The impact of intermittent exposure to ambient temperature of 40 °C, at different development stages, on the blood picture of albino lab rat

Mire Spasov, Icko Gorgoski, Metodi Stojmirov, Verica Spasova
Faculty of Natural Sciences and Mathematics, "Ss. Cyril and Methodius" University
PO box 162, 100 Skopje, Macedonia
Tel: 389-70-718-606 E-mail: mire.spasov@ugd.edu.mk

Abstract

In this paper, we investigated the effects of hyperthermic stress, involved in the intrauterine and early postnatal period, in the blood like the inner space, in fact in erythrocytes, hemoglobin, hematocrit and leucocytes.

For this purpose we used albino laboratorial rat of the Wistar kind.

The testing results led us to conclusion about the harmful influence of the hyperthermic stress in all of the investigated parameters, especially if it is applied in continuity. It's manifested by increased number of erythrocytes and hematocrit value and change in the number of the leukocytes, which in state of hyperthermic stress, is reduced. Changes occur in relation between lymphocytes and neutrophilic leukocytes. Treatment in the intrauterine life has very small negative influence.

Keywords: hyperthermic stress, blood picture, leukocytes, intrauterine and postnatal development, rat

Introduction

Homoiothermal organisms have the ability to maintain their body temperature at constant level, regardless of the ambient temperature. Such ability is known as power to thermoregulate. However, if the ambient temperature is significantly high, the temperature itself represents a stress factor whose activity causes changes at all levels of biological organization (Collins and Werner, 1968). When staying longer in conditions of high ambient temperature, the organisms react appropriately in order to maintain the inner homeostasis. In that case, the leading role is played by the processes at nerval, endocrine, metabolic homeostasis level, as well as the homeostasis of body temperature and liquids. All of these processes happen in order to adjust the organisms to the new ambient temperature (Edwards at al.,1974; Edwards, 1993). Changes that occur during such processes are dependent on duration and the manner of exposure to the appropriate thermal environment, the height of the actual temperature of the ambient, sex and generally, on the physiological condition of the organism. Especially interesting for research is the hyperthermic stress applied at the earliest development stages, and its teratogenic effect on the newborn. The first reaction of the homoiothermal organism when

staying in an ambient with high temperature, is to increase the body temperature, which is followed by loss of water in organism, change of skin circulation, loss of body mass (Bedrak et al. 1971; Dimovska, 1986), changes in lymphoidal cells and organs (Murzenok and Natukova, 1991), changes in mother-placenta blood supply (Petrova, 1991). Such manifestations, as well as a number of other manifestations, are only pre-condition for increased heat release by the organism, which in different homoiothermal organisms is manifested in different manners and with different intensity (Haslag and Hertzman, 1965; Azhaev, 1972; Wilmore et al. 1975; Hiley, 1976). In conditions of high ambient temperature, great number of tissue and organs suffer in the newborn, which all together results in anatomical malformation and retardation in body weight at birth, compared to the mass of the newborn after normal physiological pregnancy (Hendrix et al., 1979; Nazarova, 1991; Edwards, 1993). Especially sensitive to temperature increase are the proliferative cells during early embryogenesis, due to which, in such conditions they may be significantly damaged (Edwards et al., 1974).

Hyperthermic environment in homoiothermal organisms leads to impairment of their homeostasis, at which, among all, it may also lead to significant physiological changes in blood as a body fluid, which is especially important since as a most active part of the inner environment, blood has many functions, such as respiratory, nutritive, excretory, protective and it has a role in humoral regulation. The tests have shown that exposure to high ambient temperature, in homoiothermal organisms, also leads to significant changes in blood circulation in relation to mother-placenta-newborn, which conditions reduced creation of heat in the newborn, through reduced metabolic processes and reduction of gradient between body temperature of the newborn and the mother (Oakes et al. 1976). This means that the heat stress is regarded by the organism as a special condition which requires activation of certain protective mechanisms which also lead to shift in the values of the blood parameters, since even the normal physiological processes in the organism are suspended. At the same time it is indisputable that the duration of the hyperthermia has its own effect (Edwards, 1993).

Based on the data that undoubtedly and confirmatively point out to great number of pernicious activities of high ambient temperature upon the newborn, during the intrauterine and postnatal period, we set our goals upon analyzing the impact of 40° C high ambient temperature at different levels of development on the following parameters in the albino lab rat: number of erythrocytes, hemoglobin concentration, hematocrit value, number of leukocytes and leukocyte formula.

