AUTORADIOGRAPHIC INVESTIGATION OF DNA SYNTHESIS IN CELLS OF EXPERIMENTAL BRAIN TUMORS PRESERVED AT − 78°

R. I. Aniskina

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Freezing, prolonged keeping at −78°, and thawing of an oligodendroglioblastoma reduce its transplantability and depress the ability of the tumor cells to synthesize DNA. Under the same conditions DNA synthesis by cells of a dedifferentiated astorcytoma was reduced, but the transplantability of this tumor remained as before.

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A previous investigation [1] demonstrated that four strains of mouse glioblastoma and dedifferentiated rabbit astrocytoma can be kept for long periods at −78° [5]. Freezing and thawing were found to reduce the transplantability of the investigated tumors by up to 30-50%, but to have no effect on their morphological structure. Prolonging the period of keeping to 3 months in a frozen state did not influence the experimental results.

The object of the present investigation was to obtain objective data concerning the viability of glioblastomas when kept for various periods of time in a frozen state. Proliferative activity of the tumor cells after thawing was judged from their ability to synthesize DNA in vitro, investigated by autoradiography.

The study of DNA synthesis as a criterion of viability was first suggested by Ferrebee and coworkers in 1957 [7] and used by them successfully to investigate optimal freezing conditions for bone marrow [8, 9]. Later, other workers [6, 10] also reported favorably on this method and described a high degree of correlation between death of the cell and its inability to synthesize DNA.

EXPERIMENTAL METHOD

The test material consisted of an oligodendroglioblastoma of mice, possessing high malignancy and a short latent period of growth (13 days), and a dedifferentiated rabbit astrocytoma – a tumor with a long latent period of development (50-60 days).

Freezing and thawing of the tumor suspensions were carried out as described previously [1]. One part of the test material after centrifugation was transplanted into the brain of animals, while the rest was washed to remove glycerol by Ferrebee's method [5] and incubated for 4 h at 37° in medium No. 199 containing thymidine-H^3 of Soviet manufacture (specific activity 0.2 Ci/mmole) in a concentration of 1 μCi/ml. Experiments in which incubation with thymidine-H^3 continued for a shorter period were unsuccessful, although in the intact tumors the number of labeled cells was sufficient for analysis. An unfrozen tumor suspension was investigated at the same time as a control. Cell residues were fixed for 1 h in Carnoy's fluid and embedded in paraffin wax. Sections, 5 μ in thickness, were coated with type M (NIKFI) liquid photographic emuslion and exposed for 1-3 months. After development the sections were stained with Mayer's hematoxylin. To determine the percentage of labeled cells, 5000 cells were examined in regions of highest concentration of tracks on the autoradiograph. The results were subjected to statistical analysis by the Fisher – Student method and the ratio between experimental and control values calculated in each series of experiments.

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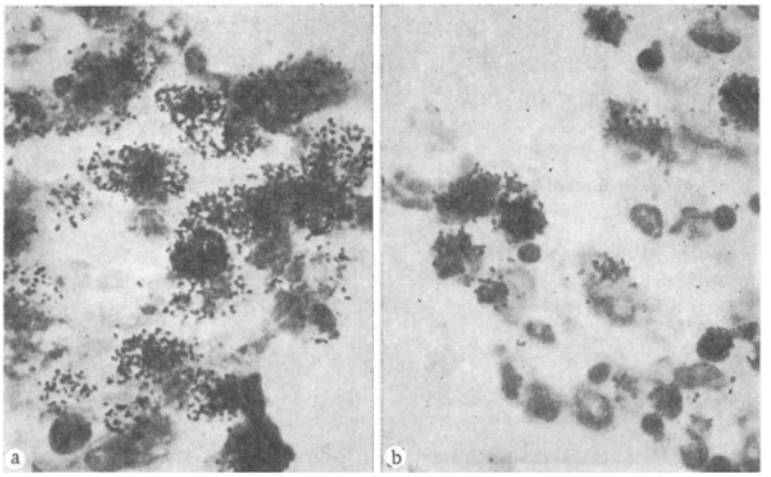

Fig. 1. Autoradiographs of oligodendroglioblastoma cells. a) Intact tumor; b) tumor after keeping for 7 months at −78°. Mayer's hematoxylin, 420×.

TABLE 1. Effect of Duration of Keeping at −78° on Ability of Cells of Dedifferentiated Astrocytome (A) and Oligodendroglioblastoma (O) to Synthesize DNA

Time of keeping at −78°	Tumor	Labeling index (in %)		Experiment/control (in %)
		expt. $M \pm t$	control $M \pm t$	
15 min	A	1,86±0,21	5,4±0,94	34,5
	O	7,51±0,88	17,9±0,82	42,8
48 h	A	2,07±0,32	6,0±0,61	34,6
	O	6,08±0,25	15,9±1,3	38,6
7 months	A	1,10±0,25	6,5±0,63	16,9
	O	12,9±1,1	33,1±0,63	39,1

EXPERIMENTAL RESULTS

Three series of experiments were carried out with each tumor and their results are shown in Table 1 and Fig. 1. Freezing and thawing caused a marked decrease in the number of cells synthesizing DNA in both glioblastomas (Table 1). Keeping for a further 48 h caused no change in the results. However, keeping for 7 months at −78° led to a marked decrease in the number of labeled cells in the dedifferentiated astrocytoma ($P < 0.01$) although it did not affect the ability of the oligodendroblastoma cells to synthesize DNA ($P > 0.05$).

Simultaneous experiments on animals showed that freezing and thawing reduced the transplantability of the oligodendroglioblastoma on the average by 21.4% compared with the control. After keeping for 7 months at −78°, the transplantability showed virtually no further decrease, remaining at a mean level of 73.8%. The latent periods of development of the tumors were lengthened with an increase in the duration of keeping. Experiments with the dedifferentiated astrocytoma revealed no injurious action of freezing, keeping, and thawing on this tumor. Regardless of the time of preservation, the number of successful transplantations was 50-60%, the same as in the control.

The results of this study of DNA synthesis in cells of experimental brain tumors subjected to freezing thus showed that most of the harmful action on the cell was due to the actual processes of freezing and thawing. Nevertheless, the different tumors evidently exhibited individual sensitivity to prolonged keeping at −78°. The later appearance of labeled cells compared with the control in tissues subjected to freezing, together with the increase in the latent period of their development in the animal brain were probably associated with the occurrence of a period of reactivation of the cells after thawing. This phenomenon has been noted by other workers [2-4].

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