Scanning Microscopy

Volume 10 | Number 3 Article 18

2-25-1996

Effects of Ionizing Radiation on Cartilage: Emphasis on Effects on the Extracellular Matrix

M. Cornelissen
University of Ghent

H. Thierens
University of Ghent

L. De Ridder
University of Ghent

Scanning Microscopy, Vol. 10, No. 3, 1996 (Pages 833-840)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/96$5.00+.25

EFFECTS OF IONIZING RADIATION ON CARTILAGE: EMPHASIS ON EFFECTS ON THE EXTRACELLULAR MATRIX

M. Cornelissen[1], H. Thierens[2] and L. De Ridder[1*]

[1]Department of Anatomy, Embryology and Histology, [2]Department of Biomedical Physics
University of Ghent, Ghent, Belgium

(Received for publication November 17, 1995 and in revised form February 25, 1996)

Abstract

In this report, we review data dealing with radiation effects on cartilage. More specifically, we emphasize on alterations caused in the extra-cellular cartilage matrix. Although radiation studies predominantly describe the effect on the structure of DNA and on the mitotic activity of cells, alterations caused by the effect on the non-mitotic activity can also be important. Cartilage, having an extracellular matrix composed of 2 major components, aggrecan and collagen, provides a good model to study this kind of radiation effects. The following topics concerning literature data are summarized: effects on the amount of matrix synthesized, effects on the activity of certain enzymes and effects on the structure and morphology of the matrix. Some new findings concerning the radiation effect on the size distribution of aggrecan-aggregate populations, *de novo* synthesized by chondrocyte cultures, either derived from calcifying or from non-calcifying cartilage, are given.

Key Words: Embryonic cartilage, ionizing radiation, extracellular matrix, aggrecan-aggregates, molecular morphology, electron microscopy.

*Address for correspondence:
L. De Ridder
Department of Anatomy, Embryology and Histology,
University of Ghent,
Louis Pasteurlaan 2,
B-9000 Gent, Belgium
Telephone number: (32) 9 224 02 24
FAX number: (32) 9 224 04 99

Introduction

Although radiation studies predominantly describe damage caused by ionizing radiation on the structure of DNA and alterations in the mitotic activity of cells, alterations caused by the effect on the non-mitotic metabolism can also be important. Cartilage provides a good model to study this kind of radiation effects. The main metabolic activity of chondrocytes is the synthesis of extracellular material, consisting of two major components: the glycoprotein collagen II and the cartilage specific proteoglycan aggrecan. Collagen II is present in the matrix as fine, 20 nm thick fibrils. The aggrecans are mainly present in the matrix as large aggregates, composed of a central hyaluronan backbone to which several aggrecans are attached. Alterations in the metabolic activity, caused by irradiation, should be observable as alterations in the synthesis and/or the structure of these matrix molecules.

Literature dealing with the effect of irradiation on cartilage, however, is scarce and contradictory. This effort represents a mini-review dealing with radiation effects on cartilage, not intending to be all inclusive, but rather reflecting the work of others as it impacts ongoing work of our laboratory. For this reason, we will emphasize data dealing with alterations caused by ionizing radiation in the "metabolic", non-mitotic activity of cartilage and in the structure and morphology of the cartilage matrix.

Literature Survey

Alterations in the "metabolic" activity caused by ionizing radiation

First, we consider data dealing with the effect of ionizing radiation on the amount of matrix synthesized post-irradiation. Next, we consider data dealing with radiation effects on the activity of certain enzymes.

Data dealing with the effect of irradiation on the amount of matrix synthesized are contradictory. Using *in vivo* experimental models, some authors describe an increase of matrix synthesis after irradiation. Engstrom