Materials and methods

During the research we used albino lab rats of the Wistar strain. In the pre-preparation period and during the experiment, the animals were acclimatized at room temperature (18-22 °C), fed with standard lab rat food (produced by Veterinary Institute-Zemun) and water - ad libitum. After the confirmation of the first day of pregnancy, according to the classic formula, the female rats were separated and divided into thee experimental groups: Group 1: the group, during the whole experiment (pregnancy, lactation, and up to the 50^{th} day of postlactation period), stayed at room temperature of 18

to 22 °C (control). Group 2. Newborn by mothers that only during the pregnancy were to be exposed for 2 hours on daily basis at high ambient temperature of 40 °C in thermostat, after which they were to be placed at room temperature. Group 3. Newborn that only in post-lacation period (without mothers, after the separation of the newborn from the mother 21 day after birth), were to be exposed to ambient temperature of 40 °C in thermostat. After the birth, newborn were left with the mother during the whole lactation period, approximately until the 21st day after birth. The day of the birth was considered as the first day of lactation that lasts until the 21st day, and the 21st day was considered as the first day of postlactation. After this period the newborn was separated from the mothers and that was considered to be the postlactation period.

1. Determination of the number of erythrocytes

The number of erythrocytes was determined by standard lab method using Thoma-haemocytometer. For that purpose, right after the decapitation of the animal, one drop from the blood was put on a watch glass and using a blood-diluting pipette for erythrocytes we took blood up to the 0,5 mark and the blood was diluted 200 times by Haem-solution (1g NaCl; 5g Na_2SO_4; 0,1g $HgCl_2$ diluted in 200 ml distilled water). The content from the blood-diluting pipette was mixed between the thumb and the index finger for 3-4 minutes. The second drop from the blood-diluting pipettete was dropped on the net of Thoma-chamber and under a microscope we counted erythrocytes in a volume of 1/4000 of mm3 (in 4 big squares, each consisting of 16 smaller squares). The number of erythrocytes was expressed by one litre of blood, and it was calculated by using the appropriate formula.

Number of Er = Er in 4 big squares / 64 x 200 x 4000 x 10^6

2. Determination of hemoglobin concentration

The determination was carried out by using cyanmethhemoglobin method. The principle is that the hemoglobin in a presence of Potassiumfericyanide oxidizes into methhaemoglobin, which in a presence of potassiumcyanide turns into cyanmethhaemoglobin, which has relatively stable colour. In a clean test-tube, using a pipette we took 5 ml of freshly prepared Drapkin solution (50mg KCN; 200mg $K_4Fe(CN)_6$; 140mg KH_2PO_4 and 0,5ml Steroh dissolved in1000 ml of distilled water) and added 0,02 ml of full blood. The test-tube was stirred and then left to stay for about 20 minutes, after which we measured the absorption of the analysis at 540-546 nm wave length, opposite to the blank assay from the Drapkin solution. Along with the analysis, we also carried out a standard one with 18g/100ml concentration. The calculation was carried out by the appropriate formula.

g Hg/l = Ea/Es x Ks

3. Determination of hematocrit value

The hematocrit was determined by standard method using hematocrit tubes. Such method presents percental volume ratio between blood plasma and formative elements. In heparized blood, under the influence of centrifugal force, the formative elements sediment, and the blood plasma stays over them. Two thirds of heparized tube (dimensions 0,1 x 100 mm) was filled with full blood. One end of the tube was closed by plasticine or burner. Such tube was taken to hematocrit centrifuge, where it was centrifuged for 10 minutes at 8-10000 rounds per minute. After the centrifuge, we routinely calculated the percental ratio between blood elements and blood plasma.

4. Determination of number of Leukocytes

We performed a standard lab method, according to which the counting of leukocytes is based on removing the erythrocytes, which are in great number and may disable the counting of leukocytes. For that purpose, we used acetic acid that hemolizes the erythrocytes, and in order to make leukocytes visible, we stained them with gentian violet. We took full blood using blood-diluting pipette for leukocytes up to the 0,5 mark, and then up to the mark 11 we added Turk-solution (2ml CH_3COOH; 3ml 1% gentian violet solution, dissolved in 300 ml of distilled water). The blood, together with the solution, was mixed for 3-4 minutes, after which the second drop from the blood-diluting pipette was placed on a net of Thoma-chamber, and under a microscope we counted the leukocytes. The counting was performed on the whole net, in 1/80 mm3 volume. The number of leukocytes was expressed in L/blood and it was calculated using the formula:

Number of Le/l = counted leukocytes/ 400 x 20 x 400 x 10^6

5. Determination of leukocyte formula

The determination was performed using the Maj Grunwald-Gimza method. The following required reagents and equipment were used: 1. Basic Maj Grunwald stain solution that was prepared using 0,25 g powder stain that was dissolved in 100 ml of absolute methyl alcohol, in which there must not be traces of acetone. The mixture was heated at 60 °C in order to achieve total dissolving of the stain, after which the solution was filtrated into a clean bottle that afterwards should be tightly sealed. 2. Maj Grunwald working solution was prepared by dissolving 100 ml of basic solution into 150 ml phosphate buffer which is pH = 6,8 (50,8 ml KH_2PO_4 1M solution and 42,9 ml Na_2HPO_4 1M solution). 3. Basic solution of Giemsa stain was prepared by dissolving 1g powdered stain in 66 ml glycerol. Such mixture was heated at 60 °C and left to boil for 2 hours. Such solution received 66 ml absolute methyl alcohol while continuously being mixed. The prepared solution was left to stay for 7-14 days directly exposed to light. Prior to usage, the solution was filtrated. 4. Giemsa working solution was prepared by diluting 100 ml of the basic solution with 400 ml phosphate buffer.

The procedure for preparation of blood smears took place on dry and extremely clean slides. Then, they were dried at room temperature for 1-2 hours, and afterwards they were fixed for 5 minutes in

pure methyl alcohol. Such smears were be stained by Maj-Grunwald working solution, a stain that was left to stay for 3 minutes. Without staining the smear, we added distilled water that was left to stay for 1 minute. The mixture of stain and water was poured out of the preparation and it was rinsed several times with mild stream of water, and afterwards we stained the smear with Giemsa stain working solution which was left to stay for 15 minutes. Giemsa stain was rinsed several times with phosphate buffer, and after that, with mild stream of water. Preparation was left to dry at room temperature for 4-5 hours, and after that, different types of leukocytes could be recognized and counted under immersion objective. At least 200 leukocytes should be counted, and based on the individual characteristics, different types of leukocytes were grouped and their percentage was calculated, i.e the relative leukocyte formula. If the absolute number of leukocytes on given sample is known, than it would be easy to calculate the relative leukocyte formula.

6. Statistical analysis

Average values were calculated from the individual values of the results obtained, according to the appropriate formula, whereas the standard error was calculated using different formula. The significance was determined by Student t-test, in a manner that when making comparison between groups with same number of animals we used the appropriate formula, while making comparison between groups with different number of animals we used the specific formula, The values were compared in special table, and values smaller than $p< .050$ were to be considered as significant.

Results

The effect of exposure to 40 °C high ambient temperature on the number of erythrocytes

The results we obtained from the testing are shown graphically in Figure 1. It can be seen from the chart that the number of erythrocytes changes differently in individual experimental groups. In the control group, the number of erythrocytes during the first post-lactation period (on the 30th day of life) significantly reduces (Fig.1, A:A1, P<.005), and in the days that followed the number is back again to values obtained at the end of the lactation (Fig. 1, A:A2, n.s.). In the group of animals that were exposed to 40 °C ambient temperature, only during the fetal development, to the 30th day of life, there is a significantly increased number of erythrocytes (Fig.1, A:B, P<.005, A1:B1, P<.001). On the 50th day of life, the number of erythrocytes in this group is not much different than the values obtained in the control group of animals (Fig.1, A2:B2, n.s.). More distinctive is the stimulating effect of the intermittent exposure to 40 °C ambient temperature in the postlactation period on the number of erythrocytes, which can be especially seen on the 50th day of life when the values are significantly higher compared to the values obtained from control group animals (Fig.1, A2:C2, P<.001).

