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Structure of Zonulae Occludentes and the Permeability of the Epithelium to Short-Chain Fatty Acids in the Proximal and the Distal Colon of Guinea Pig

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Summary. Absorption of short-chain fatty acids has been studied in the proximal and the distal colon of anaesthetized guinea pigs. Segments were perfused with a solution similar in chemical composition to that of normal colonic fluids. In the proximal colon the permeability of the mucosa was similar for acetate, propionate and butyrate. For acetate the permeability was significantly higher in the proximal than in the distal colon, and the reverse was seen for butyrate. In the distal colon the short-chain fatty acids seem to be absorbed mainly in the undissociated form due to their lipid solubility; a paracellular pathway for the dissociated molecules is of no major importance. In the proximal colon, on the other hand, a considerable portion of acetate and propionate disappears in the ionized form. Light microscopy (semithin sections) and electron microscopy (freeze-fracture replicas) showed remarkable morphological differences between the proximal and the distal colon. "Leaky spots" with only few strands were present in the zonulae occludentes between the epithelial cells at the surface of the proximal colon. In the distal colon the junctions between the cells were more compact, and significantly more strands separated the lumen from the intercellular space. These results suggest that short-chain fatty acids could be absorbed by a paracellular pathway in the proximal colon, and not in the distal colon. In the proximal colon the number of strands of the zonulae occludentes between surface cells and that between cryptal cells was similar. On the contrary, in the distal colon significantly more strands were present between surface cells than between cryptal cells. Morphological and physiological considerations suggest that absorption of short-chain fatty acids in the crypts is negligible.

Key Words colonic epithelium · guinea pig · short-chain fatty acids · zonulae occludentes · freeze fracture · permeability

Introduction

In the large bowel by far most of the anions present are the weak electrolytes acetate, propionate and butyrate (Engelhardt & Rechkemmer, 1983*a*). These short-chain fatty acids (SCFA) are produced by anaerobic microbial fermentation of undigested food residues, mainly cellulose and pectins. The absorption of SCFA is of considerable nutritional significance in herbivorous animals.

These SCFA may pass through the intestinal epithelium by transcellular and by paracellular routes. It is assumed that the transcellular pathway can be used mainly by nonionized weak electrolytes; the ionized form may cross the epithelium via the paracellular route, if no special transport system is present, like the monocarboxylic acid carrier in the proximal tubule (Ullrich, Rumrich & Klöss, 1982).

Recent studies indicate that in the guinea pig marked differences exist in the absorption rates of SCFA in different sections of the colon (Engelhardt & Rechkemmer, 1983*b*). We were interested in the routes of the SCFA passage across the colonic epithelium. Therefore, the permeability of this epithelium for SCFA was investigated by *in situ* perfusion in the proximal and in the distal colon of the guinea pig. Since paracellular permeability differences between epithelial depend on the tightness degrees of the intercellular junctions (Bullivant, 1981; Powell, 1981) we compared the features of zonulae occludentes of these epithelia in freeze-fracture replicas.

Materials and Methods

Animals and Anaesthesia

Male guinea pigs weighing 350 to 500 g were used. A pelleted standard guinea pig feed (Altromin, Lage, NO. 3022) was fed *ad libitum*. Water was always available.

Anaesthesia of the animals started with an i.m. injection of a 1:1 mixture of ketaminehydrochloride (Vetalar®, Parke-Davis) and xylazine (Rompun®, Bayer). Anaesthesia was maintained after tracheotomy with halothane and nitrous oxide/oxygen in a closed system (Rechkemmer & Engelhardt, 1981).

Anatomy of the Guinea Pig Colon

The proximal colon was defined as the section between the caecum up to the attachment of Treitz's ligament. The transverse colon is located dorsal to the stomach. The distal colon begins where the transversal colon turns in a caudal direction. The last 5 to 10 cm of the large intestine is the rectum (Cooper & Schiller, 1975).

In situ Perfusion Studies

Experimental Procedure

The anaesthetized animals were fixed on a heated plate (39°C). The abdomen was shaved and opened along the linea alba. Ligatures were placed between caecum and proximal colon as well as between transverse and distal colon. Tubes (PVC, outer diameter 3 mm) were inserted through a small incision in the upper side of the proximal and of the distal colon, and ligated in the lumen. Special care was taken not to disturb the blood supply of the segments. A small incision was made in the aboral end of the proximal as well as the distal colon. After that, the segments were carefully cleansed through the inserted tubes with a perfusion solution heated to 38°C with a pressure that was never above 10 cm H_2O. When the outflowing fluid from the bowel segments was clean a second tube was placed into the distal incisions. Thereupon the continuous perfusion was started. Both segments were perfused with a solution similar in chemical composition to that of the natural fluid in the proximal colon of the guinea pig (Na 110; K 20; Ca 2.5; Mg 7.5; Cl 30; HCO_3 20; HPO_4/H_2PO_4 30; acetate 60; propionate 10; butyrate 10 mmol · liter^{-1}). The pH was adjusted to 6.1, and the osmolarity to 300 mosmol · liter^{-1} by adding mannitol. This solution contained 1 g · liter^{-1} polyethyleneglycol (PEG 4000, Merck) and 2.5 μCi ^{14}C-PEG 4000 (New England Nuclear, FRG). The perfusion rate was 30 ml · hr^{-1}. The outflowing fluid from each segment was collected anaerobically under liquid paraffin in 20-min intervals. The perfusion periods lasted 3 to 4 hours.

During the perfusion the abdomen was closed with clamps. After the end of the perfusion period animals were killed by an intracardiac injection (T61®, Hoechst). After that, the intestinal segments were placed in 0.9% NaCl at 20°C. The segments were opened on the mesenteric side, flattened, and the mucosa was scraped off with a glass slide. Mucosa scrapings were freeze-dried.

Chemical Analysis and Calculations

SCFA were analyzed by gas-chromatography. To 1-ml samples 0.1 ml 96% formic acid was added, thoroughly mixed and centrifuged. Analysis was done at isotherm conditions (130°C) on chromosorb W-AW with 20% NPGS and 2% phosphoric acid, detector temperature 230°C, injection temperature 220°C (gaschromatograph Hewlett-Packard 5750 G, with electronic integrator 3370 G, and sampler 7670 A). ^{14}C-PEG was estimated by liquid scintillation counting (Packard, Tri-Carb 2420). All samples were corrected for quenching by the external standard method.

