

Stage-Specific Apoptosis in the Rat Seminiferous Epithelium: Quantification of Irradiation Effects

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ABSTRACT: The effects of 3 Gy local X-irradiation on the adult rat testis were studied together with exact determination of the radiation dose distribution in the testis. Seminiferous tubule segments were isolated 8–66 hours postirradiation (p.i.), squashed between a microscope slide and a coverslip, and the exact stage of the seminiferous epithelial cycle was identified under a phase-contrast microscope. The squash preparations were subjected to *in situ* end labeling (ISEL) for visualization and quantification of apoptotic cells. In controls, the highest numbers of apoptotic cells were scored in stages XII–XIV and I. *In situ* end-label staining of cells was observed in A₃–A₄ spermatogonia, spermatocytes at zygotene, pachytene, and meiotic division phases, as well as in early spermatids. In irradiated testes, from 8 hours p.i. and onward, intermediate- and B-type spermatogonia were sensitive at stages II–VI. At 42 hours, in stage I,

elevated numbers of degenerating spermatocytes were seen. Most of them had not undergone meiotic divisions at stage XIV and showed an apoptotic type of degeneration at stage I. At the time of irradiation, the cells were in stage XIII, suggesting that diakinetically spermatocytes are particularly sensitive to irradiation. Also, preleptotene–zygotene spermatocytes in stages VII–XII were sensitive to irradiation. Apoptotic-type of cell degeneration was confirmed by living cell squash preparations, electron microscopy, and DNA electrophoresis. In conclusion, irradiation may provide a useful model system for studying apoptosis, and its control in spermatogonia and meiotically dividing cells.

Key words: Testis, rat, apoptosis, meiosis, irradiation.

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The sensitivity of the mammalian testis to irradiation was first observed in 1903 (Albers-Schönberg, 1903), not long after the discovery of X-rays. Ever since, abundant literature (for reviews see Ellis, 1970; Oakberg, 1975; Bianchi, 1983) on the topic has been produced. The kinetics of germ cell killing after irradiation, the endocrinological changes, and the mutation effects have been thoroughly studied. There are different levels of sensitivity to irradiation-induced effects in strains of the same species and between different species. Man is approximately three times more sensitive than the mouse (Clifton and Bremner, 1983), doses of 0.35 Gy inducing aspermia, which may be permanent after doses of more than 2 Gy (Ogilvy-Stuart and Shalet, 1993). In mammals, the most sensitive spermatogenic cells seem to be intermediate and/or B-type spermatogonia (Bianchi, 1983). We used a dose of 3 Gy, which destroys most of the cycling spermatogonia in the rodent but leaves the other cell types unaffected. The radiation-resistant noncycling spermatogonia

eventually repopulate the seminiferous epithelium (Dym and Clermont, 1970). In another stem cell model, proposed by Huckins (1971), the stem cells are cycling, and depending on the phase of the cell cycle in which they were at the time of irradiation, repopulation of the epithelium varies (van Beek et al, 1986).

Radiation and radiomimetic cytotoxic drugs, having an effect on DNA, greatly increase apoptosis in various rapidly proliferating tissues (Kerr and Searle, 1980). Apoptosis has been suggested to serve as a mechanism preventing amplification of genetic errors (Wyllie et al, 1980). In the testis, spermatogonia undergo apoptosis after irradiation (Allan et al, 1987), a feature also present under normal conditions. An attempt to quantify the irradiation-induced apoptosis has been performed (Bianchi, 1983), but data concerning the whole cycle of the seminiferous epithelium is lacking. For studying irradiation-induced apoptosis, we first measured the accurate dose distribution in the testis, and utilized a quantitative non-radioactive *in situ* end-labeling (ISEL) method of squashed tubule segments from defined stages of the cycle of the rat seminiferous epithelium (Henriksén et al, 1995a,b). Apoptotic germ cells were also visualized in living cell squash preparations. To confirm the apoptotic type of cell death, isolated DNA from defined stages was run on an agarose gel. Additional morphological evidence was obtained by electron microscopy.

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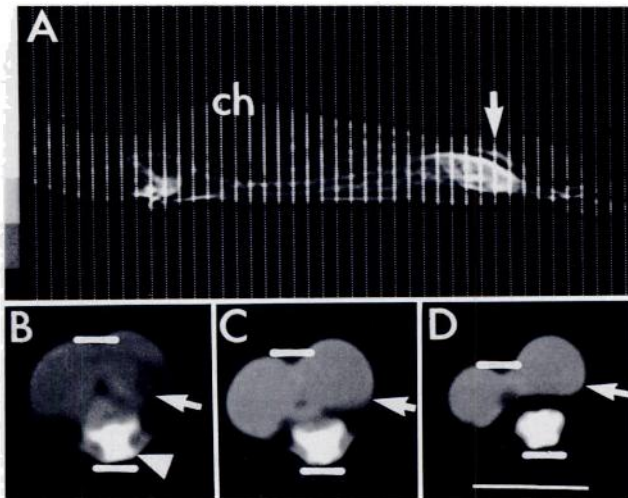


FIG. 1. Computer tomography X-ray images of an adult male rat showing the levels of the axial sections. (A), The rat was lying on its back (ch = chest, the arrow indicates the level of the midpoint of the testes). The axial slices of the testis (arrows) were taken from its proximal (B), middle (C, arrow in A) and the distal part (D). The tail bone is indicated by an arrowhead. Short lines in B–D indicate the locations of the LiF-dosimeters and the longer line in D is a scale bar equivalent to 20 mm.

Materials and Methods

Animals and Treatment

Sprague Dawley rats of 2–3 months age were used. They were anesthetized with 1.0 ml/kg fentanyl-fluanisone (Hypnorm, Janssen Pharmaceutical Ltd., Oxford, UK) and 0.3 ml/kg diazepam (Diapam 5 mg/ml, Orion, Espoo, Finland) before irradiation. They were locally irradiated (without a buildup layer, field size 5×5 cm, dose rate 2 Gy/min) by 3 Gy using 4 MeV X-rays produced by a Clinac 4/100 linear accelerator (Varian, Palo Alto, California). Controls received anesthesia and were sham irradiated. Treated rats were killed 8, 16, 42, and 66 hours postirradiation (p.i.), controls after 16, 42, and 66 hours. Each treatment group consisted of three to six animals.

