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MORPHOLOGICAL CRITERIA FOR COMPARING EFFECTS OF X-RAYS AND NEON IONS ON MOUSE SMALL INTESTINE

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Abstract

Several techniques have been used to assess changes in different parts of mouse small intestine three days after a single dose of either 16.5 Gy X-rays or 11 Gy neon beam. The doses were chosen to be approximately equivalent in terms of their effect on the number of microcolonies present.

In qualitative terms, villous damage was seen after both types of radiation exposure: collared crypts, similar to those seen in biopsies taken from patients suffering from coeliac disease, were conspicuous after neon irradiation.

In semi quantitative terms the doses used, although estimated from previous work to give biologically equivalent damage, produced a greater drop in microcolony numbers after X-irradiation. This makes all the more important the fact that significantly greater changes were seen after neon irradiation - a greater drop was seen in the number of villous profiles and the number of goblet cells per villus. There was also greater breakdown in the integrity of the villous basement membrane.

Different responses after the two types of irradiation are therefore seen in the cryptal and villous compartment. Progress is being made towards identifying and quantitating radiation induced changes in different populations of cells or tissues in the small intestine.

Key words: Scanning electron microscopy, light microscopy, X-irradiation, neon rays, small intestine.

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Introduction

The small intestine has been much used as a model for the investigation of the effects of irradiation (Potten and Hendry, 1983). Most attention has been concentrated on the effects on the epithelial population, initially in qualitative terms, in that changes are seen in the structure of the intestinal cells. Ultrastructurally, most information is available on changes in the epithelial cell population (Hugon and Borgers, 1966, 1968; D'Souza et al, 1974; Becciolini et al, 1976; Ciecura et al, 1976), although some authors do comment on changes within tissues which have a supportive role with respect to the epithelium (Chomette et al, 1977; Lieb et al, 1977).

In quantitative terms, most information is available on changes in the epithelial compartment, in that "crypts" (or microcolonies as they may be called during the recovery period after irradiation) drop in number after treatment, giving a technique whereby survival curve parameters can be computed, (Withers and Elkind, 1970).

These crypt count data have been used to give relative biological effectiveness (RBE) estimates, where one type of radiation is compared to another. However, crypt cell response, with its main damage seen three days after irradiation, reflects the response of only one compartment. There have already been reports of different villous responses after doses of different qualities of radiation (such as neutrons and X-rays) chosen to give identical crypt count effects (Carr et al, 1983). Likewise, it has been shown that one cell type of the crypt, the endocrine cell, has a measurable drop in cell numbers one day after irradiation (Wyatt et al, 1985).

It is clear, therefore, that while crypt counting is a useful technique, it reflects only one of many possible

responses seen in the gut to radiation. This paper aims to explore ways in which quantitative or semi-quantitative data can be obtained from assessment of damage to different intestinal cells, tissues or compartments, as previously briefly described (Carr et al, 1986).

The two types of irradiation chosen are X-rays and neon particles. There is a large literature on the effects of X-rays: some of the references have been cited above and the literature has been reviewed (Carr, 1981; Potten and Hendry, 1983). There has been less work done on heavy ions which are so relevant to space research as well as to therapeutic regimes in anti tumour therapy (Leith et al, 1982). There have been some reports of heavy ion effects on small intestine: these have concentrated on crypt survival (Alpen et al, 1980) or late ultrastructural effects (Fatemi et al, 1985). In the latter case, no major differences were seen between the effects of heavy ions and gamma rays, except with respect to basement membrane changes.

The present paper uses the model of small intestine three days after irradiation with either neon ions or X-rays as a vehicle to explore how many of the irradiation induced changes can be quantitated. Since this multifactorial approach would make it difficult to compare the two irradiation qualities with each other and obtain an RBE, each change is always related to the control situation. This makes the compilation of a total composite figure reflecting overall damage (Carr et al, 1986) a straightforward matter, limited only by considerations of how many variables have so far been assessed and of lack of theoretical knowledge on how to give different weightings to the figures for the available variables.

Materials and Methods

Animals

LAF_1 mice were used. They were maintained in standard animal house conditions and fed standard chow ad libitum.

Irradiation

The mice were total body irradiated with either 225 kVP X-rays at 15 mA, or a ^{20}Ne 670 MeV/nucleon plateau neon beam at the Lawrence Berkeley Laboratory's BEVALAC. Doses used were 1600 or 1720 cGy for X-irradiation and 1000 or 1240 cGy for neon ions.

