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SIMULATION WITH PHARMACOLOGICAL AGENTS OF RADIATION DAMAGE TO SMALL INTESTINAL VILLI

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Abstract

Irradiation induces damage to intestinal villi, resulting in a progressive decline in villous height and changes in topography. Gamma and neutron radiation are reported to cause changes in the structure of smooth muscle and nerve twigs of the intestinal wall. It is possible, therefore, that villous collapse may be due partly to changes in the underlying stromal elements as a result of damage to nerve or muscle.

To test this hypothesis, mice were treated with the drug reserpine which is known to affect the neural control of intestinal smooth muscle function and the small intestine was examined for topographical and histological changes. Two dose levels of reserpine were used and a group of mice were exposed to a single dose of whole body 15 Gy X-irradiation. Comparable villous collapse was observed in each group. Resin embedded semi-thin sections revealed changes in the smooth muscle cells of the muscularis externa after each treatment, suggesting a correlation between villous collapse and smooth muscle damage in response to both irradiation and drug treatment.

KEY WORDS: Scanning, electron microscopy, light microscopy, irradiation, reserpine, intestine.

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Introduction

Irradiation effects on the gastro-intestinal tract of laboratory animals such as mouse and rat vary from damage to the proliferative capacity of the crypt cells of the small intestine (Quastler, 1956; Quastler & Hampton, 1962; Hugon & Borgers, 1968; Hornsey, 1970; Becciolini et al., 1976; Ciecura et al., 1976; Lieb et al., 1977; Potten, 1977; Potten et al., 1978, 1983) to intestinal metaplasia in the pyloric glands (Watanabe, 1978).

The effect of radiation damage to mouse small intestine examined by scanning electron microscopy (SEM) was reported to consist of a progressive change of villous shape leading to flattened irregular mucosa (Carr & Toner, 1972). Similar observations were reported in the rat intestinal mucosa (Anderson & Withers, 1973). Carr et al. (1983b) postulated that such villous collapse could be partly due to damage to intravillous pegs and pericryptal plates of stroma which are composed of connective tissue, blood vessels and smooth muscle cells. There are already reports of irradiation damage to neural and muscular elements in other situations in the body. For example, single dose whole body exposure to 15 Gy X-irradiation resulted in dysplastic changes of the choroid plexus in mice (Heinzmann, 1982). Other effects of X-irradiation on the nervous system include inhibition of microtubular protein assembly in bovine brain (Coss et al., 1981), destruction of granule cells of the dentate gyrus in rats (Lee et al., 1982) and reduction in Schwann cell proliferation in injured sciatic and posterior tibial nerves (Love, 1983). Alterations in the cellular ionic environment (Roizin et al., 1974) and damage to neurones, such as alteration of shape, swelling of axons (d'Amelio et al., 1982); swelling of mitochondria, endoplasmic reticulum and Golgi, degeneration of myelinated processes or synaptic structures (de Estable Puig & Estable Puig, 1973) and damage to cells within the spinal cord (Mastaglia et al., 1976) are also included in the reported changes after irradiation.

Irradiation damage to muscle includes reduction of regenerative capacity in mouse dystrophic leg muscles (Wirtz et al., 1982), myofibrillosis, accumulation of debris and lipid and

capillary degeneration in cardiac muscle (Stearner et al., 1979). Damage to the myocardial mitochondrial membrane resulted in fibrosis (Maeda, 1982) and damage occurred in the smooth muscle component of the coronary artery (Yang & Ainsworth, 1982).

Thus, irradiation has been observed to produce damage to nerve and muscle in various sites as well as to epithelium, which is the tissue most commonly implicated in post-irradiation damage in small intestine: recent work (Carr et al., 1984a) has reported changes in connective tissue cells and organisation as well as in nerve and muscle. There is no report of how any damage to nerve and muscle might contribute to the overall radiation effects in the intestinal wall. The aim of this study, therefore, was to explore the possibility of simulating radiation damage to villi with a drug known to affect the neural control of the gastro-intestinal tract smooth muscle and hence the stromal compartments of the villi. Reserpine was chosen, since by impairing storage of noradrenaline within sympathetic nerves, it causes transmitter depletion and loss of sympathetic adrenergic inhibitory control of intestinal muscle (Carlsson, 1966). Reserpine also affects storage of 5-hydroxytryptamine and, to a lesser extent, histamine and adrenaline.

The overall effect of reserpine in the gastro-intestinal tract is to increase motility and secretion. Since the villus is crucial to these physiological functions, it might be expected that reserpine would alter villous morphology and provide some insight into the mechanisms underlying the changes induced by irradiation.

Materials and Methods

Two groups of male BALBC mice (30 g weight) were used; the first group was exposed to irradiation and the second group was drug-treated.

Irradiation

The mice were placed in a perspex jig and exposed to a single dose of 15 Gy X-rays. Tissues were taken for examination 18 hours and 3 days after irradiation. The irradiation times were chosen to include the period up to maximum intestinal damage, which is seen about three days after treatment.

