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# MORPHOLOGY OF CONTRACTILE CARDIOMYOCYTES AND VESSELS IN PREGNANT RATS AND THEIR PUPS EXPOSED TO HEMIC HYPOXIA

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## Summary

The performed morphological analysis of cardiomyocytes and myocardial vessels in 12 pregnant rats and 16 newborn pups exposed to the hemic hypoxia induced by $NaNO_2$ revealed some similar degenerative and destructive changes which were more pronounced in pups. The main morphological characteristics of hypoxic myocardial damage in pregnant and newborn rats were presented in the form of phenomena of mixed dystrophy, swelling and destruction of endothelial cells and contractile cardiomyocytes along with the lysis of myofibrils, perivascular edema, capillary hyperemia, emptiness and spasm of the arterioles and contracture changes. Revealed pathological changes in the myocardium reflect the onset of myocardiodystrophy processes in pregnant rats and their newborn pups.

**Keywords: hemic hypoxia, cardiomyocytes, vessels, myocardium, pregnant rats, pups.**

## Background

In the modern world a successful pregnancy depends on increasing exposure to adverse pathogenic exogenous factors [4, 9]. Due to the fact that main biological effect of adverse factors is realized in the form of various diseases and that

embryonic developing and pregnancy course is strongly related to a lack of oxygen in the tissues of the mother and the fetus, the reaction of maternal-placental-fetal system towards hemic hypoxia have particular interest of research. According to WHO, the incidence of hemic (anemic) hypoxia in pregnant women in countries with different levels of life nowadays has increased considerably and ranges from 21 to 80% [4, 8]. It is believed that the reason for hemic hypoxia onset is not only iron deficiency or abnormal development of erythrocytes but also the intake of a large number of nitro compounds by pregnant women due to their abundance in the drinking water, food, the air, as well as in pharmaceuticals [2]. The main pathogenic effect of those substances is associated with the conversion of hemoglobin into methemoglobin , formation of NO-heme complex in blood (hemoglobin) and tissues (myoglobin), blockage of respiratory enzymes and enzymes of the antioxidant system involving regulatory proteins, hyperactivation of the secondary messengers and cellular synthesis, e.t.c. [1, 2]. There is no doubt of the fact that the origins of many chronic, disabling or fatal pathological conditions in adults, including diseases of the cardiovascular system originate in the antenatal period and represent a prolonged fetal abnormalities [6, 8, 9]. It is known that hypoxia leads to disruption of the autonomic regulation of coronary vascular deterioration of the energy exchange with a sharp decrease in the formation of energy-rich compounds in the mitochondria of cardiomyocytes [5, 10]. However, these studies did not reveal the entire cause-and-effect relationship between hemic hypoxia of mother and formation of myocardial dysfunction in neonates [3, 6].

The aim of this article was to investigate the morphological features of cardiomycytes and vessels in pregnant rats and their newborn pups exposed to chronic hemic hypoxia induced by sodium nitrite

## Material and methods

The experiment is conducted on 12 three-month old female white Wistar rats weighing 180-200 g All manipulations on animals were carried out in strict accordance to the "Quality Rules of Clinical trials and Preclinical trials in the Russian Federation" (approved by MoH and valid from 1 January 1999), Annex 3 to the Order of the Ministry of Health of the USSR No 755 of 10.08.1977, the provisions of the Declaration of Helsinki (2000) and the recommendations of the European Community Directive (No 86 / 609ES). For the induction of pregnancy rats of both sexes were kept together at the ratio of two males to four females. During pregnancy and for 21 days after delivery (end of the period of milk feeding) females

were injected intraperitoneally daily sodium nitrite NaNO2 at a dose of 5 mg / 100 g body weight (dose causing hypoxia of moderate severity) [7]. During the first 16 days of life the hearts of pups fed by above females after pericardiotomy were extracted under ether anesthesia and immediately placed in a cardioplegic solution (0.9% KCl at a temperature of $0^0$C) to achieve the cardiac diastole. For histological examination tissue samples of left ventricle were fixed in 10% neutral formalin, and after fixation were embedded in paraffin sections. Histological sections were stained by GFP method (hematoxylin- fuchsin-picric acid) to detect areas of acute ischemic and metabolic damage and fibrogenesis, Microslides were investigated on a microscope Olympus CX-31 (Japan). For electron microscopy tissue samples of the left ventricle were fixed in 2.5% glutaraldehyde solution followed by fixation with 1% osmium tetroxide. Ultrathin sections were made on ultratome UMTP-7 and contrasted by Reynolds [5]. Studies were conducted on an electron microscope Selmi (Ukraine) at an accelerating voltage of 125 kV.