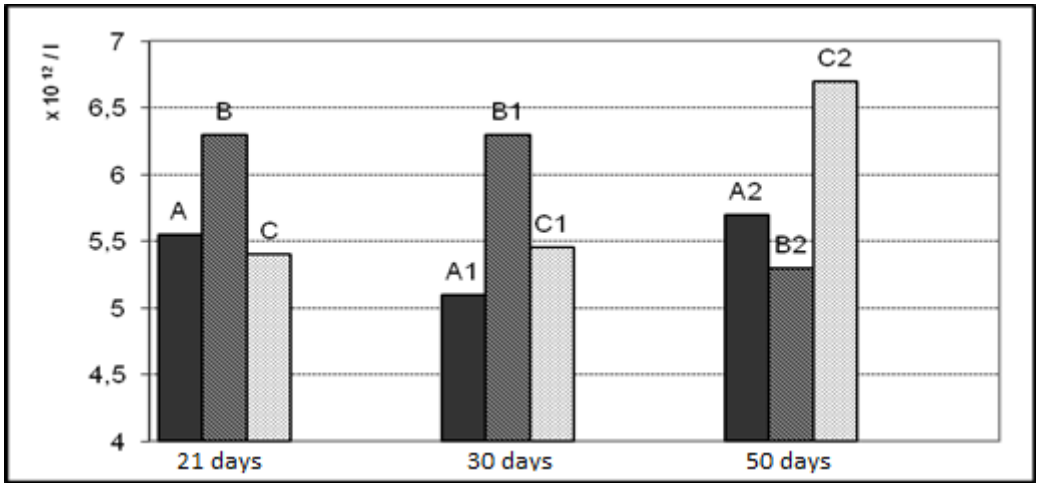

Figure 1. The effect of exposure to high ambient temperature on the number of erythrocytes on the 21st, 30th and 50th day of life.

A/A1/A2 – control group (continuously at room temperature, from pregnancy to the 50th day).

B/B1/B2 – newborn by mothers that only during the pregnancy was exposed to 40 °C ambient temperature.

C/C1/C2 - newborn that only during the postlactation period, without the mothers, was exposed to 40 °C ambient temperature.

The effect of exposure to 40 °C high ambient temperature on the hemoglobin content

The results from the research on the effect of exposure to 40 °C high ambient temperature upon the hemoglobin content of the blood in the three experimental groups are given in Fig. 2. It can be seen from the results that the hemoglobin content in the control group at three test points doesn't change significantly (Fig.2, A:A1:A2, n.s.). Exposure to high ambient temperature during fetal development in rats is the cause at the end of the lactation period for increased hemoglobin level in blood compared to the level in the control group of animals (Fig. 2, A:B, P<.001). On the day 30 of the experiment, the hemoglobin content in blood in this group returns to the level almost the same as the one of the control group (Fig.2, A1:B1, n.s.), and on the day 50 it significantly decreases (Fig. 2, A2:B2, P<.001). It can be seen from the results that there is almost identical dynamics of change of hemoglobin content in the third group of animals that were exposed only during the postlactation period. Namely, in this group during the first ten days of exposure, there is significant increase of the hemoglobin level (Fig. 2, A1:C1, P<.001), and in the next ten days the hemoglobin level significantly decreases compared to the control group (Fig. 2, A2:C2, P<.001).

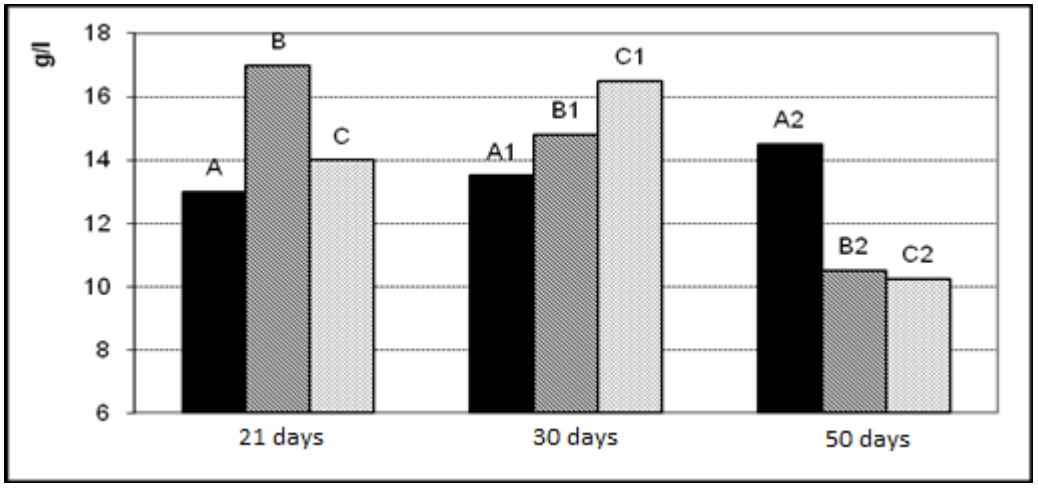

Figure 2. The effect of exposure to high ambient temperature upon the hemoglobin content on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1)

