

EFFECT OF ACUTE HEAT STRESS ON CERTAIN IMMUNOLOGICAL PARAMETERS IN ALBINO RATS

I. MINI JOSEPH, N. SUTHANTHIRARAJAN AND A. NAMASIVAYAM*

Department of Physiology,
Dr. A.L.M. PG Institute of Basic Medical Sciences,
University of Madras,
Taramani, Madras - 600 113

(Received on April 17, 1991)

Abstract : The effect of acute heat stress on certain immunological parameters were studied in male albino rats. The test rats were exposed to an ambient temperature of 40 degrees celsius for 30 minutes and sacrificed immediately. Total WBC count, Differential count, Phagocytic index, NBT reduction, organ weight body weight ratio of spleen, thymus, and popliteal lymph nodes, and soluble immune complex levels were measured in control group and the heat stressed animals. The heat stressed animals show decrease in total WBC count, and neutrophilia, eosinophilia, and lymphocytopenia. The phagocytic index showed a significant increase whereas the avidity index showed a decrease from the control value. NBT reduction was also significant. The soluble immune complex level was not altered. The heat stressed animals showed a decrease in the thymus and spleen weight/body weight ratio while the lymph node/body weight ratio showed an increase compared to the control animals.

Key words :

heat stress

immune system

INTRODUCTION

The term stress is ambiguous and precludes accurate definition. Biological stress of various types leads to General Adaptation Syndrome (1). The effect of stress on the immunological functions in human beings, resulting in psychosomatic disorders has been an important observation (2). Heat stress is encountered by all of us in every day life. When an individual is exposed to temperatures above the zone of thermal neutrality, changes in pituitary-adrenal axis occur which may influence the immune mechanisms. Since the available literature is sparse in this field, this investigation was undertaken to study the influence of acute thermal stress on certain immune parameters.

METHODS

Healthy Wistar strain male albino rats, weighing 120-130 g, were used in this study. The animals were housed (5/cage) in standard plastic cages (30 cm ×

22 cm × 14 cm) and provided with routine pellet feed and water *ad libitum*. Ten animals were used as the control group and another ten animals were used as the experimental group. Except the heat stress, the test and control groups were maintained under identical conditions.

Heat stress procedure : The rats were placed individually in an insulated wooden box heated by a thermostatically controlled infra red lamp. The box temperature was maintained at 40° Celsius and the test rats were individually exposed to this temperature for 30 minutes. The control rats were also placed in the same chamber to attain identical conditions for the same period of time, but without switching on the infra red lamp.

Immediately after the exposure, the rats were sacrificed by exsanguination from jugular vein under light ether anesthesia and blood thus collected was used for the different investigations. Total

*Corresponding Author

WBC counts and differential counts were determined following routine methods. The phagocytic activity of the neutrophils was determined by using heat killed candida (3). Microbicidal activity of the neutrophils was determined by the NBT reduction test (4). Soluble immune complex levels were determined by the method of Seth and Srinivas (5). Animals were then sacrificed by an overdose of ether and the wet weights of spleen, thymus and popliteal lymph node determined and expressed as mg/g of body weight.

RESULTS

The effects of acute heat stress on the various parameters studied are shown in Tables I, II and III.

TABLE I : Changes in total WBC and differential count in acute heat stress. (N = Neutrophil, L = Lymphocyte, E = Eosinophil, M = Monocyte, B = Basophil) values are mean of 10 animals +/-SEM.

Group	Total WBC count/ c.mm	Differential count in %				
		N	L	E	M	B
Control n=10	18,635	24.5	70.0	2.4	5.1	0.3
	+/- 1,342	+/- 1.5	+/- 1.54	+/- 0.3	+/- 1.04	+/- 1.15
Acute heat stressed animals n=10	8,840	28.6	59.2	9.1	2.5	0.1
	+/- 807 (P<0.001)	+/- 0.77 (P<0.05)	+/- 1.16 (P<0.001)	+/- 0.4 (P<0.01)	+/- 0.26 (P<0.05)	+/- 0.03 NS

TABLE II : Candida phagocytosis, NBT reduction and soluble immune complex in acute heat stressed albino rats.

Groups	Phagocytic Index	Avidity Index	NBT Reduction	SIC Index
Control	56.2	2.937	11.2	17.0
	+/- 2.14	+/- 0.17	+/- 0.71	+/- 2.9
Acute heat stressed animals n=10	70.6	2.259	6.5	11.0
	+/- 2.21 (P<0.001)	+/- 0.16 (P<0.02)	+/- 0.31 (P<0.01)	+/- 1.0 NS

Values are mean of ten animals +/- SEM.

TABLE III : Lymphoid organs weight changes in acute heat stress (Organ Weight/Body Weight Ratio).

Groups	Spleen	Thymus	Lymph Node
Control n=10	3.927	1.07	0.083
	+/- 0.3318	+/- 0/095	+/- 0.01
Acute heat stressed animals n=10	4.033	0.954	0.128
	+/- 0.154 NS	+/- 0.67 NS	+/- 0.11 (p<0.01)

Values are mean of ten animals +/- SEM.

Acute heat stress decreased the total WBC count ($P < 0.001$). Such a stress increased the Neutrophil count ($P < 0.05$) with a concomitant decrease in Lymphocyte count ($P < 0.001$). The Monocyte count also decreased significantly ($P < 0.05$), while the eosinophils showed significant increase ($P < 0.001$). The Basophils showed no change.

