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Acetic acid-induced colitis results in bystander ileal injury

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Abstract

The extent of the small intestinal injury following experimental acetic acid induction of colitis in rats was examined. Following intraluminal colonic administration of radiolabelled acetic acid, high levels of radioactivity were identified in the colon and in the liver, while low background levels were found in jejunum, ileum, caecum, and heart. The increased level of radioactivity in the liver relative to that of the heart suggests that a significant portion of the colonic intraluminal acetic acid was absorbed directly into the portal circulation. The colon, which was the only segment of intestine in direct contact with the acetic acid, had the highest levels of radiolabelled acetic acid, demonstrated a marked macroscopic mucosal ulceration, an enhanced myeloperoxidase activity, and a fall in *in vivo* fluid absorption. The jejunum, which demonstrated low levels of radiolabelled acetic acid was normal without evidence of injury. In contrast, the ileum, which displayed the same levels of radiolabelled acetic acid as did the jejunum, also demonstrated a significant fall in *in vivo* fluid absorption but showed no mucosal ulceration or increased myeloperoxidase activity.

These studies have shown that acetic acid induction of colitis produces evidence of ileal injury but that this injury is not the result of inadvertent delivery of acetic acid or recruitment of neutrophils to the ileal mucosa.

Introduction

To date, considerable animal research on the potential involvement of numerous inflammatory mediators in inflammatory bowel disease has been conducted. The variety of models of inflammatory bowel disease (IBD) has helped both to gain an understanding of the disease and to search for more effective therapies for the treatment of the disease. While none of the animal models of IBD exactly mimics the human disease, many individual features of these models enable investigators to explore the role played by particular inflammatory cells and mediators in the inflammatory process.

One of the more commonly employed models is that of an acetic acid-induced colitis. Intracolonic infusion of a dilute solution of acetic acid causes acute colitis characterized by edema, ulceration, and neutrophil infiltration in the rat [1–4], rabbit [5], and guinea pig [6]. This experimentally induced model of colitis is similar to human colitis which generally involves acute inflammation and neutrophil infiltration [7–10] and an increased formation of arachidonic acid metabolites [11, 12].

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Although 10% to 15% of the humans with idiopathic ulcerative colitis who have pancolitic involvement will also have some superficial inflammation of the terminal ileal mucosa associated with edema and spasm (revealed by an abnormal radiographic appearance of the terminal ileum known as "backwash ileitis"), ileal injury following the induction of acetic acid colitis has not, to our knowledge, been examined.

In the present study we, thus, investigated whether or not acetic acid induction of colitis produced evidence of ileal injury and whether or not this injury occurred as a consequence of inadvertent delivery of the acetic acid to the ileal mucosa.

Material and methods

Materials

Acetic acid was purchased from Fisher Scientific, Nepean, ON, Canada and prepared to a 4% solution in water (pH 2.4). ^{14}C-labelled acetic acid was purchased from New England Nuclear.

All remaining chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Methods

Induction of colitis. Acetic acid colitis was induced as previously described [2]. Nonfasting, male Sprague–Dawley rats (250–270 g, Biotron, University of Alberta, Edmonton, AB, Canada) were anaesthetized by intraperitoneal administration of ketamine (70 mg/kg) and xylazine (7.5 mg/kg). Through a sterile midline incision, the colon was isolated, and the junction of the caecum and ascending colon was occluded with a reversible ligature; this procedure was carried out without compromise of neural or vascular supply. The integrity of the occluding ligature was tested, and on each occasion the ligature completely prevented reflux of methylene blue dye from the ascending colon into the caecum or ileum (data not shown). The colon was cleansed of its luminal contents with a warm 154 m*M* sodium chloride solution, and the residual fluid was manually expressed through the rectum. Two millilitres of 4% acetic acid were injected into the lumen of the colon through a 26-gauge needle passed obliquely through the colonic wall just distal to the occluding ligature. Immediately thereafter, 10 ml of air were injected through the needle in order to clear most of the acetic acid from the colon. The occluding ligature was removed, and the midline incision was closed. The animals were allowed to recover from anaesthesia and were housed in a light-cycled room which provided free access to standard rat chow pellets (5001, Purina Mills Inc, St. Louis, MO, USA) and water. Two days later, the rats were killed with a pentobarbitol overdose (240 mg/kg i.v.), and their intestines were removed for experimentation.

