GROWTH AND CONVERSION OF LENTICULAR EPITHELIUM IN IMPLANTS AFTER SUBLETHAL WHOLE-BODY X-RAY IRRADIATION OF THE DONOR

P. V. Dunaev and V. A. Agarkov

UDC 612.6.03 : 612.844.1-06 : 612.014.481

The lenticular epithelium of sexually mature albino rats retains its power of growth and atypical differentiation when homoimplanted by F. M. Lazarenko's method after whole-body x-ray irradiation of the donor in a dose of 800 R. The equatorial and pre-equatorial zones of the epithelium are most radiosensitive.

Previous investigations demonstrated differences in the biological potencies of different parts of the lenticular epithelium in rabbits and rats at the principal stages of their ontogenesis when implanted by Lazarenko's method [1, 2].

The central zone of the epithelium of the growing intact organ, after implantation characteristically forms stratified undifferentiated layers of cells, while the epithelium of the pre-equatorial zone differentiates into multiple lens anlagen, and the equatorial epithelium into lenticular fibers.

The character of growth and differentiation of different parts of the lenticular epithelium when implanted after whole-body x-ray irradiation of rats in a sublethal dose was studied in this investigation with the aim of determining the effects of particular doses of radiation on its biological potency.

EXPERIMENTAL METHOD

Two series of experiments were carried out on adult noninbred male albino rats weighing 110-130 g. In series I the lenticular epithelium of unirradiated animals was implanted by the classical method of Lazarenko [3]. This was the control series.

In series II, the material implanted into intact recipients consisted of the minced lens obtained from donors after whole-body irradiation on the RUM-17 x-ray apparatus under the following conditions: voltage 220 V, current 15 mA, filters 1 mm Al and Cu, skin-focus distance 50 cm, dose rate 100 R/min, total dose 800 R.

The implantation was carried out 24 h after irradiation. The implants were obtained at a strictly definite time of day at stages from 1 to 90 days, fixed in Carnoy's fluid, and embedded in paraffin wax. Serial sections, 5-6 μ thick, were stained with Mayer's hematoxylin and eosin and with Heidenhain's azan; histochemical reactions for DNA, RNA (Feulgen and Brachet) and for neutral and acid mucopolysaccharides (Ritter–Oleson) were accompanied by the appropriate controls.

EXPERIMENTAL RESULTS

The tissue cultures passed through a series of consecutive states: depression, progressive destruction, activation and growth, organogenesis and differentiation, and regression. On the first day the implants were in a state of depression. The implant was permeated with exudative fluid. Cytologically (series II)

Department of Histology and Embryology, Tyumen' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 71, No. 3, pp. 91-94, March, 1971. Original article submitted August 5, 1970.

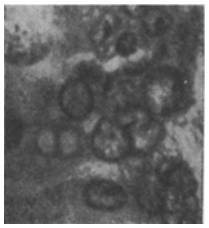

Fig. 1. Implant of lenticular epithelium after whole-body irradiation of donor in a dose of 800 R (stage of 15 days). Hematoxylin-eosin, 630×.

the lenticular epithelium showed a picture of radiation damage to the cells: oxyphilia and vacuolation of the cytoplasm, enlargement of the nucleoli, and a decrease in the mass of chromatin in the nuclei.

The depressive period was replaced at the end of the second day by a period of destructive and progressive changes, in which the destructive changes predominated. Pieces of tissue in the center of the implant showed complete destruction and lysis. At the periphery of the implant the epithelium formed irregularly shaped clusters including many vacuolated cells with pycnotic nuclei, features of karyolysis, and Feulgen-positive granules in their cytoplasm. Atypical mitoses were found occasionally. By the end of the 3rd-4th day, the destructive processes were replaced by progressive changes; the implant was invaded by blood vessels, and an intercelloidin connective tissue was formed. During this period the epithelial cells came into contact with the newly formed connective tissue. Among the connective-tissue cells there were many fusiform fibroblasts with basophilic cytoplasm. Other cells frequently seen were mast cells. These contained large Hale-positive granules in their cytoplasm, not disappearing after treatment of the sections. Mast cells undoubtedly play an important role in the formation of the intercellular ground substance of the intercelloidin connective tissue.

Typical mitoses appeared 6 days after irradiation in the clusters of epithelial cells. As a result of growth, the epithelium formed undifferentiated stratified layers and burrowing bands. Whereas in the control series at this stage the lenticular epithelium differentiated in three directions – stratified protective layers, microorgan structures, and lenticular fibers – this was not observed after irradiation of the donor.

