

# EXPERIMENTAL CHRONIC TOXIC HEPATITIS AND HEMATOLOGICAL FEATURES IN THE DYNAMICS OF MOTHER'S AND THE OFFSPRING LACTATION

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## **ABSTRACT**

*The aim of the research was to study the occurrence of autoimmune processes in chronic hepatitis and its effect on the hematological parameters of the mother and offspring in the dynamics of lactation. Material and research methods - the work was carried out on white outbred female rats, in which, after heliotrine intoxication before pregnancy, the presence of antihepatic antibodies in female rats was carried out by a passive hem agglutination reaction according to Boyden's method, as well as hematological parameters in the mother and offspring in the dynamics of lactation by conventional methods. The presence of antihepatocytic autoantibodies during lactation was established, mainly in blood serum, and in small amounts in the milk of female rats, therefore, antihepatic antibodies of the mother in toxic hepatitis are not a pathological agent for offspring during breastfeeding. In the body of female rats and offspring, anemia develops, progressing until the second week of lactation, that is, the transition of pups to a mixed diet, therefore, it is more expedient to carry out therapeutic measures before this period.*

*Key words: chronic hepatitis, lactation, mother, offspring, anti-hepatic antibodies, anemia.*

## **Introduction**

It is known that the normal development of a child depends on the process of conception, i.e. from the gene pool X and Y of the chromosomes of both parents, then in the future it is directly related to the state of the mother's body during pregnancy and breastfeeding. That is, the growth and development of the future newborn in the embryonic and fetal periods of development depends on the full functioning of the mother - placenta - fetus system, then after birth this scheme changes to the mother - mammary gland - newborn. Consequently, the adaptation of the developing offspring to environmental factors begins even in the embryonic period of development, where immunoglobulins, hormones and bioactive substances begin to flow to it through the placenta along with the necessary nutrients. And yet, a person, like many species of mammals, brings immature offspring, the formation and functioning of the life-support systems of which occurs further during the period of breastfeeding. However, this harmonious system of relationships between mother and offspring is disrupted in case of mother's illness.

It should be noted that the frequency of various extra genital pathologies in women of childbearing age is still significant. First of all, the risk of chronic damage to the hepatobiliary system increases as a consequence of the transferred viral or toxic hepatitis, which, under the influence of unfavorable environmental conditions, often take on a chronic, protracted form [3, 4]. It should also be noted that multiparous women also have a high risk of hepatobiliary pathology. The possibility of transmission of the hepatitis virus from women carriers of HBsAg to newborn babies, the development of HBsAg persistence in some children, the formation of primary chronic hepatitis is beyond doubt [5, 8]. In particular, experimental studies have established that chronic intoxication with heliotrin contributes to the occurrence of interstitial hepatitis, which further progresses to cirrhosis of the liver [4]. Along with this, in recent years, during intoxication with heliotrin, the presence of microcirculatory disorders in other organs was found, which indicates the presence of interorgan and intersystem relationships in the body of experimental animals [1]. One of the liquid media that binds the body into a single system is blood, in which “its general condition is reflected as in a mirror”. At the same time, the significance of the autoimmune processes unfolding in the maternal body during hepatitis, in the dynamics of lactation and their influence on the development of the offspring during breastfeeding and how they are reflected in the hematological parameters of the mother and offspring, remain poorly understood.

**The aim** of our research was to study the effect of chronic heliotrine intoxication of female rats before pregnancy on autoimmune processes in the mother's body and on the hematological parameters of the mother and offspring and in the dynamics of lactation.

