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Proinflammatory Effects of Local Abdominal Irradiation on Rat Gastrointestinal Tract

MIKAEL G. BUELL, PhD, MD, and R.K. HARDING, PhD

Although the role of inflammatory processes in the genesis of late changes in the gastrointestinal tract following exposure to ionizing irradiation has been extensively studied, few studies have concentrated on the presence of an acute inflammatory response in the period immediately following radiation. We therefore examined, in rats, whether the local application of 10 Gy cobalt-60 irradiation to the abdomen led to changes in the gut within the first 24 hr that were consistent with an acute inflammatory response. In stomach, small intestine, and colon, local irradiation led to a significant increase in the accumulation of plasma within the tissue by 4–8 hr following irradiation. This increase in tissue plasma volume, indicative of an increased microvascular permeability, was then sustained until the end of the 24-hr assessment period in all tissues examined. Concurrent with this was a consistent transient increase in tissue red blood cell volume, suggestive of vasodilation. Of particular note, a significant increase in the number of mucosal neutrophils was also observed between 2 and 12 hr following irradiation. This elevation in mucosal neutrophils was particularly marked in the pericryptal or deep mucosal regions of small intestine and colon and consistently preceded the vasodilation and enhanced permeability. Furthermore these pathophysiological alterations occurred at a time when histological changes in the mucosa consistent with an impaired mucosal microcirculation (ie, edema of the lamina propria and subepithelial bleb formation) were present. These results support the hypothesis that an inflammatory response occurs in the gut during the first 24 hr following abdominal irradiation. Such changes may then further exacerbate the damage initiated by the ionizing radiation.

KEY WORDS: inflammation; neutrophils; ionizing radiation; radiation enteropathy; small intestine; colon.

Application of local doses of ionizing radiation to the gastrointestinal tract is often an unavoidable consequence of radiotherapy or brachytherapy of abdominal, retroperitoneal, and pelvic malignancies. The sequelae of such gastrointestinal exposure to radiation ranges from the acute gastrointestinal syndrome seen within hours to days of irradiation (1, 2) to a subacute or chronic radiation enteropathy months to years later (3). Whereas the acute syndrome generally correlates temporally with changes in the intestinal mucosa resulting from inhibition of mitosis and disruption of cell proliferation (4), the chronic or subacute enteropathy occurs at a time when chronic transmural inflammation, fibrotic change, and vasculitis predominate.

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From the Defence Research Establishment Ottawa, and the Departments of Physiology and Medicine and the Gastrointestinal Diseases Research Group, School of Medicine, University of Ottawa, Ottawa, Ontario, Canada.

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Address for reprint requests: Dr. R.K. Harding, Protective Sciences Division, Defence Research Establishment Ottawa, Ottawa, Ontario, Canada, K1A 0Z4.

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Descriptions of the acute changes in the intestinal mucosa following irradiation have focused mainly on the impairment in cell proliferation and subsequent disruption of mucosal integrity (5–10). Concurrent with these changes in cell kinetics and epithelial renewal within the first one to four days following irradiation are histological changes consistent with an inflammatory response (4, 11, 12). To date however there is a scarcity of studies concentrating on the morphological and pathophysiological correlates of an acute inflammatory response in the gut within the first 24 hr following local abdominal irradiation. Examining the sequence of inflammatory events in this largely neglected period may allow one to further understand the mechanism whereby ionizing radiation leads to disturbances in gastrointestinal function in the acute period following exposure. Furthermore, by examining the initial steps in this acute pathophysiological process following irradiation, it is possible to gain some insight into the mechanism whereby chronic inflammatory and fibrotic change occurs and to provide a basis for therapies to modify these responses.

MATERIALS AND METHODS

Male Sprague-Dawley rats, 250–300 g in weight, were used in all experiments. All animals were allowed free access to rat chow and water *ad libitum* throughout the duration of the experiments.

Preparation of Animals. On the day prior to experiment, rats were anesthetized with sodium pentobarbital, 35 mg/kg intraperitoneal, and the right jugular vein was exposed by a 1-cm cervical incision. A heparin–saline-filled PE-50 cannula was subsequently placed into the lumen of the vessel and tied in place with 4-0 silk ligatures. The catheter was then tunneled subcutaneously and exteriorized at the nape of the neck. The incisions were closed with interrupted 4-0 silk sutures, the catheters were flushed and sealed, and the animals were allowed to recover. This catheter was subsequently used for the injection of anesthetic and radiolabeled red blood cells and plasma as described below.

Experimental Protocol. To assess the time course of the acute effects of ionizing radiation on gastric, jejunal, ileal, and colonic microvascular parameters and neutrophil accumulation, animals (six per group) were studied 2, 4, 8, 12, and 24 hr after local abdominal irradiation according to the following procedures.

Just prior to irradiation, each rat was anesthetized with an intravenous injection of Saffan (alphaxolone/alphadolone acetate, Glaxovet, Montreal, Quebec), 10–12 mg/kg, resulting in a brief 12- to 15-min period of light surgical anesthesia and relaxation. The animals were then placed in lateral recumbancy, the thorax and pelvic girdle were shielded using 5-cm-thick lead blocks, and the abdomen was irradiated using an AECL Theratron 780 cobalt-60 unit at a dose rate of 96.89–99.04 cGy/min. Parallel opposed pairs (POP) geometry was used in all experiments such that the abdomen received a nominal dose of 500 cGy laterally per side (resulting in a total abdominal dose of approx. 1000 cGy).* A constant source-to-surface distance of 80 cm was employed with a standard field size of 6.5 × 9.5 cm. Animals were then returned to their individual cages following irradiation and allowed to recover from the anesthetic in a quiet darkened environment. Control animals ($N = 6$) were treated in an identical fashion but were not irradiated.

