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WEIGHT CHANGE OF DIAPAUSING OR DEVELOPING PUPA IN SAMIA CYNTHIA PRYERI

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Abstract

Weight of the pupa of *Samia cynthia pryeri* was measured throughout diapausing period and developing period. The weight of the pupa decreased during its diapausing period and its developing period. The rate of weight decrease was definite according to the states of the pupa respectively. The value of rate of weight decrease per day was larger during the chilling period than during the 26°C-storage period. The value of the developing pupa was the largest one and it changed with a somewhat definite pattern according to the developing process. The changing pattern of the rate of weight decrease in the artificially developing pupa with ecdysteroid injection was similar to that of the developing pupa with 26°C-incubation. The change of the rate of respiration seems mainly to be responsible for the change of the rate of weight decrease during the developing process, for the changing pattern of the former was similar to the latter. The rate of weight decrease of the dead pupa was abnormally large, and the rate of weight decrease of the pupa being die during storage changed to the larger one with death. This seems to suggest some unknown mechanisms to protect the pupa from the weight decrease during diapause.

Introduction

Near Matsumoto city with cold winter climatic conditions, it is known that a giant moth, *Samia cynthia pryeri*, winters with a diapausing pupa, then develops to a moth in next early summer. The pupa does not change its feature for a long diapausing period. When the pupae are stored in an incubator kept to 26°C, diapausing pupae survive for over a year. In a laboratory condition, it is required for development of a pupa to a moth that the diapausing pupa is subjected to the long exposure to the low temperature, for over 90 days, then it is incubated with 26°C. It is known that the pupa does not develop to the moth by the 20°C-incubation (KOENUMA, A. '85).

The pupa never eats. So it is able to consider that the weight of pupa never increases during its development. Since formation of imago is carried out during pupal

stage, it is expected that metabolic rate increases corresponding to the adult development of the pupa progresses. It is expected that the weight decrease rate of a pupa represents the metabolic level of the pupa. So in this paper, weight of the pupa was measured in the various cases, and discussions were made on the relation between the weight decrease rate and the respiratory rate of the pupa during the pupal development.

Material and Methods

Pupae used in these experiments were those of *Samia cynthia pryeri* which were collected from an outdoor rearing place about 10-day-old pupae. They were transferred to outdoor durdng the third instar from a laboratory where they hatched from the eggs laied by a female moth developed from the pupa wintered in a laboratory. Pupae were weighed by an electronic balance with one or three day interval after removal their cocoons. A "relative weight decreasing rate" was represented as a percent value of the weight decrease per day per gram fresh weight of pupa.

Pupae were weighed in following cases: (1) the case in which pupae stored in 26° C.; diapausing pupae, (2) the case in which pupae were stored in 5°C, (3) the case in which pupae were stored in 20°C after the long period 5°C-storage, over 90 days, (4) the case in which developing pupae with the 26°C-incubation after the 5°C-long period storage followed various periods of the 20°C-incubation, and (5) the case in which the artificially developing pupae with α-ecdysone injection during 20°C-incubation. In the following cases, the case (2), the case (3) and the case (4), the weight of the given individual pupa were measured through an experiment respectively. In the cases of (1) and (2), the weight changes of the injured pupae were also measured.

Respiration rates of pupae were determined by a system for the determination of photosynthesis assembled by KOITO Co. LTD. The respiration rates were estimated from the discharged CO_2. The respiration was measured in the period during the completion of the cocoon to the 35th day from pupal ecdysis. The respiration was also measured with the developing pupae with the α-ecdysone injection. The respiration rate represents the discharged CO_2 per minute per gram fresh weight of the pupa.

Results

Each typical change of the fresh weight of the two pupae was present in the figure 1, one of which was kept intact with a 20°C-incubation after the 5°C-storage for over 90 days and the other one developed with a 20°C-incubation by the α-ecdysone injection after the 5°C-storage.

