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Changes in the Pattern of Protein in the Haemolymph and Fat Body of the Pink Bollworm *Pectinophora gossypiella* Saunders During the Active and Diapause Phases of the Larva

ZAKY MOHAMMAD FATHY ROSTOM*, MOHAMMAD SAYED SALAMA, AHMAD MOHAMMAD SALEM and AKILA MOHAMMAD EL-SHAFEI
Faculty of Science, Ain Shams University, Cairo, Egypt

ABSTRACT. The concentration of total protein in the haemolymph and fat body of the fourth instar larva of *Pectinophora gossypiella* Saunders increased in diapause and in early ages of termination of diapause relative to that in the active phase. In late ages of termination of diapause, a drop took place. The haemolymph and fat body of the active, diapause and 7 ages during termination of diapause of the larva were electrophoretically separated into 15 and 12 protein bands for both tissues, respectively. The different phases of the larva showed 2-7 protein bands whose concentration varied greatly during induction and termination of diapause. Some of these bands in both tissues examined were simple proteins, others were glycoproteins, lipoproteins or lipoglycoproteins. Some of these bands in both tissues were identical, while others were non-identical or specific for each tissue. During diapause, 3 characteristic protein bands appeared in each of the haemolymph and fat body, of which only one band was identical in both tissues. During termination of diapause some protein bands appeared in the haemolymph before their appearance in the fat body, or were never detected in the fat body, which suggested that some tissues, other than the fat body, might be involved in synthesizing haemolymph protein. The haemolymph and fat body during late ages of diapause termination regained many of the characteristic protein pattern of the active phase.

* *Present address :* College of Education, King Faisal University, Al-Ahsaa, Saudi Arabia.

Introduction

In the present work, the haemolymph and fat body of the fourth instar larva of the pink bollworm *Pectinophora gossypiella* Saunders were analyzed for estimating changes in the pattern of protein during induction, maintenance and termination of larval diapause. An understanding of such changes is fundamental in clarifying the sequence of physiological events leading to the induction and termination of diapause in this pest, which would be of great value in its control.

Material and Methods

The following phases and ages of the mature fourth instar larva of *P. gossypiella* were used: a) active feeding non-diapause phase: "A", b) diapause phase of ages 40 and 80 days in diapause: "D1 and D2", kept at 18°C, and c) larva during termination of diapause phase at 34°C, of ages 2, 4, 6, 8, 10, 12 and 14 days: "T2, T4, T6, T8, T10, T12 and T14, respectively". From about 500 larvae of each of these phases and ages, haemolymph and fat body samples were collected, pooled and kept at 18°C until required. Details of techniques of obtaining the larvae, and haemolymph and fat body samples, were described earlier[1]. Just before use, weighed samples of the pooled fat body were homogenized in a buffered saline solution (0.75% NaCl), centrifuged at 6000 rpm for 20 min at 4°C, and the supernatnat fluid was used for direct analysis. Haemolymph was diluted with distilled water just before use. Total protein was estimated by the Follin Ciocalteau's reagent according to the method of Lowry *et al.*[2]. Bovin serum albumin (BSA) was used as a standard Polyacrylamide gel electrophoresis was used for fractionating protein bands in the haemolymph and fat body using 6% gel at pH 8.4[3]. Simple protein bands were visualized by staining the gels with Commassie brilliant blue R250 (COBB: 0.2% in a mixture of methanol, water and acetic acid, in the ratio of 5:5:1, respectively). Destaining of the background was carried out in a solution of 10% methanol and 7.5% acetic acid. Glycoprotein bands were stained with periodic acid Schiff's reagent (PAS). Lipoproteins were stained with Sudan black B stain (SBB). Both glyco- and lipo-proteins were estimated according to the method of Chippendale and Beck[4]. The different stained protein bands were scanned using a DCD-16 Gelman gel scanner, equipped with a type 6 optic unit and a 625 nm filter.

This work was carried out at the Department of Entomology and Biochemistry, Faculty of Science, Ain Shams University, Cairo, in the period between 1982-1985.

Results

1. Total Protein (Table 1)

The concentration of the total protein in the haemolymph of "A" phase of larvae was considerably high (6.98 ± 0.06 gm / 100 ml). In "D1 and D2" ages a successive noticeable rise took place and the concentration reached about 1.5 times that in "A" phase. This high level was maintained during the early period of diapause termination, then increased to reach a maximum value at "T6" (more than double the

amount in "A" phase). Later on, the concentration decreased to reach a level close to that in "A" phase.

