Cellular damage and recovery of the early developing mouse eye following low dose irradiation. II. X-Rays on day 9 of gestation

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Summary. Mouse embryos on day 9 of gestation were exposed in utero to 90 rad X-rays. At different time intervals after treatment the eye primordia were examined for cell death. The irradiation caused an altered necrosis pattern compared with day 8, and massive cell killing during a limited time period. The rapid recovery from the pronounced damage points to a high restitution efficiency of the involved tissue.

In a previous report[2] we mentioned that programmed necrosis in certain areas of exposed optic vesicles after irradiation on day 8 was inhibited. Since cell degeneration is a prerequisite for an unobstructed morphogenesis[3], the disturbance of the normal cell-death distribution may initiate structural abnormalities[4]. However, due to the changing regeneration capacity during the prenatal period the significance of radionecrosis in the pathogenesis of malformation depends largely on the developmental stage[5]. Regarding presumptive ocular tissue, the reconstitution potencies from radiation damage are of importance. The early developing eye consists of neuroblasts which represent the most radiosensitive cell type in the embryo and fetus[6].

The purpose of this study was to gain detailed information concerning a) the dynamics of the early reaction to radiation insult, b) the pattern of cell death, and c) the regeneration powers of the presumptive ocular tissue at day 9, a more advanced stage.

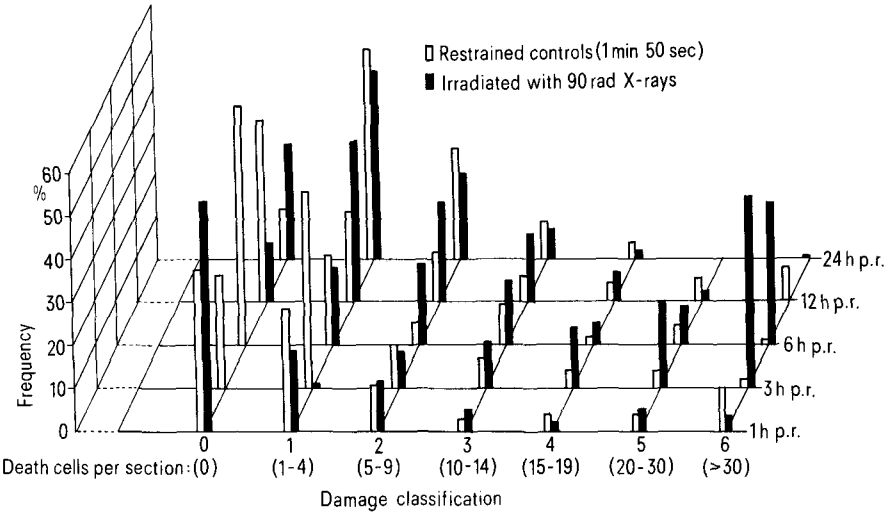
Material and methods. Pregnant NMRI mice were whole-body irradiated with 90 rad X-rays on day 9 post conception. Appropriate groups of fertilized females restrained in the plexiglass phantom during the total dose exposure time ('sham-irradiation') served as controls. The experimental conditions have been described formerly[2].

Embryos were removed 1, 3, 6, 12 and 24 h following irradiation or restraining and the heads sectioned transversally at 5 µm. In about 600 sections (60 sections per group) the total number of dead cells in the eye primordia was determined. The degenerated cells scored were arranged in 7 damage categories (classes 0–6) and the frequency of each class in relation to the investigation interval was plotted (fig.). Information referring to the spatial and temporal distribution of pycnotic loci as well to the regeneration capacity of single eye regions was obtained by the topographical partition of the whole primordium into 4 zones: the ventral, distal and dorsal zone, and the lens placode.

Results. On day 9 of gestation, the analysis of the physiological cell death in control animals yielded an agglomeration tendency of pycnotic cells in the ventral and distal zone of the optic vesicle. On day 10, for the first time, degeneration was observed in the lens cup. The fluctuating proportion of the programmed necrosis in primordia during the different time intervals (fig.) illustrates the intensified destruction of ocular tissue during morphogenetcally active periods. The 1- and 12-h-intervals represent such necrotically 'productive' phases, with extensive and massive cell death (classes 5 and 6) reaching peak incidence of 14%. Altogether, the amount of degeneration can be considered rather modest. The classes 0–2 with over 70% incidence make up the greatest part of the overall damage.

In the irradiated group, the rates of extensive and massive necrosis were of main interest. The maximum values for these high-damage categories were registered 3 and 6 h after exposure. 3 h post irradiation the frequency of massive cell death was more than 20 times higher than that of the control group (co: 2%, 90 rad: 45%) and a great part of the primordial neuroblast-population was destroyed. It is worth noting that even the less sensitive dorsal vesicle zone and lens placode were markedly involved.