### **Material and research methods**

For the experiment, 3 month old sexually mature white outbred female rats (72) weighing 120-140 grams were used. The animals were kept on a normal laboratory diet and were quarantined for two weeks prior to the experiment. We used chronic heliotrine intoxication as a model of hepatitis [1]. After the quarantine period, the females of the experimental group (O) were injected with heliotrin at a dose of 0.05 mg / gram of body weight per 0.5 ml of physiological solution subcutaneously weekly for 6 weeks, the animals of the control group (C) were injected only with saline. 10 days after the last injection, males were added to the females. For the study, females of the experimental (40) and control groups (32) were selected on days 1, 3, 7, 15, 21 and 30 of lactation. Determination of antihepatocytic antibodies was performed by RPHA according to Boyden's method. To carry out serological reactions, blood serum (0.5 ml) and milk samples (0.2 ml) taken from female rats during the above study periods were used. Sheep erythrocytes were prepared according to the generally accepted method. As an antigen, we used a sample of the liver of an intact female rat in the amount of 2.0 g, the preparation of which was carried out by water-chloroform extraction according to the method of T.A. Alekseeva [8]. The antigen content was controlled by the amount of protein, which was brought to 1%. For more accurate calculation and convenience in comparison, the results obtained were expressed in  $\log_2$  [7]. For the study, we used the blood of females from the control and experimental groups, as well as their pups on days 1, 3, 7, 14, 21 and 30 of lactation. The following hematological parameters were studied in the work: the amount of hemoglobin (Hb in gram%) according to the generally accepted method using a Sali hemometer, the total number of erythrocytes ( $\times 10^{12} / l$ )

and leukocytes ( $\times 10^9 / l$ ) using a Goryaev chamber. The obtained data were processed according to Fischer-Student, differences satisfying  $P < 0.05$  were considered significant.

## Results

As a result of our study, a certain dynamics of changes in the titer of antihepatocyte antibodies in blood and milk of rats with toxic hepatitis in the dynamics of lactation was established. In the blood serum of females of the experimental group, immediately after giving birth, autoantibodies are found that reach a titer of 1: 128, this trend persists up to 3 days of lactation, while in the control group of animals the titer of autoantibodies is 1: 8 and 1: 4 on days 1 and 3 after childbirth, respectively. In the subsequent periods of lactation, serum at a dilution of 1:64 was seropositive until the end of the lactation period. On the contrary, in the control group, antibodies are found in females only up to 7 days of breastfeeding, not exceeding a titer of 1: 4, in the subsequent periods of lactation only traces were observed.

In contrast to blood serum, during serological examination of milk samples from females of the experimental group on days 1-7 after delivery, autoantibodies were determined at a dilution of 1: 8. In subsequent periods, anti-hepatic antibodies were not always detected even at a dilution of 1: 4. In the control group, the indices of serological studies of milk during breastfeeding did not show significant differences from the experimental group.

To conduct more accurate studies and the possibility of comparing changes in the titer of antihepatocyte antibodies in the blood serum and milk of female rats in the dynamics of lactation, the results of serological studies were expressed in  $\log_2$  (see Table 1). According to these data, in toxic heliotrinic hepatitis, the appearance of autoantibodies against the liver is characteristic only of blood serum, which are present until the end of the breastfeeding period. While it was noted in milk samples obtained from females with toxic hepatitis, an insignificant titer of autoantibodies did not differ significantly from those in females of the control group.

**Table 1**  
**Changes in the activity of antihepatic antibodies in the blood and milk of female rats with chronic heliotrinic intoxication in the dynamics of lactation**

(data are expressed in  $\log_2$ ,  $M \pm m$ )

Investigated material	Group	Lactation terms (in days)				
		1	3	7	14	21
Blood	K	1,68±0,158	0,75±0,161	0,68±0,149	0,48±0,142	0,33±0,104
	O	<b>6,03±0,089</b>	<b>5,45±0,127</b>	<b>5,10± 0,114</b>	<b>4,90±0,120</b>	<b>4,73±0,114</b>
Milk	K	1,60±0,206	1,48±0,089	1,30±0,082	0,83±0,196	0,78±0,164
	O	3,75±0,133	3,45±0,158	3,43±0,212	2,00±0,199	1,95±0,225

Note: - significant differences relative to control at  $P < 0.05$  are highlighted in bold.

