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A PRELIMINARY STUDY OF THE ROLE OF GASTROINTESTINAL ENDOCRINE CELLS
IN THE MAINTENANCE OF VILLOUS STRUCTURE FOLLOWING X-IRRADIATION

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Abstract

The mechanism of gastrointestinal villous damage following ionizing irradiation is complex. Various compartments within the gastrointestinal tract have in turn been considered important for the maintenance of normal villous structure. To date, however, evidence for a single overriding regulator of epithelial well-being is lacking.

In this study, the role of the gastrointestinal (enteroendocrine) cells is explored and comparison made between endocrine cell number and villous structure.

Experiments were organised using both control and irradiated groups of mice. Two time points (1 and 3 days) and three radiation doses (6, 10 and 18Gy) were employed. A simple method for endocrine cell identification and subsequent quantification is described. Endocrine cell number was then compared with villous surface detail, as seen with a scanning electron microscope (SEM).

Results indicated a decrease in the endocrine cell number at all three radiation doses. Whereas at low doses endocrine cell recovery occurred between 1 and 3 days, at medium and high doses further decline was noticed. A similar pattern was seen when considering villous surface structure.

It is suggested that both scanning electron microscopy and endocrine cell number provide a more sensitive indicator of gastrointestinal radiation damage than do current crypt counting techniques. In addition, a link between endocrine cell number and villous structure is proposed.

KEY WORDS: Scanning electron microscopy, light microscopy, X-irradiation, enteroendocrine cell.

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Introduction

The gastrointestinal epithelium is constantly undergoing rapid renewal under the influence of a variety of factors. Bizzozero predicted this mechanism as early as 1888, but it was not until the arrival of autoradiographic methods in the late 1950s that details of epithelial cell kinetics were fully appreciated (Hughes et al., 1958; LeBlond and Messier, 1958; Quastler and Sherman, 1959). More recently a clearer picture has built up as to the pattern of gastrointestinal renewal and of the sequential events occurring during the processes of cell proliferation and maturation (Lipkin, 1965). Interest has also stimulated research into the factors responsible for the regulation of gastrointestinal renewal, and how this renewal may be altered by physiological, pharmacological and physical stimuli, as well as by disease processes (Eastwood, 1977).

The regulation of gastrointestinal epithelial proliferation is complex. Structural changes are noted both during gestation (Hermos et al., 1971) and at weaning (Koldovsky et al., 1966), and it is probable that early intimate contact with intra-luminal micro-organisms may influence subsequent epithelial growth (Abrams et al., 1963; Cook and Bird, 1973).

Food, itself, may play an important role in the regulation of epithelial renewal (Willems et al., 1971), with starvation depressing both DNA synthesis and cell proliferation, in addition to altering the number of proliferating cells (Brown et al., 1963; Altmann, 1972).

Precise mechanisms for such gastrointestinal adaptive changes are as yet unclear. For several years the role of the intrinsic gastrointestinal hormones as modulators of both structural and proliferative responses within the gut has been explored (Johnson, 1974; Oscarson et al., 1977), and evidence for the "trophic action" of many of these hormone groups have already been presented (Johnson and Guthrie, 1974; Bloom et al., 1979; Williamson et al., 1978; Willems et al., 1980). If it is accepted that the gastrointestinal endocrine cells do exert a regulating effect upon the growth and proliferation of the gastrointestinal epithelium, then in circumstances when this epithelium is damaged, the relative survival and

continued hormone production from this specific cell group may itself be instrumental in the subsequent survival and continued proliferation of a healthy epithelial lining.

The gastrointestinal epithelium, being a rapidly renewing tissue, is highly susceptible to damage from ionizing irradiation and has provided a popular model for the study of both acute and chronic irradiation effects. Although current knowledge of both the gross and microscopic changes encountered within the gastrointestinal tract is highly advanced (reviewed by Potten et al., 1983), few reports have appeared concerning the effects of ionizing irradiation on either the number or morphology of the gastrointestinal endocrine cells. In addition, their role in the subsequent repair and regeneration of X-ray damaged tissue is not known.

The present study was designed to investigate the relationship between endocrine cell number and epithelial damage following X-irradiation of mouse duodenum. Epithelial damage was assessed as an alteration in crypt number or morphology, or as changes to villous topography.

Materials and Methods

52 female mice (Hacking and Churchill Ltd.) were housed in a controlled laboratory environment and provided with food and water 'ad libitum'. At 12 weeks the animals were allocated to either a control or an irradiated group. The control group was divided into seven subgroups, according to diet control, sham-irradiation and ether sedation (Table 1). The irradiated group was divided into six subgroups according to radiation dose (Table 1). Animals were killed at 1 and 3 days by cervical dislocation.

