Absorption of Iron Following X-Irradiation of the Exteriorized Small Intestine (33664)

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The radiosensitivity of the intestine is well established and the injury produced is manifest both morphologically and functionally. The most conspicuous morphologic alterations occur in the mucosa and are accompanied by changes in intestinal motility and absorption. Intestinal absorption of a variety of substances is either increased or diminished following radiation of either the whole body, the abdomen alone, or the exteriorized small bowel (1–4). Irradiation of the exteriorized intestine excludes the effect on intestinal absorption of radiation-induced changes in body metabolism. The discontinuous nature of the morphologic lesions in the irradiated exteriorized gut which was described recently (5) allows for the study of intestinal absorption *in vivo* in an animal model which includes areas of intestinal radiation injury alternating with areas which are essentially intact or hypertrophic.

In the present experiments gastrointestinal absorption of radioiron was studied in the rat following X-irradiation of the exteriorized small intestine. The results indicate that there was an increase in radioiron absorption and suggest that this increase was due to augmented absorption in those areas of the small intestine which did not develop the morphologic characteristics of severe radiation injury.

Materials and Methods. Female Wistar rats (180–200 g) housed individually in wire bottomed cages, fed a standard diet[2] and allowed water *ad libitum* were used in all experiments. The rats were anesthetized with pentobarbital (5 mg/100 g of body wt.) prior to all surgical procedures or radiation exposures and were necropsied following an overdose of pentobarbital. Gastric intubation was performed following light ether anesthesia. Control rats were subjected to the same procedures but were not irradiated. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Irradiation Procedures. The small intestine, the cecum, and part of the colon of the rats were gently teased out through a 5-cm midline incision and a small forked holder was placed under the root of the mesentery to prevent spontaneous retraction during radiation exposure. Rats were positioned on their side under shields so that only the exteriorized gut, covered with gauze saturated with warmed 0.9% saline, was irradiated. The intestines were exposed to an air dose of 2000 R at 143 R/min, as determined by a Victoreen R meter delivered by a 250 kVp X-ray unit with a 0.5 mm copper and a 1.0 mm aluminum filter. Shielding was provided by a 0.25-in. thick sheet of lead. After irradiation, the bowel was reinserted and the incision was closed with Michelle clips.

I. In a first series of experiments performed daily, for the first 5 days following irradiation, rats were given 1 μCi of ^{59}Fe/20 mg of ^{56}Fe sulfate in 1 ml of physiological saline by stomach tube. Following the administration of radioiron, the total radioactivity of each animal was determined by means of a small animal body counter.[3] After 2 hr the rats were killed and the gastrointestinal tract and the liver were removed. Dissection was carried out on absorbent paper and the removed organs were blotted free of blood with this absorbent paper to minimize discrepancies in total radioactivity due to blood loss. The gastrointestinal tract was then opened

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[2] Rat and Mouse Baked Biscuits, Band G Laboratory Animal Food, Frederick, Maryland.

[3] Packard Armac scintillation detector.

