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Effects of irradiation on intestinal cells *in vivo* and *in vitro*

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Summary. The effects of irradiation on intestinal epithelial cells were analyzed *in vivo* and *in vitro*. The *in vivo* study was carried out on the rat small intestine and for the *in vitro* study the intestinal crypt cell-line IEC-6 was used. Rat intestine and IEC-6 cells were irradiated with X-ray doses ranging between 1-16 Gy. Energy-dispersive X-ray microanalysis was used for detection of the elemental changes in the cells. Cell morphology was investigated in the scanning electron microscope, DNA-synthesis by autoradiography of ^{3}H-thymidine incorporating nuclei and proliferation by cell counting. Our results indicate that *in vivo*, in the crypt cells, the increasing doses of irradiation led to increased sodium and lowered potassium and phosphorus concentrations. Corresponding ion shifts were found in the irradiated IEC-6 cells. Cells continued to proliferate up to the dose of 8 Gy, although the proliferation rate became lower with increasing dose of irradiation. The increasing dose of irradiation significantly reduced DNA-synthesis (16 Gy decreased DNA-synthesis by 50%) which resulted in a complete inhibition of cell proliferation. Analysis of goblet cells also showed characteristic radiation-dependent elemental changes. Scanning electron microscopical investigation of cells in culture revealed that most of the control cells were flat and had rather smooth cell membranes. Irradiation led to the appearance of numerous different membrane manifestations (microvilli of varying length and distribution, and blebs). Frequency of differences in the topology of the cells was related to the dose of irradiation. Our study clearly demonstrates that even low doses of irradiation cause changes in the ionic composition of the cells and inhibit DNA-synthesis and cell proliferation. The effects observed in the crypt cells *in vivo* were the same as in the intestinal cell line *in vitro*, which indicates that IEC-6 cells can be used for investigation of side effects of radiation to the abdomen.

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Introduction

Gastrointestinal symptoms often appear in humans and animals exposed to irradiation (Trier and Browning, 1966; Bloomer and Hellman, 1975; Donaldson et al., 1975; Baverstock and Ash, 1983; Langberg et al., 1996; Fraser et al., 1998; Denham et al., 2000). This has been shown both in individuals exposed incidentally to external irradiation and in patients undergoing therapeutic irradiation of malignant intraabdominal or pelvic tumours. The reactions of the small intestine vary, depending on the dose and the time elapsed thereafter, from slight mucosal inflammation with diarrhoea to excessive formation of fibrosis, causing adhesions and strictures of the bowel (Localio et al., 1979; Hauer-Jensen, 1990). Clinical observations in patients exposed to irradiation from the nuclear power plant in 1986 in Chernobyl showed that even exposure to low doses led to long-standing gastro-intestinal problems with fibrosis, mal-absorption and abdominal discomfort (Gale, 1987; Gottlober et al., 1997). Elevated cancer rates were shown after the Three Mile Island nuclear accident in 1979 (Hatch et al., 1991). The pathogenesis of radiation induced intestinal fibrosis is not fully understood (Hauer-Jensen, 1990) and there are no methods by which development of fibrosis can be prevented. Surgical treatment of fibrosis is complicated and often followed by delayed or incomplete healing, anastomosis and fistulae formation (Dirksen et al., 1977). Dysmotility following abdominal irradiation of small intestine in rat has been demonstrated by Fraser et al. (1998).

Moderate irradiation causes transient changes in the mucosa and permanent changes in the submucosa. Radiation has been shown to affect the stem cells in the intestinal epithelium (Potten et al., 2001). The first signs of epithelial cell damage already appear after a dose of 9 Gy and remain histologically detectable after 3 days. In the submucosa the changes are detectable first after 10

days (own observations, Rubio and Jalnas, 1996). Changes in the water content and loss of sodium were found in whole body irradiated rats (Geraci et al., 1987). To our knowledge no analytical data have been reported on the ionic changes at the cell level.

The aim of the present study was therefore to investigate if histologically detectable epithelial cell damage caused by irradiation is preceded by ionic changes in the cytoplasm of irradiated cells. The cells most sensitive to irradiation are epithelial cells in the crypts. We have therefore chosen to study *in vivo* the effects of varying doses of radiation on the crypt cells of the rat small intestine. For analysis under better defined experimental conditions an *in vitro* system consisting of the intestinal crypt cell line, IEC-6, was used.

Materials and methods

In vivo studies

30 male Sprague-Dawley rats, weighing approximately 350 g were used. Anesthesia was given by an intramuscular injection of Hypnorm (0.3 ml/kg) and Valium (0.2 ml/kg). Through a short midline incision the distal part of the ileum was lifted out and a standardized length of the gut marked with non-absorbable sutures. The marked area was exposed to X-irradiation at 250 kV and 15 mA. The distance from radiation source to the intestine was 15 cm. The dose rate was 1.3 Gray (Gy)/min and a 0.5 mm copper filter was used. The gut and the mesentery were protected by a special lead-chamber. Immediately after irradiation the bowel was pulled back intra-abdominally and the incision was closed with stitches. The radiation doses used were 9, 12 and 15 Gy. Ten days after irradiation the rats were anesthetized and portions of ileum were quickly dissected out from the rats. These doses and the time period used were chosen based on preliminary experiments showing that the effect of doses below about 8 Gy was small in *in vivo* experiments. The samples were plunged into liquid Freon 22, cooled by liquid nitrogen. Normal ileum was taken from 8 unirradiated control rats. Ileum was also taken from proximal part to the irradiated area from 22 experimental rats. Samples were stored in liquid nitrogen.

Semithin sections of frozen intestine (2-4 µm thick) were cut on a cryostat at -30 °C. They were picked up on specially designed carbon specimen holders for X-ray microanalysis (Wroblewski et al., 1978, 1983). For light microscopical examination, the adjacent sections were mounted on glass slides and stained with hematoxylin-eosin.

In vitro studies

The rat intestinal cell line IEC-6 (Quaroni and May, 1980; Sjaastad et al., 1992) was obtained from the American Type Culture Collection (Rockville, MD, USA) at passage 14. The cells were propagated in 75-cm^2-flasks from Costar (Cambridge, MA, USA) in DMEM (Dulbecco's Modified Eagles Medium) containing 5% FCS (fetal calf serum), 10 µg/ml insulin, 25 U/ml penicillin and 25 µg/ml streptomycin (standard medium) and maintained at 37 °C in water saturated atmosphere with 5% CO_2. The cells were harvested by using Trypsin-EDTA solution. The experiments were performed during passages 15-25.

Cells cultured on different growth supports (depending on type of analysis) were irradiated at the same time. The radiation source and protocols were the same as in the *in vivo* experiments. The irradiation doses used were 1, 2, 4, 8 and 16 Gy. This was based on the fact that cultured cells are more sensitive to irradiation than intestine *in vivo* and therefore lower doses and shorter times were used. During irradiation precaution was taken to keep the temperature of the incubation medium constant (37 °C) throughout the experiment. Control cells were treated identically (brought to the irradiation source and back) except for irradiation.