The effect of exposure to 40° C high ambient temperature on the hematocrit value

The changes that were observed during the testing in conditions of high ambient temperature on the hematocrit value in the three experimental groups of animals, in three phases of their development, have been graphically shown in Figure 3. This figure shows that the hematocrit value after the lactation period significantly changes up to the 30th day, after which it maintains the same level. High ambient temperature during the fetal development seems to cause premature reach of the adult level of hematocrit value (Fig.3, A1:B, n.s.). the results from our experiment show that the group that was exposed to high ambient temperature in postlactation period, in post-hyperthermic period it has increased hematocrit value (Fig. 3, A1:C1, P<.001).

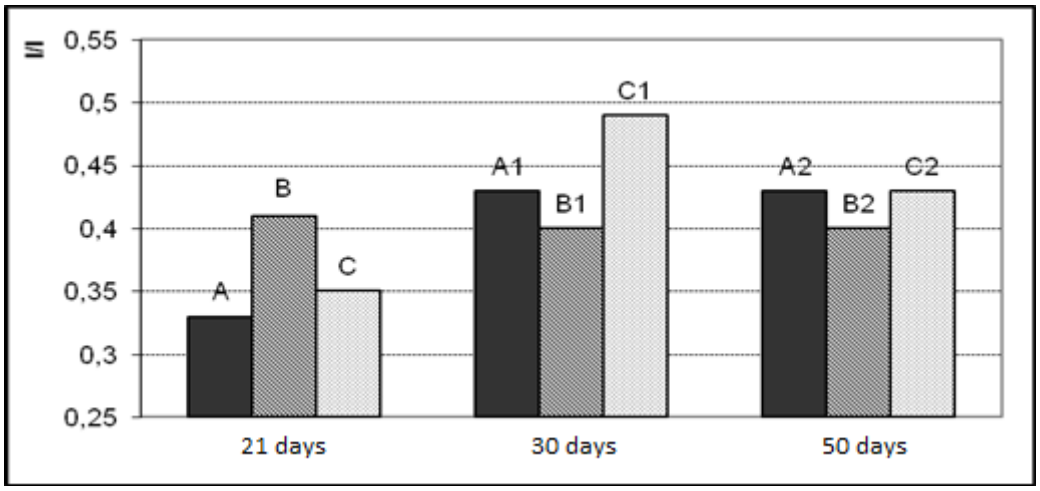

Figure 3. The effect of exposure to high ambient temperature on the hematocrit value on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1).

The effect of exposure to 40° C high ambient temperature on the number of leukocytes and leukocyte formula

Along with the other blood parameters, we also observed the change in number of leukocytes in actual experimental conditions. Our results showed that the heat stress causes pronounced leucopenia in albino lab rat (Fig. 4). The results from the research on leukocyte formula in the three experimental groups at the three observed ages, are graphically shown in Figures 5, 6 and 7. From the results shown in these Figures, it can be seen that there is significant decrease in the number of lymphocytes (Fig. 7), and in eosinophilic granulocytes (Fig. 6), in animals exposed to 40 °C in the phase of fetal development, or in the postlactation period. Unlike the aforementioned granulocytes, the relative number of neutrophile granulocytes is almost unchanged or slightly increased in both groups that were exposed to high ambient temperature (Fig. 5).