The clearance of SCFA from the colonic perfusion solution was calculated from net absorption of SCFA and from SCFA concentrations.

Net flux of SCFA $= C_i \cdot V_i - C_o \cdot V_i \cdot \mathrm{cpm}_i : \mathrm{cpm}_o$;

clearance = net flux : $((C_i + C_o) : 2)$;

where C_i and C_o are SCFA concentrations, and cpm_i and cpm_o are the ^{14}C-PEG activities in the inflow and outflow, respectively; V_i is the perfusion rate.

Morphological Studies of the Colonic Mucosa

From three anaesthetized animals mucosal samples were taken from the proximal and from the distal colon, 4.5 to 5.0 cm distal to the caecum and about 10 cm proximal from the rectum. Samples were transferred immediately into a fixative solution containing formaldehyde 2%, glutaraldehyde 2.5%, and 0.1 M Na-cacodylate buffer, pH 7.2, where they were minced and kept for 2 hr.

Light Microscopy

Parts of the segments were washed in 0.1 M Na-cacodylate buffer containing 0.22 M sucrose. Post-fixation occurred for 90 min in 2% OsO_4 solution in the same buffer. Dehydration was done in ethanol and embedding in Epon. One- to two-micrometer-thick sections were cut with an ultramicrotome (LKB Ultratome 4800 III) and stained with an alkalized toluidine blue solution.

Freeze Fracture

After aldehyde fixation some of the samples were cryoprotected (60 min in 30% glycerol diluted in Ringer's solution) and oriented on specimen holders to obtain the fracture perpendicular to the epithelial surface. The samples were frozen in liquid Freon 22 (monochlorodifluoromethane) at about −150°C and transferred into a Balzers 360 M apparatus (equipped with an electron beam gun) for fracturing and replication. The replicas (about 2.5-nm thick) were cleaned in hypochlorite bleach and chromic acid, repeatedly washed in distilled water, collected on formvar-carbon membrane (Dowell, 1964) and examined with an electron microscope (Siemens Elmiskop 101).

Quantitative Analysis of Zonulae Occludentes

The zonulae occludentes of the epithelial cells bordering the internal surface of the organ and those lining the crypts were considered separately. Columnar epithelial cells at the luminal surface were identified due to the regular length and uniform distribution of their microvilli. Goblet cells were identified due to their granules and their peculiar form. In the crypts, where numerous cell types occur (*see below*), a complete identification of the cells was often impossible. Electron micrographs with a final magnification of 40,000× were prepared. On these micrographs 150 cm of zonulae occludentes of the surface epithelium and 230 cm of zonulae occludentes of the crypt epithelium were evaluated in each colonic region. The number of superimposed strands, the depth of the zonulae occludentes and the number of strands per linear unit were estimated at reference points 1 to 1.5 cm apart from each other. A magnifying glass (Agfa 8×) was occasionally used to facilitate estimation.

In the calculation of the depth of the zonulae occludentes only the strands forming the junctional belt were considered, while irregular and mostly free-ending strands extending on its abluminal side were not taken into account.

Values are given as means and standard deviations. Differences between values were tested with the Student's *t*-test.

Results

Permeability of the Mucosa of the Proximal and the Distal Colon of the Guinea Pig for SCFA

SCFA were rapidly absorbed in the proximal as well as in the distal colon. The clearance of acetate, propionate, and butyrate from the perfusion solution was used as a parameter for comparing the permeability of the mucosa for these three SCFA.

In the proximal colon the chain length of SCFA had only a minor effect on the permeability of the mucosa for the SCFA (Fig. 1). In the distal colon, however, clearance increased with increasing chain length; the clearance was about doubled with each additional CH_2 group.

In the proximal colon acetate clearance was significantly higher as compared to the distal colon. For butyrate the contrary was observed, and for propionate no difference was seen.

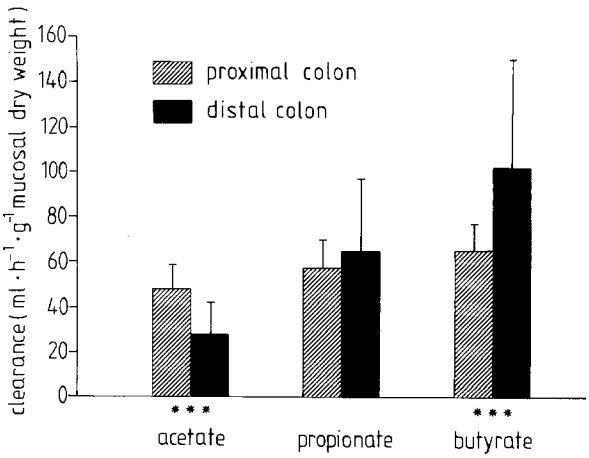


Fig. 1. Clearance for acetate, propionate and butyrate in the proximal and in the distal colon of guinea pig. The highly significant differences ($P < 0.001$) between the proximal and distal colon are indicated by asterisks

Morphology

Light Microscopy

Between proximal and distal colon of the guinea pig some characteristic morphological differences were observed (Fig. 2*a,b*): (1) The mucosa of the proximal region was about 185-μm thick, in the distal region about 230 μm. (2) In the proximal colon each crypt emanated from a long funnel-like foveola; this was very short in the distal colon. (3) In the proximal colon the crypts were shorter than in distal colon, and they showed bifurcations. (4) The surface epithelium mainly consisted of columnar cells of 40 to 45 μm height in the proximal region and of 30 to 35 μm in the distal. (5) Few goblet cells were intermingled between the columnar cells in the proximal colon surface; they were more frequent in the distal colon. (6) The crypts in the distal colon contained considerably more mucus-producing cells than those in the proximal region. Epithelial cells in mitotic division were frequently seen in the crypts of both regions.

