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RESEARCH ARTICLE

The Effect of Ethanol on the Fetal Heart Muscle

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Abstract

The survey goal was to study the effect of antenatal intoxication with ethanol on embryogenesis of the fetal cardiac muscle. The experiment was conducted on 48 female rats weighing 180-200 grams. To study the embryotoxic effect of ethanol, 2 groups were created. In group I, 18 rats throughout the pregnancy received a 15% ethanol solution daily instead of water. 18 animals of the control group were kept under normal vivarium conditions. On the 13th, 18th and 21st day of pregnancy, the females were killed, removed from the cauls of living fetuses, their length, body weight and heart weight were measured.Histological preparations stained with hematoxylin and eosin were prepared from the hearts of the fetuses. The nuclear cytoplasmic ratio, capillarydensity of the capillary network and cardiomyoblasts, procardiomyocytes and cardiomyocytes were measured. The data obtained were statistically processed. In group II, 6 animals during pregnancy (from 6 to 21 days) were injected daily with an endogastric solution of 30% ethanol at a dose of 5 g / kg of body weight, and 6 animals of the control group received physiological salt solution in equivalent amounts.On the 18th day of pregnancy, the females were killed, the material taken (cardiac muscle of the fetus) was studied by electron microscopy. Our study showed that antenatal intoxication with ethanol has a pronounced embryotoxic effect on the heart muscle, which manifests itself at the cellular and subcellular levels. The value of the obtained results lies in the fact that for the first time at the electron microscopic level, ultrastructural changes in the fetal cardiac muscle during prenatal intoxication with ethanol are shown.

Keywords: *Fetal alcohol spectrum disorder, Cardiac muscle, Electron microscopy.*

Introduction

The problem of alcoholism is relevant, attracts attention and is actively studied in various aspects [1, 4]. With alcoholism, almost all organs and systems are affected. Most experimental and clinical surveys are devoted to changes in the central nervous system [5, 6]; changes in the cardiovascular [7, 8] and digestive systems [9, 10] are well studied. A special role is given to alcoholism in the family issue. The presence of an alcoholic in the family gives rise to many economic, psychological and other problems [11, 14].

Among these problems in connection with the increase in female alcoholism, the problem of the birth of children with fetal alcohol spectrum disorder (FASDs) comes to the fore [15, 16]. FASD is characterized by a combination of face dysmorphism and various intrauterine malformations. Among malformations of internal organs, heart defects are often found [17, 18]. Alcohol taken by a future mother, regardless of the duration of pregnancy, easily penetrates the placental barrier. Moreover, its concentration in the blood of the fetus corresponds to that in the blood of the mother. Due to the immaturity of enzyme systems that metabolize ethanol in the liver (alcohol dehydrogenase), the latter is able to remain in the fetal blood and tissues for a long time unchanged.

In addition, alcohol can accumulate in the amniotic fluid and remain there for a long time, thereby creating the prerequisites for prolonged direct exposure to organs and tissues of the fetus, including the heart muscle. There are a number of studies on the features of the development of fetal organs and tissues in alcoholism of the mother.

It was established that antenatal intoxication with ethanol causes a complex of changes in the placenta and developing fetal tissues [19, 24]. A research gap is the lack of works on the features of intrauterine development of the fetal heart in case of mother's alcoholism. The contribution of this article to world science lies in the fact that for the first time an electron microscopic examination of the fetal heart muscle was carried out in the experiment and it was shown that with antenatal intoxication of the fetus with ethanol after 18 days of embryonic development, pronounced dystrophic and destructive changes in the myocardial structural elements, phenomena inside and intercellular are observed edema, beginning fibrosis of the interstitial space. Along with destructive changes, compensatory and adaptive reactions developed aimed at restoring the structure and function of muscle cells. The purpose of our survey was to study the effect of antenatal intoxication with ethanol on embryogenesis of the fetal cardiac muscle.

Methods

Depending on the objectives of the study, various versions of the experiment were performed with female rats weighing 180-200 grams. The first day of pregnancy was considered the day of detection in the vaginal smears of sperm cells. In the first version of the experiment, animals were divided into 2 groups. In the experimental group of 18 rats throughout the pregnancy, instead of water, they received a 15% ethanol solution daily. 18 animals of the control group were kept under normal vivarium conditions. On 13, 18 and 21 days of pregnancy, the females were killed by decapitation under mild ether anesthesia. Extracted from the cauls of living fruits, measured their length, body weight and heart weight.Histological preparations stained with hematoxylin and eosin were prepared from the hearts of the fetuses. The nuclear cytoplasmic ratio, specific gravity of the capillary network and cardiomyoblasts, procardiomyocytes and cardiomyocytes were measured. To evaluate histological preparations, the morphometric method was used: determination of the relative number of individual structures per unit area using an ocular grid according to Avtandilov [25].

