16. J. Metha and P. Metha, Am. J. Cardiol., 48, 366 (1981).
17. A. Shen and R. Jennings, Am. J. Pathol., 67, 417 (1972).

CHANGES IN STAINING PROPERTIES OF PURKINJE CELLS IN MICE WITH PROTEIN AND CALORIC DEFICIENCY AND DURING REHABILITATION

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Individual details revealed by the study of the cytologic structure of Purkinje cells of the cerebellum after exposure to various unfavorable conditions are largely associated with changes in their staining properties. In the ganglion-cell layer of the cerebellar cortex, besides pale neurons, dark, pyriform neurons are constantly found [12, 15]. An increase in the number of dark cells has been observed by many workers under a very wide range of experimental conditions, such as hypoxia [3], increased physical exertion [14], and protein and caloric deficiency [7]. There is also evidence that the number of dark neurons increases with an animal's age [11]. However, the question of the origin and functional role of the dark cells has not yet been finally explained. This is largely due to the fact that they are comparatively few in number if the brain is fixed by the perfusion method, but the number rises sharply if the brain is fixed by the immersion method [12].

Experimental results obtained by the writers previously [7] show no correlation between an increase in the number of dark cells and the specific character of the conditions to which the animal is exposed. An increase in the number of dark cells can most probably be regarded as a change in the type of their metabolism under different extremal conditions.

The aim of this investigation was to study changes in the staining properties of cerebellar Purkinje cells during modification of intracellular free fatty acid metabolism by the drug carnitine, in the course of dietary rehabilitation after prolonged underfeeding in early postnatal development.

EXPERIMENTAL METHOD

Experiments were carried out on 40 CBA mice. Protein and caloric deficiency was produced by reducing the concentration of nutrients in the experimental diet by 50% compared with the control. The casein content of the diet in the control group was 10%, in the experimental group 5% [5]. In the experiments of series I the cerebellum of young mice aged 40 days (n = 6) was studied immediately after a month of underfeeding. In the experiments of series II, underfed mice (n = 6) were transferred to a balanced diet from the 41st through the 70th day of life. In series III underfed mice (n = 6) received a balanced diet from the 41st through the 70th day of life, to which carnitine had been added. In all series of experiments mice (n = 12) receiving a synthetic balanced diet served as the control.

The animals were perfused with Karnovsky's fixative through the ascending aorta under pentobarbital anesthesia. The time from laparotomy until the beginning of fixation of the brain did not exceed 40 sec. Sagittal sections through the cerebellar vermis were postfixed in 2% OsO_4 solution, dehydrated, and embedded in Araldite. Under the light microscope, in semithin sections stained with methylene blue, the pale and dark pyriform neurons in the ganglion-cell layer of the cerebellar cortex were studied. The numerical results were subjected to statistical analysis by the method described in [4].

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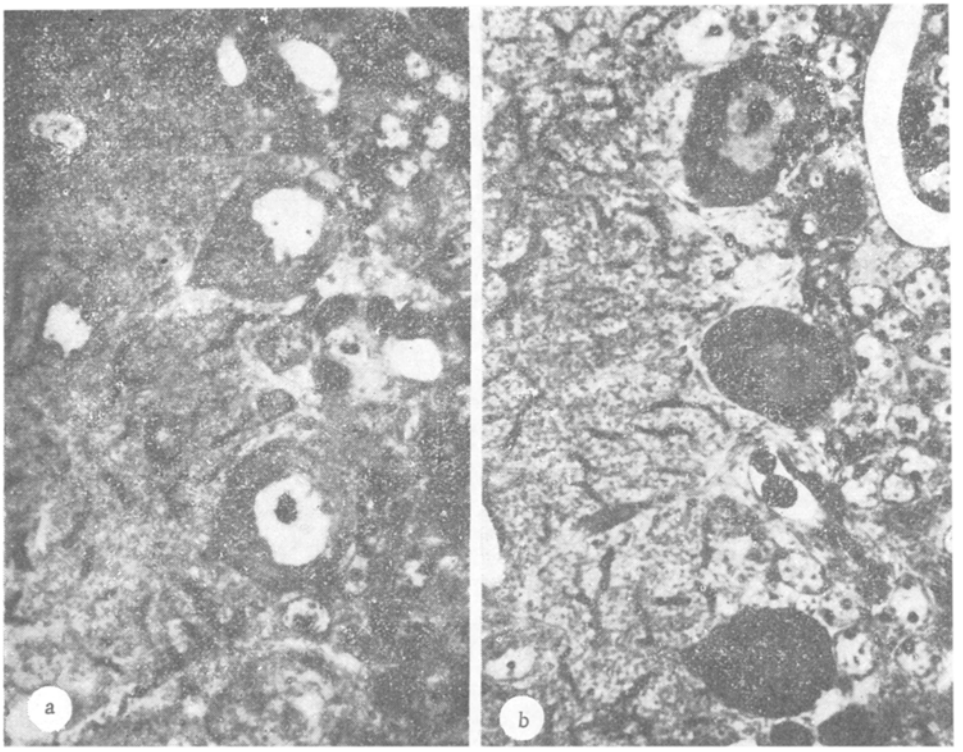

Fig. 1. Purkinje cells in cerebellar cortex of control (a) and underfed (b) mice aged 40 days.