Chronic heliotrinic hepatitis also contributes to the occurrence of certain changes in hematological parameters, both in the females themselves and in their offspring (see Tables 2 and 3). In female rats, there is a significant decrease in the amount of hemoglobin starting from the first week after birth and up to 14 days of lactation. Although from the first days of lactation there is a relative decrease in the number of erythrocytes in females in the experimental group

than in the control group, the decrease in the number of erythrocytes becomes significant only on the 14th day of lactation.

**Table 2**  
**The effect of toxic hepatitis on the hematological parameters of female rats in the dynamics of lactation (M ± m, n = 10)**

Investigated parameters	Group	Lactation terms (in days)					
		1	3	7	14	21	30
Hb gram %	K	11,2± 0,02	11,2± 0,03	11,1± 0,09	11,0± 0,01	11,2± 0,17	11,8± 0,05
	O	10,8± 0,29	10,8± 0,25	<b>10,7± 0,12</b>	<b>10,6± 0,22</b>	10,9± 0,25	11,4± 0,33
Erythrocytes x10 <sup>9</sup> /l	K	5,28± 0,11	5,21± 0,05	5,20± 0,08	5,13± 0,06	5,23± 0,11	5,41± 0,08
	O	5,00± 0,12	4,90± 0,15	4,85± 0,20	<b>4,78± 0,09</b>	5,15± 0,17	5,3± 0,14
Leukocytes x10 <sup>6</sup> /l	K	9,21± 0,17	9,06± 0,05	9,44± 0,21	9,60± 0,24	9,61± 0,16	9,72± 0,15
	O	<b>8,42± 0,28</b>	<b>8,21± 0,18</b>	<b>8,43± 0,26</b>	<b>8,71± 0,29</b>	9,11± 0,36	9,20± 0,38

**Table 3**  
**Influence of toxic hepatitis in female rats on changes in hematological parameters of offspring in the dynamics of early postnatal ontogenesis (M ± m, n =10)**

Investigated parameters	Group	Terms of postnatal development (in days)					
		1	3	7	14	21	30
Hb gram %	K	10,2±0,08	9,5±0,06	9,5±0,03	9,3±0,05	9,1±0,07	8,9±0,12
	O	<b>9,1±0,10</b>	<b>9,1±0,07</b>	<b>9,0±0,06</b>	<b>8,9±0,08</b>	8,8±0,18	8,8±0,17
Erythrocytes x10 <sup>9</sup> /l	K	3,65±0,05	3,25±0,0 5	3,30±0,0 4	3,35± 0,05	3,56±0,0 5	3,82±0,0 6
	O	<b>3,25±0,14</b>	<b>3,02±0,0 5</b>	<b>3,01±0,0 3</b>	<b>2,90±0,0 7</b>	3,16±0,2 2	3,60±0,1 8
Leukocytes x10 <sup>6</sup> /l	K	12,62±0,1 6	9,96±0,0 3	9,70±0,0 8	9,29±0,1 0	9,10±0,1 5	9,02±0,0 8
	O	<b>11,02±0,0 8</b>	<b>8,76±0,1 3</b>	<b>8,61±0,0 8</b>	8,91±0,1 6	8,80±0,6 4	8,92±0,4 6

In the study of the total number of leukocytes, leukopenia was established, which stably lasts up to 14 days of lactation. In the subsequent periods of lactation, a relative normalization of hematological parameters is noted.

The study of blood parameters of rat pups in the dynamics of early postnatal ontogenesis made it possible to establish that in the body of newborns of the experimental group there is a decrease in the amount of hemoglobin and erythropenia, which persist up to 14 days after birth. A decrease in the total number of leukocytes, observed in rat pups on day 1 after delivery, persists up to 7 days of development. On the 21st and 30th days, the blood indices approach the lower limit of these rat pups of the control group.