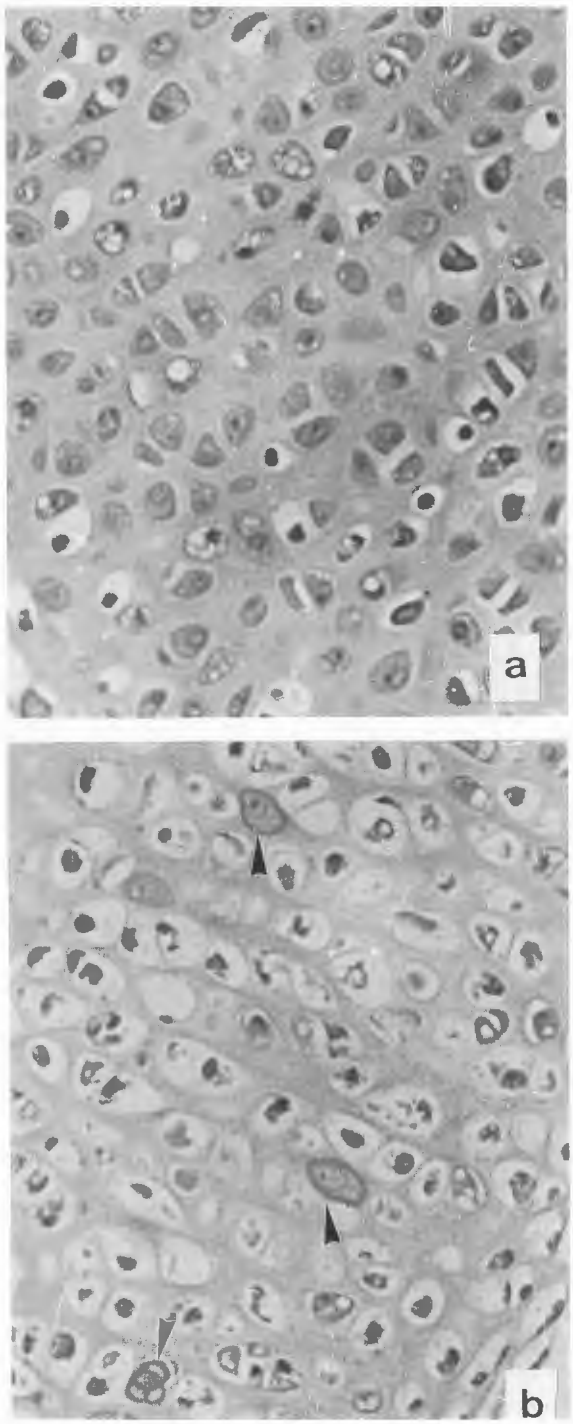

Figure 1. Light micrographs of non-calcifying sternal cartilage of a stage 41 chick embryo, cultured for 14 days: (**a**) sham-irradiated control; and (**b**) irradiation with 50 Gy. After irradiation, some of the remaining viable cells show an enhanced staining with Alcian blue around their surface, indicative for an enhanced GAG-synthesis (arrows). This enhanced staining is absent in the control cartilage.

et al. (1983b), Macpherson *et al.* (1962), and Tefft (1972) reach this conclusion based on histological observations. They describe an increase in deposition of extracellular material between the cell columns in epiphyseal cartilage. Engstrom *et al.* (1983b) mention that an increase is already observable 1 day post-irradiation for radiation doses of 5.8 and 10 Gy, but not for a radiation dose of 2 Gy. Using a radioactive matrix precursor ($Na_2S^{35}O_4$), Myers *et al.* (1989) describe that *in vitro* organ cultures from *in vivo* irradiated articular cartilage show an increase in GAG-synthesis of 21%. Others, also using *in vitro* cultures derived from *in vivo* irradiated cartilage, have reached different conclusions concerning the effect on the amount of matrix synthesis. Hugenberg *et al.* (1989) describe a decreased synthesis. Mosier *et al.* (1983) mention that an increased or decreased synthesis, of collagen as well as of proteoglycans, depends on the post-irradiation time. For experiments where not only the incorporation of radioactive precursors, but also the irradiation was performed *in vitro*, Cornelissen *et al.* (1990) and De Craemer *et al.* (1989) describe that the matrix synthesis, the collagen synthesis as well as the proteoglycan synthesis, is a radioresistant process. Data are based on short post-irradiation periods (3 days) for cultured cartilaginous chick-tibiae, derived from 7 day old chick embryos. Even radiation doses of 50 Gy had only a limited effect on the amount of matrix synthesized (a decrease of about 25% was observed). The same conclusion can be reached from data provided by Biggers and Gwatkin (1964) and De Ridder *et al.* (1988a,b) who describe the radiation effect on the lengthening of 7-day old embryonic chick tibiae. Even after a radiation dose of 50 Gy, tibiae increase equally in length during the first 3 days after irradiation. After a longer post-irradiation time, a decrease of 25% is reported by Biggers and Gwatkin (1964). According to these authors, a significant decrease at this time can be observed after administration of 6.4 Gy. Nijweide *et al.* (1978, 1980), on the other hand, describe that a dose of 5 Gy results in a significant decrease in lengthening of radii and metatarsal bones of 14-17 day old mice-embryos, already 2.5 days post-irradiation. Using organ cultures of non-calcifying cartilage, derived from the caudal part of embryonic chick sterna of 14 day old chick embryos, Cornelissen *et al.* (1993a) also show that after a longer post-irradiation period, the effect on the matrix synthesis is limited. A radiation dose of 50 Gy caused a decrease of about 35% fourteen days post-irradiation. Histological observations showed that after irradiation, some of the remaining "viable" cells, showed an enhanced matrix synthesis (Fig. 1).

