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SURFACE STUDIES OF DUODENAL LESIONS INDUCED BY THORACIC IRRADIATION

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Abstract

Acute duodenal ulcers are produced in mice as a remote ("abscopal") effect of irradiation to the lower mediastinum. Such lesions have been examined with scanning electron microscopy at 5, 8 and 28 days after irradiation with 18 Gy of X-rays. All the ulcers occur within the first 1 cm long segment of the duodenum which is endowed with Brunner's glands. The single lesions vary in size, shape and position. The damaged area often includes much of the duodenal circumference and is distinguished by conical or rudimentary villi, or even by the complete absence of villi. In contrast, around the periphery of the ulcer the villi are mostly vertical. Although the floor of these lesions appears to be covered with a continuous epithelial layer, during the first 4 weeks after irradiation the severity of the focal duodenal damage seems to increase gradually with time. The lesions have been compared with specimens from unirradiated mice and also with samples taken 3 days after partial thoracic irradiation when little damage is seen. The pattern of fully developed duodenal lesions differs greatly from that seen after direct irradiation where damage has not included localised ulceration in the samples of jejunum so far examined.

The lesions induced by partial thoracic irradiation may be related to radiation injury to vascular or autonomic nerve targets in the lower mediastinum. Such injury could result in malfunction of the pyloric sphincter or could alter the secretion by Brunner's glands and thus lead to duodenal ulceration.

KEY WORDS: Scanning electron microscopy, light microscopy, thoracic X-irradiation, duodenal lesions.

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Introduction

Thoracic irradiation of mice results in acute local damage including oesophagitis, and in distant mucosal lesions of the stomach and intestines exemplifying the so-called abscopal effects of partial body irradiation (Michalowski et al., 1983a). The site of duodenal lesions depends on the thoracic field irradiated: single proximal duodenal ulcers are produced by exposure of the lower mediastinum to X-ray doses of 14-30 Gy, while the same doses delivered to the lateral parts of the thorax give rise to solitary lesions in the vicinity of, or involving, the duodenal papilla (Michalowski et al., 1983b). After irradiation of the entire thorax, only the proximal ulcers appear and then take up to 8 days to develop (Michalowski and Burgin, 1982). The current paper sets out to describe at the ultrastructural level the lesions in the mouse duodenal mucosa at 3, 5, 8 and 28 days after irradiation of the lower mediastinum with 18 Gy X-rays, and to compare the damage seen with that produced by direct irradiation of the intestine by either whole body or abdominal exposure as already reported in the literature (Carr et al., 1983).

The direct radiation damage to the small intestine as seen with the scanning electron microscope (SEM) has been fairly fully investigated over the last ten years. The initial qualitative descriptions (Carr and Toner, 1972; Anderson and Withers, 1973) were followed by more quantitative assessment of change in villous shape after irradiation (Friberg, 1980; Carr, 1981; Carr et al., 1983). The damage to the surface of murine jejunal mucosa can be described in terms of a graded villous response, with the villi changing from the erect, finger-like shape in control animals through vertical, conical and rudimentary stages to a mucosa with absent villi (Carr, 1981). A scoring system has been developed (Carr et al., 1983) linking these stages of mucosal damage with the change in villous surface area after irradiation (Altmann, 1974). It has been found that irradiation of the abdomen

produces widespread and fairly uniform intestinal damage. The scoring system has therefore been used to quantify the whole of the portion of jejunum studied. The application of this system to the ulcers described in the current paper will be the first use of the method to assess changes in mucosal surface structure of a well circumscribed lesion contained in a larger specimen.

The studies on direct effects of radiation have so far concentrated mostly on jejunum and ileum. The current paper has used specimens taken from the proximal part of the duodenum, between the gastroduodenal junction and the ampulla of Vater where the pancreatic and biliary secretions enter the duodenum. These specimens therefore, unlike previous samples, have two fixed landmarks which allow for greater reproducibility in comparisons between different animals and for more accurate quantification. The damaged area can be compared with the secretagogue-induced ulcers studied previously in rats (Carr et al., 1979). Comparison of these two types of lesion may lead to the modification of the use of the term "ulcer" for the abscopal effects. The current paper will attempt to describe in detail the surface structure of the lesions, to discuss changes in surface structure with time after irradiation, and to explore the terminology and quantitative methods employed as well as possible pathomechanisms involved in the formation of the mucosal lesions.

Materials and Methods

Specimen preparation

Female CFLP mice were irradiated supine with a single dose of 18 Gy X-rays at 10-18 weeks of age, under general anaesthesia (0.06 mg Sagatal per gram body weight i.p.). The X-rays (250 kV, H.V.L. 1.35 mm Cu, dose rate 1.68 Gy min^{-1}, target distance 39 cms) were used to irradiate the lower mediastinum (field size, 12 x 10 mm^2), with the rest of the body shielded with lead. (Details of exposure and dosimetry are described in Michalowski et al., 1983b.)

