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# Vitamin A deficiency inhibits intestinal adaptation by modulating apoptosis, proliferation, and enterocyte migration

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**Swartz-Basile, Deborah A., Lihua Wang, Yuzhu Tang, Henry A. Pitt, Deborah C. Rubin, and Marc S. Levin.** Vitamin A deficiency inhibits intestinal adaptation by modulating apoptosis, proliferation, and enterocyte migration. *Am J Physiol Gastrointest Liver Physiol* 285: G424–G432, 2003. First published April 23, 2003; 10.1152/ajpgi.00524.2002.—In a prior study, vitamin A-deficient rats subjected to submassive small bowel resections did not mount a normal intestinal adaptive response by 10 days postoperatively, although adaptive increases in crypt cell proliferation were not attenuated and there were no differences in apoptotic indexes. The present study was designed to address the mechanisms by which vitamin A status effects adaptation by analyzing proliferation, apoptosis, and enterocyte migration in the early postoperative period (16 and 48 h) in vitamin A-sufficient, -deficient, and partially replenished sham-resected and resected rats. At 16 h postresection, apoptosis was significantly greater in the remnant ileum of resected vitamin A-deficient rats compared with the sufficient controls. Crypt cell proliferation was increased by resection in all dietary groups at both timepoints. However, at 48 h postresection, proliferation was significantly decreased in the vitamin A-deficient and partially replenished rats. By 48 h after resection, vitamin A deficiency also reduced enterocyte migration rates by 44%. This occurred in conjunction with decreased immunoreactive collagen IV at 48 h and 10 days postoperation. Laminin expression was also reduced by deficiency at 10 days postresection, whereas fibronectin and pancadherin were unchanged at 48 h and 10 days. These studies indicate that vitamin A deficiency inhibits intestinal adaptation following partial small bowel resection by reducing crypt cell proliferation, by enhancing early crypt cell apoptosis, and by markedly reducing enterocyte migration rates, which may be related to changes in the expression of collagen IV and other extracellular matrix components.

short bowel syndrome; small intestinal resection; retinoids; rats; extracellular matrix

SHORT BOWEL SYNDROME CAN RESULT from small intestinal disorders such as Crohn's disease, celiac sprue, intestinal ischemia, small bowel volvulus, and neonatal enterocolitis. This syndrome is characterized by diarrhea, weight loss, dehydration, and malabsorption resulting in malnutrition. The small intestine has the ability to adapt and thereby compensate for the loss of functional surface area. However, because this process is limited, many patients with short bowel syndrome require parenteral nutrition with its associated morbidity and costs.

Rodents subjected to partial small bowel resections have been used as experimental models to address the mechanisms responsible for intestinal adaptation. The adapting intestines in these models are characterized by increased villus heights and crypt depths occurring in conjunction with enhanced crypt cell proliferation and enterocyte differentiation, as well as changes in enterocyte migration and programmed cell death (11, 16, 21, 26). Examples of nutritional and nonnutritional factors that have been proposed as putative regulators of intestinal adaptation include glucagon-like peptide 2, glutamine, epidermal growth factor, insulin-like growth factor I, and short-chain fatty acids (reviewed in Refs. 2 and 6). In addition, our studies support a physiological role for retinoids in the adaptive response (25, 29). Administration of retinoic acid stimulated crypt cell hyperplasia very early following resection in the adapting gut (29), and vitamin A deficiency blunted the later (i.e., 10 days postoperatively) morphological changes that are phenotypic of the adaptive response (25).

To investigate the mechanistic basis by which vitamin A exerts its proadaptive effects, we used the rat resection model to study the impact of vitamin A deficiency on the early adaptive response (i.e., 16–48 h). These studies show that enhanced crypt cell apoptosis and decreased enterocyte migration rate may be responsible for the diminished adaptive response seen in vitamin A-deficient rats.

## MATERIAL AND METHODS

*Preparation of retinoids.* For oral dosing, all-*trans* retinoic acid (50 μg all-*trans* retinoic acid in 0.025 ml cottonseed oil) and retinyl palmitate (150 μg retinyl palmitate in 0.030 ml cottonseed oil) from Sigma (St. Louis, MO) were prepared as described previously (25).

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*Diets, animals, and experimental design.* The vitamin A-deficient basal diet was purchased from Dyets (Bethlehem, PA) and was formulated according to the AIN-93G rodent diet recommendations (19). The composition of the diet was specifically modified by using cottonseed instead of soybean oil, vitamin-free casein and by the omission of vitamin A from the vitamin mix. The vitamin A-sufficient diet was prepared by supplementing the basal diet with vitamin A palmitate (1.2 retinol equivalents/g diet) (25).

Sprague-Dawley dams with 10-day-old male rat pups were obtained from Sasco (Omaha, NE). The dams were provided a vitamin A-deficient diet and water ad libitum and maintained on a 12:12-h light-dark cycle. At 19 days of age, male weanlings were randomly divided into two experimental groups and given free access to pelletized vitamin A-deficient or -sufficient diets. Rats were housed individually in stainless steel wire-bottomed cages and allowed free access to food and water. Food consumption was measured every 2 days, and body weight was measured once a week. Rats were observed daily for clinical evidence of vitamin A deficiency (18, 24). As individual rats became vitamin A deficient, retinoic acid was administered daily (50 μg/day) on the back of the tongue. Rats in the other experimental groups received the vehicle control (cottonseed oil). After simultaneous discontinuation of retinoic acid to synchronize the experimental group, all the deficient rats reached a second growth plateau in 4 days. Within each dietary group, rats assigned to the two surgical groups were paired based on body weight. Seventy percent of small bowel resections or sham transections (control) were performed when serum retinol levels of those on the deficient diet were ≤0.1 μM (see below). One cohort of deficient rats was maintained on the deficient diet postoperatively. Another was treated 20 h preoperation with oral retinyl palmitate (150 μg) to replenish hepatic vitamin A stores and was then placed on the control diet postoperatively. Retinyl palmitate was used because it is readily hydrolyzed in the gut, leading to efficient absorption of retinol and restoration of hepatic vitamin A stores (1, 25). The rats were replenished before surgery rather than after surgery to minimize dosage effects resulting from differences in absorptive efficiency following small bowel resection. Rats were killed 16 (5–6 rats per surgical group) and 48 h (9–10 rats per surgical group) after surgery. Intestinal segments were removed from each region of the gut for analyses. Vitamin A status was confirmed using high-performance liquid chromatography to analyze extracts of serum and liver as previously described (25). All animal experimentation was conducted in conformity with the *Guiding Principles for Research Involving Animals* and was approved by the Animal Studies Committees of Washington University and the Medical College of Wisconsin.