In a second part, data concerning the radiation effect on enzymes are considered. Literature data describe

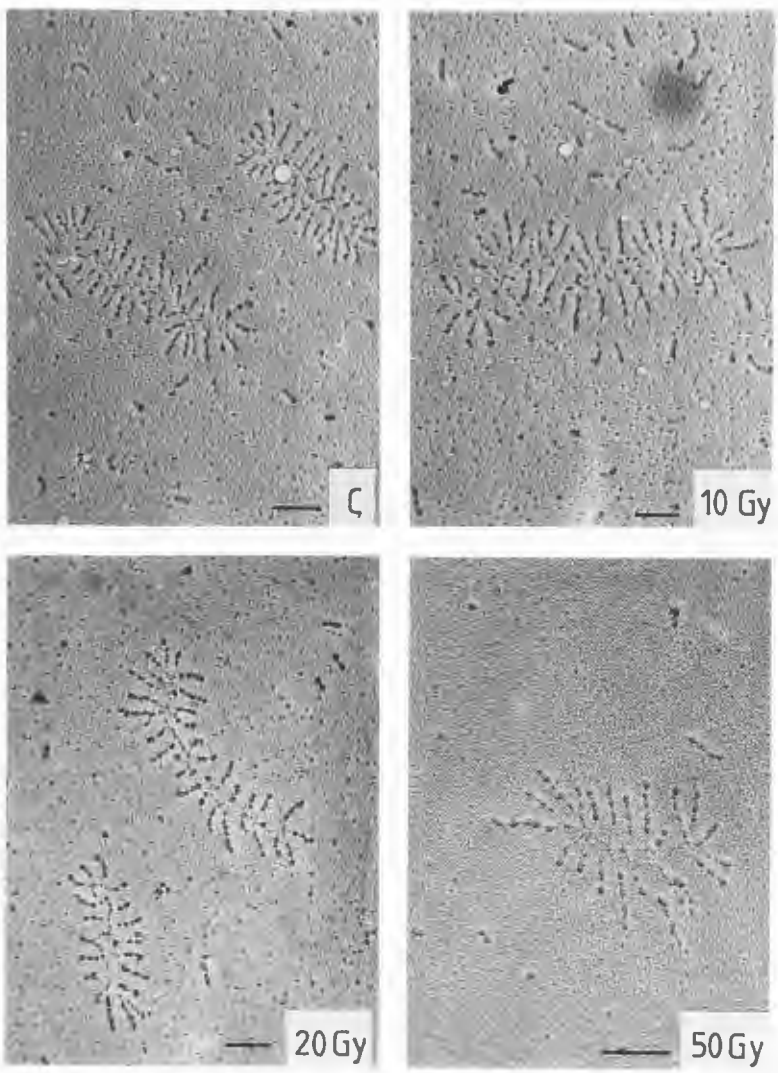

Figure 2. Electron micrograph of aggrecan-aggregates, synthesized by chondrocytes isolated from stage 41 embryonic chick sterna during a 7 day culture period. Controls (C) and radiation doses of 10, 20 and 50 Gy are given. No structural alterations due to irradiation are observed (bar = 200 nm). Aggregates consist of a central hyaluronan back bone to which several aggrecans are attached. Also, some free, non-attached aggrecans are seen.

alterations in the activity of enzymes as well as alterations in the distribution of enzymes in different regions of the cartilage tissue. According to Melanotte and Follis (1961), alkaline phosphatase, normally restricted to the chondrocytes of the late hypertrophic area, is also found in the epiphyseal chondrocytes after irradiation. According to Engstrom *et al.* (1983a,b), a decreased or increased activity is dependent on the post-irradiation time. They describe alterations in the activity of certain enzymes in the epiphysis of tibiae. One day after irradiation, a decrease in alkaline phosphatase but an increase in acid phosphatase and glucose-6-dehydrogenase is observed (8-10 Gy, *in vivo*). Three days after irradiation, all three enzymes were increased to regain the normal level 30 days post-irradiation. Concerning the radiation effect on the acid phosphatase activity, Cornelissen and De Ridder (1990, 1991) not only found variations due to the post-irradiation time, but also dependent on the type of cartilage tissue. They describe a different behaviour in calcifying and in non-calcifying cartilage. In calcifying cartilage, radiation seems to have a stimulatory effect on the activity of acid phosphatase: the number of acid phosphatase positive cells increased with the radiation dose and the post-irradiation time. In non-calcifying cartilage, no correlation between radiation and acid phosphatase activity was observed.