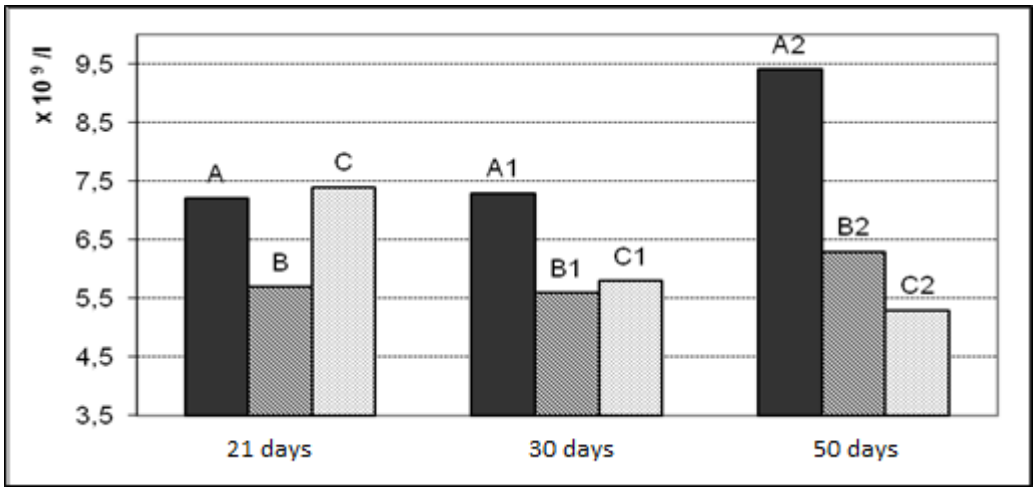

Figure 4. The effect of exposure to high ambient temperature on the number of leukocytes on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1).

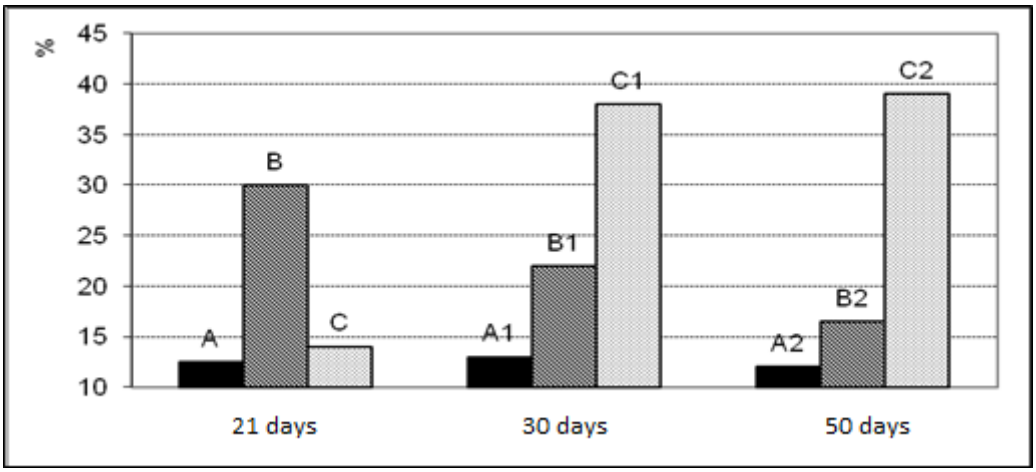

Figure5. The effect of exposure to high ambient temperature on the relative content of neutrophilic leukocytes on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1).

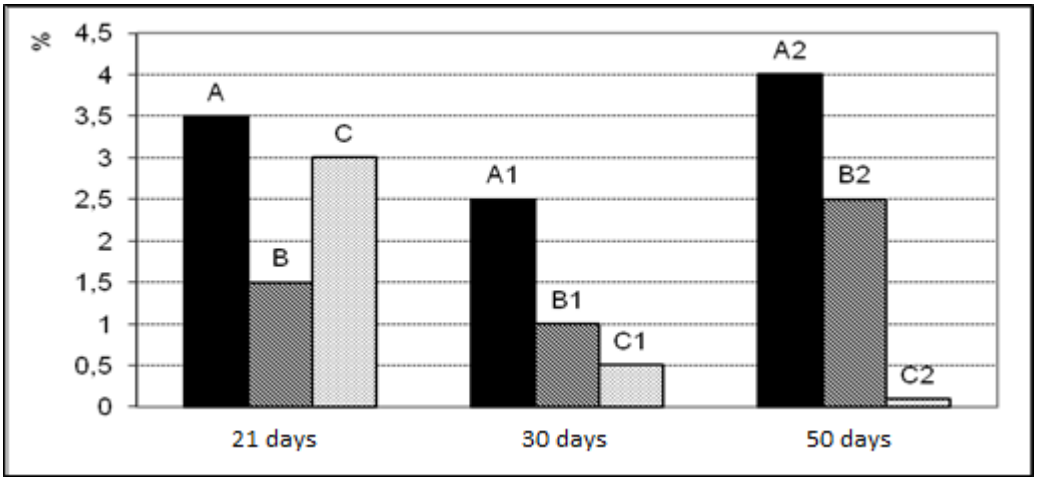

Figure 6. The effect of exposure to high ambient temperature on the relative content of eosinophilic leukocytes on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1).