For X-ray microanalysis, the cells were grown directly on titanium grids (Agar Aids) or on specially designed carbon plates (Wroblewski and Wroblewski, 1990, 1992). The grids and the carbon plates were coated with Formvar film. The film was evaporated with carbon which increases the adherence of the cells and is also more stable under the electron beam. The grids and carbon plates (sterilized by UV-irradiation or a rinse in 70% ethanol) were placed on the bottom of dry culture dishes and droplets of the cell suspension were deposited on the Formvar film. After the cells had attached to the growth support, more medium was added to the dishes. The same irradiation protocols were used for X-ray microanalysis, scanning microscopy, DNA-synthesis and proliferation assays. For X-ray microanalysis the cell-covered grids and carbon plates were quickly rinsed in redistilled water (4 °C) 48 h after irradiation, blotted with filter paper and rapidly frozen in Freon cooled by liquid nitrogen (Wroblewski and Wroblewski, 1992). In a pilot experiment we compared rinsing in water with rinsing with mannitol and found no significant differences. However, as mannitol left some residue, both on the Formvar film and on the cells, we decided to rinse the specimens with water only. After freezing the cells were freeze-dried and slowly brought to room temperature and atmospheric pressure. Prior to microanalysis cells were evaporated with carbon.

X-ray microanalysis

The cryosections designated for X-ray microanalysis were examined in a JEOL 1200CX electron microscope equipped with a scanning attachment and a TRACOR energy dispersive X-ray spectrometer. The specimens were examined in the scanning transmission mode at either 100 or 120 kV. The total counting time (life-time) was 50-100 seconds. A short time of analysis was used in order to reduce elemental loss; even though this yielded inferior counting statistics. Quantitative analysis

Table 1. Elemental concentration (in mmol/kg dry weight, mean and SEM) in nuclei and cytoplasm of crypt enterocytes from the non-irradiated rat, n= 8.

ELEMENT	Na	Mg	P	S	Cl	K	Ca
Nucleus	72 (6)	51 (5)	863 (54)	281 (15)	191 (16)	596 (29)	29 (8)
Cytoplasm	83 (5)	44 (2)	742 (19)	296 (7)	216 (7)	514 (11)	29 (3)

Table 2. Comparison of ratios of sodium, chlorine and potassium in intestinal absorptive crypt cells *in vivo* after irradiation with 9 and 12 and 15 Gy (data from Fig. 2).

DOSE (Gy)	Na/K	Na/Cl	K/Cl
0	0.12	0.30	2.62
9	0.12	0.34	2.81
12	0.47	0.69	1.47
15	0.39	0.59	1.52

was carried out using salt-gelatine standards (Wroblewski et al., 1983). The cultured cells designated for X-ray microanalysis were examined in the same way as cryosections. One way analysis of variance (ANOVA) was used to determine the significance between irradiated and control samples. A value of $p<0.05$ was considered significant.

Cell proliferation and DNA-synthesis assay

For cell counting (proliferation assay), IEC-6 cells were plated in 12-well culture plates from Costar ($1x10^4$ cells/well) and grown in DMEM supplemented with FCS 5% and antibiotics. After 48 h the cells were irradiated with varying doses, ranging from 0 to 16 Gy and a dose-rate of 1.3 Gy/min. The same X-ray source as in the *in vivo* experiments was used. Cells were counted 48 h and 120 h after irradiation, using a Coulter counter. All experiments were done in quadruplicate.

For studies of DNA-synthesis, subconfluent IEC-6 cells were grown on glass coverslips in 12-well plates (Costar). After 48 h, the cells were irradiated. Thereafter they were cultured for 48 or 120 hours. ^{3}H-thymidine (2 µCi/ml; Amersham) was added for the last 24 h of culture. The cells were subsequently fixed in 3 % glutaraldehyde, dehydrated and covered with photographic emulsion (NTB2, Kodak). After a 3-day exposure at 4 °C the cells were developed in D19 (Kodak) and 300-400 randomly selected cells were counted on each glass for determination of the fraction of ^{3}H-thymidine labelled nuclei. All counts were done blindly in triplicate.

Scanning electron microscopy

Cells were cultured on plastic cover slips (Thermanox) placed on the bottom of 12-well plates. 48 h after irradiation, the cells were fixed in glutaraldehyde in a phosphate buffer followed by OsO_4, were dehydrated in a graded series of ethanol and Freon 113, and were critical point dried. Samples were thereafter evaporated with gold. Scanning electron microscopy was then performed at an accelerating voltage of 20-30 kV. Different morphological parameters such as cell dimension, shape, height, surface and edge of the normal and irradiated cells were examined. All data were recorded in a database.

Results

X-ray microanalysis

X-ray microanalysis of cells *in vivo*

In the *in vivo* study X-ray microanalysis was done on cryosections displaying good morphological preservation. Cryosections of the control distal intestine showed well preserved muscle tissue, connective tissue and epithelium. Enterocytes with electron lucent and basally located nuclei, and goblet cells were easily identified (Fig. 1a,b). The apical surface of enterocytes was covered with an electron dense brush border (Fig. 1b). The goblet cells were filled with electron dense vesicles containing mucus. Most morphological features observed in the cryosections designated for X-ray microanalysis were also present in the adjacent sections stained for light microscopy, except for goblet cells which lost mucus during specimen preparation. Ice crystal damage was more frequently found in villus enterocytes of irradiated intestine, compared to control.

The elemental content of normal enterocytes is shown in Table 1. The elemental concentrations were very similar in nuclei and cytoplasm. Only the potassium and phosphorus content was significantly higher ($p<0.05$) in the nucleus. The data in Table 1 are from the intestine of four normal (non-irradiated) animals and the enterocytes in both the villi and the crypts were analyzed. As the elemental content, especially of mobile ions, was similar in nuclei and cytoplasm, only data obtained on the cytoplasm are presented for the irradiated cells (Fig. 2). The analysis of enterocytes from the shielded ileum from irradiated animals showed the same elemental content as enterocytes from the control animals.

Analysis of absorptive crypt cells (enterocytes) from irradiated intestine showed that already at 9 Gy elemental changes occurred in the cytoplasm of these cells. Changes became more prominent at 12 Gy, with significantly lowered phosphorus and potassium

