J. King Saud Univ., Vol. **4**, *Agric. Sci.* (1), pp. 87-98, Riyadh (A.H. 1412 / 1992).

A Novel Equation for Estimating Corpus Allatum Activities during the Last Larval Instar of the Eri Silkworm

M. Idriss and Wagiha Hussein

Department of Plant Protection, Faculty of Agriculture University of Alexandria and Plant Protection Research Institute, Agricultural Research Centre, Alexandria, Egypt

Abstract. An equational model to evaluate the juvenile hormone (JH) level in the haemolymph of the last larval instar of the Eri silkworm *Philosamia ricini* has been designed. The model is based on morphological responses obtained after injecting 5th instar larvae with triprene at a dose of 1μg, time of injection (in days), larval duration, reprogramming day of the epidermal cells and weights of injected larvae. The equational percentage of cropus allatum activity (JH-level) is given by:

$$\frac{J_{ms} - [J_{os} + LD_p + \sqrt{(D_t - D_r)^2}]}{J_{ms}} \times W \times 100$$

where J_{ms} is the maximal score of juvenilization (50 points), J_{os} is the obtained score of juvenilization after injecting a larva with 1μg triprene, LD_p is the prolongation of larval duration (in days), D_t is the time of injection (in days), D_r is the time of reprogramming of epidermal cells (5^{th} day), W is the weight of an injected larva in grams.

To test the proposed equation, the fluctuation in the JH level in the haemolymph of the last larval instar of *P. ricini* was studied using the equational model. Simultaneously the fluctuations in the JH level were studied using well established assays such as the histometrical studies of the corpora allata (CA) during the 5^{th} larval instar or experimentally, by evaluating JH-level in the extracts from larval haemolymph using scoring assay of *P. ricini* pupae. It seems clear that there is a general similarity between the results obtained from the above-mentioned techniques and those obtained from the equational model.

The present study presents a novel equation which has faciliated (i) a comparison of juvenile hormone (JH) titres of Eri-silkworm larvae treated with different compounds; (ii) a study of fluctuation in the JH level during last larval instar of *P. ricini* under control conditions or different treatments.

Introduction

In an early comparative morphological study of corpora allata (CA) Nabert clearly expressed the opinion that the CA are endocrine in function [1]. The active role of the CA hormone (juvenile hormone; JH) in producing larval characters was recog-

nized [2]. This discovery was confirmed by a number of papers dealing with various insect species [3-5]. Recently, analogues and antagonists of JH have been used to control pest populations [6]. The commercial application of JH analogues (JHA) against lepidopterous insects has been delayed because the insect endocrine dynamics are far from the simple model system which was speculated.

The major classic methods to study an insect endocrine gland such as the CA are divided into morphological, experimental and chemical methods. The morphological-method contains histometrical and histochemical studies of the gland. Changes in the volume of CA during larval development have been studied [7]. The pattern of growth in the CA differs according to insect species. In lepidopterous insects the number of cells in the CA remains constant but the CA volume is approximately doubled in each larval instar as a result of cell enlargement [8]. The maximal increase in CA volume occurs at the time of ecdysis [8]. The increase of CA volume is an indicator of the increase of its secretory activity and JH release [9]. The experimental approach to study the CA activity involves transplantation and allatectomy experiments [10], cross-circulation experiments [11] scoring assay [12, 13] and wax cuticle tests [12]. Chemically, the JHs have been identified [14 - 16]. The industrial interest in juvenoids was initiated by the discovery of JH activity in American paper products [17]. Hundreds of juvenoids have been synthesized or extracted from plants [18].

The Eri-silkworm *Philosamia ricini* Boisd. is one of the most extensively investigated insects in our laboratory. During the present investigation a novel technique for estimating CA activity has been added to our previous assays [19-21]. Data based on the experimental approach were used to generate an equation which gave an expression of the percentage of JH activity. Moreover, to evaluate the efficiency of this equation, the calculated JH activities were compared with data obtained from well established techniques.

Materials and Methods

Experimental insects

All experiments were performed on the Eri silkworm *Philosamia ricini* Boisd. (Lep., Saturniidae). According to the covnentional method [22], *P. ricini* were reared on castor oil leaves at normal laboratory conditions of 25±2°C with 75±5% RH.