Zonulae Occludentes of the Epithelial Cells at the Colonic Surface

In the *proximal* region the zonulae occludentes between columnar cells were composed by superimposed and interconnecting strands. The number of strands changed from 1–2 to 5–8 (mean 4.4 ± 0.5; *see* Table) within a short distance and in the same junction. However, this great variability in the number of strands did not alter the depth of zonulae occludentes (mean depth 358 ± 20 nm), but was due to changes in the orientation of the strands. Where strands were numerous (5 to 8 strands) they were anastomosed to each other forming a junction with polygonal meshes; where they were few in number, they bordered "passages" or "leaky spots" crossing the height of the junction. In these locations only 1 to 3 strands separated the colon lumen from the intercellular space (Fig. 3*a*).

The zonula occludens between the few goblet cells and the columnar cells at the surface showed similar morphological features. However, they were characterized by strands of various length emanating from its abluminal side and forming, at a deeper level, a second low and discontinuous junctional belt (Fig. 4*a*). Furthermore junctions of the goblet cells located in the foveolae showed numerous highly interconnected strands which formed a deep network (Fig. 4*c*).

In the *distal* region the belt formed by the zonulae occludentes between columnar cells was thinner (mean 285 ± 13 nm) than of the proximal colon. The number of the strands varied from 5 to 8 (mean 7.4 ± 0.2, Table) and their arrangement was constant

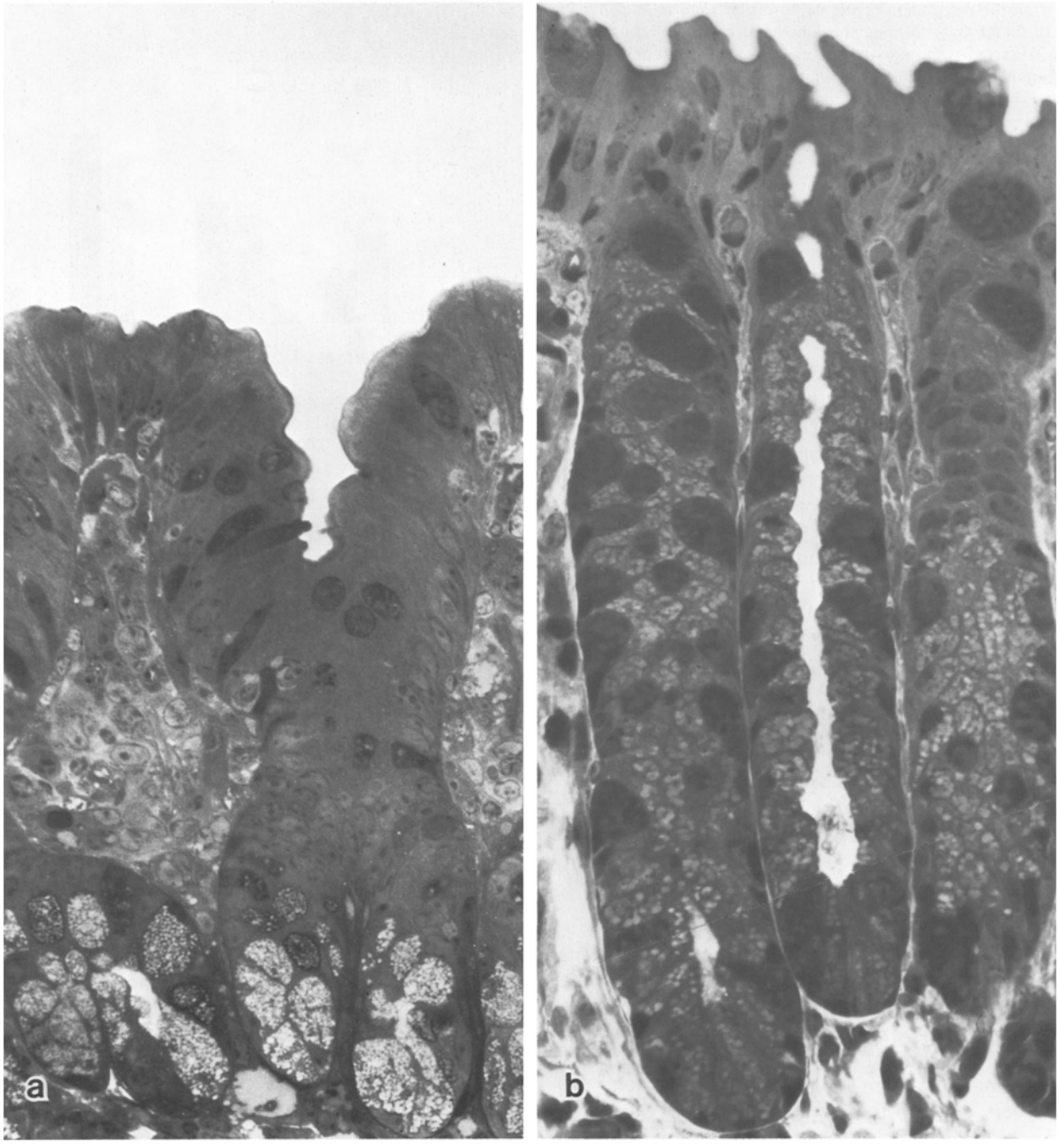


Fig. 2. Comparison by light microscopy of the colonic mucosa in the proximal (*a*) and in the distal (*b*) colon of guinea pig. Deep foveolae lined by surface epithelium and short crypts are seen in (*a*); very short foveolae and long crypts in (*b*). (*a*,*b*: 625×)

along the junctions. The ridges (face P) and the meshes they bordered were frequently covered by remnants of the E face of the adjacent plasma membrane (Fig. 3*b*). These morphological features gave to these junctions a compact uniform appearance independently of interposed goblet cells (Fig. 4*b*).

Zonulae Occludentes of the Crypt Epithelial Cells

Zonulae occludentes of the crypt epithelial cells were composed in the proximal as well as in the distal colon by the same number of superimposed strands; in mean four strands were seen (Table).

