EFFECT OF MATERNAL PROTEIN-ENERGY DEFICIENCY ON HISTOGENESIS OF THE CEREBRAL CORTEX IN EMBRYONIC MICE

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Alimentary insufficiency is a widespread environmental factor with a significant effect on growth and development [9]. The brain is especially sensitive to the action of malnutrition [2, 4, 6]. Although many investigations have been devoted to the study of the effect of protein-energy deficiency on development of the mammalian brain, some aspects of this still important problem remain unresolved. For instance, there have been few studies [5, 11] of the morphogenetic development of the cerebral cortex during restricted food intake during the period of embryogenesis. Yet it is in the prenatal period that histogenesis of the mammalian cerebral cortex takes place: Proliferation of cortical neurons has already finished at birth [3].

The aim of this investigation was to study histogenesis of the neocortex in mice during malnutrition in the period of embryonic development.

EXPERIMENTAL METHOD

Food limitation was created by feeding pregnant mice with a low-protein synthetic diet containing 5% of casein. After the first day of pregnancy, control animals received a normal balanced diet containing 10% of casein. At the 17th day of pregnancy the mice were given an intraperitoneal injection of [^{3}H]thymidine in a dose of 5 μCi/g body weight. The animals were decapitated 1, 2, 4, 6, 8, 12, 16, and 25 h after injection of the isotope. The fetal heads were fixed in Carnoy's fluid and embedded in paraffin wax. After standard treatment the structure, parameters of cellular proliferation, and death of the cells in the wall of the cerebral vesicle were studied in frontal sections cut through the parietal region of the brain anlage. All measurements were made in the dorsolateral part of the wall of the lateral ventricle of the embryonic brain. Material obtained from 23 embryos of the experimental group and 21 of the control group was studied. In another series of experiments the distribution of intensively labeled nuclei in horizontal layers of the cortex was analyzed in the parietal region of the neocortex of 20-day old mice, whose mothers had received [^{3}H]thymidine on the 13th or 17th day of pregnancy.

EXPERIMENTAL RESULTS

Under the experimental conditions used a teratogenic effect of malnutrition was observed. The frequency of resorption of the fetuses of food-deprived mothers reached 60%, whereas in the control it did not exceed 10%.

Histological study of the anlage of the telencephalon in embryos developing under conditions of alimentary insufficiency revealed the following distinguishing features (Table 1): 1) considerable development of the ventricular zone: it was 1.5 times thicker than normal; 2) weaker development of the subventricular zone, which was 2.1 times thinner than in the control; 3) the smaller area of the cortical lamina, which was 73% of the corresponding value in the control embryos.

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TABLE 1. Thickness of Zones in Wall of Lateral Cerebral Ventricle of 17-Day-Old Mouse Embryos

Zone	Thickness of zone, μ		P
	control	malnutrition	
Ventricular	63,6±1,7	97,0±2,4	<0,001
Subventricular	113,5±5,2	53,6±2,3	<0,001
Intermediate	127,4±5,7	115,1±5,8	Not significant
Cortical lamina	111,3±5,0	81,0±5,6	<0,01
Marginal	38,2±2,1	31,6±1,7	<0,05

TABLE 2. Parameters (in %) of Proliferation in Ventricular Zone of Brain of 17-Day Mouse Embryos

Parameter	Experimental conditions		P
	control	malnutrition	
MI	2,20±0,13	2,86±0,16	<0,01
ILN	18,1±1,9	24,8±1,3	<0,01
Proliferative pool	52,8	69,2	

TABLE 3. Parameters of Mitotic Cycle of Ventricular Cells

Phase of mitotic cycle	Duration, h	
	control	malnutrition
$t\left(G_1+\frac{1}{2}M\right)$	9,3	10,9
tS	6	7,6
$t\left(G_2+\frac{1}{2}M\right)$	2,2	2,7
T	17,5	21,2

The value of the mitotic index (MI) and index of labeled nuclei (ILN) and the size of the proliferative pool in the ventricular zone of embryos of the experimental group were greater than in the control group (Table 2).

The duration of the generative cycle of the ventricular cells, calculated from the curve of labeled mitoses, increased appreciably on a low protein diet, up to 21.2 h, which is 21% longer than normal (Table 3). All phases of the cell cycle were lengthened, but the duration of the synthetic period was lengthened the most (by 27%).

The number of cells with pycnotic nuclei in the anlage of the telencephalon is normally very small: The highest value of the pycnotic index observed in the subventricular zone was 0.09%, and in the ventricular zone 0.06%. In the remaining zones no cells with pycnotic nuclei could be found. The pycnotic index in embryos developing under conditions of malnutrition was 2.5 times higher than normally. On the whole the level of dystrophic changes in this case remained low: The pycnotic index did not exceed 0.17%. In view of the fact that pycnosis of the nuclei takes place immediately after the cell was passed through mitosis [8], and considering data in the literature on the time of existence of pycnosis [7], the mortality rate among the newly formed cells can be calculated. In the malnutrition experiment not more than 1.3% of newly formed cells in the ventricular zone were found to have died, whereas in the subventricular zone the incidence of dystrophic changes did not exceed 1.8%.

It can be concluded from these experimental results that keeping pregnant mice on a low protein diet leads to delayed development of the anlage of the telencephalon of their fetuses. The structure of the wall of the cerebral ventricle in 17-day embryos developing under conditions of malnutrition was retarded in its degree of maturity compared with normal and corresponded to earlier stages of embryogenesis. This was shown by the considerable development of the ventricular zone with a higher value of MI and with a larger proliferative pool than normal, the weak development of the subventricular zone, and the thinner cortical lamina. From the 13th to the 20th day of embryonic development of rodents MI in the matrix zones of the brain is known to fall, the proliferative pool becomes smaller, the area of the ventricular zone contracts, whereas the subventricular zone appears and develops considerably, and the thickness of the cortical lamina increases [1, 10, 12].

It is possible that delayed development of the embryonic brain observed under conditions of malnutrition is the result of a decrease in the rate of cell proliferation, due to lengthening of the mitotic cycle of the ventricular cells. The conclusion regarding delayed development of the neocortex following exposure of the fetus to alimentary insufficiency is confirmed by analysis of the distribution of intensively labeled nuclei of neurons in the parietal cortex of 20-day-old mice developing under conditions of malnutrition in the prenatal period, after injection of [^{3}H]thymidine on the 13th or the 17th day of embryonic life. Data on the distribution of intensively labeled nuclei in the layers of the cortex can be used to judge the time of formation of neurons in the corresponding neocortical layers. The results of analysis show that under normal conditions mainly the neurons of neocortical layer V are produced in the germinative zones of the brain on the 13th day of embryogenesis, whereas under conditions of malnutrition neurons of the deep sublayer of layer VI of the cortex begin to be formed at this time. On the 17th day of prenatal development under normal conditions the future neurons of layer II are formed in the ventricular zone. Under the influence of malnutrition mainly neurons of deeper layers of the neocortex (III and IV) are formed at these same times.

Protein-energy deficiency, acting during embryonic development, thus reduces the rate of proliferation of the ventricular cells, with consequent delay of development of the cerebral cortex of the mice.

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