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Absorption of iron in rats with experimental enteritis*

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Abstract

Inflammatory conditions of the gastrointestinal tract and iron-deficiency anemia are very common in humans. Acute intestinal inflammation was pathologically established in rats by intraluminal administration of acetic acid into the duodenum and the proximal jejunum. The study included two control groups of intact (untreated) rats and sham-operated (saline-treated) rats for each intestinal segment. A third group of rats received acetic acid. The acetic acid-induced inflammatory process was established histopathologically and biochemically. Two days after treatment, iron absorption was measured using ligated 10-cm loops of proximal jejunum or ligated duodenum in which ^{59}Fe was injected intraluminally ($n = 6$ in each group). In another four control groups (intact and sham-operated for each intestinal segment) and two acetic acid-treated groups, serosal-luminal secretion of ^{59}Fe was measured after intravenous injection ($n = 5$ in each group). ^{59}Fe transfer from the lumens of the duodenum and jejunum to the portal system was significantly lower in those rats in whom inflammation was induced by acetic acid. There was no apparent serosal-luminal secretion of intravenously injected ^{59}Fe in any of the studied groups. We conclude that acetic acid-induced intestinal inflammation significantly reduces iron absorption by the duodenum and the proximal jejunum.

Introduction

An estimated 30% of the world's population are anemic, with the highest prevalence in developing countries. Of all the causes of anemia, iron deficiency, which affects between 0.5 and 0.6 billion people, is by far the most frequent (Baynes & Bothwell 1990). Iron deficiency develops when the amounts of iron absorbed from the diet by the gastrointestinal tract are insufficient to meet normal or increased requirements.

The net iron influx depends not only on the nutritional iron and its bioavailability, but also on the anatomical and functional integrity of the iron-absorption mechanisms and, to a lesser extent, on iron excretion. Since excretion through saliva, sweat, urine, bile, and pancreatic and intestinal secretions is minimal, the small intestine remains the major regulatory organ of iron balance (Lerner & Iancu 1988). Iron malabsorption has been reported in malabsorptive states such as celiac disease, Crohn's disease, giardiasis, cow's milk intolerance, and post-infectious diarrhea (de Visia *et al*. 1992).

Although a variety of experimental approaches and designs have been applied to studying iron absorption in animals (Christophersen & Balcerzak 1976; Raja *et al*. 1987; Johnson & Jacobs 1990), iron-absorption studies in an animal model with gastrointestinal inflammatory process are lacking. The aim of the present study was to compare, quantitatively, the uptake of Fe^{3+} by the duodenum and proximal jejunum and to examine specifically the effect of the inflammatory process on iron absorption using tied-off segments of rat intestine.

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Materials and methods

Animals, diets and reagents

Male Sprague-Dawley rats (Technion Faculty of Medicine, Haifa, Israel) weighing 200 ± 25 g were maintained on tap water and a commercial non-purified rat diet (Koffolk Ltd., Tel Aviv, Israel), which were freely available. Care and treatment of experimental animals received prior institutional approval and followed recommended guidelines (National Research Council 1985). All chemicals were at least reagent grade. Glacial acetic acid was obtained from Frutarom Laboratory Chemicals (Haifa, Israel). $^{59}FeCl_3$ was purchased from Amersham International (Amersham, Bucks, UK).

Model for intestinal inflammation

Rats were anesthetized by intraperitoneal injection of ketamine HCl (75 mg kg^{-1} body weight; Rhône Merieu, Lyon, France) and acepromazine maleate (0.75 mg kg^{-1} body weight; C-VET Ltd., Bury St Edmunds, UK). Following laparotomy, the duodenum or the proximal jejunum was flushed with 3 ml of warm 154 mM NaCl (saline) and 3 ml of air. The air was manually expressed. Two milliliters of saline or 2 ml of 0.833 mM (5%, v/v) acetic acid (pH 2.58) were injected into the flushed intestinal segment. This method of inducing acute small-bowel injury was developed by our group (Naveh *et al*. 1993) and was adapted from Fedorak *et al*. (1990) and Miller *et al*. (1991). The abdominal muscles and the skin were sutured. This surgical procedure produced a transient depression in food intake in all treatment groups that lasted 24 h.

