of bone marrow with data of other workers [2, 4], the results were adjusted to exclude bone marrow cells from the coccygeal vertebrae. Both agreement and also substantial differences will be seen for individual bones and parts of the skeleton. These disagreements may evidently be attributed to differences both in the animals and in the technique used.

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EFFECT OF MATERNAL HYPOXIA ON NEUROGENESIS OF THE CEREBRAL CORTEX OF THE PROGENY IN RATS (AUTORADIOGRAPHIC STUDY)

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On the 15th day of pregnancy rats were exposed for 2 h to the action of hypoxia equivalent to an altitude of 8000 m, and on the 18th day of pregnancy they were given three injections of [^{3}H]thymidine. A quantitative autoradiographic study was made of the cerebral cortical neurons of the progeny at the age of 30 days. Rats surviving intrauterine hypoxia were shown to have a significantly higher percentage of labeled nerve cells in layers II, III, and V of the sensomotor cortex than in the control. A difference in the intensity of labeling also was found. It is suggested that maternal hypoxia can delay differentiation and maturation of cerebral cortical neurons in the progeny.

KEY WORDS: maternal hypoxia; brain; histoautoradiography.

Clinical evidence of the role of fetal hypoxia in the pathogenesis of mental retardation has now accumulated [1, 8, 9]. It has been shown experimentally that fetal hypoxia leads to disturbances of brain development and of conditioned-reflex activity in animals [3-7, 12]. However, the mechanism of the embryotoxic action of hypoxia has not yet been explained.

The critical period in the development of the cerebral cortex in rats is the 15th day of embryogenesis, when the gradual transition from proliferative processes to differentiation is complete [2].

In the present investigation a histoautoradiographic method was used to study the effect of intrauterine hypoxia at this period on neurogenesis of the sensomotor cortex in rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred female rats weighing 200 g or thereabouts. On the 15th day of pregnancy the rats were exposed to hypoxia. Hypoxic hypoxia was created by "raising" the animals in a pressure chamber to an altitude of 8000 m (267.4 mm Hg, 7.5 vol.%

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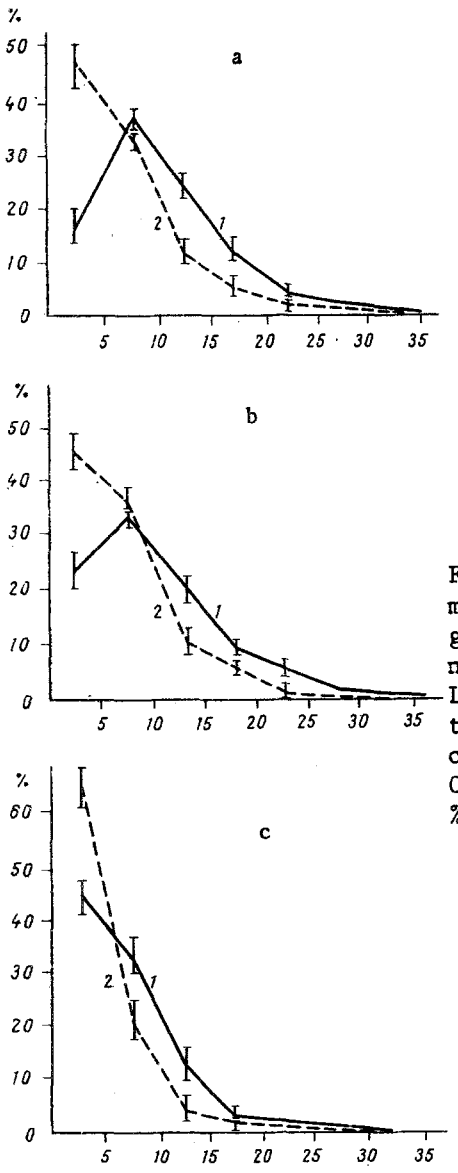

Fig. 1. Distribution of intensity of thymidine label as reflected in number of grains of silver per nucleus of labeled neurons. 1) Control, 2) experiment. a) Layer II, b) layer III, c) layer V of cortex. Abscissa, classes of cells depending on number of grains of silver per nucleus. Ordinate, frequency of classes (fraction in %).

TABLE 1. Number of Labeled Neurons (fraction in %) in Cerebral Cortex of 30-day-old Rats After Injection of [^{3}H]-Thymidine on 18th Day of Pregnancy

Layers of cortex	Control		Experiment*		Difference (d) between control (c) and experimental (e)	
	number of labeled cells	% of labeled cells (M±m)	number of labeled cells	% of labeled cells (M ± m)	$d_{c,e}$, %	P
II	1133	22,7±0,85	1648	33,0±0,50	47,5	0,999
III	1148	23,0±0,50	1639	32,8±0,25	42,6	0,999
V	1043	20,9±1,75	1525	30,5±0,10	45,9	0,999

*Brain of progeny from mothers exposed to hypoxia (8000 m, 2 h) on 15th day of pregnancy.
†Number of labeled cells per 5000 cells counted from five animals.

O_2). The "ascent" took place at a speed of 25 m/sec. Exposure of the animals in the pressure chamber lasted 2 h from the time of attaining the assigned altitude. On the 18th day of pregnancy the experimental and control female rats were given three subcutaneous injections (at intervals of 6 h) [^{3}H]thymidine in a dose of 1 μCi/g. The brains of rats aged 30 days were taken for quantitative autoradiographic investigation. Material was fixed in Carnoy's mixture. Pieces of the sensomotor cortex of the young rats were embedded in pairs (control and experimental) in the same paraffin wax block. Sections 7 μ thick were amounted on slides. Type M nuclear emulsion was applied to the dewaxed sections. The autoradiographs were kept in a refrigerator at 4°C for 20-25 days. After development and fixation of the autoradiographs the sections were stained with thionine. On microscope investigation the number of labeled nuclei of the neurons in 1000 cells counted in each layer of the cortex (II, III, V) of five control and five experimental animals was determined. The intensity of labeling also was determined in the labeled cells by counting the number of grains of silver above the nuclei. Statistical analysis of the results was carried out by Fisher's "φ" method. The experimental results are given in Table 1 and Fig. 1.

EXPERIMENTAL RESULTS

The number of labeled neurons (in % of the total) in the cerebral cortex of 30-day-old rats after injection of [H^3]thymidine on the 18th day of embryogenesis is given in Table 1. The results show that the percentage of labeled neurons in layers II, III, and V of the sensomotor cortex of the experimental group of rats was significantly greater than in the control.

The results of a study of the intensity of labeling based on the number of grains of silver per nucleus of labeled cortical neurons in the brain of 30-day-old rats are given in Fig. 1. Clearly the percentage of labeled cells in the experimental group in classes with diluted label was significantly higher than that in the control in cortical layers II, III, and V.

The results may be evidence that proliferative processes in the cortical matrix of the experimental animals continue rather longer after the 18th day of embryogenesis than normally, so that neuroblasts migrate from the matrix layer later. It can tentatively be suggested that maternal hypoxia on the 15th day of pregnancy delays differentiation of the cerebral cortical neurons of the progeny and, as a result, it delays maturation of the neurons. In mouse embryos from mothers exposed to hypoxia during pregnancy [10, 11], the labeling index in cells of the matrix of the prosencephalon falls immediately after exposure (death of some of the matrix cells has also been described) and rises a few days later. The results of the present experiments suggest that maternal hypoxia on the 15th day of pregnancy causes death of some cells of the matrix layer in the brain, and the embryo responds to this by attempting to make good this cell deficit.

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