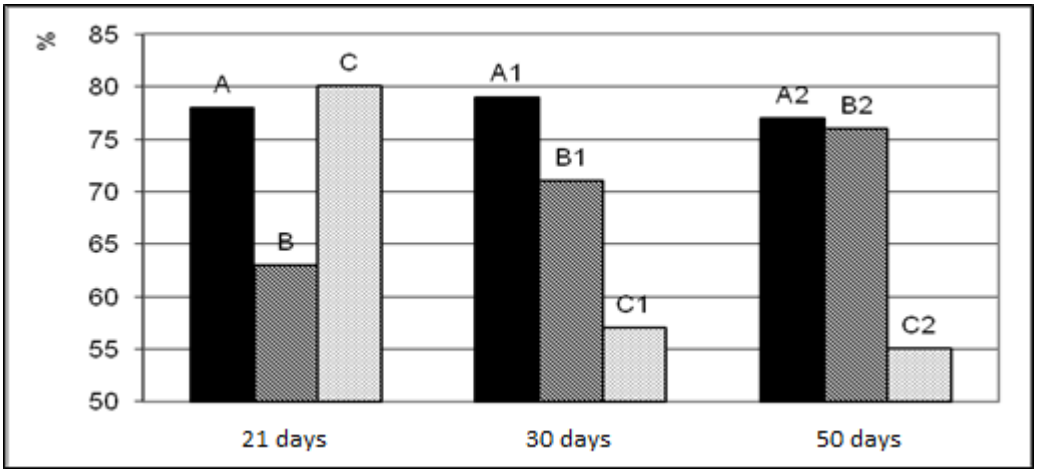

Figure7. The effect of exposure to high ambient temperature on the relative content of lymphocytes on the 21st, 30th and 50th day of life. (The legend is the same as in Figure 1).

Discussion

There are many references that point out to the impact of the high ambient temperature on the number of erythrocytes. In that manner, Bondarev et al.,(1985) point out that the number of erythrocytes in posthyperthermic period increases. But, despite such references, there is also data about another completely different effect of the moderate hyperthermal surrounding. Namely, four-month-long three-hour exposure on daily basis at 35° C temperature, causes slight decrease of the number of erythrocytes, but their volume significantly rises, as well as their average diameterp (Zaharov et al., 1986). The results from our tests on intermittent exposure to high ambient temperature on the number of erythrocytes in experimental animals are graphically shown in Figure 1. The decrease of the number of erythrocytes in control group during the period from the 21st to the 30th day of life, most probably, is a result of the increased decomposition of erythrocytes. The results we obtain show stimulating effect of intermittent exposure at 40 °C temperature, on the number of erythrocytes in the postlactation period, which can especially be seen on the 50th day of life when the values are significantly higher, compared to the values obtained in animals of the control group (A2:C2, P<.001). Such increase of the number of erythrocytes in posthyperthermic period is in accordance with the results obtained from previous research (Bondarev et al., 1985). The increase of the number of erythrocytes in groups of animals that were exposed to high ambient temperature during the fetal development and the postlactation period, probably is a result of increased secretion of erythropoietin. The erythropoietin affects the basic cells which are important for erythropoiesis (Zaharov et al., 1986), and it affects most probably the control and structural genes necessary for hemoglobin synthesis, which provides erythropoietic cell to receive the first morphological features of the erythroblast.

The references related to the impact of high ambient temperature on hemoglobin content in rat blood are almost contradictory. Namely, if, according to the research by Bondarev et al., (1985), in the posthyperthermic period there is an increased level of hemoglobin, according to Zaharov et al. (1986), days-long intermittent stay in an environment with higher temperature has no effect on hemoglobin content. The resultst from our research on hemoglobin content in the control group at the three test points suggest that they haven't changed significantly (A:A1:A2, n.s.). Exposure to 40°C temperature, during the fetal development, causes in rats an increased level of hemoglobin in blood at the end of lactation period, compared to the control group of animals (A:B, P.<.001.). On the 30th day of the experiment, the hemoglobin content in this group returns approximately to the level as in the control group (A1:B1, n.s.), so on the 50th day it could significantly decrease (A2:B2, P<.001). There is also almost equivalent dynamics of change in hemoglobin content in the group of animals that were exposed only during the postlactation period. In this group, during the first ten days of the exposure, there is a significant increase of hemoglobin level, and in the following twenty days the hemoglobin level decreases significantly, below the level of the control group. The significant decrease of hemoglobin content in groups exposed to high ambient temperature is in accordance with some authors (Bodarev et al., 1985). Such results, obtained on the 50th day of the experiment, are probably in relation to the effect of long-term exposure to high temperature that causes denaturation of proteins, due to destruction of their tertiary structure (Hahn et al.,1991). An important fact is also the data about the almost suspended protein synthesis during the hot-shock response (Lindquist and Craig, 1988). Knowing the function of hot-shock proteins in protecting the proteins from denaturation at high ambient temperatures, their longer production seems to cause a sort of tiredness of the hot-shock genes' activity, at which the organism is left without protection, i.e. the result is their intensive denaturation (Edvards, 1993).