For the heavy ion exposures, the mice were anaesthetized with Nembutal, and placed on their backs with their right sides exposed to the beam. X-ray exposures were similar except that the X-ray source was above the mouse and not parallel to the floor as in the heavy ion exposures.

Tissue collection

The animals were killed by Nembutal overdose three days after irradiation. Pieces of jejunum were fixed in 2.5% glutaraldehyde by inflating a piece of gut gently with fixative, then tying the proximal end with black thread and the distal end with white thread. Four animals were exposed to X-irradiation, four to neon irradiation and there were four control mice.

Microscopy

Scanning electron microscopy.

A piece of tissue was cut from the fixed sample in such a way that proximal, distal and mesenteric regions were all identified. This piece was postfixed in 1% aqueous osmium tetroxide and dehydrated in a series of ethanols prior to critical point drying from carbon dioxide. The specimen was then mounted on a stub with silver paint and sputter coated with gold, prior to screening in a JEOL T300 scanning electron microscope. Montages were prepared of all specimens under standard conditions.

Resin histology.

Whole circumferences of the gut tube were dehydrated in a series of ethanols prior to embedding in Spurr's resin. Sections were cut on a Reichert Jung Autocut and stained with toluidine blue, Periodic Acid Schiff (PAS) or silver, using methods adapted from routine wax histology staining techniques.

Quantitation

(a) After scanning electron microscope montages had been prepared, villous scoring was done on a score of 0-10 according to the method described previously (Carr et al, 1983)

(b) Crypts were counted by identifying on PAS - stained sections all crypt regions with 10 epithelial cells or more. Note was made of the total number of crypts per circumference and also the subtotal of heavily stained crypts in the irradiated specimens.

(c) Villous profiles were counted on PAS stained sections. The total number of villous profiles per circumference was noted, as was the subtotal of villi which showed an obvious lamina propria compartment and were also seen to be attached to the intervillous basin: the latter group was designated ALP villi (attached, with lamina propria).

(d) The number of goblet cells was counted on PAS stained sections. The total number of goblet cells per circumference was estimated, as was the number for the total villous

compartment, the ALP villous compartment and the cryptal compartment.

(e) An estimate was made of basement membrane integrity, using the silver stained sections. Four membrane compartments were assessed separately - (i) villous (within an ALP villus), (ii) intervillous basin, (iii) longitudinally cut crypts, (iv) transversely cut crypts. A basement membrane scoring system as follows was used -

0 - absent or nearly absent
1 - present but incomplete
2 - complete or near complete basement membrane.

The results for the three groups (control, X-irradiated, neon irradiated) were compared for the total basement membrane assessment and also for each basement membrane subcompartment separately.

Statistics

In general the control values were compared with the X-ray or neon irradiated values using Student's t-test to determine whether there had been any significant change produced by either radiation quality. Thereafter, values from the two different qualities were compared in a similar way. Because the figures obtained for the basement membrane section, described in (e) above, were percentages rather than actual values, they were analysed using a Cruskal - Wallis programme.

Results

Crypt assessment

Since the doses of heavy ion and X-irradiation had been chosen, based on an assessed RBE figure of 1.4 - 1.6, to give approximately equal damage in terms of depletion of crypts, it is most appropriate to describe first the data obtained from assessment of this part of the work. Both the total number of crypts and the number of heavily stained crypts (Figure 1) are reduced after irradiation (Tables 1,2). There are substantially fewer crypts (either total, or heavily stained) in the samples which have been irradiated with X-rays than in those treated with neon ions. Figures giving the percentage ratio of surviving irradiated crypts by comparison with the number in the control specimens are given in Table 3.