Diet control

A single subgroup of control animals was selected for diet control. This was designated the 'starved' group. Each animal was kept separate from food for 24 hours prior to killing. Subsequent results were compared with those from a non-fasted or fed animal group.

Ether sedation

Prior to either real or sham-irradiation, each animal was sedated with laboratory ether. To identify any effect the ether itself may have on subsequent results, experiments were repeated using ether sedation and sham-irradiation alone (Table 1).

X-irradiation

Ether sedated mice were restrained in a jig consisting of a perspex base onto which four animals were pegged by their legs. Lead sheets (3mm thick) shielded the mice leaving their abdomens exposed. Total doses of 6, 10 and 18 Gy of X-irradiation were delivered ventro-dorsally at a dose rate of 1.366Gy/min. using a Marconi X-ray machine operating at 250kVp (filtration 0.3mm Cu, 1.0mm Al; HVL 1.2mm Cu). The width of abdomen exposed to the radiation beam was 3.5cm with the abdominal centres lying on a 9cm radius from the beam central axis. Sham-irradiated control mice were restrained in an identical manner for equivalent lengths of time.

Tissue Preparation

Immediately after killing laparotomy was performed and the small intestine isolated. The duodenum was removed, fixed in 5% glutaraldehyde (Carr and Toner, 1972) and processed for light and scanning electron microscopy.

Scanning Electron Microscopy

The proximal duodenum was isolated, opened along its antepancreatic border and pinned onto cork boards. Following a 1 hour post-fixation in 1% osmium tetroxide, the samples were dehydrated through an ethanol series and saturated with amyl acetate (Carr et al., 1981). They were then critically point dried with liquid CO₂ before being mounted on aluminium stubs with graphite paint and placed in a desiccator to dry overnight. The dried, mounted tissue was sputter coated with gold and examined with a JEOL T 300 Scanning Electron Microscope. Photomicrographs were taken at a constant accelerating voltage of 25kV., using a standard spot size, and with the stage set at 45°. Standard magnifications were employed.

Light Microscopy

Following fixation, rings of tissue 3mm thick were removed from the mid-points of each duodenum and placed in Millonig's buffer (pH = 7.3). Specimens were then hydrated through alcohols (Carr et al., 1981) and soaked in two changes of propylene oxide (P.O.) for a total of 1 hour. They were then transferred to a 1:1 mixture of P.O./ Spurr resin for 3 hours and finally placed in a 1:3 mixture overnight at room temperature. The following morning they were embedded in Spurr resin and cured overnight in an oven at 40°C. The temperature was then increased to 60°C for 48 hours, by which time the resin had hardened sufficiently to allow sectioning.

The resultant resin block was precisely orientated in the jaws of a Cambridge microtome and 1.5µm semi-thin transverse tissue sections cut. Sections were stained with Azur II (a basic dye)

In order to assess the number of tissue sections required to give a representative tissue sample, a cumulative mean plot was constructed per animal. Using these plots, the minimal sample size was estimated to be 15. For each animal, a total of 15 sections were cut, leaving at least 20µm between sections.

Tissue Examination and Quantitative Histology

Semi-thin sections were examined using a Vickers binocular microscope fitted with X10 eyepieces and objective lenses of X4, X10 and X40. The structure of both villi and crypts was recorded for each animal and irregularities noted. Photomicrographs were prepared for each subgroup using an Orthoplan light microscope fitted with a Leitz Vario-orthomat meter and shutter.

For each tissue section, counts were made of the number of crypts and endocrine cells per circumference. Crypts were counted according to the criterion initially proposed by Withers and Elkind (1970): "The presence of 10 or more clustered chromophile cells per crypt section, excluding Paneth cells, is considered as one crypt, whether or not it be longitudinally, transversely or obliquely sectioned". Endocrine cells occurring within the crypt epithelium were

also counted. All endocrine cells were included, irrespective of plane of section. Mean values were calculated per mouse.

In a comparative study, values for endocrine cells/circumference are meaningful only if the crypt number per circumference remains constant. This problem was overcome by calculating the number of endocrine cells/crypt using the formula,

$$\text{Endocrine cells/crypt} = \frac{\text{Endocrine cells/circ.}}{\text{Crypts/circ.}}$$

The values given in the text are means, with standard errors from 4 mice. A one way analysis of variance was employed to determine the statistic 'F'. When a significant difference was identified, a Tukey's HSD test was employed to detect the magnitude of this difference.

Results and Discussion

The quantitative, histological and morphological findings are described and discussed for both control and irradiated groups. Scanning electron microscopy has been extensively utilised over the past 20 years in order to build up a more precise picture of the alterations in villous surface structure occurring after different types, doses and regimes of irradiation (Carr et al., 1981). The present study, although primarily confirming previous observations, was able to provide additional information as to the nature of villous tip damage, which had initially been noted with light microscopy.

