

# The Effect of Overpopulation on Neutrophile/Lymphocyte Ratio and WBC Values, in Normal and Propranolol Treated Rats

Gabriel COTOR.\*, GAJAILA G., DOBREA Mimi, GAJAILA Iuliana

The Faculty of Veterinary Medicine, Bucharest, Romania; cotorg@yahoo.com

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## Abstract

Our study evaluate the effects of the overpopulation stress on some leucogram parameters in normal and Propranolol treated rats. In this purpose 18 adult Wistar rats were separated in standard cages, for 6 days, in 3 experimental groups,. The density of rats varies: Group I = 3 rats/cage; Group II = 6 rats/cage; Group III = 6 rats/cage. The rats belonging to the Group III were injected with Propranolol (5 mg/kg, s.c.). In the 6<sup>th</sup> day of the experiment blood samples were collected. The WBC count and N/L ratio were determined. The WBC values were significantly increased ( $P < 0.05$ ) in Group II, compared to Group I (6.77 %) and III (7.71%). This leukocytosis was an adaptive response to overpopulation stress. These results suggest that in this response is involved predominantly the sympatho-adrenal mechanism. The N/L ration were significantly increased ( $P < 0.05$ ) in Group II, compared to Group I (16.94 %). This parameter was increased in Group II, compared to Group III (6.15%). But this increase was not significant, statistically speaking. According to our results the overpopulation in rat seems to be a very stressful factor. Given that Propranolol had a weak effect on N/L ratio, means that, in this case, the sympatho-adrenal mechanism is not the only involved mechanism.

## Keywords

*overpopulation stress; leukocyte; N/L ratio; rat*

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**Introduction:** Overpopulation is a well known stress factor in many species. In stress response the increased plasma corticosterone induces significant changes in leukocyte profile in mamals and bird. (Davis, 2008; Dhabhar, 1994). So, differential white blood cell counts from blood smears became a reliable and widely used method of measuring physiological stress (Cirule, 2012). In stress response increases the number of neutrophils (N) and decreases the number of lymphocytes (L). So, the ratio N/L increases, becoming a reliable indicator of stress response (Pruet, 2003). The stress response imply: sympathoadrenal system, and hypothalamo-pituitary-adrenocortical axis. The share of adrenergic mechanism in this response has not yet been clarified.

**Aims and objectives:** The aim of this study was to evaluate the effects of the overpopulation stress on some WBC parameters in normal and Propranolol treated rats. In our experiment Propranolol (a well-known beta blocker) was used as an indicator of the involvement of the sympatho-adrenal mechanism (Shilov, 2001).

**Materials and methods:** 18 adult Wistar rats were used in our study. The rats were separated in 3 experimental groups (n=6). For 6 days the animals were maintained in standard cages. The density of rats varies: Group I = 3 rats/cage; Group II = 6 rats/cage; Group III = 6 rats/cage. In the 5<sup>th</sup> and 6<sup>th</sup> days of the experiment the rats belonging to the Group III were injected with Propranolol (5 mg/kg, s.c.). The rats belonging to Group I

and Group II were injected in the same manner with physiological saline. In the 6<sup>th</sup> day of the experiment blood samples were collected from the tail vein, into heparinised plastic tubes. The blood smears were made and stained with May-Grünwald and Giemsa. The leukocyte formula and N/L ratio were determined. The blood smears were made on duplicate glass slides. These smears were stained with May-Grünwald and Giemsa. The stained preparations were examined using light microscope. In this purpose were used oil immersion lens objective. Two hundred leukocytes (neutrophils, lymphocytes, monocytes, basophils and eosinophils) were counted on each

stained slide. The N/L ratio was calculated in both duplicate stained preparations and the means were calculated for each rat. Leukocyte counts were performed within an hour after blood sampling, using a manual haemocytometer method (Turk-Zeiss chambers). In this purpose we used dilutions (1: 20) with Turk solution. The statistical significance of data was determined by the t test (Student).  $P < 0.05$  was considered significant.

**Results and discussion:** Results on haematological determinations performed in rats, during the study period are given in next table:

Group no.	WBC ( $1 \times 10^9/L$ )	N/L ratio
1	5.741	0.59
2	6.13*	0.69**
3	5.691	0.65

\* $P < 0,05$  (compared with group I and III), \*\* $P < 0,05$  (compared with group I)

The WBC values were significantly increased ( $P < 0.05$ ) in Group II, compared to Group I (6.77 %) and III (7.71%). This leukocytosis is an adaptive response to overpopulation stress. Given that Propranolol determined the maintenance of this parameter to low values, means that in this response is involved predominantly the sympatho-adrenal mechanism. The N/L ration were significantly increased ( $P < 0.05$ ) in Group II, compared to Group I (16.94 %). The same parameter was increased in Group II, compared to Group III (6.15%). But this increase was not significant, statistically speaking. N/L ratio has been indicated to be an objective stress evaluation method (Davis, 2008), so, according to our results the overpopulation in rat seems to be a very stressful factor. Given that Propranolol had a weak effect on N/L ratio, means that, in this case, the sympatho-adrenal mechanism is not the only involved mechanism.

**Conclusion:** The overpopulation stress induced the increasing of WBC values and N/L ratio. The Propranolol has led to maintain to low levels these parameters, highlighting the involvement of the sympatho-adrenal mechanism.

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