartment of the Ussing chamber and the solutions were gassed with a O_2/CO_2 (95/5%) gas mixture (5). Rotors with a stirring speed of approximately 300 rpm were used to obtain effective stirring of the bathing solutions. The chambers were heated to 37°C by water jackets. Before an experiment, the electrical parameters of the intestinal segments were allowed to stabilize for 30–40 minutes to allow the tissue to recover from the preparation and to equilibrate in temperature (5). Any tissue with a potential difference (PD) <4 mV (small intestine) or <6 mV (colon) was omitted before the start of an experiment, as presented earlier (6,7,8), (these segments constitute approx. 4% of all segments in this study).

Electrical Measurements

Electrical parameters were recorded during the experiments using a four electrode system. PD was recorded using calomel electrodes connected through agar/NaCl-bridges. At each measuring time, five current (I) pulses (0, ±15, ±30 μA) were sent (9). The current was given through AgCl electrodes through the chamber and the voltage response (U) was measured. The current (I)/voltage (U) pairs form a linear plot. PD was obtained from the intersection with the voltage axes, i.e., when I = 0, the resistance (R) of the tissue segments was calculated from the slope of the line. The R calculated is the total tissue resistance; this includes epithelial resistance and subepithelial resistance and was corrected for external adjustable resistance, e.g., the R of the bathing solution (10,11). The short-circuit current (SCC) was calculated from Ohm's law (I = U/R) (5,9).

Permeability Experiments

Start of Experiment

The KBR in each chamber was replaced by ten ml preheated KBR (to avoid temperature differences when starting the experiment) (5). To the mucosal side, trace amounts of the radiolabeled mannitol and propranolol were also added.

Sampling

The intestinal segments were studied using 4 different experimental protocols: 0, 60, 120, and 180 minutes. The segments representing 0 minutes were used as controls. Following equilibration in the chamber, the tissues were immediately fixed in 3.8% formaldehyde, e.g., these segments were not started. For the rest of the experimental protocols, 50 μl aliquots were withdrawn from the serosal side at regular time intervals and was replaced with fresh KBR. The aliquots were placed in scintillation vials for further analysis of content of radioactivity. At the end of the experiments, 3.8% formaldehyde was added to both sides, and the segments were fixed for 1 hour. Each experimental protocol was repeated 4–6 times for each experimental time and part of the intestine.

Methods of Analysis

Four ml of OptiScint Hisafe (Wallac Scintillation Prod.) was added to each scintillation vial and sample was analyzed in a liquid scintillation counter (Wallac, Finland).

Calculations

The apparent permeability coefficients (P_{app}) were calculated using equation 1 (3).

$$P_{app} = dQ/dt * 1/AC_0 \quad (1)$$

where dQ/dt represent the steady-state appearance rate of mannitol or propranolol on the serosal side, C_0 the initial concentration of the two-marker molecules on the mucosal side, and A the gross surface area of the membrane (3).

Preparation for Microscopy

Following the initial fixation for one hour in the chamber, the holder with the tissue was additionally put into a beaker with formaldehyde and fixed over night. Tissues were then dehydrated with ethanol, imbedded in paraffin, cut and stained with Periodic-acid Schiff (PAS) (to distinguish goblet cells) and Mayer's hematoxylin solution (MHS) (for coloring of cell nuclei).

Microscopy

On each glass slide two sections from each tissue were prepared and all slides were investigated in this study. All slides were coded to avoid the observer 'bias' (12) and were observed at a magnification of ×400 using a light microscope. To be able to describe possible changes in the structure of the tissue during the experiment in the Ussing chamber, tissues were studied and evaluated for morphological changes using the following parameters:

Nudeo-Apical Distance

Measurement of the distance between the nucleus and the apical membrane in enterocytes with a measuring-rod (13). Five measurements in each villus and five villi were measured in each slide. The villi were randomly chosen and measurements were all conducted at the tips of the villi.

Villus/Crypt Index

Villus index was used for duodenum, jejunum and ileum and crypt index for colon. Measurement of height (villi) or depth (crypt) and width (one-half of height (14) or depth) was done with a measuring-rod of five villi/crypts in each slide that were randomly chosen. Height/depth was divided with width to receive the index.

Morphological Scoring

Evaluation of the condition of the segment, including the epithelial cell layer, thickness, oedema, villus height or crypt

depth and amount of cells and mucus in lumen. A scale from 0–4 was used, where 0 indicates a normal undamaged segment and 4 a severely damaged segment (Table I) (modified from ref. 12 and 15).

Morphometric Measurements

Calculation of the amplification factor of the absorptive area of the villus was performed by a morphometric method using a cycloid frame (16). Micrographs were taken of all segments and a reference line was drawn at the basal lamina and a cycloid frame was randomly placed on the pictures. The number of intersections of cycloid arches and villi apical membranes were counted and this number was divided with the number of intersections with the reference line. This was repeated five times for each experimental protocol for duodenum, jejunum and ileum.