Controls

Histological appearances were very similar in all specimens (Fig 1). The villi were tall and erect, each with a normal pattern of villous folding. Goblet cells were clearly visible, with mucus wisps occasionally observed extruding from their mouths. Occasional endocrine cells were noted within the villous epithelium of all specimens, although in much reduced numbers than in the crypts. This is in keeping with the work of other authors, who with the aid of autoradiographic methods have established that most of the gastrointestinal endocrine cells are present within the cryptal compartment (Ferreira and LeBlond, 1971).

The cryptal compartment of all the control groups were relatively constant in their appearance (Fig 2). At their bases Paneth cells were readily identified by virtue of their apical dark secretory granules. The enteroendocrine cells appeared as clear pyramidal cells, outlined against a dark blue background. Granule staining was not intense but around the nucleus, and especially within the basal cytoplasm, a mottled or foamy appearance was noted, indicating the presence of contained secretory granules.

Other pale staining cell types within the duodenal epithelium were identified as :-

(i) intra-epithelial lymphocytes; these were generally smaller than the endocrine cells, possessing little if any cytoplasm and darker nuclei.

(ii) extra-epithelial connective tissue cells such as fibroblasts.

In the "sham/ether/1 day" group, the frequency of occurrence of endocrine cells was

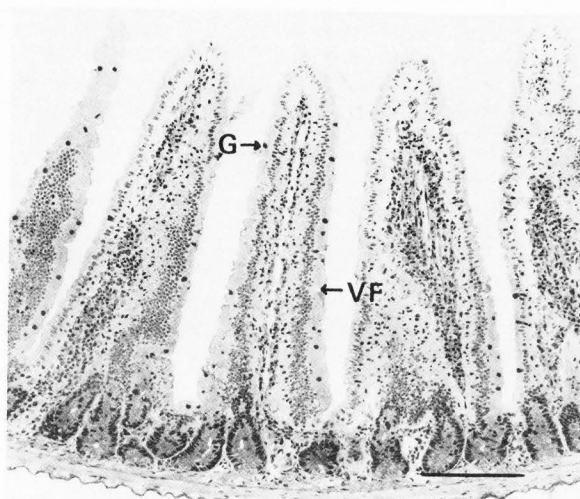


Figure 1. Light micrograph of absolute control mouse duodenum. The villi are tall and erect with a normal pattern of villous folding (VF). Goblet cells are clearly shown (G). Bar = 10µm.

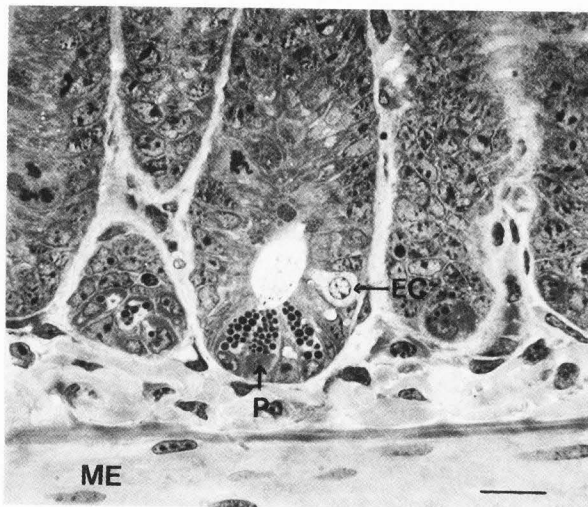


Figure 2. Light micrograph showing a high powered view of absolute control mouse duodenum. A single enteroendocrine cell is present within the epithelium of the central crypt (EC). Paneth cell (P), muscularis externa (ME). Bar = 1µm.

significantly increased in all 4 animals (Table 2, $F = 14.15$, $q = 5.69$, $p < 0.01$). Since both sham-irradiation and ether sedation alone failed to produce this significantly increased endocrine cell count (Table 2), the observed rise must have been due to the combination of the two factors. The cause is not understood at present.

Ether is a noxious substance, capable of inducing a hyperexcitable state when administered to both laboratory animals and humans (Crossland, 1980). This hyperexcitability, when combined with the stressful situation of restraint within the irradiation jig, may result in the degranulation

of a hidden element of the endocrine cell population, hitherto undetected using Azur II. If this is so, then endocrine cell counts within the control group are an underestimation.

No qualitative differences were found between animals starved or fed for 1 day prior to sampling (Table 2). This suggests that any post-irradiation anorexia is unlikely to influence significantly the final results.

The appearance of control villi visualised using scanning electron microscopy has been well documented (Toner and Carr, 1969; Carr and Toner, 1972; Friberg, 1980; Stenling et al., 1984). In the present study, except for a random bacterial presence on a number of villi, resulting in occasional villous epithelial damage, the majority of the villi appeared normal. In many of the specimens lateral collapse was noted, a common finding in control animals (Carr, 1981).