Pathology

Four animals each from the control group, the sham-operated (saline-treated) group, and the acetic acid-treated group in both experiments were treated as mentioned above and killed after 2 days to obtain intestinal samples in order to establish pathologically and biochemically the degree of inflammation induced with this new experimental model. Individual specimens were placed in 100 g l^{-1} buffered formalin overnight, washed, dehydrated, cleared, and embedded in paraffin. The tissues were then sectioned at a thickness of 5 μm and stained with hematoxylin and eosin for light microscopy.

Histomorphometry

Histomorphometric determinations were performed using an Olympus Cue 2 image analysis system with appropriate morphometry software (Olympus Corp., Lake Success, NY, USA). The system consists of a Zeiss Universal R photomicroscope (10× objective) fitted with a Panasonic WV-CD50 camera with the video image seen on a Sony 14″ color monitor and an IBM-compatible PC. Measurements were made in standardized ‘windows’ (fields) with an area of 271,538 μm^2.

Determination of myeloperoxidase activity

Duodenal or jejunal mucosal scrapings (200 mg) removed by means of glass slides were homogenized three times for 30 s at 4 °C with a Polytron (Kinematica GmbH, Kriens-Luzern, Switzerland) in 1.0 ml ice-cold 5 g l^{-1} hexadecyltrimethylammonium bromide in 50 mM phosphate buffer, pH 6.0. The Polytron probe was rinsed twice with 1.0 ml of the buffer, and the washings were added to the homogenate. The homogenate was then sonicated for 30 s, freeze-thawed three times, and centrifuged for 15 min at 40,000 *g* (Rachmilewitz *et al*. 1997). An aliquot of the supernatant was taken for determination of the enzyme activity, according to Bradley *et al*. (1982).

Ligated loop procedure

Two days after treatment, fed rats were anesthetized as above and iron absorption was measured as described previously (Hempe & Cousins 1989, Naveh *et al*. 1993). Briefly, loose ligatures were placed 1 cm distal to the pylorus and 1 cm proximal to the Treitz ligament, and 10 cm apart at the proximal jejunum. The proximal ligature of the jejunum was placed 1 cm distal to the Treitz ligament. Care was taken to keep the blood supply intact without restriction of blood flow. The intestinal segment to be used was flushed with warm saline and air, and the loose ligatures were secured after manual expression of the intestinal contents. ^{59}Fe (18.5 kBq) in 0.5 ml of a solution of 0.11 mM $FeSO_4 \cdot 7H_2O$ and 4 mM of ascorbic acid (pH 5.0) was injected into the isolated intestinal segment. The intestine was returned to the abdominal cavity. At 30 min following introduction of ^{59}Fe, the intestinal segments were rapidly excised and the contents collected in a tube, the lumen was flushed with 3 ml of saline (which was also collected), followed by 3 ml of air, and finally weighed.

Mucosal uptake (disappearance from the lumen), mucosal retention (presence in intestinal segment tissue) and transfer (mucosal uptake – mucosal retention) of ^{59}Fe were calculated as previously described (Hempe & Cousins 1989, Naveh *et al*. 1993). The percentage of ^{59}Fe transferred to the carcass was calculated as follows: (transfer/mucosal uptake) $\times 100$. ^{59}Fe in the intestinal preparations was determined with a gamma spectrometer (Compu Gamma LKB/Wallac, Turku, Finland).

Design of absorption studies

Ligated loops from which ^{59}Fe absorption was measured were as follows: experiment 1: a 10-cm segment of proximal jejunum (1 cm distal to the Treitz ligament); experiment 2: the whole duodenum except for 1 cm distal to the pylorus and 1 cm proximal to the Treitz ligament. Each experiment consisted of three comparison groups: two control groups and one experimental group ($n = 6$ per group). In the first control group, rats were not subjected to treatment of the intestine; in the second control group, saline was injected into the segment 2 days before absorption studies. In the third group, 2 ml of 0.833 mM acetic acid was injected into the segment 2 days before absorption studies as described above.