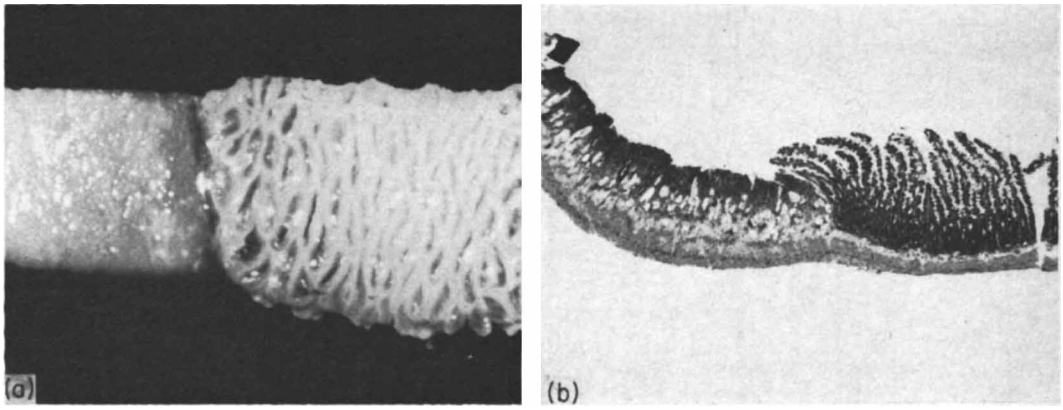


FIG. 1A. Gross photograph of a segment of ileum showing the sharp demarcation between the atrophic mucosa at left and an area with the ridge-like villi that are normal for the rat. The atrophic mucosa stains diffusely for alkaline phosphatase activity, while only the crest of the villi show any activity (×13). (B) Section from specimen in (A) above showing the completely flattened mucosa on the left and the long villi on the right; H and E ×42.5.

longitudinally and repeatedly washed in cold isotonic saline solution until its radioactivity remained constant.

The carcass with the absorbent paper used in blotting was first counted alone, and then following the addition of the liver and finally following the further addition of the washed gastrointestinal tract. The percentage of the administered dose of radioiron absorbed as well as the distribution of absorbed radioiron in the liver and intestinal tract were calculated from these data using the initial radioactivity of each intact animal as measure of the administered dose of radioactivity.

II. In another group of rats (6 irradiated and 2 control) killed 4 days after irradiation and 2 hr after administration of radioiron via gastric intubation, the intestines were excised, opened, and washed carefully as in the first experiments. They were then pinned flat and fixed in 10% cold buffered formalin solution. Following staining for alkaline phosphatase activity, a procedure that facilitates recognition of mucosal lesions (6), the specimens were photographed. Autoradiographs were then prepared by exposure to medical X-Ray film (7). During exposure specimens were separated from the film by a sheet of Saran-Wrap to avoid moisture damage. After 2 weeks of exposure the autoradiographs were developed using standard techniques and were superimposed on the photographs of the gross specimens. This allowed the comparison of the morphologic characteristics with the topographical distribution of radioiron.

III. In a final series of experiments performed 4 days after irradiation, 1 μCi of ^{59}Fe/1 μg ^{56}Fe sulfate was administered intravenously (via the tail vein) to groups of irradiated and control rats. Total body radioactivity was determined in each animal by means of a small animal body counter.[3] Two

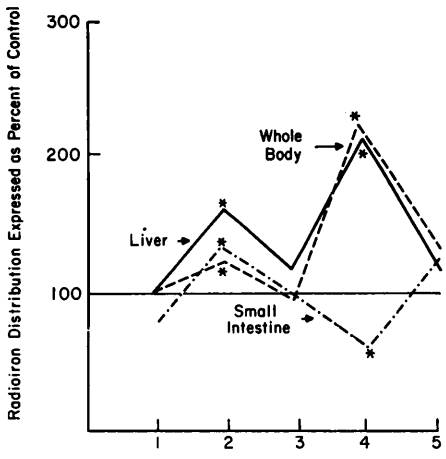


FIG. 2. Intestinal iron absorption and distribution in rats following X-irradiation of the exteriorized intestine. Each point represents the results of determinations on 14–18 rats. The asterisk indicates values with a $p \leftarrow 0.05$ as determined by Student's t test.