*Surgical procedure and tissue procurement.* Before all surgical procedures, rats were food deprived overnight and allowed free access to water. Rats were anesthetized with pentobarbital sodium (40 mg/kg ip), atropine (0.4 mg/kg ip), and inhalational methoxyflurane. Seventy percent of the resections were performed by removing the small bowel from 5 cm distal to the ligament of Treitz to 15 cm proximal to the ileocecal valve as previously described (5, 21, 25). The small intestine was reanastomosed end to end using 6-0 silk interrupted sutures. In sham-resected animals, small bowel transection and reanastomosis were performed 5 cm distal to the ligament of Treitz. During abdominal wall closure, all rats received gentamicin (4 mg in 6 ml normal saline) intraperitoneally and were then allowed free access to 50 g/l sucrose in water for the first 24 h. All rats were weighed and then killed by pentobarbital sodium overdose (150 mg/kg ip) at 16 and 48 h postoperatively. The small intestine of each rat was harvested, and the remnant ileum was used for analyses. Tissues used for light microscopy and immunohistochemistry were fixed by immersion in Bouin's solution.

*Crypt cell proliferation.* Crypt cell proliferation was assessed using 5-bromodeoxyuridine (5-BrdU) incorporation to identify the number of S-phase cells per crypt. Rats were injected intraperitoneally with 120 mg/kg 5-BrdU (8 g/l 5-BrdU and 0.8 g/l 5-fluorodeoxyuridine) 90 min before being killed. 5-BrdU was detected with a monoclonal anti-BrdU antibody and a streptavidin-biotin-staining system (Zymed Laboratories, San Francisco, CA). The number of labeled cells in 5–10 well-oriented, longitudinal crypts per ileal section was determined by using light microscopy and was reported as the percentage of 5-BrdU-labeled cells per total number of cells in the crypt.

*Analysis of apoptosis.* The identification and quantification of apoptotic cells were performed using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assays (In situ Cell Death Detection Kit, Roche Diagnostics, Brussels, Belgium) and morphological assessment. Terminal deoxynucleotide transferase was used to label the 3′-hydroxyl ends of apoptosis-induced DNA strand breaks with fluorescein-labeled dUTP. The addition of the secondary antifluorescein-peroxidase conjugate and further development with diaminobenzidine allowed for histochemical identification of apoptotic cells by light microscopy. Sections treated

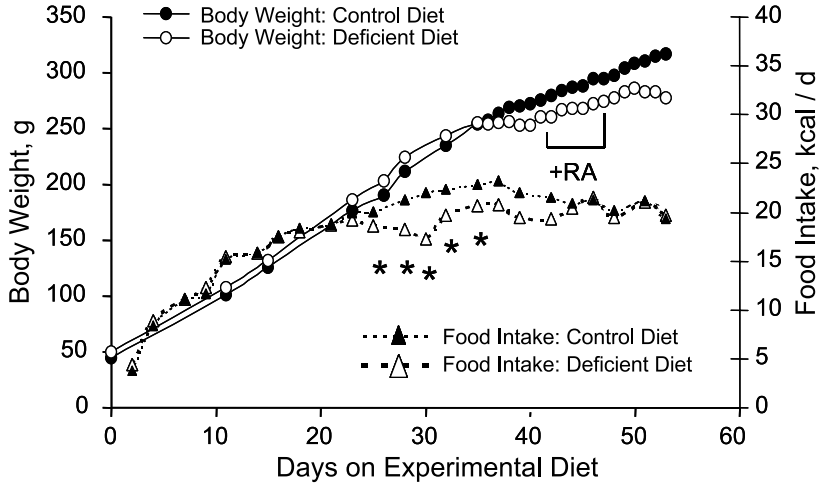


Fig. 1. Body weight and food intake on vitamin A-deficient and control diets. Rats fed the deficient or control diet were weighed, and food intake was measured at regular intervals. As rats became vitamin A deficient, food intake decreased and then body weight plateaued. The growth curves (solid lines) shown are from a representative pair of rats demonstrating the weight plateau occurring on the deficient diet (*~day 38*) and the resumption of growth following administration of retinoic acid (RA; 50 μg/day per oral). RA was removed from all of the rats 6 days before surgery. As illustrated, at the time of surgery, the growth of the deficient rats had again plateaued, indicating that they were clinically deficient. The food intake curves (broken lines) represent the mean food intake for all of the rats in each dietary group (control diet, $n = 20$; deficient diet $n = 40$). Thus the effects of RA administration and subsequent withdrawal on food intake for individual rats are not illustrated. Significant differences in mean food intake are indicated ($^*P < 0.05$).