Radiation Dose

The radiation dose in the testis was determined mathematically using a computer tomography (CT)-based CADPLAN radiation planning system (Varian-Dosetek, Espoo, Finland). The rat was CT-imaged (Fig. 1A), and the radiation field was planned from above on the area of the testis (Fig. 1B–D), to mathematically achieve optimal mean dose and dose distribution in the testis (4 MeV found to be optimal).

The actual exact dose was measured by eight 5×0.5 -mm thermoluminescence crystals (lithium fluoride embedded in plastic, Rados Technology, Turku, Finland) situated above and below the testes (Fig. 1B–D). The cumulated radiation dose was measured by DOSACUS apparatus (Rados Technology, Turku, Finland).

The Transillumination Technique and Fixation of Squash Preparations

Segments (1 mm in length) of seminiferous tubules were dissected in Dulbecco's phosphate-buffered saline (PBS, pH 7.2) under a transillumination stereomicroscope, transferred onto a microscope slide in 10 μ l of medium, and carefully squashed under a coverslip to avoid air bubbles. Germ and Sertoli cells are squeezed out of the tubule and form a homogenous layer of cells on the microscope slide. The stage of the seminiferous epithelial cycle was identified under a phase-contrast microscope (Kangasniemi et al, 1990a). Apoptotic cells were either photographed (Henriksen et al, 1995a, 1996), or the slides were briefly frozen in liquid N₂ for fixation. The coverslip was removed and the frozen slide was briefly dipped in ethanol (-20°C), fixed in formalin for 10 minutes, and washed in PBS for 2×5 minutes (Henriksen et al, 1995a,b). Slides were postfixed in ethanol:acetic acid (2:1, -20°C), washed in PBS 2×5 minutes, dehydrated, air dried, and stored at -70°C for ISEL.

ISEL

Slides were treated as described earlier (Henriksen et al, 1995a,b). After hydration, the samples were incubated with terminal transferase buffer (Promega, Wisconsin) for 10 minutes before 1 hour incubation at 37°C with terminal transferase enzyme (0.3 U/ μ l, Boehringer, Mannheim, Germany), dideoxy-digoxigenin-UTP (5 μ M, Boehringer), dideoxy-ATP (45 μ M, Pharmacia, Uppsala, Sweden), terminal transferase buffer, and CoCl₂ (both Boehringer). After washing (3×10 minutes), the slides were incubated with blocking buffer for 30 minutes (Boehringer), thereafter with anti-dig-AB (1:4,000, Boehringer) conjugated to alkaline phosphatase. After 2 hours in room temperature, the slides were washed 3×10 minutes and treated with alkaline phosphatase buffer. The apoptotic cells were visualized after exposure of the slides in the dark with nitro-blue tetrazolium and X-phosphate (Boehringer). The number of apoptotic cells per 1 mm of tubule was scored from ISEL-stained squash preparations. Stained cells were tentatively identified according to their relative size and nuclear morphology from staged squash preparations. The existence of defined germ cell types in each stage of the cycle was used as a further assistance in identification.

Electron Microscopy

The stage of tubular segments (42 hours p.i.) was identified by 0.5-mm-long squash preparations from both ends of the segment, whereafter the segment was fixed in 5% glutaraldehyde in *s*-collidine buffer (0.16 M, pH 7.4) at 20°C . Segments were post-fixed with 1% osmium tetroxide in 1.5% K-ferrocyanide, embedded in epoxy resin (Glycidether 100, Merck, Germany), and sectioned at 70 nm (Reichert E Ultramicrotome, Reichert Jung, Vienna, Austria). They were stained with uranyl acetate and lead citrate and examined with a JEOL 100 SX electron microscope (JEOL, Tokyo, Japan).

Autoradiography

Tubular segments, 5 mm in length (5 cm/pool), from stages II–V were isolated under a transillumination stereomicroscope (Par-