At varying times following anesthetization and irradiation (1, 3, 7, 11, 23 hr), each animal received an intravenous injection via the indwelling jugular vein catheter of approximately 5 μCi chromium-51-labeled red blood cells (see below) suspended in 0.5 ml normal saline and 1 μCi iodine-125-labeled bovine serum albumin (New England Nuclear) in 0.2 ml rat plasma, respectively, as markers for tissue red blood cell volume and plasma volume. This was followed immediately by the intravenous injection of 0.1 ml monastral blue B suspension (3% phthalocyanine blue in 0.85% NaCl; Sigma, St. Louis, Missouri) per 100 g body weight as a marker for altered microvascular permeability (13). These were subsequently flushed in with heparinized normal saline and allowed to circulate for 1 hr. At the end of this 1-hr period, each animal was euthanized by the bolus injection of an overdose of sodium pentobarbital via the jugular vein catheter (ie, at 2, 4, 8, 12, and 24 hr following irradiation).

Following sacrifice, a 0.5- to 1.0-ml sample of blood was obtained via cardiac puncture. The abdomen was then opened, and the stomach, jejunum, ileum, and colon were rapidly excised. A small section of each tissue was taken, briefly rinsed in normal saline to rid the lumen of contents and transferred to 10% ethanolic formalin for subsequent histological processing and morphometric analysis of mucosal neutrophils (see section on Histological Preparation and Morphometric Analysis below). The remaining portions of stomach, jejunum, ileum, and colon were then placed in 10% phosphate-buffered formalin and allowed to fix for at least 2 hr (to ensure retention of intravascular contents) prior to subsequent processing as described below.

Chromium-51-Labeling Procedure for Red Blood Cells. Red blood cells (RBCs) were labeled with chromium-51 according to a modification of the procedure of Sterling and Gray (14). Briefly, 3–5 ml of arterial blood were withdrawn into a heparinized syringe from donor rats prepared with a carotid artery cannula. This was subsequently transferred to a polypropylene tube, 30 μCi of [^{51}Cr]sodium chromate (New England Nuclear) were

*Dosimetry was carried out in three cadaver animals by placing small thermoluminescence dosimeters (TLDs) within the lumina of the stomach, jejunum, ileum, and colon. Although a nominal dose of 1000 cGy was applied to the abdomen, dosimetry with TLDs demonstrated that the viscera in question received the following doses of gamma irradiation (in cGy): stomach 762 ± 62, jejunum 927 ± 9, ileum 916 ± 39, colon 914 ± 29. As a result, subsequent reference to the radiation dose to individual organs will refer to those measured by thermoluminescence dosimetry.

added, and the suspension was incubated 40 min at room temperature. The labeled RBCs were then pelleted by centrifugation and the plasma and buffy coat aspirated. The RBC pellet was subsequently centrifuged and washed three times with an equal volume of normal saline. Following the final centrifugation, the labeled cells were resuspended to a volume of 3 ml with normal saline for injection into the recipient animal (0.5 ml/animal).

Determination of Tissue Red Blood Cell Volume and Plasma Volume. The blood sample and tissues obtained postmortem from each animal were processed as follows for the determination of tissue red blood cell volume (RBCV) and plasma volume (PV).

The blood sample was divided into three aliquots: 20 μl was used for the determination of ^{51}Cr counts per minute per microliter whole blood; the second aliquot was used for the determination of hematocrit (Hct); the third aliquot was centrifuged 5 min in a microcentrifuge. Twenty microliters of the plasma was then decanted and used for the determination of ^{125}I counts per minute per microliter plasma.

Following fixation in formalin, the gastric, jejunal, ileal, and colonic tissues were trimmed of perimesenteric fat and the luminal contents were extruded. Intestinal tissues were then opened along their transverse axes, and any adherent intraluminal contents were removed by blotting. The stomach was opened along its greater curvature, luminal contents were removed, and the forestomach was excised and discarded. The corpus–antral region was then blotted free of adherent luminal contents. All tissues were then diced, placed in preweighed gamma counting vials to a constant geometry, and wet weight was determined. All tissue samples were prepared in duplicate.

Tissue, whole blood, and plasma samples were then counted differentially in an LKB 1282 Compugamma gamma counter equipped with multiisoptope option for the automatic spill correction of counts in each of the ^{125}I and ^{51}Cr channels. Tissue RBCV (in μl/g wet tissue) was then calculated as:

$$\frac{^{51}\text{Cr cpm}_{TS} / [(^{51}\text{Cr cpm}_{BS}/20) \times \text{Hct}]}{\text{wet wt of tissue (g)}}$$

where $^{51}Cr\ cpm_{TS}$ is chromium-51 cpm in tissue sample, $^{51}Cr\ cpm_{BS}$ is chromium-51 cpm in 20 μl whole blood, and Hct is whole blood hematocrit.