Animals were killed on days 3, 5, 8 and 28 after irradiation. Unirradiated mice were also used to obtain control specimens. The animals were starved for 17-24 hrs before they were anaesthetised by the i.p. injection of 0.2 ml of Sagatal and then exsanguinated through the subclavicular vessels. The peritoneal cavity was opened and approximately 0.5 ml of 5% buffered glutaraldehyde was injected into the lumen of the duodenum and stomach, which were then removed en bloc and cut open along the line of the greater curvature. Subsequently, the specimen was pinned flat to the supporting surface with the mucosal aspect upwards and submerged in glutaraldehyde. After preliminary fixation the mucosal surface was gently washed with glutaraldehyde using a Pasteur pipette and examined with a dissecting microscope for the presence of duodenal lesions.

The specimens were washed in distilled water and then immersed in 1% buffered aqueous osmium tetroxide for 30 minutes and washed in several changes of distilled water. They were then dehydrated through an ethanol series and critical point dried in CO_2 from amyl acetate. They were then mounted on large stubs (3.2 cms) and coated with gold in a sputter coater. Scanning electron microscopy was carried out using both Cambridge Scientific Instrument Company S600 and JEOL T300 instruments operated at 20-30 kV. Only the duodenal ulcer-positive specimens from mice dissected on days 5 to 28 were submitted to SEM analysis, while those obtained on day 3 (all ulcer-negative) and from control animals were investigated at random. At least three irradiated mice were thus studied at each point.

Quantitation

Specimens were screened at a microscope tilt of 20° and a constant magnification and photographed and printed to a constant size. An exception to this was that two of the 8 day specimens were screened at 40° tilt and at a higher microscope magnification. This was due to these two specimens being screened as part of a pilot run while experimental parameters were being established. They were unable to be rescreened in the standard situation since they had been embedded for resin histology. However, since the exact tilt angle of any villus is dependent on the flatness of the specimen as well as the stage tilt angle, all four specimens taken eight days after radiation were included in analysis, thereby allowing comparison with the important eight to nine day data previously discussed (Michalowski et al., 1983b). The final magnification however remained the same in all cases. A grid 31 cms x 21 cms (selected for microcomputer analysis) was drawn on acetate sheet and placed over each print. Each grid square was assessed according to the villous scoring system described in detail by Carr et al. (1983) which covers a range from 0 for erect, control villi to 10 for a specimen which is entirely denuded of villi and has accompanying ulceration. Each square was assigned a number corresponding to the most common villous shape within it. At the 16.5-fold linear magnification used for mapping there were approximately three to six villi per square. The scores were then transferred to different grey levels (for black and white recording). Areas covered with debris, where villous detail was obscured, were given a different colour or grey level or number. The maps were produced using a Sinclair Spectrum microcomputer and recorded from the television screen or using a printer. The proportion of each ulcer map taken up by each type of villus was then

computed. By multiplying these proportions by the corresponding scores, and totalling the figures obtained, an overall score of damage was recorded for each lesion. An alternative score was produced using the total number of grid squares taken up by each type of villus. This gave a score which took account of the size of the lesion. A final ulcer score was calculated using both the percentage and area scores, weighted equally. The score for the proximal area of the duodenum, which often showed damage, was compared with the corresponding score for the distal area which was usually relatively unaffected as a result of lower mediastinal irradiation.

Results

Control specimens

Scanning electron microscopy allows a good review of the villous pattern of control specimens from the gastroduodenal junction (Figure 1) to the region of the duodenal ampulla of Vater, although this last structure is sometimes obscured by debris or folding of the specimen. The poor quality of some of the images reflects the difficulties of examining such large areas of tissue covered with projections which render coating difficult.

The villi are almost invariably finger shaped and erect; they are moderately closely packed, with areas of closer or wider spacing sometimes caused by slight wrinkling of the whole specimen during mounting. The most proximal portion of the duodenum, up to 4 mm from the boundary with the stomach, may contain villi which are more leaf shaped than finger shaped (Figure 1) and which may also show clearly defined intervillous basin areas between them.

Three days post irradiation

Specimens at this time show no areas which would be identified as "ulcerated" with the aid of a dissecting microscope. There are, however, some subtle changes which are clearly observed by scanning electron microscopy. These changes can be considered under two headings, depending on the distance of the area examined from the gastro-intestinal junction.

Gastroduodenal junction. This is the region of the duodenum where ridge shaped villi were clearly seen in two of the three control specimens. Three days after thoracic irradiation, the villi still show a large degree of separation, with marked intervillous basins (Figure 2). The villi vary from ridge shaped to floppy finger shaped with many of them leaf shaped. In one of the specimens, there was an area, about 1 mm by 0.5 mm in size and beginning approximately 1 mm from the gastroduodenal junction, where the villi were markedly lower than elsewhere (Figure 2).

Proximal duodenum proper. The rest of the proximal duodenum showed no areas of localised damage, although the villi were less closely packed in all three specimens than they were in the control. In shape, the villi were either erect or vertical.