Drug Treatment

Reserpine (1 mg/kg or 16 mg/kg) was administered by intraperitoneal injection in a volume of 0.2 ml ascorbic acid (4%). Tissues were taken for examination 1, 3 and 18 hours after reserpine administration.

The reserpine doses were chosen since a dose of 1 mg/kg would give a maximum depletion of neuronal stores of catecholamines and a dose of 16 mg/kg, like the dose of irradiation used, lay at the upper range of the level of tolerance. The time intervals were selected to observe what damage occurred early after drug treatment and at 18 hours when noradrenaline depletion would be well developed and there was overlap with the time schedule of the irradiation experiments. Mice which received either no injection or ascorbic acid only were used as control groups.

Specimen Preparation

Mice were killed by cervical dislocation. The stomach and intestines were exposed by a midline abdominal incision and were removed from the animal. The entire stomach with the proximal 1 cm of duodenum was isolated. 5% glutaraldehyde was injected into the fundus, taking care to avoid distension of the intestine. The stomach was opened along the greater curvature up to and then along the mesenteric border of the duodenum, mounted on cork and rinsed in 5% glutaraldehyde prior to fixation for at least 24 hours in glutaraldehyde. The tissues were prepared in the following way:

Samples were exposed for 30 minutes to 1% aqueous osmium tetroxide and taken through a series of alcohols, starting at 70% and ending with two changes of absolute alcohol. They were then transferred to two changes of amyl acetate prior to critical point drying and mounting on stubs. These were coated with gold for 4 minutes in a sputter coater before examination in a JEOL T300 scanning electron microscope.

The proximal first centimetre of the duodenum of each sample was scanned at 20 kV whenever specimen quality permitted, at a tilt of 40° to the horizontal. Series of micrographs were taken at a low magnification, moving distally from the gastro-duodenal junction.

The scanned samples were re-processed and embedded in resin for histological examination. A 4 mm square piece of duodenum was removed from the stub and taken through four changes of absolute alcohol, being left for 8 hours in each, with the exception of the second change in which they were left overnight. The specimens were then taken through two changes of propylene oxide, being left for 30 minutes in each before being transferred to a mixture of equal amounts of propylene oxide and Spurr's solution for 3 hours. Thereafter they were steeped overnight in a mixture of one part propylene oxide and three parts Spurr's solution. Samples were then taken through two changes of Spurr's solution, being left for 48 hours in each, prior to embedding in Spurr's resin.

The embedded samples were placed in a cold oven overnight. The temperature was increased to 45°C for 3 hours, before being raised to 60°C for 24 hours to allow the resin to harden. 1.5µm thick sections were stained with Azur II blue for histological examination.

Results

Direct Observations

Gross effects of both irradiation and reserpine treatment were observed. Mice 18 hours and 3 days after irradiation showed little spontaneous activity. Control mice and those treated with ascorbic acid were fully active as were those receiving reserpine (1 mg/kg) after 1 and 3 hours. However, mice were lethargic and showed no spontaneous activity 18 hours after treatment with 1 mg/kg reserpine and at all time intervals after treatment with 16 mg/kg reserpine. They were cold to the touch and showed nasal

Table 1

Summary of scanning electron microscopic observations on tissue following irradiation. Ranges for villous score are given in parentheses.

Experiment	Dose	Time after Exposure	Scanning Electron Microscopic Observations		
			Villous Structure	Debris	Villous Score
Control	-	-	Closely packed vertical, lateral collapse	-	0.2 (0 -0.3)
Whole Body Single Dose X-irradiation	15Gy	18 hrs	Vertical and lateral collapse	+	0.5 (0.5-0.5)
	15Gy	3 days	Horizontal, lateral and conical collapse and rudimentary villi	+	1.4 (1 -2.3)

The number of mice in each group was 3 or more

congestion, ptosis, pilo-erection and diarrhoea. After irradiation the stomach was shrivelled and empty. Ascorbic acid treated animals did not differ from controls, whereas in reserpinised animals, the fundus was prominent and gastro-intestinal motor activity was increased. The spleen was also engorged and the blood vessels appeared dilated.

Topographical Observation

Scanning electron microscopic observations allowed comparison between finger shaped control villi (Figure 1) and those after treatment (Figures 2 - 6). Montages were prepared from the micrographs which were examined for alterations in the shape of the villi and scored as follows, for villous structure (Carr et al., 1983a). A score of zero was given to normal villi which are vertical and erect, but occasionally show lateral collapse. The villous score increased cumulatively with severity of damage. Villi showing vertical collapse (i.e., erect but shorter and broader based) or horizontal collapse (lying flat on the intervillous basin) were scored as one. the next stages in the progressive damage are conical villi, scored as 3.5, followed by rudimentary villi (villi present only as clumps of epithelial cells), scored as 6, and then complete disappearance of villi (flat mucosal surface) scored as 8.5. Individual scores for villous topography were calculated for each animal and the mean was taken for each experimental group. These values are given in Tables 1 and 2, and represented in a histogram, Figure 7. Although high scores were recorded for some areas (e.g., for the presence of severely damaged villi) the final score for each group attempted to take into account the range of each villous shape and was, therefore, much lower than the maximum score for the worst damaged villi.