Phagocytic index in heat stressed animals showed a highly significant increase ($P < 0.001$) whereas the avidity index was significantly decreased ($P < 0.02$). NBT reduction was also significantly decreased ($P < 0.01$). The soluble immune complex levels however did not show any significant change.

Thymus and Spleen did not show any weight changes after heat stress while the weight of popliteal Lymph node was significantly increased ($P < 0.01$).

DISCUSSION

In the present study certain immunological parameters were studied after an acute exposure of rats to heat stress. The results suggest that such a stress influences certain immune parameters.

Our previous studies with cold stress had also shown a decrease in the total WBC count (6). Selye (1) had observed leucopenia in stressed animals. Jensen and Rasmussen (7), and Ramsey (8) and many others, have shown the involvement of adrenals in influencing the white cell contents of blood. Hence the observed decrease in white cell count of stressed animals may be attributed to the action of adrenal steroids. However, the possibility of some local chemotactic factors, causing a shift of the circulating leucocyte

population into the reservoirs, cannot be ruled out. The increase of adrenal steroids, while explaining the decrease in total WBC count, fails to offer a satisfactory explanation for the observed eosinophilia. Corticosteroids are known to produce lymphocytopenia and eosinopenia; whereas in this study while lymphocytopenia was observed, there was eosinophilia on the other hand. Similar observations have also been reported by Lee (9) in cold stressed rabbits and by Sheela and Namasivayam (10) in rats after acute cold swimming stress. In this connection it is pertinent to point out that stress induces both elevation of catecholamines and corticosteroids. Samuel (11) reported a primary and secondary leucocyte changes after the injection of epinephrine. Probably the increase in eosinophils reflects the primary response to the increase in catecholamines in the stressed animals. The absence of eosinophilia in chronically heat stressed animals (unpublished observation) also lends support to this concept of catecholamine action.

Published work on neutrophil function during stress is scanty and controversial. The increase in phagocytic index observed in this study is probably due to the action of stress produced hormones acting on the phagocytic cell receptors, which alter the metabolic activity of these cells. Similar results were reported by us when using subacute cold stress (6). While there is an increase in phagocytic index, the avidity index however decreases. This indicates that while more phagocytic cells take part in removing the

Candida, the number of *Candida* particles taken up by each cell is decreased. This is in contrast to our observation made with cold stress where both the phagocytic index and the avidity index were shown to be increased. There may be different mechanisms operating in cold and heat stress. However, the NBT reduction seen in the heat stressed animals is similar to that observed in the cold stressed animals (6).

Though there is a slight decrease in the soluble immune complex levels in heat stressed animals, it is not statistically significant. On the other hand our previous observations with cold stress had shown a significant decrease in the levels of soluble immune complex (6). Similar to avidity index this parameter also differs from that of the general stress effect.

In the heat stressed animals the primary lymphatic organs like thymus and spleen undergo involution, which form a part of the general adaptation syndrome. On the other hand lymph node is less susceptible to the stress/cortisol effect, probably because of its greater capacity to oxidize corticosteroids (12). It may be suggested that under the influence of steroid hormones of the adrenal cortex, the lymphocytes may be redistributed circulating to peripheral lymphoid organs. This phase may well be a transitory one, for leucopenia of acute stress is often followed by leucocytosis, and the lymph node under the influence of elevated steroid levels may gradually undergo involution like the primary lymphoid organs.

REFERENCES

1. Selye H. The physiology and pathology of exposure to stress. Acta Inc Medical Publishers, Montreal, Canada. 1950; 12-75.
2. Keller SE, Weiss JM, Schleifer SJ, Miller NE, Stein M. Suppression of Immunity by stress : Effect of a graded series of stressors on lymphocyte stimulation in the rat. *Science* 1981; 213:1397-1400.
3. Wilkinson. In : Techniques in clinical Immunology. Ed. by R.A. Thompson, First edition. Blackwell Sci Publication 1977.
4. Gifford RH, Malawista SE. A simple rapid micromethod for detecting chronic granulomatous disease of childhood. *J Lab Clin Med* 1970; 75:511-519.
5. Seth P, Srinivas RV. Circulating immune complexes in cervical cancer. Simple method for detection and characterisation. *Indian J Med Res* 1981; 73: 926-929.
6. Sundaresan G, Suthanthirarajan N, Namasivayam A. Certain Immunological Parameters in subacute cold stress. *Indian J Physiol Pharmacol* 1990; 34:57-60.
7. Jensen MM, Rasmussen AS Jr. Stress and susceptibility to viral infection. Response of adrenals, liver, thymus and peripheral leucocyte counts to sound stress. *J Immunol* 1963; 90:17-20.
8. Ramsey JM. Basic Pathophysiology. Modern stress and the disease process. Addison-Wesley Publishing Co., California, 1982; 94-25.
9. Lee, Byung O. Studies on effect of heat and cold stress on blood picture, values of serum protein and blood glucose in rabbits. *Ilanguk, Chukson Hakhoechi* 1982; 29(5): 199-207.
10. Sheela Devi R, Namasivayam A. *Candida* phagocytosis in acute stress. *Indian J Med Microbiol* 1986; 4: 271-277.
11. Samuels A. A primary and secondary leucocyte changes following the intramuscular injection of ephinephrine. *J Clin Invest* 1951; 3:941.
12. Dougherty TF, Berliner ML, Scheneebli GL, Berliner D. Hormonal control of Lymphatic structure and Function. *Ann N Y Acad Sci* 1964; 113:825-843.