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Altered Jejunal Permeability to Macromolecules During Viral Enteritis in the Piglet

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We studied the macromolecular permeability of segments of jejunum from 2-wk-old piglets after the animals had been experimentally infected with an invasive enteric virus, transmissible gastroenteritis virus. Jejunal segments were mounted in Ussing chambers at stages of the infection, and permeability was measured using three probe molecules of differing molecular weights. In control tissue, permeability to horseradish peroxidase was 2.6 times higher across segments with Peyer's patches than across segments without Peyer's patches, whereas polyethylene glycol 4000 and mannitol permeabilities were the same in patch and nonpatch segments. Twelve hours after infection, when virus had invaded the mucosa causing a structural lesion, and before diarrhea had begun, horseradish peroxidase permeability increased in non-patch-containing segments to equal that across patch-containing tissue. At this early 12-h stage, polyethylene glycol 4000 and mannitol permeation were unchanged in patch-containing segments compared with controls. Ninety-six hours after transmissible gastroenteritis infection, when diarrhea was severe, horseradish peroxidase permeability in patch-free segments had

returned to normal and patch-containing tissue permeability was diminished below control levels. Increased macromolecular permeability appears to occur only in the very early invasive stage of this viral enteritis and only in patch-free segments. Any consideration of the immunologic relevance of these complex phenomena must take into account the specialized function of the Peyer's patch regions of the small intestine.

The small intestine, particularly its Peyer's patch region, is capable of assimilating antigenically significant amounts of macromolecules (1,2). This study was designed to determine whether normal patterns of macromolecular absorption are distorted by mucosal disease. We studied piglets experimentally infected with transmissible gastroenteritis (TGE) virus, an invasive enteric corona virus that causes an acute self-limited diarrheal illness closely resembling human rotavirus enteritis in infants (3-6). We measured mucosa-to-serosa permeation rates of three molecules of widely differing weight across jejunum from piglets with TGE and from matched controls using an in vitro technique previously used in our laboratory to compare patch-containing with patch-free segments (2).

Materials and Methods

We measured mucosa-to-serosa permeation rates of horseradish peroxidase (HRP), 40,000 mol wt; polyethylene glycol (PEG), 4000 mol wt; and mannitol, 182 mol wt across stripped piglet jejunal mucosal segments, mounted in Ussing chambers and studied under zero transport conditions.

Conventional York Landrace piglets, obtained at 14 days

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Abbreviations used in this paper: HRP, horseradish peroxidase; TGE, transmissible gastroenteritis.

of age from a local herd known to be free of TGE and weaned to an evaporated cow milk formula for 3–5 days, were studied after infection with a standard oral dose of Purdue strain of TGE virus, and the findings were compared with those from uninfected matched controls (3). All TGE-infected animals were housed in an isolation unit and studied either 12 h (5 piglets) or 96 h (6 piglets) after they were inoculated with virus. The 12-h interval was selected to coincide with the time at which TGE virus is known to invade the upper small intestinal mucosa (4). At 96 h, our own studies (6) and others in the literature (4) demonstrate that diarrhea is well established but that the virus-laden epithelial cells have been shed into the lumen. In the present experiments, the piglets consistently developed vomiting and anorexia within 12 h of receiving TGE virus; diarrhea began within 24 h and peaked between 40 and 96 h.

