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Secretory Immunoglobulin A, Intestinal Mucin, and Mucosal Permeability in Nutritionally Induced Bacterial Translocation in Rats

Georg Spaeth, M.D.,* Thomas Gottwald, M.D.,† Robert D. Specian, Ph.D.,‡
Mark R. Mainous, M.D.,§ Rodney D. Berg, Ph.D.,‖ and Edwin A. Deitch, M.D.¶

From the Department of Surgery, Rheinische Friedrich-Wilhelms-University, Bonn, Germany; the Department of Surgery, Eberhard-Karls-University,† Tuebingen, Germany; the Departments of Cellular Biology,‡ Surgery,§ and Microbiology and Immunology,‖ LSU School of Medicine, Shreveport, Louisiana; and the Department of Surgery, UMDNJ-N.J. Medical School,¶ Newark, New Jersey*

Objective
The authors investigated the role of mucin and secretory immunoglobulin A (sIgA) in a model of nutritionally induced bacterial translocation.

Background
Parenteral and certain elemental diets have been shown to impair intestinal barrier function, whereas fiber has been shown to protect against nutritionally induced bacterial translocation. However, the factors responsible for these phenomenon have not been fully determined.

Methods
Intestinal mucin levels, mucosal protein content, sIgA, intestinal morphology, and permeability to horseradish peroxidase, bacterial translocation, and intestinal bacterial population levels were measured in rats 7 days after receiving total parenteral nutrition (TPN) solution (28% glucose, 4.25% amino acids; 307 kcal/kg/day) enterally (ORAL-TPN) or parenterally (IV-TPN) with or without enteral bulk fiber supplementation. Chow-fed rats served as control subjects.

Results
The incidence of bacterial translocation in the ORAL-TPN and IV-TPN groups was reduced significantly by the provision of fiber ($p < 0.05$). Mucosal protein, sIgA, and insoluble mucin levels were decreased in the jejunum of the ORAL-TPN and IV-TPN groups, with mucosal protein levels being decreased to a greater extent than sIgA or mucin. Although similar decreases in these parameters were observed in the fiber-fed groups, fiber appeared to improve intestinal barrier function as measured by horseradish peroxidase permeability.

Conclusions
The provision of bulk-forming fiber improves intestinal barrier function as measured by peroxidase permeability and bacterial translocation, but does not restore mucosal protein content, intestinal mucin, or sIgA levels to normal.

The gut is a complex organ, a primary function of which is the digestion and absorption of nutrients. Another equally important function, and one that often is not fully appreciated, is that of acting as a barrier in preventing the spread of intraluminal bacteria and endotoxin to systemic organs and tissues, a process termed bacterial translocation.[1] Because of the potentially important relationship between nutrition and gut barrier function, this area has received increased clinical and experimental attention during the last several years. That parenteral and certain enteral (elemental) diets are associated with loss of intestinal barrier function, manifested as bacterial translocation, has been documented by several investigative groups, including our own.[2-7] Although the mechanisms by which parenteral or certain enteral diets promote bacterial translocation are complex and not completely understood, we previously have documented that certain therapeutic maneuvers, such as the provision of bulk-forming fibers,[3,8] will limit or reverse diet-induced bacterial translocation. However, the mechanisms by which fiber protects the gut are unknown. Because fiber stimulates the production and release of various intestinal trophic factors[9,10] in addition to exerting a direct effect on intestinal mucus, epithelial cells and the gut flora,[11-13] its protective effect could be direct, indirect and mediated by secondary factors, or both. Recently, in a murine model of elemental diet-induced bacterial translocation, we found evidence suggesting that the protective effect of fiber against bacterial translocation was, to a large extent, hormonally mediated.[14] The goal of the current study is to further delineate the mechanisms of nutritionally induced bacterial translocation by measuring the effects of nutritional modulation on intestinal permeability, mucin levels, and secretory immunoglobulin A (sIgA).

MATERIAL AND METHODS

Animals

Male specific pathogen-free Sprague-Dawley rats weighing between 250 and 300 g were purchased from Charles River Laboratories (Portage, MI). They were housed under barrier-sustained conditions and allowed to recover for at least 5 days after arrival before use in the experiments. During this acclimatization period, the rats were fed Purina rat chow #5001. The animals were maintained in accordance with the recommendations of the *NIH Guide for the Care and Use of Laboratory Animals,* and the experiments were approved by the Louisiana State University Medical Center, Shreveport, Animal Care Committee.

Experimental Design

During the experiments, the rats were housed in suspended wire-grid cages to limit coprophagy and to eliminate the need for bedding. The first experiment was performed to test the hypothesis that elemental diet-induced mucosal atrophy and bacterial translocation are associated with alterations in intestinal mucin and sIgA levels. Three groups of rats were studied. The first group received water and rodent chow *ad libitum* for 7 days (chow group), the second group received total parenteral nutrition (TPN) solution intravenously (IV-TPN group), and the third group received sterile TPN solution (ORAL-TPN group). The IV-TPN and ORAL-TPN groups were pair fed. All three groups had jugular venous catheters placed and were tethered to swivels as described previously.[3] After 7 days, the rats were killed, and their mesenteric lymph node (MLN) complex was removed for bacteriologic culture. The small intestine from the ligament of Treitz to the ileocecal valve was excised. Two samples each of distal jejunum and ileum were removed to quantitate bacterial population levels and for histologic examination. The luminal contents of the remaining proximal (jejunal) and distal (ileal) small intestine were collected, and luminal (soluble) mucin and sIgA levels were quantitated in these intestinal washings. Thereafter, the washed small intestines were processed for mucosal protein and insoluble mucin content.