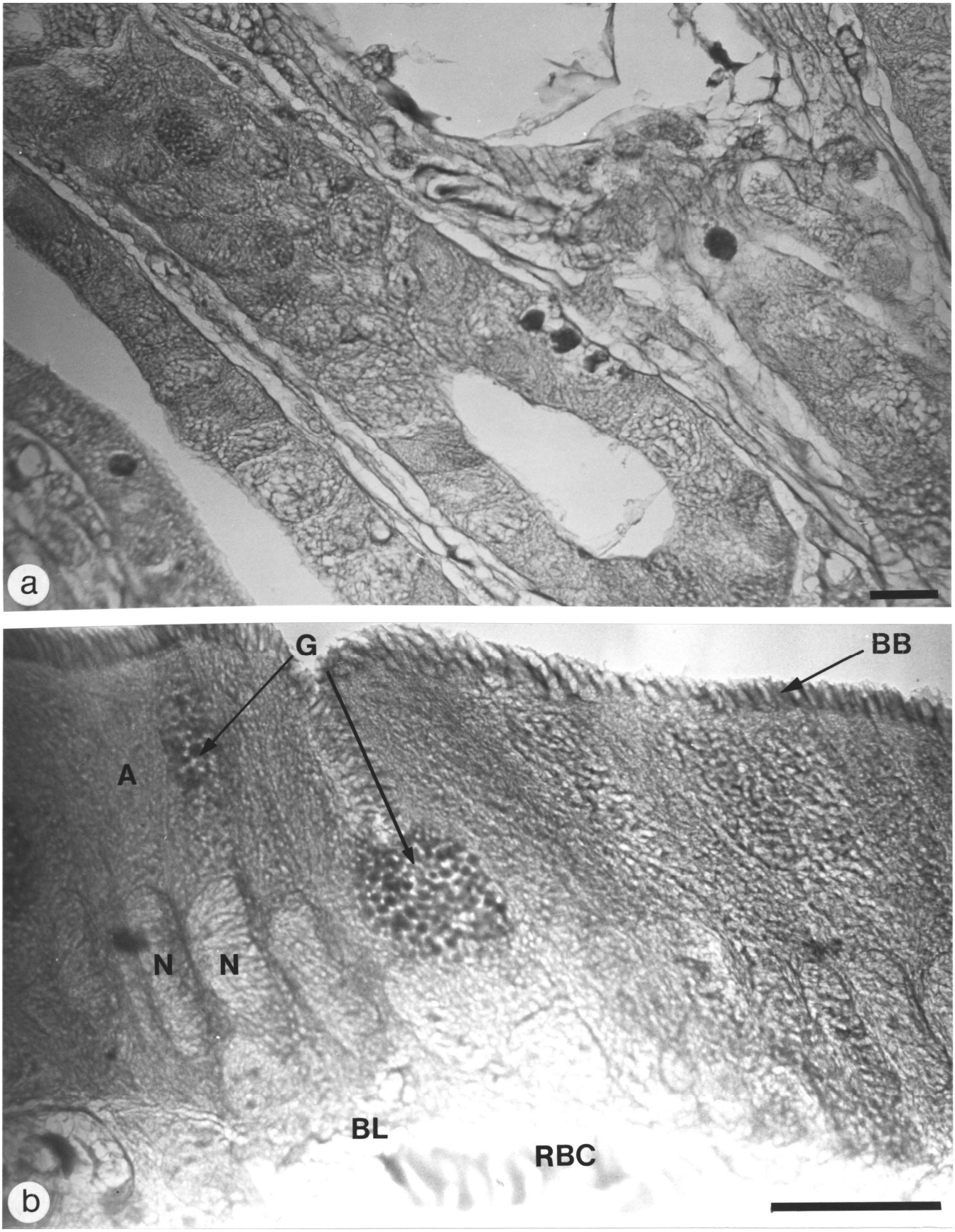


Fig. 1. a-b. Scanning transmission electron micrographs (STEM) of uncontrasted cryosections from rat intestine showing the quality of the images obtained from sections used for X-ray microanalysis. Numerous epithelial absorbtive cells (A) and goblet cells (G) at a resolution of single mucigen droplets can be seen. Brush border (BB) and nuclei (N) of the epithelial cells can be easily visualized. Basal lamina (L) and capillaries filled with red blood cells are present under the epithelial cells. Irradiation dose: 4 Gy. No morphological changes can be detected. Bars: 10 µm.

concentrations and elevated sodium concentration (Fig. 2). The ratios between the elements representing mobile ions (Table 2) indicated clearly that marked changes appear at doses higher than 9 Gy, although a slight reduction in the phosphorus and potassium concentration was already observed at 9 Gy (Fig. 2).

Analysis of goblet cells from irradiated intestine (Fig. 3) showed significantly lowered phosphorus, sulphur and potassium concentrations and elevated sodium concentrations. The analyses were performed in the areas occupied by secretory mucigen droplets. The elemental changes in goblet cells were similar to those in absorptive cells.

X-ray microanalysis of cultured cells

Culture of cells on the grids did not involve any special problems, although analysis was more difficult because grid bars sometimes contributed to the signal. When a hole in the film stretched over the grid was analyzed, signals from the grid (Ti) and sometimes Cu (from the interior parts of the microscope column) were detected. Therefore some of the cells were instead seeded on carbon specimen holders which did not contribute to the elemental spectrum. This allowed full quantitative analysis without additional correction for the extraneous signal contribution. The only problem with the use of carbon plates was that the film easily broke during cell culture or cryo-fixation. IEC-6 cells were found equally distributed over the film on grids and on the holes in the carbon plates.

The preservation of ultrastructure of the IEC-6 cells (aimed for X-ray microanalysis) was very good (Fig. 4 a,b). The control cells were very thin and therefore analysis periods of up to 100 seconds were required to obtain a sufficient number of counts.

X-ray microanalysis of the cultured cells (Fig. 5) revealed that increasing doses of irradiation caused in a dose-dependent manner a decrease of the phosphorus and potassium concentration. Already at 2 Gy, the concentration of phosphorus was significantly lowered, while the level of potassium was reduced first at 4 Gy. 8 Gy irradiation caused an increase of sodium to four times the normal concentration and further reduction of potassium concentration by 40%. There were no differences in the elemental composition between the group irradiated with 8 and with 16 Gy.

Cell proliferation and DNA-synthesis

The decrease in cell number due to irradiation was already noticeable after 48 h, but was more pronounced after 120 h (Fig. 6). Only cells irradiated with doses 1-8 Gy continued to proliferate between 48 and 120 h, while a higher dose (16 Gy) prevented an increase in cell number. The data in Fig. 6 show clearly a decrease in cell number from 84 to 14% with doses from 1 to 16 Gy. The most pronounced decrease was found at doses higher than 2 Gy. Considerable cell death at higher doses

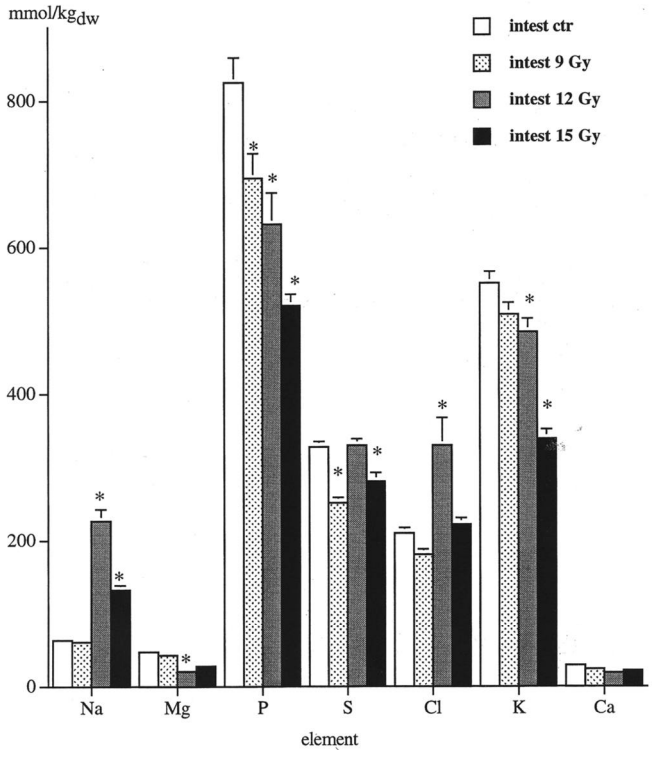


Fig. 2. Effects of irradiation on elemental concentrations of enterocytes after irradiation doses of 0-15 Gy. Elements are marked with their chemical symbols. Mean and SEM are given. Asterisks indicate statistical significance of the difference between normal and irradiated cells: *, p<0.05.