For the macromolecular permeation studies, the animals were killed after an overnight fast with 325 mg of pentobarbital sodium given parenterally; the proximal portion of the small intestine was then quickly removed. Fifteen-centimeter lengths of piglet jejunum were flushed with ice-cold normal saline, slipped over 5-ml pipettes for identification of Peyer's patches, stripped of muscular and visceral peritoneal layers, and opened into sheets. For light-microscopic studies, segments containing Peyer's patches and adjoining segments without patches were fixed in Bouin's solution, then in 95% ethanol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. For transport studies, adjacent segments were immediately mounted in conventional Ussing chambers (exposing an area of 1.27 cm²). Three pairs of tissue segments, each consisting of a patch-containing and a contiguous patch-free segment, were mounted in the chambers, using six chambers for each experiment. Chambers were perfused with oxygenated chamber buffer (Krebs–Ringer bicarbonate, pH 7.4 at 37°C with 1 mg/ml porcine serum albumin and 3 mM D-glucose). After a 10-min equilibrium period, HRP (Sigma type II, Sigma Chemical Co., St. Louis, Mo.) was added to the mucosal compartment at a final concentration of 0.5 mM (20 mg/ml); in some experiments [³H]mannitol, 10 μCi/chamber, final concentration 0.5 mM or [¹⁴C]polyethylene glycol 4000, 10 μCi/chamber, 0.69 mM final concentration, or both, were added to the mucosal chamber also. Preliminary studies had established that neither of these latter two compounds altered HRP transport. Serial samples were taken from the serosal reservoir for measurement of the macromolecules added to the mucosal side of the tissue. To eliminate any effects of transmucosal potential difference (PD) on HRP movement, a short-circuiting current was applied throughout the experiment to reduce the PD to zero; PD and the short-circuiting current were monitored at 10 min. In previous studies (2), we found that HRP did not alter the short-circuiting current, but HRP transport was ~10% higher across short-circuited tissue than across tissue that was not short-circuited.

Horseradish peroxidase was assayed kinetically as previously described (2) using o-dianisidine as the indicator. Radioactivity was measured using Aquasol 2 scintillation fluid (New England Nuclear, Boston, Mass.), in a Beckman

L57500 Liquid Scintillation System equipped with a data reduction accessory (Beckman Instruments Inc., Irvine, Calif.) for double-label analysis. Mucosa-to-serosa transport rates were determined from the slope of the line describing probe appearance in the serosal chamber in the steady-state time period, 40–80 min after addition. Serosa-to-mucosa transport rates were not studied because of an apparent toxic effect of HRP when presented at the serosal surface (2). Statistical comparisons were made using Student's *t*-test.

The molecular weight distribution of the peroxidase activity appearing in the serosal chamber was examined by gel chromatography on a 1.5 × 27-cm column of Sephadex G-75 (fine) (Pharmacia Fine Chemicals, Piscataway, N.J.), as previously described (2). The peroxidase activity from the serosal chamber moved as a single peak identical to that seen for native HRP in studies of both control and infected animals, patch and nonpatch segments.

Horseradish peroxidase (grade II), porcine serum albumin (fraction V), bovine serum albumin (fraction V), o-dianisidine, cytochrome c (practical), Sephadex G-75 (fine), and NaF were obtained from Sigma Chemical Co. [¹⁴C]Polyethylene glycol 4000 (sp act, 0.78 mCi/mg) and [³H]mannitol (sp act, 17 Ci/mmol) were obtained from New England Nuclear. All other chemicals were of reagent grade and obtained from local suppliers. Deionized water, prepared with a MilliQ apparatus (Millipore, Bedford, Mass.) was used throughout.

Results

Light Microscopy

Measurements of jejunal crypts and villi from the three study groups (Table 1) show that TGE-associated structural abnormalities were confined to

Table 1. Jejunal Mucosal Measurements

	Crypt depth	Villus height
Patch-free segments		
Control pigs	161 ± 5 (8)	383 ± 9 (8)
TGE pigs		
12 h	161 ± 13 (6) NS	183 ± 33 (6) p < 0.001
96 h	226 ± 16 (8) p < 0.005	246 ± 19 (8) p < 0.001
Patch-containing segments		
Control pigs	176 ± 16 (4)	248 ± 12 (4)
TGE pigs		
12 h	172 ± 18 (4) NS	208 ± 31 (4) NS
96 h	211 ± 32 (5) NS	166 ± 48 (5) NS

Measurements of mucosal dimensions (in microns) in patch-containing and patch-free segments of piglet jejunal mucosa after experimental transmissible gastroenteritis infection (mean ± SEM). Statistical comparisons with controls by Student's *t*-test. Only well-oriented blinded specimens were measured. Number of animals is given in parentheses. NS, not significant; TGE, transmissible gastroenteritis.