Statistics

Values are presented as means ± one standard deviation (SD). Significance of influence of experimental time for the whole experiment was tested for most morphological and permeability parameter by a one-way variance analysis (ANOVA). In ANOVA, one analysis was performed for each part of the intestine and in each case, H_0 (time has no effect), was rejected if $p < 0.05$. Morphological scoring, on the other hand, was subjected to a Spearmans correlation test instead since this parameter is subjective. A test of linear relationship between scoring and time was performed and the closer to 1, the stronger this relationship; scoring increases with increased experimental time. All data were analyzed using SPSS (release 7.0) and StatXact-3 (version 3.0.2) software.

RESULTS

Electrical Measurements

With regard to the electrical parameters; PD, SCC and R, the different regions of the rat intestinal tract showed differences. Before the start of the experiments, the small intestinal segments showed in general a PD at t = 0 of 4–8 mV while in colon this was as high as 6–10 mV. SCC varied in the small intestine between 100–250 $\mu A/cm^2$ and in colon between 40–80 $\mu A/cm^2$. Finally, the resistance values at t = 0 in the small intestine varied between 20–50 $\Omega \times cm^2$ and in the colon between 100–180 $\Omega \times cm^2$. Figure 1 (a–c) shows the electrical parameters for the 180 min experimental protocol in the different segments which are representative for the over time variations for all experimental times. Results from the PD measurements can be seen in Fig. 1a. In general, for the small intestinal segments the PD decreased by time. For the colon segment the PD decreased within the first hour and thereafter increased. Variations in SCC with time for the different segments are shown in Fig. 1b. A marked difference in SCC initially between the different tissues was observed. However, after 180 minutes in the Ussing chamber all segments except duodenum had similar SCC values. In general, duodenum showed much higher and more stable SCC values throughout the experiment. Tissue resistance values (R) for the different intestinal regions are shown in Fig. 1c. All intestinal segments showed stable R values throughout the experimental period and the R values in colon were 3–4 times higher than in the small intestinal segments.

Permeability Experiments

The permeability coefficients (P_{app}) for propranolol and mannitol in the different segments can be seen in Fig. 2 (a-b). The permeability values of propranolol increased along the intestinal tract in the ranking order: duodenum < jejunum < ileum < colon, while the permeability values for mannitol showed lower values in colon than in the small intestine. According to one-way variance analysis, P_{app} for propranolol and mannitol in duodenum and P_{app} for propranolol in ileum was significantly influenced by the length of the experiment in the Ussing chamber. As judged from P_{app} values obtained for propranolol, a steady state condition was reached after 120 minutes in duodenum and jejunum, and in colon after 60 minutes (Fig. 2a). For mannitol, stable permeability values were obtained in jejunum, ileum and colon segments after 60 minutes (Fig. 2b).

Morphological Studies

Micrographs of duodenum and colon after the experimental times of 0 and 180 minutes are shown in Fig. 3 a, b and 4 a, b respectively. In these micrographs all morphological parameters described below can be viewed.

Nucleo-apical distance was measured in duodenum, jejunum and ileum (Fig. 5). A decreased nucleo-apical distance with increased experimental time could be observed in all three

Table I. Morphological Scoring of Tissue Damage[a]

Grade of damage	Description of damage	
	Epithelium	Villus
0	No damage	No damage
1	Single cells extruded	Slight oedema
2	Groups of cells extruded and/or thin epithelium	Villus height decreased and/or several villi show oedema
3	Sheets of cells extruded and/or thin epithelium	Villus height greatly reduced and/or crypts dilated and/or severe oedema
4	Total loss of epithelium	Villus structure flattened and/or severe oedema

[a] Adapted from Ref. 12 and 15.