EXPERIMENTAL RESULTS

In semithin sections obtained from the experimental and control animals, pale cerebellar neurons were characterized by a delicate blue color, comparable in intensity with the surrounding neuropil. The dark cells were sufficiently sharply distinguished by the deep blue color of their cytoplasm and nuclei, similar in its saturation with the color of the chromatin in the nuclei of the granule cells (Fig. 1a, b).

In the present investigation, conducted on semithin sections, no differences were found in the structure of the pale and dark pyriform neurons in the control and experimental animals. However, the ratio between the numbers of these cells varied significantly depending on the experimental conditions (Table 1).

After dietary rehabilitation for 1 month the weight of the animals underfed from the 10th through the 40th day after birth still lagged significantly behind the weight of mice of the control group, whereas the weight of the mice receiving a balanced diet in the rehabilitation period with the addition of carnitine exceeded the weight of the control animals a little. The number of dark pyriform neurons after dietary rehabilitation was almost twice their number in the control 70-day-old animals.

Meanwhile, after dietary rehabilitation with the addition of carnitine to the diet, the number of dark cells was less than half their number in the control animals of the same age, and less than a quarter of their number in mice receiving dietary rehabilitation alone.

The results of these experiments confirm observations in the literature showing that the number of dark pyriform neurons increases with the animal's age [11], for they were three times more numerous in control 7-day animals than at the age of 40 days [7]. Meanwhile, in the present investigation, a considerable decrease in the number of dark neurons in the cerebellar cortex during dietary rehabilitation with the use of carnitine was discovered for the first time. This effect can evidently be explained on the grounds that during underfeeding gluconeogenesis is stimulated [1] and the concentration of free fatty acids in the brain is increased [16]. At the same time, we know that carnitine promotes transport of higher fatty acids during their passage through the mitochondrial membrane. In that way it accelerates oxidation of fatty acids in the mitochondria, and ATP formation, and it ultimately stimulates intracellular synthetic processes requiring expenditure of ATP.

TABLE 1. Individual and Mean Statistical Values of Body Weight and Numbers of Pale and Dark Pyriform Neurons in 70-Day-Old Control Animals and in Mice after Dietary Rehabilitation Alone, and after Dietary Rehabilitation with Carnitine

Group of animals	Weight of animals, g	Number of cells	
		dark	pale
Mice receiving balanced synthetic diet from 10th through 70th day after birth (control)	23,2	23,4	76,6
	21,5	20,6	79,4
	16,5	30,0	70,0
	19,7	25,6	74,4
	17,6	27,1	72,9
	24,6	18,7	81,3
$M \pm m$	20,5±1,45	24,2±4,8	75,8±8,5
Mice receiving low protein diet from 10th through 40th day after birth and balanced diet from 42nd through 70th day (dietary rehabilitation)	19,2	39,5	60,5
	19,7	34,6	65,4
	13,2	45,4	54,6
	13,4	44,6	55,4
	20,2	28,0	72,0
	12,2	57,2	42,8
$M \pm m$	16,3±1,46	41,6±6,5	58,4±7,4
P (compared with control)	<0,01	<0,005	<0,005
Mice receiving low protein diet from 10th through 40th day after birth and balanced diet with carnitine from 41st through 70th day (dietary rehabilitation with carnitine)	20,1	8,8	91,2
	23,5	8,7	91,3
	19,0	13,7	86,3
	20,2	12,2	87,8
	20,0	8,3	91,7
	24,1	7,1	92,9
$M \pm m$	21,2±0,92	9,8±3,1	91,2±9,5
P (compared with control)	<0,01	<0,001	<0,01

The increase observed in the number of dark cells in the cerebellar cortex during underfeeding and subsequent dietary rehabilitation was thus due, in our opinion, to elevation of the level of intracellular metabolism, due to an increase in the concentration of free fatty acids in these cells. Exogenous carnitine, introduced into the body during dietary rehabilitation, significantly lowered the intracellular free fatty acid concentration, and this evidently was the cause of the decrease in number of dark pyriform neurons.