Table 1. Characteristics of aggrecan-aggregate populations, synthesized after different radiation doses during a 7 day post-irradiation period for the caudal (non-calcifying) and the cephalic (calcifying) cultures. The average aggregate (expressed as the number of aggrecans per aggregate) and the number of aggregates containing more than 50 aggrecans (promille) are given. For each experiment, 900 aggregates were studied.

	control	10 Gy	20 Gy	50 Gy
caudal				
average	27	26	25	25
> 50	120	106	97	100
cephalic				
average	20	21	15	14
> 50	65	59	30	25

Structural, morphological alterations caused by ionizing radiation

In growing long-bones, abnormal arrangements of the chondrocytes in the columns are described (Engstrom *et al.*, 1983a,b; Melanotte and Follis, 1961; Nijweide *et al.*, 1978). Other authors describe cartilage necrosis, due to irradiation. Nuclear pyknosis is often used as a criterium. Macpherson (1962); Melanotte and Follis (1961); Rubin *et al.* (1972) describe that nuclear pyknosis is predominantly occurring in the dividing chondrocytes of the proliferative zone. Non-proliferative cartilage, evaluated on the occurrence of necrosis, on the other hand, seems to be more radioresistant. Articular cartilage, irradiated with 50 Gy, and observed during a 3 to 9 months post-irradiation period, showed no degenerative alterations (Takahashi *et al.*, 1992). The elastic cartilage in the ear of the rabbit even did not show necrosis up to a dose of 150 Gy, whereas the surrounding connective tissue was already damaged after a dose of 10 Gy (Langler *et al.*, 1982).

Also, the quality of the cartilage can change due to irradiation. Fragmentation of the matrix, decrease in metachromatic staining and aggregation of collagen fibrils have been described (Langler *et al.*, 1982; Melanotte and Follis, 1961; Mosier *et al.*, 1983; Nijweide *et al.*, 1978; Tefft, 1972). Early ossification due to irradiation is also mentioned. Tefft (1972) describes a slight increase in ossification in long bones. Mosier *et al.* (1983) show an increase in the number of matrix vesicles, indicative for a premature calcification. Others (Nijweide *et al.*, 1980; Wientroub, 1990), however, describe an inhibitory effect of ionizing radiation on ossification.

Finally, direct irradiation of aqueous solutions of matrix molecules results in structural alterations. Hyaluronan as well as aggrecan-aggregates and collagen are described to depolymerize (Balazs *et al.*, 1967; Bates *et al.*, 1984; Chung *et al.*, 1984; Greenwald and Moy, 1976, 1980; Lamberts and Alexander, 1964; Roberts *et al.*, 1987). Cornelissen *et al.* (1993c) used a combination of *in vitro* agarose cultures of isolated chondrocytes and a specific technique to liberate the synthesized aggrecan-aggregate populations to discuss the radiation effects on the molecular structure and on the size-distributions of *de novo* synthesized aggregate-populations. They describe that after single radiation doses of 10, 20 and 50 Gy no structural alterations in the molecular morphology could be detected (Fig. 2). However, radiation doses of 20 and 50 Gy caused a decrease in the number of large aggregates, synthesized by the chondrocyte cultures. Only about 65 promille of the aggregates contained more than 50 aggrecans, compared to about 125 promille for the control and the 10 Gy irradiated cultures. An average aggregate contained about 20 aggrecans in cultures irradiated with 20 or 50 Gy and about 27 aggrecans in control cultures and cultures irradiated with 10 Gy.

To put these findings in a proper perspective, some new data concerning the radiation effect on aggrecan-aggregates, *de novo* synthesized after irradiation are given. We discuss the size-distributions in aggrecan-aggregate populations, synthesized during a 7 days post-irradiation period by irradiated chondrocytes. For this reason, chondrocytes were enzymatically isolated from cartilaginous embryonic chick sterna (stage 41 according to Hamburger and Hamilton, 1951). One population was derived from the calcifying cephalic part, and the other from the non-calcifying caudalic part. Isolation was performed as described earlier by Verbruggen *et al.* (1990). Single radiation doses of 10, 20 and 50 Gy were used. After the 7 days culture period, a specific technique was used to liberate and visualize the aggrecan-aggregates (Cornelissen *et al.*, 1993b). Size-distributions of the newly synthesized aggrecan-aggregate populations were arrived at by expressing the number of aggrecans per aggregate. For each radiation dose, 3 experiments were performed. For each experiment, 300 aggregates were counted for the irradiated, as well as for the control cultures. Results are given in Table 1.