In an analogous fashion, tissue PV (in μl accumulated/hr/g wet tissue weight) was calculated as:

$$\frac{^{125}\text{I cpm}_{TS} / (^{125}\text{I cpm}_{PS}/20)}{\text{wet wt of tissue (g)}}$$

where $^{125}I\ cpm_{TS}$ is iodine-125 cpm in tissue sample, and $^{125}I\ cpm_{PS}$ is iodine-125 cpm in 20 μl of plasma.

Histological Preparation and Morphometric Analysis. As stated above, tissue samples were also taken for histological examination and the quantification of PMN infiltration. These tissues were fixed 20–22 hr in 10% neutralized ethanolic formalin, snap frozen in liquid nitrogen, then stored at −70°C until processed. For the histochemical detection of peroxidase granules in neutrophils (PMNs), tissue samples were stained *en bloc* using Graham's alpha-naphthol pyronine stain as modified by Ritter and Oleson for paraffin sections (15). Following dehydration and clearing, the stained blocks of tissue were embedded in Paraplast, sectioned at 5 μm in a rotary microtome, and mounted on albuminized slides. The sections were subsequently counterstained using a routine hematoxylin–phloxine–saffron (HPS) staining method (16). Combining the *en bloc* peroxidase reaction and the routine HPS stain yielded sections in which PMNs were readily identified as intensely staining red granular cells with polymorphic nuclei. As a result, such sections were readily used for both routine histological examination and the morphometric quantitation of PMNs.

To quantitate mucosal PMNs, a two-stage sampling procedure was applied (17) in which six sections from each tissue from each animal were microscopically examined with a Leitz Laborlux II microscope (Leitz Wetzlar GMBH) in single-blind fashion using 1000× magnification and oil immersion. In the case of small intestinal tissues, the number of mucosal PMNs was determined in both crypt and villus regions by counting the number observed in 5–10 high-power fields (HPF) of each tissue region. Results were then expressed as number of PMNs per 10 HPF villous or pericryptal regions. In the case of colonic and gastric tissues, an analogous approach was used in which the number of PMNs per 10 HPF was determined for both deep and superficial mucosal regions.

For estimating changes in vascular labeling with the monastral blue B (as an index of changes in microvascular permeability), 20 μm sections of the *en bloc*-stained tissues were cut and mounted on albuminized slides. These were subsequently cleared in xylene and mounted under cover slips with Permount. Sections were then examined to assess the degree and site of any increase in vascular labeling with the dye.

Statistical Analysis. Differences between control values and those obtained at various times following irradiation for all parameters were examined using a two-tailed *t* test for unpaired data. Values were considered significantly different from control at $P < 0.05$.

RESULTS

Effect of Local Irradiation on Microcirculatory Parameters

Stomach. The time course of the changes in gastric plasma volume (PV) and red blood cell volume (RBV) are shown in Figure 1A. Local abdominal irradiation led to a progressive and significant increase in PV, reaching a peak at 12 hr. Although the PV then gradually declined after 12 hr, it was still significantly greater than control 24 hr following irradiation. Gastric RBCV followed a similar trend postirradiation to that described for PV, although the magnitude of the change was less. RBCV gradually increased following irradiation, reaching a peak of 12 hr. As a result, RBCV was

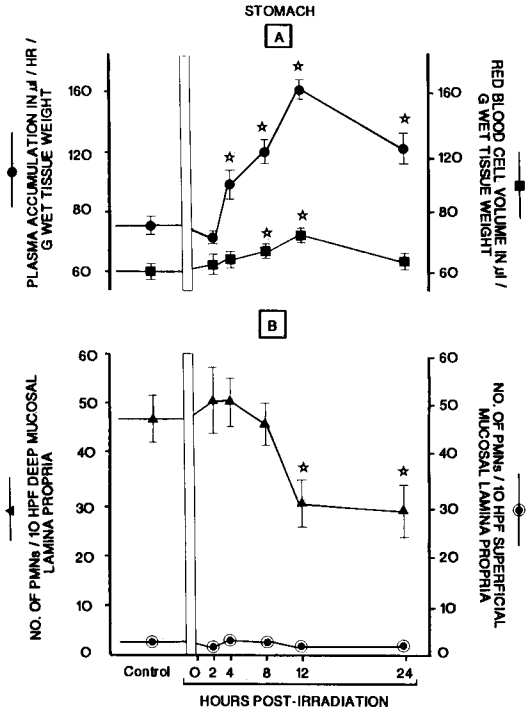

Fig 1. Acute effects of 762 cGy local abdominal ^{60}Co irradiation on gastric microcirculatory parameters and neutrophil accumulation. The upper panel (A) shows the changes in gastric tissue plasma accumulation (—●—) and red blood cell volume (—■—) in the first 24 hr following irradiation, as determined using, respectively, ^{125}I-labeled serum albumin and ^{51}Cr-labeled red blood cells. Plasma accumulation was expressed in microliters of plasma accumulated per hour per gram of wet tissue weight (left ordinate) whereas red blood cell volume was expressed in microliters per gram wet tissue weight (right ordinate). The lower panel (B) shows the changes in the number of mucosal neutrophils (PMNs) in the first 24 hr following irradiation as determined by histomorphometry. The left ordinate represents the number of PMNs per 10 high-power fields (HPF) of deep mucosal lamina propria (—▲—), while the right ordinate indicates the number of PMNs per 10 HPF of superficial lamina propria (—◉—). The abscissa shows the time points at which parameters were estimated following irradiation in hours. The open perpendicular bar immediately preceding 0 hr on the abscissa indicates the point at which irradiation was carried out. All values show the mean ± SEM of six animals per time period. Stars above the graph indicate values significantly different from control at $P < 0.05$.