TABLE 1. Total protein concentration in the haemolymph and fat body of 10 phases and ages of the pink bollworm (in gm / 100 ml haemolymph or gm / 100 gm fat body); A = active phase of larva; D_1 and D_2 = early and late ages of diapause phase of larva (40 and 80 days, respectively); FB = fat body; H = haemolymph; T_2, T_4, T_6, T_8, T_{10}, T_{12} and T_{14} = larvae during termination of diapause phase of ages 2, 4, 6, 8, 10, 12 and 14 days, respectively.

Larval phase	Item	
	H	FB
A	6.98 ± 0.06 (6.45 – 7.39)	0.708 ± 0.011 (0.669 – 0.759)
D_1	10.66 ± 0.12 (9.38 – 11.41)	0.887 ± 0.002 (0.874 – 0.897)
D_2	11.33 ± 0.09 (10.75 – 11.78)	0.458 ± 0.003 (0.444 – 0.468)
T_2	11.80 ± 0.18 (10.86 – 12.62)	0.743 ± 0.002 (0.722 – 0.75)
T_4	10.1 ± 0.12 (9.28 – 11.1)	0.852 ± 0.002 (0.845 – 0.861)
T_6	14.51 ± 0.15 (13.25 – 15.18)	1.121 ± 0.004 (1.107 – 1.156)
T_8	5.77 ± 0.07 (5.30 – 6.0)	3.457 ± 0.017 (3.376 – 3.528)
T_{10}	5.72 ± 0.07 (5.14 – 6.05)	1.753 ± 0.003 (1.741 – 1.769)
T_{12}	7.05 ± 0.04 (6.71 – 7.28)	0.649 ± 0.002 (0.636 – 0.658)
T_{14}	7.16 ± 0.13 (6.36 – 8.13)	0.846 ± 0.003 (0.835 – 0.856)

The fat body of "A" phase had a high concentration of total protein (0.708 ± 0.011 gm). An increase of about 25% was recorded in "D1", followed by a drop in "D2". During diapause termination a succesive rise was obserrved that reached a maximum at "T8" (about 5 times that in "A" phase), though decreased thereafter to reach a level close to that found in "A" phase.

Accordingly, it is clear that the concentration of the total protein in the haemolymph was much higher than that in the fat body in 10 phases and ages of larvae examined. In both tissues, the concentration of the total protein was considerably high in "A" phase. A pronounced increase took place during diapause, though a drop occurred in "D2". Deuring early ages of diapause termination, a further increase was recorded, to drop at late ages reaching a level close to that found in "A" phase.

2. Protein bands as Separated by Electrophoresis

2.1 Haemolymph (Table 2 and Fig. 1 and 3)

The haemolymph protein of the different phases and ages of larvae used was electrophoretically separated into 15 different bands by using COBB stain. The different larval phases showed 4-7 bands whose concentrations varied greatly during induction, maintenance and termination of diapause, as shown from the densitometric scanning in Fig. 3.

In "A" phase, 6 bands were identified (number 1-6). Bands number 2 and 3 constituted more than two thirds of the total amount of protein. In "D1" larvae four bands were evident (number 1,2,7 and 8) while in "D2" age 6 bands were detected (number 2,7,8,9,10 and 11). In "D1" age, bands number 2 and 7 constituted the highest concentrated bands, while the corresponding bands in "D2" were number 7 and 8. In both "D1 and D2" ages, protein bands number 7 and 8 seem to be characteristic of diapause, as these bands never showed up in either "A" larvae or in any of the other 7 ages during termination of diapause (T2 to T 14). In "D2" age, an additional characteristic diapause band, number 11, was present, though in comparatively lower concentration than those of bands number 7 and 8. Band number 7 constituted a considerable percentage of the total protein during diapause (42 and 33.6% in D1 and D2, respectively). The concentration of band number 8 increased pronouncedly from "D1" to "D2".

During termination of diapause, 4-7 bands were detected in "T2" to "T14". Of these bands, four new specific bands, number 12 to 15, were observed for the first time. The concentration of the different bands varied greatly in the different ages, though band number 3 constituted one of the major bands in all of these ages.