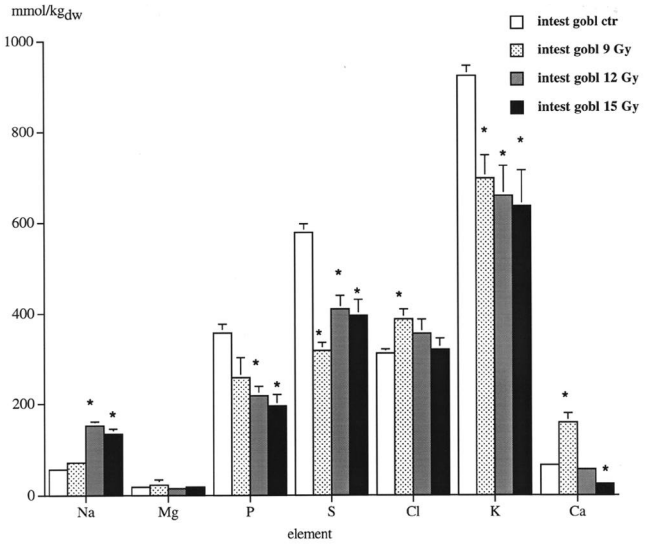


Fig. 3. Effects of irradiation on elemental concentrations of goblet cells after irradiation doses of 0-15 Gy. Elements are marked with their chemical symbols. Mean and SEM are given. Asterisks indicate statistical significance of the difference between normal and irradiated cells: *, p<0.05.

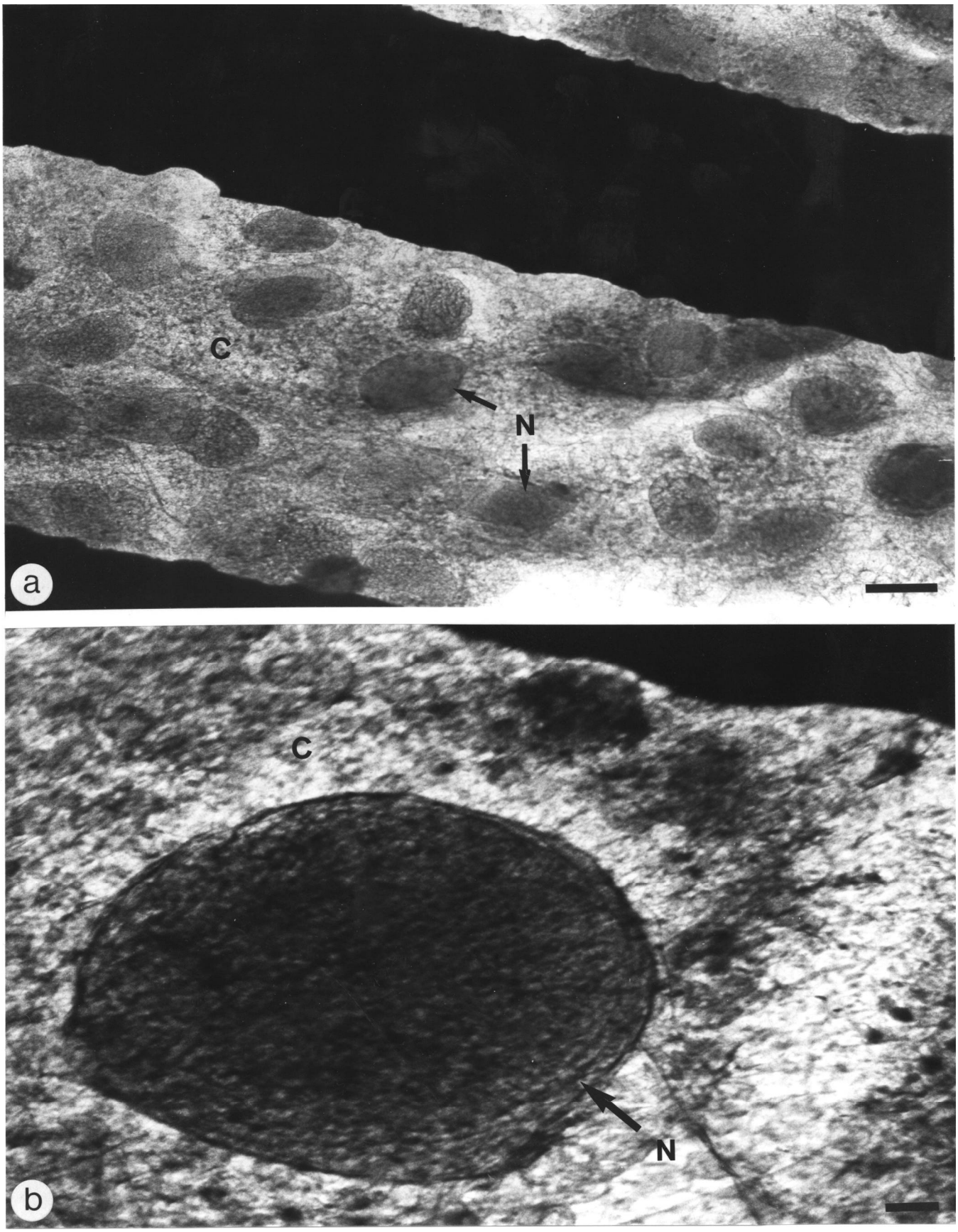


Fig. 4. Scanning transmission electron micrograph (STEM) of uncontrasted IEC-6 cells cultured on a Ti-grid. **a.** Low magnification micrograph shows numerous cells between grid bars (B). Irradiation dose 4 Gy. **b.** Higher magnification of IEC-6 cell. Nucleus (N) and cytoplasm (C) are clearly seen. No morphological structures such as microvilli or blebs, that are clearly seen in scanning micrographs, can be recorded in scanning transmission images. Irradiation dose: 2 Gy. No chromatin condensation characteristic for an apoptotic appearance can be detected in the nuclei. Bars: 10µm.

can be inferred from the data in Fig. 6.

When ^{3}H-thymidine incorporation into the nuclei was analyzed as a measure of DNA-synthesis, no significant differences were found in labelling index (73-74%) between control and cells irradiated with 1 Gy. From 2 Gy on, the labelling index decreased with an increasing dose both after 48 and 120 h. Thereafter the labelling index (LI) decreased with increasing doses of irradiation. The measurements performed after 48 h incubation had a higher percentage of labelled nuclei.

