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HAEMOCYTE COUNT OF THE CUT WORM, *Agrotis ipsilon* LARVAE AND EFFECT OF HORMONES

Elshabrawy, H. A. and G. Elsayed
Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

Haemocytes increased gradually from the first instar to late fifth instar larvae of the cut worm, *Agrotis ypsilon*.The haemocyte number declined during the prepupal stage. Ligation and decapitation decreased the haemocyte number in the posterior part of the larvae. Injection of brain homogenate either from early last instar larvae of *A. ypsilon* or nymphs of *Schistocerca gregaria* into decapitated larvae, caused increase in haemocyte count. Injection of 20-hydroxyecdysone into the posterior half of thorax ligated larvae caused a significant increase in haemocytes, while application of juvenile hormone alone had no effect.

INTRODUCTION

During the post-embryonic development the total haemocyte count (THC) shows remarkable variation from one stage to another (Rao *et al.*, 1984 ; Lackie, 1988). Development and metamorphosis in insects is controlled by an interplay of two major hormones: Juvenile hormone (JH) and 20- hydroxyl ecdysone (20-HE) (Riddiford, 1981, 1985; Lozano *et al.* 1989). Variations in THC during different physiological states may be regulated by the change in hormonal titre (Salem *et al.*,1980 a,b,c).

However, little information is available on the effect of endocrine glands on the population of haemocytes in lepidopterans (Hoffman, 1970 ; Jones, 1970 ; Rao *et al.*, 1984). Injection of 20-HE elevates the THC in ligated larvae of *Spodoptera litura* (Rao *et al.*, 1984) and starved larvae of *Corcyra cephalonica* (Sujatha and Dutta-Gupta, 1991) and in ligated larvae of *C. cephalonica* (Sujatha and Dutta-Gupta, 1993). On the contrary, factors from the corpora cardiaca, have been shown to influence the THC in adults of *Halys dentata* (Pathak, 1983).

MATERIALS AND METHODS

Insects: *A. ypsilon* larvae from a laboratory routine culture were used as a source of the different instars . The last instar was classified into three different sub stages ; early, mid and late last instar. One day old pupae were categorized as early pupae, 10 days as old pupae.

Decapitation and ligation: For decapitation, early last and late last instar larvae were ligated behind the head capsule. For ligation early last instar larvae were ligated behind the first pair of prolegs. The tissues anterior to the ligature were cut off and sealed with a wax-resin mixture after application of

an antibiotic mixture (Streptomycin: Penicillin: Phenyl thiourea- 1:1:2). These insect preparations were maintained under moist conditions to avoid desiccation.

Preparation and injection of brain homogonate: The super oesophageal ganglia were dissected out from early last and late last instar larvae of *A. ypslion* and also from the nymphs of *S. gregaria*, and were homogonized in cold insect Ringer solution. The homogenate obtained from *A. ypsilon* was injected into decapitated larvae at a dosage of one brain / larvae in 3 ىا volume. The brain homogenate from *S. gregaria* (two brains / 50 ىا of insect saline) was injected at a dosage of 5 ىا / decapitated larvae. The control insects received an equal volume of insect Ringer.

Hormone treatment: JH-1 (Hoffman-La Roche, N.J.,U.S.A) was diluted in acetone, and 5 ىg (in 5 ىا) was topically applied on the ventral surface of the ligated insects. 20-HE(Rohto Pharmaceutical Co., Japan) was dissolved in 10% ethanol (1 mg/ml) and 5 ىg of 20-HE was injected into both decapitated and thorax ligated insects. The control insects received an equal volume of the carrier.

Homocyte counting: Cell counting was carried out according to Clark and Jones (1980). Calculation of THC was carried out using the formula of Jones (1962) and expressed as the number of cells / mm^3 of haemolymph.

Statistical analysis: Student 's t-test was used.

RESULTS AND DISCUSSION

Total Haemocyte count :

Table (1) shows that the THC was gradually increased from the first instar to the late last instar larvae , where it reached its maximum. There after , it declined gradually during the pupal development and reached a low value in late pupa .