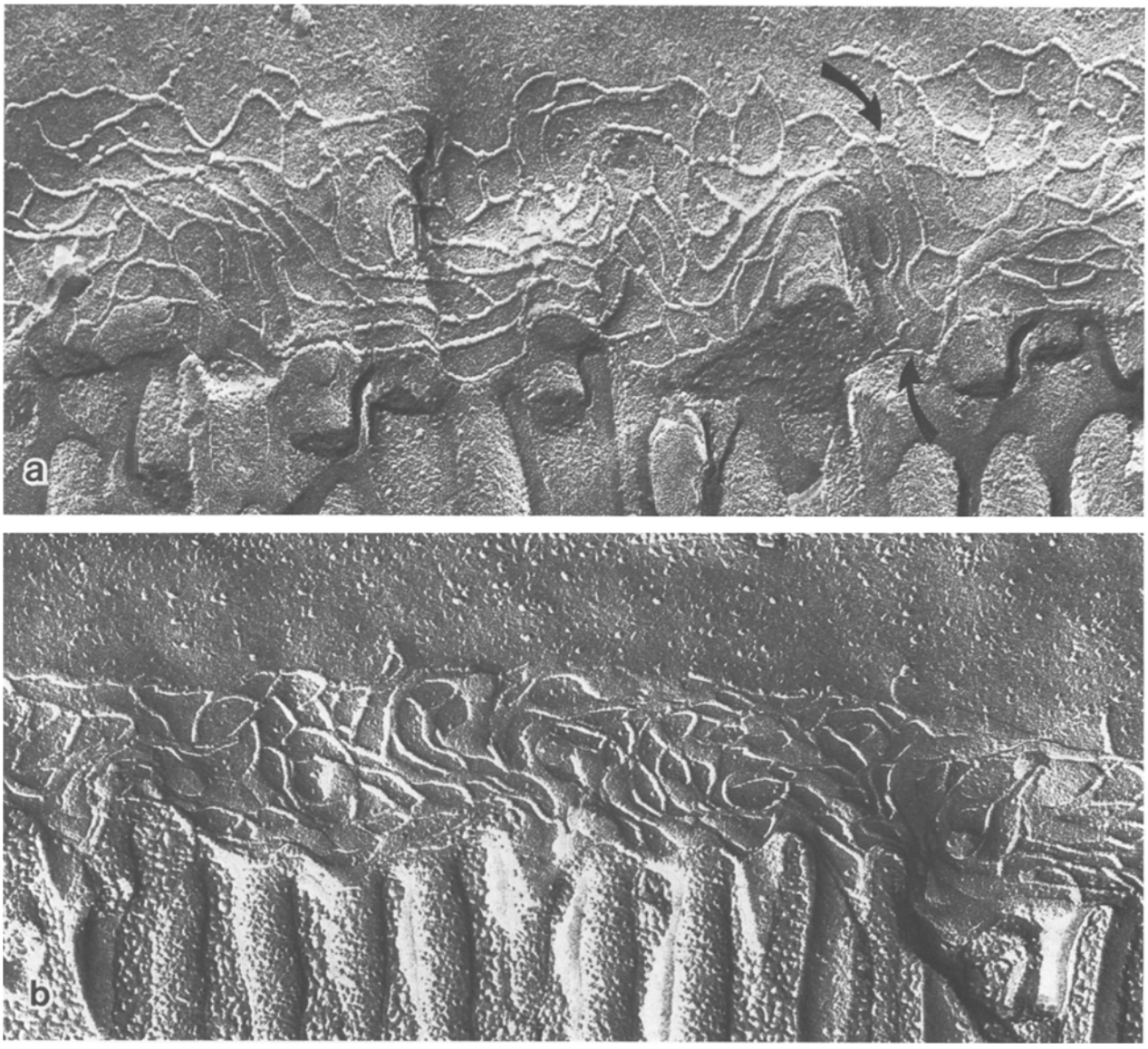


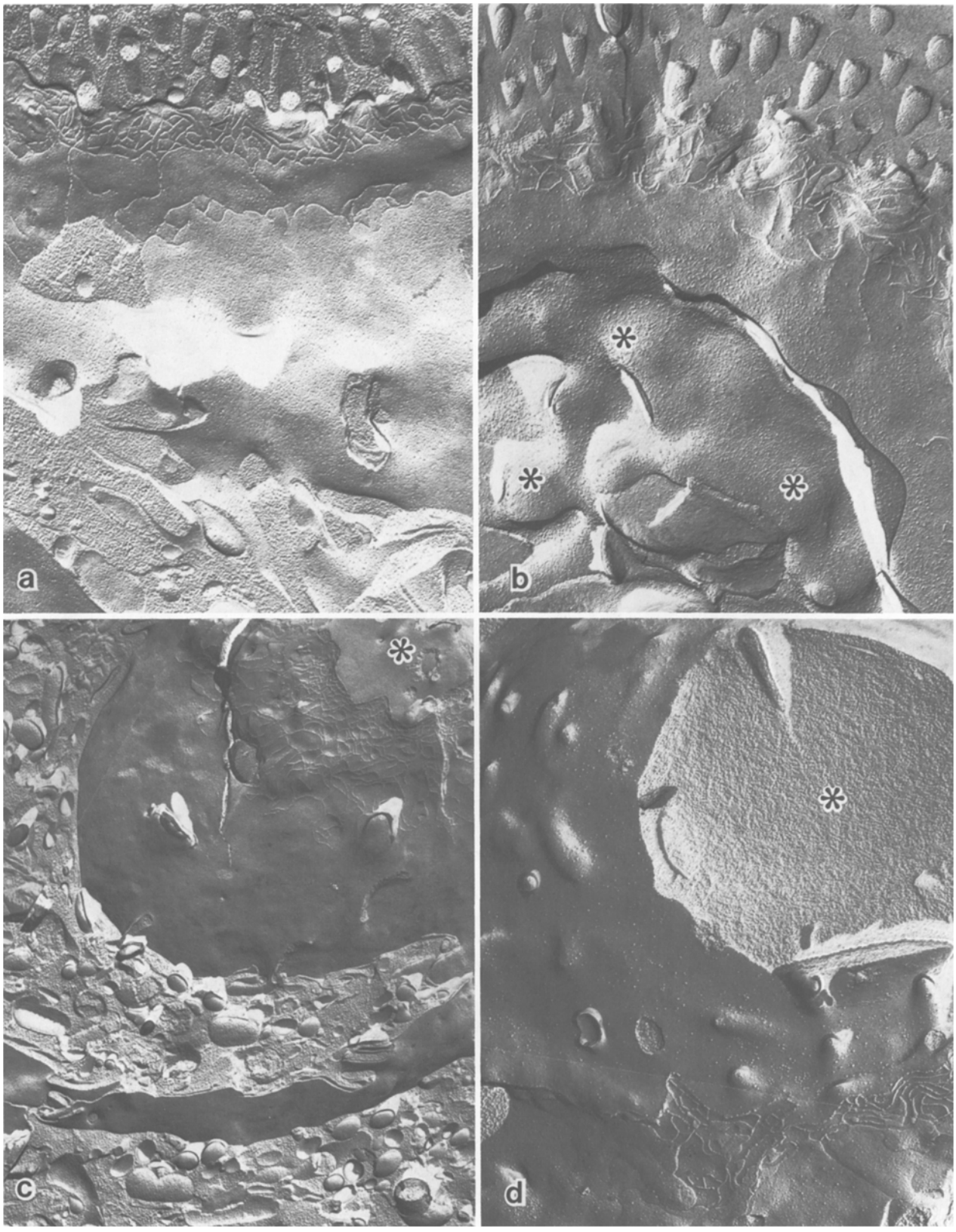
Fig. 3. Freeze-fracture replicas showing the zonulae occludentes between columnar cells of the surface epithelium in the proximal colon (*a*) and in the distal colon (*b*). Arrows in (*a*) point to sites where two strands separate the organ lumen from the intercellular space. Similar "leaky spots" are absent in (*b*). (*a*: 96,000×; *b*: 81,000×)