The data obtained were statistically processed. In the second version of the experiment, the animals were also divided into 2 groups. 6 animals of the experimental group during pregnancy (from 6 to 21 days) were daily administered endogastrally with a 30% solution of ethanol at a dose of 5 g / kg 6 animals of the control group were daily injected with physiological salt solution in equivalent amounts. On the 18th day of pregnancy, the females were killed by decapitation under mild ether anesthesia. To conduct electron microscopy, the taken material (fetal cardiac muscle) was fixed in a 2.5% glutaraldehyde solution in 0.2 M Millonig`s phosphate buffer (pH 7.4).

Semi-thin sections, 1-3 μm thick, were prepared on a «Tesla» ultramicrorotome. Semi-thin sections were stained with methylene blue - azure II and basic fuchsin. In this case, glycogen inclusions were crimsoned, and lipid droplets in olive greencolour. Ultrathin sections were prepared on LKB and Reichert ultra-microtomes, then contrasted with uranyl acetate and lead citrate. Observations and shooting of ultrathin sections of tissue were performed using an EVM-100 electron microscope.

Data, Analysis and Results

Our survey showed that antenatal intoxication with ethanol causes a decrease in the weight of the embryo (Table 1), its length (Table 2), and heart weight (Table 3) in experimental animals.

Table 1: The dynamics of changes in the mass of rat fetuses at different stages of embryonic development

Experiment Conditions / Experiment timeline	Control group	Ethanol
13 day	1,264±0,052	1,118± 0,043
18 day	2,217± 0,093	1,934± 0,073
20 day	3,672± 0,125	3,254± 0,133

Table 2: The dynamics of changes in the length of rat fetuses at different stages of embryonic development (mm)

Experiment Conditions / Experiment timeline	Control group	Ethanol
13 day	11,23±0,472	10,12±0,385
18 day	23,69± 0,734	19,47±0,564
20 day	34,72±1,249	29,73±1,248

Table 3: The dynamics of changes in the heart mass of rat fetuses at different stages of embryonic development (mg)

Experiment Conditions / Experiment timeline	Control group	Ethanol
13 day	12,43± 0,447	11,57± 0,335
18 day	21,74± 0,412	19,11± 0,706
20 day	38,73±1,356	35,62± 1,318

A study of the nuclear-cytoplasmic ratio (N:C ratio) of cardiomyoblasts, procardiomyocytes, and cardiomyocytes showed that in control animals this indicator decreases as the fetus grows. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the intensity of decrease in control animals is higher, which indicates more active processes of differentiation of muscle cells (Table 4).

Table 4: The dynamics of changes in N: C ratio in the cardiac muscle cells of rat fetuses at different stages of embryonic development (c.u.)

Experiment Conditions / Experiment timeline	Control group	Ethanol
13 day	0,351±0,013	0,394± 0,018
18 day	0,247± 0,009	0,265± 0,012
20 day	0,182±0,009	0,200±0,008

A study of the dynamics of changes in the specific density of cardiac muscle cells of rat fetuses at different stages of embryonic development showed that in control animals this indicator increases with the growth of the fetus. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the intensity of increase in control animals is higher, which indicates more active processes of differentiation of muscle cells and growth of the heart muscle (Table 5).

Table 5: The dynamics of changes in the specific density of cardiac muscle cells of rat fetuses at different stages of embryonic development (c.u.)

Experiment Conditions / Experiment timeline	Control	Ethanol
13 day	39,54 ± 1,321	35,63 ± 1,724
18 day	58,63 ± 2,697	55,49 ± 1,984
20 day	61,82 ± 2,411	58,80 ± 2,234

A study of the dynamics of changes in the specific gravity of the capillary network of the ventricular myocardium in rat fetuses at different stages of embryonic development showed that in control animals this indicator increases as the fetus grows. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the increase in control animals is higher, which indicates more active processes of the development of the capillary network, and therefore, the creation of more favorable trophic conditions for the development of cardiac muscle cells (Table. 6).

Table 6: Dynamics of changes in the specific density of the capillary network of the myocardium of rat fetuses at different stages of embryonic development (c.u.)

