

Rapid Transepithelial Antigen Transport in Rat Jejunum: Impact of Sensitization and the Hypersensitivity Reaction

M. CECILIA BERIN,* AMANDA J. KILIAAN,† P. C. YANG,* JACK A. GROOT,† JAN A. J. M. TAMINIAU,§ and MARY H. PERDUE*

*Intestinal Diseases Research Program, McMaster University, Hamilton, Ontario, Canada; †Institute of Neurobiology, University of Amsterdam, Amsterdam, The Netherlands; and §Department of Pediatric Gastroenterology, Academic Medical Centre, Amsterdam, The Netherlands

See editorial on page 1034.

Background & Aims: Intestine from sensitized rats develops a rapid secretory response to luminal antigen challenge that depends on activation of subepithelial mast cells. The aim of this study was to determine the timing and route of the transepithelial protein antigen transport. **Methods:** Rats were sensitized to horseradish peroxidase (HRP). After 10–14 days, jejunal segments were resected, mounted in Ussing chambers, and challenged with HRP on the luminal side. **Results:** Electron microscopy of tissue specimens fixed at 2 minutes (before mast cell activation) showed enhanced endocytic uptake of HRP in enterocytes of HRP-sensitized rats compared with ovalbumin-sensitized or saline-injected controls. At this time, HRP was distributed throughout epithelial cells and was already evident in the lamina propria. In contrast, HRP was restricted to the apical region of enterocytes in controls. At 30 minutes (after mast cell activation), in HRP-sensitized rats only, HRP was also located within tight junctions and the paracellular region between epithelial cells. Tissue conductance was increased in HRP-sensitized rats beginning 30 minutes after HRP addition and correlated with the overall flux of HRP across the tissue. **Conclusions:** The results show that specific sensitization enhances the initial uptake and transcytosis of antigen across intestinal epithelium. Subsequent to activation of mast cells, antigen transport is further enhanced by penetration through the paracellular pathway.

Animal models of intestinal hypersensitivity to food proteins have shown that antigen challenge of the sensitized intestine produces alterations in ion transport, permeability, and motility.¹ Antigen absorbed into the mucosa cross-links immunoglobulin E bound to FcεRI receptors on the surface of mucosal mast cells, resulting in the release of a number of active mediators, including histamine, serotonin, and prostaglandins that stimulate net ion secretion from epithelial cells.² Studies involving

the inhibition of mast cell mediators^{2,3} and the use of genetically mast cell-deficient mice and congenic controls,⁴ have clearly testified to the central role of the mast cell in mediating the effects of intestinal anaphylaxis.

In our previous experiments using rats sensitized to ovalbumin (OVA), we showed that antigen added to the luminal side of isolated segments of jejunum evokes a secretory response, beginning within approximately 3 minutes.² This rapid secretion cannot readily be explained by our current understanding of macromolecular transport across the epithelium by either the paracellular or the transcellular pathway. The paracellular pathway between adjacent epithelial cells has been shown to be impermeable under normal circumstances to macromolecules such as protein antigens.^{5,6} Kinetic studies of the transcytotic pathway in polarized epithelial cells have indicated that transport across the epithelial barrier takes approximately 20 minutes.⁷ Antigen sampling M cells are not required for this rapid secretory response, because Peyer's patch-free intestinal segments are routinely used in these experiments. Therefore, the route by which luminal antigens gain access to mast cells in the subepithelial compartment of the mucosa in such a short time is not known.

The present study was designed to investigate the effect of (1) specific sensitization, (2) nonspecific sensitization, and (3) the immediate hypersensitivity reaction on antigen transport across the intestinal epithelium. For these experiments, we sensitized rats to a model protein antigen, horseradish peroxidase (HRP), because the intact protein can be measured by kinetic enzymatic assay⁸ and its reaction product visualized by electron microscopy.⁹ We determined HRP uptake into endosomes and its rate of transport across epithelial cells in rats sensitized to HRP and compared results with those from rats sensitized to an irrelevant antigen, OVA, or nonsensitized

Abbreviations used in this paper: HRP, horseradish peroxidase; IL, interleukin; Isc, short-circuit current; OVA, ovalbumin.

© 1997 by the American Gastroenterological Association
0016-5085/97/\$3.00

saline-injected controls. We found that sensitization of rats to HRP profoundly altered the transport kinetics of this protein antigen across the epithelium. HRP was transported via a transcellular pathway in both enterocytes and goblet cells such that it reached effector cells in the lamina propria by 2 minutes. In addition, our study showed that after activation of mast cells, a large increase in the flux of HRP across the tissue occurred that correlated with increased conductance, and was associated with penetration of antigen into the paracellular region.

Materials and Methods

Animals

Pathogen-free male Sprague–Dawley rats (250–275 g; Charles River Breeding Laboratories, St. Constant, Quebec, Canada) were housed in cages equipped with filter hoods. Rats were sensitized to HRP (type II; Sigma Chemical Co., St. Louis, MO) by a subcutaneous injection of 1 mg of HRP in 1 mL alum (10% $\text{AlK}[\text{SO}_4]_2 \cdot 12 \text{H}_2\text{O}$) plus an intraperitoneal injection of 1 mL of *Bordetella pertussis* vaccine (Connaught Laboratories, Willowdale, Ontario, Canada) (HRP group). Control rats were either sensitized (using the same protocol) to OVA (grade V; Sigma) as an irrelevant antigen (OVA group), or injected with saline alone (Saline group). Rats were studied 10–14 days after sensitization. All animal experiments were conducted with approval from the McMaster University Animal Care Committee.