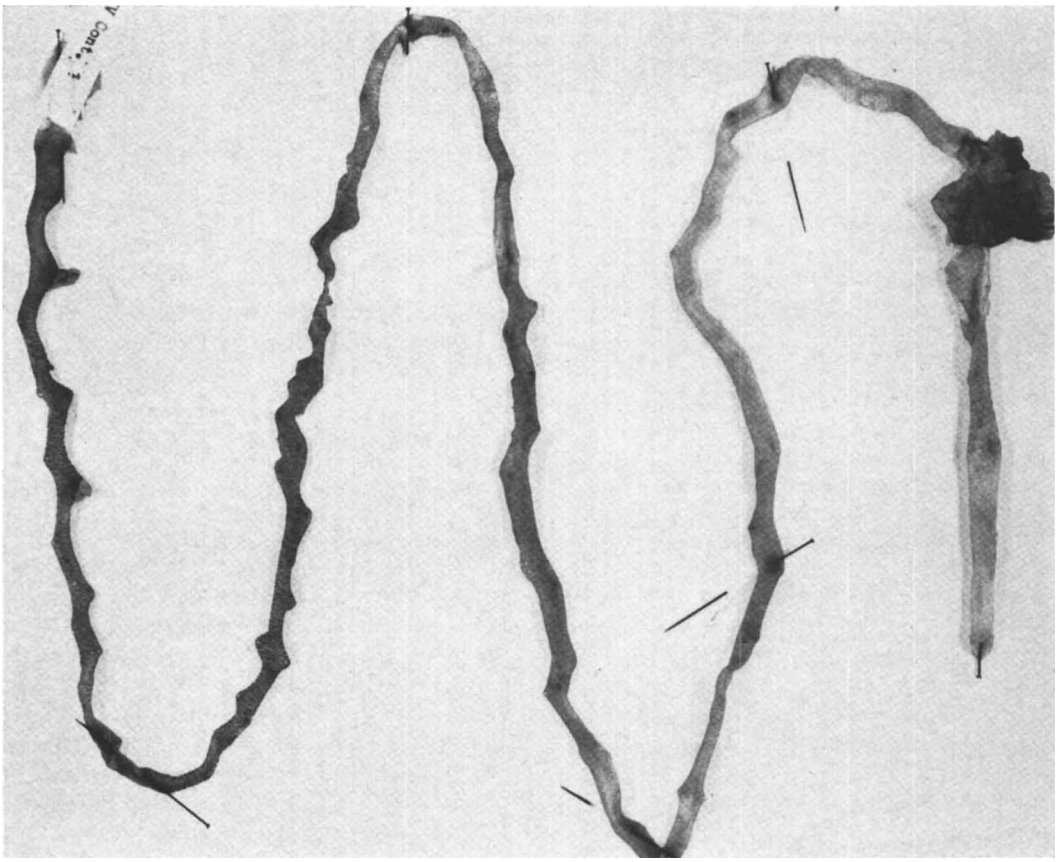
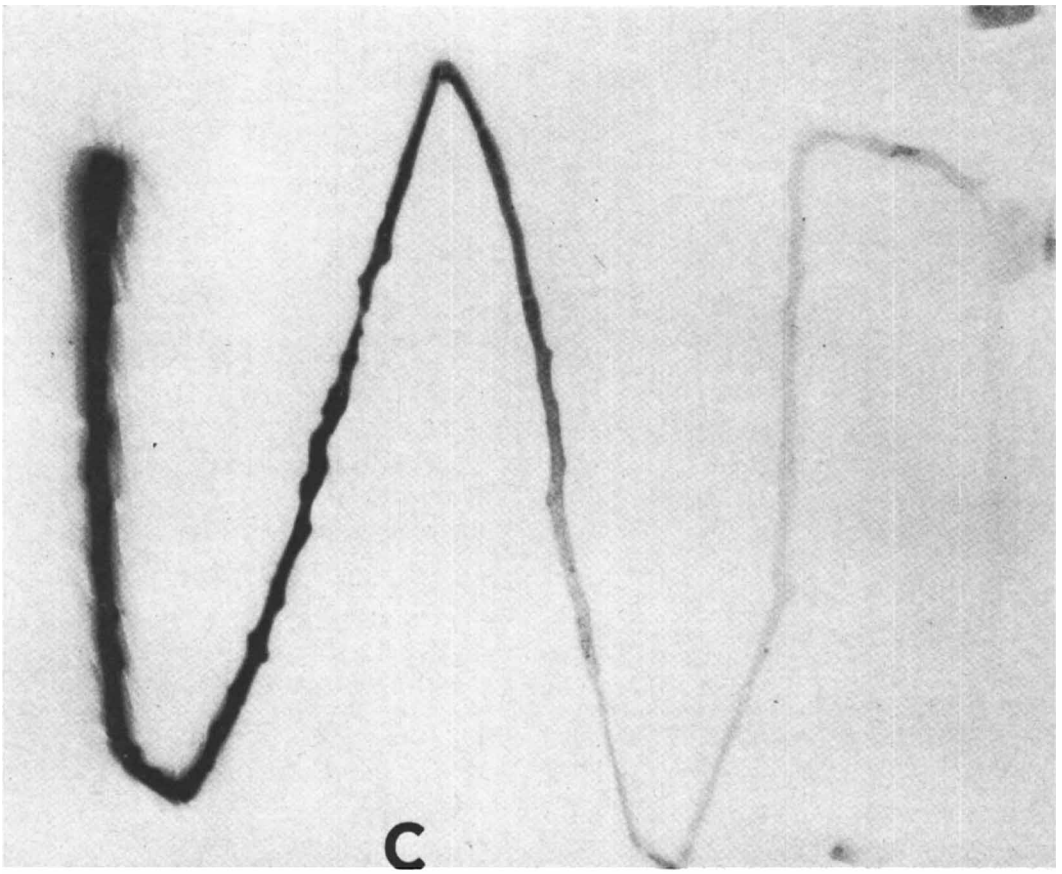