However, compared with the junctions of the surface epithelium, the number of strands per μm was greater in the crypts of the distal region than in the proximal. The very heterogeneous aspect of the junctions of the crypt epithelium is obvious. Indeed the epithelium of the crypts showed several cell types (Figs. 4*d* and 5*a–e*): cells of the same type with a different arrangement of the strands (Fig. 5*c* and *d*), as well as cells with a very irregularly deep belt and discontinuities (Fig. 6*a* and *b*). This last aspect of the junction probably occurred in cells in mitotic division as previously described by Tice, Carter and Cahill (1979) in the rat duodenum.

Common characteristics of the zonulae occludentes between the cryptal epithelial cells were long (up to 1250 nm), free-ending strands emanating from the abluminal side of the junctions (Fig. 5*a–e*).

Quantitative Analysis of the Zonulae Occludentes

Estimates in the Table indicated that strands forming junctional complexes between surface epithelial


a
b
c
d

cells in the proximal region were significantly less numerous than in the distal region; such differences were not seen between the junctions of the epithelial cells of the crypts. Figure 7 indicates the great variability in the number of superimposed strands (from 1 to 8) between the surface epithelial cells. The relative frequency showed an even distribution, 2 to 7 strands being constantly counted with a frequency of 13 to 16%. In contrast, between the surface epithelial cells of the distal colon 63% of the junctions show 7 to 8 superimposed strands, with a range from 4 to 11 strands.

The distribution of the number of superimposed strands between the crypt epithelial cells was not different between the proximal and the distal colon (Fig. 7). A maximum of the relative frequency was seen in both regions at 4 strands. That is highly significantly different from the surface cells in the distal colon, where the maximum observed was 7 strands.

The depth of zonulae occludentes of the surface epithelial cells was more or less equally distributed between 250 and 450 nm in the proximal colon (Fig. 8). In the distal colon a maximum was seen at 200–250 nm. In the crypt region the depth measurements of zonulae occludentes scattered considerably. Especially in the distal colon the lesser depth in the crypts compared to the surface was obvious.

Discussion

Short-chain fatty acid concentrations in colonic contents of all mammals studied so far are high; most of the anions present in the colon are SCFA. Absorption in the colon is rapid, it is passive and increases linearily with a corresponding increase in concentration (Ruppin et al., 1980; McNeil, 1982; Rechkemmer & Engelhardt, 1982). In the blood SCFA concentrations are low (<2 to 3 mmol · liter^{-1}), therefore a favorable concentration gradient for passive diffusion is present. Another factor favoring passive SCFA permeation is the blood-side positive electrical potential difference present in all segments of the large intestine.