## Results and discussion

According to light microscopy in the myocardium of all pregnant rats exposed to chronic hemic hypoxia we observed a phenomenon of hemodynamic instability manifested in the form of perivascular edema, venous plethora, emptiness and spasm of the arterioles. In this case the nucleus of endothelial cells of occluded arterioles were visually shifted to the lumen of the vessel, apparently due to cell swelling. In the perivascular space we determined thin layer of connective tissue along with round cell moderate proliferation (Fig. 1A). Microvascular changes were vibrant and commonly manifested by a phenomenon of erythrocyte stasis in the capillaries, arterioles and precapillaries (Fig. 1B). Pericapillary edema of varying severity was detected in the myocardium of almost all females. In the cytoplasm of cardiomyocytes, which were located in the vicinity of vessels, there was diffuse accumulation of fuchsine substrate which was confirmed by GFP staining. Those changes were indicators of ischemic damage of cardiomyocytes by sodium nitrite which was probably aggravated by their decreased nutrition and oxygen supply.

Cardiomyocytes of female rats exposed to hypoxia during pregnancy had pronounced nuclear polymorphism. Sarcolemma of cardiomyocytes was unclear with undulating contours and that, according to the literature, can be a morphological substrate for the development of cardiac arrhythmias [6]. Sarcoplasm of such cardiomyocytes contained fuchsinophillic substrate - an indicator of ischemic damage accumulated near the nuclei, as well as non-uniform thin layers of connective tissue in the interstitium (Fig. 1A).

