cus faecalis inhibited the growth of HeLa cells in vitro whereas *Streptococcus mutans* stimulated it[16]. Nevertheless, the extracellular matrix appeared to be disturbed, with fragmentation of collagen fibrils and the presence of a large number of non-striated microfilaments. Similar bundles of fine filaments, closely bound to collagen fibrils, have been described in periodontal diseases[17] or in the pulpal border zone of teeth affected by early caries lesions[5]. They were then considered to be oxytalan fibers, a pre-elastic component. In this investigation, the use of anti-type I collagen antibodies, the specificity of which has been thoroughly established elsewhere[11], clearly demonstrates the collagenous nature of these filaments. In addition, the non-periodical arrangement of peroxidase deposits is different from previous data[11,13], suggesting changes in the molecular order or helicity of collagen. Such splitting of fibrils probably represents the 1st stage of extracellular collagen degradation. Sometimes, this degradative process occurs within the vacuolar apparatus of various cell types such as fibroblasts in vivo or in vitro[18]. In the present experiment, phagocytosis of collagen fibrils was never found, although it exists under physiological conditions[10]. So, the collagen fibrils elaborated by dental pulp cells in culture might have been altered partly by the direct action of *Actinomyces viscosus* and partly by the possible activation of a latent collagenase detected in various explant culture systems[19]. The use of tissue culture as a model, therefore, most closely approximates the in vivo state of the tissue. In this regard, our results lead us to believe that *Actinomyces viscosus* may actively participate in the destruction of the organic matrix of the dentine or the pulp beneath carious lesions.

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Cellular damage and recovery of the early developing mouse eye following low dose irradiation. I. X-rays on day 8 of gestation

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Summary. The eye region of mouse embryos, irradiated with 90 rad X-rays on day 8 of gestation, was examined 24 h later for cellular damage. Besides the overall developmental retardation, the radiation insult decreased the proliferation rate and altered the mitotic phase ratio. Due to the limited extension of necrotic zones, a reduced number of dead cells was found in the irradiated optic vesicles.

The high radiosensitivity of the developing eye has been confirmed in several experimental studies. Recently, Michel and Fritz-Niggli[2] observed an enhanced rate of microphthalmia in mouse fetuses after irradiation of early organogenetic stages with low doses. Concerning the microscopical investigation of the embryonic eye, only insufficient data exist as yet with regard to the cellular changes following low dose-application.

The aim of this study was to examine the influence of X-irradiation on the cellular damage and regeneration capacity of the early eye primordium. Thereby, particular attention was given to the primary biological events (chromosome damage, mitotic delay and cell death) as the possible causes of malformations.

Material and methods. Female virgin NMRI mice, 2.5 months old, were mated overnight with fertile males of the same stock. The day when a vaginal plug was found was designated as day 0 of gestation. On day 8, pregnant mice were divided into 3 groups: 1. unrestrained, 2. sham-irradiated, 3. irradiated. The sham-irradiated group, consisting of animals restrained in a plexiglass phantom during the total dose exposure time, served as control to the irradiated one. The exposed group was whole-body irradiated with a single dose of 90 rad (200 kV, 12 mA, 1 mm Al + 0.5 mm Cu, HVL 0.93 mm Cu, 40 cm target-to-object distance and dose rate 47.5 R/min).

The embryos were examined histologically on day 9 using the following parameters: relative eye size, mitotic activity and phase distribution, anaphase aberrations and cell death. At this developmental stage, the neural tissue consists of a homogenous population of undifferentiated neuroblasts. The 24-h time lapse between irradiation and observation corresponds to 2 division cycles of the cells under study, with a total generation time of 10.5 h[3]. The relative eye size was defined as the number of sections through the eye region multiplied by 5 µm (section thickness). The mitotic index was determined by counts of all mitotic figures and non-proliferating cells in the 1st section of the optic vesicles with continuous stalk lumen. The anaphase aberrations and cell death were recorded by

Histological analysis of the eye primordia 24 h after irradiation of 8-day mouse embryos

	Control Non-restrained	Restrained 1 min 50 sec	Irradiated 90 rad X-rays
Number of eyes examined	24	24	25
Relative eye size (μm ± SEM)	199 ± 5	197 ± 5	164 ± 7*
Proliferation:			
Total cells counted	2607	2402	1660
Mitotic index	187 (7.2%)	162 (6.8%)	91 (5.5%)**
Number of prophases	73 (39%)	54 (33%)	38 (42%)
Number of metaphases	94 (51%)	92 (57%)	34 (37%)*
Number of anaphases	10 (5%)	11 (7%)	11 (12%)
Number of telophases	10 (5%)	5 (3%)	8 (9%)
Chromosome aberrations:			
Total anaphases counted	243	328	193
Anaphases with bridges	24 (10%)	27 (8%)	7 (4%)
Anaphases with fragments	40 (16%)	68 (21%)	47 (24%)
Total aberrant anaphases	64 (26%)	95 (29%)	54 (28%)

* Significantly different from the restrained control group, $p < 0.01$; ** significantly different from the restrained control group, $p < 0.05$.

inspecting every 2nd section of the whole eye region. The data were analyzed statistically with the Wilcoxon-test and the t-test.

