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STATUS OF COAGULATION SYSTEM IN ADOLESCENT RATS EXPOSED TO HYPOXIA DURING PRENATAL DEVELOPMENT

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The goal of the research was to investigate the physiological indicators in the hemostasis of adult offspring developed under prenatal hypoxia conditions. Chronic hypoxia conditions were created during different periods of the pregnancy. Three-month-old white rats that had completed the period of puberty were used in the experiments. Clinical analyses of the main indicators of the coagulation system were performed to assess the state of the hemostasis system. The effect of intrauterine hypoxia on the "mother-fetus" relationship was manifested by prolonged pathological changes in the blood coagulation and expressed changes in the liver parenchyma at the later stages of development.

Keywords: pre- and postnatal ontogenesis, hypoxia, hemostasis, coagulation.

INTRODUCTION

Supplying the body with oxygen has the main physiological role during embryonic development. Thus, a number of fetal processes, such as hemopoiesis, chondrogenesis, and placenta formation, are regularly controlled by the presence of oxygen entering the body (1). The normal level of oxygen regulates the main fetal processes during fetal development. Therefore, a lack of oxygen has a negative stress effect on the developing body and is accompanied by several dysfunctions. Intrauterine hypoxia can be caused by various reasons (mother's health condition, placental insufficiency, abnormal development of the fetus, etc.). Regardless of the cause and origin, acute or chronic hypoxia has a negative effect at any stage of development, causing metabolic and hematological deficiency in the body and leading to irreversible functional changes. Acute or chronic hypoxia, which affects the fetus at any stage, can cause developmental defects. For this reason, defects in the mother-fetal system are considered the main stress factor, accompanied by serious damage to the development dynamics of the fetus (3, 5). It has been determined by a number of studies that recovery reactions aimed at maintaining the stability of the hemostasis system take place in the body under the conditions of hypoxic influence observed during embryonic development. According to some researchers, the long-term effect of the factor causes structural and functional changes in the body, and in most cases, irreversible pathological changes are manifested (4, 6). Studies prove that the physiological dysfunctions observed during the pathological course of the "mother-fetus" relationship are initially reflected in the blood system (7, 8). Taking into account what has been said, the main goal of our research was to

determine deviations in physiological criteria in the blood system during individual development in newborn animals exposed to hypoxia during embryonic development.

MATERIAL AND METHODS

The experiments were conducted on 60 three-month-old rat pups obtained from 20 rats that passed their pregnancy in normal and hypoxic conditions. All experiments were carried out in accordance with the International Convention of the European Union (November 13, 1987, Strasbourg) and taking into account the principles of animal protection. Hypoxia conditions were created based on the method of Khvatova (1978). In the initial series of experiments, female rats that had completed the period of puberty and were mated were divided into two groups: control and experimental.

The animals from the control group spent the gestation period under normal conditions, and the animals from the experimental group were exposed to the hypoxia factor in a special barochamber for 20 minutes daily during the embryonic, pre-fetal, and fetal periods of prenatal ontogeny. Laboratory tests, such as blood coagulation indicators (total blood coagulation time, plasma recalcification time, active partial thromboplastin time, prothrombin time, thrombin time, fibrinogen amount), platelet activity, and liver histopathology studies, were conducted to reveal the adverse effects of hypoxia in three-month-old offspring. Total blood coagulation time was determined by the Moravits method, plasma recalcification time by the Howell method, prothrombin time by the Kwik method, thrombin time by the Sirmai method, plasma tolerance to heparin by the SIG method, fibrinogen richness by air drying, and thrombus test by the Fuenta-Ita method (2).

The determination of platelets and the study of the main platelet indices were performed on the DIRUI BCC-3600 blood analyzer.

Histological examination of the liver was carried out by Hematoxylin and Eosin staining.