Control Specimens

Villi in control samples were finger-like and erect and received a score of 0.2 (Figure 1). Samples from ascorbic acid treated mice showed a clean surface with less mucus than in controls (Figure 2): apart from some horizontally and vertically collapsed villi 18 hours after treatment there were few differences from control mice.

Irradiated Specimens

At 18 hours after irradiation, most villi showed vertical collapse. Horizontal and conical collapse, and rudimentary villi were restricted to the 3-day time point (Table 1, Figure 3). Although much particulate debris was seen, mucus could not be distinguished from it.

Reserpine-treated Specimens

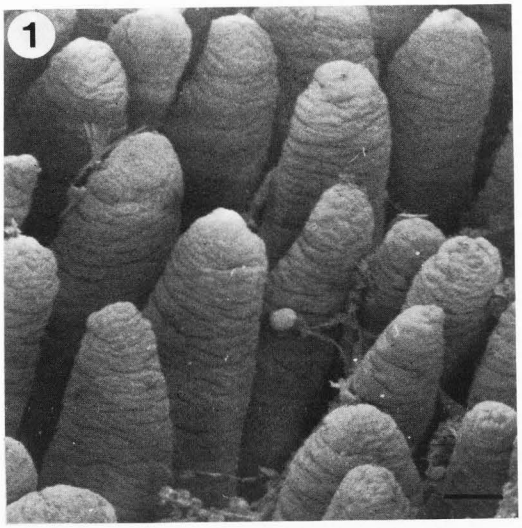
Specimens from low dose (1 mg/kg) reserpine treated animals contained villous damage ranging from vertical to horizontal collapse (Figure 4), though at the 1 and 18 hour time points, areas of clumping of villi and small villi could be seen (Table 2). Distinguishing features after 3 and 18 hours were large, fused villi (Figure 5, Table 2) and gaps between individual villi as opposed to the closely packed appearance in the control samples. Varying amounts of mucus and debris were seen in all the reserpine treated samples, particularly those at 1 and 18 hours (Table 2).

In the high dose reserpine (16 mg/kg) treated samples a similar pattern of damage, namely vertical, horizontal and conical collapse, was seen (Figure 6). The control and reserpine observations are summarized in Table 2.

Histological Observation

Finger shaped villi were seen in the control samples. In both irradiated and reserpine treated samples alterations in villous shape were seen, confirming the changes in shape observed with the scanning electron microscope. Treated samples from both groups were clearly distinguishable from controls (Figures 8 - 13). The most marked changes were in the muscle cells, and these changes were found where large, broad villi or short villi occurred.