Because cellulose fiber prevents diet-induced bacterial translocation, as we documented in previous studies,[3,8] the second experiment was performed to characterize the effect of cellulose fiber administration on bacterial translocation, cecal bacterial population levels, intestinal mucosal protein content, mucin levels, and sIgA. Four groups of rats were studied. The groups receiving IV-TPN only or ORAL-TPN only served as the positive control groups. The two experimental groups consisted of IV-TPN or ORAL-TPN diet-fed rats receiving supplemental cellulose-based fiber (Unifiber, Dow B. Hickam Inc, Sugar Land, TX). In this pair of experiments, only the IV-TPN-fed animals received jugular venous catheters. After 7 days, the rats were killed and studied as described previously.

Feeding Protocol

The chow-fed group received water and rodent chow *ad libitum.* Because previous studies with rats of the

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Address reprint requests to Edwin A. Deitch, M.D., Department of Surgery, UMDNJ-N.J. Medical School, 185 South Orange, G506, Newark, NJ 07103.
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same strain, age, and weight had shown that their *ad libitum* intake of Purina Chow was 307 kcal/kg body weight/day,[3] the rats that were pair fed the elemental diet by either route received on average 307 kcal/kg/day (230 mL/kg/day) of sterile TPN solution. The solution contained a final concentration of 4.5% amino acids (Travenol Laboratories Inc., Deerfield, IL) and 28% glucose, in addition to electrolytes and vitamins.[3] It contained 1333 kcal/L, and the nonprotein calorie:nitrogen ratio was 151:1 kcal/g N. The TPN solution was administered to the IV-TPN groups via the jugular catheter and to the ORAL-TPN groups via sterile, calibrated feeding bottles with fitted sippers. The fiber-supplemented groups received 3.3 g of cellulose powder per day. To avoid wastage and contamination, the cellulose powder was placed in the cages inside special powder feeders (Lab Products Inc., Aberdeen, MD).

Harvesting of Specimens

Before being killed, the animals were weighed and anesthetized with intramuscular ketamine and rompum (44/6 mg/kg). The skin was prepared with 70% alcohol and dissected asceptically from the thoracic and abdominal walls. The mediastinum was swabbed with sterile cotton-tipped applicator sticks, and the swabs were cultured at 37 C for 48 hours in brain-heart infusion. After a 0.2-mL sample of blood was collected by heart puncture for culture, the catheter tip was excised from the superior vena cava. Both the blood and catheter tip were cultured in brain-heart infusion to detect bacterial contamination. Next, the MLN was excised, weighed, and transferred to a sterile grinding tube containing 1 mL of brain-heart infusion. Before and after excision of the MLN, the peritoneal cavity was swabbed with a sterile, cotton-tipped applicator stick that was cultured in brain-heart infusion to detect bacterial contamination. Animals with positive cultures of either the mediastinum, blood, catheter tip, or peritoneal cavity, indicating bacterial contamination, were excluded from analysis. The incidence of contaminating cultures was 11%.

The small intestine from the ligament of Treitz to the ileocecal valve was excised and placed on a sterile sheet and divided in half. A piece of the terminal segment of each half was rinsed free of contents and placed in vials containing fixation solution for histologic analysis. In the first experiment, 0.2- to 0.5-g samples of the distal portion of each intestinal segment was placed into grinding tubes containing 9 mL of brain-heart infusion for quantitative bacterial cultures. The remaining intestinal sacs of upper and lower small bowel were rinsed with 5 mL of chilled phosphate-buffered saline (PBS), and the rinses were individually collected in calibrated tubes sitting in an ice bath. The lengths of the intestinal segments were determined under vertical extension with a 5-g weight; then they were stored in chilled PBS while awaiting further processing. Finally, segments of the cecum were harvested for quantitation of bacterial population levels and histologic analysis.

Testing for Bacterial Translocation

The MLN was cultured for translocating bacteria as described previously.[15] One half of the MLN homgenate was plated onto three blood agar plates, and the second half was plated onto three MacConkey's agar plates. The plates were examined after 24 and 48 hours of incubation at 37 C. Mesenteric lymph nodes containing a total of more than ten colony-forming units were classified as positive. Serial dilutions of the jejunal, ileal, or cecal homogenates plated onto blood agar and MacConkey agar plates and the plates were examined after 24 and 48 hours incubation at 37 C to quantitate intestinal bacterial population levels. Translocating bacteria were identified by standard procedures as described previously.[15]

Mucosal Protein

Mucosal protein samples were collected by scraping the mucosa off the ileal and jejunal segments with a glass slide. The mucosal scrapings from each mucosal segment were individually frozen in PBS and stored at −70 C until assayed. After thawing, the mucosal samples were homogenized for 30 seconds at 30,000 rpm. The homogenates were centrifuged, and the supernatants were assayed spectrophotometrically (596 nm) for protein by the Coomassie blue method (Bio Rad Laboratories, Richmond, CA).[16] Bovine plasma gamma-globulin was used as the standard. Mucosal protein content was expressed as mg of protein per cm gut length to correct for differences in gut length between the animals.