In addition to crypt counting, qualitative information on crypt structure was obtained by study of the montages obtained using scanning electron microscopy. The advantage of the technique as described in the Materials and Methods section is that a good understanding can be obtained of the spatial relationships of individual villi and their surrounding features. This follows from the fact that all montages were taken with the proximal end at the top of the specimen and the mesenteric attachment in the same

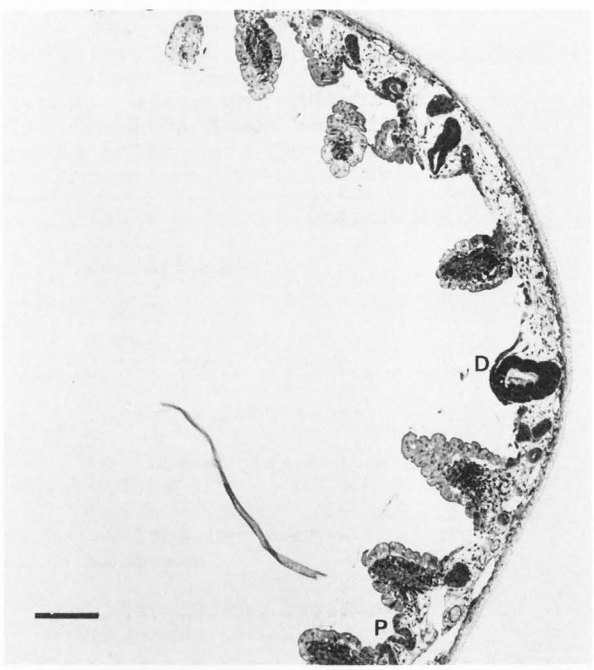


Figure 1. Light micrograph of neon irradiated section showing crypts of different staining intensities. D is a dark crypt and P is a pale crypt. Bar = 0.14mm.

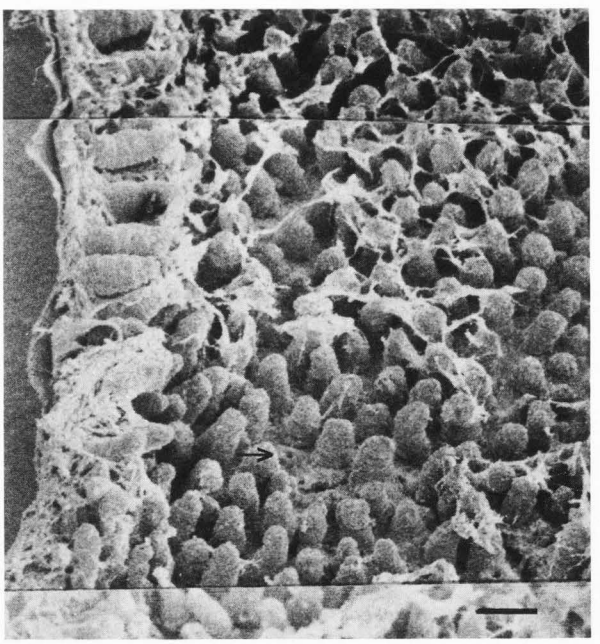


Figure 2. SEM photomontage of neon irradiated small intestine, from which collared crypts can be identified (arrow). Such montages were also used to obtain the villous scores. Bar = 0.15mm.

Table 1

Raw data of results from X-ray / neon experiments

Variable	Control	X-ray (1600-1720cGy)	Neon (1000-1240cGy)
Total crypts / circumference	88.0±8.1*	2.19±2.14	15.5±10.2
Dark crypts / circumference	**	1.1±1.0	10.7±7.3
Villous number / circumference	68.0±6.8	42.9±6.8	38.3±3.8
Villous score	0.3±0.33	1.1±0.1	1.7±0.6
Total goblet cells / circumference	666.3±79.6	184.8±35.6	144.8±72.5
Cryptal goblet cells/crypt	3.6±0.6	38.1±27.1+	6.7±7.1
Villous goblet cells/villus	5.3±1.5	3.2±0.7	2.3±0.8
Total basement membrane (% damage)	65.4±5.0	38.8±17.7	46.2±12.0
Villous basement membrane (% damage)	96.6±2.6	40.1±26.3	61.6±12.0
Intervillous basement membrane (% damage)	70.3±7.3	24.7±16.8	27.11±12.8

* Second number in each case is standard deviation.

** In control specimens, there was no distinction between dark and light stained crypts.

\+ The figure 38.1 is correct. It is high, not because the number of cryptal goblet cells is high, but because the number of crypts is small.