The major difference between the various control groups appeared to be diet related. In the fasted animals individual villi were thin and well separated; their surfaces were clean and relatively free from food or debris (Fig. 3). In the fed group, because the villi were in the process of active nutrient absorption, they tended to be more swollen and tightly packed together (Fig. 4). In addition, much food and mucous debris was present. A similar picture has been described by Friberg (1980), although his work concerned rat ileum rather than duodenum.

Irradiated

1 day. Crypt counts remained normal 1 day after either 6, 10 or 18Gy of X-irradiation. Following all three radiation doses the villi remained erect, although the pattern of villous folding gradually reduced with increasing dose. Epithelial-stromal stripping (Fig.5) was noted after 10 and 18Gy. This is thought to reflect radiation damage to the basal laminae of both epithelial and endothelial structures (Carr et al., 1984)

The numbers of endocrine cells were significantly reduced from the absolute control value at 1 day after all three radiation doses ($F = 40.36$, $q = 5.5$, $p < 0.01$). There was, however, no significant difference between the individual irradiated groups themselves (Table 2). Crypt epithelial cell death, although not quantified, became more noticeable with increasing dose of radiation. Although this is likely to be a direct radiation effect, the possibility that the loss of epithelial stimulation due to decreasing levels of gastrointestinal hormones may be modifying the picture must be considered. To date, the extent of this modification is unknown.

3 days. After 6Gy of X-irradiation epithelial damage was minimal. Although dead and dying cells were readily identifiable within the crypt bases and mitotic figures were plentiful, both crypt counts and endocrine cell numbers remained constant (Fig. 6, Table 2).

It is interesting to compare this minimal amount of cell damage, occurring 3 days after X-irradiation, with the normal compliment of crypt endocrine cells. Indeed, at a slightly lower dose of 4Gy, Pentilla et al. (1975) have shown that, along with the relative radioresistance of duodenal endocrine cells, cytoplasmic serotonin

Table 1

Experimental Groups	n.	Time killed
<u>Controls</u>		
Absolute	4	1 day
'Starved'	4	1 day
Fed	4	1 day
Sham irradiation/ether	4	1 day
Sham irradiation only	4	1 day
Ether only	4	1 day
<u>Irradiated</u>		
6 Gy	4/4	1 day/3 days
10 Gy	4/4	1 day/3 days
18 Gy	4/4	1 day/3 days

Table 2

Experimental groups	Crypts/circ.	E cells/crypt
<u>Controls</u>		
Absolute	138.7 ± 5.64	0.291 ± 0.022
Fed/1 day	127.9 ± 3.39	0.282 ± 0.031
Starved/1 day	129.2 ± 5.40	0.282 ± 0.008
Sham/Ether/1 day	136.3 ± 5.00	0.484 ± 0.019
Ether only/1 day	138.7 ± 4.59	0.289 ± 0.026
Sham only/1 day	130.6 ± 5.85	0.331 ± 0.022
Sham/Ether/3 days	136.9 ± 3.31	0.339 ± 0.021
<u>Irradiated</u>		
6Gy/1 day	127.4 ± 5.45	0.162 ± 0.010
10Gy/1 day	128.7 ± 3.50	0.141 ± 0.012
18Gy/1 day	129.0 ± 4.50	0.125 ± 0.007
6Gy/3 days	133.5 ± 7.23	0.258 ± 0.021
10Gy/3 days	107.4 ± 5.20	0.109 ± 0.014
18Gy/3 days	24.7 ± 0.82	0.064 ± 0.027

(5HT) levels decrease. This decrease may be due to increased hormone secretion, the released 5HT being actively responsible for protecting the surrounding epithelial structures against radiation damage (Gray et al., 1952; Melching et al., 1958). Although levels of 5HT have not been studied at higher radiation doses, a similar mechanism is thought to exist, thus resulting in an appreciable modification to any radiation induced effect by the surviving compliment of endocrine cells.

As the radiation dose was increased, epithelial damage increased. Villous folding was reduced and epithelial-stromal stripping became more apparent at the villous tips. The crypts showed a progressive destruction both in number and structure. Endocrine cell counts were also greatly depressed (Table 2) with both the 10Gy/3 day and the 18Gy/3 day groups varying significantly from the absolute control and the 6Gy/3 day groups ($F = 36.36$, $q = 5.5$, $p < 0.01$).