By using PAS stain, the haemolymph protein bands number 1, 2, 3, 4, 7, 8, 10, 12 13 and 14 were stained. Staining with SBB gave positive reactions with bands number 1, 2, 3, 4, 6, 7, 8, 9 and 13. Combining results of staining behavior of the protein bands with COBB, PAS and SBB revealed that bands number 5, 11 and 15 were simple proteins, bands number 10, 12 and 14 were glycoproteins, bands number 6 and 9 were lipoproteins, and bands number 1, 2, 3, 4, 7, 8 and 13 were lipoglycoproteins (Tables 2 and 3).

2.2 Fat body (Table 2 and Fig. 2 and 4)

Staining of electrophoretically separated protein bands using COBB stain revealed 12 different bands at the different phases and ages of larvae used. These bands were numbered 1' to 12' in the Tables and Figures. The different larval phases showed 2-7 bands whose concentration varied greatly during induction, maintenance and termination of diapause, as indicated from the densitometric scanning in Fig. 4.

Phase "A" possessed 5 bands, numbered 1' to 5'. In diapause phase, these five disappeared and five new bands showed up, number 6', 7' and 8' in "D1" and number 7', 9' and 10' in "D2". Bands number 6' and 8' constituted 42.4 and 28.3%, respectively, of the total protein concentration in D1, while band 9' constituted 60.8% in "D2"

TABLE 2. Changes in the pattern and percentage of protein bands in the haemolymph and fat body of 10 phases and ages of larvae of the pink bollworm; bands number 1 to 15 are those of the haemolymph and number 1' to 15' of the fat body; a = simple protein; b = glycoprotein; c = lipoprotein; d = lipo-glycoprotein; other abbreviations as in Table 1.

Larval phase	Tissue	Number and character of protein bands																																			
		d 1	d 1'	d 2	d 2'	d 3	b 3'	d 4	d 4'	a 5	c 5'	c 6	a 6'	d 7	d 7'	d 8	c 8'	c 9	c 9'	b 10	b 10'	c 11	a 11'	a 12	b 12'	a 12'	d 13	13'	b 14	14'	a 15	15'					
A	H	6		33		36		8.9		4.5		12																										
	FB		13.8		20.4		18.8		24.2		22.8																										
D_1	H	10		35										42		13																					
	FB												42.2		29.2		28.3																				
D_2	H			12										33.6		31.9		6.3		7.2		9														
	FB														34				60.8		5.2															
T_2	H					32.5												45.1		7.9				6.2		8.3										
	FB														44.7						55.3															
T_4	H					44.1												3.3		3.1				18		22		5.2		4.3						
	FB								53.2												46.8															
T_6	H					35.3		4.1				4.5						31.9		11.4				5.4		7.4										
	FB		5.1		11.5				51.3												32.1															
T_8	H					28.1		33.4												27.5				4.3		6.7										
	FB		20.2		24.8		29.7		21.2		4.4																										
T_{10}	H			6.5		33.3		42												18.2																
	FB		14.9		33.2		26.9		20.1		4.9																										
T_{12}	H	5.4		15.9		53.3		17.5												7.9																
	FB		8.9		18		14.3		22.8		11.9													12.1		12										
T_{14}	H	7		34.6		39.9		11.5				7																								
	FB		29.4		29.8		13.6		22.4		4.8																										

TABLE 3. Staining behavior of the haemolymph (H) and fat body (FB) protein bands with Commassie brilliant blue R250 (COBB), periodic acid Schiff's reagent (PAS) and Sudan black B (SBB) stains.

Type of protein bands	Staining behaviour			Number of protein bands	
	COBB	PAS	SBB	H	FB
Simple protein	+	–	–	5, 11, 15	6', 11', 12'
Glycoprotein	+	+	–	10, 12, 14	3'
Lipoprotein	+	–	+	6, 9	5', 8', 9', 10'
Lipoglycoprotein	+	+	+	1, 2, 3, 4, 7, 8, 13	1', 2', 4', 7'

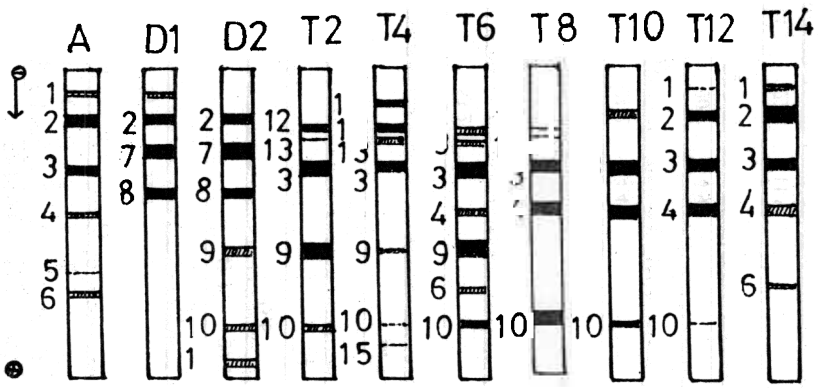


FIG. 1. Electrophoretic protein pattern of the haemolymph of 10 phases and ages of larvae of the pink bollworm stained with COBB.