Secretory Immunoglobulin A

The concentration of sIgA in the gut washings was measured by enzyme-linked immunosorbent assay. In this assay, the plates were coated with polyclonal goat anti-rat sIgA antibody raised against the secretory piece and the heavy chain components of sIgA (ICN, Eschwege, Germany). The sIgA standard was purified originally from rat bile[4] and was the generous gift of Dr J.C. Alverdy (Michael Reese Hospital, Chicago ILL.). The peroxidase-conjugated, polyclonal second antibody was raised in goats against rat IgA (Fc portion) (Biogenzia-Lemania, GmbH, Bochum, Germany). The enzyme-linked immunosorbent assay was specific for secretory IgA, and it did not react with rat serum IgA. The plates were read in an automatic Titertek Multiscan reader

Table 1. EFFECT OF DIETS ON WEIGHT GAIN AND PROTEIN CONTENT OF THE SMALL INTESTINAL MUCOSA

Group	n	Weight Gain (g)	Mucosal Protein (mg/cm length)	
			Jejunum	Ileum
Chow control	12	43 ± 6*	0.93 ± 0.19*	0.87 ± 0.17*
IV-TPN	12	12 ± 5	0.42 ± 0.14*	0.33 ± 0.18†
ORAL-TPN	12	10 ± 9	0.71 ± 0.21*	0.49 ± 0.15

* $p < 0.01$ *vs.* both other groups.
† $p < 0.05$ *vs.* ORAL TPN and $p < 0.01$ *vs.* control.
All values are expressed as mean ± SD.

(Flow Laboratories Inc., Meckenheim, Germany). The sIgA levels were normalized for the length (μg sIgA/cm gut length) and for the mucosal protein content (μg sIgA/mg mucosal protein) of each intestinal segment.

Soluble and Insoluble Mucin

An indirect enzyme-linked immunosorbent assay was developed following the methods of Mantle and Thakore,[17,18] with a standard mucin purified from rat mucin and an antimucin antibody raised against that standard in New Zealand white rabbits. Briefly, the wells of one microtiter plate were coated overnight at 4 C with 500 ng of purified mucin standard in 50 μL of PBS. In a second plate, 75 μL of the test samples or 75 μL of the mucin standards (0–500 ng) were preincubated with 75 μL antimucin antibody. After the incubation period, the mucin-coated plate was washed with 0.1% Tween in PBS. The wells were blocked with 200 μL of bovine serum albumin at 20 mg/mL in PBS for 1 hour and washed with PBS-Tween.

Fifty microliters of each preincubated sample and standard were transferred to wells of the mucin-coated microtiter plate. After a 1-hour incubation at room temperature, each well was washed with PBS-Tween. Fifty microliters containing 50 ng of horseradish peroxidase-conjugated Protein A in PBS was added to each well and allowed to bind to the antigen-bound antibody for 1 hour at room temperature. Then each well was washed with PBS-Tween to remove all unbound enzyme. The substrate (150 μL of 8.8 mM H_2O_2 and 0.6% ortho-phenylenediamine) then was added to each well. After 30 minutes, the reaction was terminated with 50 μL of 2.5 M H_2SO_4 and the absorbance quantitated at 492 nm.

The mucin levels for experiment one and two were batch run on 2 separate days. They were normalized for the length of the respective intestinal segment and for the mucosal protein content.

Morphologic Structure

The mucosal structure of the terminal ileum and jejunum of three animals from each experiment group was examined by light microscopy. The samples were recovered immediately after death and fixed by luminal perfusion and immersion in 2% paraformaldehyde-2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4 overnight at 4 C. The tissue then was dehydrated to 95% ethanol and embedded in glycol methacrylate (JB-4 Polysciences Inc., Warrington, PA). Semithin (2–4 μm) sections were cut on glass knives and stained with 1% toluidine blue O.

Horseradish Peroxidase Permeability

Permeability of the terminal ileum to macromolecules was measured by the intraluminal injection of horseradish peroxidase (40,000 d) in two additional animals from

Table 2. EFFECT OF DIETS ON BACTERIAL TRANSLOCATION (BT) TO THE MLN AND INTESTINAL BACTERIAL POPULATION LEVELS

Group	n	Incidence of BT	Bacterial Population Levels (Log_{10} CFU/g wet weight)					
			Gram-Negative Enterics			Total Aerobes		
			Jejunum	Ileum	Cecum	Jejunum	Ileum	Cecum
Chow control	12	0%*	4.5 ± 0.7	6.4 ± 0.9	6.3 ± 0.9†	6.0 ± 0.9†	7.3 ± 0.9	7.3 ± 0.6†
IV-TPN	12	58%	3.9 ± 1.6	7.6 ± 1.5‡	8.4 ± 0.6	4.4 ± 1.5	7.8 ± 1.4	8.7 ± 0.4
ORAL-TPN	12	58%	4.3 ± 1.4	6.9 ± 1.0	7.9 ± 0.8	4.5 ± 1.3	7.2 ± 1.0	8.3 ± 0.5

* $p < 0.01$ *vs.* both other groups.
† $p < 0.01$ *vs.* both other groups.
‡ $p < 0.05$ *vs.* chow control.

the chow-fed and each of the TPN diet groups with or without fiber supplementation. The ileal specimens were processed and evaluated by light microscopy as described by Rhodes.[19]

Statistical Analysis

Translocation incidence (discontinuous data) was evaluated by chi-square analysis with the Yates correction. Body weight, log-transformed bacterial counts, and mucosal protein contents were analyzed by analysis of variance with the post hoc Neuman-Keuls test. Secretory immunoglobulin A and mucin data were analyzed by the Mann-Whitney *U* test. Probabilities less than 0.05 were considered significant, except for the sIgA and mucin data of the first experiment, in which a Bonferroni correction was made, so that p values less than 0.016 were considered significant. All data are expressed as mean ± SD unless otherwise specified.