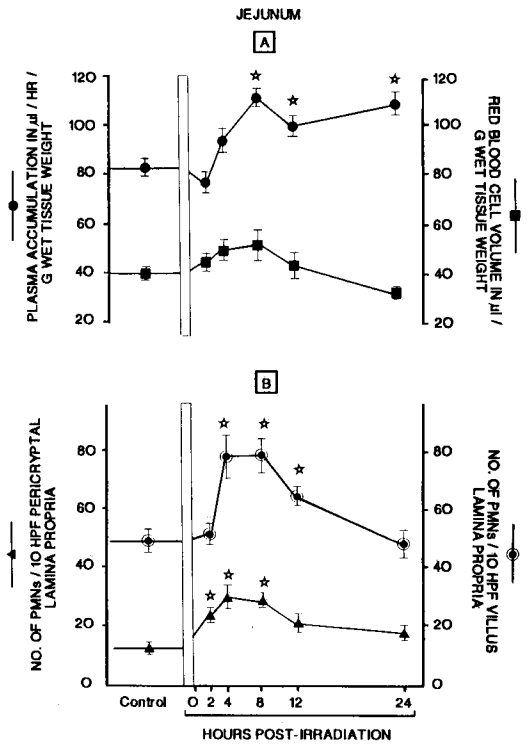

Fig 2. Acute effects of 927 cGy local abdominal ^{60}Co irradiation on jejunal microcirculatory parameters and neutrophil accumulation. The upper panel (A) shows the changes in jejunal tissue plasma accumulation (—●—) and red blood cell volume (—■—) in the first 24 hr following irradiation, as determined using, respectively, ^{125}I-labeled serum albumin and ^{51}Cr-labeled red blood cells. Plasma accumulation was expressed in microliters of plasma accumulated per hour per gram wet tissue weight (left ordinate) whereas red blood cell volume was expressed in microliters per gram wet tissue weight (right ordinate). The lower panel (B) shows the changes in the number of mucosal neutrophils (PMNs) in the first 24 hr following irradiation as determined by histomorphometry. The left ordinate represents the number of PMNs per 10 high-power fields (HPF) of pericryptal lamina propria (—▲—), while the right ordinate indicates the number of PMNs per 10 HPF of villous lamina propria (—◉—). The abscissa shows the time points at which parameters were estimated following irradiation in hours. The open perpendicular bar immediately preceding 0 hr on the abscissa indicates the point at which irradiation was carried out. All values show the mean ± SEM of six animals per time period. Stars above the graph indicate values significantly different from control at $P < 0.05$.

significantly elevated over control at 8 and 12 hr. RBCV then gradually returned towards control from 12 to 24 hr postirradiation.

Jejunum and Ileum. Both jejunal and ileal PV and RBCV followed similar trends postirradiation (Figures 2A and 3A). With both jejunum and ileum, a brief nonsignificant decline in PV was noted at 2 hr postirradiation. Plasma accumulation then rose over the subsequent 6 hr to become significantly elevated at 8 hr. This increase in PV accumulation was then sustained to the end of the 24-hr assessment period. A transient and consistent (although statistically nonsignificant) increase in RBCV was noted in both jejunum and ileum following irradiation. In both tissues, RBCV reached a peak 8 hr postirradiation then gradually declined.

Colon. The results of local irradiation on colonic PV and RBCV are shown in Figure 4A. As was observed in small intestine and stomach, colonic PV accumulation became elevated over the first 8 hr postirradiation. In contrast however, PV became

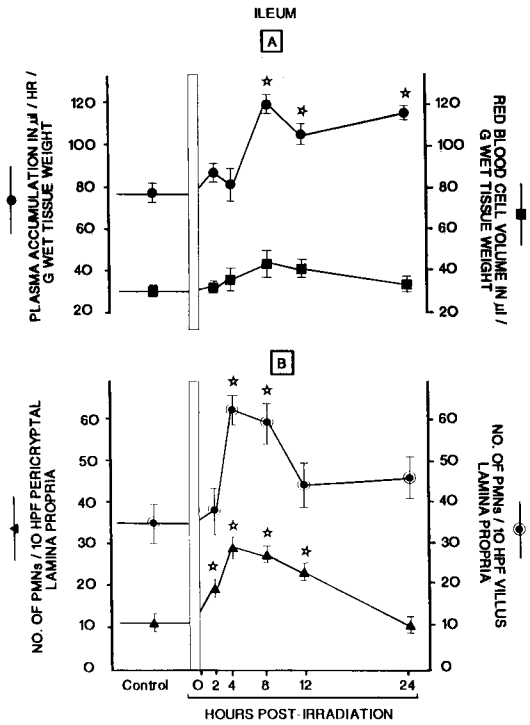

Fig 3. Acute effects of 916 cGy local abdominal ^{60}Co irradiation on ileal microcirculatory parameters and neutrophil accumulation. The upper panel (A) shows the changes in ileal tissue plasma accumulation and red blood cell volume in the first 24 hr following irradiation, while the lower panel (B) shows the changes in the number of mucosal neutrophils (PMNs) in the first 24 hr following irradiation as determined by histomorphometry. The legend and description of the axes are identical to that described for Figure 2. Again, values show mean ± SEM of six animals per time period. Values significantly different from control are indicated by a star above the data point.