Extraction procedure

To study fluctuations in the JH level during the last larval instar of *P. ricini,* 15 ml haemolymph of larvae at different ages (0, 2, 4, 6, 8 and 10 days) were collected.

During the present study, the extraction procedure to prepare crude JH extracts outlined by Williams [23] was followed out. The haemolymph was extracted with several portions of diethyl ether. The pooled ether extracts were washed with several volumes of saturated sodium chloride solution to prevent the formation of emulsions. The ether extract was evaporated, and the residual golden oil was dried in vacuo at 60°C to remove the last trace of ether [12].

Scoring assay of *P. ricini* pupae [13]

The extracted oily material was dissolved in one ml peanut oil for injecting 20 pupae of *P. ricini* (12 ± 6 hr old). A pupa was injected with 50 μl and incubated at 20 ± 2°C with 65 ± 5% RH to emerge (Table 1). This bioassay is based on points awarded for the degree of juvenilization. The assay is based on eight easily recognized morphological characters. These include the antenna, the head capsule, the compound eye, the antenna cleaner, the tarsus, the thoracic tergites, the abdomina and the male genitalia. The emerged individuals were carefuly examined. From 0 to 5 points are awarded for the degree of juvenilization of each of those eight characters. After adding total points obtained by an emerged insect, the percentage of juvenilization was calculated. Since 1980 this assay has been employed routinely in our laboratory and it has faciliated detection of juvenilizing effect of an extract and a comparison of the JH activities of different extracts.

Measurement of the CA volume

It is generally known that the surface area of a body and its volume vary with the square and cube of its radius respectively. So measurement of the CA surface area has been considered an indicator of its volume. To study fluctuations in the corpora allata (CA) volume during 5th larval instar of *P. ricini*, selected larvae at different ages (0, 2, 4, 6, 8 & 10 days) were dissected and CA were permenantly mounted. The mean area of CA for each selected age was calculated from planimeter measurements of top-view camera lucida drawings of 20 glands.

Juvenoid

Triprene a JHA (ethyl-11 methoxy-3, 7, 11-trimethyldodeca- 2, 4 dienethiolate) was kindly provided by Dr. G.B. Staal Zoecon Research Institute, Sandoz Crop Protection, California, USA. The JHA was dissolved in peanut oil [12] and a tested larva was injected with 5 μl.

Statistical analysis

The present data are expressed as means ± SD.

Table 1. The effect of the injection of Triprene into last instar larva of *P. ricini* at different ages

Measured parameters	Time of injection (in days)										
	1	2	3	4	5	6	7	8	9	10	11
Mortality (%)	31.25	62.50	43.75	50.00	43.75	62.50	87.50	37.50	31.25	37.50	31.25
Larval duration (days)	19.6	22.5	19.9	20.7	18.4	18.2	16.0	18.8	19.3	18.9	19.1
Extra larval instar %	0.00	0.00	0.00	12.50	56.25	31.25	0.00	0.00	0.00	0.00	0.00
Larval-pupal intermediates %	68.75	37.50	50.00	37.50	0.00	6.25	12.50	56.25	56.25	31.25	62.50
Pupation %	0.00	0.00	6.25	0.00	0.00	0.00	0.00	6.25	12.50	31.25	6.25

Results and Discussion

Fluctuations in the JH level during the last larval instar of *P. ricini*

The JH titre in the haemolymph fluctuates during the last larval instar of lepidopterous insects. Two peaks of JH have been detected, the first at the beginning of the instar and the second before the prepupal stage [24].

To study the fluctuations in the JH level during the last larval instar of *P. ricini*, the hormone was extracted from haemolymph of 5th instar larvae (0, 2, 4, 6, 8, & 10 days old) and juvenilizing activities of these extracts were evaluated using the scoring assay for *P. ricini* pupae. As indicated in Fig. 1, after the fourth ecdysis (0 day-old)