Results. The microscopic analysis of embryos revealed a developmental retardation of the central nervous system, including the eyes, in the irradiated group. More than 50% of the exposed forebrains were still open in their most apical section, and the dorso-lateral protrusion of the optic vesicles was absent. Besides these qualitative findings, the decrease of the eye dimension could be confirmed numerically (table). Compared with the restrained control, the difference in size was about 17% ($p < 0.01$). The determination of the mitotic index and phase ratio (table) showed the following differences between irradiated animals and controls: a) the diminution of the eye size was expressed by the decrease of the total cell number; b) the proliferation activity in the irradiated tissue was lowered, the mitotic index (5.5%) being significantly reduced ($p < 0.05$); c) the mitotic phase ratio was altered. Compared with the control, the proportion of prophases, anaphases and telophases was enhanced along with significantly lowered incidence of metaphases (−20%, $p < 0.01$). In respect to the chromosomal damage appearing in anaphases, high total aberration rates were seen in both control groups, and in the exposed one the irradiation did not cause any observable effect on the overall aberration rate. Regarding the frequency of the anaphase bridges and fragments separately, contrary trends have been observed. The incidence of chromatid fragments increased from the nonrestrained to the irradiated group, whereas the incidence of bridges declined. In the exposed eye primordia, the proportion of anaphase bridges was extraordinary low (4%), particularly if compared to the maximum fragmentation rate (24%).

In order to evaluate the extent of cytological radionecrosis, the location and number of dead cells were recorded for all 3 groups investigated. Concerning the location of cellular death, a ventral and a distal pycnotic zone with accumulated dead cells were distinguished. The quantitative information was obtained by arranging the scored degenerated cells into 4 damage categories; class 1: no or sporadic dead cells (0–9 dead cells per section); class 2: small local necrotic sites (10–19 dead cells per section); class 3: extensive necrosis (20–30 dead cells per section); class 4: massive cell death (> 30 dead cells per section). For each class the percentage frequency was ascertained.

In both control groups the quantities of dead cells reached almost identical levels, indicating that the restraining stress (1 min 50 sec) did not produce an increase in cell lethality. Class 1, with an incidence of 59%, represented the most abundant damage category. It is noticeable that the categories with extensive and massive necrosis occurred with a total frequency of about 20%. Interestingly, in the irradiated optic vesicles massive cell death was completely absent and the rate of extensive necrosis (class 3) declined to only 1%.

Discussion. The dose applied in the present study elicited a significant diminution of the eye size along with a delay in the brain development, which may result in severe morphological and functional irregularities in the postnatal period[4]. Furthermore, the suppressive effect of X-rays on cell division was expressed by a significant decrease of the mitotic index. A more precise understanding of the biological events following irradiation results from analysis of the distribution pattern of the mitotic phases. The significantly reduced metaphase frequency in the exposed optic vesicles points to a radiation induced block in the $G_2 \rightarrow$ mitosis transition, as also reported by other authors[5,6]. Moreover, no correlation between the chromosomal aberrations and mitotic delay was established; this agrees with the findings of Scott and Zampetti-Bosseler[5].

In the chain of radiation-induced primary biological processes, the last step is represented by cellular death. Our analysis yielded a remarkable constancy with respect to the degeneration sites both in the control and exposed eyes. Only in the diminished anlagen was the ventral pycnotic zone strongly reduced or absent. The increased proportion of extensive necrosis observed in many control vesicles demonstrates the importance of this process for ocular normogenesis. The failure of massive pycnosis following irradiation is based perhaps on developmental delay, demonstrated by the majority of exposed eye vesicles. Up to now, it is mainly excessive cell death that has been considered to be teratogenic[7,8], but reduced or inhibited necrosis may cause severe pathological changes as well[9]. Fritz-Niggli[10] emphasized that the temporal and spatial coordination of the different developmental processes is essential for normal ontogenesis. This statement is applicable particularly to ocular morphogenesis, which occurs in precise sequences of interacting pycnotic areas[11]. As our study showed, an inhibition of programmed necrosis may lead to a delay in the developmental progress. Hence, in the present investigation the teratogenic effect of irradiation seems to be based more on distorted interactions and decreased proliferation activity of the affected tissue than on chromosomal damage and cellular death.

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