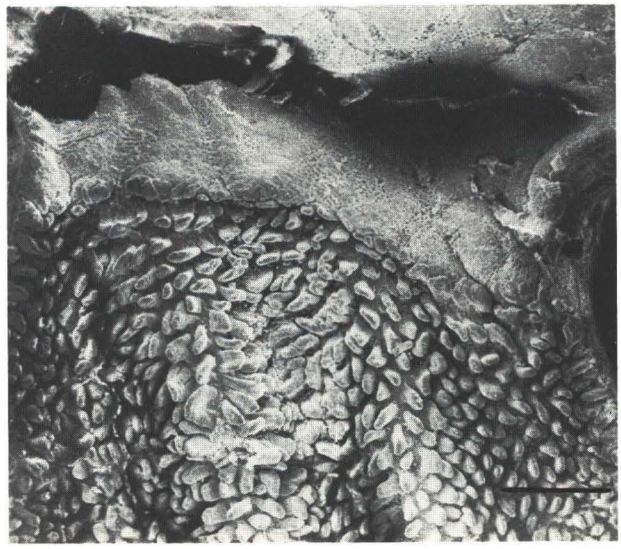

Figure 1. SEM of control gastroduodenal junction, showing leaf shaped villi, with intervillous basins. Bar = 1000 μm.

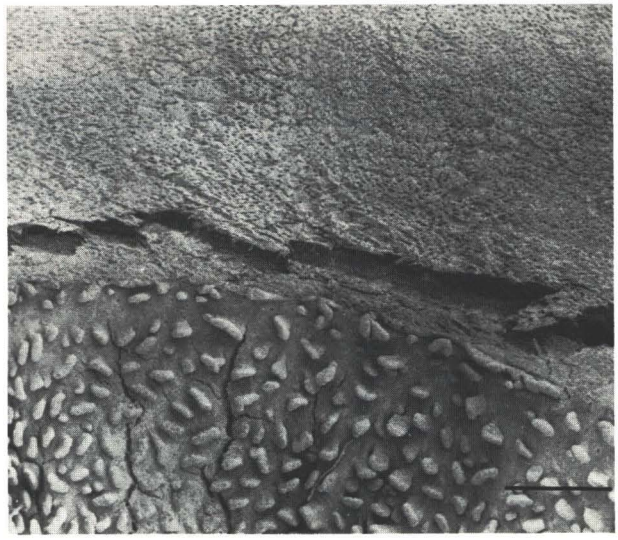

Figure 2. SEM of gastroduodenal junction 3 days irradiation . The villi are widely spaced. They are mostly leaf shaped, but a small number of them are considerably lower. Bar = 1000 um.

Five days post irradiation

By this time the damage to the duodenal mucosa is well established and each of the three specimens examined showed one well-defined and large area where the villous pattern was highly abnormal. In each case the damage extended from the gastroduodenal

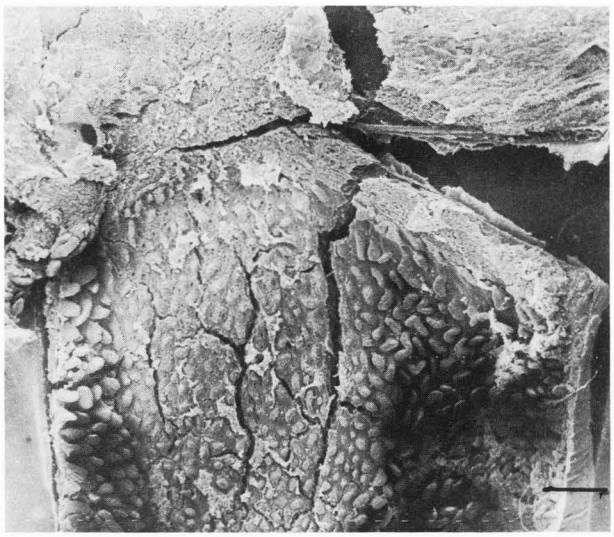

Figure 3. SEM of gastroduodenal junction 5 days after irradiation. There is a small amount of debris on the mucosal surface and the villi are low and abnormal for much of the circumference of the duodenum.
Bar = 1000 μm.

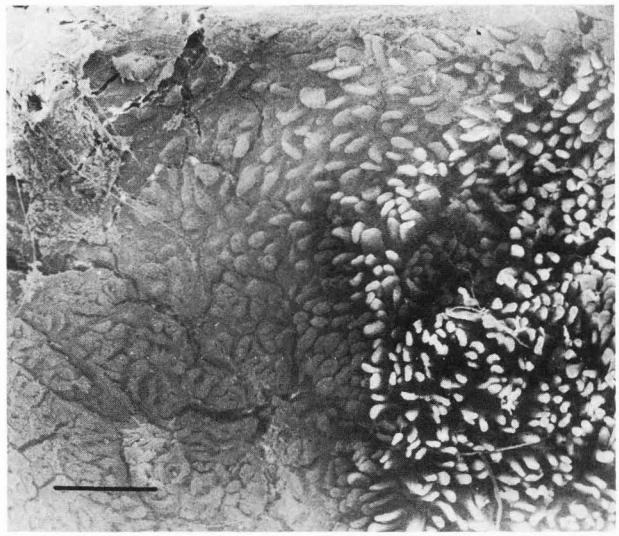

Figure 5. SEM of lesion at gastroduodenal boundary 8 days after irradiation. The mucosa is almost lacking in villi in part of the lesion, while there are many normal, finger shaped villi lateral to it.
Bar = 1000 μm.