RESULTS AND DISCUSSION

In order to monitor the dynamics of long-term changes observed in the process of hemocoagulation, studies were conducted on animals that had completed the period of puberty (3 months old). The analysis of the results obtained revealed the hypercoagulation nature of the hemostasis system in three-month-old animals exposed to hypoxia during different critical periods of embryonic development. Thus, in comparison with the control group, the coagulation time was slightly shortened in the experimental group. Acceleration of coagulation potential was recorded during the initial and final phases of hemocoagulation. According to our results, the 3-month-old rats in the control group had an average blood clotting time of 135±8.7 seconds, whereas the total blood clotting time (19-23% ($p<0.05$)) was slightly shorter in the animals in the experimental group. A comparative study conducted between separate stages of intrauterine development showed that blood clotting time was 110.2±14.6, 113.8±6.5, and 117.6±6.4 seconds in rats exposed to hypoxia in the embryonic, pre-fetal, and fetal periods of prenatal development, respectively.

Similarly, in 3-month-old rats of the control group, the plasma recalcification time (PRT) was 101±7.9 seconds, while in the animals exposed to hypoxia during the embryonic period, it was slightly shortened (13% ($p<0.05$) and amounted to 88.4±5.8 seconds. Besides, PRT was 89.4±5.9 seconds in rats exposed to hypoxia during the pre-fetal period and 90.5±5.4 seconds in rats subjected to hypoxia during the fetal period of antenatal development. At the same time, an increase in thromboplastic activity of 15-21% ($p<0.001$) was recorded in experimental group animals. Thus, the obtained results confirmed the fact that the coagulation potential increases in the initial phase of coagulation (Fig. 1 A). At the same time, the number of platelets in this age group and the study of platelet indices proved the accuracy of our results. Thus, in animals exposed to the hypoxia factor at different periods of embryonic development, compared to

the control group, an average increase of 10-15% ($p<0.001$) of PLT was determined. An increase in the number of platelets in animals from the experimental group is an indicator of a decrease in their functional activity.

