ULTRASTRUCTURAL FEATURES OF THE MYOCARDIUM OF NEWBORN MINI-PIGS AFTER CHRONIC PARENTAL ALCOHOLIZATION

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The reproductive function is disturbed in women who are chronic alcoholics, and frequently give birth to infants with various disturbances. Actively, these disturbances, which include delayed antenatal and postnatal development, craniofacial dysmorphia, and visceral anomalies, have been described as the fetal alcoholic syndrome (FAS) [3, 6, 10, 13]. It is now considered firmly established that FAS is linked with alcoholism in women, and that severity depends on the duration of alcohol consumption. However, the successive changes of the embryotoxic action of ethanol and the pathological changes caused by it in the progeny have not yet been adequately studied in relation to the duration of alcohol consumption. The characteristic pathology observed in the progeny of alcoholic parents, especially mothers, is the development of alcoholic cardiomyopathy. The study of the pathogenesis of these cardiomyopathies is very difficult because they are inadequately represented by traditional models.

The aim of this investigation was to create a model in which the conditions of alcohol poisoning would produce as closely as possible the fetal alcohol syndrome.

EXPERIMENTAL METHOD

The model was created on mini-pigs of the Svetlaya Gora population. It has been shown on this model that about 50% of pigs of both sexes readily consume a 25% solution of ethanol in water together with their food in a dose of 2.5-3.0 g/kg live weight, calculated as 100% ethanol. It has also been discovered that between 1 and 1.5 years after regulatory voluntary consumption of this dose of alcohol, several features of alcohol dependence are formed in the pigs. The animals know and await the time of receiving ethanol, they drink it actively and greedily, and quickly fall asleep after taking alcohol. The study of the newborn progeny and, in particular, of the action of alcohol consumed by the mother, on the fetal myocardium began 2 years after regular consumption of alcohol by the animals. Experiments were carried out on the male offspring of mini-pigs, the parents having received the above dose of ethanol 5 times a week for 2 years. Ethanol was given once each day at the same time. The interval between cycles was 2 days. The mother continued to receive ethanol regularly throughout pregnancy. Offspring of healthy parents, which had never taken alcohol, and which were of the same weight and age as the alcoholic mothers, served as the control. Altogether 10 experimental and three control sows took part in the experiments. The average weight of the experimental animals was 700-800 g and of the controls 800-900 g. The animals were killed on the 4th day after parturition by immediate decapitation under ether anesthesia. Within 3 min thoracotomy was performed and a fragment of tissue excised from the left ventricle of the working heart, and placed in 2.5% glutaraldehyde solution in 0.05 M cacodylate buffer with sucrose. Subsequent treatment of the material was in accordance with the usual laboratory technique. The material was embedded in a mixture of Epon and Araldite. For orientation in the block, semithin sections were cut to a thickness of 1-2 μm and stained with toluidine blue; after selection of the appropriate area, ultrathin sections were cut and examined in the IEM-100C electron microscope.

