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MORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN INTERCELLULAR JUNCTIONAL COMPLEXES IN EPITHELIAL CELLS OF MOUSE SMALL INTESTINE UPON X-IRRADIATION: CHANGES OF RUTHENIUM RED PERMEABILITY AND CALCIUM CONTENT

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Abstract

Changes of calcium-content and permeability of tight junction following X-irradiation were investigated in mouse intestinal epithelial cells by electron microscopy. In the control animals the lower parts of tight junctional area as well as the other junctional elements and the intercellular space are labeled by pyroantimonate precipitates, which contain calcium as revealed by electron spectroscopy and electron energy loss spectrometry. X-irradiation, parallel with morphological changes, lead to rapid decrease of pyroantimonate precipitable calcium content and increase of the permeability of tight junctions indicated by the penetration of ruthenium red into the intercellular space. These changes were readily reversible following 0,5 Gy doses of irradiation however, they persisted up to 24 hours following 5 Gy irradiation. We conclude that irradiation at the applied doses can transiently destabilize the tight junctions in the epithelial layer of the small intestine, presumably through a calcium dependent mechanism.

Key Words: small intestine, X-irradiation, tight junction, ruthenium red permeability, pyroantimonate precipitable calcium, electron spectroscopy, electron energy loss spectrometry.

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Introduction

The plasma membranes of neighboring epithelial cells exhibit a specialized junctional region that plays a role in cell-cell adhesion, control of transepithelial permeability, and lateral mobility of membrane domains and contribute to intercellular transport of small molecules (cell communication). The junctional complex of polarized epithelium consists of several components including tight junction, intermediate (adherens) junction, desmosome and gap junction, which are arranged in a well defined order and characterized by distinct molecular composition, ultrastructure and function (Farquhar and Pallade, 1963; Staehelin, 1974; Dragsten et al., 1981; Peracchia and Bernardini, 1984; Garrod, 1986; Cereijido et al., 1988; Parry et al., 1990; Geiger and Ginsberg, 1991). The tight junctions are found at the apical (luminal) part of adjacent epithelial cells (Farquhar and Pallade, 1963; Staehelin, 1974; Hauer-Jensen, 1990). The neighboring membranes fuse with one another at one or more points form a belt around the cells. According to freeze-fracture images the intra-membrane proteins of tight junction appears as fibrils which fuse with the corresponding ones on the adjacent cells like a zipper (Fig. 1) (Staehelin and al., 1969; Staehelin, 1974; Wade and Karnovsky, 1974; Bullivan, 1978; Suzuki and Nagano, 1991). Two proteins, ZO-1 and cingulin associated with tight junctions were recently described (Stevenson et al., 1986; Citi et al., 1989; Citi, 1992). The tight junctions are essential in the establishment of a selective permeability barrier for passage of ions and small molecules between luminal and intercellular spaces (Gumbiner,1987; Cereijido et al., 1988), and thus regulate the resistance (Madara and Dharmsthaporn, 1985; Madara, 1990). They restrict lateral mobility of membrane domains in the adjacent plasma membranes and in this way contribute to the maintenance of membrane polarity (Hoi-Sang et al., 1980; Dragsten et al., 1981; Tournier et al., 1989; Parry et al., 1990). Several lines of evidence indicate that the tight junctions are dynamic in structure and functions (Meyer et al., 1988; Shivers et al., 1988; Kan and Coleman, 1990; Nilsson et al., 1991; Citi, 1992; Jinguji and Ishikawa, 1992), i. e. they are modulated by physiological and pathophysiological effectors (reviewed by Nilsson et al., 1991), physical agents,

such as mechanical stress (Rhodes and Karnovsky, 1971; Pitelka and Taggart, 1983), ambient pressure on blood vessels (Majack and Bhalla, 1981; Mühleisen et al., 1989), temperature (Shivers et al., 1988; Cohen et al., 1990) and pharmacological treatments (Bentzel et al., 1980; Mullin and McGinn, 1987; Hecht et al., 1988; Kan and Coleman, 1990; Mullin and Snock, 1990). The mechanism(s) of regulation of tight junction permeability is not known exactly. However, there are data suggesting that multiple factors including cytoskeletal elements (Bentzel et al., 1980; Meza et al., 1982; Hecht et al., 1988), the cAMP system (Jacobson, 1979; Duffey et al., 1981), the activity of protein kinase C (Mullin and McGinn, 1987; Citi, 1992), and the calcium content of the cells are involved (Sedar and Forte,1964; Meldolesi et al., 1978; Palant et al., 1983; Pitelka et al., 1983; Kan and Coleman, 1988; Nilsson, 1991). Permeability changes of tight junction were observed after facilitated movements of calcium across the plasma membranes (Kan and Coleman, 1988), and following removal of the calcium by chelating agents (Sedar and Forte, 1964; Meldolesi et al., 1978; Palant et al., 1983; Pitelka et al., 1983; Nilsson, 1991). Several reports indicate that the intercellular contacts are targets of ionizing radiation (Durand and Sutherland, 1972; Hinz and Dertinger, 1983; Rofstad and Sutherland, 1988; Kwok and Sutherland, 1991). Some experimental data are available about functional alterations. Increased vascular permeability of macromolecules and loss of the intestinal barrier for bacteria, bacterial toxins and proteolytic enzymes point to changes in tight junctions following irradiation (Quasler, 1956; Levin et al., 1979; Hopewell, 1980; Evans et al., 1986; Spence et al., 1987). Irradiation is known to alter cell-cell contacts and cell substratum adhesion, probably via structural changes of adherens junctions and desmosomes responsible for mechanical stability of cell contacts (Somosy et al.1987). The radiation induced changes of gap junction mediated cell communication may result in tumorous transformation of cells (Trosko et al. 1990) and there is a relationship between the changes in intercellular transport via gap junctions and repair capacity of murine B16 melanoma cells after irradiation (Madhoo and Blekkenhorst, 1989). The present study was undertaken to examine the radiation induced permeability changes in parallell with the distribution and amount of calcium in the junctional complex of the highly polarized cells of mouse small intestine in which the functional consequences of irradiation have already been described (Quasler, 1956; Hauer-Jensen, 1990; Carr et al., 1991).