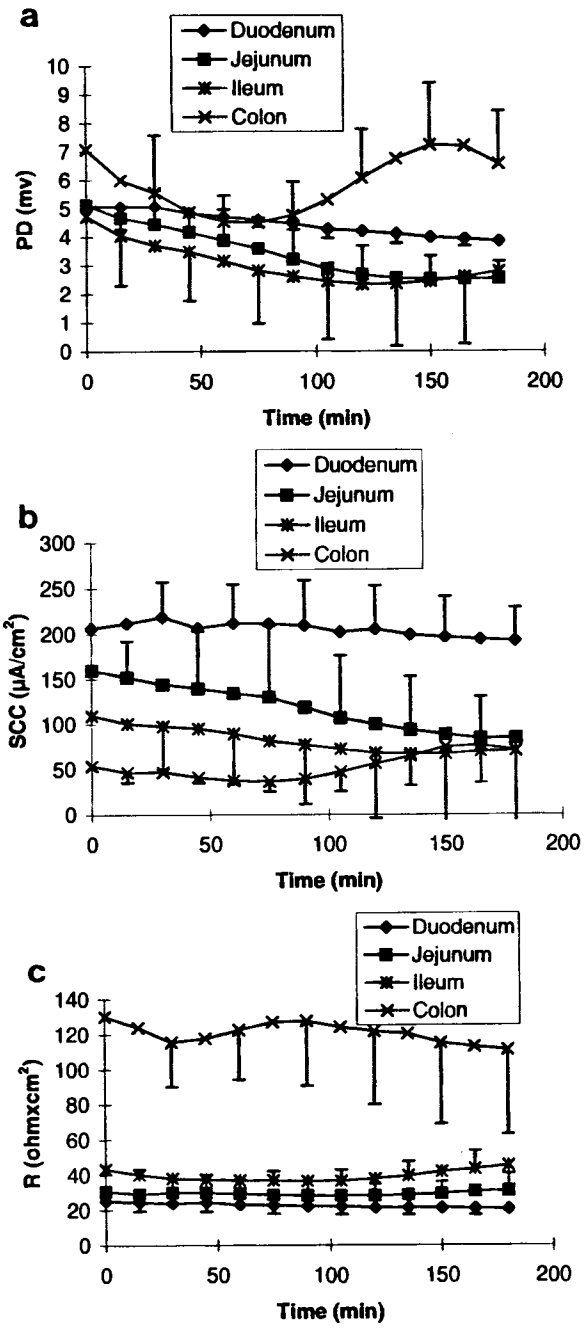

Fig. 1. Mean electrical parameter changes, PD (a), SCC (b), and R (c), for rat duodenum, jejunum, ileum, and colon during 180 minutes experimental time.

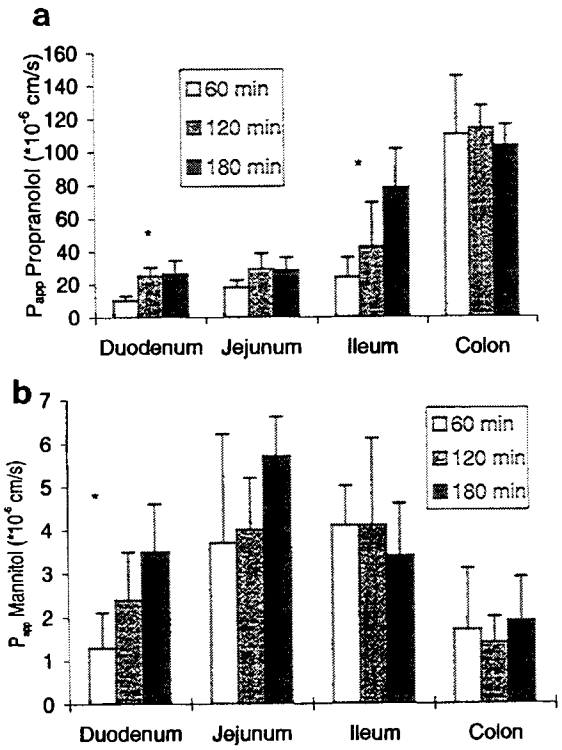

Fig. 2. Apparent permeability coefficients (P_{app}) of propranolol (a) and mannitol (b) in duodenum, jejunum, ileum, and colon of the rat at different experimental times. Values are presented as the mean P_{app} values ± SD (N = 4–6). Statistical significance for effect of experimental time was obtained for duodenum (propranolol and mannitol) and ileum (propranolol), $p < 0.05$.

regions. According to the one-way variance analysis, a significant influence of the length of the experiment was observed for duodenum and for ileum ($p < 0.05$).

Villi index calculated for the small intestinal segments and *crypt index* calculated for colon are presented in Fig. 6. A small tendency towards decreased index could be seen, however, no significance of time related changes was detected for any of the segments.

Morphological scoring results are shown in Fig. 7. All segments showed a correlation between scoring and time; i.e. scoring increased with increased experimental time. The Spearman's correlation test runs from −1 to 1, where 0 is the indicator of no correlation. All segments in this study where above 0, which means there was a positive correlation ($p < 0.05$). The morphological scoring value at time point 0 was not actually a true zero value since there was already morphological damage at t = 0.

Morphometric measurement results are shown in Table II. A cycloid frame was used to calculate the villi amplification factor. The amplification factor for duodenum and jejunum were twice as large as for ileum. For the small intestinal segments, a decreased amplification factor (approximately 50% of the initial amplification) could be seen with increased experimental time. No statistical analysis was performed of this parameter because only two measurements were performed for each segment and experimental time. The segments were, however, randomly chosen among representative segments taken from the same time point.

DISCUSSION

Electrical Measurements

Electrical parameters are widely accepted for monitoring the viability and integrity of segments in the Ussing chamber. In general, PD is said to reflect the voltage gradient generated by the tissue, R the tissue integrity and SCC reflects the ionic fluxes across the epithelium.