Ussing Chamber Experiments

Rats were anesthetized with Urethane (Aldrich Chemical Company, Milwaukee, WI) and a 15-cm segment of jejunum was excised (beginning 5 cm distal to the ligament of Treitz). The external muscle layers were stripped off, leaving the submucosal plexus and mucosa intact. From each rat, eight pieces of intestine were mounted in Ussing chambers (WPI Instruments, Narco Scientific, Mississauga, Ontario, Canada). Care was taken to avoid tissue containing Peyer's patches. The chamber opening exposed 0.6 cm² of serosal surface area to 8 mL of circulating oxygenated Krebs' buffer (pH 7.35) at 37°C. The serosal buffer contained 10 mmol/L glucose osmotically balanced with 10 mmol/L mannitol in the luminal buffer. The tissue was clamped at 0 V using a W-P Instruments automatic voltage clamp (Narco Scientific, Downsview, Ontario, Canada). The short-circuit current (I_{sc} ; in microamperes) was recorded continuously, and at regular intervals, the I_{sc} was turned off to record potential difference, so that tissue conductance (milli-seconds per square millisiemens) could be determined. Tissues were allowed to equilibrate until I_{sc} stabilized, and then HRP was added to the luminal buffer.

HRP Flux

HRP was added to the luminal buffer at a final concentration of 5×10^{-5} mol/L. Duplicate samples of serosal buffer (500 μL) were obtained at 0, 30, 60, and 90 minutes, and were replaced with Krebs' buffer. HRP activity was determined by

assaying enzyme activity using a modified Worthington method.¹⁰ Briefly, 150 μL of sample was added to 800 μL of phosphate buffer containing 0.003% H_2O_2 and 80 $\mu\text{g/mL}$ *o*-dianisidine (Sigma). Enzyme activity was determined from the rate of increase in optical density at 460 nm during a 2-minute period. Fluxes were calculated according to standard formulae and were expressed as picomoles per square centimeter per hour.

Electron Microscopy

To determine the route and timing of transepithelial transport of HRP, tissues from 4 rats in each group were obtained for electron microscopy at 2, 5, and 30 minutes after HRP addition to luminal side of tissue. Jejunal segments were fixed in 2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 2 hours at room temperature, washed and left overnight at 4°C in the same buffer, and washed three times for 5 minutes each in 0.05 mol/L Tris buffer (pH 7.6). Tissues were incubated for 30 minutes in 5 mg of 3,3'-diaminobenzadine tetrahydrochlorine (Sigma) in 10 mL of 0.05 mol/L Tris buffer and 0.01% H_2O_2 (pH 7.6, 22°C), and were subsequently processed for routine electron microscopy. Tissues were oriented such that villus epithelial cells were cut longitudinally. Photomicrographs were prepared from the midvillus region. As an indication of the rate of transcytosis, the incidence of HRP was recorded at 2 and 30 minutes after addition of HRP within specific regions of enterocytes: apical (above the nucleus), mid (beside the nucleus), or basal (below the nucleus). Five enterocytes per rat, 20 per rat group, were used. The percentage of HRP-positive enterocytes was calculated for each rat, and the mean and SE were calculated for each rat group. To determine the amount of HRP initially taken up by enterocytes, HRP-containing endosomes within a $4 \times 6\text{-}\mu\text{m}$ window in the apical region of the cell (immediately below the microvilli) were counted and measured in coded photomicrographs.⁹ Twenty photomicrographs were examined per rat, 80 for each rat group. Analysis was performed by one investigator (A.J.K.), who was unaware of the treatment group. The area of HRP within endosomes was calculated and averaged for each rat before determining group means.

As a further control, intestine from rats sensitized to HRP but not challenged with HRP was fixed for electron microscopy, and the epithelium was examined for endogenous peroxidase activity. There was no HRP evident in this group, indicating that endogenous peroxidase was not a factor in these experiments.

Statistics

Statistical significance was tested by analysis of variance (ANOVA) with the Neuman–Keuls used for posthoc analysis. Pearson's correlation coefficient was used to examine the linear relationship between HRP flux and conductance measurements. A *P* value of <0.05 was considered significant.

Results

Electrophysiological Parameters

Baseline I_{sc} did not differ between the rat groups (19.5 ± 3.3 , 26.0 ± 7.0 , and $15.9 \pm 1.7 \mu\text{A/cm}^2$ for

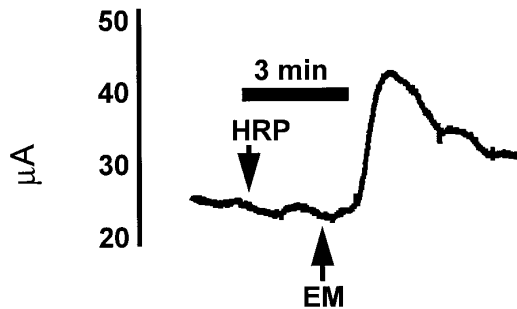


Figure 1. A representative tracing of Isc in intestine from a rat sensitized to HRP and challenged with 50 $\mu\text{mol/L}$ HRP on the luminal side of the tissue. In experiments examining the transepithelial transport of antigen before the ion secretory response, tissues were removed from the Ussing chamber and fixed for electron microscopy 2 minutes after HRP addition.

saline, OVA, and HRP groups, respectively). Intestine from rats sensitized to HRP showed a marked increase in Isc in response to luminal antigen challenge with HRP. A representative Isc tracing is shown in Figure 1. The increase in Isc at 5 minutes after HRP challenge was $32.1 \pm 2.5 \mu\text{A}/\text{cm}^2$ (mean $\pm$ SEM), and the time from antigen challenge to initial increase in Isc was 3.4 ± 0.4 minutes. The magnitude of this response was similar to that of tissue specimens from OVA-sensitized rats challenged with OVA. In contrast, HRP challenge to tissue specimens from either control groups (saline or OVA) produced no change in Isc.