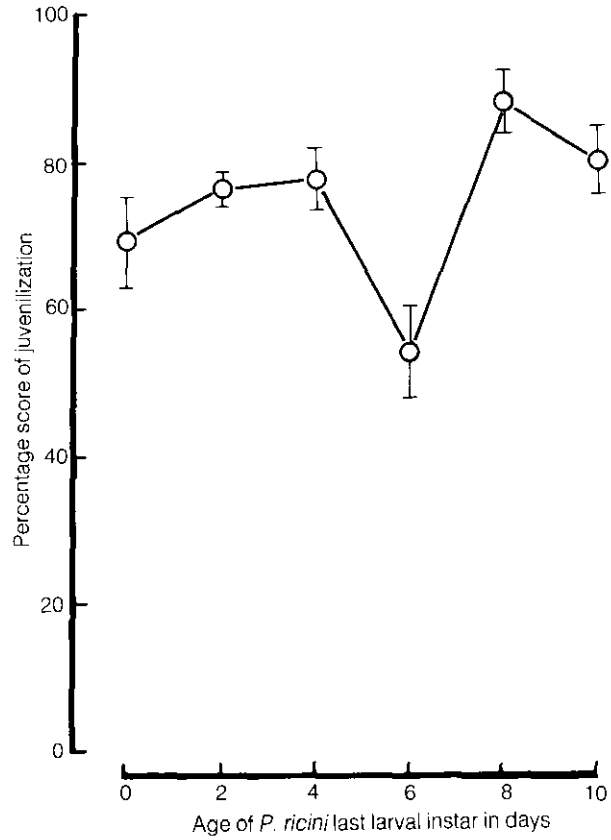

Fig. 1. Evaluation of fluctuations in the JH level in the haemolymph during the last larval instar of *P. ricini*. Scoring assay of *P. ricini* was used to estimate the percentage of juvenilization in the haemolymphal extracts

the JH started to increase to its first peak (4 days old) then decreased to the minimal level (6 days old). The JH level rapidly increased to its second peak (8 days old) then decreased just before the prepupal stage (10 days old). These data were similar to those obtained by other authors [8, 24].

On the other hand it is well established that a CA volume is an indicator of its secretory activity [9]. So, the histometrical study of the CA is routinely used in our laboratory to evaluate JH level. As shown in Fig. 2 the fluctuations in the CA surface area completely confirmed the data experimentally obtained (Fig. 1).

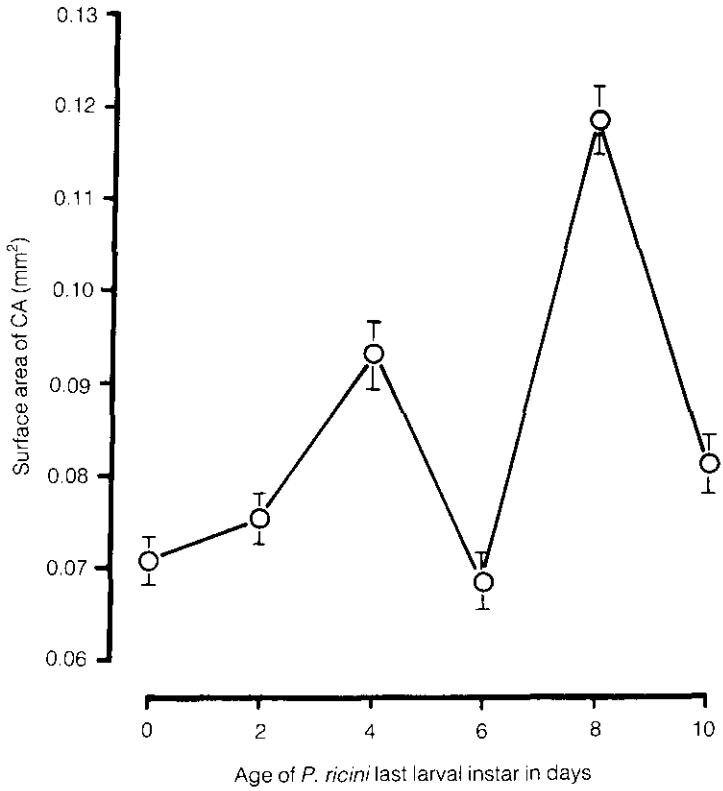

Fig. 2. The fluctuations in the CA volume during the last larval instar of *P. ricini*

From both histometrical and expeimental methods which were used, one can figure the fluctuations in the JH level during the last larval instar of *P. ricini*. Also,

these data confirmed that there is a positive correlation betwen the CA volume and its activity in larvae of the Eri-silkworm.

Determination of the epidermal cells reprogramming period of *P. ricini* last larval instar

Many authors reported that the epidermal cell genetically reprogrammes itself at a certain stage in its cycle during a larval instar or the pupal stage [6, 18, 25]. This period is called "reprogramming period." It is assumed that ecdysone forces the epidermal cells to reprogramme themselves while during this period the genetic system is influenced by JH titre [6].