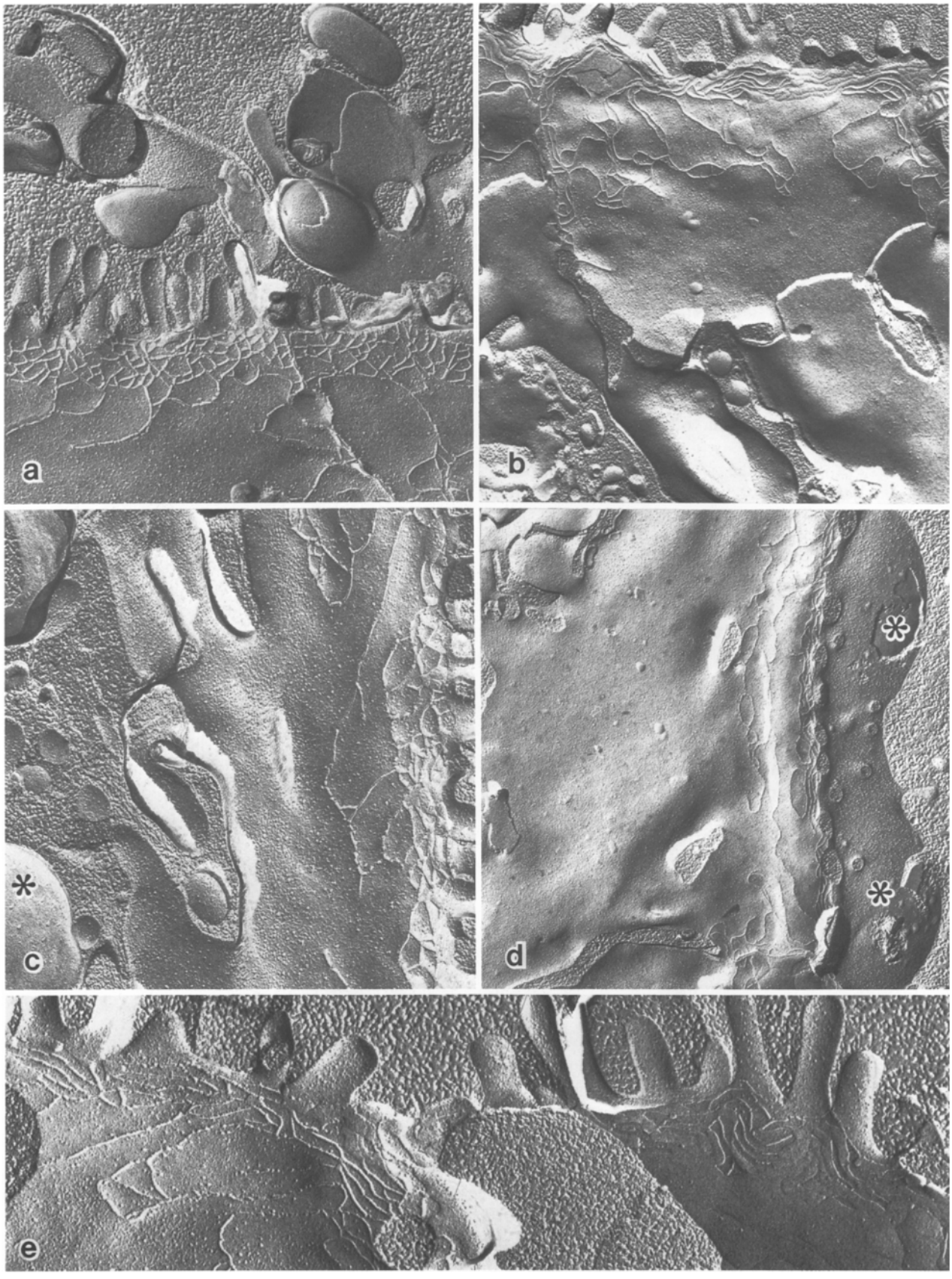
At normal pH in colonic contents (pH ~ 7) about 99% of SCFA are present as anions. However, a rapid passage across the epithelium is assumed to occur only in the undissociated, lipid-soluble form. The pK values for acetate, propionate and butyrate are similar (4.75; 4.87; 4.81, respectively). Therefore differences in lipid solubility between these SCFA depend mainly on the chain length; oil-water partition coefficients increases by a factor of 2.8 with each CH_2 group (Danielli et al., 1945). Thomson and Dietschy (1981) in a review article interpreted findings of Westergaard and Dietschy (1974), and they concluded from these experiments with small intestine mucosal cells that each CH_2 group of SCFA increases the passive permeability coefficient of the cell membrane by a factor of 1.58.

Assuming an absorption of SCFA in the undissociated lipid-soluble form exclusively, the permeability for butyric acid should be approximately twice as high as that of propionic acid, and propionic acid twice that of acetic acid. Indeed, in the distal colon of the guinea pig in our experiments the clearance of acetate, propionate and butyrate increases with each CH_2 group by a factor of 1.5 to 2. From these findings we conclude that in the distal colon of the guinea pig SCFA are absorbed in the undissociated form mainly, and passage of dissociated molecules through the epithelium is restricted.

The permeability characteristics of the proximal colon of the guinea pig, on the other hand, are significantly different in two aspects: (1) The permeability of the epithelium in the proximal colon is not significantly different for the three SCFA. (2) The permeability of acetate is significantly higher in the proximal than in the distal colon, and the reverse is seen for butyrate (Fig. 1). We conclude from these findings that in the proximal colon a portion of SCFA is absorbed in the dissociated form, the rate of absorption reflecting the difference in molecular size (absorption for acetate > propionate > butyrate). However, the major part of the absorption may still occur in the undissociated form.

Our data for the proximal colon are in good agreement with the findings of Ruppin et al. (1980) who perfused the human colon and found that 60% of colonic SCFA absorption was the result of nonionic diffusion. Similar to our findings in the proximal colon of the guinea pig, and also in the proximal colon of the rabbit (Clauss, 1978), in the human jejunum (Schmitt, Soergel & Wood, 1976) as well as in

Fig. 4 (*facing page*). Zonulae occludentes on plasma membrane P face of goblet cells. (*a*) and (*c*) proximal colon epithelium at the surface and at the foveola, respectively; (*b*) and (*d*) distal colon epithelium at the surface and at the crypt, respectively. Bulgings of mucus granules are marked by asterisks. (*a*: 34,000×; *b*: 40,000×; *c*: 16,000×; *d*: 30,000×)


a
b
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e

Table. Characteristics of zonulae occludentes of the surface and crypt epithelium in the proximal and distal colon of guinea pig (means ± standard deviation are given)

Epithelium	Colon	Number of strands	Total counts	Depth (nm)	Total counts	Number of strands · μm^{-1}
Surface:						
	proximal	4.4 ± 0.5	124	358 ± 20	95	12.4 ± 2.1
	distal	7.4 ± 0.2	120	285 ± 13	114	26.0 ± 1.9
Crypt:						
	proximal	4.0 ± 0.3	165	274 ± 22	154	14.7 ± 2.4
	distal	4.0 ± 0.4	146	179 ± 17	141	22.5 ± 4.3

the rat jejunum (Barry & Smyth, 1960), the permeability for SCFA is independent from the respective chain length; these epithelia may be considered as leaky (small intestine) or moderately leaky (proximal colon). In the caecum (Leng, 1978) and in the distal colon of the rabbit (Clauss, 1978) clearance of SCFA increases linearly with increasing chain length; that is similar to our findings in the distal colon of the guinea pig. These epithelia may be considered as moderately tight epithelia. They generate *in vivo* a transepithelial electrical potential difference of up to 40 mV, lumen side positive with respect to blood (Wrong, Edmonds & Chadwick, 1981).

The absorption of SCFA in the undissociated lipid-soluble form may occur mainly via transcellular routes. For the passage of ionized SCFA, very likely, a paracellular pathway is a most likely explanation. The specialized intermembranous structure of zonulae occludentes can limit the paracellular passage of solutes. Our interpretation of SCFA absorption data in the proximal colon of the guinea pig would require that the zonulae occludentes allow the passage of SCFA ions. The possibility is supported by our morphological observations for surface epithelial cells in the proximal colon, but not for surface epithelial cells in the distal colon.

