The arrest of intestinal epithelial 'turnover' by the use of x-irradiation

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SYNOPSIS Mitotic activity and hence cell turnover has been abolished by x-irradiation with 1,000 r. in segments of the rat's gut. The whole stomach, the ascending colon, and 5 cm. of jejunum and ileum were irradiated and the changes observed histologically up to three days. Cessation of cell turnover in the small intestine is followed by cell death and desquamation within 24 hours, leading to denudation of the epithelium and collapse of villous structure. The pylorus and colon showed similar but less marked changes. In contrast the fundus and body of the stomach were unaffected by the absence of mitosis over a period of three days. It is concluded that cell turnover is, in part, necessary to maintain the mucosal structure, particularly the villous pattern in the small intestine.

The gastro-intestinal epithelium is a dynamic structure. Although it appears an intact and lush membrane to histological examination, it is being continuously replaced and desquamated extremely quickly. This replacement of cells results from intense division in the crypts, the cells so formed migrating up the glands and villi to be shed from the surface and villous tips. In the mouse the mucous epithelial cells of the body of the stomach, the pylorus, ileum, and large intestine are replaced daily while those of the duodenum and jejunum are replaced every two days, and those of the squamous epithelium of the fundus of the stomach are replaced every five days (Creamer, Shorter, and Bamforth, 1961). Similar figures have been obtained from the small intestine in other species, including the cat, rat, and man (McMinn 1954; Leblond and Stevens, 1948; Bertalanffy and Nagy, 1958). In the rat stomach, however, the turnover time of the surface epithelium of the body of the stomach was estimated by Stevens and Leblond (1953) to be approximately 68 hours and that of the neck cells to be six and a half days.

Irradiation or the administration of antimetabolic drugs will arrest mitosis and the effect of this on the small intestine has been described (Dustin, 1950; Sullivan, Hackett, Marks, and Thompson, 1958). We decided to use the effect of irradiation on the stomach and small segments of the intestinal tract, including the colon, to review the effects of arresting cell turnover in the rat. This use of small segments obviates the more generalized effects of the 'intestinal radiation syndrome' (Quastler, 1956).

METHODS

Wistar rats of either sex weighing 200 g. were used. The animals were anaesthetized with intraperitoneal pentobarbitone sodium B.P. The segment of gut to be irradiated was delivered through an abdominal incision and its extent marked by fine silk loops. The ascending colon, the whole of the stomach, or 5 cm. of jejunum or ileum were the areas chosen for irradiation in any one animal.

In all instances, after irradiation had been completed, the exposed portion of the gastro-intestinal tract was returned to the abdominal cavity and the peritoneum and anterior abdominal wall were then closed with interrupted sutures. In no case did either the peritoneum or the abdominal wound become infected. After operation the animals were kept in free running cages with full access to chow and water.

Thirty-six animals were used and the animals from each group (stomach, jejunal, ileal and colonic irradiation) were killed at intervals of 24, 48, and 72 hours after irradiation.

The animals were killed by ether anaesthesia and the tissues were dissected immediately. For jejunal, ileal, and colonic sampling the irradiated areas, marked by the silk loops, were removed together with lengths of jejunum, ileum, and colon from non-irradiated areas; samples of stomach were also taken from some animals. The entire stomach was removed from the animals exposed to gastric irradiation. The tissues were fixed in Susa's fixative and subsequently blocked in paraffin wax. Multiple sections were cut at 4 μ thickness and stained by haematoxylin and eosin. The sections were examined by light microscopy.

IRRADIATION TECHNIQUE The anaesthetized animals were placed under a lead shield 1.7 mm. thick so con-

FIG. 1. Apparatus used for exposure of gut to irradiation; loop of jejunum in position.

structed that the loop of bowel could be brought out onto a platform (Fig. 1). The loop lay in a wax trough and the mesenteric vessels were shielded by a lead semi-circle. The bowel was covered by a wax sliver, 2 mm. in thickness, ensuring even back scatter and therefore a homogeneous distribution of the dose. A minor variation in display of the gut was necessary for the stomach.

The technical factors were: x rays at 250 KVp, 15 MA, added filter 0.25 mm. Cu + 1 mm. AL, H.V.L. 1mm. Cu, 8 cm. $\times$ 10 cm. applicator, 40 cm. F.S.D., utilizing the central axis beam. The dose delivered on the top surface of the loop of gut was 1,000 r. The total thickness of the gut varied between 2 mm. and 4 mm., giving a dose variation of a maximum of 3%.

RESULTS

Irradiation appeared to have strikingly different effects in various parts of the gastro-intestinal tract. In all areas mitotic activity was almost totally abolished by 1,000 r. Throughout the gut there was no histological evidence of vascular damage attributable to the irradiation. Wherever epithelial changes occurred an inflammatory exudation was seen, the cellular components of which consisted mainly of histiocytes and eosinophil leucocytes. As the main purpose of this study was to note epithelial changes, no further comment will be made on this inflammatory response.

The histological changes observed have been analysed with respect to mitotic activity, epithelial appearances, and the architecture of the mucosa.

