207. Incorporation of ^{14}C -phenylalanine or ^{14}C -tyrosine
into the Hypodermal Cuticle of the Silkworm. I^{*)}

Lethal Lemon Larvae

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The senior author had reported (Tsujiita, 1956) that three genes, $+^{lem}$, lem and lem^l , form a multi-allelic series at 22.3 unit on chromosome 3 of the silkworm and that their dominant recessive relation is $+^{lem} > lem > lem^l$.

Embryos homozygous for lem^l gene produced from sib-mating of individuals having the genotype $+^{lem}/lem^l$ develop normally to black young larvae which are able to hatch out by chewing up their egg chorion and grow normally until the 1st instar. Immediately after the 1st moulting normal larvae exhibit a blackish coloring owing to the presence of melanin in the hypodermal cuticle. On the contrary, moulted lethal lemon larvae (lem^l/lem^l) become distinctly yellow. A large amount of strikingly yellow pteridine granules containing sepiapterin is observed in their skin cells, but melanin pigment is scarcely produced in their hypodermal cuticle. In addition they can not chew up mulberry leaves and starve to death. It has been assumed that this inability of taking food is due to incomplete hardening and imperfect differentiation of hypodermal cuticle, especially mandibular cuticle, of the mouth organ.

In order to substantiate this assumption the present experiments were carried out.

Materials and methods. Normal $+^{lem}/+^{lem}$ ($+^{lem}/lem^l$), lemon (lem/lem) and lethal lemon (lem^l/lem^l) in the 1st instar were used as materials.

In order to examine the incorporation of ^{14}C -phenylalanine and ^{14}C -tyrosine as metabolites into the cuticular layer of the hypodermis of lethal lemon larvae, experiments were carried out.

Although newly hatched young larvae homozygous for lem^l gene are black just like normal larvae, they become slightly yellowish at the middle stage of the 1st instar, and can be distinguished from normal larvae. At this stage lethal lemon larvae were separated from normal larvae and used for radioactivity experiments. Lemon (lem/lem) larvae were taken as materials from a lemon strain also at

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Table I. Six experimental lots

Strain	¹⁴ C-labeled amino acids	¹⁴ C-phenylalanine	¹⁴ C-tyrosine
Normal larvae (+ <i>lem</i> /+ <i>lem</i> , + <i>lem</i> / <i>lem</i> ^l)		100 larvae	100 larvae
Lemon larvae (<i>lem</i> / <i>lem</i>)		100 „	100 „
Lethal lemon larvae (<i>lem</i> ^l / <i>lem</i> ^l)		100 „	100 „

Six experimental lots as Table I shows were prepared.

the same middle stage of the 1st instar.

0.02 μ c per head of each of the two ¹⁴C-labeled amino acids were given to 100 normal larvae (+*lem*/+*lem*, +*lem*/*lem*^l), 100 larvae homozygous for *lem* gene (*lem*/*lem*) and 100 larvae homozygous for *lem*^l gene (*lem*^l/*lem*^l) by feeding them with mulberry leaves painted with ¹⁴C-amino acid solution for about one and a half days at a later stage of the 1st instar. As larvae at the same growth stage in the 1st instar were used in each lot, it is assumed that they grew up to the moulting stage while eating almost the same amount of mulberry leaves contaminated with ¹⁴C-labeled amino acid.

After the 1st moulting each larva was cut into two parts, head and body (thorax and abdomen). Samples from both parts obtained from each lot were homogenized in a glass homogenizer together with 7 ml of 0.1 M borate buffer at pH 7.0, and the homogenate was kept in a thermostat at 55°C for 42 hours.

Water-soluble, alkali-soluble and alkali-insoluble proteins were prepared by the procedure shown in Fig. 1a and Fig. 1b and the radioactivity of each protein was recorded.

Experimental results. Table II shows that fairly strong radioactivity was detected in water-soluble protein of each lot. The figures in parentheses indicate the relative value of radioactivity of protein of normal and mutant larvae. No significant difference could be found between the radioactivity of water-soluble protein of normal larvae and that of mutant larvae.

Table III shows that a weak radioactivity was detected in alkali-soluble protein of normal and mutant larvae. In this case also no significant difference could be found among six experimental lots.

On the contrary, Table IV shows the radioactivity of alkali-insoluble protein containing hard cuticle protein, scleroprotein, as principal component. An important and remarkable difference was found; the radioactivity of alkali-insoluble protein of normal larvae was strongest in both phenylalanine and tyrosine lots. Next followed

Fig. 1a. Procedure of a preparation of alkali-insoluble protein.

1b. Pre-treatment procedure of the alkali-insoluble protein prepared from the procedure shown in Fig. 1a before measurement of radioactivity.

Abbreviations used: sup: supernatant, ppt: precipitate.

