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Multiplicity of tissue esterases in Pieris brassicae (L.) (Lepidoptera, Pieridae)

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Tissue esterases of *Pieris brassicae* were classified into 14 groups according to their relative electrophoretic mobilities in polyacrylamide gel. The haemolymph, midgut, fat body, and integument showed marked differences in the esterase patterns so produced. The different regions of the larval midgut contained esterases in different titres. The tissue containing the greatest number of esterase isozymes (11) observed at any stage of development was the integument of late fifth instar larvae. At this stage the dorsal parietal fat body and haemolymph contained 8—10 esterases. The haemolymph esterase pattern underwent distinct changes during development: ecdysing fourth-fifth instars had only four esterases, late wandering fifth instars eight, and 2-day pupae five. Slowly migrating esterases were prominent in the haemolymph and more rapidly migrating esterases in the midgut.

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1. Introduction

Because of their wide range of substrate specificities and their great heterogeneity, insect esterases have recently been subjected to extensive study. These studies have revealed that the esterase pattern of insects undergoes striking changes during development (LAUFER 1961, VELTHUIS & VAN ASPEREN 1963, COOK & FORGASH 1965, SIMON 1969, BRIEGEL & FREYVOGEL 1971, AHMAD 1977) and that differences exist between different tissues (CLEMENTS 1967, BRIEGEL & FREYVOGEL 1973). Much of the recent interest in insect esterases is based on the demonstrated role of certain carboxylesterrases in the degradation of endogenous juvenile hormone towards the end of the last larval instar (WHITMORE *et al.* 1972, WEIRICH *et al.* 1973, KRAMER & DE KORT 1976a, 1976b).

In the present study polyacrylamide gel electrophoresis was used to examine developmental changes in haemolymph esterases and esterase variability in some metabolically critical tissues of the cabbage butterfly, *Pieris brassicae* (L.). The study complements an earlier one on soluble esterases during the embryogenesis of *P. brassicae* (TURUNEN 1977a).

2. Material and methods

Pieris brassicae were from our laboratory-reared stock (16L : 8D, 23°, 65 % R. H.) maintained on a recently modified artificial diet (TURUNEN 1978). Larvae of a culture reared under a diapause-inducing regime were fed on the artificial diet at 11° under natural light: dark conditions (September—November inclusive, 60° N).

Haemolymph samples were obtained from ecdysing fourth-fifth instar larvae, 2-day post-ecdysis fifth instar larvae, late wandering fifth instar larvae, 2-day pupae and 1-day adults. The samples were diluted to 1 : 20 with cold 40 % sucrose solution and used for electrophoresis. Parietal (dorsal, pigmented) and perivisceral (ventral, non-pigmented) fat bodies and the integuments of late wandering male larvae were dissected under cold 0.9 % NaCl solution. Similarly, four consecutive sections of the larval midgut of 2-day post-ecdysis fifth instar males were dissected under the saline and rinsed clean of contents. All samples were homogenized in the saline, and centrifuged at 10000 g for 30 min at 4°. The supernatants were used for electrophoresis. The samples (3 µl of haemolymph in 60 µl, 1/4 integument in 100 µl, 1/25 midgut in 50 µl, and 1/5 of each midgut section in 50 µl) were spotted to each tube in a 40 % solution of sucrose. All techniques used in acrylamide

gel electrophoresis and esterase staining have been described (TURUNEN & CHIPPENDALE 1977). Cholinesterases were determined after the method of KARNOVSKY & ROOTS (1964).

3. Results

Distinct differences were seen between the electrophoretic patterns of haemolymph esterases at different stages of metamorphosis (Fig. 1). For comparison, the two major esterases detectable in newly oviposited eggs are shown (Fig. 1A). At the time of the last larval ecdysis the haemolymph contained only four esterases, with relative mobilities (Rm) between 0.25 and 0.51 (Fig. 1B). Early fifth instar larvae (already feeding) also had only four haemolymph esterases; the three most rapidly migrating of these had mobilities identical with those present in mature fifth instar larvae (Fig. 1C). Comparison of cultures reared under diapause-inducing and diapause-inhibiting conditions suggested little or no difference in the haemolymph esterase pattern of early fifth instar larvae. In larvae nearing the end of the fifth instar and preparing for metamorphosis, the number of haemolymph esterases was greater. The most notable change was the presence of several slowly migrating esterases in the haemolymph of mature fifth instar larvae (Fig. 1D). The concentrations of the slowly migrating esterases decreased again after pupation, but the complete absence of these enzymes in pupae was not verified (Fig. 1E). The haemolymph of newly emerged adults (Fig. 1F) contained two major esterases (Rm 0.33 and 0.38) apparently identical with the two major esterases of pupal haemolymph.

