DNA CONTENT IN PURKINJE CELLS OF THE CEREBELLUM OF RATS SPONTANEOUSLY INFECTED WITH KILHAM VIRUS

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The DNA content in the Purkinje cells of the cerebellum of rats spontaneously infected with Kilham virus and of rats which were not carriers of the injection was determined cytospectrophotometrically in preparations stained by the Feulgen method. The frequency of discovery of cells with hyperdiploid nuclei was the same in the two groups. It is concluded that a nonmultiple increase in the DNA content in Purkinje cells of the rat cerebellum is evidently not connected with virus infection.

KEY WORDS: Purkinje cells of the cerebellum; DNA; Kilham virus.

The DNA content in Purkinje cells of the cerebellum was investigated in rats spontaneously infected with Kilham's rat virus (RV) and rats that were not carriers of that infection. Kilham's rat virus is known to be the most widespread virus of wild and laboratory rats [2, 11]. It attacks cells actively synthezing DNA and dividing by mitosis and it multiplies in them [8, 10]. The main cytopathic action of this virus of the cerebellum is manifested as selective destruction of the outer granular layer [6, 8-10]. With respect to Purkinje cells, the statement is made in all these papers that their uniform distribution in the molecular layer is disturbed and sometimes they degenerate. All these changes take place in the cerebellum of animals with clinical manifestations of cerebellar ataxia. It was decided to study whether a nonmultiple increase in the DNA content in the Purkinje cells could be one of the manifestations of latent infection in normally developing animals. The possibility that a nonmultiple increase in the DNA content in the cells can take place under the influence of virus infection has been demonstrated, for example, in human lung cell cultures [12].

The object of this investigation was to test the earlier hypothesis that a virus lesion could be the cause of a nonmultiple increase in the DNA content in the Purkinje cells of the rat cerebellum [1].

EXPERIMENTAL METHOD

Noninbred albino rats aged 2-3 months were used. The discovery of antibodies against RV in the blood serum was used as the method for detecting infection of the animals. Serum from 22 animals was tested. Antihemagglutinins were discovered (titers 1:20, 1:40, and, in one animal, 1:160) in 12 of them. In four of these rats antibodies of the macroglobulin series, sensitive to 2-mercaptoethanol, were found, possibly indicating an infection in the recent past [3]. No antibodies were found in 10 animals. These rats were used as the control. Squash preparations were made from the cerebellum by the method of Lodin et al. [7]. Air-dried preparations were fixed with 96° ethanol and stained by Feulgen's method with hydrolysis for 12 min in 5 N HCl at 37°C. The DNA content in the nuclei was determined by the Vickers M86 scanning integrating microdensitometer in the 540 nm region. Granule cells were used as reference cells with a known diploid DNA content [1]. Altogether 450-460 Purkinje cells for the control and infected animals and about 350 granule cells were measured.

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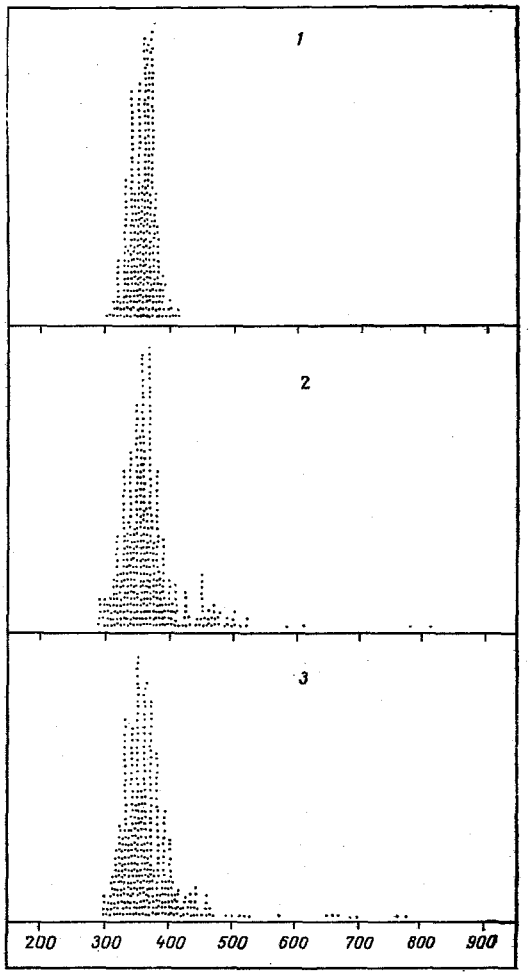

Fig. 1. Distribution of quantity of DNA – fuchsin: 1) granule cells; 2) Purkinje cells of cerebellum of rats spontaneously infected with Kilham virus; 3) Purkinje cells of control animals. Each dot represents one cell. Quantity of DNA – fuchsin in relative units shown along horizontal axis.

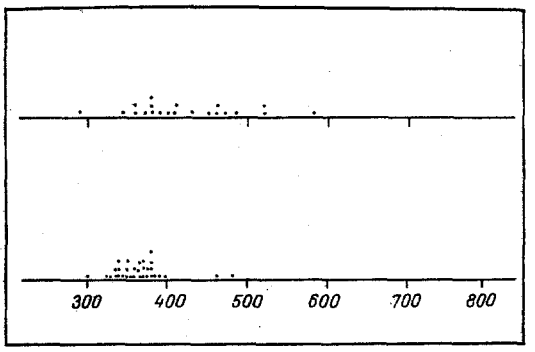

Fig. 2. Distribution of quantities of DNA – fuchsin in Purkinje cells in two preparations from one animal (infected rat No. 10 in Table 1). Legend as in Fig. 1.

