DNA CONTENT OF THE CORNEAL EPITHELIAL CELLS OF RATS AFTER X-IRRADIATION

(UDC 617.7-001.29-07:612.713-018.7-008.939.6)

M. N. Veselkina

Laboratory of Cell Morphology, Institute of Cytology, USSR Academy of Sciences, Leningrad

(Presented by Academician N. A. Kraevskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 5, pp. 95-99, May, 1965
Original article submitted February 22, 1964

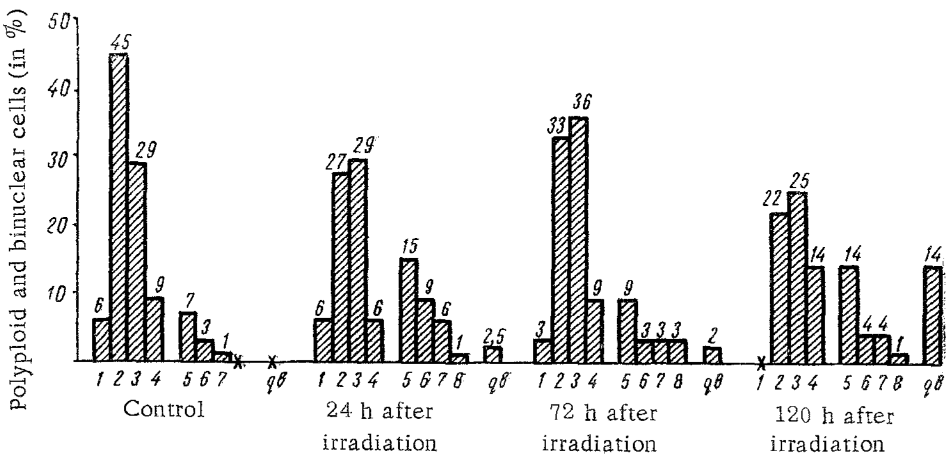
It is known that after x-ray irradiation mitoses disappear from the corneal epithelium as from other tissues [1, 3, 6, 7, etc]. When mitotic activity gradually begins to be restored atypical mitoses appear, chromosome aberrations arise and nuclear polymorphism rises markedly. In this connection, together with cells which contain large nuclei there appear cells composed of one large nucleus and one or more small satellite-nuclei, and the percentage of binuclear cells rises markedly. At various periods after irradiation multipolar mitoses arise. There are a number of reports in the literature [8, 11] which concern the appearance of polyploid cells after irradiation.

In this article the amount of DNA in the different nuclei of corneal epithelium was measured after x-ray irradiation.

METHODS

Investigations were performed on 8 rats of 120-140 g in weight. Six of these were totally irradiated in a RUM-2 apparatus; the total dose was 700 R. The rats were decapitated at 24, 72, and 120 h after irradiation. The corneas were fixed in Carnoy's fluid, embedded in paraffin and sections five microns thick were stained by the Feulgen method. The amount of DNA in spermatid nuclei of control animals served as the standard for determining the degree of ploidy of the epithelial nuclei.

The investigations were performed by the photographic method. Individual nuclei were photographed on discs in the ultraviolet MUF-6 microscope with a 9-fold reduction in the green line of the spectrum at a wave length of



Percentage of polyploid and binuclear cells in the normal state and after irradiation with 700 R (at different times).

TABLE 1. Nuclear Volume and Ploidy in the Normal State and After Irradiation

Period after irradiation In h	2 n	4 n	7 n
Control	2 800±420	6 375±685	7 000
24	2 300±280	4 255±1200	7 355±260
72	2 250±800	3 885±1250	9 970±610
120	2 330±600	3 420±700	7 880±2785

TABLE 2. Ratio of Volumes (V) and Ploidy in Nuclei and their Satellites

Nucleus		Satellite	
V (in mm^3)	ploidy (in n)	V (in mm^3)	ploidy (in n)
2 704	2,5	67,1	0,04
2 450	2,0	99,2	0,35
1 127	1,25	121,2	0,13

546 millimicrons (ob. 90 × 1.25 oc. ×). Photographs of the nuclei were scanned photometrically in a MF-4 microphotometer. Calculation of the optical density (D) was made according to the curve obtained using the recording equipment of the apparatus. The quantity of DNA in the total volume of the nucleus (V) was calculated as proportional to the product $(r_1 \times r_2)^2$, where r_1 and r_2 are the radii of the nucleus ($r_1 > r_2$). The radii of nuclei in millimeters were measured by pictures of the nucleus obtained from the negatives with a photomagnifier and magnification 15 times.

RESULTS

Preliminary study of whole mounts [2] established that the maximum number of chromosomal aberrations (47%) was observed 24 h after irradiation, while at 120 h the number of binuclear cells had markedly increased. On the basis of these data the times were selected for the cytophotometric measurement of DNA in the nuclei.