Table 2

Summary of results of X - ray and neon experiments

Variable	X-ray (1600-1720cGy)	Neon (1000-1240cGy)	Statistics C/X	C/Ne	*X/Ne	+Ne/X
Crypt count						
total	R	R	S	S	S	-
dark	R	R	S	S	S	-
Villi						
L.M. number	R	R	S	S	-	S
Villous collapse	I	I (with collared crypts)	S	S	-	-
Goblet cells						
Total	R	R	S	S	-	-
Cryptal/crypt	I	I	S	-	S	-
Villous/villus	R	R	S	S	-	S
Basement membrane						
Total	R	R	-	-	-	-
Villous	R	R	S	S	-	S
Intervillous	R	R	S	S	-	S
Crypts T.S.	-	-	-	-	-	-
Crypts L.S.	-	-	-	-	-	-

Key:
C = Control
X = X-ray irradiated
Ne = Neon irradiated
I = Increased
R = Reduced
S = Significant, using routine statistical tests
- = Data showed no significant changes
* = X-irradiated showed more damage than neon irradiated
\+ = Ne-irradiated showed more damage than X-irradiated

position. In specimens irradiated with neon, collared crypts were observed (Figure 2). These were readily observed in the montages as areas where the crypt opening was situated in a symmetrical or asymmetrical swelling in the intervillous basin (Figure 3). This area is wide after irradiation, allowing easy inspection of the crypt. Collared crypts were seen in two of the four neon irradiated specimens and in none of the X-irradiated samples; the close packing of the villi in controls made comparison with the normal situation difficult , but previous study of this area has not revealed such distorted crypts in untreated material. The collared crypts are easily seen when they are present.

Villous assessment

Counting of villous profiles

The data for these assessments include total villous profiles and also the number of ALP villi (Figure 4); in each case the number given is for each whole circumference. The data are summarised in Table 1. Both total and ALP villi differ to a statistically significant extent when either neon or X-irradiated specimens are compared with controls. Only the total number, however, differs in this way when neon and X-irradiated samples are compared with each other, with X-irradiated samples showing more damage than neon irradiated specimens.

Scoring of villous topography

The scores for the control group are fairly low, whereas those for both irradiated groups are significantly higher, although there is no such difference between the two irradiated groups. The data for comparison of the two groups are given in Table 1.

Goblet cell counts

Three different sets of data are given in Table 1. They compare, for the three groups, either total goblet cell count per circumference, cryptal goblet cells per crypt or villous goblet cells per villus (Figures 5, 6). The comparison figures are given in Table 2, which shows that significant differences are seen using all three comparison methods when control and X-irradiated data are compared, whereas there is less of a pattern when the other groups are studied.

Basement membrane integrity

The data for this variable are shown in Table 1. Although there is no significant difference between any of the groups when the total score is computed, when the scores are broken down into their subgroups (Figure 7), it is found that the villous and intervillous regions (Figure 4) show basement membrane which is damaged significantly more in the neon irradiated material (Table 2).

Comparison of data

Tables 1 and 2 have summarised the trends in the raw data. Table 1 displays the raw data. Table 2, summarising the trends from these values, highlights any statistically significant changes between the groups. Table 3 shows the percentage reductions in score for each treatment schedule compared with the relevant control data. In each case, the control data are taken as a maximum figure; to present the villous score results in this way, the data now read on a 10 to 0 scale, rather than a 0 to 10 scale.

Table 3 * Comparison of X-ray and Neon results with control data

Compartment	X-rays as % of controls	Neon as % of controls
Total crypt number	2.4	17.6
Total villous number	63.0	56.3
Villous goblet cells/ villus	60.4	43.4
Villous Basement/ membrane	41.5	63.8
Intervillous basement membrane	35.1	38.5

* All of these values are taken from Table 1, using only data where the X-ray and neon irradiated samples were statistically different from each other. The figures quoted are percentages, obtained by comparing the irradiated with the control situations.

Discussion

The aim of this paper is to comment only briefly on the significance of the response to irradiation of each individual small intestinal compartment: instead the emphasis will be on the general concept of comparing each irradiated result to the relevant control and of bringing these together to form totals or subtotals. To this end, therefore, there will be a brief discussion of each of the factors in the order in which they are given in the Results section. This will be followed by some comments on the comparison between the results for irradiated and control samples.

