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Effect of Short-term Heat-induced Stress on Complete Blood Counts (CBCs) in the Swiss Albino Mice (*Mus musculus*)

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Abstract

Sixty sexually mature, 15- to 22-week-old, male and female laboratory-bred Swiss albinos weighing 21 to 43g each were used in the present study to find out the effect of exposing mice to 35 to 40C0 for 4 hours on their blood parameters using Complete Blood Counts (CBC) as indicators. The experimental design was 4 groups of 2 X 2 factorial arrangements for genders and heat treatments. The genders were: 30 male mice and 30 female mice per treatment. The heat treatments were: Control: 30 mice (15 males and 15 females) kept for four hours at room temperature (25 ± 2 C0), and the heat stress: 30 mice (15 males and 15 females) kept at 35 to 40 0C ambient temperature for four hours. The variables measured were: white blood corpuscles (WBC), red blood corpuscles (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets count (PLT). Exposure to the heat produced a profound effect on the blood. Differences in levels of WBC, MCHC, and PLT in males and females heat exposed groups were not significant, but those of the rest (RBC, HGB, HCT, MCV, and MCH) were significant.

Keywords: Albino mice, *Mus musculus*, heat stress, Complete Blood Count

Introduction

Hyper- and hypothermia of warm-blooded animals is the loss of thermal homeostasis due to being exposed to ambient temperatures above or below their optimum limits for an effective period, resulting in the inability of an animal to practice its normal life. Such loss of homeostasis is called stress *(1-5).* Stress triggers a wide range of body changes called General Adaptation Syndrome (GAS). Productive stress aimed at regaining the homeostasis, such as sweating and cessation of feeding in hyperthermia, is called Eustress

while harmful stress such as strenuous physical activity, loss of muscular strength, or panic is called Distress. The stimuli, which produce GAS, are called the Stressors *(6)*. Hyperthermia is known to affect the physiology of animals, often causing multiple organ dysfunction syndromes *(7, 8)*. System problems include blood, liver, and kidney malfunctioning and tissue damage (5, 8-10). The extent of these problems is usually measured by blood profile tests such as CBC and liver and kidney function tests, urine analysis, and histological examination of different body tissues.

Li *et al*. *(5)* exposed male mice to the elevated ambient temperature of 42°C daily for 2 h during the period of twelve consecutive days; mice were sacrificed on days *1, 2, 4, 8,* and *12* immediately following heat exposure. Rectal, scrotal surface and body surface temperatures were significantly increased after *2* h heat exposure at all five-time points. Mice suffered heat stress when the rectal temperature approached 40°C, characterized by excessive drinking, increased respiratory and restlessness. The objective of the present study was aimed to assess the effect of short-term exposure of mice to relatively high-temperature doses.

Materials and Methods

The experimental design was 4 groups of a 2 X 2 factorial arrangement for control and heat treatment. The control consisted of 30 mice: 15 males and 15 females kept separately in two isolated cages for 4 hours at room temperature (25 ± 2 ^{0}C). The heat treatment consisted of 30 mice: 15 males and 15 females kept for 4 hours in 2 separate cages of ambient temperature ranging from 35 to 40 ^{0}C. At the end of the 4 hours, the mice were partly slaughtered, exiting blood was collected from each mouse in a separate vial for measuring CBC: white blood corpuscles (WBC), red blood corpuscles (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets count (PLT).

Results

In the present study exposure to heat resulted in three stages:
Stage 1: mice were normal during the first one to one and half hour after commencement of the exposure,
Stage 2: After that they became restless, digging in the sawdust lining the bottom, attempting to escape out of the cage, and stopped feeding and drinking, and their heartbeat and respiration rate increased. This continued for about half an hour. Their fur was wet during this stage.
Stage 3: After that, they became dormant and lied in a corner of the cage. Stage 4: During some preliminary preparatory experiments, mice were exposed to 35-40 0C to find out whether they will survive the four-hour heat exposure or not. These mice were not sacrificed but returned to normal ambient temperature (25 ± 2 0C) where they regained normal activity after 20 to 30 minutes.

Mice were found normal during the first one to one and half hour of the exposure to the heat, after that they became restless, digging in the sawdust lining the bottom of the cage, attempted to escape out of the cage, stopped feeding and drinking and their heartbeat and respiration rates increased. This continued for about half an hour. After that, they became dormant and lied in a corner of the cage.

Levels of the CBC parameters were shown in Table 1. The normal ranges (NR) reported in the literature for the mouse are given at the top of the Table. All CBC values obtained in the present study were either within or close to the normal range except for PLT of the control male and female groups which recorded higher values. All CBCs differences between male and female control groups were not statistically significant. Also, differences between males and females heat exposed groups regarding WBC, MCHC and PLT were not significant, but those of the rest (RBC, HGB, HCT, MCV, and MCH) were significant. Values

of WBC and PLT of the control groups and the heat exposed groups were significantly different. Values of MCHC of all treatments were not significantly different.

Table 1: Values of CBC parameters. Means with different superscripts along columns are significantly different (P≤0.05).