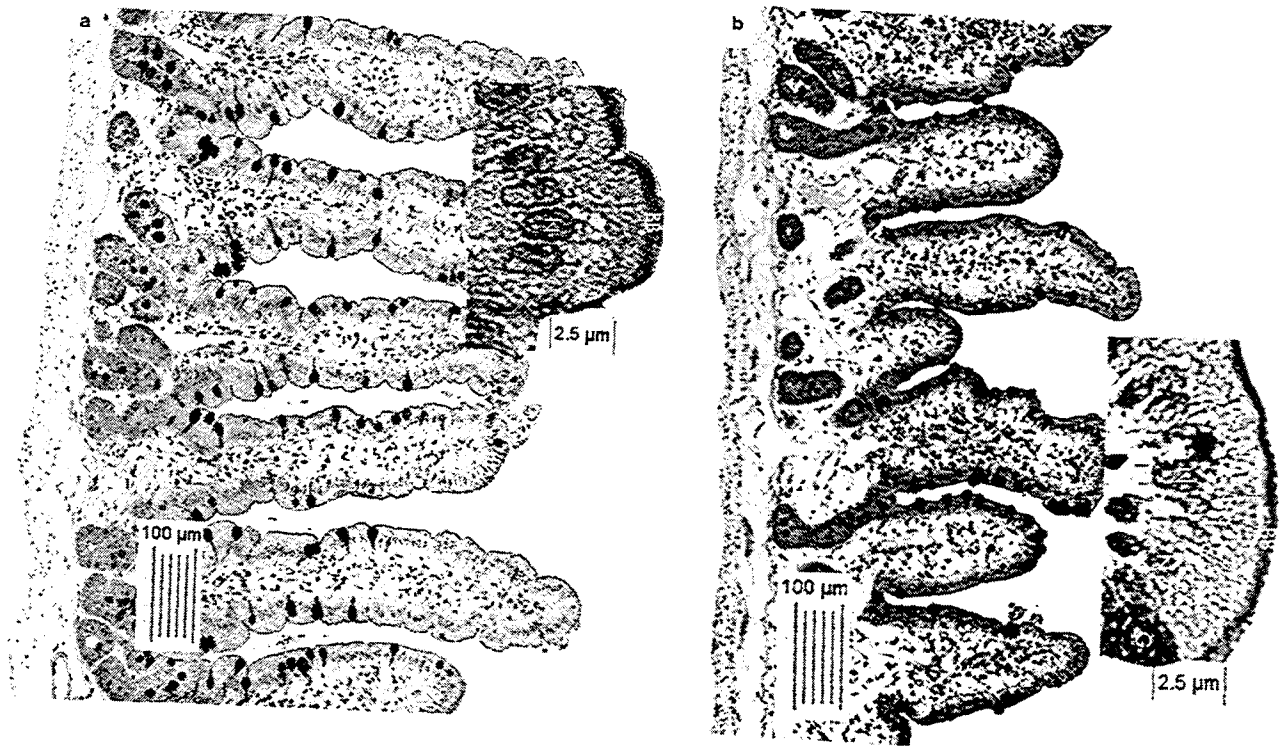

Fig. 3. Light micrograph of duodenum, as representative for the small intestinal segments, after the experimental times of 0 minutes (a) and 180 minutes (b) respectively, approximately ×50 magnification. Inset: close up view of the epithelium; N, nucleus; G, goblet cell; BB, brush border membrane; approximately ×400 magnification.

Compared to studies in humans (17), the PD values for the rat intestine in the present study show the same profile; i.e., the PD becomes more lumen negative the further down the intestine the measurement is performed and these results are comparable to earlier reports (3,11,17). Our experience points out that occasionally large differences in tissue viability with time, represented by PD, can be seen using the Ussing chamber technique. For some experiments, as shown in the present study, both PD and SCC values decreased from the base-line value with time, except for the colon segments, which all showed an increased PD with time after an initial 60 min steady value.

The resistance (R) was stable for all small intestinal segments throughout the experiments. Thus, the alterations in morphology as well as permeability of the markers during the experiments were not reflected in changes in resistance, but rather to changes in PD and SCC. However, the underlying nature of R measured from these type of experiments is probably more complex than generally known, i.e., as a measure of tissue integrity. The measured R value represents the total tissue resistance and is the result of total epithelial surface area as well as the resistances over individual cells and tight junctions (18). When the different resistances are added the resulting total R may very well seem to be constant with time. In our experiments the morphometric changes observed; i.e., the reduction in total epithelial surface area of approximately 50%, will theoretically result in a doubling of the measured resistance per se. On the contrary, increased permeability for mannitol indicates a structural tight junctional alteration which may lead to a decreased R. These two effects added together may result in an approximately constant measured R. Thus, R does not reflect tissue integrity alone. The complexity of the factors influencing R during the experiments therefore needs further evaluation.

The colon segments behaved differently from the small intestinal segments, with an increased PD after 60 min and towards the end of the experiment, 180 min. What this electrical behavior indicates is not known, but could be speculated as a much later equilibrium of the tissue segment (>60 min) or a secretion of chloride into the lumen as a response to stressful stimuli. This secretion of an anion would result in a more lumen negative PD and an increased SCC if the resistance remains unchanged.