Serosal-luminal secretion of iron

Secretion of iron was examined in two intact, sham-operated, and acetic acid-treated groups to detect possible secretion of iron from the inflamed mucosa into the lumen ($n = 5$). Two days following operation and treatment, the animals were anesthetized, the appropriate intestinal segment was flushed, and 0.5 ml of a solution of 0.11 mM $FeSO_4{\cdot}7_2O$ and 4 mM ascorbic acid (pH 5.0) was injected into the isolated duodenum or a 10-cm segment of proximal jejunum as described above. Then 37 kBq of ^{59}Fe in 0.5 ml of a solution of 0.11 mM $FeSO_4{\cdot}7H_2O$ and 4 mM ascorbic acid was injected into the ventral tail vein. After 30 min, the isolated intestinal segment was excised, and the radioactivity of the luminal fluid and the intestinal segment was measured as described above.

Statistical analysis

The data were analyzed statistically by a non-parametric one-way analysis of variance (Kruskal-Wallis test), which examines whether several independent samples come from the same distribution, and Mann-Whitney non-parametric alternative *t* test based on ranks.

Results

Histopathological examination of the acetic acid-treated group compared with sham (saline-treated) controls revealed marked inflammatory responses developing in the small intestine (Figure 1). Both the duodenum and jejunum were very severely affected by the acetic acid treatment, with the jejunum showing a much greater pathological response. In both the treated duodenum and jejunum the apical portions of the villi were grossly expanded and showed acute inflammation (Figure 1B). The mucosa was found to be edematous, with major infiltration and accumulation of inflammatory cells (polymorphonuclear leukocytes or granulocytes) that occupied much of the lamina propria. In addition, the blood vessels of the lamina propria were greatly dilated and local hemorrhages were found, indicating a pre-ulcerous state or early ulceration. In the case of the jejunum, the mucosal epithelial cells were atrophied, squamous, or detached (Figure 1B). The epithelial erosion resulted in exposed areas of the lamina propria.

Histomorphometric determinations showed that the lamina propria of villi of both the jejunum and duodenum were greatly enlarged in comparison with the sham (saline-treated) controls (Figure 2). The mean area ($\pm$ SEM) of the lamina propria in the acetic acid-treated jejunum increased to $61,121 \pm 4,268$ μm^2 vs. $30,458 \pm 9,144$ μm^2 in the sham (saline-treated) controls. A similar increase in the area of the lamina propria in the duodenum was also induced by acetic acid treatment [$68,148 \pm 7,459$ μm^2 vs. $32,133 \pm 1,652$ μm^2 in the sham (saline-treated) controls].

In rats treated with 0.833 mM acetic acid, myeloperoxidase activity in jejunal and duodenal mucosa measured 48 h after administration was significantly increased ($P < 0.0001$) in comparison with both control groups (Figure 3).

Data from ^{59}Fe absorption measurements for two experiments are summarized in Table 1. Quantitation of the data with reference to 1 g of tissue manifested significant differences between the mucosal uptake and transfer of the sham and acetic acid-treated groups in both experiments. The reduction transfer from the proximal jejunum and duodenum was 58% and 53%, respectively. The proportion of iron that was transferred to the carcass was also reduced when the sham groups were compared with the acetic acid-treated groups in both experiments ($P < 0.05$).

Negligible amounts of ^{59}Fe with no statistically significant differences were detected in the luminal