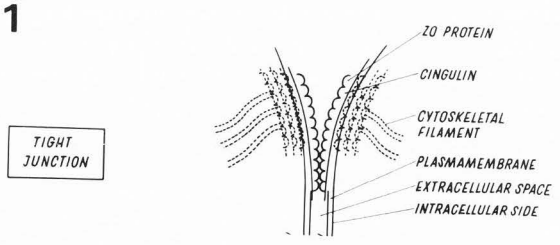

Figure 1. Model of tight junction based on the recent literature data.

Materials and Methods

Animals:
The animals (CFLP male mice) were maintained under laboratory standard conditions. They were arranged in groups of 3 animals and irradiated as indicated below. The experiments were carried out in two parallels.

Irradiation:
The mice were total body X-irradiated with 0,5 and 5 Gy doses and killed by cervical dislocation, 30 minutes, 1, 3 and 24 hours after irradiation. X-irradiation was performed with a THX-250 machine. Conditions: 200 kV, half value layer 1.0 mm Cu, source-surface distance 90 cm, dose rate 0,317 Gy water.min^{-1}.

Electron microscopy:
From each animal, three pieces of small intestine from the duodenal region (about 0.5 cm) were taken for investigation. One of them were fixed for conventional electron microscopic investigation by 0.1 M phosphate buffered 5 vol.% glutaraldehyde (pH 7.3) at 4 C, and postfixed in 1 wt.% OsO_4, buffered by same buffer. The other two samples were fixed for as described below. After fixations the tissues were dehydrated through a graded series ethanol to propylene oxide, and embedded in Durcupan AC (Fluka). The sections cut with diamond knives on an LKB ultratome and mounted onto 400-mesh zinc grids for electron spectroscopic imaging (ESI) and electron energy loss spectrometry (EELS) investigations and on 300-mesh copper grids for transmission electron microscopy. The ESI and EELS investigations were carried out in a Zeiss CEM 902 electron microscope, and JEOL 100CX electron microscope were used for routine examinations.

Cytochemistry:
1. The permeability changes of epithelium were investi-gated by the tracer molecule ruthenium red, (Luft, 1971; Hurtando de Mendoza and Moreno, 1991; Clough, 1991). The tissues were fixed in a mixture of 2.5 vol.% glutaraldehyde and 0.5 wt.% ruthenium red (Fluka) in 0.1 M cacodylate buffer, pH 7.4 for 2 to 4 hours and postfixed in 1 wt.% OsO_4 and 0.5 wt.% ruthenium red in the same buffer for 2 to 4 hours.
2. The localization of calcium was demonstrated by the potassium pyroantimonate method according to Eisenmann et al. (1979). The pieces of small intestine were immersed in ice cold 2% glutaraldehyde (Merck) buffered with potassium acetate (Reanal), and containing 0.05M potassium pyroantimonate (Merck) for 1-2 hours, rinsed in potassium acetate buffer and postfixed in 1% OsO_4 containing 0.05 M potassium pyroantimonate for 1 hour.

Microanalysis:
Since the specificity of the potassium pyroantimonate reaction is poor, and the elemental content of reaction product is influenced by the reaction conditions (buffer, pH, time) (Simson and Spicer, 1975), it was necessary to check the calcium content of reaction products by independent methods. For this purpose we used electron spectroscopic imaging and electron energy loss spectro-metry.
1. Electron spectroscopic imaging: Ultrathin sections (40-50 nm) were cut, using a diamond knife, on a LKB ultratome.

The sections were mounted onto 400-mesh zinc grids and without additional staining analyzed in a Zeiss CEM 902 electron microscope equipped with a digital-image analysis system (IBAS 2.0, Kontron). The images were collected at instrumental magnification 20,000x and 50,000x, using 80 kV accelerating voltage, an objective lens diaphragm 90 μm, a spectrometer entrance diaphragm of 650 μm and a slit-diaphragm with 9-10 eV. During image recording fixed pre-sets of TV camera setting (gain, kV, black level) were used, in "manual" remote control mode of the camera (SIT DAGE), to guarantee constancy of the parameters during recording of images. Electrons with energy-loss value of 250 eV were selected for structural imaging, while the net calcium distribution was derived in each case from images recorded at 360 eV and 330 eV energy-loss values. Shading correction for uneven illumination was performed in each individual image using a defocused image. Corrected images, recorded below and at the calcium edge, were then subtracted in order to obtain the net calcium distribution at a given field of view.

