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HEMOCYTES INTERRELATIONSHIPS CHANGES DURING THE DIFFERENT PERIODS OF DEVELOPMENT IN LAST LARVAL INSTAR OF THE SILKWORM, *Bombyx mori* (L.)

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ABSTRACT

Changes of total hemocyte counts (THC) and blood volume (BV) were studied in order to estimate the modification of absolute hemocyte counts (AHC) during the different developmental periods of the last larval instar of the mulberry silkworm *Bombyx mori*.

The THC reached its peak during the early spinning period whereas, the BV and AHC presented their peaks on the 10th day. An inverse relationship was noted between the THC and blood volume on the 4th and 9th days, and also between the 11th day and until the prepupal period. No correlation was observed between the BV and the THC during the periods between the 4th and the 5th days, and between the 9th and 10th days. A striking decrease of AHC is accompanied by a decline of THC and BV during pupation.

INTRODUCTION

In silkworm, *Bombyx mori*, several works on the classification of hemocytes at the light-microscopic level are available (Iwasaki, 1930; Nittono, 1960). Also, numerous studies concerning the ultrastructure of *B. mori* hemocytes by scanning and transmission electron microscope were

published (Akai and Sato, 1973 & 1976). Such studies are likely to supplement the information obtained by light microscope. In B. mori, functions of hemocytes have been studied by (Kawase, 1960; Akai and Sato, 1971, 1973; Sato et al., 1976; Wago, 1983 and Ashida et al.,1988).

There is no doubt that insect hemocyte population do respond to internal changes during development (Shapiro, 1979). Fewer investigations have involved total hemocyte count and blood volume in B. mori during development (e.g., at ecdysis).

This study will deal with changes in total hemocyte count and blood volume in order to estimate the absolute hemocyte counts in circulation during the development of last larval instar of Bombyx mori.

MATERIALS AND METHODS

- Animals

Investigations were carried out on the Korean hybrid 155 x 156 silkworm B. mori at laboratory conditions of 24 + 2°C and relative humidity 65-70%. Mulberry silkworm larvae were reared in trays (150 x 80 x 10 cm) and fed fresh mulberry leaves. Last larval instar has been divided into several periods according to Calvez (1981) and Shimizu (1982).

- Total hemocyte counts (THC).

Total hemocyte counts were taken after larvae were heat-killed in a hot-water bath (56-58°C). Hemolymph was withdrawn by cutting a proleg on the sixth abdominal segment with fine scissors, or by puncturing the abdomen of pupae. Blood was quickly drawn into a Thoma white blood pipette and diluted with (2% versene plus a trace of methylene blue) as used by (Wittig, 1966). The

pipette was shaken gently for several minutes, and the first four drops were discarded. The fifth drop was transferred to a Nauber double-lined hemocytometer and cells were counted in four corners and central squares. THC's were calculated according to the formula of Jones (1962).

- Blood volume (BV).

Hemolymph volume was determined by exsanguination by weighing insects, removing haemolymph as possible and reweighing the insects(Richardson et al., 1931 ; Arnold and Hinks, 1976 and Terra et al.,1982).

- Absolute hemocytes counts (AHC).

From the preceding methods, absolute hemocyte counts within the entire insect can be calculated when THC values are multiplied by the hemolymph volume.

The data were statistiçally analysed by calculating the means and standard deviation. Analysis of variance were carried out to check the significant difference between the treatments (Goulden, 1952).

RESULTS

Total hemocyte counts (THC).

Total hemocyte counts were increased gradually after ecdysis to the last larval instar during the first three days. Significant decrease was noted at the end of the obligatory feeding period (the 4th day). Another gradual increase was exhibited during the facultative feeding period. This period is (between the 4th and the 8th days). A significant decrease was observed on the 9th day. Between the 9th and 12th days, the THCs were increased gradually, and reached its maximum (8928

Table 1. Total hemocyte counts, blood volume and absolute hemocyte counts during the last larval instar of *B. mori*.

Days	Total hemocyte counts ($cell/mm^3$) mean ± SE	Blood volume (ul) mean ± SE	Absolute hemocyte counts (cells/ insect).
0	3540 ± 452	93.66 ± 6.23	331556
1	3840 ± 109	75.22 ± 2.99	288844
2	4288 ± 800	86.90 ± 3.66	372627
3	4720 ± 947	105.51 ± 11.80	498007
4	976 ± 119	183.44 ± 7.24	179037
5	2240 ± 190	156.20 ± 8.48	349888
6	3024 ± 516	229.63 ± 13.89	694401
7	5040 ± 1178	227.02 ± 8.68	1144180
8	6080 ± 1480	210.34 ± 7.00	1278867
9	3136 ± 285	286.41 ± 6.15	898181
10	5568 ± 1172	301.75 ± 28.50	1680144
11	5936 ± 1316	237.52 ± 12.00	1409918
12	8928 ± 1082	181.57 ± 8.09	1621057
13	4320 ± 706	233.81 ± 10.80	1010059
14	7712 ± 1242	207.66 ± 5.33	1601473
15	1920 ± 194	154.46 ± 9.68	296563

± 1082 cells/mm^3) on the 12th day. A significant decline was noted in the middle of spinning stage (4320 ± 706 cells/mm^3). During the prepupal stage a significant increase was observed, followed by a sharp decrease at pupation (Table 1).

Blood volume (BV).

Blood volume was decreased during the first 24 hours. Then, it presented a gradual increase until the end of the obligatory feeding period. This peak of the 4th day was about (183.44 ± 7.24 ul). Between the 4th and 5th days a slight and significant decrease occurred. A constant level of blood volume was observed between the 6th and the 8th days. The maximum value of blood volume was noted on the 10th day (301.75 ± 28.5 ul). From the tenth to the 12th day a gradual and significant decline was noted. During the middle spinning stage, blood volume exhibited another increase (From 181.57 ± 8.09 ul to 233.81 ± 10.8 ul). However, during prepupal period and pupation blood volume was decreased (Table 1).

