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EFFECT OF ALCOHOLIC INTOXICATION OF FEMALE RATS BEFORE PREGNANCY ON NEURONAL ULTRASTRUCTURE OF THE SENSOMOTOR CORTEX IN THE PROGENY

É. N. Popova

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Many clinical studies have shown that developmental disturbances characteristic of the fetal alcohol syndrome may sometimes arise in the offspring of mothers with alcoholism. The role of maternal alcoholic intoxication before pregnancy in the pathology of development of the fetus and offspring has received little study, and available data are contradictory. There is evidence that the average body weight of newborn infants of mothers taking alcohol before pregnancy is less than in the case of nondrinking women [9, 10]. According to other researchers, alcohol consumption before pregnancy (100 g alcohol per week) reduces the risk of the newborn infants being underweight [14]. In the case of alcohol abuse before pregnancy, in 30% of cases various abnormalities were found in the newborn infants; correlation has been found between the level of alcohol consumption and lowering of the mental development of children [11]. It has been shown experimentally that prolonged alcohol consumption by female rats before pregnancy leads to early embryonic mortality in some animals, to reduction of the body weight of the offspring at the age of 3 weeks, a tendency toward increased excitability of their CNS at the age of 1 month, inhibition of motor activity in the open field test, and some

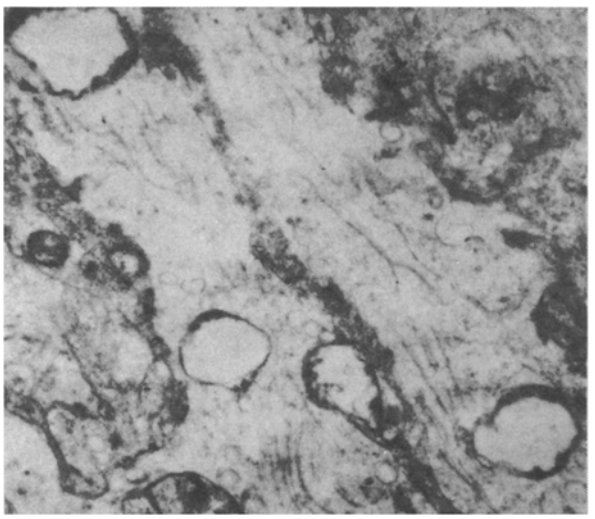

Fig. 1. Changes in ultrastructure of mitochondria in dendrites of sensomotor cortex of experimental rat aged 14 days (5800 ×).

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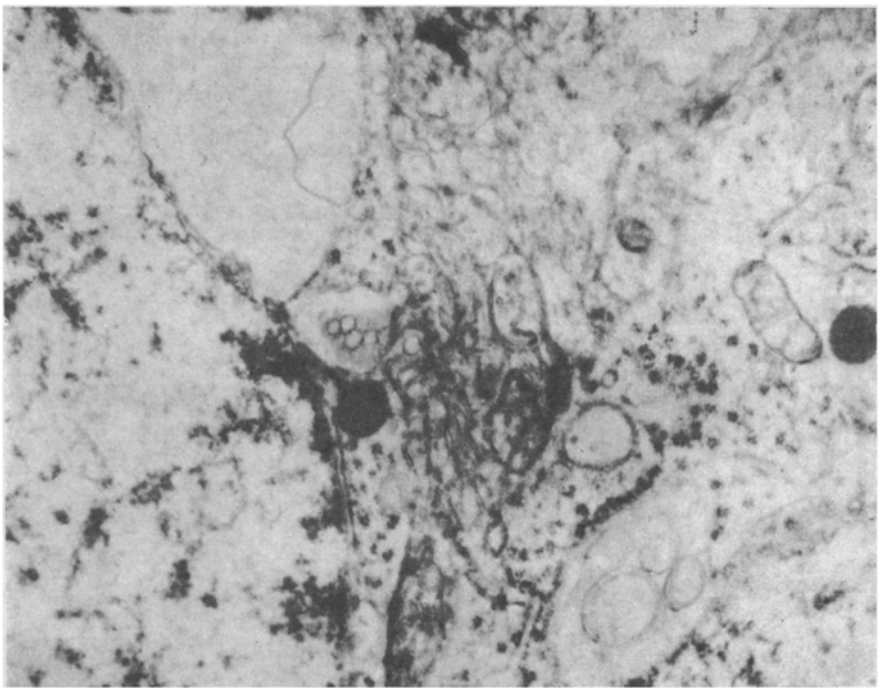

Fig. 2. Foci of destruction in sensomotor cortical neurons of experimental rat aged 21 days (7000 ×).

enhancement of the investigative reaction at the age of 2 months [7]. The aggressive reaction of experimental rats aged 1 month is considerably depressed, and the rate of formation and consolidation of a defensive conditioned reflex is reduced in the absence of morphological changes in brain structures [3].

The aim of this investigation was to study neuronal ultrastructure of the sensomotor cortex in the progeny of female rats receiving alcohol before pregnancy.