Although ionizing irradiation was itself directly responsible for many of the observed microscopical changes in epithelial structure at both 1 and 3 days post-irradiation, the role of the surviving endocrine cells in modifying such damage remains obscure. In this study, a gradual decline in endocrine cell number occurred with increasing radiation dose. Figure 7 illustrates the dose response curve for the number of

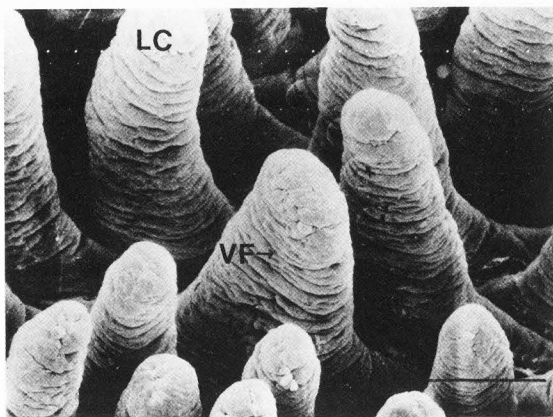


Figure 3. Scanning electron micrograph showing control villi in fasted animals. Individual villi are thin and well separated. Their surfaces are relatively free from food or debris. A normal pattern of villous folding (VF) is shown along with lateral collapse (LC). Bar = 10 μ m.

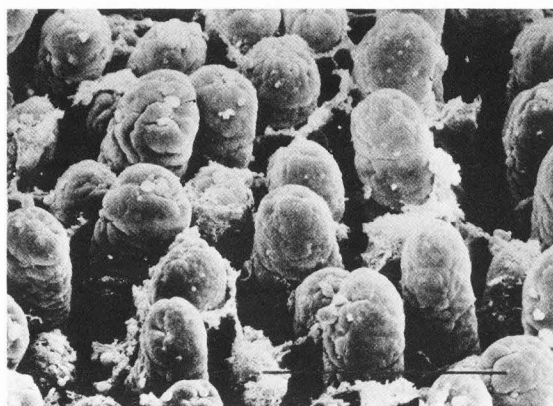


Figure 4. Scanning electron micrograph showing control villi in fed animals. The villi are swollen and closely packed. Much debris is apparent. Bar = 10 μ m.

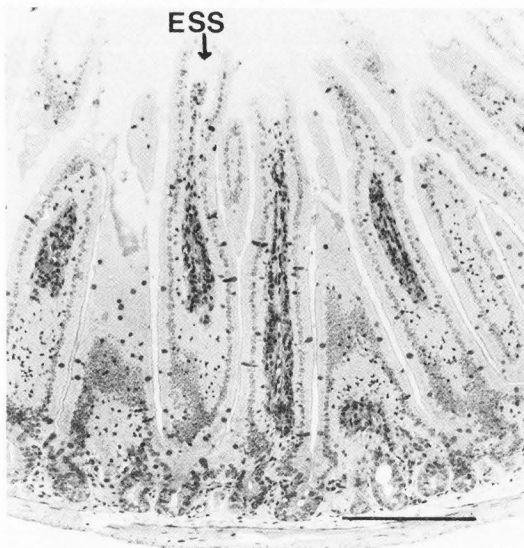


Figure 5. Light micrograph of mouse duodenum one day after 10Gy of X-irradiation. The villi remain erect, but villous folding is less apparent. Epithelial-stromal stripping (ESS) is noticeable. Bar = 10 μ m.

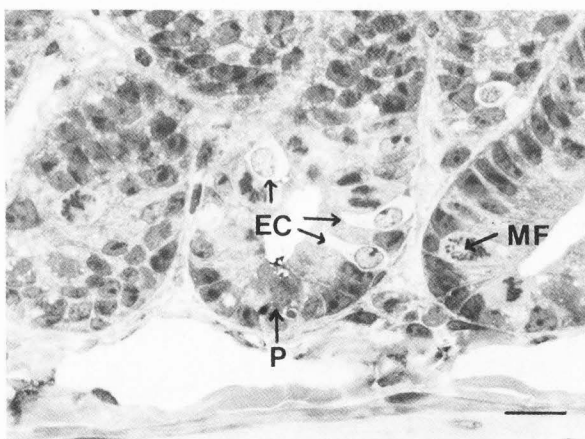


Figure 6. Light micrograph showing a high power view of mouse duodenum 3 days after 6Gy of X-irradiation. Endocrine cells (EC) are clearly visible within the crypts. Mitotic figures (MF) are prominent. Paneth cells (P) remain unchanged. Bar = 1 μ m.

endocrine cells/crypt at each time tested. As can be seen, there was some recovery 3 days after 6Gy, whereas following 10 and 18Gy a further decline was noted with time. At the point where the two curves meet, i.e. approximately 9Gy, crypt counts were just beginning to fall (Hume and Marigold, 1986).

When considering qualitative changes induced by ionizing irradiation, it was of interest that a similar pattern occurred. Beyond 9Gy, i.e. 10 and 18Gy, severe epithelial destruction was observed. Below 9Gy, however, villous damage was minimal and often not recognisable with the light microscope alone.