Crypt assessment

The doses of the two types of radiation were chosen hoping to give equivalent damage in terms of reduction in the number of crypts present: this was based on previous work (Alpen et al, 1980). However, the X-irradiated group shows a greater reduction, irrespective of whether total crypt numbers, or heavily stained crypts are counted. Discussion of the relationship between these two populations of crypts can therefore be deferred, and the simple conclusion drawn that, when the two schedules were compared, the X-irradiated samples suffered more crypt damage so RBE was misestimated. If this variable could be used as an indicator representative of damage to all compartments of the intestine, it would therefore be expected that this pattern of greater damage to the X-irradiated samples would be followed throughout the results for other areas.

The topographical results on crypt structure, although not relevant to the semi-quantitative approach so central to the theme of this paper, are worthy of brief comment. The collared crypts are easily identified in the neon irradiated group. These structures are not well understood, although they have been seen in previous studies. The previous observations cited them as unusual occurrences at the end of a fractionated regime of X-irradiation (Carr et al, 1979). They were also seen, but more often, in the neutron irradiation experiments used to comment on possible spiral movement patterns of epithelial cells from crypt to villus (Carr et al, 1981). Although comparative quantitation and further experimentation using heavier ions are both required, the hypothesis can be put forward that these collared crypts are the results of local deposition of large quantities of radiation energy and that these are most common, or most damaging, after bombardment with high LET radiations such as neutrons or neon ions.

Finally, in the crypt compartment, there are changes in the goblet cell component, with X-irradiated crypts showing a significantly greater increase in cell counts than non irradiated ones. This trend is in line with that seen for the overall counting data on the number of crypts.

Villous assessment

The total number of villous profiles is significantly reduced in both irradiated samples, with the neon ion irradiated specimens showing greater damage. This trend reverses that seen in the cryptal compartment and is therefore in agreement with previous work which indicated that the two regions respond independently to irradiation (Carr et al, 1979; Carr et al, 1984).

This trend is not confirmed by villous scoring, although the fact that the surface damage appears quantitatively the same after two regimes which produce different changes in the crypt population implies that, if the doses had been equivalent in cryptal terms, there might have been different villous responses. Villous goblet cell results, however, do confirm the presence of significantly greater changes after neon irradiation, as do basement membrane estimates for the villous and intervillous populations. The basement membrane changes underline again the importance of the interface regions in radiation damage, both in terms of changes at the epithelial/stromal zone (Carr et al, 1984) and also the endothelial/stromal boundary (Lieb et al, 1977; Fatemi et al, 1985).

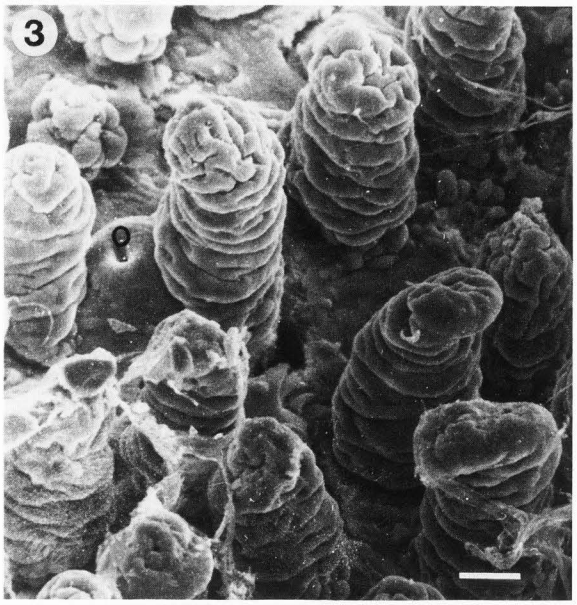
Comparison of radiation qualities

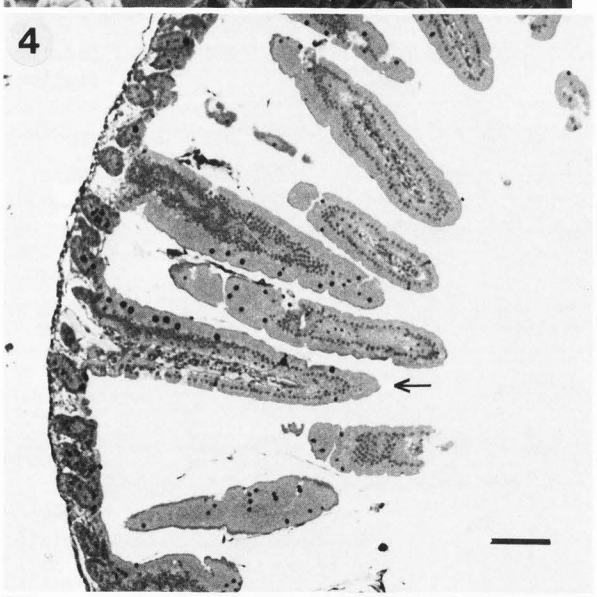
Before discussing the results obtained using this approach, it is important to comment on sampling. The experiments described here were designed to obtain information on surface