In this figure, the line with the points [A] represents the weight change of the former, and the line with the points [B] represents the weight change of the latter. In

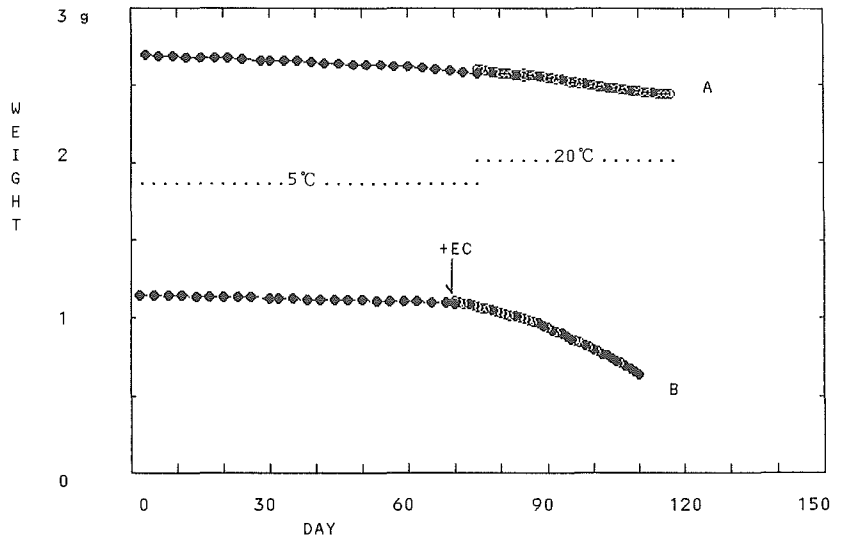

Fig. 1 Changes of the fresh weight in a diapausing pupa and a developing pupa (*Samia cynthia pryeri*)

the former case, the pupa did not change morphologically, while in the latter, the weight of the pupa decreased immediately following the ecdysone injection, then it developed to the moth for 40 days. No increase of the weight occurred in both cases.

The change of the rate of decrease in the pupal weight per day pey gram weight of the pupa was present in the figure 2. The abscissa of this figure represents the days

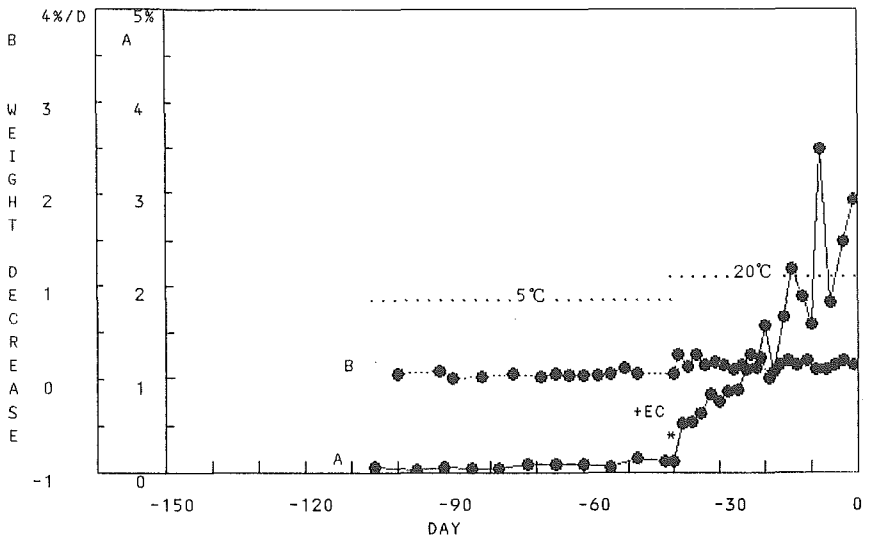

Fig. 2 Relative weight decreasing rates in a diapausing pupa and a developing pupa (*Samia cynthia pryeri*)

gone back from the finishing day of the observation and the ordinate represents the percent of weight-decrease. In this figure, each value of [B] indicates 1% larger value than each real value to avoid an overlapping each value of [A] with that of [B].

The line [A] in this figure represents the relative weight decreasing rate of the pupa with the change of the weight illustrated in the line [B] of the figure 1, while the line [B] in this figure represents the change of the weight of the pupa represented in the line [A] of the figure 1. These lines show that the weight of both pupae decrease with a definite rate less than 0.1%/day/gram respectively during the 5°C-storage period. In the [A] pupa, the relative weight decreasing rate increased rapidly according to the development during the 20°C incubation after the α-ecdysone injection. The rate became to the value of about 3.5%/day at a little before the imagination. Then the rate became the something lower just before the imagination. On the other hand, the weight of the pupa showing as [B] in this figure decreased constant with about 0.2% per day throughout the 20°C-incubating period.