It is, therefore, clearly apparent that both villous structure and crypt morphology are closely related to changes occurring within the cryptal endocrine cell population. If following irradiation endocrine cell recovery occurs, epithelial damage remains minimal. If, however, the radiation

dose is severe enough to produce continued endocrine cell destruction, both cryptal and villous epithelial elements show progressive degenerative changes, eventually leading to the animal's death.

All of the irradiated specimens exhibited a number of changes in surface appearance, which became more marked with both increasing dose and time.

After low dose irradiation the changes observed were subtle. For example, many of the villous tips became smooth, losing their pattern

of surface folds (Fig. 8). The observation of villous damage occurring at these low irradiation doses was originally presented by Hamlet et al. (1976), who reported slight distortion of the villous tips with swelling and broadening of their bases after a dose as low as 3Gy of gamma irradiation. For the 6Gy of X-irradiation used in the present work, a combination of the above appearances was noted, both villous swelling and tip distension occurring. With increasing irradiation dose, the villi become swollen and tightly packed together (Fig. 9). The mechanism for this swelling is unclear; the combination of increasing epithelial oedema, epithelial-stromal stripping and vertical collapse may all contribute to the eventual appearance.

A further feature of increasing dose was that villous creasing or folding became less prominent. With increasing time a similar pattern was observed except at 6Gy where, although altered from the controls, further loss of villous folding did not occur between 1 and 3 days. To date, no satisfactory explanation has been proffered for the occurrence of villous folding. Transmission electron microscopy (TEM) shows that at their deepest parts the folds have shorter than average epithelial cells; it has been suggested that each crease or fold may be produced by the existence of rows of such short cells (Carr et al., 1982). Removal of this creasing pattern by irradiation suggests that the villus has lost part of its epithelial-stromal integrity, a condition similar to that of epithelial-stromal stripping observed at the villous tips.

Carr et al. (1982) have further suggested that the creasing was caused by strong linear attachment of the inner aspect of the basal lamina of the villous epithelial sheet to the connective tissue or smooth muscle cells of the stromal compartments of the villus. It is already known that the smooth muscle cells within the intravillous connective tissue may contribute to the pumping motion of the villi (Sessions et al., 1968) If they also contribute towards the maintenance of villous structure, then the epithelial-stromal stripping produced by ionizing irradiation may be responsible for the decreased villous creasing patterns noticed with both light and scanning electron microscopy.

Comparison between histological and surface change.

This study has shown that both scanning electron and light microscopy are able to provide valuable information concerning the effects of ionizing irradiation on the intestinal villi. The light microscope clearly has advantages when commenting on both cryptal and stromal reactions. It is also able to provide information concerning the full thickness of the villous epithelial sheet, rather than the luminal surface alone. Also, the appearance of epithelial-stromal stripping and details concerning crypt numbers and morphology are not appreciated with scanning electron microscopy. The scanning microscope does, however, have its advantages. It is able to provide a more precise appreciation of villous surface detail, including the size and shape of the villus, its degree of swelling and its state

of erectness. At higher magnifications it is able to provide valuable information concerning epithelial cell structure, maturation and eventual cell death.

It is, however, at lower radiation doses that scanning electron microscopy realises its greatest advantage. At radiation doses below the threshold of crypt counting techniques the scanning electron microscope is able to show certain qualitative alterations in villous structure not apparent with light microscopy (Hamlet et al., 1976). This important role for the scanning electron microscope has been highlighted in the present study, changes in villous structure being clearly visible both at 1 and 3 days after 6Gy of X-irradiation.

Although light microscopy was not able to show similar alterations to villous structure after 6Gy of X-irradiation, an isolated cell type, namely the enteroendocrine cell within the epithelium of both crypts and villi, was seen to respond. At both time points crypt counts remained normal.

Since many of the endocrine hormones secreted by these enteroendocrine cells are known to stimulate epithelial growth (Johnson and Guthrie, 1976), and since a relationship between endocrine number and subsequent epithelial damage has been shown, it is plausible that these two observations are related. If villous regeneration is dependent upon a healthy epithelial endocrine cell population, then in situations when endocrine cell numbers are not maintained, epithelial cell damage would be seen to increase. This precise situation was observed at 10 and 18Gy of X-irradiation. At each dose endocrine cell counts continued to fall from 1 to 3 days paralleling several epithelial degenerative changes. Although this study has provided much circumstantial evidence in favour of the enteroendocrine cells role in the maintenance of normal villous structure following X-irradiation, further work is required in confirmation.

