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Studies on the Tyrosinase System in Lepidopterous Insects
III. Effects of SH Reagents on the Melanosis of the Body Fluid of *Samia cynthia* and *Bombyx mori*

By

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In our previous paper (HARADA and KATO, in press), it was established that SH reagents can accelerate the tyrosinase activity in the body fluid of *Philosamia cynthia ricini*. This acceleration seems to be due to the removal of the endogenous SH compound which is inhibiting the tyrosinase action.

The present investigation was undertaken to get further information favourable to our assumption that the tyrosinase activity would be inhibited by the naturally occurring SH compound in the body fluid of insects.

Materials and Methods

The body fluid of *Samia cynthia* and *Bombyx mori* (race: J122×C115) at the period of metamorphosis was used. The body fluid was collected into tubules previously cooled in the ice-water. The tyrosinase activity was estimated by the oxygen uptake measured by means of the Warburg apparatus and the darkening rate of the fluid measured by means of the Duboscq colorimeter just as in the case of the body fluid of *Philosamia* (HARADA and KATO, in press).

Experimental Results

1. *Effect of p-chloromercuribenzoic acid (PCMB) on the melanosis of the body fluid of* Samia cynthia: The wild silkworm, *Samia cynthia*, is an allied species of *Philosamia cynthia ricini*. They were collected from the field. However, it was rather difficult to collect ample number of the insects in the vicinity of Kyoto. Therefore, only the effect of PCMB was tested, for it is regarded as the most strong SH reagent. The result is given in Table 1 and Fig. 1.

PCMB (final concentration, 1/6 saturated) can induce a conspicuous increase of the tyrosinase activity in the body fluid collected whenever after the maturation of larvae, while it cannot enhance the oxygen uptake and the darkening of the body fluid taken from the feeding larvae. These results are quite

Table 1. Effect of PCMB on the melanosis of the body fluid of *Samia cynthia* at various stages.

Developmental stage	O_2 uptake (μl)/15 min.		Percentage of increase in O_2 uptake	Percentage of increase in darkening	Acceleration of tyrosinase activity
	Control	PCMB added			
5th larval	8	8	0%	0%	—
	4	4	0	0	—
	14	14	0	0	—
Prepupal	40	61	+54	+50	+
	60	100	+69	+60	+
	50	68	+36	+40	+
	56	73	+30	+22	+
	36	43	+21	+14	+
Early pupal	78	103	+33	+22	+
Late pupal	22	30	+39	+71	+

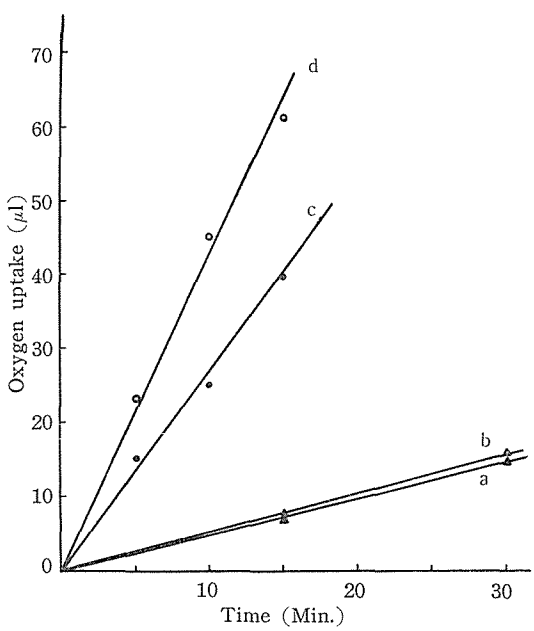

Fig. 1. Time course of the effect of PCMB on the oxygen uptake of the body fluid of *Samia cynthia*; a, in the absence of PCMB (larvae); b, in the presence of PCMB (larvae); c, in the absence of PCMB (prepupae); d, in the presence of PCMB (prepupae).

Table 2. Effect of monoiodoacetate (IAA) on the melanosis of the body fluid of *Bombyx mori*.