Claude and Goodenough (1973) suggested that the paracellular permeability of solutes is inversely correlated with the number of strands of the zonulae occludentes. As in the gallbladder of the rabbit (Claude & Goodenough, 1973), the zonulae occludentes at the surface of the proximal colon of guinea pig show short segments of belt-like junctions which are composed of 2 to 3 strands only. These "leaky spots" alternated with segments showing a large number of strands and could be candidates for the localization of a shunt pathway.

Beyond that, zonulae occludentes in the proximal colon surface areas are significantly less compact than those in the distal colon of the guinea pig. Although the depth of zonulae occludentes is somewhat larger in the proximal colon, twice as many strands are present per μm depth in the distal colon. This may be a further reason for the higher permeability in the proximal colon.

We may ask what is the purpose of these zonulae occludentes in the proximal colon with mostly a rather high number of strands and among them "leaky spots." It is assumed that a certain structural strength is necessary in the colon to sustain the marked volume changes and the alterations in the interluminal pressure in the course of vigorous motility events. It is well known that tight junctions do not only affect the degree of tightness of the paracellular pathway, but they are also of considerable importance for the strength of the mechanical attachment between cells (McNutt & Weinstein, 1973). In order to achieve sufficient attachment as well as high permeability, a sieve-like structure of zonulae occludentes with "leaky spots" could be an appropriate design. In the gallbladder, an organ with a storage function, conditions may be somewhat comparable to that of the proximal colon. Structural peculiarities of zonulae occludentes in these two tissues are obviously similar.

Fig. 5 (*facing page*). Heterogeneity of the zonulae occludentes between the epithelial cells lining the crypts in the proximal colon (*a* and *c*) and in the distal (*b*, *d* and *e*). In (*a*) freeze-fractured bacteria are present in the crypt lumen above the microvilli of an unidentified epithelial cell. The strands of its zonula occludens form large polygonal meshes. In (*b*), a replica of an unidentified cell of the crypt, the meshes of the junctional belt are close together and elongated. (*c*) and (*d*) show the different arrangement of the strands on plasma membrane P face of mucus-producing cells ("vacuolated cells"). The granules of these cells are marked by asterisks. (*e*) Further example of zonulae occludentes between unidentified cells. The arrangement of the strands in these junctions is different than in *a–d*. (*a*: 40,000×; *b*: 25,000×; *c*: 46,000×; *d*: 28,000×; *e*: 52,000×)

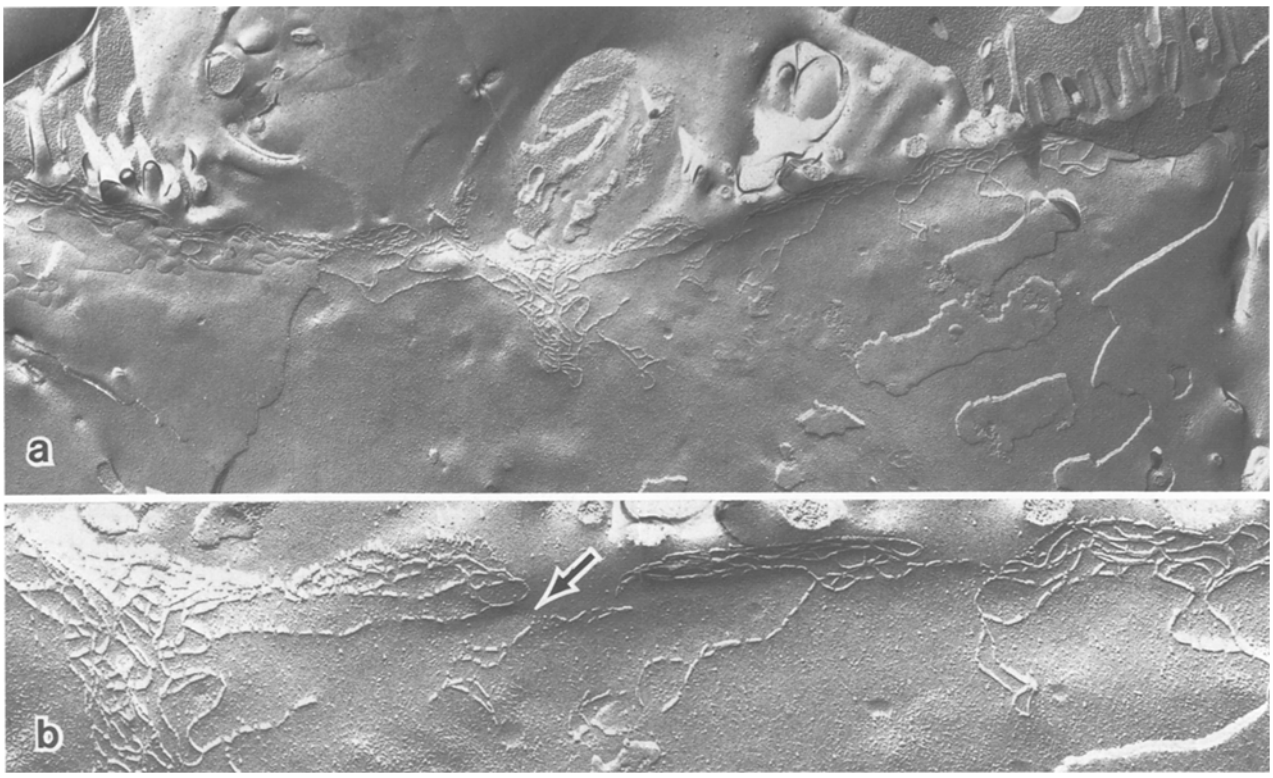

Fig. 6. Zonula occludens of an epithelial cell presumably in mitotic division; crypt of the distal colon. The junction is very irregularly deep (*a*). As seen in the higher magnification (*b*) it is discontinuous (arrow) and occasionally composed by only one strand. (*a*: 21,000×; *b*: 40,000×)