Experiment Conditions / Experiment timeline	Control	Ethanol
13 day	24,32 ± 1,037	19,64 ± 0,783
18 day	27,51 ± 1,045	22,44 ± 0,695
20 day	28,62 ± 1,031	23,13 ± 0,879

As a result of electron microscopy, it was found that in control animals on the 18th day of myocardial embryonic development contains muscle cells of various degrees of differentiation: cardiomyoblasts, cardiopromiocytes and cardiomyocytes

Cardiomyoblasts were characterized by a polygonal or oval shape and contained a large nucleus with smooth contours of the nuclear membrane (Fig. 1). The distribution of condensed chromatin was primordial in some areas of the karyoplasm. The perinuclear space is narrow. The cytoplasm contained a large number of free ribosomes, polyribosomes, and glycogen rosettes. Narrow and long, single tubules of the granular endoplasmic reticulum communicated with the perinuclear space and were equipped

with a large number of fixed ribosomes.Mitochondria were few, large, irregular in shape with a matrix of medium electron density and a small number of cristae. Krista was located transverse to the axis or randomly. Cardiomyoblasts located usually loosened. Intercellular connections were represented by simply arranged contacts of little-developed cell membranes. Small cell vesicles and short randomly located myofilaments were occasionally found in the cytoplasm of cells.

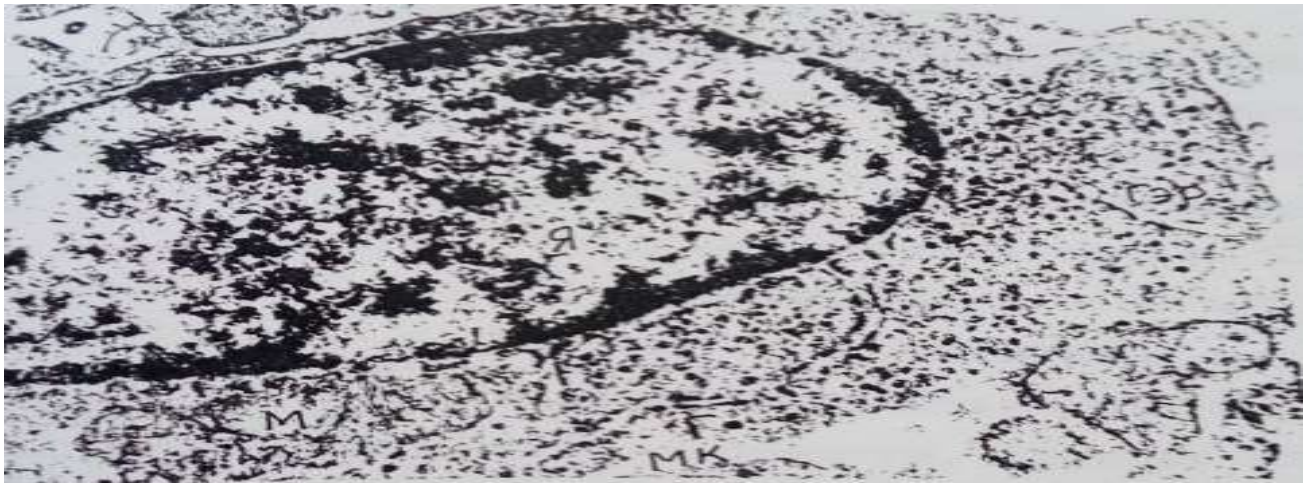

Figure 1: 18th day. Control group. Embryonic development. Cardiomyoblast. The abundance of polyribosomes (PR) and glycogen granules (G), Я- nucleus, RER - rough endoplasmic reticulum, M - mitochondria, MK - intercellular simple cell junctions. Electron diffraction pattern. x7 800

Cardiopromiocytes were characterized by an elongated shape and contained an oblong nucleus with wavy contours of the nuclear membrane (Fig. 2). The distribution of granular chromatin was mostly primordial. The perinuclear space is narrow. The cytoplasm contained a significant number of ribosomes, polyribosomes and glycogen granules, especially along myofibrils. The number of organelles, compared with cardiomyoblasts, increased. Mitochondria had a polymorphic shape, a matrix of uneven electron density, long convoluted or transverse cristae. The tubules of the rough endoplasmic reticulum were narrow with a large number of fixed ribosomes. Often they were arranged in orderly long parallel rows. Mostly near the nucleus were lamellar and vesicular structures of the Golgi complex. Also near the nucleus were short bundles of myofilaments with primary Z - membranes, which are presumably the growth points of the myofibrillar apparatus. Between the enlarging gaps of neighboring cells, simple cell junctions of cell membranes were visible.