Developmental stage	O_2 uptake (μl)/15 min.		Percentage of increase in O_2 uptake	Darkening after 15 min.	
	Control	IAA added		Control	IAA added
Mature	56	56	0%	++	++
	43	43	0	++	++
	52	52	0	++	++
Early prepupal	51	47	− 8	++	++
	78	78	0	++	++
	76	69	− 9	++	++
	59	58	− 2	++	++
	75	67	−11	++	++
Early pupal	119	119	0	+++	+++
	113	103	− 9	+++	+++

similar to those obtained with the *Philosamia* body fluid.

2. *Effect of monoiodoacetic acid on the melanosis of the body fluid of* Bombyx mori: As is shown in Table 2, monoiodoacetic acid (final concentration, $2/3\times10^{-2}$M, and neutralized with 1 N NaOH) can cause the appreciable increase neither of the oxygen uptake nor of the darkening rate of the fluid throughout the whole metamorphosing period from the mature-larval stage to the early pupal one. This is in line with the result obtained by ITO (1953), but inconsistent with the result in our previous case in which the *Philosamia* body fluid was used (HARADA and KATO, in press).

3. *Effect of PCMB on the melanosis of the Body fluid of* Bombyx mori: The result is tabulated in Table 3. The table reveals that PCMB (final con-

Table 3. Effect of PCMB on the melanosis of the body fluid of *Bombyx mori*.

Developmental stage	O_2 uptake (μl)/15 min.		Percentage of increase in O_2 uptake	Percentage of increase in darkening	Acceleration of tyrosinase activity
	Control	PCMB added			
Mature	10	9	−10%	0%	—
	23	25	+ 9	0	—
Early prepupal	30	32	+ 7	0	—
	46	45	− 2	0	—
Middle prepupal	17	19	+12	0	—
	14	13	− 7	0	—
	8	9	+12	0	—
Just after pupation	104	104	0	+5	—
	126	121	− 4	+2	—

centration, 1/6 saturated) is also unable to enhance the tyrosinase activity. This result is different from that previously obtained with the *Philosamia* body fluid.

Discussion

As demonstrated above, the tyrosinase activity in the body fluid is accelerated by addition of SH reagent in the case of the *Samia* body fluid but not in the case of the *Bombyx* body fluid. This seems to mean that the tyrosinase activity in the *Samia* body fluid is inhibited by the endogenous SH compound, but it is doubtful in the *Bombyx* body fluid.

In this connection the following two informations are interesting, in which KATO and MIURA (1959) has established that the content of SH compound in the body fluid is considerably lower in *Bombyx mori* than in *Philosamia cynthia ricini*, and SAKAGUCHI (1959) has revealed that the tyrosinase activity in the body fluid of ty-strain of *Bombyx mori* is much lower than in the body fluid of other strains, while SH compound is more involved in the former than in the latter. From these findings we can surmise the following two possibilities: One is that in the body fluid of *Bombyx mori*, except for ty-strain, the tyrosinase action is not, somehow or other, inhibited by the endogenous SH compound. The other is that in the *Bombyx* body fluid the actual inhibition by SH compound occurs as in the *Philosamia* body fluid, but the content of SH compound is so low that only a small limited part of the tyrosinase molecules is inactivated in the intact body fluid, and therefore the blocking of SH compound by SH reagents cannot be estimated by the present method of measurement. It is now impossible, however, to determine which alternative is valid. But, the latter possibility seems probable to the author, for it seems difficult to assume a quite different inhibiting mechanism in the *Bombyx* body fluid from that in the *Samia* and *Philosamia* body fluids. The interest and implications of the problem prompt a further inquiry into this matter and cautious investigation is now being carried on.

Summary

1. The effect of SH reagents on the melanosis in the body fluid of *Bombyx mori* and *Samia cynthia* was examined.

2. PCMB was able to enhance the tyrosinase activity in the body fluid taken from the mature-larvae, prepupae and pupae of *Samia cynthia*, but it exerted no significant effect on the body fluid of larvae at the feeding stage.

3. Monoiodoacetic acid and PCMB failed to cause the increase of the tyrosinase activity in the body fluid of *Bombyx mori*. The reason for it was discussed.

4. Thus, the inhibition of the tyrosinase activity by the endogenous SH

compound is evident in the *Samia* body fluid, but it is still uncertain in the *Bombyx* body fluid.

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