Effects of irradiation on DNA-synthesis are summarized in Fig. 7 a,b. Medium collected from irradiated cells and added to the non-irradiated cells had no effect on DNA-synthesis of IEC-6 cells (data not shown).

Scanning electron microscopy

Different morphological parameters, such as shape, height, surface and edge of the cells were examined. We also attempted to characterize contacts between cells, but the variation in number and type of contacts within the sample was too large to use the data and show characteristic changes due to irradiation. The total number of cells examined was approximately 400. A rigorous sampling of cell populations characterized by several independent means was necessary to ensure valid interpretation of the SEM images.

Control cells were flat and stretched, thus often allowing detection of nucleus and nucleoli and other fine structures under the cell membrane (Fig. 8a). In general, the control cells were morphologically more homogeneous. Half of the cells had a smooth surface and were round and the other half had sparse, short microvilli (0.3-0.5 µm), often limited to the nuclear area.

Irradiated cells (Figs. 8b,f, 9a,b) showed an increased number of changes in cell structure. With increasing doses of irradiation the cell-populations became more heterogeneous. The number of cells exhibiting microvilli of different length and blebs increased (Fig. 10). The range extended from naked to long microvilli. Cell populations irradiated with 1-2 Gy had a higher number of cells with short/medium and medium length of the microvilli (approx. 1µm). The frequency of the cells with longer microvilli (≥ 1.5 µm) was low. Blebs occurred in higher frequency than in control cells. The number of naked cells was about 20% of the population.

Cells irradiated with doses of 4-8 Gy were covered by short to long microvilli; in each cell many types of microvilli were present. Blebs were found in 8% of the cells. This is approximately 4% more than in control cells and 8% less than in the group irradiated with 1-2 Gy (Fig. 10). There were no morphological differences

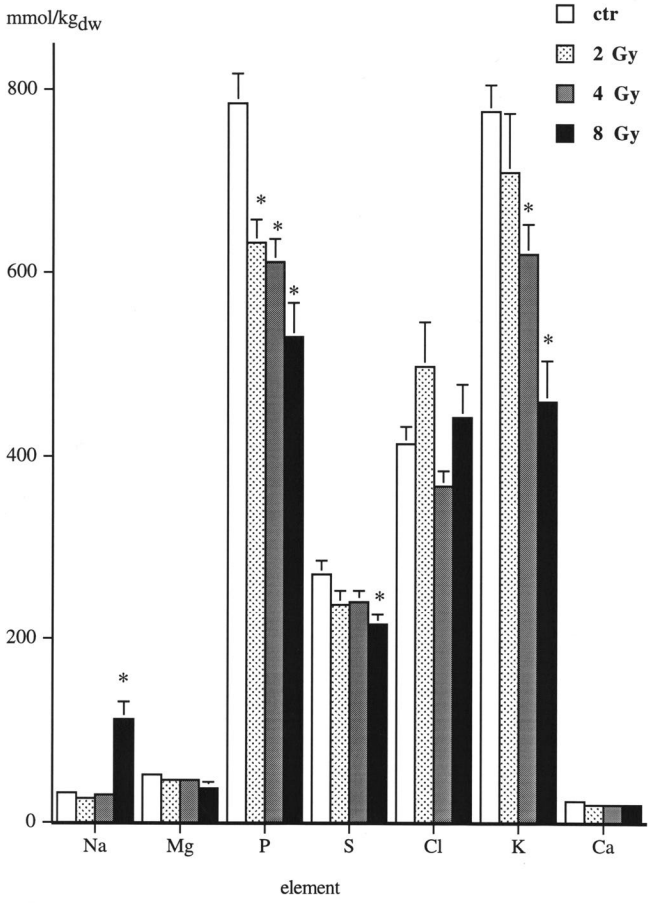


Fig. 5. Effects of irradiation on elemental concentrations of IEC-6 cells. Elements are marked with their chemical symbols. Mean and SEM are given. Asterisks indicate statistical significance of the difference between normal and irradiated cells: *, p<0.05.

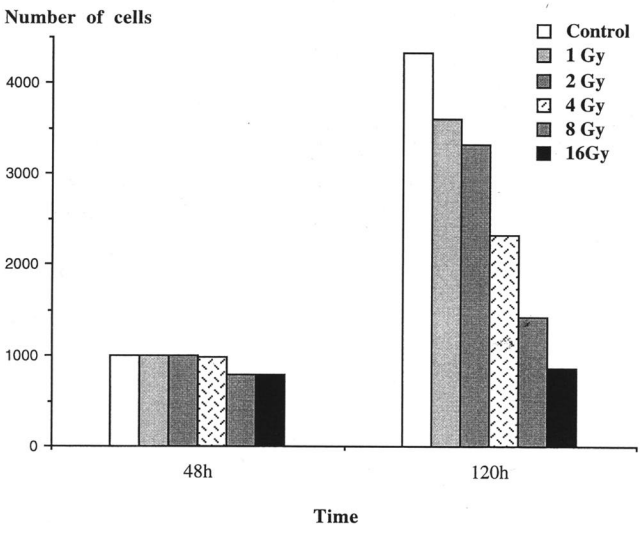
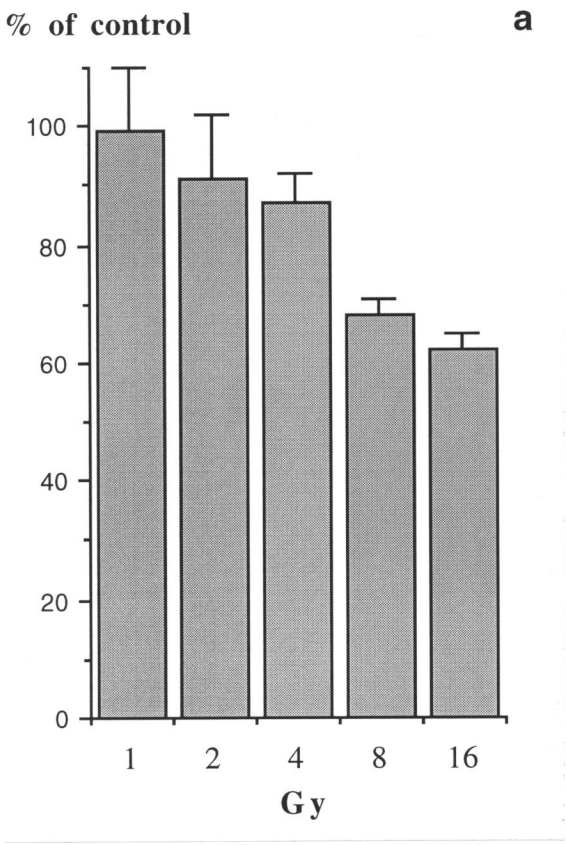


Fig. 6. Effects of irradiation (1-16 Gy) on IEC-6 cell proliferation. Cell-counts were done at 48h and 120h after irradiation. Each point represents the average of three wells. The experiment was repeated three times with similar results. All counts were done blind and in triplicate. Data are given as the percentage of the values measured in the non-irradiated, control cells.



between the group irradiated with 8 and with 16 Gy.