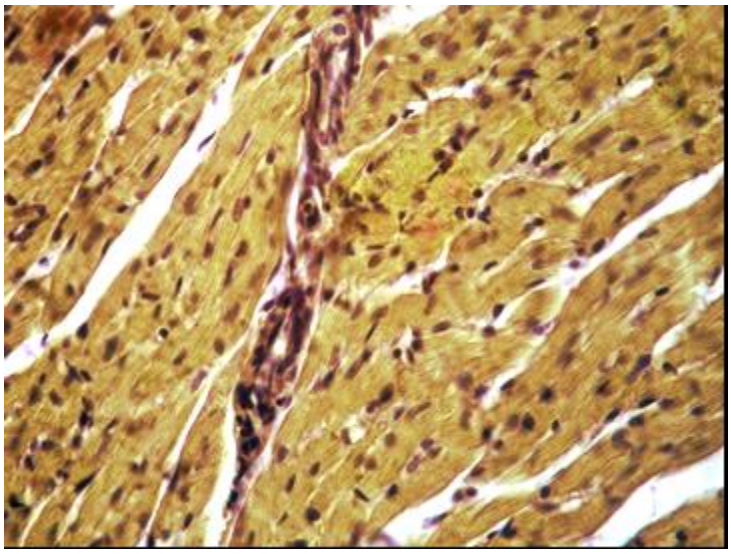

***Fig. 1A.*** *Vivid perivascular edema and spasm of the arterioles (arrows). Staining: GOFP method. Mag. x 400.*

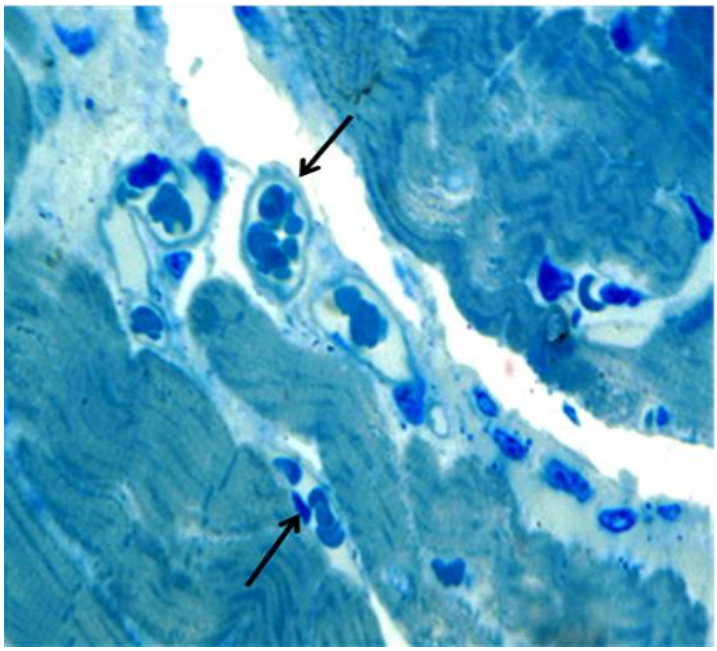

***Fig. 2B.*** *The phenomenon of erythrocyte stasis (arrows). Semithin sections. Toluidine blue staining. Mag. x 1000.*

Hypoxic changes in the vessels and cardiomyocytes in newborn rats, as well as in females were also polymorphic. Arterioles were rare and greatly transformed: their walls were partly thickened and homogenized. The lumen of such vessels has been slit, which apparently happened due to the swelling of endothelial cells (Fig. 2A). Full-blooded capillaries had unevenly colored contours, the distance between the capillaries and cardiomyocytes was significantly enlarged and that could probably exacerbate the transport of energy substrates and oxygen supply from the bloodstream directly to the contractile cardiomyocytes. In addition, almost all vessels

and cardiomyocytes were shrouded in some fine fibers of immature loose connective tissue, indicating t the initial stages of fibrogenesis and creating the so-called "vicious circle": hypoxic damage of cardiomyocytes in turn promotes fibrogenesis and fibrogenesis even more exacerbates cardiomyocyte damage due to the violation of cellular transport.

Moreover, neonatal cardiomyocytes contained diffuse fuchsine substrate and looked uneven or hypertrophy, or dramatically thinned. Near some cores or within oxyphilous met inclusion, similar to apoptotic bodies. Most cardiomyocytes were intensively violet fragmented, isolated from each other by layers of interstitial edema and had contracture changes, indicating the development of dystrophic and prenecrotic changes (Fig. 2A, 2B)

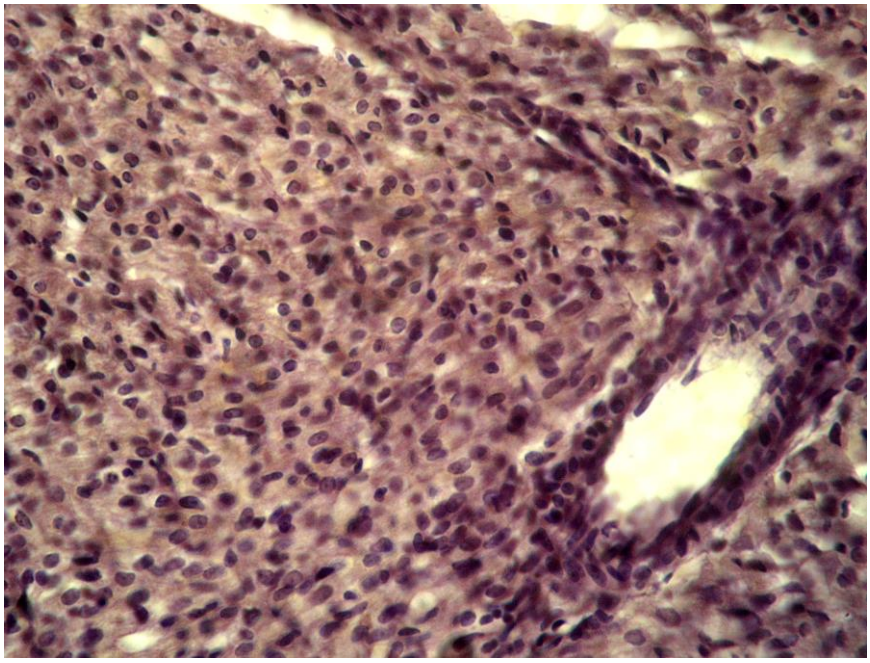

***Figure 2A.*** *Vivid ischemic changes of cardiomyocytes, perivascular and interstitial edema. Staining: GBFP method. Mag. x 400.*