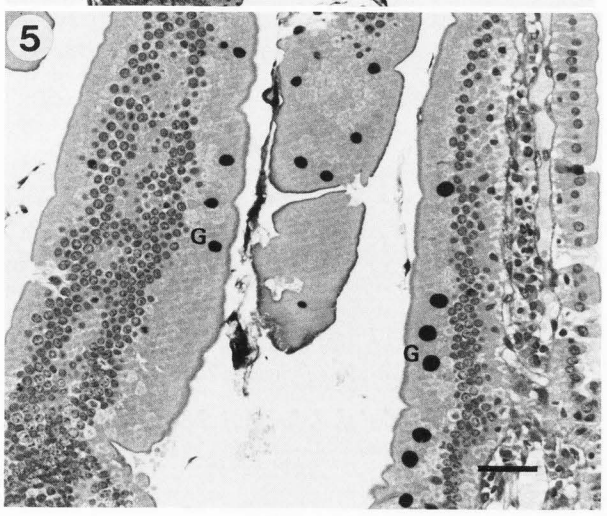
Figure 3. Topographical detail of collared crypt from neon irradiated animal, showing unusual swelling around the opening (O) of the crypt. Bar = 40μm.

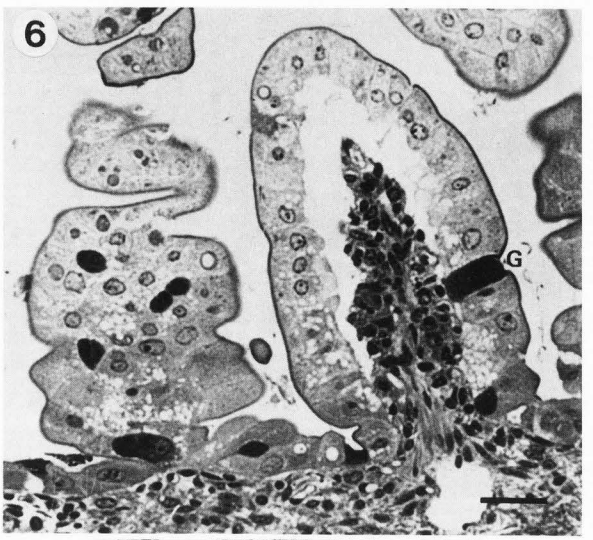
Figure 4. Light micrograph of resin section of control tissue, stained with periodic acid-Schiff stain. The arrows indicate ALP villi (attached, lamina propria visible). Bar = 0.1mm.

Figure 5. Light micrograph of control resin section stained with PAS, to illustrate typical distribution of goblet cells (G) in the villous compartment. Bar = 38.5μm.









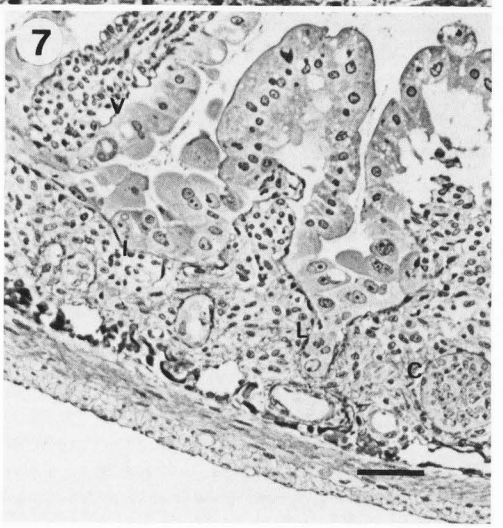


Figure 6. Light micrograph of X-irradiated section, prepared in the same way as Figure 5. The goblet cell (G) population is reduced. Bar = 30.8μm.