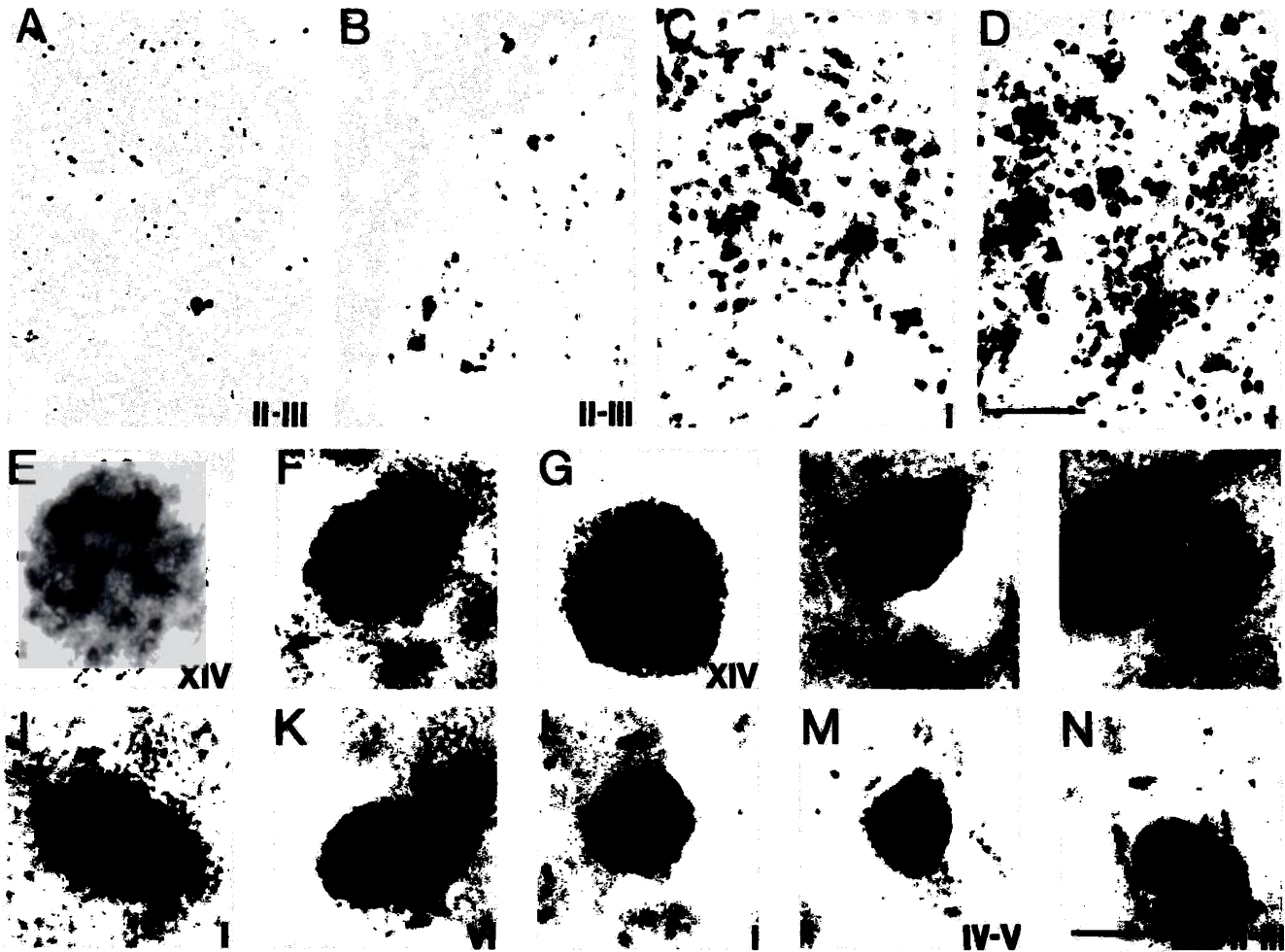


FIG. 2. Photomicrographs of ISEL-stained squash preparations, with the stage of the seminiferous epithelial cycle indicated in the right lower corner of each figure. Low power photomicrographs of control stages II-III (A) and I (C), and after 16 and 42 hours of 3 Gy local X-irradiation (B and D), respectively. High power photomicrographs (E-N), show that many spermatocytes did not accomplish their meiotic divisions (E,F), or degenerated during divisions (G, 1st meiotic division), and H-J (metaphase II—telophase). B, Spermatogonia degenerating at mitotic telophase (K). In later phases of apoptosis nuclear condensation in apoptotic spermatogonia increased (L-N). Bars = 10 μ m (A-D) and 100 μ m (E-N). A-D have been lightly counterstained with hematoxylin.

vinen and Vanha-Perttula, 1972) and frozen in liquid N₂. DNA isolation, 3'-end labeling, and autoradiography were performed as previously described (Tilly and Hsueh, 1993). Five hundred nanograms of DNA was labeled with [³²P]dideoxy-ATP (Amersham, Aylesbury, UK) with terminal transferase (Boehringer). Half (250 ng) was run on a 2% agarose gel (6 V/cm) for 3.5 hours. The gel was exposed to Konica MG SR X-ray film and exposed. From the gel, each lane (DNA < 20 kb) was cut out and the radioactivity was determined in a beta-counter (Wallac Rackbeta II, Wallac, Turku, Finland).

Statistical Analyses

Analysis of variance and the Student's *t*-test with Bonferroni corrections were utilized for statistical analyses, and a *P*-value below 0.05 was considered significant. For ISEL, two to eight replicates were analyzed from three to six animals. For autoradiography, three animals per treatment were analyzed.

Results

Radiation Dose

Figure 1 shows a CT X-ray picture of an adult male rat at the level of the testes, used for planning of the irradiation protocol. The radiation doses measured by LiF crystals were 2.6 ± 0.3 Gy above and 3.2 ± 0.2 Gy below the testes.

Identification of Cell Degeneration From ISEL Squash Preparations

Stained degenerating germ cells, presumed to be apoptotic, in an advanced stage of pycnosis were not always identifiable, but the majority of cells could be identified. In stage I controls (Fig. 2C), the most frequent cells to degenerate were pachytene spermatocytes, followed by