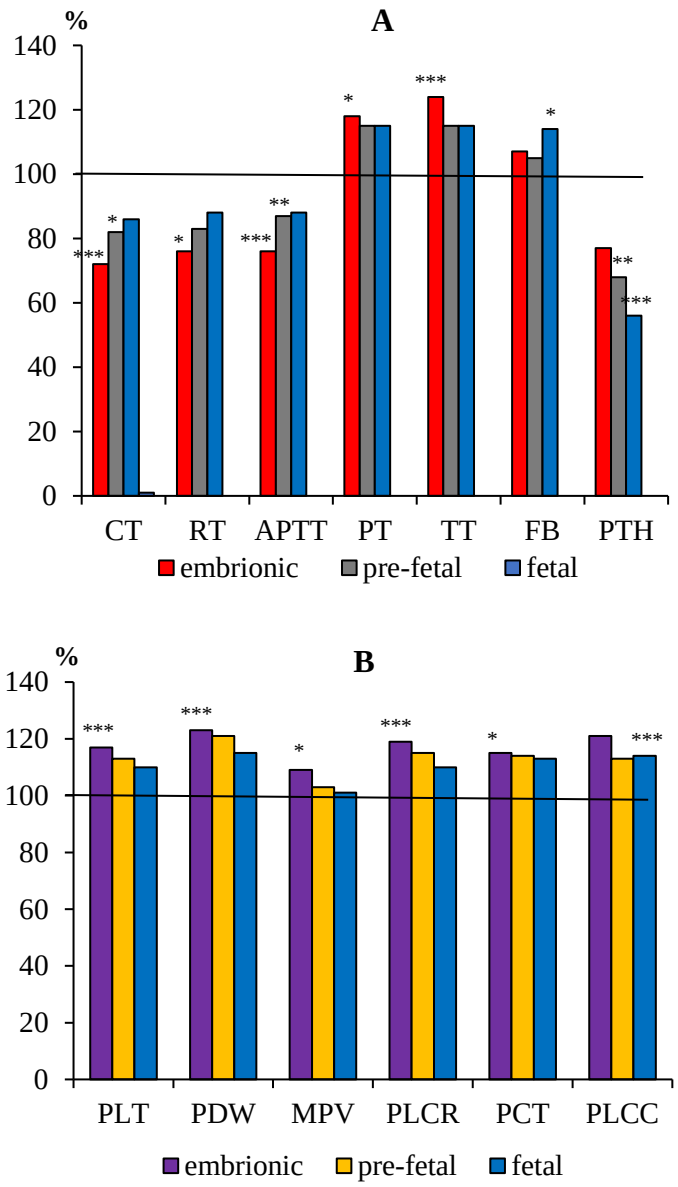

Figure 1. Percentage indicators of blood coagulation dynamics (A) and platelet indices (B) in three-month-old rats exposed to hypoxia during different periods of prenatal development (*-$p<0.05$; **- $p<0.01$; ***-$p<0.001$).

The variability in the number of platelets was reflected in the thrombocrit (PCT) indicator, which is the main platelet index; similarly, PCT increased by 12-14% ($p<0.05$) in experimental animals. Accordingly, a 13-18% ($p<0.001$) increase was recorded in the fraction of large platelets (P-LCC) compared to the control group. Thus, in 3-month-old animals, this indicator was in the range of $45\div55\cdot10^9$/l in the control group, while in trial animals it was recorded at the level of $57\div65\cdot10^9$/l. At the same time, although a slight increase in P-LCR (coefficient of large platelets) was recorded, statistically reliable results were not obtained in determining the mean corpuscular volume (MPV) of platelets (Fig. 1 B). An increase in the number of platelets in the peripheral blood, an increase in the thrombocrit index, and an increase in the fraction of large platelets in the experimental group indicated a tendency to thrombosis in the animals and deficiencies in the hemocoagulation process against the background of increased coagulation potential.

It should be noted that no statistically significant changes were observed in the second phase. In contrast, in the animals of the experimental group, as well as the initial phase, the last phase was slightly shortened and the coagulation potential increased. Thus, a 15% ($p<0.001$) decrease in thrombin activity, which is the most important indicator of the last phase, and correspondingly an increase in the level of fibrinogen confirmed this fact. The results of the thrombus test also confirmed the above results and indicated a tendency to thrombosis in the animals of the experimental group.

The long-term effects of hypoxia in the liver tissue, which plays an important role in the regulation of blood coagulation function, were monitored and histologically studied. During the comparative analysis of histological studies, no statistically reliable different results were obtained in morphometric indicators in the liver of animals of this age group. However, in 80% of the experimental animals, severe blood stagnation was observed in the liver parenchyma, and disorders in the hemodynamic sphere were detected (Figs. 2, 3).

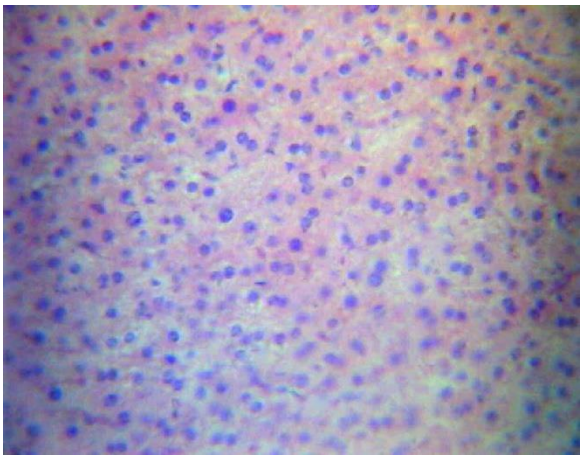

Figure 2. Liver tissue of control three-month-old animals.