TABLE 1. Number of Hyperdiploid Purkinje Cells in Rat Cerebellum

Index	Uninfected rats, № 1	2	3	4	5	6	7	8	9	10
No. of measured nuclei of Pukinje cells	70	60	63	52	29	23	59	46	30	22
Number of hyperdiploid nuclei: absolute	4	8	8	9	4	—	2	8	—	—
%	6	13	13	17	14	—	3	17	—	—

Index	Infected rats, № 1	2	3	4	5	6	7	8	9	10	11	12
No. of measured nuclei of Pukinje cells	26	25	63	52	27	26	23	26	61	53	25	28
Number of hyperdiploid nuclei: absolute	5	5	12	7	—	1	—	3	3	12	—	2
%	19	20	19	13	—	4	—	11	5	23	—	7

EXPERIMENTAL RESULTS

Cytophotometric determination of the DNA content showed that the DNA contents measured for the granule cells were grouped in a monomodal distribution (Fig. 1). Most of the measured Purkinje cells were diploid, for the maxima of the distributions coincided with those for the granule cells (Fig. 1). However, in the groups of animals compared there were some Purkinje nuclei with a hyperdiploid DNA content. On the right of the 95% confidence limit established for the granule cell distribution there were 43 nuclei for the control animals (9.5%) and 50 for the infected animals (11%). The number of these nuclei was the same in the two groups of rats compared.

The distributions just examined give an idea only of the total Purkinje cell population as a whole. It is no less important to examine how often hyperdiploid cells were found in individual animals in each group (Table 1).

It will be clear from Table 1 that the number of hyperdiploid Purkinje nuclei in both groups varied from 3 to 23%. In some rats no such nuclei were found whatever. The control and infected groups thus were indistinguishable as regards both the frequency of rats with hyperdiploid Purkinje cells in their cerebellum and the number of such cells in individual animals.

It can accordingly be concluded from these results that the increase in the DNA content in the Purkinje cells of the rat cerebellum was not connected with virus injection. However, this conclusion is still premature.

To prove it strictly the virus must be isolated from the tissue of the cerebellum, for RV is not neurotropic and antibody production may have been the result of its multiplication in another organ [6].

The question of the mechanism of formation of the hyperdiploid Purkinje cells was thus still unresolved. Distinguishing functional features of these cells likewise have not yet been explained. However, comparison of the results of the present investigation with earlier findings of the present writers [1] and other workers [4, 5] shows that the problem of the intensity of the phenomenon in the Purkinje cell population both of a single animal and in the groups of animals ought to be solved first. The number of hyperdiploid cells in the cerebellum in fact can vary from 0 to 50%. In most animals studied there were few such cells. According to other observations, Purkinje cells of adult animals contain a tetraploid DNA content. Furthermore, determination of the DNA content in the Purkinje cells of a single animal showed that the number of hyperdiploid nuclei differed in two preparations (Fig. 2). This suggests that different lobes of the cerebellum may differ with respect to this feature. Only if a certain regular pattern, connected with a certain condition (strain of animals, lobe of the

cerebellum, group of rats in the same strain, etc.), of appearance of groups of animals in whose cerebellum the number of hyperdiploid or tetraploid Purkinje nuclei is large can be discovered is it likely that the problem indicated above will be successfully investigated.

LITERATURE CITED

1. V. Ya. Brodskii, L. S. Agroskin, E. A. Lebedev, et al., Zh. Obshch. Biol., No. 6, 917 (1974).
2. E. M. Petruchuk, "The study of latent infection in rats with Kilham virus and the development of methods for its indication," Author's Abstract of Candidate's Dissertation, Moscow (1974).
3. E. M. Petruchuk, N. V. Shalunova, S. G. Dzagurov, et al., Vest. Akad. Med. Nauk SSSR, No. 2, 57 (1973).
4. A. Bregnard, A. Knüsel, and C. C. Kuenzle, Histochemistry (Berlin), 43, 59 (1975).
5. S. Fujita, T. Hattori, M. Fukuda, et al., Develop. Growth Different., 16, 205 (1974).
6. L. Kilham and G. Margolis, Am. J. Path., 49, 457 (1966).
7. Z. Lodin, I. Faltin, and K. Sharma, Acta Histochem. (Jena), 26, 244 (1967).
8. G. Margolis and L. Kilham, Res. Publ. Ass. Nerv. Ment. Dis., 44, 113 (1968).
9. G. Margolis and L. Kilham, in: The Cerebellum in Health and Disease (ed. by W. S. Fields and W. D. Willis, jr.), Green, St. Louis (1970), pp. 353-379.
10. G. Margolis and L. Kilham, in: Progress in Neuropathology, Vol. 1 (ed. by H. M. Zimmerman), Grune, New York (1971), pp. 168-201.
11. R. S. Robey, D. R. Woodman, and F. M. Hetrick, Am. J. Epidem., 88, 139 (1968).
12. L. R. Trusal, A. Anthony, and J. J. Docherty, J. Histochem. Cytochem., 23, 283 (1975).