2. Electron energy loss spectrometry: Spot analyses were performed on selected electron-dense precipitates in order to provide additional analytical proof to the two-dimensional distribution data obtained by the ESI method. The same sections which served for electron-spectroscopic imaging were utilized for EELS measurements. The electron microscopic parameters were as: 80 kV accelerating voltage, 50.000x primary magnification, analyzed area of $\leq 0{,}59\ \mu m^2$, 90 μm objective diaphragm, 300 μm spectrometer entrance diaphragm and 2 eV slit width. The transmitted electrons were detected with a photomultiplier tube installed into the photocamera chamber of the EM. All spectra were recorded in the same energy loss range, between 300 eV and 360 eV with a dwell time of 3s per channel of 2 eV. The spectrum recording and evaluation were performed with the EELS 1.3 program of the image analyzer.

Results

Specificity of pyroantimonate reaction:

The ESI technique was applied in order to demonstrate the co-distribution of the electron dense precipitates and its calcium contents. Figure 2a is the zero-loss image taken around 0-10 eV. Figure 2b is the calcium net intensity distribution image, acquired by subtraction of the pre-edge image take at 330 eV from the post-edge image acquired at 360 eV. Images are taken directly after each other with the calcium-net intensity image (Fig. 2b). Comparing Fig. 2b with Fig. 2a, it turned out, that in can be assumed in Fig. 2b represent the calcium inside the pyroantimonate precipitate in Fig. 2a as some of the particles present in Fig. 2b are co-distribution with these with Fig. 2a. The spot analyses of electron dense precipitates performed on the same areas (as in Fig. 2a) by electon energy loss spectrometry strengthen this conclusion (Fig. 2c).

Morphology and potassium pyroantimonate reaction:

The epithelium of the small intestine consists of one single layer of polarized columnar epithelial cells (Fig. 3A). Among which these ones single goblets cell (Fig. 3A) were dispersed. Rarely, apoptotic cells were also seen (Fig. 3B). The apical (luminal) surface of the epithelial cells is covered by numerous microvilli (brush border) (Fig. 3A.). The tight junctions were situated at the apicolateral transition of plasma membrane, and appeared as multiple bands of the so-called membrane fusion between the opposed plasma membranes (Fig. 4). Immediately below the tight junction the adherents junction is located. It is characterized by an about 20 nm wide intercellular space, and a dense, fibrillar material covering the cytoplasmic side of the plasma membrane. Beneath adherent junctions one or more typical desmosomes can be observed (Fig. 4). The lateral plasma membranes of the cells form interdigitations (Fig. 3A, Fig. 4 insert). The calcium containing pyroantimonate precipitates were found on the microvilli (Figs. 4, 6), on the basal lamina (not shown) and in the intercellular space between adherents junctions and desmosomes (Fig. 4). The interdigitations of the lateral plasma membrane also showed a strong labeling by the pyroantimonate reaction (Fig. 3A, Fig. 4 insert). Rarely, small deposits of the reaction products were seen at the bottom part of the tight junctions (Fig. 4). Inside the cells the reaction products are usually localized at the cytoplasmic side of desmosomes (Fig. 4) and intermediate junctions (not shown). In addition, a small number of electron-dense reaction products are seen on the mitochondria and scattered in the cytoplasm. Apoptotic epithelial cells (Fig. 3B) and goblet cells (Fig. 3A) were heavily labeled by pyroantimonate precipitates. 1-3 hours after X-irradiation at 0.5 and 5 Gy doses slight dissociation of tight junctions and dilatation of intercellular spaces between the intermediate junctions were seen (Fig. 5A, B). Parallel to these morphological changes, the X-irradiation leads to a decrease and/or disappearance of pyroantimonate reaction products from intermediate junctions, desmosomes and from other regions of intercellular spaces (Figs. 5A, B). The reduced staining pattern is observable during 24 hours following 5 Gy irradiation (Fig. 6). However, 24 hours after 0.5 Gy irradiation, the ultrastructure of the junctional region, and the distribution and amount of pyroantimonate precipitates became similar to the control (Fig. 7). It is obvious that, in parallel both radiation-induced decrease of the extracellular calcium level, the cytoplasmic calcium content increased. Particularly, dense deposits were seen on mitochondria and the reaction products on the internal face of plasma membrane were observed (Figs. 5, 6).

Penetration of ruthenium red:

In the control animals ruthenium red produces intense staining on the luminal surface of the small intestine. However, this cationic dye does not enter into the intercellular space (Fig. 8A). On the other hand the electron dense deposits of ruthenium red appear in the tight junctional area (Fig. 8B) as well as in the intercellular space after 0.5 or 5 Gy X-became similar to the control (Fig. 7). It is obvious that, in parallel both radiation-induced decrease of the extracellular calcium level, the cytoplasmic calcium content increased. Particularly, dense deposits were seen on mitochondria and the reaction products on the internal face of plasma membrane were observed (Figs. 5, 6).