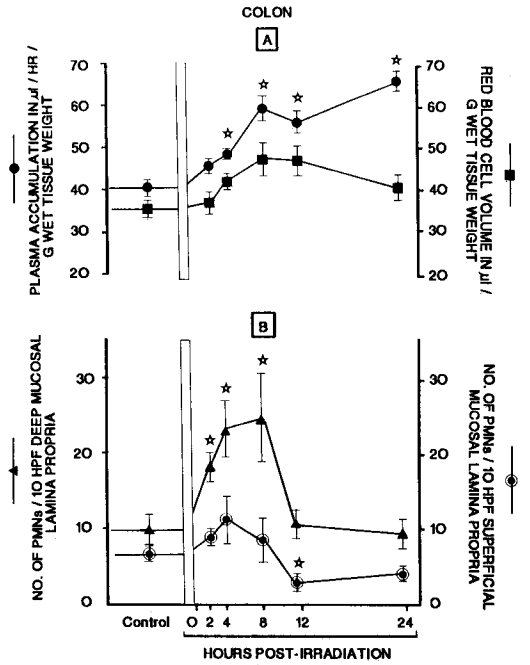

Fig 4. Acute effects of 914 cGy local abdominal ^{60}Co irradiation on colonic microcirculatory parameters and neutrophil accumulation. The upper panel (A) shows the changes in colonic tissue plasma accumulation and red blood cell volume in the first 24 hr following irradiation, while the lower panel (B) shows the changes in the number of mucosal neutrophils (PMNs) in the first 24 hr following irradiation as determined by histomorphometry. The legend and description of the axes are identical to that described for Figure 1. Again, values show mean ± SEM of six animals per time period. Values significantly different from control are indicated by a star above the data point.

significantly greater than control as early as 4 hr after irradiation. PV accumulation then reached a peak at 8 hr and remained significantly elevated for the subsequent 16 hr. Colonic RBCV gradually increased postirradiation, reaching a maximum at 8–12 hr. This trend in RBCV (similar to that observed in stomach and small intestine) was not statistically significant, however.

Effect of Local Irradiation on Mucosal Neutrophils

Stomach. PMN numbers gradually declined in the deep mucosal region of the gastric antrum during the first 12 hr following irradiation, becoming significantly depressed at 12 hr (Figure 1B). This decline in PMN numbers was then sustained until the end of the 24-hr assessment period. In contrast, no change was seen in PMNs in the superficial mucosa during the 24-hr assessment period.

Small Intestine. Similar changes in mucosal PMN numbers were observed in both jejunum and ileum following local abdominal irradiation. As shown in Figures 2B and 3B, the number of villous PMNs rapidly increased following irradiation becoming significantly elevated at 4 hr and remaining elevated for the subsequent 4–8 hr. Following this, the number of villous PMNs decreased towards control values. Changes in pericryptal PMN numbers observed postirradiation in both jejunum and ileum were even more marked than those observed in villus. By 2–4 hr postirradiation, the number of pericryptal PMNs had undergone a significant two- to threefold increase over control values. After reaching a peak at 4–8 hr, the number of pericryptal PMNs then declined, returning toward control levels by 24 hr.

Colon. The effects of local abdominal irradiation on colonic PMN numbers are shown in Figure 4B. A similar trend to that observed in jejunum and ileum was also observed in colon following irradia-

tion. Deep mucosal PMN numbers showed a rapid, transient increase within 2 hr following irradiation, reaching a peak at 8 hr. Following this significant two- to threefold increase in deep mucosal PMNs, numbers rapidly returned to control levels by 12 hr postirradiation. Although the number of superficial mucosal PMNs showed a similar initial trend to that described for deep mucosa, no statistically significant elevation was noted.

Effect of Local Abdominal Irradiation on Vascular Labeling

As described in Materials and Methods, *in vivo* vascular labeling with monastral blue B dye was used to assess the site of changes in microvascular permeability. Using this technique, no diffuse or marked change in permeability (ie, "bluing" of the tissue) following irradiation was detected in any of the tissues examined grossly or microscopically. Despite this, occasional localized areas of minimal labeling were observed more frequently in tissues (particularly the small intestine) obtained from irradiated animals. This labeling with monastral blue was more prevalent at 4–12 hr postirradiation (ie, the time when ^{125}I-labeled plasma protein accumulation was maximal) and tended to be restricted to the mucosa.

Effect of Irradiation on Gastrointestinal Morphology

In all four tissues examined (stomach, jejunum, ileum, colon), morphological changes observed at the light microscopic level following irradiation were restricted solely to the mucosa, with submucosa and muscularis being indistinguishable from control.

Stomach. The changes in gastric mucosal morphology following radiation were restricted to mild edema of the lamina propria (observed from 8 to 24 hr postirradiation) and isolated foci of vascular congestion 24 hr following irradiation. At all time periods assessed up to 24 hr, the gastric epithelium remained contiguous and intact with no evidence of mucosal sloughing, necrosis, or ulceration.