The changes we obtained from our results, regarding hematocrit value, show that after lactation period they significantly increase up to the 30th day of life, after which they maintain the same level. Such results are in accordance with previously carried out research. Namely, high ambient temperature during the fetal development seems to cause premature reach of the adult level of the hematocrit value (A1:B, n.s.). The knowledge that in posthyperthermic period there is an increased hematocrit value (Bondarev et al., 1985), have been confirmed from our experiment in the group that was exposed to high temperature during the postlactation period (A1:C1, P<.001). By increasing the environment temperature, the organism dehydration rate increases (Buzalkov, 1980). Namely, in conditions of hyperthermia, the body temperature increases, also (Dimovska et al., 1986) and there is an increased loss of water from the organism, then changes in plasma electrolytes occur that also affect the osmolarity (Dimovska et al., 1986), and all the previously mentioned, probably contributes to the increase of hematocrit value. It is known, also, that the dehydration of the organism leads to a hemoconcentration in the plasma, i.e. by hematocrit increase, too (Sapin, M.R., Samoilov, M.V., 1988).

Along with the other blood parameters we observed the change in number of leukocytes in given experimental conditions. Our results suggest that heat stress causes pronounced leucopenia in albino

lab rat. In order to see if leukocyte decrease is proportionally manifested in all kinds of leukocyte cells, we determined the leukocyte formula in animals of the three experimental groups at the three tested ages. The results are graphically shown in Figures 5, 6 and 7. From the charts of the results it can be seen that there is a significant decrease in the number of lymphocytes and eosinophilic granulocytes in animals that were exposed to 40 °C at fetal development phase, or in postlactation period. Unlike them, the relative number of neutrophilic granulocytes is almost unchanged or slightly increased in both groups that were exposed to high ambient temperature. Lymphocytopenia and eosinopenia in groups exposed to heat stress in fetal development are probably a result of inhibitory activity of high temperature upon proliferative cells in fetus (Upfold et al., 1989). Specifically, high temperature prevents production of interleukine 2 from T-lymphocytes, and it is known that interleukine is the main factor that conditions proliferation of lymphocytes. Also, it is known that the heat stress stimulates the thermal receptors in hypothalamus, the place where CRF is produced from, which via the portal blood circulation reaches the adenohypophysis, activating cells for synthesis of ACTH, which has stimulating effect on the cells of the *outer layer of the adrenal glands* for secretion of increased level of corticosteroids. The increased level of corticosteoirds in blood, on the other hand, among other things, also decreases the level of total number of leukocytes, lymphocytes and eosinophilic granulocytes. It is also known that these hormones affect lymphocyte organs, too, where they cause degenerative histological changes, which is another proof for their lymphocytopoiesic effect. The lymphocytopenia itself is considered to occur as a result of the mitosis inhibition, reduced release of lymphocytes from the depots in the circulation or because of fast cell destruction in the lymphatic tissue. Eosinopenia, also, is a result of high ambient temperature that affects hypothalamic-hypophisial-adrenal system. Despite this, the destruction of eosinophils also depends on the condition of the reticulo-endothelial system which is activated in stress conditions. Activated reticulo-endothelial system performs inhibition of release of mature eosinophils from the bone marrow (Sapin, M.R., Samoilov, M.V., 1988).

Conclusion

From the tests and the results obtained regarding the impact of intermittent exposure to lethally high ambient temperature of 40 °C, at different development stages of albino rat, based on some parameters of the blood picture, the following conclusions may be drawn:

- Intermittent exposure to lethally high ambient temperature leads to increase of the number of erythrocytes;
- High ambient temperature leads to increase of hemoglobin level in blood.
- Hematocrit value in animals exposed to 40 °C significantly increases;

Leukopenia, lymphocytopenia and eosinopenia were observed in rats exposed to heat stress activity at different development stages.

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