Macroscopic studies. The colon was rapidly excised, opened along its mesenteric border, and gently rinsed of its luminal contents with an iced solution of 154 m*M* sodium chloride. The colon was then placed, mucosal surface upwards, on a glass plate chilled to 4 °C and photographed.

In vivo intestinal fluid absorption studies. Nonfasting rats were anaesthetized with pentobarbitol and atropine and kept warm with a thermostatic heat lamp. The intestinal tract was exposed through a midline abdominal incision. An occluding ligature was placed at the ligament of Trietz, and the luminal contents were flushed out of the rectum with a warm 154 m*M* sodium chloride solution instilled via a cannula inserted through an incision just distal to the proximal occluding ligature; residual saline was emptied by gentle manual expression. Three intestinal loops of ~15 cm in length, one beginning 2 cm below the ligament of Trietz and extending distally, one beginning 2 cm above the ileocaecal valve and extending proximally, and one beginning 2 cm below the caecal–colonic junction and extending distally to the peritoneal reflexion, were created with ligatures. In isolating the loops, care was taken not to compromise mesenteric, vascular, or neural continuity. A 27-gauge needle was inserted obliquely through the outer muscle layer along the antimesenteric border, and 2 ml of 154 m*M* sodium chloride, prewarmed to 37 °C, were instilled into each empty loop. No fluid leakage was detected, and the loops were only mildly distended. The viscera were returned to the abdominal cavity and the incision was closed. The rats were allowed to recover from the anaesthesia. Sixty minutes after abdominal closure, the animals were killed by pentobarbitol overdose, and the intestinal loops were removed. The length of each loop was measured and each was weighed, both full and emptied, to determine the residual intraluminal volume.

Results were expressed as the difference between initial and residual luminal loop volume per centimetre of bowel.

Myeloperoxidase activity. An assay of intestinal myeloperoxidase (MPO) activity was used to quantitate neutrophil infiltration [13]. Lengths of intestine taken from the caecal-ascending junction to the rectum, from the 10 cm of ileum distal to the ileal–caecal valve, and from the 10 cm of jejunum proximal to the ligament of Treitz were homogenized with a polytron three times for 30 s each at 4 °C in 5 ml of 0.5% hexadecyltrimethylammonium bromide in 50 m*M* phosphate buffer (pH 6.0). The homogenate was then sonicated for 10 s and assayed for MPO activity spectrophotometrically; 0.1 ml of supernatant was combined with 2.9 ml of 50 m*M* phosphate buffer (pH 6.0) containing 0.167 mg/ml *o*-dianisidine hydrochloride and 0.0005% hydrogen peroxide, according to the method used by Bradley et al. [13]. Change in absorbance at 416 nm was measured with a Beckman DU-6 spectrophotometer. One unit of MPO activity is defined as that which degrades 1 m*M* of peroxide per minute at 25 °C.

Radiolabelled mapping of acetic acid delivery. In some experiments, induction of colitis was carried out as described above using ^{14}C-labelled 4% acetic acid (4×10^6 cpm/ml). Care was taken to remove all residual ^{14}C-labelled acetic acid from the colon prior to removing the caecal-ascending colon occluding ligature. Two hours following induction of colitis, the animal was killed with a pentobarbitol overdose (240 mg/kg i.v.) and experimentation carried out. The whole heart, liver, caecum, and the entire colon from the caecal-ascending junction to the rectum, the 10 cm of ileum proximal to the ileal–caecal valve, and the 10 cm of jejunum distal to the ligament of Treitz were removed and cut into 1 cm segments. Each organ or 1 cm intestinal segment was then placed in 3 ml of 2.5% SDS for 24 h at room temperature. The tissues were then vortexed for 1 min, and the entire 3 ml of solution was placed in scintillation fluid and assayed for radioactivity.

Statistical analysis. Values represent mean ± SEM of n animals. The Student's t-test was used to determine differences between groups, with $p < 0.05$ representing a significant difference.

Results

Distribution of acetic acid

Colon. Figure 1 gives the locations and levels of radioactivity seen in the colon following radiolabelled acetic acid delivery. Radioactivity is present throughout the entire length of the colon. The region of greatest radiolabelled activity, 6 and 7 cm from the caecal-ascending junction (the area of the distal transverse and proximal descending colon), is also the region of most severe gross macroscopic injury. The reason for this unequal distribution of radioactivity and injury is unclear, although it may represent a pooling of residual acetic acid in a dependent segment of colon during anaesthetic recovery.