Small Intestine. Both jejunum and ileum exhibited similar changes in mucosal morphology following local abdominal irradiation (Figures 5A–D). As early as 2 hr postirradiation, vacuolar changes in the crypt enterocytes consistent with cellular degeneration and necrosis were detectable (Figure 5D). Mucosal morphology 4 hr following irradiation was similar to that observed at 2 hr, except for an increase in accumulation of PMNs within the lamina propria. In contrast,

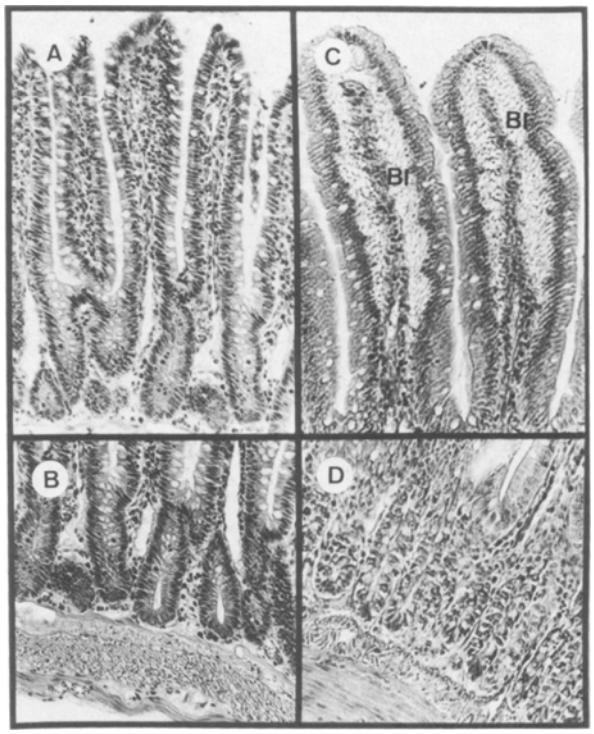

Fig 5. Changes in small intestinal mucosal morphology in the 24 hr following local abdominal irradiation (916 cGy). (A) Control ileal villi (100×). (B) Control ileal crypts (100×). (C) Ileal villi 24 hr postirradiation (100×); note the subepithelial blebs (Bl) suggestive of edema and fluid accumulation. Despite this accumulation of fluid, the overlying epithelium remains intact. (D) Ileal crypts 8 hr postirradiation (100×); note the vacuolar changes in the crypt enterocytes suggestive of cellular degeneration and necrosis.

more marked changes were observed by 8–12 hr postirradiation. At these times, as well as the previously described changes, alterations in the lamina propria suggestive of edema were also seen (Figure 5C). In particular, subepithelial blisters or blebs were noted in which the villus epithelium had been separated from the subjacent lamina propria by a flocculent acidophilic material (Figure 5C). Despite the presence of these blebs, the epithelium overlying the villus remained intact. Moderate vascular congestion was also noted in several villi 8 and 12 hr following irradiation, although no areas of extravasation or frank hemorrhage were seen. All of the previously described changes persisted at 24 hr postirradiation. Of particular note, even 24 hr following irradiation the enterocytes covering the villi appeared normal, with no evidence of epithelial denudation or sloughing.

Colon. Histological changes in the colon following irradiation were generally similar to those described above for small intestine and are shown in Figures 6A–C. In brief, only mild vacuolization of

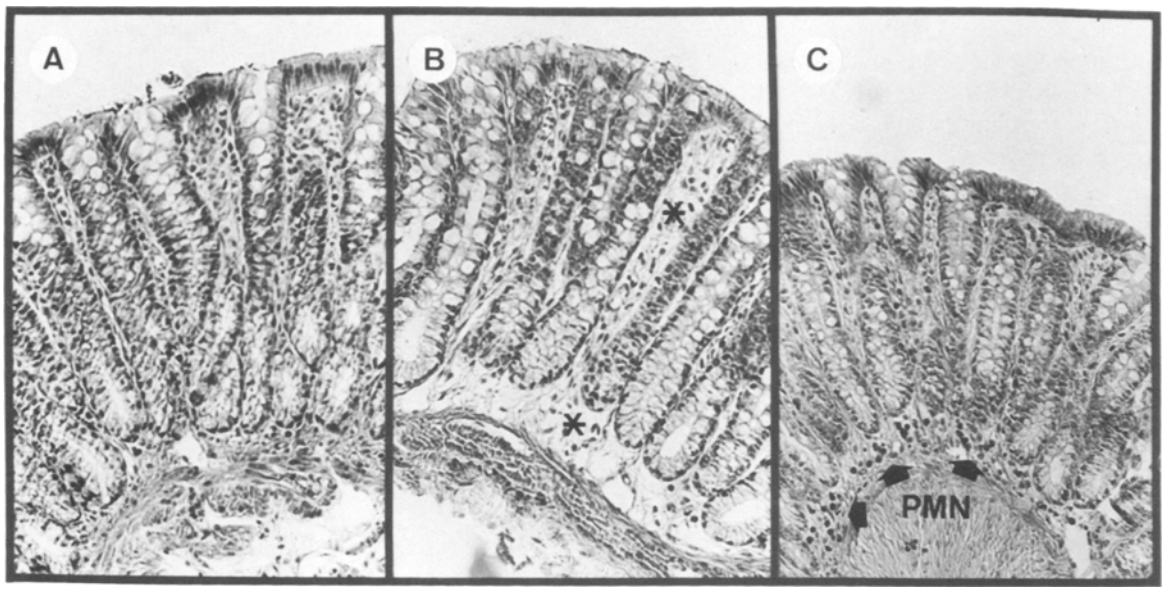

Fig 6. Morphological changes in the colonic mucosa in the 24 hr following local abdominal irradiation (914 cGy). (A) Control colon (100×). (B) Colon 12 hr postirradiation (×100); in contrast to Figure 6A, note the swelling of the periglandular lamina propria (*), suggestive of edema. As well, in comparison to control, the colonic glands 12 hr following irradiation appear mildly dilated. Despite these changes, the colonic epithelium appears normal with no evidence of disruption. (C) Colon 8 hr postirradiation (100×); in contrast to Figure 6A (control), an increased accumulation of PMNs (indicated by arrows) may be seen in the interstitium surrounding the glands.