Fig. 3 showed the relative weight decreasing rate of the pupae without imagination in various cases in which the pupa stored with different condition respectively. The abscissa of this figure represents the day gone back from the finish of the observation, and the ordinate represents the %-value of the rates. The lines [B] and [D] in this figure were shown by the 1% higher value than the real value to avoid an overlapping to those [A] and [C]. The line [B] represents the relative weight decreasing rate of the 26°C-stored pupa with an injury. In this case, the rate was kept to the same level with that of the intact diapausing pupa except the period just after the injury. The line

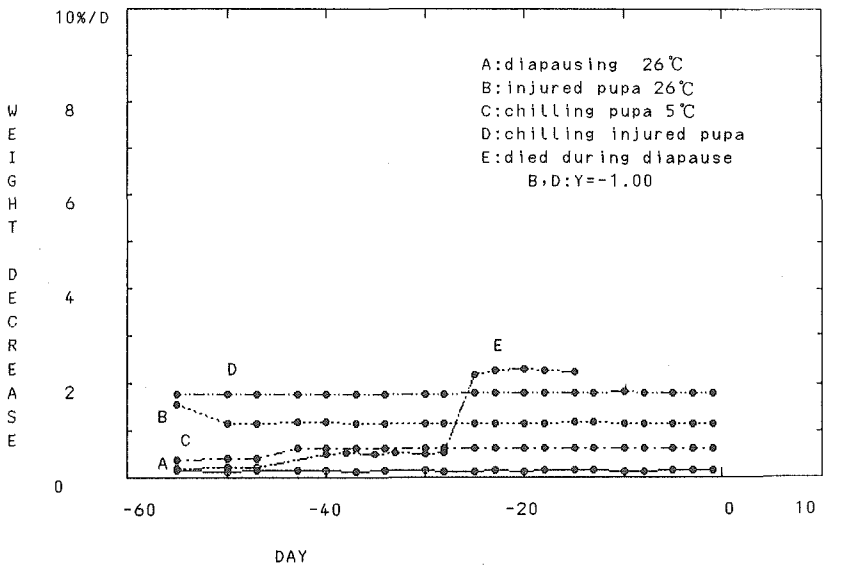

Fig. 3 relative weight decreasing rates of the pupae with various storage conditions

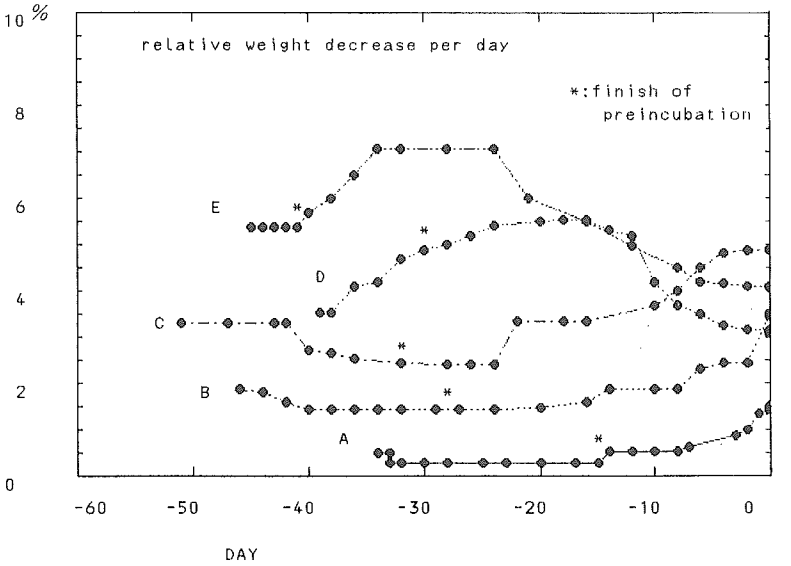

Fig. 4 Effect of length of the 20°C-storage on the weight decreasing rate of the pupae

[C] represents the relative weight decreasing rate of the 5°C-stored pupa of which chilling was initiated from the day 20 after the pupation. In this case, an increase of the rate occurred with the initiation of the 5°C-storage, then it was kept that rate during the observation. The line [D] represents the weight decreasing rate of the 5°C-stored pupa with injury. The line [E] in this figure represents an abnormal rete of weight decrease. In this case, the rate increased rapidly after the 25th day of the 26°C-storage then the pupa became hard with desiccation after the 35th day of storage, and was confirmed as death. The pupa may die at the period that the rate increased.