Initial baseline conductance was also not different between groups. However, conductance was significantly increased in HRP-sensitized rats at 90 minutes, both compared with the original baseline and with values in saline and OVA rats at 90 minutes (Table 1). Conductance in the HRP-sensitized group was stable for the first 10 minutes after HRP challenge, then slowly increased such that it was significantly increased at 30 minutes, and rapidly increased thereafter (Figure 2). In contrast, saline and OVA rats maintained a stable conductance throughout the experimental period.

Table 1. Impact of Intestinal Hypersensitivity on Jejunal Conductance

	Initial (mS/cm^2)	Final (mS/cm^2)
Saline	31.9 ± 1.9	33.5 ± 2.4
OVA	32.7 ± 1.1	34.5 ± 1.9
HRP	29.9 ± 1.8	48.8 ± 3.9^a

NOTE. Values represent the means $\pm$ SEM for 6 rats per group, 2–4 tissues per rat. Readings were taken after equilibration (time = 0 minutes) and at the end of the experiment (time = 90 minutes).

^a $P < 0.05$ compared with HRP-sensitized rats at 0 minute and compared with saline control and OVA-sensitized rats at 90 minutes.

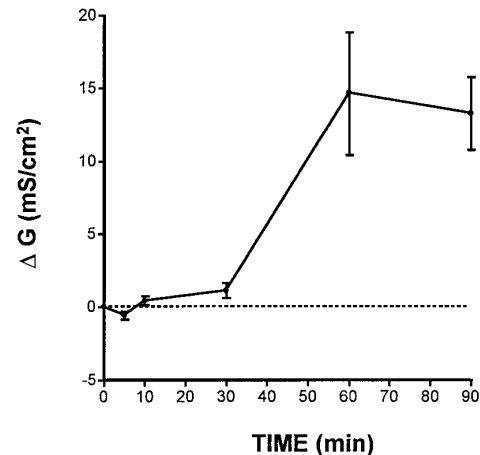


Figure 2. Change in conductance in response to HRP challenge in HRP-sensitized rats. Conductance was measured immediately before (zero time) and at intervals after luminal HRP challenge. The change in conductance at each time period was determined, and data are expressed as mean change $\pm$ SEM for 8 rats, 2–4 tissues per rat.

HRP Flux Measurements

To obtain an overview of transepithelial transport of antigen, luminal to serosal flux of HRP across the tissues was measured over a 90-minute period. The flux of HRP reached steady-state at 30–60 minutes in both control groups, but flux across intestine from HRP-sensitized rats continued to increase throughout the 90-minute experimental period (Figure 3). The HRP flux value was not significantly different between groups during the initial 30-minute period, but was significantly increased in the HRP-sensitized group during the 30–60-minute period compared with control groups (32.9 ± 10.6 vs. 10.4 ± 2.5 and $8.6 \pm 1.7 \text{ pmoles} \cdot \text{cm}^{-2} \cdot \text{hr}^{-1}$

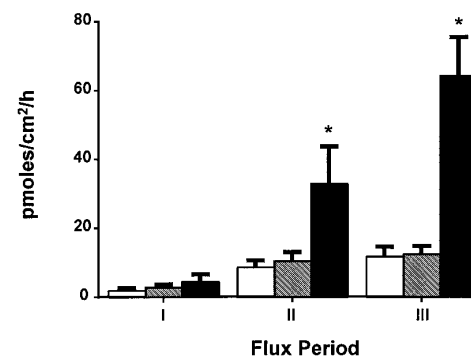


Figure 3. Effect of sensitization and antigen challenge on mucosal to serosal flux of HRP. Flux of HRP was measured across intestine obtained from rats injected with saline ($\square$), rats sensitized to OVA ($\square$), or rats sensitized to HRP ($\blacksquare$). HRP was measured in serosal buffer by kinetic enzymatic assay at 0–30 (I), 30–60 (II), and 60–90 (III) minutes after addition of 50 $\mu\text{mol/L}$ HRP to the luminal buffer. Data are expressed as mean $\pm$ SEM for 8 rats per group, 2–4 tissues per rat. * $P < 0.05$ compared with saline controls.

[mean $\pm$ SEM] for HRP sensitized, OVA, and saline, respectively), and was fivefold greater (64.3 ± 11.0 vs. 12.4 ± 2.4 and 11.9 ± 2.6 pmoles $\cdot$ cm $^{-2}$ $\cdot$ hr $^{-1}$) at the 60–90-minute flux period. The control groups did not differ from each other in each time period. The relationship between conductance and flux was examined by correlation analysis with data from the three treatment groups in the final flux period, resulting in a correlation coefficient of 0.80 ($P < 0.001$).