Summarizing: an average aggrecan-aggregate, synthesized by chondrocytes of the non-calcifying part of the sternum (caudal part) contained 27 aggrecans, an average aggrecan-aggregate synthesized by chondrocytes of the calcifying sternal part (cephalic part) contained 20 aggrecans. Both populations contained a number of large aggregates, with more than 50 aggrecans. For the non-calcifying culture, this number was 120 promille;

for the calcifying culture, 65 promille. Irradiation mainly caused alterations in the cephalic cultures. After irradiation with 10, 20 and 50 Gy, an average aggregate contained 21, 15 and 14 aggrecans, respectively. Sizes of aggrecan-aggregates synthesized by the caudal, non-calcifying chondrocytes, were practically not influenced by irradiation. An average molecule after irradiation with 10, 20 and 50 Gy contained respectively 26, 25 and 25 aggrecans. Also, concerning the presence of large aggregates alterations due to irradiation were mainly observed in the cephalic populations, starting from a radiation dose of 20 Gy. The numbers of these aggregates were reduced to about 25 promille in the cephalic cultures and only to about 100 promille in the caudal cultures.

Discussion and Concluding Remarks

Data from the literature, dealing with radiation effects on cartilage, are difficult to compare. Often, experiments are performed under very different experimental conditions. Nevertheless, the following concluding remarks can be made. Compared to other tissues, cartilage is a radio-resistant tissue. Under experimental *in vivo* conditions, very high doses are used to observe disturbances in the cartilage. For example, Langler *et al.* (1982), using single radiation doses of 10 to 430 Gy, describe that cartilage remains intact even when the surrounding connective tissue is already damaged. The fact that cartilage is devoid of blood vessels and has a large amount of inert extracellular material, separating cells, with a low mitotic activity, may lie at the basis of it's radio-resistance. Only proliferative cartilage seems to be more radiosensitive. A retardation in bone growth is observed due to the damage of the cells of the proliferative zone. This retardation is only temporary and can be restored by some of the remaining viable cells that start to divide again (Kember, 1965, 1967). Even after administration of a single dose of 20 Gy, 10 to 15 days post-irradiation, the few surviving cells of the proliferative zone of an irradiated rat tibia start to produce "recovery" clones to repopulate the cartilage plate. From *in vitro* radiation experiments on long bones derived from embryo's, it can be deduced that the effect on the lengthening depends on the type of the long bone. According to Wolpert (1981), the degree of inhibition depends on the contribution of proliferative cells to the lengthening. Long bones with an important contribution of proliferation to the lengthening seem to be more radiosensitive. This is supported by our own results concerning radiation experiments with embryonic chick tibiae (De Ridder *et al.*, 1988a,b). In the tibia, where the contribution of cell proliferation to the lengthening is also minimal (no correlation was found between thymidine incorporation and increase in length), the radiation effect to the lengthening was restricted.

As already mentioned, the metabolic activity of a chondrocyte predominantly involves the synthesis of the extracellular matrix. This matrix synthesis seems to be a radioresistant process. Some authors even mention a temporary increased synthesis after irradiation. Others however, mention a decreased synthesis. Especially, the post-irradiation time and, of course, the radiation dose are important variables which determine that radiation either results in an increased or in a decreased synthesis. According to our own findings, difficulties in interpretation may also be due to the fact that radiation may affect different cells in the same tissue in a different way. In tissue cultures of irradiated sternal (non-calcifying) cartilage, we observed that irradiation (50 Gy) results in the death of a large number of chondrocytes (evaluated by the occurrence of pyknotic nuclei). Beside the dead cells, randomly distributed in the tissue, some viable cells remained, some of which showed an enhanced synthesis (Cornelissen *et al.*, 1993a). Stimulation of some of the remaining viable cells after irradiation could explain the limited radiation effect on the matrix synthesis.

Also, causing part of the effect on the non-mitotic metabolism, are the radiation effects on the synthesis and on the activity of certain enzymes. Especially, the effect of irradiation on lytic enzymes (for example, acid phosphatase) is discussed in literature. Also, radiation effects on enzymes, involved in the calcification process (alkaline phosphatase), are subject of discussion. Both enzymes, acid phosphatase as well as alkaline phosphatase, are described which may increase shortly after irradiation; after a longer post-irradiation time, they reach their normal levels again . According to our own results concerning the radiation effect on the acid phosphatase activity, a different behaviour is observed in cephalic calcifying cartilage compared to caudalic non-calcifying cartilage (Cornelissen *et al.*, 1990; Cornelissen and De Ridder, 1990, 1991). In calcifying cartilage, a correlation between radiation and acid phosphatase activity (lysosomal activity) is found; in non-calcifying cartilage, no correlation exists. So, unlike the description in earlier literature, the radiation effect on the acid phosphatase activity is not only dependent on the radiation dose and the post-irradiation time, but also on the type of cartilage (calcifying versus non-calcifying). This can be explained by the fact that in calcifying cartilage, cells become hypertrophic before endochondral ossification starts. According to Matsuzawa and Anderson (1971), this hypertrophy is characterized by an enhanced activity of acid phosphatase. The enhanced activity of acid phosphatase could reflect a premature degeneration accompanying a premature ossification as a consequence of irradiation. Also, the findings of Melanotte and Follis

