THE REACTION OF THE CORNEAL EPITHELIUM TO LOCAL IRRADIATION WITH VARIOUS DOSES OF SOFT X-RAYS

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Of all the vital processes, mitosis is the most radiosensitive [1, 3, 5, 9]. With an increase in the dose of irradiation, the more radioresistant cytoplasmic processes, namely those responsible for the immediate viability of the cell, also begin to be affected, and this may cause cell destruction.

In the present paper we analyze the changes taking place in the corneal epithelium of an albino mouse after irradiation with soft x-rays.

EXPERIMENTAL METHOD

The corneas of albino mice were irradiated locally by means of a Dermamobil apparatus (20 kV, focus distance 10 cm, filter 0.1 mm Al) in doses of 100 r (dose rate 296 r/min) 700 r (296 r/min), and 2000 r (800 r/min). The hardness of the radiation was so selected that it was completely absorbed in the cornea. The irradiated mice were sacrificed by decapitation at the same time as control mice, at 10 A. M. on the 1st, 3rd, 5th, 7th, and 9th days after irradiation (at each time 10 experimental and 5 control mice were sacrificed). The material was fixed in Bouin's fluid and total preparations of the cornea were obtained. These were stained with Weigert's hematoxylin and the number of cells in a field of vision of the microscope and the number of normal and pathological mitoses in 100 fields of vision were counted with a magnification of: eye-piece 7×, objective 60 × 2.5. The mean value of the counts in two corneas was calculated. For all the animals we calculated the index of normal and pathological mitoses. The mean value for the group of animals sacrificed at any one time was used in the mathematical analysis of our results, which was along the lines previously followed in our analysis of cell injury due to radiation [2]. The mean error of the numerical results cited below was ±15%.

EXPERIMENTAL RESULTS

One field of vision of the microscope (magnification: eye-piece, 7×; objective, 60 × 2.5) contained on the average 196 cells of the cornea of the unirradiated animals. The mean mitotic index during the morning, at a time of maximal mitotic activity, was 8.3%. It is clear from Figs. 1, 2, and 3 that ionizing radiation in the doses investigated suppressed mitotic activity and led to pathological mitoses, in the form of chromosome bridges, splitting of chromosomes, irregular division of chromosomes, etc., and to the appearance of giant, multinuclear cells; the number of cells per unit area of the cornea was reduced.

All three doses of irradiation (see Fig. 1) caused complete suppression of mitotic activity immediately after irradiation; the differences were in the speed and character of the recovery process. The fate of the cells injured by ionizing radiation could be judged from the number of pathological mitoses and the number of cells in a field of vision, and their comparison with the number of normal mitoses, from which the intensity of cell renewal can be estimated. The curves showing the changes in the number of pathological mitoses are shown in Fig. 2. Each consists of an ascending and descending part, and the peak value rose as the dose of irradiation was increased. Comparison of the ascending gradients of the curves of the changes in the numbers of normal and pathological mitoses (see Figs. 1 and 2) showed that the probability of cells with normal and abnormal mitotic apparatuses starting to undergo mitosis was approximately the same. This means that delay in the onset of mitosis in an injured cell was due not to a disturbance of the mitotic apparatus, but to a reversible inhibition of synthesis of products essential for division.

The degree of injury to the mitotic apparatus of the cell determined the outcome of the pathological mitosis, and could be estimated from the analysis of the gradient of the curve showing the decrease in the index of pathological

mitosis. It may be concluded from a comparison of the curves illustrated in Figs. 1 and 2 that after irradiation in a dose of 100 r the number of chromosomal aberrations decreased at a rate corresponding to the intensity of cell renewal. Hence it follows that the damage to the mitotic apparatus was irreversible, and the cells entering into pathological mitosis did not divide but formed giant, multinuclear cells. The rate of decrease of the number of pathological mitoses after irradiation in doses of 700 and 2000 r was greatly in excess of the intensity of physiological regeneration. Two hypotheses may be put forward to explain this phenomenon. The simpler of the two is to suggest that irradiation in doses of 700 and 2000 r caused such serious injury to the mitotic apparatus of the cell that it died when entering into pathological mitosis. However, grounds also exist for the suggestion that at the time of a sharp decrease in the number of pathological mitoses, cells with injury to their mitotic apparatus grow old and die naturally. To explain the observed changes in the number of pathological mitoses we must postulate that the mean potential life span of the corneal epithelial cell is approximately 7 days. We shall give below the arguments in support of the second hypothesis.

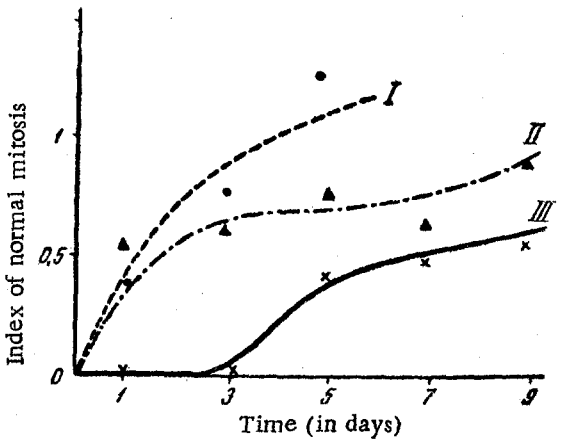

Fig. 1. Change in the number of normal mitoses after irradiation. I) Irradiation in a dose of 100 r; II) in a dose of 700 r; III) in a dose of 2000 r.