Table 1. *Vitamin A levels*

| | Vitamin A Sufficient | | Deficient Replenished | | Vitamin A Deficient | |
|---|---|---|---|---|---|---|
| | Sham | Resected | Sham | Resected | Sham | Resected |
| 16 H Postoperation | | | | | | |
| Serum retinol, μmol/l | 0.83 ± 0.08 | 0.83 ± 0.08 | 0.39 ± 0.04 | 0.40 ± 0.12 | ND | ND |
| Serum, %sufficient sham | 100.0 | 100.0 | 47.0 | 48.2 | <0.2 | <0.2 |
| Liver, %sufficient sham | 100.0 | 122.8 | <0.2 | <0.2 | <0.2 | <0.2 |
| 48 H Postoperation | | | | | | |
| Serum retinol, μmol/l | 1.16 ± 0.16 | 0.82 ± 0.09 | 0.69 ± 0.09 | 0.47 ± 0.10 | ND | ND |
| Serum, %control sham | 100.0 | 69.0 | 59.5 | 40.5 | <0.2 | <0.2 |
| Liver, %control | 100.0 | 111.4 | 17.3 | 27.4 | 1.3 | 0.2 |

Values are means ± SE for serum retinol. Serum and liver samples were obtained when the rats were harvested at 16 or 48 h postoperation and analyzed by HPLC as described in MATERIAL AND METHODS. Levels for serum retinol and for liver retinol plus retinyl esters are also presented as percentages of the vitamin A-sufficient sham-resected control rats. ND, not detected

with DNase I served as a positive control, and sections incubated without terminal transferase served as a negative control. The apoptotic index is the percentage of positively stained cells per 500 crypt cells. Apoptotic cells were also identified in hematoxylin-stained sections based on the presence of nuclear condensation and cell shrinkage.

*Enterocyte migration rate.* Enterocyte migration rates were evaluated at 48 h postresection. Briefly, five rats from each group received an intraperitoneal injection of 5-BrdU at either 90 min or 24 h before death. Ileal segments (~1 cm) were harvested at 8 cm proximal to the ileal-cecal junction and processed for immunoperoxidase staining to detect BrdU-labeled cells as described above. Because no changes in cell size occurred with changes in vitamin A status or with partial resection, the distance from the base of the crypt to the foremost-labeled cell at 1.5 and 24 h after injection was measured and used to estimate the enterocyte migration rate (change in μm/22.5 h).

*Immunohistochemical detection of laminin, collagen IV, fibronectin, and cadherin.* Paraffin-embedded tissue sections were incubated with rabbit anti-laminin (1:200; Sigma), goat anti-collagen IV (1:40; Chemicon, Temecula, CA), rabbit anti-fibronectin (1:200; Sigma), or rabbit anti-pan-cadherin (1:40; Sigma) for 1 h. For laminin, goat anti-rabbit immunogold-labeled IgG was added (1:40 dilution; Amersham Biosciences, Piscataway, NJ) followed by silver enhancement. For fibronectin and cadherin, goat anti-rabbit biotinylated IgG (1:2,000; NEN Life Science, Boston, MA) and streptavidin-horseradish peroxidase (1:1,000; DakoCytomation, Golstrup, Denmark) were then added for 30 min. Tyramide enhancement (NEN Life Science Products) was added for 10 min followed by streptavidin-horseradish peroxidase (1:1,000) for an additional 30 min. Diaminobenzidine (Sigma) was used for visualization. All slides were rinsed between steps with phosphate-buffered saline for 3 min. For collagen IV, anti-goat rhodamine red (1:200; Jackson Immuno Research Laboratories, West Grove, PA) was added for 30 min. The slides were mounted with Fluorescent Mounting Media (DakoCytomation) and viewed under fluorescence.

For collagen IV semiquantitation of immunohistochemical sections, three fields (×50) for each of three rats per group were photographed and analyzed using Scion Image Software (Scion Image beta 4.02, Scion, Frederick, MD).

*Statistical analysis.* Statistical analyses were performed using SigmaStat (Version 2.03, SPSS, Chicago, IL). Data are presented as means ± SE. Two-way ANOVA was performed to examine the effects of surgical resection and vitamin A status. Significant differences between groups were determined using Student-Newman-Keuls or Tukey's posttest, with $P \leq 0.05$ employed as nominal criterion of statistical significance.

## RESULTS

*Evidence of vitamin A deficiency.* By *days 25-30*, rats fed the vitamin A-deficient diet had significantly decreased their food intake (~90% of sufficient rats) and by *day 35,* displayed other signs of vitamin A deficiency including plateaued growth curves (Fig. 1), periocular porphyrin deposits and ruffled fur. As expected, vitamin A-deficient rats resumed growing after retinoic acid was administered. When retinoic acid was withdrawn, the growth rates decreased and plateaued within 4 days for all the deficient rats, thereby indicating successful synchronization of this group. Seventy percent of small bowel resections were performed at this time. At the time of surgery, the average body weights of the deficient and replenished rats were 80

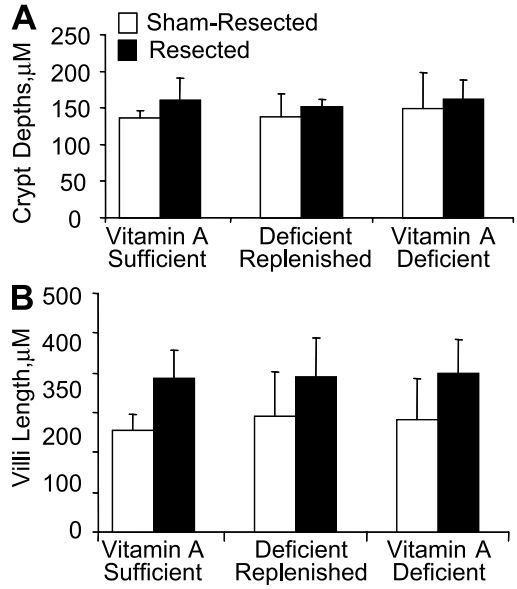


Fig. 2. Effects of altering vitamin A status on crypt depth and villus height 48 h postoperation. As described in MATERIAL AND METHODS, crypt depths (*A*) and villus heights (*B*) were measured 48 h after rats were subjected to 70% partial small intestinal resections or sham resections ($n$ = 3 for each group).