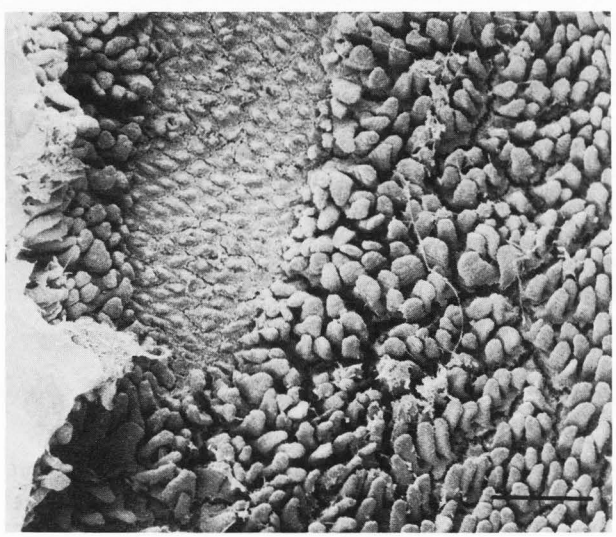

Figure 4. SEM of the distal end of duodenal lesion 5 days after irradiation. There is a sharp boundary between the abnormal villi within the lesion and the area of the duodenum covered with normal shaped villi.
Bar = 1000 μm.

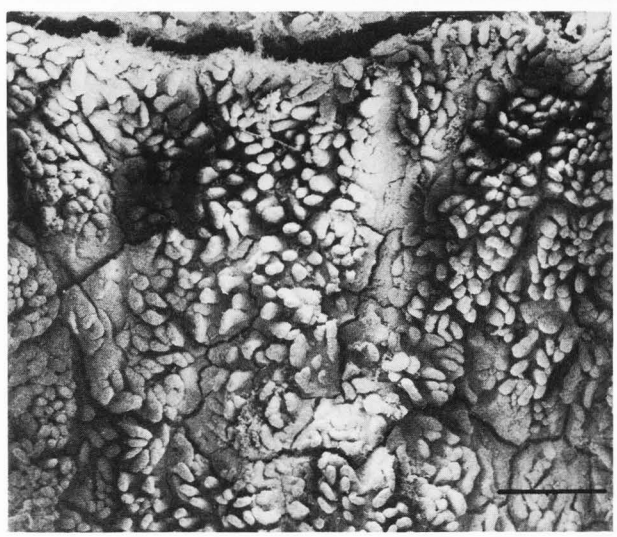

Figure 6. SEM of gastroduodenal junction 28 days after irradiation. Although some areas seem to have substantially recovered, there are patches with residual damage.
Bar = 1000 μm.

junction (Figure 3) but the lesions differed in shape and orientation. One extended around the whole circumference of the duodenum to a distance of 9 mm. The other two were long and narrow in outline (Figure 4), with a short axis of about 2 mm, and the long axis parallel to that of the duodenum and approximately 11 mm in length. There was, however, little variation in the nature of damage. Each lesion contained villi which were conical or rudimentary in shape or lacking entirely in some areas. All three lesions showed very abrupt boundaries with apparently normal mucosal surface, the villi progressing rapidly from the low profiles to vertical or erect structures (Figure 4). The remaining mucosal surface was for the most

part covered with erect villi which looked virtually normal.

Eight days post irradiation

In this group the size, orientation and definition of outlines of the lesions varied greatly. One of the specimens had only a small affected area near to, but not at the gastroduodenal junction. The lesion contained rudimentary or no villi, with a gradual transition to the surrounding normal mucosa. In each of the other three specimens examined at this time, there was a single, large area of damage present. One of these three specimens had almost all of the duodenal circumference affected, while the other two resembled those with the longitudinally oriented lesions described earlier. In all three lesions the range of villous damage was great, with patches of absent villi alongside areas of rudimentary and conical villous collapse (Figure 5). At the boundary, the transition to normal villi was sharp, but the outline of the lesions was less regular than at day 5.

Twenty eight days post irradiation

At this late time after treatment, there was a large variation in the severity of damage. In one specimen the damage was very patchy, with absent or rudimentary villi in multiple small circumscribed areas interspersed with regions of normal mucosa. In this case two of the patches were longitudinally orientated (Figure 6) and may echo the presence of a pre-existing single large lesion. The second specimen had a lesion near the gastroduodenal junction and had conical villi over much of the mucosa. The third specimen showed much greater damage, with flat mucosa extending from the gastroduodenal junction and involving almost the entire circumference of the duodenum. There were few signs suggestive of recovery in the last specimen.

Ulcer mapping

Ulcer mapping was done on sets of micrographs prepared specifically for the purpose. All montages had the same final magnification, which does not necessarily