In mature fifth instar larvae slowly migrating esterases appeared simultaneously in the integument and haemolymph (Fig. 2A). In these larvae, the integument contains both enzymes that migrate slowly and several that migrate rapidly, their total number being 11, i.e. higher than in the other tissues analysed. A comparable pattern was observed in the dorsal parietal (pigmented) fat body (Fig. 2B), although there the slowly migrating esterases were found in relatively low concentrations. The parietal fat body contained esterases with the same mobilities as those of the ventral perivisceral (non-pigmented) fat body. However, the two tissues appeared to differ in the relative titres of the esterases, and some enzymes found

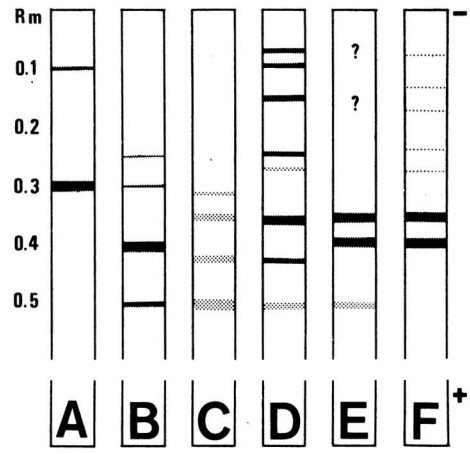

Fig. 1. Ontogenetic changes in the haemolymph (B—F) esterase pattern of *Pieris brassicae*. A: Eggs (1 day after oviposition), B: male larva at the time of the 4th-5th larval ecdysis, C: 2-day 5th instar male larva (diapause culture), D: wandering 5th instar male larva, E: 2-day male pupa, F: 1-day male adult.

in the parietal dorsal tissue appeared to be lacking from the perivisceral ventral tissue or present only in minute amounts (Fig. 2C).

Of all the larval tissues the midgut probably contains the highest esterase titre. A typical pattern of midgut esterases of a 2-day fifth instar larva is shown in Fig. 2D. The esterases are not evenly distributed along the midgut. When the four quarters were examined separately, overall esterase activity was highest in the anterior quarter, decreasing in the following order: posterior quarter, third quarter, second quarter (Fig. 2E, F, G, and H). A slowly migrating esterase was found in highest titre in the posterior quarter (Fig. 2 H).

Six esterase bands were distinguished in the soluble fraction of the adult head (Fig. 2I). With acetyl thiocholine iodide as substrate, at least four bands exhibited activity. Two of these had the same mobility as the esterases shown with α-naphthyl acetate (Rm 0.23 and 0.30). The other cholinester- hydrolysing bands moved more slowly (Rm 0.007 and 0.02). The functions of these esterases may concern the degradation of cholinester transmitters in the central nervous system.

The esterases found in the different tissues of *Pieris brassicae*, with α-naphthyl acetate as substrate, may be grouped according to their mobilities in acrylamide gel. Such a grouping

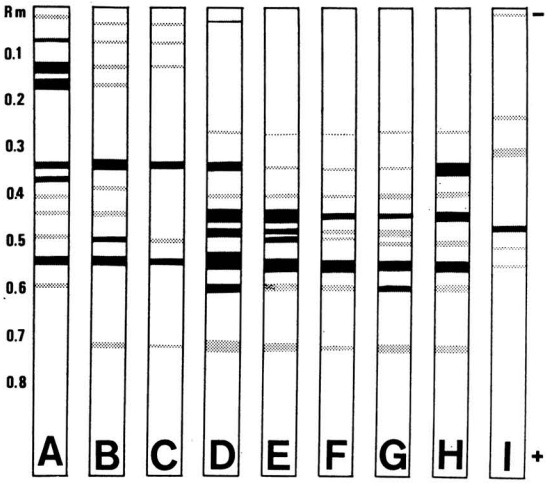

Fig. 2. Esterases in various tissues of 5th instar larvae of *P. brassicae*. A: pooled integuments of 2 late wandering male larvae, B: pooled parietal fat bodies of 3 late wandering male larvae, C: pooled perivisceral fat bodies of 3 late wandering male larvae, D: midgut of a 2-day 5th instar male larva, E: 1st, F: 2nd, G: 3rd, H:4th quarters of the midgut of a 3-day 5th instar male larva, I: 1/5 of the pooled heads of 4 one-day post-ecdysis female adults.

is arbitrary and has been made here merely to emphasize the differences between the tissues (Table 1). Some groups may include two distinct esterase isozymes present in a single tissue (for example, group IX in the integument, group X in both midgut and integument). Further, it is not suggested that the esterase isozymes classified in any group are identical in the different tissues; in fact, different esterases may have similar migration rates. According to the electropherograms, slowly migrating esterases are prominent in the haemolymph, and rapidly migrating esterases in the midgut. The esterase patterns in the fat body and the integument were closer to the pattern in the haemolymph than to that found in the midgut. Towards the end of the last larval instar slowly migrating esterases were observed in the midgut tissue also, although not in as high a concentration as in the haemolymph.