the deep colonic glands was noted up to 4 hr postirradiation. By 8–12 hr, edema of the lamina propria (Figure 6B) was observed as was increasing numbers of deep mucosal PMNs (Figure 6C). Twenty-four hours following irradiation, occasional dilated colonic glands could be seen. As was observed in stomach and small intestine, the colonic epithelium remained intact over the entire 24-hr assessment period with no evidence of epithelial sloughing.

DISCUSSION

Patients undergoing local abdominal irradiation for the treatment of pelvic, abdominal, and retroperitoneal malignancies may experience a variety of symptoms within the first weeks following exposure to ionizing radiation including nausea, vomiting, crampy abdominal pain, diarrhea, and a mucoid or bloody rectal discharge. Endoscopically, the colorectal or intestinal mucosa is hyperemic and edematous with areas of ulceration, friability, and hemorrhage. Histopathologically, the mucosa appears edematous and hyperemic with an inflammatory cell infiltrate. The picture of these clinical, pathological, and endoscopic changes in the bowel following exposure to ionizing radiation has been likened to that of the acute onset of idiopathic ulcerative colitis (18). Despite these similarities, most of the emphasis on the acute effects of irradiation on gastrointestinal structure and function has been on the disruption of cell proliferation and epithelial maturation, rather than on an examination of the inflammatory events occurring postirradiation. Therefore the present study was undertaken to examine in quantitative terms, the magnitude and time course of the acute inflammatory changes in the stomach, small intestine, and colon in the first 24 hr following irradiation.

Histologically, the changes observed in the present study following abdominal irradiation are generally consistent with those reported previously by others (11, 12, 19). Of note, all changes observed in the first 24 hr were restricted solely to the mucosa, with no demonstrable change histologically in the submucosa or muscularis externa. In jejunum, ileum, and colon, the first detectable morphological change following irradiation was the appearance of vacuoles and dense refractile granules within the epithelial cells lining the small intestinal crypts and deep colonic glands. These changes were observed as early as 2 hr post-irradiation and were sustained until the end of the 24 hr assessment period. Such acute radiation-induced changes are consistent with those previously described in the small intestine of

rats (4) and man (12) and are thought to represent evidence of enterocyte degeneration and necrosis. By 8–12 hr, edema formation within the lamina propria was evident in both small intestine and colon, such changes being sustained for the subsequent 12–16 hr. In the case of the former, this manifested itself both as a swelling of the lamina propria and in the formation of subepithelial blisters or blebs in which the villus epithelium had been separated from the subjacent lamina propria by an acidophilic flocculent material.

Such subepithelial bleb formation has previously been described in situations of a impaired villus microcirculation and has been attributed to a increased extravasation of fluid into the villus interstitium (20–25). Although mucosal edema has been described previously within the first 24 hr following irradiation in the rat small intestine (4), we are unaware of any description of subepithelial bleb formation similar to that described above. This may relate to the fact that most studies to date have concentrated on the morphological changes occurring more than 24 hr postirradiation (ie, at a time when one is already starting to get disruption in epithelial integrity).

No subepithelial blebs were noted in the colon, although marked swelling of the lamina propria suggestive of edema was observed. These edematous morphological changes in both small intestine and colon were accompanied by a concurrent transient increase in the number of mucosal PMNs (discussed below). As reported previously in dogs receiving comparable doses (11), dilated small intestinal crypts and deep colonic glands were noted by 24 hr postirradiation. Despite the presence of the morphological alterations described above, the mucosal epithelium tended to remain in continuity for the duration of the 24-hr assessment period. This absence of epithelial disruption is again consistent with observations by others in rats using comparable doses (19). In contrast to the histological changes observed in jejunum, ileum, and colon following local irradiation, no morphological changes were seen in stomach. This observation may be partly related to the fact that in our study the dose of radiation delivered to the stomach, as determined by TLD dosimetry, was approximately 17% less than that delivered to the small intestine and colon.

To assess changes in microvascular permeability in the current study, two commonly used approaches were employed. First, quantitation of the leakage of plasma into the interstitium was accomplished by assessing the accumulation of intravascularly administered ^{125}I-labeled serum albumin in the gastrointestinal tissues. Second, in an attempt to identify sites of extravasation, the particulate dye monastral blue B was employed as described by Joris and coworkers (13). In all four regions of the gastrointestinal tract examined (stomach, jejunum, ileum, and colon), there was a significant and sustained increase in the accumulation of radiolabeled plasma within the tissues postirradiation. Despite this enhanced accumulation of ^{125}I-labeled albumin, monastral blue B dye failed to reveal any sites of markedly enhanced vascular labeling. This latter method mainly labels areas in which increased vascular permeability has been caused by venular endothelial cell retraction (26) or direct severe injury to the entire microcirculation (ie, respectively, the "immediate–transient" and "immediate–sustained" patterns of vascular injury). From the present results it would not be unreasonable to conclude that the enhanced leakage of radiolabeled albumin into the tissue postirradiation is largely independent of either direct severe injury to the microcirculation or the release of permeability-enhancing vasoactive amines (since these mediators increase permeability by stimulating endothelial cell retraction).