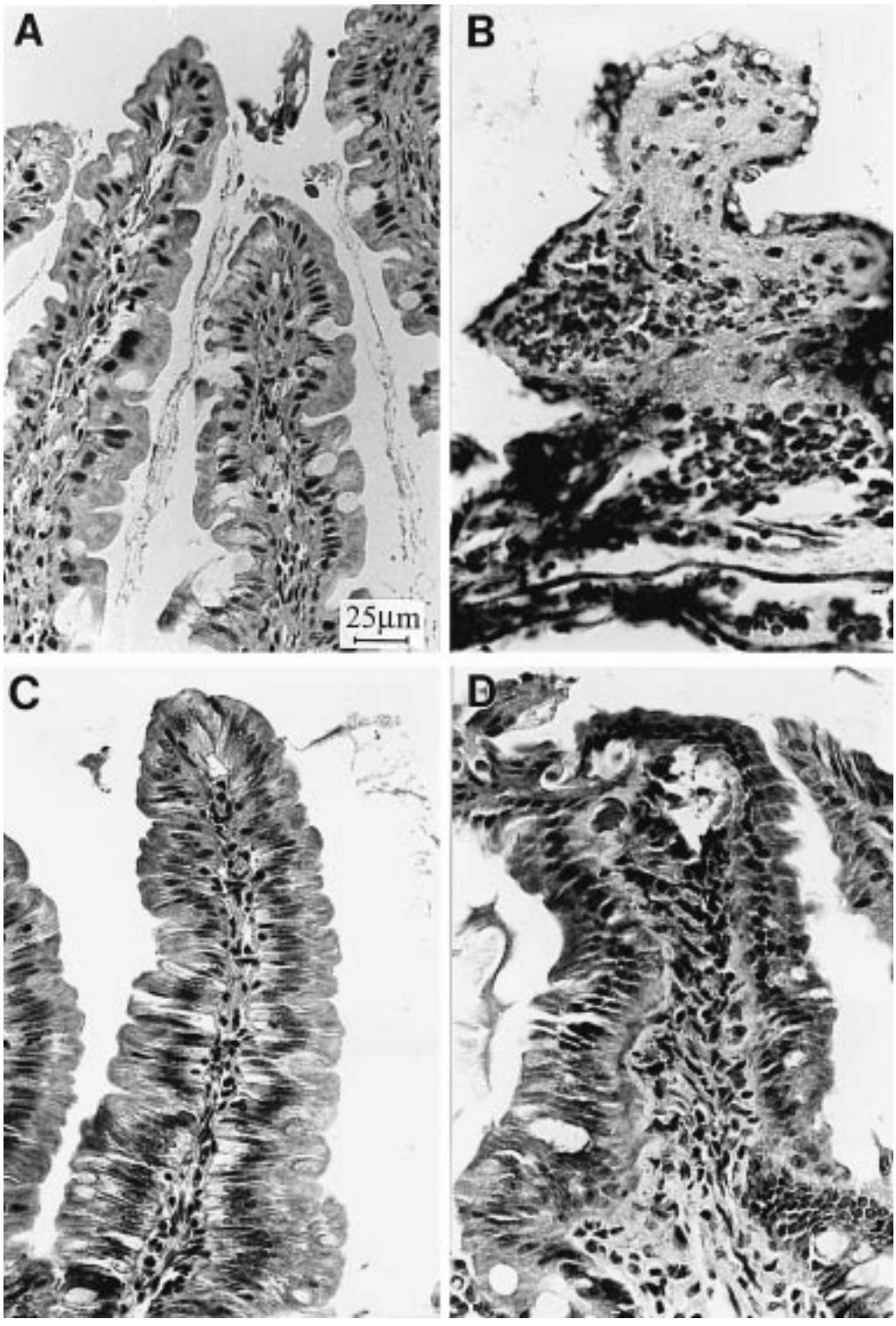

Fig. 1. Histological changes in typical villi of jejunum and duodenum after experimentally induced enteritis. (A) Jejunal mucosa from sham (saline-treated) rat. (B) Jejunal mucosa after acetic acid-induced enteritis, showing major histopathological changes. The epithelial cells are squamous or atrophied, and the lamina propria is dilated with signs of acute inflammation. (C) Duodenal mucosa from sham (saline-treated) rat. (D) Duodenal mucosa after acetic acid treatment. Whereas there is also an inflammatory response, the pathology is less marked than that of the jejunum. Wax sections (5 μm), hematoxylin and eosin staining.

