

## EFFECT OF CHRONIC VIBRATION ON THE IMMUNE STATE OF ALBINO RATS

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**Abstract :** A study was undertaken to investigate the effects of whole body chronic vibration on the immune system. Albino rats were exposed to whole body horizontal vibration acceleration 5.0 g, frequency 20 Hz for 3 hours per day for 3 months and changes were observed in plasma corticosterone level, total leucocyte count and differential leucocyte count. Neutrophil functions were accessed by candida phagocytosis and Nitroblue tetrazolium reduction test. The total leucocyte count was significantly decreased. A marked lymphopenia was observed in the differential count of the leucocytes. A significant increase in the plasma corticosterone level, candida phagocytosis and Nitroblue tetrazolium reduction was observed, indicating chronic whole body vibration to be a potent stressor in albino rats.

**Key words :** vibration  
leucocyte

corticosterone  
neutrophil function

### INTRODUCTION

Homeostatic mechanisms are geared toward counteracting the everyday stresses of life. If stresses are extreme, unusual or long lasting, however, the normal mechanism may not be sufficient. In this case the stress triggers the wide ranging set of body changes called the general adaptation syndrome GAS (1). Unlike the homeostatic mechanisms, the GAS does not maintain a normal internal environment, but in fact, it does just the opposite for instance, it raises the blood pressure and blood sugar level above normal. The purpose

of these changes in the internal environment are to gear up the body to meet an emergency.

It should be pointed out that it is impossible to remove all stress from our everyday lives. In fact some stress prepares us to meet certain challenges. Thus some stresses are productive whereas others are harmful stress. Recent findings suggest that the harmful stress temporarily inhibits certain components of the immunological system (2). The stress that produce the GAS are called stressors. A stressor may be any disturbance like heat, cold, noise, surgery,

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vibration etc. A number of reports have shown several physio-pathological changes both in human and animal after exposure to vibration.

The problem of long term low frequency vibration of whole body in the production of a "Vibration disease" has been reported (3). The disorders of the nervous, circulatory and digestive systems in this condition were interpreted not to be whole body vibration specific but were related to the totality of the working condition to which the human or animals were exposed. It has been reported that long exposure to whole body vibration can contribute to back disorders and increased prevalence of low back pain, probably in a dose related fashion (4) Rats exposed to whole body vibration have shown increased plasma corticosterone along with whole brain 5HT and 5HIAA level, (5). The same author has also shown decreased brain nor-epinephrine level in rats exposed to various whole body vibration. The decrease was also observed in hypothalamus and hippocampus (6). Recent observations have shown the effect of mechanical vibration on the behaviour of serum immunoglobulin and lymphocyte transformation (7).

From the above reports it is clear that vibration produces wide-spread disturbances in immunological, biochemical and physiological activities of human and animals. However, a perusal of literature has shown only few reports on the chronic effect of vibration on the immunological parameters. Hence, this study was undertaken with a view to elucidate some of the immunological alteration after chronic vibration in albino rats.

## METHODS

The study was conducted in healthy Wistar albino rats of either sex (150-170 gms), housed under standard laboratory conditions with food and water provided ad libitum. The animals were divided into two groups of 10 rats each.

**Control group :** Rats were kept under standard laboratory conditions.

**Experimental group:** Rats were subjects to whole body horizontal vibration acceleration 5.0 g frequency 20 Hz for 3 hours for 3 months.

After completion of the vibration exposure period the experimental animals were rapidly anesthetized with ether, within two minutes, according to the stress free procedure of Feldman and Conforti (8) which does not cause stress to the animals as evidenced by stable corticosterone level in blood. This procedure also causes no changes in the hematological parameters studied in this investigation. Blood samples were collected from the jugular vein in both the groups after ether anesthesia for the following estimation-total leucocyte count, differential leucocyte count, neutrophil function tests and plasma corticosterone.

The total and differential leucocyte counts were estimated by standard methods. The phagocytic ability of neutrophils was studied by noting the candida phagocytic activity (9). This method relies on the uptake of particles by phagocytic activity over a brief period of time. The leucocyte

suspension was obtained from buffy coat of 0.5 ml heparanized blood sample. Heat killed candida albicans was added and incubated at 37°C for 15 min and centrifuged at 2000 rpm for 5 min. Smear were made using the sediment and stained with Leishmans stain. This test relies on the uptake of heat killed candida albicans by phagocytes over a brief period of time. The intracellular candida, strain intensely and can be identified and counted inside the neutrophils. The number of neutrophils positive for candida ingestion in 100 neutrophils gives the phagocytic index (PI) and the total number of candida albicans counted within 100 positive cells divided by 100 gives the avidity index (AI).

The killing ability of neutrophil was assessed by the nitroblue tetrazolium (NBT) reduction test (10) Granulocytes isolated from 0.5 ml heparinised blood are taken on a clean microscopic slide and incubated at 37°C for 30 minutes. At the end of 30 minutes the slide was washed and to the neutrophils adhering to the slide was added NBT and incubated at 37°C for 30 minutes. The slide was fixed, stained and examined under oil immersion. When the neutrophils are exposed to the yellow dye nitroblue tetrazolium (NBT) it is taken by the cells into phagosome and intracellular reduction of the dye converts it to an insoluble blue crystalline form (formazan crystals). The blue crystals are visible in light microscope and can be counted. Percentage of NBT positive cells containing blue formazan crystals were determined by counting 200 neutrophils.

The plasma corticosterone level was estimated by the spectrophotometric method

(11) Statistical analysis was done by the student's t test.  $P < 0.05$  was considered statistically significant.

## RESULTS

Some of the possible immunological changes in response to chronic vibration have been assessed in this study.