### **Discussion**

It is known that chronic heliotrine intoxication leads to the development of toxic hepatitis, which generally tends to progress, i.e. is a model of aggressive chronic hepatitis [1,11]. With active forms of hepatitis, changes in the immune status occur, in particular, profound changes in the T and B systems of immunity are noted and an increase in the titer of immunoglobulins of various classes, an increase in the gamma fraction of globulins, are criteria for assessing the activity of a developing pathological process. In cases of transition of the process into a chronic form against the background of a slight decrease in T-helpers, the number of T-suppressors (killers) significantly decreases, which contributes to the formation of anti-hepatic antibodies and activates the pathological process [3,9,10]. Along with this, we believe that the violation of the detoxification function of the mother's liver is of great importance here. The fetal liver is not yet ready for sufficient detoxification of metabolic products. It is clear that in this case, the accumulation in tissues, including the liver, of substances that have a cytotoxic effect is possible. Another reason for pathological changes in offspring, apparently, is certain immune pathological changes in the body, since protein products of disintegrating hepatocytes cause an auto allergic reaction. In particular, the results of our study once again showed that heliotrinic intoxication of female rats before pregnancy is a trigger mechanism for an auto allergic process that progresses over time. Along with this, the relatively low titers of anti-hepatic antibodies in the blood serum are apparently associated with changes in the mother's body in the dynamics of pregnancy and lactation, stimulating regeneration processes. The almost two-fold excess of the titer of autoantibodies immediately after childbirth, relative to the further lactation period (1: 128 on day 1 and 1:64 in the subsequent periods of lactation), may be explained by the effect of a fetus foreign to the mother's body and, of course, the loss of the immunosuppressive actions of placental hormones during pregnancy, which have ceased to work after childbirth.

When comparing the results of hematological studies of the blood of females with chronic heliotrine intoxication and their offspring with the literature data, we can assume that profound metabolic changes occurring in the mother's body in toxic hepatitis, in particular, a violation of protein and vitamin metabolism, as well as detoxification function of the liver. Along with this, if we take into account the lag in the development of the gastrointestinal tract and the immune systems of newborn rat pups [1,4]. All these factors most likely contribute to the onset of anemia, not only in the mother, but also in the offspring. The progression of anemia in the female's body on the 14th day of lactation is apparently associated with the growth of rat pups and an increase in the need for milk, and, consequently, the stress of the mother's body. The immunodeficiency condition of the mother with hepatitis leads to the fact that her milk does not completely eliminate the immunodeficiency in the offspring's organism [2], which is probably the reason for leukopenia in pups. The transition of pups to a mixed diet, on the one hand, helps to reduce the load on the mother's body, on the other hand, to a decrease in the intake of

hepatotoxins [3] and anti-hepatic antibodies [5] in rat pups, and probably as a result, by the end of the breastfeeding period, a relative normalization of the blood parameters of the mother and offspring occurs.

Thus, in the blood of female rats with toxic hepatitis after childbirth and in the Dynamics of lactation, anti-hepatic antibodies are determined, but during breastfeeding, they are transmitted to rats through milk in insignificant quantities and, most likely, are not the main reason for the lag in the formation of the organs of the digestive and immune systems offspring. Along with this, with chronic heliotronic hepatitis, both in the mother's body and in the offspring, anemia is revealed, which progresses to the transition of rat pups to a mixed diet, therefore, therapeutic measures would be more effective before this period of development.

### **Conclusions:**

1. In chronic toxic hepatitis in the body of a lactating female, autoimmune hepatitis occurs, as evidenced by the presence of antihepatocytic antibodies in the blood serum. Small amounts of antibodies in milk indicate that they are not the main pathological factor for the infant.

2. Chronic hepatitis contributes to the occurrence of anemia, both in the body of female rats and in offspring, which progresses to the transition of rat pups to a mixed diet.

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