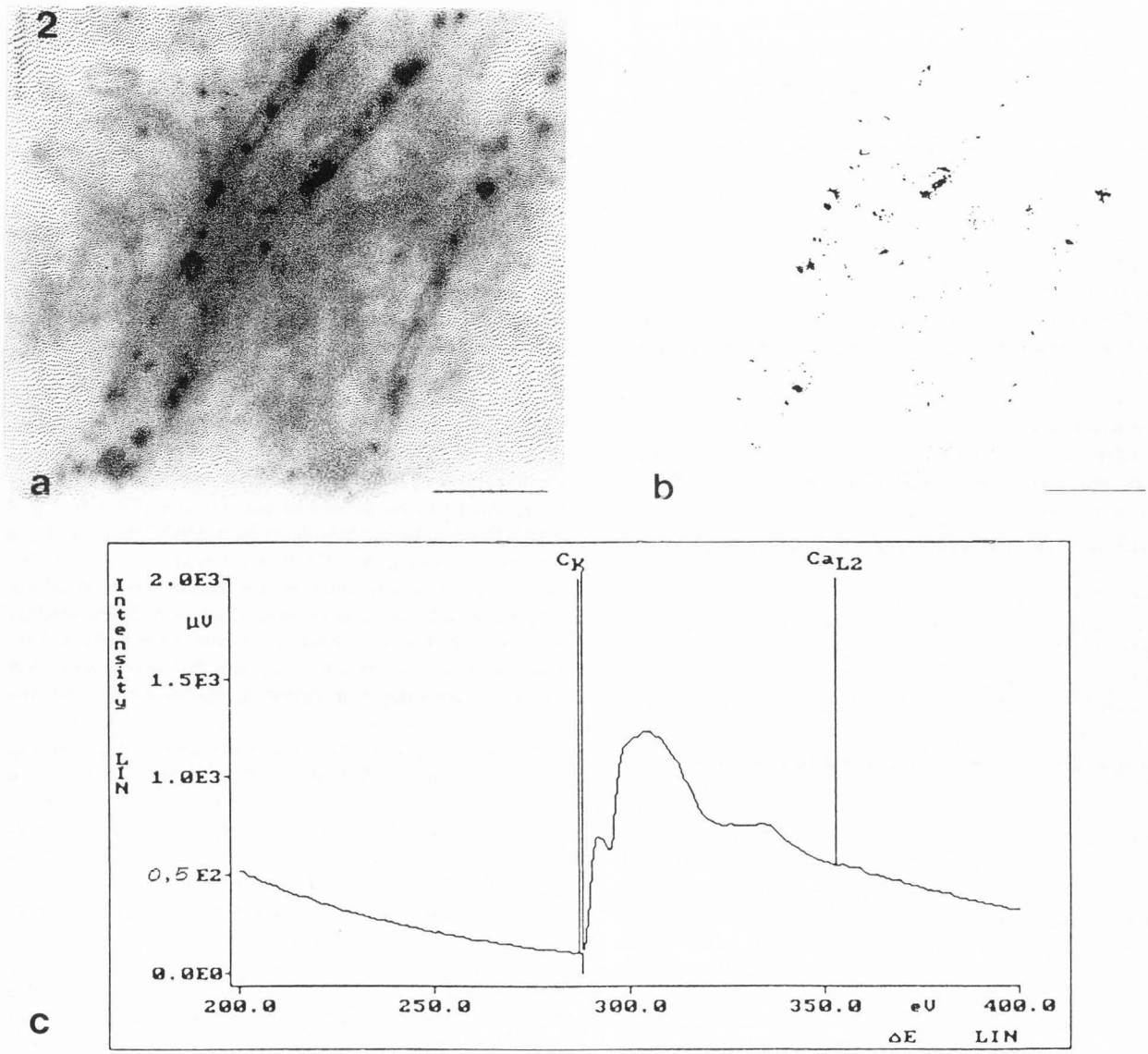

Figure 2. Electron spectroscopic images (a,b) and electron energy loss spectrum (c) of pyroantimonate precipitates. The 2a zero-loss image, 2b calcium net-intensity image, in 2a a circle indicate analyzed area from which spectrum 2c is acquired. Bars = 0.5 µm.

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Discussion

The plasma membrane is one of the primary targets of radiation in cells (Köteles et al., 1983; Edwards et al, 1984; Somosy et al. 1987) and ionizing radiation can induce changes in the function and/or supramolecular organization of the membrane (Szekely et al., 1982; Köteles et al., 1983; Edwards et al., 1984; Somosy et al., 1987; 1988; Seed and Niiro, 1991; Gorodetsky et al., 1992;).The pyroantimonate reaction is widely used histochemical method for the determination of calcium in cells and tissues (Satir and Gilula,