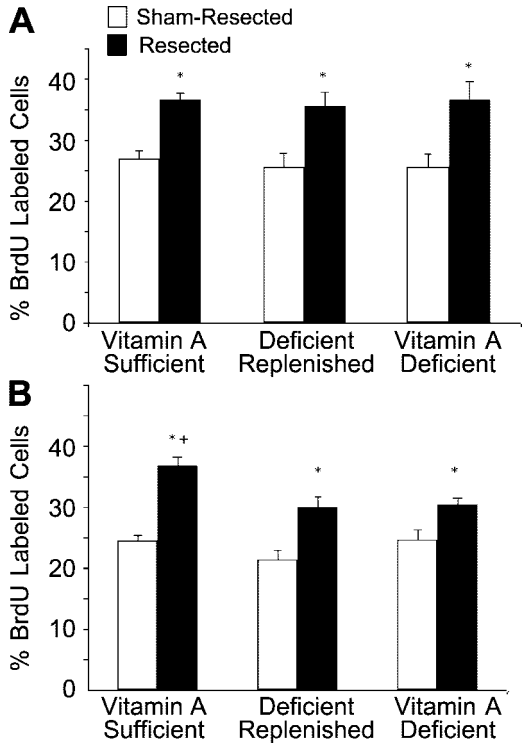


Fig. 3. Effect of altering vitamin A status on 5-bromodeoxyuridine (5-BrdU) incorporation at 16 (*A*) or 48 h (*B*) postresection. As described in MATERIAL AND METHODS, 5-BrdU incorporation into DNA was used as a marker of crypt cell proliferation. Vitamin A-sufficient, -replenished, and -deficient rats were subjected to sham resection or 70% small bowel resections and were injected with 5-BrdU 90 min before being killed. 5-BrdU was detected with a monoclonal anti-BrdU antibody and streptavidin-biotin amplification. The number of cells labeled with a monoclonal anti-BrdU antibody was determined in 5–10 well-oriented, longitudinal crypts per ileal section using light microscopy. Data are presented as the mean percentages (± SE) of 5-BrdU-labeled cells per total number of cells in the crypt for rats killed at 16 ($n$ = 5–6 rats per group) or 48 h ($n$ = 9–10 rats per group) postoperation. *$P < 0.05$ resected vs. sham-resected rats within dietary groups; +$P < 0.05$ resected rats compared across dietary groups.

and 90% of the control rats, respectively. Vitamin A status was confirmed by analysis of serum retinol and hepatic retinyl esters and retinol. Serum retinol and hepatic retinyl palmitate were undetectable in the vitamin A-deficient rats, whereas serum vitamin A levels were restored to ~50% of control levels in the replenished groups, whereas hepatic levels were still undetectable at 16 h and were <25% of control levels at 48 h postresection (Table 1).

*Morphology.* As expected at 48 h postoperation, there were no significant changes in crypt depth in the remnant intestine compared with the sham-resected controls (Fig. 2*A*). There were also no differences related to vitamin A status. By 48 h, although villus heights tended to increase to comparable levels in all of the resected groups compared with the sham controls, there were no significant changes identified by pairwise comparisons with Tukey's posttest analyses (Fig. 2*B*; for surgical effect, $P < 0.02$, for vitamin A status and the interaction between surgical and vitamin A status, $P$ was not significant).

*Cellular proliferation.* To assess the effects of vitamin A status on crypt cell proliferation, the incorporation of 5-BrdU into DNA was examined at 16 and 48 h postresection. At both time points, cell proliferation was significantly increased in resected rats compared with sham-operated controls regardless of the dietary treatment (Fig. 3; by 2-way ANOVA, $P < 0.0001$ for surgical effect at both time points). However, vitamin A status had an independent effect on cell proliferation at 48 h but not at 16 h ($P < 0.01$ for vitamin A status at 48 h, no significant interactive effect at either time point). At 48 h postresection, BrdU incorporation was significantly reduced in resected vitamin A-deficient and replenished rats compared with resected vitamin A-sufficient controls (i.e., BrdU incorporation was 20% higher in resected sufficient controls compared with vitamin A-deficient or -replenished rats; $P < 0.05$).

*Cellular apoptosis.* At 16 h postresection, crypt cell apoptosis significantly increased (~2-fold) in the remnant ileum of resected rats compared with sham controls, regardless of dietary treatment (Fig. 4*A*). However, resected vitamin A-deficient rats had a significantly greater apoptotic index compared with either the replenished or the vitamin A-sufficient resected groups (by 2-way ANOVA, $P < 0.002$ for effects of

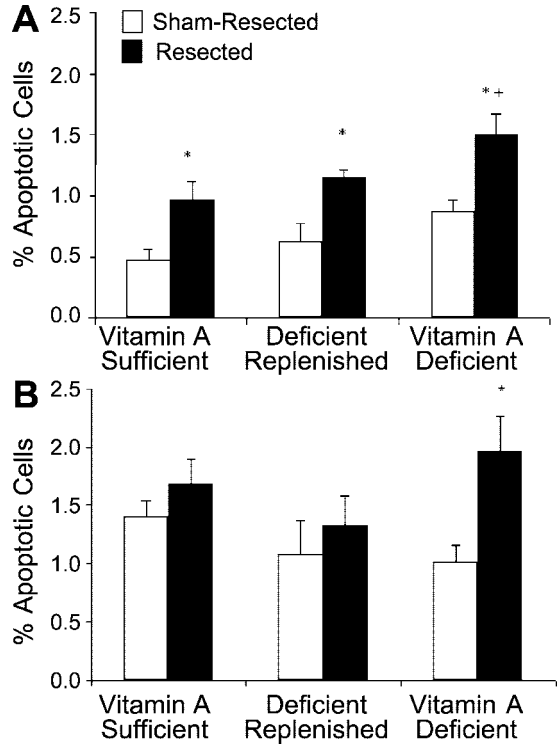


Fig. 4. Effect of altering vitamin A status on crypt cell apoptosis at 16 (*A*) and 48 h (*B*) postresection. The identification and quantification of apoptotic cells were performed using terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assays and morphological assessment (see MATERIAL AND METHODS). The percentages of apoptotic cells (±SE) are presented for each of the surgical and dietary groups at 16 ($n$ = 5–6 rats per group) and 48 h ($n$ = 9–10 rats per group) postoperation. *$P < 0.05$ resected vs. sham-resected rats within dietary groups; +$P < 0.05$ resected rats compared across dietary groups.