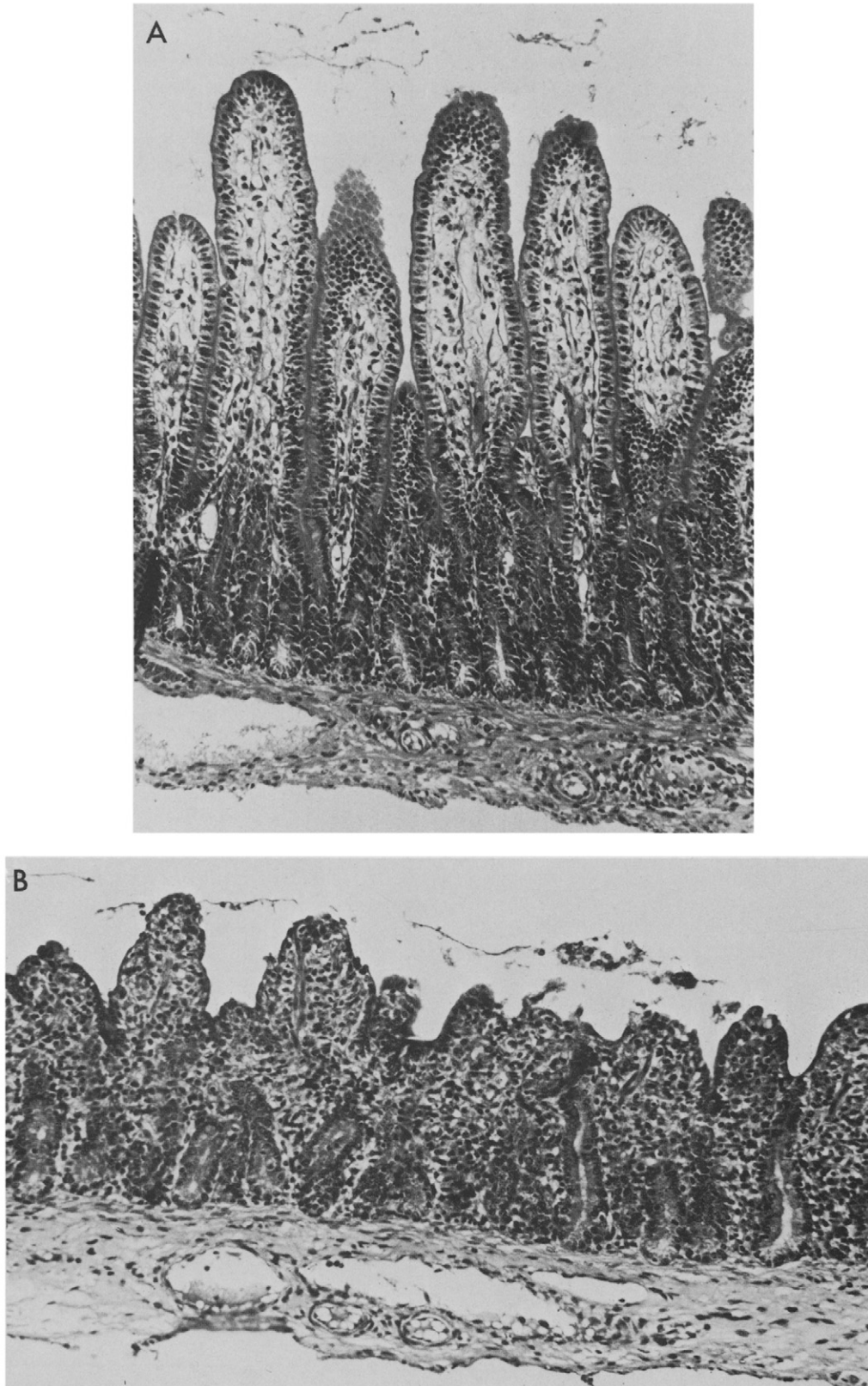


Figure 1. Light photomicrographs of piglet jejunal mucosa taken from patch-free segments. H & E, $\times 200$. A. Control animals, age 18 days, normal crypt and villus structure with intact columnar epithelium. B. An 18-day-old pig, 12 h after receiving transmissible gastroenteritis (TGE) virus. Severe lesion with shortened villi, ulcerated epithelium, and intraluminal exudate. C. Another 18-day-old pig, 12 h after TGE infection, with shortened villi but only minimal disturbance of epithelial continuity—a less severe lesion than that shown in B. D. A 20-day-old pig, 96 h after TGE infection. Villi are shortened, crypts elongated. Epithelium is intact, but somewhat cuboidal.