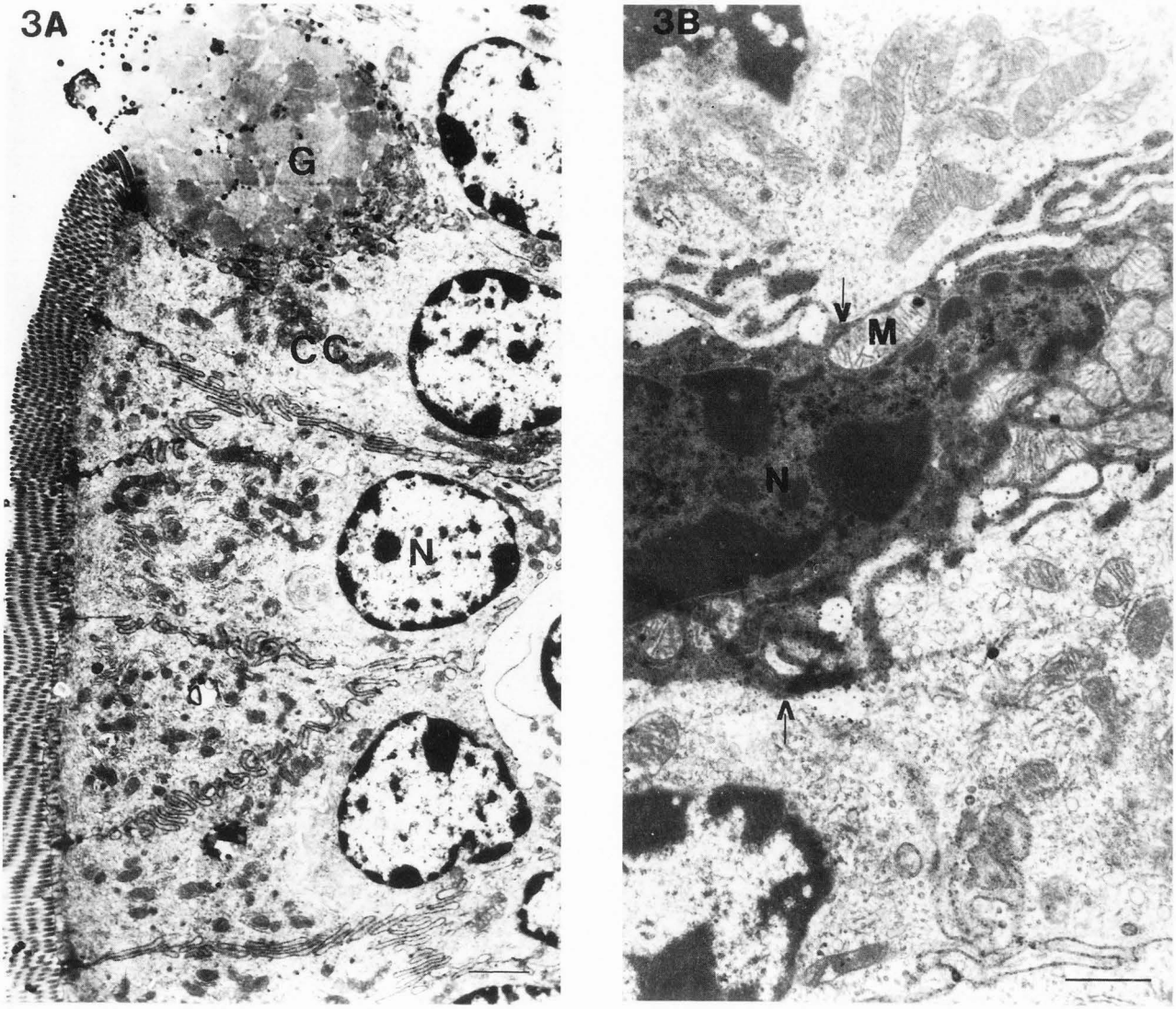


Figure 3. 3A: Overview of unirradiated small intestine. MV = microvilli, N = nucleus, GC = goblet cell, CC = columnar epithel cells. 3B: The apoptotic cell (arrows) contain a large amount of pyroantimonate precipitable calcium in the nucleus (N), in mitochondria (M) and in the cytoplasm. Bar : A = 1 μm, B = 0.5 μm.

1970; Clark and Ackerman, 1971; Simon and Spicer, 1975; Eisenmann et al., 1979; Appleton, 1988; Kogaya and Furuhashi, (1988). The ESI and EELS results shown here are indicative for the presence of calcium in the pyroantimonate precipitates. These results are in accordance with similar (ESI and EELS) observation of other objects in biology(Peute et al, 1990; Zivkovic et al, 1990, DeBruijn et al, 1993). In spite of the criticism that can be attributed again the method applied (viz. subtraction of two images) the presence of calcium seems to be shown positively, as this method underestimates the net-intensity values. The variations in calcium positive granules might be due to this underestimation or due to absence of calcium in some granules. Earlier studies about the calcium concentration in the intestinal mucosa have used X-ray microanalysis, (von Zglinicki and Roomans, 1989) and histochemical techniques based on the pyroantimonate reaction (Satir and Gilula,1970). Calcium is localized on and/or between the lateral plasma membranes of a wide variety of epithelial cells (Kogaya and Furuhashi, 1988; Mentré and Halpern, 1989; Satir and Gilula, 1970; Oschman and Wall, 1972; Eisenmann et al., 1992). The presence of calcium binding proteins in the adherents junctions and desmosomes is also known (Garrod, 1986; Geiger and Ginsberg, 1991). Our observations, on the co-localization of Ca^{2+}- containing pyroantimonate precipitates agree with these data. There is a problem concerning the possible translocation of water-soluble calcium in the cells or tissues during the fixation and histochemical reaction. The observed high Ca^{2+} content of the goblet and apoptotic epithel cells is not likely to be caused by diffusion of Ca^{2+} during this procedure. As the known