RESULTS

The chow-fed rats gained more weight over the 7-day experimental period than the IV-TPN or ORAL-TPN groups (Table 1). The jejunal and ileal mucosal protein content was much lower in the TPN-fed rats than the chow-fed rats, with the greatest decrease observed in the IV-TPN group (Table 1). The enteral or parenteral administration of TPN promoted bacterial translocation to the MLN and was associated with changes in intestinal bacterial population levels that varied according to the intestinal segment (Table 2). Intestinal bacterial overgrowth was observed in the cecum, whereas the jejunal population levels of the total aerobic bacteria were decreased in the TPN-fed rats.

Luminal sIgA, when expressed as μg/cm gut, was decreased in both the IV-TPN and ORAL-TPN groups but this decrease only reached statistical significance for the jejunum (Table 3). In the case of IV-TPN, the decrease in sIgA was less than the decrease in the mucosal protein content, so that the relative sIgA level, expressed as μg/mg mucosal protein, was increased in the IV-TPN group. Soluble mucin was decreased only slightly and inconsistently in the TPN-diet groups when normalized for gut length (Table 4). When the soluble mucin values were normalized for mucosal protein content, they were increased in the jejunum of the IV-TPN group and in the ilia of both groups fed the TPN solution. Although the absolute amounts of insoluble mucin were lower in the TPN-fed groups, when the values are expressed as μg of mucin per mg of mucosal protein, it appears that the diet-induced decrease in mucosal protein was relatively greater than the decrease in insoluble mucin in the IV-TPN-fed rats (Table 5).

The provision of dietary fiber to the IV-TPN or ORAL-TPN-fed rats did not influence body weight gain

Table 3. SECRETORY IgA LEVELS IN SMALL INTESTINAL WASHINGS

		Secretory IgA			
		Gut Length (μg/cm)		Mucosal Protein (μg/mg)	
Group	n	Jejunum	Ileum	Jejunum	Ileum
Chow control	11	12.8 ± 5.4*	32.4 ± 23.3	13.1 ± 5.3	45.3 ± 36.1
IV-TPN	12	5.3 ± 4.8	23.2 ± 16.2	17.9 ± 28.1	87.3 ± 72.5†
ORAL-TPN	12	3.2 ± 2.2	17.5 ± 9.6	5.2 ± 4.3‡	39.1 ± 25.2

* $p < 0.005$ *vs.* both other groups.
† $p < 0.016$ *vs.* oral TPN.
‡ $p < 0.016$ *vs.* both other groups.

Table 4. SOLUBLE MUCIN LEVELS IN SMALL INTESTINAL WASHINGS

		Soluble Mucin			
		Gut Length (ng/cm)		Mucosal Protein (ng/mg)	
Group	n	Jejunum	Ileum	Jejunum	Ileum
Chow control	11	10.0 ± 8.1	23.3 ± 16.1	1.2 ± 1.1	3.0 ± 2.4†
IV-TPN	12	8.5 ± 4.1	16.4 ± 8.2	2.5 ± 2.1‡	6.6 ± 4.9
ORAL-TPN	12	6.7 ± 3.5	27.9 ± 9.5*	1.0 ± 0.5	6.0 ± 2.9

* $p < 0.005$ *vs.* intravenous TPN.
† $p < 0.016$ *vs.* both other groups.
‡ $p < 0.016$ *vs.* oral TPN and 0.021 *vs.* chow.

Table 5. INSOLUBLE (STORED) MUCIN LEVELS IN HOMOGENATES OF THE SMALL INTESTINAL MUCOSA

		Insoluble Mucin gut length (μg/cm)		Insoluble Mucin mucosal protein (μg/mg)	
Group	n	Jejunum	Ileum	Jejunum	Ileum
Chow	12	1.33 ± 0.34*	1.40 ± 0.72	0.14 ± 0.02	0.19 ± 0.09
IV-TPN	12	0.88 ± 0.29	1.16 ± 0.58	0.25 ± 0.18*	0.45 ± 0.29‡
ORAL-TPN	12	0.86 ± 0.39	1.01 ± 0.38	0.13 ± 0.06	0.24 ± 0.16

* $p < 0.01$ *vs.* both other groups.
† $p < 0.01$ *vs.* chow control.

Table 6. EFFECT OF DIETARY FIBER ON WEIGHT GAIN, MUCOSAL PROTEIN CONTENT AND BACTERIAL TRANSLOCATION

Group	n	Weight Gain (g)	Bacterial Translocation Incidence	Mucosal Protein gut length (mg/cm)	
				Jejunum	Ileum
IV-TPN	10	14 ± 17	80%	0.48 ± .08	0.39 ± .11
IV-TPN + fiber	9	14 ± 10	0%	0.44 ± .12	0.42 ± .07
p value			<0.005		
ORAL-TPN	15	22 ± 13	60%	0.66 ± .16	0.50 ± .09
ORAL-TPN + fiber	15	22 ± 10	13%	0.65 ± .09	0.47 ± .06
p value			<0.05		

or mucosal protein content, although fiber did decrease the incidence of bacterial translocation to the MLN (Table 6). Fiber supplementation statistically reduced the magnitude of IV-TPN and ORAL-TPN-induced cecal gram-negative enteric bacterial overgrowth, but the magnitude of this reduction was only 0.9 and 0.5 $\log_{10}$ colony-forming units, respectively (Fig. 1). The levels of sIgA in the intestinal washings were not different between the fiber and nonfiber-supplemented groups (Table 7). Although the levels of soluble mucin were higher in the second experiment, the addition of fiber was associated with a consistent reduction in jejunal and ileal soluble mucin levels (Table 8). Although the soluble mucin levels were lower in the rats receiving fiber, the insoluble mucin levels tended to be higher (Table 9). However, this difference did not reach statistical significance. (Please note that the soluble mucin levels are expressed in ng, whereas insoluble mucin levels are expressed in μg.)