(1961) indicate that irradiation of epiphyseal chondrocytes results in a premature degeneration. They describe that alkaline phosphatase, normally restricted to the hypertrophic chondrocytes after irradiation, is also found in epiphyseal chondrocytes. Also, in general, beside cell death, acceleration of the differentiation process is known to be one of the possible effects caused by irradiation of cells (Tubiana *et al.*, 1990). Moreover, in calcifying cartilage, where changes in acid phosphatase activity could already be detected after irradiation (before the occurrence of other morphological alterations), detection of acid phosphatase can be considered as an early marker of radiation damage.

Our new data concerning the size-distributions of aggrecan-aggregate populations, *de novo* synthesized after irradiation (presented in Table 1 here), also indicate a different radiation effect on cephalic calcifying and caudalic non-calcifying chondrocytes. Also, under normal conditions, the appearance of smaller aggregates is described to accompany the process of calcification: the cephalic calcifying area (hypertrophic area) is characterized by the presence of smaller aggregates, compared to the epiphysis and the proliferative zone (Buckwalter, 1983). Our data, on the average size of an aggregate synthesized by a calcifying or non-calcifying culture (respectively containing 20 and 27 aggrecans), indicate that under *in vitro* conditions these characteristics are retained also. Our data, which describe the average aggregate-sizes after irradiation, show that alterations in size occur in the cephalic calcifying culture and not in the caudalic non-calcifying culture. We deduce that, as with the changes in acid phosphatase activity, this reduction in aggregate-size reflects a premature ossification after irradiation. The underlying mechanism for the basis of the decrease in aggregate-size (under normal physiological conditions) is not completely understood. It is accepted that smaller aggregates are not the result of an impaired synthesis but that they are the result of an extracellular degradation of existing aggregates (Poole *et al.*, 1982). Proteolytic degradation of aggregates with proteases, acting on the core protein of the aggrecans, is not accepted as the mechanism since the process of calcification is not accompanied with a shortening of the subunits (Buckwalter and Rosenberg, 1981). Only degradation of the hyaluronan back-bone seems to occur. As a possible agent for the hyaluronan degradation, enzymatic as well as non-enzymatic mechanisms are mentioned in literature. As non-enzymatic mechanism, effects of free radicals are mentioned (Bates *et al.*, 1984; Chung *et al.*, 1984; Greenwald and Moy, 1976, 1980; Roberts *et al.*, 1987). However, degradation by free radicals seems unlikely in our *in vitro* system since free radicals have only a short activity time and since their formation is restricted to the short period of irradiation. At that moment, no extracellular matrix is present around the chondrocytes. On the other hand, hyaluronidase, the enzyme capable of cutting the hyaluronan chain in pieces, could decrease aggregate-size without changing the structure of the other subunits of the aggregates. However, this enzyme has not yet been detected in cartilage and moreover, it has a low pH optimum (Sapolsky *et al.*, 1974). Maybe, in our irradiated system however, acid hydrolases could be the basis of this degradation. In calcifying cartilage, the correlation of radiation and acid phosphatase activity on one hand, and radiation and decrease in aggregate-size on the other hand, gives an indication in that direction.

References

Balazs EA, Davies JV, Philips GO, Young MD (1967) Transient intermediates in the radiolysis of hyaluronic acid. Rad Res **31**, 243-255.

Bates EJ, Harper GS, Lowther DA, Preston BN (1984) Effect of oxygen-derived reactive species on cartilage proteoglycan-hyaluronate aggregates. Biochem Int **8**, 629-937.

Biggers JD, Gwatkin RBL (1964) Effects of X-rays on the morphogenesis of the embryonic chick tibiotarsus. Nature **202**, 152-154.

Buckwalter J (1983) Proteoglycan structure in calcifying cartilage. Clin Orthop **172**, 207-232.

Buckwalter J, Rosenberg LC (1981) Proteoglycan aggregate structure in mineralizing cartilage. Orthop Trans **5**, 220-231.

Chung MH, Kesner L, Chan PC (1984) Degradation of articular cartilage by copper and hydrogen peroxide. Agents Actions **15**, 328-335.

Cornelissen M, De Ridder L (1990) Dose- and time-dependent increase of lysosomal enzymes in embryonic cartilage *in vitro* after ionizing radiation. Scanning Microsc **4**, 769-774.