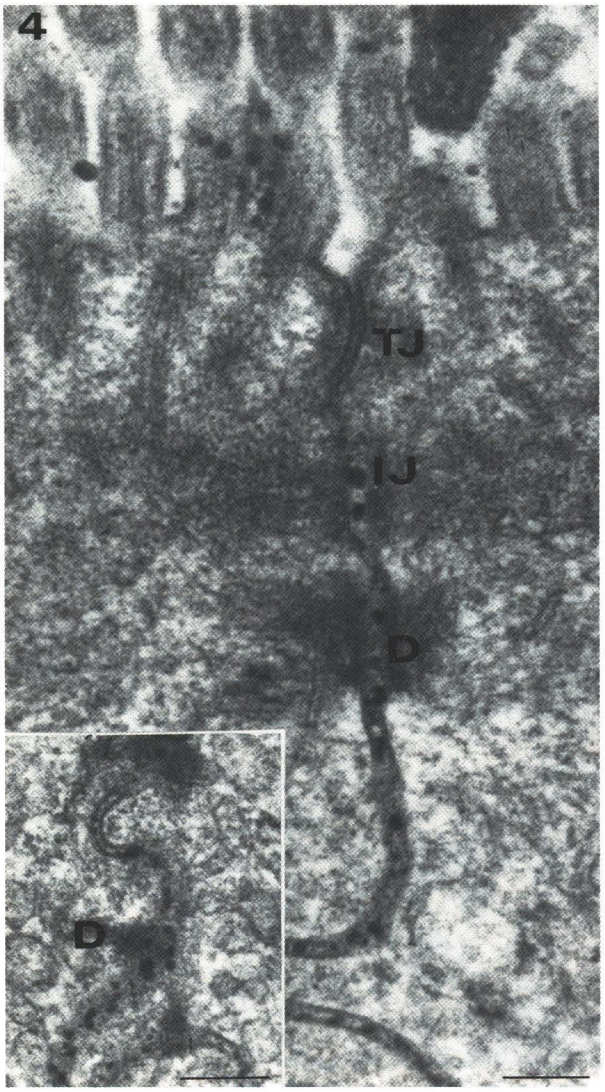


Figure 4. Cell junctional complex, untreated control. TJ = tight junction, IJ = intermediate junction, D = desmosome. Pyroantimonate precipitates are seen at bottom part of TJ, and the intercellular space of IJ and D, and other regions. The cytoplasmic side of desmosomes is rarely labelled with pyroantimonate precipite (insert). Bars: = 0.15, insert 0.2 μm.

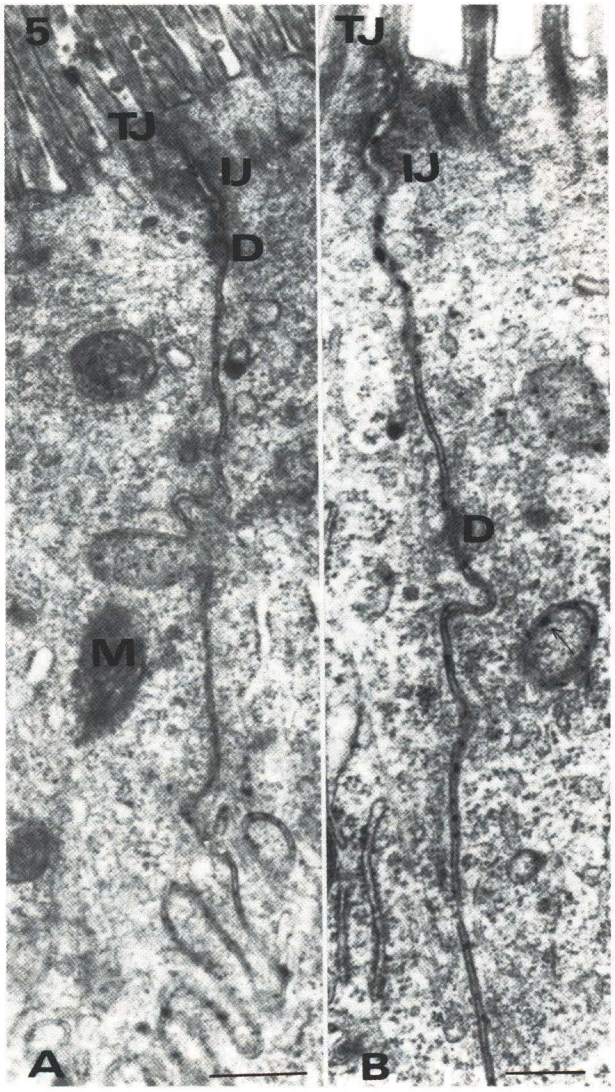


Figure 5. Cell junctional complex in irradiated (A: 0.5 Gy, 1 hour), 5 Gy, 1 hour (B) small intestine. The amount of pyroantimonate precipitates is decreased, the intermediate junctions (IJ) are dilated (A, B). D = desmosome, M = mitochondrium, TJ = tight junction. The arrow indicate the pyroantimonate precipitates located on the iner face of plasma membrane. Bars: = 0.5 μm.

increase in calcium content of apoptotic cells with increased membrane permeability could be by this histochemical reaction, this suggests that the calcium content and distribution in other cells or intercellular spaces is not changed by the preparatory procedure.

Calcium as a known intracellular mediator is involved directly or indirectly in some essential physiological processes. Among others, the changes of local calcium concentration can modulate the functions and/or structure of the elements of junctional complex (Sedar and Forte, 1964; Meldolesi et al., 1978; Palant et al., 1983; Pitelka et al., 1983; Garrod, 1986; Geiger and Ginsberg, 1991; Nilsson, 1991). However, the exact relationship between the decreased calcium content and the loss of barrier function is not clear, since in this part of the junctional complex calcium binding structural proteins are absent. We assume in agreement with Nilsson (1991), that leafiness of tight junctions following the extracellular Ca^{2+} depletion is secondary to the disruption caused by calcium-dependent adherents junction. In addition to Ca^{2+}-dependent mechanisms the structural and functional integrity of tight junctions may be regulated by protein kinase C (Mullin and McGin, 1987; Mullin and Snock, 1990), cyclic AMP signal