Table (1). Total haemocyte count in *A. ypsilon* during different stages of larval and pupal devotement.

Stage	No. of cells /mm^3 of haemolymph
1 *st* instar	858±86
2 *nd* instar	876±96
3 *rd* instar	1080±27
4 *th* instar	1160±73
5 *th* instar	1184±41
Early last instar	1207±53
Mid last instar	1287±91
Late last instar	1798±37
Early pupae	1302±105
Late pupae	1171±63

Each value: Mean ± SD of 15-20 determinations. For each determination ,heamolymph was pooled from 5 individuals .

Effect of decapitation and ligation :

Decapitation lead to removal of brain and corpora allata (ca), while in thorax ligated insects the prothoracic gland was also removed along with the brain and ca. Data in Table (2) show that THC declined drastically 24 h after decapitation in early last instar larvae when compared either with normally fed controls or starved controls. On the other hand , decapitation for 24 h in late last instar larvae did not show any effect on haemocyte count . In ligated early last instar, haemocyte counts were made from the posterior half of the larvae and THC showed a drastic decline 24 h after ligation and remained only 38-41% of the control insects (Table 2)

Table (2). Effect of decapitation and thorax ligation for 24 h on THC in early last and late last instar of *A. ypsilon* larvae

Treatment	Stage	No. of cells/mm^{3}of haemolymp
Decapitated	Early last instar	476±71
Normal control	Early last instar	1171±115
Starved control	Early last instar	1091±86
Decapitated	Late last instar	1511±98
Starved control	Late last instar	1560±116
Thorax ligated	Early last instar	506±17
Normal control	Early last instar	1298±159
Starved contol	Early last instar	1230±191

Each value : Mean ±SD of 15 determinations , each pooled from 5 larvae.

Effect of brain homogenate on decapitated larvae:

The THC of larvae which were injected with brain homogenate from early last instar of *A. ypsilon* showed a significant increase when compared with the controls.On the contrary, the brain homogenate injection from late last instar larvae of *A. ypsilon* did not produce any effect on the THC. The brain homogenate injection from the nymphs of *S. gregaria* also showed a remarkable stimulating effect and THC was significantly high than the carrier treated controls (Table 3).

Table(3). Effect of brain homogenate injection 24 h on THC in *A. ypsilon* larvae.

Brain homogenate source	No. of Cells/mm^3	
	Control	Experiment
A.ypsilon early last instar	472+86	1016+120
A.ypsilon late last instar	436+59	519+119
S. gregaria nymphs	302+96	971±107

Each value: Mean±SD of 10 determinations for each haemolymph of 5 individuals was pooled.

Decapitation and 20 – HE treatment:

The larvae were injected with 20-HE, 24 h after decapitation and haemocytes were counted 24 h after hormone treatment. 20-HE treatment caused a significant increase in THC with the haemocyte number in experimental larvae showing a two-fold increase over the carrier injected controls (Table 4).

Table(4). Effect of 20-HE for 24 h on THC in decapitated larvae of *A. ypsilon*

Treatment	No. of cells/mm^3
Control	496±61
20-HE	910±112

Each value: Mean±SD of 10 determinations, each 5 individuals were pooled.

Effect of JH-1 and 20-HE on THC in thorax ligated larvae:

Data in Table (5) show that there was no effect of JH-1 on haemocyte number after 24 h of treatment and the THC remained more or less the same as that of carrier treated controls. In contrast to this, 20 – HE treatment for 18 and 24 h caused a significant increase in THC, although this effect was less pronounced with the longer treatment of 48 h. To find out the combined effect of JH-1 and 20–HE treatment, 24 h ligated early last instar larvae were topically administrated with 5 ىg of JH–1 and were also injected with 5 ىg of 20–HE. This conjunction partially blocked the increase in THC and the number of haemocytes remained fairly low in this treatment when compared to the 20–HE treatment (Table 5).

Table(5). Effect of hormones on THC in the early last instar larvae of *A. ypsilon*

24 h after ligation .