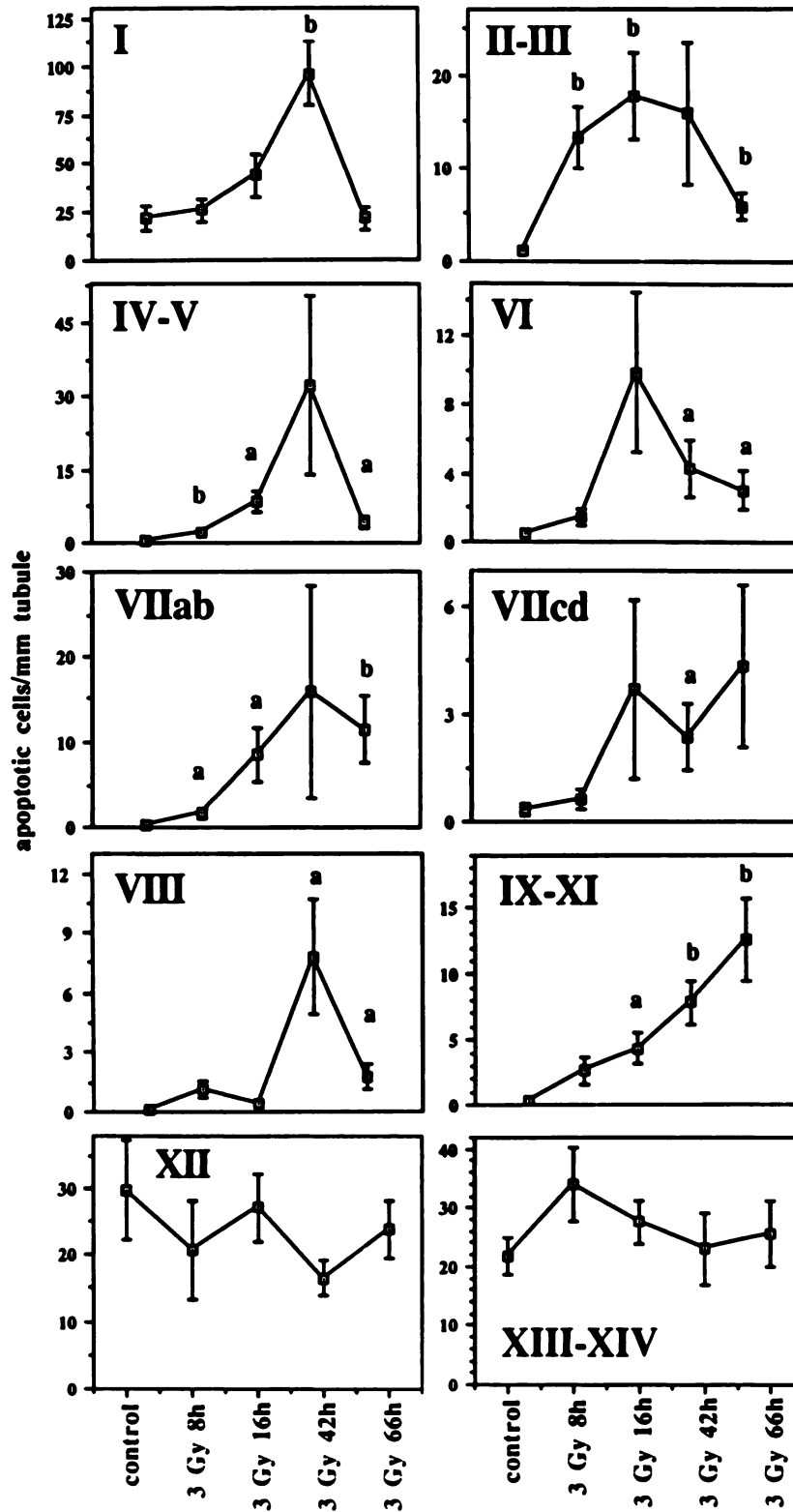


FIG. 3. Quantification of stage-specific apoptosis from ISEL-stained squash preparations at different times after irradiation, expressed as apoptotic cells per 1 mm/semiferous tubule (mean \pm SE, $n = 9-31$ [no. of squash preparations]). a, $P < 0.05$; b, $P < 0.01$ vs. respective control.