Though 6 h following treatment the proportion of massive necrosis was still considerably higher than in restrained animals (co: 2%, 90 rad: 34%), the recovery reaction of the ocular tissues had already set in. While the ventral and distal optic vesicle consisted of predominantly necrotic material, the remaining tissue did not exhibit dead cells any more. Moreover, the appearance of numerous cell fragments discharged into the eye lumen indicated further evidence of the progressing regeneration process. Already 12 h after radiation the incidence of massive cell death declined to the zero level, indicating an almost complete recovery of eye primordia (fig.). One day after exposure the amount of necrosis was not only reduced to sporadic dead



Cell death in the eye anlage of mouse embryos at different time intervals post-irradiation (p.r.). Treatment at day 9 of gestation.

cells but also the eye lumen was entirely free from cellular debris.

Discussion. The present study showed intensified and extended pycnotic zones in optic primordia after exposure on day 9 compared to the suppressed cell death following treatment on day 8[2]. The extreme rise in the radiosensitivity of the more advanced stage may be attributed to the differentiation of the radioresistant neuroectoderm cells (day 8) into highly susceptible neuroblasts. Furthermore, the analysis showed that the recovery process does not comprehend all parts of the eye primordium simultaneously. The areas containing only a slight amount of endogenous cell death during normogenesis (dorsal ventricle, lens placode) apparently regenerate faster than the regions with an intense morphogenetic activity (eye stalk, ventral and distal optic vesicle). Moreover, the decrease in the incidence of extensive and massive necrosis (classes 5 and 6) from 65 to 2% within only 9 h demonstrates the restitution efficiency of the early developing eye. However, in view of the large cellular deficiency the massive destruction of the exposed tissue 3 h p.r. may lead to serious long term effects. It has been emphasized[6–8] that despite the high regenerative potency, cell death induced exogenously always has negative consequences for the embryo. Hence the question arises whether the proliferative capacity of surviving cells will be sufficient to restore conditions corresponding to those of the control groups. One way to approach this problem would be to ascertain the total cell number in affected eyes after the completed differentiation. Finally, besides cell killing one can assume only a sublethal damage of cells (e.g. after irradiation with low doses) which in spite of the absence of necrosis produces developmental disorders[9].

The particular complexity of the necrosis pattern during early mammalian organogenesis[10] implies that further studies are necessary to clarify the still obscure role of cell death in the teratogenic pathways.

1 Supported by the Swiss National Foundation for Scientific Research. The technical assistance of Mrs E. Frei is gratefully acknowledged.

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0014-4754/83/010095-02$1.50 + 0.20/0

Cyclic nucleotides phosphodiesterase activity changes in early chick development

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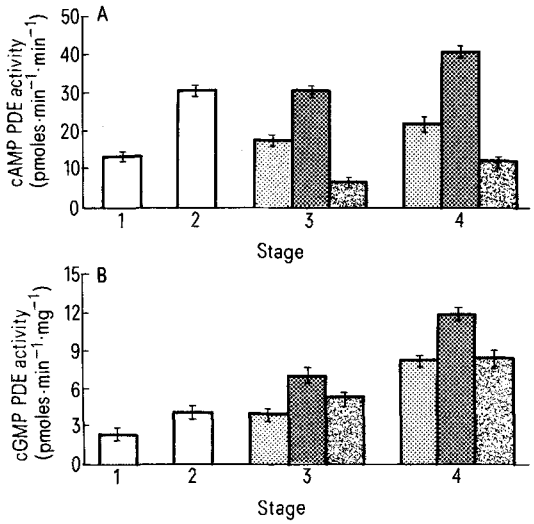
Summary. Differences in the activity of cyclic nucleotides phosphodiesterase develop in different germ layers during the gastrulation of the chick embryo.

In recent years it has been shown that the addition of external cAMP phosphodiesterase (PDE) disturbs the gastrulation process[2] and that reaggregation of chick embryo cells is cAMP-depent[3]. Also, it has been assumed that the direction of the primitive streak elongation in chick embryo depends on cyclic AMP (cAMP)[2,4].

In this paper the results of a study of the changes in cAMP and cyclic GMP (cGMP) phosphodiesterase (EC 3.1.4.17) activities in the different germ layers of the early chick embryo are reported.

Materials and methods. 8-^{3}H-cyclic-AMP, 8-^{3}H-cyclic-GMP were purchased from Amersham, England; cyclic-AMP, cyclic-GMP, 5′-AMP, 5′-GMP from 'Reanal' (Hungary), TLC plates 'Silufol' from 'Kavalier' (Czechoslovakia). Fertile White Leghorn eggs were incubated at 38.5 °C to obtain embryos at stages 1–4 of development[5].

For PDE activity measurements the following areas of embryos were used: at stages 1 and 2 - all area pellucida, at stages 3 and 4 - primitive streak, epiblast and meso- and endoblast together. Embryos were dissected with tungsten needles and separated parts of embryos were rinsed in Dulbecco-Fogt physiological saline, collected in the homogenization buffer containing 40 mM TrisHCl, pH 7.8, 5 mM $MgCl_2$ and homogenized in a Dounce microhomogenizer ('Kontes'). cAMP and cGMP PDE activities were



Changes of cAMP phosphodiesterase (A) and cGMP phosphodiesterase (B) activities (pmoles · min^{-1} · mg of protein^{-1}) during early chick development from stage 1 to 4 (□, area pellucida ▨, primitive streak; ▧, epiblast; ▩, meso- and endoblast). The activities are expressed as means ± SD for 10 separate experiments.