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STUDY OF HEMATOLOGICAL PARAMETERS OF RABBIT OFFSPRING SUBJECTED TO PRENATAL HYPOKINESIA

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Changes in the blood parameters of newborn rabbit offspring whose prenatal development occurred under conditions of hypokinesia at various stages of pregnancy were explored. The changes in blood parameters were also observed to affect the viability of the offspring, depending on the pregnancy stage affected by hypokinesia. It was found that the embryonic stage is the most sensitive period, as the number of offspring born during this stage was lower compared to the control group. Based on our experiments, it can be stated that the most resilient stage in terms of offspring number is the fetal stage of pregnancy. The reproductive performance of female rabbits exposed to hypokinesia during this stage was similar to that of the control group and was approximately 90 %. It should be noted that the reduced viability of the newborn offspring is associated with profound changes in the vascular system of the "mother-fetus" system. Unlike pregnant rabbits that underwent gestation under normal conditions, newborns whose prenatal development occurred under hypokinetic conditions at different stages of pregnancy showed alterations in leukocyte formula parameters, platelet concentration, and erythrocyte sedimentation rate. The results of our research indicate that the investigation of erythrocyte and leukocyte formulas during pregnancy may be useful for implementing early preventive measures to mitigate the adverse effects of hypokinesia. These findings provide an important scientific foundation for studying the impact of restricted movement – hypokinesia – on reproductive health in space and for preserving the reproductive functions of animals and humans during future space missions.

Key words: hipokinesia, embryo, pre-fetal growth, prenatal period.

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ДОСЛІДЖЕННЯ ГЕМАТОЛОГІЧНИХ ПОКАЗНИКІВ ПОТОМСТВА КРОЛИКІВ З ДОСВІДОМ ПРЕНАТАЛЬНОЇ ГІПОКІНЕЗІЇ

Досліджено зміни показників крові новонародженого потомства кроликів, пренатальний розвиток яких відбувався в умовах гіпокінезії на різних етапах вагітності. Відзначено, що зміни в параметрах крові також впливають на життєздатність потомства залежно від стадії вагітності, яка зазнала гіпокінезії. Встановлено, що ембріональний етап є найбільш чутливим періодом, оскільки кількість народженого потомства в цей період була нижчою порівняно з контрольною групою. На підставі проведених експериментів можна стверджувати, що найбільш стійким з точки зору кількості потомства є фетальний етап вагітності. Репродуктивна здатність самок кроликів, які зазнали гіпокінезії в цей період, була порівнянна з контрольною групою і становила близько 90 %. Слід зазначити, що зниження життєздатності новонародженого потомства пов'язане з глибокими змінами в судинній системі «мати-плід». На відміну від вагітних кролиць, які виношували потомство в нормальних умовах, новонароджені, пренатальний розвиток яких проходив в гіпокінетичних умовах на різних етапах вагітності, демонстрували зміни в параметрах лейкоцитарної формули, концентрації тромбоцитів і швидкості осідання еритроцитів. Результати нашого дослідження свідчать про те, що вивчення еритроцитарної та лейкоцитарної формул під час вагітності може бути корисним для реалізації ранніх профілактичних заходів з метою пом'якшення несприятливих наслідків гіпокінезії. Ці дані є важливою науковою основою для вивчення впливу обмеженої рухливості – гіпокінезії – на репродуктивне здоров'я в космосі та збереження репродуктивних функцій тварин і людини під час майбутніх космічних місій.

Ключові слова: гіпокінезія, ембріон, префетальний розвиток, пренатальний період.