In general, the results of the electrical parameters are also dependent on the overall prechamber treatment (determined from previous results in this laboratory), from the anesthesia of the rats to the mounting of intestinal segments in the Ussing chamber and are also indicated by the results of the morphological scoring (see below).

Permeability Experiments

The results in the present study show a distinct regional difference in permeability for mannitol and propranolol which are in accordance with results reported earlier by Artursson *et al.* (3) and Ungell *et al.* (1). The permeability coefficient of propranolol in the present study increased aborally to the small intestine. For high permeability compounds like propranolol, the absorption occurs mainly at the tip of the villus. This regional variation could therefore not be attributed to differences in the

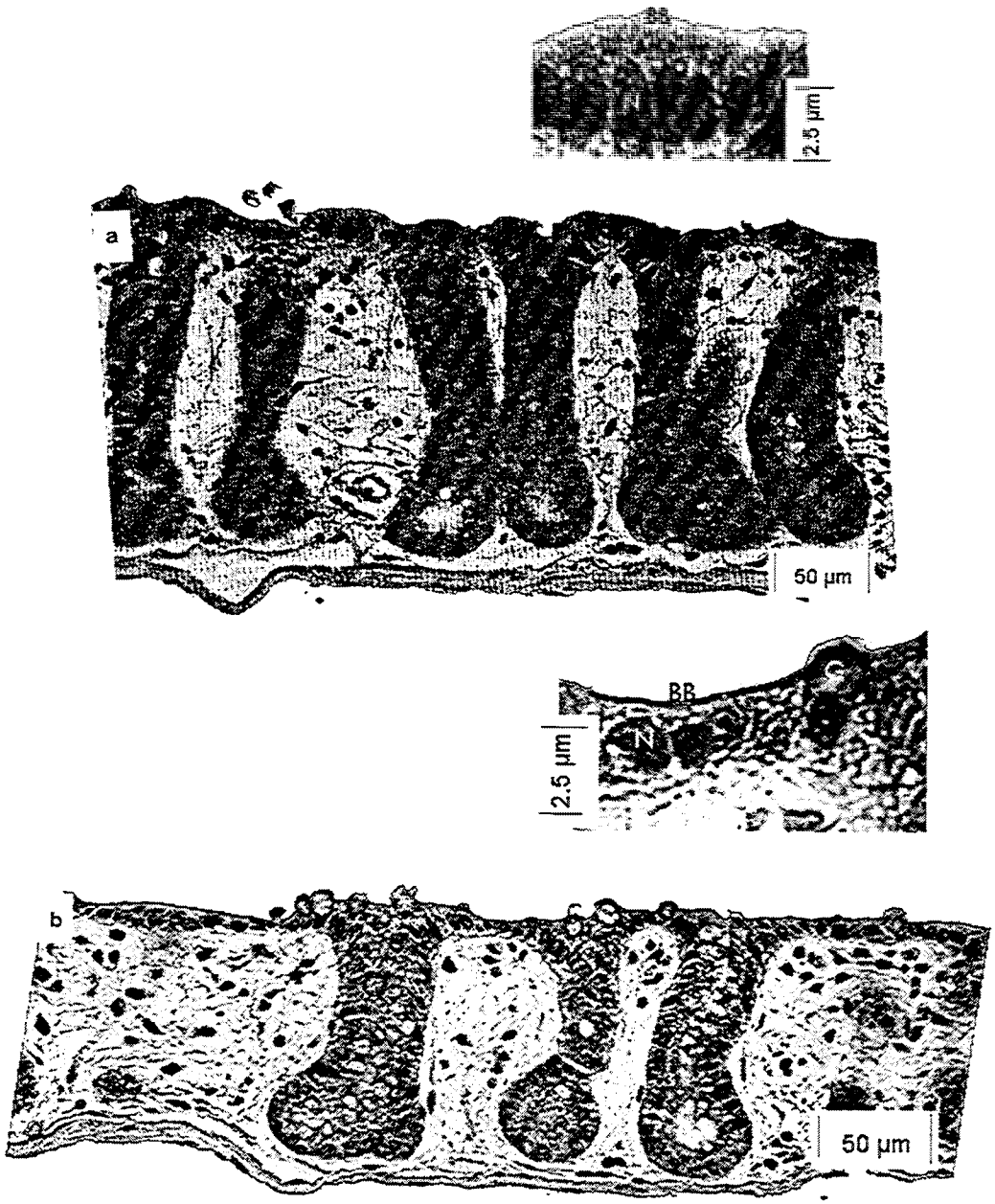

Fig. 4. Light micrograph of colon after the experimental times of 0 minutes (a) and 180 minutes (b) respectively, approximately ×100 magnification. Inset: close up view of the epithelium; N, nucleus; G, goblet cell; BB, brush border membrane; approximately ×400 magnification.

effective absorptive surface area along the intestinal tract (19), but rather to differences in the lipid composition (1), indicating a more favorable surface for hydrophobic drugs in the colon. Furthermore, small intestinal segments showed increased permeability for propranolol with increased experimental time, an increase which was highest in ileum. This increased permeability with change in experimental time in ileum can be explained by an increased pH of the microclimate adjacent to the epithelium (20) which could enhance the absorption for propranolol, which have a pKa value of 9.3. Also, the collapse of the villi structure (see below) could favor an equilibration in pH between bulk pH and the lower parts of ileal mucosa.