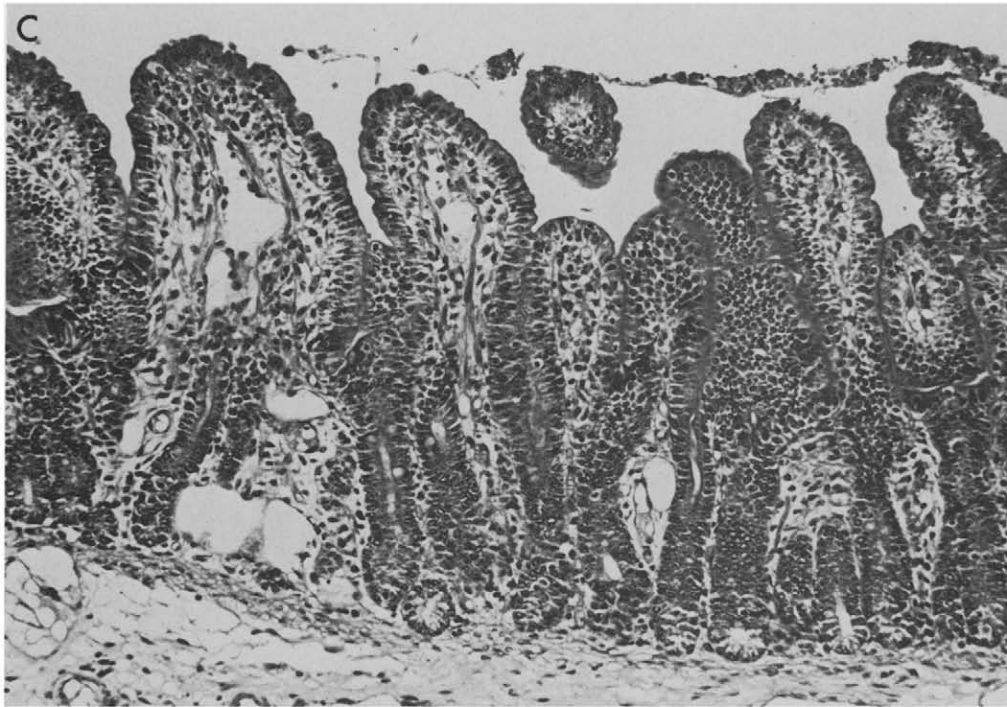


Figure 1. Continued.

segments that did not contain Peyer's patches. At 12 h, villi were shortened significantly but the specific features of the lesions varied considerably between the 5 pigs in the group. A severe lesion characterized by inflammatory exudate and small epithelial ulcerations was seen in 3 pigs; in the other 2 pigs, presumably seen earlier in the evolution of this rapidly progressive disease, there were shortened villi but only minimal epithelial disruption (Figure 1). In pigs studied 96 h after receiving the TGE virus, when diarrhea was severe, the structural findings were identical to those seen previously during the same stage of this disease: shortened villi, deepened crypts, but an intact epithelium. Previous studies in our laboratory have shown that, at this 96-h stage of TGE, the virus-infected cells are shed and the epithelium is composed of cells that have migrated up from the crypts at an accelerated rate but in a relatively undifferentiated stage (6). No alterations could be identified at the light-microscopic level in the dome epithelium overlying Peyer's patches from pigs after infection.

Horseradish Peroxidase Permeation

In control piglet jejunum the pattern of appearance of peroxidase activity in the serosal chamber after placing HRP in the mucosal chamber resembled that observed in a previous study (2). Horseradish peroxidase appeared first at 30–40 min and a steady state was maintained between 50 and 80 min when transport rates could be calculated. Mean permeability rates in Peyer's patch-containing and patch-free jejunal segments from control, 12-h, and 96-h TGE piglets are summarized in Figure 2. In control piglets, the mean rate was 2.6 times higher across patch-containing tissue ($p < 0.001$) than non-patch-containing segments. At the early viral invasion stage of TGE, 12 h after infection, peroxidase permeability (free) across patch-free tissue greatly exceeded control values ($p < 0.05$), and was similar to that occurring in patch-containing tissue from the same group and from controls. By 96 h after TGE infection, although the piglets had a mucosal lesion and diarrhea, peroxidase permeability had returned to normal in non-patch tissue. In patch-containing tissue from these 96-h piglets, HRP permeability actually was reduced compared with controls ($p < 0.05$), although it remained significantly greater than that occurring in patch-free segments. From the spread of the data summarized in Figure 2, it is clear that between piglets, variation was much greater in 12-h than in 96-h piglets. Within the 12-h group of piglets, we observed the highest permeation rates in the jejunum judged to have the most severe epithelial disruption, but we could not correlate these rates