Hypokinesia is characterized by a prolonged and considerable reduction in the range of movement [13]. From both a biological and medical perspective, the issue of mobility is particularly important during pregnancy. Summarizing the results of studies on the responses of the nervous and muscular systems, it should be noted that under conditions of experimental hypokinesia, distinct and polymorphic neurological disorders develop in a specific sequence. When analyzing the effects of hypokinesia, the functional changes it induces in the cardiovascular system have consistently attracted the interest of researchers. Based on the findings and conclusions of some researchers, it can be stated that prolonged hypokinesia may cause alterations in both cardiac activity and the circulatory system. Erythrocytes of mammals are small, anucleated, and biconcave (discocytes) cells that function to carry hemoglobin [3]. Hypokinesia affects the cellular and biochemical composition of the blood, leading to changes in blood glucose levels expressed as a percentage. Leukocytes continuously utilize oxygen and generate reactive oxygen species to destroy pathogens [8]. Among the parameters of a complete blood count, platelets are both important and, at the same time, among the most challenging cellular elements to analyze. A new indicator within the leukocyte series is the presence of immature granulocytes. According to the literature, this serves as a useful marker for the diagnosis and prognosis of inflammatory conditions, enabling better detection of inflammatory diseases and facilitating the monitoring of therapy . As is well known, changes in the dynamics of the blood's serum composition and formed elements are adaptive in nature. They determine the functional stability of the body's systems and serve as indicators of adaptive mobility. In turn, functional stability depends on individual polymorphism and genotype, the specificity of the factors affecting the organism, sex, age, psychophysiological potential, health status, and the degree of the organism's reactivity [4]. Under modern intensive livestock farming conditions, many components of technological regimes are considered natural stress factors for animals. These include reduced physiological comfort (such as holding in small spaces, restricted movement, and hypodynamia), transportation, group formation, regrouping, etc. [11]. Experimental and clinical studies conducted to date on the effects of prenatal hypokinesia have demonstrated that hypokinesia during pregnancy can negatively affect the course and outcome of pregnancy, leading to the development of pathological conditions. The impact of the hypokinesia factor on mothers during pregnancy may result in several abnormalities during the perinatal period. Moreover, a high incidence of complications during labor and the early postpartum period, preterm birth, and perinatal infant mortality has been recorded [1]. Long-term studies conducted under the supervision of A. G. Gaziyev in the "Environmental factors and development of analyzers" laboratory at the Institute of Physiology named after academician A. Garayev have shown that the impact of factors such as chronic hypoxia, hypokinesia, and electromagnetic radiation during critical periods of embryonic development leads to significant developmental delays and increased mortality rates among newborn rats [6, 7, 5].

The purpose of the study was to investigate how prenatal hypokinesia at different stages of pregnancy affects hematological parameters and the viability of newborn rabbit offspring, in order to identify the most sensitive periods of development and explore the implications for reproductive health under conditions of restricted movement, such as in space missions.

Materials and methods. The analysis of the research findings indicates that a prolonged reduction in muscle activity causes specific alterations in the physiological state of animals. Thus, in such animals, the hemoglobin content decreases, which is associated with a reduction in energy consumption and a weakening of redox reactions, resulting in decreased intensity of erythropoiesis in the bone marrow. In our studies, the main objective was to observe changes in blood parameters of rabbit offspring that underwent prenatal development under conditions of hypokinesia. The research was conducted on young rabbits of the Chinchilla breed. A total of 24 female rabbits and their newborn offspring were used in the study. The gestation period in rabbits lasts 28–30 days, and they typically give birth to 6–8 kits. An adult rabbit measures 45–50 cm in length and weighs between 900 and 1250 grams, while newborn kits measure 25–35 cm and weigh between 500 and 700 grams. E.A. Kovalenko and N.N. Gurovsky [10] concluded that one of the methods used to induce hypokinesia in experimental studies is to keep the small animal in a cage that is reduced in area and volume.According to the sizes of the rabbits, the cages used in the experiments measured 25 cm (length) × 16 cm (width) × 23 cm (height). These dimensions significantly restricted the movement of the rabbits and effectively created a state of hypokinesia. The animals were kept in a dry, heated room with good natural and artificial lighting. Female and male rabbits were housed in separate cages for planned pregnancy. All pregnant rabbits were divided into two groups: control and experimental. The control group animals were kept under standard vivarium conditions, while the experimental group was subjected to hypokinesia at different stages of pregnancy. The animals were regularly fed, provided with water, and maintained under normal temperature conditions. Female rabbits (n=24, with 24 animals in each of the control and experimental groups) were subjected to a defined fertilization period and then experienced various stages of pregnancy under hypokinesia conditions. The animals in the control group were kept under standard vivarium conditions. After the period of intrauterine exposure to hypokinesia was completed, the pregnant animals were removed from the experimental cages and transferred to regular cages for normal delivery. For analysis, blood samples were collected from the marginal vein of the ear.