Ileum. Figure 2 illustrates the level of radioactivity found in the distal ileum following radiolabelled acetic acid delivery. The level of ileal radioactivity in each region is significantly lower ($p < 0.01$) than that seen in the colon and is constant throughout the entire length of ileum measured. This degree of radioactivity likely represents acetic acid which reached the ileum via the systemic or portal circulations. It is unlikely to represent reflux of acetic acid through the occluding ligature at the caecal-ascending junction since the occluding ligature was found to be impermeable to methylene-blue-labelled acetic acid (data not shown).

Jejunum. The level of radioactivity in the jejunum (Fig. 3) is nearly identical to that seen in the ileum and likely represents the amount of radioactivity which reached the jejunum via systemic and/or portal circulation.

Caecum. As with the levels found in the jejunum and ileum, mean caecal radioactivity (Fig. 4) is significantly lower ($p > 0.01$) than that seen in the colon and once again likely represents background radioactivity.

Extra-intestinal tissues. Figure 4 shows the mean levels of radioactivity seen in heart and liver, as well as in the entire length of each intestinal segment. The heart displays a background level of radioactivity which is similar to that seen in the caecum, ileum, and jejunum and which likely represents radioactivity which reached this organ via systemic circulation. Radioactivity in the liver, however, is as

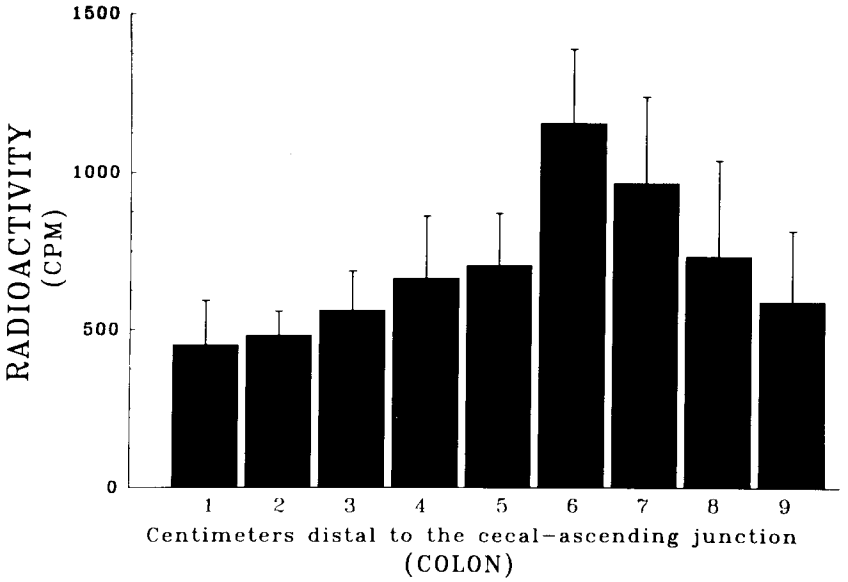

Figure 1
Location and level of radioactivity in the colon following radiolabelled acetic acid delivery. Values represent mean $\pm$ SEM of $n \geq 4$ animals.