To determine this period, 5th instar larvae of *P. ricini* were grouped at different ages (from 1 to 11 days old). Each group consisted of 32 larvae at the same age. From a preliminary experiment a dose of 2 μg triprene/larva was chosen for injecting all the tested larvae. Mortality (death before ecdysis), larval duration, ecdysis to an extra larval instar, abnormalities and pupation were recorded. As shown in the table the reprogramming period was at the 5th day of the last larval instar whereas 56.25% of the tested larvae (100% of alive insects) moulted to an extra larval instar. From tabulated data, it is apparent that when JHA was injected too long a time before the reprogramming period it was ineffective. Also as would be expected, the injection of a juvenoid too long after the period was likewise ineffective.

An equational model evaluates JH level during last larval instar of *P. ricini*

One of the prerequisites needed for our continuing studies on insect endocrinology was to find out a reliable, faster and easier technique which helps to evaluate the level of JH and its fluctuations during the last larval instar of *P. ricini*.

This technique was mainly based on the morphological response which resulted from injecting a 5th instar larva with 1 μg triprene. Fig. 3 illustrates the morphological forms obtained after treatment with JHA. Those insects showing normal pupal characters (Fig. 3, F) receive no points while those exhibiting a completely larval characters (an extra larval instar, Fig. 3, A) receive 50 points. Larval-pupal intermediates B,C,D and E (Fig. 3) receive 40, 30, 20 and 10 points respectively. Also larval duration, time of injection, reprogramming day and larval weights were considered.

On light of the negative correlation beween the natural JH titre in an insect haemolymph and the degree of juvenilization resulted from a treatment with a JHA, we proposed a model of an equation for estimating the JH-level in haemolymph of last larval instar of *P. ricini*. The equational percentage of CA activity is given by:

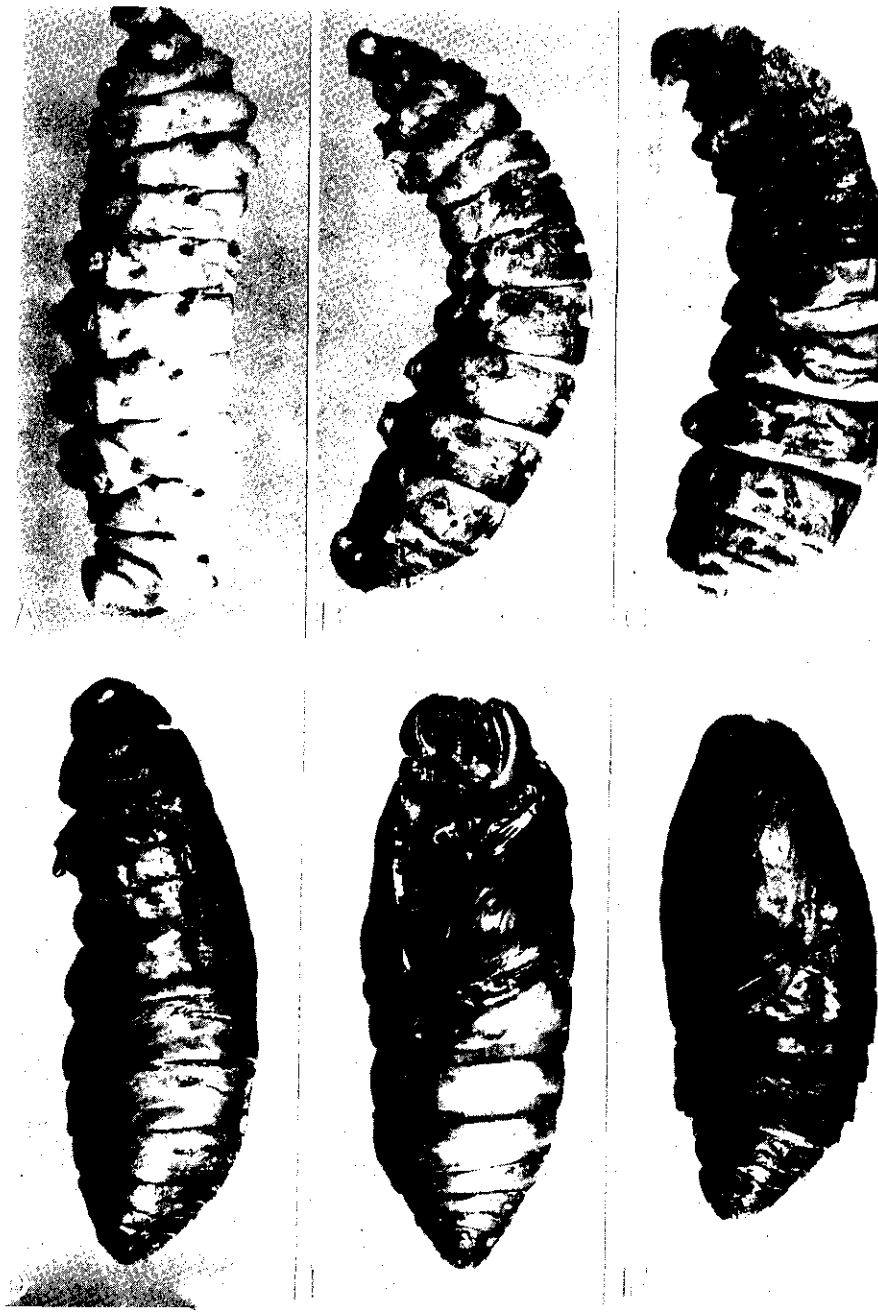

Fig. 3. Morphological forms obtained after the treatment of the last larval instar of *P. ricini* with a juvenoid, (A) an extra larval instar, (B-E) larval pupal intermediates and a normal pupa (F)

$$\frac{J_{ms} - [\, J_{os} + LD_p + \sqrt{(D_t - D_r)^2}\,]}{J_{ms}} \times W \times 100$$

where:

J_{ms} = maximal score of juvenilization (50 points).

J_{os} = obtained score of juvenilization after injecting a larva with 1μg triprene.

LD_p = prolongation of larval duration (duration of an injected larva-mean duration of control larvae) in days.

D_t = Time of injection (in days)

D_r = Time of reprogramming the epidermal cells (5^{th} day).

W = Weight of an injected larva in grams.

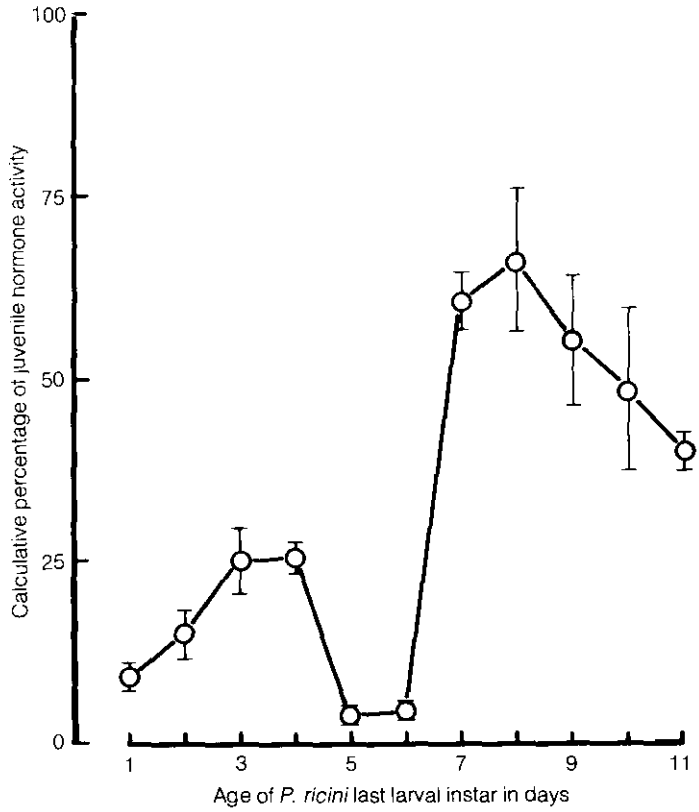

Fig. 4. the fluctuations in the JH activity during the last larval instar of *P. ricini*. JH level was evaluated using the proposed equational model

Tests of the equational model

To test the equation, fluctuations in the JH-level during last larval instar of the Eri silkworm were studied. Larvae at different ages (from 1 to 11 days old) were grouped. Each group consisted of 20 larvae at the same age. Larvae were injected with a dose of 1μg triprene/larva. The degree of juvenilization, larval duration, and weights of injected larvae were recorded. By equating this information, fluctuations in the JH level were illustrated (Fig. 4). Comparing the results of fluctuations in the JH-level obtained from the equational model (Fig. 4) with the results obtained from both the histometric method (Fig. 2) and experimental method (Fig. 1), it seems clear that there is a general similarity between them. This comparison indicates the reliability of the equation.