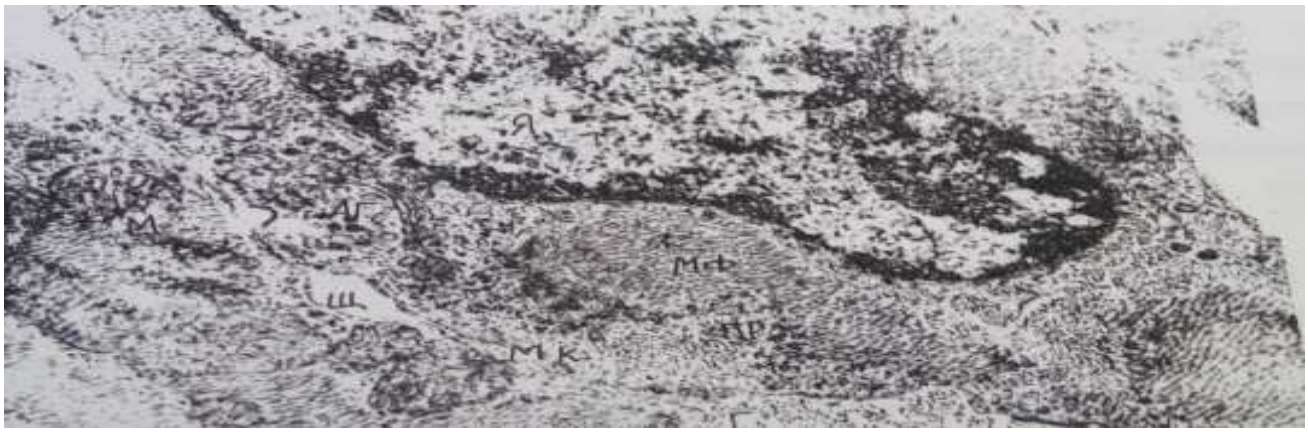

Figure 2: 18th day. Control group. Embryonic development. Cardiopromyocyte. Short bundles of myofilaments (MF), polyribosomes (PR), Golgi appendix vesicles and membranes (AG), glycogen granules (G). Between the gaps (A) of neighboring cells are simple cell junctions (CJ). Electron diffraction pattern. x14 400

Cardiomyocytes had an elongated shape and a higher degree of differentiation (Fig. 3). In the center was a large, oblong nucleus with smooth contours of the nuclear envelope and a primordially located condensed chromatin. The perinuclear space was narrow. In the cytoplasm, the number of myofilament bundles located near the nucleus and along the periphery of the cell increased. Transverse striation of fibrils due to I - and A

- disks was detected. Mitochondria took an ordered arrangement along bundles of filaments, around the nuclear envelope, under the plasmalemma. They had a matrix of medium electron density and a large number of transversely arranged cristae. Cisternae of the Golgi complex, represented by flattened membranes and small vesicles, were noted. In addition, weakly developed loose tubular and vesicular components of the sarcoplasmic reticulum were observed in the cytoplasm. The intercellular spaces increased. Direct insertion plates were formed in the form of desmosome-like structures, providing more durable contacts of muscle cells.

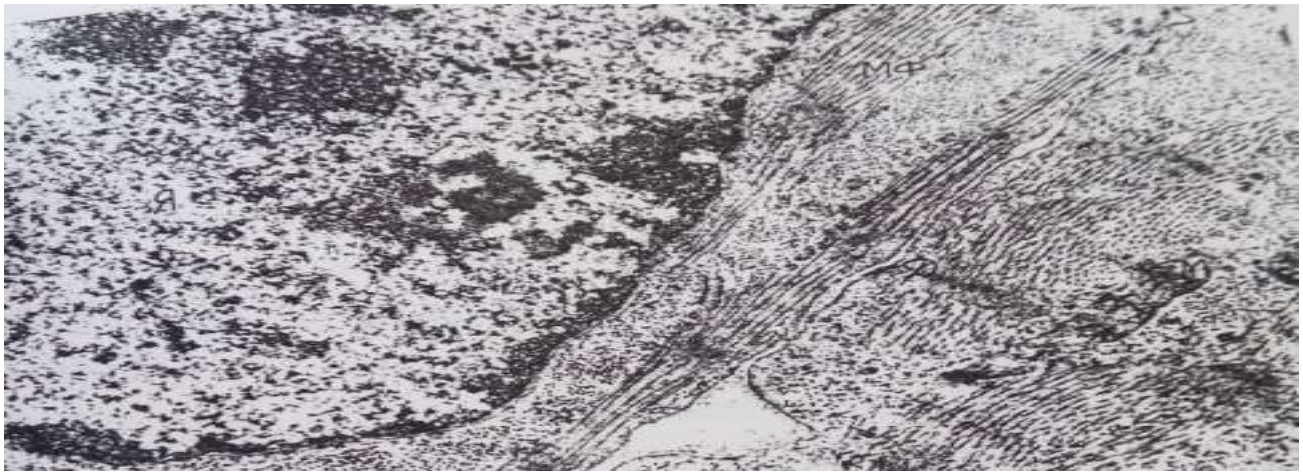

Figure 3: 18th day. Control group. Embryonic development. Cardiomyocyte In the cytoplasm there are bundles of myofibrils (MF), mitochondria (M), ribosomes and glycogen granules. D - desmosome-like bonds between cells. Я-nucleus. Electron diffraction pattern. x14 400