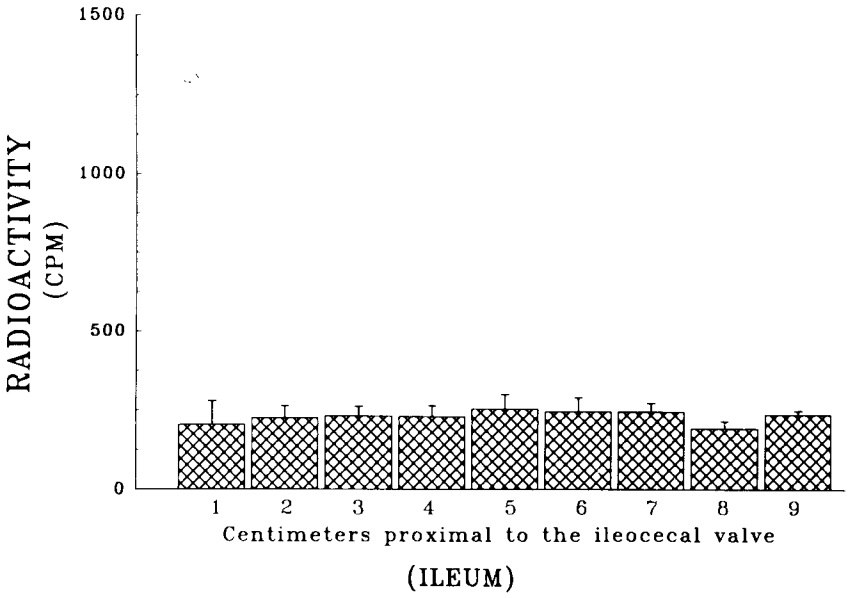

Figure 2
Location and level of radioactivity in the ileum following radiolabelled acetic acid delivery to the colon. Luminal reflux of acetic acid did not occur across the caecal-ascending colon occluding ligature. Values represent mean $\pm$ SEM of $n \geq 4$ animals.

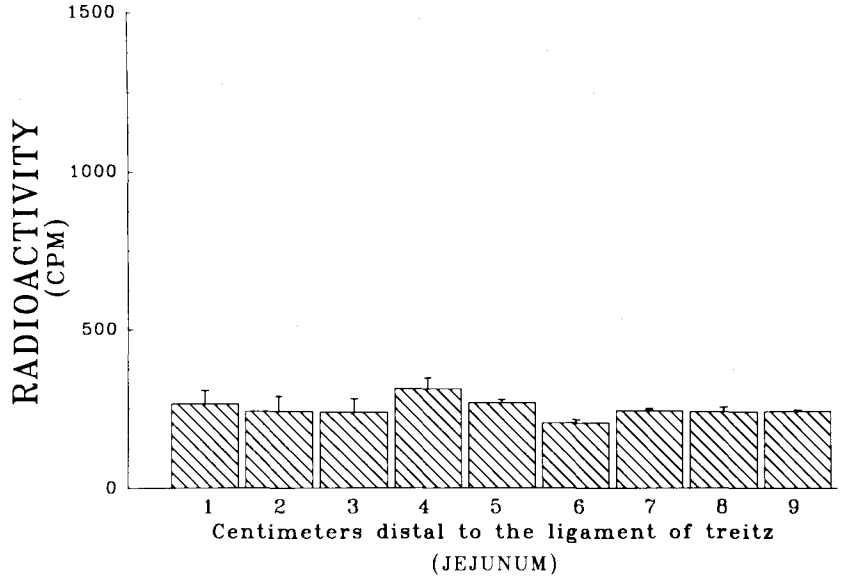

Figure 3
Location and level of radioactivity in the jejunum following radiolabelled acetic acid delivery to the colon. Values represent the mean ±SEM of $n \geq 4$ animals.

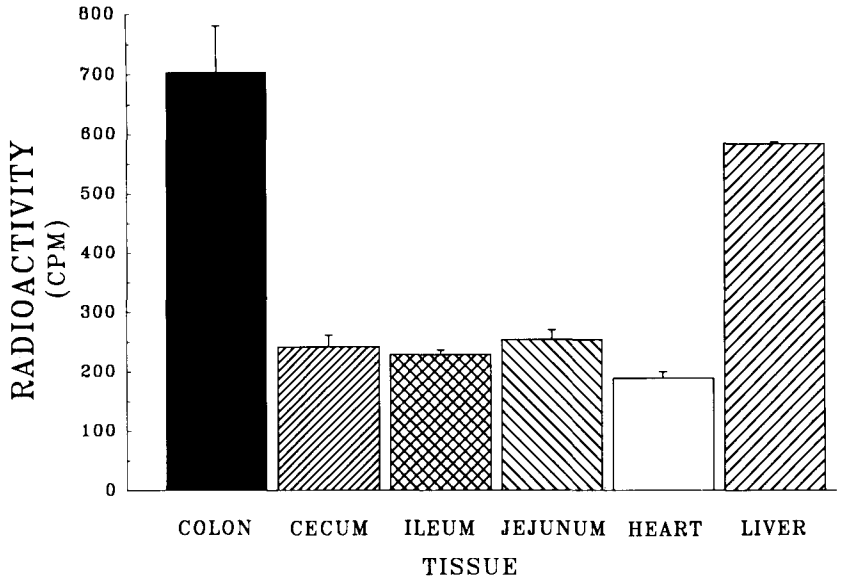

Figure 4
Tissue levels of radioactivity following radiolabelled acetic acid delivery to the colon. Values represent mean ± SEM of the whole organ or in the case of the intestine, the entire 10 cm of each respective segment. $n \geq 4$ animals.

high as in the colon, indicating that a significant amount of radioactivity was absorbed from the colon via portal circulation into the liver.