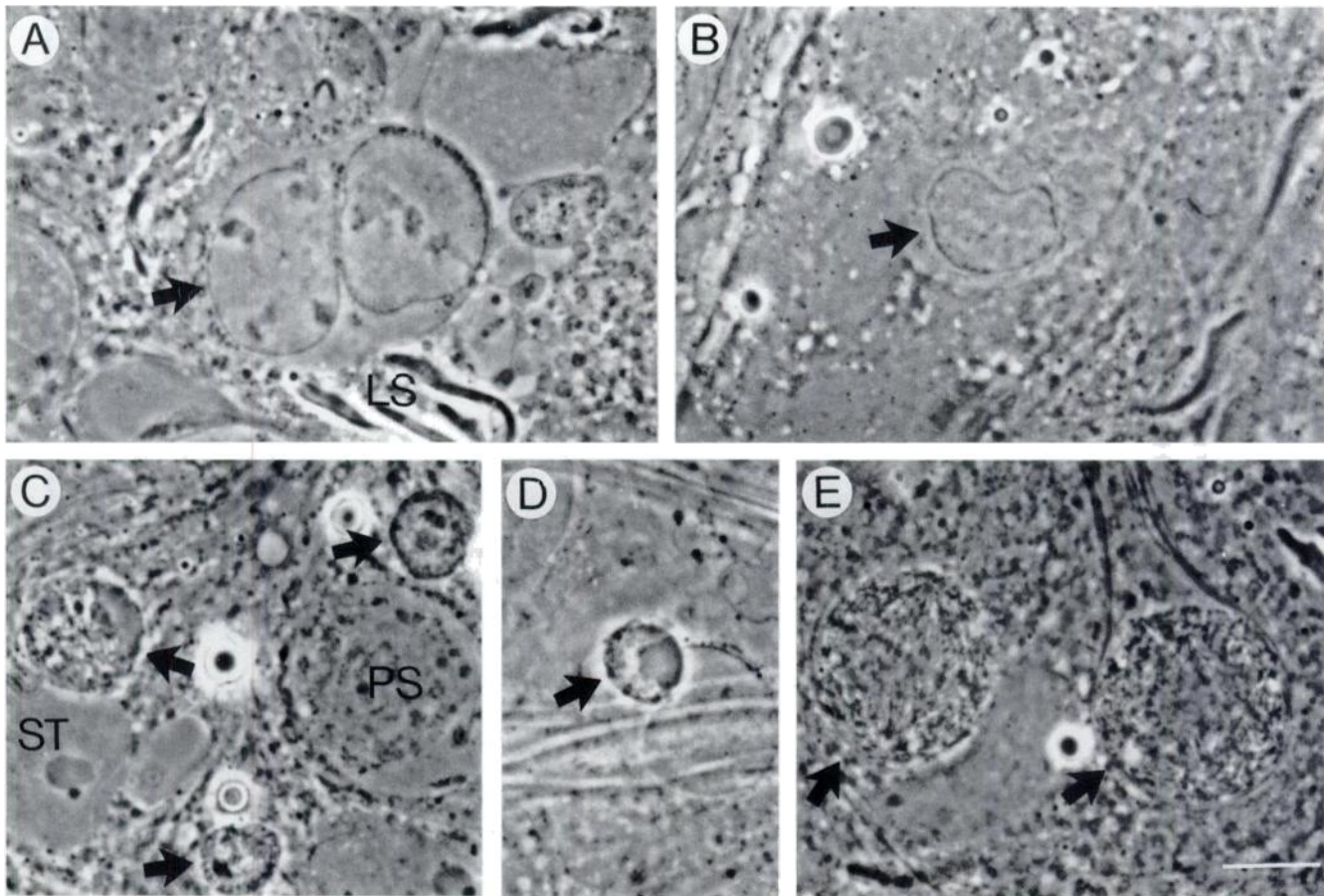


FIG. 4. Living cell preparations showing normal spermatogonia (A, arrow). Apoptotic spermatogonia (42 hours p.i.) showed finely granular chromatin clumping (B, arrow), and became pycnotic (C, D, arrows). LS = late spermatids, ST = Sertoli cell, PS = pachytene spermatocyte. At stage I, degenerating meiotic divisions were seen, with remnants of the meiotic spindle (E, arrows). All figures are from stage I, except D (stage V). Bar = 10 μ m.

step 1 spermatids, meiotically dividing cells delayed from stage XIV, and spermatogonia. At 8 hours p.i. there was an increase of death in spermatogonia and in meiotically dividing cells (Fig. 2H–J). After 16 hours many degenerating step 1 spermatids and spermatogonia were detected. After 42 hours mainly meiotically dividing cells and some early spermatids were degenerating ($P < 0.01$ vs. control, Fig. 2D; Fig. 3). Most of the cells in meiosis had not successfully undergone divisions (Fig. 2E–F). After 66 hours the number of degenerating cells returned to control levels. In control stages II–XI very few degenerating cells were seen (Fig. 2A). After irradiation, spermatogonia (Fig. 2K–N) were the main cells to degenerate in stages II–V (Fig. 2B), but some degenerating round spermatids were also seen. From stage VI a shift occurred, and preleptotene and leptotene spermatocytes were the main degenerating cells detected, together with some round spermatids and spermatogonia. In stage XII, mainly late pachytene spermatocytes, followed by zygotene cells and spermatogonia were seen to be labeled in

control ISEL preparations but degeneration of zygotene spermatocytes increased after irradiation. In stages XIII–XIV, spermatocytes at diakinesis or in division were most frequently labeled in control preparations, also early pachytene and spermatogonial cells were detected. Labeling of spermatogonia increased at all time points after irradiation, and degeneration of meiotic divisions increased particularly at 16–42 hours (Fig. 2G). Normal cells undergoing mitosis and meiosis also show chromatin condensation, but these cells were never observed to be labeled with ISEL (not shown).

Living Cell Preparations

Degenerating spermatogonia in different phases of death were seen in stages I–V 42 hours p.i. Different classes of spermatogonia exhibited a morphologically similar death. Compared with normal spermatogonia (Fig. 4A), they showed nuclear and cytoplasmic shrinkage typical for apoptosis. In an early phase of death, a slight condensation of the nucleus was seen (Fig. 4B), which became

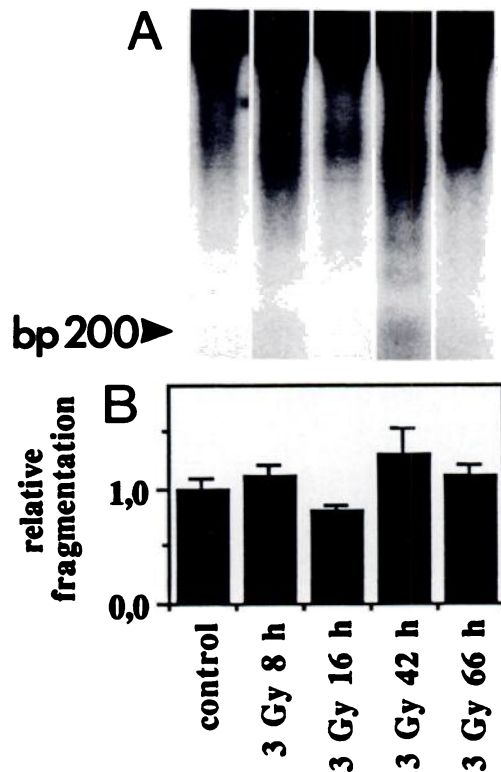


FIG. 5. (A), Autoradiography of ^{32}P -3'-end-labeled DNA from stages II–V electrophoresed on an agarose gel ($n = 3$). Apoptotic DNA fragmentation is most clearly seen at 42 hours. (B), The quantification of DNA <20 kb supported the autoradiographic findings. The bars representing different treatments did not significantly differ from each other.

more prominent in later phases of degeneration (Fig. 4C–D). Cells in a late phase of death also exhibited a bright phase negativens. Degenerating spermatocytes at meiotic divisions (Fig. 4E) were detected at stage I p.i. Some cells, although degenerating, showed remnants of the spindle apparatus.

Autoradiography

DNA isolated from stages II–V showed a nucleosomal-type of cleavage in irradiated testes (Fig. 5A). Quantification of low molecular weight DNA (Fig. 5B) did not show significant differences between p.i. time points.

Electron Microscopy

At 42 hours, most degenerating cells were seen situated near the basal lamina and were often surrounded by Sertoli cell cytoplasm. At stage I, several cells had not accomplished their meiotic divisions, and degenerated (Fig. 6A). They also showed abnormal condensation of the chromatin. Spermatogonia were seen in different phases of death; assumed mid-phase in the process with clumping of chromatin (Fig 6B). Cells in late phases of degeneration were phagocytosed by Sertoli cells (Fig. 6C–D).