In conclusion we have proposed an equation to evaluate a JH-level in the haemolymph. This equation has faciliated: (i) a comparison of the JH titre of the Eri silkworm larvae treated with different compounds; (ii) a study of the fluctuations in the JH level during last larval instar of *P. ricini* under control conditions or different treatments. Though the equation was developed for *P. ricini* last larval instar we anticipate it can easily be adapted to other lepidopetrous insects.

References

[1] Nabert, A. "Die Corpora Allata der Insekten". *Z. Wiss. Zool.*, 104 (1913), 181-358.
[2] Wigglesworth, V.B. "Function of the Corpus Allatum in Insects." *Nature Lond.*, 136 (1935), 338.
[3] Bounhiol, J.J. "Metamorphose Apres Ablation des Corpora Allata Chez le ver a Soie *(Bombyx more)." C.R. Acad. Sci. Paris,* 203 (1936), 388-389.
[4] Piepho, H. "Nicht-Artspezifische Metamorphose-Hormone bei Schmetterlingen." *Natur Wissenschaften,* 26 (1938), 363.
[5] Bodenstein, D. "Untersuchungen Sum Metamorphoseproblem. II. Entwicklungsrelationen in Verschmolzenen Puppenteilen." *Arch. Entw-Mech. Org.*, 139 (1938), 636-660.
[6] Schneiderman, H.A. Insect Hormones and Insect Control. In: *Insect Juvenile hormone.* Menn, J. and Beroza, M. (Eds.) New York and London: Academic Press,1972.
[7] Pflugfelder, O. *Entwicklungsphysiologie der Insekten,* 490 p Leipzig: Akademische Verlagsgesellschaft, Geest & Portig, 1958.
[8] Legay, J.M. "Note sur l'Evolution des Corpora Allata au Cours de la vie Larvaire de *Bombyx mori." C.R. Acad. Sci. (Paris),* 144 (1950), 512-513.
[9] Engelman, F. "Endocrine Control of Reproduction in Insects." *Ann. Rev. Ent.*, 13 (1968), 1-26.
[10] Fukuda, S. "Hormones in Molting and Metamorphosis, Silkworm." *J. Fac. Sci. Tokyo Imp. Univ.*, 6 (1944), 477-532.
[11] Wigglesworth, V.B. "The Function of the Corpus Allatum in the Growth and Reproduction of *Rhodnius prolixus. Quart." J. Microscop. Sci.*, 79 (1936), 91-119.
[12] Gilbert, L.I. and Schneiderman, H. "The Development of Bioassay for the Juvenile Hormone of Insects." *Trans. Am. Microsc. Soc.*, 74 (1960), 38-67.
[13] Idriss, M. and Abdellatif, M. "Detection of Juvenile Hormone Active from Invertebrate and Verterbrate Tissues by Using the Scoring Assay of *Philosamia ricini* pupae." *Proc. Egyp-Hung. Conf. Plant Protec.*, (1982), 78-90.