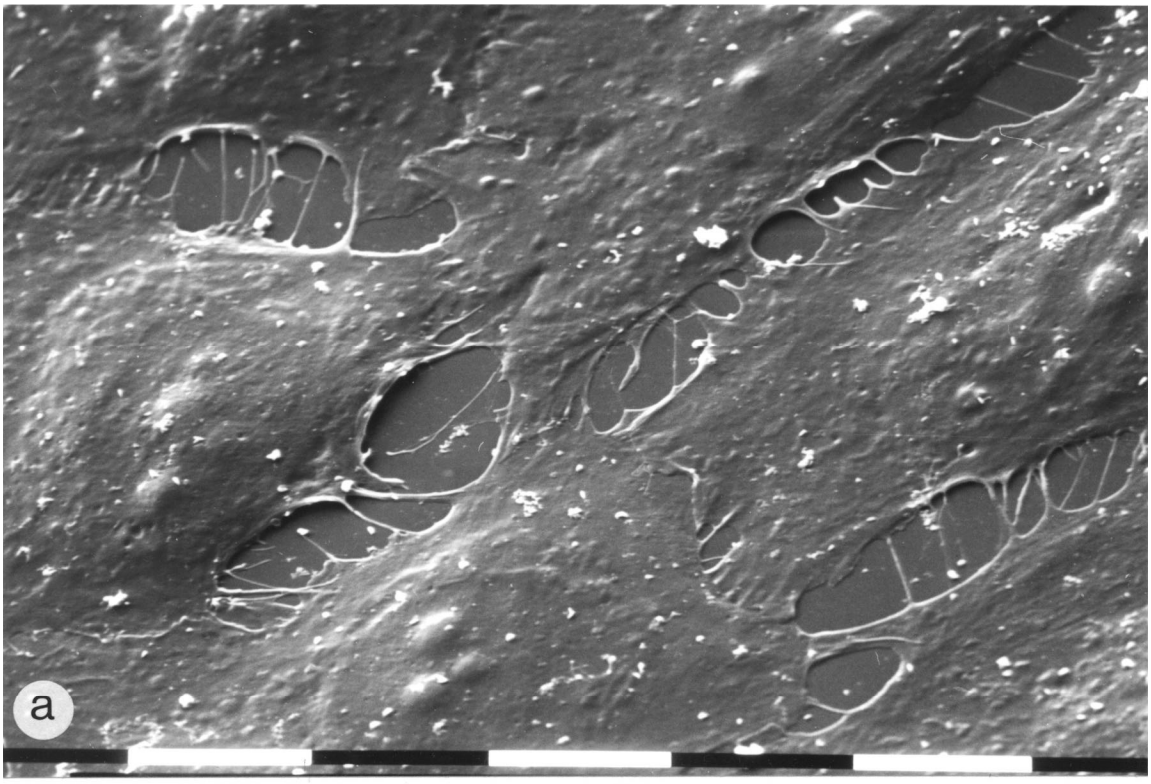
Discussion

In order to understand the role of therapeutic irradiation in causing various symptoms from the abdominal tract, changes in the elemental content and mitotic activity of the intestinal cells were investigated. *In vivo* and *in vitro* systems were used. X-ray microanalysis of semithin cryosections of control and irradiated intestine showed that irradiation caused morphological changes in the villous enterocytes while enterocytes in the crypts remained unaffected. Changes consisted mostly in increased ice crystal formation in the villous cells, which could have been caused by increased hydration of these cells. As these cells are more differentiated their regeneration potential post irradiation may be lower than that of the crypt cells. The scanning transmission image was easily matched with the light microscopical image. However, in the histological slides the mucus content of the goblet cells was lost during dehydration of the sections in ethanol.

By means of X-ray microanalysis we have shown that the potassium and phosphorus concentrations were higher in the nuclei than in the cytoplasm of the enterocytes *in vivo*. An opposite relationship has been reported by von Zglinicki and Roomans (1989). However, these authors did not distinguish between villus and crypt cells and performed analysis on ultrathin frozen sections, which could explain the differences between the results.

Changes in the elemental composition became more prominent when the irradiation dose was increased, which clearly shows that the elemental content of the cells is directly influenced by the dose of X-rays. Most pronounced were changes occurring in the cells irradiated with high doses (12-15 Gy), with significantly elevated sodium and reduced potassium concentrations. These changes may reflect disturbances in the function of Na/K pumps. High intracellular sodium concentrations may slow down uptake of glucose and thereby affect the energy metabolism in the cell. This would then inhibit synthesis of ATP which would lead to impaired function of the ATP-dependent Na/K ATPase. In the *in vitro* study, the lowered concentration of potassium in the irradiated cells was accompanied by a lower mitotic index. Loss of intracellular potassium has been previously reported to be associated with reduced cell viability, or age-induced changes in membrane permeability or membrane potential. Increase in the chlorine concentration, detected with high doses of

Fig. 7. Effects of irradiation on ^{3}H-thymidine incorporation into nuclei of IEC-6 cells. 48 h after seeding the cells are irradiated with 0-16 Gy. 24 h **(a)** or 96 h **(b)** later, the cells are incubated with ^{3}H-thymidine (2 µCi/ml) for 24 h. In each specimen, 300-400 randomly selected cells are counted to determine the labeling index (LI). Data are given as the percentage of the labeled control cells. All counts were done blind and in triplicate.