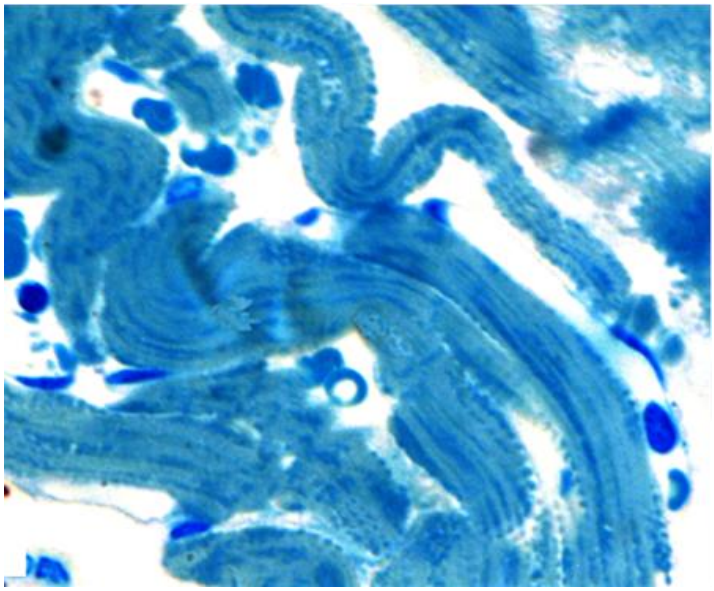

***Fig.2B.*** *Contracture changes and focal fragmentation of muscle fibers of the myocardium (as a sign of a possible heart rhythm disturbances). Semithin sections. Toluidine blue staining. Mag. x1000.*

According to electron microscopy investigation endothelial cells of capillaries of pregnant rats exposed to hypoxia were thinned, the level of micropinocytosis in them was reduced. In some capillaries we revealed vivid tortuosity of the luminal surface (Figure 3). Lumen of numerous vessels was compensatory enlarged, there was observed in some sludge of erythrocytes and adhesion of blood cells to the wall of the capillaries, resulting apparently by disrupting the structure of the basement membrane. The space between the capillaries and the sarcolemma was usually extended and filled predominantly by fibrous component composed out of loose connective tissue, which was mainly compose out of collagen fibers and contained some amount of amorphous substance identical by density to the blood plasma in the lumen of the capillary with separately located microfibrills. The majority of capillary walls were intensively osmiophillic presumably due to their impregnation by the blood plasma.

Cardiomyocytes which were located near those vessels were characterized by small focal and diffuse myofibrillar lysis - so-called "melting" of myofibrils. I-bands were lysed to a large extent up to thin (actin) filaments. Myofibrils become less dense and in some cases their total lysis was observed along with significant degradation sarcomeres in intercalated disks and the expansion of perinuclear area (Fig. 3A).