FIG. 3. Alkaline phosphatase stain and ^{59}Fe gross autoradiographs of the normal and irradiated small intestine: (A) nonirradiated small intestine, alkaline phosphatase stain; (B) nonirradiated small intestine, ^{59}Fe autoradiograph; (C) irradiated small intestine, alkaline phosphatase stain; and (D) irradiated small intestine, ^{59}Fe autoradiograph.

hr later the animals were killed, the gastrointestinal tract was removed intact, opened, and repeatedly washed as in the other experiments. The washed intestine was then replaced in the carcass and the amount of radioactivity was determined again. This procedure allowed the determination of radioiron loss through the intestinal mucosa.

Results and Comment. The discontinuous nature of the lesion observed in the irradiated exteriorized small intestine was reported in detail previously (5). The sharp demarcation between atrophic and normal mucosal areas is shown in Fig. 1.

Figure 2 illustrates the whole body absorption of radioiron as well as the distribution of radioiron within the liver and small intestine. By the second day after irradiation iron absorption was increased and the amount in the small intestine and liver was also increased when compared to that of control sham-irradiated animals. On the third day following irradiation there was no essential difference. By the fourth day the total body radioiron had increased markedly as had the percentage of iron in the liver. Interestingly, at this time the amount of the administered dose of radioiron remaining in the wall of the small intestine was decreased, a finding which is in agreement with the findings of Prasad and Osborne (8). By the fifth day the amount of radioiron present in the liver and intestinal wall was approaching the level observed in the control animals.



B

Figure 3 shows a control small intestine and one collected 4 days after irradiation with 2000 R X-rays, with the corresponding autoradiographs. The autographs of the control intestine (Fig. 3B) indicates that the most iron absorption in the rat normally takes place in the upper small bowel with progressively less absorption distally in a descending gradient. These results are in essential agreement with those of Wack and Wyatt (7). In the irradiated intestine (Fig. 3D) the areas which concentrated radioiron and which were presumably responsible for absorption were those areas which had been spared and were hypertrophic, the intensity of the label being actually higher than in the corresponding levels of the control intestines. In contrast, the areas of mucosal atrophy, did not contain radioiron.

The increased absorption of radioiron shows a correlation with the morphological data previously described (5, 9). Villous hypertrophy and the return to normal of various enzymes on the fourth day following irradiation (9) correlate well with the apparently augmented iron absorption seen at this time. The diminished amount of radioiron in the intestinal wall as determined by differential counting reflects the decreased surface through which the iron is absorbed, since the autoradiographs indicate an increased content of radioiron in the absorbing areas. An alternative explanation is that the diminished amount of radioiron in the small intestine at this time is suggestive of a rapid transport of radioiron through the small intestinal mucosa.