[14] Roller, H., Dahm, K.H., Sweely, C.C. and Trost, B.M. "The Structure of the Juvenile Hormone." *Angew. Chem.*, 6 (1967), 179-180.
[15] Meyer, A.S., Schneiderman, H.A., Hanzman, E. and Ko, J.H. "The Two Juvenile Hormones from the Cecropia Silk Moth." *Proc. Nat. Acad. Sci. U.S.*, 60 (1968), 853.
[16] Judy, K.J., Schooly, D.A., Dunham, L.L., Hall, M.S., Bergot, B.J. and Siddal, J.B. "Isolation, Structure and Absolute Configuration of a New Nutural Insect Juvenile Hormone from *Manduca Sexta Proc.*" *Natl. Acad. Sci.* USA, 70 (1973), 1509-1513.
[17] Slama, K. and Williams, C.M. "Paper Factor" as an Inhibitor of the Embryonic Development of the European Bug, *Pyrrhocoris apterus*." *Nature*, 210 (1966), 329-330.
[18] Slama, K., Romanuk, M. and Sorm, F. *Insect Hormones and Bio Analogues*. NY: Springer Verlag Wien, 1974.
[19] Idriss, M., El Gayar, F. and Rawash, I. "Operated *Philosamia ricini* (Diu.) (Lep. Saturniidae) and *Bombyx mori* (L.) (Lep. Bombycidae) Larvae as Brain Hormone Bioassay Test Organisms." *Z. ang. Ent.*, 92 (1981), 371-374.
[20] Idriss, M. "Prothoraeic Gland Activities during the Last Larval Instar of Lepidopterous Insects." *Pest Sci.*, 23 (1988), 358-362.
[21] Idriss, M. "Action of Juvenile Hormone and Ecdysone in the Metamorphic Endocrine Centres." *Insect Sci. Aplic.*, (in press), (1990).
[22] Gomaa, A. "Biological Studies on the Eri-silkworm *A. Eixini.*" *Z. ang. Ent.*, 74 (1973), 120-126.
[23] Williams, C.M. "The Juvenile Hormone of Insects." *Nature*, 178 (1956), 212-213.
[24] Yagi, S. The Role of Juvenile Hormone in Diapause and Phase Variation in Some Lepidopterous Insects. In: *The Juvenile Hormones*. Gilbert, L. (Ed.) NY: Plenum press, 1976.
[25] Wigglesworth, V.B. *Insect Hormones*, London: Freeman and Co., 1970.

معادلة جديدة لتقدير نشاط غدة الجسم الكروي (كوربوراالاتا) خلال العمر اليرقي الأخير لدودة حرير الخروع

ممدوح أدريس و وجيهة حسين

قسم وقاية النبات، كلية الزراعة، جامعة الأسكندرية ومعهد بحوث وقاية النبات

مركز البحوث الزراعية، الأسكندرية، مصر

ملخص البحث. تم استنباط نموذج معادلي لتقويم مستوى هرمون الشباب في العمر اليرقي الأخير لدودة حرير الخروع. ويعتمد هذا النموذج على صفات شكلية ناتجة عن حقن اليرقات بمشابه هرمون الشباب «تراى برين» بجرعة قدرها واحد ميكروجرام. وكذلك على وقت الحقن بالأيام وعلى عمر الطور اليرقي الأخير وعلى ميعاد تقبل خلايا البشرة لفعالية الهرمون (الذي عبر عنه ببرمجة خلايا البشرة) وعلى وزن اليرقة المحقونة.

وقد وضعت المعادلة على النحو التالي:

$$\frac{ت_{او} - [ت_{وم} + ع\ ي_{ط} + \sqrt{7}\ (ي_{ح} - ي_{ب})^{2}]}{ت_{او}} \times و \times ١٠٠$$

حيث $ت_{او}$ عبارة عن أعلى وحدات لتأثير الهرمون التي عبرنا عنها «بقوة اليرقة على الاحتفاظ بمظاهر الشباب» (٥٠ وحدة)، $ت_{وم}$ وحدات التشبب المتحصل عليها نتيجة حقن اليرقة بجرعة واحد ميكروجرام تراي برين، ع $ي_{ط}$ الزيادة في طول العمر اليرقي، $ي_{ح}$ يوم الحقن، $ي_{ب}$ يوم البرمجة في خلايا البشرة (اليوم الخامس)، و وزن اليرقة المحقونة.

لاختيار هذه المعادلة تم دراسة التذبذب في مستوى هرمون الشباب في دم يرقات العمر الخامس لدودة حرير الخروع باستخدام هذه المعادلة المقترحة. وفي الوقت نفسه تم دراسة التذبذب في مستوى هذا الهرمون بطريقتين قياسيتين مختلفتين، الأولى دراسة التذبذب في حجم غدة الجسم الكروي خلال العمر اليرقي الأخير، والثانية طريقة استخلاص هرمون الشباب من الدم على فترات مختلفة واختباره بطريقة «اختبار الوحدات» فكان هناك تطابق كامل بين النتائج المتحصل عليها من الطريقتين القياسيتين وتلك التي تحصلنا عليها بالمعادلة المستنبطة. وقد سهلت هذه المعادلة (١) دراسة مستوى هرمون الشباب في يرقات عوملت بمواد مختلفة، (٢) دراسة التذبذب في مستوى هرمون الشباب تحت الظروف القياسية والمعاملات المختلفة.