EXPERIMENTAL METHOD

The model of long-term alcoholic intoxication of female rats was developed in the Laboratory of Drug Toxicology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, where physiological investigation of the offspring was carried out as a first step. Experiments were carried out on female rats weighing initially 180-200 g, which received a 5% solution of alcohol for 1 month instead of water before mating, followed by a 10% solution of alcohol for the next 2 months. The sensomotor cortex of the progeny was investigated at the ages of 14 and 21 days. Material was treated by the standard formula; ultrathin sections were stained with uranyl acetate and lead citrate and examined and photographed in the Hitachi HV-11E electron microscope (Japan).

EXPERIMENTAL RESULTS

Prolonged alcohol consumption by female rats before pregnancy did not cause any developmental anomalies in the progeny. With respect to physical development (growth of hair, opening of the ears and eyes, and of the vagina in females) the experimental young rats did not differ significantly from intact animals, as other workers also have observed [3, 7]. However, morphological investigation of the brain of the experimental offspring revealed ultrastructural disturbances of the sensomotor cortical neurons. Membranous inclusions, bounded by a single or, more frequently, a double membrane, with transparent or moderately osmiophilic contents, were identified in the nuclei of many cortical cells of experimental rats aged 2 weeks. These intranuclear structures were found in different numbers, more or less close to one another, and more often at the periphery of the nucleus. Their appearance in the nucleus is regarded as a sign of dystrophic changes in the CNS [6]. Since membranous structures are observed in neocortical nuclei of old rats [5], their presence in the experimental rats at the age of 14 days points to depression of functional activity of the nucleus — the main component of the protein-synthesizing system — and, consequently, depression of the function of

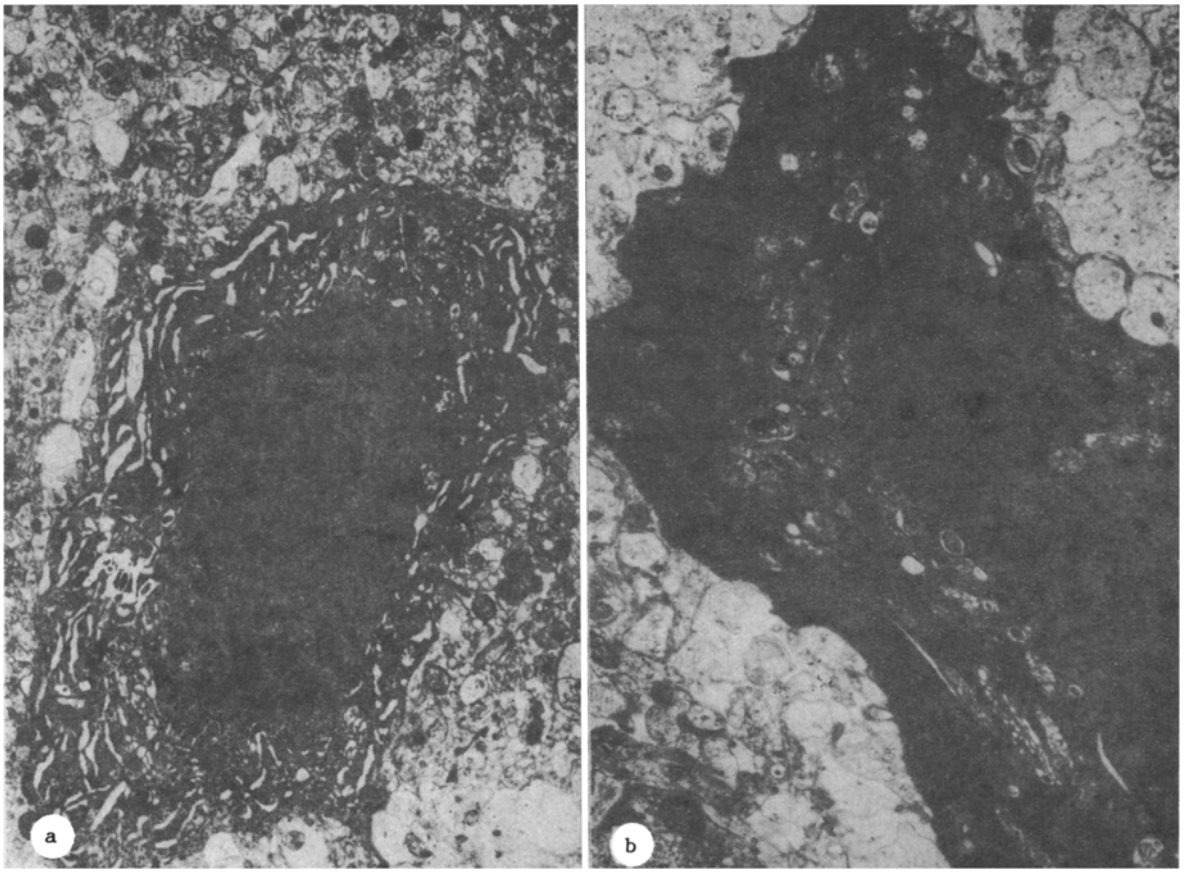

Fig. 3. Hyperchromic type II (a) or type III (b) neurons in sensomotor cortex of experimental rat aged 21 days (4800 ×). N) Nucleus.

a high proportion of cortical neurons in the offspring of females with alcoholic intoxication before pregnancy. Dystrophic changes affecting the nucleus are often accompanied by disorganization of the Golgi apparatus, the lamellar complex of which may become curved in shape, while the individual cisterns (or most of them) swell considerably until edema develops, with destruction of the limiting membrane and with blurring of the outline.

