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Metabolism of Leucine During the Early Pupal Stage of Cabbage Worm, *Pieris rapae* L.

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배추흰나비 (*Pieris rapae* L.)의 초기 용시기에 따른 Leucine의대사

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적 요

배추흰나비 (*Pieris rapae* L.)의 용시기 (pupal stage)의 큐티클 형성 및 경화에 따른 leucine 의 대사를 규명하기 위하여 ^{3}H-leucine 또는 ^{14}C-tyrosine 을 용화직후 혈림프에 주입시켰다.

혈림프내의 leucine 은 탈피후 3시간 동안에 걸쳐 큐티클 단백질 합성에 관여하였고 또한 혈림프, 지방체, 장 및 큐티클 사이를 자유로이 이동하였는데 이것은 leucine 대사에서 지방체 및 장이 혈단백질의 합성 및 저장에 관여하고 있다는 자료를 암시하여 준다.

INTRODUCTION

The incorporation of haemolymph amino acids into cuticle protein is known to extend to various time after ecdysis according to kinds of insects. Kristensen (1966, 1968) indicated that in locusts tyrosine is taken up by the cuticle during the first 5 hr post-ecdysis, and Mitlin *et al.* (1968) reported that ^{14}C-lysine is incorporated into cuticle protein of adult boll weevil during the first 8 hr of post-ecdysis. However, Fox and Mills (1971) showed that the incorporation of ^{14}C-leucine into cuticle protein takes place within the first 2 hr following ecdysis.

Simmons and Mitchell (1962) suggested that labelled non-essential amino acids enter rather slowly into protein molecules, but the radioactive essential amino

acids such as leucine is relatively fast incorporated.

The present paper is carried out to determine the incorporation of tyrosine and leucine into cuticle proteins of *Pieris rapae* during post-ecdysial stage when cuticle formation and sclerotization are occuring. The incorporation of tyrosine into tissues and organs (haemolymph, gut, and fat body) is also followed to trace possible places for protein storage and synthesis.

MATERIALS AND METHODS

Cabbage worms (*Pieris rapae* L.) were used in all experiments. Two groups were used for each series of injection; one group was given 0.1 μCi of U-^{3}H-leucine (200 mCi/mmol), and the other group was given 0.1 μCi of U-^{14}C-tyrosine (531 mCi/mmol) as control for sclerotizatien. The pupae at ecdysis were injected radioactive amino acids, using a Hamilton microsyringe through the dorsal cuticle, and sacrificed at the end of the appropriate time interval (1/4, 1/2, 1, 2, 3, 4, 5, and 6 hr after injection).

Blood was removed through a severed tip of the head and collected in the microsyringe. Cuticle, gut, and fat body were dissected, rinsed in water, placed on absorbant paper, and then dried. After weighing, they were crushed to mix with 10 ml of scintilation fluid. The radioactivity of all samples were determined in a Beckman LS-100 C Liquid Scintilation Counter.

RESULTS

Leucine is normally limited to protein synthesis while tyrosine is converted not only into protein but into N-acetyldopamine for sclerotization, therefore both ^{3}H-leucine and ^{14}C-tyrosine were used to determine the period and the rate of cuticle protein deposition. The haemolymph, gut, and fat body were also observed to trace the possible sites for storage and/or metabolizing route. The results obtained from the above tissues are shown in Table 1.

The labelled leucine in the haemolymph shows a initial peak during the first 30 min and then decreases to a low level at 2 hr, and maintains a low level until 5 hr. Fox and Mills (1971) suggested that there is a high rate of protein synthesis in the haemolymph in the first hour after ecdysis. Therefore, the initial decrease suggests that protein is being synthesized and taken up by other organs during this period. The labelled leucine in gut has a peak at 2 hr, then decreases to a low level at 4 hr, and then reaches a steady level thereafter.

The radioactive leucine level in fat body shows a sharp decline until first hour, rises to a little high point at 2 hr, and then followed by a constant level

Table 1. Radioactivity in four organs at various post-ecdysial times.

Time (hr)	^{3}H-Leucine				^{14}C-Tyrosine
	cuticle	fat body	blood	gut	cuticle
¼	59	238	104	165	123
½	90	180	103	165	127
1	132	60	59	132	136
2	147	125	28	277	246
3	188	136	34	148	312
4	178	125	34	91	483
5	198	110	28	105	504
6	205	100	52	101	573

*The values for the blood are in counts/min per μl and all others are expressed as counts/min per mg.

thereafter.