In the figure 4, the weight decreasing rates of the five pupae which were incubated with 26°C after the long 5°C-storage and each different period of 20°C-storage. The fate of each pupa in this figure was different, some pupae developed and others dead without imagination. The pupae experienced the 20°C-storage over 15 days developed to the moths, while the pupae experienced the 20°C-storage for 5 or 10 days did not develop. In this figure, the lines [A],[B] and [C] represent the rates of the developed pupae and the lines [D] and [E] represent the rates of the pupae failed to imagine. In this figure, each value of the line [B] was 1% higher than each value of the line [A], one of the line [C] was also 1% higher than the one of the line [B], that of the line [D] was 1% higher than that of the line [C] and that of the line [E] was 1% higher tan that of the line [D] respectively to avoid their overlap. In this figure too, the rates of weight decrease of the dead pupae increased rapidly at given period of the incubation. The similar patterns of the change of the rate among the developing pupae were observed.

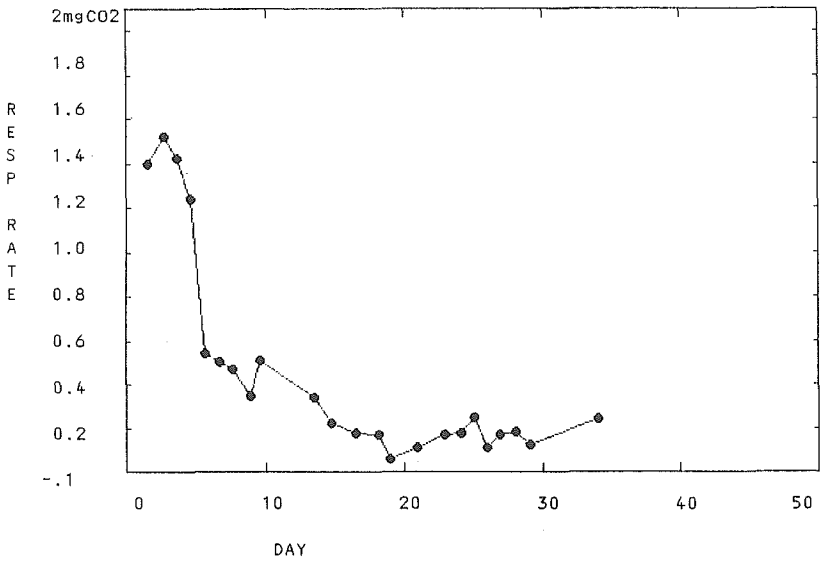

Fig. 5 Change of the CO_2 discharge rate in a larva with cocoon completion

A change of the respiratory rate with the pupation in the larva at the cocoon completion was shown in the figure 5. The level of the respiration became stable with a very low level after an initial high level. About 15 days from the beginning of the respiratory depression were required for its stabilization.

A feature of CO_2 discharge of the 19-day old diapausing pupa was shown in the figure 6. This pupa was stored with 26°C from its pupation. In this figure, abscissa represents the time and ordinate represents the difference in concentration of CO_2