The first microorgan structures appeared from the epithelium of the pre-equatorial zone on the 9th day of the experiment. These structures were smaller than in the controls and showed evidence of radiation damage.

Later, at the stage of 12-15 days, the greatest degree of activation of the surviving epithelial cells was observed. The implant contained the largest number of atypical microorgan structures and lenticular fibers. The writers consider that atypical differentiation can be attributed to the direct action of ionizing radiation on both the nucleus and the cytoplasm of the living cell. The morphological reflection of this action was disappearance of the nucleoli and chromatin in the nucleus (with the appearance of optically empty nuclei) and the appearance of vacuoles in the cytoplasm (Fig. 1). As a result of the direct action on the nuclear apparatus, atypical mitoses appeared, and these also led to incorrect differentiation.

Many glycogen granules accumulated in the cytoplasm of the epithelium in the control implants before differentiation of the epithelial cells, and these were subsequently utilized as energy-yielding and building material. In the irradiated animals, no accumulation of glycogen in the cytoplasm of the epithelial cells took place (Fig. 2).

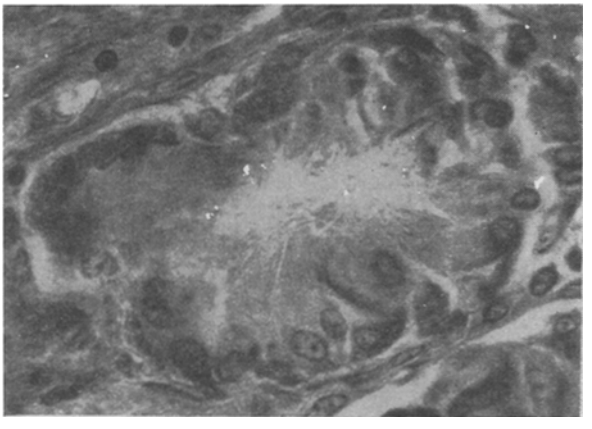

Fig. 2. Implant of lenticular epithelium after whole-body irradiation of donor in a dose of 800 R (stage of 15 days). Ritter–Oleson, 630×.

Fig. 3. Implant of lenticular epithelium after whole-body irradiation of donor in a dose of 800 R (stage of 45 days). Hematoxylin-eosin, 420×.

The much weaker leukocytic response of the recipient to the newly formed lenticular fibers of the irradiated donors compared with that in the control series of experiments was noteworthy.

By the 18th day after operation, the newly formed epithelial structures in the control implants were undergoing destruction. At this stage in the implants of the irradiated lenses, the epithelial cells remained viable. Numerous mitoses were seen. However, the characteristic feature of burrowing growth with the formation of undifferentiated epithelial bands in the intercelloidin connective-tissue layers of the implant was not found. In subsequent stages the epithelium differentiated with the formation of atypical microorgan structures. No signs of radiation damage could be seen in the newly formed organ structures at this stage. This indicates that cells of the implanted lens which have suffered primary radiation damage die. Only those cells which were more resistant to the dose of x-rays given remained capable of progressive changes.

Until 45 days, the epithelium in the implants of irradiated lenses remained viable. A large proportion of the epithelial cells did not undergo organ-specific differentiation. Only a small proportion of cells differentiated into atypical lenticular fibers (Fig. 3).

In both the experimental and control series no significant changes took place in the lens capsule before the stage of 90 days, and it constantly gave a strongly positive PAS-reaction.

Analysis of these results shows that the lenticular epithelium remains capable of growth and differentiation after irradiation in a dose of 800 R and implantation. Differentiation of newly formed epithelial structures in the implants of irradiated lenses is delayed and occurs later than in the control because of the extensive destruction of the implanted tissue. Atypical differentiation can be attributed to the direct action of x-rays on both nuclear and cytoplasmic components of the cell. The synthesis of glycogen, required for the formation of biologically active compounds, is disturbed during organ-specific differentiation. The equatorial and pre-equatorial zones of the lenticular epithelium are most sensitive to x-rays.

LITERATURE CITED

1. V. A. Agarkov, in: Morphogenesis and Regeneration [in Russian], Tyumen' (1970), p. 75.
2. P. V. Dunaev, in: Problems in Theoretical and Practical Medicine [in Russian], Tyumen' (1964), p. 19.
3. F. M. Lazarenko, Principles Governing Growth and Conversions of Tissues and Organs during Cultivation (Implantation) in Vivo [in Russian], Moscow (1959).