We know from the literature that the content of nonesterified fatty acids rises sharply during physical exercise [2] and cerebral ischemia [6]. It has also been observed that the intensity of oxidation of fatty acids decreases with age in most tissues of the body, while the rate of their synthesis remains the same [10].

In this connection it is a very interesting fact that the most drastic disturbance of phosphorylation processes in the mitochondria takes place between the first and second minutes of ischemia [6]. It is this fact which can evidently explain the difference mentioned above in the number of dark cells after fixation by the perfusion and immersion methods.

The work of the cell under β-oxidation conditions demands long-term reorganization of its enzyme system [1], and it is this which in all probability explains the raised level of functional activity of the dark cells [8] and intensification of intracellular regeneration associated with it [9].

LITERATURE CITED

1. E. K. Alimova, A. T. Astvatsatur'yan, and L. V. Zharov, Vopr. Med. Khim., No. 5, 451 (1974).
2. S. P. Anikeeva and Yu. M. Shteinberg, Vopr. Med. Khim., 27, 935 (1981).
3. N. N. Bogolepov, in: Adaptive Functions of the Brain [in Russian], Baku (1980), p. 30.
4. I. N. Bronshtein and K. A. Semendyaev, A Guide to Mathematics [in Russian], Moscow (1981), p. 701.
5. G. M. Erastov, D. I. Medvedev, K. Yu. Reznikov, et al., in: Current Problems in Etiology, Pathogenesis, Clinical Features, and Treatment of Tropical Diseases [in Russian], Moscow (1976), p. 170.

6. Yu. V. Lazarevich, M. D. Maevska, and I. Streshnaider, Anesteziol. Reanimatol., No. 5, 39 (1980).
7. D. I. Medvedev, I. I. Babichenko, I. Z. Eremina, and A. I. Kravtsova, Byull. Éksp. Biol. Med., No. 3, 108 (1983).
8. E. Ya. Sanovich, A. A. Pal'tsyn, and V. P. Tumanov, Byull. Éksp. Biol. Med., No. 4, 498 (1981).
9. D. S. Sarkisov, Regeneration and its Clinical Importance [in Russian], Moscow (1970), p. 59.
10. A. A. Pashkova, in: The Circulation and Old Age [in Russian], Kiev (1965), p. 125.
11. D. A. Chetverikov, S. N. Toporova, and I. N. Ulybina, in: Neuronal Mechanisms of Integrative Activity of the Brain [in Russian], Erevan (1979), p. 31.
12. I. N. Ulybina and S. P. Tsyndrenko, Tsitologiya, 16, 30 (1974).
13. V. N. Yarygin and A. S. Pylaev, Byull. Éksp. Biol. Med., No. 6, 105 (1975).
14. E. R. Meitner, Folia Morphol. (Prague), 25, 2 (1977).
15. S. Mehta, N. K. Relan, and S. K. Nain, Ind. J. Exp. Biol., 19, 238 (1981).

HYPERPRODUCTION AND INTRAVITAL REJECTION OF SURPLUS EPITHELIUM OF INTESTINAL VILLI

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KEY WORDS: intestinal villi; intestinal epithelium; rejection of surplus epithelium; sources of epithelium in deposits on luminal surface of small intestine.

During the study of intestinal biopsy material from dogs [9, 11] accumulations of epithelial and villous cells were found in the surface layer of mucus and patterns of formation and rejection of surplus structures of the crypt epithelium and of pinching off and rejection of the villi and their distal fragments were described. Several membrane enzymes were discovered in the epithelium covering the rejected villi and in the mucus [1, 3, 4, 7, 12], suggesting that the phenomenon of rejection of structures of the small intestinal mucosa plays a definite role in premembrane contact digestion. The origin of some of the rejected structural elements has not been explained. Gaps in the data on surplus formations on the surface of the villi in dogs also required to be filled.

Since the facts on intravital loss of layers of eptihelium outside the "extrusion zone" are of fundamental importance, it was decided to study the sources of epithelium in deposits on the luminal surface of the small intestine in some other species of animals and in man.

EXPERIMENTAL METHOD

The small intestine was removed in its entirety or segments were taken from different parts of it from anesthetized animals (cats, albino rats and mice, guinea pigs, rabbits) for morphological investigation, cut lengthwise and, together with its contents, fixed with 12% neutral formalin solution. The mucosa was dissected after fixation. Additionally, 25 biopsy specimens of mucosa from various parts of the small intestine unaffected by disease were obtained from patients after resection of the stomach or small intestine (for ulcer or fistula). The biopsy material was obtained and fixed without any mechanical procedures on the surface of the mucosa. The native mucosa was studied under a binocular loupe, using only atraumatic micromanipulations with instruments. Serial microscopic sections were prepared by a special method [10] from the various forms of villi, cut off the mucosa or isolated from the layer of deposits.

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