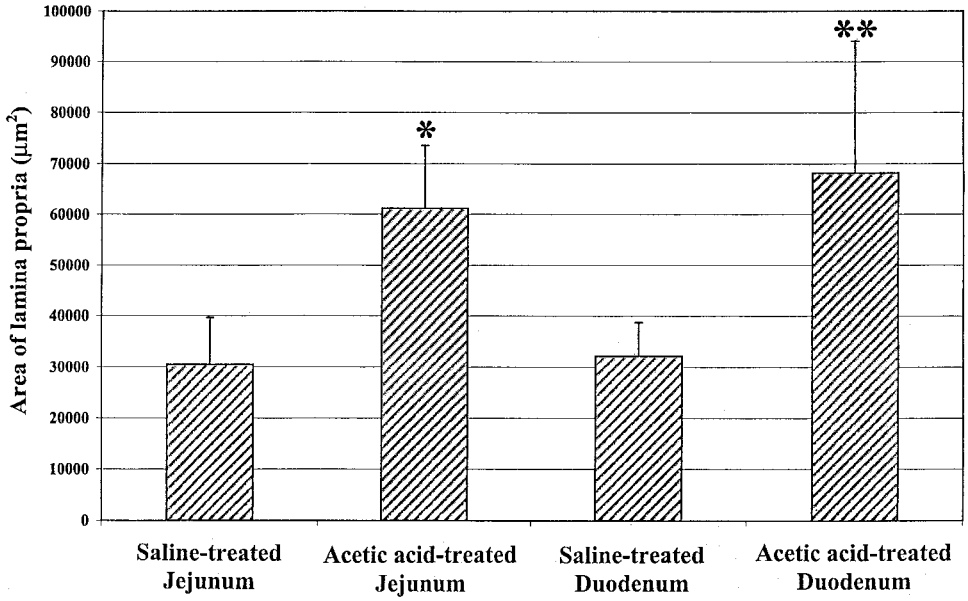

Fig. 2. Histomorphometry of area of lamina propria of jejunal and duodenal mucosa in acetic acid-induced enteritis. $^{*}P < 0.003$, $^{**}P < 0.001$ compared with sham (saline-treated) rats.

fluid and the intestinal mucosa following intravenous injection of ^{59}Fe to the control, sham-operated, and acetic acid-treated groups. Thus, mucosal secretion of ^{59}Fe into the lumen could not have altered the absorption values reported in Table 1.

Discussion

Individual variability in iron absorption is large, even in normal, healthy subjects (Magnusson *et al.* 1981). Despite much research over the past 50 years, the precise details of intestinal iron absorption remain unclear. Most nutritional iron is absorbed in the duodenum and proximal jejunum because of the apparently abundant iron receptors on the proximal rather than distal intestinal absorptive mucosal cells (Huebers *et al.* 1971). In a physiological setting, iron absorption involves the digestion of food with interactions between free iron and food components and the uptake of various forms of iron by the upper intestinal mucosa (‘mucosal uptake’), mucosal processing of iron, and finally the movement of iron from the mucosal cell to the body interior (‘transfer’). Iron uptake in the mucosa is more rapid than its release. Thus, the transfer of iron from the enterocyte to the portal system is the rate-limiting step in the iron-absorption process (Huebers 1986).

The results of our experimental enteritis model show significant histopathological changes in both the jejunal and duodenal mucosa. Further confirmation of the inflammatory processes is provided by the increased levels of myeloperoxidase activities. Our animal model was first developed to examine zinc absorption in rats with acetic acid-induced enteritis and colitis (Naveh *et al.* 1993). Histological evaluation of the small intestine and colon revealed that the acetic acid treatment initiated an inflammatory response. The inflammation caused a reduction in zinc absorption in comparison to the corresponding control groups, whereas, using the same measures of absorption as were utilized for zinc, we could not detect a significant difference in the absorption of [^{3}H]leucine between the sham-operated group and the acetic acid-treated group.

In the present research study, when the data were quantitated with reference to unit weights, a reduction of 53% and 58% in ^{59}Fe transfer was noted in the duodenum and proximal jejunum, respectively, of the acetic acid-treated groups in comparison to the sham groups. These results are comparable to those of de Vizia *et al.* (1992), who evaluated iron absorption 1–3 months after metronidazole therapy in children affected by giardiasis and 2–6 months after initiation of gluten-free diet in patients with celiac disease and showed a striking change from malabsorption of iron

Table 1. Mucosal uptake, retention, and transfer of ^{59}Fe by ligated segments of rat intestine.

Treatment group	Mucosal uptake (% of dose g^{-1} tissue)	Mucosal retention (% of dose g^{-1} tissue)	Mucosal transfer (% of dose g^{-1} tissue)	Mucosal transfer (% of mucosal uptake)
Experiment 1 (proximal jejunum)				
Control	98.21 ± 9.88*	30.87 ± 7.02	67.33 ± 7.04	69.18 ± 5.50*
Sham	81.82 ± 8.59*	27.06 ± 7.18	54.76 ± 7.93†	68.02 ± 5.64*
Acetic acid-treated	53.98 ± 7.28	31.04 ± 7.72	22.94 ± 1.00	46.14 ± 7.33
Experiment 2 (duodenum)				
Control	133.77 ± 12.67†	6.96 ± 1.52	126.80 ± 13.40†	94.42 ± 1.34†
Sham	101.15 ± 9.87†	9.99 ± 1.73	91.16 ± 10.44†	89.62 ± 2.21*
Acetic acid-treated	57.01 ± 4.62	14.11 ± 3.15	42.90 ± 3.18	75.96 ± 3.99