Electron Microscopy

Two-minute time point. To determine if antigen was reaching cells in the lamina propria before mast cell activation and the subsequent ion secretory response, tissues were fixed 2 minutes after HRP challenge (Figure 1). Electron photomicrographs of enterocytes and the underlying lamina propria were used to assess the distribution of HRP (Figure 4). In HRP-sensitized rats, HRP was found to be distributed throughout enterocytes such that 65% of enterocytes examined had HRP within endosomes in the apical region, 50% were positive for HRP in the midregion, and 45% of enterocytes contained endosomes positive for HRP in the basal region of the cell, adjacent to the lamina propria (Figure 5). OVA rats also had HRP distributed throughout enterocytes, but the incidence of HRP-containing endosomes was much lower than that observed in the HRP-sensitized group. HRP-positive endosomes in the apical region were found in 45% of enterocytes in OVA rats, 20% contained HRP in the midregion, and only 15% contained HRP in the basal region. Saline rats had a more limited distribution of HRP within enterocytes than the HRP- or OVA-sensitized rats. Enterocytes from saline rats did contain HRP-positive endosomes, but these were predominantly in the apical region. Of the enterocytes examined, 30% contained HRP within the apical region, 10% were positive for HRP in the midregion, but there was zero incidence of HRP within the basal region of enterocytes. In addition to enterocytes, HRP was also observed within goblet cells (Figure 4) and Paneth cells (not shown) of HRP-sensitized rats. Intestinal epithelial cells from rats sensitized to HRP but not challenged showed no HRP reaction product in any location.

To determine if sensitization altered the amount of HRP taken up into enterocytes, the diameter of HRP in endosomes within the apical region was measured (Figure 6). The mean area of endosomal HRP was significantly greater in HRP-sensitized rats compared with saline rats (257 ± 80 nm ^{2} vs. 72 ± 16 nm ^{2}). OVA-sensitized rats did not significantly differ from either group (111 ± 45 nm ^{2}).

At 2 minutes after HRP challenge, HRP was already

present in the lamina propria (Figure 7A) in 40% of photomicrographs examined from the HRP-sensitized group, compared with 10% and 0% of photomicrographs from OVA and saline rats, respectively (Figure 5). At 5 minutes after HRP challenge, there was more HRP evident within the lamina propria, and mast cells showed early signs of activation (Figure 7B). The source of lamina propria HRP seemed to be transcellular antigen transport, because there was no evidence for paracellular transport of HRP. Tight junctions were negative for HRP in all 3 groups at the 2-minute postchallenge time point.

Thirty-minute time point. In tissues fixed for electron microscopy analysis at 30 minutes after HRP challenge, mast cells were obviously activated. Transcellular movement of HRP across the epithelium was again observed, with HRP distributed throughout enterocytes in all 3 treatment groups (Figure 8). HRP-sensitized rats had an increased incidence of HRP in the basal region of enterocytes compared with OVA and saline rats (50% of enterocytes positive for HRP-containing endosomes compared with 25% and 20%, respectively). HRP was observed in the paracellular region and tight junctions between adjacent epithelial cells in 3 of 4 rats sensitized to and challenged with HRP (Figure 9). Epithelium from OVA-sensitized or saline rats showed no HRP crossing the tight junctions or within the paracellular region.

Discussion

Previous experiments in our laboratory have shown that intestine from rats sensitized to OVA responds to antigen challenge on the luminal side of the tissue with a rapid (beginning in approximately 3 minutes) increase in *I*_{sc} that is caused by chloride ion secretion.^{2,11} Mast cells are required for the response.^{2,4} These findings suggest that antigen is transported across the epithelial barrier to activate subepithelial mast cells within a very short time. However, the recognized kinetics of transepithelial protein movement indicate that approximately 20 minutes are required for transcytosis.⁷ The aim of the current study was to determine the route and timing of antigen transport across the intestinal epithelium in sensitized rats. We showed that protein transport across the intestinal epithelium was increased by nonspecific sensitization, and dramatically enhanced by specific sensitization such that antigen gained access to effector cells in the lamina propria within 2 minutes after its addition to the luminal surface. Our finding that transcytosis was maximal for the sensitizing antigen suggests that there may be a component of recognition in the antigen transport process. In addition, morphological documentation of HRP between tight junctions and adjacent epithelial cells together with increased tissue con-