The morphologic appearance of the intestinal mucosa of both the IV-TPN and ORAL-TPN-fed rats was grossly normal (Fig. 2), although occasionally ileal villi had evidence of limited areas of subepithelial edema or superaged villous tip cells (Fig. 3). Additionally, cell desquamation appeared perturbed in the animals fed fiber-free TPN because cells in the process of exfoliation from the villous tips was observed in these animals, but not in the other groups (Fig. 4). Despite these abnormal extrusion zones, macroscopic gaps in the epithelial lining were quite rare. These minor mucosal abnormalities were not present in the IV-TPN or ORAL-TPN animals receiving supplemental fiber. The relative number and volume of the goblet cells in the ileum and jejunum were similar between the chow-fed groups and all the TPN-fed groups (data not shown). To extend these light microscopic studies, we examined intestinal permeability using horseradish peroxidase as a permeability probe. Ileal mucosal permeability to horseradish peroxidase was increased in the IV-TPN and ORAL-TPN animals (Fig. 5A). In these TPN-fed animals, the peroxidase had penetrated between the epithelial cells, and the basal membranes of the villi were stained intensely. The magnitude of peroxidase staining of the intercellular space and of the lamina propria was significantly improved by fiber

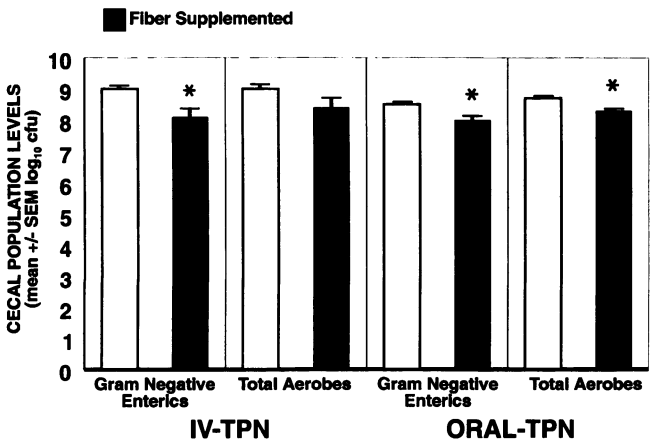

Figure 1. Fiber supplementation was associated with a modest decrease in intestinal bacterial population levels. *p < 0.05

Table 7. SECRETORY IgA LEVELS IN SMALL INTESTINAL WASHINGS

Group	n	Secretory IgA			
		Gut Length (μg/cm)		Mucosal Protein (μg/mg)	
		Jejunum	Ileum	Jejunum	Ileum
IV-TPN	10	6.3 ± 4.9	29.2 ± 13.3	13.9 ± 13.2	82.2 ± 38.4
IV-TPN + fiber	8	4.8 ± 2.7	24.1 ± 14.9	10.0 ± 4.9	59.2 ± 39.3
ORAL-TPN	9	3.8 ± 1.5	14.1 ± 6.0	7.1 ± 6.2	30.0 ± 18.8
ORAL-TPN + fiber	10	3.3 ± 1.2	18.3 ± 14.1	5.0 ± 1.7	39.9 ± 33.3

Table 8. SOLUBLE MUCIN LEVELS IN SMALL INTESTINAL WASHINGS

		Soluble mucin			
		Gut Length (ng/cm)		Mucosal Protein (ng/mg)	
Group	n	Jejunum	Ileum	Jejunum	Ileum
IV-TPN	10	79.0 ± 64.6	93.5 ± 35.4	18.3 ± 17.5	28.2 ± 14.7
IV-TPN + fiber	8	27.8 ± 20.4	58.6 ± 35.7	6.6 ± 4.1	14.0 ± 8.8
p value		<0.05	<0.05	NS	<0.05
ORAL-TPN	9	62.0 ± 34.3	126.6 ± 56.3	9.3 ± 3.9	25.1 ± 9.2
ORAL-TPN + fiber	10	26.5 ± 16.4	78.7 ± 37.5	3.9 ± 2.0	16.5 ± 7.7
p value		<0.01	<0.05	<0.01	<0.05

supplementation of the TPN diets (Fig. 5B), but still was greater than that observed in the chow-fed rats (Fig. 5C).

DISCUSSION

Because many intensive care unit or immunocompromised patients receive TPN and this nutritional regimen is associated with alterations in intestinal structure[20–22] and function,[23,24] we and others have investigated the effects of parenteral nutrition on intestinal barrier function and bacterial translocation.[2–4] These studies have suggested that the ability of the intestinal mucosa to exclude bacteria is compromised in TPN-fed rats and in animals receiving certain enteral diets,[2–8] and that bacterial translocation induced by feeding rats TPN solution intravenously or enterally can be prevented by the administration of bulk-forming fiber.[3,8] Because bulk fiber exerts a direct stimulatory effect on intestinal mucus, epithelial cells, and the gut flora,[11–13] one goal of the current study was to test the hypothesis that diet-induced bacterial translocation is associated with an alteration of intestinal mucin and sIgA content and that fiber might prevent bacterial translocation through its ability to maintain normal intestinal mucin and sIgA levels. The