The permeability coefficient for the hydrophilic molecule mannitol decreased in the ranking order; small intestine > colon confirming the results by Artursson *et al.* (3) and Palm *et al.* (6), who showed similar result for PEG-400, mannitol, creatinine and the hydrophilic beta blockers atenolol and practolol. Argenzio and co-workers (21) showed that when colon epithelia were exposed to bile salts, a marked increased permeability to mannitol could be observed due to epithelial damage. The present study showed no increase in mannitol transport in colon with time, which indicates that there was restricted damage to this epithelium, also seen by the data from the morphological scoring and in the micrographs (Fig. 3–4). Duodenum and

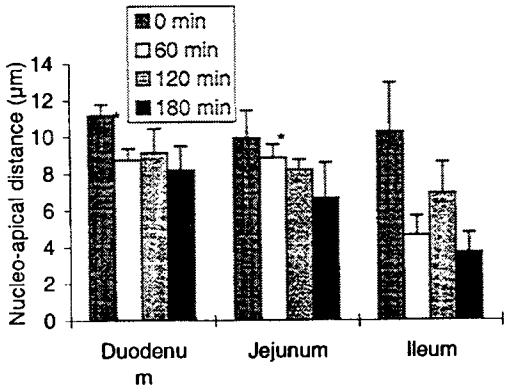

Fig. 5. Nucleo-apical distance (µm) for duodenum, jejunum and ileum for different experimental times, presented as mean values ± SD (N = 4–6). Statistical significance for effect of experimental time was obtained for duodenum and ileum, $p < 0.05$.

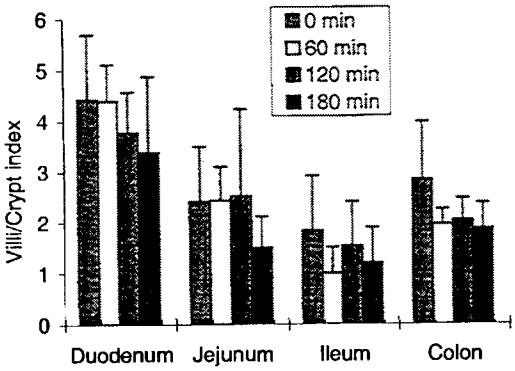

Fig. 6. Villi index for duodenum, jejunum and ileum, and crypt index for colon, for different experimental times, presented as mean values ± SD (N = 4–6).

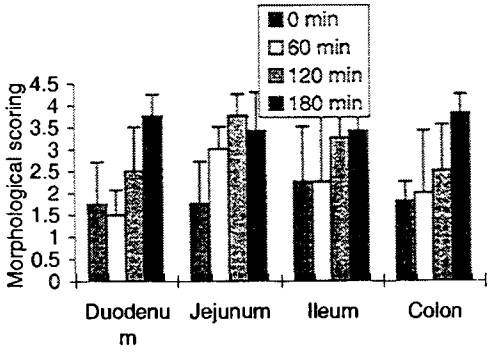

Fig. 7. Morphological scoring for duodenum, jejunum, ileum, and colon for different experimental times, presented as mean values ± SD (N ± 4–6). A correlation between increased scoring with increased experimental time was observed for all segments due to values above 0 in Spearman's correlation test, $p < 0.05$. The main cause for scoring values obtained at the different experimental times were; 0-60 min: oedema and cell extrusion, 120 min: extensive cell extrusion and loss of villi/crypt structure, and 180 min: loss of villi/crypt structure and a thin epithelium.

jejunum showed a significant increased mannitol transport with increased experimental time. The epithelium, however, remained intact according to the morphological scoring. One reason for the increased mannitol transport could be the extensive reparative process induced by the sloughing of cells or the widening of the space between villi (see below). This is also indicated by the reduction in villi index and nucleo-apical distance which indicate a reduction in number of cells. As the segment *in vitro* is able to start the restitution process by closing the damaged area by movement of adjacent cells, the epithelium becomes intact (22). No increase in mannitol transport could be observed in the ileum segments with increased length of experiment, which means that this part of the small intestine seems less sensitive to morphological or structural changes.