Macroscopic changes

Intracolonic application of acetic acid produces an immediate blanching and a mild muscular contraction of the colonic mucosa which resolves spontaneously over 20–30 min. At the time of death (2 d after acetic acid instillation), the jejunum and ileum were grossly normal with no evidence of erythema or ulceration. In contrast, the colon demonstrated gross macroscopic ulceration (Fig. 5).

Myeloperoxidase activity

Intestinal myeloperoxidase activity levels quantify intestinal neutrophil content [13]. As shown in Fig. 6, acetic acid-induced colitis significantly elevates myeloperoxidase activity in the colon but not in the jejunum or ileum.

In vivo fluid absorption

As shown in Fig. 7, colitic animals demonstrate a spontaneous net colonic fluid and electrolyte secretion relative to the levels seen in sham-operated controls. This significant alteration in colonic fluid absorption is associated with both macroscopic ulceration and with the myeloperoxidase activity changes described above. The Ileal net fluid absorption in acetic acid-induced colitic animals is also significantly lower than the levels seen in sham-operated controls; however, unlike the situation in the colon, these alterations occur in the absence of gross macroscopic ulceration (Fig. 5) or myeloperoxidase activity (Fig. 6) changes. In contrast to colonic and ileal changes, jejunal fluid absorption is unchanged from the absorption levels found with sham-operated controls.

Discussion

The present study was undertaken to investigate the jejunal and ileal morphologic and functional

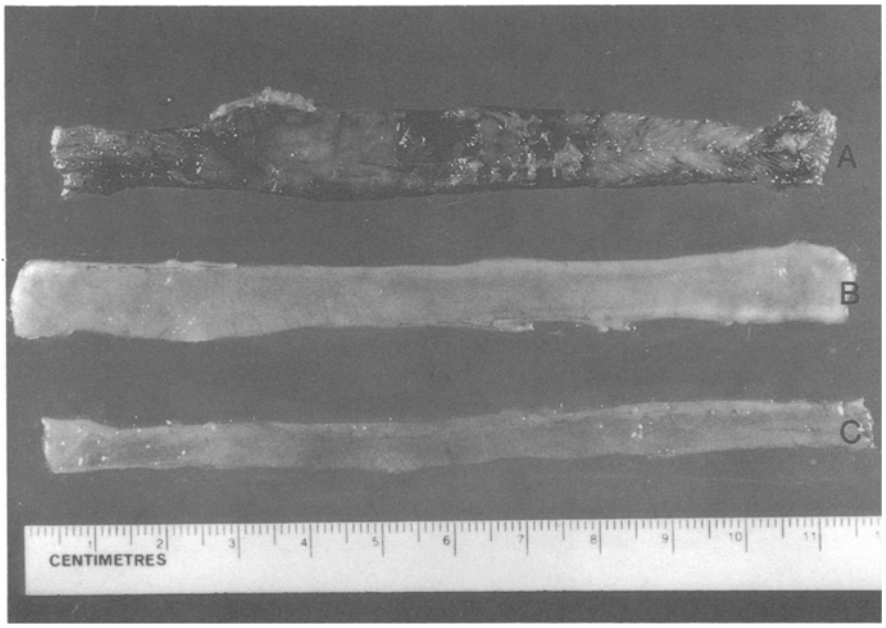

Figure 5
Macroscopic mucosal appearance following 4% acetic acid induction of colitis: (A) colon; (B) ileum; (C) jejunum. The colon demonstrates gross hemorrhage, exudate and ulceration, while ileum and jejunum are grossly normal.