Table I presents the data on the loss of an intravenously administered dose of radioiron.

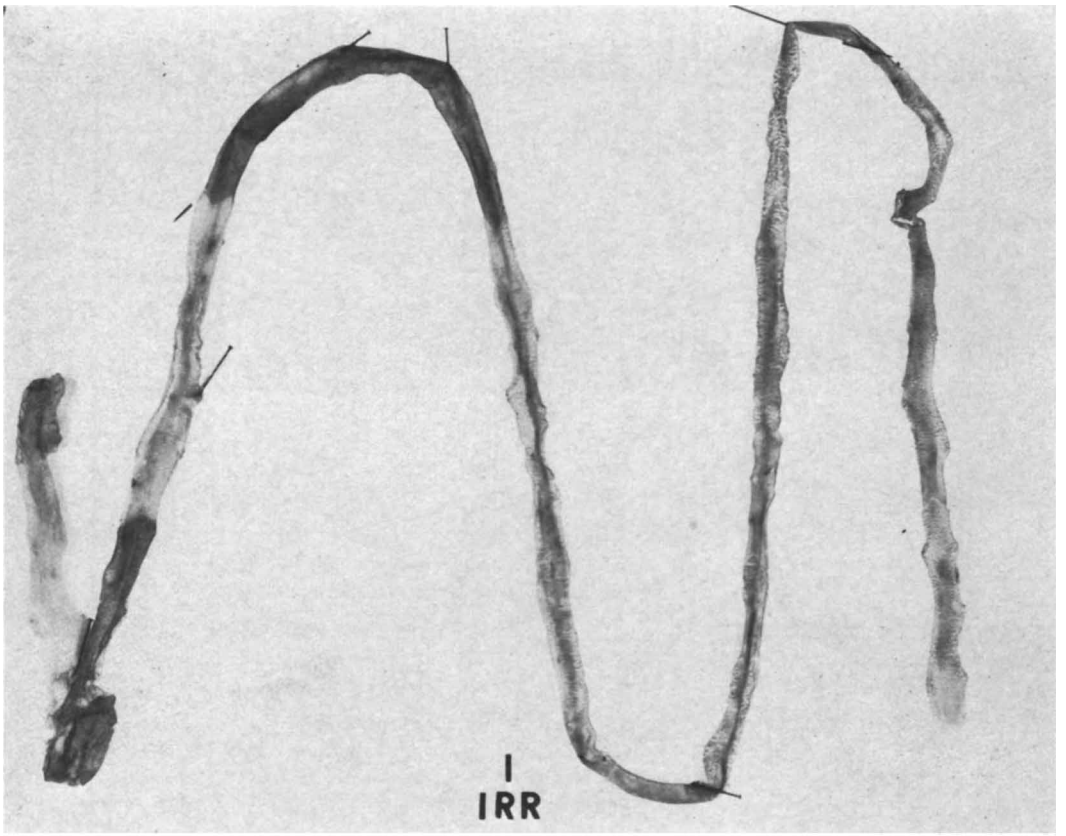


TABLE I. Gastrointestinal Loss of Radioiron.