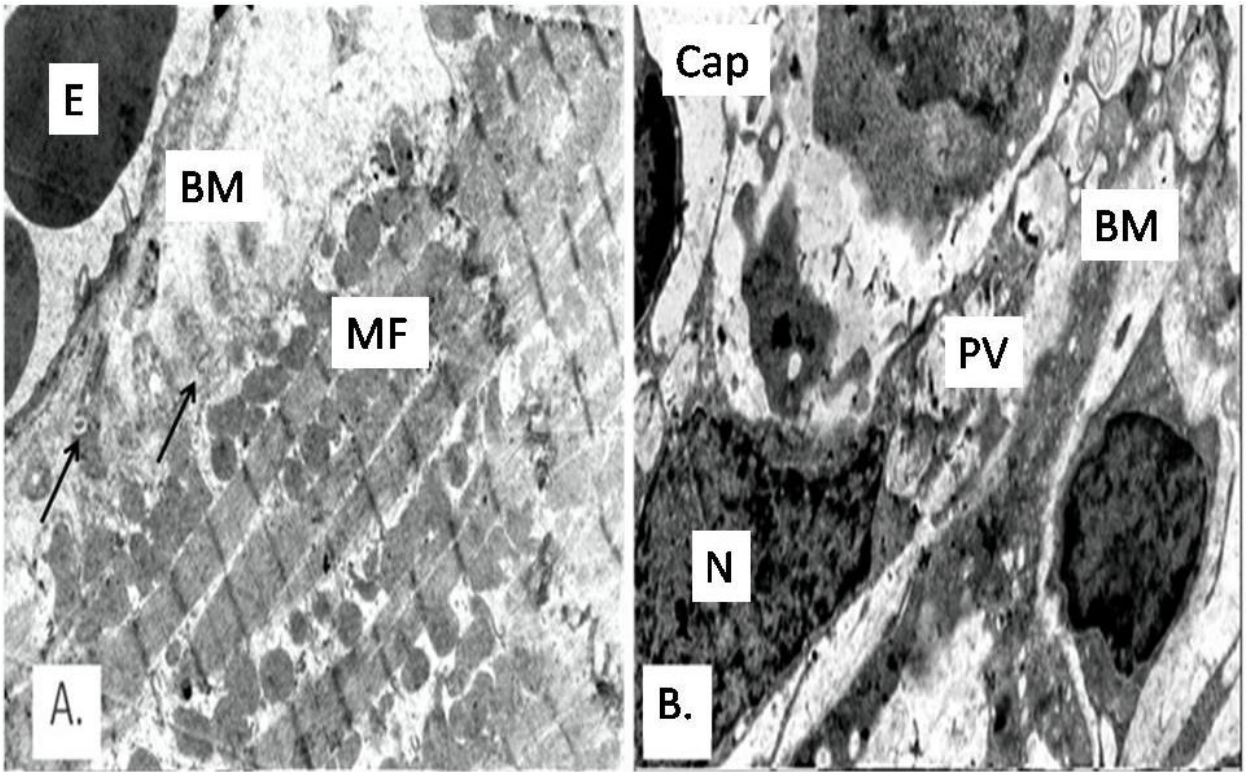


***Fig. 3A .*** *Pericapillary space enlarged (arrows). Diffuse myofibrillar lysis (MF). BM - basal membrane. E - erythrocyte. TEM. x 12000.*

***Figure 3B.*** *Dense endotheliocyte with micropinocytic vesicles (PP), the nucleus of endotheliocyte (N) has pointed invaginations. Basement membrane (BM). In the lumen of the capillary (CAP) a large number of white blood cells. TEM. x 18000.*

The study of electronograms of newborn rats revealed that the matrix of the cytoplasm of endothelial cells in the same capillary was vividly enlightened in some cells, indicating the swelling of the cytoplasm, while other cells become electronically dense. In some endothelial cells where matrix was enlightened, in their cytoplasm we detected abundant single gated micropinocytic vesicles of small size mainly located on the free edge of basal membrane. In the case of compaction of endothelial matrix cytoplasmic micropinocytic vesicles of various types were located on the free edges of the basal membarne as well as in the cytoplasm itself. Capillary basement membranes looked unevenly thickened and multi-layered. We often determined unusual for myocardium fenestrated capillaries which originated probably due to partial destruction of endothelial cells. In the cytoplasm of endothelial cells there were also observed numerous folds and bays on the luminal surface of the cells, sometimes with a margin of endothelial protrusions into the lumen of the vessel. In endothelial cells and adjacent cardiomyocytes there were present plenty of mitochondria with enlightened matrix and destroyed cristae, indicating the development of mitochondrial dysfunction.

Thus, the analysis of the morphology of cardiomyocytes and vessels of the myocardium in pregnant and newborn rats exposed to hemic hypoxia revealed similar degenerative and destructive changes which demonstrated greater degree of severity in neonatal rats.

## Conclusions

1. The main morphological characteristics of hypoxic myocardial damage in pregnant rats and their newborn pups were similar and represented by mixed dystrophy, swelling and destruction of endothelial cells and together with lysis of myofibrills and contracture changes in the contractile cardiomyocytes.

2. The negative ischemic and hypoxic changes were more pronounced in the myocardium of pups .

3. Morphological changes in vessels of pregnant rats and their pups revealed a perivascular edema, capillary hyperemia, emptiness and spasm of the arterioles. Perivascular space was filled by the thin layer of connective tissue, which can probably exacerbate of substrate and oxygen transportation from the bloodstream to the cells during their physical loading and exacerbate hypoxic and ischemic changes in the contractile cardiomyocytes.

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