Cornelissen M, De Ridder L (1991) Stimulation of lysosomal enzymes in embryonic cartilage after ionizing radiation. Prog Histochem Cytochem **23**, 141-145.

Cornelissen M, Thierens H, De Ridder L (1990) Dose- and time-dependent radiation inhibition of RNA and glycosaminoglycan synthesis in embryonic cartilage: an *in vitro* study. Int J Rad Biol **587**, 1007-1016.

Cornelissen M, Thierens H, De Ridder L (1993a) Radiation effects on the matrix synthesis in non-ossifying embryonic cartilage *in vitro*: a functional and morphological study. Tissue Cell **25**, 343-350.

Cornelissen M, Verbruggen G, Malfait AM, Veys EM, Broddelez C, De Ridder L (1993b) The study of representative populations of native aggrecan aggregates synthesized by human chondrocytes *in vitro*. J Tiss Cult Meth **15**, 139-146.

Cornelissen M, Thierens H, De Ridder L (1993c) Effects of ionizing radiation on the size distribution of proteoglycan aggregates synthesized by chondrocytes in agarose. Scanning Microsc **7**, 1263-1268.

De Craemer D, Thierens H, Cornelissen M, Beele H, Lepercque S, De Ridder L (1989) The effects of ionizing radiation on the glycosaminoglycan synthesis in embryonic cartilage *in vitro*. Cytotechnology **2**, 19-25.

De Ridder L, Thierens H, Cornelissen M, Segaert O (1988a) Effects of ionizing radiation on the metabolism and longitudinal growth of cartilaginous embryonic chick tibiae *in vitro*. Int J Rad Biol **53**, 965-975.

De Ridder L, Thierens H, Cornelissen M (1988b) Growth inhibition of cartilaginous embryonic chick tibiae by ionizing radiation: An *in vitro* study. Cytotechnology **1**, 223-231.

Engstrom H, Jansson JO, Engström C (1983a) Effect of local irradiation on enzyme activities of growing rat bone. A tetracycline labelling investigation. Acta Radiol **22**, 129-133.

Engstrom H, Magnussen BC, Turessen I (1983b) Effect of 50 kV irradiation on enzyme activities of growing rat bone. A histopathologic and enzyme histochemical investigation. Acta Radiol Oncol **22**, 65-70.

Greenwald RA, Moy WW (1976) Degradation of cartilage proteoglycans and collagen by superoxide radical. Arthrit Rheum **19**, 799.

Greenwald RA, Moy WW (1980) Effects of oxygen derived free radicals on hyaluronic acid. Arthrit Rheum **23**, 455-463.

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol **88**, 49-92.

Hugenberg ST, Myers SL, Brandt K (1989) Suppression of GAG synthesis by articular cartilage, but not of HA synthesis by synovium, after exposure to radiation. Arthritis Rheum **32**, 468-474.

Kember NF (1965) An *in vivo* cell survival system based on the recovery of rat growth cartilage from radiation injury. Nature **207**, 501-503.

Kember NF (1967) Cell survival and radiation damage in growth cartilage. Br J Radiol **40**, 496-505.

Lamberts HB, Alexander P (1964) Post-irradiation changes in solutions of hyaluronic acid exposed to X-rays. Biochim Biophys Acta **88**, 642-644.

Langler AJ, Michaels L, Kember NF (1982) Radiation necrosis of cartilage: A comparison of experimental and clinical results. Br J Radiol **55**, 942-943.

Macpherson S, Owen M, Phil D, Vaughan J (1962) The relation of radiation dose to radiation damage in the tibia of weanling rabbits injected with strontium 90. Brit J Radiol **35**, 221-234.

Matsuzawa T, Anderson HC (1971) Phosphatases of epiphyseal cartilage studied by electron microscopic cytochemical methods. J Histochem Cytochem **19**, 801-808.

Melanotte PL, Follis RH (1961) Early effects of X-irradiation on cartilage and bone. Am J Pathol **39**, 1-15.

Mosier HD, Sondhaus CA, Deraden LC, Zuniga OF, Janssons RA, Good CB, Roberts RC (1983) Cartilage metabolism during growth retardation following irradiation of the head of the neonatal rat. Proc Sci Exp Biol Med **172**, 99-106.

Myers SL, Slowman SD, Brandt KD (1989) Radiation synovectomy stimulates glycosamino-glycan synthesis by normal articular cartilage. Lab Clin Med **114**, 27-35.

Nijweide PJ, Gaillard PJ, Hekkelman JW, Hermann-Erlee MPM, van der Plas A, Vegt GB, Mellinck JH (1978) The effects of γ-irradiation on embryonic bone and cartilage *in vitro*. Rad Res **73**, 234-250.