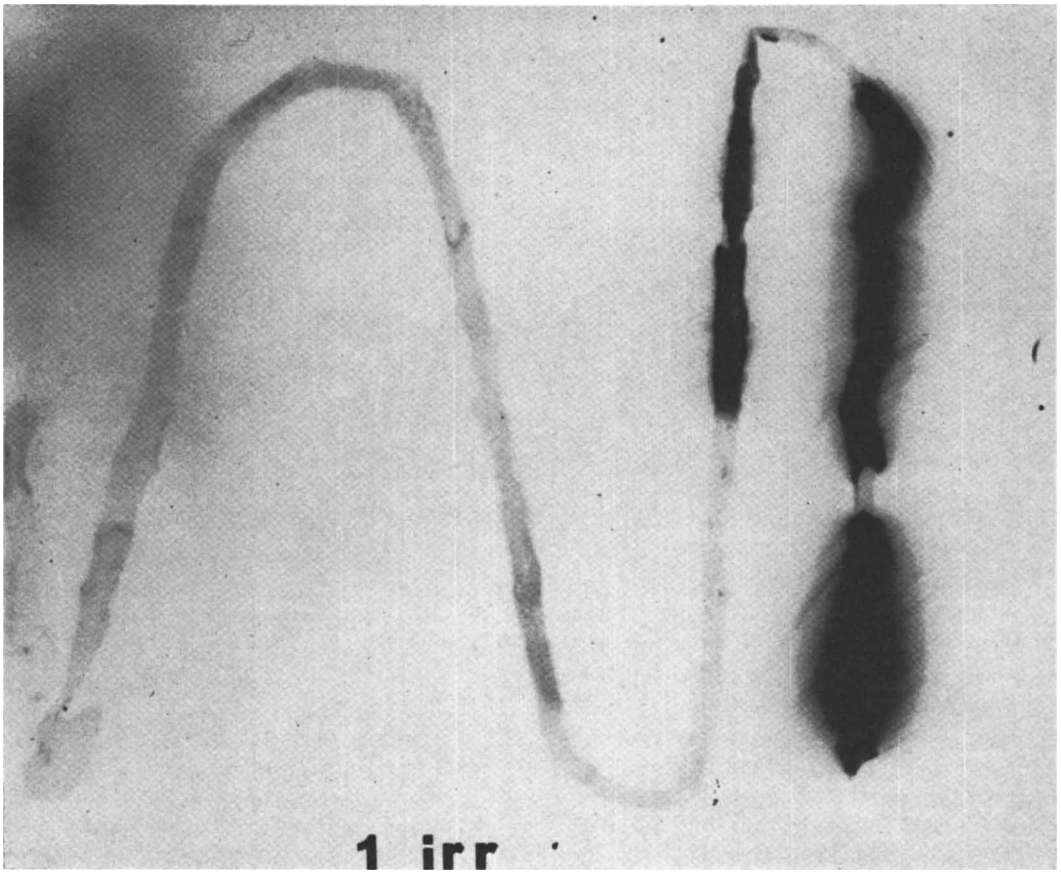
	No. of rats	Body retention (%) in 2 hr ± SD
Control	8	98.4 ± 1.2
Irradiated	9	96.3 ± 2.9

Four days following radiation to the exteriorized gut there was no significant loss of radioiron into the gut lumen suggesting that the epithelial surface of the atrophic mucosa, however defective, may still act as a barrier, and prevents leakage of iron through the intestinal wall.

These data suggest that intestinal absorption of iron is increased following irradiation of the exteriorized small intestine. They further suggest that a functional mucosa is necessary for such absorption as the areas that are responsible for this increased absorption are areas which have escaped the full effects of the irradiation and are actually hypertrophic. The abnormal epithelial cells of the atrophic mucosa, on the other hand, while useless in the absorptive process are still effective as a barrier.

The explanation for the augmented absorption of radioiron in those areas of the exteriorized irradiated intestinal tract which are practically unaffected by radiation is not clear at present. Possible hypotheses include: the actual over-shoot phenomenon resulting in hypertrophy of the villi accompanied by an increase in certain enzymes (9). Alternatively, one may postulate a nonspecific growth stimulator, which affects the mucosa and iron absorption in the same manner as the "growth stimulator" to hepatic growth described by Mendel (10), also increases iron absorption. These possibilities are presently being evaluated in this laboratory.

Summary. The intestinal absorption of ra-



dioiron was determined following X-irradiation of the exteriorized small intestine in the rat. The morphologic lesion resulting was discontinuous, with areas of intestinal radiation injury alternating with areas which were essentially intact or hypertrophic. The results indicate that there was an increase in radioiron absorption following irradiation and suggest that this increase was due to augmented absorption in those areas of the small intestine which did not develop the morphologic characteristics of severe radiation injury. Possible mechanisms are discussed.

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