The control smooth muscle cells did not show any signs of damage and did not vary in staining density (Figure 8). The damage seen after irradiation or reserpine treatment varied from gaps between the cells to abnormalities in the cytoplasm. In the irradiated samples the cytoplasm was streaky and densely stained (Figures 9 and 10). After treatment with the low dose of reserpine gaps were seen between individual cells in addition to some dense patches in the cytoplasm (Figures 11 and 12). In the high dose reserpine treated specimens the density of staining varied and the cytoplasm showed dark areas (Figure 13).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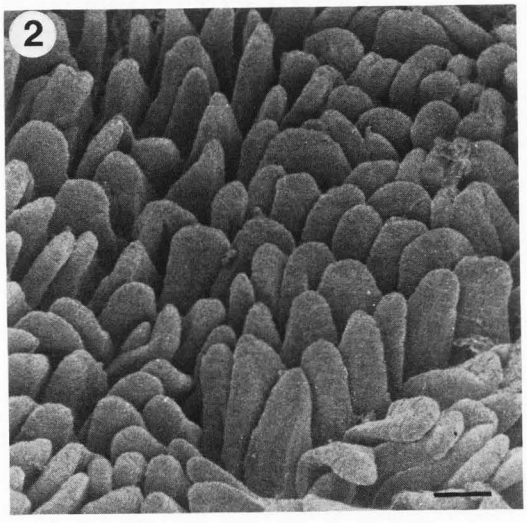
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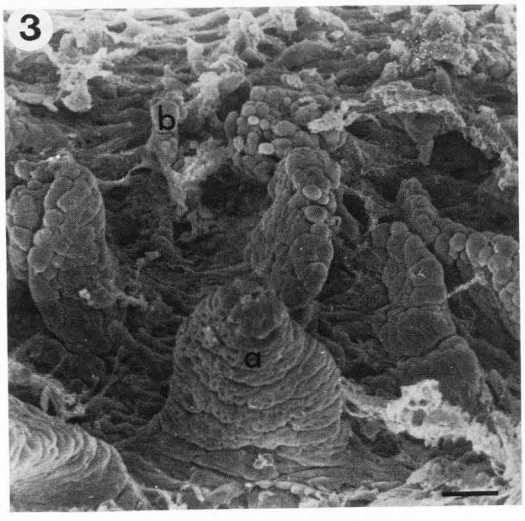
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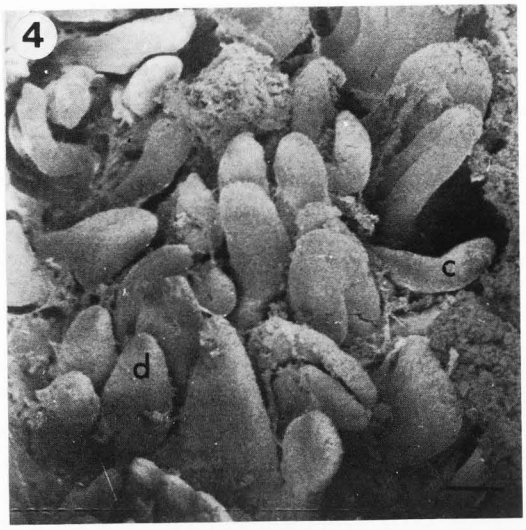
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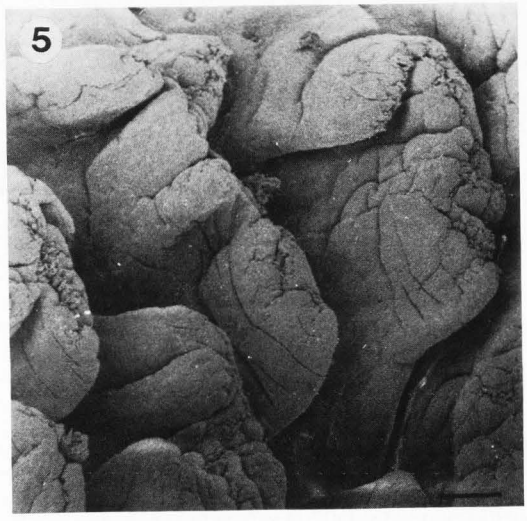
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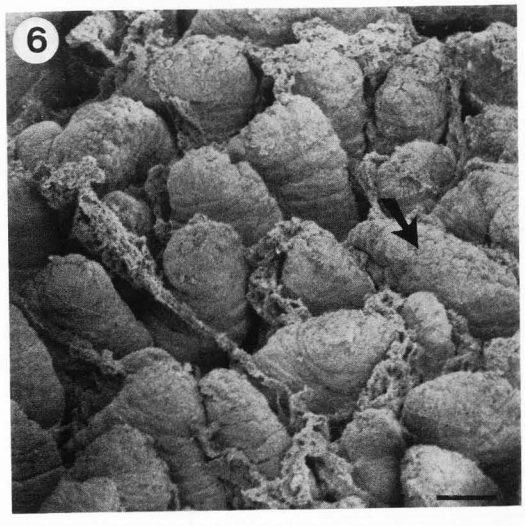
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Figure 1. Scanning electron micrograph of finger shaped control villi. Bar = 0.6 mm.

Figure 2. Scanning electron micrograph of villi after treatment with ascorbic acid. The surface is free of mucus. Some laterally and vertically collapsed villi are seen. Bar = 15 mm.

Figure 3. Scanning electron micrograph of villi three days after exposure to 15 Gy X-irradiation:
a) conically collapsed villus
b) rudimentary villus
The surface is severely damaged. Bar = 0.6 mm.

Figure 4. Scanning electron micrograph of villi one hour after treatment with reserpine (1 mg/kg):
c) horizontally collapsed villus
d) vertically collapsed villus
The surface shows debris and mucus. Bar = 15 mm.

Figure 5. Scanning electron micrograph of villi eighteen hours after treatment with reserpine (1 mg/kg). The villi are fused, as described in the text. Bar = 0.6 mm.

Figure 6. Scanning electron micrograph of villi eighteen hours after treatment with reserpine (16 mg/kg). Arrow indicates fused villus. The remaining villi are vertically or conically collapsed. Bar = 0.6 mm.

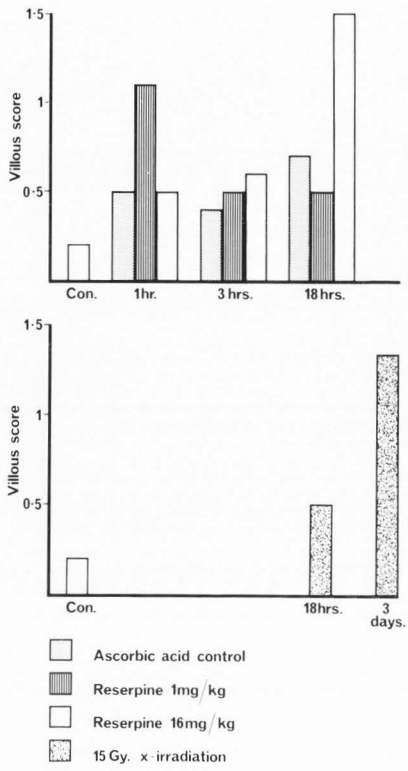


Figure 7. Histogram of mean villous scores against time after irradiation or treatment with ascorbic acid or reserpine as compared with controls.