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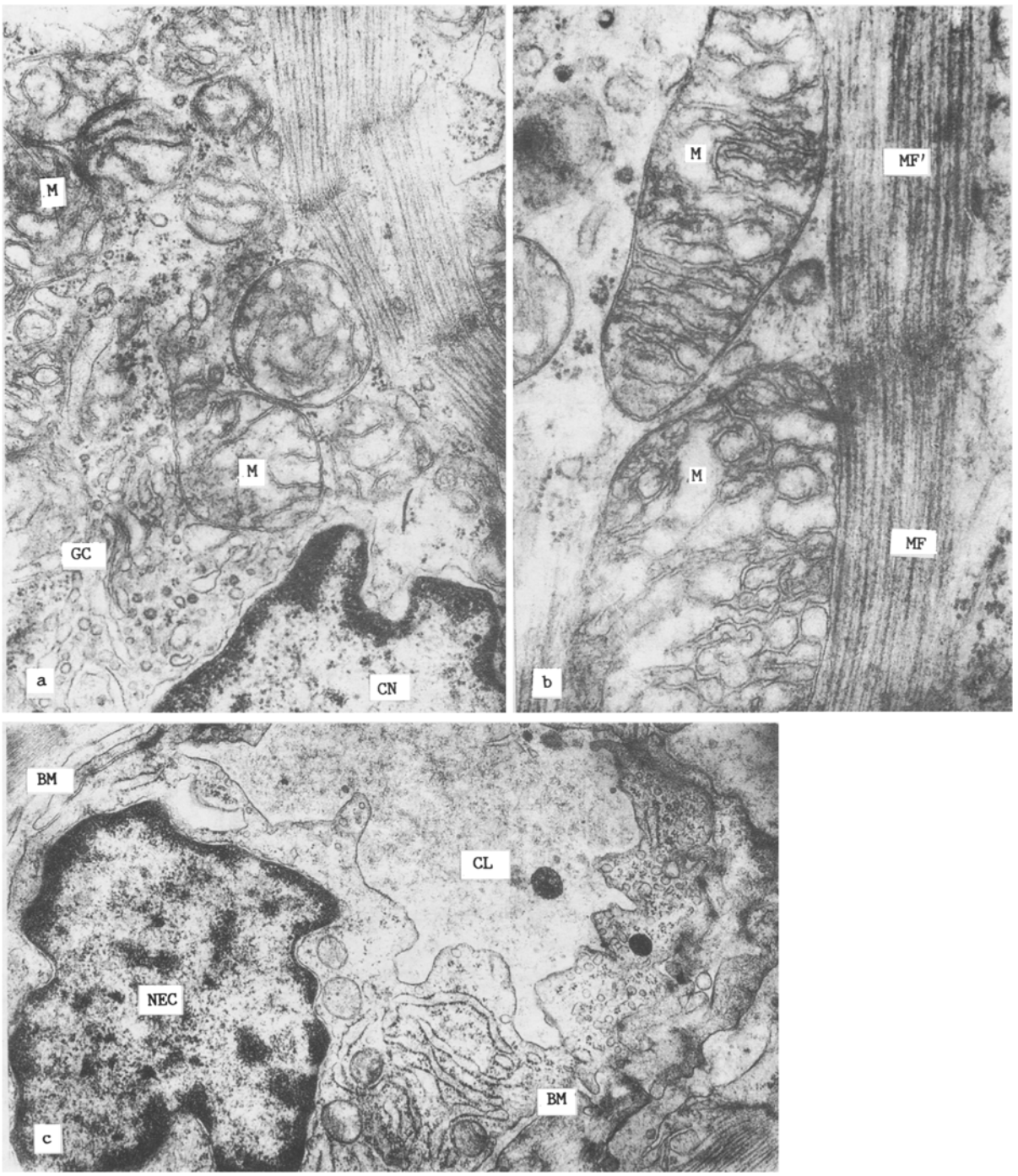

Fig. 1. Ultrastructure of myocardium of a control mini-pig. a) Fragment of myocardium of newborn piglet. Control. CN) Cardiomyocyte nucleus, M) mitochondria, GC) Golgi complex, 30,000×. b) Fragment of myocardium of newborn piglet. Control. M) Mitochondria of cardiomyocyte, MF) myofibrils. 50,000×. c) Fragment of myocardium of newborn piglet. Capillary. Control. NEC) Nucleus of endothelial cell, BM) basement membrane, CL) capillary lumen. 20,000×.

EXPERIMENTAL RESULTS

In the control group the heart muscle of the newborn piglets consisted of cardiomyocytes, in the central part of which was located a long or oval nucleus, containing one or two dense nucleoli. The chromatin formed irregular concentrations, being located mainly near the inner nuclear membrane (Fig. 1a). The perinuclear space was a little wider than normally. Myofibrils

