METABOLIC STABILITY OF DNA IN BRAIN NERVE CELLS

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It has been shown by means of autoradiography that radioactive labels in DNA of glial cells and neurons of the mouse brain remain there for long periods of time (up to one year).

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Many experiments have shown that the DNA molecule possesses high metabolic stability. Confirmation of this view is given by preservation of label in the DNA molecule until death of the cell. During division, the DNA label is distributed uniformly between the two cells [14]. This fact has been confirmed in bacteria [8, 10], in tissue cultures [9, 12], and in mammalian cells [4, 6, 11]. However, most evidence has been obtained by biochemical methods incapable of strictly localizing the labeled DNA or on objects with a short cycle of generation. Interest in the investigation of DNA metabolism in cells with a long generation cycle has been aroused by the recent publication of researches in which doubt has been cast on the metabolic stability of DNA [5, 14].

The most suitable model for investigating DNA metabolism is mammalian nerve cells, division of which is very rarely observed in the postnatal period, yet at the same time they stand out sharply among other cells in the level of their metabolic processes [3].

The object of the present investigation was to determine the content of H^3-thymidine label in DNA of nerve cells at various times after administration of labeled nucleoside.

EXPERIMENTAL METHOD

Two series of experiments were performed. In series I thymidine-H^3 was injected intraperitoneally in a dose of 5 μCi/g into CC17BR mice on the 12th to 15th day of pregnancy. The progeny of one litter was divided into groups (not less than 2 animals in each) and sacrificed 3, 40, 230, and 360 days after birth.

In the experiments of series II, thymidine-H^3 was injected subcutaneously in a dose of 4 μCi/g into mice of the same line on the 3rd, 7th, 32nd, and 70th days after birth. These animals were sacrificed 12, 32, 130, and 230 days after injection of the isotope.

The histological treatment, preparation of the autoradiographs and scheme of the analyses of the specimens were identical in both series of experiments. The brain was fixed in Carnoy's fluid and embedded in paraffin wax. Serial sections of tissue 5 μ in thickness were dewaxed and covered with NIKFI type M nuclear emulsion. Exposure lasted 80 days at 4°. After development, the specimens were stained with azure-eosin and methyl green-pyronine. During analysis of the autoradiographs the index of labeled nuclei, the intensity of label (number of silver grains over the nucleus of the labeled cells), and the distribution of labeled cells by intensity of label were determined. The following types of nerve cells were chosen for analysis: pyramidal cells from cortical layers V and VI, large pyramidal cells from the cornu ammonis, Purkinje cells of the cerebellum, and mitral cells of the olfactory lobe. The index of labeled nuclei was counted in 5000 cells for each animal. Curves of distribution of cells against intensity of label were plotted from the results of analysis of 500 labeled cells for each type studied. The mean number of grains above the nucleus of the labeled cell was determined for the same number of cells.

EXPERIMENTAL RESULTS

When thymidine-H^3 was injected in the postnatal period, labeled glial cells and neurons were found. Labeled nerve cells were located in the fascia dentata, in the outer and inner molecular layers of the

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TABLE 1. Index and Intensity of Labeling of Mouse Brain Nerve Cells at Various Periods after Birth*

Female mouse No.	No. of mouse from the same litter	Day of pregnancy when thymidine-H^3 injected	Age of sacrificed animals (in days)	Type of nerve cells							
				pyramids of cortical layer V		pyramids of cortical layer VI		pyramids of cornu ammonis		mitral cells	
				labeling index	no. of silver grains	labeling index	no. of silver grains	labeling index	no. of silver grains	labeling index	no. of silver grains
1	1	15th	3	39,4	8,3	39,6	7,8	14,2	5,9	8,4	6,6
	2			39,8	8,6	40,2	6,9	14,3	5,9	6,9	7,0
	4		40	39,2	7,3	40,0	7,0	13,9	6,0	—	—
	5			39,6	6,7	39,5	7,8	14,0	5,8		
	6			39,3	8,3	39,3	7,2	14,3	5,8		
	7		360	39,3	6,8	39,6	6,8	14,8	6,3	8,0	6,8
	8			39,9	8,4	39,8	7,4	14,3	5,8		
	9			39,3	8,3	39,2	8,0	13,7	5,9	8,1	6,8
2	1	15 th	3	40,3	8,9	39,9	9,0	14,3	7,1	8,1	6,5
	2			39,8	9,3	38,4	8,9	14,2	6,0	8,0	7,0
	3			39,5	9,0	39,7	8,5	14,5	6,5		
	4		230	40,1	9,0	39,9	7,8	14,4	7,3	8,3	7,7
	5			39,2	8,9	39,8	9,3	14,7	6,0	7,8	7,0
	6			38,6	8,0	40,6	8,5	14,4	6,7		
	7		360	39,8	8,5	40,3	8,9	14,3	6,8	8,5	7,5
	8			39,3	9,0	39,3	9,0	14,2	6,6	8,0	6,8
3	1	12 th	3							12,5	5,6
	2									11,8	5,8
	3									10,9	6,0
	4		40							10,2	6,0
	5									12,0	5,6
	6		360							10,6	5,0
	7									12,0	5,4
4	1	12 th	3							9,8	5,4
	2									10,0	6,0
	3									12,0	5,1
	4		360							11,5	5,4
	5									9,9	5,4

*Thymidine-H^3 injected into female mice on 12th and 15th day of pregnancy.

cerebellum, and in the granules cells of the olfactory bulb. The labeled cells were most numerous after injection of the isotope on the 3rd and 7th day after birth. Besides nerve cells of the types given above, solitary labeled cells were also found in other brain structures, although the staining methods used did not allow these cells and the astrocytes to be strictly differentiated. The pyramidal cells of cortical layers V-VI and the cornu ammonis and also the Purkinje cells and the mitral cells of the olfactory lobe did not contain label. If labeled nucleoside was given during the embryonic period of development, mainly nerve cells were labeled.