Table 1

Villous patterns in proximal duodenal areas

Specimen	E	L	L^1	V	C	R	A	D	(X)	Total normal	Total (-X)
Con 1	158	122	1	10	0	0	0	0	(67)	281	291
2	180	229	0	0	0	0	0	0	(0)	409	409
3	282	92	0	0	0	0	0	10	(35)	374	384
Mean	207	148	0	0	0	0	0	3	(34)	355	361
3d 1	160	40	0	46	1	7	2	23	(30)	200	279
2	179	111	0	51	7	4	0	24	(9)	290	376
3	206	138	0	14	0	0	0	0	(32)	344	358
Mean	182	96	0	37	3	4	1	16	(24)	278	338
5d 1	25	71	0	14	22	57	18	82	(25)	96	289
2	55	1	0	65	25	24	13	96	(5)	56	279
3	126	89	0	81	61	10	7	27	(36)	215	401
Mean	69	54	0	53	36	30	13	68	(22)	122	323
8d 1	204	57	0	14	13	79	51	28	(22)	261	446
2	145	0	0	71	8	11	52	108	(52)	145	395
3	96	87	0	17	4	5	3	0	(18)	183	212
4	39	2	0	77	33	40	51	39	(0)	41	281
Mean	121	37	0	45	15	34	39	44	(23)	158	334
28d 1	0	0	0	214	87	60	46	4	(27)	0	411
2	131	0	0	125	16	63	67	39	(59)	131	441
3	128	51	0	138	29	29	4	8	(58)	179	387
Mean	86	17	0	159	44	51	39	17	(48)	103	413

E - erect villi
L - lateral collapse
L^1 - leaf shaped villi
V - vertical collapse
C - conical collapse
R - rudimentary villi
A - absent villi
D - debris
(X) - cracks, folds

Areas including cracks and folds have not been included in the total counts. Areas covered by debris have been included in the total but not scored, since it is difficult to estimate what type of mucosa they obscure.

correspond to those illustrated in the figures.

The grid used had 31 x 21 squares, each 1 cm square. Since there was a difference between the proximal duodenum, where villous damage was more common and severe, and the distal area where it was absent or mild, the data for these two areas have been presented separately. The largest ulcer was found to extend from the gastroduodenal junction almost as far as 25 squares on the grid, so this distance was taken as delineating the proximal part while the area covering the remaining 5 squares was considered as distal. This arbitrary subdivision is based on the SEM description of a small number of lesions. From the comparison of lesions produced by lower mediastinal (51 cases) as opposed to lateral thoracic irradiation (47 cases) it seems that a more appropriate dividing line would lie slightly proximal to the current one (Michalowski et al., 1983b and unpublished observations). For the purposes of describing the lesions examined with the SEM, the line has, however, been drawn to include the full length of the lesions mapped. In some cases, the specimen did not extend quite as far as 25 squares, but the shortfall was not a large one.

Tables 1 and 2 show for each specimen the total number of squares dominated by villi of a given type in the proximal and distal areas of the duodenum, respectively. The reading for "normal" villi includes erect, finger-shaped villi, as well as a few showing lateral collapse and the moderate number of leaf shaped villi in the proximal segment of the duodenum, just beyond the gastroduodenal junction. The percentage of each type of villus was then found and multiplied by the score relevant for that type. These figures were then totalled to give a final score for each specimen, and finally the mean for each group of mice. The final scores can be related to the maximum possible number for a fictitious specimen which would have 100% ulcerated surface assigned 10 on the scale, and thus the final score of 1,000.

The figures based on the percentage of each specimen taken up by the various types of villi, reflect the averaged severity but not the extent of mucosal damage. Further scores were therefore calculated, this time based on the absolute number of squares occupied by each villous type (area scores). Whereas the maximum possible percentage score would be 1,000, the maximum area score would be 6510. (31 x 21 squares in the grid times 10 score for maximum damage, i.e. loss of the epithelial cover.) The final area score was therefore divided by 6.51 to give equal weight to both types of score. A final total score for each ulcer was then computed by adding together the percentage score and the area score, with equal weight, and dividing the total by 200. The last score would therefore have a maximum of 10, as does the

Table 2

Villous patterns in distal duodenal areas

Specimen	E	L	L[1]	V	C	R	A	D	(X)	Total normal	Total (-X)
Con 1	55	2	0	0	0	0	0	0	(24)	57	57
2	72	0	0	0	0	0	0	0	(5)	72	72
3	80	1	0	0	0	0	0	0	(12)	81	81
Mean	69	1	0	0	0	0	0	0	(14)	70	70
3d 1	41	0	0	0	0	0	0	0	(16)	41	41
2	75	0	0	10	0	0	0	0	(0)	75	85
3	70	0	0	0	0	0	0	0	(5)	70	70
Mean	62	0	0	3	0	0	0	0	(7)	62	65
5d 1	18	0	0	0	0	0	0	44	(9)	18	62
2	39	0	0	1	0	0	0	2	(9)	39	42
3	69	0	0	5	5	0	0	1	(10)	69	80
Mean	42	0	0	2	2	0	0	16	(9)	42	61
8d 1	0	0	0	0	0	0	0	0	(6)	0	0
2	52	0	0	1	0	0	0	0	(11)	52	53
3	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-
28d 1	17	0	0	58	6	0	0	0	(12)	17	81
2	9	0	0	1	0	0	0	0	(15)	9	10
3	59	0	0	0	0	0	0	0	(21)	59	59
Mean	28	0	0	20	2	0	0	0	(16)	28	50

original villous scoring system (Carr et al., 1983). The final scores for both proximal and distal areas of duodenum are shown in Table 3. Both percentage scores and area scores are plotted in Figure 7.