The complete blood analysis was performed using the "Mindray BC-2800 Vet" hematology analyzer, manufactured in 2007 by Shenzhen Mindray Bio-Medical Electronics. The experiments were carried out in strict compliance with the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), as well as the resolution adopted at the First National Bioethics Congress (Kyiv, 2001).

Table 1

Comparison of blood parameters in offspring born to mothers experiencing prenatal hypokinesia (Control Group)

	Measured Parameters	Unit	Embryonic period	Pre-Fetal period	Fetal period
			Control Group	Control Group	Control Group
1.	Mean Corpuscular Volume (MCV)	fL	70.4875±5.639	70.4875±5.639	72.9625±5.837
2.	Mean Corpuscular Hemoglobin (MCH)	pg	21.1±1.688	21.1±1.688	20.6375±1.651
3.	Mean Corpuscular Hemoglobin Concentration (MCHC)	g/l	295.375±23.63	295.375±23.63	283.25±22.66
4.	Red Cell Distribution Width (RDW)	%	14.5625±1.165	14.5625±1.165	15.8875±1.271
5.	Platelets (PLT)	10^9/l	97.75±7.82	97.75±7.82	305.125±24.41
6.	Mean Platelet Volume (MPV)	fL	4.725±0.378	4.725±0.378	5.6375±0.451
7.	Erythrocyte Sedimentation Rate (ESR)	mm/s	4.5±0.36	4.5±0.36	4.5±0.36
8.	Leukocytes (White Blood Cells)	10^9/l	4.7±0.376	4.7±0.376	6.0625±0.485
9.	Lymphocytes	10^9/l	0.7875±0.063	0.7875±0.063	2.0625±0.165
10.	Monocytes	10^9/l	0.4625±0.037	0.4625±0.037	0.4125±0.033
11.	Granulocytes	10^9/l	3.5875±0.287	3.5875±0.287	3.5875±0.287
12.	Lymphocytes LYM	%	19.1625±1.533	19.1625±1.533	33.85±2.708
13.	Granulocytes	%	75.4±6.032	75.4±6.032	6.6875±0.535
14.	Monocytes	%	8.2875±0.663	8.2875±0.663	59.4625±4.757
15.	Erythrocytes	10^12/l	5.50875±0.4407	5.50875±0.4407	4.91875±0.3935
16.	Hemoglobin (HGB)	g/l	114.25±9.14	114.25±9.14	101.75±8.14
17.	Hematocrit (HTC)	%	38.3±3.064	38.3±3.064	35.8375±2.867

Note: P<0.01 (**), <0.05 (*),<0.001 (***) indicate statistically significant differences between mean values;ns = not significant.

Table 2

Comparison of blood parameters in offspring born to mothers experiencing prenatal hypokinesia (Experimental Group)

	Measured Parameters	Unit	Embryonic period	Pre-Fetal period	Fetal period
			Experimental Group	Experimental Group	Experimental Group
1.	Mean Corpuscular Volume (MCV)	fL	72.9625±5.837^{ns}	74.0625±5.925^{ns}	69.9125±5.593^{ns}
2.	Mean Corpuscular Hemoglobin (MCH)	pg	20.6375±1.651^{ns}	20.3375±1.627^{ns}	20.725±1.658^{ns}
3.	Mean Corpuscular Hemoglobin Concentration (MCHC)	g/l	283.25±22.66^{ns}	274.875±21.99^{ns}	298.625±23.89^{ns}
4.	Red Cell Distribution Width (RDW)	%	15.8875±1.271^{ns}	15.775±1.262^{ns}	15.075±1.206^{ns}
5.	Platelets (PLT)	10^9/l	305.125±24.41 ***	227.5±18.2***	304.29125±24.3433^{ns}
6.	Mean Platelet Volume (MPV)	fL	5.6375±0.451 *	5.925±0.474**	4.525±0.362^{ns}
7.	Erythrocyte Sedimentation Rate (ESR)	mm/s	5.5±0.44 *	5.5±0.44*	6±0.48**
8.	Leukocytes (White Blood Cells)	10^9/l	6.0625±0.485 **	4.55±0.364^{ns}	9.8±0.784**
9.	Lymphocytes	10^9/l	2.0625±0.165 ***	2.125±0.17***	2.225±0.178^{ns}
10.	Monocytes	10^9/l	0.4125±0.033^{ns}	0.3125±0.025**	0.4125±0.033^{ns}
11.	Granulocytes	10^9/l	3.5875±0.287^{ns}	2.1125±0.169***	9.725±0.778***
12.	Lymphocytes LYM	%	33.85±2.708 ***	46.625±3.73***	22±1.76**
13.	Granulocytes	%	6.6875±0.535 ***	46.325±3.706***	73.1625±5.853***
14.	Monocytes	%	59.4625±4.757 ***	7.05±0.564*	4.775±0.382***
15.	Erythrocytes	10^12/l	4.91875±0.3935^{ns}	4.93625±0.3949^{ns}	4.7425±0.3794^{ns}
16.	Hemoglobin (HGB)	g/l	101.75±8.14^{ns}	100.625±8.05^{ns}	100.125±8.01^{ns}
17.	Hematocrit (HTC)	%	35.8375±2.867^{ns}	36.525±2.922^{ns}	33.2375±2.659^{ns}