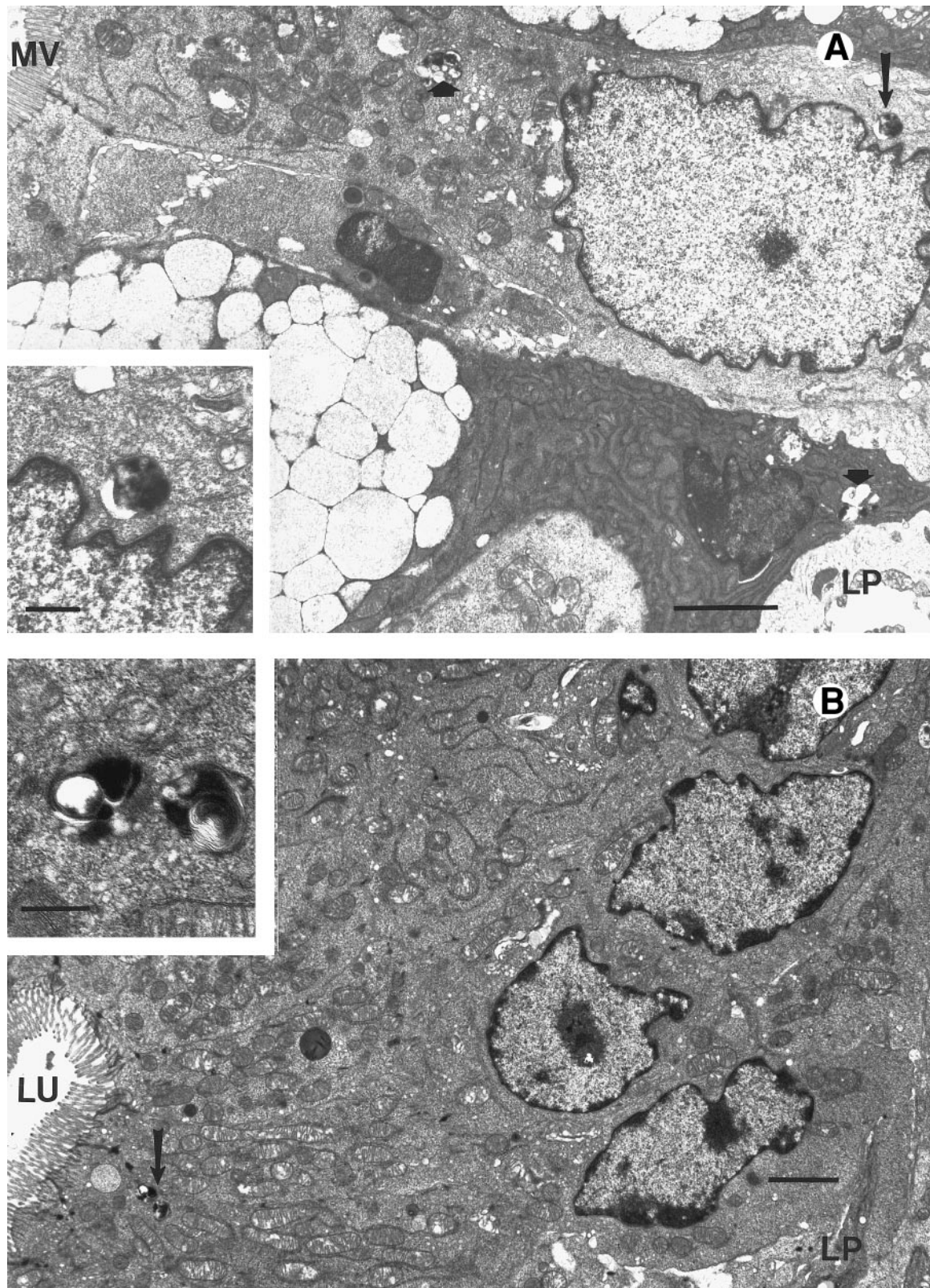


Figure 4. Representative photomicrographs prepared from tissues fixed 2 minutes after addition of HRP to the lumen, showing HRP-containing endosomes within epithelium from (A) HRP-sensitized rats and (B) OVA-sensitized rats. The lumen (LU) and microvilli (MV) are indicated on the left side of the photomicrographs. *Short arrows* indicate the presence of HRP-containing endosomes; *long arrows* indicate the endosomes shown in the higher magnification insets. Bars = 2 μm (inset bars = 0.5 μm).

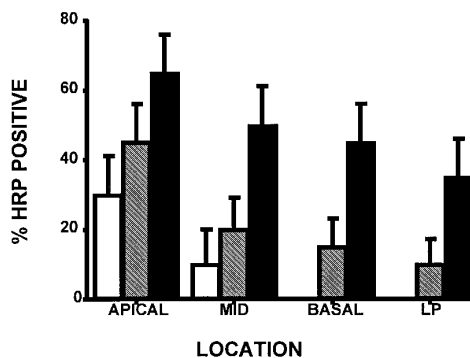


Figure 5. Distribution of HRP within enterocytes and in the lamina propria. Tissues from saline (□), OVA-sensitized (▨), and HRP-sensitized (■) rats were fixed for electron microscopy 2 minutes after luminal HRP addition. Coded photomicrographs (20 for each of 4 rats/group) were examined for incidence of HRP (percentage of sections with HRP-containing endosomes) in the apical, mid, or basal region of enterocytes, or in the lamina propria (LP), and data are expressed as percentage (mean $\pm$ SEM) of photomicrographs examined in each group that were positive for HRP.

ductance suggest that after activation of effector cells, antigen penetrated the epithelium via the paracellular pathway.

To examine antigen transport across the epithelium, we used HRP (a protein that can be readily measured by enzymatic assay and visualized by electron microscopy) as a sensitizing antigen. Intestine from HRP-sensitized rats responded to HRP challenge with similar ion transport responses, as we previously observed with rats sensitized to OVA,² with respect to the magnitude of the *I*_{sc} increase and the timing of the secretory response.

To determine the effect of sensitization on initial antigen uptake (i.e., before mast cell activation), tissues were fixed for electron microscopy at 2 minutes, approximately 1 minute before the beginning of the *I*_{sc} increase induced by luminal HRP antigen challenge. Quantification of the amount of HRP within a fixed apical region of enterocytes revealed that there was significantly greater uptake of HRP into enterocytes of HRP-sensitized rats compared with OVA-sensitized and nonsensitized control rats. Although there was a trend showing increased uptake of HRP in OVA sensitized rats, this did not reach the level of statistical significance. However, when the distribution of HRP within enterocytes was examined, HRP was located in endosomes throughout enterocytes and in the lamina propria in both HRP-sensitized and OVA-sensitized rats, although the incidence of HRP in all regions was lower in OVA sensitized rats. In contrast, HRP within the epithelium of saline-control rats was confined to the apical region of enterocytes. These findings indicate that the rate of antigen transport across epithelial cells and into the lamina propria was altered

by specific sensitization, and that the rapid initial uptake of antigen may have been influenced by specific recognition. The data suggest that nonspecific sensitization enhances transepithelial antigen transport, but not to the same extent as specific sensitization. These results agree with previous *in vivo* experiments from our laboratory showing that intestine from OVA-sensitized rats had a greater baseline transport of ⁵¹chromium-ethylenediaminetetraacetic acid than control rats.¹² Taken together, these studies imply that sensitization alone alters the barrier properties of the intestine.