Primary capillaries were characterized by a low degree of differentiation and consisted only of thickened endothelium (Fig. 4). Large nuclei occupied almost the entire volume of the central part of the endothelial cell and were characterized by an even distribution of lumps of condensed chromatin. The nuclear envelope had wavy contours. The outer nuclear membrane was equipped with a large number of ribonucleoproteins.Numerous organoids were visible in the cytoplasm: narrow tubules of the rough endoplasmic reticulum, large, round mitochondria, cisternae of the Golgi complex, ribosomes and polyribosomes. The number of pinocytotic vesicles was insignificant. The apical surface of the endothelium was smoothed with flattened cytoplasmic outgrowths. Rare intercellular compounds belonged to the type of simple contact. Along the basal part of the plasmolemma, the basement membrane was absent; occasionally, we noted a loose fibrillar component.

Figure 4: 18th day. Control group. Embryonic development. The peripheral part of the capillary endothelium is thickened and contains a large number of ribosomes (RS) and tubules of the rough endoplasmic reticulum (RER). Electron diffraction pattern. x14000

Electron microscopy revealed that on the 18th day of embryonic development with antennal ethanol intoxication, the cardiomyoblasts had a round or slightly oblong shape with uneven contours of the plasma membrane (Fig. 5). The oval or slightly irregular nucleus contained a moderate amount of chromatin evenly distributed in the karyoplasm. The perinuclear space was narrow, in some areas

expanded and passing into the tubules of the granular endoplasmic reticulum. Medium-sized polymorphic forms of mitochondria had a matrix of medium electron density and vacuolated cristae. Many mitochondria underwent myelination, homogenization with the formation of autophagolysosomes. The cisternae of the granular endoplasmic reticulum were narrow or slightly dilated. Their gaps were filled with flaky material. The tubules of the rough endoplasmic reticulum located on the periphery of the cell lost fixed ribosomes. The plasma membrane had uneven contours and, in some areas, underwent microclasmosis and destruction. Cardiomyoblasts were located loosely. Celljunctions were very rare compared to normal. The contacting surfaces of the cells occupied a small contact area.

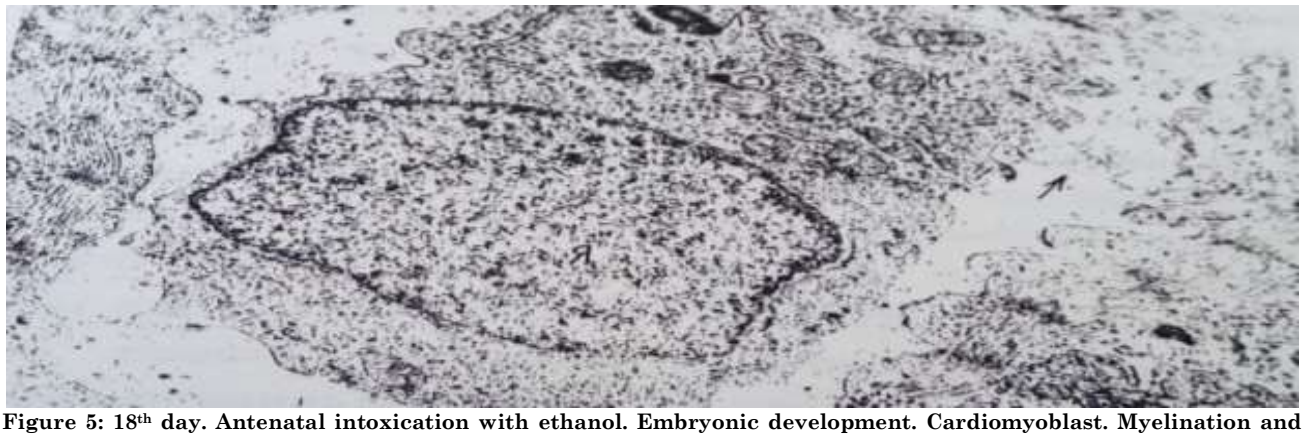

Figure 5: 18th day. Antenatal intoxication with ethanol. Embryonic development. Cardiomyoblast. Myelination and homogenization of mitochondrial cristae (M), the appearance of lysosomes (LZ). Microclasmatosis (indicated by arrow). Я- nucleus. Electron diffraction pattern. x15 600

Cardiopromiocytes were characterized by apparent intracellular edema (Fig. 6). A small oval-shaped nucleus was located in the center. Chromatin was dispersed, a thin strip of condensed chromatin was primarially located. Most of the hyaloplasm was enlightened and filled with edematous fluid, pushing myofibrils and organelles to the periphery of the perinuclear region. Myofibrillar fibers located along the plasmalemma underwent lysis and the disappearance of transverse striation. Mitochondria were large. Cristas of most mitochondria underwent destruction, lysis, myelination. Some mitochondria swelled with matrix enlightenment and vacuolization of cristae. The tubules of the rough endoplasmic reticulum were single, partially vacuolated and lacked fixed ribosomes. The number of glycogen granules, free ribosomes and polyribosomes sharply decreased. The number and extent of cell junctions decreased. Often we noted the phenomena of pronounced intercellular edema.