Conclusions

1. Qualitative light microscopy is relatively unreliable in detecting epithelial damage at low doses of radiation.
2. Both scanning electron microscopy and cryptal endocrine cell counts provide a more sensitive indicator of gastrointestinal radiation damage than do current crypt counting techniques.
3. A link between cryptal endocrine cell number and gastrointestinal epithelial structure is proposed.

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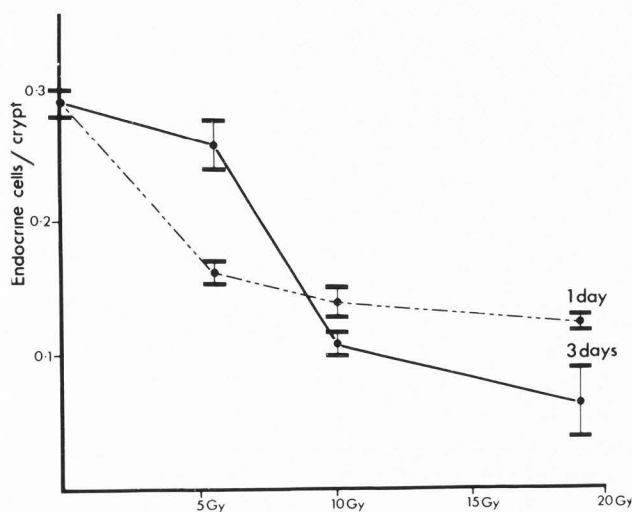


Figure 7. Graph showing the number of endocrine cells/crypt for the irradiated experimental groups at 1 and 3 days (n = 4/group).

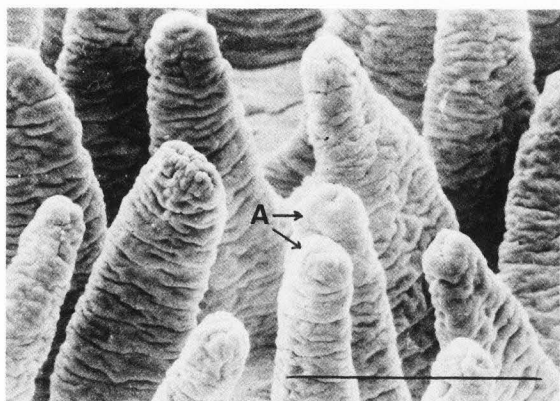


Figure 8. Scanning electron micrograph showing duodenal villi 1 day after 6Gy of X-irradiation. The villous tips are smooth and swollen (A). Bar = 10µm.

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Figure 9. Scanning electron micrograph showing duodenal villi 1 day after 18Gy of X-irradiation. Surface debris is abundant. The villous tips are smooth and swollen. Bar = 10µm.

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Discussion with Reviewers

C.P. Sigdestad: The authors show a clear correlation between morphology and endocrine cell number. What is less clear, however, is if the cited correlation relates in a significant fashion to the maintenance of villous integrity after radiation. Said in another way, can the correlation exist and still have no cause and effect relationship with the morphological changes noted after irradiation?

Authors: In this preliminary study we have shown that, in addition to changes in crypt morphology and villous topography, the number of gastrointestinal endocrine cells also decreases following X-irradiation. Since many of the hormones secreted from this specific cell group

are known to have a trophic action within the gastrointestinal tract (Johnson and Guthrie, 1976; Jacobs et al., 1976; Bloom et al., 1979; Creutzfeldt et al., 1971), it would seem highly probable that the above observations are related. To date, however, there is no hard evidence to support categorically this 'cause and effect' relationship and further work is required in its substantiation.

C.P. Sigdestad: One troublesome area is that there appears to be a synergistic effect (at 1 day) in the number of endocrine cells per crypt in the sham irradiated and ether sedated mice. In addition, the dose response is non-existent at 1 day and covers only $\frac{1}{3}$ of a decade on day three post-irradiation.

Authors: We agree that this apparent synergism seen in the sham irradiation/ ether sedation/ 1 day group is difficult to explain and do not ourselves fully understand its significance. Nevertheless, it does represent an increase in endocrine cell number as opposed to the decrease noted following irradiation. If this elevated value (the true control with which to compare the ether sedated/ irradiated/ 1 day animals) had been used in the calculations rather than the more believable absolute control value, a significant decrease in endocrine cell number would have been noticed after all three radiation doses. However, since this 'control' value was so elevated, rightly or wrongly it was disregarded and the absolute control used for subsequent statistical analysis.

C.P. Sigdestad: It is difficult for the reader to follow the argument that the trophic action of the endocrine cells is responsible for crypt regeneration. It is difficult to envisage how surviving crypts can enlarge to 2.5 times their normal size, as we have shown on numerous occasions three days after irradiation, when the cells allegedly responsible are reduced to approximately $\frac{1}{3}$ of the number noted in the controls.