4. Discussion

The changes in the isozyme pattern of the haemolymph esterases of *Pieris brassicae* during the fifth instar suggest that the slowly migrating esterases have a specific function or functions before pupation. Such esterases (Rm 0.07 and Rm 0.15—0.16) were not found in larval haemolymph during the fourth-fifth larval ecdysis or early fifth instar, and their concentrations appeared to decrease markedly after pupation. In late fifth instar larvae slowly migrating esterases are present in other tissues beside the haemolymph, e.g. in the integument

Table 1. Tissue esterases of *Pieris brassicae*.

Esterase group[1]	Observed mobility	Tissue and stage of development studied					
		Egg	Haemolymph	Midgut	Fat body	Integumen	Head
I	0.01—0.02	—	—	—	—	—	adult[2]
II	0.02—0.04	—	5th instar	5th instar	—	5th instar	—
III	0.06—0.07	2 & 3-day	5th instar	—	5th instar	5th instar	—
IV	0.09—0.11	1 — 3-day	5th instar	—	5th instar	—	—
V	0.13—0.15	2 & 3-day	5th instar	—	5th instar	5th instar	—
VI	0.16—0.18	—	—	—	—	5th instar	—
VII	0.20—0.25	—	5th instar	5th instar[3]	—	—	adult[2]
VIII	0.27—0.29	—	5th instar	—	—	—	—
IX	0.30—0.37	1 — 3-day	5th instar	5th instar	5th instar	5th instar	adult[2]
X	0.36—0.44	3-day	2day pupa	5th instar	5th instar	5th instar	—
XI	0.41—0.48	3-day	5th instar	5th instar	5th instar	5th instar	adult
XII	0.47—0.54	3-day	5th instar	5th instar	5th instar	5th instar	—
XIII	0.52—0.59	—	—	5th instar	5th instar	5th instar	adult
XIV	0.65—0.72	—	—	5th instar	—	—	—

[1] Each group may include more than one type of esterase (see text).
[2] Probably a cholinesterase.
[3] Midgut esterases VII—XIII have been tentatively characterized as carboxylesterases (Turunen 1977b).

and fat body. These esterases (or esterase) may be the enzymes responsible for the hydrolysis of juvenile hormone (JH) observed to occur before pupation in several insects (WHITMORE *et al.* 1974, SANBURG *et al.* 1975, KRAMER & DE KORT 1976a, VINCE & GILBERT 1977, BROWN *et al.* 1977). JH-hydrolysing esterases could contribute to the rapid decrease in haemolymph JH activity observed in *P. brassicae* at the time of the larval-pupal transformation (VARJAS *et al.* 1976).

The different tissues of fifth instar larvae exhibited characteristically different patterns of esterase isozymes. These data complement the previous results of CLEMENTS (1967), obtained with starch gel electrophoresis, and provide details of esterase distribution within metabolically critical tissues. The present results showed about 11 esterase isozymes in the integument of fifth instar larvae, in contrast to the 3 found by CLEMENTS. The data suggest that the majority of the tissue esterases in the midgut, haemolymph and integument are carboxylesterases (CLEMENTS 1967, TURUNEN 1977b).

The esterases of the dorsal parietal and ventral perivisceral fat bodies also differed in fifth instar larvae of *P. brassicae*. Possibly these differences are only quantitative. The ventral tissue (adjacent to the intestine) is white, whereas the dorsal tissue (lining the body wall) is pigmented, the green to yellow hue probably resulting from dietary carotenoids and the colour of the aureomycin added to the artificial diet. This selective accumulation possibly indicates some metabolic difference between the two tissues.

Several of the midgut esterases are probably concerned with extra- as well as intracellular digestion. The three major gut wall esterases of *P. brassicae* were found by CLEMENTS (1967) to be secreted into the intestinal lumen, and comparable secretion of midgut esterases has been observed in other species of insects (COOK *et al.* 1969, TURUNEN & CHIPPENDALE 1977).

References

AHMAD, S. 1976: Larval and adult housefly carboxylesterase: isozyme composition and tissue pattern. — Insect Biochem. 6:541—547.

