

## The Activity of Succinic Dehydrogenase During the Metamorphosis of the Pine Moth, *Dendrolimus spectabilis* BUTLER

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松蟲의 變態에 따른 Succinic Dehydrogenase 의 活性度

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### 摘 要

松蟲(*Dendrolimus spectabilis*)의 變態에 따른 succinic dehydrogenase 의 活性度를 Thunberg 管法을 利用하여 測定하였다.

一般的으로 活性度の 變動은 器官發生과 密接한 關係를 가지고 있으며 各 器官에 있어서의 活性度는 아래와 같다.

1. Gut 는 여러 器官中 第一 높은 活性度를 보여 주며 fat body 와 더불어 U 字모양 curve 의 活性度를 나타내고 있다.
2. Brain 과 testis 는 上昇의 活性度를 보여 주었다.
3. Body wall 과 nerve cord 의 活性度는 不規則的이었다.

### INTRODUCTION

It is well known that succinic dehydrogenase plays an important role in the respiratory processes of most living cells taking part in the chain reactions in oxidation of fats, carbohydrates and proteins.

The activity of the succinic dehydrogenase during the metamorphosis was described by many workers for the various species of holometabolous insects (Wolsky, 1941; Ludwig and Barsa, 1955; Ludwig and Barsa, 1958; Agrell, 1949 and Diamantis, 1962). Krogh (1914) described that the O<sub>2</sub> consumption of holometabolous insects follows a U-shaped curve during metamorphosis. Wolsky (1941) showed that the rate of the activity of succinic dehydrogenase has been correlated with that of respiratory metabolism and Ludwig and Barsa (1955) reported that metamorphosis of the Japanese beetle is associated with a U-shaped change in the activity of succinic dehydrogenase.

So far as the authors know, there is no other report of the study on the change of activity of succinic dehydrogenase in various organs during the metamorphosis of the insect, except for the qualitative study on the succinic dehydrogenase activity in different organs of the sawfly, *Cephalacia arbutis* L. during diapause and development (Slama, 1959). The present study was undertaken to determine whether the various organs during the metamorphosis of the pine moth, *Dendrolimus spectabilis* BUTLER are associated with the change of the activity of succinic dehydrogenase.

### MATERIALS AND METHODS

The larvae were collected from the vicinity of Seoul city and maintained at room temperature (approximately 26°C) in rearing box containing pine needles.

The larvae were examined daily and at the desired age the samples were obtained from the box. The insects obtained were dissected under the stereomicroscope and the organs amputated for the study were gut, fat body, brain, testis, body wall and nerve cord. One g of the samples were homogenized in 30 ml. of phosphate buffer (0.02 M, pH 7.2) and

centrifuged at  $1,500 \times g$  for 10 minutes to remove the cell debris. The supernatant was further centrifuged at  $10,000 \times g$  for 20 minutes and the precipitate was suspended in 10 ml. of phosphate buffer. This suspension in the phosphate buffer was used as enzyme solution. The activity of succinic dehydrogenase was determined by the Thunberg method as given by Umbreit, Burris and Stauffer (1957) and triphenyltetrazolium chloride was used as the hydrogen acceptor.

In the main tube of Thunberg's tubes 1 ml. of  $10^{-5}$  M triphenyltetrazolium chloride, 1 ml. of 0.2 M succinate and 2 ml. of phosphate buffer (0.02 M, pH 7.2) were added and in the side tube 2 ml. of the enzyme solution was added. After the evacuation, the contents of both tubes were mixed and incubated at  $30^{\circ}\text{C}$ .

Triphenylformazan thus formed was extracted with 3 ml. of ethyl acetate and its optical density was determined at  $480 \text{ m}\mu$  using a Beckman spectrophotometer Model B.

### OBSERVATION

The changes in the activity of succinic dehydrogenase in various organs during metamorphosis are shown in Table 1.