age. Bands 6', 8' and 9' characterized diapause only, and did not show up in any other phase or age of the larvae. During "T2" to "T14", two to seven protein bands were detected, of which bands number 11' and 12' were specific for diapause termination phase.

By using PAS stain, bands number 1', 2', 3', 4', and 7' gave positive reaction, while with SBB stain bands number 1', 2', 4' 5', 7', 8', 9' and 10' were stained. Compiling results of the three stains used revealed that bands number 6', 11' and 12' were simple proteins, number 3' was glycoprotein, number 5', 8', 9' and 10' were lipoproteins, and number 1', 2', 4' and 7' were lipoglycoproteins (Table 2 and 3).

Table 3 summarizes the staining behavior of the different protein bands in both the haemolymph and fat body towards COBB, PAS and SBB stains.

Comparing the relative mobilities of the protein bands found in both the haemolymph and fat body revealed that the haemolymph protein bands number 1, 2, 3, 4, 5 and 7 were identical with the fat body protein bands number 1', 2', 3', 4', 5' and 6', respectively (Table 4). The non-identical bands in the haemolymph (number 6, 8,

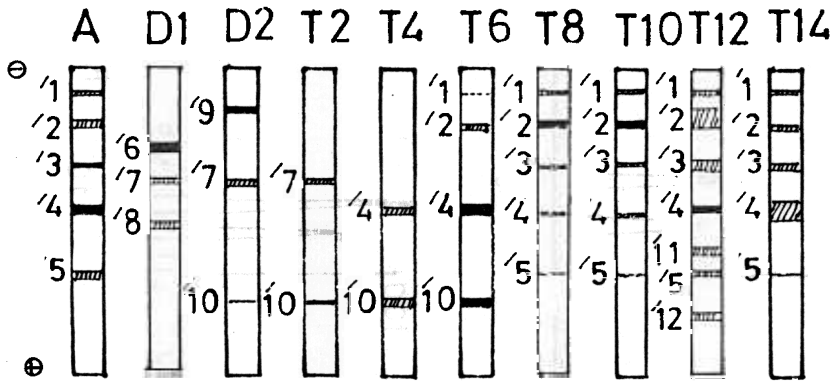


FIG. 2. Electrophoretic protein pattern of the fat body of 10 phases and ages of larvae of the pink bollworm stained with COBB.

9, 10, 11, 12, 13, 14 and 15) and in the fat body (number 7', 8, 9', 10, 11' and 12') seemed to be specific for either tissue.

TABLE 4. Identical (similar R_f) and non-identical (different R_f) electrophoretic protein bands in the haemolymph and fat body of 10 phases and ages of larvae of the pink bollworm; abbreviations as in Tables 1 and 2.

Identical bands		Non-identical bands	
H	FB	H	FB
1	1'	6	7'
2	2'	8	8'
3	3'	9	9'
4	4'	10	10'
5	5'	11	11'
7	6'	12	12'
–	–	13	–
–	–	14	–
–	–	15	–

Discussion

Changes in the haemolymph and fat body protein levels reflect the balance between synthesis, storage, transport and degradation during ontogeny, as well as a response to particular ecological and physiological conditions[5]. In the present work, the high concentration of the haemolymph and fat body protein observed in "A" larvae of *P. gossypiella* is a common phenomenon observed in some other insect species[6-15].