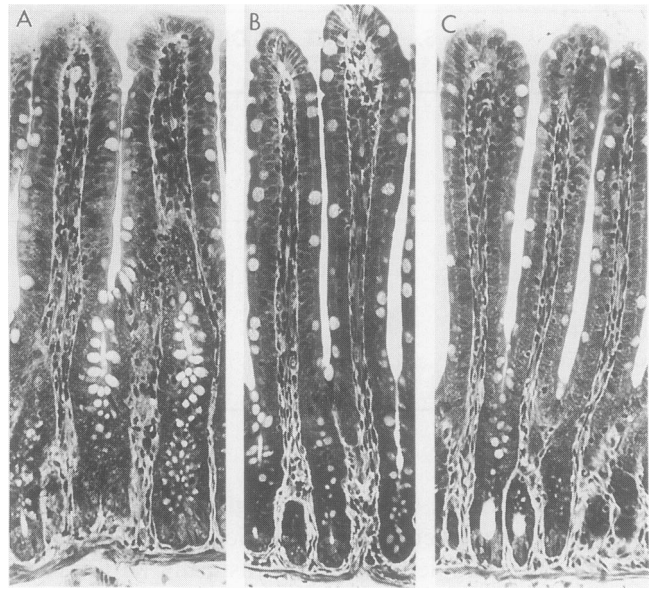

Figure 2. Rat distal ileum comparing the effects of varying diets on mucosa architecture. Stained with toluidine blue. The left panel illustrates the normal architecture after feeding with animal chow.(A) The mucosa from the middle panel is from an animal maintained on IV-TPN; (B) the panel on the right is from an animal fed ORAL-TPN solution. (C) Although mucosal protein was decreased in both of these samples when compared with chow-fed control subjects, the mucosa is of normal height. Despite the apparent normal height of the mucosa, there is an apparent decrease in the number of goblet cells in both the IV-TPN and ORAL-TPN groups. All micrographs × 160.

Table 9. EFFECT OF FIBER ON INSOLUBLE (STORED) MUCIN LEVELS IN HOMOGENATES OF THE GUT MUCOSA

		Insoluble Mucin Gut Length (μg/cm)		Insoluble Mucin Mucosal Protein (μg/mg)	
Group	n	Jejunum	Ileum	Jejunum	Ileum
IV-TPN	7	0.59 ± 0.20	0.63 ± 0.20	122 ± 57	185 ± 81
IV-TNP + fiber	9	0.72 ± 0.51	0.83 ± 0.80	172 ± 118	212 ± 225
ORAL-TPN	8	0.68 ± 0.10	0.76 ± 0.15	111 ± 29	157 ± 35
ORAL-TPN + fiber	12	0.99 ± 0.45	0.78 ± 0.41	152 ± 63	164 ± 82

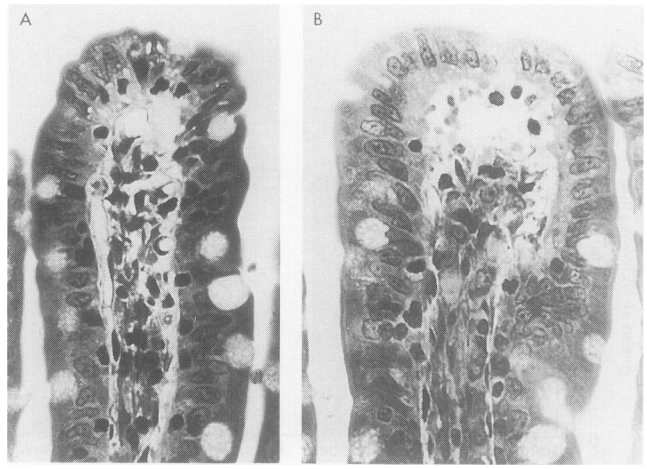

Figure 3. In animals maintained on either IV-TPN (A) or ORAL-TPN (B), there are no macroscopic breaks in the epithelial barrier, although a modest amount of villous tip interstitial edema is apparent. Micrographs × 430.

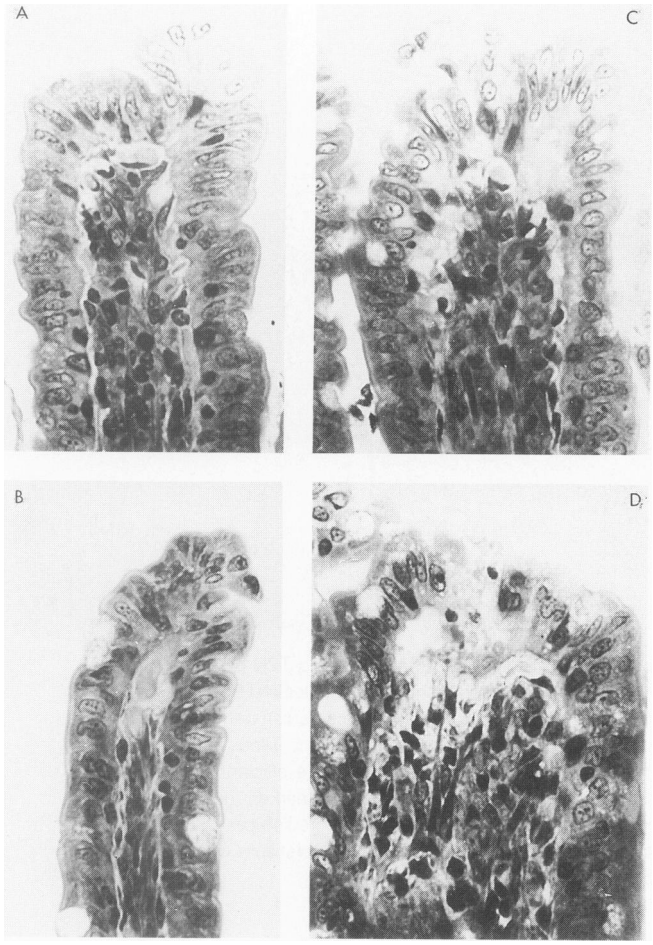

Figure 4. In animals receiving an oral diet without fiber (ORAL-TPN), one apparent consequence is a perturbation of the normal process of cell desquamation. These villi appear to accumulate cells in the process of exfoliation, perhaps because of the lack of normal dietary stimuli from luminal material (A–C); ×420. Despite these unusual extrusion zones, macroscopic gaps indicative of missing cells, and thus, a dysfunction epithelial barrier, are seldom seen (D); ×510.