Discussion

Radiation Changes

The current report presents scanning electron microscopic observations showing alterations in the mucosal surface of the small intestine after whole body X-irradiation. These observations include a progressive decline from finger shaped erect villi (18 hours after exposure) and conical and rudimentary villi (3 days after irradiation). These findings are similar to previous reports on villous morphology, both for control samples (Carr & Toner, 1968; Marsh et al., 1968; Asquith et al., 1970; Burke & Holland, 1973), and for irradiated villi (Carr & Toner, 1972; Anderson & Withers, 1973; Carr et al., 1981, 1983a).

The mean villous score increased from 0.5 at 18 hours after irradiation to 1.5 at 3 days. These values indicate that the severity of damage increased with time. Compared with previously published villous scores of 0.6 three days after a single dose of 16 Gy X-irradiation (Carr et al., 1983a), the current score of 1.5 is substantially higher. The difference in the scores for the two sets of data may be attributed to the use of two different strains of mice, in agreement with the previous suggestions that villous damage may be strain dependent (Carr et al., 1984b).

Light microscope studies revealed extrusion of cells from villous tips and the presence of dead cells in crypts, both at 18 hours and 3 days. In addition, at 3 days, the surface epithelium consisted of very short cells. These observations on epithelial damage are consistent with earlier reports (Quastler & Hampton, 1962; Frieburg, 1980; Carr et al., 1984b) and underline the importance of the response of the mobile epithelial compartment in the total expression of radiation injury.

However, in the current study changes were also seen in smooth muscle. The changes included gaps between individual cells and densely stained cytoplasm with areas of dense patches. These can be compared with the ultrastructural observations of Carr et al. (1984a), where gaps between smooth muscle cells were seen after gamma irradiation. In addition, previous reports of irradiational damage to muscle in other situations of the body (Stearner et al., 1979; Maeda, 1982; Yang & Ainsworth, 1982; Wirtz et al., 1982) confirm the possibility that cells of this type may suffer irradiation damage expressed as morphological changes.

The smooth muscle damage reported here seemed to be related to areas of occurrence of either large or short villi, thus suggesting a possible link between villous morphology and smooth muscle structure. It seems, therefore, that the role of smooth muscle in the expression of irradiation damage in the intestinal wall has indeed been underestimated and requires further consideration.

Table 2

Summary of observations as seen in the scanning electron microscope. Ranges for the villous score are in parentheses.

Treatment	Time after Treatment (hours)	Scanning Electron Microscopic Observations		
		Villous Structure	Mucus/ Debris	Mean Villous Score
Control	-	Closely packed, vertical lateral collapse	-	0.2 (0 -0.3)
Ascorbic Acid Control	1	Closely packed. Some horizontal and vertical collapse	+	0.5 (0.5-0.5)
	3	Closely packed. Some horizontal and vertical collapse	-	0.4 (0.3-0.5)
	18	Closely packed. Horizontal and vertical collapse	-	0.7 (0.5-1.0)
Reserpine 1 mg/kg	1	Closely packed. Vertical collapse. Some fusion. Areas of clumping, small villi present	++	1.1 (0.5-2.3)
	3	Separated and vertical, lateral and horizontal collapse. Small villi present	+	0.5 (0.5-0.6)
	18	Separated and vertical, horizontal and lateral collapse. Some fusion. Areas of clumping. Small villi present	++	0.5 (0.5-0.6)
Reserpine 16 mg/kg	1	Closely packed. Vertical and lateral collapse. Some fusion	+	0.5 (0.5-0.5)
	3	Separated and vertical, horizontal and lateral collapse. Some fusion. Areas of clumping	++	0.6 (0.5-0.7)
	18	Separated and vertical, horizontal and lateral collapse. Some conical collapse. Some fusion. Areas of clumping. Small villi present	+++	1.5 (1.0-2.3)

The number of mice in each group was three or more

Reserpine Changes

Reserpine, at both doses, produced topographical and histological changes similar to those seen after irradiation. The high dose of reserpine produced maximum damage at 18 hours, the extent of which was similar to that seen after irradiation at 3 days (Table 2); the villous scores were 1.5 for reserpine and 1.4 for irradiation. It may be, therefore, that the changes in function observed after either reserpine or irradiation are linked to the high villous scores. The low dose of reserpine also produced damage, resulting in a score of 1.1. Reserpine produces maximum depletion of stores of catecholamines in 18 - 24 hours (Anden & Henning, 1966), and the observed changes may reflect the impairment of sympathetic nerve function and withdrawal of inhibition from cholinergic motor nerves in the intestinal nerve plexuses (Gershon, 1981).