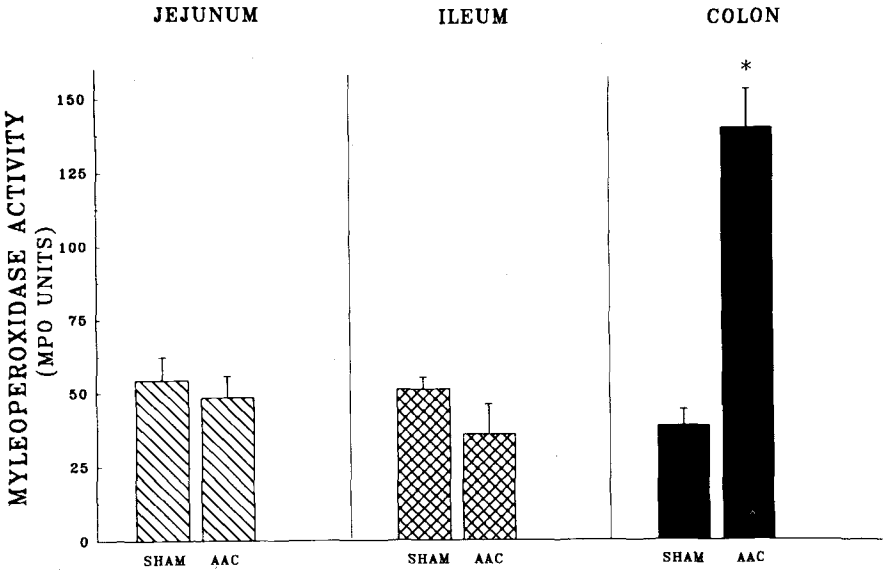

Figure 6
Myeloperoxidase activity in jejunum, ileum, and colon from acetic acid-induced colitic and sham-operated control animals. Values represent mean ± SEM of $n \geq 6$ animals. * $p < 0.01$ relative to sham-operated control.

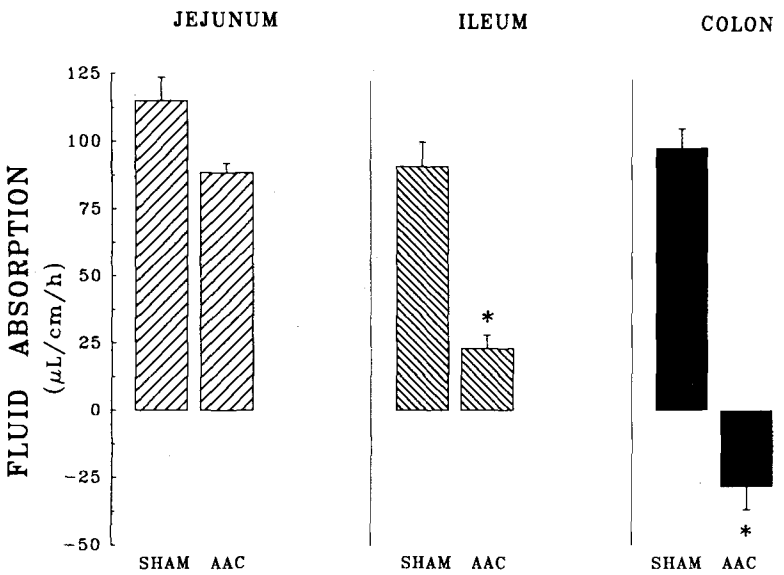

Figure 7
In vivo fluid absorption in jejunum, ileum, and colon from acetic acid-induced colitic and sham-operated control animals. Values represent mean ± SEM of $n \geq 6$ animals. * $p < 0.01$ relative to sham-operated control.

fluid absorptive changes which follow acetic acid induction of colitis. This study examined these changes in relation to local and systemic exposures to acetic acid.

Various animal models of experimentally induced colitis have been developed; these models mimic, to a certain degree, human ulcerative colitis. Similarities in the patterns of arachidonic acid metabolism seen with acetic acid-induced colitis and human inflammatory bowel disease, as well as the simplicity of acetic acid application, have led to the frequent use of acetic acid-induced colitis as an animal model. Recently, the trinitrobenzene sulphonic acid-induced and acetic acid-induced ileitis which occurs following direct application of these noxious chemical agents to the ileal mucosa has been described [14, 15]. Nevertheless, the extent of the small intestinal injury following experimental induction of colitis with direct application of acetic acid to the colon has not been explored. Acetic acid is known to cause massive epithelial cell injury *in vivo*, which appears to be dependent on the protonated (lipophilic) form of the acid, since neither HCl (pH 2.3) nor sodium acetate (pH 7.0) produces colitis in rats [16]. Since the acid is lipophilic, it is membrane-permeable and has free access to all the layers of the mucosa and sub-mucosa, where it equilibrates its conjugate base to generate large quantities of H^+ and acetate anions. As in human inflammatory bowel disease, the pathogenesis of acetic acid-induced colitis is not completely understood. Altered colonic blood flow [17], increased mucosal permeability [18] with enhanced bacterial cell wall [19] or chemotactic peptide transport [20], increased arachidonic acid metabolism [11], increased generation of oxygen-derived free radicals [21], and altered colonic carboxypeptidase activity [20] have all been implicated.