rationale for investigating intestinal mucus is that under physiologic conditions, the mucus layer slows the penetration of particles[25] and may function as a mechanical barrier that limits microorganisms present in the intestinal lumen from reaching and adhering to gut epithelial surfaces.[25–27] Mucins, which are high-molecular weight glycoproteins, are the primary components of the mucus gel. They account for the viscosity and elasticity of this gel[28] and provide binding sites for bacteria and immunoglobulins, particularly sIgA.[29] Although mucins produced by goblet cells are the major component of the mucus gel, with the exception of one study suggesting that intestinal mucin levels are significantly decreased throughout the small bowel in protein-malnourished rats,[30] little direct information is available on diet-induced alterations of intestinal mucin composition or content. Consequently, we quantitated intestinal mucin content by measuring the soluble fraction of mucins in washings of the intestinal lumen and the insoluble fraction of mucins in homogenates of the mucosa from IV-TPN or ORAL-TPN-fed rats and chow-fed controls. The insoluble fraction is composed of the mucus gel covering the epithelium plus the mucin stored in the goblet cells and comprises 97% to 99% of the total mucin content of the bowel.

In the jejunum of the IV-TPN and ORAL-TPN groups, insoluble mucin levels and sIgA levels were decreased, and the respective ileal values showed a similar tendency. Because the intestinal flora is a major stimulus for sIgA production[31,32] and the intestinal flora was increased in the TPN-fed groups, one would have expected an increase rather than a decrease in luminal sIgA levels. Such an increase was not observed, even when the values were corrected for intestinal atrophy by expressing them as μg sIgA/mg mucosal protein. Because the primary function of sIgA is to bind to luminal bacteria and block their attachment to epithelial cell receptors and thereby prevent mucosal colonization and bacterial translocation,[31] these findings of decreased sIgA and insoluble mucin levels, in combination with increased ileal and cecal gram-negative enteric population levels, are consistent with the hypothesis that the oral or intravenous administration of TPN solution promotes bacterial translocation via an imbalance between host defense factors and bacterial overgrowth.

Our observation that the absolute levels of intestinal sIgA are decreased in both IV-TPN and ORAL-TPN-fed rats differ somewhat from the studies of Lim et al.,[33] who measured biliary sIgA levels in ORAL-TPN and IV-TPN-fed rats. They observed reduced sIgA levels in the bile of the IV-TPN-fed but not the ORAL-TPN-fed animals and concluded that enteral stimulation is important for the production of normal amounts of sIgA. Although bile is a major source of sIgA in the rat, there also is evidence that under some circumstances, such as in self-filling blind loops, that biliary sIgA contributes only marginally to the overall amount of sIgA detectable in the gut.[34] Thus, the fact that we measured luminal intestinal rather than biliary sIgA levels may explain why we observed a decrease in sIgA levels in the ORAL-TPN-fed rats.

To further test the hypothesis that altered levels of intestinal mucin and sIgA contributed to bacterial translocation, the effects of fiber administration on mucin levels, sIgA levels, and bacterial translocation in rats receiving IV-TPN or ORAL-TPN were determined. Fiber supplementation effectively reduced bacterial translocation but was not associated with significant increases in sIgA or insoluble mucin levels. Moreover, surprisingly, soluble mucin levels both in the jejunum and ileum were