Absolute hemocyte counts (AHC).

Absolute hemocyte counts exhibited a slight decrease during the first 24 hours. It then increased gradually until the third day. This increase from the first to the third day was about 72.4%. A decline was noted between the 3rd and the 4th days. This decline was about 46%. During the facultative feeding period two peaks were recorded. The first peak was detected on the 8th day (1278867 cells/larva) and the second on the tenth (1680144 cells/larva), which was the maximum value of absolute hemocyte counts. From the 10th to the 11th day a slight decline was noted. At the early spinning stage absolute hemocyte counts returned again to about its maximum value (1621057 cells/larva). During the middle spinning period a decrease of about 37.69% occurred. An increase of absolute hemocyte counts was noted

during prepupal stage. The number of hemocytes during the early spinning period was equal to that during the prepupal stage. At pupation a striking loss of absolute hemocyte counts was mentioned (From 1601473 cells/larva to 291620 cells/larva) which about 81.79%, (Table 1).

DISCUSSION

The cyclic changes in the population of hemocytes have been frequently demonstrated by many authors during growth and development. In B. mori attention was attributed to hemocyte types and their fine structure during the last larval instar; whereas, less attention was attributed to their population interrelationships changes due to the different periods of development in last larval instar.

In the present data THC and absolute hemocyte counts reached their peaks at the early spinning period while blood volume exhibited its peak at mature larvae. Pathak (1984) suggested that there is a negative correlation between the BV and THC. In contrast Shapiro (1966) reported that the THC was increased without a significant decrease in blood volume. Thus, it appears from the present data that the negative correlation between the THC and BV was not recorded at all studied times. This negative correlation has been detected between BV and THC on the 4th and 9th days, and also between the 11th day and until the prepupal stage. On the other hand, the BV decreased about 14.8% while the THC increased about 129.5% between the 4th and the 5th days. Also, between the 9th and the 10th days blood volume was increased about 5.3%, but the THC increased 77.5%. A constant level of BV from the 6th to the 8th days was accompanied by a significant increase of the THC. These results indicate that another factor (s) may regulate the absolute number of hemocytes in B. mori last instar.

The moulting is a result of important changes in hormonal reactions, Arnold (1974) pointed out that the variation in the hemogram during ecdysis may be influenced by the titre of ecdysone in the blood. In B. mori Calvez et al., (1976) noted that the ecdysteroid titre began to increase on the 8th day and reached its peak (prepupal peak) just before spinning. Thereafter, in several lepidopterous insects another peak has been detected (commitment peak) during the last instar (Lafont et al.,1977; Bollenbacher et al., 1978; Agui and Hiruma, 1982; Kallenborn and Mosbacher, 1983; Gelman and Brents, 1984; Plantevin et al., 1984; Essawy 1985 and Zimowska et al.,1985). Dean et al.,(1980) reported that the absence of this peak in Bombyx mori may be the result of time increments between titre points or the subtlety of the changes in the titre. Consequently, the increase of THC between the 4th and 5th days, and between the 9th and the 10th may be directly due to the ecdysone release which stimulate hemocytes mitotic divisions. However, a possible existence of an indirect effect on the hemopoietic organs should be taken into account. Akai and Sato (1971) concluded that at the time of moulting, the hemopoietic organs release all types of hemocytes into the hemolymph.

The present observation in B. mori indicate that the absolute hemocyte counts was striklingly decreased and it was accompanied by a decline in THC and BV during pupation. Such a result was observed in Ephestia kuhniella (Arnold, 1952).A more or less similar situation was obtained in Spodoptera litura (Prasada et al.,1984). On the other hand, in B. mori the hemocytes "gained" during last larval stage are the ones that are "lost" at pupation. This observation had been also suggested by Arvy et al.(1948) in Chrysomela decemlineata. The disappearance of hemocytes at the end of the last larval instar may be due to the assistance of hemocytes in the removal of autolysed tissues during the late prepupal and

early pupal stage of development (Walker, 1966; Sass and Kovacs, 1977 and Essawy et al., 1985).

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تغيرات العلاقات التداخلية لخلايا الدم فى العمر اليرقى الاخير لدودة ان الحرير خلال فترات التطور المختلفة

لتقدير التغيرات فى العدد المطلق لخلايا الدم فى الحشرة و علاقته بالتغيرات التى تحدث فى الفترات المختلفه أثناء التطور فى العمر اليرقى الاخير لدوده حرير القز ، فقد تم دراسة العدد الكلى لخلايا الدم و كذلك حجم الدم فى كل فترة من فترات التطور .

أوضحت النتائج أن العدد الكلى لخلايا الدم يصل الى أقصى معدل له أثناء الطور المبكر لغزل الشرنقة فى حين ، أن حجم الدم و العدد المطلق لخلايا الدم يصل الى أقصى معدل له فى اليوم العاشر .

وقد أظهرت الدراسه أن هناك علاقه عكسيه بين العدد الكلى لخلايا الدم و بين حجم الدم فى اليوم الرابع و التاسع ، و كذلك من اليوم الحادى عشر و حتى طور ماقبل العذراء . لم تلاحظ علاقه بين حجم الدم و العدد الكلى لخلايا الدم فى الفترة ما بين اليوم الرابع والخامس و كذلك ما بين اليوم التاسع و العاشر . لوحظ أيضا أثناء التعذر انخفاض حاد فى العدد المطلق لخلايا الدم يكون مصحوبا بانخفاض فى العدد الكلى لخلايا الدم و حجم الدم .