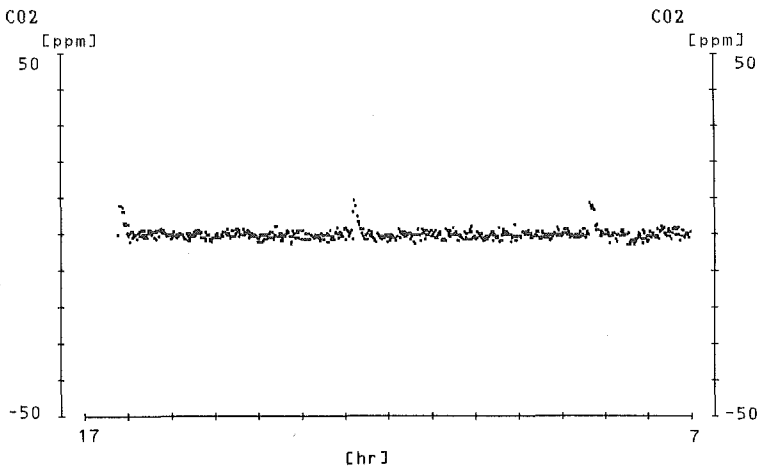

Fig. 6 CO_2 discharge of the 19-day old diapausing pupa

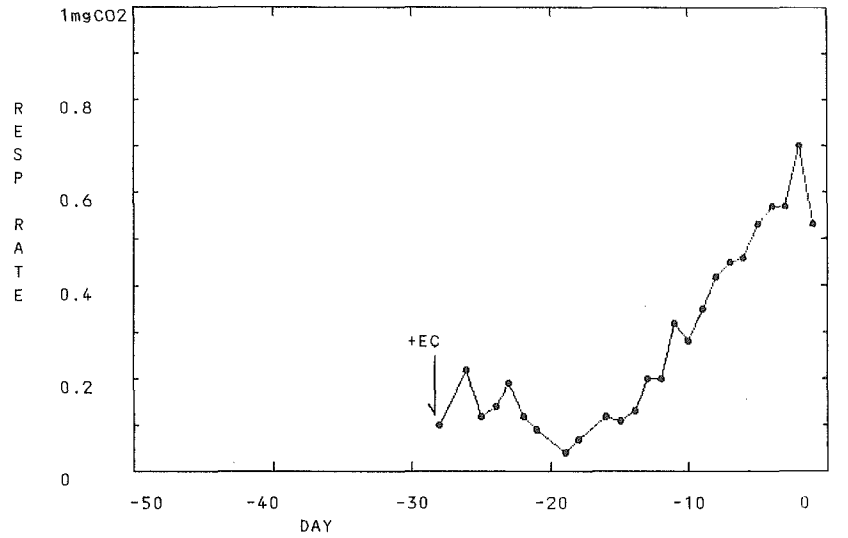

Fig. 7 Change of the CO_2 discharge of the ecdysone-injected pupa as development preceeded

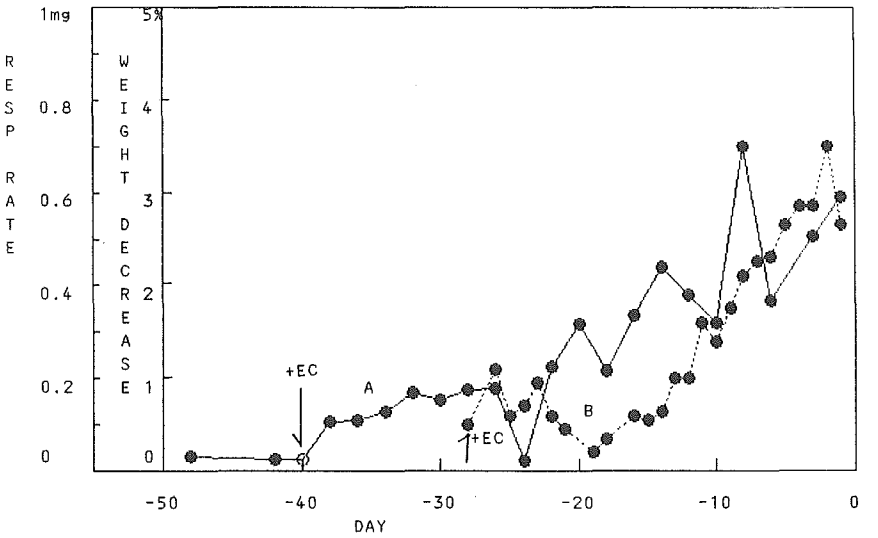

Fig. 8 Relation between the change of the CO_2 discharge rate and the rate of weight decrease in the developing pupae

between the inlet and the outlet of the respiration chamber. The CO_2 discharge in pulse is able to be observed in this case.

A change of respiration along with the development in the ecdysone injected pupa with 26°C-incubation is indicated in the figure 7. The abscissa in this figure represents days gone back from the imagination and the ordinate represents the CO_2 discharge

rate. CO_2 discharge rate decreased gradually till the 15-day after the ecdysone injection then increased rapidly to the maximal level at 1 day brfore the imagination. At the imagination day itself, the CO_2 discharge rate decreased a little. The maximum level of it reached to the about 8 times higher level than that of the minimum.

In the figure 8, the change of the CO_2 discharge rate along with the development of the ecdysone injected pupa was indicated together with the change of the rate of weight decrease of the ecdysone injected pupa. In this case, the pupa measured respiration and the pupa weighed were not the same individual. In this figure, abscissa represents the day before the imagination and the one of the ordinates and the other represents the CO_2 discharge rate and the rate of weight decrease respectively. Each day of the injection is marked by each arrow. Although the days required for the imagintion from the injection differed in both pupae, similar patterns of changes were shown between the CO_2 discharge and the weight decreasing rate of the pupae along with their development.

Discussion

The pupa of insect never eats. So it is expected that the weight of pupa never increases during its development. As it is indicated in the figure 1, the weight of pupae decreased in both cases, one continued the diapause and another developed. This is the just expected result.