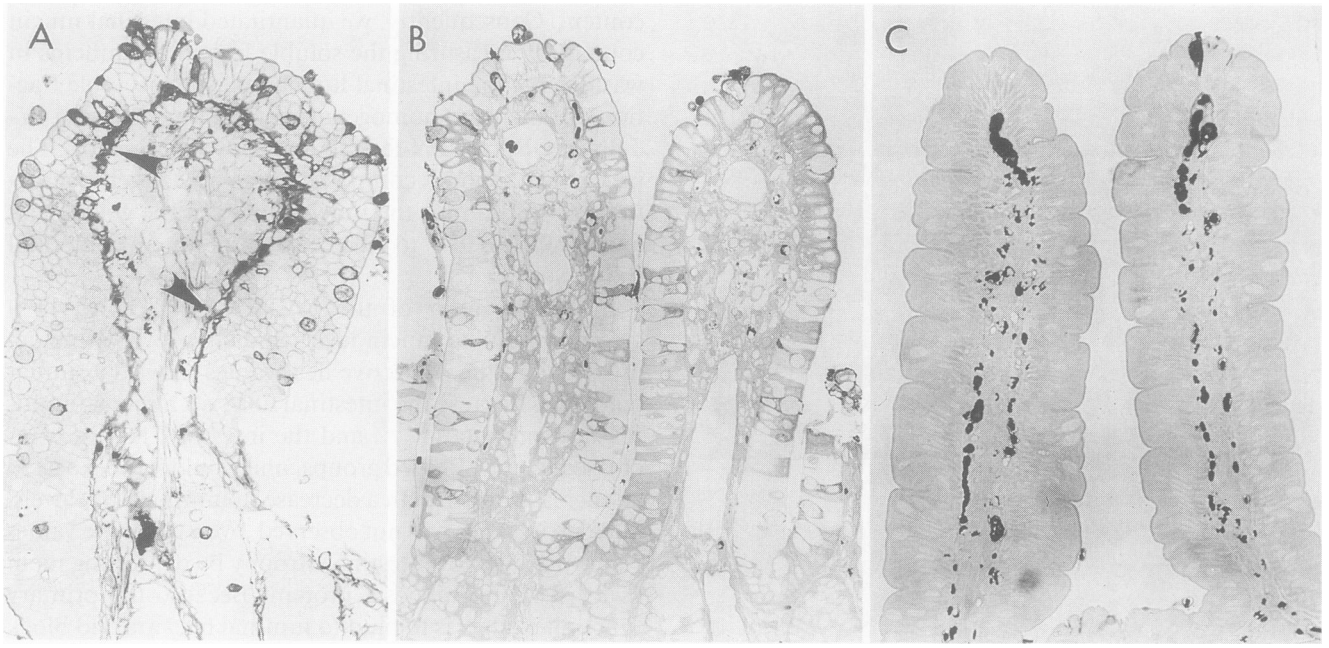

Figure 5. Using horseradish peroxidase as a luminal marker, the epithelium in animals receiving TPN (A) or ORAL-TPN solution (not shown) failed to maintain a permeability barrier. Horseradish peroxidase leaked freely into the interstitium of the villus, passing between the epithelial cells. The accumulation of horseradish peroxidase in the interstitium is extensive throughout the villus (arrows), but more pronounced toward the villous tip. When animals on ORAL-TPN were supplemented with fiber (B), the epithelium was much more efficient at excluding the horseradish peroxidase. Although some cells were outlined by the leakage of the marker, there was no apparent accumulation in the interstitium. In rats maintained on control chow (C), horseradish peroxidase always was excluded by the tight junctions between the epithelial cells. The label in the interstitium was from intravascular red blood cells and granulocytes. All micrographs × 160.

reduced consistently when fiber was added to IV-TPN or ORAL-TPN, independent of whether the soluble mucin levels were normalized for gut length or for mucosal protein content. One potential explanation why the soluble mucin levels were lower in the fiber-fed rats receiving IV-TPN or ORAL-TPN may be that the presence of fiber enhanced the washout of the soluble mucin fraction. Indeed, the soluble mucin levels (expressed as ng/mg mucosal protein) was increased in the jejunum of the IV-TPN and in the ileum of both the IV-TPN and ORAL-TPN groups compared with chow-fed rats. Thus, the fiber content of the chow or the provision of the dietary fiber preparation to the TPN-fed rats may have enhanced the washout of the soluble mucin fraction. This possibility is consistent with experiments using radio-labeled glucose and sulfur showing that the synthesis and turnover of mucin glycoproteins is increased when cellulose fiber is added to a fiber-free diet.[35] Although results from studies of mucin kinetics and our results of altered soluble mucin levels indicate that the dietary fiber content has an important influence on gut mucins, the changes in mucin and sIgA levels do not appear to fully explain the phenomenon of TPN-induced bacterial translocation or the protective effect of fiber.

Because physical disruption or injury of the intestinal mucosa resulting in increased intestinal permeability is one of the major factors associated with bacterial translocation,[1] we investigated the effect of the TPN diets with and without fiber supplementation on intestinal morphology and permeability. The lack of histologic evidence of significant intestinal atrophy of both TPN-fed groups in the current experiments is consistent with our previous studies.[3] This seeming contradiction between our biochemical results documenting that mucosal protein content was decreased while morphologically the mucosal thickness and villous height was normal is best explained by the fact that the circumference of the gut decreases as an adaptation to the parenteral[20] or enteral[36] administration of an elemental diet. Although there was no histologic evidence of gross mucosal injury, the mucosa was not fully normal. Some of the villous tip cells had become rounded, their nuclei were pyknotic, and in some villous tips, there was increased evidence of cell shedding. These observations are consistent with previous studies documenting that intestinal cell shedding is increased in rats receiving parenteral or enteral low residue diets[36] and that the villous tip cells of TPN-fed animals are considerably older than animals fed a regular

diet.[21] Because fiber-free diets reduce the rate of epithelial cell turnover and crypt cell production and prolong crypt-villous transit times,[11,35,38,39] we tested whether tight junction integrity of these aged cells was decreased using horseradish peroxidase (40,000 d) as a permeability probe. Intestinal mucosal permeability to horseradish peroxidase was increased in the IV-TPN and ORAL-TPN animals along the entire length of the villus. The administration of fiber significantly reduced the extent and magnitude of peroxidase staining of the intercellular space and the lamina propria of the TPN-fed rats. These permeability studies indicate that by maintaining a more normal rate of epithelial renewal, fiber may have restored the barrier function of the mucosa.

These studies indicate that the oral or intravenous administration of TPN solution causes profound changes in intestinal composition and function. These changes include decreased mucosal protein and mucin content (insoluble fraction), altered luminal levels of sIgA and mucin (soluble fraction), increased ileal and cecal levels of bacteria, loss of tight junction integrity, and bacterial translocation. The provision of bulk-forming fiber improves intestinal barrier function as measured by peroxidase permeability and bacterial translocation, but does not restore mucosal protein content, mucin, or sIgA levels to normal. Based on these and other experimental studies[3,5,6,8] showing a beneficial effect of bulk-forming fiber on intestinal barrier function, the addition of bulk-forming fiber to fiber-free enteral diets may be clinically beneficial. Further experimental and clinical studies are required to determine the exact mechanisms underlying the protective effect of fiber on intestinal barrier function and its clinical utility.

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