Table II. Radioactivity of water-soluble protein

Genotype	Organs	¹⁴ C-phenylalanine	¹⁴ C-tyrosine
		cpm/mg of protein (index)	cpm/mg of protein (index)
<i>+lem/+lem</i> <i>+lem/lem¹</i>	Head	310' (100)	225 (100)
	Thorax and abdomen	568 (100)	395 (100)
<i>lem/lem</i>	Head	412 (132.9)	418 (185.8)
	Thorax and abdomen	274 (48.2)	223 (56.5)
<i>lem¹/lem¹</i>	Head	99 (31.9)	247 (109.8)
	Thorax and abdomen	333 (58.6)	481 (121.8)

Table III. Radioactivity of alkali-soluble protein

Genotype	Organs	¹⁴ C-phenylalanine	¹⁴ C-tyrosine
		cpm/mg of protein (index)	cpm/mg of protein (index)
<i>+lem/+lem</i> <i>+lem/lem¹</i>	Head	5 (100)	2 (100)
	Thorax and abdomen	75 (100)	71 (100)
<i>lem/lem</i>	Head	3 (60.0)	1 (50.0)
	Thorax and abdomen	86 (114.7)	47 (66.2)
<i>lem¹/lem¹</i>	Head	1 (20.0)	3 (150.0)
	Thorax and abdomen	48 (64.0)	96 (135.2)

that of lemon larvae and the weakest radioactivity was detected in lethal lemon larvae.

It seems that the head is the better part of the larvae for the estimation of the incorporation of the two ¹⁴C-labeled amino acids as metabolites into the cuticular scleroprotein of larval hypodermis. Namely, the head of the normal lot had the strongest radioactivity for both ¹⁴C-phenylalanine and ¹⁴C-tyrosine derivatives; cpm per mg protein of ¹⁴C-phenylalanine derivatives was 644 (index: 100) and that of ¹⁴C-tyrosine derivatives was 366 (index: 100). Next follows the lemon lot which shows much weaker radioactivity. For the head part, the index of ¹⁴C-phenylalanine lot is 33.7 and that of ¹⁴C-tyrosine lot is 44.3. On the contrary both amino acid metabolites were scarcely incorporated into the head sample of *lem¹* larvae: the index of ¹⁴C-

Table IV. Radioactivity of alkali-insoluble protein

Genotype	Organs	^{14}C -phenylalanine	^{14}C -tyrosine
		cpm/mg of protein (index)	cpm/mg of protein (index)
$+^{lem}/+^{lem}$ $+^{lem}/^{lem^l}$	Head	644 (100)	366 (100)
	Thorax and abdomen	817 (100)	595 (100)
$^{lem}/^{lem}$	Head	217 (33.7)	162 (44.3)
	Thorax and abdomen	303 (37.1)	594 (99.8)
$^{lem^l}/^{lem^l}$	Head	4 (0.6)	8 (2.2)
	Thorax and abdomen	26 (3.2)	55 (9.2)

phenylalanine lot being only 0.6 and that of ^{14}C -tyrosine lot only 2.2.

The radioactivity of the body of lemon larvae in the phenylalanine lot is much weaker (index: 37.1) than that of normal larvae, but cpm in tyrosine lot is almost the same as that of normal larvae (index: 99.8). On the contrary, the radioactivity of the body of lethal lemon larvae is conspicuously weak: the index of ^{14}C -phenylalanine lot is only 3.2 and that of ^{14}C -tyrosine lot only 9.2.

Consideration. Injection is a better method for incorporation experiments with radioactive compounds. However, lethal larvae are too small and feeding with those compounds was tried in place of injection. It is considered from the experimental results that ^{14}C -phenylalanine and ^{14}C -tyrosine are permeable through the epithelial cells of the mid-gut of normal, lemon and lethal lemon larvae and are absorbed into hypodermal cells as important materials for the formation of hard cuticle protein and melanin biosynthesis.

In previous paper Tsujita (1953, '56, '61) reported that the young $^{lem^l}/^{lem^l}$ larvae developed within eggs laid by a $^{lem}/^{lem^l}$ moth can not chew the chorion and starve to death. These larvae, when liberated from the eggs by cutting through the shell, showed partly yellowish brown and partly yellow color and moved around but could not feed on mulberry leaves and consequently starved to death.

Melanin formation in the hypodermal cuticle (Nagashima 1952) and production of pteridine granules containing isoxanthopterin (Tsujita unpublished) in the pigmentation stage of the body and just after hatching of normal larvae have been demonstrated. However, in hypodermal cells of the young lethal lemon larvae a large amount of pteridine granules containing sepiapterin is formed and

melanin is scarcely produced in their skin cuticle.

It was assumed that incomplete development of hypodermal cuticle and defective melanization are caused by an abnormal phenylalanine metabolism in young lethal lemon larvae. If incorporation experiments with radioactive compounds in later embryonal stages were possible, their slight incorporation into hypodermal cuticle of young lethal lemon larvae would be detected just like in the present experiments with lethal lemon larvae which were segregated immediately after the 1st moulting.

Summary. Incorporation experiments with ^{14}C -phenylalanine or ^{14}C -tyrosine as metabolites into the hypodermal cuticle of normal and mutant (lemon and lethal lemon) larvae were carried out. It was found from the experimental results that in the lethal lemon larvae the radioactivity of alkali-insoluble protein containing as principal component hard cuticle protein, scleroprotein, is conspicuously weaker in both head and body than in normal larvae.

References

- Tsujita, M.: Ann. Rep. Nat. Inst. Genet., **3**, 24-27 (1952).
——: Jap. J. Genet., **30**(2), 107-117 (1955).
——: Jap. J. Genet., **36**(9-10), 337-246 (1961).