Studies in control animals have shown that there is significant transcytosis of macromolecules to the basal membrane in polarized epithelial cells, but that the time from apical uptake of protein to exocytosis across the basal membrane is slow, requiring at least 20 minutes.⁷ This is consistent with our observations of antigen transport across intestine from saline-control rats. It is not clear how sensitization induces changes in the kinetics of transcytosis, but it is possible that cytokines released from immunocytes could regulate endocytic transport. Atopy is associated with an increase in T_{H2} cell cytokines, particularly interleukin (IL)-4, IL-5, and IL-10.¹³ Although a number of cytokines have been shown to have profound effects on ion secretion and epithelial barrier resistance, the impact of these immunological mediators on the transcytotic pathway has yet to be addressed. Inflammatory cytokines (IL-1, tumor necrosis factor α) have been shown to up-regulate the endocytic rate in hepatic endothelium,¹⁴ indicating that immunological regulation of endocytic pathways is possible. In addition,

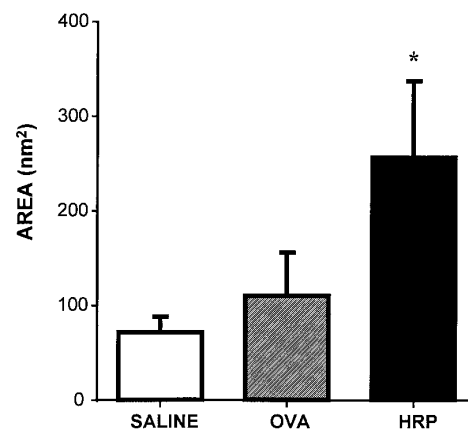


Figure 6. Effect of sensitization on uptake of HRP into enterocytes. The area of endosomal HRP within $4 \times 6\text{-}\mu\text{m}$ windows beneath the microvilli was determined in coded electron photomicrographs taken from intestine of saline-injected, OVA-sensitized, or HRP-sensitized rats. Tissue was fixed 2 minutes after luminal addition of HRP, and 20 photomicrographs per rat (4 rats per group) were used for quantification. *Significance at $P < 0.05$ compared with saline-injected control rats.

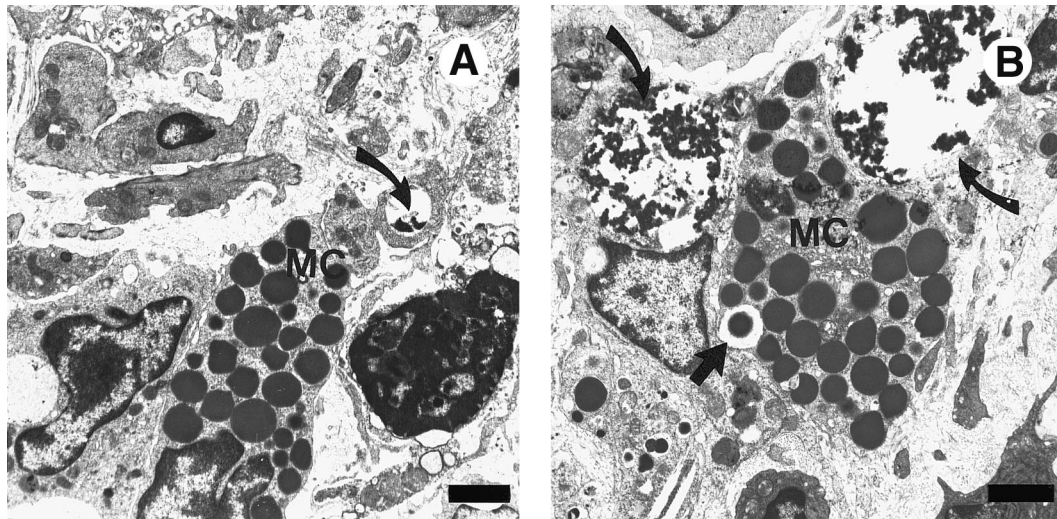


Figure 7. Representative photomicrographs showing HRP within the lamina propria in HRP-sensitized rats at (A) 2 minutes and (B) 5 minutes after luminal addition of HRP. Curved arrows indicate the presence of HRP; the straight arrow indicates a clear area surrounding a mast cell (MC) granule, an early sign of activation. Bars = 2 μ m.

milk protein has been shown to stimulate secretion of cytokines (including tumor necrosis factor α) from peripheral blood mononuclear cells obtained from infants with cow's milk allergy, and epithelial cells cultured with supernatants from these cells demonstrated increased flux of intact HRP.¹⁵

The specificity of the increased uptake of antigen into the enterocyte raises the intriguing possibility that there is recognition of antigen by the enterocyte, possibly through an antibody-mediated mechanism. If uptake of HRP was occurring through a receptor-mediated transport mechanism, this could explain both the specificity and increased rate of transport observed. Binding to a

receptor might reduce lysosomal degradation of the protein and thus explain the increased incidence at 30 minutes of HRP in endosomes in HRP-sensitized rats compared with the other rat groups.