Nijweide PS, Burger RH, van Delft JL, Kawilarang-de-Haas EWM, Wassenaar AM (1980) Effects of γ-irradiation on cartilage matrix calcification. Rad Res **84**, 64-76.

Poole A, Pidoux I, Rosenberg L (1982) The role of proteoglycans in endochondral ossification. J Cell Biol **92**, 249-255.

Roberts CR, Mort JS, Roughley PJ (1987) Treatment of cartilage proteoglycan aggregate with hydrogen peroxide. Biochem J **247**, 349-357.

Rubin P, Casarett G, Farrer P (1972) The effect of intra-articular ^{198}Au instillations on articular cartilage. Radiology **103**, 685-690.

Sapolsky A, Keiser H, Howell D, Woessner J (1974) Metalloproteases of human articular cartilage that digest cartilage proteoglycan at neutral or acid pH. J Clin Invest **58**, 1030-1039.

Takahashi S, Sugimoto M, Kotoura Y, Oka M, Sasai K, Abe M, Yamamuro T (1992) Long-lasting tolerance of articular cartilage after experimental intraoperative radiation in rabbits. Clin Orthop **275**, 300-305.

Tefft M (1972) Radiation effect on growing bone and cartilage. In: Frontiers of Radiation Therapy and Oncology. Radiation Effect and Tolerance, Normal Tissue. Vol. 6. Vaeth JM (ed.). Karger, Basel, Switzerland. pp. 289-311.

Tubiana M, Dutreix J, Wambersie A (1990) Cellular effects of ionizing radiation. Cell survival curves. In: Introduction to Radiobiology. Taylor and Francis, London. pp. 86-125.

Verbruggen G, Veys EM, Wieme N, Malfait AM, Gijselbrecht L, Nimmegeers J, Almquist KF, Broddelez C (1990) The synthesis and immobilisation of cartilage-specific proteoglycan by human chondrocytes in different concentrations of agarose. Clin Exp Rheum **8**, 371-378.

Wientroub S, Weiss JF, Catravas GN, Reddi AH

(1990) Influence of whole body irradiation and local shielding on matrix-induced endochondral differentiation. Calcif Tissue Int **46**, 38-45.

Wolpert L (1981) Cellular basis of skeletal growth during development. Brit Med Bull **37**, 215-219.

Discussion with Reviewers

L. de Saint-Georges: What is the interest of the cartilage as a model for the study of the effect of ionizing radiation to a non-mitotic metabolism system? The radiation effect on a non-mitotic system will only be detected at very high doses, used in radiotherapy to damage locally transformed cells. The study of such doses on normal cells might present a rather limited interest.

Authors: Indeed, at the start of our work, only after administration of very large doses of ionizing radiation, alterations could be observed on the metabolic activity or on the structural components in our cartilage model. Effects were only found with radiation doses of more than 150 Gy (de Ridder *et al.*, 1988a). Later on, when looking at the effect on the molecular structure of newly synthesized proteoglycan molecules, effects were already visible at a dose of 20 Gy after a short post-irradiation period (7 days) (Cornelissen *et al.*, 1993c). From the observed differences between the newly synthesized molecules from cephalic calcifying and caudalic non-calcifying cartilage cultures, it could be deduced that ionizing radiation in our cartilage model results in an acceleration of the differentiation process.

T.M. Seed: What is the statistical significance of the noted differences in the data listed in Table 1 as well as those described in the text?

Authors: The noted differences concern the populations of very large aggregates, containing more than 50 aggrecans per aggregate. Applying Poisson's statistics, no significant differences were observed after administration of 10 Gy compared to the controls. After a radiation dose of 20 and 50 Gy, significant differences (95% confidence limit) were found.

P.J. Nijweide: What is the molecular basis of the finding that cartilage cells of calcifying cartilage do respond to irradiation with the production of smaller aggregates, while cells of non-calcifying cartilage do not?

Authors: The appearance of smaller aggregates in cartilage is occurring under normal conditions (e.g., with increasing age and during the process of endochondral ossification) as well as under pathological conditions (e.g., in chondrosarcomas, osteoarthritis and rheumatoid arthritis). Looking at the molecular morphology of the aggrecans and aggrecan-aggregates after irradiation, it was seen that alterations occurred in the size of the aggregates, without changing the length of the aggrecans itself. These alterations are also described during the process of endochondral ossification for the aggregates found in the hypertrophic cartilage area (Buckwalter, 1983; Buckwalter and Rosenberg, 1981). The described alterations do not fit with those described for chondrosarcomas (larger aggregates and longer aggrecans) and arthritis (shorter aggrecans due to the cleavage of the protein-core).