Discussion

The present data support the concept that spermatogonia are sensitive to irradiation, but the high rate of death in meiotically dividing primary spermatocytes was unexpected because diakinesis and metaphase I have been suggested to be the most irradiation-resistant spermatocyte phase (Oakberg, 1975). The effect was primarily seen at stage I of the cycle of the seminiferous epithelium, particularly at 42 hours p.i. At the time of irradiation, the cells that subsequently degenerated were in stage XIII (Clermont and Harvey, 1965), suggesting that the diaknetic spermatocytes are very sensitive to irradiation-induced DNA damage. At 66 hours p.i., ISEL of stage II–III squash preparations did not reveal any of these cells, probably due to efficient phagocytosing activity of Sertoli cells. Indeed, in electron microscopic photographs 42 hours p.i., groups of these cells were situated near the basal lamina and Sertoli cells (not shown). The observations support the view that irradiation induces a cell cycle arrest, and the cells pass through stage XIV without dividing, and finally degenerate at stage I. The abnormal nuclear condensation seen in ISEL preparations and electron micrographs suggests that the death could be mediated through apoptosis. The lack of a nuclear membrane and tetraploid genome in meiotically dividing cells might be one reason explaining the atypical morphology in these degenerating cells. Also somatic cells are sensitive and arrest in their cell cycle p.i.; for example HL-60 cells arrest in G2/M and undergo apoptosis (Gorczyca et al, 1993). Some of the spermatocytes in the present study entered meiosis but degenerated during different phases of divisions (Fig. 2G–J). The nuclear condensation in the cells mentioned is in agreement with findings that cells in mitotic divisions can degenerate through an apoptosis-type of mechanism (Milas et al, 1995).

Re-examination of old data, and electron microscopy of irradiated testes, led Allan et al (1987) to conclude that spermatogonia degenerate via apoptosis in the rat. This concept was further confirmed in this study; degenerating cells in living cell squash preparations showed nuclear pycnosis and a bright phase negative appearance compared to normal cells. Such cells are rapidly phagocytosed by Sertoli cells (Parvinen et al, 1993), a feature also seen in electron micrographic pictures in the present study. The compact nuclei in apoptotic spermatogonia could also be demonstrated in ISEL preparations. DNA autoradiography from stages II to V containing sensitive spermatogonia showed nonsignificantly increased low molecular weight DNA labeling 42 hours p.i. In ISEL preparations apoptotic cells can be quantitated at a single cell level and have been suggested to give a more sensitive quantitative estimate of apoptosis than DNA autoradiography

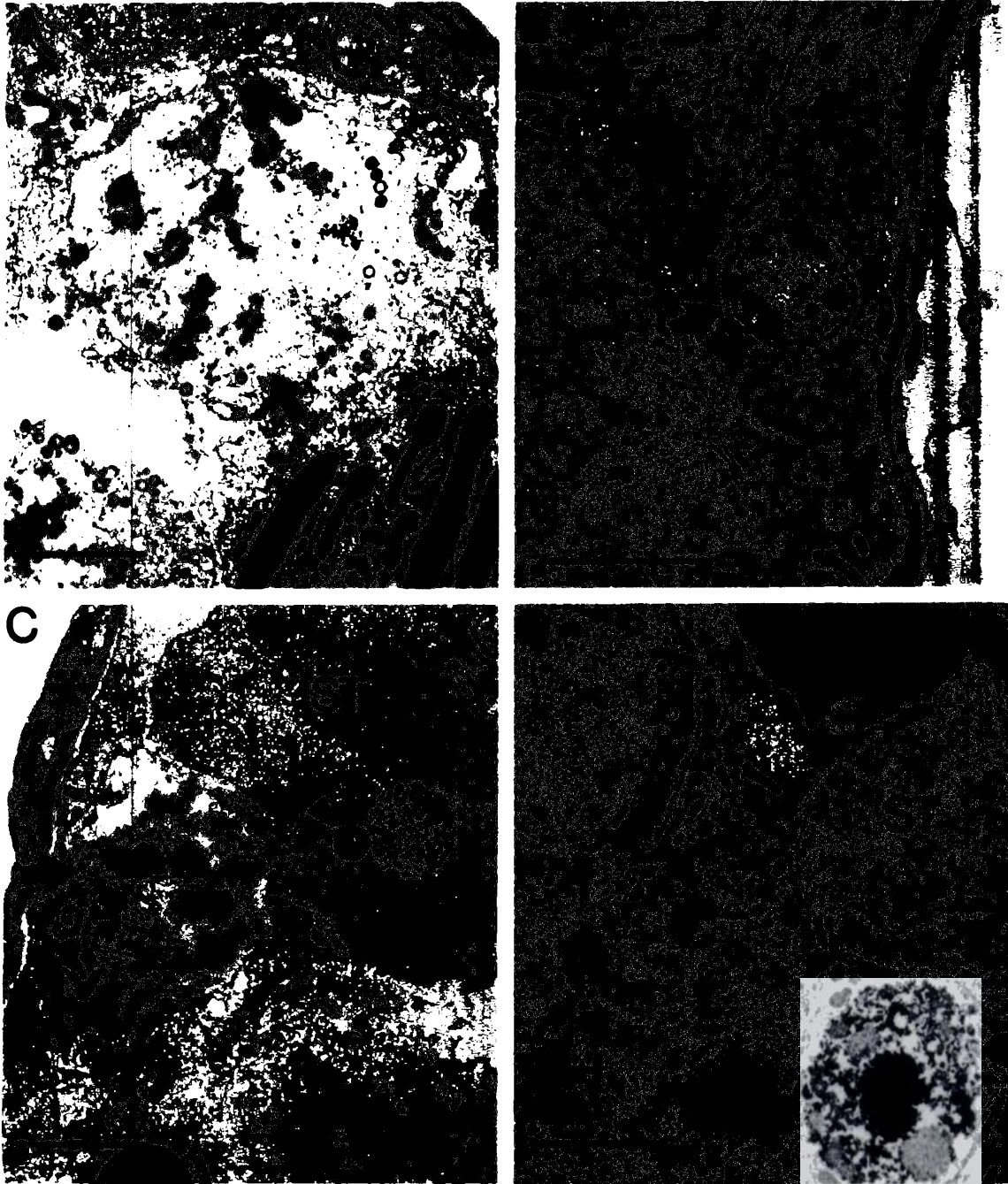


FIG. 6. Electron microscopy of apoptotic cells 42 hours p.i. Most of them were situated near the basal lamina (labeled by an asterisk). At stage I, degenerating spermatocytes were seen (arrow in **A**). In **B–D**, different steps of the apoptotic chromatin condensation are seen (arrows). ST = Sertoli cell, arrowhead = Sertoli cell satellite karyosome. All figures are from stage I, except **B** (stages III–IV). Bars = 5 μ m.