BRIEGEL, H. & FREYVOGEL, T. A. 1971: Non-specific esterases during development of Culicine mosquitoes. — Acta Tropica 28:291—297.

BRIEGEL, H. & FREYVOGEL, T. A. 1973: Non-specific esterases in several organs of Aedes aegypti (L.) during female adult life. — Comp. Biochem. Physiol. 44B: 371—380.

BROWN, J. J., CHIPPENDALE, G. M. & TURUNEN, S. 1977: Larval esterases of the southwestern corn borer, Diatraea grandiosella: temporal changes and specificity. — J. Insect Physiol. 23:1255—1260.

CLEMENTS, A. N. 1967: A study of soluble esterases in Pieris brassicae. — J. Insect Physiol. 13:1021—1030.

COOK, B. J. & FORGASH, A. J. 1965: The identification and distribution of the carboxylic esterases in the American cockroach, Periplaneta americana (L.). — J. Insect Physiol. 11:237—250.

COOK, B. J., NELSON, D. R. & HIPPS, P. P. 1969: Esterases and phosphatases in the gastric secretion of the cockroach, Periplaneta americana. — J. Insect Physiol.15:581—589.

KARNOVSKY, M. J. & ROOTS, L. 1964: A "direct-coloring" thiocholine method for cholinesterases. — J. Histochem. Cytochem. 12:219—221.

KRAMER, S. J. & DE KORT, C. A. D. 1976a: Age-dependent changes in juvenile hormone esterase and general carboxylesterase activity in the hemolymph of the Colorado potato beetle, Leptinotarsa decemlineata. — Molecular Cellular Endocrinol. 4:43—53.

KRAMER, S. J. & DE KORT, C. A. D. 1976b: Some properties of hemolymph esterases from Leptinotarsa decemlineata Say. — Life Sci. 19:211—218.

LAUFER, H. 1961: Forms of enzymes in insect development. — Ann. N. Y. Acad. Sci. 94:825—835.

SIMON, I. P. 1969: Esterase isozymes in the mosquito Culex pipiens fatigans. Developmental and genetic variation. — Ann. Entomol. Soc. Amer. 62:1307—1311.

SANBURG, L. L., KRAMER, K. J., KEZDY, F. J. & LAW, J. H. 1975: Juvenile hormone-specific esterases in the haemolymph of the tobacco hornworm, Manduca sexta. — J. Insect Physiol. 21:873—887.

TURUNEN, S. 1977a: Changes in soluble proteins and esterases during embryogenesis in Pieris brassicae (L.) (Lepidoptera, Pieridae). — Ann. Zool. Fennici 14:185—190.

TURUNEN, S. 1977b: Food utilization and esterase activity in Pieris brassicae during chronic exposure to lindane-containing food. — Entomol. Exp. Appl. 21:254—260.

TURUNEN, S. 1978: Artificial diets and lipid balance: a quantitative study of food consumption and utilization in Pieris brassicae. — Ann. Entomol. Fennici 44:27—31.

TURUNEN, S. & CHIPPENDALE, G. M. 1977: Esterase and lipase activity in the midgut of Diatraea grandiosella: digestive functions and distribution. — Insect Biochem. 7:67—71.

VARJAS, L., PAGUIA, P. & DE WILDE, J. 1976: Juvenile hormone titers in penultimate and last instar

larvae of Pieris brassicae and Baratha brassicae in relation to the effect of juvenoid application. — Experientia 32:249—251.

VELTHUIS, H. H. W. & VAN ASPEREN, K. 1963: Occurrence and inheritance of esterases in Musca domestica. — Entomol. Exper. Appl. 6:79—87.

VINCE, R. K. & GILBERT, L. I. 1977: Juvenile hormone esterase activity in precisely timed last instar larvae and pharate pupae of Manduca sexta. — Insect Biochem. 7:115—120.

WEIRICH, G., WREN, J. & SIDDALL, J. B. 1973: Developmental changes of the juvenile hormone esterase activity in haemolymph of the tobacco hornworm, Manduca sexta. — Insect Biochem. 3:697—407.

WHITMORE, D. Jr., WHITMORE, E. & GILBERT, L. I. 1972: Juvenile hormone induction of esterases: A mechanism for the regulation of juvenile hormone titre. — Proc. National Acad. Sci. U.S.A. 69:1592—1595.

WHITMORE, D. Jr., GILBERT, L. I. & ITTYCHERIAH, P. I. 1974: The origin of hemolymph carboxylesterases 'induced' by the insect juvenile hormone. — Molecular Cellular Endocrinol. 1:37—54.

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