INCORPORATION OF H^3-THYMIDINE IN CELLS OF THE STRATIFIED SQUAMOUS EPITHELIUM OF RATS AFTER X-RAY IRRADIATION

(UDC 612.015.33:612.312.3.014.1]-06:612.014.481.1-08)

L. Ya. Zhorno

Laboratory of Cell Morphology, Institute of Cytology, USSR Academy of Sciences, Leningrad
(Presented by Academician N. A. Kraevskii)
Translated from Byulleten' Éksperimental'noi Bioligii i Meditsiny, Vol. 59, No. 5,
pp. 99-103, May, 1965
Original article submitted February 22, 1964

A detailed study of DNA synthesis in irradiated tissue has been carried out by the method of autoradiography using tritiated thymidine on small bowel epithelium [4, 6-8] and a number of other tissues [1, 2, 5]. These investigations have established the dissimilar effect of radiation on different periods in the mitotic cycle.

The present paper is a study of DNA synthesis in the nuclei of various cells of the stratified squamous epithelium of the rat tongue during the depression of mitotic activity produced by x-irradiation. In addition, the morphologic changes in the epithelium at different periods after irradiation were studied and a connection was established between binuclear elements and cells which were undergoing mitosis.

METHODS

The experiments were performed on 34 white rats of weight 150-180 g. The experimental animals were subjected to total single doses of x-ray of 700 R from a RUM-3 apparatus. Tritiated thymidine (specific activity 4.5 curies/Mmole) was injected subcutaneously into all animals in a dosage of 0.5 microcurie per gram of weight. The experimental rats received the isotope at different periods after irradiation: at 1, 24, 48, 96, and 240 h. Animals—both controls and experimentals—were killed at 4, 24, and 48 h after injection with tritiated thymidine. To obtain autographs on paraffin sections stained with Feulgen reagent, a liquid emulsion of type R (NIKFI) was used. The exposure period for the autographs was 15 days. In parallel with this, preparations were made that were stained with hemalum and iron hematoxylin. In the autographs the percent of labelled nuclei in the epithelial cells and the intensity of the labelling are determined. In addition, we calculated the number of layers of epithelium that contain labelled nuclei at the different times after thymidine administration.

RESULTS

In control animals at 4 h after injection of tritiated thymidine (Table 1) the greatest number of nuclei which contained the label were encountered in cells of the basal layer—14.9% (Fig. 1a, d). In the stratum malpighii where mitoses are observed very rarely, only isolated, single-nucleated cells appeared labelled (0.7%). No significant difference was detected between the intensity of labelling nuclei from cells of the basal and malpighian layers.

At 24 h after isotope injection the percentage of labelled nuclei in cells of the malpighian layer rose to 10.5. Not only uninuclear but also binuclear malpighian cells, which are mainly distributed in the layer lying adjacent to the basal layer, were labelled (Fig. 1b). The average intensity of labelling in the basal and malpighian cells was almost the same and was slightly less than at 4 h after injection of tritiated thymidine. Such an increase in the number of labelled nuclei of the malpighian layer at 24 h after isotope injections is related to the transport of cells which had previously incorporated tritiated thymidine from the upper strata of the basal layer after their next division.

At 48 h after isotope injection (Fig. 1c) the number of malpighian cells containing incorporated tritiated thymidine is still more markedly elevated in comparison to the preceding periods of investigation (see Table I).

In irradiated animals the most marked alteration in DNA synthesis in epithelial nuclei, as shown in Table 1, is noted when the isotope is injected at 1 and 96 h after irradiation. When the isotope is given at 1 h after irradiation

TABLE 1. Incorporation of H^3-Thymidine into Nuclei of the Stratified Epithelial Cells of the Tongue at Different Times after Irradiation and Isotope Administration

Time of administration of H^3-thymidine after irradiation (in hours)	Time of fixation of material after H^3-thymidine irradiation (h)	Basal layer		Malpighian layer		No. of epithial layers containing labelled nuclei
		labelled nuclei (in %)	Aver. intensity of labelling	labelled nuclei (in %)	Aver. intensity of labelling	
Control	4	14,9	15,9	0.7	14,0	2
1	4	4,5	14,0	0,5	19,0	2
24	4	11,6	9,0	0,8	11,0	2
48	4	8,4	15.9	0,7	20,7	2
96	4	24,3	21,2	0,5	15,7	2
240	4	7,5	17,1	0,5	16,7	2
Control	24	12,5	9,2	10,5	10,2	2
1	24	3,3	10,6	0,7	10,8	2
24	24	22,8	7,1	11,0	12,2	3
49	24	4,6	8,4	0,4	8,0	2
96	24	38,5	8,1	19,3	14,0	5
240	24	6,6	10,3	3,1	8,5	2
Control	48	10.8	9,7	23,8	11,2	5
1	48	0,7	7,0	3,8	8,7	5
24	48	8,1	10,6	4,8	12,8	3
48	48	9,0	10,1	7,0	14,5	4
96	48	18,6	9,8	33,8	14,0	6

TABLE 2. Normal and Atypical Mitoses, Binuclear Cells and Cells with Atypical Nuclei in Stratified Epithelium of the Tongue in Rats after Irradiation (in %)