During induction and maintenance of diapause, a successive rise in the haemolymph protein was detected in *P. gossypiella* larva, as such cases of larvae of *Cydia pomonella*[12] and *Diatraea grandiosella* (Dyar)[9,11]. Such rise in haemolymph

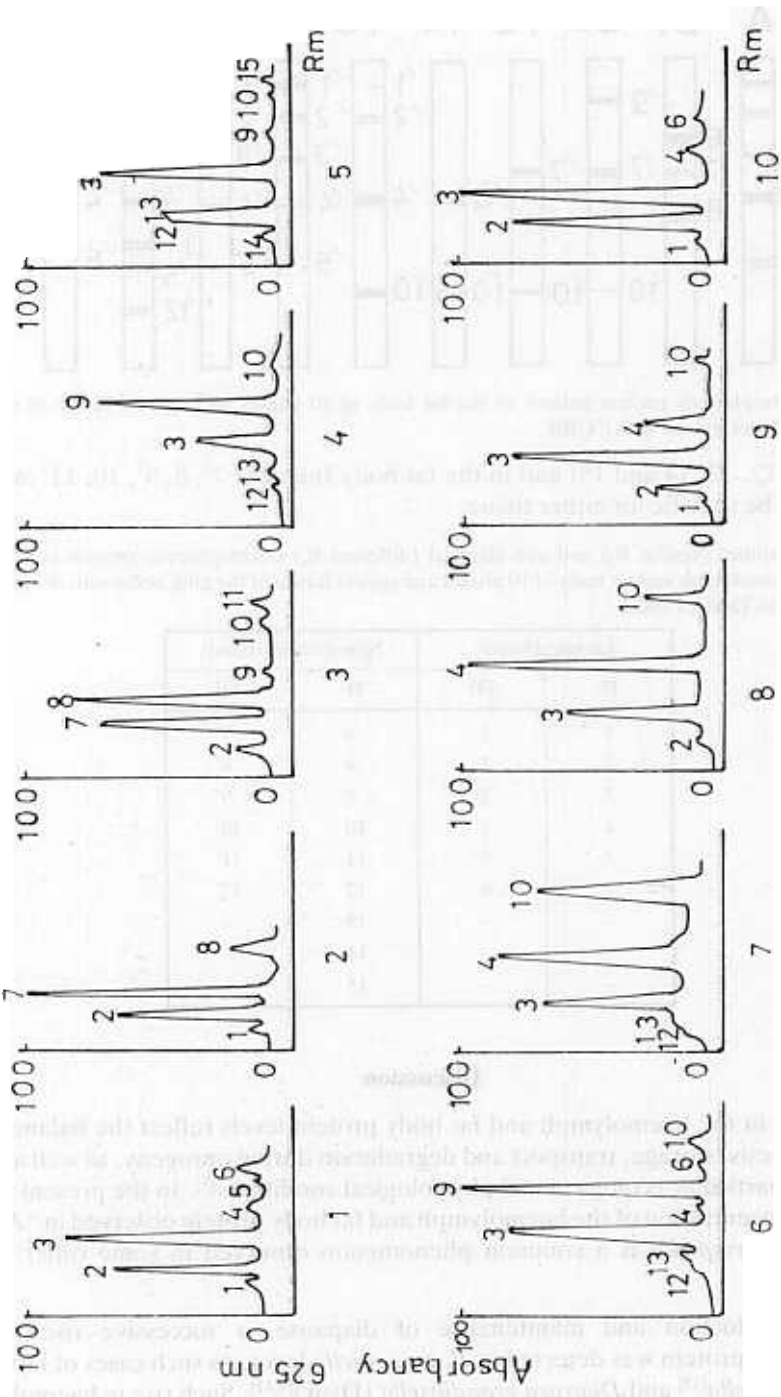
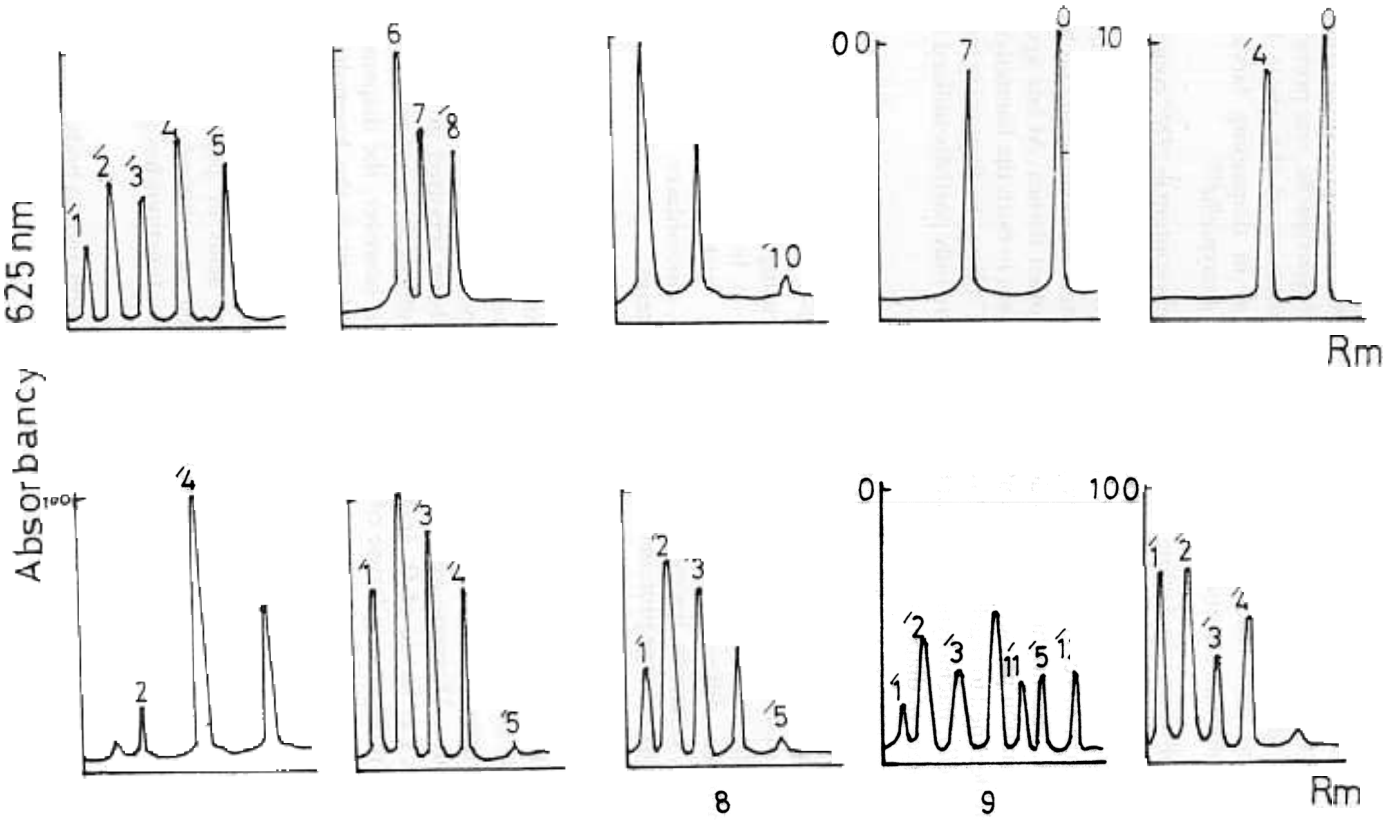