The histological changes seen after reserpine treatment were similar to those observed after irradiation in that smooth muscle was affected. The changes in smooth muscle itself after reserpine would not be anticipated from the

Figure 8. Light micrograph of control sample:
A - inner, circular muscle layer
B - outer, longitudinal muscle layer
Bar = 10 μm.

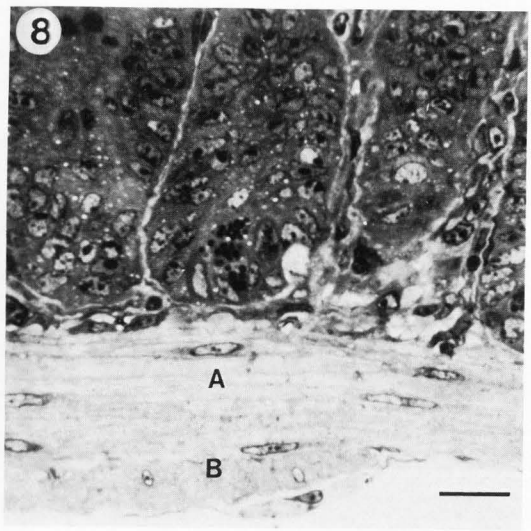
Figure 9. Light micrograph of samples eighteen hours after exposure to 15 Gy X-irradiation:
T = dense patches in the cytoplasm
Bar = 10 μm.

Figure 10. Light micrograph of samples eighteen hours after exposure to 15 Gy X-irradiation:
X = streaky, dense cytoplasm
Bar = 10 μm.

Figure 11. Light micrograph of samples one hour after treatment with reserpine (1 mg/kg):
P = areas showing gaps in the muscle layer
Bar = 10 μm.

Figure 12. Light micrograph of samples one hour after treatment with reserpine (1 mg/kg):
R = dense patches in the cytoplasm
Bar = 10 μm.

Figure 13. Light micrograph of samples eighteen hours after treatment with reserpine (16 mg/kg):
S = granularity in the smooth muscle layer
Bar = 10 μm.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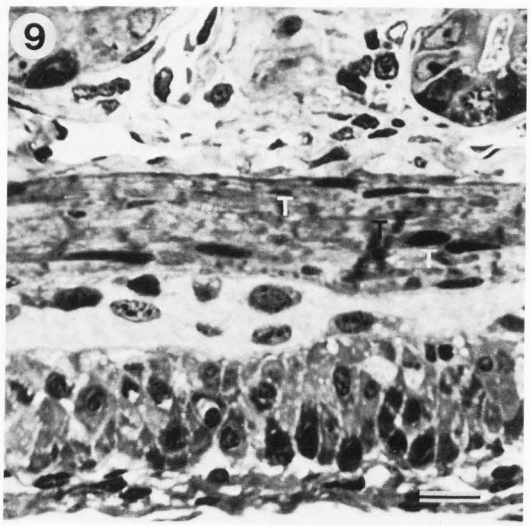
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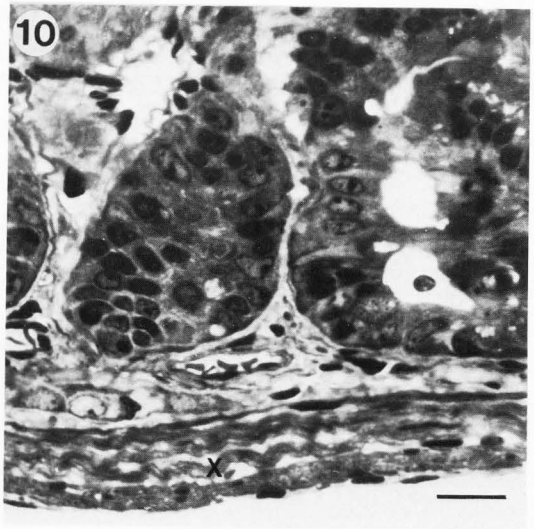
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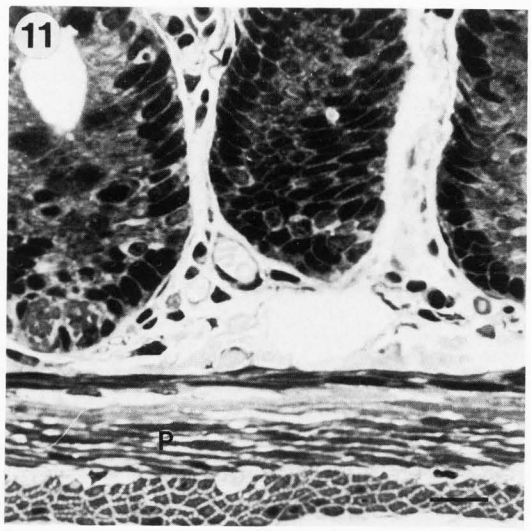
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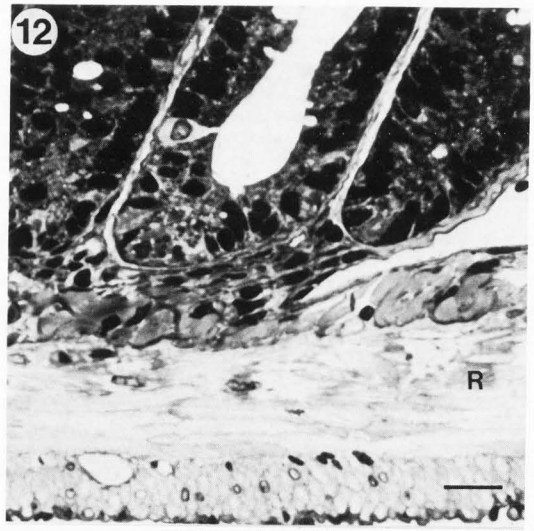
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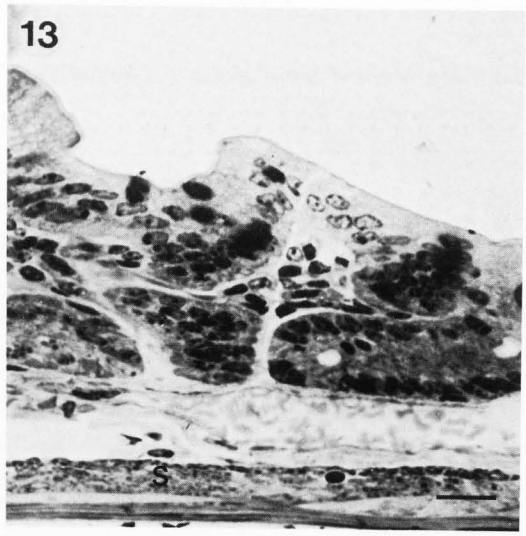
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drug's known ability to impair sympathetic neurone function. The changes could be due also to the doses of reserpine used, particularly that of 16 mg/kg which may have been exerting non-specific effects. However, Casteels & Login (1983) have reported a direct depressant action of reserpine on the smooth muscle cell membrane. It has also been reported that reserpine can affect cell proliferation in developing rat brain (Patel, et al., 1979) and the reduction may be partly due to a direct action on the proliferating ependymal cells. Thus the observed changes in villous morphology reported in the present paper may be due to the combined effects of reserpine on neuronal function and smooth muscle.