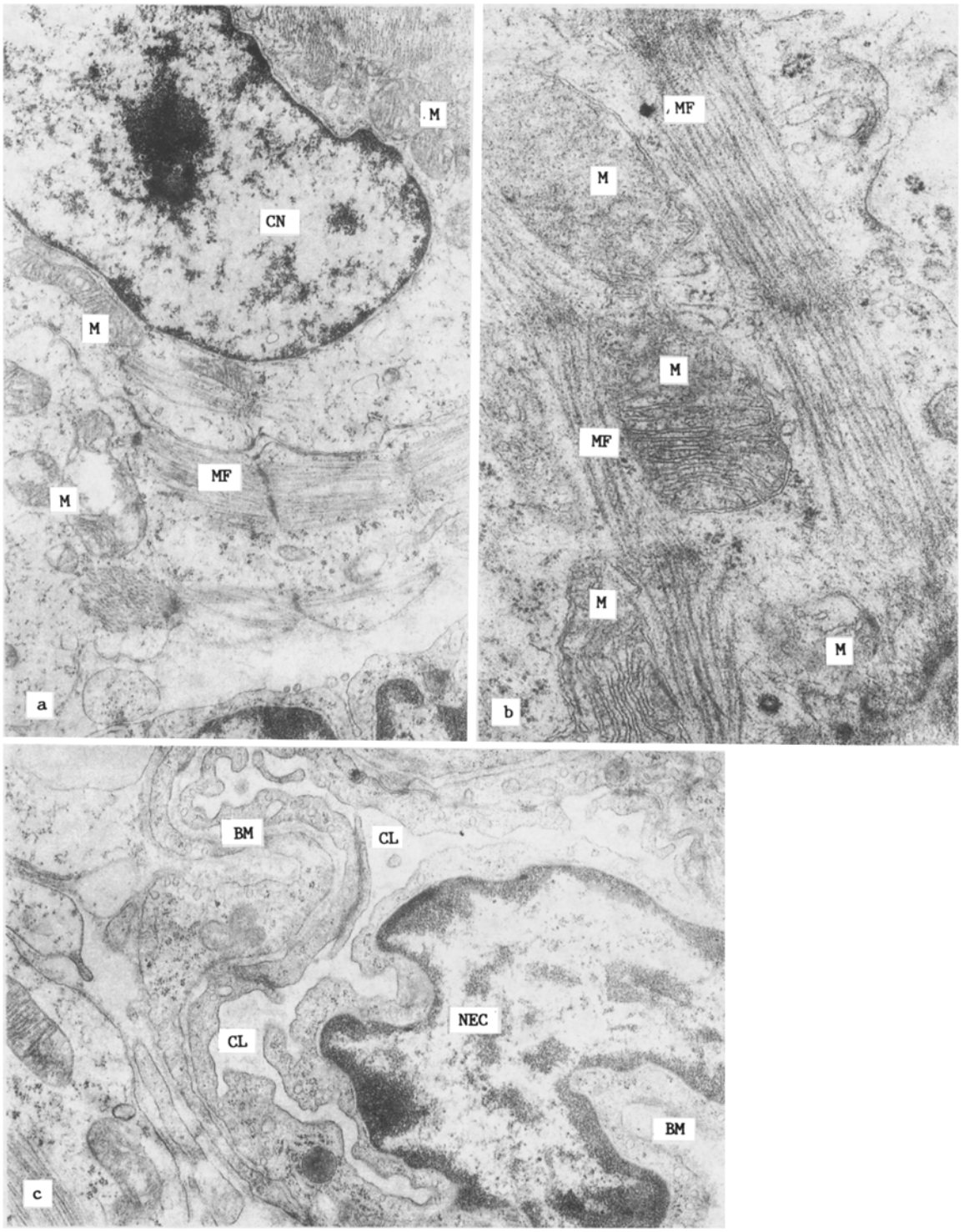

Fig. 2. Ultrastructural changes in myocardium of experimental mini-pig. a) Fragment of myocardium of newborn piglet. Experiment. CN) Cardiomyocyte nucleus, M) mitochondria, MF) myofibrils. 12,000×. b) Fragment of myocardium of newborn piglet. Experiment. M) Mitochondria of cardiomyocyte, MF) Myofibrils. 40,000×. c) Fragment of myocardium of newborn piglet. Capillary. Experiment. NEC) Nucleus of endothelial cell, BM) basement membrane, CL) capillary lumen. 16,000×.

occupied a large part of the cardiomyocyte. Between them lay mitochondria with a translucent matrix. Their tubular cristae were twisted and branched. Spaces between the cristae were wide (Fig. 1a, b). Between the myofibrils were concentrations of glycogen. The cytoplasmic reticulum was well developed and formed aggregations at the level of the T system. Many ribosomes were found

in the sarcoplasm, mainly in groups. The Golgi complex was close to the nucleus and consisted of a system of tubules and vesicles, including rough forms (Fig. 1a). At the level of z-disks the myocardial cells were in close contact with one another. Blood capillaries, their lumen often gaping, lay next to the cardiomyocytes (Fig. 1c). The basement membrane was clearly identifiable. Nuclei of the endothelial cells projected into the capillary lumen. Chromatin was distributed in clumps. The luminal border of the cytoplasm of the endothelial cells had uneven outlines. Solitary processes of cytoplasm projected into the capillary lumen. Adjacent endothelial cells made contact with one another either through interdigitation of their processes or through the formation of tight junctions. The cytoplasm of the endothelial cells contained the ordinary set of organelles, and also numerous pinocytotic vesicles, some of which were rough.

Evidence of intracellular edema in the heart muscle was found in the experimental group in the offspring of alcoholic parents. Meanwhile edema of the interstitial connective tissue also was present, so that the intercellular spaces were considerably widened (Fig. 2a).The nucleoplasm of the cardiomyocytes was edematous. The nuclei were translucent. Small concentrations of chromatin were scattered all over the nucleus, and the inner nuclear membrane formed a narrow electron-dense band.