The results of determination of the index and intensity of labeling of the nerve cells are shown in Table 1. Pyramidal cells from the cerebral cortex gave similar results both for the index and intensity of labeling. Lower values were obtained for the pyramidal cells of the cornu ammonis and mitral cells. These differences were evidently attributable to a difference in the duration of premitotic replication of DNA in the ependymal cells and to the fact that cells of the ependymal layer do not all begin to differentiate at the same time [2]. Another possible cause which cannot be ruled out is variations in the blood supply of different parts of the brain and the ability of thymidine-H^3 to penetrate into them [1].

The index and intensity of labeling remained constant for the cell types described above at all stages of the experiment.

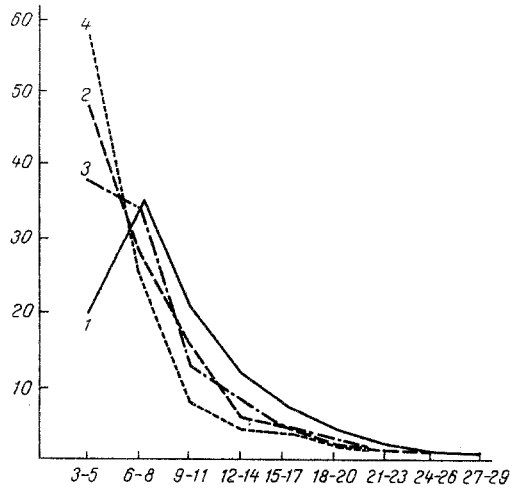

Fig. 1. Distribution of silver grains over nuclei of various types of nerve cells in the mouse brain on the 3rd day of life. Abscissa – number of silver grains above nucleus; ordinate – number of labeled cells (in %); 1) distribution in neurons of cortical layer V; 2) distribution in neurons of cornu ammonia; 3) distribution in mitral cells; 4) distribution in Purkinje cells.

The frequency of distribution of the labeled nuclei of the cortical pyramidal cells by intensity of labeling differed from that of their distribution in the pyramidal cells of the the cornu ammonis, Purkinje cells, and the mitral cells (see Fig. 1). In the last types mentioned asymmetry of the distribution curve was more marked. On the other hand, distribution of label in the nerve cells at various times after injection of isotope was identical in homologous structures with the distribution of silver grains in the animals aged 3 days. Individual variations in the frequency of distribution of labeled cells were minimal. Hence, the differentiated cells of the nervous system, in which growth and intensive metabolic processes take place, maintained a constant content of DNA labeled with thymidine-H^3. The phenomenon of liberation of label observed by Chang and Vetrovs [5] and others in fibroblast tissue cultures and in human amnionic cells was not observed in the differentiated, nondividing mammalian cells.

Under our experimental conditions the cells were evidently not damaged by radiation. We observed no morphological changes in the labeled nerve cells or changes in the distribution of label within them.

The results of this investigation thus indirectly confirm the metabolic stability of DNA. However, before this problem is finally solved, the possibility of reutilization of labeled products of DNA metabolism within the metabolic reserves of the cell must be ruled out. This possibility is discussed fully by Cowey and Walton [7].

LITERATURE CITED

1. S. M. Blinkov and I. I. Glezer, The Human Brain in Figures and Tables [in Russian], Leningrad (1964), p. 272; English Translation, New York (1968).
2. N. D. Gracheva, in the book: Investigation of Cell Cycles and Nucleic Acid Metabolism during Cell Differentiation [in Russian], Moscow-Leningrad (1964), p. 90.
3. Yu. M. Zhabotinskii, Normal and Pathological Morphology of the Neuron [in Russian], Leningrad (1965).
4. L. L. Bennett, Jr., L. Simpson, and H. E. Skipper, Biochim. Biophys. Acta, 42, 237 (1960).
5. R. S. Chang and H. Vetrovs, Science, 139, 1211 (1963).
6. S. L. Commerford, Nature, 206, 949 (1965).
7. D. B. Cowey and B. P. Walton, Biochim. Biophys. Acta, 21, 211 (1956).
8. Y. Fijisawa and A. Sibatani, Experientia (Basel), 10, 178 (1954).
9. G. M. Healy, L. Siminovitch, L. S. Parker, et al., Biochim. Biophys. Acta, 20, 425 (1956).
10. A. D. Hershey, J. Gen. Phys., 38, 145 (1954).
11. G. Hevesy and A. Forssberg, Strahlentherapie, 116, 324 (1961).
12. W. Hull and P. L. Kirk, J. Gen. Physiol., 33, 335 (1950).
13. S. R. Pelc, J. Cell. Biol., 22, 21 (1964).
14. J. H. Taylor (Editor), Molecular Genetics, New York (1963).