Morphological Studies

The nucleo-apical distance decreased with increased experimental time in all small intestinal segments (Fig. 5). In duodenum and ileum this decrease was statistically verified. The decreased distance was probably caused by the restitution process when cells flatten out to compensate for the extensive cell sloughing observed (12,23,24). This process can occur very rapidly on the villi tips *in vitro* and is not dependent on the cell division (22). The sloughing of cells increased with time in the Ussing chamber experiments for all small intestinal segments although not statistically significant. The villus index was calculated for the small intestinal segments. The proximal part of the small intestine had the longest and thinnest villi, while distally the villi were shorter and thicker as reported earlier by Thomson (14,18). As the decreased surface area available for drug absorption is inversely correlated to an increase in the apparent permeability coefficient, an indication that a larger absorptive surface along the crypt-villus-axis between the villi becomes available is suggested. It has previously been shown by Rafter *et al.* (13) that the crypt depth in rat colon decreased as a function of increased damage. This indicates that there is no progressive damage going on in the colon segments in the present study after the initial 60 minutes.

Our data suggest that there are no significant changes in villi/crypt index parameters for respective segment although the micrographs show a change. Therefore, in our opinion, morphometric measurement seems to be a better measurement of changes in surface area than villi/crypt index. A different pattern for all segments, depending on the length of experimental time, could be seen in the morphological scoring. Generally, for short experiments (0 and 60 min), the reason for the scoring results was oedema and at longer experimental times (120 and

Table II. The Different Calculated Amplification Factors Shown for each Experimental Time in the Small Intestine

Experimental time	Duodenum	Jejunum	Ileum
0	5.0	4.7	2.4
60	4.3	3.0	2.4
120	3.7	2.7	1.8
180	2.4	1.7	1.1
Total decrease from 0 min to 180 min (%)	52.0	63.5	54.9

180 min), the main cause was a decreased villus surface area. The scoring value of 1.5 obtained for the 0 minutes reading are explained by the fact that there was an initial handling, transfer of segments and equilibration time prior to each experiment. Thus the 0 minutes reading by definition is the time for start of an experiment in the Ussing chamber and does not represent an unaffected segment.

It is well known that villus amplification factors vary with intestinal location (18,25). In proximal small intestinal regions the amplification is reported to be sevenfold (26,27) and in distal ileum it is threefold (27) *in vivo*. Corresponding amplification values in the present study are 5 and 3 for jejunum and ileum respectively showing good agreement with earlier reports. Marcial *et al.* (28) have shown that the amplification is reduced about 50% when the segments are mounted in the Ussing chamber. The reason for the decreased villi amplification factor in the present study was the reduction in villus surface area (Table II) probably due to epithelial cell loss. At 180 minutes the regional difference were still valid although jejunum was the segment with the largest decreased villi amplification factor. The results of Bastie *et al.* (29) showed that jejunum was the segment with the largest decreased amplification due to the largest reduction in villus surface area.

CONCLUSIONS

The present morphological investigation of excised rat intestinal segments used for experiments in the Ussing chamber, adds information to the overall understanding of the different viability and integrity parameters used. The time dependent changes in permeability coefficients of mannitol and propranolol are suggested to be related to changes in electrical parameters and morphological alterations. The main morphological changes observed were decreased nucleo-apical distance, decreased villi amplification factor, initial oedema, cell sloughing, and epithelial restitution.

Presented data illustrates the nature of the time dependent alterations of segments and keeping these alterations in mind will facilitate correct experimental design and interpretation of permeability data using the Ussing chamber as a technique for drug absorption studies.