A total of 410 nuclei (98 control cells; 89 measured at 24, 64-72, 77-120 h after irradiation; 3 nuclei with satellite-nuclei and 80 spermatids). In this number were included 13 epithelial nuclei which contained an amount of DNA which corresponded to less than 2n. These, evidently, were incomplete, broken at the time of preparation of the mounts. The distribution of nuclei according to the amount of DNA they contained is depicted in the figure. Out of 98 epithelial nuclei from control corneas, six contain less than 2n DNA, 44–2n; 28 nuclei–3n; 9 nuclei–4n. The remaining 11 nuclei contain 5-7n DNA. Starting from this figure it may be considered that in normal conditions about 10% of nuclei are polyploid, since 2n and 4n reflect change in the quantity of DNA in the normal nuclear cycle. At 24 h after irradiation the number of nuclei which contain 5n-7n DNA equals 27, which is 30% of the total number of measured nuclei. The percent of polyploid nuclei remains increased in comparison to the control and in the subsequent periods after irradiation as well: at 72 h–18%, at 120 h–23% (see figure). Polyploid nuclei are found both in the basal and in the surface layer. At 24 h the number of binuclear cells (normally single) increases and reaches a maximum at 120 h. In the majority of cases the nucelar size is proportional to the DNA content (Table 1), which is in accord with the data in the literature [10] but nuclei may be encountered of large size with a small DNA

TABLE 3. Ratio of Size and DNA Content (in Units of Ploidy) in the Nuclei of Binuclear Cells

Nucleus "a"		Nucleus "b"		Nucleus "a"		Nucleus "b"	
V (in mm^3)	ploidy (in n)	V (in mm^3)	ploidy (in n)	V (in mm^3)	ploidy (in n)	V (in mm^3)	ploidy (in n)
1 150	1	945	1	3 136	2	2 912	2
1 470	1	1 848	1,5	1 296	2	1 296	2
1 008	1	1 875	2	2 304	2	2 788	2,5
1 296	1	1 680	2	3 885	2,5	3 780	2,5
1 408	1	2 808	2,5	8 208	3	11 040	5
704	1,5	736	1,5	4 410	3,5	4 590	3,5
1 215	1,5	1 980	2	4 116	3,5	5 795	3,5

content (7200 –3n) and the opposite (2788-5n). Therefore, the increase in number of large nuclei (see figure) after irradiation may be considered as the increase in number of polyploid cells.

In addition to large nuclei photometry was made of 3 nuclei with satellites (Table 2).

As seen from Table 2, satellite-nuclei contain a negligible amount of DNA and, evidently, are formed from the single chromosomes or parts of them (bridges) during atypical mitoses.

Binuclear cells were also subjected to special study; these are rarely encountered in the normal state but they increased markedly in number after irradiation. Only 14 binuclear cells were subjected to photometry—those which fell completely within the section. The size of their nuclei correspond to the DNA content. The ploidy of nuclei from binuclear cells is varied and extremely different combinations are encountered. The smallest is 1n + 1n, and the greatest 3n + 5n. The ratio of nuclear size and DNA content in each nucleus of binuclear cells is presented in Table 3.

The existence of binuclear cells with nuclei of different degrees of ploidy indicates the asynchrony of DNA synthesis occurring within the cells. This is confirmed by the work [4] in which binuclear cells are studied after administration of C^{14}-adenine. In this experiment among the binuclear cells are seen cells in which one nucleus is labelled and the other not, which confirms the asynchrony of DNA synthesis therein.

The existence of binuclear cells with ploidy (1n + 1n) is observed in the dorsal epidermis of the rat [10], where a large number of instances have been noted when each of the 2 nuclei of the binuclear cell contains almost exactly half of the normal quantity of DNA. It is true, the author feels, that the formation of a binuclear cell occurs at the expense of amitosis and there are no data concerning changes in DNA with amitosis.

We are inclined to suggest that the formation of binuclear cells occurs as a result of the disruption of the mitotic process after irradiation. Several other authors [5] also hold this point of view.

SUMMARY

In a period of 24-120 h after the irradiation of rats with x-rays in a dose of 700R there is a marked nuclear polymorphism. In the corneal epithelium there appear cells with satellite nuclei and a larger number of binuclear cells. The results of investigations carried out by means of cytospectrophotometry showed that after irradiation the number of polyploid nuclei containing DNA in quantities of 5-8 respectively grows 30% whereas normally it is only 10%. The satellite nuclei contain an insiginficant amount of DNA, which points to their formation from chromosome fragments during atypical karyokinesis. The amount of DNA in the nuclei of binuclear cells varies extensively; the minimum quantity being 1n + 1n, the maximum we have noted being 3n + 5n.

LITERATURE CITED

1. I. B. Bychkovskaya, Concerning the biological action of x-rays in small doses [in Russian], Diss. kand., Leningrad (1953).
2. M. N. Veselkina, Tsitologiya, No. 5 (1962), p. 571.
3. G. P. Gruzdev, Radiobiologiya, No. 1 (1961), p. 52; No. 3, p. 407.
4. L. N. Zhinkin, V. Ya. Brodskii, and G. S. Lebedeva, Tsitologiya, No. 5 (1961), p. 514.
5. L. Ya. Zhorno and L. P. Ovchinnikova, Dokl. AN SSSR, 144, No. 4 (1962), p. 907.
6. G. S. Strelin, Vestn. rentgenol., Nos. 1-2 (1934), p. 98.
7. Idem, Dokl. AN SSSR, Novaya seriya, 73, No. 6 (1950), p. 1283.
8. Idem, Med. radiol., 2, p. 77 (1960).
9. K. W. Cleland, Nature, 191 (1961), p. 504.
10. J. Fautrez, E. Pisi, and G. Cavalli, Ibid., 176 (1955), p. 311.
11. G. Politzer, Pathologie der Mitose. Berlin (1934).