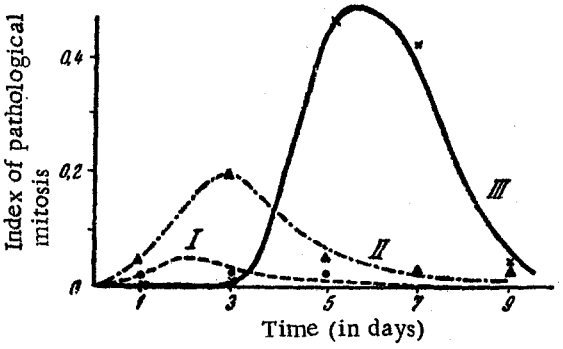

Fig. 2. Change in the number of pathological mitoses after irradiation. I) Irradiation in a dose of 100 r; II) in a dose of 700 r; III) in a dose of 2000 r.

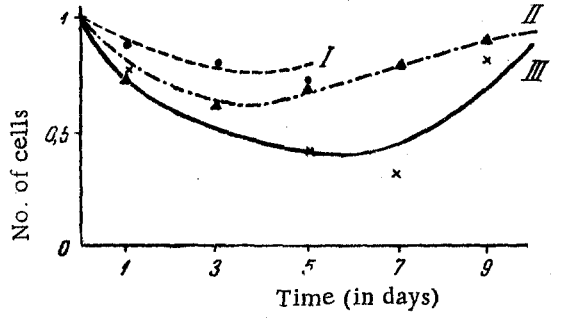

Fig. 3. Change in the number of cells per field of vision after irradiation. I) Irradiation in a dose of 100 r; II) in a dose of 700 r; III) in a dose of 2000 r.

Our analysis of the radiation injury to the tissue would be incomplete if we did not examine the question of death of the cell in the interphase stage. By determining experimentally the rate at which cells appear in the tissue (as a result of normal mitoses) and the change in the number of cells in unit volume of tissue (from the change in the number of cells in a field of vision of the microscope, Fig. 3), we may determine the intensity of the loss of cells from the cornea.

If we accept that the duration of mitosis in the corneal epithelium is 30 min, and the amplitude of the diurnal variations in the number of mitoses is approximately 1.5-2 [4, 6, 8], then the constant of renewal of the cornea of the albino mouse, according to Leblond and Walker [7], will be 0.3 per diem (time of renewal 3.4 days). In unirradiated tissue, the intensity of removal of cells from the tissue is equal to the intensity of renewal. After irradiation in doses of 100 and 700 r, on account of the sharp decrease in the number of normal mitoses, the rate of removal of cells from the tissue falls to 0.15 per diem. As the mitotic activity approaches normal, the intensity of removal of cells from the tissue approaches the value of 0.3 per diem characteristic of unirradiated tissue. These facts suggest that viable cells are being ousted from the unirradiated corneal epithelium. The mean potential life span of the corneal epithelial cell of the albino mouse is approximately 6.7 days. This period is almost twice the time of renewal. This factor must evidently increase the resistance of the tissue to external influences.

We observed a different situation after irradiation in a dose of 2000 r. Despite the total suppression of mitotic activity on the 1st-3rd day after irradiation (see Figs. 1 and 2), the intensity of removal of the cells from the tissue on the first day was 0.32, and it fell to a level corresponding to natural death only on the 3rd day after irradiation. This means that for 24 h after irradiation of the cornea in a dose of 2000 r intensive destructive processes took place in the corneal cells in a stage of interphase.

Hence, the corneal cells may be detached as a result of one of the following processes: 1) Ousting of cells from the tissue by newly formed cells as a result of division; 2) death of cells as a result of natural aging; 3) radiation destruction of cells. In unirradiated tissue the first process is most intensive, and it determines the rate of removal of cells from the cornea, overshadowing the role of death of the cells from natural aging. After irradiation of the tissue, however, when the mitotic activity falls sharply, the rate of removal of cells from the tissue is determined by the intensity of natural dying. As the dose of irradiation is increased, the effect of direct radiation destruction of the cells is brought into play.

SUMMARY

An inquiry was made into the changes occurring in the corneal epithelium of albino mice following soft x-irradiation in doses of 100, 700 and 2,000 r. These x-ray doses depress the mitotic activity, cause the appearance of pathological mitosis and reduction of the number of cells per unit of corneal area. The affection of the mitotic apparatus, underlying pathological mitosis, blocks irreversibly the passing of the cell through mitotic stages. Of the doses investigated only 2,000 r caused a direct radiation destruction of the cell.

LITERATURE CITED

1. E. Ya. Graevskii and I. M. Shapiro, Uspekhi Sovr. Biol. 17, 2, 185 (1959).
2. A. D. Strzhizhovskii, Radiobiologiya 1, 1, 104 (1961).
3. I. A. Utkin, Vopr. Onkol. 1, 4, 3 (1955).
4. Ya. L. Shekhtman, Izvest. Akad. Nauk SSSR, Seriya Biol. 2, 172 (1959).
5. E. P. Cronkite, Radiobiology at the intra-cellular level (London, 1959).
6. N. P. Knowlton and W. R. Winder, Cancer Res. 1950, v. 10, N 1, p. 59.
7. C. P. Leblond and B. E. Walker, Physiol. Rev. 1956, v. 36, N. 2, p. 255.
8. C. C. Lushbaugh, J. Histochen. a. Cytochen. 1956, v. 4, N. 6, p. 499.
9. R. H. Mole, Brit. J. Radiol. 1959, v. 32, N 380, p. 437.

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