Results are presented as means ± SEM ($n = 6$ for each treatment group). Values of the acetic acid-treated groups were compared with those of the unoperated control groups and the sham (saline-treated) groups. Within an experiment, values with an asterisk or dagger are significantly different from values for the acetic acid-treated group: $^{*}P < 0.05$, $\dagger P < 0.01$.

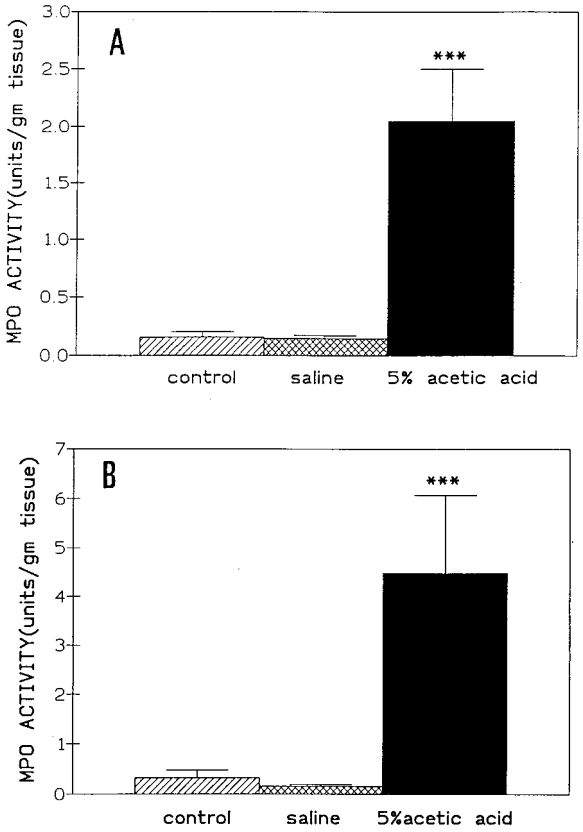

Fig. 3. Myeloperoxidase (MPO) activity in proximal jejunum (A) and duodenal mucosa (B) of the control (intact), sham (saline-treated), and experimental (acetic acid-treated) groups measured 48 h after operation. Results are means ± SD ($n = 4$). $^{***}P < 0.001$, experimental vs. both control groups.

to nearly normal absorption of iron. The increment of serum iron after an iron load, once the intestinal mucosal damage was presumably repaired, was almost three times that observed during the initial evaluation.

The damage caused by acetic acid to the intestinal mucosa resulted in reduction of mucosal uptake of ^{59}Fe scoring to 34% in the proximal jejunum and 43% in the duodenum in comparison with the sham groups. Brush-border protein with transport properties and fatty acids responsible for the translocation of iron from the intestinal lumen to cytoplasm (Skikne & Baynes 1994; Lombard *et al.* 1997) may have been injured following the acetic acid treatment of the intestine.

The increased retention of iron in the jejunal loops as compared with the duodenal loops may have been due to the fact that the surface area (and hence the paracellular routes) in the thinner jejunal segment is greater than in an equal unit weight of the thicker duodenal segment. However, the capacity to transfer iron taken up by the enterocytes is reduced on moving down the gastrointestinal tract, and this is in keeping with our data demonstrated by the difference in ^{59}Fe transfer expressed as percentage of the uptake by the jejunal and duodenal mucosae [69.2 vs. 94.4 in the control and 68.0 vs. 89.6 in the sham (saline-treated) group], respectively. The minimal serosal-luminal secretion and the minimal mucosal retention following intravenous injection of ^{59}Fe do not seem to explain the reduced absorption of iron from the duodenum and proximal jejunum in the acetic acid-treated groups.

In conclusion, acetic acid injected into different segments of rat intestine induces an inflammatory process of the treated segments, and this process sig-

nificantly reduces iron absorption in the duodenum and proximal jejunum.

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