Table 3

Final proximal and distal duodenal scores

Specimen		Score (proximal)	Score (distal)
Con	1	0.03	0.00
	2	0.00	0.00
	3	0.00	0.00
	Mean	0.01	0.00
3d	1	0.28	0.00
	2	0.21	0.07
	3	0.03	0.00
	Mean	0.17	0.02
5d	1	1.46	0.00
	2	1.04	0.01
	3	0.83	0.16
	Mean	1.11	0.06
8d	1	1.83	0.00
	2	1.51	0.01
	3	0.27	not available
	4	2.21	not available
	Mean	1.46	-
28d	1	2.52	0.55
	2	2.15	0.05
	3	0.92	0.00
	Mean	1.86	0.20

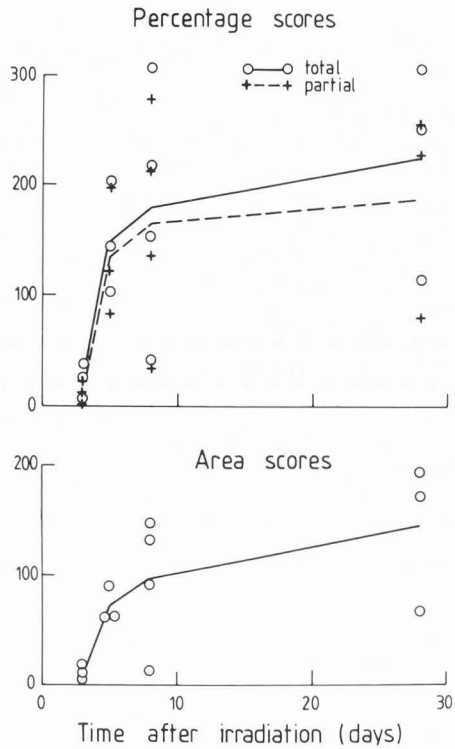

Figure 7. Graph to illustrate percentage scores and area scores, plotted against time after irradiation.

Discussion

This account of the surface structure of the duodenum in thorax x-irradiated mice deals with the nature, extent, localization and patho-mechanism of the remote damage thus induced. Each of these facets of the duodenal lesions will be discussed in turn.

Nature of abscopal duodenal lesions

The lesions appear as ulcers when studied by dissecting light microscopy (Michalowski and Burgin, 1982; Michalowski et al., 1983b). The greater resolution of the scanning electron microscope allows a close examination to be made of the covering of the damaged areas. These are seen as extensive, often sharply delineated mucosal regions whose chief distinguishing feature is the presence of villi which have lost height to a varying degree. In some cases, the villi are so damaged that the mucosal surface is flat, with crypt openings scattered over it. Parts of the affected mucosa may be covered with debris to a greater extent than is seen in control animals. Without further preparative manipulation it is impossible to see below this debris with the SEM. With the possible exception of these obscured areas, none of the damaged mucosa so far examined shows discontinuity of the epithelial covering. This implies that the nature of the damage is different from that described previously in rat duodenal ulcers produced by subcutaneous infusion of pentagastrin and carbachol (Carr et al., 1979) where, at the early stages, the epithelium was entirely absent over much of the ulcer floor. The two types of acute lesion are thus different, and the term "ulcer", which includes an implication of an epithelial breach, is applicable only to the rat secretagogue-induced duodenal damage, while the term "lesion" is preferable for the focal changes in villous structure of the murine duodenum, as induced by thoracic irradiation.

During the development phase also the two pathologies are not identical, with the honeycomb pattern seen during re-epithelialisation of the secretagogue-induced ulcers (Carr et al., 1979) not in evidence when the villous pattern in the thoracic radiation-induced lesions changes as time goes on. Thus, in both development and 'recovery', there exist structural differences between the lesions induced by thoracic irradiation in mice, on the one hand, and the secretagogue-induced ulcers in different animal species (Kowaleski, 1974;

Konturek et al., 1974; Ridley et al., 1974; Carr et al., 1979) and spontaneous human ulcers (Gregory et al., 1982), on the other.

Extent of abscopal duodenal lesions

The villous scoring system used in the present study has already been fully described (Carr et al., 1983). There are various ways in which it can be applied to the description of localised lesions. Any of these is useful in allowing some quantitative comparison of the lesions at different stages to be made. However, some discussion of the data in the tables is necessary before final conclusions can be drawn.

There are two problems in applying the scoring system: firstly, the handling of the data involving vertical villi and, secondly, the use of percentage and area scores to give a final score.

The inclusion or omission of scores from the least understood stage of villous collapse (vertical) has been considered. The data indicate that it makes little difference to the final progression of the numbers which method is used. Since this is the case, the total score has been used in Table 3.

The use of percentage and area scores has also been considered. The percentage score gives information on an averaged damage in the entire specimen, while the area score reflects both the absolute size and severity of the lesion. Both types of information are needed for a quantitative description of the lesions. Accordingly, the two scores were added after they had been adjusted to give both equal weighting. The final scores then allow a comparison between the proximal and distal areas (Table 3). The distal areas show much less damage than the proximal areas at each time point.

The most marked feature is the way in which the proximal scores increase steadily even up to 28 days after irradiation. This factor is outstanding when comparison is made with secretagogue induced ulceration, where many of the specimens showed marked healing by 10 days after ulceration (Carr et al., 1979). It is also a different pattern from the villous changes seen after direct abdominal irradiation (Carr and Toner, 1972; Anderson and Withers, 1973; Friberg, 1980; Carr et al., 1983), where damage is widespread and not confined to small localised lesions and where much of the damage is healed at times later than 5 days post irradiation. These abscopal lesions then are remarkable in their localisation and timing of development and are not similar to direct radiation damage to the epithelial and stromal compartments of the small intestine, suggesting that an entirely different pathomechanism is involved.