FUNDUS OF THE STOMACH Mitoses were absent at 24 and 48 hours but a few mitotic figures were seen at 72 hours after irradiation. Epithelium appeared normal throughout. The architecture was unchanged.

BODY OF THE STOMACH In the normal animal, mitoses are seen at the junction between the parietal

cells and the cells of the surface epithelium. No mitotic activity was present in this area in any of the irradiated specimens examined.

There was no significant change in the appearances of the epithelium. The architecture was unchanged.

PYLORUS OF THE STOMACH No evidence of mitotic activity was seen after 24 to 48 hours but an occasional cell in mitosis was seen in one animal at 72 hours.

Twenty-four hours after irradiation degenerative changes were seen in the surface epithelial cells, and in the cells of the depths of the glands. Similar changes were present after 48 hours and were more marked after 72 hours. Cells had been shed and free cells were seen in the lumen of the stomach but there was no denudation of the surface. The architecture was unchanged.

JEJUNUM A photomicrograph of normal rat small intestine is included for comparison (Fig. 2).

No mitoses were seen and the cells in the crypts appeared necrotic.

Profound changes were present throughout the epithelium. After 24 hours the epithelial cells over the villi appeared irregular and necrotic (Fig. 3). In some villi massive desquamation of cells had occurred, and the epithelial sheet was separated from the basement membrane by oedema so that the villi were almost entirely denuded. Shed epithelial cells were present in the lumen of the gut. After 48 hours much of the stroma was denuded of epithelium. In other areas villi retained an intact envelope of degenerate epithelial cells separated by oedema from the core. At 72 hours some of the collapsed villi were still covered by very irregular and degenerate epithelium but in other areas the stroma was bare. Large numbers of shed epithelial cells were present in the intestinal lumen at this stage (Figs. 4 and 5).

After 24 hours the villi were still recognizable structures though some showed cores distended with oedema fluid. By 48 hours, however, many villi were completely collapsed. The villi from which the epithelial covering had been lost appeared either as stubby projections or shrunken cores. After 72 hours this collapse was widespread so that villous structure had almost completely disappeared and the surviving mucosa appeared flattened and irregular. Crypts were reduced in depth and greatly diminished in numbers 72 hours after irradiation.

ILEUM The changes in the ileum were identical with those described in the jejunum.

COLON In the colon no mitoses were seen in any of the specimens examined.

FIG. 2. *Rat: Normal small bowel. Haematoxylin and eosin.*

FIG. 3. *Rat: Small intestine 24 hours after exposure to 1,000 r; degeneration of cells, oedema, and early desquamation. Haematoxylin and eosin.*

FIG. 4. Rat: Small intestine; 48 hours after irradiation; marked degenerative epithelial changes with massive shedding are present. Haematoxylin and eosin.

FIG. 5. Rat: Small intestine; 72 hours after irradiation; gross architectural disorganization and denudation of stroma. Haematoxylin and eosin.

FIG. 6. Rat: Colon; 72 hours after irradiation; area of ulceration. Haematoxylin and eosin.

After 24 hours no change was seen in the appearances of the epithelium but at 48 hours the surface cells were irregular and degenerate. Shed cells were present in the lumen of the gut. After 72 hours these changes were more marked with most of the epithelial cells showing necrotic changes.

The normal architecture was retained at 24 hours but after 48 to 72 hours the glands were reduced in depth and ulceration was present in some areas (Fig. 6).

DISCUSSION

These experiments were planned to observe the effects of the arrest of epithelial cell turnover in the gut. The tool we used to arrest mitosis was x -irradiation. It is known that the observable effects of ionizing irradiation are maximal in dividing cells and we have assumed that although there was an effect on mature cells this would not play much part. This assumption is supported by the findings of Dustin (1950) and Baserga and Morsiani (1958) who have described the effects of antimetabolic drugs on the small intestine. Their findings are almost identical

with those produced by the dosage of x -irradiation used in these experiments.

The results show a great variability in response in different areas. The fundus and body of the stomach showed minimal histological response whereas the small intestine exhibited the most marked changes. In order to explain this two factors seem of importance.

First there is apparently a difference in the potential cell life in various areas. When mitosis is stopped, the continuous migration of cells presumably ceases. The epithelial cells of the body of the stomach appear to remain viable up to three days whereas in the small intestine and pylorus the older cells are dead within one day, and all the cells are necrotic within two days. There is some relation here to the normal turnover time because the cells of the fundus and body of the stomach turn over at a slower rate than elsewhere. This would suggest that in the healthy small intestine the cells are at the end of their life when they have reached the extrusion zones whereas in the body of the stomach this does not appear to be the case. In the colon the cells

appear to have at least an extra day of life in hand when they have reached the extrusion zones.

Secondly there is a difference in behaviour of the dead or dying cells in various areas. Shedding from the basement membrane generally occurs but is strikingly apparent in the small intestine. Here the villi are denuded by massive desquamation, whereas in the pylorus and colon fewer cells are lost.

From these findings it is clear that the villi are uniquely vulnerable to epithelial loss. The collapse and disappearance of the normal villus shape after cell desquamation shows that its form is almost entirely due to an intact epithelium.

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