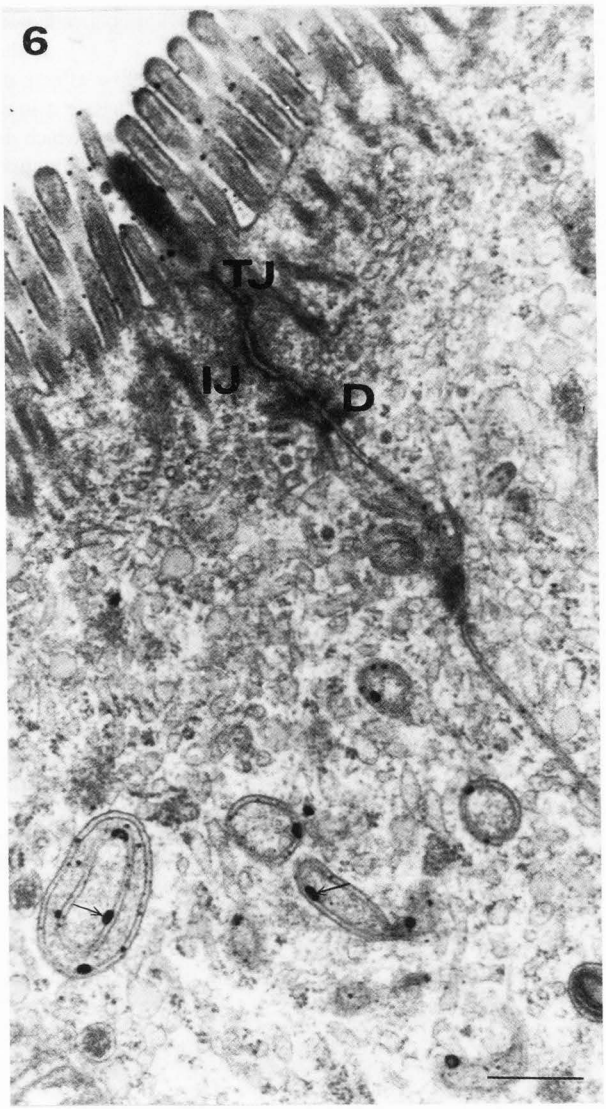


Figure 6. Cell junctional complex in irradiated (5 Gy, 24 hours) small intestine. The amount of pyroantimonate precipitates decreased, the intermediate junction (IJ) dilated. TJ = tight junction, D = desmosomes. Pyroantimone deposits located at the inner surface of lateral plasma membrane are indicated by an arrow. Bar: = 0.5 μm.

Figure 7. Cell junctional complex of irradiated (0.5 Gy, 24 hours) small intestine.The fine structure and calcium content of junctional region similar is to that of the control. TJ = tight junction, IJ = intermedate junction, D = desmosome, M = mitochondria. Bar: = 0.25 μm.

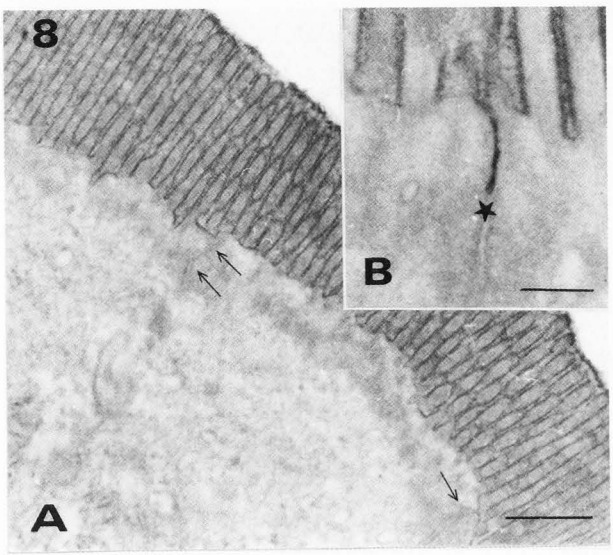
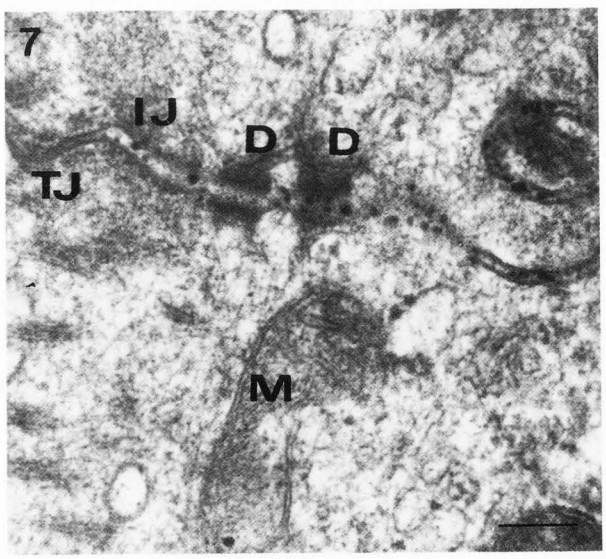


Figure 8. Ruthenium red staining of control (A) and X-irradiated (0,5 Gy, 1 hour) small intestine (B). The dye is excluded from the intercellular space (→) of control tissue, but is present between the cells of X-irradiated animals (*). Unstained material. Bars: A = 0.5 μm, B = 0.3μm.