The myofibrils were disunited and thin. Their pattern was disturbed (Fig. 2b). In many areas some myofibrils were broken off at the z-disk level. The mitochondria varied in shape: rodlike, circular, triangular. Some mitochondria preserved their usual structure, but total or partial edema could be seen in many of them (Fig. 2a). The matrix of the preserved mitochondria was condensed and the cristae in many of them were erect and arranged densely one against the other in the form of a palisade (Fig. 2a, b). Tubules of the rough cytoplasmic reticulum were fragmented and considerably dilated. The membranes of the rough cytoplasmic reticulum were free from ribosomes over large areas. The number of free ribosomes visible in the sarcoplasm was less than in the control group. The Golgi complex was located near the nucleus and consisted of dilated tubules and vesicles of varied diameter, including some of a rough kind. Concentrations of glycogen were present in the cytoplasm. Sometimes pale and dark cells could be seen in the preparations. The impression was created that the changes described above in the organelles were more marked in the pale cardiomyocytes.

Most blood capillaries were in a collapsed state (Fig. 2c). The endothelial lining was reduced in thickness. The nuclei of the endothelial cells almost completely occupied the lumen of the capillary. Coarse-dispersed chromatin was arranged in large clumps. The luminal border of the cytoplasm of the endothelial cells was uneven in outline, forming folds and outgrowths. The intercellular spaces were often dilated. Many pinocytotic vesicles, some of them rough, were seen in the cytoplasm of the endothelial cells, especially near their luminal border. The basement membrane was widened in some places and its noncellular component was reduced in density.

The pathogenesis of the FAS has not yet been adequately studied. It is assumed than ethanol, which has a lipotropic action, passes through the placenta into the fetal blood. Ethanol is not hydrolyzed in the fetus and its excretion is considerably reduced, as a result of which toxic acetaldehyde accumulates in the fetal tissues, and this may be the cause of development of the FAS. There is also evidence that ethanol and its toxic metabolites can induce spasm of the umbilical vessels, leading to a disturbance of transplacental transport of nutrients and fetal hypoxia [7]. Ultrastructural changes found in the cardiomyocytes of the offspring of alcoholic mini-pigs also have been observed in adult alcohol-consuming experimental animals and in human alcoholics [2, 4, 8, 11, 12]. They indicate that ethanol has a toxic effect on the myocardium. Most investigators consider that the toxic action of ethanol on the heart and viscera is based on activation of lipid peroxidation [1, 5, 9, 14], increased membrane permeability, and uncoupling of oxidation and phosphorylation in the mitochondria, which is accompanied by depression of protein synthesis and of the intensity of cellular respiration [9, 15, 16]. Besides changes in the mitochondria, there was also a clearly visible decrease in the number of ribosomes both on membranes of the rough cytoplasmic reticulum and in the sarcoplasm. The state of myocardial hypoxia in all probability was aggregated by narrowing of the capillary lumen, and collectively these changes facilitate the development of total or partial edema of the cardiomyocyte as a whole, including the mitochondria.

The morphological changes observed thus point to the formation of an alcoholic cardiomyopathy, and the investigation showed that mini-pigs of the Svetlaya Gora population can be used for models of fetal alcoholic cardiomyopathies.

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TREATMENT OF BURNS WITH CULTURED FIBROBLASTS

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An important problem in the treatment of burns is the search for effective methods of treatment of extensive burns, for which skin autografting is difficult because of a deficiency of the patient's skin. New opportunities in this field have been opened up by recent achievements in biotechnology, making the obtaining of skin substitutes a real possibility. There have been several independent reports of positive results of the use of skin substitutes based on cultures of epidermis [3-5, 8]. However, a long time (over 3 weeks) is needed to obtain the principal component of substitutes of this kind, namely a cultured layer of epidermis [5], and its use does not exclude the possible development of a graft rejection reaction [6]. Wound infection, which is the rule with extensive burns, greatly reduces the efficacy of skin substitutes [7]. The use of autologous epithelial cells for the production of cultured epidermal layers makes it virtually impossible to create "banks" of these substitutes.

For the reasons mentioned above, the search for new approaches to the solution of this problem is necessary. We therefore studied the possibility of using cultured fibroblasts for the treatment of extensive burns. By using a culture of fibroblasts to treat burns, we took into account the regulating effect of fibroblasts on wound healing [2] and also Sarkisov's hypothesis [1] of the polypotency of the properties of certain cells of mesenchymal origin and, in particular, the cells known as pericytes.

EXPERIMENTAL METHOD

Fibroblasts obtained by culture were transplanted to 11 patients at the All-Union Burns Center. The area of the burn wounds varied from 30 to 75% of the body surface. No special preparation of the wounds was carried out before transplantation of fibroblasts. The treatment program was worked out in the department. In nine cases transplantation was carried out on burns of the IIIa-IIIb and IIIb degree, with fine pink granulations and with no evidence of suppuration, bleeding, edema, or hypergranulation, and in two cases on skin donation sites slow to heal. The area of the grafts varied from 28 to 280 cm^2. A graft of fibroblasts was applied to the wound at a distance of 0.5-1 cm from its edges, and the graft was then covered with petrolatum

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