Time after irradiation (in hours)	Basal layer			Malpighian layer		
	normal mitoses	atypical mitoses	cells with atypical nuclei	binuclear cells with normal nuclei	binuclear cells with atypical nuclei	uninuclear cells with atypical nuclei
Control	1,6	—	—	17,3	—	—
0,25	1,1	—	—	9,2	—	—
0,5	0,5	—	—	24,6	—	—
1	0,2	—	—	41,6	—	—
2	—	—	—	19,4	—	—
5	—	—	—	45,0	—	—
24	1,4	—	—	12,8	—	—
48	0,8	0,7	4,0	11,6	22,8	2,1
75	0,7	0.3	7,9	7,9	21,2	9,7
96	0,3	0,1	11,7	7,9	27,0	7,6
144	1,8	0,6	0,1	12,1	1,0	0,2
240	1,5	—	—	17,0	—	—

there is observed (mainly) a sharp fall in the percentage of labelled nuclei. In addition, the mean intensity of nuclear labelling in most instances remains almost the same as in control animals. When the isotope is administered at 96 h after irradiation, a sharp rise in the percentage of labelled nuclei (Fig. 2) in the epithelium of experimental animals killed at different periods after receiving the isotope is noted, but in some cases the intensity of labelling both in the basal and malpighian layers, and even the number of labelled nuclei is noted to rise. More intense labelling, evidently, is related to the rate of DNA synthesis and an increase in the number of labelled nuclei—to the shortening of the G_1 period. At 240 h after irradiation a decrease in the percentage of labelled nuclei is again noted.

Changes in the mitotic index in the epithelium were studied at 15 and 30 min., 1, 2, 5, 24, 48, 71, 96, 144, and 240 h after irradiation. A fall in mitotic activity in the epithelium was detected already at 30 min after irradiation. At 3 and 5 h mitoses were completely absent. At 24 h the mitotic activity had been restored almost to normal, but then fell again and finally, at 240 h once more reached the initial level (Table 2).

The fall in mitotic activity produced by irradiation was accompanied in the majority of instances by an increase in the number of binuclear cells and, contrariwise, restoration of mitotic activity led to normalization of the number of binuclear epithelial elements. These results agree entirely with the data of other workers [3].

Comparing the disruption of DNA synthesis with the mitotic activity after irradiation, it may be noted that the alterations in these processes are not always parallel. Thus, for instance, at 96 h after irradiation an increase in

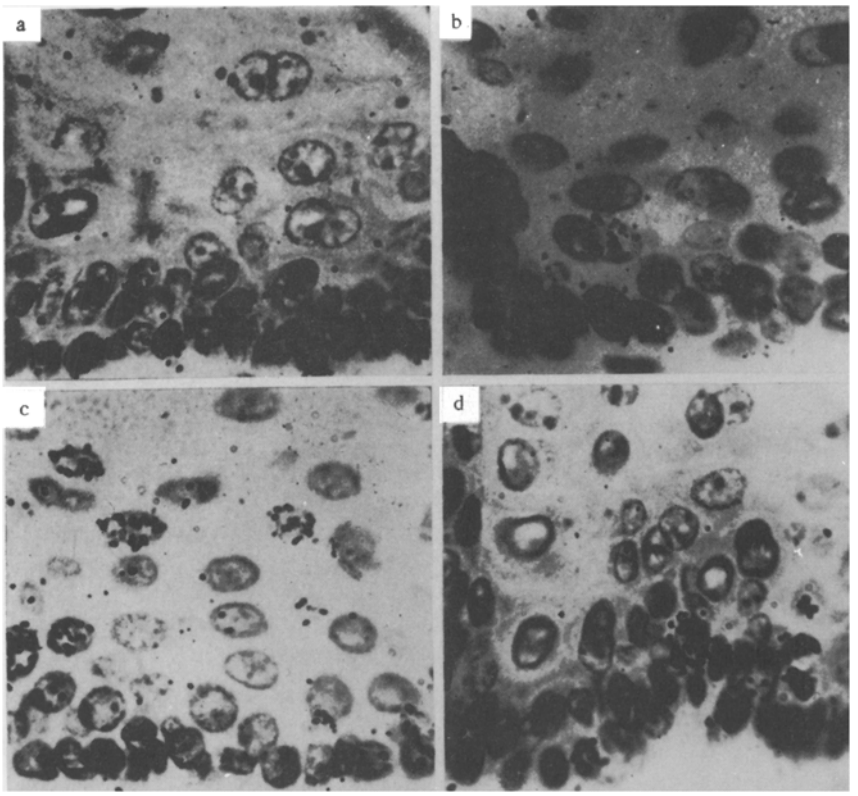

Fig. 1. Incorporation of H^3-thymidine into nuclei of cells from different layers of the tongue epithelium in control rats. a) At 4 h; b) at 24 h; c) at 48 h after administration of isotope; d) labelled anaphase in basal layer of epithelium (4 h after isotope). Autoradiograph exposure 15 days. Stain: hematoxylin (a, d), Feulgen reaction (b, c). Magnification: Ob. 90× , oc. 7× .