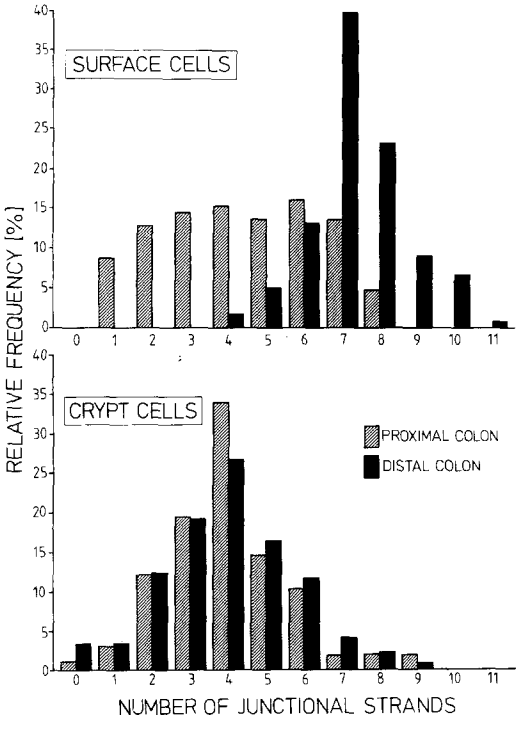

Fig. 7. Relative frequency distribution of the number of junctional strands in the proximal and in the distal colon of surface and of crypt cells

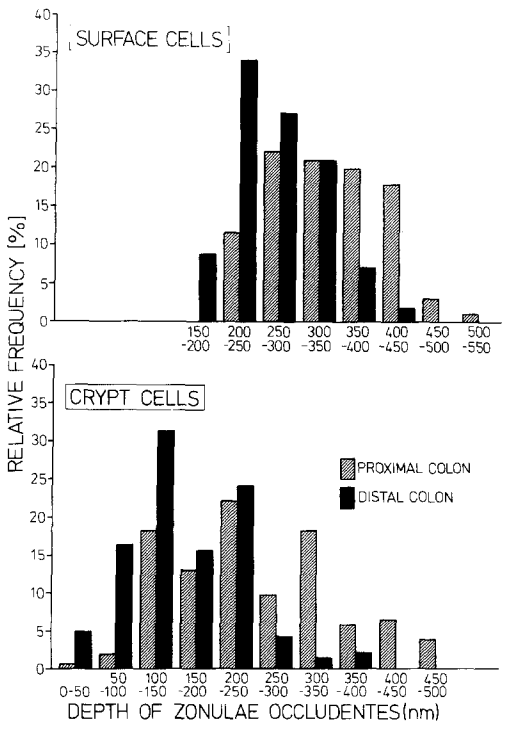

Fig. 8. Relative frequency distribution of depth measurements (50 nm intervals) of zonulae occludentes in the proximal and distal colon of surface and of crypt cells

It should be emphasized that in the discussion so far we have focused on junctions between epithelial cells at the *surface* of the colonic mucosa. This absorbing epithelium may be treated as two resistances in parallel, the lipid component and aqueous channels either in the membrane ("pores") or between cells ("tight junctions"). SCFA may pass through both parallel resistances (Thomson & Dietschy, 1981). We have schematically compiled our observations for the proximal and the distal colon of the guinea pig in Fig. 9. The parallel channel model (Jackson, Tai & Steane, 1981) agrees with our observations in the proximal colon; in the distal colon the pH partition model seems to give an appropriate explanation.

Light microscopy shows clear differences between the crypts of proximal and distal colonic regions. Our freeze-fracture studies demonstrate a wide variability in architecture and extension of the zonulae occludentes which is due, in both regions, to the different epithelial cell types and to the cells in mitotic division. In addition, the quantitative data indicate that no consistent difference in number of strands can be recorded. However, the depth of the junctions significantly differs between proximal and distal regions (Table, Figs. 7 and 8); the functional implication of this result remains to be explained.

Recently, freeze-fracture investigations on the goblet cells of the rat small intestine show high variability in strand number and depth of the zonulae occludentes. The zonulae of the goblet cells are considered as focal sites which increase the permeability of the epithelium (Madara & Trier, 1982). In the guinea pig, the goblet cells of the proximal and distal colonic regions of the surface epithelium show similar morphological characteristics as junctions between neighboring columnar cells (Fig. 4*a,b*). Therefore the presence of these goblet cells should not affect the tightness of the epithelium at the colonic surface.

Considering the tightness of the zonulae occludentes, we may conclude that in the proximal region the permeability properties of the surface and of the crypt epithelium are similar, whereas in the distal colon the surface epithelium is considerably tighter than that of the crypts. Our morphological findings would indicate a low paracellular resistance in the crypts. A comparatively high paracellular permeability for small water-soluble molecules therefore should be expected. Our findings in the distal colon do, however, illustrate that no major absorption of ionized SCFA due to their molecular size does occur in the crypts. Three conditions may be responsible for that: (1) Openings from the lumen into the crypts are very small in respect to the total surface area; thus only a limited number of molecules may enter. (2) Due to the high permeability of the epithelium the few SCFA molecules that enter could be rapidly absorbed already in the foveola or immediately below. (3) Crypts are filled with mucus which might retard the diffusion of SCFA considerably, as was shown for H^+ ions in gastric mucus (Williams & Turnberg, 1980).

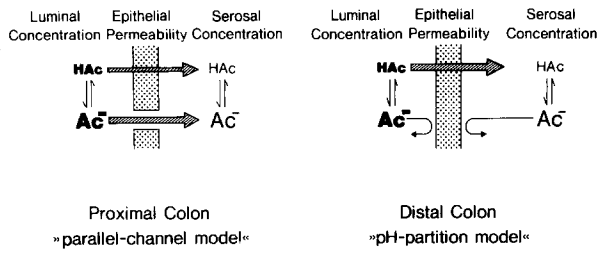

Fig. 9. Model for transepithelial passage of acetate in the proximal and the distal colon of guinea pig. Findings (Fig. 1) indicate that in the proximal colon a paracellular transport takes place. The permeability is indicated by the thickness of respective arrows. The thicker letters indicate a higher concentration of acetate at the luminal side. Only about 1 to 2% of acetate is present in the undissociated form on both sides of the epithelium, the smaller letters for HAc shall indicate these small concentrations. In the distal colon all SCFA are absorbed in the undissociated, lipid-soluble form

We assume that SCFA are absorbed across the surface cells of the colonic epithelium, and not in the crypts. The crypts seem to have mainly a secretory function for chloride (Welsh et al., 1982) or macromolecules (Sakata & Engelhardt, 1981; Gorelick, Sarras & Jamieson, 1982); very likely crypts do not play a major role in absorption.

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