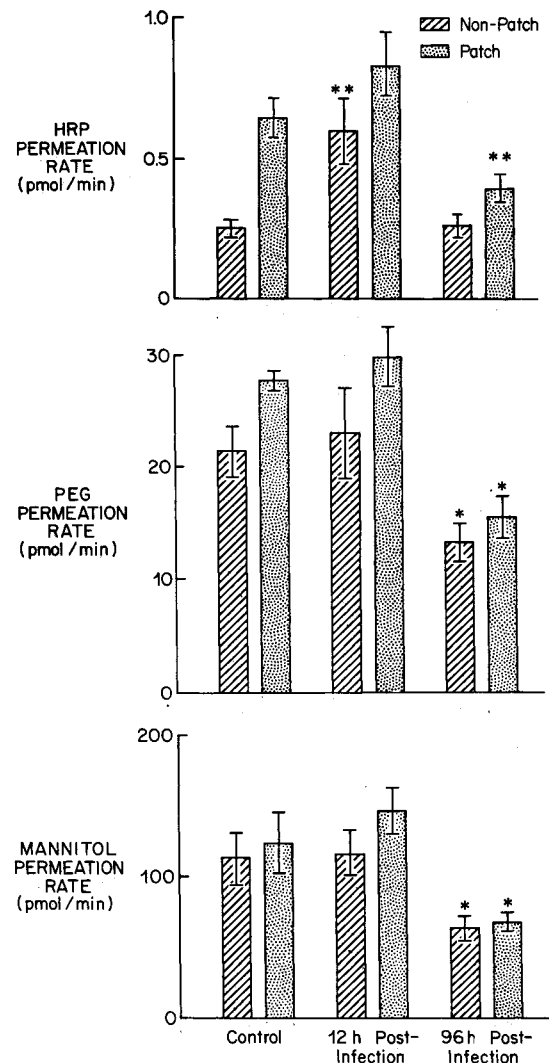


Figure 2. Permeation rates for three macromolecules, horseradish peroxidase (HRP) (mol wt 40,000), polyethylene glycol (PEG) 4000 (mol wt 4000), and mannitol (mol wt 182) across jejunum, comparing Peyer's patch-containing tissue with patch-free segments from control, 12-h, and 96-h transmissible gastroenteritis pigs (mean \pm SEM). Significant differences from controls, * $p < 0.05$, ** $p < 0.005$.

with any measurable features of the jejunal lesion (i.e., villus height, crypt depth, or mucosal thickness). The lag between addition of HRP to the mucosal chamber and appearance in the serosal chamber tended to be shorter in the group of piglets with severe lesions (0–36 min) than those with milder lesions (39 min), supporting a role for epithelial disruption in increased permeability rates.

Polyethylene Glycol 4000 and Mannitol Permeation

In Figure 3, appearance of PEG and mannitol in the serosal chamber after their addition to the mucosal chamber are compared with that of peroxi-

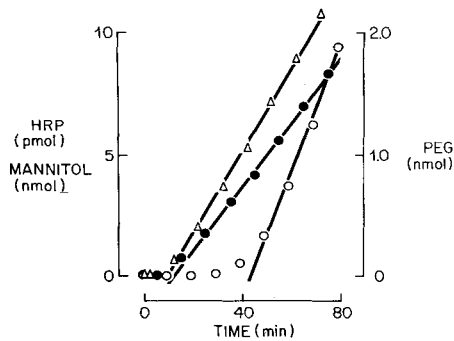


Figure 3. Time-courses for permeation of three macromolecules, horseradish peroxidase (HRP) (mol wt 40,000), ○; polyethylene glycol (PEG) 4000 (mol wt 4000), ●; and mannitol (mol wt 182), △, added to mucosal reservoir of Ussing chamber at zero time. Note that units for rate calculations are adjusted for each of the molecules. Lag period before first appearance of molecules in the serosal chamber are for mannitol, 9 min; PEG 4000, 10 min; and HRP, 43 min. Lines derived by least-squares analyses.