Chronic vibration group resulted in a statistically significant decrease in the total white blood cell count. ( $P < 0.001$ ) (Table I). In the differential count a marked lymphopenia was observed in chronic vibration group, which is statistically ( $P < 0.05$ ) significant after exposure to vibration, but no significant change was seen in neutrophil, eosinophil, basophil and monocyte counts. (Table I). A significant increase in the plasma corticosterone level ( $P < 0.001$ ) was observed in the vibration group (Table II). In neutrophil function test the phagocytic index (PI) ( $P < 0.001$ ) and avidity index (AI) ( $P < 0.001$ ) were significantly raised in chronic vibration

TABLE I: Total leucocyte count and differential leucocyte count in chronic vibration.

Parameter	Control (n = 10)	Chronic Vibration (n = 10)
Total leukocyte count (per cu.mm)	18480±1170.71	12979±1572.63*
Differential count (%)		
Lymphocyte	59.1±4.39	51.4±3.26*
Neutrophil	31.0±4.4	31.3±4.2
Monocyte	7.6±0.8	7.4±1.28
Eosinophil	5.5±1.02	5.7±1.10
Basophil	1.2±0.87	1.37±0.64

Values are expressed as mean ± SD; Significance: \* $P < 0.001$ .

group animals when compared with the control animals. The percentage of NBT positives containing blue formazon deposit was determined. NBT reduction showed a significant increase ( $P < 0.001$ ) in chronic vibration. (Table III)

TABLE II: Plasma corticosterone level in chronic vibration

Parametre	Control (n = 10)	Chronic Vibration (n = 10)
Plasma corticosterone ( $\mu\text{g}$ )	92.06 $\pm$ 1.2	122.25 $\pm$ 5.9

Values are expressed as mean  $\pm$  SD; Significance: \* $P < 0.001$ .

TABLE III: Neutrophil function in chronic vibration.

Parametre	Control (n = 10)	Chronic Vibration (n = 10)
Phagocytic Index (PI)	74.6 $\pm$ 1.5	88.8 $\pm$ 2.48*
Avidity Index (AI)	2.89 $\pm$ 0.1	3.82 $\pm$ 0.35*
NBT reduction test (%)	10.4 $\pm$ 1.1	17.7 $\pm$ 1.00*

Values are expressed as mean  $\pm$  SD; Significance: \* $P < 0.001$ .

## DISCUSSION

The neural and hormonal system play a role in homeostatic regulation and much of the control exercised in response to stressors stems directly from the activity of autonomic nervous system and adrenal gland. In our study certain immunological changes were observed after exposure to chronic vibration for three months. The data suggest that vibration acts as a significant stressor with respect to the overall body physiology thought most of the effects are indirect being manifested through activation of the autonomic nervous system and hypothalamic pituitary adrenal axis.

Chronic vibration produced a significant decrease in total white cell count. Similar results were also obtained in rabbits exposed to heat stress. Recent report indicates that rats exposed to noise also showed similar results (12). It could be due to the excessive release of corticosterone in vibration stress which is known to cause leukopenia.

The significant elevation in the plasma corticosterone level observed in the vibration group could be due to norepinephrine acting at corticotropin releasing factor (CRF) neuron in the paraventricular nucleus of hypothalamus to directly stimulate CRF release which stimulate the anterior pituitary to release ACTH, which stimulate the adrenal cortex to release corticosterone (13) our findings are also in agreement with elevated plasma corticosterone during noise stress (14).

Our present study shows that chronic vibration causes lymphocytopenia. This is in agreement with results reported in the literature (15). Plasma corticosterone was elevated during chronic vibration. The corticosteroids have extensive complex effect on the immune system. Increase corticosterone diminish mitogen induced lymphocyte stimulation (16). It has been demonstrated that recirculation of lymphocyte traffic in humans is sensitive to corticosteroids (17). From the earlier studies it was recognised that elevated glucocorticoids production in many species of animal and man was associated with adrenal hypertrophy, thymic involution and lymphocytopenia (18).

A significant enhancement in the neutrophil functions has been observed in our study. The phagocytic ability of the neutrophils as indicated by the phagocytic index and avidity index has been significantly increased in vibration. Scanty literature evidence exists on neutrophil function tests in vibration. However reports have shown that increase phagocytic index and avidity index during chronic vibration may be due to the action of stress hormone corticosterone on phagocytic cell receptors which alter the metabolic activity of the phagocytic cell.

In our study an increase corticosterone level has been observed which could have mediated the changes in the neutrophil functions via the receptors present on the leucocytes. Phagocytes have beta adrenergic receptors and possess receptors for neuro peptides. Thus phagocytes can be affected by the neuro transmitters. In our study killing ability of the neutrophil as indicated by the NBT reduction has been significantly increased. Reports have shown that phagocytosis is an energy mediated phenomenon and 3-5 cyclic adenosine monophosphate (cAMP) acts as a second

messenger. The cAMP regulates selective extrusion of lysosomal enzymes in phagocytic neutrophil and impair the neutrophil ability to kill candida albicans. The phagocytic cells contain alpha and beta adrenergic receptors which when stimulated can elevate cellular cyclic guanosine mono phosphate (cGMP) where by alteration in the endocrine environment (or) autonomic output could modulate the neutrophil function. Sympathatic neurohormones (Catecholamines) acts through cAMP, while parasythetic neurotransmitters (Acetylcholine) act through cGMP (19). The changes observed in neutrophil function in this study may be due to the interaction of these different factors.

From our study it is clear that chronic vibration causes various physiological changes like leucopenia a significant elevation in the corticosterone and an enhancement of neutrophil functions. Based on these it can be concluded that chronic vibration (acceleration 5.0 g, frequency 20 Hz for 3 hours per day for 3 months) is a potent stressor and the elevated plasma corticosterone level lend support to this concept.

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