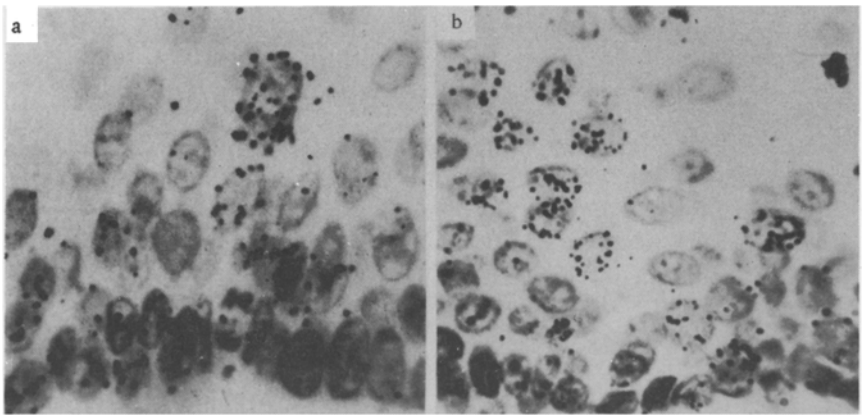

Fig. 2. Incorporation of H^3-thymidine into nuclei of cells from different layers of the tongue epithelium in irradiated rats. Increase in number of labelled nuclei. a) At 96 h after irradiation and 48 h after isotope. Autoradiograph exposure 15 days. Feulgen reaction. Obj. 90× , oc. 7× .

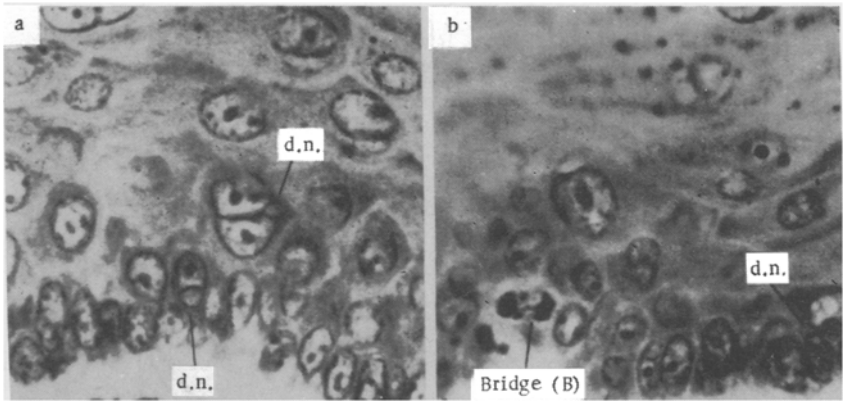

Fig. 3. Tongue epithelium of irradiated rat. a, b) Increased nuclei and small supplementary nuclei (d. n.) adjacent to the membrane of the "main" nucleus; b) atypical anaphase with bridge and chromosome fragments (B) at 48 h (a) and 72 h (b) after irradiation. Stain: hematoxylin. Obj. 90×, oc. 7×.

the percentage of nuclei which are synthesizing DNA is observed when there is a significant decrease in mitotic activity. Besides this, beginning at 48 h after irradiation atypical mitotic figures are encountered in the epithelial cells of the basal layer: bridges, fragments and non-disjunction of chromosomes (Fig. 3b). In addition, in epithelium damaged by irradiation cells with atypical nuclei were observed both in the malpighian and basal layers. This is manifested by considerable increase in their size and in the appearance of one or several small nuclear satellites which lie directly against the nuclear membrane of the "large" nucleus (see Fig. 3). When complete restoration of mitotic activity occurs both the atypical mitotic figures and cells with atypical nuclei disappear.

SUMMARY

In the epithelium of the tongue in albino rats, mitoses disappear after irradiation and the percentage of cells synthesizing DNA markedly declines. In 96 h, the number of labelled nuclei sharply increases as compared to the control ones. Consequently, irradiation interferes with the transition of the cells from the G_1 period to the S period and the G_2 period. With the advent of a compensatory process, all periods of the mitotic cycle apparently become shorter.

LITERATURE CITED

1. M. N. Veselkina, Tsitologiya, No. 5 (1962), p. 571.
2. A. L. Zosimovskaya, In book: Material from the Third Conference on the Problems of Regeneration and Cellular Reproduction [in Russian], Moscow (1962), p. 69.
3. G. N. Orlova, Tsitologiya, No. 1 (1961), p. 67.
4. M. G. Chumak,Radiobiologiya, No. 6 (1963), p. 866.
5. L. G. Lajtha, R. Oliver, and T. Kumatori et al., Radiat. Res., 8 (1958), p. 1.
6. S. Lesher, R. J. M. Fry, G. A. Sacher, Exp. Cell. Res., 24 (1961), p. 398.
7. H. Quastler, J. P. M. Bensted, and B. Chir et al., Brit. J. Radiol. 32 (1949), p. 501.
8. F. G. Sherman and H. Quastler, Exp. Cell Res., 19 (1960), p. 343.