Note: P<0.01 (**), <0.05 (*),<0.001 (***) indicate statistically significant differences between mean values; ns = not significant.

Results of the study and their discussion. In the blood analysis of offspring born to mothers who experienced the embryonic stage of pregnancy under hypokinesia conditions, changes were observed in the leukocyte formula indicators. Thus, total leukocyte count increased to (6.0625±0.485**)×10⁹/L, and total lymphocyte count rose to (2.0625±0.165***)×10⁹/L. No significant changes were observed in total granulocyte count (3.5875±0.287^{ns})×10⁹/L and total monocyte count (0.4125±0.033^{ns})×10⁹/L. LYM% increased significantly to (33.85±2.708***)%, while the percentage of granulocytes decreased to (6.6875±0.535***). The percentage of monocytes increased to (59.4625±4.757***). Erythrocyte concentration in the blood was (4.91875±0.3935^{ns})×10¹²/L, and HGB concentration was 101.75±8.14^{ns} g/L. Mean corpuscular hemoglobin (MCH) was 20.6375±1.651^{ns}, and mean corpuscular volume (MCV) was 72.9625±5.837^{ns} fL – both remained unchanged. However, a significant increase was observed in the platelet count, which reached 305.125±24.41***×10⁹/L. Red cell distribution width (RDW) was 15.8875±1.271^{ns}%, and hematocrit (HCT) was 35.8375±2.867^{ns}% – no significant changes were observed. However, the erythrocyte sedimentation rate (ESR) showed a slight increase, reaching 5.5±0.44 (mm/h)*. However, the blood analysis of offspring born to mothers who experienced the fetal stage of prenatal development under hypokinesia conditions showed that the total leukocyte count remained unchanged at (4.55±0.364ns)×10⁹/L. In contrast, total lymphocyte count increased to (2.125±0.17***)×10⁹/L, while total granulocyte count decreased to (2.1125±0.169***)×10⁹/L, and total monocyte count also decreased to (0.3125±0.025**)×10⁹/L. The percentage of lymphocytes (LYM%) increased to 46.625±3.73***%, while the percentage of granulocytes decreased to 46.325±3.706***%. The percentage of monocytes showed a slight change, reaching (7.05±0.564*)%. Erythrocyte concentration in the blood was 4.93625±0.3949^{ns}×10¹²/L, hemoglobin concentration (HGB) was 100.625±8.05^{ns} g/L, the mean corpuscular hemoglobin concentration (MCHC) was 274.875±21.99ns g/L, Mean corpuscular hemoglobin (MCH) was 20.3375±1.627^{ns} pg, and mean corpuscular volume (MCV) was 74.0625±5.925^{ns} fL – both remained unchanged. However, the platelet count increased significantly, reaching 227.5±18.2*** ×10⁹/L. Red cell distribution width (RDW) was 15.775±1.262$^{ns\%}$, and hematocrit (HCT) was 36.525±2.922^{ns}% – both showed no significant changes. Erythrocyte sedimentation rate (ESR) was 5.5±0.44* mm/h, indicating a slight increase. However, the analysis of blood samples from offspring born to mothers who underwent the fetal period of prenatal development under hypokinesia conditions revealed the following results: total leukocyte count increased to (9.8±0.784**)×10⁹/L, total lymphocyte count was (2.225±0.178^{ns})×10⁹/L, and total monocyte count remained unchanged at (0.4125±0.033^{ns})×10⁹/L. Total granulocyte count significantly increased to (9.725±0.778***)×10⁹/L. The percentage of lymphocytes (LYM%) decreased to 22±1.76**%, while the percentage of granulocytes increased to (73.1625±5.853***)%. The percentage of monocytes markedly decreased to 4.775±0.382***%. The erythrocyte concentration in the blood was 4.7425±0.3794^{ns}×10¹²/L, showing no significant change. The erythrocyte sedimentation rate (ESR) increased significantly to 6±0.48** mm/h. Hemoglobin concentration (HGB) was 100.125±8.01^{ns} g/L, mean corpuscular hemoglobin concentration (MCHC) was 298.625±23.89^{ns} g/L, mean corpuscular hemoglobin (MCH) was 20.725±1.658ns pg, and mean corpuscular volume (MCV) was 69.9125±5.593^{ns} fL. The platelet count was 304.29125±24.3433^{ns}×10⁹/L. Red cell distribution width (RDW) was 15.075±1.206^{ns}%, and hematocrit (HCT) was 33.2375±2.659^{ns}%, also showing no significant change (Table 1, Table 2).