Although early studies using trypan blue (27–29) have demonstrated an increased vascular permeability in rat small intestine starting 18–24 hr after local abdominal irradiation at a dose of 1500 R (15 Gy), no data on permeability changes prior to this time has been available. It was postulated from the results of these studies (27–29) that the increase in intestinal vascular permeability during the first 24 hr following irradiation was mediated via the release of a vasoactive amine (probably histamine) whereas that 24–72 hr postirradiation was due to an esterase- or protease-mediated system. The current results are not inconsistent with those described above, although they do tend to mitigate the early hypotheses that histamine plays a pivotal role in the early permeability changes. Of note, the time course of the increased accumulation of ^{125}I-labeled albumin described above more closely resembles a "delayed–prolonged" permeability response since it starts 4–8 hr after the initial insult (irradiation) and then is sustained for a further 16–20 hr. Although the mechanism whereby microvascular permeability is enhanced in a "delayed–prolonged" response remains elusive, neutrophil accumulation has been

implicated as playing a possible role, presumably through the release of PMN lysosomal contents (30).

In this light, the current study has also demonstrated a significant increase in the number of mucosal PMNs in the small intestine and colon at a time just preceding and accompanying the increase in microvascular permeability. Although the observation that the rise in mucosal PMNs consistently preceded the increase in vascular permeability does not prove a causal relationship, it does indicate a possible association of the two events. At present, we do not know the mechanism whereby exposure of the abdomen to local irradiation leads to an increase in mucosal PMNs within the small intestine and colon, although two hypotheses may be relevant. First, since crypt cell degeneration is present as early as 2 hr postirradiation, the concurrent marked rise in pericryptal or deep mucosal PMNs may reflect a natural response to the presence of intraluminal microorganisms entering the intestinal interstitium at the level of the crypts or deep colonic glands. As reported recently (19), this breaching of the mucosal barrier by bacteria postirradiation may occur at a time well before any evidence of significant histological damage to the mucosa. The second hypothesis relates to recent evidence indicating a significant alteration in prostanoid metabolism postirradiation. Dunn and coworkers (31) have demonstrated that exposure of cultured vascular endothelial cells to a dose of 5 Gy gamma radiation leads to the generation of a neutrophil chemoattractant within 24 hr following irradiation and an increased adherence of PMNs to endothelial cells as early as 4 hr postirradiation. More recent studies (32) have further demonstrated the generation of a lipoxygenase pathway-dependent neutrophil chemoattractant by cultured vascular endothelium as early as 1 hr postirradiation. It is well known that leukotrienes, particularly leukotriene B_4, are potent neutrophil chemoattractants (33–35) and potential mediators of the inflammatory response. Based upon the results of these studies, it is not unreasonable to postulate that the rapid generation of a leukotriene such as LTB_4 in the period immediately following exposure to ionizing radiation may play a major role in eliciting the increased accumulation of PMNs observed in the present study. Furthermore, the increased vascular permeability and vasodilation observed by us in the first 12 hr following irradiation are both consistent with the effects of an increased local generation of leukotrienes (34, 36).

In summary, the present study has demonstrated that local exposure of the abdomen of rats to a single, nominal 10-Gy dose of ionizing radiation leads to changes in the gastrointestinal tract consistent with the classical features of an acute inflammatory response. Such alterations, occurring within the first 12 hrs following local irradiation, included a significant extravasation of plasma into the gastrointestinal interstitium, vasodilation, and a significant increase in the number of mucosal neutrophils. Furthermore, these inflammatory events occurred at a time when the mucosal epithelium of affected regions remained apparently intact. Although the observed increase in mucosal neutrophils may be occurring as a natural defensive response to an increased number of intraluminal microorganisms breaching the mucosal barrier, it is also possible (and not mutually exclusive) that the elevated PMN levels may be secondary to the nonspecific generation of chemoattractants by stromal elements (ie, endothelial cells) of the irradiated lamina propria. In the former case, PMNs would presumably be executing a beneficial role by limiting the spread of potentially pathogenic organisms entering the mucosal interstitium. In the latter case, the increased mucosal neutrophils could in fact be detrimental by further exacerbating the damage initiated by the ionizing radiation. This proinflammatory response within the gut could thus play an etiologic role in the development of symptoms classically associated with the acute gastrointestinal syndrome (such as nausea, vomiting, and diarrhea). Perhaps more importantly, this response may increase the severity of mucosal injury in the gastrointestinal tract exposed to radiation. Since mucosal injury has been implicated as a major causal factor in the genesis of late fibrotic change after therapeutic radiation (37–39), pharmacological manipulation of the currently reported acute inflammatory response may be of potential benefit in reducing the incidence and severity of this adverse sequela to radiotherapy. Subsequent studies will be required to further explore the potential therapeutic advantage of such interventions.

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