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Stress induced alterations in the hemocyte population of *Periplaneta americana* (L.)

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Abstract. Alterations in the hemocyte population of *Periplaneta americana* (L.) during starvation and desiccation were studied for 11 days. Total hemocyte count showed increasing trend throughout the starvation period. Changes in differential hemocyte count were evident. Plasmatocytes and granulocytes decreased marginally. Increase in coagulocyte count occurred from 6th day onwards. Two-fold increase in the total hemocyte and differential count was pronounced during subsequent days. During the combined stress period total hemocyte count was significantly higher. Differential hemocyte count was also altered. Degenerative changes set in during late phases of the experiment. The causal factors for such changes in hemocyte population under experimental stress are discussed.

Keywords. Roach; *Periplaneta americana*; hemocytes; desiccation; starvation; total and differential counts.

1. Introduction

Knowledge of the insect hemocyte population is essential for physiological and other studies. Data on total and differential counts in insects are available. The hemocyte counts vary during development, physiological status, starvation, wounding, infection etc. (Jones and Tauber 1952; Shapiro 1966, 1967, 1968, 1979; Jones 1967; Arnold and Hinks 1976; Gupta 1985; Sonawane 1985). Inadequate information on starvation and almost no information on desiccation induced changes in insect total hemocyte count (THC) and differential hemocyte count (DHC) are available. The above stresses are known to induce certain physiological changes. Present work is aimed at finding out the effects of the above two metabolic stresses, separately and in combination, on THC and DHC of *Periplaneta americana*.

2. Material and methods

Adult roaches of the same age and with no regard to sex, were collected from a single den. Four batches, each of 50 roaches were used for the experiments. Batch I served as control. It was kept under laboratory conditions. For starvation, roaches of batch II were employed. They were supplied with water but no food. Batch III was exposed to desiccation by keeping the roaches in plastic containers having a number of holes in them. Such containers were kept in desiccators containing anhydrous $CaCl_2$. The roaches were, however, supplied with food. For combined effect, the roaches (batch IV) were kept as above and to them neither food nor water was supplied.

The THC and DHC of the roaches of the above batches were done every 24 h, for 11 days. All the roaches were found dead on 12th day. The hemolymph was obtained

by amputation of antennae. After dilution (in Thoma-Zeiss hemocytometer) the hemocytes were counted in Neubaur's hemocytometer (Witting 1966). Following formula of Jones (1962) was adopted for calculations.

$$\frac{\text{Hemocytes in} \times 1\text{ mm squares} \times \text{dilution} \times \text{depth of the chamber}}{\text{Number of 1 mm squares counted}}$$

For the DHC, fixed stained monolayers (Sonawane 1985) were used.

3. Results

3.1 *Starvation*

During the entire starvation period the THC remained higher (control, 22, 291 ± 262). On 5th day the count was the highest (40, 935 ± 1067) and on the termination day it was 32,031 ± 692 (table 1).

Prohemocytes (PRS) increased to 6% (control, 3%) after 2 days of starvation. Percentage of plasmatocytes (PLS) and granulocytes (GRS) decreased marginally. No change in the count was seen in the coagulocytes (COS) and spherulocytes (SPS). On the 3rd day PLS increased to 65% (control, 53%). Amongst them the spindle shaped were dominating. They were almost more than half the total number of PLS. On the 5th day PLS decreased to 45% and the GRS increased in their count to 60% (control, 40%). At this stage, even the PLS contained granules which were basophilic and hence similar to the granules in GRS seen under normal conditions. Increase in the COS count commenced from the 6th onwards (control, 3%). On the 7th day their count was 5·5%. From the 8th day onwards distinct morphological changes were

Table 1. Effect of starvation, desiccation and starvation and desiccation (combined) on THCs of *P. americana.*

Experimental period (days)	Experimental condition (THC, mean ± SE)		
	Starvation	Desiccation	Starvation and desiccation
1	—	—	—
2	36,250 ± 883	42,500 ± 2,041	60,062 ± 1,662
3	38,125 ± 255	25,312 ± 598	62,406 ± 487
4	38,750 ± 510	22,500 ± 510	88,687 ± 514
5	40,935 ± 1,067	91,000 ± 4,102	62,375 ± 625
6	35,625 ± 555	74,437 ± 1,165	56,125 ± 1,586
7	34,343 ± 257	68,093 ± 2,634	54,000 ± 797
8	32,968 ± 1,032	27,500 ± 2,041	63,750 ± 510
9	33,656 ± 271	11,093 ± 553	57,562 ± 482
10	33,843 ± 180	8,875 ± 525	44,487 ± 1,067
11	32,031 ± 692	—	52,031 ± 781

Mean THC of control insects was 22,291 ± 261.
Differential hemocyte count of control insects was PRS, 3%; PLS, 53%; GRS, 40%; SPS, 1%; COS, 3%.

evident in some of the GRS. In some such cells, the granules were pushed towards the periphery. The nuclear volume showed increase. Their chromatin was loosened and dispersed. Perinuclear cisternae were quite evident in them. The GRS remained the dominating hemocyte type from 5th onwards. They were followed in number by PLS, COS, PRS and SPS in that order.

On the 10th day the count of different hemocytes was GRS 56%, PLS 30%, COS 6%, PRS 4·5% and SPS 3·5%.