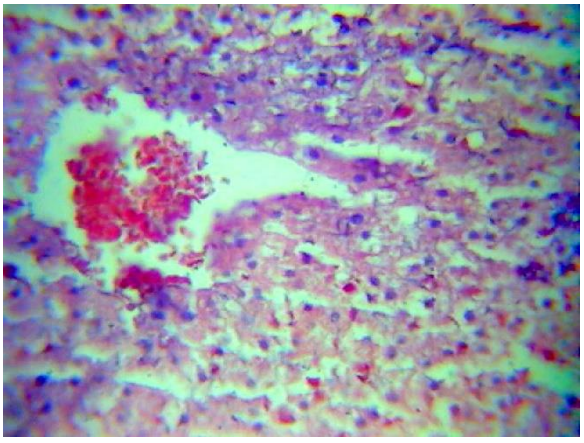

Figure 3. Liver tissue in three-month-old animals exposed to hypoxia during embryonic development. Blood stasis observed in the parenchyma.

The mentioned pathologies were mostly recorded in the venous structures of the liver. Thus, in the liver parenchyma of animals from the experimental group, the detection of numerous venous stagnations and expansions of sinusoids caused by deficiencies in hemodynamics showed traces of the negative effect of prenatal hypoxia and determined the stable nature of the changes.

CONCLUSION

Hereby, our results revealed serious dynamic dysfunctions in the hemostasis system of the new generation as a result of hypoxic exposure of the fetus during antenatal development. The obtained results proved that the embryonic period of prenatal development is more sensitive to antenatal hypoxia, and they determined the continuous nature of these changes. At the same time, it has been determined by experiments that the dysfunctions observed in the condition of intrauterine hypoxia in the blood coagulation system, which is the main component of the hemostasis system, are caused by deficiencies in the platelet ring and liver parenchyma.

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СОСТОЯНИЕ СИСТЕМЫ КОАГУЛЯЦИИ У ПОЛОВОЗРЕЛЫХ КРЫС, ПОДВЕРГНУТЫХ ГИПОКСИИ В ПЕРИОД ПРЕНАТАЛЬНОГО РАЗВИТИЯ

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Целью работы было изучение физиологических показателей в системе гемостаза организма в индивидуальном развитии после воздействия внутриутробной гипоксии. В опытах были использованы трехмесячные белые крысы, завершившие период половой зрелости. Условия хронической гипоксии были созданы в разные периоды пренатального развития. На потомстве в соответствующем возрасте были проведены клинические анализы процесса свертывания крови и отслежена динамика основных показателей. В результате влияние внутриутробной гипоксии на взаимоотношения «мать-плод» проявляется стойкими патологическими изменениями динамики свертывания крови и выраженными изменениями паренхимы печени на поздних сроках развития.

Ключевые слова: пре- и постнатальный онтогенез, гипоксия, гемостаз, коагуляция.

PRENATAL İNKİŞAF DÖVRÜNÜ HİPOKSİYA ŞƏRAİTİNDƏ KEÇİRMİŞ CİNSİ YETİŞKƏN SİÇOVULLARDA LAXTALANMA SİSTEMİNİN VƏZİYYƏTİ

Gülnar Qurban qızı Cəfərova

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Tədqiqat işində əsas məqsəd dölün prenatal hipoksiyası şəraitinin fərdi inkişaf zamanı orqanizmin hemostazında fizioloji göstəricilərin araşdırılması olmuşdur. Eksperimentlərdə cinsi yetişkənlik dövrünü başa vurmuş 3 aylıq ağ siçovullardan istifadə edilmişdir. Heyvanlarda boğazlığın ayrı-ayrı dövrlərində xroniki hipoksiya şəraiti yaradılmış və alınmış yeni nəslin müvafiq yaş ərəfəsində qanda laxtalanma prosesinin kliniki analizləri aparılaraq, əsas göstəricilərin dinamikası izlənilmişdir. Nəticə etibarı ilə prenatal hipoksiya şəraitinin "ana-döl" münasibətlərinə müdaxiləsi inkişafın sonrakı mərhələlərində qanın laxtalanma dinamikasında normadan kənara çıxan nəticələrin davamlı təzahürü və qaraciyər parenximasındakı ciddi dəyişikliklərlə özünü biruzə verir.

Açar sözlər: pre- və postnatal ontogenez, hipoksiya, hemostaz, laxtalanma.

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