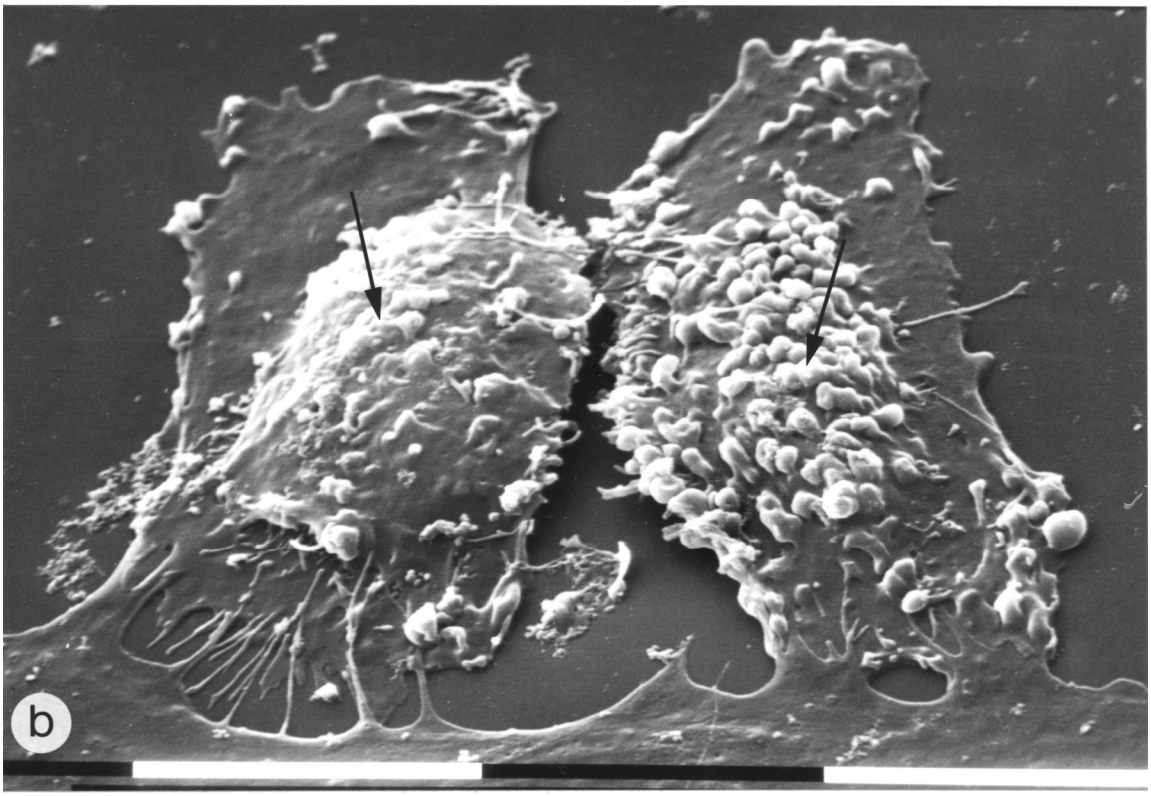


Fig. 8. Scanning electron micrograph (SEM) of IEC-6 cells cultured on plastic coverslips and fixed 48 h after irradiation. **a.** Non-irradiated cells. Both cells with smooth surface and cells with surface covered with numerous microvilli are present. **b.** At irradiation dose of 1 Gy numerous blebs (arrows) are present on the cell surface in the region corresponding to the position of the cell nuclei. Magnification bar (10 µm) is located at horizontal axis of the micrographs. Quantitative data on cell morphology (surface topography) after different irradiation doses are given in Fig. 10.
Bars: 10µm.

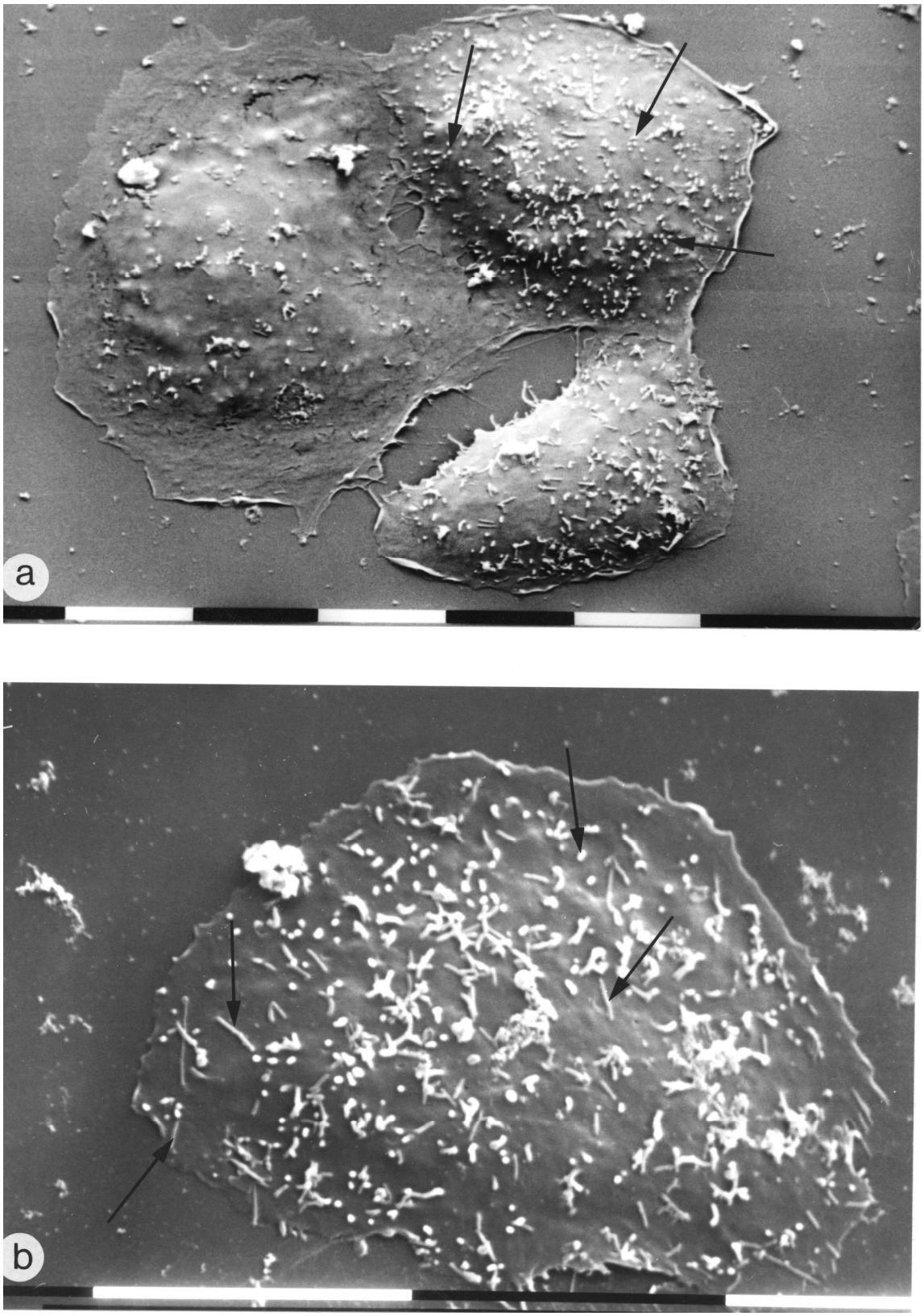


Fig. 9. Scanning electron micrograph (SEM) of IEC-6 cells irradiated with 2 and 4 Gy, respectively. **a.** At irradiation dose of 2 Gy numerous short microvilli (arrows) are present on the cell surface **b.** Microvilli ranging from short to short-medium length are characteristic for 4 Gy irradiated cells (arrows). Quantitative data on cell morphology (surface topography) after different irradiation doses are given in Fig. 10. Bars: 10µm.

irradiation, was probably caused by leakage of chloride down its electrochemical gradient. In the cultured cells elemental changes occurred at lower doses of irradiation. There are at least two alternative explanations for the differences in the sensitivity between the cells *in vivo* and *in vitro*: (1) in the intestine *in vivo*, the cells were protected by the surrounding connective tissue, while *in vitro* the cells were directly exposed to the surrounding medium; (2) *in vivo*, the enterocytes coexist with cells of connective tissue, that may synthesize different growth factors contributing to faster regeneration/recuperation of enterocytes after injury caused by irradiation; and (3) the differentiation process is divergent for the cells *in vivo* and *in vitro*. Fernandéz-Segura et al. (1999) studied changes in elemental content and morphology induced by UV irradiation. The authors found that cultured nonoblastoid cells (U937) after irradiation show several changes associated with apoptotic cell death such as plasma membrane blebbing and elemental changes in Na, Cl and K content. The recorded changes in nonoblastoid cells already appeared 1 hour after irradiation. Lowering of potassium levels and elevated sodium as well as blebbing are also found in irradiated IEC-6 cells. However, in the IEC-6 cells it is likely that numerous morphological and ionic manifestations caused by activation/inhibition of different cytoplasmic enzymes (proteases) are slower. It has been shown that cultured intestinal epithelial cells can withstand washing with distilled water prior to cryofixation without any change in ionic composition (Zhang and Roomans, 1998). It could be argued that this resistance might be diminished after irradiation damage. This might exaggerate the effects of irradiation on elemental content. However, the intestinal cells *in vivo* were cryofixed directly without rinsing and showed a similar pattern of changes.