Figure 7. Light micrograph of control resin section stained with silver, to illustrate the different situations of basement membrane - villous (V), intervillous (I), longitudinal crypts (L) and transverse crypts (C). Bar = 43.9μm.

topography (using scanning electron microscopy) and ultrastructure (using transmission electron microscopy). These techniques are both fairly time consuming: as a result, animal groups of three or four, as used here, are usually regarded as adequate. Recent developments in resin section staining, using techniques for wax histology, allow study of material which has been particularly well fixed, embedded and stained. These techniques are still

being improved and require careful standardisation. As a result, they are also fairly time consuming. The silver and PAS stained material used here was this type of resin embedded, specialist stained material. The procedures were carried out on the relatively small populations of animals available for the electron microscope studies. The data should therefore be regarded as preliminary, but it should be noted that, particularly for the silver stained material, the changes may be observable solely because of the good images obtained using these techniques. It may be that the results would not be reproducible using routine wax histological preparations, such as those used on large groups of animals for crypt counting (Withers and Elkind, 1970). It may be that the best way to confirm and extend these results will be to report on ultrastructural changes at the epithelial/stromal boundary; such work is nearing completion.

The protocol for these experiments was based on an assumed RBE range of 1.4-1.6 for neon ions as opposed to X-rays. The crypt survival data given here indicate that the assumption was not entirely accurate for the strain of mice used; this stresses the importance, in morphological studies such as these, of carrying out crypt counting on the tissue being used instead of relying on previously collected data.

Although after X-irradiation there was greater quantitative damage in the cryptal compartment, the collared crypts were only seen after neon irradiation. The villous compartment was also more damaged after neon irradiation: the number of villi, the number of villous goblet cells per villus and the basement membrane component of the villus all being affected. It seems therefore that the RBE may be greater than 1.4-1.6 for the villous findings, and also for collared crypts, but not for crypt survival. The results suggest that the radiation response depends on the cell or tissue examined and it seems that there may be qualitative as well as quantitative differences between the effect of X-rays and neon ions. In conclusion, it is most important that such morphological information be derived wherever possible from experiments designed to study the effects of radiation on tissues.

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Discussion with Reviewers

S. Siew: How do the doses used in your experiments correspond to those used in human beings?

Authors: The doses used in experiments were higher than therapeutic doses.

S. Siew: Could you describe more precisely how you preserved the identification of proximal, distal and mesenteric regions in the scanning electron microscopy material?

Authors: A piece of gut was cut from previously isolated tissue with the black thread intact at the proximal end. It was then incised along the mesenteric border. The gut tissue thus opened up was pinned on to cork immediately and the proximal and distal ends were labelled. This labelling was retained to the stage of screening in a scanning electron microscope.

S. Siew: Could you give the details of your staining techniques, PAS and silver, on resin embedded material?

Authors: PAS METHOD: Remove resin by immersing in saturated NaOH in absolute ethanol for 20 min followed by 3 changes of absolute ethanol, 5 min in each change. Rehydrate through descending series of ethanol beginning from 90% ethanol followed by 70% ethanol and then wash in running tap water. The slides should be left for 5 min in each of the above stages. Follow the underlying technique immediately after washing: immerse in 1% periodic acid (10 min); wash in running water (10 min); steep in Schiff's reagent (20-30 min); wash in running water (15-30 min); transfer to Weigert's haematoxylin (5-10 mins); differentiate in acid alcohol; blue in Scott's solution (1 min); wash in water. Dehydrate through ascending series of ethanol beginning with 70%, through 90%, to 100% (15 secs in each change), followed by a further minute in a second change of 100% ethanol. Immerse in carbol xylol (5 min), then xylol (5 min). Mount and examine.
SILVER METHOD: Remove resin and hydrate to water as before. Impregnate for 1-2 hours in 2% aqueous $AgNO_3$ at 50°C. *Subsequent steps to be performed at room temperature*: Rinse for 1-2 sec in water and develop to a light brown tint in the following solution : 3% gelatin (40 ml), 2% $AgNO_3$ (10 ml). Mix well and add 4 ml 1% hydroquinine while still stirring. Wash through 3 changes of water and tone in 1% gold chloride for 10-15 sec. Wash sections in water for 5 min. Immerse in 2% oxalic acid for 3-5 min. Wash in water. Dehydrate, clear and mount.