The radioactive leucine is incorporated into the cuticle during the first 3 hr after ecdysis while the incorporation of labelled tyrosine occurs at a steady rate for 6 hr (Fig. 1). These results suggest that cuticle protein synthesis

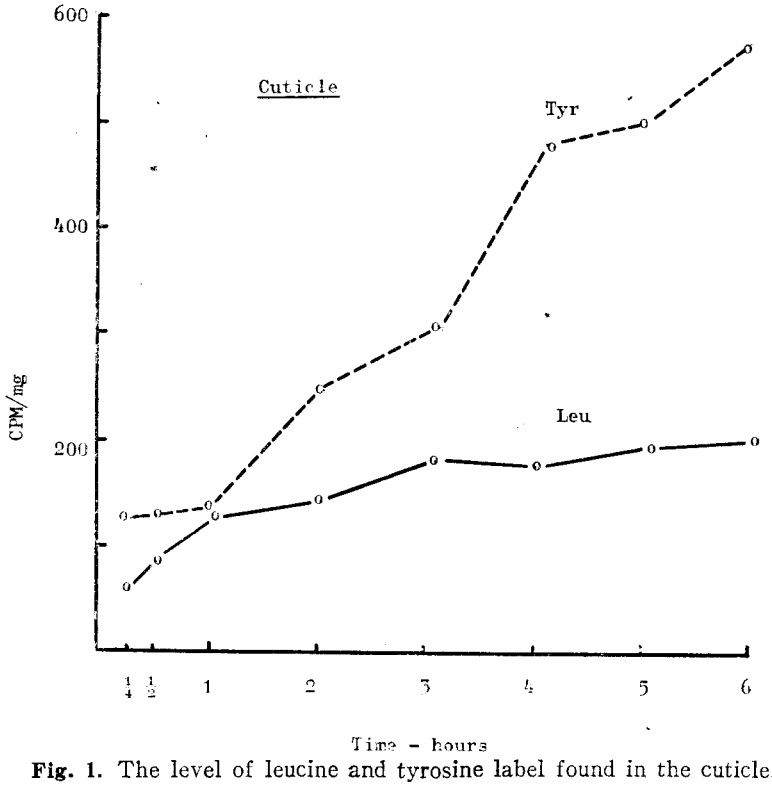

Fig. 1. The level of leucine and tyrosine label found in the cuticle.

mainly takes place within 3 hr after ecdysis while tyrosine and/or metabolite are moved into the cuticle over a rather extended hour for sclerotization process.

Different tissues have been compared on the basis of the radioactive leucine incorporation (Fig. 2). There is an initial low level in all tissues, indicating that the label is being moved into other organs. The autoradiographic study shows that muscle contains a considerable amount of label within the first hour. While the radioactivity of fat body, gut, and cuticle increases until 2 hr, that of blood shows a decrease during that time, indicating that free leucine and/or protein are moving from blood to gut, fat body, and the cuticle. However, during 3 to 6 hr there is a constant level in all organs, suggesting that there may be little movement of leucine between these organs