Position and possible cause of abscopal lesions

Previous descriptions of these lesions make it clear that their general position is dependent on the thoracic field irradiated, with lower mediastinal exposure, as used here, producing damage in the proximal duodenal region (Michalowski et al., 1983b). Study of this region with the scanning electron microscope confirms that there is damage in this proximal region and none in the region of the duodenal papilla, as produced by irradiation of the lateral thoracic fields (Michalowski et al., 1983b).

Further comment can be made on the exact parts of the proximal duodenum which are involved as a result of lower mediastinal irradiation. Before commenting on the development of the lesions, variations in villous shape in control animals should be discussed. These involve dividing the duodenum into a junctional region, which extends up to 4 mm from the gastroduodenal junction and the duodenum proper. The first of these regions (junctional region) has villi shaped like leaves or short ridges and extensive intervillous basins, while the second has finger shaped villi, as seen in the jejunum (Carr and Toner, 1972).

The lesions can be seen to occur at 5 days after irradiation, from the junction itself, right through the junctional region and well into the non-junctional region, to a final total length of up to 11 mm. This comprises the proximal area referred to in the tables. At the earlier stage of 3 days post irradiation, what little damage there was occurred only in the junctional region, approximately 1 mm from the junction itself. It is clear therefore that the areas most involved are at or near the gastroduodenal junction with the pyloric sphincter.

Another feature of interest is that the lesions vary in the extent to which they spread around the circumference of the duodenum. At five days after irradiation there are two patterns of damage, one in which the single lesion extends right around the circumference and the other confined to a narrower portion of the duodenum. Both the position of the damage, just distal to the pyloric sphincter, and the variation in its extent, point to a likely cause of the lesions: such a pattern would be produced by variable damage to the sphincter itself. This would cause an increase in sphincteric relaxation, thereby allowing acid stomach contents to enter the duodenum, with resulting mucosal damage. The two patterns of damage (circumferential or elongated lesions) could be caused by complete or partial sphincteric relaxation, respectively. A further contributing factor might be an impaired secretion by Brunner's glands reducing protection to the duodenal mucosal surface. The glands' product is alkaline, affording protection independent of that of the mucus.

Both sphincteric relaxation and/or reduced duodenal secretion could be caused by radiation damage to targets in the lower mediastinum. Two such targets are envisaged and damage to either or both could affect the sphincter. These are the autonomic nerve

supply and the blood supply. Damage to the latter in the thorax might be expected to lead to more widespread lesions than are seen, but cannot be ruled out. Damage to the autonomic supply could produce the described effects, if the sympathetic nerves which normally contract the sphincteric muscle, were affected. This would involve radiation damage to the sympathetic trunk in the region T_5 - T_{12}, or to the splanchnic nerves, either of which might fall within the lower mediastinal field.

While there are some problems in fitting this hypothesis in with the theories of stomach emptying, at this stage it is the most likely explanation for the presence of the abscopal lesions in their specific location and characteristic extent.

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Discussion with Reviewers

Reviewer I: In tables 1 and 2 the authors mention a total (-x) corrected in order to avoid the effects of cracks and folds of the specimens. These effects and debris are still remaining in the score and a systematic error is introduced by dividing the final area score by a 6.51 factor which comes from the full area (31 x 21 squares) containing cracks, folds and debris. How do you correct the wave effect due to flattening of the specimen?

T.D. Allen: What is the ulcer mapping actually telling us - conclusions do not seem to have been drawn?

Authors: The ulcer mapping is allowing the use of topographical changes as studied using the S.E.M. to compare damage in comparatively small areas of the gut. This helps to offset the dangers of subjectivity which may creep in when surfaces are being compared qualitatively. Thus the specimens 28 days after treatment were regarded as showing only moderate damage when they were examined without the scoring system: only after

scoring (and the use of mapping to adapt the scoring for small areas instead of an entire specimen as previously done) did the true extent of the residual damage become clear. This important final result has made the disadvantages of the technique seem worth tolerating. The problems of cracks, folds and debris and the wave effect were fully appreciated, but no sound method for dealing with them has yet been suggested.

Reviewer I: The Figures at x 16.5 magnification apparently do not cover the whole diameter of the duodenum. What about the area not shown and the region systematically neglected of the incision zone before flattening of the specimens?
Authors: The whole circumference was examined and scored, although not necessarily illustrated in the Figures. The neglected area of the incision zone was felt to be standard from specimen to specimen and was ignored.

D. Baldetorp: Perhaps the quality of the specimens could have been somewhat higher, what was the composition of the fixative vehicle?
Authors: The quality of the specimens was influenced by the comparatively large areas being scanned. With such areas, coating a surface covered in villous projections is difficult to do well: it was noted that the image quality was better over the flatter, damaged areas than in the more normal regions. This is a familiar problem, but with smaller specimens it is easier to optimise the adhesion and coating conditions. The fixative was prepared in Millonig's buffer solution.