S. Siew: Do these techniques work as well in other resins or only in Spurr's?

Authors: These techniques can be recommended for Spurr's resin. The authors' experience on previously scanned reprocessed material (Indran et al, (1985) Scanning Elect. Microsc., I, 1165-1175), suggests that Spurr's resin produces well stained sections unlike epoxy resin, although modifications of the technique should produce good results with other resins.

S. Siew: At what thickness did you section the resin embedded material?

Authors: The sections were obtained at a thickness of 1.5 micrometers.

S. Siew: It would appear that strands of mucus are present in Figure 2. You do not describe the removal of mucus in the Materials and Methods section. Was it not done?

Authors: No attempts were made with respect to removal of mucus. The degree of mucus retention may indicate qualitative surface changes that might reflect tissue response to different radiation schedules. We felt it was important not to remove any surface mucus.

S. Siew: What was the appearance of the "collared crypts" in the resin embedded material?

Authors: The collared crypts will be reprocessed in the future for light microscopic examination.

S. Siew: Did you do goblet cell counts on the scanning electron microscopy material?

Authors: We have not counted the goblet cells. It is not always easy to identify these cells by SEM.

S. Siew: What was the incidence of collared crypts in the neon irradiated material?

Authors: No attempt has yet been made to count these structures, although further work is planned to cover this point. More recent studies on iron ion irradiated small intestine showed some specimens with a very large number of these structures.

R. Laschi: The authors should more precisely describe the morphological characteristics of collared crypts and explain why they are considered abnormal. What is the SEM aspect of normal crypts?
Secondly, the authors state that collared crypts are more easily seen in neon irradiated mice with respect to X-ray irradiated mice. Could this be merely due to the thinning of villi prevailing in neon irradiated animals?

Authors: The collared crypts form part of a mound in the intervillous basin: this has an obvious crypt mouth in its centre. The collared crypts themselves are such obvious structures that it is felt that they would be visible even if the villi were closer together as in control or X-irradiated samples.

T. M. Seed: Would the authors care to speculate as to the nature and mechanisms of induction of the very interesting "collared" crypts noted followed only after heavy neon ion, but not X-irradiation?

Authors: The collared crypts are seem after neon irradiation, occasionally after X-irradiation and are similar to structures seen in some conditions of gastroenteropathy. Further to what is suggested in the text, it is possible that the structure identified by the topography as collared crypts may be related to the dark crypt seen in resin sections. We hope to explore the possible link by reprocessing the scanned specimens. At this stage we would only repeat our suggestion that the collared crypts may be caused by local deposition of large quantities of radiation energy.

T.M. Seed : My only real concern and comment is directed towards the authors' stated intention to irradiate the intestinal tissues with 'biologically equivalent' doses (ie 1600-1720 cGy X-rays and 1000-1240 cGy heavy neon ions) based on intestinal crypt survival. Clearly, the X and neon irradiation doses were not equivalent (as stated in the Results and Table 3). In the authors' opinion, does such nonequivalence diminish in any way the strength of the observations made ?

Related to the above, I see that the results of Alpen's study in 1980 were used as the basis of the targetted RBE value of 1.5. Can the authors offer a reasonable explanation as to why such difference in crypt survival was noted between the original study and the current one ?

Authors: We feel the point of the difference between our results and those of Alpen (1980) can best be discussed by looking at the different way in which the experiments were done. Our results, unlike those of Alpen, were not based on complete dose response data for our particular model: it is difficult to make valid comparisons between our single dose data and his results, which came from a complete set of D_o and D_{200} measurements. In order to compare the two sets of results for the particular end-point at issue, i.e. crypt counting, we would have had to collect more data. It is also worth noting that the energies of the neon beams were different and the methods of assessing crypt survival were not the same. These factors may partly explain the fact that our crypt counting results were not entirely as predicted by Alpen's work.
At the same time, if we look at our results, as summarised in Table 3, we find that, although the drop in crypt numbers is not as expected, and is quite

different for the two radiation treatments given, the results of some of the other end-points do show approximate equivalence. For example, total villous number and intervillous basement membrane end-points show changes from the control values which are roughly equivalent. Since the conclusion that we drew from our work is that different results may be obtained for each end-point studied, the fact that the crypt count figures differ does not, in our view, diminish in any way the strength of the observations made. An ideal continuation of the work would permit the construction of dose response curves for all end-points, which could then be compared individually.