The observation that both irradiation and reserpine alter villous structure supports the hypothesis that villous collapse may be due partly to changes in the underlying stromal elements as a result of damage to nerve or muscle. The findings that the time courses of the effects of the two treatments differed and that rudimentary villi were not seen after reserpine treatment, may indicate that different mechanisms of action underly the treatments. Further information as to the effects of irradiation and reserpine can be obtained from study of the ultrastructure observed with transmission electron microscopy. Such a study may reveal evidence of neuronal damage due to irradiation as has been reported already in the literature.

Ascorbic Acid Changes

In ascorbic acid treated animals at 18 hours, the villous score was 0.7, and there was separation of the smooth muscle cells, again supporting the view that an effect on smooth muscle may be involved in changes in villous morphology. After ascorbate treatment, the intestinal surface was free of mucus. A similar picture has been reported in fixed intestinal samples treated with ascorbic acid prior to dehydration (Carr et al., 1984b). Thus ascorbic acid both *in vivo* and *in vitro* results in the removal of mucus, possibly by directly flushing out previously secreted mucus.

Since villous damage resulting in a score of 0.7 and some separation of smooth muscle cells were both seen 18 hours after giving ascorbic acid, some of the effects seen after reserpine treatment may be partly attributable to the ascorbic acid which was used as a vehicle to dissolve the reserpine.

Ascorbic acid could affect mucosal structure either by virtue of its low pH or by more specific effects. The former could be studied further by examining the effects on the internal structure of the intestinal wall of a range of substances with different pH levels. One possible specific effect would be the ability of ascorbic acid to enhance the effects of dimethyl phenyl piperazine (DMPP) which by stimulating the ganglion cells of Auerbach's plexus increases acetylcholine release and hence increases gastrointestinal motor activity (Hayashi et al., 1983). It seems then that the current observation of villous collapse produced by ascorbic acid alone requires further study to determine its precise mechanism of action.

Conclusions

The effectiveness of reserpine was confirmed by the observations of the external appearance of the mice, their reduced spontaneous activity and the dilatation of blood vessels and increased intestinal motility, indicating the loss of sympathetic nerve function. The presence of similarities in villous damage and histological changes after both reserpine and radiation treatment supports the hypothesis that changes in the muscular component of the intestinal wall may contribute to the post-irradiation changes of villous structure which have been so widely reported. Transmission electron microscopic studies are required to investigate further the changes in nerve and muscle in the intestinal wall after these two different forms of treatment, to determine whether the two mechanisms are in any way similar or are producing similar mucosal surface damage by totally different underlying mechanisms.