vitamin A status and of surgery with no significant interaction). By 48 h postoperation, two-way ANOVA indicated that surgery ($P = 0.02$) but not vitamin A status had a significant independent effect on apoptosis and there was no significant interaction between surgery and vitamin A status (Fig. 4*B*). However, post hoc analysis demonstrated that the apoptotic index was only significantly increased by resection in the vitamin A-deficient group.

*Enterocyte migration rate.* The pathogenesis of the reduced villus heights and crypt depths observed with vitamin A deficiency was further explored by analysis of enterocyte migration rates (Fig. 5). Both the absolute distances traveled by the foremost-labeled cells at 24 h postresection and the rates of enterocyte migration were significantly decreased in the remnant ileums of the vitamin A-deficient (179.3 ± 10.0 μm, 4 μm/h) and partially replenished rats (199.6 ± 9.0 μm, 4.2 μm/h) compared with the vitamin A-sufficient rats (256.6 ± 24.1 μm, 7.2 μm/h). There were no significant differences between the vitamin A-replenished and -deficient groups.

*Collagen IV, laminin, fibronectin, and cadherin.* Enterocyte migration from crypt to villus tip depends on epithelial-basement membrane/extracellular matrix interactions. To begin analyzing how vitamin A deficiency affects enterocyte migration, the expression of basement membrane components at 48 h and 10 days postoperation were assessed by immunohistochemical techniques. The 10 day-old tissues were obtained from rats reported in Ref. 25. At 48 h postoperation, collagen IV expression was altered by vitamin A deficiency (Fig. 6). As noted in Fig. 6, *A–B*, collagen IV is normally expressed in the mucosal and villus core lamina propria. In vitamin A-deficient gut (Fig. 6, *D–E*), collagen IV expression is patchy or absent in the villus core. This effect persisted at 10 days postoperation (compare Fig. 6*C* with *F*). However, regardless of dietary group, collagen IV expression appears more abundant in the villus core lamina propria in resected compared with sham-resected ileum (compare Fig. 6*A* with *B* and *D* with *E*). These observations were confirmed by semiquantitative analyses, which demonstrated that vitamin A deficiency reduced collagen IV levels by 40–60% (see Fig. 6*G*; by 2-way ANOVA, $P < 0.05$ for surgical and vitamin A status effects and not significant for their interaction). In contrast, the vitamin A status at 48 h postoperation had no effect on laminin (Fig. 7), fibronectin (Fig. 8), or cadherin (Fig. 8), although laminin expression was decreased by vitamin A deficiency at 10 days postresection (Fig. 7, *C* and *F*).

## DISCUSSION

We previously showed (25) that vitamin A deficiency blunts the adaptive response without modifying crypt

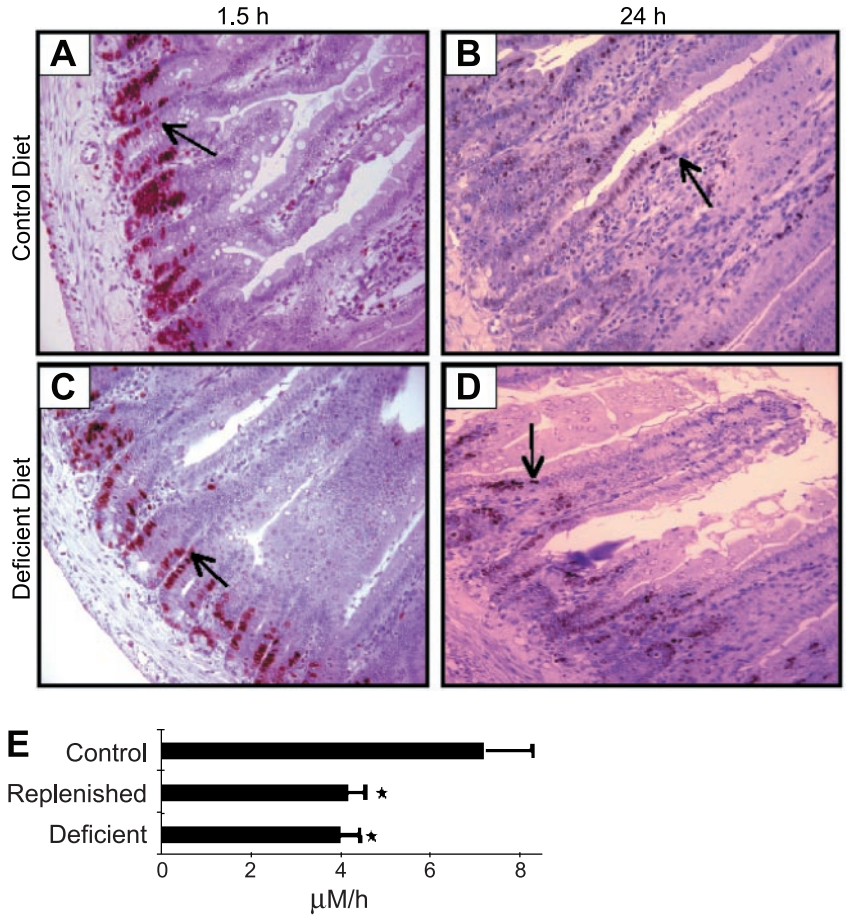


Fig. 5. Effects of altering vitamin A status on enterocyte migration following 70% partial small bowel resection. Rats maintained on the vitamin A-sufficient (control) diet (*A* and *B*) or the vitamin A-deficient diet (*C* and *D*) were subjected to intestinal resection and killed at 48 h postoperation. At either 1.5 or 24 h before death, rats ($n = 5$ for each injection timepoint) were injected with 5-BrdU (see MATERIAL AND METHODS and Fig. 1). The distance between the foremost-labeled ileal cells at 1.5 (*A* and *C*) and 24 h (*B* and *D*) were measured and used to determine the migration rate. Representative sections from rats injected at each time point are presented (magnification ×200). Arrows indicate the front of labeled cells used for calculating the enterocyte migration rate. The data expressed as micrometers per hour (means ± SE) are summarized in the bar graph (*E*). *$P < 0.05$ vs. control.