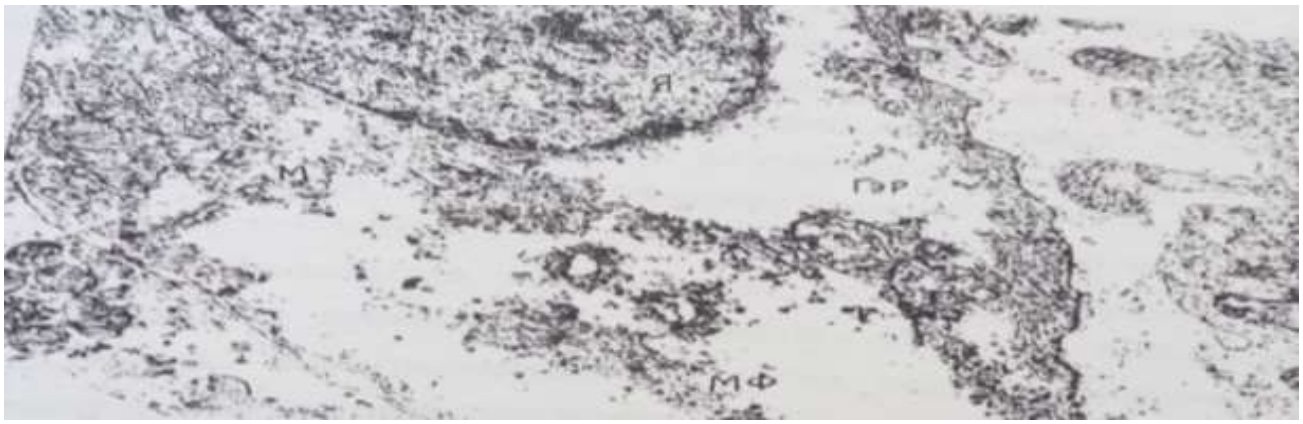

Figure 6: 18th day. Antenatal intoxication with ethanol. Embryonic development. Severe edema of the cytoplasm of the cardiopromyocyte with enlightenment of the hyaloplasm, myofibrils (MF) are lysed. Destruction and myelination of mitochondrial cristae (M), reticulum vacuolization (RER). Я- nucleus. Electron diffraction pattern. x14 400

Cardiomyocytes were also characterized by symptoms of apparent intracellular edema. The rounded nuclei possessed smooth contours of the nuclear envelope and dispersed chromatin distribution. The outer nuclear membrane was poor in ribosomal granules. In the sharply clarified hyaloplasm, mitochondria polymorphic in size and shape with a matrix of medium electron density and a large number of cristae were located.

The tubules of the roughendoplasmic reticulum were expanded, most of them lost ribosomes and turned into small vacuoles. The Golgi complex was in a state of atrophy and was represented by small vesicles. Myofibrils underwent severe osmiophilia or lysis. The number of free ribosomes, glycogen granules was reduced. The plasmalemma had uneven contours with small protrusions and partial loosening. The intercellular space has been expanded. Cells were connected by single desmosome-like junctions. Individual cardiomyocytes underwent partial (Fig. 7) or complete destruction. In this case, the integrity of the plasma membrane was interrupted with the release of organelles, membrane and granular material into the intercellular space. Collagenoblasts with high collagen-producing activity, collagen fibrils were found in the intercellular space.