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Discussion with Reviewers

C.S. Potten: I do not completely understand the scoring system. The scores can go as high as 8.5. Some of the pictures show rudimentary villi which would be scored as 6. Why is it that the scores in the tables never exceed 1.5? Does this mean that in the most severely damaged cases many villi are still relatively normal?
Authors: The score of 1.5 is a mean of the average scores of all samples in that group. The average score for each sample was obtained by scoring each type of collapse found in that sample. Normal villi were seen in all but the most severely damaged samples and their presence contributed to a reduction in the average score.

J.R. Poley: Ultrastructurally, what is the difference between the villous surface in figure 1 (control) and figure 2 (ascorbic acid)? Macroscopically the surface is free of mucus (figure 2) and the morphologic aspect of the villi is somewhat different.
Authors: There were no topographical differences between control and ascorbic acid treated microvilli, although sharper micrographs were obtained more easily in the latter case.

J.R. Poley: Is there a possibility that radiation or reserpine may damage the brush border cytoskeleton which may then collapse and lead to a reduction of villous height?
Authors: We have no information on the brush border cytoskeleton.

J.R. Poley: Are the mucosal changes following radiation/reserpine compatible with those seen after colchicine?
Authors: From the literature, the mucosal changes following colchicine treatment include a decrease in the transport of ^{3}H-Fucose-labelled glycoproteins to the brush border and an increase of radiolabel in the Golgi apparatus, apical vesicles and tubules, lysosome-like bodies and lateral plasma membrane [Block, J, Ginsel, LA et al. (1981) The effect of colchicine on the intracellular transport of ^{3}H-Fucose-labelled glycoproteins in the absorptive cells of cultured human small intestinal tissue. Cell and Tissue Research, 215, 1-12]. With the information available so far we are unable to comment on reserpine-induced changes in the Golgi apparatus or plasma membrane.

J.R. Poley: What is the state of the crypts in mucosal specimens shown in figure 3? Hypoplastic? Hyperplastic?
Authors: No attempt has been made to classify the crypts as hypoplastic or hyperplastic.

J.R. Martinez: Although the authors report several previous findings indicating that irradiation damages both neural and smooth muscle structures in several tissues, the exact connection between changes (densely stained cytoplasm, areas of dense patches, gaps between muscle cells) in the smooth muscle cells of the muscularis externa and villous collapse in the intestine is not made clear. What evidence can the authors provide that such alterations in smooth muscle cells actually have a causal relationship with villous collapse and other changes in intestinal villi?
Authors: The changes in smooth muscle were seen chiefly underlying collapsed villi after radiation or reserpine treatment. There is no evidence that there is any causal relationship between villous collapse and damage to muscle. However, the results do not disprove the hypothesis that such a link may exist. The results are encouraging, therefore, in indicating the need for further work.

J.R. Martinez: Is there any reference on the effects of reserpine on intestinal catecholamines?
Authors: The effects of reserpine on the gastrointestinal tract were first described by Gillespie and Mackenna (Gillespie, JS & Mackenna, BR. (1961) The inhibitory action of the sympathetic nerves on the smooth muscle of the rabbit gut, its reversal by reserpine and restoration by catecholamines and by DOPA. J. Physiol. 156, 17-34.) and the consequences of this action in increasing motor activity are reviewed by Gershon (1981).

Reviewer IV: 5% glutaraldehyde was used as the fixative. What buffer did you use? Is 5% not a rather high concentration of glutaraldehyde?
Authors: The buffer used was Millonig's buffer. The concentration of glutaraldehyde used was 5%. It is rather high but it is the concentration that has been used for a number of years in these experiments. It was chosen for perfusion fixation rather than immersion fixation, but we still use it to allow comparison between all specimens, no matter when they were prepared.

Reviewer IV: Could you explain what is meant by "the fundus was prominent"?
Authors: The fundus appeared large and could be distinguished easily.

Reviewer IV: Could you state precisely which part of the duodenum was examined?
Authors: The proximal 1 cm of duodenum was examined beginning at the gastroduodenal junction.

Reviewer IV: Could you explain what is meant by "the low cell position" in the jejunum?
Authors: The low cell position is a term used by Potten and his co-authors in their description of crypt cells. The "low cell position" cells are the crypt cells adjacent to the Paneth cells (Potten et al., 1978).

Reviewer IV: Could you state what difference, if any, there was in the degree of intestinal epithelial damage in the various groups?
Authors: There were no noticeable changes in epithelial structure after any of the treatments apart from an increase in apparent cell extrusion after irradiation.

Reviewer IV: What vascular lesions were present on histological examination? Do you not consider that vascular damage may contribute to the lesions of the smooth muscle cells ?
Authors: Vascular lesions were not identified on histological examination, but it is possible that smooth muscle damage could be secondary to damage to blood vessels or nerves.