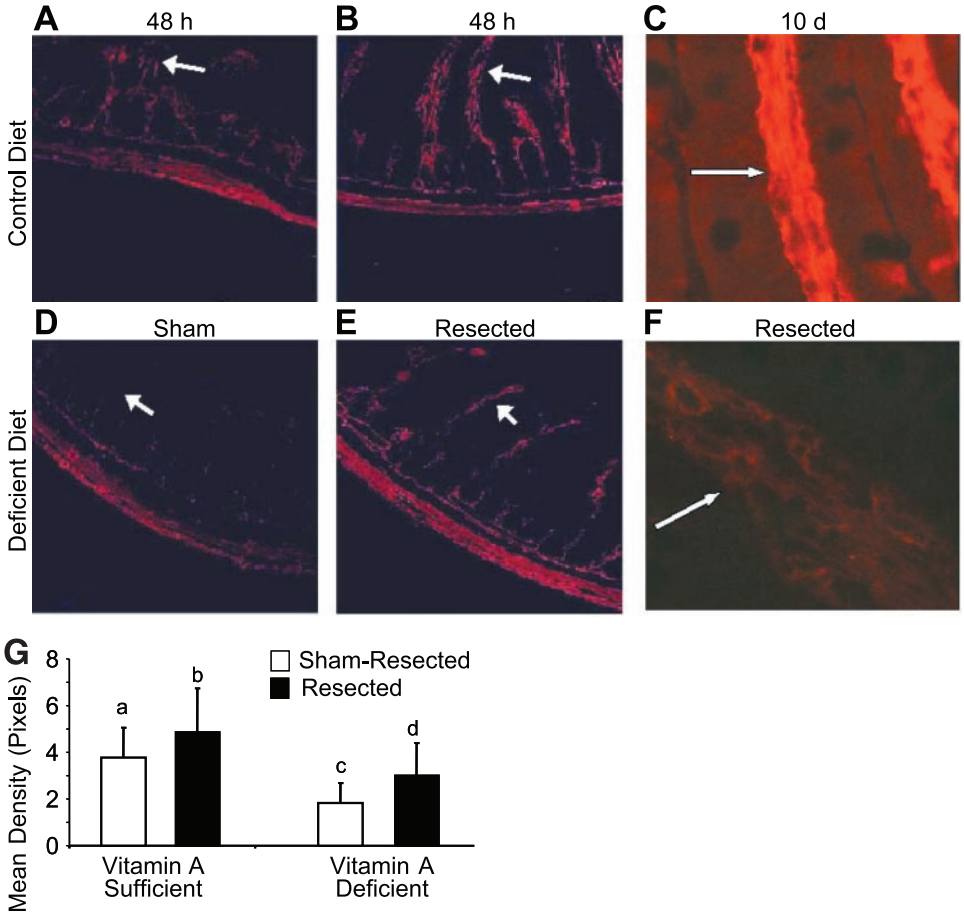


Fig. 6. Effects of intestinal resection and altering vitamin A status on immunoreactive collagen IV. Representative photomicrographs of ileal cross sections obtained at 48 h or 10 days after sham operation (*A* and *D*) or partial small bowel resection (*B*, *C*, *E*, *F*). The sections were incubated with goat anti-collagen IV and then anti-goat rhodamine and viewed under fluorescence as described in MATERIAL AND METHODS. Arrows indicate the villus core lamina propria. Magnifications for *A*, *B*, *D*, and *E* are ×50 and for *C* and *F*, ×400. *G*: results of semiquantitative analysis of sections from at least 3 fields from each of 3 rats per group. Significant differences ($P < 0.05$) were obtained for the main effects of vitamin A status and for surgery as indicated by the letters. There was no significant interaction between surgery and vitamin A status.

cell proliferation or apoptosis when studied at 10 days postresection. This was surprising, because the determinants of villus height and crypt depth in the adapting remnant clearly include compensatory changes in the rates of these processes as well as epithelial cell migration (16; also Y. H. Tang, unpublished data). The growth-promoting intestinal effects of endogenous or exogenous vitamin A or the inhibitory effects of vitamin A deficiency are likely to result from changes in each of these basic processes. Thus the goal of this study was to determine whether changes in cell proliferation, apoptosis, and/or enterocyte migration occur-