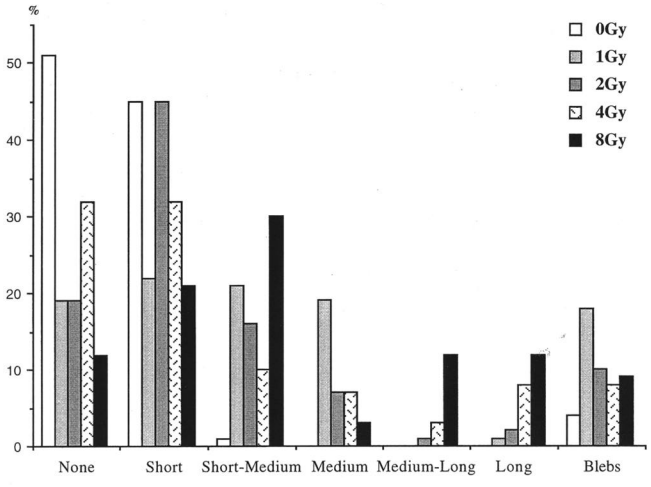


Fig. 10. Effects of irradiation on IEC-6 cell-morphology. The cells are irradiated with 0-8 Gy. The diagram shows expression of different morphological parameters such as length of microvilli and occurrence of blebs. The counts are normalized and shown as percent of all analyzed cells. The groups are 1, naked cells; 2, cell with short microvilli; 3, cells with short-medium long microvilli; 4, cells with medium microvilli; 5, cells with medium-long microvilli; 6, cells with long microvilli; 7, cells with blebs.

The elemental changes in goblet cells are reminiscent of those in absorptive cells and the results probably represent changes in the elemental content of the secretory mucigen droplets. Increased sodium and decreased potassium due to irradiation mirrored the changes in the absorptive cells. A decrease in phosphorus and sulfur was found to almost the same extent in all irradiated goblet cells without correlation with the irradiation dose. This was in contrast to the findings in the absorptive cells. It is likely that irradiation causes a disturbance in the synthesis and sulfation of mucus protein and polysaccharides. The changes in calcium levels in goblet cells probably mainly reflect changes in the production of (calcium-rich) mucus.

In the IEC-6 cells, the elemental changes were accompanied by inhibition of DNA-synthesis. When thymidine incorporation data were compared with proliferation rate data it was evident that irradiation caused lowering of the proliferation rate due to an inhibition of DNA-synthesis as earlier described by Chwalinski and Potten (1986). Cells irradiated with 16 Gy stopped proliferating as there was no increase in the cell number between 48 h and 120 h, a time period during which at least 2 cell cycles should have taken place. Part of the population of irradiated cells might have been stopped at G0 phase, which often happens when cultured cells lack hormones or nutrients needed. Changes in DNA windings after irradiation such as found in CHO-cells and cultured bovine lens epithelial cells (Affentranger and Burkart, 1992; Baumstark-Khan et al., 1992a,b) might have also caused the decreased proliferation rate.

The structural changes in the cell membrane often correlate with altered permeability of the cells. This can lead to changes in the intracellular concentrations of ions involved in the control of cell proliferation. It is also possible that ionic changes could induce phenotypic modulations leading to increased synthesis of the extracellular matrix and intestinal fibrosis. We have not investigated the effects of irradiation on the submucosal fibroblasts, but it can not be excluded that these cells are less sensitive to irradiation than the epithelial cells, and, contrary to the enterocytes, are triggered by X-rays to proliferate and secrete extracellular matrix, thus leading to expansion of the connective tissue component. However, it is still unknown if only submucosal fibroblasts are responsible for fibrosis formation or if epithelial stem cells are also involved.

Scanning electron microscopical investigations showed that irradiated cells presented numerous distinct membrane manifestations in the form of microvilli of different sizes and blebs. This might be a sign of apoptosis; however, no direct determination of apoptosis was carried out. Increasing irradiation doses were shown to cause an increase in the number of microvilli. Cells

irradiated with higher doses than 2 Gy presented microvilli of different sizes. It was also shown that the number of blebs increased with an increased dose of irradiation. The blebs may represent cyst formations filled with extracellular fluid which could explain the high sodium and chlorine and low potassium values in these cells.

Porter et al. (1973) and Wetzel et al. (1978) studied by light microscopy (on Feulgen-stained cells) and by scanning electron microscopy on the same cells, structural changes of the cell surface in correlation with different phases of the cell cycle. Wetzel et al. (1978) found that smooth-surfaced cells were non-mitotic, approximately 60% of fully rounded cells covered with microvilli were in metaphase or anaphase, elongated cells were in anaphase, and paired cells with many blebs were probably either in telophase or newly arrived in interphase. This classification can hardly be valid in the present investigation, because the highest percentage of smooth cells was found in the non-irradiated cells with the highest proliferating rates. We therefore conclude that in the present investigation, the membrane manifestations were not correlated to the different stages in the cell cycle. It is therefore more probable that changes described by us are a direct effects of irradiation.

Numerous studies have shown that the extent of the changes might, apart from the radiation dose also depend on other factors such as the environment of the cell (Burna and Rozengurt, 1984). or its genetic characteristics. Skwarchuk and Travis (1998) studied the development of radiation-induced fibrosis of the colorectum and observed a difference in histology and fibrogenic cytokines in irradiated colorectum of two murine strains. Ettarh et al. (2000) studied streptozotocin-diabetic and non-diabetic mice that were exposed to 10 Gy. The authors found that the expression of radiation damage in the diabetic mouse was less severe than in the non-diabetic mouse. Effects of acute and protracted radiation on small intestinal morphological parameters were studied by Brennan et al. (1998) who found that the number of villous enterocytes and goblet cells changed during different procedures and that the epithelial cells undergoing a protracted radiation schedule appeared to be returning to normal within 3 days of the cessation of radiation, a finding which was in contrast with the results obtained with an acute dose.

The present study shows that irradiation of the intestinal epithelial cells both *in vivo* and *in vitro* induces inhibition of DNA-synthesis and cell proliferation together with changes in the elemental content of the cells. The cultured cells and cells *in vivo* show similar changes which means that IEC-6 cells can be successfully used in studies on the effects of irradiation on the intestinal mucosa.

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