At the early time point (2 minutes), it is also possible that a small number of HRP molecules, too few to be detected morphologically, could have leaked across the tight junctions. This possibility cannot be ruled out in this study, although the conductance data suggest that there was no opening of tight junctions until after 5

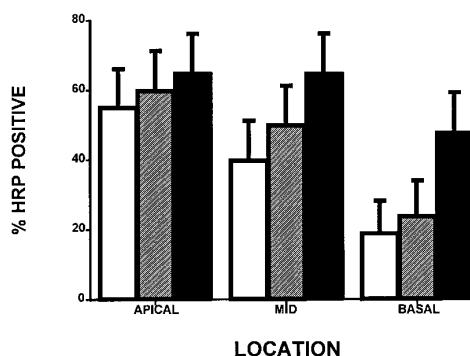


Figure 8. Distribution of HRP within enterocytes after effector cell activation. Tissues from saline ($\square$), OVA-sensitized ($\square$), and HRP-sensitized ($\blacksquare$) rats were fixed for electron microscopy 30 minutes after luminal HRP addition. Coded photomicrographs (20 for each of 4 rats/group) were examined for incidence of HRP (percentage of sections with HRP-containing endosomes) in the apical, mid, or basal region of enterocytes; data are expressed as percentages (mean $\pm$ SEM) of photomicrographs examined in each group that were positive for HRP.

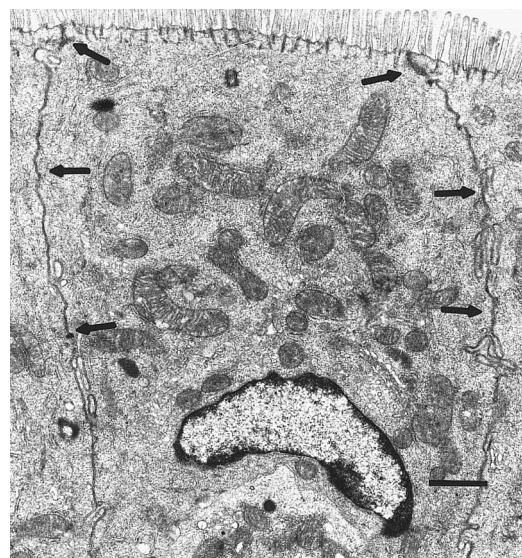


Figure 9. Representative photomicrograph of an enterocyte from an HRP-sensitized rat 30 minutes after addition of HRP to the luminal buffer. Arrows indicate the paracellular space between enterocytes, which is filled with HRP. Bar = 1 μ m.

minutes, at the earliest. Further studies are in progress to accurately determine the contribution of each pathway to transepithelial antigen transport before mast cell activation.

Although numerous studies have shown an increased flux of specific antigen,¹² bystander antigens,^{16–19} or small molecular weight probes¹² during intestinal anaphylaxis, the route of the uptake has not been clearly delineated. Therefore, we examined the uptake of HRP across the intestine by electron microscopy 30 minutes after HRP challenge, well after the mast cell–mediated increase in *Isc*. As indicated previously, HRP transport via endosomes was enhanced by specific antigen challenge. In addition, the antigen-induced hypersensitivity response was associated with an increase in tissue conductance and the presence of intact HRP in the paracellular regions between enterocytes. HRP within the lateral spaces could be attributed to either penetration through tight junctions and/or exocytosis of intracellular HRP. High magnification electron microscopy demonstrated HRP within the tight junctional area. Although paracellular HRP was not present in all sections examined from HRP-sensitized rats, HRP in the paracellular regions was never documented in any sections from saline or OVA rats. In addition, the conductance was elevated in HRP, but not saline or OVA rats. Measurements of conductance, believed to reflect the permeability of the tight junction,¹⁹ correlated highly with the flux of HRP across tissues from all three treatment groups. This suggests that the increased flux in specifically sensitized and challenged rats was related to the increased conductance of the tight junctions, and that the pathway of HRP transport included a paracellular component.

Previous studies in our laboratory have shown that receptor antagonism of mast cell mediators or neural blockade attenuates the ion secretory response,^{2,20} indicating that release of mast cell and nerve products occurs after antigen challenge. Rat mast cell protease II has been shown to decrease epithelial barrier function.²¹ In addition, several studies have established that neural activation or neurotransmitters can alter epithelial permeability to macromolecules. Stimulation of rat intestine with cholinergic agonists^{6,9} increased tight junctional permeability, and stress was also shown to impair barrier function via cholinergic mechanisms.²² In those studies, electron microscopy indicated HRP within the tight junctions and paracellular regions. Neural blockade with tetrodotoxin and atropine inhibited macromolecular transport across the normal small intestine.²³ Evidence also suggests that substance P and neuropeptide Y can modify the epithelial barrier.^{2,24} In our study, because HRP was observed in the paracellular space only after

the ion secretory response, we hypothesize that activation of mucosal mast cells and/or their interaction with nerves mediated the paracellular permeability to antigen.

Mucosal to serosal flux of HRP was significantly elevated in rats specifically sensitized to HRP compared with OVA-sensitized and saline control rats. This is consistent with previous findings that demonstrated increased serum levels of antigen after anaphylaxis.¹² This could be caused by enhanced uptake via both the transcellular and paracellular routes. In addition, the flux of HRP continued to increase throughout the entire experimental period in rats sensitized to HRP, whereas in the other rat groups, the flux of HRP reached equilibrium after 30 minutes. The escalating rate of antigen transport may indicate that after crossing the epithelial barrier, HRP was activating mast cells and perhaps nerves, thereby continuing to augment the epithelial barrier defect.

In summary, transepithelial antigen transport in sensitized rats appeared to be a highly complex process composed of two phases. The initial phase occurred before mast cell activation. This phase involved enhanced endocytic uptake of specific antigen into enterocytes and its rapid transcytosis into the lamina propria. The second phase occurred after mast cell activation and was associated with a markedly increased conductance, the presence of antigen in tight junctions and paracellular regions, and increased overall flux of antigen across the tissue. Further experiments are necessary to define fully the mechanisms responsible for phase I and II of transepithelial antigen transport.