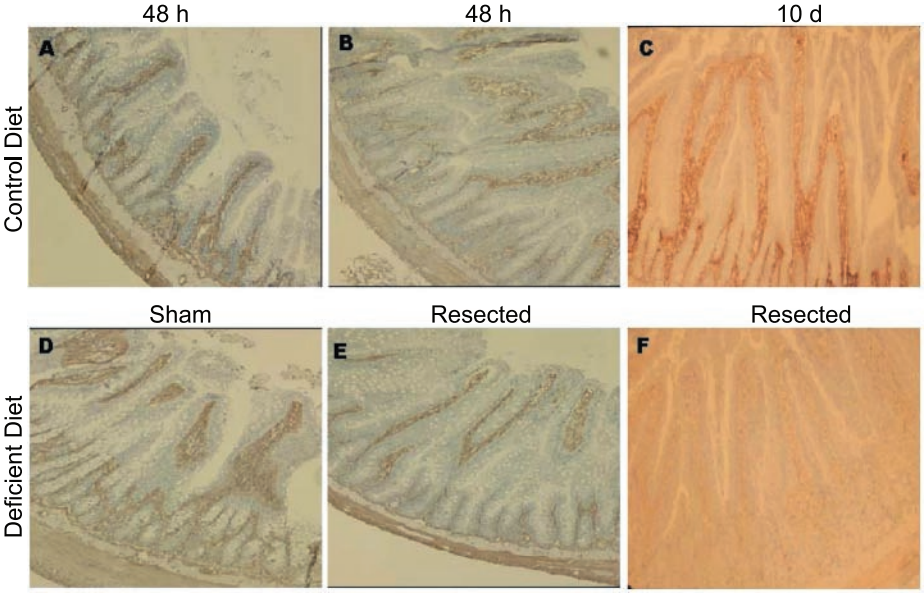


Fig. 7. Effects of intestinal resection and altering vitamin A status on immunoreactive laminin. Representative postoperative ileal cross sections obtained at 48 h or 10 days after sham operation (*A*, *D*) or partial small bowel resection (*B*, *C*, *E*, *F*) were incubated with rabbit anti-laminin, then goat anti-rabbit immunogold-labeled IgG followed by silver enhancement as described in MATERIAL AND METHODS. Magnification for all sections ×50.

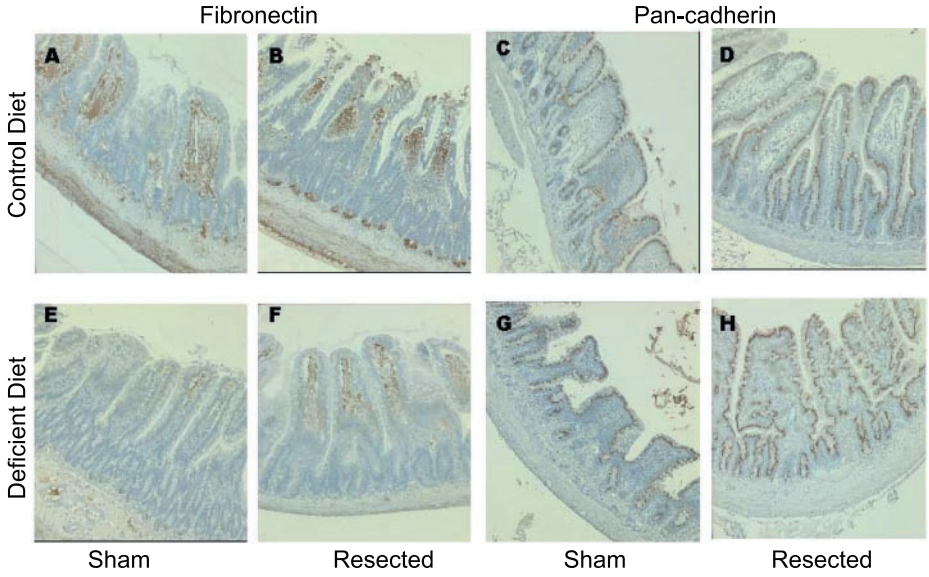


Fig. 8. Effects of intestinal resection and altering vitamin A status on fibronectin and cadherin expression. As indicated, representative ileal cross sections from rats on the control or deficient diets obtained at 48 h after sham operation or partial small bowel resection were incubated with rabbit anti-fibronectin or anti-pancadherin, then goat anti-rabbit biotinylated IgG and streptavidin-horseradish peroxidase, which were followed by tyramide enhancement and diaminobenzidine as described in MATERIAL AND METHODS. Magnification for all sections ×50.

ring in the early postoperative period could account for the failure of the vitamin A-deficient intestine to adapt. Rats were studied in the first 48 h of the adaptive response because of evidence that vitamin A could modulate early adaptation by enhancing crypt cell proliferation as early as 6 h postresection (29) and evidence that the expression of genes that are regulated by vitamin A and/or involved in its metabolism is increased within 48 h postresection (5).

As expected, at both 16 and 48 h postresection, crypt cell proliferation in the ileal remnant was increased in resected compared with sham control rats. However, at 48 h postresection, BrdU incorporation was significantly decreased in vitamin A-deficient compared with -sufficient resected rats. Partial restoration of serum retinol levels (i.e., the deficient replenished rats) did not increase postresection proliferative levels to control levels by 48 h postoperation. These data, in conjunction with the demonstrated ability of retinoic acid to stimulate crypt cell proliferation at 6 h postresection (29), are consistent with an active role for vitamin A in the initiation stages of the adaptive response. Vitamin A deficiency did not inhibit proliferation when assessed at 16 h postresection or at 10 days postresection (25). These differences between the prokinetic effects of administered vitamin A (29) at 6 h, the effects of vitamin A deficiency at 48 h, and the failure of vitamin A depletion to inhibit crypt cell proliferation at 16 h or 10 days postoperation suggest that there are temporal changes in the mechanisms by which retinoids and vitamin A deficiency impact on adaptation. These data also suggest that the effects of exogenous retinoic acid may be distinct from those of endogenous retinoids.