FIG. 3. Densitometric scanning of the haemolymph protein bands of 10 phases and ages of larvae of the pink bollworm; 1 = "A" larvae; 2 = "D1" larvae; 3 = "D2" larvae; 4 = "T2" larvae; 5 = "T4" larvae; 6 = "T6" larvae; 7 = "T8" larvae; 8 = "T10" larvae; 9 = "T12" larvae; 10 = "T14" larvae.



boll

phases and

pupal

larva

larva

protein might add to the concentration of solutes, which in turn, lowers the freezing point of the haemolymph, that enables the species to withstand unfavorable low temperature during diapause. A parallel rise in the concentration of other components, such as free amino acids in the haemolymph of *P. gossypiella,* was previously recorded[1]. An increase in the total protein concentration of the whole body homogenate was also recorded in other cases, *e.g.* in diapausing larvae of *Trogoderma granarium* Everts[17] and in 3 strains of *P. gossypiella*[18].

In the pink bollworm, the decline of the fat body protein content in "D2" coincided with a further rise of the haemolymph protein in this age, which might refer to an active release of protein from the fat body. The early ages of termination of diapause in the pink bollworm was characterized by a rise in both the haemolymph and fat body protein content. This might be due to depletion from other tissues. At late ages of diapause termination phase, the concentration of protein in both the haemolymph and fat body declined to reach a level close to that in "A" phase. In diapausing larvae of *D. grandiosella,* haemolymph and fat body protein are only partially utilized during the later stages of diapause[19].

With respect to protein fractionation, it is known that the number of protein bands separated electrophoretically depends on the resolving power of the technique used. In some insect species 10-30 bands have been identified[20]. Turunen[21] obtained the highest number of bands (60) by using the isoelectric focusing method, while by using other methods the number of bands ranged between 30-45. In general, haemolymph protein includes different types of compounds, such as enzymes (*e.g.* phenoloxidases, esterases, lysozymes, amino oxidases, glucosidases, ...*etc.*), lipid-transport proteins, storage proteins and antifreeze proteins.