(Henriksen et al, 1996). Rapid phagocytosis of apoptotic cells *in vivo* may also account for the rather small increase in DNA fragmentation after irradiation. Of spermatogonia, types A_3 – A_4 , B, and intermediate were the most radiation-sensitive cells, as expected from the literature. However, the reason for their sensitivity is not known. In stages VII–XII, preleptotene–zygotene primary spermat-

ocytes were the main cells that became degenerated p.i. Some of the stained and quantified cells at stages VII–XI might have been type A_1 – A_2 spermatogonia. Preleptotene spermatocytes have been reported as the most sensitive spermatocytes to degenerate after irradiation (Oakberg, 1975). We also suggest stages VII–VIII preleptotene spermatocytes to be affected because the most pronounced

apoptosis was calculated 42–66 hours p.i. in stages IX–XI. Spermatids and spermatozoa are the most resistant cells to irradiation (Bianchi, 1983), and only a few of them were seen to undergo apoptosis in stages I–VII after irradiation in the present study. In controls, except for stage I, apoptotic round spermatids were rarely detected.

Spermatogenic cells seem to show very different levels of irradiation sensitivity. Spermatogonia were the most sensitive (increase in death 8 hours p.i.), whereas stage XIII spermatocytes were in a degenerative state 16–42 hours p.i. On the other hand, stage XII spermatocytes seem to be more resistant because at 66 hours p.i. stage XII have developed into stage I, where degenerating meiotically dividing cells were at control levels, and the observations do not support them to be deleted earlier either, i.e., in stages XIII–XIV. Even within a cell type the sensitivity may vary depending on the phase of the cell cycle. In spermatogonia, type A and intermediate degenerate during interphase–early prophase and type-B cells during anaphase/early telophase (Bianchi, 1983). It might explain why some stage II–VI squash preparations showed a very large number of apoptotic spermatogonia, and some very few. This was evident particularly in stages IV–V, 42 hours after irradiation. Large variations were noted also in autoradiographic quantitations (Fig. 5B). Further, the progression of the stages must also be taken into account; for example, 66 hours after irradiation some stage II tubules have developed into stage VI tubules, but there are few type B spermatogonia/preleptotene spermatocytes left to become apoptotic if they were earlier deleted as intermediate spermatogonia in stages II–III. The turnover due to rapid phagocytosis by Sertoli cells may also affect the present quantitative data.

There are several factors that makes the comparison with earlier data difficult concerning the effects of irradiation; the dose and the dose rate are certainly influencing the response of the testis. Local irradiation of the testes has the advantage of avoiding systemic effects seen after whole body irradiation, and by the use of high voltage X-rays a relatively homogenous effect is achieved (Kangasniemi et al, 1990b). The fact that the variation between measured and expected dose was below 10% in the whole testis volume is partially due to a backscattering effect from the underlying material.

Both the qualitative and quantitative data, concerning apoptosis in the control testes, are in agreement with earlier findings (Kerr, 1992; Henriksen et al, 1995a,b; Billig et al, 1995; Brinkworth et al, 1995). The reasons for the spontaneous death have been reviewed elsewhere (Sharpe, 1995), but they still remain largely unclear.

Irradiation as well as many radiomimetic cytotoxic drugs induce apoptosis in the testes (Allen et al, 1987), and probably provide a useful model for studying apoptosis and its genetic regulation in germ cells. The genes

and their products involved in spermatogenic apoptosis have not yet been clarified. One possible candidate gene involved in irradiated testes could be p53, a tumor suppressor gene. DNA damage rapidly increases levels of p53 (Kastan et al, 1991) and appears to be an important component of the G1 arrest and apoptosis that follow DNA damage (Kuerbitz et al, 1992). Emerging evidence also favors a role for p53 in the G2/M exit and following apoptosis (Liebermann et al, 1995). For studying apoptosis and its regulation in spermatocytes and spermatids other apoptosis inducers can be employed: gonadotropin releasing hormone treatment (Billig et al, 1995; Sinha-Hikim et al 1995, Brinkworth et al, 1995), ethane dimethane sulfonate administration (Henriksen et al, 1995b), cryptorchidism (Shikone et al, 1994; Henriksen et al, 1995a), 2-methoxyethanol (Ku et al, 1995), or simply *in vitro* conditions (Henriksen et al., 1996). In conclusion, the present results show that irradiation with 3 Gy induces apoptosis in spermatogonia. In addition, preleptotene–leptotene spermatocytes and meiotically dividing cells, with the initial damage at stage XIII, seem to undergo an apoptosis-type of cell death after irradiation.