**Table 1.** The activity of succinic dehydrogenase in various organs during the metamorphosis of the pine moth, *Dendrolimus spectabilis*.

Stages	Organs					
	Gut	Fat body	Brain	Testis	Body wall	Nerve cord
8-h instar larva	0.638	0.035	0.032	—	0.074	0.010
prepupa, one day	0.610	0.029	0.036	—	0.065	0.009
prepupa, four days	0.420	0.018	0.037	—	0.070	0.011
pupa, two days	0.318	0.017	0.037	0.046	0.064	0.014
pupa, five days	0.412	0.025	0.046	0.084	0.050	0.010
adult, five days	0.643	0.029	0.055	0.098	0.058	0.012

Each value is an average of three determinations. The fat body and gut exhibited U-shaped curves for the activity while the brain and testis showed increasing values.

Thy body wall and nerve cord, however, showed the irregular activity. The changes in the activity were closely associated with the development of various organs. As shown in Table 1, the activity in the gut was high at 0.638 in the larva, decreasing to 0.318 in the 2-day pupa, and increasing rapidly during the remainder of the pupal stage reaching 0.643 in the adult. Nerve cord could not be obtained in sufficient quantity to study and the extirpation of testis from the larvae and prepupae was impossible. No study was made in the enzyme activity of male and female insects.

### DISCUSSION

As can be seen in Table 1, the various organs are characterized by different succinic dehydrogenase activity.

The values for activity in the gut and brain generally were higher in the larva, prepupa and pupa, especially reaching maximum values in the adult. On the contrary, however, the activity in the fat body was maximum in the larva and decreased to a minimum in the two-day pupa, then increased again in the five-day pupa. The results show the activity is closely correlated with the changes in growth of individual organs at different developmental stages.

Wolsky (1941) with *Drosophila melanogaster*, Agrell (1949) with the blow fly, *Calliphora erythrocephalla* and Ludwig and Barsa (1955) with the Japanese beetle, *Popillia japonica* and the mealworm, *Tenebrio molitor*, reported that succinic dehydrogenase exhibited U-shaped activities during metamorphosis. Fink (1925) correlated the descending portion of curve with the histolysis of larval tissue and the ascending portion with the histogenesis of adult structures.

The present experiment is in agreement with that of Jones (1964). He stated that the activity of malic and isocitric dehydrogenases in the gut and fat body of the mealworm showed the U-shaped curves.

In the pine moth, the muscle attached to the body wall, in general, was well developed in the larva and nearly absent in the pupa. The irregular activity of the body wall may be associated with the changes in the underlying muscle mentioned above.

Jones (1964) indicated that the nerve cord of the mealworm exhibited U-shaped curve for the activity of cytochrome oxidase and Slama (1959) stated that during the pupal development of the sawfly, rapid increase of succinic dehydrogenase activity was observed in the nerve cord.

But in this experiment, the nerve cord exhibited low values and showed the irregular activity, whereas it is difficult to mention the activity of it because the nerve could not be obtained in sufficient quantity to study at extirpation. Perez (1910) and Murray and Tigs (1935) reported that the heart and the nerve cord in the blow fly persist relatively unchanged through metamorphosis, while Whitten (1961) observed that the nerve cord of the house fly undergoes considerable changes of structure during metamorphosis. Our observations were in agreement with that of Whitten (1961). The brain and the testis showed increasing activities with the growth of the insect. The increases of this activity seem to be associated with the development of brain and the maturation of testis.

### SUMMARY

The activity of succinic dehydrogenase during metamorphosis of the pine moth, *Dendrolimus spectabilis* BUTLER was determined by the Thunberg's method.

In general, the changes in the activity were closely associated with the development of organs. The results of activity obtained were as follows:

- 1) The gut showed the highest activity and exhibited U-shaped curve with the fat body.
- 2) The brain and the testis showed increasing activities.
- 3) The activities in the body wall and the nerve cord were irregular.

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