In addition to blood parameters, our studies also examined the reproductive outcomes of pregnant rabbits and the viability of their offspring. We found that the factor of hypokinesia affects both the ability of mothers exposed to hypokinesia during different stages of pregnancy to give birth and the survival rate of their offspring.

During the experiments, in the control group at the embryonic stage, 67 offspring were born from 11 pregnant rabbits, and 7 of these offspring did not survive. In the experimental group, 7 out of 11 pregnant rabbits carried their pregnancies to term. However, 27 of the offspring lost their viability. At the pre-fetal stage, in the control group, 58 offspring were born from 9 pregnant rabbits, and 4 of them did not survive. In the experimental group, 8 out of 9 pregnant rabbits carried their pregnancies to term, and 11 offspring did not survive. However, during the fetal period, 56 offspring were born from 8 pregnant rabbits in the control group, and 3 of them did not survive. In the experimental group, all 8 pregnant rabbits gave birth, and 3 offspring lost their viability (Figs. 1, 2).

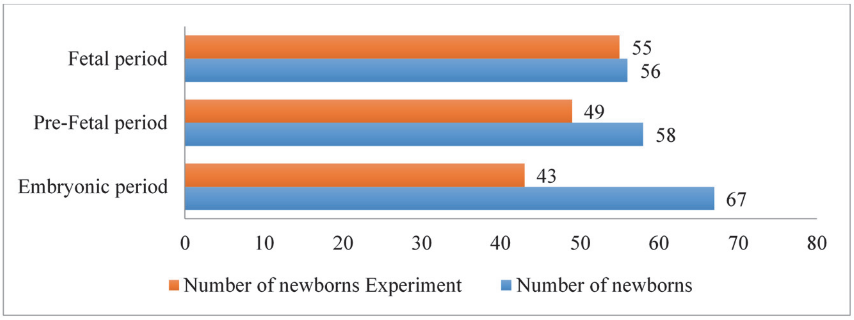

Fig. 1. Newborn counts during embryonic, pre-fetal, and fetal periods.

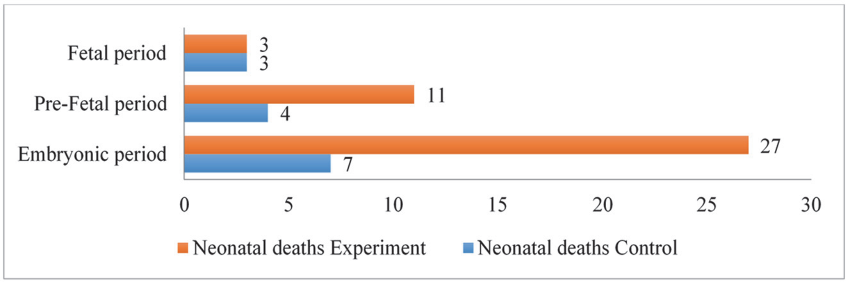

Fig. 2. Neonatal death counts during embryonic, pre-fetal, and fetal periods.