Authors: Although the actual number of endocrine cells within the cryptal epithelium is reduced following X-irradiation, the relative activity and subsequent hormone production from the surviving cell fraction may be adequate to produce such changes. These highly specialised 'switched on' cells may themselves be directly responsible for directing subsequent epithelial growth and regeneration.

F. Bonvicini: As regards crypt morphometry, what is the authors' opinion on the importance of evaluating the radiation effects not only on the number of crypts but also on their depth, and, in particular, on the extension of the proliferative compartment?

Authors: Crypt depth is being studied in relationship to radiation dose and will form the basis of a future presentation. In addition, we are looking at changes in endocrine cell distribution within the cryptal compartments following X-irradiation and trying to compare these differences with alterations to both crypt size and number.

F. Bonvicini: Would it be interesting to study not only duodenum but also more distal parts of the intestine, and therefore the cells secreting enteroglucagon, another hormone which is presumed to have a trophic effect on intestinal mucosa?

F. Bonvicini: Do the authors not think it would be interesting to verify whether there is one type of endocrine cell in particular which decreases in number? For example, what about the immunohistochemical identification of cells secreting gastrin, a hormone to which a major role in mucosal trophism has been attributed?

Authors: Now that a decrease in endocrine cell number has been demonstrated, we agree that the use of more specific methods to detect individual cell types would be helpful. Assays for both gastrin and enteroglucagon would primarily be involved.

M.S. Al-Tikriti: Please comment on why you used mid-duodenal samples for LM and proximal samples for SEM. Why were not both areas studied using both microscopes and their data compared?

Authors: Mid-duodenal samples were used for endocrine cell quantification in order to standardise the results. When constructing the experimental method we were extremely aware of the differences in endocrine cell distribution encountered at different levels within the intestine (Josephson and Altmann, 1973). In comparing individual animals it was therefore important to select a constant portion of the gastrointestinal tract. The duodenal mid-point provided that confidence. Samples for SEM were taken immediately adjacent to this area in order to compare endocrine cell number and villous structure within the individual as well as for the experimental group. However, morphological differences within this small area are not significant and will not affect the result.

M.S. Al-Tikriti: Is there any preference for using females only?

Authors: No. Animals were matched for age and sex primarily to ensure reproducibility of results.

M.S. Al-Tikriti: What was the idea behind using 6, 10, 18Gy, and would it be appropriate to use 6, 12, 18Gy so we can say double or triple dose?

Authors: The response of both villous structure and crypt number to irradiation, although dose dependent, is not linear (Hume and Marigold, 1986; Carr et al., 1984). Radiation doses were chosen to lie on the three main portions of the crypt survival curve for X-rays, namely the plateau, shoulder and exponential portions (Hume and Marigold, 1986). A dose of 12Gy, replacing 10 Gy would not have met this criteria.

M.S. Al-Tikriti: Why were no counts of mitotic figures taken and analysed?

Authors: The analysis of mitotic figures, although interesting, was not considered of primary importance for this study.

M.S. Al-Tikriti: What is the first signal for

hormone production? Does the death of epithelial cells trigger EC proliferation or is the endocrine cell stimulated by other factors to proliferate?

Authors: As endocrine cell numbers do not increase following X-irradiation, it is unlikely that they are stimulated to proliferate. Indeed, several weeks after irradiation, their numbers are still reduced from pre-radiation values (unpublished data). We postulate that those surviving cells are in fact 'turned on' by some as yet unidentified factor to produce certain hormones themselves responsible for gastrointestinal trophism.

M.S. Al-Tikriti: It appears that it would be difficult to find 10 chromophilic cells per crypt. Please comment on this procedure.

Authors: We found the procedure described by Withers and Elkind (1970) to be straightforward and easily reproduced. We experienced no specific difficulties identifying such cells, except perhaps at 3 days following 18Gy of radiation when crypt numbers were severely depleted.

M.S. Al-Tikriti: Please comment on why the separation of epithelium in figure 5 is located only on the top of the villus and not along the sides also. This appears very similar to fixation artifact.

Authors: The epithelial-stromal stripping seen in this experiment has been described in some detail in previous publications (Carr et al., 1982). It is thought unlikely that this effect, only seen in the irradiated groups after specific doses, is fixation artefact.

T.M. Seed: The difference in number of villus vs. cryptal endocrine cells following irradiation is due to inhibition of production rather than differential radiosensitivity of mature vs. immature cell types?

Authors: The absence of endocrine cells from the villous epithelium following X-irradiation is interesting. In theory the mature villous cells should be less radiosensitive than the immature cryptal cells. More precise quantification of villous endocrine cells is required if meaningful comparison is to be made. We speculate, however, that endocrine cell extrusion rates are increased following irradiation and that these 'lost cells' are not being replaced by migration of 'new cells' from the crypt bases.

Additional References

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