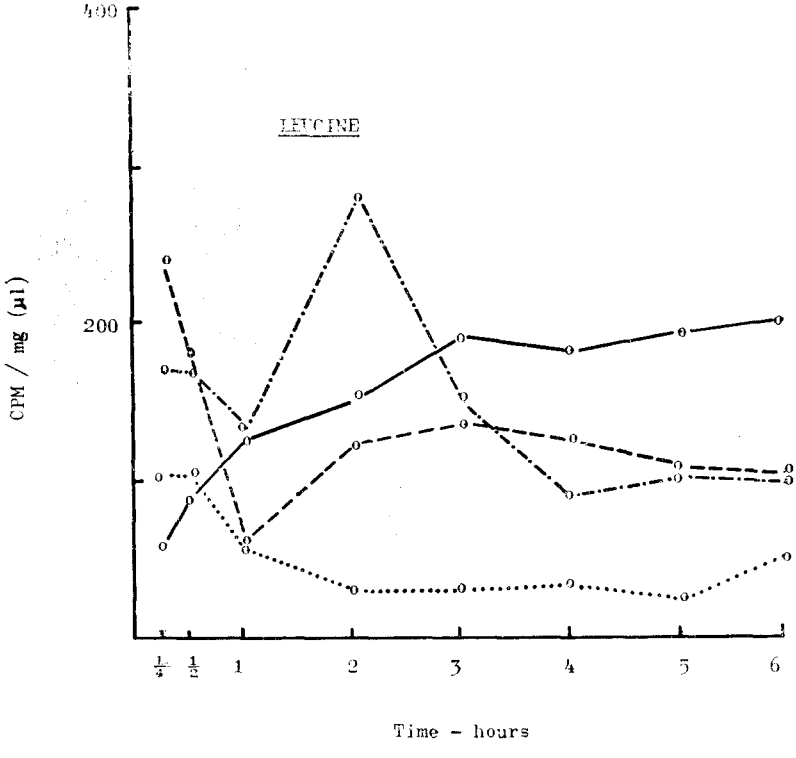

Fig. 2. The radioactive leucine incorporation into the tissues at various post-ecdysial times. ···o··· blood; -··o··- -gut; --o-- fat body; ——o —— cuticle.

DISCUSSION

Hill and Goldsworthy (1968) showed that protein reserves in the fat body and blood are lost during the moulting cycle, suggeting that haemolymph

proteins are mobilized into newly synthesized cuticle. Tobe and Loughton (1969) have also shown that blood proteins are taken up by the cuticle, and Fox and Mills (1968) indicated that some blood proteins are similar if not the same as some cuticle proteins.

The present results also showed that during 3 hrs post-ecdysis the radioactive leucine in cuticle continues to increase but labelled leucine in haemolymph continues to decrease, suggesting that leucine in haemolymph is being taken up by the cuticle for the formation of cuticle protein during this period.

Wirtz and Hopkins (1977) suggested that tyrosine is stored as some bound form during larval growth in *L. maderae* or *P. americana* for utilization in sclerotization and melanization of new cuticle after ecdysis. Levenbook *et al.* (1969) showed that ß-alanyl-L-tyrosine accumlates in the haemolymph of the fleshfly *Sarcophaga bullata* during larval feeding. After the formation of the white puparium, the concentration of the peptide decreases rapidly with concomitant increases in the levels of free ß-alanine and tyrosine as a result of the action of an ecdysterone-stimulated peptidase responsible for the mobilization of haemolymph ß-AT (Bodnaryk, 1971b; Bodnaryk and Levenbook, 1969). The liberated ß-alanine is covalently incorporated into the forming puparium and the tyrosine is metabolized to dopamine and used for sclerotization (Bodnaryk, 1970; Bodnaryk and Levenbook, 1969; Bodnaryk, 1971 a).

Fox and Mills (1971) showed that the incorporation of labelled leucine into the cuticle occurs within a short time post-ecdysis while radioactive tyrosine is incorporated at a constant rate for several hours, suggesting that most of the cuticle protein synthesis is taking place within a short time after ecdysis whereas tyrosine and/or metabolites for the sclerotization are moved into the cuticle over rather extended hours.

The present results also indicated that the radioactive leucine is incorporated into the cuticle during the first 3 hrs after ecdysis while the incorporation of labelled tyrosine occurs at a steady rate for 6 hr, suggesting that cuticle protein is synthesized within 3 hr after ecdysis while the incorporation of tyrosine into cuticle for sclerotization takes place over a rather extended hour.

Geiger *et al.* (1977) suggested that blood proteins can be freely exchanged between the serum and the haemocytes, and protein from either can be incorporated into the cuticle. Fox and Mills (1971) suggested that the fat body may be storing tyrosine temporally, and both the fat body and gut could be synthesizing proteins.

Recently, Dortland (1978) indicated that the fat body is the main producer of haemolymph proteins in *Leptinotarsa decemlineata*. The present results showed

the free movement of radioactive leucine between fat body and haemolypmh, supporting even partly this kind of role of fat body in *Pieris rapae*, although the amounts of radioactive leucine as free amino acid and protein molecules were not measured separately. The present results are providing only the evidence for involvement of fat body and gut in leucine metabolism in *Pieris rapae*. The exact roles of fat body and gut, the relation between serum and haemocytes in leucine metabolism in *Pieris rapae* will be next problems to be solved.

SUMMARY

To determine the metabolism of leucine during the cuticle formation and the sclerotization process in *Pieris rapae* L., U-^{3}H-leucine or U-^{14}C-tyrosine is injected into the haemolymph of newly molted pupa through dorsal cuticle of heart area.

The results show that leucine as a common amino acid participates in the synthesis of cuticle protein over the first 3 hr after ecdysis. It is also shown that leucine in the haemolymph at ecdysis is freely being moved between major internal organs during the short time period post-ecdysis, providing the evidence for some involvements including haemolymph protein synthesis and storage of fat body and gut in metabolism of leucine.

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