As the figure 2 showed, the weight decreasing rates of the pupae failed to develop kept constant to the definite low level, while the weight decreasing rates of the pupae developed to imagines changed with the common pattern correspond to their development. In the latter cases, the rate increased gradually in the early stage of the pupal development, then it increased the more rapid as the more later developmental stage, and it reached the maximum rate at the one day before the imagination and finally it decreased a little at the day of imagination. This specific changing pattern in the weight decreasing rate is ascribed to the metabolic rate of the pupa changing with a specific pattern during development. On the other hand, the constant weight decreasing rate of the diapausing pupa is ascribed to the constant metabolic state of the diapausing pupa.

A long period storage with a low temperatwre is required for development of diapausing pupae in *S. c. pryeri* (KOENUMA, A. '85). This fact suggests that during this storage period the pupae develop without any morphological change. In fact, the weight decreasing rate of the 5°C-storage pupa was somewhat higher than that of the 26°C-storage pupa. These results mean that the 5°C-storage pupa is kept to a higher metabolic level than the 26°C-storage diapausing pupa. Therefore, it is reasonable to interpret the 5°C-storage pupa as the one of the states of developing pupa.

A fact has been reported that fates are different according to the period in which

pupae are incubated with the 20°C before the 26°C-incubation (KOENUMA, A. '85, KOENUMA, A., TSUBOUCHI, M. and KONNO, T. '86). On the one hand, the pupae failed in the normal development in the cases in which the 5°C-storage pupae were incubated with 26°C after 5-days or 10-days of 20°C-incubation. On the other hand, they developed normally in the cases in which the 5°C-storage pupae were incubated with 26°C after 15 days or 20 days of 20°C-incubation. In the former cases, the weight decreasing rates of the pupae changed with rapid increases during incubation, while in the latter cases, the weight decreasing rates of the pupae changed with a common pattern during incubation along with the process of the development. This fact is possible to interpret as follows : in the former cases, the physiological changes of the pupae with the developmental process failed in the normal occurrence, while in the latter cases, they occur normally.

Though the time required for imagination was variable according to the various conditions preceded the 26°C-incubation, the weight decreasing rates changed with a common pattern in the later developmental process. These results suggest that adult development progresses on the common temperature dependent process after the diapause of the pupa terminates. It has been reported that fluctuation in the time required for imagination of the pupa with the 20°C-incubation was smaller than that of the pupa incubated directly (KOENUMA, A. '85). This fact is well explicable as that some kinds of physiological changes are required before the initiation of adult development of the chilled pupa, and the pupae experience such physiological changes during the 20°C-incubation, then develop simultanenusly with the 26°C-incubation.

When the respiration rate of the individual was measured every day during the period from the completion of cocoon till the completion of possibly stable diapause, it resulted as follows : it decreased rapidly from the first high level to the very low but stable level for about 15 days. Since there reported that floxinophile granules of the lateral neurosecretory cells of the pupal brain began to decrease as soon as the pupation and reached in the minimum for about 15 days (KOENUMA, A. '85), the fact that the respiratory rate of the pupa become to the minimum for about 15 days after pupation suggests that the completion of the stable diapause occurred in this period. With a consideration about the fact that pupation is required 3 days after the completion of cocoon, a high level in respiratory rate at first seems to be correspond to progress of the pupation.

Pulsatile CO_2 discharge was recorded in the diapausing pupa. Such pulsatile CO_2 discharge has ever been reported by SCHNEIDERMAN, H.A. and WILLIAMS, C.M. in the diapausing cecropia pupa ('53). Both worms belong to a common family, Saturniidae, (Lepidoptera), accordingly pulsatile CO_2 discharge might to be an unique feature during pupal diapause.

Diapausing pupa develops by α-ecdysone injection (KOENUMA, A. '85, '87). Since a

close relation was obtained between the change of weight decreasing rate and the change of CO_2 discharge rate during development of the α-ecdysone injected pupa, it suggested that the metabolic rate of pupa was responsible for the weight decrease of the pupa.

The changing pattern of the weight decreasing rate of the pupae dead on the way of development were different from those of both the surviving pupae without imagination and the developed pupae to imagines. In these dead pupae, the pupal weight decreased rapidly on the way of development, and each of them became an extreme desiccated state. Since increase of the metabolic rate would not occur in the dead pupae, it is probable to regard the cause of these rapid decrease of the pupal weight as the increase of evaporation of water. In the surviving diapausig pupae, these results suggest a possibility of existence of something unknown mechanism for protection from desiccation.

Acknowledgement

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