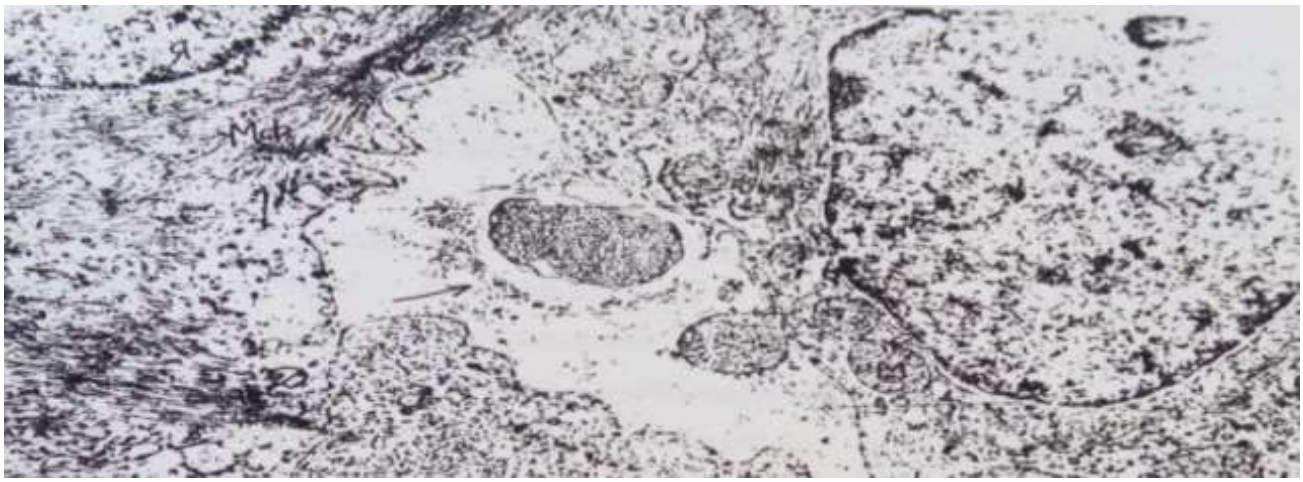

Figure 7: 18th day. Antenatal intoxication with ethanol. Embryonic development. Destruction of the plasmalemma (indicated by the arrow) of the cardiomyocyte with the release of organelles and granular material into the intercellular space. Я- nucleus. Lysis, excretion and osmiophilia of myofibrils (MF). Desmosome-like junctions (D) are single. Electron diffraction pattern. x16 800

The vascular wall of the capillaries was characterized by the appearance of a loose basement membrane, which is absent in the normal range. We regard this phenomenon as a compensatory reaction of capillaries to the toxic effects of alcohol. The capillary endothelium was characterized by hypertrophy and swelling of mitochondria with lysis and myelination of cristae, partial vacuolization of the rough endoplasmic reticulum, fuzzy contours, microclazomatosis of the apical surface, sharp loosening of the basement membrane (Fig. 8).

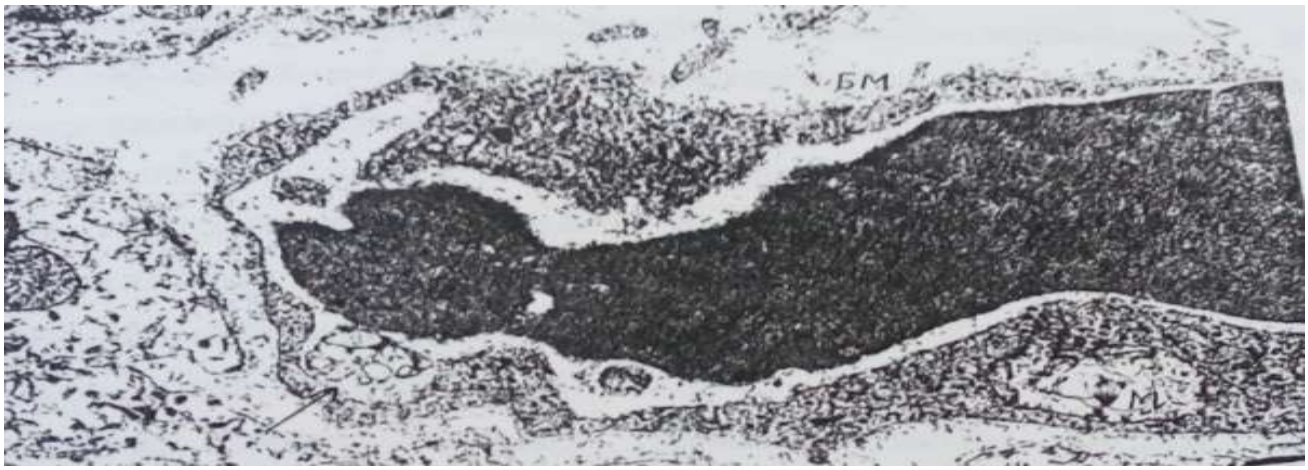

Figure 8: 18th day. Antenatal intoxication with ethanol. Embryonic development. Capillary. Visible is the myelination of mitochondrial cristae (M), the phenomena of microplasma (indicated by an arrow). The basement membrane (BM) is loosened. Electron diffraction pattern. x12000

Discussions

An experimental study of fetal cardiac muscle embryogenesis showed that antenatal intoxication with ethanol causes a delay in fetal development, which is manifested by a decrease in their weight (Table 1), length (Table 2) and heart weight (Table 3). The difference between the indices of the experimental and control groups is observed on the 13th day; it persists on the 18th and 20th days of embryogenesis.

A study of the nuclear-cytoplasmic ratio (N: C ratio) of cardiomyoblasts, procardiomyocytes, and cardiomyocytes showed that in control animals this indicator decreases as the fruit grows. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the intensity of decrease in control animals is higher, which indicates more active processes of differentiation of muscle cells (Table 4).