References

1. Crowe SE, Perdue MH. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992; 103:1075–1095.
2. Crowe SE, Sestini P, Perdue MH. Allergic reactions of rat jejunal mucosa: ion transport responses to luminal antigen and inflammatory mediators. *Gastroenterology* 1990;99:74–82.
3. Zhang S, Myers S, Castro GA. Inhibition of anaphylaxis-evoked intestinal fluid secretion by the dual application of an H_1 antagonist and cyclooxygenase inhibitor. *Gastroenterology* 1991;100: 922–928.
4. Perdue MH, Masson S, Wershil BK, Galli SJ. Role of mast cells in ion transport abnormalities associated with intestinal anaphylaxis: correction of the diminished secretory response in genetically mast cell-deficient W/W^c mice by bone marrow transplantation. *J Clin Invest* 1991;87:687–693.
5. Walker WA, Cornell R, Davenport LM, Isselbacher KJ. Macromolecular absorption: mechanism of horseradish peroxidase uptake and transport in adult and neonatal rat intestine. *J Cell Biol* 1972; 54:195–205.
6. Phillips TE, Phillips TL, Neutra MR. Macromolecules can pass through occluding junctions of rat ileal epithelium during cholinergic stimulation. *Cell Tissue Res* 1987;247:547–554.
7. Bomsel M, Prydz K, Parton RG, Gruenberg J, Simons K. Endocytosis in filter-grown Madin-Darby canine kidney cells. *J Cell Biol* 1989;109:3243–3258.
8. Heyman M, Ducroc R, Desjeux J-F, Morgat JL. Horseradish peroxi-

- dase transport across adult rabbit jejunum in vitro. *Am J Physiol* 1982;242:G564–G588.
9. Bijlsma P, Kiliaan AJ, Scholten G, Heyman M, Groot JA, Taminiau JAJM. Carbachol, but not forskolin, increases mucosal-to-serosal transport of intact protein in rat ileum in vitro. *Am J Physiol* 1996;271:G145–G147.
 10. Maehly AC, Chance B. The assay of catalases and peroxidases. In: Glick D, ed. *Methods of biochemical analysis*. New York: Wiley-Interscience, 1954:357–398.
 11. Forbes D, Patrick MK, Perdue MH, Buret A, Gall DG. Intestinal anaphylaxis: in vivo and in vitro studies of rat proximal colon. *Am J Physiol* 1988;255:G201–G205.
 12. Crowe SE, Soda K, Stanis AM, Perdue MH. Intestinal permeability in allergic rats: nerve involvement in antigen-induced changes. *Am J Physiol* 1993;264:G617–G623.
 13. Daser A, Meissner N, Herz U, Renz H. Role and modulation of T-cell cytokines in allergy. *Curr Opin Immunol* 1995;7:762–770.
 14. Martinez I, Sveinbjornsson B, Vidal-Vanaclocha F, Asumendi A, Smedsrod B. Differential cytokine-mediated modulation of endocytosis in rat liver endothelial cells. *Biochem Biophys Res Comm* 1995;212:235–241.
 15. Heyman M, Darmon N, Dupont C, Dugas B, Hirribaren A, Blaton M-A, Desjeux J-F. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor- α , altering intestinal function. *Gastroenterology* 1994;106:1514–1523.
 16. Zhang S, Castro GA. Boosted mucosal immune responsiveness in the rat intestine by actively transported hexose. *Gastroenterology* 1992;103:1162–1166.
 17. Turner MW, Boulton P, Shields JG, Strobel S, Gibson S, Miller HRP, Levinsky RJ. Intestinal hypersensitivity reactions in the rat. I. Uptake of intact protein, permeability to sugars and their correlation with mucosal mast-cell activation. *Immunology* 1988;63:119–124.
 18. Bloch KJ, Bloch DB, Stearns M, Walker WA. Intestinal uptake of macromolecules. VI. Uptake of protein antigen in vivo in normal rats and in rats infected with *Nippostrongylus brasiliensis* or subjected to mild systemic anaphylaxis. *Gastroenterology* 1979;77:1039–1044.
 19. Madara JL. Loosening tight junctions, lessons from the intestine. *J Clin Invest* 1989;83:1089–1094.
 20. Kosecka U, Marshall JM, Crowe SE, Bienenstock J, Perdue MH. Pertussis toxin stimulates hypersensitivity and enhances nerve-mediated antigen uptake in rat intestine. *Am J Physiol* 1994;267:G745–G753.
 21. Scudamore CL, Thornton E, McMillan L, Newlands GFJ, Miller HRP. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995;182:1871–1881.
 22. Saunders PR, Kiliaan AJ, Groot JA, Perdue MH. Stress-enhanced epithelial permeability to antigenic proteins is mediated by cholinergic mechanisms and occurs via both transcellular and paracellular pathways [Abstract]. *Gastroenterology* 1995;108:A911.
 23. Kimm MH, Curtis GH, Hardin JA, Gall DG. Transport of bovine serum albumin across rat jejunum: role of the enteric nervous system. *Am J Physiol* 1994;266:G186–G193.
 24. McKay DM, Berin MC, Fondacaro JD, Perdue MH. Inhibition of antigen-induced ion secretion by neuropeptide Y in rat jejunum. *Am J Physiol* 1996;271:G987–G992.

Received January 13, 1997. Accepted May 19, 1997.

Address requests for reprints to: Mary H. Perdue, Ph.D., Intestinal Disease Research Program, Health Sciences Centre 3N5C, McMaster University, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. e-mail: perdue@fhs.mcmaster.ca; fax: (905) 522-3454.

Supported by a grant from the Medical Research Council of Canada.

The authors thank Michelle Benjamin and Greet Scholten for their expert technical assistance.