Sex – Temp. effect	WBC (10^3 cells /µl)	RBC (10^6 cells/mm3)	HGB (g/dl)	HCT (%)
NR	3.0 -14.2	5.0 -9.5	10.9 -16.3	38.5 -45.1
Male control	7.14 ± 0.55^{a}	8.86 ± 0.26^{a}	14.53 ± 0.38^{ab}	45.4 ± 1.3^{ab}
Female control	6.28 ± 0.41^{a}	8.86 ± 0.26^{a}	14.71 ± 0.39^{b}	46.2 ± 1.4^{b}
Male heated	4.67 ± 0.58^{b}	8.03 ± 0.25^{b}	13.59 ± 0.36^{a}	41.6 ± 1.4^{a}
Female heated	4.90 ± 0.42^{b}	8.78 ± 0.24^{a}	15.63 ± 0.26^{c}	49.2 ± 1.5^{b}
Sex – Temp. effect	MCV (fl)	MCH (pg/cell)	MCHC (g/dl)	PLT (10^3 cells/µl)
NR	48.0 -56.0	11.9 -19.0	25.9 - 35.1	160 – 410
Male control	51.3 ± 0.29^{a}	16.3 ± 0.15^{a}	31.8 ± 0.22^{a}	886.3 ± 54.1^{a}
Female control	52.2 ± 0.42^{a}	16.6 ± 0.17^{a}	31.9 ± 0.23^{a}	911.8 ± 44.7^{a}
Male heated	51.8 ± 0.32^{a}	16.9 ± 0.23^{a}	32.8 ± 0.47^{a}	399.8 ± 58.7^{b}
Female heated	56.1 ± 0.27^{b}	17.9 ± 0.39^{b}	31.9 ± 0.71^{a}	411.2 ± 37.0^{b}

NR: normal CBC ranges published in the international literature.

Discussion

It seems that during the first stage, the heat exposure did not produce a noticeable effect. In the second stage, the mice suffered from the heat and were trying to find the least hot place in the cage by moving restlessly around, digging in the sawdust, and trying to escape out of the cage. The signs of heat stress commonly reported in the literature depend on species but may include animals bunching, seeking shade, panting, slobbering or excessive salivation, foam around the mouth, open-mouth breathing, lack of coordination, and trembling, which are aimed at strengthening the organism against the hyperthermia. In the third stage, the mice discovered that this behavior was futile and that it was better to lie dormant in one side of the cage as a continuation of the physical activity observed in the second stage would result in the production of more metabolic heat which would aggravate the situation further. Previous studies reported that stressed animals attempt to make abnormal or extreme adjustments in their physiology, behavior, or interception in order to cope with adverse aspects of the stress *(1-5)*. That mice regained normal activity after 20 to 30 minutes (fourth stage) after the termination of the heat exposure means that the exposure did not cause long-term or permanent physiological disorder. All CBC values obtained in the present study were either within or close to the normal range reported in the literature except for PLT of the control male and female groups which recorded much higher values. The cause for this anomaly could not be inferred. It might be due to the type of food presented for the mice during the raising period. The feed consisted of a mixture of barley, concentrate pelted feed for white hen breed, and broken pieces of dry white bread. The composition of the concentrate is unknown to us. It may be that the unavoidable delay in taking the blood

samples had led to the breakdown of megakaryocytes into platelets. All CBCs values between male and female control groups were not statistically significant. This is in spite of the observation that males were fighting for dominance most of the time and often injuring each other. Exposure to heat, however, had a profound effect on values of the CBC parameters which may differ significantly between males and females. Females tend to be more affected by heat than males.

It is not possible to account for all CBC differences between the heat exposed groups and the control groups of the present study. Exposure to heat decreased the concentration of WBCs of male and female mice. This is in agreement with previous findings that reported this decrease could be related to atrophy of all lymphoid organs (thymus, bursa, spleen, or liver) as their weights were significantly reduced by heat stress *(11-15)*. This could have been a result of the reduction in feed intake, thereby providing fewer nutrients for the proper development of these organs. Another study reported that exposure of birds to high environmental temperature causes an increase in the plasma corticosterone which subsequently depresses the activity of the lymphoid organs and total leucocytes count *(16)*. In the present study, a significant decrease in the RBC count of the heat exposed mice exposed compared to that of the control group was observed. This is in agreement with the findings that reported that heat stress in mammals decreases the level of Adrenocorticotropin Hormone (ACTH), which might then result in decreases in RBC counts, HCT, and HGB concentration *(17)*. In addition, the depression of HCT during the hot season was also reported to be related to a reduction in cellular oxygen, a requirement for reducing metabolic heat production in order to compensate for the elevated environmental heat load *(18-20)*. The decrease in the number of RBCs may be due to the inhibition effect of heat stress on the life span of the present RBCs as well as the production of new RBCs from the bone marrow. A significant decrease in packed cell volume was recorded in the male heat exposed mice compared to the control groups or the heat exposed female group. This is in agreement with studies mentioned that heat stress led to a significant decrease in mononuclear cells (*13,15,21,22*). Meanwhile other suggested that reduced blood HCT can be attributed to hemo-dilution (*23,24*).

In the present study, a significant decrease in HGB of the Male heat exposed mice in comparison with the Female control group and the Female heat exposed group was observed. Vo, *et al. (24)* concluded that heat stress leads to a decrease in RBCs numbers, the mean corpuscular hemoglobin concentration, and the life span of red blood cells. This leads to decreased hemoglobin concentration in the blood due to the positive relation between the RBCs number and hemoglobin concentration in blood. The MCH value estimated the average hemoglobin content of each red cell. A significant increase in MCH of the Female heat-stressed mice in comparison with control groups and the Male heat-exposed group. However, others reported that heat stress decreases the mean corpuscle hemoglobin value *(12,13,15,25)*. The results in Table (1) indicated an indirect relation between the degree of temperature used in heat stress and the increase in MCV value in the female heat-stressed mice. Similar results were obtained previously *(12,13,24,26)*. MCHC is an index of the proportion of hemoglobin per average red blood corpuscle. The results in Table (1) showed that MCHC level was not affected by exposure to heat.

Conclusion

Short-term exposure of albino mice to heat produced profound effect on their blood CBC.

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