In this experiment, we found the following: after the intraluminal administration of radiolabelled acetic acid, a significant level of radioactivity was identified in the colon and in the liver, while constant background levels were found in jejunum, ileum, caecum, and heart. The high level of radioactivity found in the colon is likely a consequence of radiolabelled products migrating to an intramural position. The colonic epithelium provides a selective barrier to the transmucosal exchange of solutes and fluids, and it restricts the migration of the potentially noxious agents that normally reside within the gut lumen. Acetic acid-induced disruption of this protective barrier and the subsequent increase in mucosal permeability which has been described [17] would allow for the inappropriate introduction of toxins, antigens, and bacteria into the lamina propria and, possibly, into the portal and systemic circulations. In addition, injury to the colonic mucosa would impair normal fluid and electrolyte absorption. Indeed, we found that all rats exposed to acetic acid developed net *in vivo* colonic fluid secretion. The presence of higher levels of radioactivity in the transverse and proximal descending colon, than in other segments of the colon likely relates to the prone position in which the animals were placed during anaesthetic recovery. In this position, the transverse colon is the most dependent part of the colon and serves as a catchment area for any residual luminal acetic acid. The increased concentration and contact time of acetic acid in this region would account for the increased severity of ulceration [2].

The increased level of radioactivity in the liver relative to that of the heart suggests that a significant portion of the intramural acetic acid, or its metabolites, were absorbed directly into the portal circulation, either because of increased epithelial permeability or because of increased endothelial permeability. Indeed, Leung and Koo [22] have recently shown that within minutes after acetic acid contact, colonic ischemia develops, followed shortly by death of vascular endothelial cells. The fact that the jejunum and ileum demonstrated similar levels of radioactivity to those seen in the heart suggests that the levels found in the small intestine are not the result of radiolabelled acetic acid reflux from the colon through the lumen or portal circulation, but instead likely represent radioactivity which reached the small intestine through the systemic circulation. The colon, which was in direct contact with the acetic acid and had the highest levels of radioactivity, demonstrated a marked macroscopic mucosal ulceration, an enhanced myeloperoxidase activity, and a fall in *in vivo* fluid absorption. The jejunum, which showed low systemic levels of radioactivity, demonstrated no ulceration, and no changes in myeloperoxidase activity or fluid absorption. In contrast, the ileum, which displayed the same levels of radioactivity as did the jejunum, demonstrated a significant fall in *in vivo* fluid absorption but showed no mucosal ulceration or increased myeloperoxidase activity. It is, thus, unlikely that either acetic acid itself or neutrophil infiltration is implicated in the ileal fluid absorption abnormality. Instead, other mediators

which have inflammatory and immunoregulatory potential, and which perhaps come from the injured colon, may be the agents responsible for the transport abnormalities identified in the ileum. The work of Sartor et al. [19] which demonstrates that luminal bacterial cell wall polymers with pro-inflammatory potential can cross injure colonic epithelia, and are capable of initiating and potentiating intestinal inflammation, supports this contention. Why this abnormality should occur only in the ileum and not in the jejunum remains to be determined. Pro-inflammatory bacterial peptides synthesized by colonic bacteria are present in the bile and it is possible that they are concentrated in the ileum at the site of bile reabsorption [20].

While we did not characterize the molecular species in which the radiolabel existed at the time of our analysis, it is unlikely to have remained as ^{14}C-acetic acid. More likely, the ^{14}C-acetic acid was metabolized by colonic flora to ^{14}C-labelled acetate, absorbed across the colonic mucosa in this form, and was further metabolized in the liver.

Our study has shown that acetic acid induction of colitis produces evidence of ileal injury (lowered *in vivo* fluid absorption) but that this injury is not the result of inadvertent delivery of acetic acid, or its metabolites, or recruitment of neutrophils to the ileal mucosa; it may, in fact, be the result of the activity of certain, as yet unknown, mediators.

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