dase in a study of a patch-free segment of jejunum from a control pig. The lag times for these smaller molecules (mannitol, 9 min and PEG 4000, 10 min) were much less than that for the larger HRP molecule (43 min). All three molecules reached a steady state rate of appearance that varied inversely with molecular weight during the 80-min study. Mean permeability rates for the three groups of piglets seen in the lower panels of Figure 2 show patterns for PEG 4000 and mannitol quite different from those found for HRP in the upper panel. Patch-containing segments did not differ from patch-free segments in their permeability to PEG 4000 or mannitol in control or TGE piglets. Permeation of these smaller molecules was unchanged from controls at 12 h after TGE infection and significantly diminished in patch and nonpatch segments at 96 h ($p < 0.05$).

Electrical Properties of the Tissue

During the 90-min permeation studies, the electrical properties of the tissue remained stable (Table 2). In control piglets PD across patch-contain-

ing segments was significantly less than that across patch-free segments. Normally, PD across the intestinal epithelium is generated by the Na/K adenosine triphosphatase in the basal lateral membrane of the mature villus enterocyte, whereas conductance, or permeability of the tissue to ions, reflects tissue surface area and integrity of the tight junctions (7). The reduced PD but unchanged conductance in patch tissues most likely reflects the reduced proportion of mature villus enterocytes in patch epithelium.

Transmural PD was significantly decreased across patch-free segments from piglets after TGE infection; previous studies have shown that this decrease is accompanied by diminished Na/K adenosine triphosphatase activity in TGE jejunum (8). Conductance was altered significantly only at 96 h after TGE infection, when it was decreased, presumably due to reduced mucosal surface area. The electrical properties of patch and nonpatch tissue did not differ after infection, except at 96 h when the conductance of patch tissue was significantly lower than that of nonpatch tissue ($p < 0.05$).

Discussion

Very early in the course of invasive viral enteritis, absorption of HRP appears to be enhanced. Our column chromatography studies indicate that only intact HRP protein was detected under all study conditions. Macromolecule permeation has been found to be inversely related to molecular weight (9), a finding with which our *in vitro* control data agree. The anatomic site of size selectivity has not been identified. It has been modeled by a group of pores of varying size; most just large enough to admit mannitol, a small fraction large enough to admit PEG, and a still smaller fraction capable of admitting HRP (9). If epithelial disruption opened up defects sufficiently large to admit HRP, as we suspect is this case, HRP passage might be affected relatively more than the passage of PEG or mannitol, which have numerous

Table 2. Electrical Properties of Piglet Jejunal Mucosa

	Potential difference (mV)		Conductance (mMho/cm ²)	
	Patch-free	Patch	Patch-free	Patch
Control pigs	8.11 ± 0.59 (13)	5.96 ± 0.48 (12)	18.9 ± 1.07 (13)	18.37 ± 0.81 (12)
TGE pigs				
12 h	5.96 ± 0.66 (5) <i>p</i> < 0.05	5.04 ± 0.68 (5) NS	19.4 ± 1.76 (5) NS	16.9 ± 1.20 (5) NS
96 h	5.07 ± 0.83 (6) <i>p</i> < 0.01	4.57 ± 0.92 (6) NS	15.6 ± 0.89 (6) <i>p</i> < 0.05	14.0 ± 0.71 (6) <i>p</i> < 0.005

Electrical properties of stripped piglet jejunal mucosa measured in Ussing chambers during course of transport studies (mean ± SEM). Peyer's patch-containing and patch-free segments studied from control pigs and from pigs 12 h after experimental transmissible gastroenteritis infection. Statistical comparison with controls by Student's *t*-test. Number of animals is given in parentheses. NS, not significant; TGE, transmissible gastroenteritis.

alternate pathways. It is unlikely that at the 12-h stage the disease significantly stimulated active processes associated with macromolecular uptake, as our previous experiments showed these processes to be confined to the Peyer's patch regions (2) where the disease had no impact on permeation at 12 h. Altered unstirred water layer resistance, presumed to be greater over nonpatch segments than over the dome epithelium, and therefore more influenced over nonpatch segments by mucosal disease, also could have changed HRP permeation. Again, however, some impact on PEG and mannitol movement would be expected if this mucosal surface phenomenon were a major factor. A relative increase in the vulnerability of 12-h TGE tissue to damage from the *in vitro* experimental technique, causing an artificial increase in permeability, is also unlikely. Tissues maintained electrical stability during the study period, without increasing conductance, suggesting that tissue integrity was well-preserved in the chambers.