3.2 *Desiccation*

Alterations in THC during desiccation are presented in table 1. Two-fold increase in the count occurred during the first two days. On the 4th day it appeared to be normal. There was an abrupt increase (91,000 ± 4102) on the 5th day. The count started decreasing from the 6th day onwards. On the 10th day it was 8,875 ± 825. Most of the PLS observed on the 3rd day were spindle shaped. They became polymorphic on the 4th and 5th day. The DHC remained unchanged upto the 8th day. From this day onwards, the degenerative changes commenced in all the hemocyte types. Such changes were frequent and pronounced in the GRS. Because of this, some of them resembled the COS. Cell fragmentation was rapid on the 10th day and it was impossible to distinguish the cell types on subsequent days.

3.3 *Starvation and desiccation*

The THC was significantly higher in *P. americana* during the above combined stress period. Data in table 1 reveal the highest count (88,687 ± 514) on the 4th day and minimum on the 10th day (44,487 ± 1067). The data also indicate that the fluctuations in the total hemocyte count occur but they are, however, of a smaller magnitude than during the stress conditions applied separately.

After two days, the GRS predominated. Together with the PLS they comprised the main bulk on the 4th day (80–89%). The PRS increased to about 7·10%, the COS about 3% and the SPS less than 1%. Increase in the COS was evident on the 5th day (about 6%). At the end of the 7th day, however, the COS percentage dropped below average (i.e. less than 3%). Degenerative changes in many PLS and the GRS were initiated by the end of the 8th day. Their cell membrane was inconsistent, ill defined, and lysed at many places. Enhanced degenerative changes were evident from the 9th day onwards till the termination of the experiment. On the 11th day, surprisingly, some newly formed PLS and GRS were seen along with the old degenerating hemocytes.

4. Discussion

Jones (1950) reported that no appreciable change was seen in the THC of the last instar larvae of *Tenebrio molitor* starved for 30 days. According to him there exists a tendency in them for the GRS to increase and the PLS to decrease in count. Apparent hemocytopenia sets in after starvation for 120 days. During this period the PRS increased many folds. Decrease in the THC is evident after prolonged starvation

(Jones and Tauber 1952) in the same insect. An increase in the THC is reported in *Leptinotarsa* (Arvy *et al* 1948) and *Prodenia* (Yeager 1945; Rosenberger and Jones 1960) during starvation. On the other hand a decrease in the THC is seen in the starved larvae of *Bombyx mori* (Nittono 1960) and in *Galleria* (Shapiro 1966). From the above it is apparent that in some insects there is an increase and in others decrease in the THC occurs following starvation. For the above changes in hemocyte population, variations in the blood volume (BV) are thought to be responsible. According to Arvy *et al* (1948) and Rosenberger and Jones (1960) the BV decreases during starvation. Observations made by Yeager and Munson (1950) on *P. americana* indicate no change in the BV. It is well known that under adverse conditions and at the time of experimental stress, particularly when metabolic water is not available, fluid from the tissue spaces is added to the circulating hemolymph (Shapiro 1979; Gupta 1985), thus increasing the hemocytes.

Increase in the hemocyte population has been attributed to the mitotic activity. Throughout the experiment no mitotic divisions were seen. So this reasoning fails to satisfy the reason for the increased THC.

Another possible reason for the increase in the THC is well argued by Shapiro (1966, 1979). According to him the mitotic activity (if at all it exists during starvation) would not be solely responsible for the increase in THC. However, he is of the view that the existing transitional cells differentiate into various types. In addition, the hemocytes adhering to the tissue and organ surfaces are brought into circulation which leads to increase in the THC. This view is supported by Jones (1962), Shapiro (1979) and Gupta (1985). Logically this argument seems to be acceptable for the present results. During the last phase of the present experiment degenerative processes set in and hence the THC decreased during the last phase of starvation.

An outbreak of the PLS on the 3rd day, domination of the GRS after the 5th day and increase in the COS count from the 6th day onwards suggest progressive transformation of one hemocyte type into another. This view is adequately substantiated (Shapiro 1979; Gupta 1985) for normal and experimental conditions. It has been shown that the sessile PLS enter the circulation during the experimental stress conditions and hormonal stimulation (Pathak 1983). The same PLS differentiate further into GRS. This entire process leads to the increase in THC under such situations. This view has been also put forward by Pathak (1983) and he has shown that the THC of *Halys dentata* alters under the influence of neurosecretory cells and corpora cardiaca. Decrease in the THC after the 8th day is due to the degenerative changes in the hemocytes. The GRS seem to be involved in the intermediary metabolism and the metabolism is directly or indirectly under the hormonal control (Wigglesworth 1979).

Fluctuations in the THC during desiccation most probably reflect changes in BV. As the DHC did not change, it seems obviously that desiccation during the first two days probably reduces the BV and hence, there is a rise in the THC. In the next two days some mechanism (endocrine?) might be operating to try to regulate the BV. Drastic desiccation at the end of the 5th day might not be allowing the BV to make up itself, and this might have resulted in an abrupt increase in THC. Decrease in the THC during the last phase is because of the degenerative and lytic processes. Unfortunately there are no comparable data in this regard, hence, at this juncture it is not appropriate to draw definite conclusions.

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