A study of the dynamics of changes in the specific density of cardiac muscle cells of rat fetuses at different stages of embryonic development showed that in control animals this indicator increases with the growth of the fetus. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the intensity of increase in control animals is higher, which indicates more active processes of differentiation of muscle cells and growth of the cardiac muscle (Table 5).

The study of the dynamics of changes in the specific density of the capillary network of the rat myocardium at different stages of embryonic development showed that in control animals this indicator increases with the growth of the fetus. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, it can be seen that the increase in control animals is higher, which indicates more active processes of the development of the capillary network, and therefore, the creation of more favorable trophic conditions for the development of cardiac muscle cells (Table-6). Electron - microscopically, it was found that on the 18th day of the embryonic period, the myocardium contains muscle cells of various degrees of differentiation: cardiomyoblasts, cardiopromyocytes and cardiomyocytes.

In the group with antenatal intoxication with ethanol, significant ultrastructural changes are noted in comparison with the control group. In the cardiac muscle of the fetus, pronounced dystrophic and destructive changes in muscle cells and capillary endothelium were observed, a significant decrease in the level of differentiation of muscle cells with a sharp decrease in myofibrillar fibers and intercellular connections, a phenomenon inside and intercellular edema, beginning of interstitial fibrosis. Along with destructive changes, compensatory - adaptive reactions aimed at restoring the structure and function of muscle cells also developed. The vascular wall of the capillaries was characterized by the appearance of a loose basement membrane, which is absent in the normal conditions. We regard this phenomenon as a compensatory reaction of capillaries to the toxic effects of alcohol. Assessing the slowdown in fetal growth, it can be assumed that the cause of intrauterine growth retardation is the toxic effect of alcohol.

The toxic effects of alcohol on fetal tissue are based on the characteristics of its pharmacokinetics and metabolism in the placenta. The metabolism of ethanol in the tissues of the placenta and the fetus is carried out through the same enzymes. The only isoform of alcohol dehydrogenase present in the placenta is practically not involved in the oxidative metabolism of ethanol, as a result of which it freely penetrates the bloodstream of the fetus.

Under the action of ethanol and acetaldehyde, there is inhibition of adhesion, migration and proliferation of trophoblast, as well as a violation of the processes of remodeling of the spiral uterine arteries. Ethanol has a profound vasoconstrictive effect on the vessels of the umbilical cord, while the umbilical arteries respond to lower concentrations of ethanol and are characterized by a greater degree of contractility compared to veins. Alcohol-induced decrease in uteroplacental blood flow leads to a decrease in placental mass. An increase in perfusion pressure in the vessels of the placenta contributes to the disruption of oxygen transport and the development of fetal acidosis [20], which results in a delay in fetal development. We suggest that a delay in the intrauterine development of the fetus with fetal alcohol spectrum disorder may also be due to a lack of receptors for the transforming growth factor.

It was found that the concentration of transforming growth factor in the blood of pregnant women who abuse alcohol is much higher than in women who are free from this addiction (71.7 and 6.6 ng / ml, respectively). The results obtained may indicate a deficiency of receptors for this growth factor associated with exposure to ethanol [26].

It should be noted that the delay in embryonic growth may be associated with an increase in the activity of ornithidine decarboxylase, which plays an important role in the processes of embryonic growth. Induction of the activity of ornithidine decarboxylase leads to the synthesis of polyamines, which precedes DNA replication and cell division. In chicken embryos, the activity of ornithidine decarboxylase falls to a very low level at 144 hours of incubation, while ethanol causes an increase in its activity, which affects subsequent cell division processes and, accordingly, causes a delay in embryonic growth [27].

The results concerning intrauterine growth retardation are reliable; this is confirmed by the data of statistical processing of our material, similar results obtained in the clinic and experiment [28, 30], as well as electron microscopic data obtained by us. The results obtained during the survey are of undoubted value, since the electron-microscopic data that we obtained are new data in the structure of knowledge known to mankind.

Conclusion

The article presents valuable scientific data not previously known. Electron microscopic examination revealed that antenatal intoxication with ethanol on the 18th day of embryonic development causes pronounced dystrophic and destructive changes in muscle cells and capillary endothelium, a significant decrease in the level of differentiation of muscle cells with a sharp decrease in myofibrillar fibers and cell junctions, the phenomenon of intracellular and intercellular edema, beginning fibrosis interstitial space.

Along with destructive changes, compensatory - adaptive reactions aimed at restoring the structure and function of muscle cells also developed. Antenatal intoxication with ethanol at the cellular level is manifested by a delay in the differentiation of cardiac muscle cells, a decrease in the rate of increase in their specific gravity and specific gravity of the myocardial capillary network in rat fetuses at different stages of embryonic development.

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