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REFERENCES

1. A-L. Ungell. (1997) *Drug Dev and Ind Pharm* **23**:1–14.
2. T. T. Kararli. Gastrointestinal absorption of drugs. *Crit. Rev. Therap. Drug Carrier Systems.* **6**:39–86 (1989).
3. P. Artursson, A.-L Ungell, and J.-E Löfroth. Selective paracellular permeability in two models of intestinal absorption: cultured monolayers of human intestinal epithelial cells and rat intestinal segments. *Pharm. Res.* **10**:1123–1129 (1993).
4. R. L. Oberle, T. J. Moore, and D. A. P. Krummel. Evolution of mucosal damage of surfactants in rat jejunum and colon. *J. Pharm. and Tox. Methods.* **33**:75–81 (1995).
5. A.-L Ungell, A. Andreasson, K. Lundin, and L. Utter. Effects of enzymatic inhibition and increased paracellular shunting on transport of vasopressin analogues in the rat. *J Pharm. Sci.* **81**:640–645 (1992).
6. K. Palm, K. Luthman, A-L. Ungell, G. Strandlund, and P. Artursson. Correlation of drug absorption with molecular surface properties. *J Pharm. Sci.* **85**:32–39 (1996).
7. R. B. Podesta and D. F. Mettrick. HCO_3 transport in rat jejunum: relationship to NaCl and H_2O transport in vivo. *Am. J. Physiol.* **232**:E62–68 (1977).
8. R. B. Podesta and D. F. Mettrick. HCO_3^- and H^+ secretion in rat ileum in vivo. *Am. J. Physiol.* **232**:E574–579 (1977).
9. J. Karlsson. Drug absorption in cell culture models of the intestinal epithelium. Doctoral thesis at Uppsala University, Department of Pharmacy, Division of Pharmaceutics (1995).
10. M. Hemlin, M. Jodal, O. Lundgren, H. Sjövall, and L. Stage. The importance of the subepithelial resistance for the electrical properties of the rat jejunum *in vitro*. *Acta Physiol. Scand.* **134**:79–88 (1988).
11. D. W. Powell. Barrier function of epithelia. *Am. J. Physiol.* **241**:G275–G288 (1981).
12. C. K. Henrikson, R. A. Argenzio, J. A. Liacos, and J. Khosla. Morphologic and functional effects of bile salt on the porcine colon during injury and repair. *Lab. Invest.* **60**:72–87 (1989).
13. J. J. Rafter, V. W. S. Eng, R. Furrer, A. Medline, and W. R. Bruce. Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. *Gut.* **27**:1320–1329 (1986).
14. A. B. R. Thomson, M. Keelan, M. T. Clandinin, and K. Walker. Dietary fat selectively alters transport properties of rat jejunum. *J. Clin. Invest.* **77**:279–288 (1986).
15. J. A. Fix, K. Engle, P. A. Porter, P. S. Leppert, S. J. Selk, C. R. Gardner, and J. Alexander. Acylcarnitines: drug absorption-enhancing agents in the gastrointestinal tract. *Am. J. Physiol.* G332–G340 (1986).
16. H. J. G. Gundersen, T. F. Bendtsen, L. Korbo, N. Marcussen, A. Moller, K. Nielsen, J. R. Nyengaard, B. Pakkenberg, F. B. Sorensen, A. Vesterby, and M. J. West. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Recent stereological developments for pathology.* **96**:379–394 (1988).
17. G. R. Davis, C. A. Santa Ana, S. G. Morawski, and J. S. Fordtran. Permeability characteristics of human jejunum, ileum, proximal colon and distal colon: results of potential difference measurements and unidirectional fluxes. *Gastroenterology.* **83**:844–850 (1982).
18. A. Collett, D. Walker, E. Sims, Y.-L. He, P. Speers, J. Ayrton, M. Rowland, and G. Warhurst. Influence of morphometric factors on quantitation of paracellular permeability of intestinal epithelia in vitro. *Pharm. Res.* **14**:767–773
19. A. Strocchi and M. D. Levitt. Role of villus surface area in absorption. *Dig. Dis. Sci.* **38**:385–387 (1993).
20. G. T. A. McEwan and M. L. Lucas. The effect of E. coli STa enterotoxin on the absorption of weakly dissociable drugs from rat proximal jejunum in vivo. *Br. J. Pharmacol.* **101**:937–943 (1990).
21. R. A. Argenzio, C. K. Henrikson, and J. A. Liacos. Effect of prostaglandin inhibitors on bile salt-induced mucosal damage of porcine colon. *Gastroenterology* **96**:95–109 (1989).
22. A. Nusrat, C. A. Parkos, T. W. Liang, D. K. Carnes, and J. L. Madara. Neutrophil migration across model intestinal epithelia: monolayer disruption and subsequent events in epithelial repair. *Gastroenterology* **113**:1489–1500 (1997).
23. R. Moore, S. Carlson, and J. L. Madara. Rapid barrier restitution in an in vitro model of intestinal epithelial injury. *Lab Invest.* **60**:237–244 (1989).
24. J. L. Madara. Maintenance of the macromolecular barrier at cell extrusion sites in intestinal epithelium: physiological rearrangement of tight junctions. *J. Membrane Biol.* **116**:177–184 (1990).
25. T. M. Mayhew and C. Middleton. Crypt, villi and microvilli in the small intestine of the rat. A stereological study of their variability within and between animals. *J. Anat.* **141**:1–17 (1985).

26. R. Stenling and H. F. Helander. Stereological studies on the small intestinal epithelium of the rat. *Cell Tissue Res.* **217**: 11–21 (1981).
27. T. M. Mayhew. Stereology: progress in quantitative microscopical anatomy. In V. Navaratnam and R. J. Harrison (eds.), *Progress in anatomy*, Cambridge University Press, Cambridge, 1983, pp. 81–112.
28. M. A. Marcial, S. L. Carlson, and J. L. Madara. Partitioning of paracellular conductance along the ileal crypt-villus axis: a hypothesis based on structural analysis with detailed consideration of tight junction structure-function relationships. *J. Membrane Biol.* **80**:59–70 (1984).
29. M. J. Bastie, D. Balas, J. Laval, F. Senegas-Balas, C. Bertrand, C. Frexinos, and A. Ribet. Histological variations of jejunal and ileal mucosa on days 8 and 15 after hypophysectomy in rat: morphometrical analysis in light and electron microscope. *Acta anat.* **112**:321–337 (1982).