Significant changes were observed in the mitochondria, the energy-providing system of the cells. The varied degree of mitochondrial damage in the cerebral cortex of different animals, in different cortical neurons, and within the same cell, will be noted.

A varied degree of severity of mitochondrial swelling was observed with reduction of the cristae culminating in edema of the organelles. Sometimes only remnants of cristae in a few areas of the inner membrane were left of the edematous mitochondria, or the membrane could not be detected and the organelle was converted into a vacuole. The ultrastructure of mitochondria in the processes of neurons was more severely affected (Fig. 1). Similar changes in mitochondrial ultrastructure in the rat cerebral cortex have been found in various types of experimental anoxia [1] and in old animals [5, 8], which suggests the presence of oxygen deficiency of the brain in the offspring of female rats receiving alcohol before pregnancy. Besides dystrophic changes in the neurons, signs of a compensatory—adaptive character were observed in the cerebral cortex of the experimental progeny at the age of 2 weeks. These included hypertrophy of individual mitochondria, which differed widely in shape but were most frequently circular or ring-shaped, and proliferation of dense-core vesicles, concerned with the rapid transport of newly synthesized proteins [12].

Poorly differentiated neurons were found in the experimental rats of this age group, just as in intact animals aged 2 weeks. They had a more osmiophilic nucleus, compared with the pale cytoplasm, in which single, unevenly dilated cisterns of the rough endoplasmic reticulum (RER), lacking in ribosomes and polysomes over wide areas, were scattered, together with infrequent mitochondria with a few cristae and a poorly developed Golgi apparatus. In the 21-day-old offspring of females receiving alcohol, a polymorphic pattern of sensomotor cortical

neuronal ultrastructure was found. Some animals had signs of underdevelopment of the processes of their nerve cells. Dystrophic changes were preserved in the neurons of all the young rats in the form of edema of individual cisterns of the Golgi apparatus and membranous structures in the nucleus. In addition, foci of translucency in the cytoplasm, completely free from membranes or bounded only on one side by a membrane, which have been described in hippocampal neurons in the postischemic period [13], were found in some cells. On this basis it can be postulated that alcoholic intoxication in females before pregnancy has a prolonged postanoxic action on cortical neuronal ultrastructure in the offspring. Myelin-like bodies or foci of destruction, filled with floccular material, and membranous and vesicular structures (Fig. 2) could be seen in the cytoplasm of individual nerve cells, evidence of the irreversibility of the changes in groups of neurons.

The presence of at least three types of hyperchromic neurons in the cerebral cortex of some of the experimental 21-day-old rats is interesting. The type I hyperchromic cells have nuclei and cytoplasm of the normal size. The nucleus is relatively pale, with occasional masses of chromatin, and uneven or undulating in outline. Polysomes, unevenly dilated cisterns of the RER and Golgi apparatus, and swollen mitochondria with reduction of their cristae are the dominant features in the more osmiophilic matrix of the cytoplasm. Pale multivesicular bodies and single foci of translucency, lacking membranes, also are present. These neurons may correspond to moderately hyperchromic cells revealed by light microscopy, and with increased synthetic activity of their nucleus. In the type II hyperchromic cells osmiophilia of the nucleus is intensified due to enlargement of the finely granular material and of chromatin masses. The outlines of the nucleus are undulating, and the perinuclear cistern has numerous expansions. The density of the cytoplasm is determined by the large number of ribosomes; the ultrastructure of the remaining organelles is similar to that of the type I cells (Fig. 3a). It is suggested that this hyperchromophilia is equivalent to an inhibited state [4]. Hyperchromic type II cells have high electron density of their nucleus and cytoplasm, in which ribosomes are in close contact with one another, cisterns of the RER are compressed, and the mitochondria have focal translucencies and reduction of their cristae. The vacuolar component of the Golgi apparatus and single translucencies in the cytoplasm can be distinguished. The glial membrane is absent (Fig. 3b). Such cells probably correspond to the greatly hyperchromic neurons observed under the light microscope. They have low synthetic activity and are considered to be cells in the stage preceding death [2]. Compensatory-adaptive changes are more marked in the experimental rats aged 21 days than at the age of 14 days: double neurons, enlargement of the nucleolus, an increase in areas of the lamellar component of the Golgi apparatus, and hypertrophy of individual mitochondria. However, during activation of the nucleus and a tendency toward restoration of the mitochondrial ultrastructure, destructive changes still remain, suggesting that prenatal cerebral anoxia plays a role in the pathogenesis of alcohol-induced damage to cortical neurons in the offspring in the early stages of postnatal development. This evidently causes the subsequent disturbances of brain function.

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