	Treatment	Duration	No. of cells / mm^3
A	JH – 1	24 h	511±77
	Control	24 h	463±63
B	20 – HE	18 h	1286±118
	Control	18 h	470±56
	20 – HE	24 h	1411±132
	Control	24 h	613±26
	20 · HE	48 h	917±109
	Control	48 h	702±96
C	JH-1 + 20 – HE	24 h	918±47
	Control	24 h	518±39

The values: Mean ± SD for 8-12 determinations, sample was the pool from 5 individuals .

In *A. ypsilon* larvae, the THC increases gradually and the number reaches its maximum in the late last instar. The THC declines gradually with onset of metamorphosis, and this could be attributed to the increased number of degenerating blood cells as reported in *Ephestia kuhniella* (Arnold, 1952).

The decline in THC after thorax ligation may be due to lack of new haemocytes coming from the unteriorly located haemopoietic organs (Hoffman *et al.*, 1979) commonly attached to the imaginal discs (Akai and Sato, 1971).When ligation was behind the head (decapitation), varied effects were produced according to the age of larvae. Unlike the thorax ligated, the decapitated individuals retain their haemopoietic organs. Therefore, it could be suggested that the THC in *A. ypsilon* is regulated by factors / hormones produced in the head region and the effect of these factors / hormones may be direct or indirect.

The results reported here show that the injection of brain homogenate from early last instar larvae of *A. ypsilon* significantly increases the THC in decapitated insects. However, no effect on THC was detectable if the brain homogenate from late last instar was used. This suggests that the neurosecretory cells in the brain of early last instar larvae have a factor which regulates the haemopoiesis. Injection of brain homogenate from nymphs of *S. gregaria* also causes a significant increase in THC. It is conceivable that, in the brain homogenate prothoracicotropic (PTTH) is present, which triggers the prothoracic gland of *A. ypsilon* to synthesize and release ecdysone (Bollenbacher and Granger, 1985), and this in turn regulates the haemocyte population in decapitated larvae (Knoblach and Steele, 1989).

Injection of 5 µg of 20-HE causes a significant increase in THC in both thorax ligated and decapitated larvae up to 18-24 h after hormone treatment. The effect was not persistent when the effect was pursued after 48 h. This might be due to the biodegradation of exogenous hormone in the insect systems (Koolman, 1982). The result that JH-1 treatment partially blocks the stimulating effect of 20-HE on THC, is in accordance with other findings showing that JH and ecdysone have opposing physiological effect (Wyss, 1976; Yudin *et al.*, 1982). The above results suggest that JH and 20-HE interact in a specific manner to regulate the haemocyte number during larval development of *A. ypsilon* larvae.

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تعداد خلايا الدم فى يرقات الدودة القارضة و تأثير الهرمونات

حمدى عبد الصمد الشبراوى–جمال السيد
قسم الحشرات الإقتصادية والمبيدات ــ كلية الزراعة ــ جامعة القاهرة ــ الجيزة

يزداد تعداد خلايا الدم تدريجيا من العمر الأول حتى العمر الخامس ليرقات الدودة القارضة، ثم ينخفض تعدادها بشدة حتى تختفى أثناء طور ما قبل العذراء. يؤدى إستبعاد الرأس عن طريق الربط أو القطع إلى إنخفاض عدد خلايا الدم فى الجزء الخلفى من اليرقات. ويسبب حقن يرقات الدودة القارضة مستبعدة الرأس بالقطع بممزوج المخ سواء المأخوذ من اليرقات فى عمرها الأخير أو المأخوذ من حوريات الجراد الصحراوى زيادة فى عدد خلايا الدم. يؤدى الحقن بمشابه هرمون الإنسلاخ (20-hydroxyecdysone)
فى منتصف الصدر الأمامى لليرقات منزوعة الرأس إلى زيادة معنوية فى عدد خلايا الدم بينما لم يكن للمعاملة بهرمون الحداثة منفردا أى تأثير.