In *P. gossypiella* specific diapause protein bands appear prominently in both the haemolymph and fat body. The concentration of these bands was very high in both the haemolymph (55 and 70.5% in D1 and D2, respectively) and the fat body (74.5 and 60.8% in D1 and D2, respectively) relative to the total protein concentration in either tissue. During termination of diapause, other specific protein bands showed up in both tissues. Diapause-associated protein has been reported in other diapausing insects, *e.g.* larvae of *D. grandiosella* and adults of *Leptinotarsa decemlineata*[9-11,22-26]. In diapausing larvae of *D. grandiosella,* however, the diapause-associated protein is found in the fat body only and absent in the haemolymph. Diapause-associated proteins have been suggested to play variable roles associated with diapause such as: JH-storage or -transporting protein, which is released during diapause to protect the circulating JH from being degraded; a storage molecule which might be hydrolyzed to its constituent amino acids or peptides during diapause, or being a proenzyme which is activated or released during diapause[11,22,27]. However, no definite proof of any of these functions has yet been offered.

The presence of conjugated proteins in the haemolymph and fat body, especially lipo- and lipoglyco-proteins, is of special interest. Lipids are known to be a chief source of energy. In the haemolymph of *P. gossypiella* diapause proteins were either

lipoglycoprotein (bands number 7 and 8) or lipoprotein (number 9). In the fat body, diapause protein number 6' was simple protein, while number 8' and 9' were lipoproteins.

In the pink bollworm, during termination of diapause, some of the identical protein bands were detected in the fat body (number 1', 2' and 4') before their appearance in the haemolymph (number 1, 2 and 4). This indicates their probable synthesis and/or release from the fat body into the haemolymph. On the other hand, some other haemolymph proteins appeared before their detection in the fat body (number 3 and 3'), or were never detected in the fat body (number 6, 8, 9, 10, 11, 12, 13, 14 and 15). The synthesis or storage of these haemolymph proteins by the fat body is questionable, and some other tissues may be involved. In larvae of *Pieris brassicae,* tissues like the midgut, pericardial cells and the haemocytes contribute in production of haemolymph protein[28].

During late ages of diapause termination, however, both the haemolymph and fat body re-exhibited many of the characteristic protein pattern of "A" phase. This is parallel with results obtained on whole body extract of this species[29].

In contrast, in some other insect species, *e.g. Ostrinia nubilalis* (Hubner) larva[4,30], *Protoparce quinquemaculata* larva[31], and *Pieris brassicae* pupa[28], the pattern and titre of haemolymph and/or fat body protein remain fairly constant during phases of activity, diapause and throughout diapause development.

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التغير في نموذج البروتين في الدم والجسم الدهني في بكتينوفورا جوسيبيلا ساوندرز أثناء حالتي النشاط والكمون اليرقي

زكي محمد فتحي رستم* ، محمد سيد سلامة ، أحمد محمد سالم و عقيلة محمد الشافعي
كلية العلوم ، جامعة عين شمس ، القاهـــرة ، مصر

المستخلص . ازداد تركيز البروتين الكلي في الدم والجسم الدهني للطور اليرقي الرابع أثناء الكمون وفي الأعمار المبكرة أثناء إنهاء الكمون ، ثم انخفض بعد ذلك ، بالمقارنة للموجود باليرقة النشطة . وقد تبين وجود ٢-٧ شرائط بروتينية في الأحوال اليرقية المختلفة تأرجحت تراكيزها كثيراً أثناء إحداث وإنهاء الكمون ، هذه الشرائط في كل من الدم والجسم الدهني كانت بروتينات بسيطة أو دهنية أو جليكوجينية أو دهنية جليكوجينية ، والبعض منها فقط كان متناظرًا في كلا النسيجين ، وأثناء الكمون ظهر ٣ شرائط بروتينية مميزة في كل من الدم والجسم الـدهني ، وفي الأعـمار المتأخرة أثناء إنهاء الكمون استعاد النسيجان الكثير من النموذج البروتيني المميزة للحالة النشطة لليرقة .

* العنـوان الحالي كلية التربية ، جامعة الملك فيصل ، الاحســاء ، المملكة العربية السعودية