Increased rates of enterocyte proliferation are characteristic of the adaptive response following small bowel resection and are important in the generation of increased absorptive surface area. Once the morphological adaptive response is maximized, the new homeostatic state must be maintained by balancing rates of enterocyte proliferation and loss. Whereas the exact mechanism of enterocyte loss is not known, apoptosis is increased in murine small intestinal crypts and villi following small bowel resection (11, 15, 26; also Y. H. Tang, unpublished data). These data suggest that programmed cell death plays a key role in the adaptive response. Thus crypt cell apoptosis was examined to assess its role in mediating the effects of vitamin A deficiency on intestinal adaptation. At 48 h postresection, crypt cell apoptosis was significantly increased by resection compared with sham resection in the vitamin A-deficient rats but not the sufficient or replenished resected rats. At 16 h, apoptosis was increased significantly in resected rats compared with sham controls regardless of dietary treatment. However, compared with the vitamin A-sufficient or replenished resected groups, the number of apoptotic bodies was significantly greater in the vitamin A-deficient resected rats. Thus, during early adaptation, depletion of vitamin A stores was associated with augmented cell loss via apoptosis. A similar increase in apoptosis occurred in the livers of vitamin A-deficient rats following partial hepatectomy (7). In that model of compensatory hyperplasia, the number of apoptotic bodies in the liver of vitamin A-deficient rats was increased fourfold by 30 min postoperation and was maximal at 8 h after partial hepatectomy. Replenishment with retinyl palmitate 24 or 48 h before partial hepatectomy reduced the number of apoptotic cells to the level observed in control vitamin A-supplemented rats. Thus it was concluded that vitamin A was important for the survival of hepatocytes after partial hepatectomy as well as in intact vitamin A-deficient rats (7).

Because the differences in crypt cell proliferation and apoptosis in the remnant ileum of vitamin A-deficient rats were modest compared with vitamin A-sufficient controls, changes in the rate of enterocyte migration out of the crypt and up the villus were sought as an additional mechanism to account for the inhibition of adaptation that occurred when vitamin A stores were depleted. Vitamin A deficiency reduced epithelial

cell migration rates to 44% of vitamin A-sufficient resected rats. These data are consistent with observations implying that vitamin A deficiency impaired the migration of thymidine-labeled cells out of the jejunal crypts of unoperated rats (31). Thus inhibition of enterocyte migration in vitamin A deficiency may well account for the conspicuous villus blunting observed by 10 days postresection (25). Changes in enterocyte migration could be mediated by modifications in epithelial basement membrane proteins and/or the associated cell surface adhesion molecules. For example, depletion of villus E-cadherin in mice increased migration rates up the villus by disrupting cell-cell and cell-substratum contacts (12), and forced overexpression of E-cadherin inhibited cell migration (13). In both experimental models, changes in E-cadherin levels were associated with concordant changes in α- and β-catenin levels. Although, these studies indicate that cadherins and their associated proteins may be important regulators of enterocyte migration, we did not observe immunohistochemical changes in cadherin levels resulting from intestinal resection or changes in vitamin A status.

Evidence suggesting that modulating vitamin A levels could alter the extracellular matrix, and therefore enterocyte migration, include cell culture data indicating that retinoids influence cell adhesion to basement membrane proteins and the synthesis of extracellular matrix proteins including laminin, fibronectin, and collagen IV (20). Retinoids also increase β-catenin protein levels in SKBR3 breast cancer cells (4) and activate the E-cadherin/catenin complex in human MCF-7 breast cancer cells (28). In vivo studies have demonstrated that administration of retinoic acid to adult rats upregulates β-1-integrin in aortic vascular smooth muscle cells (17). In addition, retinoids suppress the synthesis of matrix metalloproteinases that mediate the degradation of the extracellular matrix (22). Experimentally induced vitamin A deficiency has been associated with changes in the expression of extracellular matrix components. For example, vitamin A-deficient rats have increased levels of liver fibronectin mRNA (14), reduced collagen in the adventitia of small-caliber pulmonary arteries and arterioles and in the alveolar septa (3) and lung parenchyma (27) as well as in bone (8). In the eye, deficiency is associated with reduced basement membrane and corneal laminin (23) and delayed corneal epithelial migration attributed to the absence of fibronectin (9, 30). These data suggest that retinoids may also regulate cell adhesion and the synthesis of extracellular matrix proteins in the gut and thus influence epithelial cell migration and differentiation.

To address the putative relationship between the inhibition of enterocyte migration by vitamin A deficiency and changes in the extracellular matrix, we analyzed the expression of laminin, collagen IV, fibronectin, and cadherins postresection. In models of epithelial restitution following injury, a process that also involves migration of enterocytes, collagen IV and laminin have been identified as critical extracellular matrix components. For example, treatment with collagen IV-blocking antibodies profoundly inhibited migration of rat intestinal IEC-6 cells in a restitution model using wounded monolayers (10). As shown in Fig. 6, at 48 h postresection, collagen IV was increased in the remnant ileum of resected rats compared with equivalent segments from sham-resected rats. Vitamin A deficiency decreased collagen IV in both sham and resected rats without completely eradicating the resection-associated increase. At 48 h, there were no apparent differences in laminin, cadherin, or fibronectin staining attributable to changes in vitamin A status in resected rats. At 10 days postresection, vitamin A-deficient rats continued to exhibit decreased collagen IV levels and had reduced laminin levels, whereas fibronectin and cadherin levels remained unchanged (Figs. 6–8). This demonstration that vitamin A deficiency does modify the extracellular matrix in the remnant small intestine after partial resection is compatible with the hypothesis that such changes contribute to the observed inhibition of enterocyte migration and the adaptive response.

Vitamin A replenishment before surgery was able to partially reverse the effects of deficiency on apoptosis but did not restore crypt cell proliferation, enterocyte migration, or collagen IV expression within the first 48 h postoperation. Because serum retinol levels were only restored to 50% of control values, these data indicate that marginal vitamin A levels are sufficient to inhibit apoptosis but not to stimulate proliferation or to restore the extracellular matrix and thus enterocyte migration.

In conclusion, these studies demonstrate that vitamin A deficiency inhibits intestinal adaptation following partial small bowel resection by enhancing early crypt cell apoptosis, reducing crypt cell proliferation, and markedly reducing enterocyte migration. These data provide further evidence supporting a pivotal role for vitamin A in intestinal adaptation and underscore the importance of elucidating the mechanistic basis for vitamin A effects in the adapting and normal small intestine.

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## DISCLOSURES

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