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References

- Albers-Schönberg HE. Über eine bisher unbekante Wirkung der Roentgenstrahlen auf den Organismus der Tiere. *Münch Med Wochenschr* 1903;50:1859.
- Allan DJ, Harmon BV, Kerr JFR. Cell death in spermatogenesis. In: Potten CS, ed. *Perspectives on Mammalian Cell Death*. London: Oxford University Press; 1987:229–258.
- Bianchi M. Cytotoxic insult to germinal tissue. Part I: the testis. In: Potten CS, Hendry JH, eds. *Cytotoxic Insult to Tissue. Effects on Cell Lineages*. Edinburgh: Churchill Livingstone; 1983:259–307.
- Billig H, Furuta I, Rivier C, Tapanainen J, Parvinen M, Hsueh AJW. Apoptosis in testis germ cells: developmental changes in gonadotropin dependence and localization to selective stages. *Endocrinology* 1995;136:5–12.
- Brinkworth MH, Weinbauer GF, Schlatt S, Nieschlag E. Identification of male germ cells undergoing apoptosis in adult rats. *J Reprod Fertil* 1995;105:25–33.
- Clermont Y, Harvey SC. Duration of the cycle of the seminiferous epithelium in normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology* 1965;76:80–89.
- Clifton DK, Bremner WJ. The effect of testicular X-irradiation on spermatogenesis in man. A comparison with the mouse. *J Androl* 1983;4: 387–392.
- Dym M, Clermont Y. Role of spermatogonia in the repair of the seminiferous epithelium following X-irradiation of the rat testes. *Am J Anat* 1970;128:265–282.
- Ellis LC. Radiation effects. In: Johnson AD, Gomes WR, VanDemark NL, eds. *The Testis*. New York: Academic Press; 1970;3:330–376.
- Gorczyca W, Gong J, Ardel B, Traganos F, Darzynkiewicz Z. The cell

- cycle related differences in susceptibility of HL-60 cells to apoptosis induced by various antitumor agents. *Cancer Res* 1993;53:3186-3192.
- Henriksen K, Hakovirta H, Parvinen M. *In situ* quantification of stage-specific apoptosis in the rat seminiferous epithelium: effects of short-time experimental cryptorchidism. *Int J Androl* 1995a;18:256-262.
- Henriksen K, Hakovirta H, Parvinen M. Testosterone inhibits and induces apoptosis in rat seminiferous tubules in a stage-specific manner: *in situ* quantification in squash preparations after administration of ethane dimethane sulfonate. *Endocrinology* 1995b;136:3285-3291.
- Henriksen K, Kangasniemi M, Parvinen M, Kaipia A, Hakovirta H. *In vitro*, follicle-stimulating hormone prevents apoptosis and stimulates DNA synthesis in the rat seminiferous epithelium in a stage-specific fashion. *Endocrinology* 1996;137:2141-2149.
- Huckins C. The spermatogonial stem cell population in adult rats. III. Evidence for a long-cykling population. *Cell Tissue Kinet* 1971;4:335-349.
- Kangasniemi M, Kaipia A, Mali P, Toppari J, Huhtaniemi I, Parvinen M. Modulation of basal and FSH-dependent cyclic AMP production in the rat seminiferous tubules staged by an improved transillumination technique. *Anat Rec* 1990a;227:62-76.
- Kangasniemi M, Veromaa T, Kulmala J, Kaipia A, Parvinen M, Toppari J. DNA-flow cytometry of defined stages of the rat seminiferous epithelium: effects of 3 Gy of high-energy X-irradiation. *J Androl* 1990b;11:312-317.
- Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 in the cellular response to DNA damage. *Cancer Res* 1991;51:6304-6311.
- Kerr JB. Spontaneous degeneration of germ cells in normal rat testis: assessment of cell types and frequency during the spermatogenic cycle. *J Reprod Fertil* 1992;95:825-830.
- Kerr JFR, Searle J. Apoptosis: its nature and kinetic role. In: Meyn RE, Withers HR, eds. *Radiation Biology in Cancer Research*. New York: Raven Press; 1980:367-384.
- Ku WW, Wine RN, Chae BY, Ghanayem BI, Chapin RE. Spermatocyte toxicity of 2-methoxyethanol (ME) rats and guinea pigs: evidence for the induction of apoptosis. *Toxicol Appl Pharmacol* 1995;134:100-110.
- Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB. Wild-type p53 is a cell cycle checkpoint determination following irradiation. *Proc Natl Acad Sci USA* 1992;89:7491-7495.
- Liebermann DA, Hoffman B, Steinman RA. Molecular controls of growth arrest and apoptosis: p53-dependent and independent pathways. *Oncogene* 1995;11:199-210.
- Milas L, Hunter NR, Kurdoglu B, Mason KA, Meyn RE, Stephens LC, Peters LJ. Kinetics of mitotic arrest and apoptosis in murine mammary and ovarian tumors treated with taxol. *Cancer Chemother Pharmacol* 1995;35:279-303.
- Oakberg EF. Effects of radiation of the testis. In: Hamilton D, Greep RO, eds. *Handbook of Physiology*. Baltimore: Williams & Wilkins; 1975;5:233-245.
- Ogilvy-Stuart AL, Shalet SM. Effect of radiation on the human reproductive system. *Environ Health Perspect* 1993;101(suppl 2):109-116.
- Parvinen M, Toppari J, Lähdetie J. Transillumination-phase contrast microscopic techniques for evaluation of male germ cell toxicity and mutagenicity. In: Chapin RE, Heindel J, eds. *Methods in Reproductive Toxicology*. Orlando, Florida: Academic Press; 1993:142-165.
- Parvinen M, Vanha-Perttula T. Identification and enzyme quantitation of the stages of the rat seminiferous epithelial wave in the rat. *Anat Rec* 1972;174:435-450.
- Sharpe RM. Regulation of spermatogenesis. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*, 2nd ed. New York: Raven Press; 1995:1363-1434.
- Shikone T, Billig H, Hsueh AJW. Experimentally induced cryptorchidism increases apoptosis in rat testis. *Biol Reprod* 1994;51:865-872.
- Sinha-Hikim AP, Wang C, Leung A, Swerdloff RS. Involvement of apoptosis in the induction of germ cell degeneration in adult rats after gonadotropin-releasing hormone antagonist treatment. *Endocrinology* 1995;136:2770-2775.
- Tilly JL, Hsueh AJW. Microscale autoradiographic method for the qualitative and quantitative analysis of apoptotic DNA fragmentation. *J Cell Physiol* 1993;154:519-526.
- van Beek ME, Davids JA, de Rooij DG. Variation in the sensitivity of the mouse spermatogonial stem cell population to fission neutron irradiation during the cycle of the seminiferous epithelium. *Radiat Res* 1986;108:282-295.
- Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 1980;68:251-306.