T.D. Allen: Some of the Figures all suffer from charging artefact - perhaps some sort of osmium impregnation via OTO or Osmium TCH preparation would alleviate this problem?
Authors: The preferred technique always takes into account the need for plastic embedding, sectioning and staining after S.E.M. observation. While osmium impregnation would help the charging problem, it has been found in the past that it makes the subsequent procedure more difficult: it is likely that this would be a particular problem with such large specimens.

Reviewer I: Why was there selection of only ulcer positive specimens (day 5 to 28) and random investigation for day 3 and control?
Authors: The study aimed at extending earlier light microscopic observations on duodenal lesions induced by thoracic irradiation of mice (see References by Michalowski et al., 1982, 1983a and b). The average yield of duodenal lesions occurring in mice irradiated to the lower mediastinum with 16-30 Gy X rays and assessed with the dissecting microscope was approximately 50%, and the response was all-or-none in character. No lesion-negative specimens were included in the present study beyond the induction (latent) period of ulcer formation which was in excess of 3 days. Specimens with lesions were entered on the S.E.M. study at random. These lesions were compared with the duodenal surface structure during the lag period (day 3) and in controls.

D. Baldetorp: The dose 18 Gy mentioned is it a given dose or mid-point absorbed dose in the lower mediastinum? Peripheral nerves are considered to have a relatively high radiation tolerance, and very high radiation doses are needed to cause dysfunction and damage to the nerve. Do you think that structures in the sympathetic trunk are more radiosensitive than peripheral nerves in general and have you in the current study been able to demonstrate any postirradiation damage morphologically in a sympathetic nerve or ganglion?
Authors: All the experiments to date involved single exposure to X rays. The dose of 18 Gy was that absorbed in the mediastinal structures, as described in more detail in the paper by Michalowski et al., 1983b. We have carried out no studies on the nervous tissue in the irradiated volume but microscopic lesions in the sympathetic ganglia were described for thorax-irradiated rabbits [Sasaki, H. (1943) Causative mechanism of gastric and duodenal ulcer after X-irradiation of the upper thoracic spinal column. Nippon Acta Radiol. 4, 692-716].

D. Baldetorp: How was the dosimetry controlled?
T.D. Allen: There is a basic problem in abscopal effects, how can the authors be certain that the duodenum did not receive any direct irradiation?
Authors: The dose of scattered radiation absorbed in the pyloric region was measured using LiF dosimetry and found to be equal to 7.3 $\pm$ 2.7 % of that in the irradiated volume, i.e. 1.3 Gy (Michalowski et al., 1983b). This dose was not ulcerogenic and the incidence of duodenal lesions following thoracic irradiation was lowered by simultaneous exposure of the remaining parts of the body to X-ray doses of 1-5 Gy (Michalowski and Burgin, 1982). Thus the scattered radiation not only cannot be deemed responsible for the lesions, but actually acts to prevent their formation.

T.M. Seed: Were the unirradiated control animals anaesthetised in the same fashion as the irradiated test animals? What was the overall incidence, as well as the incidence of specific lesion types, at the various postirradiation time points? Similarly, what was the incidence of the 'low grade' lesions in the controls? Did the authors observe individual animals with multiple lesions in any of the animals, or were there only single lesions?

Authors: The control animals were anaesthetised along with those to be irradiated. In combined data of several experiments performed in 1981-1983 and involving a total of several hundred mice, the average incidence of proximal duodenal lesions induced by irradiating the lower mediastinum with 16-30 Gy was approaching 50%, with insignificant dose-dependence (Michalowski et al., 1983a). Beyond the induction period, the incidence did not change significantly during the 6 weeks following exposure. No lesions were ever observed in either control mice or in mice similarly irradiated to the upper mediastinum (Michalowski et al., 1983b). The lesions were as a rule single.

Reviewer I: Why were two different microscopes and different tilt angles used?
Authors: After the pilot experiments were done, a new SEM was purchased which gave better results more easily with these difficult large specimens. The original group could not be rescreened because they had been embedded in resin. However, the point at 8 to 9 days is a crucial one, since it links the current results with the previous work (Michalowski et al., 1983b). For that reason, the 8 day material was retained.

J.E. Bruni: In view of the radiosensitivity of crypt cells and their importance in the normal maintenance of villi, has disruption of proliferative events in the crypts been considered as a possible cause of the mucosal lesions? In the same context, it is difficult to understand how villi may disappear without some evidence of damage (discontinuity) in the mucosal surface. The preferred technique would have been to follow the histopathology of the lesioned mucosa by combined TEM and SEM.
Authors: Firstly, the radiosensitivity of the crypt cells is not thought to be relevant to the production of duodenal lesions by lower mediastinal irradiation. If anything, irradiation of the abdomen, in addition to exposure of the thorax, prevents lesion formation (Michalowski and Burgin , 1982). Secondly, the surface of the lesions is covered either with simple cuboidal epithelium or an inflammatory exudate, as seen with routine histological techniques [Man, WK, Michalowski, A, Li, SK, Barr, J, Burgin, J, Baron, JH and Spencer, J. Gastrointestinal histamine after thoracic irradiation in the mouse. Br. J. Exp. Path. in press]. Correlative resin histology is currently being carried out on the lesions studied by scanning electron microscopy and described in the current paper: transmission electron microscopy will be done on suitable samples.