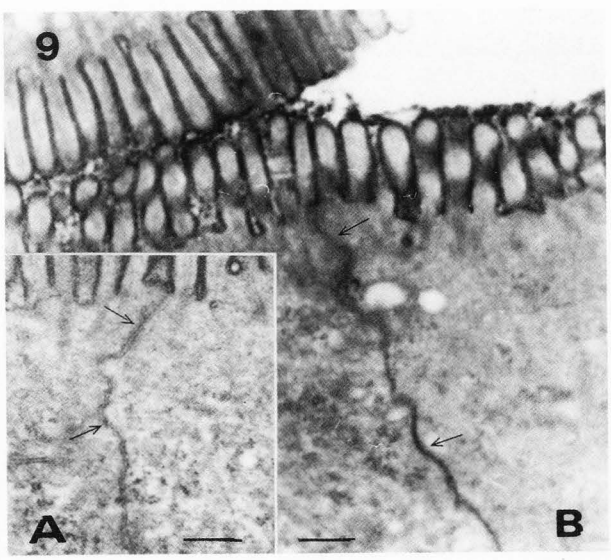


Figure 9. Ruthenium red staining 24 hours after irradiation with 0.5 Gy (A) and 5 Gy (B). The dye is absent in the intercellular space of the former sample (→), but is present in later one (→). Unstained material. Bars: 0.5 μm.

transduction systems (Jacobson, 1979; Duffey et al., 1981), histamine (Willoughby, 1960), and cytokins (tumor necrosis factor, interleukin 1, γ interferon) (Cotran and Pober, 1992; Mullin et al., 1992). The relationship between these different possible regulatory mechanisms is not clear. Recently, Citi supposed (1992), that tight junctional permeability may be regulated by the extracellular Ca^{2+} level which can act both via Ca^{2+} binding molecules of the junctional complex and through the Ca^{2+}- modulated signal transduction pathway involving cAMP and protein kinase systems. In addition, Mullin et al. (1992) suggested, that at least two different mechanisms regulate the tight junction permeability; i.e. they resulted that increase of transepithelial permeability induced by tumor necrosis factor diminished faster than the same effect was induced by protein kinase C activators (Mullin et al., 1992).

The lethal doses of irradiation caused the total loss of barrier functions of small intestine in experimental animals and made the passing of bacteria, bacterial toxins and proteolytic enzymes through the epithelial layer possible. This might be direct causes of intestinal radiation death (Quasler, 1956). Radiation induced pathophysiological changes in the vascular system may lead to the death of the organism, too (Fanger and Lushbaugh, 1967). Non-lethal doses of irradiation induced increased permeability of water, ion and proteins and damage of the junctional complex have already been described in small intestine and other vascular tissues (Levin et al., 1979; Hopewell, 1980; Evans et al., 1986; Spence et al., 1987; Fajardo., 1989; Hauer-Jensen 1990; Peterson et al., 1992). This changes were followed by segmental separation of endothelium from the basement membrane, scattered vacuoles and blebs within the endothelium, subendothelial and interstitial edema and finally caused by late radiation fibrosis (Evans et al., 1986; Hauer-Jensen, 1990).

The main result of our studies presented here is that even small dose of in vivo X-irradiation, also can alter the organization and function of tight junction in mouse small intestine, may be via the decreased calcium content. According to experimental data irradiation can act through other possible regulating factor of paracellular transport, i.e. more released histamine and cytokins (Evans et al., 1986; Fajardo, 1989; Hallahan et al,. 1989), and increased activity of protein kinase C (Hallahan et al., 1991; 1992 a, b; Kim et al., 1992). The relationship between the decreased calcium content and the effects of released biologically active mediators and activity changes of protein kinase C is not exactly clear. However, recently a few data were reported about it , i.e. the histamine induced a dose-dependent mobilization of calcium from internal stores and enhanced entry of extracellular calcium (Crawford et al., 1992), respectively, the concentration of intracellular calcium a important factor in regulation of protein kinase C activity (Sando et al., 1992). Therefore we think, that the rapid increase of intestinal permeability upon irradiation with low doses may due to a transient loss of Ca^{2+} in the junctional area, as we resulted, and conceivable the effects of tumor necrosis factor. High doses of irradiation evoke a long lasting calcium decrease, as we also show here, which is caused some indirect changes via different signal transduction systems (i.e. protein kinase C) and/or released biological mediators (histamine). As we have shown in the results, in parallel with the radiation-induced decrease of the intercellular calcium content, there is a slight increase of cytoplasmic calcium content in surviving cells, and a high calcium content in the formed apoptotic cells. Presumably this calcium is extracellular origin. We plan to investigate the mechanisms of this hypothetical calcium uptake in the future.

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Discussion with Reviewers

Reviewer I: The pyroantimonate method as used by the authors appears only to localize intercellular deposits of Ca^{++}, but what happens to internal stores of Ca^{++}? Are they mobilized and transported to the cell surface?

Authors: Pyroantimonate deposits as we shown, localized in the cytoplasmic region. The amount of calcium in internal stores smaller as in intercellular region. We plan to investigate the activity changes of calcium transporting enzymes upon irradiation.

T.M. Seed: What do the authors use as "controls" for the cytochemical assays?

Authors: The specificity of cytochemical assay was determined by electron spectroscopic imaging and electron energy loss spectrometry investigations

T.M. Seed: "The radiation induced changes of gap junction mediated cell communication may result in tumorous transformation of cells (Trosko et al. 1990)". I realize several workers have suggested this to be a possibility, however by no means is it clearly so. There are too many exceptions that one needs to account for, e. g. , radiation induced tumorgenesis of hematopoietic elements without prominent display of junctional apparatus.

Authors: The exact relationship between radiation-induced changed cell communication and carcinogenetic process, and this mechanism, absolutely not clear. May it only one factor of tumorous transformation.