Since our primary objective was to investigate the effects of prenatal hypokinesia on blood parameters, we conducted a comparative analysis by monitoring both normal conditions and the impact of hypokinesia. It is worth noting that the evidence obtained in these series confirms and aligns with the results of previous studies. The findings of our research indicate that significant changes occur in hematological parameters in offspring born to mothers who experienced hypokinesia during various stages of prenatal development. This leads to functional impairments in the organism's immune and hematopoietic systems. Similar to the findings reported by Mizoguchi Y. [12], our study also shows that mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) of erythrocytes remain unchanged. Although some researchers have reported a decrease in leukocyte count in pregnant rabbits compared to non-pregnant ones [2], and studies conducted on New Zealand white rabbits have also shown a significant reduction in white blood cell parameters during fetal development stages, we have observed instances of increased leukocyte counts [12]. The increase in white blood cell counts at the early stages of pregnancy in rabbits [9] aligns with the findings of our study. In summary, the increase in the number of lymphocytes and platelets is associated with the activation of the immune system and the intensification of inflammatory responses. The decrease in the number of granulocytes and monocytes indicates a weakening of the immune defense system, which may reduce the organism's resistance to pathogens. The observed increase in erythrocyte sedimentation rate (ESR) reflects the presence of inflammatory and stress conditions in the body. Various internal and external environmental factors during pregnancy, including restricted physical activity, trigger stress responses that can ultimately lead to mortality [14]. In our experiments, we observed decreased birth outcomes in female rabbits subjected to hypokinesia during various stages of pregnancy and reduced viability of their offspring. When examining the number of newborns and those that lost viability, it became clear that due to the effects of hypokinesia at different pregnancy stages, a certain percentage of animals failed to give birth. This phenomenon was most frequently observed in animals exposed to hypokinesia during the embryonic period. The fetal period is considered the most resilient stage. Based on our research, we can state that hypokinesia can negatively affect the organism's physiological and reproductive functions. During space missions, exposure to hypokinesia is particularly significant. Therefore, investigating the effects of hypokinesia during pregnancy on the health of both the mother and fetus in long-term space flights is an important and timely scientific issue.

Taken together, these findings underscore the detrimental impact of hypokinesia on maternal physiology and reproductive performance. The implications of these results extend beyond terrestrial biology, offering valuable insight into the risks associated with spaceflight conditions. As restricted mobility is an inherent aspect of long-duration missions in space, understanding how it affects pregnancy and fetal development is essential. Our study provides a scientific foundation for developing preventive strategies aimed at safeguarding reproductive health under such conditions, whether in animal models or future human space travelers.

These findings highlight the need for continued research into the physiological impacts of prenatal hypokinesia, especially in the context of spaceflight and other conditions involving prolonged movement restriction.

Conclusion

These results further clarify the impact of external factors on blood cell development during the prenatal period, providing a foundation for future research. Although previous studies have examined the effects of hypokinesia on the adult organism, the influence of hypokinesia on hematological parameters during different stages of prenatal development has not been extensively investigated. Our study fills this gap by systematically demonstrating how fetal hematological indicators change under conditions of prenatal movement restriction. At the same time, it reveals how hypokinesia during various stages of prenatal development affects reproductive outcomes and the viability of offspring. The results we obtained can be regarded as a novel contribution to the evaluation of the effects of hypokinesia on prenatal development and hematological parameters under space flight conditions, representing an innovation in the field of space biology. These findings play a crucial role in protecting reproductive health and developing appropriate interventions during long-term space missions. Our research highlights the necessity of developing measures to prevent and mitigate hypokinesia during pregnancy, as well as the importance of studying hematological parameters and physiological characteristics of pregnancy that are critical for life in space conditions. Thus, our study provides a valuable contribution to fundamental scientific research focused on investigating the effects of hypokinesia during different stages of pregnancy in space environments.

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