Our findings 96 h after infection were in marked contrast with those just described at 12 h. Permeation of HRP, normal in patch-free segments, indicates that during the diarrheal phase of viral enteritis, the jejunal mucosa is not excessively permeable to the macromolecule. Reduced uptake of the smaller molecules, PEG 4000 and mannitol, at the same stage, could be due to surface area and unstirred water layer changes, even though permeation of the larger and more slowly moving HRP molecule was not similarly affected. The reduced permeation of all three probe molecules through patch-containing segments at 96 h is unlikely to be related to surface area or surface water layer influences, as the epithelium over Peyer's patches was less distorted by this viral enteritis than the epithelium over patch-free segments. Alterations in epithelial uptake or subsequent processing of the probes in the epithelium or in the deeper layers could contribute to this reduced permeation at 96 h. The epithelial basement membrane, which under normal conditions is very porous in the Peyer's patch region (10), could also be affected by the disease.

Speculation on the relevance of our preliminary findings to clinical problems should be guarded. In attempting to explain the many syndromes of persisting diarrhea in young children, it has been postulated that small bowel lesions like viral enteritis

might allow enhanced uptake of antigenically significant macromolecules, which in turn might stimulate local responses capable of causing ongoing intestinal damage. Our data do not support the concept of a major breakdown in the mucosal barrier to macromolecular absorption during viral diarrhea. Increased permeation appears to occur only during the early hours of the infection when virus disrupts the epithelium and not during diarrhea when the epithelium is relatively undifferentiated but intact. Our findings do not preclude possible ongoing immune-based mucosal damage in response to macromolecular antigens which continue to be taken up in the non-patch-containing regions of the intestine at normal rates throughout the disease.

References

- Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology* 1977;72:440-51.
- Keljo DJ, Hamilton JR. Quantitative determination of macromolecular transport rate across intestinal Peyer's patches. *Am J Physiol* 1983;244:G637-44.
- Butler DG, Gall DG, Kelly MH, Hamilton JR. Transmissible gastroenteritis; mechanisms responsible for diarrhea in an acute viral enteritis in piglets. *J Clin Invest* 1974;53:1335-42.
- Pensaert M, Haelterman EO, Burnstein T. Transmissible gastroenteritis of swine: virus intestinal cell interactions I. Immunofluorescence, histopathology and virus production in the small intestine through the course of infection. *Arch Virus* 1970;31:321-34.
- Kerzner B, Kelly MH, Gall DG, Butler DG, Hamilton JR. Transmissible gastroenteritis: sodium transport and the intestinal epithelium during the course of viral enteritis. *Gastroenterology* 1977;72:457-61.
- Shepherd RW, Butler DG, Cutz E, Gall DG, Hamilton JR. The mucosal lesion in viral enteritis. Extent and dynamics of the epithelial response to virus invasion in transmissible gastroenteritis of piglets. *Gastroenterology* 1979;76:770-7.
- Schultz SG. Salt and water absorption by mammalian small intestine. In: Johnson LR, ed. *Physiology of the gastrointestinal tract*. New York: Raven, 1981:983-9.
- Kelly M, Butler DG, Hamilton JR. Transmissible gastroenteritis in piglets: a model of infantile viral enteritis. *J Pediatr* 1972;80:925-31.
- Landry CA, Axon ATR, Milton PJ, Hider RC, Creamer B. Permeability of the small intestine to substances of different molecular weight. *Gut* 1970;11:466-70.
- Low FN, McCluggage SG. Microdissection by ultrasonification: scanning electron microscopy of the epithelial basal lamina of the alimentary canal in the rat. *Am J Anat* 1984;169:137-47.